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Peroxisome proliferator–activated receptor α and enzymes of carnitine biosynthesis in the liver are down-regulated during lactation in rats

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Abstract

This study investigated the hypothesis that lactation lowers gene expression of peroxisome proliferator–activated receptor (PPAR) α in the liver and that this leads to a down-regulation of hepatic enzymes involved in carnitine synthesis and novel organic cation transporters (OCTNs). Thirty-two pregnant female rats were divided into 4 groups. In the first group, all pups were removed, whereas in the other groups, litters were adjusted to sizes of 4, 10, or 18 pups per dam. Dams suckling their litters, irrespective of litter size, had lower relative messenger RNA concentrations of PPARα, various classic PPARα target genes involved in fatty acid catabolism, as well as enzymes involved in carnitine synthesis (trimethyllysine dioxygenase, 4-*N*-trimethylaminobutyraldehyde dehydrogenase, γ-butyrobetaine dioxygenase) and OCTN1 in the liver than dams whose litters were removed ($P < .05$). Moreover, dams suckling their litters had a reduced activity of γ-butyrobetaine dioxygenase in the liver and reduced concentrations of carnitine in plasma, liver, and muscle compared with dams without litters ($P < .05$). In conclusion, the present study demonstrates for the first time that lactation leads to a down-regulation of PPARα and genes involved in hepatic carnitine synthesis and uptake of carnitine (OCTN1) in the liver, irrespective of litter size. It is moreover suggested that down-regulation of PPARα in the liver may be a means to conserve energy and metabolic substrates for milk production in the mammary gland.

1. Introduction

Carnitine (L-3-hydroxy-4-*N*-*N*-*N*-trimethylaminobutyrate) is an essential metabolite, which has a number of indispensable functions in intermediary metabolism. The most prominent function lies in its role in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix where β-oxidation takes place [1-3]. All tissues that use fatty acids as a fuel source require carnitine for normal function. Carnitine is derived from dietary sources and endogenous biosynthesis [4]. Carnitine biosynthesis involves a complex series of reactions involving several tissues [5]. Lysine provides the carbon backbone of carnitine. Lysine in protein peptide linkages undergoes methylation of the ε-amino group to yield trimethyllysine (TML), which is released upon protein degradation. The released TML is further oxidized to γ-butyrobetaine (BB) by the action of TML dioxygenase (TMLD), 3-hydroxy-*N*-TML aldolase, and 4-*N*-trimethylaminobutyraldehyde dehydrogenase (TMABA-DH). BB is hydroxylated by BB dioxygenase (BBD) to form carnitine. The last reaction occurs in rats exclusively in liver and testes [6]. Distribution of carnitine within the body and intracellular homeostasis of carnitine are controlled by novel organic cation transporters (OCTNs) that belong to the solute carrier 22A family. The OCTNs are important for the absorption of carnitine in the small intestine, distribution of carnitine between tissues, and renal reabsorption of carnitine [7,8]. Three OCTNs have been identified so far: OCTN1, OCTN2, and OCTN3 [9-11]. OCTN1 and OCTN2 are expressed in several tissues such as kidney, intestine, skeletal muscle, heart, liver, and brain [11-13]. In contrast, OCTN3 is expressed exclusively in testes, kidney, and small intestine [11,14]. Because of its high binding affinity for carnitine and its wide expression, OCTN2 seems to be the most physiologically important carnitine transporter [11]. The fact that inborn or acquired defects of OCTN lead to primary or secondary systemic carnitine deficiency demonstrates their essential role in carnitine homeostasis [8].

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Recently, it has been shown that the expression of hepatic enzymes of carnitine synthesis and OCTN is up-regulated by peroxisome proliferator–activated receptor (PPAR) α, a transcription factor belonging to the nuclear hormone receptor superfamily [15-18]. PPARα is highly expressed in tissues with high rates of fatty acid oxidation. It is activated by synthetic agonists such as fibrates or by natural agonists such as fatty acids or eicosanoids [19]. The physiologic role of PPARα lies in the mediation of metabolic responses to fasting. Upon activation, PPARα up-regulates genes involved in cellular fatty acid uptake and transport, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, and gluconeogenesis [20].

Lactation is a physiologic state characterized by a dramatic increase in the energy and nutrient requirement of the organism for milk production. This demand is usually met by an increased food intake and by the utilization of energy stores. In addition, several metabolic adaptations develop in the lactating organism aiming to conserve energy and metabolic substrates for milk production in the mammary gland [21-24]. Recently, it has been shown that down-regulation of uncoupling proteins (UCPs) 1 and 3 in brown adipose tissue and of UCP3 in skeletal muscle, leading to a decrease of metabolic fuel oxidation and thermogenesis, contributes to these metabolic adaptations during lactation [25-27]. Moreover, expression of enzymes involved in uptake and utilization of fatty acids in liver and skeletal muscle is reduced during lactation, an effect that helps spare fatty acids for milk production [28,29]. Uncoupling proteins 1 and 3 as well as enzymes involved in fatty acid oxidation are transcriptionally regulated by PPARα [20,30,31]. The finding that these genes are down-regulated during lactation suggests that gene expression and/or transactivation of PPARα is lowered during lactation, which, however, has not yet been investigated.

Under the assumption that PPARα is down-regulated during lactation, it is expected that the expression of genes involved in hepatic synthesis and cellular uptake of carnitine is reduced, too, because transcription of these genes is regulated by PPARα. The present study, therefore, aims to investigate the hypotheses that messenger RNA (mRNA) expression of PPARα in the liver is lowered in the lactating organism and that this leads to a down-regulation of hepatic enzymes involved in carnitine synthesis and OCTN, which in turn may lead to a reduction of plasma and tissue carnitine concentrations. For this end, we performed an experiment with lactating and nonlactating rats. It has been shown that expression of UCP [25] and thermogenesis in brown adipose tissue [24] decline with increasing litter sizes in rats as a result of increasing milk output. To investigate whether expression of PPARα and the respective PPARα target genes in the liver is also influenced by litter size, we adjusted litters to a low (4 pups), intermediate (10 pups), and high size (18 pups) as a way to influence the milk output by lactating dams.

2. Materials and methods

2.1. Animal experiment

Forty 11-week–old female rats were obtained from Charles River (Sulzfeld, Germany). They were kept in Macrolon cages (Uno Roestvaststaal, Zevenaar, The Netherlands) in a room maintained with controlled temperature (23°C ± 1°C), humidity (50%-60%), and lighting (6:00 AM to 6:00 PM). All the rats were mated by housing 1 male rat with 2 female rats. After pregnancy was ascertained by observation of sperm in the vaginal smears (only 32 of 40 rats), each rat was kept individually in 1 cage. At the day of parturition, designated as day 1 of lactation, rats and their litters were randomly assigned to 4 groups of 8 rats each. In the first group of dams, all pups were removed, whereas in the other groups, litters were adjusted to sizes of 4, 10, or 18 pups per dam. During the whole experiment, all the rats received a commercial standard rodent diet (Altromin, Lage, Germany) ad libitum. Food intake during the lactation was recorded. Water was available ad libitum from nipple drinkers during the whole experiment. At day 15 of lactation, the dams were killed by decapitation in the nonfasted state. Blood was collected from the opened neck into heparinized polyethylene tubes (Sarstedt, Nürnbrecht, Germany) by the use of heparinized plastic funnels. Liver, skeletal muscle, and mammary gland were quickly removed, snap-frozen in liquid nitrogen, and stored at −80°C pending analysis. Plasma was obtained by centrifugation of the blood (1100*g*, 10 minutes, 4°C) and stored at −20°C. All experimental procedures described followed established guidelines for the care and handling of laboratory animals [32] and were approved by the council of Saxony-Anhalt.

2.2. Lipid analysis

Plasma nonesterified fatty acid (NEFA) concentrations were determined using the enzymatic NEFA C kit from Wako Chemicals (Neuss, Germany, ref 99975406).

2.3. Analysis of carnitine, BB, and TML

Concentrations of free carnitine, acetyl carnitine, TML, and BB in plasma liver and skeletal muscle were determined by tandem mass spectrometry using deuterated carnitine-d_3 (Larodane Fine Chemicals, Malmö, Sweden) as internal standard [16]. Fifty milligrams of freeze-dried tissues was extracted with 0.5 mL methanol/water (2:1, vol/vol) by homogenization (Tissue Lyzer; Qiagen, Hilden, Germany), followed by sonification for 20 minutes and incubation at 50°C for 30 minutes in a shaker. After centrifugation (13 000*g*, 10 minutes), 20 μL of the supernatant was added with 100 μL methanol containing the internal standard, mixed, incubated for 10 minutes, and centrifuged (13 000*g*, 10 minutes). Plasma samples were handled at 4°C in the same manner as the supernatant after tissue extraction. The final supernatants were used for quantification of the compounds by a 1100-er series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a Kromasil 100 column (125 mm ×

Table 1
Characteristics of the primers used for real-time reverse transcriptase polymerase chain reaction analysis

Gene	Forward primer (from 5′ to 3′)	Reverse primer (5′ to 3′)	NCBI GenBank
ACO	CTTTCTTGCTTGCTTTCCTTCTCC	GCCGTTTCACCGCCTCGTA	J02752
BBD	ATTCTGCAAAAGCTCGGAAA	CTCCTTGGAGTCCTGCTCTG	NM_022629
CYP4A1	CAGAATGGAGAATGGGGACAGC	TGAGAAGGGCAGGAATGAGTGG	NM_14972
FABPpm	ACCATCCACTGCCGTCTTAC	CCCCGATGCGTAGGTATTCT	M18467
FAT/CD36	TCGTATGGTGTGCTGGACAT	GGCCCAGGAGCTTTATTTTC	L19658
FATP	GGTAGCAAATGCACCCTCAT	CTCCTGCTGTGATGTGAGGA	U89529
GAPDH	GCATGGCCTTCCGTGTTGC	GGGTGGTCCAGGGTTTCTTACTC	NM_017008
L-CPT I	GGAGACAGACACCATCCAACATA	AGGTGATGGACTTGTCAAACC	NM_031559
OCTN1	CCTCTCTGGCCTGATTGAAG	CTCCGCTGTGAAGACGTACA	NM_012930
OCTN2	AGCATTTGTCCTGGGAACAG	ACTCAGGGATGAACCACCAG	NM_022270
PPARα	CCCTCTCTCCAGCTTCCAGCCC	CCACAAGCGTCTTCTCAGCCATG	NM_013196
TMABA-DH	TTTGAGACTGAAGCCGAGGT	CACCGGGCTGACGTTATAGT	NM_022273
TMLD	GCCCTGTGGCATTCAAGTAT	GGTCCAACCCCTATCATGTG	AF374406

2 mm, 5-μm particle size; CS-Chromatographie Service, Langerwehe, Germany) and an API 2000 LC-MS/MS System (Applied Biosystems, Darmstadt, Germany). The analytes were ionized by positive ion (5500 V) electrospray. As eluents, methanol and a methanol/water/acetonitrile/acetic acid mixture (100:90:9:1, vol/vol/vol/vol) were used.

2.4. Activity of BBD

Activity of BBD in liver was determined as described previously in detail by van Vlies et al [6]. Homogenates from liver were prepared by homogenizing tissue in 10 mmol/L 3-morpholinepropanesulfonic acid buffer (pH 7.4) containing 0.9% (wt/vol) sodium chloride, 10% (wt/vol) glycerol, and 5 mmol/L dithiothreitol.

2.5. RNA isolation and real-time reverse transcriptase polymerase chain reaction

For the determination of mRNA expression levels of PPARα, cytochrome P450 A1 (CYP4A1), acyl–coenzyme A oxidase (ACO), liver-type carnitine palmitoyltransferase I (L-CPT I), fatty acid translocase (FAT/CD36), fatty acid transport protein (FATP), plasma membrane fatty acid

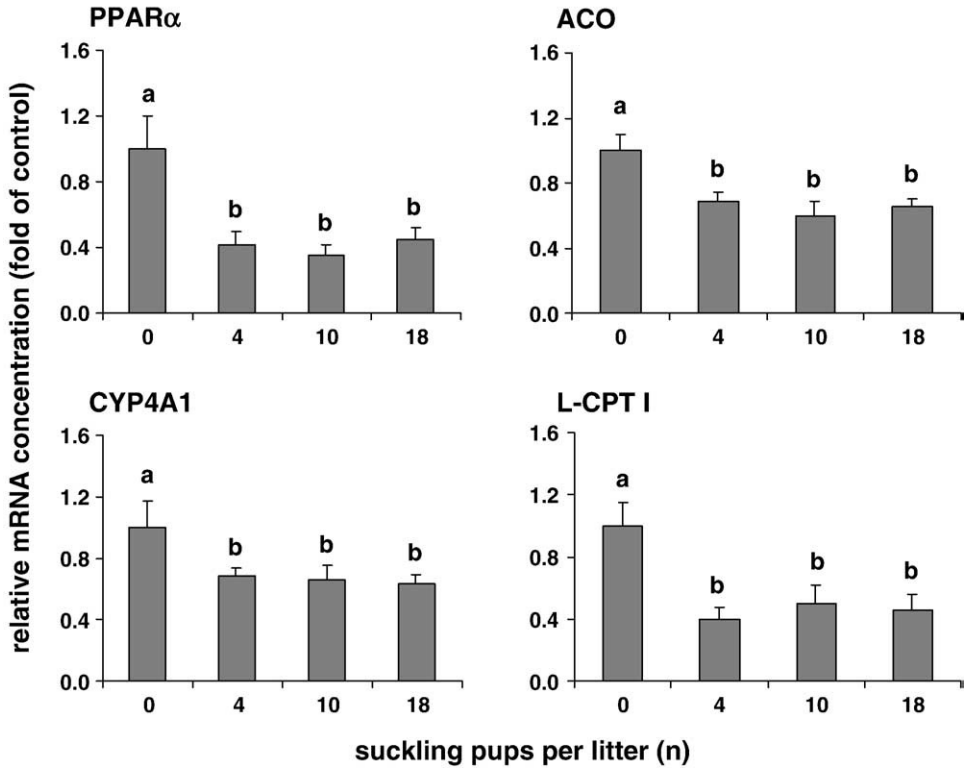

Fig. 1. Relative mRNA concentrations of PPARα, ACO, CYP4A1, and L-CPT I in the liver of lactating rats whose litters where either removed (group 0) or adjusted to 4 (group 4), 10 (group 10), and 18 (group 18) pups immediately after birth, at day 15 of lactation. Bars represent mean ± SE (n = 8 per group) and are expressed as fold of relative mRNA concentration of control (group 0). Bars without a common letter (a, b) differ ($P < .05$).

binding protein (FABPpm), OCTN1, OCTN2, TMABA-DH, TMLD, and BBD, total RNA was isolated from rat livers and rat mammary glands using Trizol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. The RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. Complementary DNA synthesis and determination of relative mRNA concentrations were performed by real-time detection reverse transcriptase polymerase chain reaction as described previously [33]. Characteristics of gene-specific primers obtained from Operon (Köln, Germany) are shown in Table 1.

2.6. Statistical analysis

Statistical analysis of the data was performed using the Minitab Statistical Software (Minitab, State College, PA). Data were analyzed by 1-way analysis of variance. For significant F values, means were compared by Fisher multiple range test. In all experiments, means were considered significantly different for P less than .05. Values in the text are means ± SE.

3. Results

3.1. Food intake of dams and litter weights

Average daily food intake of the dams during the lactation phase (day 1 to day 15 of lactation) was influenced by the litter size. Dams whose litters were removed consumed less food per day than dams suckling their litters ($P < .05$). Dams with 4 pups consumed less food per day than dams with 10 or 18 pups ($P < .05$); daily food consumption of dams with 10 and 18 pups did not differ (0 pups per litter, 22 ± 1 g/d;

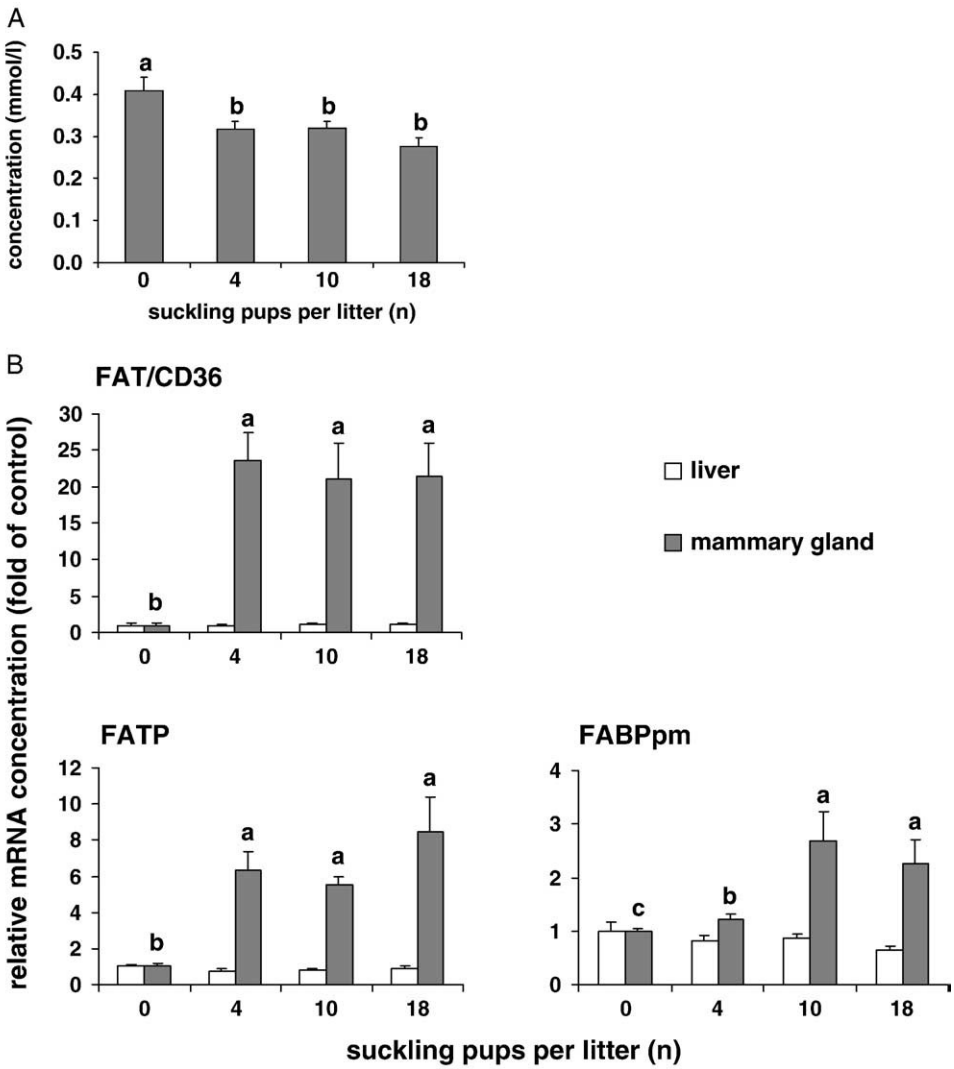

Fig. 2. A, Concentration of NEFA in the plasma of lactating rats whose litters where either removed (group 0) or adjusted to 4 (group 4), 10 (group 10), and 18 (group 18) pups immediately after birth, at day 15 of lactation. Bars without a common letter (a, b, c) differ ($P < .05$). B, Relative mRNA concentrations of FAT/CD36, FATP, and FABPpm in the liver and mammary gland of lactating rats whose litters where either removed (group 0) or adjusted to 4 (group 4), 10 (group 10), and 18 (group 18) pups immediately after birth, at day 15 of lactation. Bars represent mean ± SE (n = 8 per group) and are expressed as fold of relative mRNA concentration of control (group 0).

4 pups per litter, 38 ± 2 g/d; 10 pups per litter, 52 ± 1 g/d; 18 pups per litter, 55 ± 2 g/d). Final body weights of dams with different litter sizes did not differ (0 pups per litter, 317 ± 7 g; 4 pups per litter, 331 ± 12 g; 10 pups per litter, 331 ± 7 g; 18 pups per litter, 325 ± 9 g). Weights of whole litters at day 15 were increased with increasing litter size (4 pups per litter, 148 ± 15 g per litter; 10 pups per litter, 304 ± 8 g per litter; 18 pups per litter, 376 ± 10 g per litter).

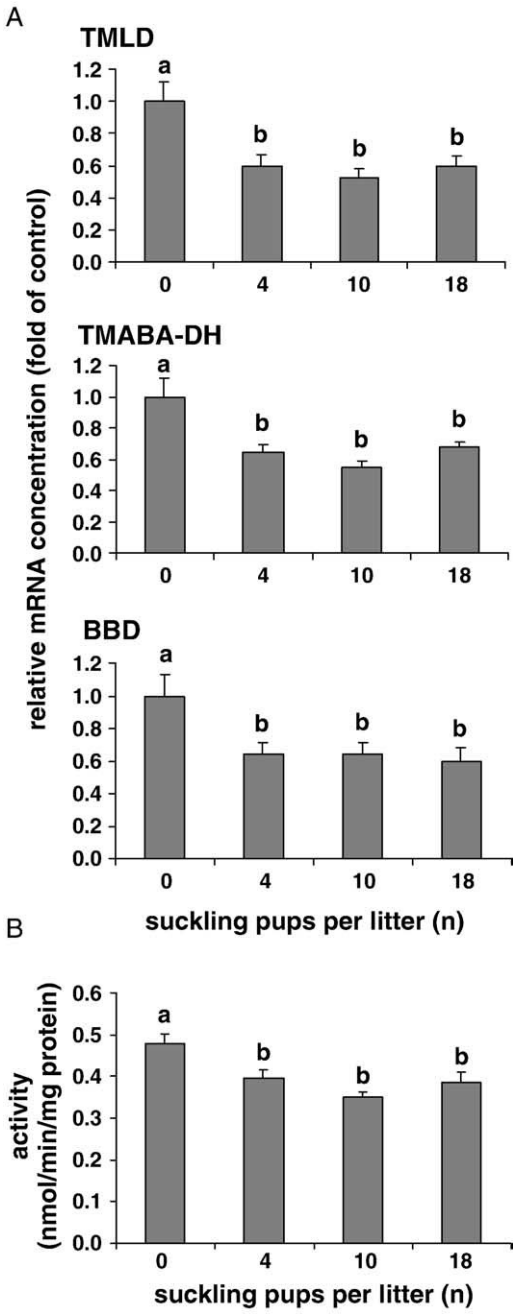

Fig. 3. A, Relative mRNA concentrations of TMLD, TMABA-DH, and BBD in the liver of lactating rats whose litters where either removed (group 0) or adjusted to 4 (group 4), 10 (group 10), and 18 (group 18) pups immediately after birth, at day 15 of lactation. Bars represent mean ± SE (n = 8 per group) and are expressed as fold of relative mRNA concentration of control group (group 0). B, Activity of BBD in the liver of lactating rats whose litters where either removed (group 0) or adjusted to 4 (group 4), 10 (group 10), and 18 (group 18) pups immediately after birth, at day 15 of lactation. Bars without a common letter (a, b) differ ($P < .05$).

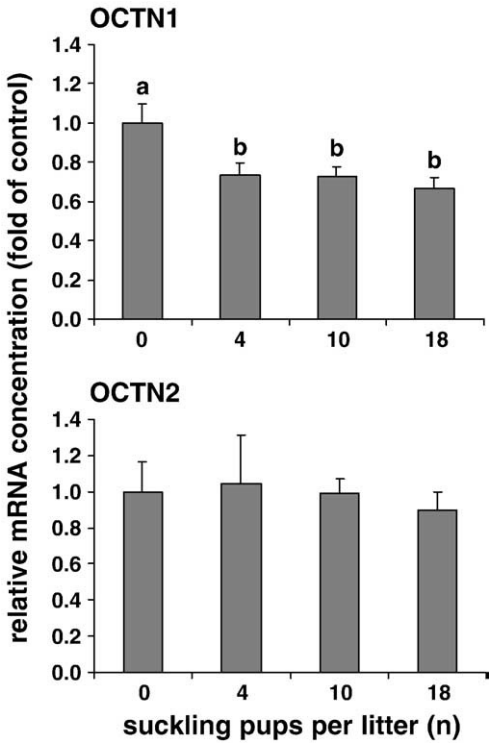

Fig. 4. Relative mRNA concentrations of OCTN1 and OCTN2 in the liver of lactating rats whose litters where either removed (group 0) or adjusted to 4 (group 4), 10 (group 10), and 18 (group 18) pups immediately after birth, at day 15 of lactation. Bars represent mean ± SE (n = 8 per group) and are expressed as fold of relative mRNA concentration of control (group 0). Bars without a common letter (a, b) differ ($P < .05$).

3.2. Expression of PPARα and target genes of PPARα in the liver

Dams with litters had lower relative mRNA concentrations of PPARα and its target genes (ACO, CYP4A1, L-CPT I) in the liver than dams whose litters were removed ($P < .05$, Fig. 1). No difference was observed in the relative mRNA concentrations of these genes between dams with 4, 10, or 18 pups, respectively (Fig. 1).

3.3. Concentrations of NEFA in plasma and expression of fatty acid transporters in liver and mammary gland

Plasma NEFA concentration was lower in dams with 4, 10, or 18 pups than in dams whose litters were removed. There was, however, no difference in plasma NEFA concentration between dams with 4, 10, or 18 pups (Fig. 2A). Dams with litters, irrespective of the litter size, had higher relative mRNA concentrations of fatty acid transporters (FAT/CD36, FATP, FABPpm) in the mammary gland than dams whose litters were removed ($P < .05$, Fig. 2B). In the liver, relative mRNA concentrations of fatty acid transporters did not differ between the 4 groups (Fig. 2B).

Table 2
Concentrations of free carnitine, acetylcarnitine, total carnitine, BB, and TML in plasma, liver, and skeletal muscle

	Litter size (pups per litter)			
	0	4	10	18
Plasma (μmol/L)				
Free carnitine	31.6 ± 2.2[a]	14.2 ± 1.0[b]	10.6 ± 0.8[b]	16.7 ± 1.2[b]
Acetyl carnitine	7.12 ± 0.62[a]	3.32 ± 0.34[b]	3.44 ± 0.43[b]	4.10 ± 0.37[b]
Total carnitine	40.0 ± 3.0[a]	17.8 ± 1.0[b]	14.3 ± 0.9[b]	21.2 ± 1.0[b]
TML	0.96 ± 0.06	0.95 ± 0.06	1.24 ± 0.10	1.19 ± 0.12
BB	0.39 ± 0.03	0.37 ± 0.03	0.36 ± 0.02	0.38 ± 0.04
Liver (nmol/g)				
Free carnitine	412 ± 36[a]	234 ± 26[b]	261 ± 16[b]	311 ± 14[b]
Acetyl carnitine	25.5 ± 2.6[a]	4.6 ± 0.2[b]	4.2 ± 1.0[b]	6.8 ± 1.5[b]
Total carnitine	441 ± 33[a]	245 ± 15[b]	278 ± 19[b]	320 ± 15[b]
TML	2.17 ± 0.17	2.36 ± 0.24	2.62 ± 0.14	2.15 ± 0.10
BB	4.98 ± 0.43[b]	4.89 ± 0.41[b]	4.90 ± 0.52[b]	8.36 ± 0.55[a]
Skeletal muscle (nmol/g)				
Free carnitine	430 ± 20[a]	342 ± 21[b]	260 ± 20[c]	322 ± 30[bc]
Acetyl carnitine	153 ± 8.0[a]	124 ± 6.8[b]	114 ± 9.3[b]	105 ± 7.6[b]
Total carnitine	585 ± 25[a]	468 ± 25[b]	375 ± 27[c]	429 ± 35[bc]
TML	12.7 ± 1.5	11.6 ± 0.6	13.5 ± 0.5	10.9 ± 0.5
BB	10.8 ± 0.4[b]	17.0 ± 0.9[a]	15.2 ± 0.6[a]	16.9 ± 0.7[a]

Data are means ± SE, n = 8 for each group. Means marked without a common superscript letter (a, b, c) differ ($P < .05$).

3.4. Expression of enzymes involved in hepatic carnitine synthesis and OCTN

Dams with litters, irrespective of litter size, had lower relative mRNA concentrations of TMLD, TMABA-DH, and BBD in the liver than dams whose litters were removed ($P < .05$, Fig. 3A). The activity of BBD in the liver was also lower in dams with litters compared with those whose litters were removed ($P < .05$, Fig. 3B). Relative mRNA concentration of OCTN1 was also reduced in dams with litters compared with those without litters ($P < .05$, Fig. 4). All these parameters, mRNA concentrations of enzymes involved in carnitine synthesis and of OCTN1 as well as activity of BBD, were however not different between dams with 4, 10, or 18 pups (Figs. 3A and 4). Relative mRNA concentration of OCTN2 in the liver did not differ between the 4 groups of rats (Fig. 4).

3.5. Carnitine concentrations in plasma and tissues

Dams with litters had lower concentrations of free carnitine, acetyl carnitine, and total carnitine in liver, skeletal muscle, and plasma than dams without litters ($P < .05$, Table 2). In liver and plasma, concentrations of total carnitine did not differ between dams nursing 4, 10, and 18 pups (Table 2). In skeletal muscle, concentration of free and total carnitine was lowest in dams nursing 10 pups ($P < .05$, Table 2). Dams nursing 18 pups did not differ in skeletal muscle free and total carnitine concentration from those nursing 4 or 10 pups (Table 2).

3.6. Concentrations of carnitine precursors in plasma and tissues

The concentration of TML in plasma, liver, and skeletal muscle did not differ between the 4 groups of rats (Table 2). Concentration of BB in plasma also did not differ between the 4 groups of rats. The BB concentration in skeletal muscle, however, was higher in dams with litters than in dams without litters ($P < .05$, Table 2). The BB concentration in liver was not different between dams with 4 or 10 pups and those whose litters were removed; in contrast, the hepatic BB concentration was higher in pups with 18 pups than in the other 3 groups ($P < .05$, Table 2).

4. Discussion

In this study, we tested the hypothesis that PPARα is down-regulated during lactation and that this leads to a down-regulation of enzymes involved in hepatic carnitine synthesis and OCTN. For this purpose, we compared lactating rats nursing 4, 10, or 18 pups with nonlactating rats whose litters were removed immediately after birth.

The data of this study confirm our hypothesis that lactation leads to a down-regulation of PPARα in the liver, some of its classic target genes (ACO, L-CPT-1, CYP4A1), as well as OCTN1 and enzymes involved in hepatic carnitine synthesis. Recently, it has been observed that gene expression of OCTN and of enzymes involved in hepatic carnitine synthesis is regulated by PPARα [15-18]. Although we have no direct proof for this, it is likely that down-regulation of OCTN1 and genes coding for enzymes involved in carnitine synthesis in the liver of lactating dams was due to the down-regulation of PPARα. In our previous experiments [15,16], OCTN2 was even more strongly up-regulated in rats by treatment with a PPARα agonist than OCTN1, suggesting that expression of OCTN2 is more sensitive toward PPARα activation than OCTN1. The finding that OCTN2, in contrast to OCTN1, was not down-regulated in response to the lowered expression of PPARα during lactation was, therefore, unexpected. It is, however, possible that baseline expression of PPARα caused already the minimum expression level of OCTN2. In this case, a reduction of the expression would not further down-regulate expression of OCTN2.

We found, moreover, that lactating rats have reduced carnitine concentrations in plasma, liver, and skeletal muscle compared with nonlactating female rats. Tissue carnitine concentrations are influenced by the rate of endogenous carnitine synthesis, the amount of carnitine absorbed from the diet, the distribution of carnitine within the body by carnitine transporters, the excretion of carnitine via the urine, and, in the lactating rat, additionally by the amount of carnitine excreted via the milk [34]. In the female rat, carnitine synthesis occurs exclusively in the liver [6]. BBD catalyzes the last step of synthesis in the liver [5]. However, it has been demonstrated in rats that the activity of BBD far exceeds that necessary to support the normal rate of carnitine synthesis [5]. Therefore, it is questionable whether the reduced activity of that enzyme, as observed in the liver of lactating rats, was responsible for the reduced plasma and tissue carnitine concentrations of lactating rats. It has been

shown that TML and its ability to penetrate to the site of TMLD are the rate-limiting factors for carnitine synthesis [35]. As TML concentration in the liver was unchanged, we assume that the availability of TML was not limiting for the carnitine synthesis during lactation.

In conclusion, the present study demonstrates for the first time that genes coding for enzymes involved in hepatic carnitine synthesis and OCTN1 are down-regulated in lactating rats. It is, moreover, shown that PPARα in the liver is down-regulated during lactation. As OCTN and enzymes of carnitine synthesis are up-regulated by PPARα, the down-regulation of PPARα may be the reason for the reduced gene expression of these proteins involved in whole-body carnitine synthesis. Down-regulation of PPARα, which also leads to an impairment of enzymes involved in hepatic β-oxidation, during lactation may be a means to conserve energy and metabolic substrates for milk production in the mammary gland.

References

[1] McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 1997;244:1-14.

[2] Brass EP. Pivalate-generating prodrugs and carnitine homeostasis in man. Pharmacol Rev 2002;54:589-98.

[3] Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Asp Med 2004;25:455-73.

[4] Rebouche CJ, Seim H. Carnitine metabolism and its regulation in microorganisms and mammals. Annu Rev Nutr 1998;18:39-61.

[5] Vaz FM, Wanders RJ. Carnitine biosynthesis in mammals. Biochem J 2002;361:417-29.

[6] van Vlies N, Wanders RJ, Vaz FM. Measurement of carnitine biosynthesis enzyme activities by tandem mass spectrometry: differences between the mouse and the rat. Anal Biochem 2006;354:132-9.

[7] Lahjouji K, Mitchell GA, Qureshi IA. Carnitine transport by organic cation transporters and systemic carnitine deficiency. Mol Genet Metab 2001;73:287-97.

[8] Tein I. Carnitine transport: pathophysiology and metabolism of known defects. J Inherit Metab Dis 2003;26:147-69.

[9] Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, et al. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. FEBS Lett 1997;419:107-11.

[10] Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, et al. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem 1998;273:20378-82.

[11] Tamai I, Ohashi R, Nezu JI, Sai Y, Kobayashi D, Oku A, et al. Molecular and functional characterization of organic cation/carnitine transporter family in mice. J Biol Chem 2000;275:40064-72.

[12] Wu X, Huang W, Prasad PD, Seth P, Rajan DP, Leibach FH, et al. Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. J Pharmacol Exp Ther 1999;290:1482-92.

[13] Slitt AL, Cherrington NJ, Hartley DP, Leazer MT, Klaassen CD. Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. Drug Metab Dispos 2002;30:212-9.

[14] Durán JM, Peral MJ, Calonge ML, Ilundáin AA. OCTN3: a Na^+-independent L-carnitine transporter in enterocytes basolateral membrane. J Cell Physiol 2005;202:929-35.

[15] Luci S, Geissler S, König B, Koch A, Stangl GI, Hirche F, et al. PPARα agonists up-regulate organic cation transporters in rat liver cells. Biochem Biophys Res Commun 2006;24:704-8.

[16] Ringseis R, Pösel S, Hirche F, Eder K. Treatment with pharmacological peroxisome proliferator–activated receptor α agonist clofibrate causes up-regulation of organic cation transporter 2 in liver and small intestine of rats. Pharmacol Res 2007;56:175-83.

[17] van Vlies N, Ferdinandusse S, Turkenburg M, Wanders RJ, Vaz FM. PPARα-activation results in enhanced carnitine biosynthesis and OCTN2-mediated hepatic carnitine accumulation. Biochim Biophys Acta 2007;1767:1134-42.

[18] Koch A, König B, Stangl GI, Eder K. PPARα mediates transcriptional upregulation of novel organic cation transporters-2 and -3 and enzymes involved in hepatic carnitine synthesis. Exp Biol Med 2008;233:356-65.

[19] Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator–activated receptors, orphans with ligands and functions. Curr Opin Lipidol 1997;8:159-66.

[20] Mandard S, Müller M, Kersten S. Peroxisome proliferator receptor α target genes. Cell Mol Life Sci 2004;61:393-416.

[21] Dewey KG. Energy and protein requirements during lactation. Annu Rev Nutr 1997;17:19-36.

[22] Smith MS, Grove KL. Integration of the regulation of reproductive function and energy balance: lactation as a model. Front Neuroendocrinol 2002;23:225-56.

[23] Williamson DH. Regulation of metabolism during lactation in the rat. Reprod Nutr Dev 1986;26:597-603.

[24] Trayhurn P, Douglas JB, McGuckin MM. Brown adipose tissue thermogenesis is suppressed during lactation in mice. Nature 1982;298:59-60.

[25] Pedraza N, Solanes G, Iglesias R, Vazquez M, Giralt M, Villarroya F. Differential regulation of expression of genes encoding uncoupling proteins 2 and 3 in brown adipose tissue during lactation in mice. Biochem J 2001;355:105-11.

[26] Xiao XQ, Grove KL, Grayson BE, Smith MS. Inhibition of uncoupling protein expression during lactation: role of leptin. Endocrinol 2004;145:830-8.

[27] Pedraza N, Solanes G, Carmona MC, Iglesias R, Vinas O, Mampel T, et al. Impaired expression of the uncoupling protein–3 gene in skeletal muscle during lactation: fibrates and troglitazone reverse lactation-induced downregulation of the uncoupling protein–3 gene. Diabetes 2000;49:1224-30.

[28] Xiao XQ, Grove KL, Smith MS. Metabolic adaptations in skeletal muscle during lactation: complementary deoxyribonucleic acid microarray and real-time polymerase chain reaction analysis of gene expression. Endocrinology 2004;145:5344-54.

[29] Zammit VA. The effect of glucagons treatment and starvation of virgin and lactating rats on the rates of oxidation of octanoyl-L-carnitine and octanoate by isolated liver mitochondria. Biochem J 1980;190:293-300.

[30] Barbera MJ, Schlüter A, Pedraza N, Iglesias R, Villarroya F, Giralt M. Peroxisome proliferators–activated receptor α activates transcription of the brown fat uncoupling protein–1 gene. A link between regulation of thermogenic and lipid oxidation pathways in the brown fat cell. J Biol Chem 2000;276:1486-93.

[31] Brun S, Carmona MC, Mampel T, Vinas O, Giralt M, Igleasias R, et al. Activators of peroxisome proliferators–activated receptor α induce the expression of the uncoupling protein–3 gene in skeletal muscle: a potential for the lipid intake–dependent activation of uncoupling protein–3 gene expression at birth. Diabetes 1999;48:1217-22.

[32] National Research Council: guide for the care and use of laboratory animals. Washington, DC: National Institutes of Health; 1985. Publication no. 85-23 (rev.).

[33] Ringseis R, Muschick A, Eder K. Dietary oxidized fat prevents ethanol-induced triacylglycerol accumulation and increases expression of PPARα target genes in rat liver. J Nutr 2007;137:77-83.

[34] Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. Ann N Y Acad Sci 2004;1033:30-41.

[35] Rebouche CJ, Lehman LJ, Olson L. Epsilon-*N*-trimethyllysine availability regulates the rate of carnitine biosynthesis in the growing rat. J Nutr 1986;116:751-9.