TECHNISCHE UNIVERSITÄT MÜNCHEN

Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Lehrstuhl für Molekulare Ernährungsmedizin

The role of fatty acid quality in a maternal obesogenic diet for adipose tissue development in offspring mice

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades

eines Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzende: Univ.-Prof. Dr. H. Daniel

Prüfer der Dissertation:

1. Univ.-Prof. Dr. M. Klingenspor
2. Univ.-Prof. Dr. R. Ensenauer,
   Heinrich-Heine-Universität Düsseldorf

Die Dissertation wurde am 14.04.2016 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 20.07.2016 angenommen.

Für Christiane, Karl Heinz und Sebastian

"Quo nos fata trahunt retrahuntque sequamur" - Vergil, Aen. I, 5.

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Summary

The increased prevalence for adiposity during the last decades resulted in more than one third of women in the reproductive age who are affected by overweight or obesity in Germany today. Human epidemiological and animal studies suggest an increased predisposition of the exposed offspring for a wide range of metabolic disturbances later in life. “Programming” of adipose tissue is considered essential; however, mechanisms are mainly unclear. Further, the impact of components of a maternal obesogenic diet on offspring adipocyte development is not well established.

Using an NMRI outbred mouse model, previous experiments of our research group demonstrated sex-specific disturbances of adipose tissue expandability and glucose tolerance especially in the female offspring, which were exposed to a periconceptional high-fat, high-calorie diet (HC, 60% kcal mainly from saturated fat) and transferred to lean foster dams immediately after birth, whereas male offspring were less affected. The **first** part of the PhD project aimed to assess mechanistic alterations which might be associated with these sex-specific differences in adipose tissue of offspring exposed to a maternal adipogenic milieu. Firstly, even in the absence of any postnatal HC diet feeding after weaning, exposure to a maternal adipogenic milieu *in utero* induced a reduction of fat mass and adipocyte size in the intra-abdominal adipose tissue of adult female offspring. This was accompanied by a downregulation of genes involved in inflammatory pathways and an upregulation of genes of *de novo* lipogenesis (DNL) resulting in a higher activity of its key enzyme, fatty acid synthase. Secondly, when females were fed a HC diet after weaning, exposure to a maternal adipogenic milieu increased their susceptibility towards obesity accompanied by an enlargement of adipocyte size and an impaired glucose tolerance.

Preventive strategies to improve such adverse effects of the maternal obesogenic milieu on the offspring adipose tissue phenotype are urgently needed. The **second** part of the PhD project aimed to improve the adipogenic maternal milieu via an isocaloric modification of the fatty acid quality of a HC diet using medium-chain fatty acids (MCFAs; 36% of total fatty acids) and omega-3 long-chain polyunsaturated fatty acids (Ω-3 LC-PUFAs; Ω-6:Ω-3 ratio: 2.2:1). Even though energy assimilation was similar to HC diet fed dams, the qualitative fatty acid modification of the obesogenic maternal diet resulted in an improvement of the dams’ gestational phenotype and a quantitative upregulation of nutrient transport genes in the female placentae. In the respective offspring, sex-specific effects were evident including a microarray-based detection of downregulated genes involved in cholesterol biosynthesis in female intra-abdominal adipose tissue at age 6 weeks, which was accompanied by lower plasma cholesterol levels and an approximation of adipocyte size to the control diet situation at an age of 16 weeks. In the males, body fat mass was reduced, while intra-abdominal adipocyte size and plasma cholesterol status was less affected.

In conclusion, exposure to a maternal adipogenic milieu appears to disturb the ability for appropriate adipose tissue expansion in the female offspring with activation of adipocyte DNL and development of adiposity when HC diet fed. Modification of the fatty acid quality of a maternal obesogenic diet using MCFAs and Ω-3 LC-PUFAs might be a promising approach to ameliorate the maternal milieu with possible beneficial effects on the offspring metabolic phenotype.

Zusammenfassung

Aufgrund des Anstiegs der Prävalenz für Fettleibigkeit sind derzeit mehr als ein Drittel der Frauen im fertilen Alter in Deutschland übergewichtig oder adipös. Tier- und Human-Studien belegen eine erhöhte Anfälligkeit der Kinder fettleibiger Mütter für zahlreiche metabolische Erkrankungen. Die „Programmierung“ des Fettgewebes scheint hierbei eine essentielle Rolle zu spielen, wobei die Mechanismen und der Einfluss einzelner Nährstoff-Kompontenten in einer mütterlichen adipogenen Diät auf die Fettentwicklung der Nachkommen noch weitestgehend unerforscht sind.

Unsere Forschergruppe zeigte am Auszucht-Mausstamm NMRI, dass es insbesondere bei weiblichen Nachkommen zu Störungen der Fettgewebsentwicklung und Glukose-Toleranz kommt, wenn die Nachkommen peri-konzeptionell einer mütterlichen Diät mit erhöhtem Fett- und Kaloriengehalt (HC, 60% kcal hauptsächlich aus gesättigtem Fett) ausgesetzt waren und von Kontroll-gefütterten Ammen gesäugt wurden. Der **erste** Teil der Doktorarbeit hatte zum Ziel, mögliche mechanistische Veränderungen für diese geschlechtsspezifischen Unterschiede zu untersuchen. Auch ohne HC-Fütterung der Nachkommen nach der Geburt, führte das mütterliche adipogene Milieu bei erwachsenen weiblichen Nachkommen zu einer Verminderung der Fettmasse und intra-abdominalen Fettzellgrößen. Dies ging mit einer verminderten Expression inflammatorischer Gene sowie einer erhöhten Expression von Genen der *De-novo* Lipogenese (DNL) einher, wobei auch die Aktivität des Schlüsselenzyms Fettsäuresynthase erhöht war. Bei zusätzlicher HC-Fütterung nach dem Absetzen erhöhte das mütterliche adipogene Milieu die Anfälligkeit von Weibchen für Übergewicht, vergrößerte Fettzellen und gestörte Glukosetoleranz.

Präventionsmaßnahmen zur Verbesserung der negativen Effekte des mütterlichen adipogenen Schwangerschafts-Milieus auf die Fettgewebsentwicklung der Nachkommen sind daher dringend nötig. Der **zweite** Teil der Doktorarbeit hatte zum Ziel, die mütterliche HC-Diät durch eine isokalorische Modifizierung der Fettqualität (Fat-mod HC Diät) zu verbessern, wobei dem Fettanteil MCFAs (36% des Gesamt-Fetts) und Ω-3 LC-PUFAs (Ω-6:Ω-3 ratio: 2.2:1) zugesetzt wurden. Trotz gleicher Energie-Assimilierung im Vergleich zur HC Gruppe bewirkte die Fat-mod HC Diät eine Verbesserung des mütterlichen Milieus und eine quantitative Erhöhung von Nährstofftransporter-Genen in den weiblichen Plazenten sowie geschlechtsspezifische Effekte bei den Nachkommen. Im intra-abdominalen Fettgewebe von 6 Wochen alten weiblichen Nachkommen ergab eine Microarray-Analyse eine Herunterregulierung von Genen der Cholesterol-Biosynthese, was im Alter von 16 Wochen mit reduzierten Plasma-Cholesterin-Werten und einer Annäherung der Fettzellgrößen an die Kontroll-Situation einherging. Bei den Männchen war das Körperfett vermindert, wobei die Fettzellgrößen nicht beeinflusst wurden.

Ein adipogenes intrauterines Milieu scheint insbesondere bei exponierten weiblichen Nachkommen die Fettgewebsentwicklung zu stören und zu einer Aktivierung der DNL im Fettgewebe zu führen, was bei HC-Fütterung Übergewicht begünstigt. Die Modifizierung der Fettsäure-Qualität einer mütterlichen adipogenen Diät mittels MCFAs und Ω-3 LC-PUFAs könnte ein Ansatz sein, um das mütterliche Milieu bei Übergewicht in der Schwangerschaft zu verbessern und den metabolischen Phänotyp der Nachkommen positiv zu beeinflussen.

Abbreviations

Abca1	ATP-binding cassette, sub-family A, member 1
Ac	acetylation
Acaca	acetyl-Coenzyme A carboxylase alpha
Acly	ATP citrate lyase
Acsm3	acyl-CoA synthetase medium-chain family member 3
Adipoq	adiponectin, C1Q and collagen domain containing
Agpat2	1-acylglycerol-3-phosphate O-acyltransferase 2
ALA	α-linolenic acid
AMPK	5' AMP-activated protein kinase
BAT	brown adipose tissue
BCA	bicinchoninic acid assay
Bhlhe40	basic helix-loop-helix family, member e40
BMI	body mass index
C/EBP-β	CCAAT/enhancer binding protein
C57BL/6	C57 Black 6
CD/CD	exposure to maternal CD + postnatal CD feeding
CD/HC	exposure to maternal CD + postnatal HC diet feeding
Cd36	Cd36 antigen
CEST	Central European Summer Time
Cidea	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A
Copg2	coatomer protein complex, subunit gamma 2
Cpt1	carnitine palmitoyltransferase 1
Csprs	component of Sp100-rs
Dci	dodecenoyl-Coenzyme A delta isomerase
DEG	differentially expressed gene
Dgat1	diacylglycerol O acyltransferase 1
DHA	docosahexaenoic acid
DNMT	DNA-methyltransferase
DOHaD	developmental origins of health and disease
Dpc	days post coitum
DTT	dithiothreitol
Ear11	eosinophil-associated, ribonuclease A family, member 11
Ech1	enoyl coenzyme A hydratase 1
EDTA	ethylenediaminetetraacetic acid
Egr2	early growth response 2
EPA	eicosapentaenoic acid
Ephx2	peroxisomal, epoxide hydrolase 2
FA	fatty acid
Fabp4	fatty acid binding protein 4
Fads2	fatty acid desaturase 2
Fasn	fatty acid synthase
Fat-mod HC	fat-modified high-fat, high-calorie
FDR	false discovery rate
FFA	free fatty acids
FFPE	formalin-fixed, paraffin-embedded
Gm	predicted gene
Got2	glutamatic-oxaloacetic transaminase 2 (also known as Fabp-pm, plasma membrane fatty acid binding protein)
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)
H2-M9	histocompatibility 2, M region locus 9
HAT	histon acetylase
HC	high-fat, high-calorie
HC/CD	exposure to maternal HC diet + postnatal CD feeding

HC/HC	exposure to maternal HC diet + postnatal HC diet feeding
Hdac	histone deacetylase
HDL	high density lipoprotein
HE	hematoxylin-eosin
HFD	high fat diet
Hoxc9	homeobox C9
HP	heat production
Il	interleukine
Il1rn	interleukine 1 receptor agonist
ipGTT	intraperitoneal glucose tolerance test
Irf4	interferon regulatory factor 4
LCFA	long-chain fatty acid
LDL	low-density lipoprotein
Lep	leptin
mat-Fat-mod HC	exposure to maternal fat-modified high-fat, high-calorie diet
mat-HC	exposure to maternal high-fat, high-calorie diet
MCFA	medium-chain fatty acid
Me	methyl group
Me1	malic enzyme 1
mESC	mouse embryonic stem cell
Mcp1	monocyte chemotactic protein 1
Mest	mesoderm specific transcript/imprinted paternally expressed gene 1 (also known as Peg1)
Mrc1	macrophage mannose receptor 1
MRI	magnetic resonance imaging
Mup	major urinary protein
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NBF	neutral buffered formalin
NEFA	non-esterified fatty acid
NMR	nuclear magnetic resonance
NMRI	Naval Medical Research Institute
Nr1h3	nuclear receptor subfamily 1, group H, member 3 (also known as Lxra, liver X receptor alpha)
Pcbd1	pterin 4 alpha carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 1
PFA	paraformaldehyde
Plin2	perilipin 2
Plp1	cytoplasmic proteolipid protein (myelin) 1
PMSF	phenylmethylsulfonyl fluoride
Pnpla2	patatin-like phospholipase domain containing 2 (also known as Atgl, adipose triglyceride lipase)
Pomc	pro-opiomelanocortin
Ppara	peroxisome proliferator activated receptor alpha
Pparg	peroxisome proliferator activated receptor gamma
Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
Ppib	peptidylprolyl isomerase B
RER	respiratory exchange ratio
RF	radio frequency
Rik	RIKEN cDNA
ROS	reactive oxygen species
Rps3a	ribosomal protein S3A
Rxra	retinoid X receptor, alpha
S100b	S100 protein, beta polypeptide, neural
Saa3	serum amyloid A 3
Scd2	stearoyl-Coenzyme A desaturase 2

SEM	standard error of the mean
Sfrp5	secreted frizzled-related sequence protein 5
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)
Slc2a1	solute carrier family 2 (known as Glut1, glucose transporter 1)
Slc38a2	solute carrier family 38, member 2
Snat2	sodium-dependent neutral amino acid transporter-2
Srebf1	sterol regulatory element binding transcription factor 1
SREBP	sterol regulatory element binding protein
TAG	triacylglyceride
Tbp	TATA box binding protein
Tbx1	T-box 1
TF	transcription factor
Tmem	transmembrane protein
TNF	tumor necrosis factor
Ube2d2	ubiquitin-conjugating enzyme E2D 2
Ucp1	uncoupling protein 1
Ung	uracil N-glycosylase
VCO_2	carbon dioxide production
VEGF	vascular endothelial growth factor
Vgll3	vestigial like 3 (Drosophila)
VMN	ventromedial hypothalamic nucleus
VO_2	oxygen consumption
WHO	world health organisation
WHR	waist-to-hip ratio
Zfp423	zinc finger protein 423
Ω-3 LC-PUFA	omega-3 long-chain polyunsaturated fatty acid

1. Introduction

1.1. Obesity as a public health enemy

Since 1980 the worldwide prevalence of overweight and obesity has been doubled resulting in 1.9 billion adults, who are overweight or obese today (WHO). Worldwide, obesity and its metabolic consequences are causative for more deaths compared to underweight.

1.1.1. Definition of obesity

Obesity is defined as excess fat accumulation resulting in increased levels of circulating fatty acids and pro-inflammatory cytokines, which are mainly secreted by the metabolically active visceral adipose tissue. This chronic low-grade inflammation (also called meta-flammation) provides the basis for several metabolic consequences, such as an increased risk for cardiovascular disease, type-2 diabetes, hypertension, cancer and psychosomatical complaints (3).

The body mass index (BMI) is mainly used as a simple and fast method for the determination of excess body fat. The calculation of BMI is based on body weight in kilograms, which is divided by height in meters squared (BMI= (weight (kg) / [height $(m)^2$).

According to the WHO classification, a BMI between 25 ≤ 30 is defined as overweight and a BMI >30 as obesity. To avoid the limitation of an artificial BMI classification caused by e.g. a high muscle mass, the calculation of waist-to-hip ratio (WHR) is also commonly applied as a predictor for obesity-associated disease risks. According to the “Deutsche Gesellschaft für Sportmedizin und Prävention”, overweight is classified as a WHR between 0.8-0.84 (women) or 0.9-0.99 (men) and obesity as WHR > 0.85 (women) and >1.0 (men), respectively.

In children, the classification of overweight and obesity is based on the reference system of Kromeyer-Hauschild (4). Using a reference database, children with a BMI exceeding the 90^{th} percentile are classified as overweight and with a BMI above the 97^{th} percentile as obese.

1.1.2. Prevalence of overweight and adiposity

Worldwide, 38% of men and 40% of women, older than 18 years, are overweight, while 11% of adult men and 15% of women are obese (WHO). In addition to the increasing number of people, who are affected by overweight or obesity in industrialized countries, the prevalence is even raising in low and middle income countries.

In Germany, the prevalence is increasing resulting in 67.1% of males and 53% of females at an age of 25 and 79 years, who are affected by overweight or obesity (5). In addition to the individual health threats, the treatment of obesity-related diseases induces immense costs for the German health care system, which are estimated to rise up to 22.4 billion euro in the year 2020 (6).

In the same context, the number of obese and overweight women in the reproductive age has been increasing steadily for the past years (Figure 1). Considering that more than 30% of women in the reproductive age are affected by overweight or obesity in Germany today, special attention should be given to the influence of the maternal obesogenic milieu on the metabolic development of the child.

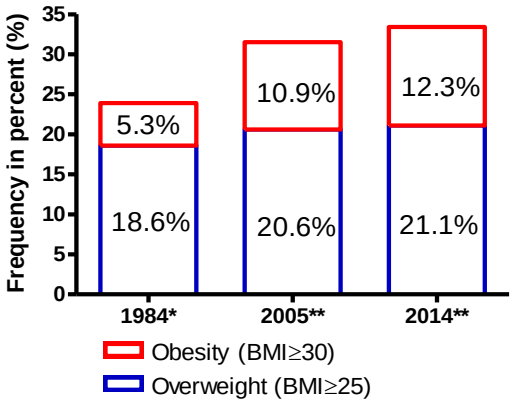

Figure 1: Increasing prevalence for overweight or obesity in women in the reproductive age (data derived from *(7) and **(8).

It is already known that the pre-conceptional BMI of women, high gestational weight gain, gestational diabetes, high birth weight, smoking and rapid postnatal weight gain negatively influence the metabolic development of children later in life (9) (10) (11). Nowadays, around 19% of adolescents (age 11 to 17 years) are affected by overweight or obesity (12), which is accompanied by an increased risk for persistent obesity, type 2 diabetes and cardiovascular disease throughout life (13).

1.1.3. Characteristics of 'fetal programming'

Fetal programming is defined as an environmental exposition during a critical phase of development, which results in an increased risk of diseases in later life.

First evidence for the critical impact of the intrauterine environment on the metabolic development of the offspring was provided by Barker et al. in the early 1990s. Based on epidemiological data in England and Wales, they showed that a low birth weight may act as a surrogate marker for an increased risk for cardiovascular disease and an up to 6-fold increased risk for type 2 diabetes later in life (14) (15). More recent studies also detected an association between an elevated birth weight and an increased predisposition for metabolic disturbances later in life (Figure 2), corroborating the decisive impact of a proper fetal development (2).

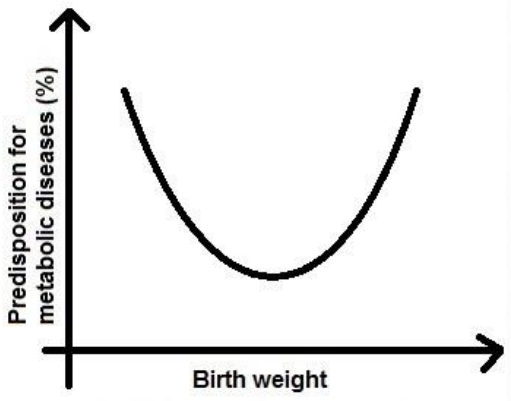

Figure 2: U-shaped relationship between a disadvantageous intra-uterine environment (induced by maternal under- or overnutrition) and an increased risk for metabolic diseases during later life of the offspring (1) (2).

The critical impact of the timing of the environmental insult was firstly indicated by the Dutch famine (16). While intrauterine exposure to caloric restriction regardless of gestational stage resulted in a disturbed glucose tolerance, very early developmental time windows seemed to be most susceptible for an adverse maternal environment. Maternal undernutrition during the first trimester of pregnancy was associated with an increased prevalence for cardiovascular diseases, an atherosclerosis-promoting plasma lipid profile and psychological strains later in life (17).

This Developmental Origins of Health and Disease (DOHaD) is supposed to be based on a mismatch between pre- and postnatal nutrient supply (18). Maternal nutrient restriction causes a predictive adaptive response during offspring development preparing the offspring for the postnatal survival under nutrient deprivation. This “programmed” thrifty phenotype of the offspring may be not appropriate in the presence of nutrient excess and lead to the development of disease.

In particular, more relevant for the current obesity epidemic, several human and animal studies have shown that maternal pre-pregnancy overweight and/or disproportionate high weight gain during pregnancy are also associated with an increased risk of adiposity and metabolic disease in the offspring (19) (20), indicating U-shaped distribution of the offspring risk for metabolic diseases (Figure 2).

Comparable to intrauterine nutrient deprivation, exposure to a maternal adipogenic milieu leads to structural and functional defects during offspring development resulting in an increased risk for metabolic diseases later in life.

1.1.4.’ Vicious cycle’ of obesity

Maternal obesity results in a ‘vicious cycle’ of obesity transmitting an enhanced risk for metabolic disturbances from the mothers to their progenies and subsequent generations.

1.1.4.1. Metabolic disturbances in obese mothers during pregnancy

In the non-pregnant state, obese women have an increased risk for metabolic disturbances such as meta-flammation, dyslipidemia, hyperinsulinemia and vascular dysfunction (21). As a

consequence of physiological adaptions, which aim to assure the optimal nutrient supply of the offspring, the disadvantageous effects of an adipogenic milieu are additionally exacerbated during obese pregnancy (Figure 3). Human placental lactogen (hPL) induces an enhanced lipolysis (22) and a higher insulin secretion promoting insulin resistance (23). Additionally, increased levels of progesterone and estrogene also affect insulin secretion by pancreatic β-cells. Further, in obese women body fat is preferentially stored as metabolically active central fat, which is known to secrete obesity-promoting adipokines and is characterized by lower insulin sensitivity and higher release of non-esterified fatty acids (NEFAs) (24). Due to the excess of free fatty acids, the mitochondrial capacity is overcharged resulting in the generation of reactive oxygen species (ROS), which is accompanied by the production of oxysterols, lipid peroxides and oxidized lipoproteins. These cytotoxic lipid metabolites may act as ligands for nuclear receptors and lead to a dysregulation of metabolic pathways and placental development (25). In addition to the adipogenic milieu at conception, these physiological adaptions during pregnancy can overcharge the metabolic capacity resulting in exacerbated gestational complications for both mother (e.g. gestational diabetes, hypertension, pre-eclampsia) and child (e.g. macrosomia, intrauterine fetal demise, congenital anormalies) (26).

1.1.4.2. Sex-specific impact on organ architecture and function in the offspring

Offspring is dia-placentally exposed to the adipogenic maternal milieu providing the basis for structural and functional defects during the intrauterine development (Figure 3). Excess glucose levels, which are transferred from the mother to the offspring, result in fetal hyperinsulinemia, which may structurally affect the ventromedial hypothalamic nucleus (VMN) resulting in a dysregulation of satiety, pancreatic insulin secretion and body weight of the offspring (27). In mice, maternal HFD feeding during lactation resulted in an impaired neuronal insulin signaling, which is supposed to disturb melanocortin projections in offspring hypothalamus (28). Further, hyperleptinemia in neonatal rats seemed to impair myocardial function resulting in a higher systolic blood pressure in adult offspring (29). In addition to the disturbances of central regulation, animal and human epidemiological studies indicate an increased predisposition for adiposity and impaired glucose tolerance of the exposed offspring (20) (30). Animal studies suggest that an intrauterine obesogenic exposure impairs insulin sensitivity and insulin secretion, which might be caused by disturbances in muscle and pancreas architecture (20). As shown in animals, maternal nutrient supply during pregnancy influences the lineage commitment of multipotent stem cells. This may result in altered promotion of myogenesis, adipogenesis or fibrogenesis disturbing the muscle physiology of the offspring (31). A predisposition of the offspring for the development of changes related to the metabolic syndrome is corroborated by the finding of an increased hepatic fat accumulation and insulin resistence in adult rat offspring exposed to maternal

obesity and gestational diabetes (32). In addition, animal and human studies suggest an increased risk for cardiovascular diseases of the exposed offspring. This may be potentially attributable to kidney dysfunction, which adds to the development of hypertension during postnatal life (33). Especially during recent years, research in animals has been focusing on the effects of an aberrant gut microbiota, which is transferred perinatally from obese mothers to their offspring, possibly affecting metabolic health during postnatal life (34).

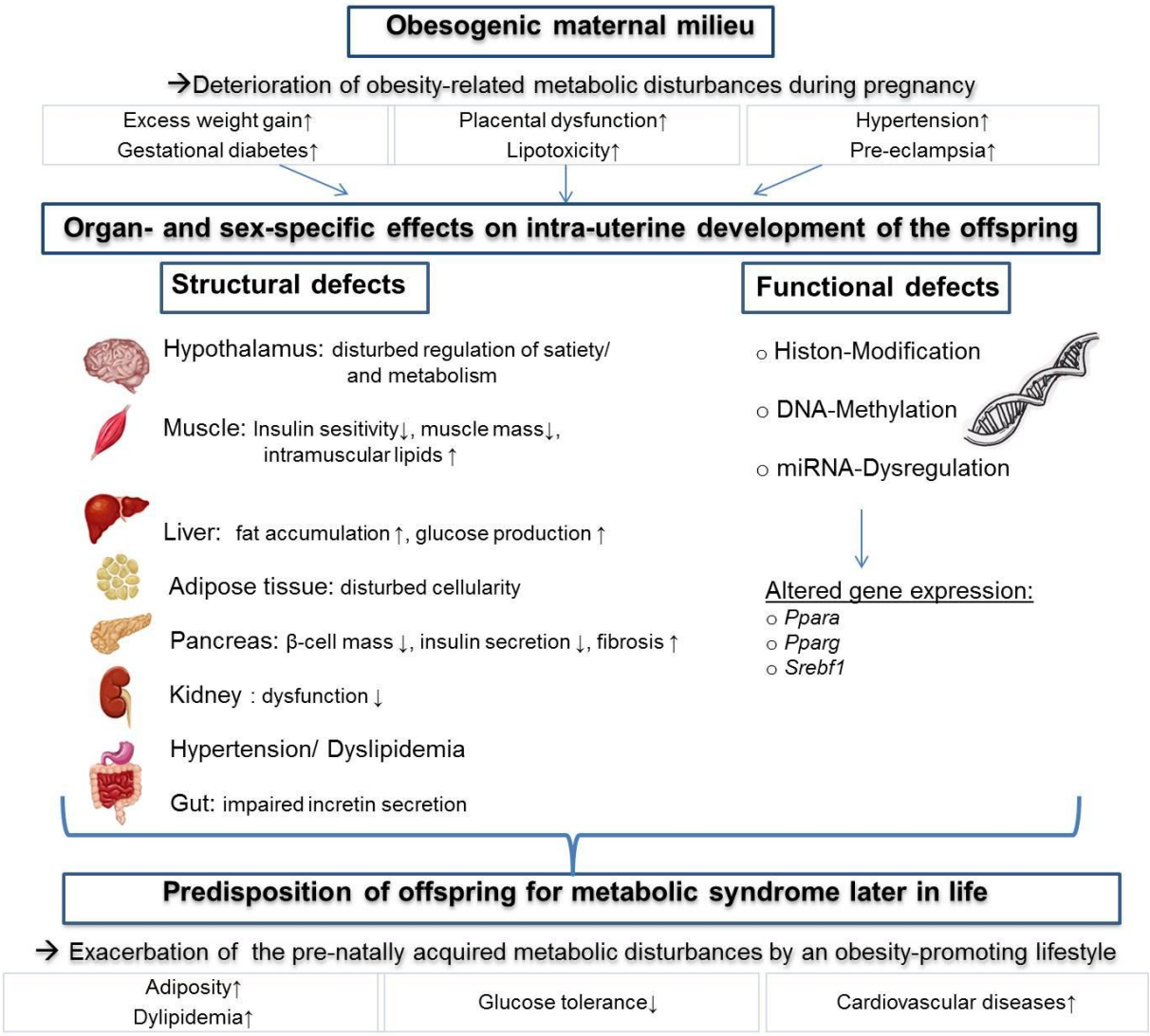

Figure 3: 'Vicious cycle' of obesity: A maternal adipogenic milieu before and during pregnancy results in numerous structural and functional defects in offspring development, which predispose the offspring for the development of metabolic diseases later on (summarizing Figure is based on (1, 20, 25, 35)).

In more recent studies, emerging attention has been paid to the sex-specific effects of a disadvantageous maternal milieu, which might be initiated even in the pre-implantation embryo (36). Looking at the placenta as the first place for the feto-maternal exchange, female placentae showed a dynamic adaptive response to Ω-3 fatty acid intake (37), maternal high fat diet and nutrient deprivation, whereas male placenta seems to be less affected (38). Accordingly, the sex-specific alterations of the dia-placental nutrient flow in response to inadequate maternal nutrient supply, directly affects the intrauterine

development in a sex-specific manner. Thus, there is an elevated risk of the male offspring for growth restriction, higher insulin and cortisol levels, hypertension, impaired nephrogenesis and deficiencies in learning abilities (39) (40). While these sex-specific developmental differences were mainly based on animal studies, the higher susceptibility of the male offspring to develop diseases later in life was also corroborated by epidemiological human studies showing an increased birth rate of girls during times of “economic” decline (41). Based on these sex differences, it is indispensable to assess the effects of the maternal milieu on offspring development separated by sex.

1.1.4.3. Epigenetic alterations in the offspring

In addition to structural defects in response to a maternal obesogenic milieu, perinatally acquired epigenetic alterations represent a pivotal point for the metabolic health of the offspring. Epigenetic regulation is based on a modification of gene expression without an alteration of the DNA sequence via DNA methylation, histone modifications and a variety of non-coding RNAs (Figure 4). It is already known that nutritional insults can affect the epigenome 1.) during the perinatal development and to a lesser extent 2.) during the later postnatal life (17): Firstly, before implantation of the embryo de-methylation of the genome takes place, which is subsequently followed by a re-arrangement of epigenetic patterns. Consequently, the “unsoiled” epigenome is highly sensitive for environmental insults during this “critical phase” of development and the maternal milieu may comprise sustainable effects on offspring development. Secondly, the epigenome can also be modified during postnatal life in response to long lasting nutrient excess or deprivation (= called “dietary transition”), whereas the regulative capacity decreases after birth when the epigenetic pattern is mostly established. Considering the high susceptibility for epigenetic modifications during very early developmental stages, the maternal periconceptional milieu appears to be most decisive for the metabolic health of the offspring.

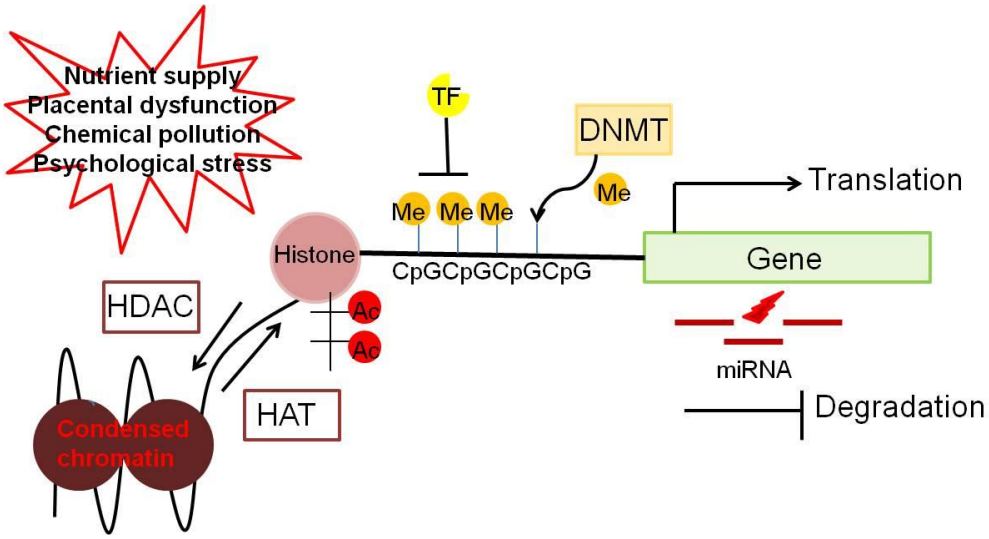

Figure 4: Epigenetic regulation is based on DNA methylation, histone modification and miRNAs: For DNA methylation, DNA-methyltransferases (DNMTs) transfer methyl-groups (Me) to cytosine within *CpG*-islands. DNA-methylation represses gene expression via inhibition of transcription factor (TF) binding, recruitment of transcriptional co-repressors and promotion of histone modification. Acetylation (Ac) of histones via histone acetylases (HAT) results in an open chromatin structure, which promotes gene expression, whereas deacetylation via histone deacetylases (HDAC) leads to a densed chromatin structure, which represses gene expression. Small non-coding RNA modulate gene expression by induction of mRNA degradation or repression of translation (summarizing Figure is based on (1) (42)).

With respect to the establishment of epigenetic alterations in the offspring, perinatal exposure to numerous environmental “threats” such as nutrient restriction or excess, placental dysfunction, chemical pollution and psychological stress can affect the epigenome of the offspring that appears to result in lasting metabolic disturbances later in life (42).

While the majority of published studies focused on the effects of maternal nutrient deprivation, several animal studies have detected various epigenetic modifications of genes in response to maternal overnutrition providing the basis for the promotion of a metabolic syndrome-like phenotype.

In rodent hypothalamus, perinatal overnutrition results in a decreased expression of pro-opiomelanocortin (POMC) via promoter hypermethylation (43) and an increased expression of dopamine and opioid-related genes caused by hypomethylation (44), which resulted in a disturbed anorexigenic gene regulation and an increased preference for highly palatable food, respectively. In offspring adipose tissue, maternal obesity leads to an altered cellularity and lipogenic capacity via histone modifications and DNA methylations of gene promotors (45). Additionally, multigenerational HFD feeding leads to DNA hypomethylation in promoter regions of several inflammatory genes in adipose tissue and was transferred across generations leading to a persistent inflammatory state (46).

Widespread epigenetic alterations (histone acetylation, DNA methylation) were detected in the liver of fetal and adult offspring in response to maternal obesity and diabetes during pregnancy (47) (48) (49).

While first studies suggest a sex-specific epigenetic regulation in female liver (50), up to now there is barely any study that assessed the epigenetic pattern separated by sex. However, the detection of global DNA hypomethylation solely in the placentae of female offspring points to a sex-specific epigenetic adaption induced by the maternal adipogenic milieu (51).

1.2. Effects of maternal obesity on adipogenesis and glycemic control in the offspring

Based on these widespread structural and functional alterations in offspring born to obese mothers, there is increasing evidence from human and animal studies determining the impact of an adipogenic milieu on glucose metabolism and adipose tissue development of the offspring.

1.2.1. Current knowledge from human studies

Considering the highly dynamic range of developmental adaptions, the particular time window of the adipogenic exposure is of major importance.

In humans, the majority of epidemiological studies determine the effects of maternal obesity before and during pregnancy (52), detecting an association between existing pre-conceptional maternal obesity and a lifelong increased risk of the offspring for adiposity (53). Recently, a higher adipogenic potential of mesenchymal stem cells was detected in the umbilical cord of children born to obese mothers, supporting an impact of maternal obesity on the very early cell lineage commitment (54). Further, an elevated BMI at conception leads to an increased birth weight and fetal insulin resistance, which is accompanied by an increased risk for adiposity and diabetes in the later life (55) (56). Additionally, also excessive weight gain during pregnancy results in an increased risk for childhood overweight and adiposity (57) (19) and seems to be most deleterious in women, who are already obese before pregnancy (58). Considering that most obese women have an elevated BMI before conception and remain obese during pregnancy, it is difficult to identify the distinct effects of an adipogenic exposure during specific critical phases of development in humans. With respect to the early postnatal life as an additional vulnerable time window, human studies suggest that breastfeeding beneficially modulate the metabolic development of the child (59). Nevertheless, especially in obese mothers the breastfeeding rates are reduced, which may deteriorate the prenatally acquired risk for metabolic disturbances in the offspring (59). Further, in humans the effects of an adipogenic intrauterine exposure may be influenced by other factors including inherited genetic pre-disposition and a common obesity-promoting lifestyle of mother and child.

1.2.2. Animal models for fetal programming of offspring obesity

In order to control the confounding effects of these influencing factors, animal studies seem to be more suitable to distinguish among the impact of the pre-gestational adipogenic milieu, gestational weight gain and the early postnatal life during lactation (60).

1.2.2.1. Differences in adipose tissue development: Man vs. mouse

Especially with respect to the adipose tissue, the developmental time window differs between mice and human resulting in a different vulnerability for environmental insults. While in larger mammals adipose tissue arrangement mainly occurs before birth, in rodents the development of early adipocyte precursors starts at the end of pregnancy comprising neural development and blood vessel formation (61). However, adipocyte maturation and lipid accumulation is completed during lactation and “early adulthood” up to an age of 14 weeks in rodents (45). In contrast, human adipose tissue development mainly occurs during pregnancy (62).

In rodents, the earliest embryonic subcutaneous adipose cells are detected at 15-16 *days post coitum* (dpc), whereas first perirenal adipose tissue appears around 12 hours before birth (63). It is supposed that adipocyte hyperplasia, which is associated with obesity, is based on a disturbed recruitment of mesenchymal stem cells to the adipocyte lineages, which results in the formation of preadipocytes followed by the proliferation and differentiation into mature adipocytes (64).

Despite immanent species differences, similar mechanisms are involved in adipose tissue expansion in altricial and precocial species (65) and thus, mice seems to be a well manageable model for the discrimination of pre- and postnatal influencing factors on adipose tissue development.

1.2.2.2. Offspring effects of maternal obesity during pregnancy and lactation

The main number of animal studies focuses on the impact of an exposure to a maternal adipogenic milieu during pregnancy and lactation detecting a metabolic-syndrome-like phenotype of the exposed offspring (20).

With respect to adipose tissue development, there is some evidence that suggests a site- and sex-specific developmental response to maternal nutrient restriction (45). However, only few studies have as yet focused on the effects of an exposure to a maternal high fat diet on adipose tissue development and glucose tolerance of the offspring in a sex-specific manner. In rats, the exposure to a maternal adipogenic milieu during pregnancy and lactation induces a quantitative and qualitative change of triglycerides stored in offspring adipose tissue, which was accompanied by increased activities of lipogenic enzymes, such as lipoprotein lipase and glycerol-3-phosphate dehydrogenase, in adulthood (66). However, regulation of adipocyte growth and function seemed to be more disturbed in the rat female offspring than

in male (67). Further, adult offspring born to obese dams showed an increased fat expansion and adipocyte hypertrophy, which were accompanied by altered mRNA expression of the peroxisome proliferator activated receptor gamma (*Pparg*) and adrenoreceptors (68). With respect to possible epigenetic adaptions in response to maternal obesity during pregnancy and lactation, adipose tissue of adult offspring exhibited a decreased leptin (*Lep*) and an increased adiponectin expression, which may be caused by alterations of histone modifications in the promoter regions of the respective genes (69).

Regarding glycemic control, the maternal obesity before and during pregnancy also affects glucose metabolism of the offspring, which can result in increased pancreatic fat content and fibrosis in adult mice (70). Insulin resistance seemed to be more pronounced in the male offspring (71). During aging, mice born to and suckled by obese mothers showed hyperinsulinemia at an age of 3 months and elevated plasma glucose levels at an age of 6 months.

In general, the predisposition of the offspring for metabolic disturbances in response to maternal obesity during pregnancy and lactation is supposed to become more apparent by an additional high fat diet (HFD) feeding after weaning. In the majority of these studies the perinatal exposure to maternal obesity exacerbates the consequence of the postnatal HFD feeding resulting in inappropriate weight gain or adiposity and a dramatically disturbed glucose/insulin regulation (30).

1.2.2.3. Offspring effects of periconceptional maternal obesity excluding exposure in the lactational period

Another experimental approach in animals is the determination of the effects of offspring exposure to maternal obesity, present at conception and during pregnancy (=which we defined as the “periconceptional period”) excluding any obesogenic exposure during lactation. An NMRI mouse model for periconceptional exposure to a maternal adipogenic milieu was established by our research group and has the critical advantage that environmental insults are limited to very early critical phases of development (72). Considering that in rodents adipocyte development is initiated *in utero*, the observed effects might be attributable to alterations during pre-adipocyte development in response to the maternal adipogenic milieu. Nevertheless, only a few studies have determined the effects of the maternal adipogenic milieu in adulthood excluding any postnatal adipogenic exposure during the suckling period by fostering using lean dams (73) (74) (75) (76) (77).

Our research group detected striking sex-specific effects of the exposure to a maternal periconceptional HC diet on offspring fat development and glucose metabolism comprising a disturbed adipose tissue expansion and glucose intolerance in the female offspring and a predisposition for adiposity, elevated liver fat accumulation and insulin resistance in the male offspring (72). These adverse effects of an intrauterine adipogenic milieu were also

corroborated by other studies detecting increased adiposity and disturbed insulin and adiponectin signaling, caused by the exclusively periconceptional fat exposure (75) (73), suggesting sex-specific differences (74).

Additionally, in rodent adipose tissue, maternal obesity results in an altered methylation pattern within *CpG* sites in the promoter region of pro-adipogenic developmental genes (such as zinc finger protein 423 (*Zfp423*), CCAAT/enhancer binding protein (*C/ebp-b*)) indicating an alteration of the intrauterine adipocyte development in offspring born to obese mothers (73). Accordingly, HFD feeding before mating and throughout gestation induces inflammatory alterations and hypertrophia in the subcutaneous adipose tissue of newborn mice, accompanied by higher fetal insulin and glucose level (78).

Already at birth, neonates exposed to maternal HFD during gestation exhibited hyperglycemia accompanied by an increased pancreatic α-cell and a decreased β-cell volume and number (79). With respect to the postnatal glycemic development of the prenatally exposed offspring, HFD exposure during gestation only, excluding any lactational influences, seems to be sufficient to induce hypoinsulinaemia in the offspring after weaning (80), whereas some recent animal studies suggest that the adipogenic effects of an intrauterine adipogenic exposure were exacerbated by a postnatal HFD feeding later on (81) (77). These studies emphasize that environmental insults even during very early phases of development may have lasting impact on the intrauterine determination of organ structure and functionality in the postnatal life.

1.3. Strategies of nutritional modification during pregnancy in rodents

In order to modulate or even prevent this prenatally acquired predisposition for metabolic disturbances, strategies for an amelioration of the maternal milieu are urgently needed (82). For the last few years, studies have focused on the distinct effects of nutritional compounds in the maternal diet which may affect fetal development in mice and humans (20) (83).

1.3.1. Effects of micronutrients

In humans, a recent randomized controlled trial indicated an association between maternal micronutrient intake (such as vitamin D, vitamin B12, magnesium, retinol, vitamin E, selenium) and neonatal size and adiposity (84). Nevertheless, the optimal micronutrient intake during overweight or obese pregnancies is far from clear in humans. At least in animal studies, the addition of genistein, quercetin or mixtures of antioxidants (such as vitamin A, C, E and selenium) to maternal obesogenic diets points to an amelioration of the obesity-associated low grade inflammatory state during pregnancy, leading to a lower prevalence for glucose intolerance, hypertension and adiposity in the offspring (85) (86), which might come along with sex-specific differences in body weight (87). Considering the effects of the prenatally acquired epigenetic effects on postnatal gene expression patterns, methyl donors

such as choline, betaine, folic acid and vitamin B12 were added to a maternal high calorie diet (45% kcal from fat) in order to counteract the effects of maternal obesity in mice (88). At least in this rodent study, this “promethylating cocktail” is supposed to influence DNA-methylation *in utero* and prevent excess postnatal fat accumulation in a sex- and age-dependent manner.

1.3.2. Impact of the carbohydrate and protein quality

With respect to the effects of the macronutrient quality, human studies suggest that a low glycemic index improves glycemic control and reduces weight gain in mothers (89), which may result in an improved environmental milieu for offspring development. Especially in the non-pregnant state, a glucose-reduced diet affects carbohydrate and lipid metabolism via activation of the 5' AMP-activated protein kinase (AMPK) leading to an increased glycolysis and fatty acid oxidation, whereas gluconeogenesis and glycogen synthesis are reduced (90) (91). An additional elevation of the dietary resistant starch content of the diet amplifies the beneficial effects of the low sugar content via its prebiotic-like properties resulting in the production of short chain fatty acids by bacterial fermentation, an enhanced secretion of satiety hormones, improved insulin tolerance and weight control (92) (93). However, there are only limited data assessing the preventive effects of an elevated starch to sugar ratio during obese pregnancy. In a previous experiment of our research group (Master Thesis: Martina Gimpfl), the improvement of the starch to sugar ratio (24:1) of a high-fat, high-calorie diet (60% kcal from fat) showed no beneficial effects on maternal milieu in our NMRI mouse model compared to an unmodified high-fat, high-calorie diet (HC) with a starch to sugar ratio of 1:24. However, sex-specific offspring effects were seen for a high maternal fructose intake (20% kcal from fructose) showing an increased hepatic fat accumulation especially in the male offspring in rodents (94). In contrast, a maternal sucrose-rich diet (26% kcal from sucrose) seems to induce glucose intolerance and adiposity in the female offspring (95). Accordingly, as suggested in a rat study, the reduction of dietary glucose and the elevation of the starch content may result in an amelioration of the maternal milieu and an improved offspring glucose tolerance (96).

The source and amount of dietary protein is considered as an important set point for the regulation of food intake, lipid and glucose metabolism via modulation of gut microbiota, stomach emptying and hormone signaling (97). In human pregnancy, a balanced protein-energy supplementation positively affects birthweight, especially in undernourished women (98). Additionally, rodent studies point to an U-shaped influence of protein intake indicating that an extremly low (10% w/w) as well as a high (40% w/w) protein intake during pregnancy and lactation might influence the metabolic development of the offspring in a sex-specific manner (99) (100), leading to disturbed glucose tolerance and body weight control, respectively. Considering these distinct effects for each individual nutritional component, it is

indispensable to use specifically matched control diets in order to guarantee a similar intake of protein, minerals, trace elements and vitamins between the experimental and the control groups and should be particularly reported for the application of HFDs.

1.3.3. Alteration of the fat quality of an obesogenic diet

Irrespective of the grade of maternal obesity, intake of a HFD (60% kcal from fat) dramatically influences offspring development in mice (101). Thus, alteration of the fatty acid profile of a maternal obesogenic diet by changing the length towards shorter chain fatty acids (medium-chain fatty acids (MCFAs)) and the degree of saturation of fatty acids (omega-3 long-chain polyunsaturated fatty acids (Ω-3 LC-PUFA)) might also influence the intrauterine development of the offspring.

1.3.3.1. Medium-chain fatty acids (MCFAs)

MCFAs consist of 8-12 carbon atoms and are mainly found in coconut oil. In the non-pregnant state, animal and human studies indicate that MCFAs lead to a reduction of body weight gain and fat mass (102) (103), whereas the effects especially on hepatic fat accumulation are highly dose-dependent (104). In contrast to long-chain fatty acids (LCFAs, ≥14 carbon atoms), MCFAs are rapidly absorbed, independent of the carnitine palmitoyltransferase 1 (CPT1) transporter system inside the mitochondrion (105). This transport mechanism of MCFAs is particularly beneficial in obesity, when the CPT1 transport system is overcharged resulting in mitochondrial stress (106). Thus, MCFAs represent an effective energy source, while only minor amounts are stored as body fat (107).

With respect to possible beneficial effects on the offspring, in the study by Dong et al. (108) exposure to a maternal MCFA-enriched diet (>30% kcal coconut oil) during pregnancy resulted in an altered hepatic expression of genes involved in lipid metabolism of the offspring. This exposure was accompanied by a lower susceptibility to HFD-induced obesity in the later life of the offspring (108).

In addition, first evidence for the advantageous effects of MCFAs on offspring outcome comes from human studies detecting an association between low dietary MCFA intake and reduced birth weight and duration of gestation (109). Despite sparse data about the mode of action of MCFAs during pregnancy, there is first evidence that MCFAs (in particular lauric acid: C12) may act as a precursor for Ω-3 LC-PUFAs, whereas myristic acid (C14) might promote the conversion of α-linolenic acid (ALA) to docosahexaenoic acid (DHA) and may additionally induce myristoylation of histones resulting in an altered gene expression in the fetus (110). Based on these promising results, further studies are urgently needed to assess the effects of maternal MCFA intake on offspring development during pregnancy.

1.3.3.2. Omega-3 long-chain polyunsaturated fatty acids (Ω-3 LC-PUFAs)

The most investigated representatives of Ω-3 PUFAs are ALA, eicosapentaenoic acid (EPA) and DHA. Vegetable oils such as flaxseed oil, walnut oil or camelina oil serve as effective sources for ALA, whereas EPA and DHA are mainly found in cold water fish such as sardines and carps or red algae. Further, DHA or EPA can be endogenously converted from ALA (111).

Over the last few years an increasing number of animal and human studies have been dealing with the preventive effects of supplementation of HFDs using Ω-3 LC-PUFAs. There is some evidence that Ω-3 LC-PUFAs are able to attenuate HFD-induced metabolic disturbances such as glucose intolerance, meta-flammation and excessive fat accumulation, just to mention the most prominent ones (112) (113). On a molecular level, Ω-3 LC-PUFAs might act as “nutrient sensors” exerting anti-adipogenic properties via binding to PPAR subclasses, suppression of nuclear receptor subfamily 1, group H, member 3 (NR1H3) and sterol regulatory element binding proteins (SREBP), resulting in an enhanced lipolysis and reduced lipogenesis, respectively (114) (115). However, especially in humans the molecular and cellular relevance of Ω-3 LC-PUFAs for the prevention of metabolic diseases are conflicting and far from clear (116). Nevertheless, at least in the non-pregnant state, *in vitro* and animal studies indicate advantageous effects of Ω-3 LC-PUFA on adiposity and body weight regulation (117) (118), whereas in both animal and human studies only very limited and inconsistent data exist about the effects of Ω-3 LC-PUFA supplementation during pregnancy (119) (120) (Figure 5).

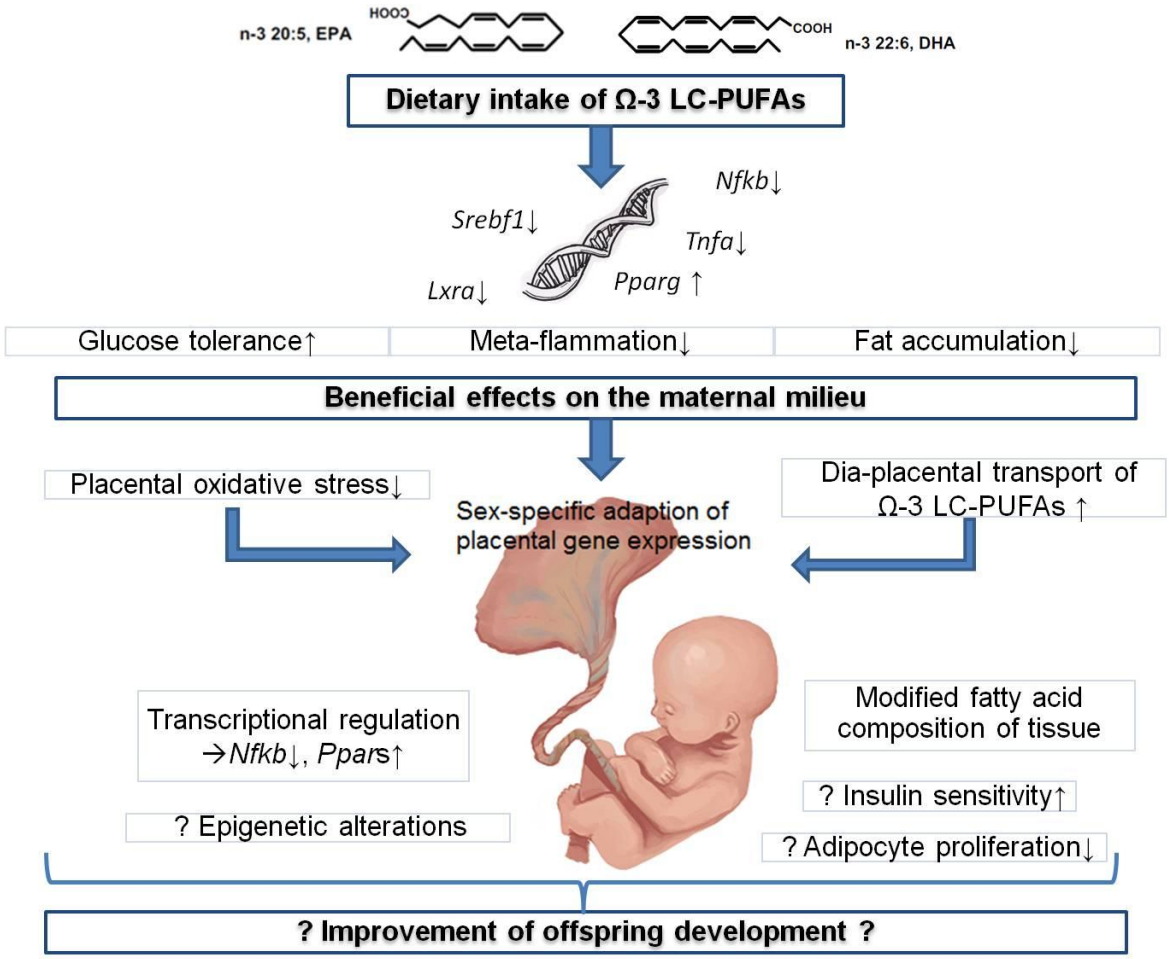

Figure 5: Possible mechanisms linking maternal Ω-3 LC-PUFA intake during pregnancy and/or lactation and metabolic development of the offspring (summarizing Figure is based on (114) (121) (122) (123)).

Animal studies indicate that an increased Ω-3 LC-PUFA content of the maternal diet (9% w/w canola oil) modulates the metabolic development of the offspring via enhanced peroxisome proliferator activated receptor-alpha (PPARA) and decreased SREBP1 expression in mouse liver (124), leading to an altered expression of target genes involved in lipid and glucose metabolism (Figure 5). In addition, in adult rat offspring endogenous PUFA synthesis seems to be affected by maternal Ω-3 LC-PUFA intake via methylation of *CpG* islands within the fatty acid desaturase 2 (*Fads2)* gene (125). Highlighting the inconstant outcomes between studies, a recent mouse study points to sex-specific effects of an Ω-3 LC-PUFA-enriched maternal diet (8% w/w fish oil + 1 % w/w sunflower oil) showing an improved insulin sensitivity and lower adiposity only in the male offspring (126). However, in another mouse study, maternal Ω-3 LC-PUFA intake (3.3% Ω-3 of total fatty acids) seemed to increase subcutaneous adipose tissue in both female and male offspring (127). As a possible explanation for these contradictory effects of maternal Ω-3 LC-PUFA intake, evidence is emerging on the essential role of a balanced Ω-6 to Ω-3 LC-PUFA ratio in the maternal diet (7% w/w soybean oil), which seems to be more crucial for adipose tissue development of the offspring in comparison to the absolute Ω-6 and Ω-3 LC-PUFA intake *per se* (128).

Furthermore, among the majority of animal studies, a huge variance is apparent in terms of the dosage and source of the applied of Ω-3 LC-PUFAs, which might responsible for the finding of inconclusive effects. The fat quantity, the source of the applied Ω-3 LC-PUFAs (krill oil vs. fish oil), the ratio of EPA:DHA and the ratio of Ω-3:Ω-6 LC-PUFAs have distinct effects on the metabolic regulation, as shown in animal studies (129). Therefore, it is indispensable to mention qualitative and quantitative variations among the applied Ω-3 LC-PUFAs in more detail, in order to allow a comprehensive interpretation of outcome parameters.

1.4. Lack of knowledge

Several animal and human studies have focussed on the critical impact of an adverse maternal milieu during early phases of development on metabolic health of the offspring. The exposure to a maternal adipogenic milieu exerts age- and organ-dependent effects on the intrauterine development of the offspring comprising structural and functional alterations of organs that may predispose the offspring to develop metabolic disturbances during postnatal life. Over the last few years, increasing evidence has pointed to sex-specific effects of the exposure to a maternal adipogenic milieu on offspring development. However, the sex-specific impact of the maternal milieu before and during pregnancy on the adipose tissue phenotype and adipocyte gene regulation is not well established. In particular, the effects of an exposure to a maternal adipogenic milieu followed by an additional postnatal HC diet feeding of the offspring on adipose tissue expansion and the consequences for each sex need further research.

In order to counteract the negative effects of an adipogenic maternal milieu, further research is urgently needed which focusses on possible preventive strategies, such as an alteration of the maternal diet or physical exercise of the mother, which may allow for modulation of the offspring development *in utero*. Emerging evidence exists for the critical role of distinct macro- and micro-nutrients in the maternal diet in obese pregnant women, such as e.g. vitamin B12, vitamin D, folic acid, fatty acids and starch, which are assumed to exert beneficial effects on the intrauterine development of the offspring. Focusing on the fatty acid quality of a maternal obesogenic diet, first promising evidence comes from human and animal studies that investigated the effects of supplementation with either MCFAs or Ω-3 LC-PUFAs on the metabolic development of the offspring.

Thus, the effects of an altered fatty acid spectrum of an obesogenic diet before and during pregnancy, using a combination of both, an enrichment with shorter-chain fatty acids (MCFAs) and a reduction of the Ω-6:Ω-3 LC-PUFA ratio, seems to be a promising approach to ameliorate the maternal milieu. However, effects of such a combined change of fat quality of an adipogenic diet on mother and offspring development have not been studied at all.

1.5. Open questions and aim

Our previous data indicated sex-specific effects of an exclusively periconceptional exposure to a maternal HC diet on the adipose tissue phenotype in the offspring, i.e. a reduced abdominal adipose tissue mass, lower adipocyte size and an impairment of fasting glucose in the female offspring. In contrast, male offspring showed an increased body fat mass, hyperinsulinemia and signs of liver steatosis (72).

Therefore, the **first research question** focused on possible mechanisms which might be associated with the development of sex-specific differences in the offspring adipose tissue phenotype and might possibly explain the disturbed fat expansion in the female offspring. We aimed to study the effects of the exposure to a maternal HC diet *in utero* on adipose tissue gene expression patterns in adult offspring in the absence of any postnatal HC diet feeding throughout life. We further aimed to corroborate candidates of dysregulated transcriptional pathways on a functional level.

Subsequently, the **second research question** addressed whether the observed effects of a periconceptional obesogenic milieu on adipose tissue and glucose metabolism in the offspring are enhanced by post-weaning HC diet feeding of the offspring. Thus, we aimed to assess the consequences of the exposure to a maternal HC diet on transcriptional regulation and cell size in adipose tissue as well as functionality and morphology of the pancreas in the adult offspring. Further, we intended to investigate effects for each sex separately.

Our previous data revealed promising effects of an alteration of the fatty acid quality of an adipogenic diet using both MCFAs and Ω-3 LC-PUFAs on maternal outcome parameters at the end of mouse pregnancy. Thus, the **third research question** of the thesis project addressed whether the modified fat quality of the maternal adipogenic diet has beneficial effects on the placenta and offspring organ development. We aimed to assess the impact of an alteration of the fatty acid quality of an adipogenic diet before and during gestation on the offspring phenotype and the organ-specific gene expression in a time- and sex-specific manner.

2. Material and Methods

2.1. Animals

Animal experiments were performed in strict compliance with the European Union recommendations for the protection of animals used for scientific purposes (Directive of the European Parliament and of the Council of 22th September 2010 [2010/63/EU]) and were approved by the Committee on Animal Health and Care of the local governmental body of the state of Bavaria. Particulars on chemicals and equipment are depicted in appendix (Table S1).

2.1.1. Origin of NMRI mice

For all experiments, three week old NMRI (Naval Medical Research Institute) mice were obtained from Janvier (Le Genest ST Isle, France). NMRI mice were originally bred in Switzerland. After transfer to USA in the year 1926, mice were maintained as an inbred strain for 51 generations and were known as NIH/PI. Afterwards, mice were introduced into the Naval Medical Research Institute giving the strain its present name. Nowadays, NMRI mice are a classical outbred strain and are characterized by an albino appearance, a fast growth rate and good mating success (Harlan Laboratories, 2008).

Compared to the inbred C57 Black 6 (C57BL/6) mice, NMRI mice remain “metabolically healthier” as a consequence of HFD feeding (130). Thus, they were chosen because we aimed to use a model of mild diet-induced maternal obesity that has less severe metabolic consequences in pregnancy and shows closer correspondence to the considerable percentage of pregnant women with overweight and obesity, but no gestational metabolic complications.

2.1.2. Housing and diets

Mouse experiments were conducted in the animal facility of Gene Center of the Ludwig-Maximilian-Universität München (LMU) (see Experiment A and Experiment B1) or at the German Mouse Clinic (GMC), Helmholtz Zentrum; München (see Experiment B2) (http://www.mouseclinic.de/).

Paws of newborn mice were tattooed. At an age of 3 weeks, mice were marked by ear piercing according to the universal mouse numbering system. Mice had free access to water and their respective diets *ad libitum*. In the closed barrier facility, the temperature was 23 °C, the humidity was 40 % and the light/dark cycle was 12 hours, turning the lights on at 7 am in the Gene Center and at 6 am in the GMC. During breeding, male and female mice were separately housed in open-top Makrolon cages type III (n=15 mice per cage) in the Gene Center or in individually ventilated cages (IVC) type II (n=2-5 mice per cage) in the GMC.

During pregnancy, dams were individually housed in Makrolon cages type II in both animal facilities.

The three experimental diets were manufactured by ssniff Spezialdiäten GmbH (Soest; Germany) and sterilized by γ-radiation. The diets were delivered via cooled transport and stored at 4°C (CD, control diet; HC diet, high-fat, high-calorie diet) and -20°C (Fat-mod HC diet, Fat-modified high-fat, high-calorie diet), respectively. Further, a standard maintenance diet (MD 1320: Altromin Spezialfutter GmbH & Co. KG, Lage; Germany) was only applied to foster dams during the pre-pregnancy period and consists of 11% of total energy from fat, 24% from protein and 65% from carbohydrates. At mating, foster dams were switched to CD during pregnancy and lactation.

The detailed composition of the three experimental diets (CD, HC diet, Fat-mod HC diet) is listed in Table 1. CD (S8102-E701, Ssniff) is the respective control to the HC diet consisting of 13% of total energy from fat, 27% from protein and 60% from carbohydrates. The HC (E15741-347, Ssniff) and Fat-mod HC (S8379-E712, Ssniff) diets were hypercaloric containing the same amount of energy. In both diets, 60% of total energy content originates from fat, 19% from protein and 21% from carbohydrates.

Table 1: Composition of the diets

	HC diet	**Fat-mod HC diet**	**Control diet**
Crude nutrients (g/100g)			
Starch	1.1	1.1	25.2
Sugar / Dextrines	24.2	24.2	27.1
Crude fat	34.6	34.6	5.1
Energy (kcal%) derived from			
Protein	19.0	19.0	27
Carbohydrates	21.0	21.0	60
Fat	60.0	60.0	13
Total energy (MJ/kg)	21.4	21.4	15.0

Fat-mod HC diet, Fat-modified high-fat, high-calorie diet; HC diet, high-fat, high-calorie diet.

The fat quality of the diets is shown in more details in Table 2 and Table S2. The fat content of the unmodified HC diet mainly consists of beef tallow, which is characterized by high amounts of saturated long-chain fatty acids (LCFA) (>C14). Soybean oil was added to assure the supply with essential fatty acids, such as linoleic acid (C18:2). The carbohydrate fraction of the HC diet contains high amounts of sucrose and glucose resulting in a low starch to sugar ratio (1:24) and a rapid metabolizability of the diet.

In the Fat-mod HC diet, the qualitative change of the fat fraction was achieved by a partial replacement of beef tallow by coconut oil, walnut oil and fish oil (called EPA oil) resulting in an increased amount of MCFAs (C8 – C12) and a lower ratio of Ω-6: Ω-3 PUFAs compared to the unmodified HC diet (Table 2). All diets were sterilized by γ-radiation. To avoid a

possible oxidation of Ω-3 PUFA caused by lasting storage at room temperature, the Fat-mod HC diet was replaced by fresh food twice a week. Similar to the HC diet, the Fat-mod HC diet was characterized by a low starch to sugar ratio (1:24).

Table 2: Characterization of fat quality

	HC diet	Fat-mod HC diet	Control diet
Fat source (g/100g)			
Beef tallow	31.5	9.5	1.0
Soybean oil	3.1	-	4.0
Walnut oil	-	3.1	-
Marine oil, Ω-3	-	2.0	-
Coconut oil	-	20.0	-
Fatty acid composition (g/100g)			
Caprylic acid (C8:0)	-	1.50	-
Capric acid (C10:0)	0.01	1.19	0.01
Lauric acid (C12:0)	0.04	8.89	0.01
Myristic acid (C14:0)	1.18	3.90	0.06
Palmitic acid (C16:0)	8.18	4.64	0.75
Palmitoleic acid (C16:1 n-7)	1.33	0.56	0.05
Stearic acid (C18:0)	6.04	2.49	0.35
Oleic acid (C18:1 n-9)	12.20	5.55	1.40
Linoleic acid (C18:2 Ω-6)	2.50	2.44	2.14
Arachidonic acid (C20:4 Ω-6)	0.08	0.04	-
Linolenic acid (C18:3 Ω-3)	0.36	0.49	0.24
EPA (C20:5 Ω-3)	-	0.37	-
Docosapentaenoic acid (C22:5 Ω-3)	-	0.05	-
DHA (C22:6 Ω-3)	-	0.24	-
Fatty acid categories (wt% of analyzed fatty acids)			
Saturated MCFAs	0.2	35.8	0.4
Saturated LCFAs	48.2	34.1	23.2
MUFAs	42.4	18.9	28.0
Ω-6 LC-PUFAs	8.1	7.7	42.8
Ω-3 LC-PUFAs	1.1	3.5	4.8
Ratio Ω-6:Ω-3 LC-PUFAs	7.2:1	2.2:1	8.9:1
Ratio EPA:DHA	-	1.5:1	-
Ratio saturated LCFAs:MCFAs	308:1	0.95:1	58:1

Beef tallow was obtained from Unimelt, Würzburg, Germany; soybean oil, marine oil (Ω-3) and coconut oil were provided by Henry Lamotte, Bremen, Germany; walnut oil was obtained from Vitaquell, Hamburg, Germany.

Fat-mod HC diet, fat-modified high-fat, high-calorie diet; HC diet, high-fat, high-calorie diet; LCFAs, long-chain fatty acids (myristic acid, palmitic acid, stearic acid); MCFAs, medium-chain fatty acids (caprylic acid, capric acid, lauric acid); MUFAs, monounsaturated fatty acids (palmitoleic acid, oleic acid); Ω-3 LC-PUFAs, Ω-3 long-chain polyunsaturated fatty acids [linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid, docosahexaenoic acid (DHA)]; Ω-6 LC-PUFAs, Ω-6 long-chain polyunsaturated fatty acids (arachidonic acid, linoleic acid).

2.2. Experimental schedule

The experimental design has already been published by our research group (72). In the PhD project, our mouse model was adapted for the assessment of the deteriorating effects of an additional HC diet feeding during the postnatal life of the offspring (2.2.1.: Experiment A) and for the determination of possible “preventive” effects of the altered fat quality of the maternal obesogenic diet on the phenotype of mothers and offspring (2.2.2.: Experiment B).

2.2.1. Exp. A: Maternal HC diet exposure followed by postnatal HC diet feeding of the offspring

The experimental schedule is depicted in Figure 6; the experiment has already been conducted by our research group before the start of the PhD project.

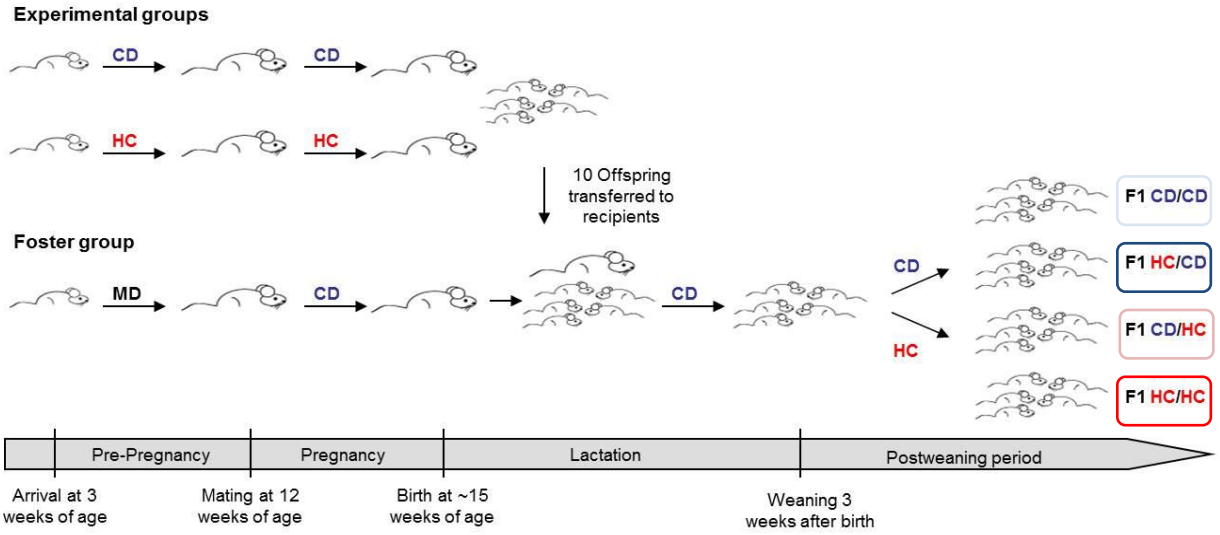

Figure 6: Experimental schedule used for the generation of maternal control diet (CD)- and high-fat, high-calorie diet (HC)-group and their offspring. Immediately after birth, each litter was reduced to a size of 10 offspring and transferred to one of the dams of the foster group, whose pups were removed. Offspring were weaned either onto CD or HC diet, generating four experimental groups: CD/CD, HC/CD, CD/HC, HC/HC which represent maternal diet versus offspring diet, respectively.
CD/CD, prenatal exposure to maternal CD + postnatal CD feeding; CD/HC, prenatal exposure to maternal CD + postnatal HC diet feeding; HC/CD, prenatal exposure to maternal HC diet + postnatal CD feeding. HC/HC, prenatal exposure to maternal HC diet + postnatal HC diet feeding.

28 female NMRI mice at the age of three weeks were randomly distributed into three groups. Two groups (n=7 mice per group) were fed by the HC diet or the CD. A third group (foster dams, n=14) received a standard maintenance rodent diet. Body weight, body fat and average food intake were assessed once per week. At the age of 12 weeks, mice were mated and screened for vaginal plugs every morning and evening. Females of the experimental groups remained on their specific diets during pregnancy. To guarantee that the suckling offspring was only exposed to CD, dams of the foster group were switched to CD during pregnancy and lactation. Food intake, body weight and body composition of the

mice were assessed every three days. All animals were allowed to give birth naturally. Within 12 hours after birth, the size of each litter of the two experimental groups (CD, HC diet) was adjusted to 10 animals. After culling of surplus pups, the experimental offspring was directly transferred to one of the foster dams, which gave birth at the same day and whose pups were removed. None of the dams were excluded due to a low offspring number or large variations in litter size. To avoid any selection influence of the experimenter, offspring were randomly chosen regardless of sex, size or other features. Offspring were weighed every three days. At day 21, offspring were weaned either onto CD or HC diet, generating four experimental groups: CD/CD, HC/CD, CD/HC, HC/HC, which represent maternal diet versus offspring diet, respectively. Male and female offspring were distributed equally between groups. Beginning at an age of three weeks, body composition and food intake were measured weekly. At the age of 5 months, an intraperitoneal glucose tolerance test (ipGTT) was performed (12h fasting, injection of 1.5g glucose/kg body weight) in subgroups of mice (CD/CD, HC/CD, CD/HC, HC/HC; n=7), as described in (72). Further, at the age of 5 months, the remaining animals were fasted for 4h, anesthetized, bled from the retroorbital plexus and killed by cervical dislocation. Organs were dissected, blotted dry, and weighed to the nearest mg. Intra-abdominal adipose tissue was removed from the abdominal cavity and comprised perigonadal, perirenal and omental fat depots. Tissue samples were immediately processed and either frozen at -80°C, fixed in 4% paraformaldehyde (PFA, pH 7.4), or fixed in RNALater (Qiagen, Hilden, Germany) and frozen at -20°C.

Using the available organs from this previous experiment, during the PhD project histological examination of the pancreas was performed in order to assess cellular alterations possibly influencing glucose tolerance. Additionally, a DNA-microarray, which was conducted in the intra-abdominal adipose tissue of the offspring, was analyzed and verified on a transcriptional level using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and on a functional level (enzyme activity).

2.2.2. Exp. B: Alteration of the fat quality of a maternal obesogenic diet

Exp. B1: Effects on dams and placental transport

The schedule of the experiment is depicted in Figure 7 and was part of the Master thesis by Martina Gimpfl (submitted in October 2012). Briefly, 30 female NMRI mice at the age of three weeks were randomly assigned to the two hypercaloric diets (experimental groups: HC diet and Fat-mod HC diet). The first group received the unmodified HC diet and the second group was fed by the Fat-mod HC diet. Body weight, body fat and food intake per group were measured weekly. At the age of 5 weeks, the individual food intake per mouse as well as the assimilated energy and energy content of feces were determined using bomb calorimetry. At the age of 12 weeks, mice were mated and screened for vaginal plugs every morning and evening. Females remained on their specific diets during pregnancy. Food intake, body

weight and body composition of the pregnant mice were determined every three days. At 16.5 dpc, an ipGTT was performed (6h fasting, injection of 1.5g glucose/kg body weight), as described in (72). After overnight fasting at 17.5 dpc, the mice were anesthetized, bled from the retroorbital plexus and sacrified by cervical dislocation and organs including 6 different fat depots (perigonadal, perirenal, omental as well as, epicardial, subcutaneous and brown adipose tissue) were dissected. Organs were weighted to the nearest mg and were either frozen at -70°C, fixed in 4% PFA (pH 7.4), or placed into RNALater (Qiagen).

In the PhD project, the maternal milieu was characterized in more detail comprising the assessment of adipocyte size of the omental adipose tissue, which is located close to the stomach extending into the ventral abdomen of the obese pregnant dams. Further, mRNA abundances in the omental adipose tissue, liver and placentae were measured (Gimpfl et. al, under review).

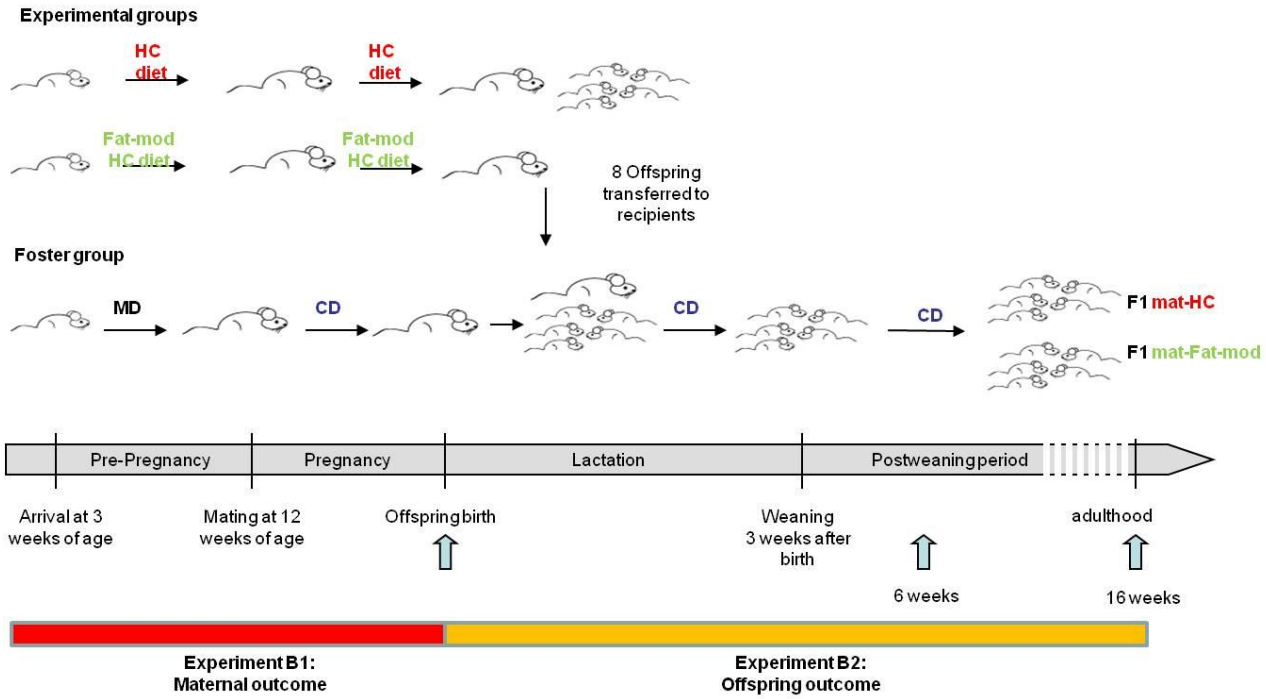

Figure 7: Experimental schedule which was used for the assessment of the effects of the maternal high-fat, high-calorie diet (HC) and fat-modified high-fat, high-calorie diet (Fat-mod HC diet) on mothers (Experiment B1) and their offspring (Experiment B2). Immediately after birth each litter was reduced to a size of 8 offspring and transferred to one of the dams of the foster group, whose pups were removed. All offspring were weaned onto CD generating two offspring groups: mat-HC and mat-Fat-mod HC representing exposure to the respective maternal diet *in utero*. Arrows depict the time of dissection of offspring subgroups at birth, 6 weeks and 16 weeks of age.

Exp. B2: Effects on the offspring

The experiment was conducted during the PhD project in cooperation with two master students (Helena Hartmann and Le Gu; Nutrition and Biomedicine, TU München), who helped to generate weight curves and data of adipose tissue development of dams/offspring.

30 female NMRI mice at the age of three weeks were randomly assigned to the two hypercaloric diets (experimental groups: HC diet and Fat-mod HC diet) and proceeded as

described above (Figure 7). Additionally, a third group (foster dams, n=30) was mated simultaneously with the two experimental groups and received CD during pregnancy and lactation. At 7.5 dpc, individual 24h food intake of all pregnant dams was determined. All animals were allowed to give birth naturally. Within 12 hours after birth, body weight and sex of the offspring were determined via assessment of the anogenital distance (Figure 8). Each litter of the two experimental groups (HC diet, Fat-mod HC diet) was adjusted to a size of 12 offspring (n=6 males; n=6 females). 4 animals (n=2 males; n=2 females per litter) were dissected immediately at birth. The remaining 8 animals (n=4 males; n=4 females per litter) were directly transferred to one of the dams of the foster group, which gave birth at the same day and whose pups were removed. Offspring were weighed every three days during the lactational phase.

At day 21, offspring were weaned to CD generating two offspring groups (mat-HC and mat-Fat-mod HC). Beginning at an age of three weeks, body composition and average food intake were measured weekly. At the age of 12 weeks, metabolic phenotyping was performed in offspring subgroups (n=10 mice per sex and subgroup; subgroups were randomly chosen) determining the physical activity, heat production and respiratory quotient of the offspring. At the age of 6 and 16 weeks, 4 animals (n=2 males; n=2 females per litter) for every time of dissection were fasted for 2-3.5 h, anesthetized by isoflurane, bled from the retroorbital plexus and killed by cervical dislocation. Organs were dissected, blotted dry, and weighed to the nearest mg. Intra-abdominal adipose tissue was removed from the abdominal cavity and comprised mainly perigonadal and a smaller amount of omental fat depots. Tissue samples were immediately processed and either frozen in liquid nitrogen, fixed in 4% neutral buffered formalin (NBF), or fixed in RNALater (Qiagen) and frozen at -20°C.

In order to assess the impact of the two hypercaloric diets (HC diet, Fat-mod HC diet) in comparison to the control situation, 15 female NMRI dams were fed the control diet before and during pregnancy, according to the experimental schedule (Figure 7). Offspring of CD-fed mothers, which were transferred to CD-fed foster dams and weaned onto CD, served as the reference group. This mat-CD offspring were used to control for the effects of exposure to the two obesogenic diets.

2.3. Sex determination of the offspring by visual inspection at birth

Immediately after birth, sex of the offspring was determined by visual inspection of the anogenital distance and the presence of milk glands (Figure 8) (Experiment B2).

The sex of the offspring, which were dissected at birth, was only based on visual inspection by two independent examiners. Sex of the offspring at weaning, when it could be recognized more clearly and unmistakably, was in agreement in 96% (mat-Fat-mod HC group) and 100% (mat-HC group) of cases between the first (at birth) and second (at weaning) assessment.

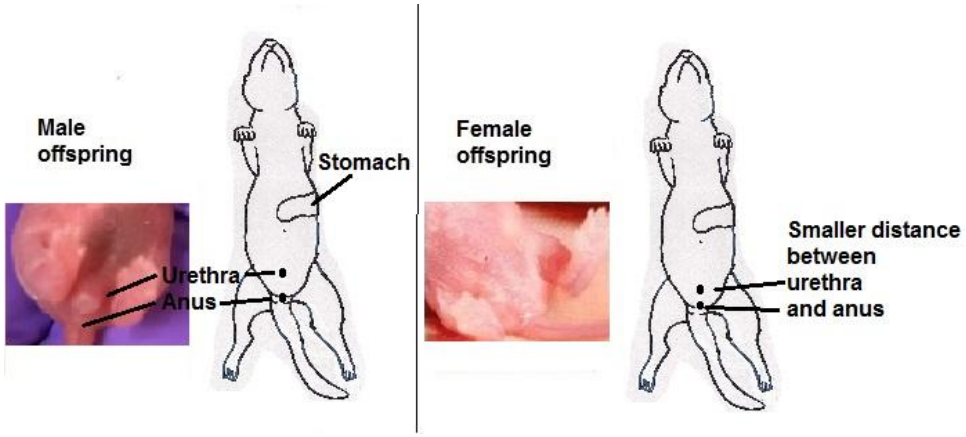

Figure 8: Sex determination at birth: Male offspring is characterized by a longer distance between anus and urethra (left). In female offspring the anogenital distance is lower (right). Additionally, in the female offspring, the presence of milk glands can be observed immediately after birth.

2.4. Body composition analysis of dams and the offspring

Once a week, fat and lean body mass were assessed by magnetic resonance imaging (MRI). The minispec LF50H Analyzer (Bruker, Karlsruhe, Germany) uses a nuclear magnetic resonance (NMR) frequency of 7.5 MHz and is appropriate for animals up to 60-70 g of weight (according to the manufacturer).

The principle of MRI is based on the application of an external homogenous magnetic field in order to determine specific interactions between an additionally applied radio frequency (RF) pulse, which is oriented perpendicularly to the external magnetic field. The resulting alterations in the resonance absorption of hydrogen nuclei (protons) are dependent on the chemical environment and indicate the body composition of the respective animal. Using this method, the fat and lean body mass of whole animals can be measured without anesthesia and without harmful radiation. Another critical advantage is the fast analysis of body composition (within 2 minutes) and the surface independency of the measurement (http://www.brukeroptics.com).

The calibration of the minispec had been carried out previously in cooperation with the technical service of Bruker. The calibration used by our research group, was based on a group of mice, which covered a wide range of distinct body weights, fat and lean masses. Prior to every analysis, a standardized sample of raps seeds (=daily check) is measured and serves for validation of the calibration. The body weight of each mouse was measured, put into the restrainer and fixed by a plastic stopper. Body composition analysis was carried out and results of fat and lean masses were related to the individual body weight.

2.5. Phenotyping of energy metabolism of the offspring by indirect calorimetry

On a regular basis we analysed the average values by measuring the food intake per cage (4-5 animals per cage) over a period of 7 days. For more detailed analyses, metabolic phenotyping was conducted in a randomly chosen subgroup of male and female offspring (Experiment B2: mat-HC and mat-Fat-mod HC, n=10 per group and sex).

At an offspring age of 12 weeks, individual energy expenditure was monitored by indirect calorimetry (Actimot2 TSE-System, 302020 series, Bad Homburg, Germany) in cooperation with Dr. J. Rozman (Institute of Experimental Genetics; GMC; Helmholtz Zentrum; München), as described in (72). In brief, CO_2 and O_2 concentrations in air volumes, flowing through a reference control cage, were compared to the alteration of concentrations in the animal cage. Based on the concurrently measured gas concentrations and the air flow through the cage, the O_2 consumption (VO_2, ml h^{-1} $animal^{-1}$) and CO_2 production (VCO_2, ml h^{-1} $animal^{-1}$) were calculated. Respiratory exchange ratio (RER) served as an indicator for metabolic fuel utilization and was calculated as the ratio VCO_2/VO_2. The calculation of the heat production (HP) is based on VO_2 and RER: HP [mW] = (4.44 + 1.43 × RER) × VO_2 [ml h^{-1}].
During the 21h-period (from 13:00 central European summer time (CEST) to 10:00 CEST next day) of metabolic phenotyping, each mouse had free access to food and water and was individually placed into a metabolic chamber, which was put into a ventilated cabinet continuously supplied with an overflow of fresh air from outside. Every 10 minutes, a sub-sample was taken from each individual mouse channel and was used for gas analysis. In addition, locomotor activity along the x-, y-, and z-axis was monitored via interruptions of laser light beams serving as a quantitative estimate for the physical activity level. In addition, body mass and food intake were analyzed individually before and after the indirect calorimetry.

2.6. Plasma analysis of biochemical parameters in the offspring

Blood was drawn in female and male offspring (Experiment B2) immediately before dissection at an age of 6 weeks and 16 weeks. After 2-3.5h of fasting, blood samples were taken from the retrobulbar plexus during anesthesia with isoflurane. Blood was treated with lithium-heparin (Kabe Labortechnik GmbH, Nümbrecht-Eisenroth, Germany) or EDTA (Kabe Labortechnik GmbH). After mixing of the blood with the anticoagulant by inversion of the sample tube, the sample was centrifugated at 5000 x g/min for 10 minutes and plasma was frozen. Lithium-heparin-plasma samples were thawed and analyzed by an Olympus AU480 autoanalyzer (Olympus, Hamburg, Germany). Adapted reagent kits from Olympus (available from Beckman Coulter, Krefeld, Germany) or Wako Chemicals GmbH (Neuss, Germany) were used for the analysis of NEFAs. Calibrations and quality controls were carried out in accordance to the protocols of the manufacturers. Blood sampling processing and analysis were performed in cooperation with Dr. B. Rathkolb (Institute of Experimental Genetics; GMC; Helmholtz Zentrum; München), according to biomedical methods outlined in (131, 132).

2.7. Morphological investigations in dams and the offspring

For the morphological investigation of liver, intra-abdominal adipose tissue and pancreas (Experiment: A), organs were fixed in 4% NBF (Bio-optica, Milano, Italy) for 24 h and were stored in 70% ethanol until further processing.

After de-hydration of the samples using a succession of ascending concentrations of ethanol, organs were embedded in paraffin (Carl Roth GmbH, Karlsruhe, Germany) and were sectioned at 5 µm by a cool-cut microtome (Thermo scientific, Wilmington, USA; HM 355). For liver and adipose tissue histology, one section per animal was put on standard microscope slides (Carl Roth GmbH, Karlsruhe, Germany). For pancreas histology, 6 sections at a distance of 50 µm were prepared for each animal and were mounted on Starfrost slides (Waldemar Knittel Glasbearbeitungs GmbH, Braunschweig, Germany), which have a better adhesive capacity. Slides were incubated at 37°C for several hours in order to guarantee that the dried tissue was stuck on the slides.

2.7.1. Staining of liver and intra-abdominal adipose tissue

For assessment of hepatic fat accumulation and adipocyte size, hematoxylin-eosin (HE) staining was performed, which is based on the physical principle of electroadsorption using the different isoelectric points of cytoplasma and nucleus (133).

At first, the organ sections on the slide were deparaffined via xylene (Carl Roth; Karlsruhe; Germany) and re-hydrated via descending concentrations of ethanol. After washing in distilled water, the organ samples were stained with HE, which results in a different colouring of cytoplasma (red) and nucleus (blue). For hematoxylin staining, the slides were incubated in Mayer's Hematoxylin (Bio-optica, Milano, Italy) for 2 minutes, rinsed by tap water (essential step for blueing) and washed by distilled water. For eosin staining, the slides were plunged in eosin (Bio-optica) for 30 seconds. After washing the slides with destilled water, the samples were dehydrated with 100% ethanol for 3 times and put into xylene again.

In a last step, the slides were embedded using mounting medium (Pertex, Medite, Burgdorf; Germany) and cover slips and were hardened overnight at 60°C. After scanning the slides by the Nano Zoomer 2.0 HT (Hamamatsu Photonics, Herrsching am Ammersee, Germany) hepatic steatosis and adipocyte size were assessed.

2.7.1.1. Assessment of liver steatosis

Grading of liver steatosis was previously performed in all dams (Experiment B1) as part of the Master Thesis (Martina Gimpfl). Hepatic fat accumulation was assed using a steatosis score (134), which was modified by Dr. A. Blutke (Institute of Veterinary Pathology at the Center for Clinical Veterinary Medicine; LMU) and has already been published by our research group (72). In brief, four liver sections per mouse were evaluated twice in a blinded manner (200x final magnification) assigning the extent of fat accumulation to 4 ascending

steatosis grades (grade 0: no steatosis - grade 4: severe macro- and microvesicular vaculation) and were additionally interpreted by an independent pathologist.

2.7.1.2. Determination of adipocyte size

Adipocyte size was assessed in all dams (Experiment B1) and a subgroup of randomly chosen female offspring (Experiment B2: mat-HC, mat-Fat-mod HC and mat-CD, n=10 per subgroup) and was determined, as described in (72) (135). In brief, images of adipose tissue were taken randomly (final magnification 200x) using a Viewing software (NDP.view2 U12388-01; Hamamatsu Photonics, Herrsching am Ammersee, Germany). After conversion of the images into binary format using ImageJ (http://rsb.info.nih.gov/ij) and GIMP (www.gimp.org), the area (µm) of 100 adipocytes per animal were determined. In case of overlapping adipocyte profiles, minor adjustments were made by hand and were matched to the original images. For Experiment B2, results were additionally validated in a subgroup (n=5 animals per group) by an independent investigator in a blinded manner revising the area of 50 adipocytes per animal again.

2.7.2. Immunohistochemistry in offspring pancreas

Immunohistochemistry is applied for the tissue-based detection of antigens using labeled antibodies. Its history goes back to the year 1941 (136), when it was particularly used for the detection of pathogens whereas the antibody specificity and mode of detection has been continuously improved ever since. Commonly, a primary antibody binds to a specific epitop (e.g. insulin) on a paraffin-fixed tissue section. After binding of an enzyme (e.g. alkaline phosphatase)-linked secondary antibody, the respective antigen can be visualized by the addition of a chromogen, which is enzymatically converted to a colorant.

2.7.2.1. Staining of pancreatic β-cells

Pancreas histology was performed in subgroups of female offspring (Experiment A: CD/CD, CD/HC, HC/HC; n=4 per group).

Pancreas sections were prepared and the staining of β-cells was conducted according to (137). Pancreas sections were deparaffinized using xylene, re-hydrated in descending concentrations of ethanol and washed in distilled water. Pancreas sections were put into 1% hydrogen peroxide solution for 15 min in order to reduce the activity of endogenous peroxidase. After 10 min washing in TBS (pH 7.4), pancreas sections were pre-incubated with normal goat serum (1:10 in TBS) for 30 min to reduce non-specific binding. For the detection of β-cells (=insulin positive cells), the slides were incubated with the first antibody (dilution: 1:500 in TBS) (Guinea pig anti-Insulin, DAKO Diagnostika, Hamburg, Germany) at room temperature in a humidity chamber for 2 h. After washing in TBS for 10 minutes, the alkaline phosphatase (AP) - conjugated second antibody (diluted 1:100 in TBS) (Goat Anti-

Guinea Pig IgG (H+L)-AP, Southern Biotech, Birmingham, USA) was applied for 1 h. The slides were washed in TBS for 10 min and immunoreactivity was visualized using the VECTOR Red AP Substrate Kit (Vector Laboratories, Burlington, Ontario, Canada). Pancreas sections were counterstained by Mayer's hemalaun solution and dehydrated in ascending concentrations of ethanol. After putting into xylene, the slides were embedded using mounting medium (HistofluidR, Marienfeld-Superior, Lauda-Königshofen, Germany) and cover slips.

2.7.2.2. Computer-based assessment of β-cell content

The determination of islet profiles was carried out on immunohistochemically stained sections using a Videoplan^{R} image analysis system (Zeiss Kontron, Eching, Germany), which was connected to a microscope by a color video camera. The cross-sectional area of the pancreas was measured by circling the cutting surface. The profiles of islets were planimetrically assessed at an 850x final magnification by defining their shape via the arrow of the digitizing tablet. The volume of the total pancreas [V(Pan)] was approached using the weight of the pancreas before embedding which was divided by the specific weight of murine pancreata (1.08 mg/mm^3). The volume density of β-cells in the pancreas [Vv(β-cell/Pan)] was determined by division of the sum of cross-sectional areas of β-cells by the pancreas area. The total β-cell volume [V(β-cell, Pan)] was calculated by multiplying [Vv(β-cell/Pan)] and [V(Pan)].

2.8. Gene expression analysis in dams and offspring

2.8.1. Microarray analysis in intra-abdominal adipose tissue of the offspring

Since the middle of the 90's, DNA-microarray analyses have been used to get a preliminary overview about the gene expression pattern, e.g. resulting from different treatment conditions.

2.8.1.1. Sample preparation and array implementation

Experiment A: DNA-microarray analysis was performed in subgroups of male and female offspring (Experiment A: CD/CD and HC/CD; n=5 per sex and group) in cooperation with the research group of Dr. Blum (Gene Center, LMU München) using Affymetrix GeneChips (Mouse Gene 1.0 ST Array, Affymetrix, Santa Clara, USA).

Adipose tissue (50-100 mg per sample) was homogenized (Silent Crusher M, Heidolph, Schwabach, Germany) in 1.2 ml of Trizol reagent (Invitrogen, Carlsbad, USA) with intermittent cooling. After removing the visible white fat layer on the top of Trizol, further preparation of total RNA was done according to the Trizol protocol. Total RNA was checked for purity and integrity and 100 ng were applied for preparation of labelled probes for microarray hybridization using Affymentrix WT cDNA synthesis, Amplification and Terminal

labelling kits (Affymetrix, Santa Clara, USA), according to the manufacturer's instruction. Briefly, total RNA was reversely transcribed into cDNA using T7 promoter-tagged random primers. cDNA was amplified by *in vitro* transcription and was reversely transcribed via random incorporation of dUTP. After fragmentation of the resulting cDNA via the Uracil N-glycosylase (UNG) and subsequently terminal labelling, probes were hybridized to Affymetrix GeneChips and scanned on an Affymetrix GeneChip Scanner 3000.

Experiment B2: In addition, RNA-microarray analysis was performed in subgroups of female offspring (Experiment B2: mat-CD, mat-HC and mat-Fat-mod HC; n=10 per sex and group) in cooperation with the research group of Prof. Beckers (Institute of Experimental Genetics; GMC; Helmholtz Zentrum; München) using Mouse Ref-8 v2.0 Expression BeadChips (Illumina, San Diego, CA, USA). After isolation of total RNA via trizol extraction, RNA was purified via RNeasy Mini Kit (Qiagen, Hilden, Germany) and RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent technologies, Waldbronn, Germany). 300 ng of high quality RNA (RIN>7) were amplified via the Illumina TotalPrep RNA Amplification kit (Ambion, Life Technologies GmbH, Darmstadt, Germany) and hybridized to the Expression BeadChips (Illumina). Staining and scanning were done according to the Illumina expression protocol. The GenomeStudioV2010.1 software (gene expression module version 1.6.0, Illumina) combined with the MouseRef-8_V2_0_R3_11278551_A.bgx annotation file was used for data processing. After subtraction of the background, the remaining negative expression values were removed by offset introduction.

2.8.1.2. Validation of the microarray

For validation of the Affymetrix microarray, a panel of 10 genes was chosen according to a consistent distribution of the calculated $\log_2$fold changes over the entire range and over all pathways, stratified by offspring sex (Appendix Table S3). After extraction of RNA from intra-abdominal adipose tissue and generation of cDNA, mRNA abundances were quantified using qRT-PCR (as described in section 2.7.2).

2.8.2. qRT-PCR analysis in adipose tissue, liver and placenta of dams and offspring

2.8.2.1. RNA extraction

Intra-abdominal adipose tissue and liver

In intra-abdominal adipose tissue, mRNA abundances were quantified in all dams (Experiment B1) and subgroups of female offspring (Experiment A: CD/CD, HC/CD, CD/HC, HC/HC; n=5 per group).

Hepatic mRNA abundances were quantified in all dams (Experiment B1). Total RNA was extracted from RNALater (Qiagen) conserved frozen intra-abdominal adipose tissue or liver samples via the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany).

Placenta

In Placenta, mRNA abundances were determined in a subgroup of 5 placenta pools per dietary group and sex (138). For sex determination, fetal DNA was extracted from tail tip (Nucleospin Tissue kit; Macherey-Nagel, Düren, Germany), according to the manufacturer. Fetal sex was assessed by PCR amplification of the sex determining region Y (SRY), which is located on the male Y-chromosome and was detected on a 1.5% agarose gel (Master Thesis: Martina Gimpfl). For subgroup selection, firstly, litters were ranked according to the weight of the dams at conception, and 5 litters were selected across the range. Secondly, placental weights of each included litter were ranked again. From each included litter, 3 placentae per sex were selected across the weight range and used for the generation of placenta pools. Each placenta was homogenized in Qiazol (miRNeasy Mini Kit, Qiagen, Hilden, Germany), and equal amounts (10 mg) of the three homogenates were pooled. Total RNA was extracted from placenta pools using the miRNeasy Mini Kit (Qiagen).

2.8.2.2. Generation of cDNA and measurement of transcriptional abundances

250 ng RNA (for adipose tissue) or 1000 ng RNA (for liver and placenta) were reversely transcribed into cDNA via the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany); according to the manufacturer's instruction.

qRT-PCR was run on the StepOnePlus System (Applied Biosystems, Foster City, USA) using Power SYBR Green (Applied Biosystems), according to the following protocol:

	Temperature	Time	Number of Cycles
Denaturation	95 °C	10 min	1x
Denaturation	95 °C	15 s	40x
Annealing	55 °C	15 s	
Extension	60 °C	60 s	
Extension	60 °C	2 min	1x

All samples were measured in duplicates and mRNA abundances were calculated relative to the mean of the reference genes ubiquitin-conjugating enzyme E2D 2 (*Ube2d2*) in intra-abdominal adipose tissue, peptidylprolyl isomerase B (*Ppib*) in liver or TATA box binding protein (*Tbp*) in placenta, as references. Details on primer sequences are shown in appendix (Table S4).

2.9. Development of a micronized FASN activity assay in intra-abdominal adipose tissue

In order to validate the results obtained on a transcriptional level, the activity of the fatty acid synthase (FASN), representing a key enzyme of *de novo* lipogenesis, was measured in

subgroups of female offspring (Experiment A: CD/CD, HC/CD, CD/HC, HC/HC; n=5 per group).

FASN, a multi-enzyme complex, catalyzes the synthesis of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA, which is accompanied by the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) (139). The principle of the assay is based on the measurement of the linear decline of NADPH by spectrophotometer reflecting the relative FASN activity. Unfortunately, published assays need high sample amounts and have not been optimized for the detection of small differences in FASN activity such as in murine tissue. Thus, we aimed to modify the original method of Nepokroeff et al. (140) and adapted the assay conditions to a 96-well format Therefore, it was necessary to reduce of the applied sample amount and to improve the sensitivity of the assay for the detection of diet-specific variations in FASN activity *in vitro* (Gimpfl et al., 2016, Methods Note in preparation).

2.9.1. Preparation of adipose tissue homogenates

Intra-abdominal adipose tissue (20-30 mg) was homogenized with 3-fold volume of ice-cooled homogenization buffer using a micropistil. To preserve the functionality of the enzyme, the homogenization of the tissue did not exceed 15-20 sec.

After centrifugation of the homogenate at 12,000 x *g* for 12 min at 4 °C, the fat phase was removed and the fat-free infranatant was aspirated with a syringe (27 G needle or insulin syringe). In a second centrifugation step at 100,000 x g and 4 °C for 1 h cell debris was removed. Immediately before the measurement of the FASN activity, a bicinchoninic acid (BCA) protein assay (Pierce BCA Protein Assay Kit; Thermo scientific, Wilmington, USA) was performed for determination of the protein amount (141), according to manufacturer's instructions.

Homogenization buffer (pH 7):
0.25 M sucrose
1 mM DTT (dithiothreitol)
1 mM EDTA (ethylenediaminetetraacetic acid)
1:50 proteinase inhibitor
1:1000 PMSF (phenylmethylsulfonyl fluoride)
→ Addition of PMSF, proteinase inhibitor and DTT at the experimental day

2.9.2. Validation of optimal reaction conditions for the fatty acid synthtase (FASN)

The following parameters were investigated and adapted in order to achieve optimal conditions for reliable measurements of the FASN activity in murine adipose tissue:

- Temperature (25 °C, 30 °C, 37 °C)
- Protein amount (10 µg – 25 µg)
- DTT concentration (final assay concentration: 0.3 mM – 1 mM),
- NADPH (final assay concentration: 0.08 mM - 0.64 mM)

For assay validation, an adipose tissue pool of MD-fed mice (n=3) was used as positive control (expected high endogenous FASN activity). In addition, an adipose tissue pool of HC diet fed mice (n=3) served as a negative control (expected low endogenous FASN activity). Assay intravariability (calculated from quintuplicates measured on one plate) and intervariability (calculated from five independent measurements of the identical sample on five plates) were used to assess the variability following determination of optimal conditions for different temperature and substrate concentrations.

2.9.3. Spectrophotometric measurement of FASN activity

After optimization of the assay conditions, FASN activity was measured in subgroups of female offspring (Experiment A: CD/CD, HC/CD, CD/HC, HC/HC; n=5 per group) in five independent measurements.

Final assay protocol:

1.) Temperature of all buffers and the measuring chamber of the heatable spectrometer (Spectrophotometer Synergy H1; Biotek, Winooski, United States) were set at 37°C.

2.) In an initial incubation step, sample homogenates, containing FASN, were re-activated at 37°C for 15 min in activation buffer and was subsequently put into 96-well plates containing pre-heated FASN buffer.

Activation buffer (pH 7.4)
500 mM potassium phosphate;
5 mM DTT
→ Addition of DTT prior to experiment

FAS-Puffer (pH7.0)
500 mM potassium phosphate,
1 mM EDTA
1 mM β-mercaptoethanol
→ Addition of EDTA and β-mercaptoethanol prior to experiment

3.) The final reaction mixture in the 96-well plate (210µl; pH 7.0) consisted of 20 µg protein, 40 mM sucrose, 400 mM potassium phosphat, 795 µM EDTA, 635 µM β-mercaptoethanol and 1 mM DTT. After addition of 160 µM NADPH, the reaction was started by adding 70 µM acetyl-CoA and 90 µM malonyl-CoA.

A blank without malonyl-CoA was simultaneously measured for each sample taking into account the aut-oxidation of NADPH. The enzymatic oxidation of NADPH was measured every 15 seconds at 340 nm over a period of 20 minutes at 37°C using a heatable spectrophotometer (Spectrophotometer Synergy H1; Biotek) equipped with the data analysis software Gen 5 1.1 (Biotek).

4.) Enzyme activity was assessed during the linear range of reaction (45-195 sec after reaction start) determining the rate of decrease in A340 per minute using $\Delta C = \Delta A/E$ [ΔC =

decrease of NADPH, ∆A = decrease of absorbance, E = extinction coefficient of NADPH (E340 nm = 6.22 mM−1*cm−1)]. Fatty acid synthase activity was expressed as nmol NADPH oxidized *min−1*mg protein−1.

2.10. Statistics

2.10.1. Subgroup selection

In Experiment A, offspring were ranked according to their relative intra-abdominal adipose tissue weight (%) adjusted to body weight and subgroups were selected across the range. In Experiment B2, offspring subgroups were randomly chosen using a randomizer (http://www.zufallsgenerator.net/).

2.10.2. Analysis of Microarray data

Affymetrix microarray (Experiment A): After normalization of the raw data using RMA1, differentially expressed genes were identified with the program LPEadj2, operating within the statistical platform R3. A false discovery rate (FDR) of 5% was set as significance threshold.
Illumina microarray (Experiment B2): After normalization of the data (quantile), the statistical programming environment R (R Development Core Team) implemented in CARMAweb (142) was used for statistical analysis. Limma *t*-test was used for identification of differentially expressed genes (FC>1.2x, p<0.05). CARMAweb was used for the generation of heatmaps and the web-tool BioVenn (http://www.cmbi.ru.nl/cdd/biovenn/) was applied for generation of Venn diagrams, which graphically represent diet-induced differentially expressed genes. Based on gene ontology (GO) categories, enrichment of diet-induced differentially expressed genes was analysed using AmiGO (http://amigo.geneontology.org/amigo). Results of the Affymetrix array and the Illumina array were additionally revised by the Database for Annotation, Visualization and Integrated Discovery (DAVID) (enrichment score> 1.3, p <0.05) or the QIAGEN's Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity), respectively. TFactsS was used to predict the transcription factors regulated by the maternal adipogenic milieu in the offspring adipose tissue (www.tfacts.org). Prediction using Tfacts was based on diet-specifically upregulated and downregulated genes compared to a database of experimentally-validated target genes (p <0.05, E-value <0.05, Q-value <0.05, FDR 5%). Additionally, KEGG (Kyoto Encyclopedia of Genes and genomes) was used for hypothesis-driven identification of differentially expressed genes (DEGs) in pathways involved in lipid metabolism and fat deposition in female offspring adipose tissue.

2.10.3. Data analysis via Graphpad and SAS

For the analysis of offspring data, differences in body weight and total fat mass were determined by analysis of variance (ANOVA) (linear mixed models; PROC MIXED) using

SAS release 8.2 (SAS Institute; Cary; NC; USA). Analysis included the fixed effects of the different maternal diets (HC diet, Fat-mod HC diet), the age, and the interaction of diet*age as well as the fixed effect of mother within diet [mother(diet)] considering that offspring of the same litter is exposed to the same maternal milieu. Individual differences were tested by Tukey's post hoc test. For analysis of placental gene expression, square root-transformed data 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 by GraphPad Prism 4.0 (GraphPad Software; San Diego; USA). Kolmogorov-Smirnov test was used for testing of normality distribution. Differences in dissection data of dams and offspring (organ and placenta weights), gene expression data (relative mRNA abundances in liver/adipose tissue/placenta, number of DEGs detected by DNA-microarray), FASN activity (specific NADPH oxidation), adipocyte size of dams, comparison of adipocyte size in offspring between an age of 6 and 16 weeks, mating characteristics (mating success, litter size, sex ratio of the offspring), plasma cholesterol status (total cholesterol, HDL, non-HDL, LDL), physical activity measured as covered distance in 15 min (cm) and respiratory quotient (VCO_2/VO_2) were analysed by unpaired t-test or non-parametric Mann-Whitney-U test. Differences in adipocyte size and plasma lipids (triglycerides, NEFAs) of the offspring (Exp.B2: mat-CD, mat-Fat-mod HC, mat-HC) and pancreatic β-cell volume in offspring (Exp.A: CD/CD, CD/HC, HC/HC) were analysed by ANOVA followed by Tukey's post hoc test.

Metabolic phenotyping data (heat production and food intake) were analysed by a linear regression model (143).

Data are presented as mean ± standard error of the mean (s.e.m.), and a p-value ≤ 0.05 was assigned as significant.

3. Results

3.1. Impact of an obesogenic diet on the maternal environment *in utero*

Our research group has already shown (72) that HC diet feeding of dams before and during pregnancy results in metabolic disturbances that we considered as “mild” (summarized in Figure 9). HC diet feeding caused an increased maternal body fat content accompanied by an impaired glucose tolerance. Further, the obese dams were characterized by elevated liver steatosis, higher concentrations of free fatty acids (FFA) and evidence of increased oxidative stress as indicated by raised levels of hydrogen peroxide in the serum. Nevertheless, dams showed no signs of inflammation or dyslipidemia. Despite these relatively “mild” metabolic alterations of maternal obesity, we previously identified long-term effects on the metabolic health of the adult offspring (72), as demonstrated in Figure 10 (blue).

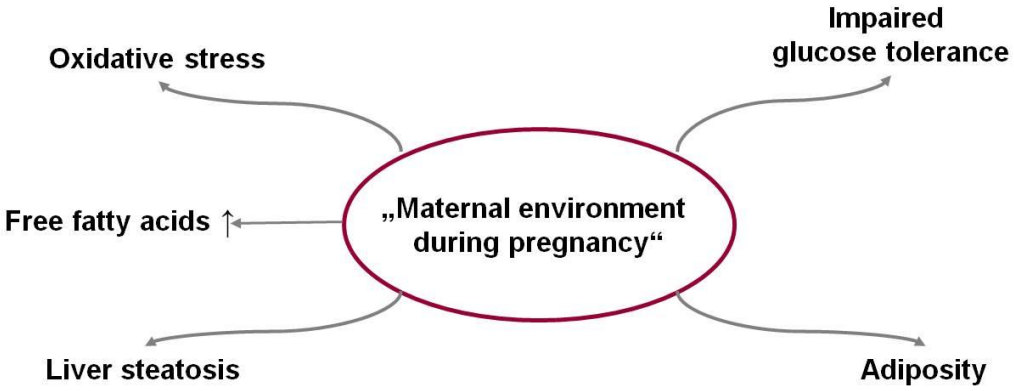

Figure 9: Adverse effects of a periconceptional adipogenic diet on dams providing the basis for offspring development *in utero* (previous data of the research group; (72)).

3.2. Offspring effects of a prenatal HC diet exposure followed by postnatal HC diet feeding

To assess the effects of periconceptional maternal obesity on the postnatal metabolic development of the progenies, the offspring was either weaned on CD or HC diet after lactation (Schedule: Experiment A).

3.2.1. Phenotypical consequences in the offspring

Offspring fed with a postnatal HC diet were compared to offspring on postnatal CD, and distinct effects are summarized in Figure 10 ((72) and Gimpfl et al., 2016; preparation of manuscript for publication).

Postnatal CD feeding: (CD/CD) vs (HC/CD)

Without any additional HC diet feeding in their entire postnatal life, adult offspring exposed to the maternal adipogenic milieu developed changes in a sex-specific manner. As shown previously by our research group, the HC/CD male offspring showed increased obesity and signs of the metabolic syndrome, such as hyperinsulinemia, hyperleptemia and

hyperuricemia compared to control males (CD/CD). Further, the male offspring (HC/CD), which were exposed to the prenatal HC diet, developed fatty liver disease in adulthood.
Contrary, the HC/CD female offspring showed fasting hyperglycemia, a reduction in total body fat mass, especially in the intra-abdominal adipose tissue, which was accompanied by a reduced mean adipocyte size (72).

Postnatal HC diet feeding: (CD/HC) vs (HC/HC)

In order to assess whether these sex-specific effects of the exposure to a maternal adipogenic milieu *in utero* are worsened by postnatal HC diet feeding, the offspring were weaned on HC diet after being nursed from lean foster dams.
In the male offspring, there was only an effect of the postnatal HC diet feeding on weight gain and glucose tolerance, whereas their exposure to maternal obesity *in utero* seemed to have only minor additional effects.
In contrast, the HC/HC female offspring showed a dramatic increase in body weight gain and fat mass, which were exacerbated by exposure to a prenatal adipogenic milieu. Additionally, the HC/HC female offspring showed a strongly impaired glucose tolerance. Details on the phenotypical characteristics of postnatally HC diet fed offspring at an age 5 months are shown in the appendix (Table S5; Gimpfl et al., 2016; preparation of manuscript for publication).

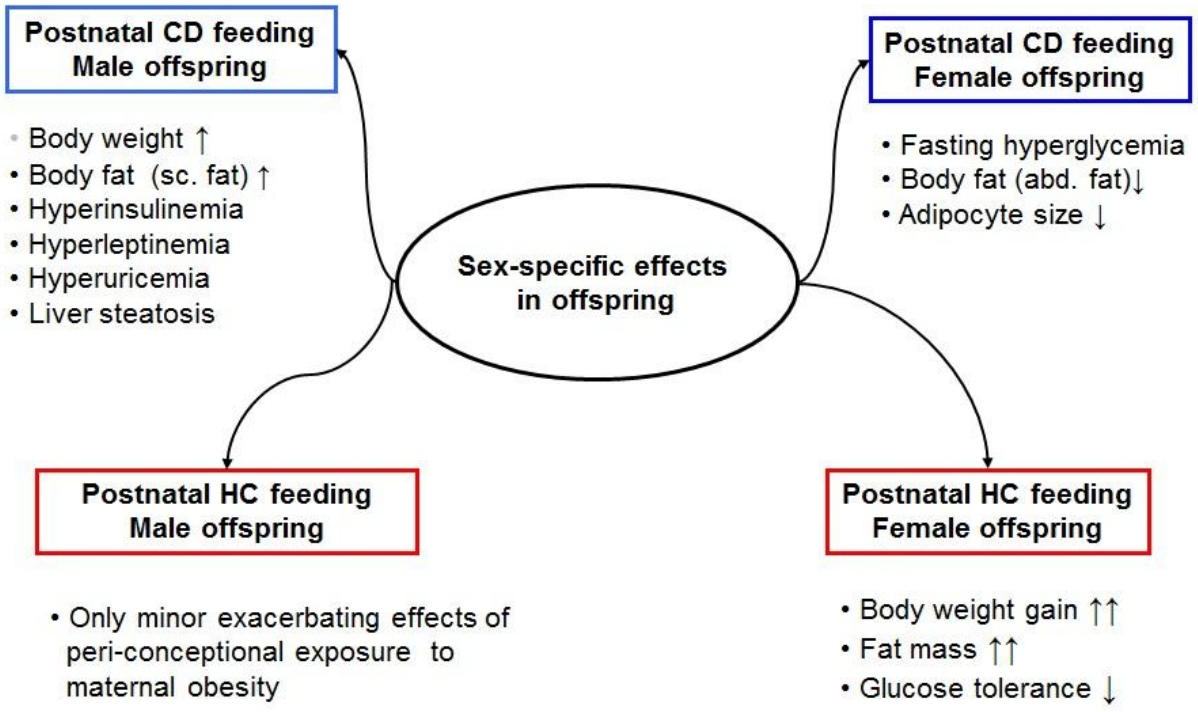

Figure 10: Adverse effects of a periconceptional exposure to a maternal adipogenic milieu on adult offspring with postnatal HC diet feeding compared to postnatal CD feeding (previous data of the research group; (72) and Gimpfl et al., 2016; preparation of manuscript for publication).

3.2.2. Transcriptional alterations in the intra-abdominal adipose tissue of the offspring

Because of the striking sex-specific consequences of the maternal adipogenic milieu for the offspring, the adipose tissue phenotype of the offspring was characterized in more detail during the PhD project.

3.2.2.1. Confirmation of the DNA-microarray results via qRT-PCR

To gain knowledge on the pure effects of a maternal adipogenic milieu on offspring adipose tissue gene regulation, we studied offspring that had not been exposed to any additional adipogenic influences throughout their postnatal life. DNA-microarray analysis was conducted in the intra-abdominal adipose tissue of the postnatally CD-fed male and female offspring at an age of 5 months. Comparison was drawn between offspring born to obese mothers (HC/CD) and to CD-fed mothers (CD/CD), separated by sex. For identification of DEGs between groups, the local pooled error method (144) was used, which has the critical advantage to be more robust towards outlier values in single genes. Due to genetic variation within our outbred NMRI strain, such outliers can be expected to occur much more often compared to an inbred mouse line. Before interpreting the microarray results, the microarray-based differences in gene expression of a selected panel of genes were evaluated using qRT-PCR. The gene panel was selected across the entire range of log_2fold changes, stratified by offspring sex. Validation via qRT-PCR showed that the direction of regulation of the majority of genes was in agreement with the DNA-microarray results (Appendix Table S3).

3.2.2.2. Annotation of differentially expressed genes in offspring adipocytes

Analysis of the DNA-microarray results separated by sex showed a comparable quantity of DEGs in response to the maternal gestational diet in the male and female offspring (128 DEGs for males and 117 for females) (Figure 11). In the male offspring, 57 genes were downregulated, whereas 71 genes were upregulated when exposed to a maternal HC diet *in utero*. In the female offspring born to obese mothers, 60 genes were diet-specifically down-regulated and 57 genes were up-regulated compared to the CD/CD females (Figure 11B). Further, there was a share of only 29 genes, which were differentially expressed in both sexes (Figure 11A, C). Only five of these DEGs (*Gm9000 /// Rps3a; 2610305D13Rik; Bhlhe40; Tmem45b; 4933409K07Rik /// Gm7819 /// Gm3893 /// Gm10590 /// LOC100503421*), which responded to maternal HC diet in males and females, revealed the same direction of regulation (Figure 11C). The majority of these similarly regulated genes were involved in RNA/DNA coding. The residual 24 diet-specifically regulated genes were inversely regulated in both sexes and were mainly attributable to transcriptional regulation. Interestingly, among the genes, which were differentially regulated in both sexes, *Cidea (*cell death-inducing DNA fragmentation factor, alpha subunit-like effector A) and *Ucp1*

(uncoupling protein 1), representing two brown adipose tissue (BAT)-specific markers, were upregulated in the HC/CD females and downregulated in the male offspring. Further, *Leptin,* as a main regulator of fat mass and energy balance, was also inversely regulated in both sexes.

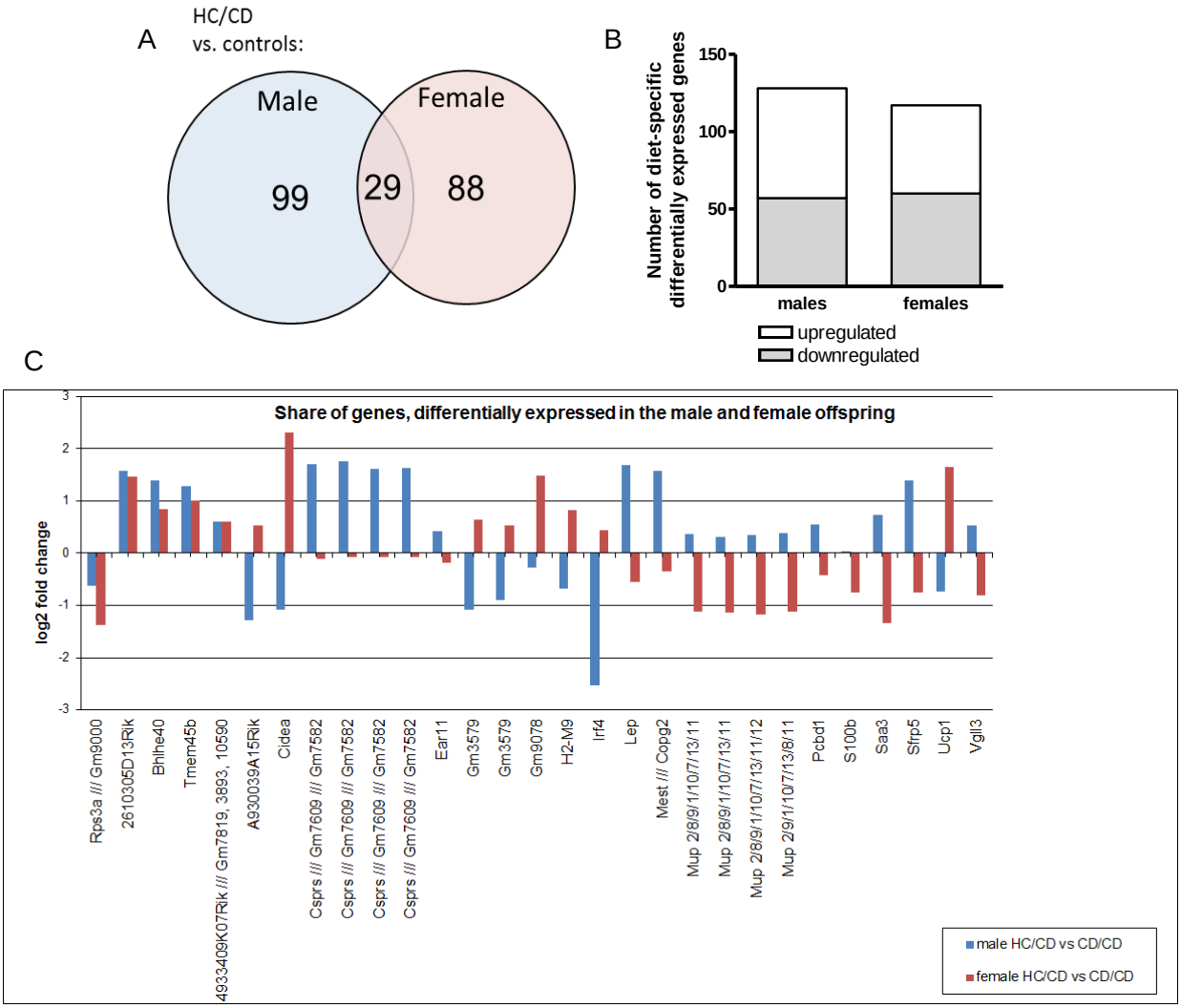

Figure 11: Microarray-based detection of sex-specific effects of a periconceptional obesogenic exposure on offspring gene expression in intra-abdominal adipose tissue at an age of 5 months: Differentially expressed genes (DEGs) in male and female offspring exposed to a maternal HC diet (n=5 per group and sex) or CD (n=5 per group and sex) *in utero* and weaned onto CD (HC/CD vs CD/CD). After hybridization of probes to Affymetrix GeneChips (Mouse Gene 1.0 ST Array, Affymetrix), DEGs were identified by LPEadj2. Calculation of log_2fold changes is based on means per group **(A)** DEGs in males (light blue), in females (light pink) or in both offspring sexes as result of a prenatal maternal HC diet exposure. **(B)** Number of up-and downregulated DEGs separated by sex. Up- and downregulated DEGs are shown as white and light grey bars, respectively. **(C)** Share of genes, differentially expressed in both sexes in response to exposure to a maternal HC diet *in utero*. *Bhlhe40*, basic helix-loop-helix family, member e40; *Cidea*, cell death-inducing DNA fragmentation factor, alpha subunit-like effector A; *Csprs*, component of Sp100-rs; Ear11, eosinophil-associated, ribonuclease A family, member 11; *Gm*, predicted gene; HC/CD, exposure to maternal HC diet + postnatal CD feeding; *H2-M9*, histocompatibility 2, M region locus 9; *Irf4*, interferon regulatory factor 4; *Lep*, leptin; *Mest ///Copg2*, mesoderm specific transcript /// coatomer protein complex, subunit gamma 2; *Mup*, major urinary protein; *Pcbd1*, pterin 4 alpha carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 1; *Rik*, RIKEN cDNA; *Rps3a*, ribosomal protein S3A; *S100b*, S100 protein, beta polypeptide, neural; *Saa3*, serum amyloid A 3; *Sfrp5*, secreted frizzled-related sequence protein 5; *Tmem*, transmembrane protein 98; *Ucp1*, uncoupling protein 1; *Vgll3*, vestigial like 3 (Drosophila).

3.2.2.3. Functional enrichment analysis of differential gene expression in offspring adipocytes

Despite a comparable quantity of DEGs in the intra-abdominal adipose tissue of the female and male offspring, the affected genes differed highly in quality.

In the male offspring, enrichment analysis of the DEGs showed no enrichment in metabolic pathways. However, HC/CD male offspring showed a downregulation of DEGs which belonged to the GO category “circadian rhythm” (GO:0007623), “circadian regulation of gene expression” (GO:0032922) and “regulation of circadian rhythm” (GO:0042752) ($p<0.01$), whereas there was no significant enrichment of upregulated DEGs. Prediction analysis for differentially regulated transcription factors showed neither significant upregulation nor downregulation in male offspring.

In contrast, in the HC/CD female offspring, DEGs were highly enriched in lipogenetic (upregulation of genes) and inflammatory pathways (downregulation of genes) (Figure 12). Prediction analysis for differentially regulated transcription factors revealed an upregulation of sterol regulatory element-binding transcription factor 1 (*Srebf1*) and 2 (*Srebf2*) in female adipocytes exposed to a maternal obesogenic milieu. *Srebf1* and *Srebf2* represent important transcription factors involved in the regulation of fatty acid biosynthesis and cholesterol metabolism, respectively, and corroborate the enrichment of DEGs in lipogenic pathways in females born to obese mothers.

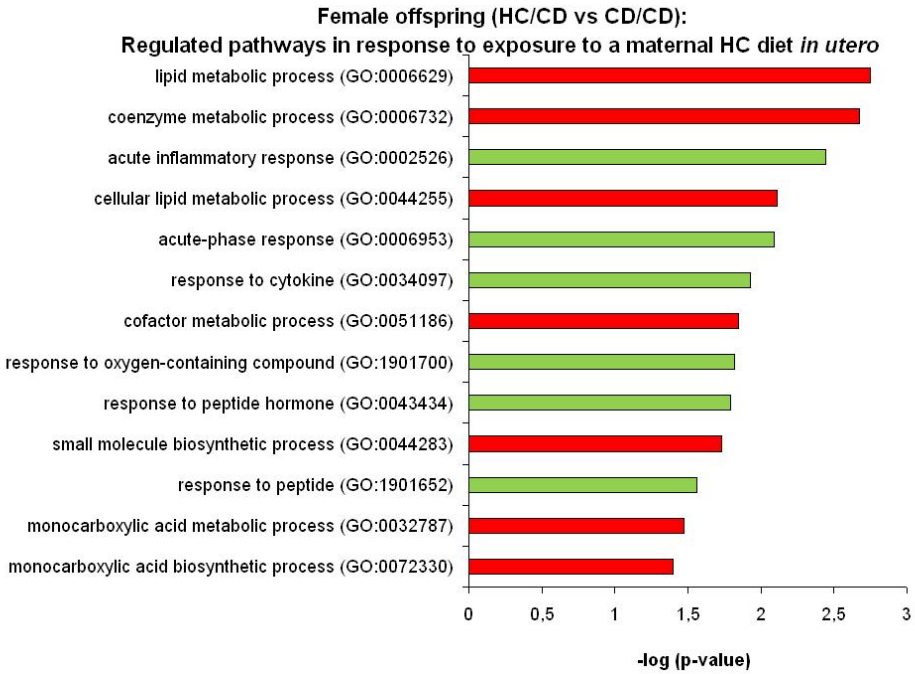

Figure 12: Effects of exposure to a maternal HC diet on enrichment of differentially expressed genes (DEGs) in the intra-abdominal adipose tissue of female offspring at an age of 5 months: Enrichment analysis was based on DEGs of female offspring exposed to maternal HC diet (n=5) or CD (n=5) *in utero* and weaned onto CD (HC/CD vs. CD/CD). DEGs were identified by LPEadj2. Enrichment analysis of DEGs was conducted via AmiGo ($p<0.05$); Significant enrichment of up- and downregulated DEGs to metabolic pathways was indicated by red or green bars, respectively.
CD/CD, exposure to maternal CD diet + postnatal CD feeding; GO, gene ontology; HC/CD, exposure to maternal HC diet + postnatal CD feeding.

3.2.2.4. Hypothesis-driven analysis of differential gene expression in offspring adipocytes

Based on the striking phenotypical alterations and DNA-microarray results, we focused on the female offspring. As described above (3.2.1., Figure 10), a maternal adipogenic milieu led to smaller adipocytes compared to the CD/CD females. This might suggest disturbances in adipose tissue development. In a hypothesis-driven approach, we intended to get first insights on pathways that could be involved in the reduction of adipose mass and cell size in female offspring (Table 3).

At **first**, we looked at major genes which are known to be involved in lipodystrophy in order to exclude a disease-associated origin of the diminished fat cell size. Apart from *Agpat2* (1-acylglycerol-3-phosphate O-acyltransferase 2), we detected no transcriptional alterations. The absence of lipodystrophy was corroborated by an about 1.7 fold upregulation of *Agpat2* in the adipocytes of the HC/CD female offspring. *Agpat2* is known to be mutated in congenital generalized lipodystrophy 1 and is required for triacylglyceride (TAG) accumulation in mature adipocytes. Thus, the diminished fat cell size in the HC/CD female offspring was at least not attributable to a lack of this enzyme, as suggested by the DNA-microarray results.

The **second** hypothesis aimed to focus on possible transcriptional alterations within the PPARG-pathway (KEGG: mmu03320), representing a main regulator of lipid storage and adipogenesis. Looking at downstream members of the PPARG pathway, the expression of the malic enzyme 1 (*Me1)*, stearoyl-Coenzyme A desaturase 2 (*Scd2*) and *Ucp1* was increased about 1.8 fold, 2.2 fold and 1.7 fold in HC/CD females, respectively. *Me1* produces NADH (nicotinamide adenine dinucleotide), which is used for fatty acid biosynthesis, via decarboxylation of malate to pyruvate. *Scd2* is also involved in adipogenesis and catalyzes the synthesis of unsaturated fatty acids.

In a **third** approach, we assessed the effects of the maternal adipogenic milieu on the abundance of genes involved in embryonic determination of adipocyte cell lineage. We detected an upregulation of early stage markers (*Egr2*, early growth response 2; *Ucp1*) and late stage markers (*Gpd1*, glycerol-3-phosphate dehydrogenase 1) of adipocyte differentiation and regulation in HC/CD females. The early stage marker *Egr2* (1.6 fold upregulation) promotes adipogenesis via CCAAT/enhancer binding protein beta (C/EBPβ) - dependent and - independent signalling pathways. In addition, *Ucp1* is also involved in the development of preadipocytes and promotes mitochondrial expansion. *Gpd1* (1.9 fold upregulation), representing a marker of mature adipocytes, is required to provide the glycerol backbone to esterify fatty acids into triglycerides. However, *Lep* was downregulated in the small adipocytes of the HC/CD female offspring.

However, in the adipose tissue of the female offspring at an age of 5 months, no DEGs were detected in pathways involved in embryonic adipose tissue determination such as “vascular

endothelial growth factor (VEGF) – signalling", "blood vessel formation" and "neuronal development" in mouse embryonic stem cells (mESCs). Further, DNA-microarray data revealed no effects of the exposure to a maternal HC diet *in utero* on transcripts involved in pathways related to "Typ II diabetes" and "β-cell dysfunction".

Table 3: Hypothesis-driven annotation of differentially expressed genes (DEGs) identified by DNA-microarray analysis:

Hypothesis	Differentially expressed genes	log_2fold change HC/CD ♀ vs CD/CD ♀	FDR	Genes per pathway
Involvement of lipodystrophy	*Agpat2*	0.73	0.012	1/ 12
Effects on triacylglycerol biosynthesis	*Agpat2*	0.73	0.012	1/16
Alteration of PPAR- signaling pathway	*Me1*	0.85	<0.001	3/59
		0.77	0.005	
	Scd2	1.11	<0.001	
	Ucp1	0.79	0.026	
Alteration of early stage markers (= Stem cells and immature adipocytes)	*Egr2*	0.70	0.047	2/14
	Ucp1	0.79	0.026	
Alteration of late stage markers (= Mature adipocytes and/or (secreted) regulatory molecules)	*Gpd1*	0.91	<0.001	2/17
	Lep	-0.70	0.036	

After hybridization of probes to Affymetrix GeneChips (Mouse Gene 1.0 ST Array, Affymetrix), DEGs were identified by LPEadj2. Calculation of log_2fold changes is based on medians per group.
Agpat2,1-acylglycerol-3-phosphate O-acyltransferase 2; CD/CD, exposure to maternal CD + postnatal CD feeding; *Egr2*, early growth response 2; FDR, false discovery rate; *Gpd1*, glycerol-3-phosphate dehydrogenase 1 (soluble); HC/CD, exposure to maternal HC diet + postnatal CD feeding; *Lep*, leptin; *Me1*, malic enzyme 1, NADP(+)-dependent, cytosolic; *Scd2*, stearoyl-Coenzyme A desaturase 2; *Sfrp5*, secreted frizzled-related sequence protein 5; *Ucp1*, uncoupling protein 1.

3.2.2.5. Quantitative validation of microarray results

As described above (3.2.2.3; Figure 12), microarray-based enrichment analysis showed a downregulation of genes involved in inflammatory pathways and an upregulation of genes of *de novo* lipogenesis in the intra-abdominal adipose tissue, when female offspring were exposed to a maternal obesogenic milieu followed by postnatal CD feeding. Thus, we investigated a panel of genes, representative for the respective pathways, by qRT-PCR (Figures 13 and 14).

In the CD-fed female offspring (CD/CD, HC/CD), quantitative analysis of several inflammatory genes did not show statistically significant differences between the HC/CD and CD/CD female adipocytes (Figure 13A). However, we detected a trend (p=0.11) towards a downregulation of two transcripts associated with the anti-inflammatory M2-macrophages: interleukine 10 (*Il-10)* and macrophage mannose receptor 1 (*Mrc1)* (145).

During postnatal HC diet feeding (CD/HC, HC/HC), mRNA abundances of inflammatory genes were significantly upregulated compared to postnatal CD-feeding, whereas the exposure to a maternal adipogenic milieu had no additional effect (Figure 13B).

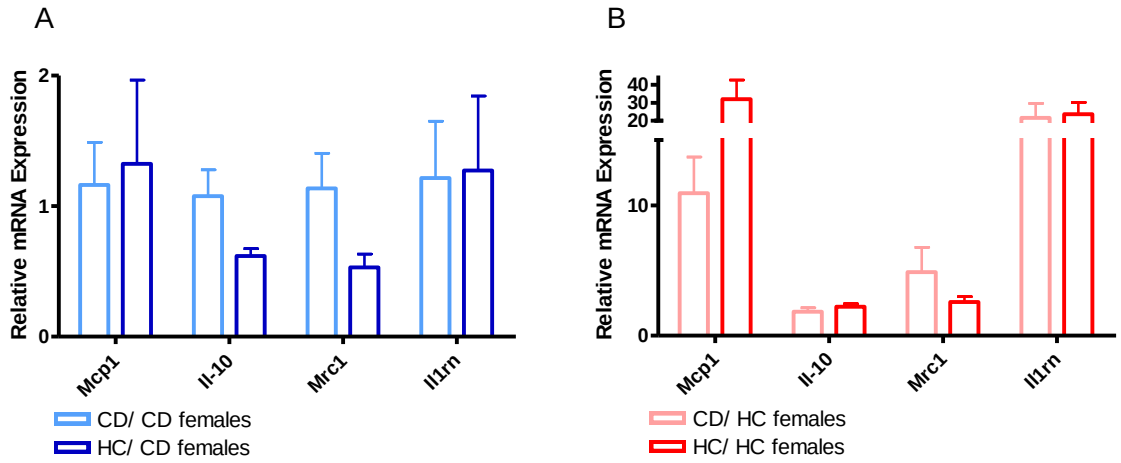

Figure 13: Impact of the exposure to a maternal HC diet on the expression of inflammatory genes in intra-abdominal adipose tissue of female offspring at an age of 5 months: mRNA abundances in adipose tissue of female offspring exposed to a maternal HC or CD *in utero* and weaned onto **(A)** CD (HC/CD, CD/CD; n=5 per group) or **(B)** HC diet (CD/HC, HC/HC; n=5 per group). mRNA expression relative to the reference gene *Ube2d2*.
Data are presented as mean ± s.e.m. and were analysed by Mann-Whitney U test.
CD/CD, exposure to maternal CD + postnatal CD feeding; CD/HC, exposure to maternal CD + postnatal HC diet feeding; HC/CD, exposure to maternal HC diet + postnatal CD feeding; HC/HC, exposure to maternal HC diet + postnatal HC diet feeding; *Il-10*; interleukine 10; *Il1rn*, interleukine 1 receptor agonist; *Mcp1*; monocyte chemotactic protein 1; *Mrc1*, macrophage mannose receptor 1; *Ube2d2*, ubiquitin-conjugating enzyme E2D 2.

Regarding the quantitative validation of lipogenetic genes in the intra-abdominal adipose tissue of the female offspring, a dysregulation in the transcriptional abundance of key enzymes involved in *de novo* lipogenesis was confirmed by qRT-PCR. Without any postnatal HC diet feeding, the maternal adipogenic milieu resulted in a significant upregulation of key transcripts involved in *de novo* lipogenesis in HC/CD females (Figure 14A), corresponding to the DNA-microarray results. We detected a significant upregulation of *Fasn*, which catalyzes the synthesis of palmitate in the presence of acetyl-CoA, malonyl-CoA and NADPH. There was also an upregulation of the acetyl-coenzyme A carboxylase alpha (*Acaca)*, which influences the fatty acid synthesis via carboxylation of acetyl-CoA to malonyl-CoA, and *Me1*, which provides NADPH for fatty acid biosynthesis. Further, there was a trend (p=0.06) towards an upregulation of the ATP citrate lyase (*Acly)*, which is involved in the generation of Acetyl-CoA, and the stearoyl-coenzyme A desaturase 2 (*Scd2)*, which is essential for the insertion of double-bonds during lipogenesis, in response to an exposure to a maternal adipogenic diet *in utero*. However, *Pparg* abundance was not significantly different between groups.

In addition to the measurement of lipogenic mRNA abundances in the female offspring without postnatal HC diet feeding (CD/CD, HC/CD), qRT-PCR was also conducted in the

intra-abdominal adipose tissue of postnatally HC diet fed female offspring (CD/HC, HC/HC). mRNA abundances of lipogenetic genes were downregulated during postnatal HC diet feeding compared to postnatal CD feeding. There was no effect of the maternal adipogenic milieu due to the strong effects of the postnatal HC diet feeding for a long period of several weeks (Figure 14B).

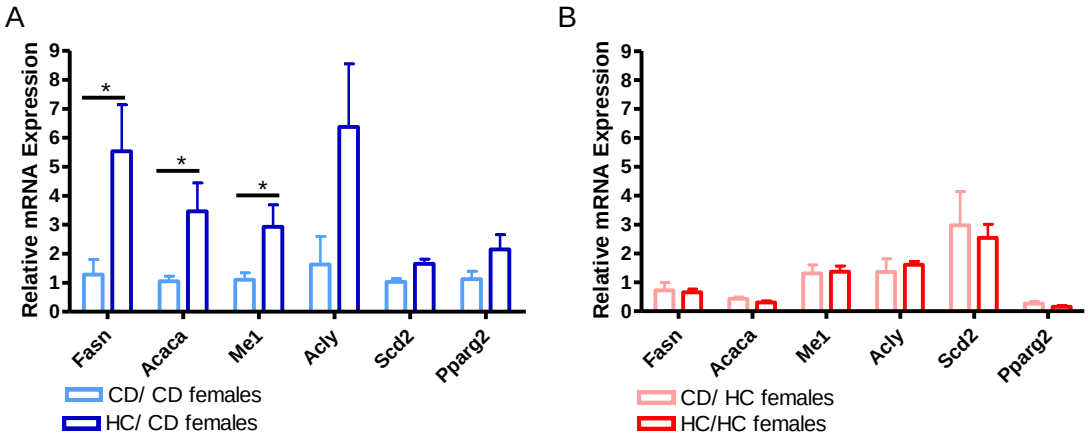

Figure 14: Impact of the exposure to a maternal HC diet on the expression of lipogenic genes in intra-abdominal adipose tissue of female offspring at an age of 5 months: mRNA abundances in adipose tissue of female offspring exposed to a maternal HC diet or CD *in utero* and weaned onto **(A)** CD (HC/CD, CD/CD; n=5 per group) or **(B)** HC diet (CD/HC, HC/HC; n=5 per group). mRNA expression relative to the reference gene *Ube2d2*. Data are presented as mean ± s.e.m. Significant differences were detected by Mann-Whitney U test; *$p < 0.05$.
Acaca, acetyl-Coenzyme A carboxylase 1; *Acly*, ATP citrate lyase; CD/CD, exposure to maternal CD + postnatal CD feeding; CD/HC, exposure to maternal CD + postnatal HC diet feeding; *Fasn*, fatty acid synthase; HC/CD, exposure to maternal HC diet + postnatal CD feeding; HC/HC, exposure to maternal HC diet + postnatal HC diet feeding; *Me1*, malic enzyme 1; *Pparg*, peroxisome proliferator activated receptor gamma; *Scd2*, stearoyl-Coenzyme A desaturase 2*; Ube2d2*, ubiquitin-conjugating enzyme E2D 2.

3.2.3. Effects of maternal HC diet exposure on enzyme function in offspring adipocytes

FASN, a key enzyme involved in fatty acid biosyntheses, was analyzed to confirm the transcriptional alterations of female adipocytes prenatally exposed to an adipogenic diet on a functional level.

3.2.3.1. Method development for analysis of FASN activity

First, the FASN activity assay was micronized to a 96-well format and assay conditions were adapted for murine adipose tissue (Gimpfl et al., 2016; Methods Note in preparation). The method development was based on the original protocol of Nepokroeff et al. measuring the FASN activity in rat liver at 30 °C in a 1 ml cuvette (140). The linear decline of NADPH was measured by spectrophotometer at 340 nm. Based on the FASN-induced synthesis of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA through the oxidation of NADPH, the decline of NADPH reflects the relative FASN activity (139).

FASN reaction:

$$\text{Acetyl-CoA} + 7\ \text{Malonyl-CoA} + 14\ \text{NADPH} + 14\ H^+ \rightarrow 1\ \text{Palmitic acid (C16)} + 7\ CO_2 + 14\ NADP^+ + 8\ \text{CoA} + 6\ H_2O$$

In order to reduce the required sample amounts and to optimize the assay sensitivity for murine adipose tissue, the reaction temperature, DTT and NADPH buffer concentrations were adapted.

For optimization of the assay condition, adipose tissue pools of MD-fed mice were used. To maintain optimum FASN activity throughout the assay, all buffers and the measuring chamber of the spectrometer were heated to a physiological temperature of 37°C. The concentration of the substrate NADPH was set to 0.16 mM NADPH in the final reaction mixture, since higher concentrations (>0.32 mM NADPH) led to an inhibition of the enzyme reaction. Optimal FASN activity was determined when using 1 mM DTT in the final reaction mixture (Appendix Figure S2). Measurement of the FASN activity as a function of the applied protein amount showed a linear increase between 15 µg and 25 µg protein (Appendix Figure S1). Using 0.16 mM NADPH, 1 mM DTT and 20 µg protein in the final reaction mixture, the intra-assay variation (CV) was 5.9% and the inter-assay variation (CV) was 18.5%.

For assay validation, an adipose tissue pool of HC diet-fed adult female NMRI mice was used as negative control and showed a low endogenous FASN activity, as expected. In contrast, in the adipose tissue pool of the MD-fed mice, we detected a significantly higher endogenous FASN activity (=positive control) (Figure 15).

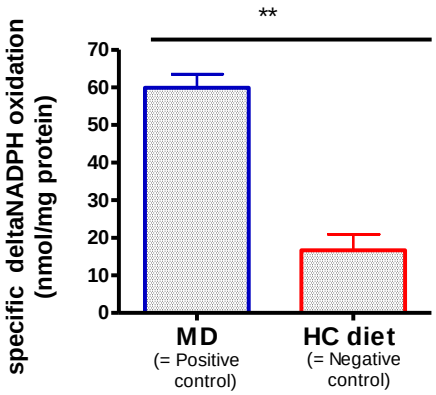

Figure 15: Assay validation: Fatty acid synthase (FASN) activity in adipose tissue of adult female mice fed a maintenance diet (MD) (n=3) or HC diet (n=3): Adipose tissue samples of three mice per dietary group were pooled for the preparation of sample homogenates, and FASN activity was determined by 5 independent measurements per group. FASN activity was reflected by linear enzymatic oxidation of NADPH at 37°C at 340nm.
Final reaction mixture contains: 20 µg protein, 40 mM sucrose, 400 mM potassium phosphat, 795 µM EDTA, 635 µM β-mercaptoethanol, 1 mM DTT, 160 µM NADPH, 70 µM acetyl-CoA and 90 µM malonyl-CoA. A blank without malonyl-CoA was simultaneously measured for each sample.
Data are presented as mean ± s.e.m. Significant differences were detected by Mann-Whitney U test; $^{**}p < 0.01$.
DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HC: high-calorie, high-fat diet feeding; MD, maintenance diet feeding; NADPH, nicotinamide adenine dinucleotide phosphate.

3.2.3.2. Measurement of FASN activity in offspring adipose tissue

After optimization of the assay conditions, FASN activity was determined in the intra-abdominal adipose tissue of the female offspring at an age of 5 months. Two groups of mice were investigated: offspring which were either fed a CD or a HC diet after weaning.

In the absence of any postnatal HC diet feeding, the increased mRNA abundance of *Fasn* in adipocytes was reflected in a significantly increased enzyme activity in the HC/CD females compared to the CD/CD females (Figure 16A). Comparable to the transcriptional downregulation, postnatal HC diet feeding led to a significant reduction of the FASN activity irrespective of the periconceptional milieu (Figure 16B).

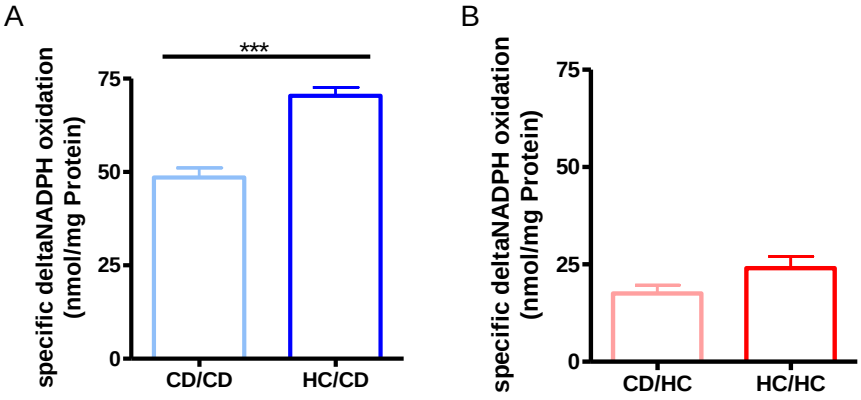

Figure 16: Effects of a the exposure to a maternal HC diet on the activity of the fatty acid synthase (FASN) in intra-abdominal adipose tissue of female offspring at the age of 5 months: FASN-activity in adipose tissue homogenates of female offspring exposed to maternal HC diet or CD *in utero* and weaned onto **(A)** CD (HC/CD, CD/CD; n=5 mice per group) or **(B)** HC diet (CD/HC, HC/HC; n=5 mice per group). FASN activity was determined by 5 independent measurements per mouse and was reflected by linear enzymatic oxidation of NADPH at 37°C at 340nm.
Final reaction mixture contains: 20 µg protein, 40 mM sucrose, 400 mM potassium phosphat, 795 µM EDTA, 635 µM β-mercaptoethanol, 1 mM DTT, 160 µM NADPH, 70 µM acetyl-CoA and 90 µM malonyl-CoA. A blank without malonyl-CoA was simultaneously measured for each sample.
Data are presented as mean ± s.e.m. Significant differences were detected by Mann-Whitney U test; ***$p < 0.001$.
CD/CD, exposure to maternal CD + postnatal CD feeding; CD/HC, exposure to maternal CD + postnatal HC diet feeding; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HC/CD, exposure to maternal HC diet + postnatal CD feeding; HC/HC, exposure to maternal HC diet + postnatal HC feeding; NADPH, nicotinamide adenine dinucleotide phosphate.

3.2.4. Impact on glucose metabolism and pancreas morphology of the offspring

As described above (3.2.1, Figure 10), exposure to a maternal adipogenic milieu led to an impaired glucose tolerance in the HC/HC female offspring at an age of 5 months, whereas the male offspring were not affected. In absence of any postnatal HC diet feeding, the exposure to a maternal adipogenic milieu *in utero* did not result in an impaired glucose tolerance neither in male nor in female offspring.

Based on the disturbed glucose tolerance test in the adult female HC/HC offspring, pancreata were examined histologically during the PhD project in order to detect any underlying alterations in insulin secretion that might potentially explain the impaired glucose tolerance.

To evaluate morphological alteration of the pancreatic cells in the HC/HC female offspring, we assessed the volume of β-cells in the pancreata of both postnatally HC diet fed female groups (HC/HC and CD/HC). The CD/CD group served as a reference reflecting the physiological β-cell amount. No significant differences were detected among the female groups: CD/CD, CD/HC and HC/HC (Figure 17).

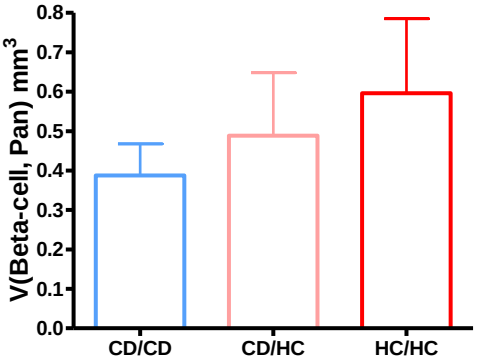

Figure 17: Effects of the exposure to a maternal HC diet on the pancreatic β-cell volume in female offspring at an age of 5 months: Immunohistochemical staining of β-cells via alkaline phosphatase reaction. Assessment of β-cell volume in pancreata of post-natally HC diet fed (CD/HC, HC/HC) and control (CD/CD) female offspring (n=4 mice per group).
Data are presented as mean ± s.e.m. and were analysed by ANOVA and Tukey's post hoc test.
CD/CD, exposure to maternal CD + postnatal CD feeding; CD/HC, exposure to maternal CD + postnatal HC diet feeding; HC/HC, exposure to maternal HC diet + postnatal HC diet feeding.

3.3. Alteration of the fat quality of a maternal obesogenic diet

Because of the observed adverse effects of a periconceptional adipogenic HC diet during gestation on dams and offspring, we aimed to improve the gestational milieu by modifiying the fatty acid quality of the adipogenic diet (see section 1.3.3.). We hypothesized that this change in fatty acid quality ameliorates the maternal milieu resulting in an improvement of the metabolic outcome in the offspring.

3.3.1. Influence of the qualitative fat change of the obesogenic diet on the maternal milieu

The fatty acid quality of the adipogenic diet was improved in an isocaloric manner by the addition of MCFAs (36% of total fatty acids) and Ω-3 LC-PUFAs (Ω-6:Ω-3 ratio: 2.2:1). In a preliminary experiment, the phenotypical effects of the Fat-mod HC diet on the maternal milieu were assessed before and during pregnancy (Master thesis: Martina Gimpfl) and are summarized in Figure 18 (Gimpfl et. al, under review).

We have previously shown that the functional compounds (Ω-3 LC-PUFAs, MCFAs) contained in the maternal Fat-mod HC diet are stable during storage at ambient temperature for at least 1 week and are not affected by irradiation.

Importantly, assessment of energy assimilation detected no significant differences per day and body weight in the HC diet and Fat-mod HC diet group.

Despite similar energy assimilaton, we detected a reduction in body weight and total fat mass in the Fat-mod HC diet fed mothers before and during pregnancy, which was accompanied by reduced weights of the subcutaneous and intra-abdominal fat pads (i.e. the perigonadal, perirenal and omental fat pads) at the end of pregnancy. Further, the dietary change in fat quality led to a reduction of liver weight and hepatic steatosis at the end of pregnancy. In addition, histological and clinical examination showed no signs of inflammation or liver injury. However, there were no effects on fetal and placental weights.

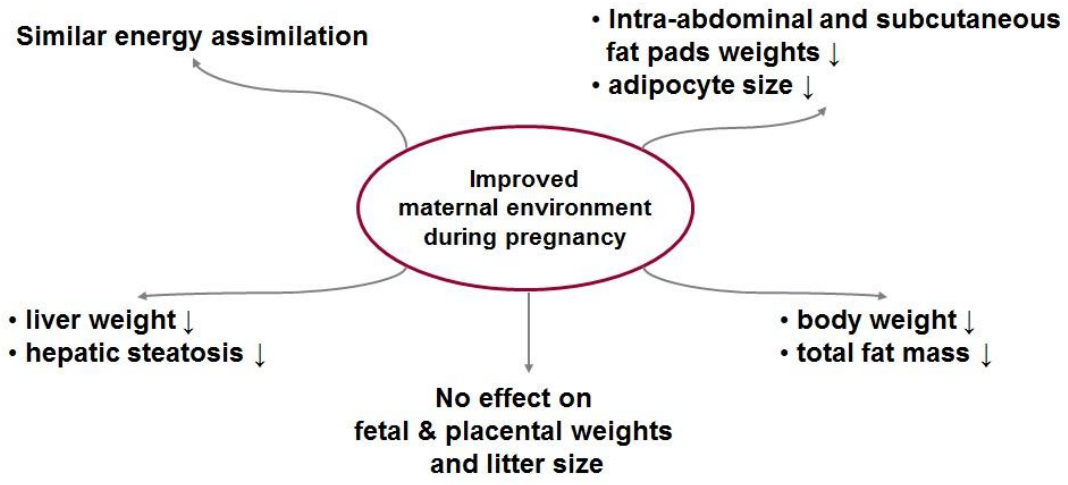

Figure 18: Preventive effects of the modified fatty acid composition of an adipogenic diet on the maternal milieu (previous data of the research group; Gimpfl et. al, under review).

Using the organ samples generated during the Master thesis (Experiment B1), the maternal milieu was characterized in more detail during the PhD project (Gimpfl et al., under review).

3.3.1.1. Effects on the maternal adipose tissue phenotype and transcriptional regulation

As part of the visceral fat depot, we studied the omental fat at the end of obese pregnancy in more detail. In addition to the reduced weight of the omental fat pad in dams fed the Fat-mod HC diet (as described before, Figure 18), their omental adipocyte size was also significantly reduced (Figure 19A, B). Analysis of adipocyte size distribution indicated a leftward shift in the histogram showing a higher proportion of smaller adipocytes in the omental fat of the Fat-mod HC diet compared to the HC diet group (Figure 19C).

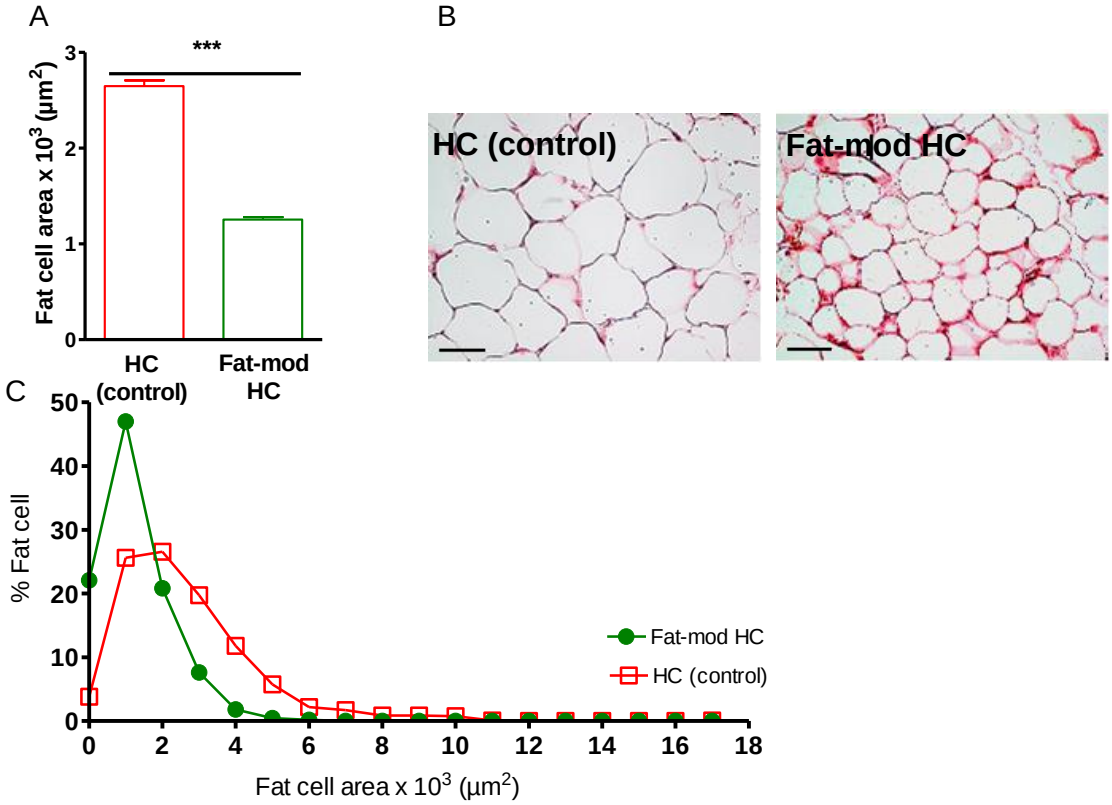

Figure 19: Impact of the periconceptional fat-modified high-fat, high-calorie diet (Fat-mod HC diet) on maternal adipose tissue at the end of gestation. **(A)** Adipocyte size of omental adipose tissue of mice at 17.5 dpc (Fat-mod HC diet: n= 12; HC diet: n= 11). Data are presented as mean ± s.e.m. Significant differences between the Fat-mod HC diet group and the HC diet group were determined by the Mann-Whitney U test; ***p < 0.001. **(B)** 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). **(C)** 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).
Fat-mod HC diet, fat-modified high-fat, high-calorie diet; FFPE, formalin-fixed, paraffin-embedded; HC diet, high-fat, high-calorie diet; HE, hematoxylin-eosin.

Next, we examined transcript abundances in the omental adipose tissue of the Fat-mod HC diet dams compared to the HC diet group. We aimed to investigate whether regulatory pathways were affected underlying the reduction of adipocyte size in the Fat-mod HC diet

dams (Figure 20). In the Fat-mod HC diet group, a significantly lower mRNA expression was identified for *Srebf1* compared to the HC diet group. Further, the transcript abundances of two other “nutrient sensors” *Pparg1* (*p*= 0.07) and *Nr1h3* (*p*= 0.09) tended to be lower than in the HC diet dams; however, these differences were not significant. In addition, the mRNA expression levels of mesoderm-specific transcript/imprinted paternally expressed gene 1 (*Mest*, also known as *Peg1*) and *Lep*, involved in mechanisms influencing adipocyte size (146) and adipose mass regulation, respectively, were strongly decreased (Figure 20). However, we detected no transcriptional differences of genes involved in lipolysis, fatty acid β-oxidation, fatty acid storage and transport.

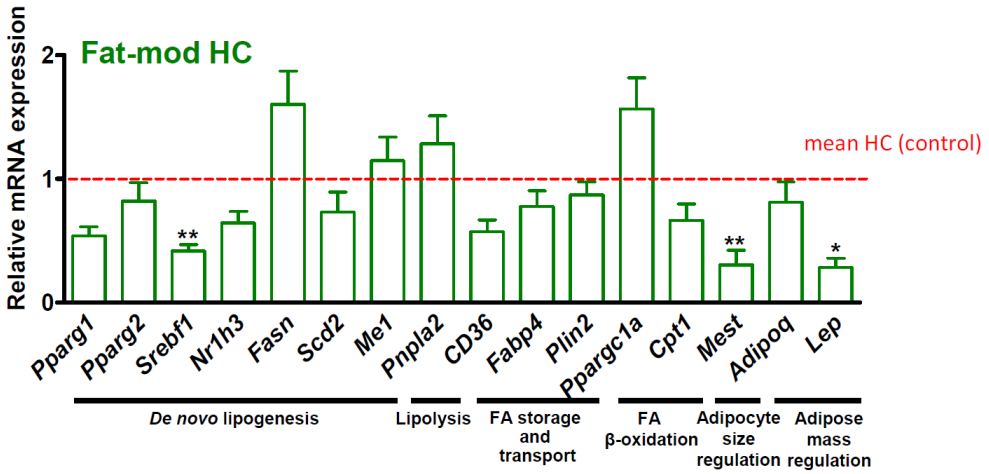

Figure 20: Impact of the periconceptional fat-modified high-fat, high-calorie (Fat-mod HC diet) diet on mRNA abundances in omental adipose tissue at the end of gestation. mRNA expression of genes involved in fatty acid metabolism, adipocyte size determination, and adipose mass regulation relative to the reference gene *Ube2d2* in omental adipose tissue of dams of the Fat-mod HC diet group (n= 9) at day 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.
Adipoq, adiponectin, C1Q and collagen domain containing; BAT, brown adipose tissue; *Cd36*, Cd36 antigen; *Cpt1*, carnitine palmitoyltransferase 1; dpc, days post coitum; FA, fatty acid; *Fabp4*, fatty acid binding protein 4*; Fasn*, fatty acid synthase; Fat-mod HC diet, fat-modified high-fat, high-calorie diet; FFPE, formalin-fixed, paraffin-embedded; HC diet, high-fat, high-calorie diet; *Lep*, leptin; *Me1*, malic enzyme 1; *Mest*, mesoderm specific transcript/imprinted paternally expressed gene 1 (also known as Peg1); *Nr1h3*, nuclear receptor subfamily 1, group H, member 3 (also known as *Lxra*, liver X receptor alpha); *Plin2*, perilipin 2; *Ppargc1a*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *Pnpla2*, patatin-like phospholipase domain containing 2 (also known as *Atgl*, adipose triglyceride lipase); *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.

3.3.1.2. Impact on gene regulation in maternal liver

Because we found differences in liver weight and hepatic fat accumulation in the Fat-mod HC diet group (3.3.1, Figure 18), the regulation of hepatic gene transcripts involved in liver steatotic and metabolic pathways was compared between the two dietary groups (Figure 21). To study whether fatty acid metabolism is impaired resulting in liver steatosis, we

investigated anabolic and catabolic pathways in the liver of the Fat-mod HC diet dams compared to the HC diet group.

In the Fat-mod HC diet group, liver mRNA abundances of *Fasn* and *Acaca*, representing two key enzymes of *de novo* lipogenesis, were significantly reduced and were accompanied by a significant downregulation of *Srebf1* and its transcriptional activator *Nr1h3* (also known as *Lxra*).

Further, the mRNA abundance of *Pparg*2, involved in the regulation of liver steatosis, was also reduced in the Fat-mod HC diet group compared to the HC diet group. Consequently, the expression of the fatty acid binding protein 4 (*Fabp4*), as one of its target genes, was also lower, whereas the hepatic expression of genes involved in mitochondrial fatty acid β-oxidation was not affected.

The transcript abundance of the hepatic ATP-binding cassette, sub-family A, member 1 (*Abca1*) representing the main modulator of HDL biogenesis, was reduced. This downregulation might be induced via a transcriptional cascade involving PPARG-mediated interaction with NR1H3.

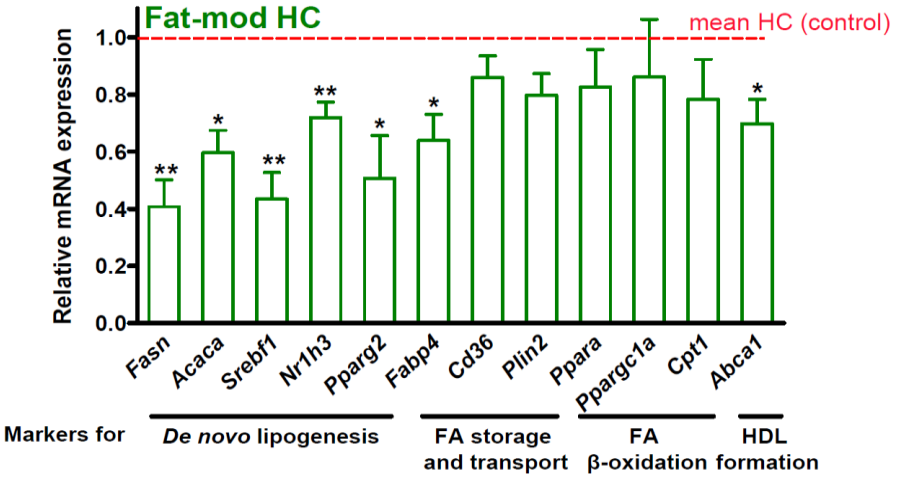

Figure 21: Impact of the periconceptional fat-modified high-fat, high-calorie (Fat-mod HC diet) diet on mRNA abundances in liver at the end of gestation. mRNA expression of genes involved in *de novo* lipogenesis, fatty acid storage, transport and β-oxidation, and HDL formation relative to the reference 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.

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; **p < 0.01.

Abca1, ATP-binding cassette, sub-family A, member 1; *Acaca*, acetyl-coenzyme A carboxylase alpha; *Cd36*, Cd36 antigen; *Cpt1*, carnitine palmitoyltransferase 1; *dpc*, days post coitum; FA, fatty acid; *Fasn*, fatty acid synthase; Fat-mod HC diet, fat-modified high-fat, high-calorie diet; *Fabp4*, fatty acid binding protein 4; FFPE, formalin-fixed, paraffin-embedded; HC diet, high-fat, high-calorie diet; HDL, high density lipoprotein; HE, hematoxylin-eosin; *Nr1h3*, nuclear receptor subfamily 1, group H, member 3 (also known as *Lxra*, liver X receptor alpha); *Plin2*, perilipin 2; *Ppara*, peroxisome proliferator activated receptor alpha; *Ppargc1a*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *Pparg*, peroxisome proliferator activated receptor gamma; *Ppib,* peptidylprolyl isomerase B; *Srebf1*, sterol regulatory element binding transcription factor 1.

3.3.1.3. Effects on transcriptional abundances of nutrient transporters in the placenta

To get first insights into possible effects of the altered fat quality of the maternal adipogenic diet on aspects of intrauterine nutrient supply towards the offspring, the placental abundances of important nutrient transporter (e.g. for fatty acid and glucose transport) were assessed and analysed separated by sex.

We detected barely any effects on the mRNA abundances in placenta pools of the male offspring, whereas in placenta pools of the female offspring the transcriptional abundances of nutrient transporters were increased (Figure 22A, B). However, no mRNA differences were detected for genes involved in lipid accumulation and HDL/LDL cholesterol transport in neither sex.

We performed ANOVA to analyze the influencing factor sex, diet and the interaction diet*sex of the offspring. The ANOVA revealed a significant effect of both the maternal diet ($p=0.0095$) and the interaction of diet*sex of the offspring ($p=0.03$) on the mRNA abundance of *Pparg1*. Further, the sex-specific expression of the retinoid X receptor alpha (*Rxra)*, representing the functional dimerization partner of PPARG, was significantly affected by the maternal diet*sex of the offspring ($p=0.03$). This was accompanied by diet-specific effects ($p<0.05$) on the fatty acid transporters solute carrier family 27, member 1 (*Slc27a1*) and member 4 (*Slc27a4*), two PPARG target genes involved in fatty acid uptake.

Interestingly, the upregulation of PPARG/RXR-activated fatty acid transporters was only evident in female placenta pools of the Fat-mod HC diet group. In addition, transcriptional abundances of the solute carrier family 2 (*Slc2a1*; also known as *Glut1*, glucose transporter 1), which is important for the placental glucose transport, were diet-specifically increased in comparison to the HC diet group ($p<0.05$). Further, we detected a diet-specifically increased mRNA expression of the sodium-dependent neutral amino acid transporter-2 (*Snat2;* also known as *Slc38a2)*, acting as transporters for small neutral amino acids, in the placenta pools of the Fat-mod HC diet female offspring compared to the HC diet group ($p<0.05$). However, male placenta pools were not affected. In contrast, the PPARG target gene *Fabp4*, which is involved in intracellular trafficking of fatty acids in the placenta, was significantly reduced by the Fat-mod HC diet exposure in both male and female placenta pools in comparison to the HC diet group.

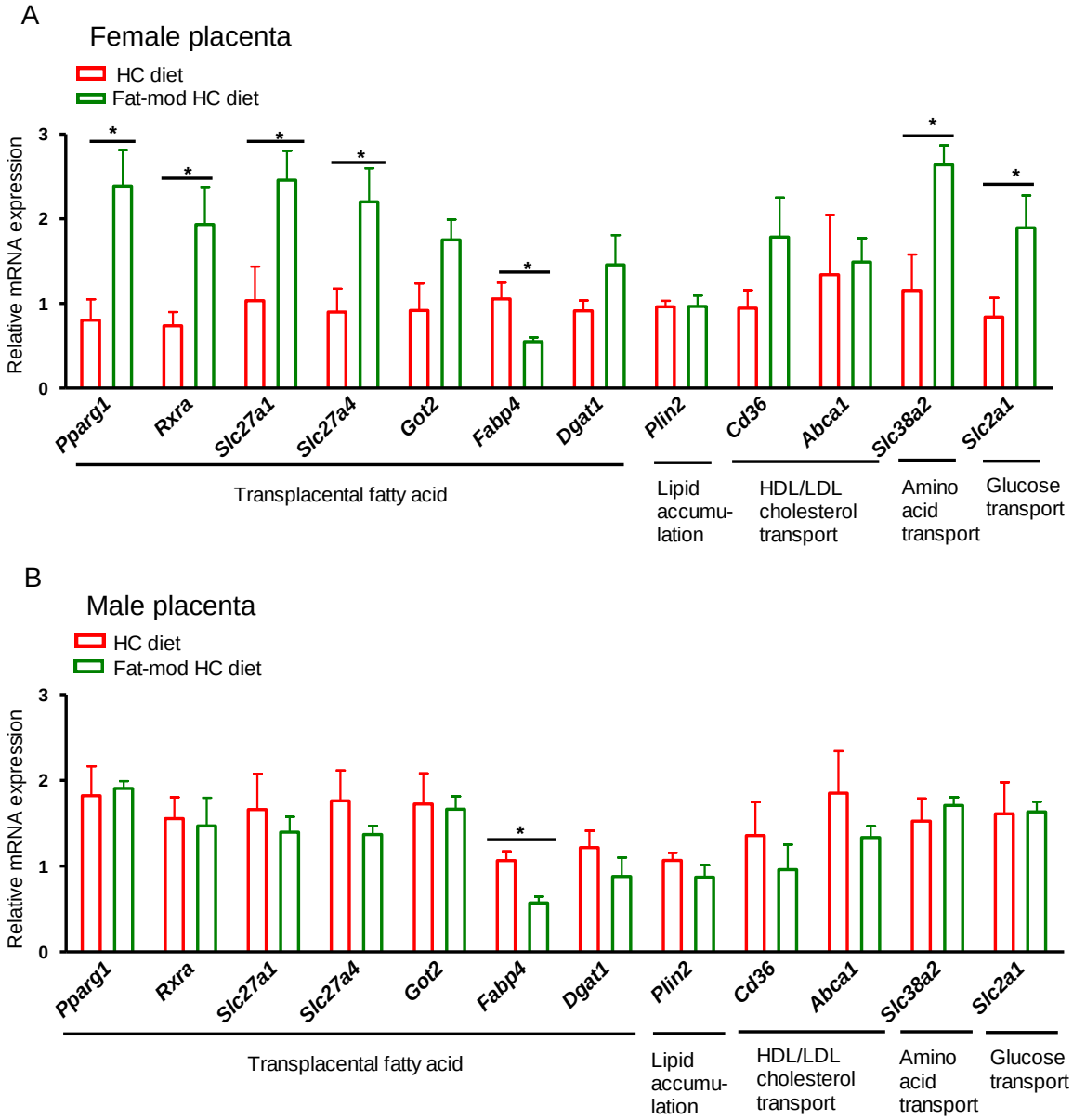

Figure 22: Impact of the periconceptional fat-modified high-fat, high-calorie (Fat-mod HC diet) diet on placental gene expression at the end of gestation. mRNA expression of genes involved in placental fatty acid transport and storage relative to the reference gene *Tbp* in placenta pools of **(A)** female offspring exposed to the Fat-mod HC diet or the HC (control) diet (n= 5/group) and **(B)** 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 Oacyltransferase 1; dpc, days post coitum; Fat-mod HC diet, fat-modified high-fat, high-calorie diet; *Fabp4*, fatty acid binding protein 4; *Got2*, glutamatic-oxaloacetic transaminase 2 (also known as *Fabp-pm,* plasma membrane fatty acid binding protein); HC diet, high-fat, high-calorie diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; *Plin2*, perilipin 2; *Pparg1*, peroxisome proliferator activated receptor gamma 1; Slc2a1, solute carrier family 2 (facilitated glucose transporter), member 1 (also known as *Glut1,* glucose transporter 1) *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),*Slc38a2;* solute carrier family 38, member 2 (also known as *Snat2*, sodium-dependent neutral amino acid transporter-2)*; Rxra*, retinoid X receptor, alpha*; Tbp,* TATA box binding protein.

3.3.2. Effects of the fat-modified adipogenic diet on mating and neonatal parameters

Because the effects of the qualitatively altered obesogenic diet were promising, in a next step we assessed the consequences of the metabolically improved maternal milieu for the development of the exposed offspring (Experiment B2). In this subsequent experiment, the amelioration of the maternal milieu by the qualitative fat change was corroborated, albeit to a weakened extent compared to Experiment B1. In the dams of Experiment B2, the altered fat composition of the maternal diet showed a trend towards a reduced liver weight (HC diet (n=14), 3.10 ± 0.16g; Fat-mod HC diet (n=13), 2.76 ± 0.07; p = 0.06) and weight of the intra-abdominal fat mass including the perigonadal and omental fat pads (HC diet (n=14), 0.74 ± 0.18g; Fat-mod HC diet (n=13), 0.50 ± 0.16; p=0.34) at the end of pregnancy.

The qualitative fat change in the obesogenic diet did not adversely affect mating success. This was supported by the lack of significant differences in fertility rate, litter size, sex ratio and neonatal mortality between both groups (Table 5).

Table 5: Mating characteristics

	mat-HC (n=14)	**mat-Fat-mod HC** (n=13)
Fertility rate (%)	93.3	86.6
Litter size	12.77 ± 0.06	13.69 ± 0.50
Sex ratio (male/ females)	1.44 ± 0.40	1.01 ± 0.13
Neonatal mortality (%)	2.63 ± 1.90	4.81 ± 0.55

Data are normally distributed and are presented as mean ± s.e.m. Analysis of significant differences between the mat-HC and the mat-Fat-mod HC group by unpaired t-test.
mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3); high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

3.3.3. Consequences of the altered maternal obesogenic diet for the offspring

Offspring were analysed longitudinally over time and according to sex.

3.3.3.1. Phenotyping of energy metabolism in the adult offspring

Phenotyping of energy metabolism was conducted at an age of 12 weeks in order to ensure that phenotypical alterations are not contributed to alterations in food intake, heat production and physical activity (Table 6). There were no significant differences between groups for any of the parameters

Table 6: Phenotyping of energy metabolism in the adult offspring: Food intake, movement activity, indirect calorimetry of mat-Fat-mod HC and mat-HC offspring at an age of 12 weeks

Parameter	Male offspring		Female offspring	
	mat-HC (n=10)	**mat-Fat-mod HC** (n=10)	**mat-HC** (n=10)	**mat-Fat-mod HC** (n=9)
Body weight (g)	44.75 ± 1.36	44.10 ± 1.42	34.85 ± 1.11	35.84 ± 1.09
Food intake (g)	2.93 ± 0.23	2.99 ± 0.23	3.35 ± 0.32	2.71 ± 0.34
Adjusted food intake (g)	3.07 ± 0.23	3.11 ± 0.22	3.20 ± 0.33	2.59 ± 0.35
Adjusted avg heat production (W)	0.72 ± 0.01	0.71 ± 0.02	0.76 ± 0.01	0.74 ± 0.01
Adjusted min heat production (W)	0.50 ± 0.01	0.48 ± 0.01	0.55 ± 0.02	0.52 ± 0.02
Adjusted max heat production (W)	1.08 ± 0.03	1.10 ± 0.03	1.12 ± 0.03	1.05 ± 0.03
Covered distance in 15 min (cm)	4222.30 ± 453.01	4975.30 ± 617.68	4036.50 ± 279.68	4600.44 ± 369.77
Respiratory quotient (VCO_2/VO_2)	0.85 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	0.85 ± 0.01

Data are normally distributed and are presented as mean ± s.e.m. Analysis of significant differences between the mat-HC and the mat-Fat-mod HC group by unpaired t-test.
Avg, average; mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet; VCO_2, carbon dioxide production; VO_2, oxygen consumption.

3.3.3.2. Development of body weight and total body fat mass in the offspring

Male offspring

The change in fat quality of the obesogenic maternal diet before and during pregnancy led to a significantly decreased birth weight in the offspring cohort of the mat-Fat-mod HC males (Table 8). While suckling at lean foster dams, their weight course became similar to that of the mat-HC male offspring. Nevertheless, after weaning onto CD, the mat-Fat-mod HC males showed a reduced body weight gain (Figure 23A), which was accompanied by a decrease in total body fat mass (Figure 23B).

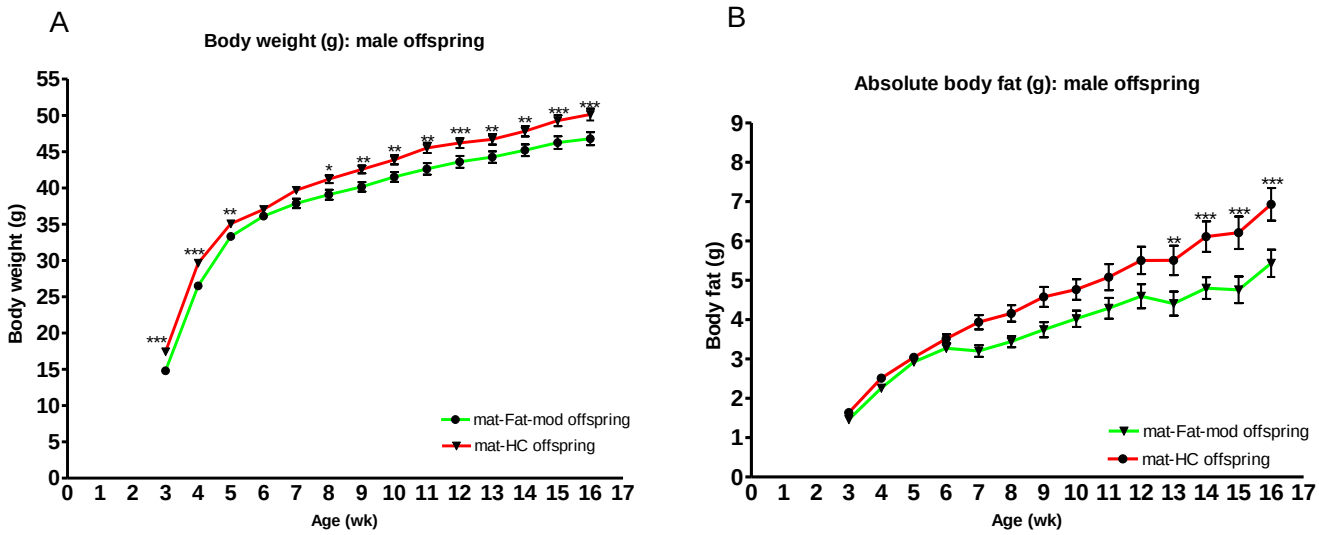

Figure 23: Impact of the periconceptional fat-modified high-fat, high-calorie (Fat-mod HC diet) diet on body composition of male offspring. (A) Body weight and (B) total body fat (g) of male offspring following periconceptional exposure to a maternal high-fat, high-calorie (mat-HC) diet or fat-modified high-fat, high-calorie diet (mat-Fat-mod HC) (animal number: 3-6 weeks/ 7-16 weeks n =54/33 mat-HC; n= 45/27 mat-Fat-mod HC).
Data are presented as mean ± s.e.m. For body weight (g) and total body fat (g), ANOVA revealed a significant effect ($p < 0.0001$) of diet, age, maternal milieu and the interaction diet*age. Significance of individual differences was tested by Tukey's post hoc test; *$p<0.05$, **$p<0.01$, ***$p<0.001$.
mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

Female offspring

Despite a reduced birth weight of the mat-Fat-mod HC female offspring (Table 8), weight differences between mat-HC and mat-Fat-mod HC females disappeared while suckling at lean foster dams. There were small but significant differences during the early post-weaning period (age: 3 to 7 weeks). Contrary to males, there were no significant differences in body weight and total fat mass between both female offspring groups from 8 weeks of age to the time of dissection at 16 weeks of age (Figure 24).

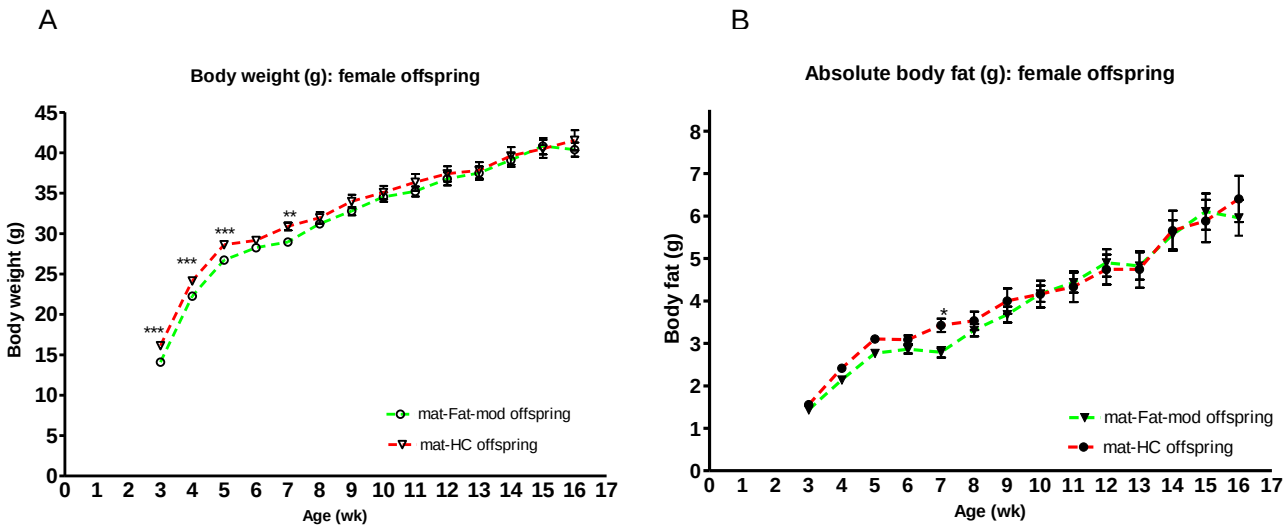

Figure 24: Impact of the periconceptional fat-modified high-fat, high-calorie diet on body composition of female offspring. (A) Body weight and (B) total body fat (g) of female offspring following periconceptional exposure to maternal high-fat, high-calorie diet (mat-HC) or fat-modified high-fat, high-calorie diet (mat-Fat-mod HC) (animal number: 3-6 weeks/ 7-16 weeks n =47/28 mat-HC; n= 56/36 mat-Fat-mod HC).
Data are presented as mean ± s.e.m. For body weight, ANOVA revealed a significant effect of diet ($p=0.0027$), age ($p<0.0001$) and maternal milieu ($p<0.0001$), but no interacting effect of diet*age ($p=0.6916$). For total body fat (g), a significant effect of diet ($p<0.0001$), age ($p<0.0001$), maternal milieu ($p<0.0001$) and the interaction diet*age ($p=0.0445$) was detected by ANOVA. Significance of individual differences was tested by Tukey's post hoc test; *$p<0.05$, **$p<0.01$, ***$p<0.001$.
mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

3.3.3.3. Longitudinal analysis of plasma lipid status in the offspring

Alteration of the fatty acid composition of the obesogenic maternal diet resulted in sex- and age- dependent effects on the plasma lipid status in the offspring.

With respect to the plasma triglyceride and NEFA concentrations, we detected effects of the maternal diet only at an offspring age of 6 weeks (Figure 25), whereas at an age of 16 weeks differences vanished among groups (data not shown). A control group of offspring, which were born to CD-fed mothers in the same experimental setting, served as reference group (mat-CD; Figure 25). At an age of 6 weeks, plasma NEFA concentrations in both male and female mat-Fat-mod HC offspring were lower than in the mat-HC and mat-CD groups (Figure 25A, B). In the 6-week-old female offspring of the mat-Fat-mod HC group, plasma triglycerides were reduced compared to the mat-HC female offspring and were similar to the control group (Figure 25B). In contrast, we detected no diet-specific differences of triglyceride concentrations in the plasma of the male offspring (Figure 25A).

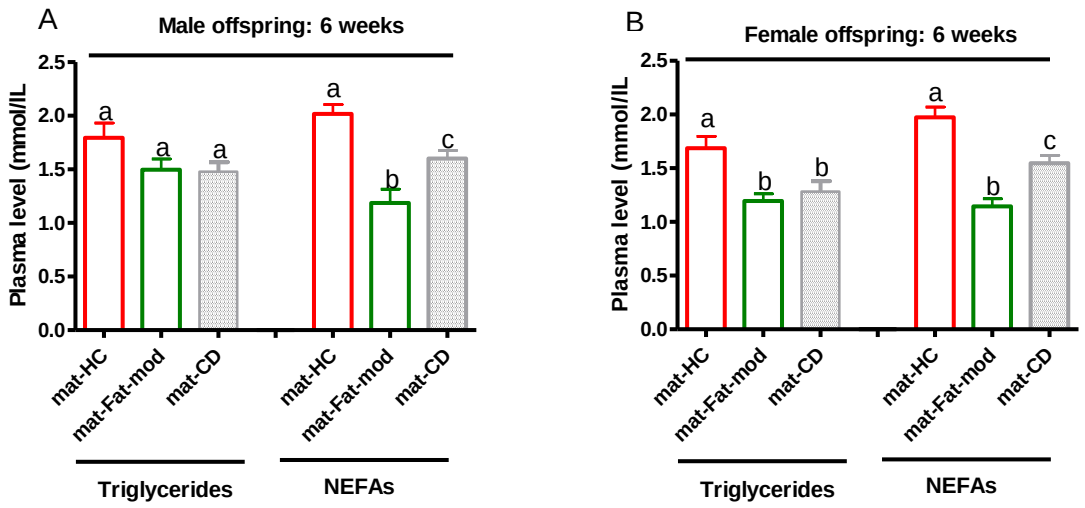

Figure 25: Triglycerides and NEFA concentrations in the plasma of **(A)** male offspring (mat-HC: n=21; mat-Fat-mod HC: n=19, mat-CD: n=19) and **(B)** female offspring (mat-HC: n=19; mat-Fat-mod HC: n=18, mat-CD: n=16) at an age of 6 weeks following prenatal exposure to different maternal dietary compositions.
Data are presented as mean ± s.e.m. and were analysed by ANOVA and Tukey's post hoc test.
mat-CD, exposure to maternal control diet (=reference); mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet; NEFA, non-esterified fatty acids.

Differences in plasma cholesterol concentrations were especially apparent in the female offspring exposed to mat-Fat-mod HC diet compared to the mat-HC group and emerged during aging (Table 7). At an age of 6 weeks, HDL-cholesterol was reduced in the mat-Fat-mod HC female offspring compared to the mat-HC diet group, whereas we detected no significant differences in any lipoprotein class in the male offspring between both groups. At an age of 16 weeks, concentrations of total plasma cholesterol, comprising HDL-cholesterol, low-density lipoprotein (LDL)-cholesterol and non-HDL-cholesterol, were reduced in the mat-Fat-mod HC female offspring compared to the mat-HC offspring. The reduction of total cholesterol in the mat-Fat-mod HC female offspring was mainly attributable to the pronounced decrease of HDL-cholesterol at an age of 16 weeks. In the mat-Fat-mod HC male offspring, only HDL- cholesterol was reduced at an age of 16 weeks.

Table 7: Plasma cholesterol concentrations of mat-HC and mat-Fat-mod HC male and female offspring at an age of 6 weeks and 16 weeks

	6 weeks		**16 weeks**	
Male offspring	**mat-HC** (n=21)	**mat-Fat-mod HC** (n=19)	**mat-HC** (n=32)	**mat-Fat-mod HC** (n=26)
cholesterol (mmol/L)	4.16 ± 0.13	4.00 ± 0.14	4.95 ± 0.20	4.44 ± 0.19
LDL- cholesterol (mmol/L)	0.55 ± 0.03	0.54 ± 0.03	0.68 ± 0.04	0.64 ± 0.03
HDL- cholesterol (mmol/L)	3.12 ± 0.08	3.01 ± 0.10	**3.36 ± 0.09**	**3.06 ± 0.12***
non-HDL- chol.(mmol/L)	1.04 ± 0.06	0.99 ± 0.04	1.49 ± 0.10	1.37 ± 0.09
Female offspring	**mat-HC** (n=19)	**mat-Fat-mod HC** (n=18)	**mat-HC** (n=28)	**mat-Fat-mod HC** (n=36)
cholesterol (mmol/L)	3.25 ± 0.10	2.94 ± 0.13	**3.69 ± 0.13**	**3.00 ± 0.13 *****
LDL- cholesterol (mmol/L)	0.44 ± 0.02	0.43 ± 0.02	**0.50 ± 0.02**	**0.41 ± 0.02 ****
HDL- cholesterol (mmol/L)	**2.41 ± 0.08**	**2.13 ± 0.11***	**2.55 ± 0.09**	**2.09 ± 0.09 *****
non-HDL- chol. (mmol/L)	0.85 ± 0.04	0.81 ± 0.04	**1.13 ± 0.07**	**0.91 ± 0.04 ****

Data are normally distributed and are presented as mean ± s.e.m. Analysis of significant differences between the mat-HC and the mat-Fat-mod HC group was performed by unpaired t-test.
mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

3.3.3.4. Longitudinal analysis of the adipose tissue phenotype of the offspring

In order to determine the effects of prenatal exposure to the differently composed maternal adipogenic diets on the offspring throughout life, dissections were performed at three specific time points - relevant to adipose tissue development in mice (at birth, 6 weeks and 16 weeks). Dissection at birth was chosen to evaluate the unadulterated effects of the maternal milieu on the newborn offspring. Considering that fat accumulation in mice mainly takes place during postnatal life (45) (62), the dissection at an age of 6 weeks was estimated as earliest date for drawing an adequate sample amount of white adipose tissue. As shown in our previous experiments (72), at an age of 16 weeks the phenotypical alterations firstly appeared in response to the exposure to a maternal adipogenic milieu, and therefore, this age was chosen as experimental end point of our study.

Newborn offspring outcomes

Body weight of newborn males and females was significantly reduced in response to the altered fat quality of the maternal obesogenic diet compared to the mat-HC group (Table 8). Dissection of the newborn offspring showed no significant differences in the weight *of M. quadriceps* and BAT between the mat-HC and mat-Fat-mod HC groups. As expected for the adipose tissue development in rodents, white adipose tissue could not be observed in any group at birth.

Table 8: Absolute and relative organ weights of mat-HC and mat-Fat-mod diet offspring at birth, at an age of 6 weeks and 16 weeks.

	birth		**6 weeks**		**16 weeks**	
Male offspring	**mat-HC** (n=15)	**mat-Fat-mod HC** (n=21)	**mat-HC** (n=21)	**mat-Fat-mod HC** (n=19)	**mat-HC** (n=32)	**mat-Fat-mod HC** (n=27)
Body weight (g)	**2.002 ± 0.024** (n=65)	**1.796 ± 0.024***** (n=66)	36.805 ± 0.734	36.824 ± 0.527	**48.763 ± 0.801**	**45.467 ± 0.949****
Intra-abdominal fat (g)	0.000 ± 0.000#	0.000 ± 0.000#	0.962 ± 0.052	0.856 ± 0.041	**2.110 ± 0.167**	**1.605 ± 0.139***
Intra-abdominal fat (g/g BW)	0.000 ± 0.000#	0.000 ± 0.000#	2.589 ± 0.112	2.329 ± 0.112	**4.269 ± 0.283**	**3.442 ± 0.232***
Subcutaneous fat (g)	0.000 ± 0.000#	0.000 ± 0.000#	**0.716 ± 0.061**	**0.552 ± 0.036***	**1.362 ± 0.120**	**0.982 ± 0.078***
Subcutaneous fat (g/g BW)	0.000 ± 0.000#	0.000 ± 0.000#	**1.925 ± 0.147**	**1.498 ± 0.093***	**2.760 ± 0.214**	**2.126 ± 0.141***
Brown adipose tissue (g)	0.013 ± 0.001	0.013 ± 0.001	**0.191 ± 0.013**	**0.144 ± 0.009****	**0.296 ± 0.018**	**0.218 ± 0.016****
Brown adipose tissue (g/g BW)	0.666 ± 0.046	0.688 ± 0.049	**0.522 ± 0.035**	**0.389 ± 0.024****	**0.606 ± 0.036**	**0.476 ± 0.032****
M. quadrizeps (g)	0.010 ± 0.001	0.007 ± 0.001	0.396 ± 0.019	0.386 ± 0.019	0.563 ± 0.017	0.530 ± 0.017
M. quadrizeps (g/g BW)	0.521 ± 0.059	0.417 ± 0.038	1.076 ± 0.050	1.056 ± 0.057	1.160 ± 0.034	1.174 ± 0.039
Carcass (g)	n.d.	n.d.	14.858 ± 0.348	14.719 ± 0.275	18.681 ± 0.281	18.105 ± 0.355
Carcass (g/g BW)	n.d.	n.d.	40.459 ± 0.744	39.945 ± 0.334	**38.381 ± 0.332**	**39.872 ± 0.272***
Female offspring	**mat-HC** (n=17)	**mat-Fat-mod HC** (n=23)	**mat-HC** (n=19)	**mat-Fat-mod HC** (n=18)	**mat-HC** (n=28)	**mat-Fat-mod HC** (n=35)
Body weight (g)	**1.839 ± 0.023** (n=60)	**1.761 ± 0.019***** (n=79)	29.537 ± 0.601	28.880 ± 0.554	40.032 ± 1.214	38.806 ± 0.883
Intra-abdominal fat (g)	0.000 ± 0.000#	0.000 ± 0.000#	0.688 ± 0.074	0.621 ± 0.054	2.225 ± 0.253	2.283 ± 0.197
Intra-abdominal fat (g/g BW)	0.000 ± 0.000#	0.000 ± 0.000#	2.290 ± 0.197	2.119 ± 0.155	5.233 ± 0.463	5.505 ± 0.389
Subcutaneous fat (g)	0.000 ± 0.000#	0.000 ± 0.000#	0.529 ± 0.051	0.509 ± 0.053	1.384 ± 0.153	1.283 ± 0.121
Subcutaneous fat (g/g BW)	0.000 ± 0.000#	0.000 ± 0.000#	1.784 ± 0.164	1.753 ± 0.174	3.307 ± 0.289	3.132 ± 0.260
Brown adipose tissue (g)	0.014 ± 0.001	0.014 ± 0.001	0.137 ± 0.006	0.166 ± 0.038	0.219 ± 0.022	0.185 ± 0.013

Brown adipose tissue (g/g BW)	0.779 ± 0.050	0.769 ± 0.032	0.465 ± 0.023	0.563 ± 0.127	0.539 ± 0.046	0.461 ± 0.029
M. quadrizeps (g)	0.005 ± 0.001	0.004 ± 0.000	0.328 ± 0.018	0.321 ± 0.018	0.461 ± 0.017	0.469 ± 0.015
M. quadrizeps (g/g BW)	0.304 ± 0.030	0.251 ± 0.017	1.102 ± 0.049	1.118 ± 0.063	1.162 ± 0.037	1.185 ± 0.035
Carcass (g)	n.d.	n.d.	11.862 ± 0.266	11.439 ± 0.235	15.493 ± 0.335	15.351 ± 0.315
Carcass (g/g BW)	n.d.	n.d.	40.168 ± 0.422	39.622 ± 0.404	39.037 ± 0.572	38.675 ± 0.386

Data are normally distributed and are presented as mean ± s.e.m. Analysis of significant differences between the mat-HC and the mat-Fat-mod HC group by unpaired t-test.; **p*<0.05, ***p*<0.01, ****p*<0.001. [#] white adipose tissue was not detectable.
BW, body weight; Body weight at birth comprises the total number of offspring which were dissected at birth or transferred to foster dams; mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet; n.d. not dissected.

Fat mass and adipocyte size of the offspring at an age of 6 weeks

Male offspring

At an age of 6 weeks, the emerging differences in total body fat of the male offspring (Figure 23) were reflected in a reduced total and relative subcutaneous fat pad weight (Table 8) of the mat-Fat-mod HC males compared to the mat-HC males. Interestingly, BAT was also reduced in the mat-Fat-mod HC male offspring, whereas intra-abdominal adipose tissue was not affected by the prenatal exposure to the mat-Fat-mod HC diet.

With respect to the adipocyte size in the intra-abdominal adipose tissue, there were no significant differences between the mat-Fat-mod HC and mat-HC male offspring (Figure 26).

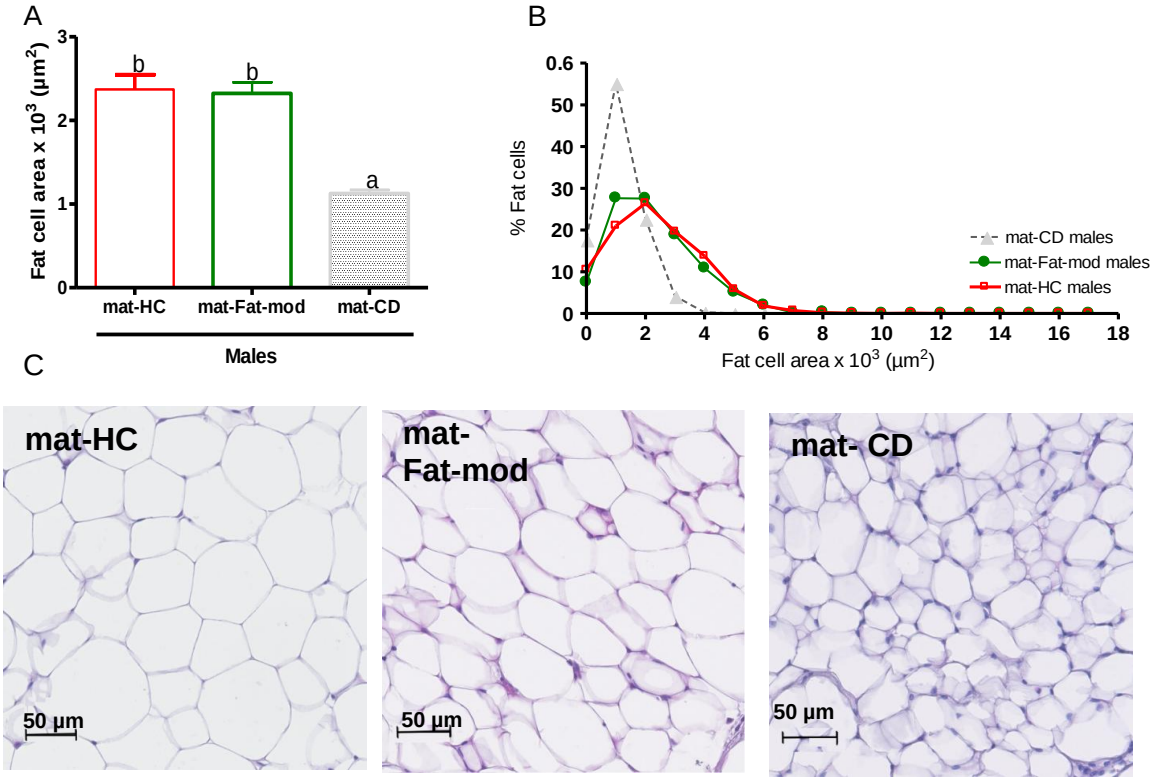

Figure 26: Impact of the periconceptional fat-modified high-fat, high-calorie diet on **(A)** adipocyte size of intra-abdominal adipose tissue of male offspring at an age of 6 weeks (n=10 mice per group; mean of 100 adipocytes per mouse). **(B)** Intra-abdominal fat pad histology, representative sections of mat-HC, mat-Fat-mod HC and mat-CD male offspring groups at an age of 6 weeks (FFPE, HE, scale bars represent 50 µm). **(C)** Fat cell area distribution of intra-abdominal fat pads at at an age of 6 weeks expressed as percentage of cells (n=10 mice per group; 100 adipocytes per animal).
Data are presented as mean ± s.e.m. and were analysed by ANOVA and Tukey's post hoc test.
FFPE, formalin-fixed, paraffin-embedded; HE, hematoxylin-eosin; mat-CD, exposure to maternal control diet (=reference); mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

In order to draw conclusions about effects of the maternal milieu on the normal range of adipocyte size, the adipocytes of a control group of male offspring, born to CD-fed mothers in the same experimental setting, were also analysed. Interestingly, the adipocyte sizes of both the mat-HC and mat-Fat-mod HC male offspring were significantly increased at an age of 6 weeks compared to the control situation, possibly indicating an acceleration of adipose tissue development during early postnatal life.

Female offspring

In the female offspring, no significant differences in organ and fat pad weights were detected (Table 8). Comparable to the male offspring, the intra-abdominal adipocytes of the mat-Fat-mod HC and mat-HC female offspring were similar in size, whereas adipocytes of both female groups (mat-HC, mat-Fat-mod HC) were significantly larger compared to the female control offspring (Figure 27).

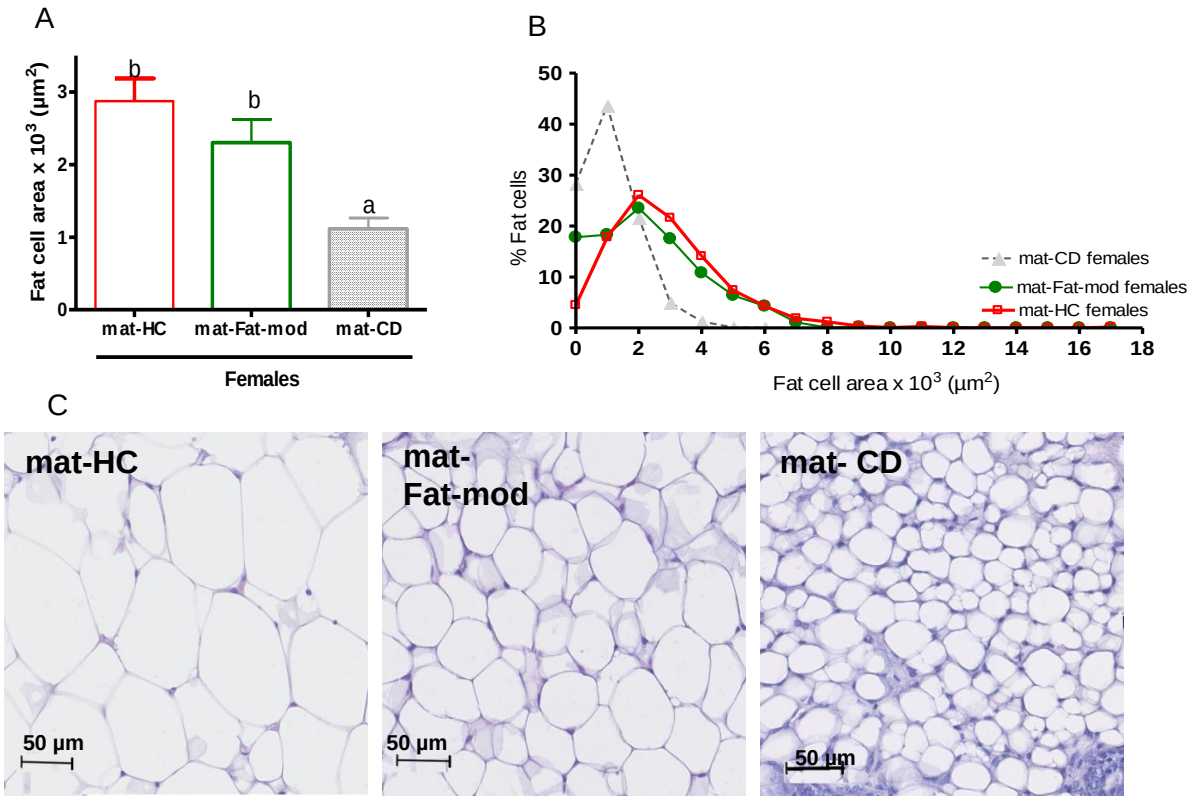

Figure 27: Impact of the periconceptional fat-modified high-fat, high-calorie diet on **(A)** adipocyte size of intra-abdominal adipose tissue of female offspring at an age of 6 weeks (n=10 mice per group; mean of 100 adipocytes per mouse). **(B)** Intra-abdominal fat pad histology, representative sections of mat-HC, mat-Fat-mod HC and mat-CD female offspring groups at an age of 6 weeks (FFPE, HE, scale bars represent 50 µm). **(C)** Fat cell area distribution of intra-abdominal fat pads at an age of 6 weeks expressed as percentage of cells (n=10 mice per group; 100 adipocytes per animal).
Data are presented as mean ± s.e.m. and were analysed by ANOVA and Tukey's post hoc test.
FFPE, formalin-fixed, paraffin-embedded; HE, hematoxylin-eosin; mat-CD, exposure to maternal control diet (=reference); mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

Fat mass and adipocyte size of the offspring at an age of 16 weeks

Male offspring

At an age of 16 weeks, body weight and weight of all fat compartments were reduced in the mat-Fat-mod HC male offspring (Table 8) corresponding to the decreased total body fat mass detected by NMR spectroscopy (Figure 23). Interestingly, the carcass to body weight ratio was significantly increased in the mat-Fat-mod HC male offspring, indicating a higher relative lean mass in response to the prenatal exposure to an altered fat composition of the adipogenic diet (Table 8).

Comparable to the adipocyte size at an age of 6 weeks, there were no significant differences between the mat-Fat-mod HC and mat-HC male offspring (Figure 28). At an age of 16 weeks adipocyte sizes of the mat-Fat-mod HC and mat-HC male offspring were similar to control.

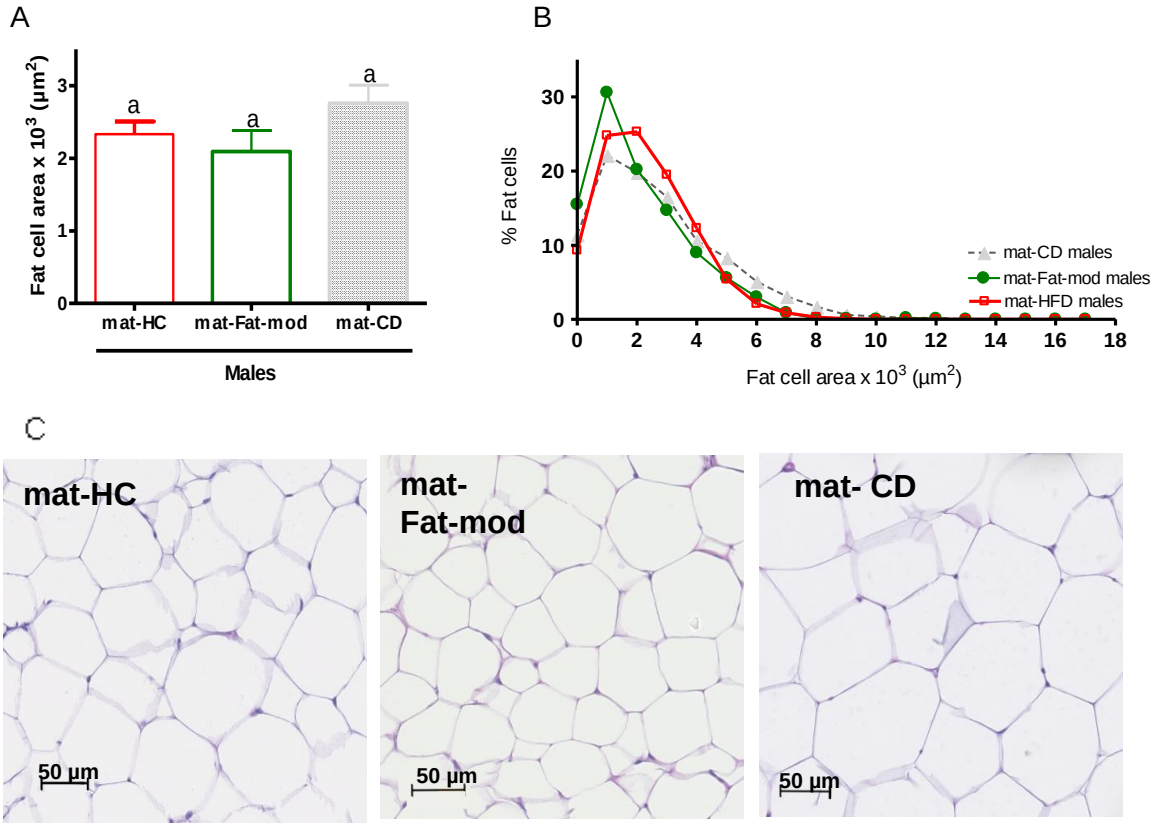

Figure 28: Impact of the periconceptional fat-modified high-fat, high-calorie diet on **(A)** adipocyte size of intra-abdominal adipose tissue of male offspring at an age of 16 weeks (n=10 mice per group; mean of 100 adipocytes per mouse). **(B)** Intra-abdominal fat pad histology, representative sections of mat-HC, mat-Fat-mod HC and mat-CD male offspring groups at an age of 16 weeks (FFPE, HE, scale bars represent 50 µm). **(C)** Fat cell area distribution of intra-abdominal fat pads at an age of 16 weeks expressed as percentage of cells (n=10 mice per group; 100 adipocytes per animal).
Data are presented as mean ± s.e.m. and were analysed by ANOVA and Tukey's post hoc test.
FFPE, formalin-fixed, paraffin-embedded; HE, hematoxylin-eosin; mat-CD, exposure to maternal control diet (=reference); mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

Female offspring

In accordance to the total body fat mass (Figure 24), no significant differences of organ weights were detected in the female offspring (Table 8).

As shown previously (72), the adipocyte size in female offspring exposed to a maternal adipogenic diet *in utero* was significantly smaller compared to the control group. In the actual experiment, the mat-Fat-mod HC female offspring showed a tendency towards an increased adipocyte size compared to the mat-HC females. Furthermore, this was accompanied by a trend towards an approximation to the adipocyte size of the control group (Figure 29).

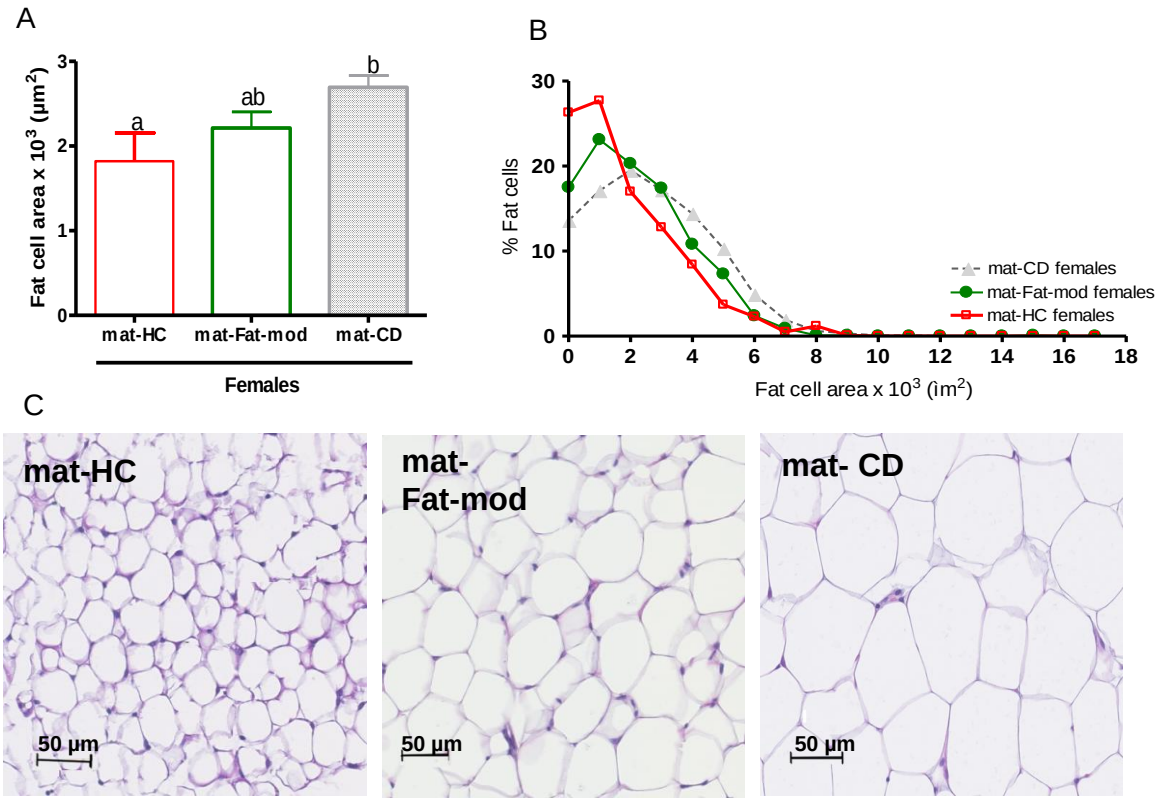

Figure 29: Impact of the periconceptional fat-modified high-fat, high-calorie diet on **(A)** adipocyte size of intra-abdominal adipose tissue of female offspring at an age of 16 weeks (n=10 mice per group; mean of 100 adipocytes per mouse). **(B)** Intra-abdominal fat pad histology, representative sections of mat-HC, mat-Fat-mod HC and mat-CD female offspring groups at an age of 16 weeks (FFPE, HE, scale bars represent 50 µm). **(C)** Fat cell area distribution of intra-abdominal fat pads at an age of 16 weeks expressed as percentage of cells (n=10 mice per group; 100 adipocytes per animal).
Data are presented as mean ± s.e.m. and were analysed by ANOVA and Tukey's post hoc test.
FFPE, formalin-fixed, paraffin-embedded; HE, hematoxylin-eosin; mat-CD, exposure to maternal control diet (=reference); mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

3.3.3.5. Time course of intra-abdominal adipocyte development of the offspring

Considering the dynamic development of adipocytes in the intra-abdominal adipose tissue during postnatal life in mice, we studied two different time points (at age: 6 and 16 weeks) in a sex-specific manner.

Figure 30A shows the physiological increase of adipocyte size during postnatal development (age: 6 to 16 weeks) for the male and female offspring of the control group. In the mat-CD male offspring, adipocyte size showed a significant increase of 145% ($p<0.0001$) between 6 and 16 weeks. In contrast, the growth of adipocyte size of male offspring of both maternal HC diet groups stagnated (mat-HC: -1.7%, $p=0.87$ and mat-Fat-mod: -10%, $p=0.48$) over the same period (Figure 30B, C).

In the female control offspring, adipocyte size also strongly increased by approximately 140% ($p< 0.0001$) between an age of 6 and 16 weeks (Figure 30A). Comparable to the male offspring, the growth of adipocyte size stagnated in the mat-Fat-mod HC females (-3.9%, $p=0.81$) between an age of 6 and 16 weeks (Figure 30C). In the mat-HC female offspring, we

even detected a significantly decline of adipocyte size (-36.7%, p=0.03) during the same period (Figure 30B).

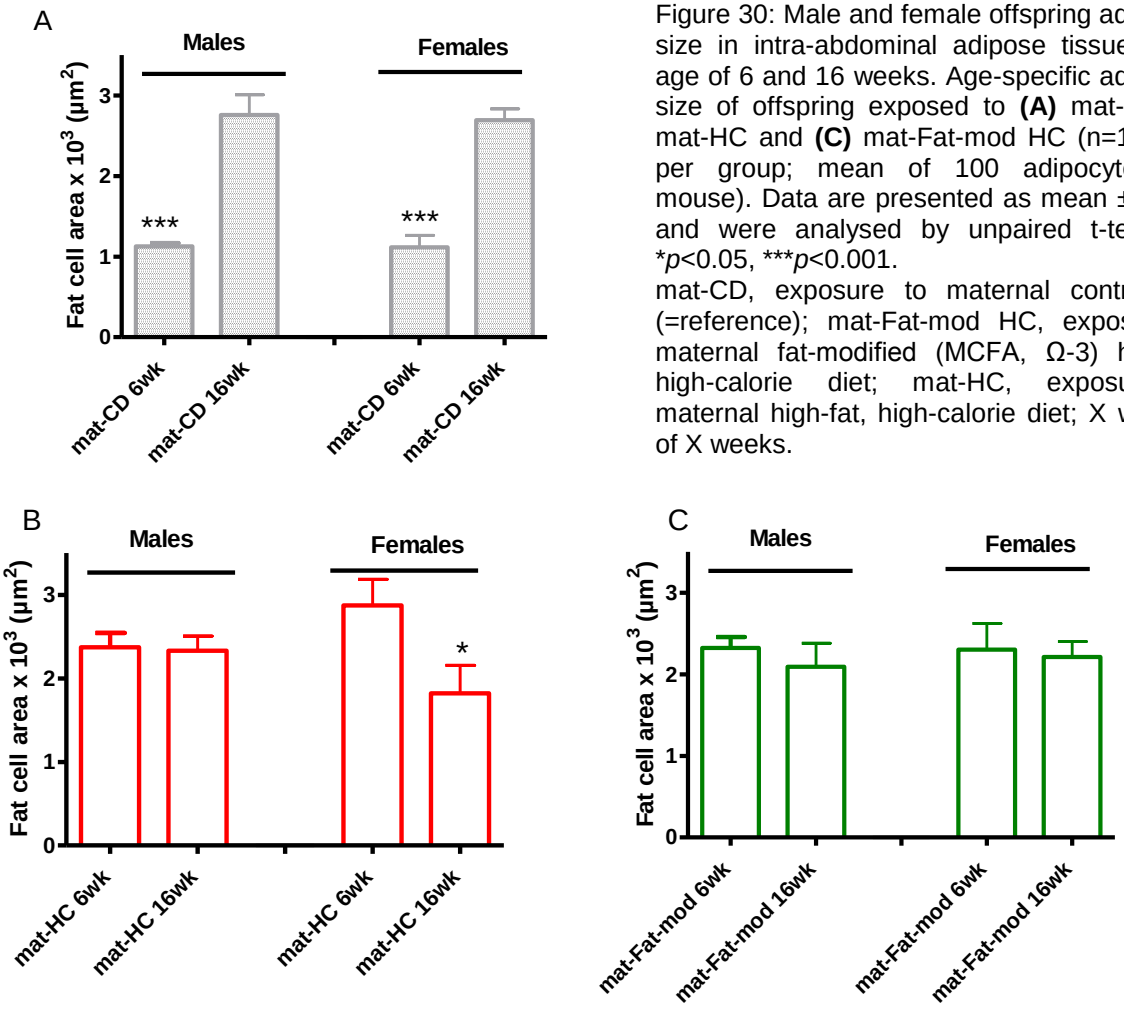

Figure 30: Male and female offspring adipocyte size in intra-abdominal adipose tissue at an age of 6 and 16 weeks. Age-specific adipocyte size of offspring exposed to **(A)** mat-CD **(B)** mat-HC and **(C)** mat-Fat-mod HC (n=10 mice per group; mean of 100 adipocytes per mouse). Data are presented as mean ± s.e.m. and were analysed by unpaired t-test and **p*<0.05, ****p*<0.001.
mat-CD, exposure to maternal control diet (=reference); mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet; X wk, age of X weeks.

3.3.4. Effects on gene expression pattern in intra-abdominal adipose tissue of the offspring

Because of the modulating effects of the prenatal exposure to an altered fat composition of the maternal adipogenic diet on the adipocyte phenotype of the female offspring (Figure 30), RNA-microarray analysis was conducted in intra-abdominal adipose tissue of mat-Fat-mod HC and mat-HC female offspring at an age of 6 weeks. **Firstly**, for each of the two offspring groups, DEGs were analysed in relation to the mat-CD offspring, which were born to CD-fed mothers in the same experimental setting. **Secondly**, gene expression of the mat-Fat-mod HC female offspring was compared to that of the mat-HC group.

3.3.4.1. Hierarchical clustering of differentially expressed genes in female adipocytes

In a first approach, we aimed to gain an overview about the gene expression pattern induced by the intrauterine exposure to the altered fat composition of the maternal obesogenic diet.

Thus, the gene expression in the intra-abdominal adipose tissue of the mat-HC and mat-Fat-mod HC female offspring was compared to that of the mat-CD female offspring. Significantly DEGs were detected as outlined above (2.10.2). A panel of genes, which was differentially expressed between mat-HC vs mat-Fat-mod HC female offspring, was determined and expression per animal was depicted in the heatmap (Figure 31A) for the three female offspring groups (mat-HC, mat-Fat-mod HC, mat-CD). Accordingly, this heatmap of DEGs showed a distinct expression pattern in the intra-abdominal adipose tissue of the mat-HC and mat-Fat-mod HC female offspring. However, the pattern of differential gene expression following exposure to the altered maternal adipogenic diet (mat-Fat-mod HC) seemed to approximate the control situation (Figure 31A).

Compared to the mat-CD female offspring, the number of DEGs in response to exposure to the maternal obesogenic diet (mat-HC diet vs mat-CD) exceeded the amount of DEGs in the intra-abdominal adipose tissue of the mat-Fat-mod HC female offspring (mat-Fat-mod HC diet vs mat-CD) (Figure 31B). In particular, the comparison of the gene expression of the mat-HC vs mat-CD female offspring revealed 578 DEGs, whereas in intra-abdominal adipose tissue of the mat-Fat-mod HC vs mat-CD female offspring only 148 genes were differentially expressed. In the group of the mat-HC female offspring, 256 genes were downregulated and 322 genes were upregulated. In contrast, in the group of the mat-Fat-mod HC female offspring, only 50 genes were downregulated, and 98 genes were upregulated, each in comparison to female control adipocytes (mat-CD) (Figure 31C).

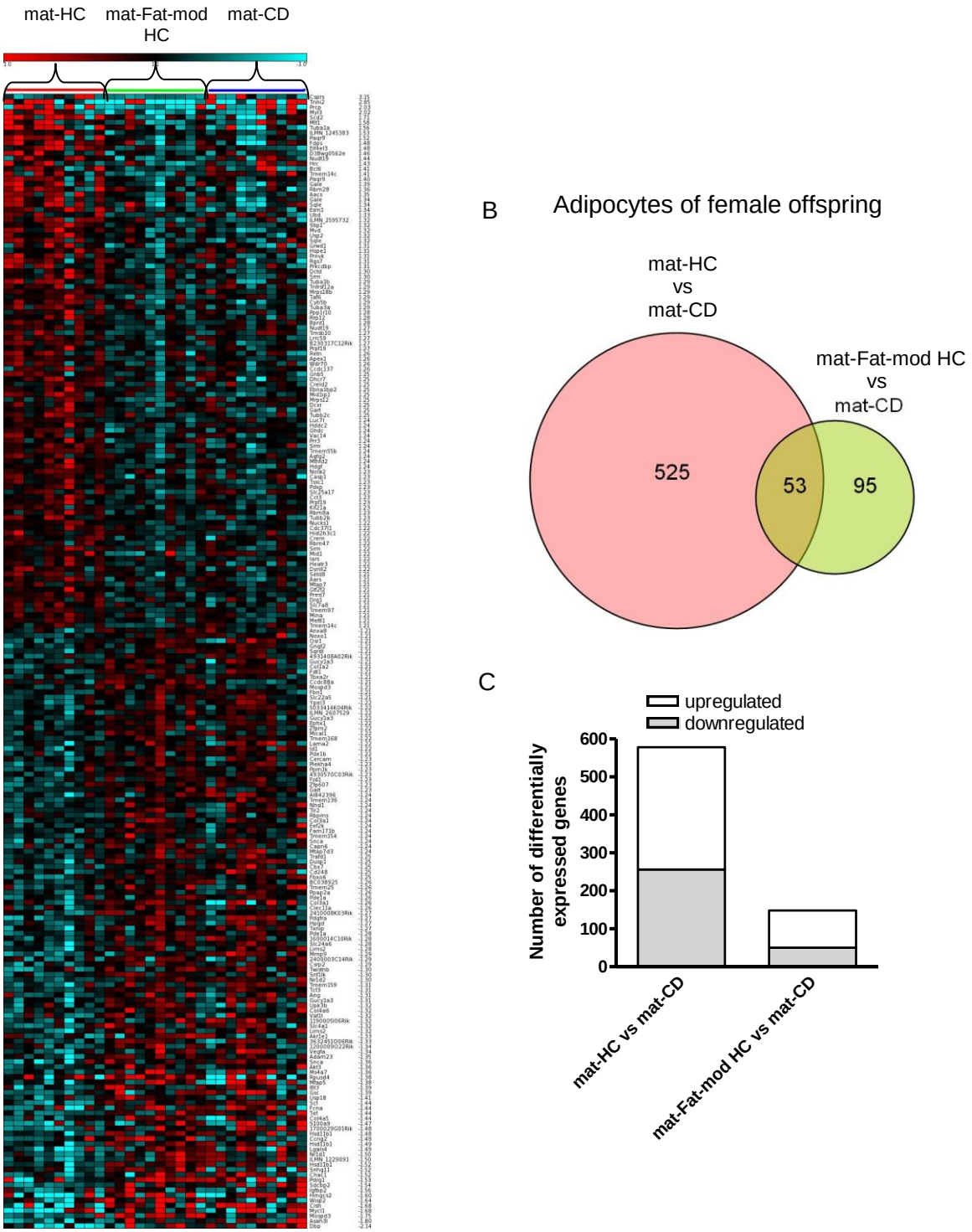

Figure 31: Microarray-based detection of effects in adipocytes of female offspring at an age of 6 weeks following exposure to the two adipogenic diets (mat-HC, mat-Fat-mod HC) *in utero* in comparison to control situation. **(A)** Hierarchical clustering of a panel of genes which were differentially expressed between mat-HC vs mat-Fat-mod HC female offspring. Expression per animal (n=10 animals per group) was depicted in the heatmap for the three female offspring groups (mat-HC, mat-Fat-mod, mat-CD. Fold changes of upregulated genes are shown in red, fold changes of downregulated genes in blue. **(B)** Differentially expressed genes (DEGs) in adipocytes of female offspring: comparison mat-HC vs mat-CD female offspring (red), comparison mat-Fat-mod HC vs mat-CD female offspring (light green) or in response to both maternal high-fat, high-calorie diets (ochre). **(C)** Number of up- and downregulated DEGs. Up- and downregulated DEGs are shown as white and light grey bars, respectively.
After hybridization of probes to Expression BeadChips (Illumina), DEGs were identified by limma t-test (fold change>1.2x, $p<0.05$).
mat-CD, exposure to maternal control diet; mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet.

3.3.4.2. Functional enrichment analysis of differential gene expression in female adipocytes

In addition to the quantitative differences, the prenatal exposure to an altered fat composition of the maternal adipogenic diet affected the type of DEGs in adipocytes of the female offspring at an age of 6 weeks.

Effects of prenatal exposure to the two adipogenic diets in comparison to the mat-CD female offspring

In a first approach, the effects in adipocytes of the female offspring were analysed following exposure to the two maternal adipogenic diets (mat-HC, mat-Fat-mod HC) in comparison to control situation. We conducted gene enrichment analysis for **1)** the share of genes which were differentially expressed in response to both adipogenic diets *in utero* (ochre intersection, Figure 31B) and **2)** DEGs which were unique either for the mat-HC female offspring (red area, Figure 31B) or for the mat-Fat-mod HC female offspring (green area, Figure 31B), each in comparison to the mat-CD female offspring.

Firstly, the small share of DEGs (n=53) in response to prenatal exposure to each of the maternal adipogenic diets (mat-HC, mat-Fat-mod HC) showed the same direction of regulation (ochre intersection: Figure 31B). Four of the DEGs were enriched in pathways of “urogenital system development” (fold enrichment: 1.86) (CD44 antigen, secreted frizzled-related protein 1, sine oculis-related homeobox 1 homolog, slit homolog 2) and five DEGs belonged to “fatty acid metabolic process” (fold enrichment: 1.33) (Table 9). Interestingly, all of these five DEGs enriched in “fatty acid metabolic process” were significantly downregulated in female offspring adipocytes in response to prenatal exposure to each of the maternal adipogenic diets (mat-HC, mat-Fat-mod HC) compared to control situation (Table 9).

Table 9: Share of differentially expressed genes (DEGs) involved in “fatty acid metabolic process” in intra-abdominal adipose tissue of mat-HC and mat-Fat-mod HC female offspring compared to control:

DEGs	Fold change	
	mat-HC ♀ vs mat-CD ♀	mat-Fat-mod HC ♀ vs mat-CD ♀
Acsm3	-1,69	-1,86
Dci	-1,33	-1,34
Ech1	-1,24	-1,22
Ephx2	-1,65	-1,41
Plp1	-1,57	-1,37

After hybridization of probes to Expression BeadChips (Illumina), DEGs in intra-abdominal adipose tissue of female offspring were identified by limma t-test (fold change>1.2x, p<0.05). Enrichment analysis was conducted via DAVID using the share of DEGs, which were differentially expressed in mat-HC and mat-Fat-mod HC female offspring (each in comparison to control).
Acsm3, acyl-CoA synthetase medium-chain family member 3; DEG, differentially expressed gene; *Dci*, dodecenoyl-Coenzyme A delta isomerase; *Ech1*, enoyl coenzyme A hydratase 1; *Ephx2*, peroxisomal, epoxide hydrolase 2; FC, fold change; mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet; *Plp1*, cytoplasmic proteolipid protein (myelin) 1.

The significant downregulation of DEGs involved in “fatty acid metabolic process” was in accordance with the reduced growth of adipocyte size between an age of 6 and 16 weeks in response to the prenatal exposure to each of the maternal adipogenic diets (mat-HC, mat-Fat-mod HC) compared to control situation (3.3.3.5.; Figure 30). When we analyzed the plasma lipid concentrations (3.3.3.3.), exposure to an altered fat composition of the maternal adipogenic diet resulted in reduced NEFA concentrations in the plasma of the mat-Fat-mod HC female offspring at an age of 6 weeks in comparison to the mat-CD female offspring (mat-Fat-mod HC diet vs mat-CD; Figure 25). This may correpond to the downregulation of DEGs involved in “fatty acid metabolic process” pathways in the intra-abdominal adipose tissue of the mat-Fat-mod HC females compared to control situation. In contrast, the plasma triglyceride and NEFA concentrations were elevated in the mat-HC female offspring at an age of 6 weeks in comparison to control (mat-HC diet vs mat-CD).

Secondly, we focused on enriched pathways which might be associated with the observed differences in adipose tissue phenotype of the female offspring prenatally exposed to the maternal adipogenic diets (mat-HC, mat-Fat-mod HC). Enrichment analysis was based on DEGs which were specifically differentially expressed in response to mat-HC female offspring (red: Figure 31B) or mat-Fat-mod HC female offspring (green: Figure 31B), each in comparison to controls (Appendix Table S6).

When we analysed DEGs specific for the exposure to mat-HC diet, we detected a highest enrichment of upregulated DEGs involved in “cholesterol biosynthetic process” in the adipocytes of mat-HC female offspring (fold enrichment: 4.09) compared to control (list of DEGs shown in appendix, Table S6).

Enrichment analysis of genes, which were exclusively differentially expressed only in the group of mat-Fat-mod HC female offspring compared to control, revealed that DEGs were

enriched in pathways such as “cell adhesion processes” (fold enrichment: 2.59) and “blood vessel development” (fold enrichment: 1.38) (list of DEGs shown in appendix, Table S6).

Effects of the altered fat quality of the adipogenic diet compared to mat-HC female offspring

In the next step, differential gene expression in the intra-abdominal adipose tissue of the mat-Fat-mod HC females was compared to that of the mat-HC female offspring (Figure 32) in order to get first insights into possible “preventive” effects of exposure to a fat-modified adipogenic diet *in utero*. When compared to the mat-HC female offspring, enrichment analysis showed that the mat-Fat-mod HC diet resulted in a downregulation of genes involved “cholesterol biosynthesic process” at an age of 6 weeks. This result of the RNA-microarray at an age of 6 weeks may correspond to the lower plasma cholesterol concentrations in the mat-Fat-mod HC female offspring at an age of 16 weeks in comparison to the mat-HC female offspring (3.3.3.3.; Table 7). In addition, genes of “developmental processes” were upregulated in the mat-Fat-mod HC female offspring compared to the unmodified mat-HC group.

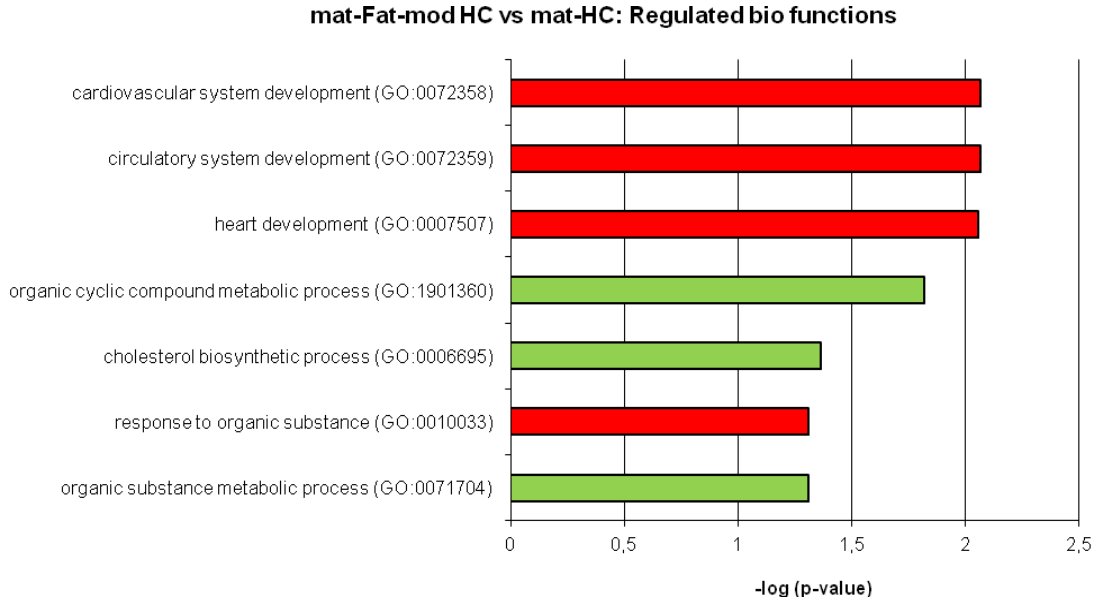

Figure 32: Enrichment analysis of differentially expressed genes (DEGs) in adipocytes of the female offspring at an age of 6 weeks following exposure to mat-Fat-mod HC diet in comparison to the mat-HC female offspring: After hybridization of probes to Expression BeadChips (Illumina), DEGs were identified by limma t-test (fold change>1.2x, $p<0.05$). Enrichment analysis was based on DEGs of female offspring exposed to maternal HC diet (n=10) or Fat-mod HC diet (n=10) *in utero* (comparison: mat-Fat-mod HC diet vs mat-HC diet). Enrichment analysis of DEGs was conducted via AmiGo ($p<0.05$). Significant enrichment of down- and upregulated DEGs to metabolic pathways was indicated by green or red bars, respectively.
mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet; GO, gene ontology.

4. Discussion

Exposure to a maternal obesogenic milieu results in a 'vicious cycle' transmitting an enhanced risk for obesity and related metabolic disturbances from the mothers to their offspring. Especially, adipose tissue is considered an important organ linking adverse metabolic exposures in pregnancy and the later health of the offspring. However, more research is needed to investigate which nutritional compounds may play a role.

The consequences of the exposure to a maternal obesogenic diet in pregnancy on the adipocyte phenotype of the offspring and the underlying gene regulation are not well established. What happens, when the offspring is not only fed an obesogenic diet during postnatal life but has additionally been exposed to a maternal obesogenic milieu *in utero*, is not clear for adipose tissue development and requires further research. Finally, whether negative effects can be counteracted by "nutritional reprogramming" strategies is currently unclear.

4.1. Influence of a prenatal adipogenic exposure on the adipose tissue phenotype and glucose tolerance in offspring

In our NMRI outbred mouse model for periconceptional maternal obesity, the offspring, which were born to obese mothers, transferred to lean foster dams and weaned onto CD (HC/CD), showed sex-specific metabolic disturbances at an age of 5 months. The exposure to a prenatal obesogenic milieu alone seems to disturb the development of fat mass and adipocyte size as evidenced in the adult female offspring, whereas adipose tissue development of male offspring appeared to be less affected. We investigated possible adipose tissue-mediated disturbances in gene regulation, which might underlie the reduced adipocyte expandybility in the female offspring born to obese mothers.

4.1.1. Alteration of *de novo* lipogenesis in adipocytes of adult offspring

We assessed the effects of the prenatal adipogenic exposure on the intra-abdominal adipose tissue of the offspring comprising perigonadal, perirenal and omental fat pads, similar to previous studies (72). Even in the absence of any postnatal HC diet feeding, microarray analysis indicated an upregulation of lipogenetic pathways in the small female adipocytes, when female offspring were exposed to a maternal obesogenic milieu. This upregulation of *DNL* in the intra-abdominal adipose tissue of HC/CD female offspring was corroborated by enhanced transcriptional abundances of *Me1* and *Acaca*, representing two important enzymes involved in *de novo* fatty acid synthesis providing NADPH (147) and Malonyl-CoA (148), respectively. In addition, *Fasn*, the key enzyme of *DNL* (149), was upregulated on a transcriptional and enzyme activity level in the intra-abdominal adipose tissue of the HC/CD females. This implies that the transcriptional upregulation results in an enhanced activity of FASN and is not attributable to a transcriptional adaptive response with no functional impact (150). The upregulation of *DNL* in small adipocytes has already been demonstraed in

humans (151) and is known to promote fat accumulation during adipose tissue development (152). The crucial impact of *DNL* on the regulation of body fat mass was demonstrated by a recent rodent study for exercise-induced weight loss (153). In adipose tissue of rats, exercise resulted in a decreased expression of lipogenic genes, such as *Fasn* and acetyl carboxylase 1, which might be involved in the prevention of body fat re-gain after weight loss. Thus, the upregulation of lipogenic genes in the small adipocytes of the HC/CD female offspring might provide the basis for disturbed fat accumulation later on. Accordingly, in a rodent model of maternal nutrient restriction, small for gestational age offspring showed an increased *DNL* in white adipose tissue at the end of weaning, which is supposed to precede the development of obesity during adulthood (154).

In our NMRI mouse model, the predisposition towards a disproportionate adipogenic capacity, caused by the exposure to a maternal adipogenic milieu, emerged especially during postnatal HC diet feeding. Female offspring which were prenatally exposed to maternal obesity and weaned onto HC diet (HC/HC) showed excess fat accumulation and increased adipocyte size. In the male offspring, there was only an effect of the postnatal HC diet feeding on body fat mass, whereas the prenatal exposure to a maternal adipogenic milieu exerted only mild additional effects on fat accumulation. In accordance to recent mouse studies, the exposure to a maternal obesogenic milieu seems to overcharge the metabolic capacity to adapt to postnatal nutrient excess (30), predisposing the exposed offspring to the development of non-communicable diseases later on. Focusing on adipose tissue expansion, rat studies indicated an increased predisposition to diet-induced obesity of offspring, born to obese mothers (155) (67). In accordance to the sex-specific effects in our study, Bayol et al. showed that postnatal junk food feeding induced a more pronounced increase of perirenal fat mass and adipocyte size in the female offspring when additionally exposed to maternal junk-food diet *in utero*. However, as expected for nutrient excess, transcriptional abundances of lipogenetic genes as well as the enzyme activity of FASN were reduced in response to postnatal HC diet feeding in our study, while downregulation was not affected by the prenatal maternal milieu.

Thus, the disturbed ability for appropriate adipose tissue expansion in the female offspring may be corroborated by the dramatic increase of fat accumulation during postnatal HC diet feeding. This might be based on an enhanced lipogenesis in the adipose tissue of female offspring exposed to a maternal HC diet, which might predispose the female offspring for disproportionate fat expansion.

4.1.2. Possible involvement of inflammation in intra-abdominal adipose tissue of the offspring

In the intra-abdominal adipose tissue of the HC/CD female offspring, microarray-based enrichment analysis showed that the reduced fat mass was also accompanied by a downregulation of inflammatory pathways.

Recent studies indicated an essential role of inflammation for adipose tissue formation. In contrast to the prevailing opinion reporting deleterious effects of systemic low-grade inflammation during obesity (156), acute local inflammation seems to be essential for correct adipose tissue formation (157). Wernstedt Asterholm et al. used three transgenic mouse models to corroborate that local inflammation is urgently needed for a proper adipose tissue function, which prevents the adverse effects of obesity-associated systemic inflammation (157). In case of reduced pro-inflammatory signalling in adipose tissue, their study showed that fat mass was diminished. In addition, HFD-feeding resulted in reduced glucose tolerance, increased hepatic steatosis and reduced adiponectin concentrations (157). Accordingly, there is evidence that the induction of angiogenesis via infiltration of macrophages plays an essential role for remodelling of adipose tissue during obesity (158), while pro-inflammatory cytokines may counteract obesity via induction of energy expenditure (159). Further, maternal IL-6 deficiency during pregnancy and lactation led to earlier onset of adiposity and reduced insulin sensitivity in the offspring suggesting a critical role of inflammatory cytokines even during the pre- and early postnatal life (160).

Thus, the microarray-based downregulation of inflammatory pathways in the intra-abdominal adipose tissue of the HC/CD females might be a possible feature of the disturbed adipose tissue physiology resulting in an impaired capability for proper adipose tissue remodeling and expansion. However in a first approach, quantitative confirmation by qRT-PCR showed no significant downregulation of a small panel of selected inflammatory genes. However, we detected a trend towards a transcriptional downregulation of *Il-10* and *Mrc1,* which are known to be associated with M2-macrophages (145). Thus, a closer look at transcript and protein levels of further pro-inflammatory cytokines (e.g. tumor necrosis factor- alpha (TNF-α), IL-1-β or IL-6), which are known to be involved in adipose tissue inflammation (161), would be an interesting approach for future reseach.

4.1.3. Other potential influences of altered pre-adipocyte development on mature adipose tissue

Using our pregnancy mouse model, excluding any additional adipogenic exposure during lactation, the phenotypical alterations in the offspring might be attributable to insults of the maternal milieu during very early stages of development *in utero*. Further, our research group showed that the delayed expansion of the visceral adipose tissue is already present in 3-week old female offspring born to obese mothers (72). This might suggest that the reduced

adipocyte expandibility is already established during the very early development of the female offspring.

Regarding the intrauterine development, an appropriate recruitment of embryonic mesenchymal stem cells to different cell fates provide the basis for the cellular composition of adipose tissue later on (162). Thus, we investigated the expression of genes involved in innervation, vasculalization and fibrosis of adipose tissue, which might contribute to the smaller adipocytes of the HC/CD female offspring. Despite a lack of transcriptional differences of genes involved in any of these pathways, two **early stage markers** (*Egr2, Ucp1)*, which are expressed by stem cells and immature adipocytes, were significantly upregulated in the adipose tissue of the HC/CD females at an age of 5 months. With respect to the *Ucp1* abundance during development, highest UCP1 levels are detected in the abdominal adipose tissue immediately after birth in mammals (163). During postnatal life UCP1 expression rapidly declines via morphological conversion of brown-like to white adipocytes. However, the higher transcriptional abundance of *Ucp1,* that we found in the intra-abdominal adipose tissue of the HC/CD female offspring at an age of 5 months, might suggest a shift of adipocyte subtypes, which might possibly result in an altered adipose tissue composition. Considering that EGR2 is an important transcription factor for the promotion of adipocyte differentiaton (164), the observed upregulation of *Egr2* possibly suggests an induction of adipogenesis in adipocytes of the HC/CD females, which persists even at an age of 5 months.

With respect to **markers of mature adipocytes**, we detected differential expression of two genes (*Leptin*, *Gpd1*), involved in the regulation of expansion of adult adipose tissue. Leptin secretion corresponds to adipocyte size and fat mass (165) and was significantly downregulated in HC/CD females according to their lower fat mass. Further, we detected a transcriptional upregulation of *Gpd1*, which catalyzes the oxidation of NADPH maintaining the electron transport chain in mitochondria (166). Further, GPD1 is more active in isolated mitochondria from BAT compared to WAT (167). This corresponds to the enhanced transcriptional abundance of *Ucp1*, as a unique selective BAT marker, in the intra-abdominal adipose tissue of HC/CD females.

In addition, the microarray-based detection of the upregulation of *Gpd1, Cidea* and *Ucp1* might possibly suggest the hypothesis that the altered adipose tissue expandibility of the exposed female offspring might be associated with enhanced **'browning'** of the adipose tissue. UCP1 is originally found in BAT and uncouples fatty acid β-oxidation from oxidative phosphorylation for heat production. While white adipocytes are mainly required for energy storage, BAT is characterized by its capability for non-shivering thermogenesis (168). Contrary to white adipocytes containing a single large lipid droplet and elongated mitrochondria, brown adipocytes consist of several lipid vacuoles and numerous, spherical

mitochondria (169). However, adipocytes with BAT characteristics are also found in WAT depots after exposure to cold or β-adrenergic agonists and are called white-like or “brite”. Due to the ability of these brite cells to express UCP1, enhanced browning of white fat is known to induce energy expenditure and reduce diet-induced obesity (170). While the developmental origin of brite and classical brown adipocytes is not fully understood, it is supposed that white adipocytes can trans-differentiate to brite adipocytes stimulated by environmental factors (168). Further, there is evidence that cell fate might be set at a pre-adipocyte level (168). If so, the maternal adipogenic milieu might affect the brite adipocyte formation and may possibly provide the basis for the increased *Ucp1* and *Cidea* abundances in the intra-abdominal adipose tissue of the HC/CD females. Accordingly, in a recent rodent study higher UCP1 levels were detected in adipose tissue of newborn mice, which were exposed to an adverse maternal milieu (171). In this study, the increased UCP1 expression was associated with a reduced gonadal fat mass of the male offspring at weaning (171), which is in accordance to the disturbed adipose tissue expansion of the HC/CD females in our study. However, on a protein level, our workgroup detected no significant differences of UCP1 expression in the intra-abdominal adipose tissue of the HC/CD and CD/CD female offspring, as assessed by IHC-staining of UCP1 in a previous experiment. Nevertheless, based on the transcriptional upregulation of *Ucp1, Cidea* and *Gpd1* in the intra-abdominal adipose tissue of the HC/CD female offspring, one may suppose that the exposure to a maternal adipogenic milieu might induce an altered trans-differentiation of white and brite adipocytes during intrauterine adipocyte commitment.

However, for a valid assessment of this hypothesis a histological and immunohistochemical confirmation of other brite-specific markers in the adiposue tissue of the HC diet exposed female offspring could be an interesting approach for future projects.

4.1.4. Influence of a prenatal adipogenic exposure on glucose tolerance later in life

Linking the ability for proper fat expansion to glucose metabolism, studies revealed the critical role of an appropriate adipose tissue accumulation reporting that both, increased as well as decreased fat mass negatively influence glucose tolerance (172, 173). These studies correspond to our mouse study, detecting a disturbed ability for appropriate adipose tissue expansion in the female offspring born to obese mothers (as described in 4.1.1.). This was accompanied by a strongly impaired glucose tolerance, detected by an ipGTT at an age of 5 months. In contrast, glucose tolerance of male offspring seemed to be less affected by the exposure to a maternal obesognic milieu (Gimpfl et al., 2016; preparation of manuscript for publication). In the literature, there is also evidence that HC diet exposure during gestation, excluding any lactational influences, seems to be sufficient to induce hypoinsulinaemia in the offspring after weaning (80). Some recent animal studies suggest that the diabetogenic

effects of an intrauterine adipogenic milieu were exacerbated by a postnatal HFD later on (81) (174).

In order to assess morphological alterations which might result in an impaired insulin secretion, the β-cell content in the pancreata of HC/HC and CD/HC female offspring were determined in comparison to the unexposed CD/CD females. However, compared to control, pancreatic β-cell content was not affected, neither by the maternal adipogenic milieu nor by the postnatal HC diet feeding and was accompanied by similar fasting insulin and glucose concentrations in serum of the HC/HC and CD/HC female offspring, as observed previously (Gimpfl et al., 2016; preparation of manuscript for publication). Considering that a defective insulin action is more decisive for postprandial hyperglycemia than a disturbed insulin secretion in humans (175), insulin response was assessed via insulin tolerance test in a previous experiment of our research group (Gimpfl et al., 2016; preparation of manuscript for publication). However, no differences in insulin response were detected between the HC/HC and CD/HC female offspring.

However, we are aware that intraperitoneal injection of glucose solution bypass incretin effects (176). Additionally, the increased glucose levels during ipGTT in the HC/HC female offspring might also be attributable to a higher glucose load in fat mice since the amount of the applied glucose solution is calculated according to body weight. With respect to the mouse strain-specific characteristics, the impact of the exposure to a maternal adipogenic milieu on glucose tolerance without profound effects on insulin secretion and insulin response in the female offspring might be a metabolic feature of NMRI mice. The absence of any deteriorating effects of a prenatal obesogenic exposure on glucose tolerance in the male offspring might additionally suggest that NMRI mice are less prone for obesity-promoting insults compared to inbred strains (177). All the more, we detected a disturbed glucose tolerance in female offspring induced by postnatal HC diet feeding subsequent to exposure to a maternal obesogenic milieu *in utero*. This might indicate a predisposition of the exposed females towards a disturbed regulation of glucose metabolism, while alterations of pancreatic morphology might be even further exacerbated during aging (178).

4.1.5. Sex-specificity of the prenatal obesogenic exposure on offspring phenotype

In different organ systems evidence emerges that an unfavourable maternal milieu during pregnancy induces sex-specific effects on the offspring development during later life. Even in the early ‘70s, there was initial evidence for a site – and sex-specific arrangement of adipose tissue cellularity (179). However, only for the last few years recent studies have considered that intrauterine development of male and female offspring is differentially affected by the exposure to an unfavorable maternal milieu (40) urging for the assessment of the impact of the maternal environment separated by offspring sex. Accordingly, data were analysed in a sex-specific manner by our research group corroborating female-specific disturbances of

glucose tolerance and fat expansion at an age of 5 months, which was also apparent in the female offspring at an age of 9 months (72).

When investigating the **prenatal period**, recent animal studies confirm that the initial hit for sex differences might have already been set *in utero* corresponding to our model of periconceptional maternal obesity. During the pre-implantation period, the maternal environment exert sex-specific effects via a different responsiveness of male and female embryos for regulatory factors, secreted by the reproductive tract of the mother (36). Considering that DNA is re-methylated shortly after implantation, the epigenome is highly sensitive for environmental insults especially during early stages of development (17). Despite some evidence for an altered sex ratio caused by litter loss during the implantation period in response to inappropriate maternal nutrient supply (180), we detected no differences of the sex ratio in any dietary group. This finding might be associated with the only mild metabolic disturbances due to gestational obesity in NMRI dams in response to HC diet feeding (177).

In addition, placenta morphology and function is also significantly influenced by offspring sex (181). In rodent studies global DNA hypomethylation was detected exclusively in the placentae of female offspring of obese mothers (51). Further, placentae of female offspring are characterized by a dynamic adaptive response to maternal nutrient supply, such as Ω-3 fatty acid intake (37), maternal HFD or nutrient deprivation (38). In contrast, male placentae showed lower adaptive capacity of transcriptional regulation (38) and higher placental inflammation (182). Accordingly, the higher adaptive response to maternal dietary modification of the female placentae was also observed during the PhD project (Experiment B1: discussed in 4.2.). Considering that the placenta is the first instance for the fetal-maternal exchange, these sex-specific alterations of placental architecture and nutrient flow might directly affect the intrauterine development of the offspring providing the basis for sex-specific metabolic changes in the offspring during postnatal life (40).

During **postnatal life**, the striking sex-specific effects of the maternal milieu on postnatal gene expression pattern were corroborated by our research group. At an age of 5 months, we detected only a small share between diet-specific DEGs in the intra-abdominal adipose tissue of the male and female offspring exposed to a maternal adipogenic milieu. Accordingly, the influence of the maternal adipogenic milieu on different metabolic pathways was also corroborated by a recent mouse study detecting only a 10% share of DEGs in the liver of 2-week-old male and female offspring in response to maternal obesity (183). In our study, enrichment analysis of DEGs showed that completely different pathways are affected by the the exposure to the maternal adipogenic milieu in the intra-abdominal adipose tissue of male and female offspring. In contrast to the exposed female offspring, in the male offspring downregulated DEGs were enriched in pathways of e.g. ‘circadian rhythm’. In

accordance to our study, a rat study showed sex-specific effects on adipose tissue expansion and function in the female offspring exposed to a maternal adipogenic milieu during pregnancy and lactation (67). Subsequently, the predisposition for adiposity of the female offspring, which were born and suckled by obese mothers, was additionally exacerbated by 'junk food' diet after weaning.

Some animal and human studies indicate that maternal obesity negatively affects insulin sensitivity especially in the male offspring (71) (184). However, there is also evidence for impaired glucose tolerance in HFD-fed female offspring, born to obese mothers, whereas males seem to be less affected (185). Accordingly, our research group detected more pronounced disturbances of glucose tolerance after postnatal HC diet feeding in mat-HC females. However, the underlying mechanisms for this sex-specific sensitivity need further research.

4.1.6. Impact of the prenatal adipogenic exposure on offspring compensatory plasticity

Considering that the observed transcriptional alterations were detected in the offspring at an age of 5 months, dynamic adaptations during postnatal life might additionally affect the transcriptional regulation of the offspring. This postnatal adaptation might possibly compensate the initially acquired insults during the periconceptional phase. With respect to the observed phenotypical alterations at an age of 5 months, the upregulation of lipogenetic genes in the HC/CD females might be attributable to the higher lipogenetic capacity and insulin sensitivity of smaller adipocytes (151). Further, it is already known that an increased fat mass is associated with a chronic low-grade inflammatory state (186), while the reduced fat mass in the HC/CD females might cause a downregulation of inflammatory pathways, accordingly. Nevertheless, this compensatory plasticity might be a secondary adaption during postnatal life and is not the primary cause of the observed phenotypical alterations, which might be acquired during prenatal development.

Focussing on the initial effects of a maternal adipogenic milieu during the early developmental life, the intrauterine exposure to environmental insults are considered to cause "predictive adaptive responses" in offspring leading to an adjustment of the metabolic regulation to the expected environment in future life (187). Based on the adaptive potential of the intrauterine milieu, one could suppose that offspring exposed to a maternal obesogenic milieu might be rather well adapted to nutrient excess during postnatal life. Nevertheless, it is already known that nutrient restriction as well as nutrient surplus during pregnancy equally predisposes the offspring for excess fat accumulation and glucose intolerance later in life (188). Comparable to nutrient restriction, a maternal obesogenic milieu is supposed to lead to reduced vascularity and nutrient transport capacity of the placenta, which might be caused by obesity-associated placental inflammation (188). Thus, maternal obesity might also prime

the offspring for life in a nutrient-restricted environment resulting in a mismatch between expected and existing postnatal nutrient availability. Another explanatory approach for the lack of adaptation during prenatal nutrient surplus suggests that overnutrition is rarely present during evolutionary history. Thus, developmental plasticity in response to maternal obesogenic exposure has not led to a survival benefit (189).

4.1.7. Impact of the duration and type of the adipogenic influences in pregnancy

In the context of a well-founded interpretation of the offspring effects observed in animal studies, the role of distinct time windows during offspring development should be considered and a comprehensive characterization of the offspring exposure to a maternal obesogenic milieu is urgently needed.

Critical role of different developmental time windows during offspring development

In contrast to humans, the adipose tissue expansion mainly occurs postnatally in rodents, whereas the recruitment of embryonic mesenchymal stem cells to the adipocyte lineages is initiated *in utero* (61). In the mouse model used by our research group, any exposure during the lactational phase is intentionally excluded. Thus, we hypothesize that the exposure to a maternal obesogenic milieu might affect lineage commitment of embryonic mesenchymal stem cells *in utero* resulting in a disturbed adipose tissue expansion during postnatal life.

In addition to the prenatal period, the lactational phase also represents a critical window for hypothalamic development and affects *e.g.* the response to leptin (190). Thus, if offspring would not be transferred to CD-fed foster dams, the early metabolic development would be additionally affected by the differential milk composition of lean and obese dams. Compared to lean rodents, milk of obese dams is characterized by 128% higher insulin, 10-fold increased leptin and 50% reduced PUFA concentrations, which might override the prenatally acquired effects (70) (191). Another critical advantage of the transfer of the offspring to CD-fed foster dams is based on the observation that offspring starts to nibble at the solid diet of the mothers at an age of 10 days (according to unpublished observations: Martina Gimpfl). Nevertheless, the impact of an additional intake of a small amount of obesogenic maternal diet by the offspring during lactation is not addressed at all in the literature. Considering that fostering *per se* might affect the offspring development in a sex-specific manner (192), we also transferred the offspring born to CD-fed dams to CD-fed foster dams in order to avoid any methodical differences between the pups born to the HC diet- and CD- fed mothers. Nevertheless, especially in the mat-HC offspring, which is exposed to an obesogenic milieu during pregnancy and transferred to a control environment during lactation, the mismatch between pre- and postnatal milieu is also supposed to affect offspring development and cannot be completely excluded in our model (193). Nevertheless, our fostering model is suitable to detect possible mechanisms during very early phases of development, which

might predispose the offspring to an altered adipocyte physiology in the later life, provided that postnatal confounding is kept as low as possible.

Critical considerations on models for maternal diet-induced obesity

In the literature, there is a huge compositional diversity for so-called “high fat diets” containing 25%-60% energy from fat, while the fat content is mostly obtained from different fat sources (194). Considering the different metabolic effects of distinct fatty acids, a detailed definition of the nutritional composition is indispensable - but is not always provided in the literature. For this reason, in our mouse study we assessed the fatty acid quality of the applied diets dependent on storage duration, temperature and irradiation in a preliminary experiment and reported the dietary composition as accurately as possible. Further, we determined the food intake, assimilated energy, heat production and movement activity of individually housed mice in order to monitor the experimental setting and exclude possible confounding effects attributable to an altered energy intake or expenditure.

Another mandatory prerequisite is the use of specifically matched control diets in order to avoid confounding effects of other nutrients (e.g. fiber, phytochemicals), which differ between a HFD and standard chow diet (195). Nevertheless, in the year 2008 only 14% of high-impact publications applied a specifically matched control diet, which only differs in its fat and carbohydrate amount compared to the respective HFD (195). In our study, a specifically matched control diet was used resulting in an adjusted intake of protein, minerals, trace elements and vitamins between control and HC diet groups. In order to induce fast weight gain, the unmodified HC diet of our research group contains 60% of mainly saturated fat originating from beef tallow and a low starch to sugar ratio.

The experimental schedule used by our studies differs from the experimental design applied in the majority of animal studies. Using our NMRI mouse model, offspring is exposed to mild diet-induced maternal obesity during pregnancy and is transferred to CD-fed foster dams immediately after birth (72). Compared to inbred C57BL/6J mice, HFD feeding of NMRI mice induces only mild metabolic disturbances comprising e.g. an impaired glucose tolerance at the end of pregnancy, increased concentrations of NEFAs and reactive oxygen species in the serum, whereas no signs of low-grade inflammation, insulin resistance, or alterations of the serum lipid profile were detected (72), Thus, the use of these outbred mice is more appropriate to assess the effects of obese pregnancy with less severe metabolic consequences.

4.2. Effects of a change in fat quality of the maternal adipogenic diet

For published reports on Ω-3 LC-PUFAs, a considerable inaccuracy and wide range exist in terms of the applied amount, the source of Ω-3 fatty acids (krill oil vs. fish oil), the ratio of EPA:DHA and the ratio of Ω-3:Ω-6 LC-PUFAs. Therefore, up to now, it is almost unfeasible to draw convincing conclusions about the effects of Ω-3 LC-PUFAs in both rodents and humans. This specifically refers to supplementation in pregnancy (119) (120). Furthermore, there is sparse data available for the effects of MCFAs during pregnancy (108). Preliminary data of studies using single components such as an increase in MCFAs and a balanced Ω-6 to Ω-3 LC-PUFA ratio were promising (128) (108). Thus, our research group modified the fatty acid profile of a high-fat, high-calorie diet via elevation of the MCFA content (using coconut oil) and via improvement of the Ω-6 to Ω-3 LC-PUFA ratio (using fish oil). Thereby, we aimed to take advantage of e.g. the rapid oxidizability of MCFAs (105) and the "nutrient sensor" capacity of Ω-3 fatty acids (115) and their advantageous effects on the maternal gestational milieu in order to test whether this leads to an improved intrauterine development of the offspring (108) (127).

4.2.1. Rationale for dosage of functional fatty acids in the maternal obesogenic diet

The addition of MCFAs is supposed to reduce body weight and fat mass in animals and humans via its rapid absorbability from gut and efficient usage for β-oxidation in mitochondria independent of the CPT1 transport system (196). Additionally, first data suggest beneficial effects of MCFAs in the maternal diet during pregnancy indicating a lower susceptibility of the offspring for HC diet-induced obesity during early adult life, which was accompanied by a downregulation of lipogenetic genes (108).

However, with respect to the most effective dosage of MCFAs in rodents and humans, there is a huge variability in the literature, possibly resulting in different metabolic effects. Especially for the impact of MCFAs on hepatic fat accumulation, a high dose-effect relationship is reported (104). Supplementation of a HFD (45% kcal from fat) with lower amounts of MCFAs (C8:0-C12:0, 50% of total fatty acids) might increase the risk for hepatic fat accumulation despite lower total body fat accumulation (196), whereas the use of a higher MCFA proportion (C8:0+C10, 90% of total fatty acids) in a HFD (45% kcal from fat) seems to reduce liver steatosis (197). However, in our study, the fat fraction of the Fat-mod HC diet consisted of 36% MCFAs (C8-C12) of total fatty acids, which was previously shown to reduce the hepatic fat accumulation in the obese dams of our study (Gimpfl et al., under review).

Regarding Ω-3 fatty acids, there is also a large range of the reported amount, source, ratio of EPA:DHA and Ω-3:Ω-6 LC-PUFAs. Despite the wide diversity of the applied amounts, the majority of studies detected a preventive effect of Ω-3 LC-PUFA consumption on obesity-associated metabolic disturbances such as glucose intolerance, dyslipidemia and chronic low

grade inflammation (117) (118) (112). With respect to a supplementation of a maternal obesogenic diet during pregnancy with Ω-3 LC-PUFAs, animal and human studies reported inconsistent effects on maternal and offspring outcome (120) (119).

The observed discrepancies might be attributable to sparse data about the critical impact of the ratios of Ω-3:Ω-6 LC-PUFAs and EPA:DHA in the diet (117). There is first evidence that not only the total amount of the enriched Ω-3 LC-PUFAs *per se* but also a balanced Ω-6 to Ω-3 LC-PUFA ratio (ratio 2:1) in a HFD (35% kcal from fat) might exert beneficial effects on the metabolic regulation in non-pregnant dams (198). In our study, we aimed to improve the Ω-6 to Ω-3 LC-PUFA ratio (ratio 2.2:1) of our HC diet (60% kcal from fat). We added relatively low amounts of Ω-3 LC-PUFAs achieved by the replacement of 3.5% of total fatty acids with Ω-3 LC-PUFAs (C18:3 Ω-3/C20:5 Ω-3/C22:5 Ω-3/ C22:6 Ω-3). In addition, the ratio of EPA:DHA should also be considered, because different effects of EPA and DHA on blood pressure, serum lipids and inflammation were detected in animals and humans (199). Thus, in our study the supplementation of the obesogenic diet with Ω-3 LC-PUFAs resulted in an EPA to DHA ratio of 1.5:1 corresponding to other rodent studies using intervention diets with an EPA to DHA ratio of 1.2:1 to 1.7:1 (200) (201). In these studies, a higher Ω-3 LC-PUFAs supplementation (25-35% of total fatty acids) of the applied HFDs (~40% kcal from fat) exerted adiposity-lowering effects were detected (201) (200). Despite a lower total amount of supplemented Ω-3 LC-PUFAs in our study, the Fat-mod HC diet dams also showed a reduced total body fat mass (Gimpfl et al., under review). Accordingly, there is also evidence for beneficial effects of the supplementation of a HFD (45% kcal from fat) with lower total DHA and EPA amounts (< 10% of total fatty acids) in mice, while the EPA to DHA ratio (ratio 1.2:1) was comparable to our study (202). Based on these studies, the altered fat composition of a maternal adipogenic diet, by combining the effects of MCFAs and Ω-3 LC-PUFAs, seems to be a promising approach to ameliorate the maternal milieu, which might possibly exert beneficial effects on offspring development *in utero*.

4.2.2. Effects on maternal phenotype and transcriptional regulation of maternal tissue

In a preliminary experiment, the comparability of assimilated energy was assured by analysis of food intake, which showed no differences between the unmodified HC and Fat-mod HC diet dams (Gimpfl et al., under review). Further, serum analysis of fatty acids in mice on the Fat-mod HC diet also reflected the change in fat quality of the maternal adipogenic diet.

In our study, the altered fat composition of the maternal adipogenic diet, taking advantage of the beneficial effects of both MCFAs and Ω-3 LC-PUFAs, resulted in an improvement of the maternal milieu. Reduced adiposity was accompanied by a decreased adipocyte size, reduced liver steatosis and lower concentrations of serum HDL cholesterol in dams at the end of pregnancy (Master Thesis: Martina Gimpfl).

During the PhD project, the effects of an altered fatty acid spectrum in the adipogenic diet of the mother were assessed in more detail and compared to the unmodified HC diet with respect to the transcriptional regulation in maternal liver, adipose tissue and placenta as well as offspring phenotypes.

Transcript abundances in the liver of dams

In liver, the altered fat composition of the maternal adipogenic diet induced a downregulation of lipogenetic genes including *Fasn* and *Acaca.* FASN represents the main regulator of fatty acid synthesis, which catalyzes the synthesis of long chain saturated fatty acids from acetyl-CoA and malonyl-CoA, whereas ACACA promotes fatty acid synthesis via carboxylation of acetyl-CoA to malonyl-CoA. The transcriptional downregulation of these two important enzymes of *de novo* lipogenesis corresponds to the reduced hepatic fat accumulation and might be mediated by the modulating effects of the fat-modified adipogenic diet on gene expression. Accordingly, in the liver of the Fat-mod HC diet dams, genes for transcription factors such as *Srebf1, Nr1h3* and *Pparg2* were also downregulated, which are known as important “nutrient sensors” and are involved in the regulation of *DNL*. At least for Ω-3 LC-PUFAs, suppressive results might be mediated via inhibition of SREBP1, which involves inhibitory binding of unsaturated fatty acids to *Nr1h3* (203). Similarly, expression of *Pparg2*, an important adipogenesis-regulating transcription factor and sensoring system for fatty acids, and one of its target genes involved in fatty acid transport, *Fabp4*, was reduced. This might likely contribute to the lower fat accumulation in the liver (204). The decreased mRNA abundance of *Pparg*, through a transcription cascade involving *Nr1h3,* also appeared to affect expression of its target gene *Abca1* (205). Hepatic *Abca1* is a major modulator of HDL formation, found to be transcriptionally repressed by Ω-3 LC-PUFAs *in vitro* (206), and its suppression has been shown to result in reduced plasma HDL cholesterol concentrations (207). Comparable to the lower HDL concentrations in the serum of the Fat-mod HC dams, a reduction in serum HDL cholesterol has also been observed in C57BL/6J mice following long-term (16 weeks) Ω-3 LC-PUFA supplementation of a standard diet with a similar EPA/DHA ratio of 1.5:1 (208). Its mechanism of action was proposed to involve an improved hepatic uptake of HDL cholesteryl esters and accelerated reverse cholesterol transport (207) (208). In mice, HDL cholesterol represents 70% of total cholesterol, which might be accompanied by an immanent resistance to diet-induced arteriosclerosis (209). In contrast, human cholesterol is characterized by a high LDL cholesterol fraction (209). Thus, possible conclusions about the arteriosclerosis risk in humans should be drawn with caution from data obtained in wild type mice. However, it is already known that reducing the LDL cholesterol concentrations and raising the HDL cholesterol levels seems to be protective against the progression of atheriosclerosis in humans (210). With respect to Ω-3 LC-PUFA intake in

humans, the risk for cardiovascular diseases is reduced via its modulating effects on plasma triglycerides, HDL and LDL cholesterol (211).

However, we detected no adaptive upregulation of key fatty acid-regulated transcription factors of liver lipid catabolism (*Ppara, Ppargc1a*) and *Cpt1,* which catalyzes the rate-limiting step of fatty acid β-oxidation. This might potentially attributable to the direct entry of MCFAs into the mitochondrion occurring independently from the CPT1 transport system.

With respect to the gene regulatory effects of MCFAs, there is first evidence for the involvement of MCFAs in Ω-3 metabolism. *In vitro* studies using liver homogenates indicate that MCFAs possibly contribute to the effects of Ω-3 LC-PUFAs via formation of ALA from lauric acid (212) (110), which is converted to EPA in the presence of myristic acid. In rats, exposure to a diet (22% kcal from fat) containing ALA (1.6% of total fatty acids) and different amounts of myristic acid (0.71 - 5.57% of total fatty acids) is supposed to induce a dose-dependent accumulation of EPA in plasma and liver indicating a conversion of ALA to EPA, which is promoted by myristic acid (213). Considering that in the Fat-mod HC diet of our study, the myristic acid and ALA content account for 11.4% and 1.43% of total fatty acids, respectively, the effects of EPA might be amplified by the elevated myristic acid and ALA content of the Fat-mod HC diet.

Transcript abundances in the omental adipose tissue of the dams

In rodents, the amount of omental adipose tissue is low (214). However, in pregnant and obese mice a relevant amount of omental fat can be dissected (215) (216). Thus, we also found a substantial amount of omental fat in our obese NMRI dams at the end of pregnancy, and therefore assessed the effects of the Fat-mod HC feeding on the omental adipose tissue, as part of the visceral fat depot. In addition to the anti-adipogenic effects of the altered fat composition of the adipogenic diet in the liver, we also detected a transcriptional downregulation of *Srebf1* in the omental adipose tissue of the dams, which is known to be mediated by the inhibition of *Nr1h3* via Ω-3 LC-PUFA binding (115). Although SREBF1 is known to induce the expression of genes involved in *de novo* lipogenesis, in our study no lipogenetic downstream genes of *Srebf1* were affected by the altered fat composition of the maternal adipogenic diet. However, the transcriptional abundances of *Mest* and *Leptin* were significantly reduced in the Fat-mod HC diet dams compared to the HC diet dams. While MEST is involved in the enlargement of adipocytes (146), LEPTIN is mainly produced in white adipose tissue and associated with fat mass (165). Thus, the observed downregulation of these genes is in accordance with the decreased adipocyte size and fat mass in the Fat-mod HC diet dams. Despite sparse data on the transcriptional regulation via MCFAs, *in vitro* studies indicate a PPARG-dependent downregulation of lipogenetic genes by octanoate (217). Further, a rat study showed a downregulation of *Pparg* and adipogenic genes in

adipose tissue, which was induced by a HFD (46.8% energy from fat) mainly consisting of MCFAs (97% of total fatty acids) (218). However, data on effects on gene regulation by MCFAs in adipose tissue are sparse and inconsistent.

Together, the maternal phenotype and gene regulatory effects may be the result of potentially additive effects or interrelationships of fatty acids of the modified adipogenic diet. Based on these findings, we next investigated effects on the placenta.

4.2.3. Effects of the altered fat composition of the maternal adipogenic diet on placental gene transcription

To gain first insights into possible effects of the prenatal exposure to an altered fat composition of the maternal adipogenic diet on nutritional supply of the offspring *in utero*, we assessed the transcriptional abundances of nutrient transporters in the placenta.

Despite a similar placental weight at 17.5 dpc between the HC- and Fat-mod HC diet group, nutrient transporter genes were sex-specifically upregulated in response to the fat-modified adipogenic diet exposure during pregnancy. The placentae of the Fat-mod HC diet exposed female offspring showed an increased transcriptional abundance of nutrient transporters involved in fatty acid uptake, which was accompanied by an upregulation of the transcription factor genes *Pparg* and *Rxra*. In rats, PPARG is essential for trophoblast differentiation and maturation during placental development (219). Especially in the presence of gestational diabetes, PPARG activation exerts beneficial effects via the reduction of nitric peroxide synthesis (220). It is already known that PPARG forms heterodimers with RXRA, which leads to transcriptional activation of its target genes, such as *Fatp1 (=Slc27a1)* and *Fatp4 (=Slc27a4)* (221). Fatty acid transport proteins (FATPs) are mainly located at placental trophoblasts and are known to be involved in the placental transport of LCFAs. Considering that an increased FATP1 and FATP4 expression is associated with high DHA levels in the maternal plasma and cord blood (222), the enhanced Ω-3 LC-PUFA content of the Fat-mod HC diet might reach fetal circulation and may potentially influence intrauterine development of the offspring. In our study, the transcriptional levels of *Fabp4* were downregulated in the placentae of both sexes. In addition to the essential role of FABP4 for intracellular lipid accumulation and trafficking in the placenta, a recent human study indicated that placental FABP4 expression is increased during maternal obesity and diabetes, while inhibition of FABP4 results in reduced triglyceride accumulation in human placental trophoblast cells *in vitro* (223). Nevertheless, we detected no differences in the transcriptional abundance of *Perilipin*, representing a marker for lipid droplet size, between the placentae of the HC- and Fat-mod HC diet group in both sexes.

With respect to MCFAs, there are only few data about the placental transport in humans and animals, whereas first evidence exists for a lower MCFA oxidation in placenta compared to liver in humans (224).

However, nutrient transporter gene expression in the male placentae seems to be less affected by the maternal diet in comparison to female placentae in our study. A sex-specific dynamic adaption in response to maternal Ω-3 LC-PUFA intake was also shown in a recent human study that detected pronounced transcriptional alterations of genes involved in the cell cycle especially in the female placentae (37).

The higher adaptive capacity of the female placentae in response to an altered maternal nutrient supply (38) (181) is known to lead to a higher abort rate of male offspring in the presence of a disadvantageous maternal environment (225). In our study, the abort rate of male offspring was not affected, as shown by a balanced sex ratio in offspring born to HC diet-, Fat-mod HC diet- or CD-fed dams. Despite a similar fetal weight at 17.5 dpc in both dietary groups (Experiment B1), mat-HC female and male offspring showed an increased birth weight (19.5 dpc) in comparison to the mat-Fat-mod HC offspring (Experiment B2). Despite possible confounding effects owing to the comparison of two independent experiments, the higher birth weight of the mat-HC offspring might be caused by an increased weight gain especially during late pregnancy resulting in macrosomia at birth, which is known as an independent prediction factor for an increased risk of childhood adiposity in humans (226). In rodents, initial differentiation of embryonic subcutaneous adipocytes occurs between 15 dpc and 16 dpc (227), suggesting that especially an excessive weight gain during late pregnancy might possibly affect early adipocyte differentiation in the mat-HC offspring of our study.

4.2.4. Sex- and age-specific effects on the offspring adipose tissue phenotype

With respect to the post-weaning period, the developmental trajectory of adipose tissue is strikingly sex-, site- and age-dependent (228) (229) (230). This led us to evaluate the effects of the fat-modified adipogenic diet of the mother (Experiment B2) on different offspring fat compartments at three critical times of adipose tissue development (at birth, 6 weeks, 16 weeks). Analyses were carried out for sex differences. Despite the absence of any white adipose tissue at birth, newborn mice were dissected in order to assess the “direct” effects of the maternal milieu on BAT development without any postnatal influences. Based on the mainly postnatal fat accumulation in rodents (45) and the extrapolation of our previous data (72), at an age of 6 weeks white adipose tissue expansion is expected to be sufficiently advanced in NMRI mice that an adequate sample of intra-abdominal adipose tissue can be drawn for microarray analysis. Dissection at an age of 16 weeks was chosen as experimental end point in order to evaluate effects of the exposure to a maternal adipogenic milieu in advance to the pronounced manifestation of phenotypical alterations from an age of 16 weeks onwards, as seen in our previous experiments (72) (Gimpfl et al., 2016; preparation of manuscript for publication).

Sex-specificity of the prenatal dietary exposure on total offspring body fat mass

Body weight and total body fat of the mat-Fat-mod HC male offspring were reduced compared to the mat-HC males. In contrast, no differences of body weight and fat mass were detected in the female offspring between groups.

In the male offspring, the observed sex-specific effects on fat mass correspond to a recent rodent study, which showed a reduction of fat mass solely in those male offspring, that were exposed to a maternal diet (fat: 9% w/w) supplemented with Ω-3 PUFAs (17.7% of total fatty acids) during early pregnancy (126). Additionally, there is also first evidence for beneficial effects of MCFAs (48% of total fatty acids) in a maternal HFD (38% kcal from fat) during pregnancy (108). This rat study indicated a lower susceptibility of the male offspring for HC diet-induced obesity during early adult life, which was accompanied by a downregulation of lipogenetic genes in the liver (108). In our study, the assessment of the mechanistic background for this male-specific phenotypical alteration will be a promising approach for future research of our research group, but was not addressed in the PhD project.

In the female offspring, we could confirm the results of our previous experiments (72) (Gimpfl et al. 2016, preparation of manuscript for publication) on a cellular level. We detected a smaller adipocyte size in the 16-week-old female offspring of the mat-HC group compared to the control group (outlined in more detail below). This alteration of adipocyte size seems to precede the reduction of total body fat mass, which emerged in the mat-HC females in our previous experiments from an age of 16 weeks onwards (72) (Gimpfl et al. 2016, preparation of manuscript for publication).

Dynamics of adipocyte size expansion during postnatal life

In order to get first insights into possible effects of a maternal fat-modified adipogenic diet on the expandability of offspring adipocytes, we looked at the expansion of adipocyte size in an age-related manner.

At **“mouse childhood phase”** (age of 6 weeks), male and female offspring of the mat-HC and mat-Fat-mod HC group showed an enlarged adipocyte size in the intra-abdominal adipose tissue compared to control. This increase of adipocyte size was evident in both sexes irrespective of the fatty acid quality of the maternal obesogenic diet in pregnancy. The critical influence of an altered adipose tissue cellularity especially during early childhood was corroborated by a recent human study (231). Landgraf et al. showed that an enlarged adipocyte size and number in obese children was accompanied by an increased adipose tissue inflammation and insulin resistance (231) indicating that adipose tissue dysfunction is initiated in early childhood. Additionally, an enlarged adipocyte size even without obesity is associated with type 2 diabetes in humans. Larger adipocytes of non-obese diabetic individuals showed a disturbed capacity for lipogenesis and differentiation of adipocyte

progenitor cells, which might negatively affect adipose tissue inflammation and insulin sensitivity (232). Thus, the larger adipocytes in the mat-HC and mat-Fat-mod HC offspring during “childhood” might possibly point to a disturbed adipocyte function providing the basis for metabolic disturbances later on.

During the growth period between the age of 6 and 16 weeks, growth of adipocytes of the mat-Fat-mod HC exposed female and male offspring stagnated. In contrast, only the adipocyte size of the female mat-HC offspring decreased in this period of time. Considering that in control offspring a physiological increase of the adipocyte size was observed between an age of 6 and 16 weeks, the reduced cell growth might possibly point to disturbed adipose tissue expandibility in response to exposure of either of the maternal adipogenic diets. However, the observed decline of growth in mat-HC female adipocytes might suggest more severe disturbances compared to the stagnation of adipocyte size of the mat-Fat-mod HC female offspring. In the literature, the “adipose tissue expandability hypothesis” indicates that a reduced storage capacity of the adipose tissue leads to an increased ectopic fat accumulation e.g. in the liver or pancreatic β-cells, resulting in increased lipotoxicity, apoptosis and insulin resistance later on (233).

At “early mouse adulthood” (age 16 weeks), the mat-HC female offspring showed a decreased adipocyte size compared to control offspring, which corroborates the results of our previous experiments (72). Most remarkably, adipocyte size of the mat-Fat-mod HC female offspring tended to approximate the control situation. In contrast, at an age of 16 weeks, male offspring of all three groups (mat-CD, mat-HC and mat-Fat-mod HC) showed a similar adipocyte size. Such striking age-dependent effects of a maternal obesogenic milieu *in utero* on the adipose tissue development of the offspring were also observed in a rat study, which showed an enlarged adipocyte size in male and female offspring at an age of 3 weeks (234). However, maternal diet-specific differences in adipocyte size disappeared during offspring aging, which corroborates the sex- and age-specificity of adipose tissue development.

4.2.5. Effects on gene expression patterns in adipocytes of female offspring

Based on our previous data showing a disturbed adipose tissue development especially in the mat-HC female offspring (72) (Gimpfl et al. 2016, preparation of manuscript for publication), the PhD project initially focused on possible preventive effects of the mat-Fat-mod HC diet on the adipose tissue phenotype of the female offspring.

Even before the onset of variations in adipocyte size at an age of 16 weeks, qualitative and quantitative differences in gene expression were detected in the intra-abdominal adipose tissue of the mat-HC versus mat-Fat-mod HC female offspring at an age of 6 weeks. Compared to the female control offspring, only few differences in gene expression were

detected in the mat-Fat-mod HC female offspring, leading to the hypothesis that the disadvantageous effects of the unmodified mat-HC diet may be alleviated.

At an age of 6 weeks, the intra-abdominal adipose tissue of mat-HC females showed an enrichment of upregulated DEGs involved in **cholesterol biosynthesis** compared to female control offspring. This transcriptional upregulation at an age of 6 weeks may have consequences for the protein level, as reflected in elevated plasma cholesterol concentrations in the mat-HC female offspring at an age of 16 weeks. Accordingly, the negative impact of exposure to a maternal high fat diet (45% kcal from fat) during pregnancy and lactation on cholesterol biosynthesis was also seen in a mouse study detecting elevated cholesterol concentrations in the male and female offspring at an age of 36 weeks (235). Additionally, there is first evidence for the effects of Ω-3 LC-PUFAs in the maternal diet of diabetic rats on lipid disorders and cholesterol concentrations in serum of offspring (121). Accordingly, in our study, the cholesterol biosynthesis was downregulated in the intra-abdominal adipose tissue of the mat-Fat-mod HC female offspring, when compared to the mat-HC females, which may indicate a protective response.

In accordance to the observed reduction of adipocyte size expandability between an age of 6 and 16 weeks, **lipogenetic pathways** were downregulated in the mat-HC and mat-Fat-mod HC female offspring, each compared to the mat-CD female offspring (Table 9). However, triglyceride and NEFA concentrations were elevated in the plasma of the mat-HC females at an age of 6 weeks, which might be a possible feature of the reduced storage capacity of adipose tissue. Accordingly, in a transgenic ob/ob mouse model, partial PPARG knock out induced a disturbed adipose tissue expandibility and hypertriglyceridaemia resulting in an increased predisposition for insulin resistance despite a leaner phenotype (236). In contrast, in the 6-week-old mat-Fat-mod HC female offspring, NEFA concentrations were reduced, possibly corresponding to the microarray-based downregulation of lipogenetic genes, when compared to both the mat-CD and mat-HC female offspring. This might possibly suggest the hypothesis that excess free fatty acids are removed from the circulation and are possibly stored in adipocytes of the mat-Fat-mod female offspring, which tend to be larger than adipocytes of the mat-HC female offspring. This may also explain the lower triglyceride concentrations in the mat-Fat-mod HC female offspring compared to the mat-HC females.

Further, the prenatal mat-Fat-mod HC exposure induced e.g. an upregulation of DEGs in intra-abdominal adipose tissue, which were enriched in pathways such as “blood vessel development” and “cell adhesion processes”. This was accompanied by an upregulation of genes involved in developmental processes such as “circulatory system development” and “heart development”. An intact vascularization and a distinct expression pattern of cell adhesion molecules are essential for proper adipose tissue formation during early adipocyte lineage commitment (237) (238). Therefore, the identified upregulation of genes in the mat-

Fat-mod HC female adipocytes might point to possible compensatory effects of the altered fat composition of the maternal adipogenic diet during early phases of development. Nevertheless, in the literature, the effects of Ω-3 LC-PUFA supplementation of a maternal diet in pregnancy and lactation on adipose tissue development of the offspring remain controversial in mice and humans (121).

However, in our study, the amelioration of the maternal adipogenic milieu via MCFAs and Ω-3 LC-PUFAs might be a promising approach to influence the prenatal cell development during early vulnerable time windows, which might positively affect the postnatal adipose tissue expandability in the female offspring.

4.3. Concluding remarks

Our results show that a maternal adipogenic diet before and during pregnancy, which mainly consists of saturated fatty acids, induces an adipogenic maternal milieu and provides the basis for long-term consequences to offspring health. In the absence of any postnatal HC diet feeding, the exposed female offspring showed a reduced inta-abdominal adipose tissue mass and lower adipocyte size, whereas the male offspring developed an increased fat mass (72). We detected exacerbating effects of postnatal HC diet feeding specifically in these female offspring, which had been exposed to a maternal adipogenic milieu *in utero*. Thus, we conclude that the prenatal exposure to a maternal HC diet might disturb the ability for proper fat mass expansion and cell size development in the female offspring. In contrast, adipose tissue development of the male offspring seemed to be less affected.

In a **first** step, we focused on possible cellular regulatory changes, which might be involved in the development of the observed sex-specific differences in the adipose tissue phenotype and also differences in glucose tolerance. One possibly relevant mechanism might be the upregulation of genes involved in *de novo* lipogenesis in the adipose tissue of adult female offspring exposed to an adipogenic milieu *in utero,* which received only CD postnatally. Upregulation of this pathway was also reflected on a functional level as measured by an increased activity of FASN, the key enzyme of *de novo* lipogenesis.

Further, DNA-microarray analysis showed a downregulation of inflammatory genes in response to the maternal adipogenic milieu in the adipose tissue of the female offspring. Considering that local inflammation is essential for correct adipose tissue formation (157), we hypothesized that the downregulation of inflammatory pathways in response to the maternal adipogenic milieu may be another mechanism that disturbs adipose tissue expandability, and might become more obvious during postnatal HC diet feeding. Due to the fact that the adipose tissue development in mice mainly takes place in the postnatal life (239), in our model of periconceptional maternal obesity, the initial hit must occur during an early phase of prenatal adipocyte development. Thus, we hypothesized that improving maternal metabolism during these early vulnerable time windows in gestational obesity might be a promising

approach to ameliorate the prenatal adipocyte development in the offspring that may have positive consequences on adipose tissue expandability during postnatal life.

In the **second** step, we therefore aimed to take advantage of the anti-obesogenic effects of an altered fatty acid quality of the maternal adipogenic diet using both MCFAs and Ω-3 LC-PUFAs. Feeding this fat-modified adipogenic diet to dams before and during pregnancy resulted in an improvement of the maternal milieu in pregnancy. In the placentae of the female offspring, transcriptional abundances of important nutrient transporters were upregulated, whereas male placentae were less affected. Thus, this study suggested that the "functional" fatty acids in the Fat-mod HC diet could possibly affect offspring organ and specifically adipose tissue development in a sex-specific manner. At an offspring age of 6 weeks, the mat-Fat-mod HC female offspring showed reduced abundances of genes involved in cholesterol biosynthesis in the intra-abdominal adipose tissue and lower plasma cholesterol concentrations at an age of 16 weeks. Further, the exposure to an altered fat quality of the maternal adipogenic diet induced an upregulation of adipocyte genes involved in pathways such as "blood vessel development" and "cell adhesion processes" in comparison to the intra-abdominal adipose tissue of the mat-CD female offspring. Considering that proper angiogenesis is essential for adipose tissue plasticity (240), this led us to hypothesize that the disturbed adipose tissue formation in the mat-HC female offspring might be ameliorated by the exposure to the altered fat composition of the maternal adipogenic diet in the mat-Fat-mod female offspring. Also, adipocyte size appeared to be influenced by the exposure to a maternal Fat-mod HC diet. In contrast to the disturbed adipose tissue expandability in the mat-HC female offspring, prenatal exposure to the mat-Fat-mod HC diet induced an approximation of the adipocyte size of the female offspring to the control situation at an age of 16 weeks. Together, we conclude that exposure to an altered fatty acid spectrum in a maternal adipogenic diet could attenuate the disadvantageous effects of an unmodified maternal adipogenic diet on offspring metabolic outcome in mice.

4.4. Future perspectives

Based on the results of the PhD project, many promising approaches for future research arise:

1. Considering that in our mouse model for periconceptional maternal obesity the early adipocyte development of the offspring might be affected *in utero*, the assessment of early developmental genes would be helpful. Thus, a closer look at possible action sites of the functional fatty acids (MCFAs/n-3 LC-PUFAs) during very early vulnerable time windows would be a promising approach for future *in vitro* experiments.
2. With respect to the detected age-dependent differences of adipocyte size development in the offspring exposed to different maternal diets *in utero*, future experiments might focus on the dynamics in the development of adipose tissue to get insights into lifetime-dependent changes of gene expression and phenotype. Besides the investigated pathways, there is also indication for other dysregulated pathways such as ‘browning’ of white adipose tissue, which would be interesting to be followed in future experiments.
3. Regarding organ-specific effects of an altered fat composition of the maternal adipogenic diet on the offspring, the examination of other organs, particularly those of metabolic relevance (muscle, liver, pancreas and lungs) is just ongoing and will be in the focus of future experiments.
4. Recent studies suggest that the exposure to a maternal adipogenic milieu *in utero* may induce long lasting alterations in adipose tissue physiology, which might be based on epigenetic mechanisms. Whether epigenetic alterations are also involved in disturbed capacities for adipose tissue expansion, and how this relates to the sex-specificity of effects, might be an interesting approach for future research.

With respect to an increasing number of obese or overweight women at reproductive age, nutritional preventive strategies are urgently needed. The findings of the altered fat composition of the maternal adipogenic diet via MCFAs and Ω-3 LC-PUFAs provide first evidence for the beneficial effects of a periconceptional intake of ”healthy“ fat compounds, which might be a promising approach to be assessed in humans in order to ameliorate the consequences of obese pregnancies on mother and child.

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Templates for self-drawn images:

- Figure 3 (depiction of organs): Painted by Robin Zenker via Photoshop, according to (1).
- Figure 5 (depiction of placenta + baby): Painted by Robin Zenker via Photoshop, using the illustration of the “Human Placenta Project” as template.

Appendix

Table S1: Particulars on chemicals and equipment

Chemicals and Kits	Supplier	Article number
4% NBF	Bio-optica	05-K01004/CO
2-Methylbutan	VWR	24872298
5x One Taq GC Reaction Puffer	New England Biolabs	B9023S
6x Gel DNA Loading solution	Thermo scientific	R0611
99% Ethanol (1-2% Butanon)	HEMA GmbH und Co. KG	642
Acetyl Coenzyme A,sodium salt	Sigma	A2056
GeneChip WT Terminal Labeling Kit	Affymetrix	900671
Aqua dest.	Millipore	
Beta- mercaptoethanol	Carl Roth	4227.3
DTT	AppliChem GmbH	A1101,0005
EDTA	Kabe Labortechnik GmbH	78035
Eosin	Bio-optica	05-10002
Ethidium bromide	AppliChem GmbH	A1152,0010
Gene Ruler 100 bp	Fermentas	SM0243
Goat Anti-Guinea Pig IgG (H+L)-AP	Southern Biotech	6090-04
Guinea pig anti-Insulin	DAKO Diagnostika	A0564
Illumina TotalPrep RNA Amplification kit	Ambion, Life Technologies GmbH	AMIL1791
LE Agarose	Biozym	840001
Malonyl Coenzyme A, Lithium salt	Sigma	M4263
Mayer’s Hematoxylin	Bio-optica	05-06002E
miRNeasy Mini Kit	Qiagen	217004
Monopotassium Phosphate	Sigma	P5655
Mounting medium (HistofluidR)	Marienfeld-Superior	6900002
Mounting medium (Pertex)	Medite	41-4010-00
Mounting medium (Tissue-Tek O.C.T. Compound)	VWR	4583
NADPH	Sigma	N7505
Nucleospin RNA II kit	Macherey-Nagel	740955
Nucleospin Tissue kit	Macherey-Nagel	740952.50
paraffin	Carl Roth GmbH	6642.6
PFA	Sigma	070M1006V
PMSF	Sigma	93482
Proteinase inhibitor cocktai	Sigma	P8340
QuantiTect Reverse Transcription Kit	Qiagen	205310
RNALater	Qiagen	76106
RNeasy Mini Kit	Qiagen	74104
Sucrose	Sigma	84097
SYBR Green	Applied Biosystems	4309155
Trizol reagent	Invitrogen	15596018
VECTOR Red AP Substrate Kit	Vector Laboratories	SK-5100
Xylene	Carl Roth	2662.4

Mice and diets	Supplier	Article number
Control diet	ssniff Spezialdiäten GmbH	S8102-E701
Fat-mod HC diet	ssniff Spezialdiäten GmbH	S8379-E712
HC diet	ssniff Spezialdiäten GmbH	E15741-347
Maintenance diet	Altromin Spezialfutter GmbH & Co. KG	MD 1320
NMRI Mice	Janvier	RjHan:NMRI

Hardware and Software	Supplier	Location
Actimot2 TSE-System, 302020 series	TSE Systems	Bad Homburg, Germany
Agilent 2100 Bioanalyzer	Agilent Technologies	Waldbronn, Germany
Aluminium round cuts	Korff	Oberbipp, Switzerland
Analysis software Gen 5 1.1	Biotek	Winooski, United States
Bacillol AF	Hartmann	Heidenheim, Germany
Calorimeter C7000	IKA	Staufen, Germany
Drawing Ink A (black)	Pelikan	Hannover, Germany
EDTA capillary tube	Kabe Labortechnik GmbH	Nümbrecht-Eisenroth, Germany
Eppi 1,5 ml PCR Clean	Sarstedt	Nümbrecht, Germany
Eppi 2,0 ml PCR Clean	Sarstedt	Nümbrecht, Germany
Falcon 10 ml, yellow	Sarstedt	Nümbrecht, Germany
Falcon 15 ml, red	Sarstedt	Nümbrecht, Germany
Falcon 50 ml, blue	Sarstedt	Nümbrecht, Germany
Filter paper cards	Becker-Olgemüller	Munich, Germany
GeneChip Scanner 3000	Affymetrix	Santa Clara, USA
GenomeStudioV2010.1 software (gene expression module version 1.6.0)	Illumina	San Diego, CA, USA
GraphPad Prism 4.0	GraphPad Software Inc.	San Diego; USA
Homogenisator (Silent Crusher M)	Heidolph	Schwabach, Germany
Insulin syringe	BD	New Jersey, USA
Lithium-heparin caplillary tube	Kabe Labortechnik GmbH	Nümbrecht-Eisenroth, Germany
Micro test plate, 96 well	Sarstedt	Nümbrecht, Germany
Microscope slides	Carl Roth GmbH	Karlsruhe; Germany
Microtome HM 355	Thermo Scientific	Wilmington, USA
Minispec LF 50	Bruker	Karlsruhe; Germany
Mouse Gene 1.0 ST Array	Affymetrix	Santa Clara, USA
Mouse Ref-8 v2.0 Expression BeadChips	Illumina	San Diego, CA, USA
Nano Zoomer 2.0 HT	Hamamatsu Photonics	Herrsching am Ammersee, Germany
Nanodrop 100; Version 3.7.0	Thermo Scientific	Wilmington, USA
Nunc® CryoTubes®	Thermo Scientific	Wilmington, USA
Olympus AU480 autoanalyzer	Olympus	Hamburg, Germany
Pierce BCA Protein Assay Kit	Thermo Scientific	Wilmington, USA
Pipette tips 0,5-2 µl Gilson	Sarstedt	Nümbrecht, Germany
Pipette tips 1000 µl, blue	Sarstedt	Nümbrecht, Germany
Pipette tips 200 µl, yellow	Sarstedt	Nümbrecht, Germany
Punch for ear tagging	EBECO	Castrop-Rauxel, Germany

QIAGEN's Ingenuity Pathway Analysis	Qiagen	Redwood City
R Development Core Team	Foundation for Statistical Computing	Vienna, Austria
SAS release 8.2	SAS Institute	Cary; NC; USA
Spectrophotometer Synergy H1	Biotek	Winooski, United States
Starfrost slides	Waldemar Knittel Glasbearbeitungs GmbH	Braunschweig, Germany
StepOnePlus System	Applied Biosystems	Foster City, USA
Thermocycler, PEQStar;	PEQLAB Biotechnologie	Erlangen; Germany
VideoplanR image analysis system	Zeiss Kontron	Eching, Germany
Viewing software (NDP.view2 U12388-01)	Hamamatsu Photonics	Herrsching am Ammersee, Germany

Table S2: Fatty acid composition of hydrogenated coconut oil and marine oil (Ω-3)

→ Functional compounds of the Fat-mod HC diet

	Coconut oil (% of analyzed fatty acids)	**Marine oil, Ω-3** (% of analyzed fatty acids)
Caprylic acid (C8:0)	5.49	-
Capric acid (C10:0)	5.15	-
Lauric acid (C12:0)	45.54	-
Myristic acid (C14:0)	19.58	6.55
Palmitic acid (C16:0)	10.80	16.85
Palmitoleic acid (C16:1 n-7)	-	8.43
Stearic acid (C18:0)	11.82	3.40
Oleic acid (C18:1 Ω-9)	1.06	12.02
Linoleic acid (C18:2 Ω-6)	0.07	3.15
Arachidonic acid (C20:4 Ω-6)	-	0.95
Linolenic acid (C18:3 Ω-3)	< 0.05	0.98
EPA (C20:5 Ω-3)	-	18.53
Docosapentaenoic acid (C22:5 Ω-3)	-	1.99
DHA (C22:6 Ω-3)	-	11.70
Others	< 0.69[1]	8.76[2]

Fatty acid composition was analyzed by gas chromatography. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; Fat-mod HC diet, fat-modified high-calorie diet.

[1]Other fatty acids in coconut oil comprise caproic acid (C6:0, 0.34%), arachidic acid (C20:0, 0.15%), and eicosenoic acid (C20:1), behenic acid (C22:0), erucic acid (C22:1), lignoceric acid (C24:0) (< 0.05% each).

[2]Other fatty acids in marine oil (Ω-3) comprise stearidonic acid (C18:4 n-3, 2.97%), gadolinium acid (C20:1, 2.01%), erucic acid (C22:1, 1.65%), pentadecanoic acid (C15:0, 0.58%), arachidic acid (C20:0, 0.48%), heptadecanoic acid (C17:0, 0.45%), docosatetraenoic acid (C22:4, 0.42%), myristoleic acid (C14:1, 0.20%).

Table S3: Verification of microarray results via qRT-PCR using a panel of 10 genes

		log_2 fold change			
		Array	qRT - PCR	Array	qRT - PCR
Gene ID	Gene name	**HC/CD ♂ vs CD/CD ♂**		**HC/CD ♀ vs CD/CD ♀**	
10379820	Acaca	-0.03	-1.6	**1.22*** **	1.9
10394538	Acaca	-0.00		**1.11*** **	
10391146	Acly	-0.40	-1.1	**1.70*** **	**3.2***
10456392	Cidea	**-1.04***	-1.9	**1.54*** **	2.4
10520362	Insig1	-0.27	-0,1	**0.70***	0.6
10536789	Lep	**1.64*** **	**5.3***	**-0.70***	-0.1
10405753	Me1	0.00	-0.9	**0.77** **	1.5
10595480	Me1	0.00		**0.85*** **	
10537062	Mest	**1.21** **	-1.4	**-1.40*** **	0.8
10529977	Pgc1a	-0.60	-2.7	0.27	0.5
10529979	Pgc1a	**-1.06*** **		0.47	
10463355	Scd2	-0.16	-1.6	**1.11*** **	1.4
10573152	Ucp1	**-1.8*** **	0.4	**0.79***	3.0

After hybridization of probes to Affymetrix GeneChips (Mouse Gene 1.0 ST Array, Affymetrix), DEGs were identified by LPEadj2. Gene panel was selected across the entire range of log_2fold changes, stratified by sex-effect. Calculation of log_2fold changes is based on medians (DNA-microarray) or means (qRT-PCR); significant differences were calculated between HC/CD vs CD/CD offspring; *p<0.05, **p<0.01, ***p<0.001.
CD/CD, exposure to maternal CD + postnatal CD feeding; HC/CD, exposure to maternal HC diet + postnatal CD feeding;
Acaca, acetyl-CoA carboxylase alpha; *Acly*, ATP citrate lyase; *Cidea*, cell death-inducing DNA fragmentation factor, alpha subunit-like effector A, *Insig1*, insulin induced gene 1; *Lep*, Leptin; *Mest*, mesoderm specific transcript; *Pgc1a*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *Scd2*, stearoyl-Coenzyme A desaturase 2; *Ucp1*, uncoupling protein 1.

Table S4: Primers for the measurement of transcriptional abundances via qRT-PCR

Transcript	**forward primer**	**reverse primer**
Acaca	CAGACTGATCGCAGAGAAAG	CTCAGGCTCACATCTGCTAC
Acly	GAAGTTGGGAAGACCACTGGGATC	AGGGATCTTGGACTTGGGACTGA
Cd36	CATGATTAATGGCACAGACG	TCCGAACACAGCGTAGATAG
Cidea	TGC TCT TCT GTA TCG CCC AGT	GCC GTG TTA AGG AAT CTG CTG
Cpt-1	TGTTTCGACAGGTGGTTTGA	GAAGAGCCGAGTCATGGAAG
Fabp4	CATGAAAGAAGTGGGAGTGG	AGTACTCTCTGACCGGATGG
Fasn	AGATCCTGGAACGAGAACAC	TCGTGTCAGTAGCCGAGTC
Insig1	ATAGCCACCATCTTCTCCTC	TCCCAGGTGACTGTCAATAC
Il-10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
Il1rn	TTGTGCCAAGTCTGGAGATG	AGAGCGGATGAAGGTAAAGC
Lep	ATCCCAGGGAGGAAAATGTGCTG	TACCGACTGCGTGTGTGAAATGTC
Nr1h3	GGATAGGGTTGGAGTCAGCA	GCTCAGCACGTTGTAATGGA
Mcp1	GGCTCAGCCAGATGCAGTTAACG	TCCTTCTTGGGGTCAGCACAG
Me1	ATAAACACCTGCAAGAAGGC	GGGTTCAGGATAAACAGTGG
Mest	AAGCCCTGAGATAGTTGTGC	ATCACTCGATGGAACCTCAG
Mrc1	CACCACTGACTACGACAAAG	GATGCCAGGTTAAAGCAGAC
Pgc1a	CGAAGAGCATTTGTCAACAG	TGCTGTTCCTGTTTTCTGTG
Pnpla2	CAAGGGGTGCGCTATGTGGA	GGTTGGTTCAGTAGGCCATTCCT
Pparg2	TCCTGTTGACCCAGAGCAT	TGCGAGTGGTCTTCCATCA
Ppara	CTCTGGGCAAGAGAATCCAC	TGATGTCACAGAACGGCTTC
Pparg1	GTGAGACCAACAGCCTGAC	TTCACCGCTTCTTTCAAATC
Scd2	GAGATCTCTGGCGCTTACTC	CTTTTCAAACTTCTCGCCTC
Srebf1	CCACTCACCATCCTACAGC	GGAAGGGTCCCTGAGAAG
Ube2b2	CACAGTGGTCTCCAGCACTA	CATTCCCGAGCTATTCTGTT
Ucp1	ACTTTGGAAAGGGACGACCCCTAA	GCAAAACCCGGCAACAAGAGC
Tbp	CAAACCCAGAATTGTTCTCCTT	ATGTGGTCTTCCTGAATCCCT
Ppib	ACGCAACATGAAGGTGCT	GGCCTACATCTTCATCTCCA
Plin2	AGAAAATTCAGGGTGCTCAG	GTACGTGACTCGATGTGCTC
Abca1	AGTGATAATCAAAGTCAAAGGCACAC	AGCAACTTGGCACTAGTAACTCTG
Slc2a1 (=Glut1)	CCAGCTGGGAATCGTCGTT	TGCATTGCCCATGATGGA
Slc38a2 (=Snat2)	GTCACCCTCACGGTCCCAGTAGTTA	GCATACCCATAGCTGTCGCAGAAGT
Rxra	GGAAATATGGCCTCCTTCAC	CTTTGCGTACTGTCCTCTTG
Slc27a1 (=Fatp1)	AGGTCAATGAGGACACGATGGAG	CTGGTACATTGAGTTAGGGTCCAAC
Slc27a4 (=Fatp4)	CTGAAGCTGCCCTGGACCCA	AGGGCATCCCGCCTAAGGTTG
Got2 (=Fabp-pm)	GTTACCGAAGCCTTCAAGAG	TGGGCAGGTATTCTTTGTCC
Dgat1	CCAGGTGGTGTCTCTGTTTC	CTGCCAGGCGCTTCTCAATC
Adipoq	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT

Table S5: Offspring effects of a periconceptional exposure to a maternal adipogenic milieu followed by postnatal HC diet feeding at an age of 5 months

Postnatally HC diet fed offspring	**Male**		**Female**	
	CD/HC (n=9)	HC/HC (n=10)	CD/HC (n=11)	HC/HC (n=10)
Body weight (g)	60.75 ± 2.95	65.32 ± 2.81	**47.56 ± 3.81**	**60.79 ± 2.66 ***
Carcasse	20.26 ± 0.65	20.78 ± 0.83	**15.95 ± 0.84**	**19.89 ± 0.71 ****
NRL	12.31 ± 0.12	12.46 ± 0.12	**11.59 ± 0.17**	**12.09 ± 0.13 ***
Liver (g)	2.98 ± 0.27	3.14 ± 0.31	1.97 ± 0.14	2.23 ± 0.15
Liver (g/g BW)	4.85 ± 0.27	4.74 ± 0.30	4.27 ± 0.27	3.64 ± 0.13
Subcutaneous fat (g)	3.91 ± 0.44	4.09 ± 0.35	**2.89 ± 0.53**	**4.74 ± 0.30 ***
Subcutaneous fat (g/g BW)	6.28 ± 0.54	6.19 ± 0.36	**5.51 ± 0.74**	**7.81 ± 0.41 ***
Intra-abdominal fat (g)	1.55 ± 0.17	1.87 ± 0.16	**5.64 ± 1.13**	**8.88 ± 0.64 ***
Intra-abdominal fat (g/g BW)	2.50 ± 0.20	2.83 ± 0.14	10.53 ± 1.71	14.46 ± 0.51
Leptin (ng/ml)	66.00 ± 2.68	63.28 ± 3.00	**36.90 ± 8.00**	**63.75 ± 5.55 ***
HOMA-IR	86.38 ± 9.45	78.54 ± 9.86	24.98 ± 8.20	51.24 ± 14.02
Fasting glucose (mmol/l)	12.46 ± 1.12	13.24 ± 1.03	8.80 ± 0.59	11.18 ± 1.09
Glucose (mmol/l) ipGTT: 80 min after ip-injection	20.12 ± 1.87 (n=7)	19.78 ± 1.41 (n=7)	**10.84 ± 0.84** (n=7)	**15.92 ± 1.67 *** (n=7)
Glucose (mmol/l) ipGTT: 160 min after ip-injection	12.05 ± 1.54 (n=7)	12.86 ± 1.86 (n=7)	**5.71 ± 0.36** (n=7)	**9.43 ± 1.24 **** (n=7)

Data are presented as mean ± s.e.m. and were sex-specifically analysed by Mann-Whitney U test.
CD/HC, exposure to maternal CD + postnatal HC diet feeding; HC/HC, exposure to maternal HC diet + postnatal HC diet feeding; HOMA-IR, homeostatic model assessment insulin resistance; ipGTT, intraperitoneal glucose tolerance test (intraperitoneal injection of 1.5 g glucose per kg body weight); NRL, nose-to-rump length.

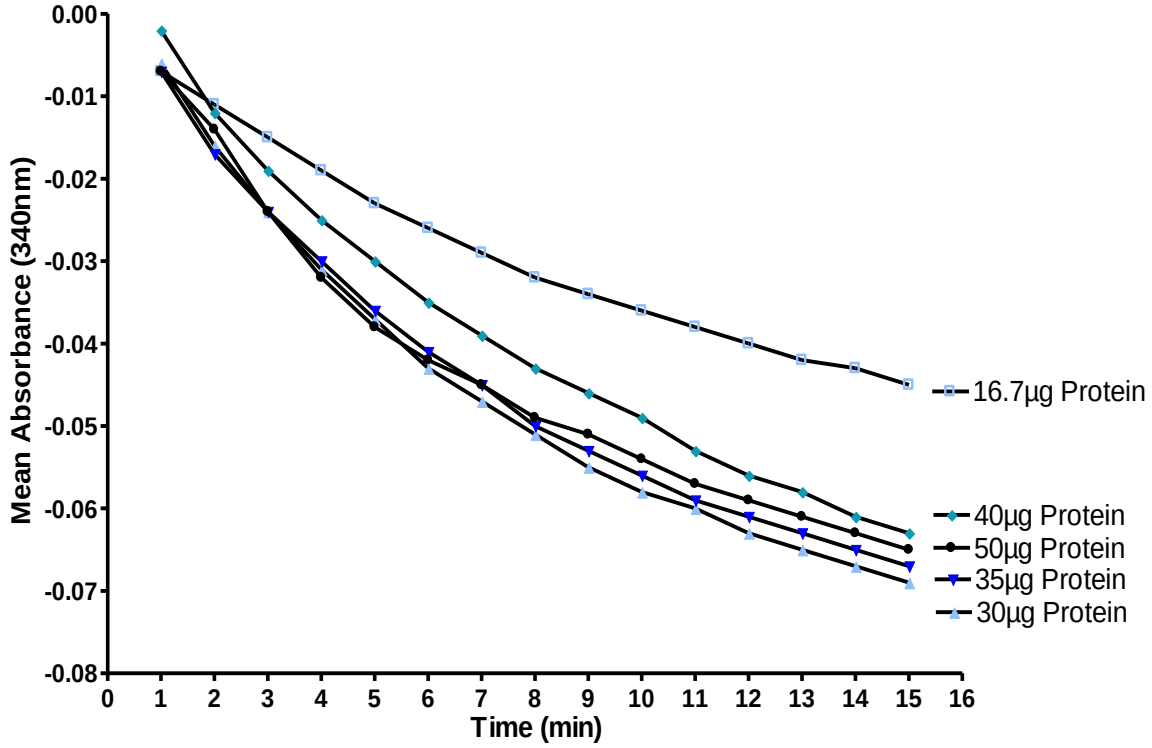
Table S6: Microarray-based enrichment analysis of differentially expressed genes in intra-abdominal adipocytes of the mat-HC and mat-Fat-mod HC female offspring

Mat-HC vs. mat-CD	DEGs	fold change	raw p-value
Sterol biosynthetic process	*Hmgcs2*	-1,85	0,021
	Dhcr7	1,36	0,004
	Nsdhl	1,38	0,014
	Cyp51	1,44	0,006
	Hsd17b7	1,29	0,002
	Insig2	-1,33	0,016
	Mvd	1,52	0,001
	Pmvk	1,35	0,019
	Oprs1	1,24	0,027
	Fdps	1,63	0,003
Mat-Fat-mod vs. mat-CD			
Blood vessel development	*Arhgap22*	1,21	0,035
	Col5a1	1,23	0,005
	Itga7	1,26	0,029
	Rtn4	-1,24	0,041
	Zfpm2	1,23	0,039
Cell adhesion	*Wisp1*	1,36	0,017
	Wisp2	1,75	0,006
	Col6a2	1,23	0,033
	Col16a1	1,29	0,027
	Cntn1	1,38	0,009
	Itga5	1,21	0,004
	Itga7	1,26	0,029
	Nid2	1,23	0,042

After hybridization of probes to Expression BeadChips (Illumina), DEGs in intra-abdominal adipose tissue of female offspring were identified by limma t-test (fold change>1.2x, p<0.05). Enrichment analysis was conducted via DAVID using DEGs, which were unique for mat-HC (vs mat-CD) and mat-Fat-mod HC (vs mat-CD) female offspring.
mat-Fat-mod HC, exposure to maternal fat-modified (MCFA, Ω-3) high-fat, high-calorie diet; mat-HC, exposure to maternal high-fat, high-calorie diet;
Arhgap22, Rho GTPase activating protein 22; *Cntn1*, contactin 1; Col16a1, collagen, type XVI, alpha 1; *Col5a1*, collagen, type V, alpha 1; *Col6a2*, collagen, type VI, alpha 2; *Cyp51*, cytochrome P450, family 51; DEG, differentially expressed gene; *Dhcr7*, 7-dehydrocholesterol reductase; *Fdps*, similar to farnesyl diphosphate synthetase, farnesyl diphosphate synthetase, predicted gene 5873, predicted gene 8163, predicted gene 3571; *Hmgcs2,* 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2; *Hsd17b7*, hydroxysteroid (17-beta) dehydrogenase 7; *Insig2*, insulin induced gene 2; *Itga5*, integrin alpha 5 (fibronectin receptor alpha) ; *Itga7,* integrin alpha 7; *Mvd*, mevalonate (diphospho) decarboxylase; *Nid2*, nidogen 2; Nsdhl, NAD(P) dependent steroid dehydrogenase-like; *Oprs1*, sigma non-opioid intracellular receptor 1; *Pmvk,* phosphomevalonate kinase; *Rtn4*, reticulon 4; *Wisp1*, WNT1 inducible signaling pathway protein 1; *Wisp2*, WNT1 inducible signaling pathway protein 2; *Zfpm2*, zinc finger protein, multitype 2.

Figures S1 and S2: Validation of FASN activity assay

A



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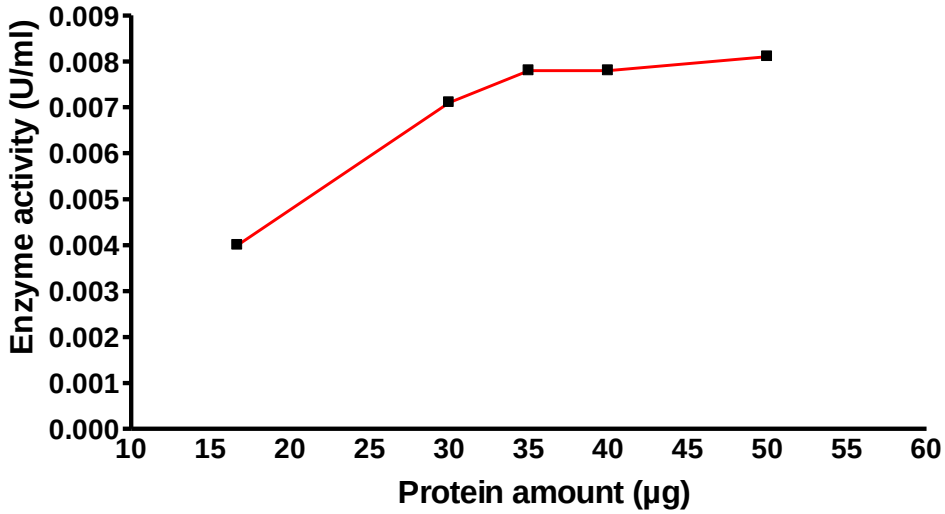
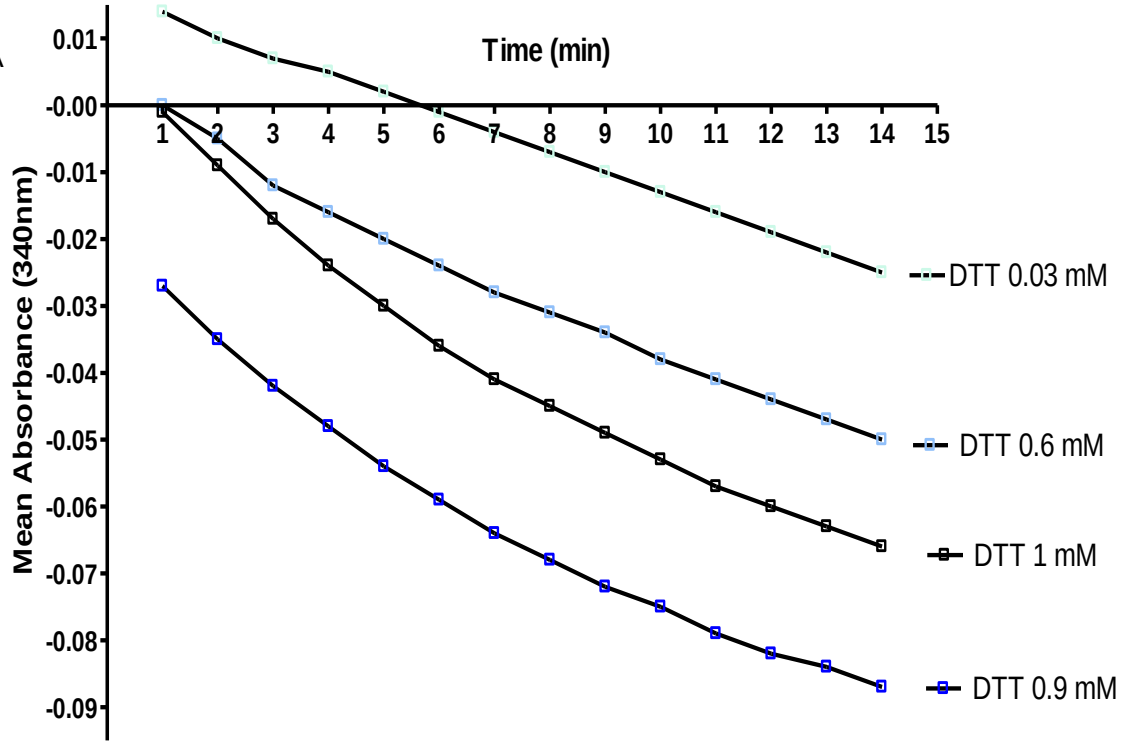


Figure S1: Validation of enzyme activity according to protein amount showed a linear increase of enzyme activity for the use of 15µg- 25µg protein: (A) Linear absorbance and (B) FASN activity for different protein amounts using 16.7 µg, 30 µg, 35 µg, 40µg, 50µg protein in final reaction mixture. Linear enzymatic oxidation of NADPH was measured at 37°C at 340nm. Final reaction mixture contains: x µg protein, 40 mM sucrose, 400 mM potassiat phosphat, 795 µM EDTA, 635 µM β-mercaptoethanol, 0.3 mM DTT, 160 µM NADPH, 70 µM acetyl-CoA and 90 µM malonyl-CoA. A blank without malonyl-CoA was simultaneously measured for each sample.

A



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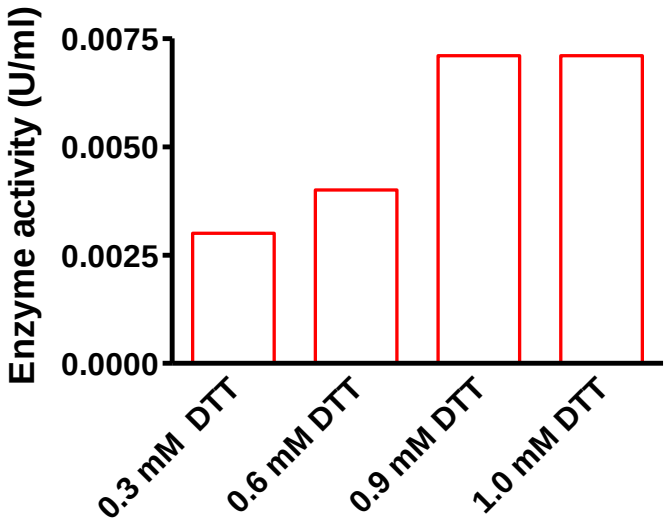

Figure S2: Validation of enzyme activity according to DTT (dithiothreitol) concentration showed optimal enzyme activity for the use of 1 mM DTT in final reaction mixture: (A) Linear absorbance and (B) FASN activity for different DTT amounts using 0.3 mM, 0.6 mM, and 0.9 mM and 1.0 mM DTT in final reaction mixture. Linear enzymatic oxidation of NADPH was measured at 37°C at 340nm. Final reaction mixture contains: 20 µg protein, 40 mM sucrose, 400 mM potassium phosphat, 795 µM EDTA, 635 µM β-mercaptoethanol, x mM DTT, 160 µM NADPH, 70 µM acetyl-CoA and 90 µM malonyl-CoA. A blank without malonyl-CoA was simultaneously measured for each sample.

Acknowledgement

My first thank goes to Prof. Dr. Regina Ensenauer for giving me the opportunity to perform my PhD project at the Institute of Molecular Nutrition at the research center of the Dr. von Hauner Children's Hospital (Ludwig-Maximilians-University, Munich).
Further, I want to thank all members of the research group of Molecular Nutrition. Special thanks to Susanne Wullinger and Maria Ostner for technical assistance.

I am also grateful to Prof. Dr. Adalbert A. Roscher for his helpful advice during the establishment of the enzyme activity assay.

Also thanks to the members of the Institute of Experimental Genetics at the German Mouse Clinic (Helmholtz Zentrum; München). Special thank goes to Dr. Jan Rozman, Dr. Birgit Rathkolb, Ann-Elisabeth Schwarz, Silvia Crowley, Sebastian Kaidel and Mareike Bamberger.

Special thanks go to Helena Hartmann and Le Gu for their great work and support during the animal experiment.

In addition, best thanks to Prof. Eckhard Wolf (Institute of Molecular Animal Breeding and Biotechnology, Gene Center, Ludwig-Maximilians-University, Munich) for his statistical support.

Special thank goes to my parents, Sebastian, Hermann, Thomas, Caro, Lisa and Maik who always backed me during difficult times.

Last but not least, best thank goes to Prof. Martin Klingenspor for the supervision of my PhD thesis.

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der promotionsführenden Einrichtung des Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel:

The role of fatty acid quality in a maternal obesogenic diet for adipose tissue development in offspring mice

am Institut für Molekulare Ernährung (Forschungszentrum des Haunerschen Kinderspitals, Ludwig-Maximilians-Universität, München) unter der Anleitung und Betreuung durch Prof. Dr. Regina Ensenauer ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Abs. 6 und 7 Satz 2 angegebenen Hilfsmittel benutzt habe.

Ich habe keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht, oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.

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Die vollständige Dissertation wurde noch nicht veröffentlicht.

Ich habe den angestrebten Doktorgrad noch nicht erworben und bin nicht in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.

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Veröffentlichungen

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