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Role of maternal tissue in the synthesis of polyunsaturated fatty acids in response to a lipid-deficient diet during pregnancy and lactation in rats

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ABSTRACT

During pregnancy and lactation, metabolic adaptations involve changes in expression of desaturases and elongases (Elovl2 and Elovl5) in the mammary gland and liver for the synthesis of long-chain polyunsaturated fatty acids (LC-PUFAs) such as arachidonic acid (AA) required for fetal and postnatal growth. Adipose tissue is a pool of LC-PUFAs. The response of adipose tissue for the synthesis of these fatty acids in a lipid-deficient diet of dams is unknown. The aim of this study was to explore the role of maternal tissue in the synthesis of LC-PUFAs in rats fed a low-lipid diet during pregnancy and lactation. Fatty acid composition (indicative of enzymatic activity) and gene expression of encoding enzymes for fatty acid synthesis were measured in liver, mammary gland and adipose tissue in rats fed a low-lipid diet. Gene expression of desaturases, elongases, fatty acid synthase (Fasn) and their regulator Srebf-1c was increased in the mammary gland, liver and adipose tissue of rats fed a low-lipid diet compared with rats from the adequate-lipid diet group throughout pregnancy and lactation. Genes with the highest ($P < 0.05$) expression in the mammary gland, liver and adipose tissue were Elovl5 (1333%), Fads2 (490%) and Fasn (6608%), respectively, in a low-lipid diet than in adequate-lipid diet. The percentage of AA in the mammary gland was similar between the low-lipid diet and adequate-lipid diet groups during the second stage of pregnancy and during lactation. The percentage of monounsaturated and saturated fatty acids was significantly ($P < 0.05$) increased throughout pregnancy and lactation in all tissues in rats fed a low-lipid diet than in rats fed an adequate-lipid diet. Results suggest that maternal metabolic adaptations used to compensate for lipid-deficient diet during pregnancy and lactation include increased expression of genes involved in LC-PUFAs synthesis in a stage- and tissue-specific manner and elevated lipogenic activity (saturated and monounsaturated fatty acid synthesis) of maternal tissues including adipose tissue.

1. Introduction

During pregnancy and lactation, females supply nutrients, e.g., long-chain polyunsaturated fatty acids (LC-PUFAs) such as arachidonic acid (AA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids for fetal and postnatal development (Rapoport et al., 2011). Human fetuses and newborns synthesize insufficient amounts of LC-PUFAs; therefore, maternal tissue and milk lipids serve as the primary source (Carlson et al., 1986; Uauy et al., 1996). LC-PUFAs are acquired from the diet, mobilized from adipose tissue reserves or synthesized de novo in the maternal organism (Del Prado et al., 2001; Rodríguez-Cruz et al., 2006, 2009). Therefore, their amount in maternal tissues is partially dependent upon the rate of synthesis (Nakamura, 2004).

Fatty acid elongation and desaturation are two key metabolic pathways for the synthesis of LC-PUFAs from their dietary precursors, the essential PUFAs linoleic acid (LA) and α-linolenic acid (ALA), by fatty acid desaturases (Fads) 1 and 2 and elongases Elovl2 and 5 (Guillou et al., 2010). The expression and activity of these enzymes change as a result of nutritional and physiological factors (Jump, 2008; Rodriguez-Cruz et al., 2011, 2006). For example, a low-fat diet significantly increases the abundance and enzymatic activity of Elovl5, Fads1 and Fads2 mRNAs in the liver and mammary gland (Jump, 2008; Qin et al., 2009; Rodriguez-Cruz et al., 2011, 2006). This mechanism is regulated mainly by sterol-regulatory element binding transcription factor 1c (Srebf-1c) activity, which is mediated by sterol regulatory elements present in the regulatory region of these target genes [desaturases, elongases and fatty acid synthase (Fasn)] (Kumadaki et al., 2008; Nara et al., 2002).

Abbreviations: LC-PUFAs, long-chain polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; ALA, α-linolenic acid; Fads1, fatty acid desaturase 1; Fads2, fatty acid desaturase 2; Elovl, elongation of very long chain fatty acids; Srebf-1c, sterol-regulatory element binding transcription factor 1c; Fasn, fatty acid synthase; LLD, low-lipid diet; ALD, adequate-lipid diet; D2Pw, day 2 of post weaning; DEPC, diethylpyrocarbonate; CHREBP, carbohydrate-responsive element-binding protein; HNF4α, hepatic nuclear factor 4 alpha; MLX, max-like factor-X; PPARγ, peroxisome proliferator-activated receptor alpha; RXR, retinoid-X receptor; LXR, liver X receptor; MFA, monounsaturated fatty acids; Scd, stearoyl-CoA desaturase; MCSFA, medium-chain saturated fatty acids.

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In response to the high lipid demands during pregnancy and lactation, metabolic adaptations are triggered, including the participation of extrahepatic tissues and increased fat storage in the adipose tissue from early pregnancy (Cetin et al., 2009). Our previous studies suggest that these tissues include the mammary gland and, together with the liver, play an important role in the uptake of LA and its conversion to synthesize AA, depending on the dietary lipid content during lactation in rats (Rodriguez-Cruz et al., 2011, 2006, 2009). For instance, expression of enzymes (Fads1 and Fads2) involved in AA synthesis (Rodríguez-Cruz et al., 2006) and conversion of LA to AA (Rodríguez-Cruz et al., 2009) were increased in the mammary gland and liver of animals fed a low-fat diet. Nevertheless, these parameters were evaluated only during the day of increased milk production (day 12 of lactation). It is significant to highlight that the demands for LC-PUFAs increase from the last period of pregnancy and throughout lactation for fetal and newborn development. We recently proposed that synthesis of LC-PUFAs is mainly conducted by the mammary gland during these physiological stages (Rodriguez-Cruz et al., 2011). These findings support the hypothesis that lactating mammary tissue and the liver can possess different checkpoint controls throughout pregnancy and lactation (Rudolph et al., 2007). Despite the fact that adipose tissue expresses the genes encoding enzymes involved in LC-PUFAs synthesis, this tissue does not appear to actively synthesize Fads1 and Fads2 (Rodriguez-Cruz et al., 2011). Consequently, adipose tissue could serve as a reservoir to supply these fatty acids to the fetus or newborn when the mother is fed an adequate-lipid diet.

According to this metabolic scheme, it appears that pregnancy and lactation cause tight coordination between the mammary gland and liver to synthesize LC-PUFAs in response to the high demand of the fetus and newborn, whereas adipose tissue works as a reservoir for such fatty acids. Nevertheless, it is more difficult for mothers fed a lipid-deficient diet to supply them sufficient LC-PUFAs to avoid alterations in fetal development (Bhatia et al., 2011). The response of tissues such as liver, mammary gland and adipose tissue mainly providing LC-PUFAs under these nutritional conditions is not well known. We hypothesized that metabolic adaptations consist of an increased expression of the enzymes involved in LC-PUFAs synthesis and their regulator Srebf-1c in the tissues supplying LC-PUFAs including adipose tissue in a mother fed a lipid-deficient diet throughout pregnancy and lactation. To test this hypothesis, in the liver, mammary gland and adipose tissue at various stages of pregnancy and lactation in rats fed a lipid-deficient diet, we analyzed the following: fatty acid composition and expression of desaturases (Fads1 and Fads2), elongases (Elovl2 and Elovl5), and Srebf-1c (and its target gene, Fasn) (Kumadaki et al., 2008). Srebf-1c was included to explore it as a possible mechanism for the regulation of desaturases and elongases. Enzymatic activity of desaturases and elongases was indirectly measured by the determination of fatty acid composition. We also measured the fatty acid composition in the milk clot to determine whether the metabolic adaptations were sufficient to reach the concentration of LC-PUFAs in the milk of rats fed an adequate-lipid diet.

2. Materials and methods

2.1. Materials

Fatty acid methyl ester standards for chromatographic analysis were obtained from PolySciences (Niles, IL). All other chemicals and solvents were reagent molecular grade from usual commercial sources.

2.2. Experimental animals and tissue collection

One hundred and fifty six female Sprague Dawley rats were obtained from the Animal Care Facility of the Centro Médico Nacional Siglo XXI, Mexican Institute of Social Security (IMSS) in Mexico City. Animals (maximum of five rats per cage) were housed at 22 ± 2 °C with a 12-h light/dark cycle and had free access to water. From weaning until 8 weeks of age, rats were fed a Chow 5008 commercial diet (Agribrands Purina, México, D.F., México) containing 24 g of protein, 61 g of carbohydrates, and 5 g of fat per 100 g of dry weight. When rats reached 8 weeks of age, they were adapted and randomly allocated to a purified diet for 6 weeks of either a low-lipid diet (2% lipids) with 22 g of protein, 67.2 g of carbohydrates and 2 g of fat or adequate-lipid diet (5% lipids) with 22.2 g of protein, 61.4 g of carbohydrates and 5 g of fat as previously reported (Rodríguez-Cruz et al., 2006, 2009). Lipids were obtained from corn oil rich in LA and whose fatty acid composition was 12.1% C16:0, 2.2% C18:0, 30.7% C18:1, 1% C18:3n − 3 and 54% C18:2n − 6. At 14 weeks of age, rats from each diet were randomly assigned to two groups: one group of nonpregnant rats (control, n = 6) and another group of rats that were mated and randomly assigned to pregnant (P) or lactating (L) groups for each diet. Nonpregnant and pregnant rats were housed individually and were sacrificed at days 0, 1, 5, 10, 14, 16, and 20 of pregnancy; days 1, 5, 10, 12, and 20 of lactation; and at day 2 of postweaning (D2Pw). Six rats from each subgroup of pregnancy or lactation were included. The day of parturition was considered as day 0 of lactation. Litters were adjusted to eight pups per dam, and food intake and body weight of pups and their mothers were registered daily (Fig. 1). A purified diet was administered ad libitum during pregnancy and lactation. The protocol was approved by the Animal Care Ethics Committee of the IMSS, Mexico.

Rats were euthanized according to the Mexican Official Norm (NOM-062-ZOO-1999). In this study, rats were decapitated after diethyl ether anesthesia, and whole mammary gland, liver, and parametrial adipose tissue were removed. Tissues were harvested, rapidly frozen in liquid nitrogen, and stored at −70 °C for subsequent RNA isolation and total lipid extraction. Pups were euthanized after diethyl ether anesthesia and their stomachs were then dissected. Milk clots were removed, weighed, mixed and stored at −70 °C for subsequent total lipid extraction and fatty acid composition.

2.3. RNA isolation, cDNA synthesis and quantitative real-time (qRT)-PCR

Frozen tissues were pulverized and total RNA was isolated from the mammary gland, liver and adipose tissue (0.1–0.2 g) of rats using a TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA concentration was determined by absorbance at 260 nm, and the samples were diluted to 1 μg/μl in RNAse-free water. RNA integrity was evaluated via electrophoresis in a 1% agarose gel with ethidium bromide staining (1.25 ng/μl, Sigma). First-strand cDNA from tissues was synthesized from 2 μg of the total RNA using the MMLV reverse transcriptase. Total RNA was preincubated with random primers and dNTPs (100 mM) at 65 °C for 5 min. The product was then incubated in a reaction buffer (250 mM Tris–HCl, 375 mM KCl, and 15 mM $MgCl_2$) and DTT (0.1 M) at 37 °C for 2 min. Finally, cDNA synthesis was performed using the MMLV reverse transcriptase. RNase-free deionized diethyl pyrocarbonate (DEPC)-treated ultrapure MB grade water (USB Corporation, Cleveland, OH) was used to achieve a final volume of 20 μl. cDNA synthesis was performed at 25 °C for 10 min followed by 37 °C for 50 min and 70 °C for 15 min. All reagents were from Invitrogen. Before qRT-PCR analysis, cDNA quality was tested by final-point PCR amplification of the reference β-actin gene.

The relative mRNA levels of the target genes and β-actin reference transcript as previously reported for mammary tissue (Kelleher and Lónnerdal, 2005; Naylor et al., 2005; Rodriguez-Cruz et al., 2011, 2006) were determined by qRT-PCR using cDNA preparation for the tissues. cDNA synthesized from the tissues was mixed with Light Cycler Fast Start DNA Master^{PLUS} SYBR Green I (Roche, Indianapolis, IN) and various sets of gene-specific forward and reverse primers (Table 1). The mixture was then subjected to RT-PCR quantification using the Light Cycler 3.5 Detection System (Roche). qRT-PCR was performed in specially designed Light Cycler capillaries in 20 μl total volume