Synthesis of long-chain polyunsaturated fatty acids in lactating mammary gland: role of Δ5 and Δ6 desaturases, SREBP-1, PPARα, and PGC-1

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Abstract The purpose of this work was to study whether rat lactating mammary gland can synthesize PUFAs through the expression of Δ5 and Δ6 desaturases (Δ5D and Δ6D), whether these enzymes are regulated by the transcription factors sterol-regulatory element binding protein 1 (SREBP-1) and peroxisome proliferator-activated receptor α (PPARα) and the coactivator peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β), and whether these desaturases are regulated by the lipid concentration in the diet. The results showed that on day 12 of lactation, ~35% of the linoleic acid in the diet, which is the precursor of PUFAs, is transferred to the mammary gland. There was expression of Δ5D and Δ6D in mammary gland, and it was regulated by the corn oil content in the diet. The higher the corn oil content in the diet, the lower the expression of both desaturases. Induction of Δ5D and Δ6D was associated positively with similar changes in SREBP-1 and PGC-1β. Expression of PPARα was barely detected and was not affected by the corn oil content in the diet, whereas PGC-1β expression increased as the corn oil in the diet increased. These results indicate that the lactating mammary gland has the capacity to synthesize PUFAs and can be regulated by the lipid content in the diet.—Rodriguez-Cruz, M., A. R. Tovar, B. Palacios-González, M. del Prado, and N. Torres. **Synthesis of long-chain polyunsaturated fatty acids in lactating mammary gland: role of Δ5 and Δ6 desaturases, SREBP-1, PPARα, and PGC-1. *J. Lipid Res.* 2006.** 47: **553–560.**

Supplementary key words sterol-regulatory element binding protein 1 • peroxisome proliferator-activated receptor α • peroxisome proliferator-activated receptor γ coactivator 1

Δ5 and Δ6 desaturases (Δ5D and Δ6D) are microsomal enzymes that form part of a three enzyme system that includes NADH-cytochrome b_5 reductase, cytochrome b_5, and Δ6D or Δ5D (1). Mammals are unable to synthesize long-chain polyunsaturated fatty acids (LC-PUFAs) from acetyl-CoA; thus, two PUFAs, linoleic acid (LA; 18:2 n-6) and α-linolenic acid (18:3 n-3), called essential fatty acids, must be supplied from the diet. Mammals are able to synthesize LC-PUFAs from their precursor PUFAs. Fatty acid desaturases introduce a double bond in a specific position of long-chain fatty acids and are required for the synthesis of highly unsaturated fatty acids (LC-PUFAs). The conversion of LA and α-linolenic acid to γ-linolenic acid (18:3 n-6) and stearidonic acid (18:4 n-3) is determined by the enzymatic activity of Δ6D. After desaturation and elongation by Δ6D and elongase, respectively, Δ5D introduces another double bond at the Δ5 position of the 20 carbon fatty acids 20:3 n-6 and 20:4 n-3 to synthesize 20:4 n-6 (arachidonic acid; AA) and 20:5 n-3 (eicosapentanoic acid), respectively. The highly unsaturated fatty acids are mainly esterified into phospholipids and contribute to the maintenance of membrane fluidity (2). These desaturases are expressed in liver, heart, and brain and are regulated by hormonal and nutritional manipulation in rodents (3, 4). In addition, studies in rats indicate that hepatic Δ6D mRNA expression and enzymatic activity are highly dependent upon the composition of dietary fat. The ingestion of diets low in essential fatty acids or LC-PUFAs results in high levels of Δ6D enzyme activity and mRNA expression (5, 6).

Diets high in PUFAs are negative regulators of lipogenesis that exert their effects primarily at the level of transcription. Two transcription factors, sterol-regulatory element binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor α (PPARα), are transcription factors that play a key role in the regulation of desaturases by PUFAs in liver. SREBP-1c is synthesized as a larger precursor protein that is anchored to the endoplasmic reticulum. After proteolytic cleavage, the

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N-terminal domain migrates to the nucleus and activates target genes by binding to sterol-regulatory elements (2). Expression of Δ6D and Δ5D mRNA is positively regulated by SREBP-1 in liver as a result of the presence of a sterol-regulatory element in the promoter region of Δ6D and probably Δ5D (7, 8). On the other hand, PPARα is a member of the steroid receptor family that contains a hydrophobic ligand binding site and a DNA binding domain. Binding of a ligand causes a conformational change of PPARα, which then forms a heterodimer with retinoid X receptor and binds to peroxisome proliferator response elements located in the promoter region of the target genes. Hypolipidemic compounds called peroxisome proliferators, such as fibrates, which are ligands of PPARα, induce the expression of enzymes involved in fatty acid oxidation as well as hepatic Δ5D and Δ6D (7).

The increase in expression of Δ6D is associated with the presence of peroxisome proliferator response elements in the promoter region of this gene (9). In addition, these transcription factors are regulated by specific coactivators that enhance or repress their activity. The family of peroxisome proliferator-activated receptor γ coactivators (PGC-1) is involved in the expression of multiple genes that are regulated by SREBP-1 and PPARα. There are two members of the PGC-1 family, PGC-1α and PGC-1β. PGC-1α is associated with the activation of fatty acid oxidation, whereas PGC-1β is associated with lipogenesis, among other functions. During lactation, there is an increase in the use of metabolic fuels to sustain high rates of lipid and protein synthesis. In the lactating mammary gland, there is a high demand of the LC-PUFAs docosahexanoic acid (DHA) and AA to incorporate them into the milk. These LC-PUFAs are essential for the normal growth and development of the brain and retina of the newborn. Lactating mothers lose ~70–80 mg DHA/day in breast milk, in addition to the amount lost by oxidation or used to fulfill the mother's own requirement (10). Thus, the maternal organism synthesizes them when they are not provided by dietary lipids. Studies in Mexican women with low intake of AA showed adequate levels of AA in their milk (11), suggesting that during lactation, there are some metabolic adaptations to synthesize adequate amounts of LC-PUFAs. However, it is not known whether these adaptations include the participation of extra hepatic tissues, such as mammary gland and/or adipose tissue, to synthesize LC-PUFAs by regulating the expression of Δ5D and Δ6D.

The purpose of the present study was to investigate the presence of Δ6D and Δ5D mRNA in mammary gland and whether or not this expression was regulated by dietary LA present in corn oil (55% LA). In addition, we explored the possible mechanism of regulation of these desaturases by SREBP-1 and PPARα during lactation. Our results indicate that Δ6D and Δ5D mRNAs are present in lactating mammary gland, liver, and adipose tissue. Also, Northern blot analysis showed that a low corn oil diet increased the mRNA abundance of these desaturases in a tissue-specific manner in liver and lactating mammary gland but not in adipose tissue. Furthermore, our results indicate that expression of these desaturases was regulated by dietary LA only in mammary gland and liver. This effect could be attributable in part to SREBP-1, because SREBP-1 mRNA expression followed the same pattern of expression of Δ5D and Δ6D and was also regulated by the presence of LA in the diet. The higher the LA content in the diet, the lower the expression of SREBP-1 in liver and mammary gland. Contrary to that, liver PPARα mRNA increased with the LA content in the diet, and there was little expression and no change of PPARα in mammary gland and adipose tissue. These results indicate that mammary gland participates in the synthesis of LC-PUFAs by increasing Δ6D and Δ5D expression when the LA content in the diet is low through reduction in the expression of SREBP-1, whereas in situations in which the presence of corn oil in the diet is high, there is an inhibition of Δ5D, Δ6D and SREBP-1 and an increase in the fatty acid oxidation through an increase in PPARα expression.

MATERIALS AND METHODS

Female Sprague-Dawley rats (8 weeks old) obtained from the Animal Care Facilities at the Centro Médico Nacional, Instituto Mexicano del Seguro Social, were housed at 22 ± 2°C with a 12 h light/dark cycle and free access to water. From weaning until 8 weeks of age, rats were fed a commercial diet (Purina, Guadalajara, México). When rats reached 8 weeks old, they were randomly assigned and adapted to one of the specific diets described below for 6 weeks before mating. When animals reached 14 weeks of age (220–280 g), they were mated. Pregnant females were housed individually and continued on the same diet until day 12 of lactation. The day of birth was considered day 0 of lactation, and litters were weighed and adjusted to eight pups per dam. No gender differentiation was done. Food intake and body weight were assessed daily. The protocol was approved by the Animal Care Committee of the Instituto Mexicano del Seguro Social.

Diets

The compositions of the 2, 5, and 10% corn oil diets used in the experimental groups are shown in **Table 1**. The composition of fatty acids in the corn oil diet was as follows: palmitic acid (C16:0), 12.1%; stearic acid (C18:0), 2.2%; oleic acid (C18:1), 31.7%; and linoleic acid (C18:2), 55.2%.

Materials

[1-^{14}C]LA (2.04 GBq/mmol) was purchased from Amersham (UK). Unlabeled fatty acid methyl ester standards were obtained from Agilent Technologies. All other chemicals and solvents were reagent molecular grade from common commercial sources. [α-^{32}P]dCTP (10 mCi/ml) was purchased from Amersham (UK).

Metabolic fate of LA in different organs of the lactating rat

Measurement of [^{14}C]LA incorporation. The metabolic fate of an oral load of [1-^{14}C]LA was determined in lactating rats at day 12 of lactation as described previously (12) in each dietary treatment. Rats had free access to food during the procedure, and the litters stayed with the rats. Briefly, after intragastric administration of [1-^{14}C]LA (0.5 g, with a specific activity of 0.44 μCi/mmol per rat), the $^{14}CO_2$ production (total oxidation of the oral fatty acid load) was measured every hour for 5 h in a

TABLE 1. Composition of experimental diets

Ingredient	LCOD	ACOD	HCOD
		g/kg diet	
Casein	222	222	222
Glucose	336	307.5	272.5
Corn starch	336	307.5	272.5
Corn oil	20	50	100
Vitamin mix[a]	10	10	10
Mineral mix[b]	40	40	60
Cellulose	36	63	63
Energy (kJ/g)	15.65	15.82	16.53

ACOD, adequate-corn oil diet (5%; n = 10); HCOD, high-corn oil diet (10%; n = 10); LCOD, low-corn oil diet (2%; n = 14).

[a]Vitamin mixture contained (per kg): *p*-aminobenzoic acid, 11.01 g; ascorbic acid, 101.66 g; biotin, 0.044 g; cyanocobalamin, 2.97 g; calcium pantothenate, 6.61 g; choline dihydrogen citrate, 349.69 g; folic acid, 0.20 g; inositol, 11.01 g; menadione, 4.95 g; niacin, 9.91 g; pyridoxine HCl, 2.20 g; riboflavin, 2.20 g; thiamin HCl, 2.20 g; dry retinyl palmitate, 3.96 g; dry ergocalciferol, 0.44 g; dry DL-α-tocopheryl acetate, 24.23 g; corn starch, 466.67 g.

[b]Mineral mixture contained (per kg): ammonium molybdate, 0.025 g; calcium carbonate, 292.9 g; calcium phosphate, 4.3 g; cupric sulfate, 1.56 g; ferric citrate, 6.23 g; magnesium sulfate, 99.8 g; manganese sulfate, 1.21 g; potassium iodide, 0.005 g; potassium phosphate, 343.1 g; sodium chloride, 250.6 g; sodium selenite, 0.015 g; zinc chloride, 0.2 g.

glass desiccator connected to a wash bottle fitted with a sintered-glass tube that contained ethanolamine-ethylene glycol to trap the CO_2. Five hundred microliters of this mix was added directly to the scintillation fluid for measurement of radioactivity. After 5 h, rats were anesthetized with pentobarbital (35 mg/kg body weight), and a sample of mammary gland, liver, parametrial adipose tissue, and the whole intestinal tract were removed. The carcass was homogenized with water. The intestinal tract was homogenized in 30 g/L $HClO_4$ (w/v). Pups were euthanized after diethyl ether anesthesia, and their stomachs were dissected. Milk clots were removed, weighed, and mixed, and the remaining pup was homogenized in water. After saponification of all tissues with 1.5 ml of 60% KOH, lipids were extracted in duplicate samples of 0.5 g of mammary gland, milk clot, liver, and parametrial adipose tissue and 5 g of intestinal tract and carcass (13). The radioactivity in the extracted fatty acids was measured, and the ^{14}C-labeled lipid incorporated in the tissues was calculated. The amount of [1-^{14}C]LA absorbed was determined as the difference between the radioactivity in the total dose and the radioactivity remaining in the intestinal tract. Analysis of the whole intestinal tract, mammary gland, liver, parametrial adipose tissue, and clot milk was performed on at least seven individual rats per dietary treatment.

Gas chromatographic analysis. Mammary gland lipids were extracted with chloroform-methanol by a modified Folch, Lees, and Sloane-Stanley method (14). Fatty acid methyl esters were methylated with 1 N HCl in methanol and dissolved in hexane. Fatty acid methyl esters were separated by gas chromatography using a Hewlett-Packard GC system and a 50 m × 0.32 mm CP-Sil 88 Chrompack capillary column with helium as a carrying gas. Identification of the fatty acid methyl esters was based upon retention times obtained for methyl ester standards from Poly Science. Also, fatty acid composition was measured in the corn oil diet.

Northern blot analysis. Total RNA was isolated from liver, mammary gland, and adipose tissue of rats on day 12 of lactation using the method of Chomczynski and Sacchi (15). For Northern analysis, 15 μg of RNA was electrophoresed on a 0.8% agarose gel containing 37% formaldehyde, transferred onto a nylon membrane filter (Hybond-N+; Amersham, Buckinghamshire, UK), and cross-linked with an ultraviolet light cross-linker (Amersham). cDNA probes for the rat hepatic Δ5D, Δ6D, SREBP-1, FAS, and PPARα were prepared by reverse transcriptase-polymerase chain reaction with the primers shown below. Probes were labeled with deoxycytidine 5′[α-^{32}P]triphosphate (110 TBq/mmol; Amersham) using the Rediprime DNA labeling system (Amersham). Filters were prehybridized with rapid-hyb buffer at 65°C for 60 min and then hybridized with the labeled probe for 2.5 h at 65°C. Membranes were washed once with 2× citrate saline solution (SSC)/0.1% SDS at room temperature for 20 min and then twice with 0.1× SSC/0.1% SDS at 65°C for 15 min each. Image digitization and quantitation of radioactive bands (cpm) in the membranes were done with an Instant Imager electronic autoradiography system (Packard Instrument, Meriden, CT). Results are expressed as arbitrary units.

The forward and reverse primers used were as follows: for Δ6D, upper (5′-TGC CTT CCG TGC CTT CCA C-3′) and lower (5′-GTG CCC GCT GAA CCA GTC ATT-3′); for Δ5D, upper (5′-TCT TGC CCA CGA TGC CAC GAC-3′) and lower (5′-CTT TGC CCC GCC TGC TTC TGA-3′); for PPARα, upper (5′-CCC CAC CAG TAC AGA TGA GTC-3′) and lower (5′-GGA GTT TTG GGA AGA GAA AGG-3′); for SREBP-1c, upper (5′-TCC CAG AGT AGC CCC TTG TCC-3′) and lower (5′-CCA GTC CCC ATC CAC GAA-3′); for FAS, upper (5′-GCT TTG CTG CCG TGT CCT TCT-3′) and lower (5′-GTG TCT GCT GGG GTC CTC GTT-3′). PCR products were sequenced with asymmetric PCR using the BigDye kit and analyzed in an ABI PRISM 377 automated sequencer (Applied Biosystems).

Real-time RT-PCR. For quantitative real-time PCR, total RNA was extracted from mammary gland according to Chomczynski and Sacchi (15) and pooled. The first-strand cDNA was synthesized from 350 ng of total RNA with the Multiscribe Master Mix (PE Applied Biosystems). Samples were subjected to quantitative real-time PCR using the TaqMan probe and primer sets for PGC-1α (Rn 00580241) and PGC-1β (Rn 00598552). The ABI PRISM 7000 system was used for the reaction and detection (Applied Biosystems). The probe was labeled with FAM at the 5′ end and with TAMRA at the 3′ end. PCR amplification was performed in a total volume of 10 μl containing 22 ng of cDNA sample, 900 nM of each primer, and 12.5 μl of TaqMan Universal PCR Master Mix. For each reaction, the polymerase was activated by preincubation at 95°C for 10 min. Amplification was then performed by 45 cycles of 95°C for 15 s and 60°C for 60 s. The PGC-1α and PGC-1β cDNA quantity in each sample was normalized to the housekeeping gene for β-actin. The probes and primers for rat PGC-1α, PGC-1β, and β-actin were obtained from PE Applied Biosystems (Pre-developed TaqMan Assay Reagents Control kits). Real-time PCR was carried out in triplicate for each sample.

RESULTS

Maternal food consumption

Daily food consumption at the end of the pregnancy was similar in low (LCOD), adequate (ACOD), or high (HCOD) corn oil diet groups (21.10 ± 6.12 g; mean ± SD) and varied very little longitudinally during pregnancy. After parturition, food consumption increased progressively, reaching ~42.98 g at day 12 of lactation in the three

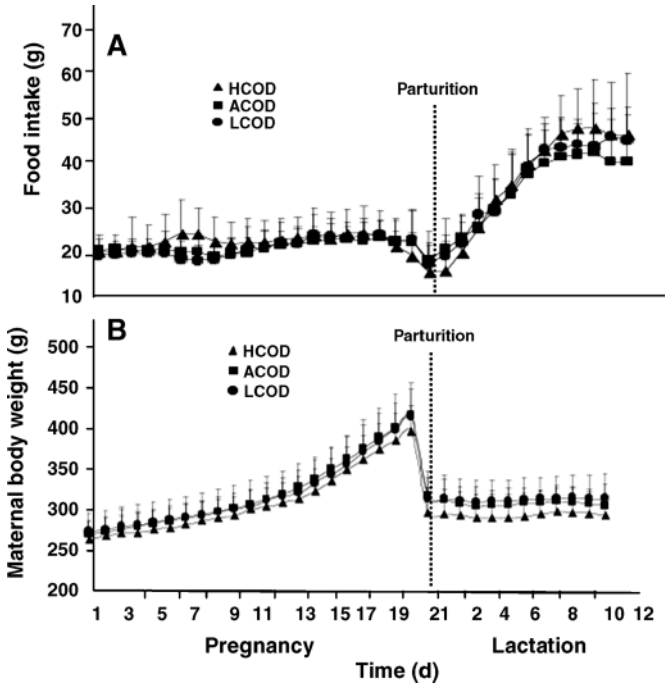

Fig. 1. Food intake (A) and maternal body weight (B) of rat dams fed a low (LCOD), adequate (ACOD), or high (HCOD) corn oil diet. Values are means ± SD (n = 14 rats per group). The diets of groups LCOD, ACOD, and HCOD contained 20, 50, and 100 g/kg corn oil, respectively.

groups, and there was no difference among groups during lactation (**Fig. 1A**).

Maternal weight gain

The body weight of dams increased progressively, and no differences were observed among groups during pregnancy (Fig. 1B). At the end of pregnancy, rats weighed ~415.75 ± 34 g (mean ± SD). After parturition, body weight decreased rapidly to 311.55 g, weight gain varied little longitudinally, and there were no differences among groups despite the different oil contents in the diets.

Measurement of [1-^{14}C]LA incorporation and total oxidation

The intestinal absorption of [1-^{14}C]LA was similar among the three groups, and the average absorption was 83.38%. The main compartment with the highest [1-^{14}C]LA incorporation was the mammary gland, followed by milk clot and litter carcass, suggesting that mammary gland plays an important role in the uptake of dietary LA to synthesize milk lipids. **Table 2** shows that ~35% of the LA in the diet is transferred to the mammary gland. The amount of [1-^{14}C]LA transferred to the mammary gland was defined as the sum of the absorbed dose divided by the total tissue in the mammary gland, milk clot, and the carcass of the pups. Other tissues that showed [1-^{14}C]LA incorporation were the liver followed by the maternal carcass; the lowest incorporation was observed in parametrial adipose tissue (**Table 3**).

The rate of oxidation of dietary LA in the lactating rat fed different concentrations of corn oil in the diet is shown in **Table 4**. These results indicate that the concentration of corn oil in the diet is directly proportional to the total oxidation of LA.

Content of LA and AA in lactating mammary gland

The percentage of LA was 15.4 ± 2.4%, 22.2 ± 0.5%, and 29.9 ± 0.9% (n = 8) in mammary gland of rats fed LCOD, ACOD, and HCOD, respectively. Values are means ± SEM and were significantly different ($P < 0.0001$). The percentage of AA was 1.7 ± 0.3%, 1.4 ± 0.2%, and 1.3 ± 0.2% (n = 8) in mammary gland of rats fed LCOD, ACOD, and HCOD, respectively, and there were no differences between groups.

Δ6D, Δ5D, SREBP-1, and PPARα expression in mammary gland, liver, and adipose tissue of lactating rat

The effect of different concentrations of corn oil in the diet was studied at day 12 of lactation on Δ5D, Δ6D, SREBP1c, and PPARα mRNA expression levels in liver, mammary gland, and adipose tissue. Northern blot analysis in liver and mammary gland identified two transcripts of Δ6D mRNA, dominant and minor, of ~4.0 and 2.2 kb, respectively (**Fig. 2**). Δ6D, Δ5D, SREBP-1, and FAS mRNA concentration decreased as corn oil concentration increased in the diet. These results indicate that the high LA content (55%) in the corn oil diet decreases lipogenesis, as can be seen through its target gene FAS mediated through SREBP-1. Interestingly, the abundance of mRNA PPARα in liver increased as corn oil in the diet increased (Fig. 2A), suggesting that the presence of high LA in the diet increases lipid oxidation. PPARα mRNA was 1.33-fold higher in the HCOD group than in the ACOD and LCOD groups (Fig. 2B).

TABLE 2. Incorporation of [1-^{14}C]LA in mammary gland, milk clot, and litter carcass of rat dams fed different concentrations of corn oil in the diet at day 12 of lactation

Experimental Group	Mammary Gland	Milk Clot	Litter Carcass	Total Transferred to the Mammary Gland (Mammary Gland + Milk Clot + Litter Carcass)
		% of absorbed dose/total tissue		
LCOD	24.3 ± 3.3	10.1 ± 2.2	5.8 ± 1.6	32.6 ± 7.0
ACOD	21.6 ± 1.3	12.1 ± 1.7	4.4 ± 0.6	37.9 ± 2.6
HCOD	22.6 ± 3.2	12.5 ± 2.5	5.1 ± 0.7	34.0 ± 3.1

LA, linoleic acid. The results are means ± SEM.

TABLE 3. Incorporation of [1-^{14}C]LA in different compartments of lactating rats fed different concentrations of corn oil in the diet at day 12 of lactation

Experimental Group	Total [1-^{14}C]LA Incorporation		
	Parametrial Adipose Tissue	Liver	Maternal Carcass
	% of absorbed dose/total tissue		
LCOD	0.54 ± 0.08	8.3 ± 1.2	7.0 ± 1.2
ACOD	0.42 ± 0.11	5.2 ± 0.7	4.5 ± 0.9
HCOD	0.65 ± 0.13	6.1 ± 1.0	5.8 ± 1.4

The results are means ± SEM.

Mammary gland plays an important role in milk synthesis; however, it is not known whether this gland participates in the synthesis of LC-PUFAS through Δ6D and Δ5D and whether these enzymes are regulated by the lipid content in the diet. The results shown in Fig. 2A, B indicate that Δ6D and Δ5D mRNA are present in mammary gland and that, interestingly, both enzymes are regulated by the corn oil content in the diet. The higher the content of corn oil in the diet, the lower the expression of both enzymes. Also, SREBP-1 mRNA was expressed in the mammary gland and decreased by the increasing concentrations of corn oil in the diet, indicating that the synthesis of LC-PUFAs through Δ5D and Δ6D is not specific to the liver and that it can be nutritionally regulated also by SREBP-1. FAS mRNA expression followed a similar pattern (Fig. 2A, B). These data indicate that consumption of corn oil decreases lipogenesis in the mammary gland. On the other hand, PPARα was undetectable by Northern blot analysis and barely detectable by real-time RT-PCR in lactating mammary gland, indicating that oxidation of fatty acid might possibly be limited.

In adipose tissue, Δ5D and Δ6D were present; however, the minor transcript of Δ6D mRNA was not observed. Δ6D and Δ5D, SREBP-1, and FAS mRNA expression were not affected by the presence of different concentrations of corn oil in the diet, and there was minimal expression of PPARα, indicating that the nutritional regulation of Δ6D, Δ5D, and SREBP-1 expression is specific for liver and mammary gland (Fig. 2A).

PGC-1α and PGC-1β

The coordinated repression of Δ6D, Δ5D, and FAS by SREBP-1 in mammary gland in response to dietary corn oil prompted us to investigate whether the coactivators PGC-1α and PGC-1β were regulated by the presence of oil in the diet. The PGC-1 coactivators have powerful transcription activity when they dock on a transcription factor. PGC-1α can bind and coactivate most members of the nuclear receptor family, such as PPARα; however, there was almost negligible expression of PPARα in mammary gland measured by Northern blot and real-time PCR. These results suggest that although PPARα concentration is insignificant, PGC-1α is absolutely required for the normal expression of a large number of mitochondrial genes (16), increasing the enzymatic capacity for fatty acid β-oxidation when animals are fed a high-oil diet. Interestingly, although PGC-1β shares many of its genes with PGC-1α, PGC-1β can interact with SREBP-1. As can be seen in **Fig. 3**, there was a decrease in PGC-1β as the concentration of oil in the diet increased, and this was associated with lower expression of SREBP-1 and its target genes FAS, Δ5D, and Δ6D; however, it was only significant in animals fed the 10% corn oil diet. These results suggest that PGC-1β is involved in the repression of lipogenic genes after consumption of the high concentration of LA contained in the corn oil diet. This response was mediated through the repression of SREBP-1 in mammary gland, contrary to that found in animals fed high saturated fats, in which PGC-1β was strongly induced (17). These results raise the interesting possibility that the induction of PGC-1β is influenced by the dietary type of fat and under different physiological conditions, such as the lactation.

TABLE 4. Total oxidation of [1-^{14}C]LA in lactating rats fed different concentrations of corn oil at day 12 of lactation

Experimental Diet (g LA/100 g)	CO_2 Production	
	% CO_2 of absorbed dose	*g LA oxidized/100 g diet*
LCOD (1.2)	18.09 ± 3.14	0.22 ± 0.04[a]
ACOD (3.0)	15.95 ± 1.85	0.48 ± 0.06[a]
HCOD (6.0)	17.43 ± 2.0	1.05 ± 0.12[a]

The results are means ± SEM. LA, linoleic acid.
[a] $P < 0.05$ by ANOVA.

DISCUSSION

During pregnancy and lactation, there is an increased need of nutrients to meet the energy and amino acid requirements for both the mother and the infant (18). To fulfill the demand of nutrients by the newborn, the mother provides them through the milk to the infant. Thereby, the lactating mammary gland has a large demand of nutrients for milk synthesis and to maintain the metabolic functions of lactocytes (19). Among the nutrients present in the milk are the LC-PUFAs, which play an important role in the neurological development of the newborn (20–22). Interestingly, low dietary intake of PUFAs in the diet results in an adequate amount of the fatty acids in the milk (11), suggesting that mammary gland could play an important role in the synthesis of LC-PUFAs. Previous reports indicated that liver was considered the primary site of AA, eicosapentanoic acid, and DHA production for peripheral tissues (23). However, in this study, we observed that ~35% of [^{14}C]LA was transferred directly from the diet to the mammary gland, and 12% was transferred to the milk independently of the lipid content in the diet, an amount similar to that reported in lactating women (24, 25).

These results indicate that mammary gland could be a temporal maternal pool of LA during lactation before it is secreted in the milk or metabolized to AA. Assuming that the transfer rate of the LA tracer is applicable to nonlabeled dietary LA, we suggest that ~35% of dietary LA is used to

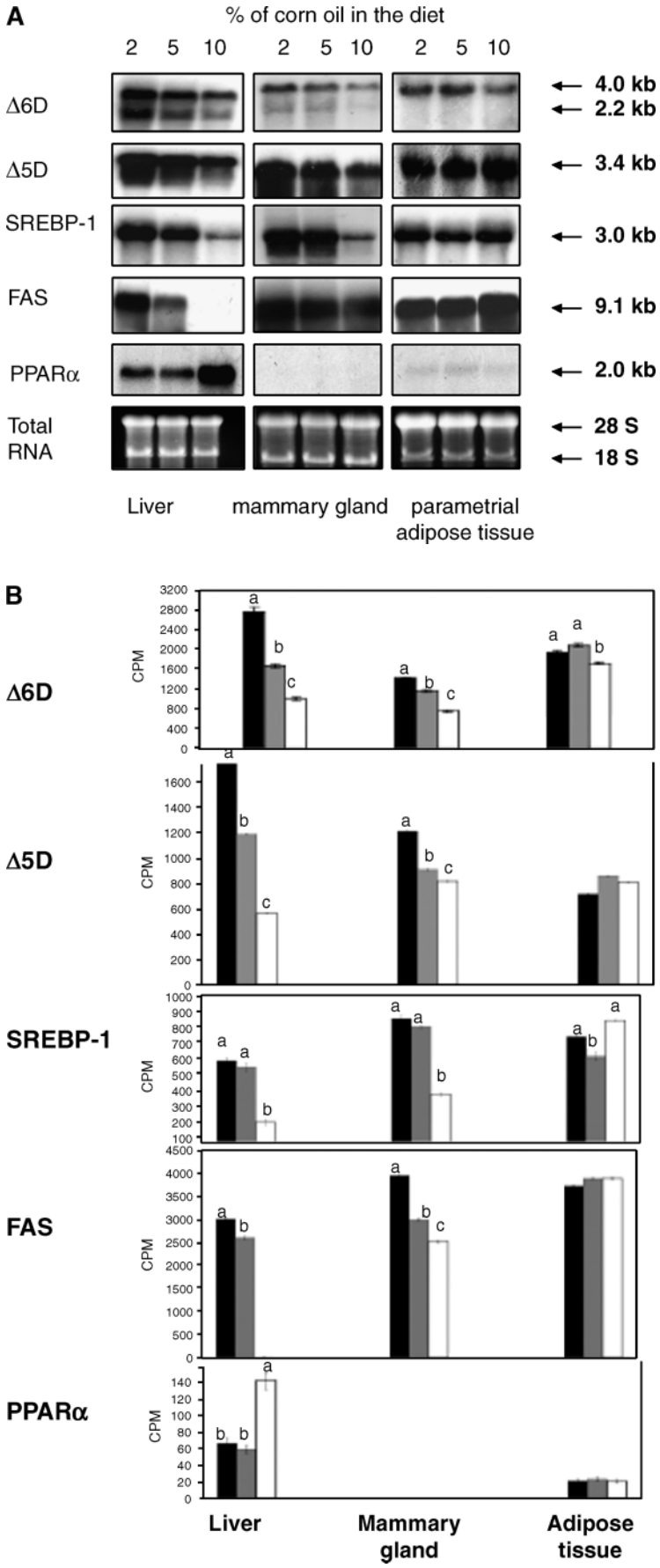

Fig. 2. A: Northern blot analysis of Δ6 and Δ5 desaturases (Δ6D and Δ5D), sterol-regulatory element binding protein 1 (SREBP-1), FAS, and peroxisome proliferator-activated receptor α (PPARα) mRNAs in liver, mammary gland, and adipose tissue of lactating rats fed the LCOD, ACOD, or HCOD. Total RNA samples (15 μg) were pooled equally from each group and electrophoresed followed by hybridization with the Δ6D, Δ5D, SREBP-1, FAS, and PPARα cDNA probes. Ethidium bromide staining of total RNA was used to confirm equal loading. B: Cpm of Δ6D, Δ5D, SREBP-1, FAS, and PPARα mRNA in liver, mammary gland, and adipose tissue. Black bars, LCOD; gray bars, ACOD; white bars, HCOD. Values are means ± SEM (n = 3). Different letters above the bars indicate significant differences among groups: a > b > c.

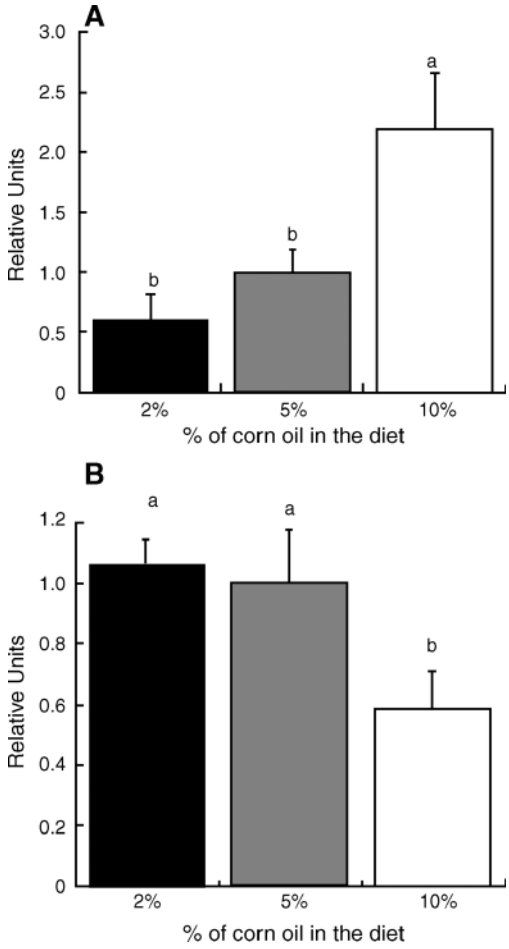

Fig. 3. Gene expression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (A) and PGC-1β (B) in lactating mammary gland of rats fed LCOD, ACOD, or HCOD as determined by real-time PCR assays. PCR amplification was performed in a total volume of 10 μl containing 22 ng of cDNA sample, 900 nM of each primer, and 12.5 μl of TaqMan Universal PCR Master Mix. Black bars, LCOD; gray bars, ACOD; white bars, HCOD. Expression of mRNA is shown as relative units. Values are means ± SEM (n = 3). Different letters above the bars indicate significant differences among groups: a > b.

synthesize lipids of milk. In addition, our results show the presence of Δ5D and Δ6D in the mammary gland, indicating the capacity of the gland to synthesize DHA and AA from essential PUFAs. Δ5D showed only one transcript of 3.4 kb in liver, mammary gland, and adipose tissue. Δ6D showed two transcripts, of 4 and 2.2 kb in liver and mammary gland, whereas adipose tissue expressed only one transcript of 4 kb (Fig. 2A). Interestingly, the expression Δ5D and Δ6D were downregulated by increasing amounts of corn oil in the diet only in liver and lactating mammary gland, indicating that besides the liver (3, 4), mammary gland plays an important role in the synthesis of LC-PUFAs during lactation. These results are in agreement with previous studies that demonstrated that n-6 and n-3 PUFAs suppress the hepatic expression of rodent Δ6D by inhibiting the rate of Δ6D gene transcription. The inhibition of Δ6D gene transcription applies to both rat and human Δ6D (9).

It has been reported that in liver, SREBP-1 activates genes of fatty acid synthesis, including all three desaturases (Δ6D, Δ5D, and Δ9D stearoyl-CoA). Sterol-regulatory elements have been identified in the promoters of Δ6D, Δ9D, and probably Δ5D, although they have not been demonstrated. It was reported that PUFAs decrease the expression of Δ5D and Δ6D in liver by downregulating SREBP-1 expression (8). In this study, we demonstrated that high concentrations of corn oil in the diet decreased the expression of SREBP-1, leading to a reduction in the expression of Δ5D, Δ6D, and FAS in the mammary gland, indicating that there is no need to synthesize LC-PUFAs.

Furthermore, there was an increase of 1.18-fold more $^{14}CO_2$ in rats fed HCOD than in rats fed ACOD, and HCOD rats expired 3.7-fold more $^{14}CO_2$ than rats fed LCOD, indicating an increase in dietary fatty acid oxidation as the concentration of dietary corn oil increased. This is in agreement with previous studies that showed a decrease in dietary fatty oxidation during lactation in rats fed a low-fat diet (12, 26). Fatty acid oxidation seems to occur preferentially in liver via PPARα, because mammary gland and adipose tissue expressed low levels of PPARα. Therefore, the higher the concentration of corn oil in the diet, the higher the expression of PPARα to increase the oxidation of fatty acids in the liver. More research is needed to clarify the mechanisms of fatty acid oxidation in the lactating mammary gland.

Thus, when the amount of PUFAs in the diet is low (2% corn oil), there is an adaptive mechanism to synthesize LC-PUFAs through an increase in the expression of SREBP-1 in mammary gland and liver, which in turn increases the expression of Δ5D, Δ6D, and FAS. At the same time, there is a reduction in the expression of hepatic PPARα, reducing the oxidation of fatty acids. These adaptations allow the mammary gland to synthesize milk with adequate concentrations of the essential and LC-PUFAs required to meet the needs of the newborn.

On the other hand, when the oil content in the diet is high (10%), there is no need to synthesize LC-PUFAs, leading to a reduction in the expression of Δ6D, Δ5D, SREBP-1, and FAS and increasing the oxidation of fatty acid mediated by PPARα in liver but not in mammary gland. The PGC-1 coactivators have powerful transcription activity when they dock on the transcription factor PPARα; however, there was almost negligible expression of PPARα in mammary gland, suggesting that although PPARα concentration is insignificant, PGC-1α is required for the normal expression of a large number of mitochondrial genes (16), increasing the enzymatic capacity for fatty acid β-oxidation when animals are fed a high-oil diet. Low expression of PGC-1β was associated with low expression of SREBP-1 and its target genes FAS, Δ5D, and Δ6D (Figs. 2A, 3). These results suggest that PGC-1β is involved in the repression of lipogenic genes after consumption of the high concentrations of LA contained in the high-corn oil diet. These results raise the interesting possibility that the induction of PGC-1β is influenced by the amount of PUFAs in lactating mammary gland.

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