Original Article

Expression Profile of Fatty Acid Metabolism Genes in Preimplantation Blastocysts of Obese and Non-Obese Mice

Cornelia Alice Irene Königsdorf Anne Navarrete Santos Juliane-Susanne Schmidt Sünje Fischer Bernd Fischer

Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany

Key Words

Obesity • Blastocysts • Lipid metabolism

Abstract

Background: Recent studies have disclosed a close relationship between maternal obesity, fetal metabolism and pre- and postnatal development. The lipid metabolism in preimplantation embryos is a possible target of metabolic programming. **Methods:** 31 genes of beta-oxidation and fatty acid and cholesterol uptake, synthesis and regulation were analyzed in day 3.5 blastocysts from NZO (obese) and C57Bl/6 (normal weight) mice by RT-PCR and semi-quantitative PCR. **Results:** The most obvious difference between both strains was the lack of the RXR gamma transcript in NZO blastocysts. In adult NZO mice, RXR gamma is detectable in most tissues. In a semiquantitative analysis, a higher transcription rate of fatty acid transport protein 4 ($p = 0.004$) and a reduced transcript number of fatty acid synthase ($p = 0.049$) was found in NZO blastocysts. Cholesterol synthesis regulation was modified in NZO blastocysts, as indicated by the ratio of sterol regulatory element-binding protein (SREBP) 2 / insulin-induced gene 1 (*Insig 1*) ($p = 0.001$). **Conclusion:** In mouse blastocysts enzymes and signal molecules of fatty acid and cholesterol metabolism resemble those expressed postnatally. Distinct differences in transcription rates of genes between blastocysts from obese and non-obese mothers indicate that preimplantation embryo development is an early target for metabolic programming.

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Introduction

Obesity is one of the major problems of the Health Care Systems in industrialized countries worldwide (obesity pandemics). The World Health Organization estimates that in some European countries as many as 20% of the children are overweight [1]. These childhood obesity rates implicate, among others, the question for the inheritance of obesity and metabolic programming. Recent studies have disclosed a close correlation between maternal nutrition during early pregnancy and fetal metabolism and development [2–4]. Obesity itself seems highly likely to program for metabolic disorders in the offspring [5].

Aim of this study was to further analyze when and how metabolic programming may start in ontogenesis. We do not know in detail the spectrum of lipid metabolism parameters in preimplantation embryos. We have performed a comprehensive transcription analysis of a broad spectrum of parameters characterizing lipid metabolism in blastocysts. By comparing embryos from obese (NZO) and non-obese mice (C57Bl/6) we address the question whether differences in lipid metabolism occur as early as during preimplantation embryo development.

As an obese animal model the New Zealand obese (NZO) [6] mouse was chosen. This is an established animal model of a polygenetically induced obesity for the analysis of metabolic syndrome and diabetic parameters. Due to leptin resistance these mice show a hyperleptinemia as part of the metabolic disruption [7]. Beside obesity, these mice show hyperlipidemia, insulin resistance and hyperinsulemia, glucose intolerance as well as arterial hypertension [8–10].

Results of Pan et al. [11] revealed a polymorphism in the NZO genome which inactivates phosphatidylcholine transfer protein (PC-TP). The expression of this cytosolic protein is regulated by PPAR alpha and is involved in lipid and energy metabolism [12]. The inactivation of PC-TP may play a role in the modified energy homeostasis in the polygenetic NZO model of obesity and diabetes.

As a control mouse strain of normal body weight, C57Bl/6 mice were employed in the current study. Using C57Bl/6 as controls for NZO mice is a common practice in metabolic studies [13] although both strains do not have the same genetic origin. The reproductive performance of the NZO mice is reduced due to an altered hormonal status and impaired ovarian function [13]. The NZO phenotype is caused by polygenetic metabolic disorders and is not the result of one single disruption. C57Bl/6 mice are a commonly used strain and do not show severe health problems; their phenotype is constantly lean.

For the molecular analysis of day 3.5 blastocysts from both strains, a great spectrum of lipid metabolism parameters was examined. Parameters of beta-oxidation (acyl-CoA-oxidase 1 (Acox1), carnitine-palmitoyl transferase 1 (Cpt1), organic cation/carnitine transporter 2 (Octn2)) were analyzed representing the catabolic metabolism of fatty acid oxidation. The fatty acid synthesis is catalyzed by fatty acid synthase (Fasn) and acyl-CoA-carboxylase (Acc). The uptake of fatty acids is assisted by fatty acid transport proteins (FATPs). Especially the long-chain-polyunsaturated fatty acids (LCPUFAS) need this transport system for cellular uptake. FATPs are active transporters, belonging to the Solute Carrier Family 27, also known as enzymes with very long-chain acyl CoA synthetase activity (ACSVL) co-localizing with acyl-CoA synthetase (Acs). This enzyme esterifies fatty acids so that they cannot diffuse out of the cell. FATPs are regulated by their substrates; increased concentration of fatty acids does enhance FATP expression. But the main signaling pathway to regulate FATP expression is the PPAR (peroxisome proliferator-activated receptors) / RXR (retinoid-X receptors) pathway. These intracellular signaling molecules form PPAR/RXR heterodimers and activate PPAR response elements at corresponding genes in the nucleus. All of the three PPAR isoforms (alpha, beta/delta, gamma; Ppara, Ppard; Pparg) are

involved in cellular lipid metabolism regulation. Pparg/ RXR alpha (Rxra) heterodimers play a role in adipocyte differentiation and cellular lipid accumulation [14, 15]. Recent studies assume that PPAR gamma / RXR alpha heterodimers play a role in the pathogenesis of the metabolic syndrome [16]. The RXR exist in three isoforms, Rxra, RXR beta and RXR gamma (Rxrg). Knockout experiments have shown that one functioning RXR allele is sufficient for normal development of mouse embryos [17, 18].

Intracellular lipid transport is facilitated by fatty acid binding proteins (FABPs), organizing the transport among the cell organelles and the supply of metabolites to different metabolic pathways. There are seven isoforms of the FABPs with different expression patterns; Fabp4 (isoform of the adipose tissue) is a biological marker for the metabolic syndrome because Fabp4 levels correlate with BMI, triglyceride level, blood glucose concentration, and insulin level [19–21].

The interaction of SREBPs and INSIGs is essential for cellular cholesterol homeostasis. If intracellular cholesterol concentration decreases, SREBP and SCAP (SREBP cleavage activating protein) induce the release of transcription factors (MBTPS1, MBTPS2). These transcription factors activate the transcription of gene sequences with a SRE region (sterol regulatory element), like HMG-CoA reductase (Hmgcr) and LDL receptor (Ldlr). If the cholesterol concentration increases, INSIGs prevent the activation of SREBP and SCAP at the endoplasmic reticulum. Embryonic tissues have an increased ratio of SREBP in relation to INSIG because they need higher cholesterol concentrations than adult tissues do [22].

Adipophilin and perilipin belong to the PAT proteins which are involved in maturing of intracellular lipid droplets. They protect lipid droplets against lipolysis. Recent studies suppose that perilipin is decreased in an obese organism and that the PAT proteins play a role in the pathogenesis of the metabolic syndrome [23–25]. Perilipin and adipophilin are expressed in fetal membranes, suggesting that these proteins are involved in fetal lipid storage mechanisms and development [26].

Material and Methods

Animals, Hormonal Stimulation, and Embryo Recovery

Mouse strains NZO (New Zealand obese, substrain NZO/HlBomDife) and C57Bl/6 were used for blastocyst recovery. The weight of the NZO dams was > 59 g [10] and clearly higher than that of C57Bl/6. Most notably was the massive intraperitoneal adiposis. Mature females of both strains (6–10 months of age) were maintained at a 12-hour light : 12-hour dark photoperiod. Males were kept separately. Females (NZO: n = 20 pregnant mice, C57Bl/6: n = 31) were treated with 7.0 IU PMSG (Sigma Aldrich, Taufkirchen, Germany) at day 1 and 7.5 IU human chorionic gonadotropin (Sigma Aldrich) at day 3 by intraperitoneal application. Mating and embryo recovery were performed as described before [27]. At day 3.5 post coitum (p.c.) blastocysts were flushed from uteri, washed three times with PBS, pooled in groups of 10–15 embryos and stored at –80 °C in PBS. The blastocysts were not sexed.

RNA Extraction and cDNA Synthesis

Messenger RNA from blastocyst pools was isolated with DYNABEADS (Invitrogen, Karlsruhe, Germany) and subsequently used for cDNA synthesis. All protocols were used according to the manufacturer's instructions and modifications as described by Tonack et al. [27]. For the reverse transcriptase reaction, Superscript™ II RT-kit and dNTPs were purchased from Fermentas (St. Leon-Rot, Germany), random primer from Roche (Mannheim, Germany) and RNase inhibitor from Promega (Mannheim, Germany). For one cDNA sample 10–15 blastocysts from different mice of one strain were pooled.

Primer Generation and Sequencing PCR

Primer sequences of the analyzed genes were derived from mouse sequences using the specific primer program Primer3 (<http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi>). Using cDNA from mouse tissues (liver, muscle, heart, brain), primers were tested by PCR. The amplified PCR products were purified

Table 1. Oligonucleotides and annealing temperature used for PCR

Name of primer	Genbank Acc. number	Bases	Sequence 5'–3'	T, °C	Fragment, bp
Acacafw	XR_033425.1	20	GCCTCTTCCTGACAAACGAG	60	235
Acaca rev		20	TGACTGCCGAAACATCTCTG		
Acox1 fw	NM_015729.2	20	CAGGAAGAGCAAGGAAGTGG	60	189
Acox1 rev		19	CCTTTCTGGCTGATCCCAT		
Acs2 fw	NM_153807.2	20	GGAGCAGAAGGCTGGTAGTG	62	300
Acs2 rev		20	TCCAGCTCTGCAGGGTAGAT		
Adipophilin fw	AF_443203.1	16	GGAGTGGAAGGCATCG	60	171
Adipophilin rev		16	TGGCATGTAGGAGCTG		
Cpt1 fw	NM_013495.1	20	GATGTGGACCTGCATTTCCTT	60	89
Cpt1 rev		20	TCCTTGTAAATGTGCGAGCTG		
Cd36 fw	NM_007643.3	20	GCTTGCAACTGTCAGCACAT	60	292
Cd36 rev		20	GAGCTATGCAGCATGGAACA		
Fabp1 fw	NM_017399.2	20	GTCAGCTGTGGAAGGAAGC	60	285
Fabp1 rev		20	GTCTCCAGTTCGCACTCCTC		
Fabp1 nested fw	NM_017399.2	20	TGCCACCATGAACTTCTCCG	62,5	96
Fabp1 nested rev		20	TGAACTCATTTGCGGACCACT		
Fabp3 fw	NM_010174.1	20	CATCGAGAAGAACGGGGATA	60	239
Fabp3 rev		20	TGCCATGAGTGAGAGTCAGG		
Fabp4 fw	NM_024406.1	20	TGGAAGCTTGTCTCCAGTGA	60	282
Fabp4 rev		20	TCGACTTTCATCCCCTTC		
Fasn fw	NM_007988.3	17	TGCTCCCAGCTGCAGGC	60	109
Fasn rev		20	GCCCCGTAGCTCTGGGTGTA		
Gapdh fw	XM_001473623.1	20	ACCCAGAAGACTGTGGATGG	60	172
Gapdh rev		20	ACACATTGGGGGTAGGAACA		
Hmgcr fw	NM_008255.1	20	TGGAGATCATGTGCTGCTTC	60	248
Hmgcr rev		20	GCGACTATGAGCGTGAACAA		
Insig1 fw	NM_153526.3	20	GTGGAGCTTGCAATCTGTGA	60	234
Insig1 rev		20	CTTCTCCGGAATAGCTCGTG		
Insig2 fw	NM_133748.1	20	TTCTGGTAGGTCCCACGTTT	60	212
Insig2 rev		20	TTCACACTCTGGCTGGTGAC		
Ldlr fw	NM_010700.2	20	CAGCTCTGTGTGAACCTGGA	57	271
Ldlr rev		20	TCAGGGCGCTGTAGATCTTT		
Lpl fw	XM_001476462.1	20	GTCGAAGTATTGGGATCCAG	60	186
Lpl rev		20	AGCAGGGAGTCAATGAAGAG		
Octn2 fw	NM_011396.2	20	ACAGTATCCCGTTGGAGACG	60	213
Octn2 rev		20	ACACCAGTCCCACTCTGTC		
Perilipin fw	NM_175640.1	20	GATCGCCTCTGAACTGAAGG	60	225
Perilipin rev		20	CTTCTCGATGCTTCCCAGAG		
Ptgis fw	NM_008968.3	20	GACAGCATGCCTGTGCTAGA	60	273
Ptgis rev		20	GCCGTTTCCCATCTTTGTAA		
Ppara fw	NM_011144.3	20	ATGCCAGTACTGCCGTTTTC	60	220
Ppara rev		20	GGCCTTGACCTTGTTCATGT		
Ppard fw	NM_011145.3	20	TGGAGCTCGATGACAGTGAC	60	161
Ppard rev		20	GTA CTGGCTGT CAGGGTGGT		
Pparg fw	NM_011146.2	16	GATGGAAGACCGCATT	60	116
Pparg rev		17	AACCATTTGGGCTCTTG		

Table 1 continued on next page

Table 1. Continued

Name of primer	Genbank Acc. number	Bases	Sequence 5'–3'	T, °C	Fragment, bp
Rxra fw	XM_001477331.1	20	GGTGGTGTCTGTGGACAGTG	60	202
Rxra rev		20	ACTAAGGCACCTGCTCCTGA		
Rxrb fw	NM_011306.3	20	CTGCAAGGGTTTCTTCAAGC	60	239
Rxrb rev		20	ATCCTGTCCACAGGCATCTC		
Rxrg fw	NM_009107.2	20	TCCTCCAGGAATCAACTTGG	60	234
Rxrg rev		20	TGAAGAAGCCTTTGCAACCT		
Rxrg nested fw	NM_009107.2	21	AGCTCCCAGCTAAATGTGGTC	60	174
Rxrg nested rev		20	TGTACACACAGTAGTGCTTC		
Slc27a1 fw	NM_011977.3	16	TGGCTGCTCTAATGTC	60	124
Slc27a1 rev		16	AGCAGAACTTGGCTCT		
Slc27a2 fw	NM_011978.2	16	CGGCCAGGAATCTAT	60	100
Slc27a2 rev		16	AAAGCTAAAGCAACCA		
Slc27a3 fw	NM_011988.1	16	AGGCTGCTCGAGTCAT	61	175
Slc27a3 rev		16	AACTTGGGTTTACCAC		
Slc27a4 fw	NM_011989.2	16	CTGTTCTCCATGCTGA	60	226
Slc27a4 rev		16	TGGCGCTGTAATTGAA		
Slc27a5 fw	NM_009512.1	16	TGGGCAGAAGCAGCAT	60	123
Slc27a5 rev		16	GCCGTGACTTGCTTGT		
Slc27a6 fw	NM_001081072.1	20	GGTCACGGTGCTGGATAAGT	60	109
Slc27a6 rev		20	CGAGGAGTGTTTCCAGGAGAG		
SREBP-1 fw	NM_011480.2	20	CCCTGTGTGTACTGGCCTTT	60	292
SREBP-1 rev		20	TTGCGATGTCTCCAGAAAGTG		
SREBP-2 fw	NM_033218.1	20	GCGGAGGAGAAAATCCTACC	60	252
SREBP-2 rev		20	GCAGTACAGGCTCTCCTTGG		
18s fw		21	AGAAACGGCTACCACATCCAA	60	89
18s rev		27	CCTGTATTGTTATTTTCGTCACCTACCT		

T = Temperature.

by separation in a preparative 1.8 (w/v) agarose gel and extracted by a gel extraction kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The purified DNA was used for sequencing with the Big Dye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences were compared at <http://blast.ncbi.nlm.nih.gov/Blast.cgi> with the established mouse gene sequences. The sequences of the primers used are listed in table 1.

PCR

Copy DNA of day 3.5 blastocysts of NZO and C57Bl/6 mice were analyzed by PCR. The same volume of blastocyst pool cDNA was used for both strains in one PCR. The PCR standard was assembled by 2.5 µl 10 × PCR buffer, 1 µl 50 mmol/l MgCl₂, 0.75 µl 10mmol/l dNTP (Fermentas), 0.75 µl primer forward, 0.75 µl primer reverse (Biomers, Ulm, Germany), 0.4 µl Taq polymerase (5 U/µl) (Invitrogen), 2, 3 or 4 µl cDNA, DEPC-H₂O ad 20 µl. The volume was incubated at 94 °C for 3 min and 40 cycles of 94 °C / 1 min, 1 min annealing temperature, 72 °C / 1min, 72 °C / 10min. Resulting PCR products were separated by electrophoresis on 1–2.2 (w/v) agarose gel and stained with ethidium bromide. For all primers the appropriate annealing temperature and resulting DNA fragments length are given in table 1. For the semiquantitative measurements the amount of cDNA and PCR cycles were adjusted to exponential range of PCR.

Densitometric Analysis

The analyses were performed with Image Analysis Software (Bio 1D Version 99; LTF, Wasserburg, Germany) determining the relative band density of the PCR products. Band densities were adjusted to GAPDH references.

Fig. 1. Gel electrophoresis illustrating RT-PCR products of transcripts for enzymes and metabolites of beta oxidation (A), fatty acid synthesis (B) and PAT proteins (C) from day 3.5 p.c. blastocysts of obese (NZO) and non-obese (C57Bl/6) mice. Two cDNA pools for each group with 10–15 in vivo blastocysts were analyzed. For primers and fragment size refer to table 1.

Statistical Analysis

Band density was independently analyzed for two or three blastocyst pools of each mouse strain. Data are reported as mean and standard error and were analyzed by independent sample t-test with 95% confidence interval (95% CI) ($p < 0.05$). For the comparison of SREBPF transcription levels to *Insig1*, the GAPDH adjusted mean values of *INSIG 1* ($N = 3$), *INSIG 2* ($N = 2$), and *SREBPF1* and *2* ($N = 2$) were used. The resulting ratios (below or over 1) were analyzed by one-sample t-test with 95% CI.

Results

Expression Profile of Fatty Acid Metabolism Genes

The transcriptional activities of 31 genes involved in lipid metabolism were analyzed in day 3.5 blastocysts from obese NZO and non-obese C57Bl/6 mice. For each gene a RT-PCR analysis was performed using specific oligonucleotides (table 1). Genes were chosen by relevance and sequence availability from databases.

Transcripts for enzymes and metabolites of beta-oxidation, fatty acid synthesis, PAT proteins (fig. 1), fatty acid uptake and transport as well as lipid and cholesterol metabolism regulation (fig. 2) were detectable and did not differ between blastocysts from both strains. Transcripts from *FATP6* and *FABP1* were not found (data not shown). An obvious difference in blastocysts from both mouse strains was the lack of *Rxrg* mRNA in NZO blastocysts (fig. 3A). In tissues of adult NZO mice *Rxrg* was detectable (fig. 3B), demonstrating a transient lack of this transcription factor during preimplantation development.

Semiquantitative Analysis of *FATP4*, *FAS*, *SREBP*, and *INSIG* Expression

To identify differences in transcript amounts, the PCR product intensities were quantified densitometrically for all analyzed parameters. There were no differences in transcription rates beside transcription numbers of *Fatp4* and *Fasn*. The RNA amount of *Fatp4* and *Fasn* was calculated relative to the house keeping gene product GAPDH. A higher transcript rate of *Fatp4* (fig. 4A; $p = 0.004$) and a lower one of *Fasn* (fig. 4B; $p = 0.049$) was found in NZO blastocysts.

For further investigation of the regulation of the cholesterol metabolism, the mean values of the SREBP transcription rates were compared in relation to *INSIG* in both strains (fig. 5). A higher ratio of SREBP to *INSIG* suggests a preponderance of cholesterol synthesis activating transcription factors. Whereas the ratio of *SREBP-1* / *Insig1* and *2* and of *SREBP-2* / *Insig2* (fig. 5) was balanced in both groups, the ratio of *SREBP-2* / *Insig1* (fig. 5A) was

Fig. 2. Gel electrophoresis illustrating RT-PCR products of transcripts of **A** fatty acid uptake and transport, **B** lipid and **C** cholesterol metabolism regulation from day 3.5 p.c. blastocysts of obese (NZO) and non-obese mice (C57Bl/6). Two cDNA pools for each group with 10–15 in vivo blastocysts were analyzed. For primers and fragment size refer to table 1.

Fig. 3. Expression of *Rxrg* mRNA in 3.5 day p.c. blastocysts (**A**) and organs (**B**) from NZO and C57Bl/6 mice. Three cDNA pools for each group with 10–15 in vivo blastocysts were analyzed. cDNA synthesis was controlled by *Gapdh* PCR. For primers and fragment size refer to table 1. No transcripts were detectable in blastocysts from NZO mice. The *Rxrg* PCR bands of figure 5A are composed of two different agarose gels (third position of NZO and C57Bl/6 PCR bands).

Fig. 4. Comparison of the relative expression levels of fatty acid transport protein 4 (Slc27a4) (A) and Fasn (B) in mouse day 3.5 p.c. blastocysts of obese and non-obese mice. Mean relative abundance of the Slc27a4 and Fasn transcripts at blastocyst stage of in vivo embryos from NZO (grey) and C57Bl/6 (white) mice determined by semiquantitative RT-PCR (N = 3, $*p_{Slc27a4} = 0.004$, $p_{Fasn} = 0.049$).

increased in blastocysts from C57Bl/6 mice, but not in NZO embryos. Thus, a difference in embryonic cholesterol metabolism regulation is detectable at day 3.5 p.c. in blastocysts from obese and non-obese mice.

Discussion

Even if final verifications on the protein levels have still to be done, our findings clearly show that lipid metabolism pathways in blastocysts closely resemble those in adult cells. Day 3.5 blastocysts depend on nutrient supply by uterine secretions and self-synthesis. Irrespective of the non-vascular supply of nutrients, all major lipid metabolism pathways are already established before implantation and placentation.

The mouse strains used in this study are not of the same genetic origin. It is not possible to link the described results to only obesity or diabetes; it has to be linked to the complete disease pattern of the metabolic syndrome. Due to the polygenetic background of their obesity, the morphology and metabolic profile of the NZO mice are not the effect of one single modification or mutation. Therefore, the obesity of the NZO mice is closer to most human diseases of the metabolic syndrome than one specific genetic disorder.

Fig. 5. Ratio of transcript amounts of Srebp1/2 to Insig1 (A) and Insig2 (B) in day 3.5 p.c. blastocysts of obese NZO (grey bars) and non-obese C57Bl/6 (white bars) mice. Analysis by independent one-sample t-test showed no significant results for the ratio of Srebp1/2 in relation to Insig2 and Srebp1 to Insig1 in both strains. The ratio of Srebp2 to Insig1 in blastocysts of the C57Bl/6 mice was increased (**p = 0.001) while it was balanced in blastocysts of the NZO mouse strain.

The transcriptional changes observed in the current study may be due to genetic changes in the NZO mothers and blastocysts. First, a direct mutation of the affected genes cannot be excluded. Secondly, an indirect feedback of the blastocysts' altered metabolism due to maternal obesity and/or diabetes on gene expression has to be taken into consideration. Recent studies have revealed a number of differences in gene expression profiles in blastocysts from obese mothers thus emphasizing the effects of maternal nutrition during the periimplantation period on early embryos. Jungheim et al. [28] reported a decrease of IGF-I receptor expression in blastocysts from mice fed a high-fat diet. The circulating maternal fatty acids showed altered insulin signaling and glucose transport in these blastocysts. Furthermore, a high-fat diet enhanced the mitochondrial activity in oocytes and zygotes of mice. The increase in fatty acids and carbohydrates due to excessive nutrient exposure did alter mitochondrial oxidation [29].

Some features of lipid metabolism have been shown to exert a decisive impact on prenatal development. The uptake of fatty acids by FATPs is an essential metabolic pathway during fetal development. Heterozygous *Fatp4* knockout mice (*Fatp4*^{+/-}) do not give birth to homozygous offspring [30]. Human embryos developing beyond the four-cell stage contain higher concentrations of linoleic and oleic acid than those with a reduced development capacity [31]. Thus, LCPUFAS are essential for preimplantation embryos. FABPs are needed for intracellular fatty acid transport and contribute to cell differentiation as shown in embryonic stem cells [32].

The generation of energy is the most important objective of beta-oxidation of fatty acids. Inhibition of *Cpt1* impairs mouse blastocyst development. In contrast, supplementation of L-carnitine during oocyte maturation enhances developmental competence, even under carbohydrate deficiency [33]. The synthesis of fatty acids is another important metabolic pathway. Birth rates of heterozygous *Fasn* mice are decreased and are not reversible by an enhanced supply of fatty acids during pregnancy. *Fasn* deletion seems to be lethal as homozygous *Fasn*^{-/-} mice were not found in the study of Chirala et al. [34].

The PPAR/RXR pathway is involved in the regulation of lipid uptake and storage in preimplantation embryos. It is noteworthy that one of the dimerisation partners, Rxrg, is lacking in preimplantation embryos from NZO mice. In adult tissues, this transcription factor is expressed. The mouse is not the only species with this transient lack in expression as Rxrg transcripts in bovine blastocysts were only detectable in some but not all analyzed embryos [35, 36]. Surprisingly, Rxrg expression is positively correlated with the developmental competence of an embryo [36]. We have analyzed the reproductive performance of both strains used in current study. Although the number of observations is necessarily limited, we found no differences in blastocyst numbers or litter size between NZO and C57Bl/6 mice (data not shown). The litter sizes of NZOs ranged between 1–12 litters (5–7 litters on average; R. Kluge, DfE Potsdam, personal communication) and do not differ compared to other mouse strains [37]. Therefore, the potential significance of Rxrg for developmental competence needs further investigation.

The spectrum of the analyzed cholesterol metabolism parameters shows that the target molecules Hmgcr and Ldlr are involved in cholesterol homeostasis in blastocysts. Cholesterol can be synthesized by eight-cell embryos [38]. A lack of cholesterol is the pathogenetic reason for the Smith-Lemli-Opitz syndrome which is accompanied by intrauterine growth retardation [19]. It is likely that the roles of Ldlr and lipoprotein lipase (Lpl) are directed towards intake and utilization of metabolites from uterine secretions by blastocysts.

Fetal cholesterol synthesis cannot be suppressed by exogenous sterol. Accordingly, the ratio of SCAP and SREBP to the INSIGs is constantly increased in fetal tissues [22]. The findings in this study support this view, but only for blastocysts from C57Bl/6 mice. In NZO blastocysts this ratio is balanced and could lead to a reduced cholesterol synthesis with progressing development. If confirmed, this would be a difference in the regulation of cholesterol synthesis in preimplantation embryos from obese mothers.

Even if the quantitative differences in Fatp4 and Fasn expression need further validation, they implicate an altered relation of fatty acid uptake and self-synthesis in blastocysts from obese mothers. The transcript rates of Fatp4 are increased ($p = 0.004$), potentially due to an enhanced supply of fatty acids in the uterine secretions. In diabetic rabbits, the concentration of glucose in uterine fluid clearly differs from that in normoglycemic rabbits [40]. It is likely that the uterine fluid of obese mothers has comparable changes in the composition of fatty acids and adipose tissue hormones. In the ewe, maternal obesity increases the expression of Fatp1 and 4 in placental tissues. Simultaneously, maternal and fetal blood triglyceride levels are enhanced [41], indicating a positive correlation of fatty acid supply and increased FATP expression. At the same time, the Fasn transcript rates are decreased in NZO blastocysts ($p = 0.049$). This could be a compensation for the potentially higher fatty acid uptake via Fatp4 in blastocysts from obese mice.

Conclusion

In blastocysts from obese (NZO) and non-obese (C57Bl/6) mice lipid metabolism enzymes and signaling pathways closely resemble those of adult cells. Rxrg could not be detected in NZO embryos. The transcript rates of Fatp4 and Fasn differ between blastocysts from obese and non-obese mouse strains, which might indicate differences in the fatty acid compositions of the uterine fluid between both strains. The ratio of SREBP-2/Insig1 is enhanced in C57Bl/6 and balanced in NZO blastocysts. This is regarded as first evidence that cholesterol synthesis may be differently regulated in preimplantation embryos of obese and non-obese mice. Subtle changes in the expression of some investigated lipid metabolism parameters of a broad and comprehensive spectrum being analyzed in current study let us suggest that blastocysts are an early target of maternal programming during preimplantation embryo development in obese mice.

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Disclosure Statement

The authors declare no conflict of interest.

References

- 1 World Health Statistics 2009 (WHO): http://www.who.int/whosis/whostat/EN_WHS09_Full.pdf, p 83.
- 2 Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ: Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 2007;30:2287–2292.
- 3 Kwong WY, Miller DJ, Wilkins AP, Dear MS, Wright JN, Osmond C, Zhang J, Fleming TP: Maternal low protein diet restricted to the preimplantation period induces a gender-specific change on hepatic gene expression in rat fetuses. *Mol Reprod Dev* 2007;74:48–56.
- 4 Wakefield SL, Lane M, Schulz SJ, Hebart ML, Thompson JG, Mitchell M: Maternal supply of omega-3 polyunsaturated fatty acids alter mechanisms involved in oocyte and early embryo development in the mouse. *Am J Physiol Endocrinol Metab* 2008;294:E425–434.
- 5 Sullivan EL, Grove KL: Metabolic imprinting in obesity. *Forum Nutr* 2010;63:186–194.
- 6 Bielschowsky M, Bielschowsky F: A new strain of mice with hereditary obesity. *Proc Univ Otago Med School* 1953;31, 29–31.
- 7 Igel M, Becker W, Herberg L, Joost HG: Hyperleptinemia, leptin resistance, and polymorphic leptin receptor in the New Zealand obese mouse. *Endocrinology* 1997;138:4234–4239.
- 8 Ortlepp JR, Kluge R, Giesen K, Plum L, Radke P, Hanrath P, Joost HG: A metabolic syndrome of hypertension, hyperinsulinaemia and hypercholesterolaemia in the New Zealand obese mouse. *Eur J Clin Invest* 2000;30:195–202.
- 9 Giesen K, Plum L, Kluge R, Ortlepp J, Joost HG: Diet-dependent obesity and hypercholesterolemia in the New Zealand obese mouse: identification of a quantitative trait locus for elevated serum cholesterol on the distal mouse chromosome 5. *Biochem Biophys Res Commun* 2003;304:812–817.
- 10 Chankiewitz E, Peschke D, Herberg L, Bazwinsky I, Mühlbauer E, Brömme HJ, Peschke E: Did the gradual loss of GLUT2 cause a shift to diabetic disorders in the New Zealand obese mouse (NZO/HI)? *Exp Clin Endocrinol Diabetes* 2006;114:262–269.
- 11 Pan HJ, Agate DS, King BL, Wu MK, Roderick SL, Leiter EH, Cohen DE: A polymorphism in New Zealand inbred mouse strains that inactivates phosphatidylcholine transfer protein. *FEBS Lett* 2006 ;580:5953–5958.
- 12 Kang HW, Wei J, Cohen DE: PC-TP/StARD2: of membranes and metabolism. *Trends Endocrinol Metab*. 2010; 21:449–456.
- 13 Radavelli-Bagatini S, Blair AR, Proietto J, Spritzer PM, Andrikopoulos S: The New Zealand obese mouse model of obesity, insulin resistance and poor breeding performance: evaluation of ovarian structure and function. *J Endocrinol* 2011;209:307–315.
- 14 Metzger D, Imai T, Jiang M, Takukawa R, Desvergne B, Wahli W, Chambon P: Functional role of RXRs and PPARgamma in mature adipocytes. *Prostaglandins Leukot Essent Fatty Acids* 2005;73:51–58.
- 15 Schaiff WT, Bildirici I, Cheong M, Chern PL, Nelson DM, Sadovsky Y: Peroxisome proliferator-activated receptor-gamma and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts. *J Clin Endocrinol Metab* 2005;90:4267–4275.
- 16 Kouidhi S, Jarbouy S, Marrakchi R, Froidevaux MS, Seugnet I, Abid H, Bchir F, Brahem M, Demeneix B, Guisouma H, Elgaai AB: Adiponectin expression and metabolic markers in obesity and type 2 diabetes. *J Endocrinol Invest* 2011;34:e16–23.

- 17 Krezel W, Dupé V, Mark M, Dierich A, Kastner P, Chambon P: RXR gamma null mice are apparently normal and compound RXR alpha +/- RXR beta +/-RXR gamma -/- mutant mice are viable. *Proc Natl Acad Sci U S A* 1996;93:9010–9014.
- 18 Mark M, Kastner P, Ghyselinck NB, Krezel W, Dupé V, Chambon P: Genetic control of the development by retinoic acid. *C R Seances Soc Biol Fil* 1997;191:77–90.
- 19 Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS: Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 2006; 52:405–13.
- 20 Haluzík M, Anderlová K, Doležalová R, Adamíková A, Haluzíková D, Housová J, Svačina S, Haluzík M: Serum adipocyte fatty acid binding protein levels in patients with type 2 diabetes mellitus and obesity: the influence of fenofibrate treatment. *Physiol Res* 2008;58:93–99.
- 21 Vural B, Atalar F, Ciftci C, Demirkan A, Susleyici-Duman B, Gunay D, Akpınar B, Sagbas E, Ozbek U, Buyukdevrim AS: Presence of fatty-acid-binding protein 4 expression in human epicardial adipose tissue in metabolic syndrome. *Cardiovasc Pathol* 2008;17:392–398.
- 22 Yao L, Jenkins K, Horn PS, Lichtenberg MH, Woollett LA: Inability to fully suppress sterol synthesis rates with exogenous sterol in embryonic and extraembryonic fetal tissues. *Biochim Biophys Acta* 2007;1771:1372–1379.
- 23 Wang Y, Sullivan S, Trujillo M, Lee MJ, Schneider SH, Brolin RE, Kang YH, Werber Y, Greenberg AS, Fried SK: Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. *Obes Res* 2003; 11:930–936.
- 24 Dalen KT, Schoonjans K, Ulven SM, Weedon-Fekjaer MS, Bentzen TG, Koutnikova H, Auwerx J, Nebb HI: Adipose tissue expression of the lipid droplet-associating proteins S3-12 and perilipin is controlled by peroxisome proliferator-activated receptor-gamma. *Diabetes* 2004;53:1243–1252.
- 25 Bell M, Wang H, Chen H, McLenithan JC, Gong DW, Yang RZ, Yu D, Fried SK, Quon MJ, Londos C, Sztalryd C: Consequences of lipid droplet coat protein downregulation in liver cells: abnormal lipid droplet metabolism and induction of insulin resistance. *Diabetes* 2008;57:2037–2045.
- 26 Meadows JW, Pitzer B, Brockman DE, Myatt L: Expression and localization of adipophilin and perilipin in human fetal membranes: association with lipid bodies and enzymes involved in prostaglandin synthesis. *J Clin Endocrinol Metab* 2005;90:2344–2350.
- 27 Tonack S, Fischer B, Navarrete Santos A: Expression of the insulin-responsive glucose transporter isoform 4 in blastocysts of C57/BL6 mice. *Anat Embryol (Berl)* 2004;208:225–230.
- 28 Jungheim ES, Schoeller EL, Marquard KL, Loudon ED, Schaffer JE, Moley KH: Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology*. 2010;151:4039–4046.
- 29 Igosheva N, Abramov AY, Poston L, Eckert JJ, Fleming TP, Duchon MR, McConnell J: Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One* 2010; 5:e10074.
- 30 Gimeno RE, Hirsch DJ, Punreddy S, Sun Y, Ortegón AM, Wu H, Daniels T, Stricker-Krongrad A, Lodish HF, Stahl A: Targeted deletion of fatty acid transport protein-4 results in early embryonic lethality. *J Biol Chem* 2003;278:49512–49516.
- 31 Haggarty P, Wood M, Ferguson E, Hoad G, Srikantharajah A, Milne E, Hamilton M, Bhattacharya S: Fatty acid metabolism in human preimplantation embryos. *Hum Reprod* 2006;21:766–773.
- 32 Schroeder F, Atshaves BP, Starodub O, Boedeker AL, Smith RR 3rd, Roths JB, Foxworth WB, Kier AB: Expression of liver fatty acid binding protein alters growth and differentiation of embryonic stem cells. *Mol Cell Biochem* 2001;219:127–138.
- 33 Dunning KR, Cashman K, Russell DL, Thompson JG, Norman RJ, Robker RL: Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. *Biol Reprod* 2010;83:909–918.
- 34 Chirala SS, Chang H, Matzuk M, Abu-Elheiga L, Mao J, Mahon K, Finegold M, Wakil SJ: Fatty acid synthesis is essential in embryonic development: fatty acid synthase null mutants and most of the heterozygotes die in utero. *Proc Natl Acad Sci U S A* 2003;100:6358–6363.
- 35 Mohan M, Malayer JR, Geisert RD, Morgan GL: Expression patterns of retinoid X receptors, retinaldehyde dehydrogenase, and peroxisome proliferator activated receptor gamma in bovine preattachment embryos. *Biol Reprod* 2002;66:692–700.
- 36 Mamo S, Ponsuksili S, Wimmers K, Gilles M, Schellander K: Expression of retinoid X receptor transcripts and their significance for developmental competence in in vitro-produced pre-implantation-stage bovine embryos. *Reprod Domest Anim* 2005;40:177–183.
- 37 Bielschowsky M, Bielschowsky F, Lindsay D: A new strain of mice with a high incidence of mammary cancers and enlargement of the pituitary. *Br J Cancer* 1956;10:688–699.
- 38 Pratt HP, Keith J, Chakraborty J: Membrane sterols and the development of the preimplantation mouse embryo. *J Embryol Exp Morphol* 1980;60:303–319.
- 39 Ramin N, Thieme R, Fischer S, Schindler M, Schmidt T, Fischer B, Navarrete Santos A: Maternal diabetes impairs gastrulation and insulin and IGF-I receptor expression in rabbit blastocysts. *Endocrinology* 2010; 151:4158–4167.
- 40 Zhu MJ, Ma Y, Long NM, Du M, Ford SP: Maternal obesity markedly increases placental fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe. *Am J Physiol Regul Integr Comp Physiol* 2010;299:R1224–1231.