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Activation of Phospholipase A2 Is Associated with Generation of Placental Lipid Signals and Fetal Obesity

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Context: Obesity and diabetes during pregnancy are associated with increased insulin resistance and higher neonatal adiposity. In turn, insulin resistance triggers inflammatory pathways with accumulation of placental cytokines.

Objective: To determine placental signals that translate into development of excess adipose tissue, we investigated the role of phospholipases A2 (PLA2) as targets of inflammatory mediators.

Setting: The study was conducted at Case Western Reserve University, Department of Reproductive Biology.

Subjects: Volunteers gave informed written consent in accordance with the Institutional Review Board guidelines. Placenta and cord blood samples were obtained at the time of elective cesarean section in 15 term pregnancies.

Intervention: Neonatal anthropometric measurements were performed within 48 h of delivery. Placentas were grouped based on neonatal percentage body fat as obese (body fat $\geq$ 16%) and lean control (body fat $\leq$ 8%).

Main Outcome Measures: The primary outcomes were placenta PLA2 expression and fatty acid concentration.

Results: Expression of PLA2G2A and PLA2G5, the main placenta phospholipases, was greater ($P < 0.05$) in placenta of obese compared with control neonates and was associated with increased 20:3 and 20:5 omega-3 polyunsaturated fatty acids. TNF-α and leptin content was increased 3-fold in placenta of obese neonates. TNF-α and leptin both induced a time-dependent activation of PLA2G2 and PLA2G5 in placental cells.

Conclusion: Accumulation of omega-3 fatty acids through secretory PLA2 activation is associated with high neonatal adiposity. We propose that the generation of placental lipid mediators through TNF-α and leptin stimulation represents a key mechanism to favor excess fetal fat accretion. (***J Clin Endocrinol Metab* 91: 248–255, 2006**)

NEONATES WHOSE BIRTH weight is not appropriate for gestational age, either underweight or overweight, have higher susceptibility to develop obesity and metabolic disorders later in life (1–3). The mechanism(s) that occur *in utero* to modify fetal development and permanently affect the offspring have yet to be determined. Observational studies in the human population as well as experimental data in animals have established that part of the size at birth is determined by the environment in which the fetus develops (4–6). the mechanism(s) that occur *in utero* to modify fetal development and permanently affect the offspring have yet to be determined. Obesity and glucose intolerance are the most common maternal metabolic abnormalities occurring during pregnancy. They are associated with an increased rate of larger neonates, which is usually recognized as a heavier birth weight but more specifically consists in a higher percentage of body fat (7). Studies employing body composition methodologies have shown that infants of obese and gestational diabetes women have excess adiposity when compared with matched controls, suggesting that the regulatory mechanisms for fat deposition are initiated *in utero* (8). Classically, 17% of the variance in neonatal fat mass can be accounted for by maternal demographic and anthropometric variables. When maternal pregravid weight and insulin sensitivity are added to the above factors, almost 30% of the variance in fat mass can be explained, suggesting that the metabolic status of the mother strongly impacts fetal growth and development (4, 8).

The placenta is the primary target of all the stimuli coming from the mother. Hence, placental transfer, metabolism, and endocrine functions may vary accordingly to the extent of changes in maternal homeostasis. We have previously reported that increased maternal insulin resistance and gestational diabetes induce multiple alterations of placental gene expression (9). Among the alterations were an increased expression of cytokines and mediators of inflammatory signaling pathways indicating that the placenta adapts to the diabetic environment by developing chronic inflammation. In turn, placental changes may further contribute to the diabetic environment by secreting factors that will increase insulin resistance, more specifically TNF-α (10, 11). Data have recently accumulated showing that inflammatory mediators and components of the immune system cross-talk with metabolic factors to generate signals triggering obesity (12, 13). The striking similarities between the adipose tissue

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Abbreviations: AA, Arachidonic acid; ALA, α-linolenic acid; cPLA2, cytosolic PLA2 group G4A; DHA, docosahexaenoic acid; EPA, 20:5 omega-3; FCS, fetal calf serum; IOD, integrated OD; iPLA2, independent PLA2 group 6; LA, linoleic acid; PLA2, phospholipase A2; PUFA, polyunsaturated fatty acid; sPLA2, secretory PLA2.

and the placenta secretome as it relates to cytokine production suggest that such interactions may also occur within the placenta (14, 15). Of the many possible cellular targets of inflammatory cytokines are proteins involved in lipid metabolism and signaling.

The phospholipase A2 (PLA2) family of lipolytic enzymes catalyzes the hydrolysis of membrane phospholipids, releasing arachidonic acid (AA), docosahexaenoic acid (DHA), and other polyunsaturated fatty acids (PUFAs). The released PUFAs are precursors of a variety of eicosanoids, 20 carbon compounds that include prostaglandins, thromboxanes, leukotrienes, and lipoxins. It is generally accepted that the series 2-eicosanoids (omega-6 family) generated from AA trigger more severe and acute inflammatory reactions than do the series 3-eicosanoids (omega-3 family). However, the biological function of each eicosanoid is tissue specific and may have opposing effect depending on the cell environment (16, 17).

The activation of PLA2s by several cytokines suggests that these enzymes function as cellular links between inflammatory pathways and lipid metabolism. To investigate molecular components of fetal obesity, we searched for targets of placental inflammatory responses. We determined the expression profile of PLA2 in placenta of obese neonates and measured the PUFA composition of several lipid moieties. In addition, we studied the effects of TNF-α and leptin on placental secretory PLA2 (sPLA2) expression and activity. Based on our findings, we propose that placental sPLA2 activation by leptin and TNF-α affect placental lipid pathways that enhance accretion of fetal adipose tissue.

Subjects and Methods

The choriocarcinoma BeWo cells were obtained from the American Type Culture Collection (Rockville, MD). Culture media, balanced salt solutions, fetal calf serum (FCS), and trypsin were from Invitrogen (Gaithersburg, MD). Human recombinant leptin was from Linco (St. Charles, MO), and TNF-α was from Sigma Chemical Co. (St. Louis, MO). Oligonucleotides were synthesized by International DNA Technology (Coralville, IA). Other reagents were from Sigma.

Study subjects

The protocol was approved by the Institutional Review Board of Case Western Reserve University. Pregnant women were randomly recruited at the time of elective cesarean section. After neonatal anthropometrics and body composition were obtained, placentas were divided into two groups based on percent neonatal body fat (Table 1). Neonates with a percent body fat more than 16 were considered obese at 38–39 wk gestation in the population recruited at MetroHealth Medical Center (Catalano, P., unpublished data). The obese neonates included in the study were born from gestational diabetes mellitus insulin-treated mothers. Neonatal anthropometric measurements were performed within 48 h of delivery. Neonatal body composition was estimated using the total body electrical conductivity (EM-Scan HP-2; TOBEC, Springfield, IL).

Placental biopsies and RNA preparation

Placental biopsies (approximately 1 cm^3) were performed within 10 min of delivery and immediately snap-frozen in liquid nitrogen. Total RNA was prepared from whole villous tissue samples using CsCl gradient (18) or from cultured cells using the Trizol procedure. After isolation, samples were electrophoresed individually to verify RNA integrity and reverse transcribed for microarray analysis.

Analysis of RNA expression

cDNA served as a template to generate cRNA that was fragmented and hybridized to U133A human oligonucleotide arrays (Affymetrix, Santa Clara, CA) as described (9). The strategy for filtering and analyzing microarray results has been described previously (9). Briefly, results were scaled to an average signal intensity of 1500 to correct for hybridization efficiency. A serial four-step analysis was completed to select the significantly modified transcripts while minimizing false positive genes. The first step excluded all probe sets with signal below the probe pair threshold (nc). Average background and scaled noise were calculated for each array and entered in the subsequent analysis: 1, genes showing an absolute call of present (P-calls) according to MAS 5 algorithm; 2, genes showing a difference call of increased or decreased; and 3, genes with a difference in signal detection at least 4.5 times the average background minus the scaled noise. The genes having satisfied these criteria were included into the fourth level of selection based on a fold change at least 2 or −2 or below, consistent in at least two comparisons. The absolute copy number was calculated by comparing individual Cp (average of duplicate determination for each sample) to a plasmid DNA standard curve of known copy number included in each PCR run. Average signal intensity was calculated after subtracting the average background plus the scaled noise. Fold change in gene expression was calculated after scaling and filtering signal intensity data as described (9).

Real-time RT-PCR analysis was performed for leptin, TNF-α, and PLA2 transcripts using a fluorescence temperature cycler (Lightcycler; Roche Molecular Diagnostics, Indianapolis, IN) to verify microarray results. Specific primers were designed within the 3′ coding region of the genes (sequences available upon request). Results normalized to β-actin were calculated as absolute copy number per microgram RNA added per PCR. PLA2 expression in primary cultured trophoblast cells was assessed by real-time RT-PCR using the same primer pairs.

Isolation of primary placental cells

Placental cells were isolated by the technique described by Kliman *et al.* (19) and modified by Phillips *et al.* (20). Approximately 30 g villous tissue was dissected from placenta, blotted on gauze, and washed with sterile saline to remove excess blood. The tissue was finely minced, and washed again with saline. The tissue was then digested three times at 37 C in a shaking water bath for 30 min each with 0.25% trypsin and 300 U/ml DNase I in Hanks' balanced salt solution, pH 7.4. Cells were centrifuged from digestion supernatants, washed with culture medium, and filtered through 100-μm nylon mesh (Becton Dickinson, Franklin Lakes, NJ). Trophoblasts were separated from mononuclear cells by Percoll (Amersham Pharmacia Biotech, Piscataway, NJ) gradient centrifugation. Cells were counted and seeded in Iscove medium supplemented with 10% FCS in six-well plates at a density of 3×10^6 cells per well.

Cell culture and in vitro stimulation

Seeded BeWo cells were grown in standard F12K medium supplemented with 10% FCS, penicillin G (100 U/ml), and streptomycin (100

TABLE 1. Neonatal anthropometrics at delivery

	Gestational age (wk)	Placental weight (g)	Birth weight (g)	Ponderal index (g/cm^3)	% Body fat
Control	38.2 ± 0.5	566 ± 32	3152 ± 83	2.62 ± 0.07	5.2 ± 0.7
Obese	39.2 ± 0.4	649 ± 58[a]	3927 ± 123[b]	3.18 ± 0.18[a]	17.1 ± 0.6[b]

Results are means ± SE, with n = 7 for control and n = 8 for obese.
[a] $P < 0.05$; [b] $P < 0.001$, control *vs.* obese.

TABLE 2. Gene expression profile of PLA2 in placenta of obese neonates

Gene	Description	Accession no.	Signal intensity	Fold change
PLA2G6	Group VI cytosolic calcium independent	NM_003560	2094 ± 170	1
PLA2G2A	Group IIA secretory	NM_000300	4683 ± 647	3
PLA2G5	Group V secretory	NM_000929	2792 ± 225	2.6
PLA2G2D	Secretory	NM_012400	Absent	
PLA2G2E	Secretory	NM_014589	Absent	
PLA2G2F	Secretory	NM_022819	Absent	
PLA2G12A	Secretory	NM_030821	Absent	
PLA2G7	Group VII lipoprotein associated	NM_005084	701 ± 118	1.1
PLA2G4A	Cytosolic calcium dependent	M_68874	263 ± 45	1
PLA2G4B	Cytosolic calcium dependent	NM_005090	Absent	
PLA2G4C	Cytosolic calcium dependent	AF_065214	Absent	
PLA2G10	Group X	NM_003561	Absent	
PLA2G1B	Group IB pancreas	NM_000928	Absent	

Average microarray signal intensity was calculated as mean ± SE for placenta of control neonates. The gene expression level in placenta of control neonates was arbitrarily set at 1. Relative fold change above average was calculated for placenta of obese (n = 8) *vs.* control neonates (n = 10).

μg/ml) at 37 C in 5% CO_2 and 95% air. Plated BeWo or primary placental cells were washed twice and starved in medium without serum and without cytokines for 16 h before the stimulation experiment. Recombinant human leptin (200 ng/ml) and TNF-α (5 ng/ml) was added for the time indicated. Stimulation was stopped by placing the cells on ice and washing twice in ice-cold PBS. For enzyme measurements, cells were scraped in PBS and centrifuged at 1000 rpm for 5 min, and the pellet was lysed in HEPES-NaCl buffer (pH 7.4) supplemented with protease inhibitors. Release of sPLA2 was assayed in the culture medium using an enzyme immunometric assay specific for human sPLA2G2A (Cayman Chemicals, Ann Arbor, MI)

Placental lipid analysis

Frozen placental aliquots were used for lipid extraction and purification (2:1) (21). Phospholipids and neutral lipids were separated as previously described (22) by eluting neutral lipids in chloroform as phospholipids were retained in Silicagel 60 (Sigma). Phospholipids were recovered in methanol. Total lipid, neutral lipid, or phospholipid fatty acids were simultaneously saponified and methylated following the method of Lepage and Roy (23). Fatty acid methyl esters were separated and quantified on a Perkin-Elmer gas chromatograph (Autosystem, Norwalk, CT). Nitrogen was used as carrier gas, and the fatty acid methyl esters were compared with purified standards (Sigma). Individual fatty acids are expressed as percentage of total fatty acids in each sample. Phospholipids were quantified after image analysis and separation by one-dimensional thin layer chromatography using the G5–700 BIOIMAGE thin layer chromatography scanner of Bio-Rad (Hercules, CA). Spots were quantified as integrated OD (IOD) against an internal standard of cholesteryl formate, included in every application. Calibration curves were constructed by plotting the IOD of phospholipid standards, corrected by the IOD of cholesteryl formate, *vs.* the amount of lipid loaded and drawn from second-order least-square regression equations.

Statistical analysis

All data are presented as mean ± SEM. Significance for statistical differences was calculated using Wilcoxon signed rank test and *t* test.

Results

Expression levels of sPLA2 are up-regulated in placenta of obese neonates

The placental characteristics of sPLA2s were obtained by gene profile analysis of human term placenta. Based on signal intensities, the high molecular mass calcium-independent PLA2 group 6 (iPLA2) and the low molecular mass sPLA2 group 2A and group 5 displayed the highest expression levels (Table 2). These two PLA2 are not selective for AA containing phospholipids. In contrast, the calcium-dependent cytosolic PLA2 group G4A (cPLA2), which preferentially hydrolyzes AA containing phospholipids, was expressed at a much lower level than sPLA2. The PLA2G1B pancreatic form and the isoforms B and C of PLA2G4 were not detected. Comparing expression levels in placenta of control and obese neonates, we found that sPLA2 group 2A and 5 were up-regulated more than 2-fold (Table 2). Up-regulation of these sPLA2 was further confirmed by quantitating their absolute levels in placental samples used in the array experiments (Fig. 1). The absolute mRNA levels of PLA2G2 and PLA2G were 2-fold higher in placenta of obese neonates ($P < 0.01$), whereas PLA2G6 mRNA levels remained unchanged.

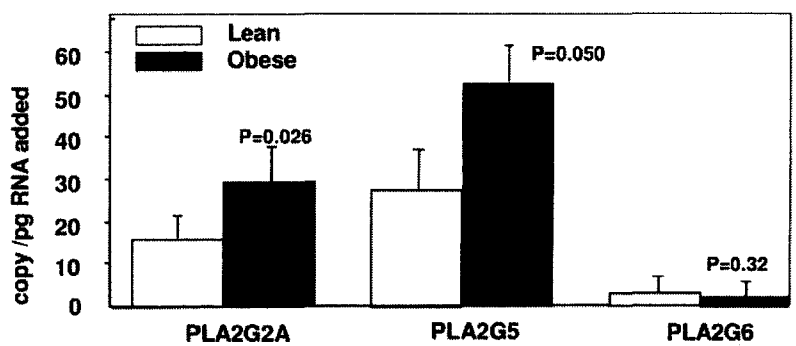

FIG. 1. Expression of placental sPLA2. The mRNA levels for the secretory enzymes PLA2G2, PLA2G5, and PLA2G6 were measured by real-time RT-PCR in placenta of control and obese fetuses. Placentas were obtained at delivery, and PLA2 abundance was calculated in gene copy number using actin as normalization control. Values are means ± SE, with n = 6 in each group.

Increased sPLA2 expression is associated with enrichment in placental omega-3 PUFAs

To investigate the functional significance of the activation of sPLA2, we determined the composition of different lipid moieties in placenta from control and obese neonates. Phospholipids represented the major lipid fraction of the placenta. Total phospholipids content was lower in placenta of obese compared with control neonates (21.4 ± 0.4 *vs.* 24.5 ± 0.9 mg/g tissue; $P < 0.02$). Table 3 shows that the proportion of linoleic acid (LA) and α-linolenic acid (ALA) was similar in lean control and obese placenta in the neutral lipid fraction and the phospholipid moiety. The composition in other omega-6 metabolites was similar in placenta from control and obese neonates. However, we observed an increase in 20:3 omega-3 and 20:5 omega-3 (EPA) in the neutral lipids as well as in the phospholipid fraction in the obese group.

Placentas of obese neonates have increased content of TNF-α and leptin

It has been postulated that increased production of cytokines provides a link between inflammation and disorders of lipid homeostasis such as obesity and diabetes. In particular, adipose mRNA expression and protein levels of leptin and TNF-α increase in obesity (24–26). We have shown that leptin and TNF-α are synthesized within the placenta and can either act locally or be released into the systemic circulation (10, 27, 28). To investigate the factors responsible for the increased release in omega-3 metabolites, we measured leptin and TNF-α in placentas of control and obese neonates. As shown in Fig. 2, TNF-α mRNA expression and protein content were increased 3- to 4-fold in placenta associated with fetal obesity, whereas leptin mRNA levels were increased 6-fold and leptin concentrations were tripled. These data indicate that the increase in placental leptin and TNF-α is associated with modification of lipid composition.

Transcriptional activation of sPLA2 by TNF-α and leptin

To explore the links between high TNF-α and leptin and lipids, we investigated the effect of TNF-α and leptin on sPLA2 in two placenta cell types, the BeWo cell line and primary cultured cells from term placenta. The kinetics of TNF-α-induced stimulation of PLA2G2 and PLA2G5 showing an early rise at 1 h with a maximum between 4 and 8 h

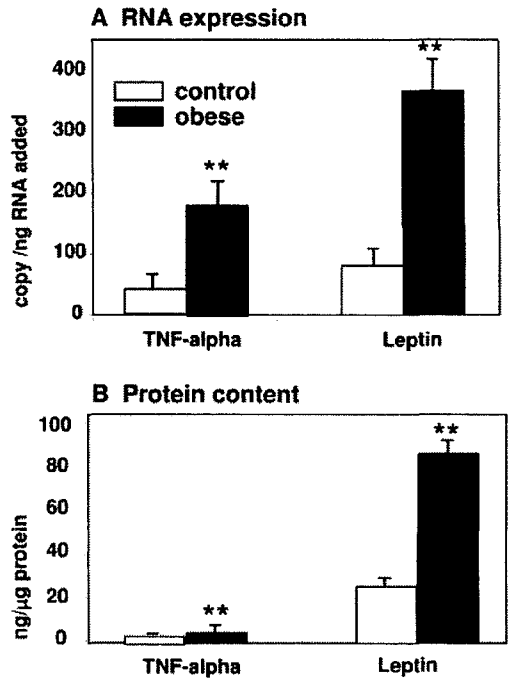

FIG. 2. Increased content of leptin and TNF-α in placentas of obese neonates. A, Absolute mRNA levels for leptin and TNF-α were measured by real-time RT-PCR using actin as normalization control. B, Protein content was determined using ELISA on 3000 × *g* supernatant of placental homogenates. Values are means ± SE, with n = 6 in each group. **, Statistical difference with $P < 0.001$.

suggests that the effects of TNF-α are transcriptional (Figs. 3 and 4). Leptin treatment of both cell types increased the expression of PLA2G2 and PLA2G5 also starting at 1 h (Figs. 3 and 4). The expression of PLA2G6 was not modified under the same stimulatory conditions (not shown). The release of PLA2G2A, the most abundant sPLA2 in placenta, was increased by incubation with leptin and TNF-α with a transient rise at 8 h, returning to basal values at 24 h (Fig. 5).

Discussion

The rate-limiting step in the generation of lipid mediators of inflammation is the release of AA, DHA, and 20 other carbon PUFAs from cellular phospholipids. This process in-

TABLE 3. Omega-3 and omega-6 PUFA composition of placenta from control and obese neonates

	Neutral lipids		Phospholipids		Total lipids	
	Lean	Obese	Lean	Obese	Lean	Obese
Omega-6 PUFAs						
LA 18:2	16.2 ± 1.09	13.9 ± 0.78	7.44 ± 0.17	7.78 ± 0.41	9.34 ± 0.60	8.34 ± 0.55
AA 20:4	7.40 ± 0.40	8.61 ± 0.60	20.1 ± 0.67	20.2 ± 1.56	18.3 ± 0.70	18.2 ± 0.66
22/4	0.72 ± 0.17	1.18 ± 0.16	1.53 ± 0.09	1.53 ± 0.09	1.48 ± 0.09	1.45 ± 0.12
22/5	0.96 ± 0.09	0.92 ± 0.19	1.61 ± 0.18	1.26 ± 0.12	1.53 ± 0.14	1.28 ± 0.12
Omega-3 PUFAs						
ALA 18:3	0	0	0	0	0.06 ± 0.01	0.06 ± 0.01
ETA 20:3	0.08 ± 0.04	0.14 ± 0.04	0.02 ± 0.01	0.28 ± 0.10[a]	0	0.04 ± 0.00[a]
EPA 20:5	0	0	0	0	0.18 ± 0.08	0.47 ± 0.09[a]
DHA 22:6	1.10 ± 0.15	1.60 ± 0.15[a]	3.80 ± 0.33	4.00 ± 0.19	3.60 ± 0.18	4.10 ± 0.19

Values are means ± SE with n = 7 in obese and n = 6 in control group. ETA, Eicosatrienoic acid.
[a] Statistical significance with $P < 0.05$ in obese *vs.* lean in each lipid moiety.

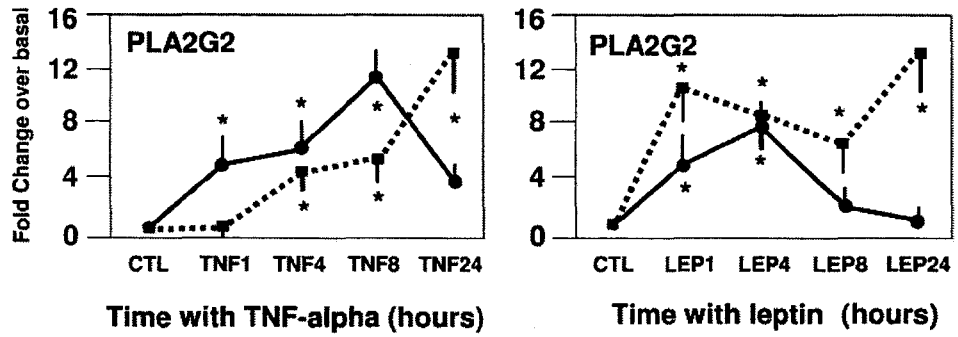

FIG. 3. Regulation of secretory PLA2G2 by TNF-α and leptin. The kinetics of leptin and TNF-α-induced changes in PLA2G2 expression was studied in BeWo placental cells (*solid line*) and primary cultures from term placenta (*dotted line*). Cells were serum deprived for 16 h before being exposed to 5 ng/ml TNF-α or 200 ng/ml leptin (LEP) for the indicated times. Total RNA was analyzed by real-time RT-PCR. Results were normalized for β-actin and expressed as fold changes over control levels. Values are means ± SE of three to five independent culture experiments. *, Statistical difference with $P < 0.01$.

volves the action of one or more phospholipases. The type of fatty acid released is tissue and enzyme specific, AA and DHA being the most abundant metabolites released by PLA2 (29). Among the 19 PLA2 isoforms cloned so far, high molecular weight cytosolic cPLA2s group IV–VI and the small secretory or extracellular sPLA2 have been best characterized (30). In addition to their intrinsic lipolytic properties, the sPLA2s are implicated in inflammatory reactions through the generation of eicosanoids and other lipid mediators for signal transduction (31).

Expression of sPLA2 is up-regulated in placenta of obese neonates

Gene expression profiling shows that the secretory proteins PLA2 group II and group V (PLA2G2, PLA2G5) and the calcium-independent cPLA2 group VI (iPLA2/PLA2G6) are the major PLA2 enzymes expressed in term placenta. In contrast, the group IV cPLA2 and the pancreatic IB were not detected at significant levels. This pattern of expression extends data showing PLA2 immunoreactivity in placenta (32, 33). Expression of sPLA2G2 and PLA2G5 was up-regulated in placenta of obese neonates, whereas PLA2G6 was unchanged. To our knowledge, this is the first report showing that the expression of placenta PLA2 is modified in pathological fetal growth. These findings are in line with the observations that the concentration of placental products of PLA2 hydrolysis such as DHA is positively correlated with placental weight (34, 35). In addition, the conversion rate of AA to PUFAs appears to be higher in placenta of type 1 diabetic than in nondiabetic women (36). Collectively, these data indicate that qualitative and quantitative modifications of placental lipid content are associated with alterations of fetal growth.

The composition in omega-3 PUFAs is enriched in placenta of obese neonates

Quantification of lipid moieties showed that 20:3 and 20:5 omega-3, two n-3 PUFAs, were enriched in placenta of obese neonates, whereas the concentration of AA, an essential omega-6 PUFA, was not modified. The preferential generation of omega-3 products may have several explanations. First, iPLA2, which generally displays a higher specificity for releasing AA than sPLA2, was not up-regulated in placenta of obese neonates. In addition, there may be a natural substrate preference of sPLA2s for EPA or DHA-containing phospholipids based on placental membrane composition (37, 38). This is supported by the lower content in total phospholipids in placenta of obese neonates. Besides being released by phospholipid hydrolysis, DHA and AA can also be derived from the two essential fatty acids LA and ALA through

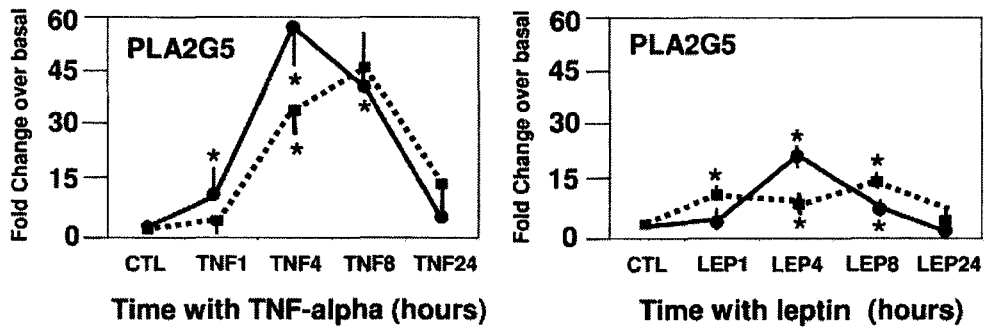

FIG. 4. Regulation of sPLA2G5 by TNF-α and leptin. The kinetics of leptin- and TNF-α-induced changes in PLA2G5 expression was studied in BeWo placental cells (*solid line*) and primary cultures from term placenta (*dotted line*). Cells were maintained in complete medium with 10% FCS for 48 h and then serum deprived for 16 h before being exposed to 5 ng/ml TNF-α or 200 ng/ml leptin (LEP) for the indicated times. Total RNA was analyzed by real-time RT-PCR. Results were normalized for β-actin and expressed as fold changes over control levels. Values are means ± SE of three to five independent culture experiments. *, Statistical difference with $P < 0.01$

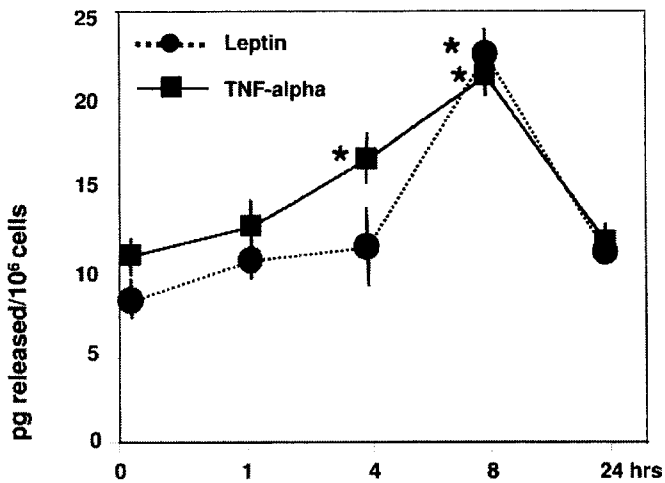

FIG. 5. Regulation of sPLA2 release by primary cultured placental cells. Plated cells were incubated in medium containing 10% FCS for 48 h before stimulation with 5 ng/ml TNF-α (*solid line*) or 200 ng/ml leptin (*dotted line*) in the absence of serum. Values are means ± SE of three culture experiments, each performed in triplicate. *, Statistical difference with $P < 0.001$.

elongation and desaturation processes. However, this does not appear to be a main pathway for the *in situ* production of PUFAs within the placenta (34). In addition, LA and ALA concentration was similar in placenta of control and obese neonates (Table 2). It is also unlikely that increased EPA results from a differential uptake of DHA from maternal circulation because plasma levels of AA and DHA were similar in mothers of control and obese neonates (data not shown).

Increased placenta TNF-α and leptin and activation of sPLA2

TNF-α is released in several cell types in response to inflammation, infection, or injury and participates in pleiotropic responses related to the progression of inflammation (39). Beyond its pivotal role in regulating whole-body homeostasis, leptin also regulates immune responses (40). It has become evident that leptin and TNF-α are instrumental in promoting the molecular cascades that link insulin resistance, obesity, and inflammation (13, 26). Both cytokines are produced in large quantity by the human placenta, and their production is up-regulated in pregnancy with diabetes (10, 41). The data reported here show that the production of leptin and TNF-α is enhanced in placenta of obese neonates, strengthening the view that their accumulation is causally linked to alterations of the *in utero* environment (9). The generation of eicosanoids through agonist-stimulated release of AA has been observed in several cell types with TNF-α, IL-1, and IL-6 being the most potent stimulators of PLA2 activity (14–17). In addition, leptin stimulated iPLA2γ in murine macrophages (42), indicating that molecules that are usually produced by the immune system also act as metabolic regulators through their ability to generate lipid mediators. We did not detect iPLA2 in placenta, but leptin clearly enhanced the expression of sPLA2 through a pattern consistent with a transcriptional mechanism. Based on our findings, it is reasonable to expect that sPLA2-induced lipolytic pathways are enhanced in placenta of obese fetuses as a result of high leptin and TNF-α content (Fig. 6). Increased placental lipolytic activity may be facilitated through paracrine mechanisms because of the close vicinity of the Hofbauer macrophages producing TNF-α and the trophoblast cells producing leptin. The differential effect between the sPLA2 isoforms may be a result of structural characteristics in their promoters such as peroxisome proliferator response element sites (43, 44).

Consequences of PLA2 activation

Our model linking chronic placental inflammation to lipid pathways suggests that PLA2 activation has multiple consequences for feto-placental metabolic homeostasis (Fig. 6). The PUFAs generated upon sPLA2 activation may participate in progression and amplification of local inflammation. Although classically, omega-3-derived metabolites are considered less potent inflammatory mediators than AA-derived metabolites, they can act as transactivators of gene promoters. In addition, some of the PUFAs and eicosanoids may be directly transferred to the fetus and serve as substrates for adipogenesis. Another consequence of enhanced sPLA2 activity may be the modification of the membrane lipid bilayer composition through changes in its phospholipid content. Long-chain PUFAs are associated with rearrangement of the cell membrane lipid bilayer leading to an increase in membrane fluidity. Our working hypothesis is that a chronic disturbance in cell membrane composition

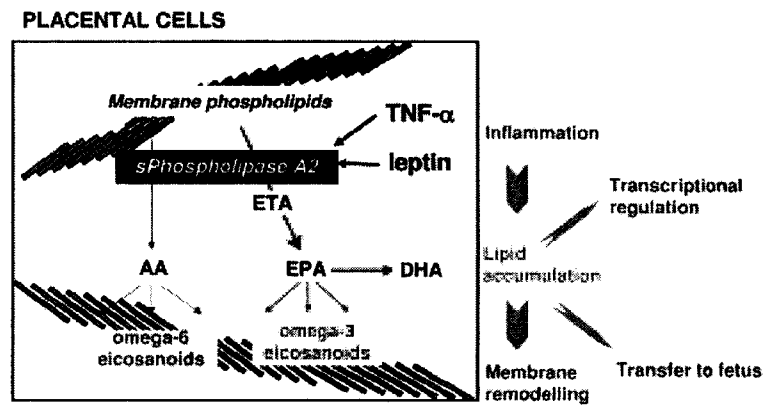

FIG. 6. Schematic model of chronic placental inflammation and lipid dysfunction. Increased placental production of TNF-α and leptin in placenta of obese neonates contributes to a state of local inflammation with activation of PLA2G2 and PLA2G5. The subsequent accumulation of omega-3 PUFAs creates an imbalance in lipid content that may have several consequences. The omega-3 fatty acids can be directly transferred to the fetus and contribute to fat accretion. They can induce modification of placental membrane fluidity, generating structural rearrangements that may enhance nutrient transport efficiency. Alternatively, PUFAs can amplify inflammatory signals through transcriptional regulation. This combination of events will be excess responsible for *in utero* programming of obesity by favoring excess fat accretion in the fetus.

leading to membrane remodeling modifies placental structure in such a way to increase the efficiency of materno-fetal nutrient transfer. This in turn would increase the availability of adipogenic substrates for the fetus as shown previously for free fatty acid transfer with advancing gestation and type 1 diabetic pregnancy (45, 46). Our model is strongly supported by animal studies, showing that mice with PLA2 null mutation have reduced adipose tissue and increased insulin sensitivity (47) as well as PLA2 mutation in diabetic individuals (48).

Collectively, our results provide experimental support to the theory that intricate immune and metabolic reactions are instrumental in the development of obesity (12, 13). We propose that the activation of placental sPLA2 resulting in an accumulation of lipid mediators in the feto-placental unit facilitates excess fetal fat accretion, thus programming of *in utero* obesity.

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