Downregulation of peroxisome proliferator-activated receptor α and its coactivators in liver and skeletal muscle mediates the metabolic adaptations during lactation in mice

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Abstract

Previous studies have shown that genes involved in fatty acid uptake, fatty acid oxidation, and thermogenesis are downregulated in liver and skeletal muscle of rats during lactation. However, biochemical mechanisms underlying these important metabolic adaptations during lactation have not yet been elucidated. As all these genes are transcriptionally regulated by peroxisome proliferator-activated receptor α (*Ppar α*), we hypothesized that their downregulation is mediated by a suppression of *Ppar α* during lactation. In order to investigate this hypothesis, we performed an experiment with lactating and nonlactating *Ppar α* knockout and corresponding wild-type mice. In wild-type mice, lactation led to a considerable downregulation of *Ppar α* , *Ppar* coactivators *Pgc1 α* and *Pgc1 β* , and *Ppar α* target genes involved in fatty acid uptake, fatty acid oxidation, and thermogenesis in liver and skeletal muscle ($P < 0.05$). *Ppar α* knockout mice had generally a lower expression of all these *Ppar α* target genes in liver and skeletal muscle. However, in those mice, lactation did not lower the expression of genes involved in fatty acid utilization and thermogenesis in liver and skeletal muscle. Expression levels of *Ppar α* target genes in lactating wild-type mice were similar than in lactating or nonlactating *Ppar α* knockout mice. In conclusion, the present findings suggest that downregulation of *Ppar α* and its coactivators in tissues with high rates of fatty acid catabolism is responsible for the reduced utilization of fatty acids in liver and skeletal muscle and the reduced thermogenesis occurring in the lactating animal, which aim to conserve energy and metabolic substrates for milk production in the mammary gland.

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Introduction

Lactation is a physiological state characterized by a dramatic increase in the energy and nutrient requirement of the organism for milk production. This demand is usually met by a markedly increased food intake and by the utilization of energy stores. In addition, several metabolic adaptations develop in the lactating animal aiming to conserve energy and metabolic substrates for milk production in the mammary gland (Trayhurn *et al.* 1982, Williamson 1986, Dewey 1997, Smith & Grove 2002). Recently, it has been shown that downregulation of uncoupling proteins (*Ucp*) 1 and 3 in brown adipose tissue and of *Ucp3* in skeletal muscle, leading to a decrease in metabolic fuel oxidation and thermogenesis, contributes to these metabolic adaptations during lactation (Trayhurn *et al.* 1982, Pedraza *et al.* 2000, 2001, Xiao *et al.* 2004a). Furthermore, expression of proteins involved in uptake and oxidation of fatty acids in skeletal muscle (Xiao *et al.* 2004b) as well as the rates of

fatty acid oxidation and ketogenesis in the liver (Whitelaw & Williamson 1977) are reduced during lactation, effects that help to spare fatty acids for milk production in the mammary gland. Both *Ucp* and proteins involved in fatty acid uptake and oxidation are transcriptionally regulated by peroxisome proliferator-activated receptor α (*Ppar α* ; Bruns *et al.* 1999, Barbera *et al.* 2000, Young *et al.* 2001, Mandard *et al.* 2004). *Ppar α* is a ligand-activated transcription factor, which is abundantly expressed in tissues with high rates of fatty acid oxidation, such as liver and skeletal muscle, and its physiologic role lies in the mediation of metabolic responses to fasting (Schoonjans *et al.* 1997, Leone *et al.* 1999, Mandard *et al.* 2004). Upon activation by either nonesterified fatty acids (NEFA) released from adipose tissue and taken up into tissues or exogenous ligands (diet-derived fatty acids or fibrates), *Ppar α* upregulates genes involved in all aspects of fatty acid catabolism including cellular fatty acid uptake and transport, mitochondrial and peroxisomal fatty acid oxidation, as well as ketogenesis (Mandard *et al.* 2004). We have

recently observed that lactating rats have reduced mRNA concentrations of *Pparα* and *Pparα*-regulated genes involved in fatty acid utilization in the liver compared with nonlactating rats (Gutgesell *et al.* 2009). This finding suggested that downregulation of genes involved in fatty acid uptake and oxidation as well as *Ucp* during lactation is mediated by suppression of *Pparα*.

The aim of the present study was to test the hypothesis that downregulation of *Pparα* mediates the reduced expression of genes involved in fatty acid uptake and β-oxidation in tissues with high rates of fatty acid utilization such as liver and skeletal muscle, which favors the availability of fatty acids for milk triacylglycerol (TAG) production in the mammary gland. For that purpose, we performed an experiment with *Pparα* knockout and corresponding wild-type mice, and studied the influence of lactation on the expression of *Pparα* and selected *Pparα*-responsive genes involved in fatty acid uptake (*Fabp_{pm}* and *Fatp*), fatty acid oxidation (*Cpt1*, *Cyp4a10* and *Mcad*), and thermogenesis (*Ucp3*) in liver and skeletal muscle respectively. It has been shown that the transcriptional activity of *Ppar* is enhanced by several coactivators, including *Pparγ* coactivator (*Pgc1α* and *1β*). These coactivators are required for the ability of *Ppar* to increase gene transcription to the maximum (Yu & Reddy 2007). In order to study whether lactation influences the expression of these coactivators, we also determined the expression of *Pgc1α* and *Pgc1β* in liver and skeletal muscle.

During lactation, an increased flow of NEFA, originating from hydrolysis of TAG by hormone-sensitive lipase in adipose tissue into the mammary gland for milk production, is observed, whereas uptake of fatty acids by lipoprotein lipase (*Lpl*) from TAG-rich lipoproteins such as chylomicrons and very low-density

lipoproteins into adipose tissue is reduced during lactation (Williamson 1986). The uptake of both fatty acids released by *Lpl* from TAG-rich lipoproteins and albumin-bound NEFA in the plasma released from adipose tissue into the mammary gland is mediated by fatty acid transporters, and is an important source for milk TAG synthesis. To obtain information about alterations in the uptake of fatty acids into the mammary gland during lactation, we also determined expression of fatty acid transporters and *Lpl* in the mammary gland as well as TAG concentrations in plasma.

Materials and methods

Animal experiment

The animal experiment was carried out with female *Pparα* knockout mice (129S4/SvJae-*Pparα*^{tm1Gonz/J}) and corresponding wild-type mice (129S1/SvImJ) obtained from Jackson Laboratory (Bar Harbor, ME, USA). They were kept in Macrolon cages in a room maintained with controlled temperature (23 ± 1 °C), humidity (50–60%), and lighting (0600 to 1800 h). All animals were fed a commercial standard diet for rodents (Altromin GmbH, Lage, Germany). The standard diet was nutritionally adequate for lactating mice according to the recommendations of American Society for Nutritional Sciences (ASNS) (Reeves *et al.* 1993), and contained 11.9 MJ/kg metabolizable energy. The composition of the standard diet was (g/kg diet): crude protein (19.0), crude fat (4.0), crude fiber (6.0), crude ash (7.0), and nitrogen-free extracts (53.0) (Altromin). Water was available *ad libitum* from nipple drinkers. All experimental procedures described followed established guidelines for the care and

Table 1 Characteristics of the primers used for RT-PCR analysis with real-time detection

Gene	Forward primer (from 5' to 3')	Reverse primer (5'–3')	PCR product size (bp)	NCBI GenBank
<i>β-actin</i>	ACGGCCAGGTCATCACTATTG	CACAGGATTCATACCCAAGAAG	87	NM_007393
<i>Cyp18</i>	GTGGTCTTTGGGAAGGTGAA	TTACAGGACATTGCGAGCAG	210	NM_008907
<i>Cyp4a10</i>	TGAGGGAGAGCTGGAAAAGA	CTGTTGGTGATCAGGGTGTG	266	NM_010011
<i>Fabp_{pm}</i>	CCAGAAAGGGAAGGACATCA	GTCTCCAGTTCGCACTCCTC	132	NM_017399
<i>Fas</i>	TGGGTTCTAGCCAGCAGAGT	ACCACCAGAGACCGTTCATGC	158	NM_007988
<i>Fat/Cd36</i>	GAGCAACTGGTGATGGTTT	GCAGAATCAAGGGAGAGCAC	207	NM_007643
<i>Fatp</i>	TGCTTTGGTTTCTGGGACTT	GCTCTAGCCGAACACGAATC	156	NM_11977
<i>Gapdh</i>	AACGACCCCTTCATTGAC	TCCACGACATACTCAGCAC	191	XM_001476707
<i>l-Cpt 1</i>	CCAGGCTACAGTGGGACATT	GAACTTGCCCATGTCTCTGT	209	NM_013495
<i>Lpl</i>	GGGCTCTGCCTGAGTTGTAG	AGAAATTTGGAAGGCCTGGT	157	NM_008509
<i>Mcad</i>	AGGTTTCAAGATCGCAATGG	CTCCTTGGTGCTCCACTAGC	152	NM_007382
<i>m-Cpt 1</i>	GTCGTTCTTCAAGGTCTGG	AAGAAAGCAGCAGTTCGAT	232	NM_009948
<i>Pgc1α</i>	AAACTTGCTAGCGGTCCTCA	TGTTGACAAATGCTCTTC	342	NM_008904
<i>Pgc1β</i>	AACCCAAACAGTCTCACAGG	TGCTGCTGTCCTCAAATACG	371	NM_133249
<i>Pparα</i>	CGGGAAAGACCAGCAACAAC	TGGCAGTGGAAGAATCG	137	NM_011144
<i>Ucp3</i>	CCACACTT CCTCTGCTCTC	GTATAGGGCGCTCAAATGGA	235	NM_009464

handling of laboratory animals (UFAW 1999, Society for Endocrinology's guidelines on the use of animals, URL: http://www.endocrinology-journals.org/misc/use_of_animals.dtl) and were approved by the council of Saxony-Anhalt.

At 14 weeks of age, the mice were mated by housing two female mice with one male mouse (129S1/SvImJ) for 6 days. At the day of parturition, designated as day 1 of lactation, wild-type mice ($n=10$) and *Ppar α* knockout mice ($n=14$) were randomly assigned to two groups. In one group of the wild-type and *Ppar α* knockout mice, all pups were removed (without litter), whereas in the other group litters were adjusted to six pups per dam (with litter). During pregnancy and lactation, female mice were kept individually in single cages, and diets fed *ad libitum*. Feed consumption was measured every day by determining the weight of the remaining diet at the day after feed administration. At day 15 of lactation, dams received the last dose of the diet at the beginning of the light cycle (0600 h) and were killed 4 h later at 1000 h by decapitation under light anesthesia with diethyl ether. Animals were killed in the nonfasted state to prevent fasting-induced *Ppar α* activation.

Sample collection

Blood was collected into heparinized polyethylene tubes (Sarstedt, Nürnberg, Germany). Plasma was obtained by centrifugation of the blood (1100 g; 10 min; 4 °C) and stored at -20 °C. Liver, mammary gland, and skeletal muscle were excised, immediately shock frozen with liquid nitrogen, and stored at -80 °C pending analysis.

Lipid analysis

Plasma TAG concentrations were measured using reagent kits obtained from Merck Eurolab (Ref. 113009990314) according to the manufacturer's protocol.

RNA isolation and RT-PCR with real-time detection

Total RNA was isolated from the liver, mammary gland, and skeletal muscle (*M. semitendinosus*) using Trizol reagent (Invitrogen) according to the manufacturer's protocol. RNA concentration and purity were estimated from the optical density at 260 and 280 nm (SpectraFluor Plus, Tecan, Crailsheim, Germany). Synthesis of cDNA and determination of mRNA abundance by RT-PCR with real-time detection (Rotor-gene 2000, Corbett Research, Mortlake, New South Wales, Australia) using Sybr Green I were performed as recently described in detail (Ringseis *et al.* 2007c).

Figure 1 Relative mRNA concentrations of *Ppar α* in the liver (A) and skeletal muscle (B) of wild-type mice, whose litters were either removed (without litter) or adjusted to 6 pups/dam (with litter) immediately after birth, at day 15 of lactation. Bars represent means $\pm$ S.E.M. for $n=5$. *Indicates a significant difference from 'without' litter group. $P < 0.05$.

Relative quantification of mRNA concentrations was performed using the $\Delta\Delta C_t$ method (Pfaffl 2001). Threshold cycle (C_t) values were obtained using Rotorgene Software 5.0 (Corbett Research). The housekeeping genes *Gapdh* (liver), β -actin (skeletal muscle), and *Cyp18* (mammary gland) were used for normalization. Different housekeeping genes were used in the various tissues, because none of the housekeeping genes tested served as an appropriate reference gene in all tissues. The existence of a single PCR product of the expected length was guaranteed by melting curve analysis and 1% agarose gel electrophoresis. Relative mRNA concentrations are expressed as fold of mRNA concentration of the wild-type without litter group. Characteristics of the primers (Eurofins MWG Operon, Ebersberg, Germany) used for RT-PCR with real-time detection are shown in Table 1.

Statistical analysis

Data were analyzed using the Minitab Statistical Software (Minitab Rel. 15, State College, PA, USA). Treatment effects were analyzed by two-way ANOVA.

Figure 2 Relative mRNA concentrations of the *Ppar* α target genes *I-Cpt I*, *Cyp4a10*, *Fabp_{pm}*, and *Fatp* in the liver of lactating wild-type and lactating *Ppar* α knockout mice, whose litters were either removed (without litter) or adjusted to 6 pups/dam (with litter) immediately after birth, at day 15 of lactation. Bars represent means $\pm$ S.E.M. for $n=5$ in wild-type mice and $n=7$ in *Ppar* α knockout mice. Bars without a common letter (a–c) differ, $P<0.05$. Significant effects ($P<0.05$) from two-way ANOVA: *I-Cpt I*: litter, genotype, and litter $\times$ genotype; *Cyp4a10*: litter, genotype, and litter $\times$ genotype; *Fabp_{pm}*: litter, genotype, and litter $\times$ genotype; *Fatp*: litter, genotype, and litter $\times$ genotype.

with classification factors being litter, genotype, and the interaction of litter and genotype. For significant *F*-values, means were compared by Fisher's multiple range test. Means were considered significantly different for $P<0.05$. Data presented in the text are shown as means $\pm$ S.E.M.

Results

Food intake, body weights of dams, and weights of litters

Dams with litters consumed more food per day than those without litters, irrespective of genotype (wild-type mice without litter: 2.0 ± 0.2 g/d; wild-type mice with litter: 7.7 ± 0.7 g/d; *Ppar* α knockout mice without litter: 2.1 ± 0.2 g/d; *Ppar* α knockout mice with litter: 7.9 ± 1.0 g/d; $P<0.05$). Body weights of dams at day 1 of lactation did not differ between groups (wild-type mice without litter: 22.2 ± 0.6 g; wild-type mice with litter: 22.1 ± 0.9 g; *Ppar* α knockout mice without litter: 22.7 ± 1.3 g; *Ppar* α knockout mice with litter: 23.7 ± 2.2 g). However, body weights at day 15 of

lactation were higher in dams with litters than in those without litters, irrespective of the genotype (wild-type mice without litter: 20.8 ± 0.6 g; wild-type mice with litter: 26.0 ± 0.7 g; *Ppar* α knockout mice without litter: 20.1 ± 0.9 g; *Ppar* α knockout mice with litter: 26.1 ± 2.1 g; $P<0.05$). Litter weights of dams of both genotypes did not differ either at day 1 or at day 15 of lactation (day 1 of lactation: wild-type mice: 11.8 ± 1.0 g; *Ppar* α knockout mice: 11.2 ± 1.1 g; day 15 of lactation: wild-type mice: 43.5 ± 2.1 g; *Ppar* α knockout mice: 44.8 ± 2.2 g).

TAG concentrations in plasma

TAG concentrations in plasma were influenced by the factors litter and genotype ($P<0.05$); mice with litters had lower concentrations of TAG in plasma than those without litters, irrespective of the genotype; *Ppar* α knockout mice had higher TAG concentrations in plasma than wild-type mice (wild-type mice without litter: 0.61 ± 0.10 mmol/l; wild-type mice with litter: 0.39 ± 0.02 mmol/l; *Ppar* α knockout mice without litter: 0.90 ± 0.10 mmol/l; *Ppar* α knockout mice with litter: 0.69 ± 0.09 mmol/l).

Figure 3 Relative mRNA concentrations of the *Ppar α* target genes *m-Cpt I*, *Mcad*, *Fabp_{pm}*, *Fatp*, and *Ucp3* in the skeletal muscle of lactating wild-type and lactating *Ppar α* knockout mice, whose litters were either removed (without litter) or adjusted to 6 pups/dam (with litter) immediately after birth, at day 15 of lactation. Bars represent means $\pm$ S.E.M. for $n=5$ in wild-type mice and $n=7$ in *Ppar α* knockout mice. Bars without a common letter (a–c) differ, $P<0.05$. Significant effects ($P<0.05$) from two-way ANOVA: *m-Cpt I*: litter and litter $\times$ genotype; *Mcad*: litter and litter $\times$ genotype; *Fatp*: genotype and litter $\times$ genotype; *Ucp3*: litter and litter $\times$ genotype.

Expression of *Ppar α* in liver and skeletal muscle

Wild-type mice with litters had markedly lower relative mRNA concentrations of *Ppar α* in liver and skeletal muscle than wild-type mice without litters ($P<0.05$; Fig. 1).

Expression of *Ppar α* target genes in the liver

Wild-type mice with litters had markedly lower relative mRNA concentrations of *l-Cpt I*, *Cyp4A10*, *Fatp*, and *Fabp_{pm}* in the liver than wild-type mice without litters ($P<0.05$; Fig. 2). Relative mRNA concentrations of all these genes in the liver were lower in *Ppar α* knockout mice with and without litters than in wild-type mice with litters ($P<0.05$; Fig. 2); however, relative mRNA concentrations of these genes in the liver did not differ between *Ppar α* knockout mice with litters and those without litters (Fig. 2).

Expression of *Ppar α* target genes in skeletal muscle

In skeletal muscle, relative mRNA concentrations of *m-Cpt I*, *Mcad*, *Fatp*, and *Ucp3* were lower in wild-type mice with litters compared with wild-type mice whose litters were removed ($P<0.05$; Fig. 3). In contrast, relative mRNA concentrations of these genes did not differ between *Ppar α* knockout mice with litters and *Ppar α* knockout mice without litters (Fig. 3). The relative mRNA concentrations of *m-Cpt I*, *Mcad*, *Fatp*,

and *Ucp3* in skeletal muscle were lower in *Ppar α* knockout mice with and without litters than in wild-type mice without litters ($P<0.05$; Fig. 3). Unexpectedly, relative mRNA concentrations of *m-Cpt I* and *Mcad* in skeletal muscle in wild-type mice with litters were even lower than in *Ppar α* knockout mice with and without litters ($P<0.05$; Fig. 3). The relative mRNA concentration of *Fabp_{pm}* in skeletal muscle did not differ between the four groups of mice (Fig. 3).

Expression of *Ppar* coactivators *Pgc1 α* and *Pgc1 β* in liver and skeletal muscle

In liver and skeletal muscle, relative mRNA concentrations of *Pgc1 α* and *Pgc1 β* were lower in wild-type mice with litters compared with wild-type mice whose litters were removed ($P<0.05$; Fig. 4). In contrast, relative mRNA concentrations of *Pgc1 α* and *Pgc1 β* in liver and skeletal muscle did not differ between *Ppar α* knockout mice with litters and *Ppar α* knockout mice without litters (Fig. 4). However, whereas relative mRNA concentrations of *Pgc1 α* and *Pgc1 β* in liver were lower in *Ppar α* knockout mice with and without litters than in wild-type mice without litters ($P<0.05$; Fig. 4), relative mRNA concentrations of these genes in skeletal muscle did not differ between wild-type mice without litters and *Ppar α* knockout mice with and without litters (Fig. 4).

Figure 4 Relative mRNA concentrations of *Pgc1α* and *Pgc1β* in liver (A) and skeletal muscle (B) of lactating wild-type and lactating *Pparα* knockout mice, whose litters were either removed (without litter) or adjusted to 6 pups/dam (with litter) immediately after birth, at day 15 of lactation. Bars represent means $\pm$ S.E.M. for $n=5$ in wild-type mice and $n=7$ in *Pparα* knockout mice. Bars without a common letter (a–b) differ, $P<0.05$. Significant effects ($P<0.05$) from two-way ANOVA: *Pgc1α* liver: litter and litter $\times$ genotype; *Pgc1β* liver: litter, genotype and litter $\times$ genotype; *Pgc1α* skeletal muscle: litter and litter $\times$ genotype; *Pgc1β* skeletal muscle: litter and litter $\times$ genotype.

Expression of fatty acid transporters, *Lpl*, and fatty acid synthase in the mammary gland of mice

Wild-type mice with litters had markedly higher mRNA concentrations of *Fat/Cd36*, *Fatp*, *Lpl*, and fatty acid synthase (*Fas*) in the mammary gland than wild-type mice without litters ($P<0.05$; Fig. 5). In *Pparα* knockout mice, the mRNA concentrations of these genes were also higher in the mammary gland of dams with litters than in those without litters ($P<0.05$; Fig. 5).

Discussion

The aim of the present study was to test the hypothesis that downregulation of *Pparα* in tissues with high rates of fatty acid catabolism (liver and skeletal muscle) is responsible for the metabolic adaptations occurring in the lactating animal, such as decreased fatty acid oxidation and diminished thermogenesis (Zammit 1980, Pedraza *et al.* 2000, 2001, Xiao *et al.* 2004a,b). Herein, we could clearly demonstrate for the first time that downregulation of *Pparα* and *Pparα* target genes involved in fatty acid uptake (*Fabp_{pm}* and *Fatp*), fatty acid oxidation (*Cpt 1*, *Cyp4a10*, and *Mcad*), and thermogenesis (*Ucp3*) occurs only in tissues of lactating wild-type mice. In *Pparα* knockout mice, in contrast, lactation did not result in a reduced expression of *Pparα* and *Pparα* target genes in these tissues. These findings strongly indicate that the metabolic adaptations in the lactating animal (Zammit 1980, Pedraza *et al.* 2000, 2001, Xiao *et al.* 2004a,b) are mediated by the diminished expression of *Pparα* in liver and skeletal muscle. As expected, expression levels of *Pparα* target genes were markedly lower in liver and skeletal muscle of *Pparα* knockout mice than in those of nonlactating wild-type mice. In this regard, it is, however, noteworthy that *Pparα* target genes, with the exception of *m-Cpt 1* and *Mcad* in skeletal muscle, were expressed in tissues of *Pparα* knockout mice at levels comparable with those of lactating wild-type mice. This indicates that lactation in wild-type mice causes a similar reduction in the expression of *Pparα* target genes as disruption of *Pparα* expression.

Although *Pparα* is clearly an important transcription factor in regulating the expression of genes involved in fatty acid oxidation, the observation that the mRNA levels of *m-Cpt 1* and *Mcad* in skeletal muscle were even stronger reduced by lactation than by the *Pparα* knockout implies that also other *Pparα*-independent mechanisms might be involved. As lactating wild-type mice had lower mRNA concentrations of *Pgc1α* and *Pgc1β*, which are required for maximal transcriptional activity of *Ppar* (Yu & Reddy 2007, Feingold *et al.* 2008), in liver and skeletal muscle than their nonlactating counterparts, we suggest that a reduced expression of

Figure 5 Relative mRNA concentrations of *Fat/Cd36*, *Fabp_{pm}*, *Fatp*, *Lpl*, and *Fas* in the mammary gland of lactating wild-type and lactating *Ppar α* knockout mice, whose litters were either removed (without litter) or adjusted to 6 pups/dam (with litter) immediately after birth, at day 15 of lactation. Bars represent means $\pm$ S.E.M. for $n=5$ in wild-type mice and $n=7$ in *Ppar α* knockout mice. Bars without a common letter (a–b) differ, $P<0.05$. Significant effects ($P<0.05$) from two-way ANOVA: *Fat/Cd36*: litter; *Fabp_{pm}*: litter and litter $\times$ genotype; *Fatp*: litter; *Lpl*: litter; *Fas*: litter.

Ppar coactivators could have also contributed to the decreased expression of *Ppar α* -regulated genes in skeletal muscle and liver during lactation. In liver, the expression of both coactivators was lower in *Ppar α* knockout than in wild-type mice, indicating that *Pgc1 α* and *Pgc1 β* are regulated by *Ppar α* in that tissue. However, in skeletal muscle, the expression of both coactivators did not differ between wild-type mice without litters and *Ppar α* knockout mice with and without litters, suggesting that *Pgc1 α* and *Pgc1 β* in skeletal muscle are regulated by *Ppar α* independently. The finding that *Ppar* coactivators in skeletal muscle were not reduced in *Ppar α* knockout mice compared with wild-type mice could provide an explanation for

the observation that some *Ppar α* -regulated genes, such as *m-Cpt I* and *Mcad*, in skeletal muscle were only slightly reduced in knockout versus wild-type mice. Reduced mRNA concentrations of *Ppar* coactivators in skeletal muscle of lactating wild-type mice compared with lactating *Ppar α* knockout mice could be responsible for the finding that the expression of some *Ppar α* target genes such as *m-Cpt I* and *Mcad* in skeletal muscle was even lower in lactating wild-type than in knockout mice.

Besides downregulation of fatty acid oxidation enzymes and fatty acid transporters in skeletal muscle, we observed downregulation of *Ucp3* in skeletal muscle of lactating wild-type mice, which is consistent with recent observations in skeletal muscle and brown

Figure 6 Working hypothesis: downregulation of *Ppar* α and its coactivators leads to a reduced uptake of fatty acids into tissues with high rates of β -oxidation, such as liver, skeletal muscle, and heart, and to a reduced rate of β -oxidation in these tissues. downregulation of *Ucps* in brown adipose tissue (*Ucp1* and *Ucp3*) and skeletal muscle (*Ucp3*) additionally diminishes thermogenesis and oxidation of fuels, which are spared for milk synthesis. As recently shown, hepatic enzymes of carnitine synthesis are concomitantly also reduced during lactation, which, in turn, leads to a reduced carnitine content in liver and skeletal muscle (Gutgesell *et al.* 2009). As carnitine is involved in β -oxidation by transferring fatty acids into the mitochondrion, inhibition of carnitine synthesis can be regarded also as a means to diminish fatty acid oxidation during lactation. On the other side, synthesis of fatty acids and TAG in the liver is enhanced during lactation, leading to an increased secretion of VLDL-TAG and an increased uptake of fatty acids from VLDL-TAG into the mammary gland due to the enhanced expression of *Lpl* (Farid *et al.* 1978, Grigor *et al.* 1982). Simultaneously, the uptake of NEFA derived mainly from lipolysis in white adipose tissue by fatty acid transporters into the mammary gland as well as *de novo* fatty acid synthesis catalyzed by lipogenic enzymes including *Fas* in the mammary gland is increased, which also enhances the pool of fatty acids in the mammary gland available for milk TAG production. *CD36/Fat*, CD36/fatty acid translocase; FA, fatty acid; *Fabp_{pm}*, plasma membrane-fatty acid-binding protein; *Fas*, fatty acid synthase; *Fatp*, fatty acid transport protein; *Hsl*, hormone-sensitive lipase; *Lpl*, lipoprotein lipase; NEFA, nonesterified fatty acids; *Pgc1* α/β , PPAR γ co-activator 1 α/β ; *Ppar* α , peroxisome proliferator-activated receptor α ; TAG, triacylglycerols; *Ucp1/3*, uncoupling protein 1/3; VLDL, very low-density lipoprotein.

adipose tissue of lactating rats (Pedraza *et al.* 2000, 2001, Xiao *et al.* 2004a,b). Since *Ucp* function to uncouple the respiratory chain and, thereby, increase heat production, downregulation of *Ucp* in brown adipose tissue of rats during lactation (Pedraza *et al.* 2001, Xiao *et al.* 2004a,b) has been suggested to reflect the need to conserve energy during lactation through a decrease in nonshivering thermogenesis (Williamson 1986, Pedraza *et al.* 2000, 2001, Xiao *et al.* 2004a,b). The finding that *Ucp3* expression was reduced in skeletal muscle of lactating wild-type mice, but not in lactating *Ppar* α knockout mice, again might indicate that the reduction of thermogenesis in skeletal muscle during lactation (Zammit 1980, Pedraza *et al.* 2000,

2001, Xiao *et al.* 2004a,b) is also mediated by the diminished expression of *Ppar* α . Nevertheless, although *Ucp3* has been proposed to play an important role in regulating energy expenditure and thermoregulation (Boss *et al.* 1997, Vidal-Puig *et al.* 1997), the exact physiological role of *Ucp3* is still elusive; i.e. observations from recent studies indicate that *Ucp3* function in skeletal muscle and heart is likely to be related to fatty acid catabolism (Muoio *et al.* 2002, Murray *et al.* 2005). In line with this assumption is the observation that *Ucp3* participates in mitochondrial antioxidant defense and serves as an 'early response' to elevated reactive oxygen species (ROS) production and potential oxidative stress by increasing uncoupling

respiration during prolonged exercise in rat skeletal muscle (Jiang *et al.* 2009). Therefore, future studies have to elucidate the exact physiological role of *Ucp3* downregulation during lactation.

Based on the findings of this study and other studies in the literature (Trayhurn *et al.* 1982, Pedraza *et al.* 2000, 2001, Xiao *et al.* 2004a,b), we propose the model shown in Fig. 6. It suggests that downregulation of *Ppar α* and its coactivators in tissues with high rates of fatty acid utilization, such as liver, skeletal muscle, and heart, and subsequently reduced utilization of fatty acids by these tissues during lactation mediates an increased flow of NEFA from white adipose tissues and TAG-rich lipoproteins into the mammary gland, and thus helps to spare energy and metabolic substrates for milk production. The physiologically increased availability of fatty acids in the mammary gland during lactation (Williamson 1986, Dewey 1997, Smith & Grove 2002) is reflected by the marked upregulation of fatty acid transporters (*Fat/Cd36* and *Fatp*) and *Lpl*, which mediate the uptake of albumin-bound NEFA and fatty acids released from TAG-rich lipoproteins respectively in the mammary gland and the reduced concentrations of NEFA (Pedraza *et al.* 2000) and TAG (Gutgesell *et al.* 2009) in plasma of lactating animals in both genotypes. In addition, expression of the lipogenic enzyme *Fas*, which is critical for *de novo* fatty acid synthesis, was strongly increased in the mammary gland of lactating mice of both genotypes. This indicates that *Ppar α* does not play an essential role for the uptake of fatty acids into the mammary gland, and that *de novo* fatty acid synthesis in the mammary gland is similar in both genotypes. This indication was not unexpected as *Ppar α* mRNA was only barely detectable in the mammary gland in the present study (data not shown) – a finding that largely confirms those of others (Gimble *et al.* 1998, Rodriguez-Cruz *et al.* 2006).

Our proposed model is supported by the finding that activation of *Ppar α* during lactation disturbs the normal metabolic adaptations during lactation. We have recently observed that activation of hepatic *Ppar α* in the lactating rat by feeding a dietary oxidized fat, a potent activator of hepatic *Ppar α* (Chao *et al.* 2001, Sülzle *et al.* 2004, Ringseis *et al.* 2007b), leads to an increased uptake of fatty acids into the liver and an enhanced β -oxidation in the liver, whereas uptake of fatty acids into the mammary gland by fatty acid transporters and *Lpl* was decreased, which, in turn, led to a dramatically reduced milk TAG content and reduced weight gains of litters during suckling (Ringseis *et al.* 2007a). Similar observations regarding an impairment of lactation-induced energy-sparing mechanisms by the administration of *Ppar α* activators during lactation have been reported from others (Pedraza *et al.* 2000).

In conclusion, the present study shows for the first time that downregulation of *Ppar α* , *Ppar* coactivators, and *Ppar α* -regulated genes, which are involved in fatty acid uptake, fatty acid oxidation, and thermogenesis, occurs only in tissues of lactating wild-type mice but not *Ppar α* knockout mice. We postulate that downregulation of *Ppar α* and its coactivators in tissues with high rates of fatty acid catabolism is responsible for the reduced utilization of fatty acids in liver and skeletal muscle and the reduced thermogenesis occurring in the lactating animal, which aim to conserve energy and metabolic substrates for milk production in the mammary gland. The mechanism through which *Ppar α* is downregulated during lactation is currently unknown, but it may be speculated that hormonal changes associated with lactation, such as hyperprolactinemia or hypoleptinemia (Xiao *et al.* 2004a), and also changes in the levels of GH or insulin, might be causative. Therefore, further studies are necessary to identify the mechanisms behind the changes in *Ppar α* expression during lactation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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