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Modulation of Fatty Acid Transport and Metabolism by Maternal Obesity in the Human Full-Term Placenta[1]

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ABSTRACT

Knowledge of the consequences of maternal obesity in human placental fatty acids (FA) transport and metabolism is limited. Animal studies suggest that placental uptake of maternal FA is altered by maternal overnutrition. We hypothesized that high maternal body mass index (BMI) affects human placental FA transport by modifying expression of key transporters. Full-term placentas were obtained by vaginal delivery from normal weight (BMI, 18.5–24.9 kg/m^2) and obese (BMI > 30 kg/m^2) women. Blood samples were collected from the mother at each trimester and from cord blood at delivery. mRNA and protein expression levels were evaluated with real-time RT-PCR and Western blotting. Lipoprotein lipase (LPL) activity was evaluated using enzyme fluorescence. In vitro linoleic acid transport was studied with isolated trophoblasts. Our results demonstrated that maternal obesity is associated with increased placental weight, decreased gestational age, decreased maternal high-density lipoprotein (HDL) levels during the first and third trimesters, increased maternal triglyceride levels during the second and third trimesters, and increased maternal T3 levels during all trimesters, and decreased maternal cholesterol (CHOL) and low-density lipoprotein (LDL) levels during the third trimester; and increased newborn CHOL, LDL, apolipoprotein B100, and T3 levels. Increases in placental CD36 mRNA and protein expression levels, decreased SLC27A4 and FABP1 mRNA and protein and FABP3 protein expression, and increased LPL activity and decreased villus cytotrophoblast linoleic acid transport were also observed. No changes were seen in expression of PPARA, PPARD, or PPARG mRNA and protein. Overall this study demonstrated that maternal obesity impacts placental FA uptake without affecting fetal growth. These changes, however, could modify the fetus metabolism and its predisposition to develop diseases later in life.

CD36, FABP, fatty acids, human full-term placenta, LPL, obesity, PPAR, SLC27A4

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INTRODUCTION

Obesity is increasing at an alarming rate in North America [1]. It has been estimated that by 2030, 86.3% of all Americans will be overweight or obese [2]. Coincident with the rise in obesity, the number of obese women who enter pregnancy has reached a peak. In the United States, at least one-third of women of reproductive age are obese [3]. In addition, obese women are more likely to suffer from chronic health issues, such as gestational diabetes [4] and reproductive disorders [5]. Maternal obesity can also affect the infant's health by increasing fetal adiposity [6], inducing early onset of metabolic disorders such as type 2 diabetes [7], and increasing the risk of birth defects [8].

Essential fatty acids (EFA) and their long-chain polyunsaturated FA derivatives (LC-PUFA) are crucial for fetal growth and development [9, 10]. Fatty acids (FA) are especially important during the third trimester of pregnancy, when the fetus intensifies its nutrient demand for exponential growth. To satisfy its need for FA, the fetus depends on the maternal diet as well as on placental transport and metabolism [11]. FA from the maternal blood circulation are present either as free FA (FFA) bound to albumin or are incorporated into lipoproteins from which they can be dissociated by placental lipoprotein lipase (LPL). FA can then be transferred to the fetus by simple diffusion or through transport proteins [12]. Several proteins involved in lipid transport have been identified in the human placenta, including CD36 (also known as FA translocase [FAT]), placental plasma membrane FA binding protein (FABPpm), members of solute carrier family 27 (SLC27As; also known as FA transport proteins [FATPs]), and intracellular FA binding proteins (FABPs). CD36 is a glycosylated FAT protein located on both the placental microvillus and the basal membranes [13]. CD36 has multiple ligands including FFA, collagen, and oxidized low-density lipoprotein (LDL) [14]. While CD36 has been shown to be involved in FA uptake in the heart and skeletal muscle [15], little is known about its function in the placenta. Human pFABPpm is present only on the microvillus membrane of the syncytiotrophoblast [16, 17] and binds preferentially to LC-PUFA and EFA [18, 19]. SLC27A proteins are part of a family of six integral transmembrane proteins. It has been shown that overexpression of SLC27As increases the rate of FA internalization [20, 21]. SLC27A1 (also known as FATP1) was the first member of this family identified in the human placenta and is localized on both the microvillus and the basal membranes [13]. Expression of *SLC27A2* to *SLC27A6* (also known as *FATP2-6*) mRNA was later confirmed in human full-term placenta [22, 23]. The strong expression levels of SLC27A1 and SLC27A4 in human placenta suggests an important role for these proteins in FA

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transfer. Moreover, the embryonic lethality caused by deletion of the *SLC27A4* gene in mice reveals its crucial role in maternal-fetal FA transport during embryogenesis [24]. However, the specificity of each SLC27A for a particular FA is either unknown or specific to the isoform [25]. The presence of all these FA carriers on placental membranes suggests a directional transport between the maternal and fetal circulations. It has been demonstrated that both placental microvillus and basal membranes are involved in the uptake of linoleic acid, with preferential transport of linoleic acid by the microvillus membrane of the syncytiotrophoblast [26]. In addition, several forms of FABPs are expressed by human trophoblasts including FABP3 (heart-FABP) and FABP1 (liver-FABP) [13, 27–29]. These intracellular proteins are involved in the uptake and trafficking of FA in the cytosol [30]. However, the exact mechanisms underlying FA uptake by transport proteins are still uncertain in the human placenta.

Placental FA transport is likely to be the target of important maternal and fetal regulation due to its preponderant contribution in weight control of the newborn either by promoting or limiting FA transfer to the fetus. We hypothesized that the maternal lipid profile influences placental FA uptake by human trophoblasts. Therefore, this study was designed to characterize the effects of high body mass index (BMI; kg/m^2) during pregnancy on maternal and newborn circulating lipids, expression of several FA carriers, and on linoleic acid uptake by trophoblasts.

MATERIALS AND METHODS

Subjects

Women participating in this study were recruited between 2002 and 2006 at their first prenatal visit (before their 10th week of pregnancy) at the Clinique Fidès (Montreal, QC, Canada) or at the Centre Hospitalier de l'Université de Montréal (CHUM)-Hôpital Saint-Luc, Montreal, QC, Canada). Each participant gave informed consent and completed an interviewer-administered questionnaire on sociodemographic characteristics, age, medical history, drinking and smoking habits, weight, and height. Of the 900 women recruited, 150 women were enrolled in this study. Selected women were Caucasian and healthy (except for obesity), and their babies were delivered vaginally. Exclusion criteria were inappropriate BMI, pregnancies with pathologies, multiple pregnancies, fetal abnormalities, smoking, medication known to interfere with lipid metabolism, untreated hypo- or hyperthyroidism, other methods of delivery (medicated or caesarean), and cancer. As a measure of adiposity, selected women were divided into two groups according to their prepregnancy BMI (kg/m^2), based on their weight and height at their first prenatal visit. Women with a BMI between 18.5 and 24.9 kg/m^2 were considered to have normal weight, and those with a BMI > 30 kg/m^2 were considered obese, based on World Health Organization criteria [31]. Data relative to the weight and height of the newborns were collected at delivery. The study was conducted with the approval of the Université du Québec à Montréal (UQÀM) and the CHUM ethics committees for research on human subjects.

Blood and Tissue Samples

Blood was collected in Vacutainer gel tubes (BD, Oakville, ON, Canada) during the first (10–17 weeks), second (22–28 weeks), and third trimesters (at delivery, 36–42 weeks) from the mother as well as from venous cord blood at delivery. Mothers fasted only for the second blood collection. Samples were centrifuged at $3500 \times g$ for 15 min less than 1 h after collection. After samples were centrifuged, plasma was recovered and stored at −20°C for further analysis. Placentas, obtained from vaginal delivery, were immediately immersed in Dulbecco modified Eagle medium (DMEM; Sigma, Oakville, ON, Canada) containing antibiotics (0.12 mg/ml penicillin, 5 μg/ml amphotericin, 50 μg/ml gentamicin; Invitrogen, Burlington, ON, Canada), and $NaHCO_3$ (90 mg/ml). After the amnion, chorion, and decidual layers were removed, villous tissue was cut into approximately 5-cm^2 pieces. Tissue was either frozen in liquid nitrogen and kept at −80°C until further analysis or directly processed to isolate cells.

Lipid and Hormone Assays

Plasma levels of total cholesterol (CHOL), LDL, high-density lipoproteins (HDL), triglycerides (TG), alpha-fetoprotein (AFP), albumin, apolipoproteins (ApoA-1 and ApoB-100), and tri-iodothyronin (T3) were individually measured at the Clinical Biochemistry Service of Hôpital Saint-François d'Assise (Québec, QC, Canada), using the Unicel 36 DX600 Synchron clinical system (Beckman-Coulter, Mississauga, ON, Canada), an Elecsys automated or Hitachi 717 device (Roche Diagnostics, Laval, QC, Canada). Total FFA plasma levels were determined using the FFA Half-Micro test (Roche Diagnostics, Laval, QC, Canada) according to the manufacturer's instructions.

Real-Time RT-PCR

Total RNA was purified from human placental tissue using the High Pure RNA tissue kit (Roche Diagnostics) according to the manufacturer's instructions. Total RNA (500 ng) was then reverse transcribed using the MMLV Reverse Transcriptase (Sigma Aldrich, Oakville, ON, Canada) and oligo(dT) primers (Roche Diagnostics) according to the manufacturer's instructions. Real-time RT-PCR assays were performed with a LightCycler 480 instrument (Roche Diagnostics) using 0.5 μM of both sense and antisense primers and 480 SYBR Green I Master kit (Roche Diagnostics) according to the manufacturer's instructions. Primers were designed using PrimerBlast, based on sequences available in GenBank (Table 1). The cycling parameters for all genes were carried out at 95°C for 5 min; 50 cycles at 95°C for 10 sec, at 60°C for 20 sec, and at 72°C for 20 sec. Melting curve analyses (65°C–95°C) were performed at the end of each run to ensure the specificity of the amplification. Each essay included negative controls with no template, and each sample was analyzed in duplicate. The standard curve (with at least five serial dilutions) method was used to evaluate expression of each gene in unknown samples. Each target gene was paired with each reference gene. The two reference genes *GAPDH* and *HPRT1* were used. The geometric mean of the resulting ratios was calculated and used for further analysis as described by Vandesompele et al. [32].

Purification of Syncytiotrophoblast Membranes

Membranes were purified from fresh placental tissue obtained from full-term vaginal delivery (CHUM-Hôpital St-Luc, QC, Canada) as described by Éthier-Chiasson et al. [33].

TABLE 1. Primers used for real-time RT-PCR.

Gene	Primer set (5′–3′)		Product size (bp)	Reference
	Sense	Antisense		
CD36	cgaaagtcactgcgacatga	ccttggatggaagaacgaatc	179	
SLC27A4	tggacccctcgctcagcctc	cagccctgtggtgccggatg	176	
FABP3	cactcacccacggcactgca	tcccggtcagtggcacctga	187	
FABP1	cggaagagctcatccagaag	ttgtcaccttccaactgaacc	193	
PPARA	acttatcctgtggtccccgg	ccgacagaaaggcacttgtga	251	[81]
PPARD	tcagaagaagaaccgcaac	taggcattgtagatgtgcttgg	207	[29]
PPARG	tcagggctgccagtttcg	ccctcggatatgagaaccc	187	[29]
LPL	atggctggacggtaacagga	gcggacactgggtaatgctc	136	[82]
GAPDH	gaaggtgaaggtcggagtcaa	ggaagatggtgatgggatttc	227	[83]
HPRT1	gaccagtcaacaggggacataa	aagcttgcgaccttgacc	167	

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Western Blotting

Tissues were homogenized in ice-cold hypertonic buffer (125 mM Tris-HCl, 2 mM $CaCl_2$, 1.4% [v/v] Triton X-100, pH 8.0) containing Complete mini-EDTA-free antiprotease cocktail (Roche Diagnostics) using a Polytron PT 3000 tissue homogenizer (Brinkmann, Canada). The homogenate was kept on ice for 30 min and centrifuged at 10 000 × *g* for 25 min at 4°C. The supernatant was recovered and stored in aliquots at −80°C until further use. The protein concentration was determined using the BCA protein assay kit (Pierce Biotechnology) according to the manufacturer's instructions. An aliquot of total proteins (50 or 100 μg depending on the protein detected) was diluted in sample buffer. Proteins were heated at 95°C for 5 min, run with appropriate SDS-PAGE, and electroblotted to a polyvinylidene fluoride membrane (Millipore, Cambridge, ON, Canada). Transfer efficiency was evaluated with Ponceau red S solution (0.6% Ponceau, 1% acetic acid). Membranes were then blocked in 5%–10% skimmed milk diluted in TBS-T (20 mM Tris Base, pH 7.6, 137 mM NaCl, 0.15% Tween-20) for 1 h at room temperature or overnight at 4°C. Thereafter, membranes were incubated with primary antibodies overnight at 4°C (SLC27A4, CD36, PPARA, LPL) or for 1–2 h at 25°C (FABP1, FABP3, PPARD, PPARG). The primary antibodies used were against CD36 (1:250 dilution; Abcam, Cambridge, MA), SLC27A4 (1:1000 dilution; Abnova Corporation, Walnut, CA), FABP3 (1:2000 dilution; GeneTex, Irvine, CA), FABP1 (1:1000 dilution; GeneTex), PPARA (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), PPARD (1:2000 dilution; Santa Cruz Biotechnology), PPARG (1:2000 dilution; Santa Cruz Biotechnology), and LPL (1:1000 dilution; Abcam). Blots were washed three times for 10 min with TBS-T and probed with a horseradish peroxidase-conjugated secondary antibody at room temperature for 60 min (1:5000 dilution; New England Biolabs, Pickering, ON, Canada). Detection was performed using the Immobilon Western system (Millipore, Billerica, MA) and visualized by autoradiography (Hyperfilm ECL; GE Healthcare, Baie d'Urfée, QC, Canada). Quantification of the bands was performed with Quantity One software (Bio-Rad Laboratories, Mississauga, ON, Canada). Normalization was done with Amido Black staining (Sigma Aldrich) [34].

Quantification of LPL Activity

Tissues were homogenized in ice-cold extraction buffer (150 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.4) using a pestle homogenizer. The homogenate was centrifuged at 10 000 × *g* for 30 min at 4°C. The supernatant was recovered and stored in aliquots at −80°C until further use. LPL enzyme activity was measured in the supernatant by the enzyme fluorescence method using the Roar LPL Activity assay kit (Roar Biomedical, NY) and SpectraMax M5 (Molecular Devices, Sunnyvale, CA) according to the manufacturer's instructions. Results were normalized to the amount of protein (nmol per mg of tissue protein). Protein concentration was determined using the BCA protein assay kit (Pierce Biotechnology) according to the manufacturer's instructions.

Isolation of Villous Cytotrophoblasts

Cytotrophoblasts were isolated from fresh human full-term placentas, as described by Daoud et al. [29]. Briefly, villous tissue was washed with 0.9% NaCl, minced, and digested four times at 37°C for 30 min. The digestion medium was composed of Hanks balanced salt solution (Sigma), 0.1 mM $CaCl_2$, 0.8 mM $MgCl_2$, 1.5 mg/ml trypsin (Sigma), and 0.2 mg/ml DNase (Roche Diagnostics). After each incubation, the supernatant was replaced with fresh medium. The supernatant recovered after each digestion (except for the first one) was layered onto calf serum (Gibco, Burlington, ON, Canada) and centrifuged at 1215 × *g* for 15 min. Pellets were pooled, resuspended in DMEM-high glucose (DMEM-HG) containing PSN 1X (Invitrogen), deposited on top of a discontinuous 5%–70% (v/v) Percoll (Sigma) gradient, and centrifuged at 507 × *g* for 25 min. Villous cytotrophoblasts were collected from the appropriate layers, washed in DMEM-HG containing 1× PSN, and seeded in a 24-well plate (Corning, Acton, MA) at a density of approximately 1.4×10^6 cells/well. The purity of preparations of cells isolated from full-term placentas was evaluated by flow cytometry as described by Daoud et al. [29], using fluorescein isothiocyanate-conjugated monoclonal antibody against cytokeratin-7, a specific marker of trophoblasts, and a FACScan system (Becton Dickinson, San Jose, CA) with Winmdi software. Cells were cultivated for 4 days at 37°C. The culture medium (DMEM-HG containing 2 mM glutamine, 10% fetal bovine serum [Gibco], and 1× PSN) was changed every day.

Linoleic Acid Uptake Assays

Linoleic acid uptake assays were carried out as described by Daoud et al. [29]. Briefly, ^{14}C-labeled linoleic acid (GE Healthcare, Baie d'Urfée, QC, Canada) was evaporated under N_2 and dissolved in 50 μl of 0.1 M NaOH. Defatted bovine serum albumin (BSA; Roche Diagnostics) dissolved in PBS was added to obtain the desired linoleic acid:BSA molar ratios (1:1) and incubated at 37°C for 15 min. The linoleic acid-BSA solution was then diluted to its final concentration with PBS. Linoleic acid uptake assays were performed with trophoblasts cultured for 1 or 4 days. Cells were washed twice with 0.5 ml of PBS and incubated with 0.25 ml of radiolabeled linoleic acid for different times at 37°C. Uptake was stopped with 1 ml of ice-cold 0.5% BSA/PBS. Then, the cells were washed three times with the same solution and twice with 0.9% NaCl to remove any surface-bound linoleic acid. Cells were solubilized by addition of 0.5 ml of NaOH (0.5 M). Aliquots (0.25 ml) were added to 4 ml of scintillation cocktail, and the cell-associated radioactivity was measured with a β-scintillation counter. Additional aliquots were used to evaluate the cellular protein content of each well by using the BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL) according to the manufacturer's instructions.

Statistical Analyses

Data were expressed as means ± SEM. Data were analyzed with an unpaired Student *t*-test or two-way ANOVA when appropriate with PRISM version 5.01 software (GraphPad Software, La Jolla, CA). A *P* value of <0.05 was set as the level of significance. The relationship between two variables was evaluated with the Spearman correlation and represented by a curve of the Pearson linear correlation.

RESULTS

Maternal, Placental, and Neonatal Characteristics

Women participating in this study were divided into two groups: those with normal weight (BMI 18.5–24.9 kg/m^2) and those who were obese (BMI > 30 kg/m^2), according to criteria of the World Health Organization. Both groups were

TABLE 2. Maternal, placental, and neonatal factors of normal weight (18.5 < BMI < 24.9 kg/m^2) and obese (BMI > 30 kg/m^2) women.[a]

Parameter	Normal	Obese
n	134	16
Mother		
Maternal BMI (kg/m^2)	**21.86 ± 0.16**	**34.62 ± 1.00*** **
Maternal weight gain (kg)	12.54 ± 0.33	10.71 ± 1.54
Maternal age (yr)	30.58 ± 0.38	31.56 ± 1.13
Gestational age (wk)	**39.31 ± 0.16**	**38.31 ± 0.30***
Newborn		
Placental weight (g)	**572.9 ± 11.71**	**780.3 ± 62.96*** **
Birth weight (g)	3299 ± 44.67	3448 ± 111.0
Birth-placenta weight ratio adjusted to gestational age	**0.15 ± 0.003**	**0.13 ± 0.01** **
Birth length (cm)	51.45 ± 0.20	51.50 ± 0.50
Ponderal index (kg/m^3)	24.25 ± 0.21	25.41 ± 0.63
Head circumference (cm)	33.92 ± 0.13	34.63 ± 0.42

[a] Results are expressed as means ± SEM (*P < 0.05, **P < 0.01, ***P < 0.001, Student *t*-test), and boldface text indicates significant results.

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TABLE 3. Maternal plasma hormonal and lipid profiles of normal weight (18.5 < BMI < 24.9 kg/m^2) and obese (BMI > 30 kg/m^2) women.[a]

	First trimester		Second trimester	
Parameter	Normal	Obese	Normal	Obese
TG (mM)	1.38 ± 0.07 (n = 90)	1.82 ± 0.21 (n = 8)	**1.92 ± 0.05** (n = 129)	**2.57 ± 0.18***** (n = 15)
FFA (mM)	ND	ND	ND	ND
CHOL (mM)	4.76 ± 0.09 (n = 90)	4.74 ± 0.23 (n = 8)	6.61 ± 0.10 (n = 130)	6.54 ± 0.30 (n = 16)
LDL (mM)	2.42 ± 0.07 (n = 90)	2.58 ± 0.26 (n = 8)	3.77 ± 0.1 (n =130)	3.63 ± 0.24 (n = 16)
HDL (mM)	**1.72 ± 0.04** (n = 90)	**1.32 ± 0.08**** (n = 8)	1.99 ± 0.05 (n = 111)	1.72 ± 0.12 (n = 15)
AFP (KIU/L)	23.08 ± 2.79 (n = 50)	14.88 ± 4.89 (n = 7)	164.3 ± 6.94 (n = 111)	149.8 ± 24.23 (n = 15)
Albumin (g/L)	39.72 ± 0.38 (n = 50)	37.71 ± 1.02 (n = 7)	36.24 ± 0.22 (n = 129)	36.20 ± 0.45 (n = 16)
ApoA1 (g/L)	1.77 ± 0.03 (n = 89)	1.68 ± 0.09 (n = 8)	2.11 ± 0.02 (n = 129)	2.06 ± 0.08 (n = 16)
ApoB100 (g/L)	0.85 ± 0.02 (n = 89)	0.92 ± 0.09 (n = 8)	1.29 ± 0.03 (n = 129)	1.41 ± 0.1 (n = 16)
T3 (nM)	**2.22 ± 0.06** (n = 50)	**2.75 ± 0.14**** (n = 7)	**2.70 ± 0.05** (n = 111)	**3.43 ± 0.16***** (n = 15)

[a] Results are expressed as means ± SEM (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student *t*-test), boldface text indicates significant results, and ND indicates not determined.

TABLE 3. *Continued.*

	Third trimester maternal plasma		Third trimester venous cord blood	
Parameter	Normal	Obese	Normal	Obese
TG (mM)	**2.72 ± 0.09** (n = 107)	**3.28 ± 0.30*** (n = 14)	0.63 ± 0.05 (n = 101)	0.74 ± 0.15 (n = 14)
FFA (mM)	0.64 ± 0.04 (n = 87)	0.69 ± 0.09 (n = 14)	0.15 ± 0.02 (n = 87)	0.20 ± 0.04 (n = 14)
CHOL (mM)	**7.19 ± 0.14** (n = 108)	**6.148 ± 0.32*** (n = 14)	**1.73 ± 0.05** (n = 102)	**2.25 ± 0.50*** (n = 14)
LDL (mM)	**4.06 ± 0.12** (n = 107)	**3.1 ± 0.31**** (n = 14)	**0.78 ± 0.03** (n = 102)	**1.16 ± 0.36*** (n = 14)
HDL (mM)	**1.88 ± 0.04** (n = 107)	**1.54 ± 0.11*** (n = 14)	0.68 ± 0.02 (n = 102)	0.75 ± 0.13 (n = 14)
AFP (KIU/L)	100.9 ± 6.25 (n = 104)	108.6 ± 16.21 (n = 12)	51900 ± 5356 (n = 98)	57070 ± 9930 (n = 12)
Albumin (g/L)	34.38 ± 0.31 (n = 106)	32.7 ± 0.83 (n = 12)	37.83 ± 0.42 (n = 100)	36.46 ± 1.46 (n = 13)
ApoA1 (g/L)	4.37 ± 0.03 (n = 102)	2.00 ± 0.10 (n = 14)	0.84 ± 0.02 (n = 88)	0.93 ± 0.11 (n = 13)
ApoB100 (g/L)	1.4 ± 0.03 (n = 103)	1.29 ± 0.10 (n = 14)	**0.26 ± 0.01** (n = 88)	**0.39 ± 0.12*** (n = 13)
T3 (nM)	**2.70 ± 0.05** (n = 105)	**3.13 ± 0.13**** (n = 13)	**1.06 ± 0.02** (n = 99)	**1.28 ± 0.13**** (n = 12)

significantly different in terms of their prepregnancy BMI ($P < 0.0001$) but were similar in terms of the weight gained, maternal age, and birth weight (Table 2). Interestingly, the gestational age was shorter ($P = 0.0331$) in obese women than in mothers with normal weight, whereas the placental weight was higher ($P < 0.0001$) in obese women than in normal weight mothers (Table 2). Also, the birth weight:placental weight ratio adjusted to gestational age was lower ($P = 0.0014$) in obese women than in normal weight mothers.

Maternal and Newborn Plasma Hormone and Lipid Profiles

During the first trimester, T3 levels were higher ($P = 0.0021$) in obese mothers than in normal weight mothers, whereas HDL levels were lower ($P = 0.0023$) in obese mothers than in normal weight mothers (Table 3). During the second trimester, plasma T3 and TG levels were higher in obese mothers than in normal weight mothers (Table 3, $P < 0.0001$). During the third trimester, T3 ($P = 0.0074$) and TG ($P = 0.0461$) levels were higher in obese mothers than in normal weight mothers, whereas plasma levels of total CHOL ($P = 0.0101$), LDL ($P = 0.0076$), and HDL ($P = 0.0105$) were lower in obese mothers than in normal weight mothers (Table 3). Plasma levels of FFA, AFP, albumin, and ApoA-1 were not at all affected by high prepregnancy maternal BMI (Table 3). In newborns, T3 ($P = 0.0058$), total CHOL ($P = 0.0222$), LDL ($P = 0.0186$), and ApoB-100 ($P = 0.0166$) plasma levels were significantly increased in obese pregnancies compared with normal weight pregnancies, while no changes were noted for TG, FFA, albumin, AFP, and ApoA-1 levels (Table 3). No correlations were found among maternal TG, CHOL, LDL, and HDL levels and gestational age or among gestational age and newborn CHOL, LDL, and ApoB-100 plasma levels.

Influence of Maternal BMI on mRNA and Protein Expression of FA Carriers in Human Full-Term Placentas

We compared expression levels of CD36, SLC27A4, FABP1, and FABP3 mRNA and proteins in placentas from normal weight women (n = 6) and obese women (n = 6). Women's characteristics were representative of the total subjects' characteristics as for their prepregnant BMI (normal, 22.78 ± 0.67 kg/m^2; obese, 33.27 ± 1.30 kg/m^2, $P < 0.0001$) weight gain (normal, 12.77 ± 1.73 kg; obese, 10.85 ± 0.48 kg), age (normal, 30.83 ± 2.50 years; obese, 31.33 ± 0.88 years), and gestational age (normal, 40.17 ± 0.17 weeks; obese, 38.50 ± 0.62 weeks; $P = 0.0265$). The placental expression of CD36 mRNA and protein was significantly higher (respectively, $P = 0.0145$ and $P = 0.0263$) in obese pregnancies than in normal weight pregnancies (Fig. 1, A and B). On the other hand, the placental expression of SLC27A4 mRNA and protein (respectively, $P = 0.037$ and $P = 0.001$) and the placental expression of FABP1 mRNA and protein (respectively, $P = 0.0407$ and $P = 0.0242$) were lower in obese pregnancies than in normal weight pregnancies (Fig. 1, A and B). Interestingly, the SLC27A4 protein was expressed by both maternal sides (brush border membranes) and fetal side purified membranes (basal plasma membranes) of syncytiotrophoblasts (Fig. 1C). Finally, FABP3 protein expression but not its mRNA was lower ($P = 0.0086$) in obese pregnancies than in normal weight pregnancies (Fig. 1, A and B). No correlation was found between FA carriers' expression (mRNA or protein) and age, birth weight, head circumference, or gestational age.

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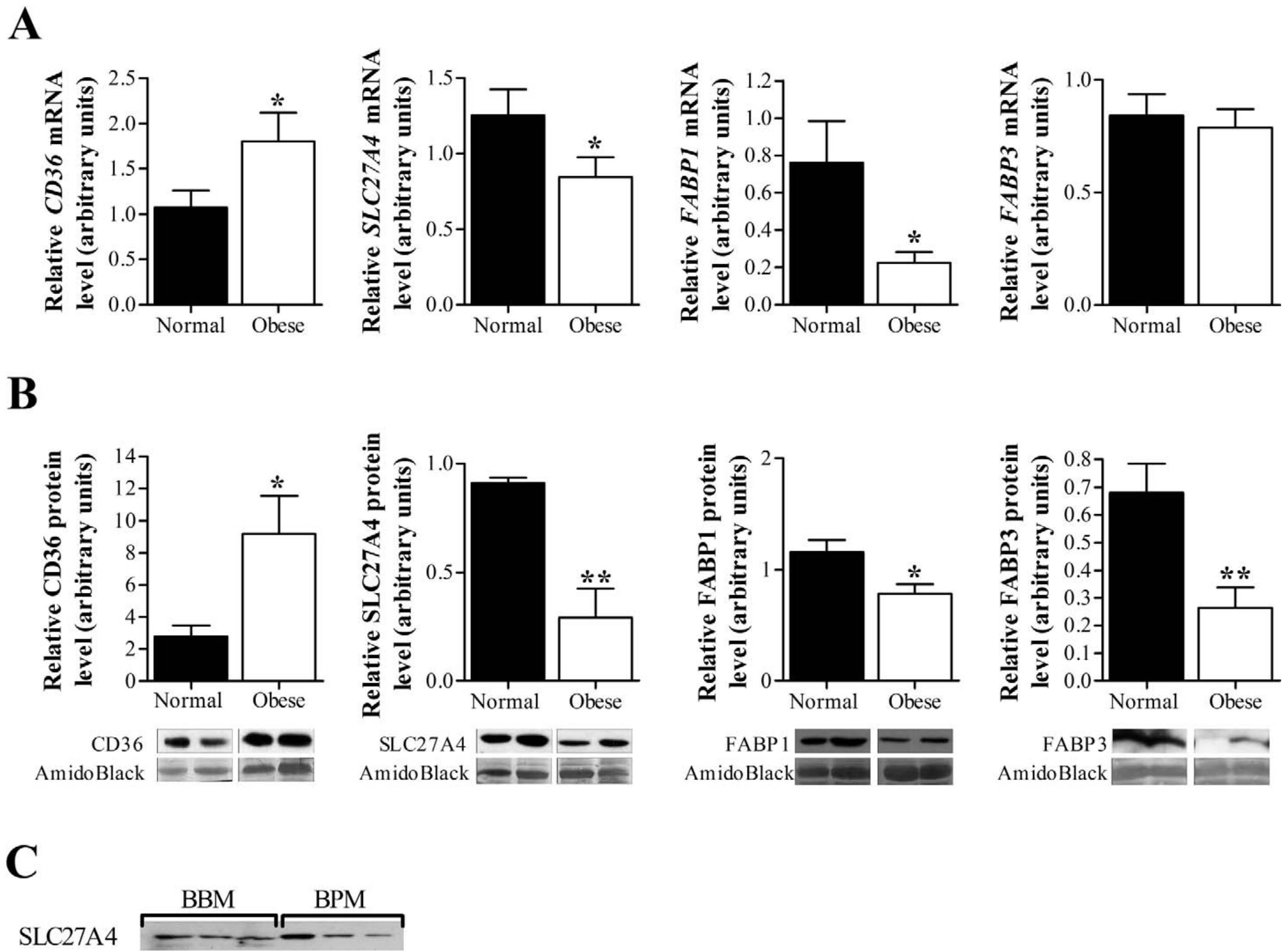

FIG. 1. Expression of FA transporters in human full-term placentas according to maternal prepregnancy BMI (kg/m^2). **A**) Placental expression levels of *CD36*, *SLC27A4*, *FABP1*, and *FABP3* mRNA were evaluated in normal weight (n = 6) and obese (n = 6) women after normalization with two reference genes (*GAPDH* and *HPRT1*). Results are expressed as geometric means ± SEM (**P* < 0.05; ***P* < 0.01). **B**) Densitometric analyses of CD36, SLC27A4, FABP1, and FABP3 protein levels were performed in normal weight (n = 6) and obese (n = 6) women after normalization with Amido Black staining. Results are expressed as means ± SEM (**P* < 0.05; ***P* < 0.01). Representative Western blots are presented. **C**) SLC27A4 expression was evaluated in human placental tissues using proteins from maternal side purified membranes (brush border membranes, BBM, n = 3) and fetal side purified membranes (BPM, n = 3).

A positive correlation was found between FABP3 protein expression and weight gain (P = 0.0328, r^2 = 0.3801). In addition, a negative correlation was found between FABP3 protein expression and placental weight (P = 0.012, r^2 = 0.4841).

Influence of Maternal BMI on the mRNA and Protein Expression of LPL and Its Activity in Human Full-Term Placentas

We compared the expression levels of LPL by real-time RT-PCR and Western blotting (Fig. 2) between normal weight women (n = 6) and obese women (n = 6). The same placentas were used to study the expression of FA carriers and LPL and activity of LPL. As previously described, their characteristics were representative of the total subjects' characteristics as for their prepregnant BMI (normal, 22.78 ± 0.67 kg/m^2; obese, 33.27 ± 1.30 kg/m^2; P < 0.0001), weight gain (normal, 12.77 ± 1.73 kg; obese, 10.85 ± 0.48 kg), age (normal, 30.83 ± 2.50 years; obese, 31.33 ± 0.88 years), and gestational age (normal, 40.17 ± 0.17 weeks; obese, 38.50 ± 0.62 weeks; P = 0.0265). As shown in Figure 2, A and B, the levels of placental LPL mRNA and protein expression did not change in obese women compared to those in normal weight women. However, LPL activity increased (P = 0.0262) in full-term placentas from obese women compared to those in normal weight women (Fig. 2C). No correlations were found among LPL activity and age, weight gain, birth weight, placenta weight, head circumference, or gestational age.

Influence of Maternal BMI on the mRNA and Protein Expression of PPARA, PPARB, and PPARG in Human Full-Term Placentas

We compared the placental expression of PPARA, PPARD, and PPARG (also known as PPARα, β/δ, and γ) by using real-time RT-PCR and Western blotting (Fig. 3) in normal weight (n = 6) and obese (n = 6) women. The same placentas were used to study the expression of FA carriers, LPL, and PPARs and the activity of LPL. As previously described, their

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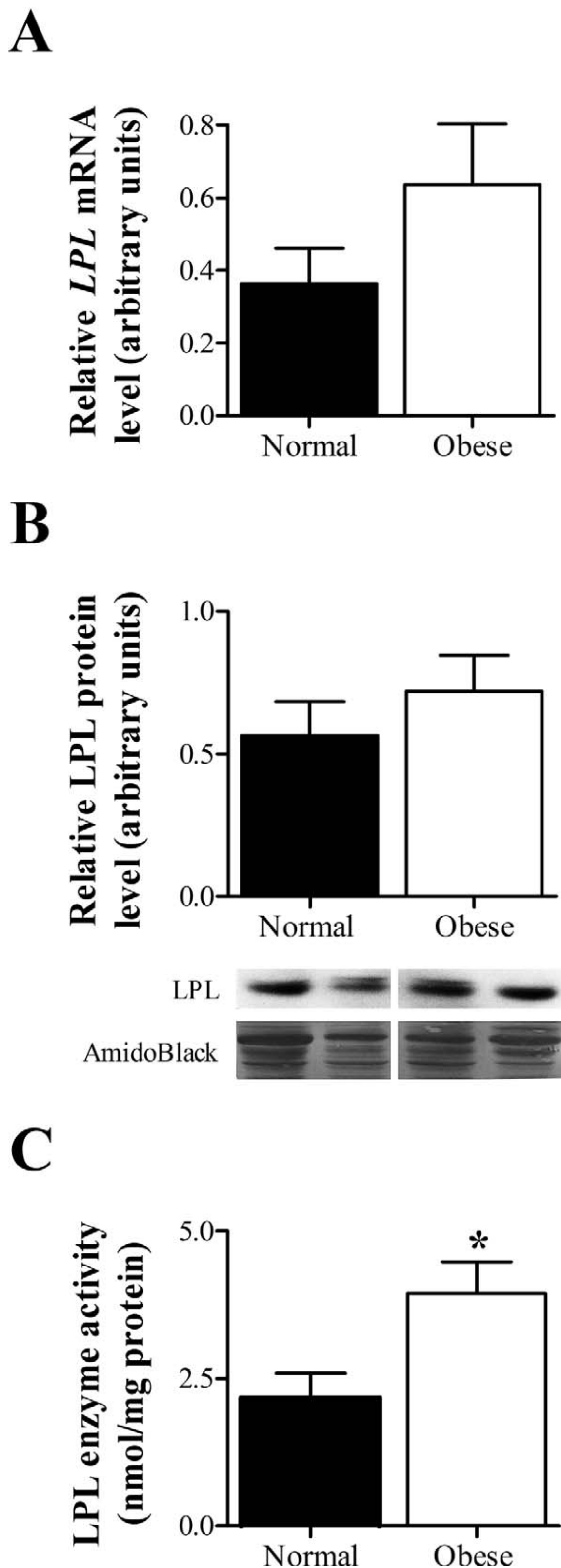

FIG. 2. LPL expression and activity in human full-term placentas according to maternal prepregnancy BMI (kg/m^2). **A**) Placental expression of *LPL* mRNA was evaluated in normal weight (n = 6) and obese women (n = 6) after normalization with two reference genes (*GAPDH* and *HPRT1*). Results are expressed as geometric mean ± SEM. **B**) Densitometric analysis of LPL protein levels was performed in normal weight (n = 6) and obese women (n = 6) after normalization with Amido Black staining. Results are expressed as mean ± SEM. **C**) Placental LPL activity (nmol/mg protein) was evaluated in normal weight (n = 6) and obese women (n = 6). Results are expressed as mean ± SEM (*$P < 0.05$).

characteristics are representative of the total subjects' characteristics as for their prepregnant BMI (normal, 22.78 ± 0.67 kg/m^2; obese, 33.27 ± 1.30 kg/m^2; $P < 0.0001$), weight gain (normal, 12.77 ± 1.73 kg; obese, 10.85 ± 0.48 kg), age (normal, 30.83 ± 2.50 years; obese, 31.33 ± 0.88 years), and gestational age (normal, 40.17 ± 0.17 weeks; obese, 38.50 ± 0.62 weeks; $P = 0.0265$). As shown in Figure 3, A and B, the mRNA and protein levels of placental PPARA, PPARD, and PPARG were not different between the placentas of obese women and those of normal weight women.

Linoleic Acid Transport in Villous Cytotrophoblasts and Syncytiotrophoblasts

Freshly isolated cells from normal weight (n = 6) and obese (n = 4) women were used to elucidate the effect of obesity on linoleic acid uptake (Fig. 4). Their characteristics were representative of the total subjects' characteristics as for their prepregnant BMI (normal, 21.14 ± 0.90 kg/m^2; obese, 34.98 ± 2.14 kg/m^2; $P = 0.0001$), weight gain (normal, 12.20 ± 1.17; obese, 11.75 ± 1.53), age (normal, 30.50 ± 1.43 years; obese, 32.50 ± 2.47 years), and gestational age (normal, 40.17 ± 0.17 weeks; obese, 39.00 ± 0.58 weeks; $P = 0.0485$). Villous cytotrophoblast cell FAT activity was lower ($P = 0.0374$) for obese women than for normal weight women (Fig. 4A). On the other hand, syncytiotrophoblast linoleic acid transport activity did not change for placentas from both groups (Fig. 4B).

DISCUSSION

In addition to other factors, such as genetic factors, the intrauterine environment is determinant for newborn and adult overall health. Several studies have demonstrated that babies born from obese women are more likely to suffer from obesity and metabolic and cardiovascular diseases [35]. The mechanisms by which maternal obesity increases the risk of developing future metabolic diseases are uncertain but likely include changes in fetal nutrients supplied by the placenta. Knowledge of the effect of maternal obesity on lipid transport and metabolism in the full-term placenta is mostly limited to animal studies.

Maternal dietary FA influence several aspects of the pregnancy, including fetal growth and development [36, 37] and FA availability in the fetal circulation [38]. It has been demonstrated that high prepregnancy BMI strongly increases the risk of having large-for-gestational age (LGA) babies by promoting the growth of fetal fat mass and increasing the percentage of body fat [6]. In this study, we found increased maternal plasma levels of TG and decreased maternal plasma levels of total CHOL, LDL, and HDL, in agreement with other studies [39, 40]. Surprisingly, we did not observe birth weight or ponderal indexes that were higher in newborns of obese women than in those of normal weight women. It could partly be due to the lack of excessive gestational weight gain in obese women and/or to the unchanged levels of TG and FFA in the venous cord blood despite the increased maternal plasma levels of TG in obese pregnancies. The rate of adverse outcomes such as hypertension and inadequate birth weight (<2500 g or ≥4000 g) is lower in obese women with low or recommended weight gain than in those with excess weight gain [41]. Alterations of transport mechanisms of other nutrients such as amino acids could also be involved [42]. Finally, a number of lipids and other nutrients could be stored in the placenta instead of being transferred to the fetus, as suggested by the higher placental weight in obese pregnancies than in normal weight

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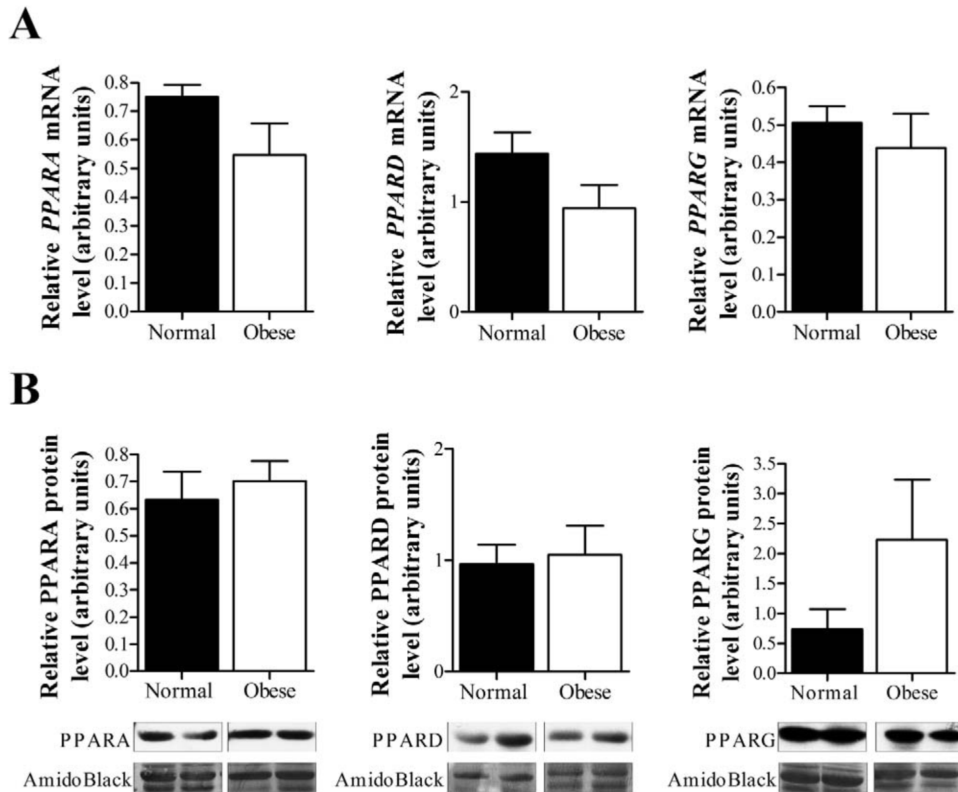

FIG. 3. PPARs expression in human full-term placentas according to maternal prepregnancy BMI (kg/m^2). **A**) Placental expression of *PPARA*, *PPARD*, and *PPARG* mRNA was evaluated in normal weight (n = 6) and obese (n = 6) women after normalization with two reference genes (*GAPDH* and *HPRT1*). Results are expressed as geometric mean ± SEM. **B**) Densitometric analysis of PPARA, PPARD, and PPARG protein levels was performed in normal weight (n = 6) and obese (n = 6) women after normalization with Amido Black staining. Results are expressed as mean ± SEM.

pregnancies. Studies have reported mostly inflammatory changes in the placenta of obese women, such as an accumulation of immune cells (macrophages and neutrophils) and an increase in the expression of several inflammatory cytokines (interleukin-1 [IL-1], IL-6, tumor necrosis factor-α [TNF-α], IL-8) [40, 43, 44]. Interestingly, high levels of IL-6 and not TNF-α stimulated FA accumulation in cultured human trophoblasts [45]. Thus, the increased IL-6 levels in obese women [46] could contribute to FA accumulation and high placental weight but also to the hypertriglyceridemia in obese women in our study. Indeed, in other tissues, IL-6 and TNF-α stimulate lipolysis [47] and TG secretion [48]. In addition, even if maternal plasma levels of total CHOL, LDL, and HDL are decreased, we demonstrated an increased total CHOL, LDL, and ApoB levels in plasma from newborns of obese mothers. If this abnormal lipoprotein profile persists through childhood, it could contribute to the higher risk of cardiovascular diseases in children born from obese mothers than in those born from normal weight mothers [49–51]. Overall, these findings may reflect changes in fetal/newborn lipoprotein metabolism, such as increased very-low-density lipoprotein (VLDL) secretion or reduced LDL-receptor-mediated clearance in the liver and/or in placental lipid transport. Roberts et al. [44] observed an increase in placental vessel muscularization in obese women. This could affect the blood flow and therefore placental nutrients transfer. The increase in placental weight and not in birth weight in our obese women also suggests an altered placental efficiency. Placental efficiency is determined by the surface area and barrier thickness but also by the number and activity of transport proteins [52]. Thus, additional alterations in the expression of placental transport proteins could disturb the transfer of nutrients across the placental barrier.

We investigated the expression of two important transport proteins present on both sides of the syncytiotrophoblast: SLC27A4 and CD36. Placental expression of SLC27A4 was decreased in obese women compared to that in normal weight women. In other tissues, the involvement of SLC27A4 in FA uptake is still controversial [53–55]. It has been proposed that SLC27A4 participates to FA uptake and metabolism as an enzyme and not as a transport protein [56]. A study by Larqué et al. [23] suggests that in the placenta, SLC27A4 is involved mainly in FA esterification. Thus, the reduced SLC27A4 placental expression in obese women would probably alter FA esterification rather than FA uptake. On the other hand, enhanced placental expression of CD36 that could be due to the high levels of T3 in obese women [57] certainly increases the FA uptake and metabolism as well as the uptake of oxidized LDL in obese women compared to that in normal weight women [58, 59]. However, it is difficult to evaluate the impact of these changes as it is still unclear if the expression of SCL27A4 and CD36 changes on both the maternal and fetal sides of the syncytiotrophoblast. Even if the FA uptake by placental cells increases on the maternal side in obese pregnancies, FA metabolism and storage in the cytoplasm of placental cells depend on the presence of cytoplasmic FABPs. Hence, the reduced placental expression of FABP1 and FABP3 in obese women in our study reveals a problem in the handling of FA in placental cells that could affect placental metabolism and delivery to the fetus. Moreover, the reduced expression of FABP1 and the increased expression of CD36 could provoke

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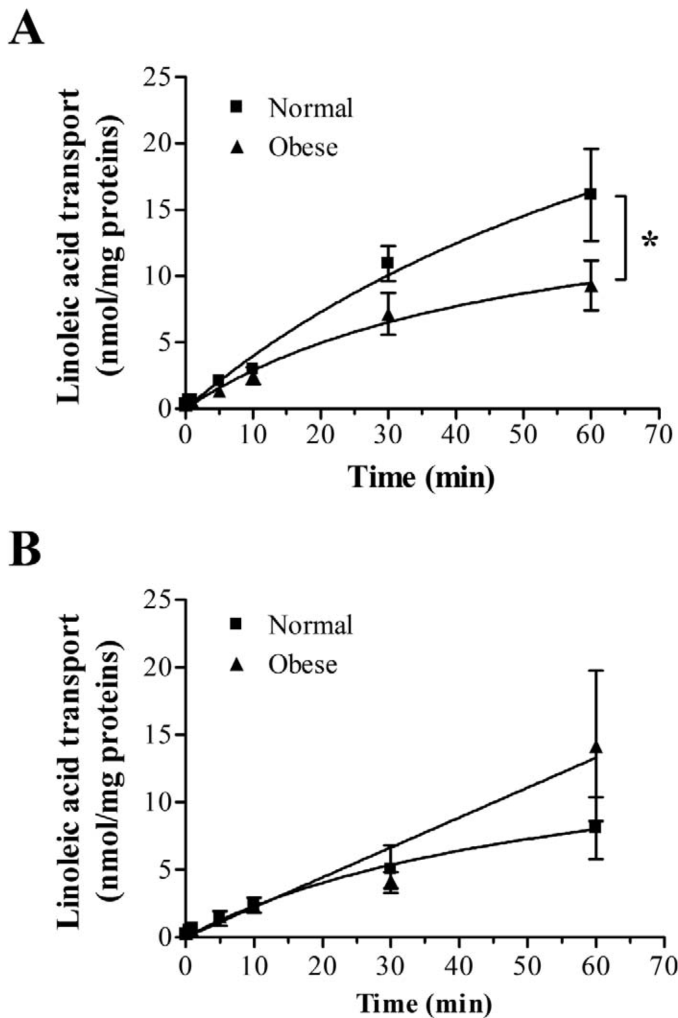

FIG. 4. Influence of maternal prepregnancy BMI (kg/m^2) on in vitro villous cytotrophoblasts and syncytiotrophoblasts linoleic acid transport kinetics. **A**) Villous cytotrophoblasts were isolated from human full-term placenta from normal weight (black squares, n = 6) and obese (black triangles, n = 4) women. Data are expressed as means ± SEM (*$P < 0.05$). **B**) Syncytiotrophoblasts were differentiated from cytotrophoblasts isolated from human full-term placenta from normal (black squares, n = 6) and obese (black triangles, n = 4) women. Data are expressed as means ± SEM.

an increase in oxidative stress in the placenta [60, 61]. This may contribute to the development of preeclampsia, a complication that is frequent in pregnant obese women [62]. However, these results do not explain the reduced linoleic acid uptake in cytotrophoblasts of obese women as Daoud et al. [29] showed that the expression of FABP1 and FABP3 was not correlated with linoleic acid uptake in human trophoblasts.

We also observed an increase in placental LPL activity in obese women compared to that in normal weight women, which could be responsible for placental TG accumulation. LPL is responsible for hydrolysis of TG-rich lipoproteins (HDL, LDL, VLDL) and interacts with lipoprotein receptors [63]. Magnusson et al. [64] reported as well an increased placental LPL activity in LGA babies of insulin-dependent diabetes mellitus pregnancies. Regulation of placental LPL activity is multifactorial and involves TG, FFA, estradiol, glucose, insulin, and cytokines [45, 65, 66]. It has been shown that tissue-specific dysregulation of LPL is implicated in obesity [67].

Placental FA uptake and metabolism are regulated partly by a family of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), through the regulation of a number of placental FA/lipid homeostasis-related proteins. PPARs form heterodimers with the retinoid X receptor (RXR) and bind to the peroxisome proliferator-response element (PPRE) in the promoter region of their target genes to control their transcription in a ligand dependent-manner [68]. PPARG and RXR agonists stimulate lipid uptake in human trophoblasts [69]. The PPAR/RXR heterodimers can be activated by either RXR (9-*cis* retinoic acid) or PPAR ligands (for example, FA and oxidized LDL) [68]. The lack of activation of PPARs could be due to the reduced expression of FABP1. In hepatocytes, FABP1 is necessary to bind and transport ligands into the nucleus for direct interaction with PPARα [70]. Even if the expression of PPARs is unaltered by maternal obesity, it could be possible that their DNA binding activity is affected [71]. Moreover, in human monocytes, it has been shown that the induction of CD36 can be dependent on RXR and not on PPAR [72]. In addition, RXR can form heterodimers with the liver X receptors (LXR) involved in lipid homeostasis through the transcriptional regulation of genes involved in FA metabolism, triacylglycerol synthesis, and CHOL homeostasis such as CD36, ABCA1, ABCG1, LPL, and SREBP-1c [73]. Interestingly, human and mouse LXRα, and not LXRβ mRNA, and promoter activity are positively regulated by T3 [74, 75], and trophoblasts have a high binding capacity for T3 [76]. High plasma levels of T3 are often associated with obesity [77]. Activation of placental LXRα in obese pregnancies by high levels of T3 could therefore affect lipid homeostasis by altering the expression of CD36, the activity of LPL, and the levels of CHOL in the fetal circulation. T3 could also impact FA uptake and metabolism by increasing the production of epidermal growth factor [78, 79].

This study has a number of limitations. First, non-fasting samples were used because of the pregnancy itself and to facilitate the collection of both maternal and newborn blood samples from the same pregnancy. Studies have used both fasting [40] and non-fasting [80] samples. This could explain discrepancies regarding lipids in obese pregnant women in the literature. Second, the gestational age differed between control and obese pregnancies. However correlations showed that gestational age had no significant effect on maternal and neonatal lipids, on the expression of CD36, SLC27A4, FABP1, or FABP3 or on LPL activity. Third, the women were obese before and during their pregnancy, therefore it is difficult to conclude about the effects of prepregnancy obesity independent of the effects of obesity. The effect of prepregnancy obesity can still be seen through the increased maternal HDL and T3 levels in obese pregnancies compared to normal-weight ones already during the first trimester.

In conclusion, maternal obesity influences placental functions, especially lipid transport and metabolism in the human full-term placenta, without affecting fetal growth (Fig. 5). Maternal obesity also affects the neonatal lipid profile, suggesting a compromised placental lipid transport and/or an altered fetal metabolism. A modified fetal metabolism could therefore increase the predisposition of the fetus to cardiovascular and metabolic disorders in its childhood and adult life. Further studies are needed to better understand the impact of altered placental expression of specific transport proteins on fetal growth and development and the cellular mechanisms involved.

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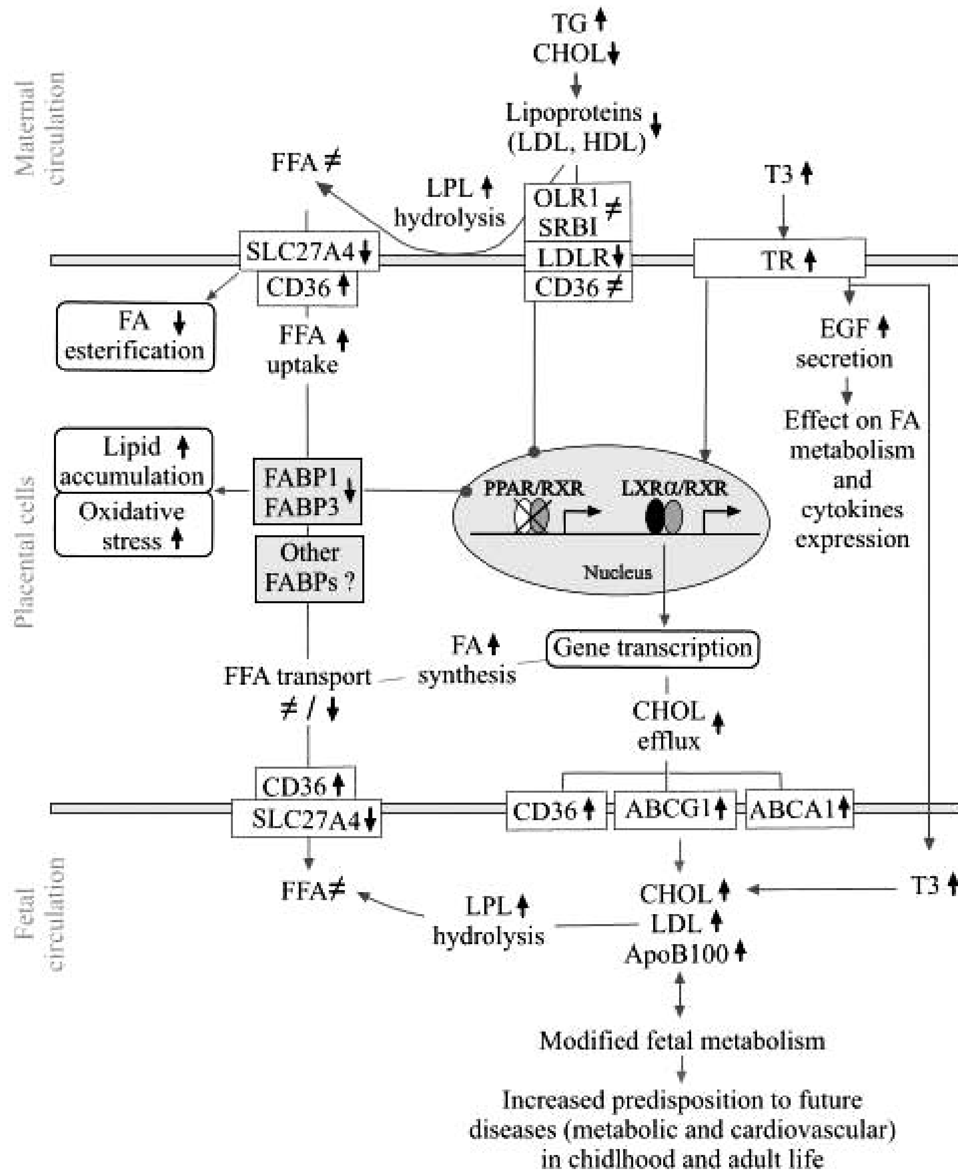

FIG. 5. Modulation of FA transport and metabolism by obesity in the full-term human placenta. FFA, free fatty acids; HDL, high density lipoprotein; LDL, low-density lipoprotein; AFP, alpha-fetoprotein; LPL, lipoprotein lipase; SLC27A4, fatty acid transport protein 4; CD36, fatty acid translocase; FABP, fatty acid binding protein; Apo, apolipoprotein; TG, triglycerides; CHOL, cholesterol; OLR1, Oxidized low-density lipoprotein receptor 1; SRBI, Scavenger receptor class B member 1; T3, triiodothyronine; TR, thyroid hormone receptors; EGF, epidermal growth factor; PPAR; peroxisome proliferator-activated receptors; RXR, retinoid X receptors; LXR, liver X receptors; ABCA1, ATP-binding cassette, subfamily A, member 1; ABCG1, ATP-binding cassette subfamily G member 1.

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