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The effect of gestational age on expression of genes involved in uptake, trafficking and synthesis of fatty acids in the rat placenta

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ABSTRACT

Gestation triggers a tight coordination among maternal tissues to provide fatty acids (FA) to the fetus through placental transport; however, there is insufficient evidence regarding regulation of proteins involved in placental transport of FA according to gestational age. The aim of this study was to determine the role of gestational age on the expression of genes involved in FA uptake, trafficking and synthesis in the rat placenta to support fetal demands. Gene expression of encoding proteins for placental transport and synthesis of FA was measured in placenta. Also, FA composition was measured in placenta, fetuses and newborns. mRNA expression of lipoprotein lipase (*lpl*) and *fatp-1* (for uptake) was 4.4- and 1.43-fold higher, respectively, during late gestation than at P14, but expression of *p-fabp-pm* decreased 0.37-fold at late pregnancy in comparison with P14. Only mRNA *fabp-4* member for trafficking of FA was 2.95-fold higher at late gestation than at P14. mRNA of *fasn* and *elovl-6* participating in saturated FA and enzymes for the polyunsaturated FA synthesis were downregulated during late gestation and their regulator *srebf-1c* increased at P16. This study suggests that gestational age has an effect on expression of some genes involved in uptake, trafficking and synthesis of FA in the rat placenta; mRNA expression of *lpl* and, *fatp-1* for uptake and *fabp-4* implicated in trafficking was expressed at high levels at late gestation. In addition, placenta expresses the mRNAs involved in FA synthesis; these genes were expressed at low levels at late gestation. Additionally, mRNAs of *Srebf-1c* transcriptional regulator of desaturases and elongases was highly expressed during late gestation. Finally, these changes in the rat placenta allowed the placenta to partially supply saturated and monounsaturated FA to the fetus.

1. Introduction

Both humans and rat possess hemochorial placentation with fetal-derived trophoblasts bathed in maternal blood and well positioned to regulate placental transport functions between maternal and fetal blood. Among transported nutrients, uptake and trafficking of fatty acids (FA) is critical for embryonic growth and neural development in all eutherians, particularly during the second half of pregnancy when the fetal/placental growth ratio is markedly increased, corresponding to increasing fetal caloric demands (Soares et al., 2012). Placental transfer of FA is a complex process involving binding to membrane proteins for uptake and cytoplasmic trafficking proteins, although the biochemical mechanisms are not fully understood. Albumin-bound FA, very-low-density lipoproteins, and chylomicrons in the maternal circulation are the major source of FA to the placenta and require the action of triglyceride (Tg) hydrolases such as lipoprotein lipase (Lpl) (Bonet et al., 1992; Magnusson et al., 2004; Magnusson-Olson et al., 2007), allowing FA to be taken up by the placenta (Magnusson-Olson et al., 2006). Several membrane proteins are thought to be responsible for FA uptake in placenta, such as placenta-specific plasma membrane-associated FA binding protein (pFabp-pm) (Campbell and Dutta-Roy, 1995) and FA translocase (Fat) (Magnusson-Olson et al., 2007; Cunningham and McDermott, 2009; Dubé et al., 2012). FA transport in the placenta is conducted by Fatp-1 and Fatp-4 (Duttaroy, 2009). Localization of Fatp-4 expression in trophoblasts and the yolk sac of the developing wild-type mouse chorioallantoic placenta suggest a role for Fatp-4 in

Abbreviations: pFabp-pm, placenta-specific plasma membrane associated fatty acid binding protein; Fat, fatty acid translocase; Fatp, fatty acid transport proteins; Fabp, fatty acid binding proteins; LC-PUFAs, long-chain polyunsaturated fatty acids; Scd, stearoyl-CoA desaturase; Elovl, elongation of very long chain fatty acids; AA, arachidonic acid; LA, linoleic acid; ALA, α-linolenic acid; Fads, fatty acid desaturase; Srebf-1c, sterol-regulatory element binding transcription factor 1c; DEPC, diethylpyrocarbonate; Tbp, box binding protein; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; HNF, hepatocyte nuclear factor; EFAs, essential fatty acids.

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fat absorption in early embryogenesis (Cunningham and McDermott, 2009). Within cells, cytoplasmic FA are bound by FA binding proteins (Fabps) thought to be involved in intracellular trafficking. Fabp-1, -3, and -4 members have been detected in the syncytiotrophoblast, but their function in intracellular trafficking of FA in trophoblasts is unknown (Mishima et al., 2011).

In addition to FA supplied by synthesis in liver and adipose tissue, endogenous FA synthesis is observed in rapidly proliferating tissues such as the placenta, although little information is available on placenta *de novo* FA synthesis (Wilentz et al., 2000). FA elongation and desaturation are two key metabolic routes for the synthesis of saturated, monounsaturated, and long-chain PUFA (LC-PUFA). Saturated FA either synthesized *de novo* in the cytosol by Fasn or derived from the diet can be further desaturated by stearoyl-CoA desaturase (Scd) to produce monounsaturated and/or elongated FA by elongases Elovl-1, Elovl-6 and Elovl-7 into long-chain FA (16C, 18C, 20C and 22C) (Guillou et al., 2010; Sánchez et al., 2014). LC-PUFA such as arachidonic acid (AA), EPA, and DHA are synthesized from their dietary precursor essential PUFA linoleic acid (LA) and α-linolenic acid (ALA) by FA desaturases (Fads) 1 and 2 and elongases Elovl-2 and Elovl-5 (Guillou et al., 2010). It has been demonstrated that the tight maintenance of cellular levels of FA (saturated, mono- and polyunsaturated) is to some extent controlled 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 *Fasn*) (Nara et al., 2002; Kumadaki et al., 2008; Rodríguez-Cruz et al., 2012). Srebfs are a family of transcriptional factors isolated as a result of their properties to join to sterol response elements (SRE). Srebfs family is formed by Srebf-1a, Srebf-1c and Srebf-2. The first two are encoded by only one gene, but transcribed by different promoters. Srebf-1c, the predominant isoform expressed in different tissues as the liver, macrophages, white adipose tissue, adrenal gland and brain. Although there is a certain superposition among different isoforms, Srebf-1c is mainly responsible for the expression of genes implicated in lipogenesis, whereas Srebf-2 activates genes implicated in cholesterol metabolism (Xu et al., 2013); however, information about the role of Srebf-1c in lipogenesis in the placenta is lacking. Because, isoform Srebf-1c is principally responsible in lipid synthesis, this information led us to propose that it could be also expressed in placenta and its mRNA expression pattern change throughout gestation in response to increasing fetal lipids demands.

In response to the high lipid demands during pregnancy, metabolic adaptations are triggered including the participation of extrahepatic tissues and increased fat storage in adipose tissue from early pregnancy (Cetin et al., 2009). Our previous studies suggest that these tissues include the mammary gland. Together with the liver and adipose tissue, they play an important role in the increased expression of genes involved in synthesis and increased uptake of FA and its conversion to synthesize LC-PUFA in a stage- and tissue-specific manner to meet the high demand of FA during pregnancy and lactation (Rodríguez-Cruz et al., 2006, 2009, 2011; Sánchez et al., 2014). According to this metabolic scheme, pregnancy causes tight coordination among maternal tissues to provide FA to the fetus; therefore, the placenta could play an important role in the supply of FA. However, mRNA expression of all placental proteins involved in uptake, trafficking and FA synthesis is largely unknown. Although transcript of only some of these proteins has been identified in placenta, their regulation is unknown during different stages of pregnancy. In particular, FA requirement increases during the last period of pregnancy due to the growth spurt of the fetus, with an increase in triglycerides (per mg placenta) at the 15th and 20th days of pregnancy (Diamant et al., 1980). This led us to hypothesize that, in order to maintain FA homeostasis, the placenta should be able to regulate transfer of FA or possibly synthesize these to adapt to constant changes in demands of its own as well as for the developing fetus. Therefore, gestational age could regulate the expression of mRNA from encoding enzymes involved in placental transfer of FA. To test this hypothesis, we established the following: to evaluate if the transcript of all elongases and desaturases involved in FA synthesis is expressed in placenta and to determine the role of gestational age on the mRNA expression of proteins involved in FA uptake, trafficking and synthesis in placenta. We then analyzed mRNA expression of *srebf-1c* as a possible regulator of those placental mRNAs at different stages of pregnancy. Finally, FA composition was measured in placenta, fetus and newborns with the purpose to identify changes in their proportion to support rapid cellular growth of the fetus.

2. Materials and methods

2.1. Materials

FA 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

Twenty one 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. A maximum of five rats per cage 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 Chow 5008 commercial diet (Agribrands Purina, Mexico, D.F.) containing 24 g of protein, 61 g of carbohydrates, and 5.0 g of fat per 100 g of dry weight. When rats reached 8 weeks of age, they were adapted to a purified diet for 6 weeks with adequate nutrients as previously reported (Table 1) (Rodríguez-Cruz et al., 2006, 2009, 2011; Sánchez et al., 2014). At 14 weeks of age, rats were mated and randomly assigned to three groups (n = 7). Pregnant rats were housed individually and sacrificed. Because fetal lipid deposition increases during late gestation, we analyzed these changes at the 14th, 16th and 20th days of pregnancy (P14, P16 and P20). A purified diet was administered ad libitum during pregnancy. 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 all placentas and

Table 1
Composition of experimental diets.

Ingredient	g/kg diet
Casein	222
Glucose	307.5
Corn starch	307.5
Corn oil[a]	50
Vitamin mix[b]	10
Mineral mix[c]	40
Cellulose	63
Energy (kJ/g)	15.82

[a] Fatty acid composition is 12.1% palmitic acid (C16:0), 2.2% stearic acid (C18:0), 30.7% oleic acid (C18:1), 1% linolenic acid (C18:3n-3) and 54% linoleic acid (C18:2n-6).
[b] 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-tocopherol acetate, 24.23 g; corn starch, 466.67 g.
[c] 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.