tapraid4/zeg-jcem/zeg-jcem/zeg99913/zeg0037-13z | xppws | S=5 | 6/6/13 | 16:42 | Art: 13-1614 | Input-ss

Decreased Concentrations of the Lipoprotein Lipase Inhibitor Angiopoietin-Like Protein 4 and Increased Serum Triacylglycerol Are Associated With Increased Neonatal Fat Mass in Pregnant Women With Gestational Diabetes Mellitus

AQ: 1 The surnames of the authors are highlighted in the text

Henar Ortega-Senovilla, Ute Schaefer-Graf, Katrin Meitzner, Michael Abou-Dakn, and Emilio Herrera

Faculties of Pharmacy and Medicine (H.O.-S., E.H.), University CEU San Pablo, E-28668 Madrid, Spain; and Department of Obstetrics and Gynecology (U.S.-G., K.M., M.A.-D.), Center for Diabetes in Pregnancy, St Joseph's Hospital, D-12101 Berlin, Germany

AQ: 2 OK

Context: Angiopoietin-like protein 4 (ANGPTL4) is an extracellular inhibitor of lipoprotein lipase (LPL) activity. No studies have been done in pregnancy in which hypertriglyceridemia and tissue-specific changes in LPL activity are present.

Objective: The objective of the study was to determine the relationship between neonatal fat mass (FM) and concentrations of ANGPTL4 and triacylglycerols (TAG) in maternal and cord serum of pregnant women with gestational diabetes mellitus (GDM) compared with controls.

Design: Maternal blood samples (control, n = 90, and GDM, n= 80) and umbilical cord blood were drawn before and after vaginal delivery, respectively. Control and GDM subjects were grouped separately into 3 subgroups, according to neonatal FM: 0–25th percentiles, 25th-75th percentiles, and 75th-100th percentiles.

Outcome Measures: Glucose, insulin, TAG, nonesterified fatty acids (NEFAs), and ANGPTL4 were determined in maternal and neonatal serum.

Results: Age and pregestational body mass index did not differ between GDM and control women in any subgroups. Maternal serum of GDM pregnant women who delivered the newborn with the highest FM showed the highest concentrations of TAG and NEFAs and lowest concentration of ANGPTL4, despite glucose and insulin concentrations being independent of changes in neonatal FM. However, cord serum of neonates of GDM patients with the highest FM showed higher concentrations of insulin and lower concentrations of TAG than those with lower neonatal FM but no significant differences in NEFAs or ANGPTL4 concentrations.

Conclusions: In well-controlled GDM pregnancies, decreased maternal ANGPTL4 concentrations and a gradient of TAG toward the fetus are related with higher neonatal FM. However, in GDM fetuses with the highest FM, the potential effect of ANGPTL4 inhibiting adipose tissue LPL activity could be overcome by their hyperinsulinemia.

Placental transfer of lipids is increased during the second half of pregnancy as the fetus deposits fat rapidly (1, 2). Essential fatty acids for the fetus (ie, polyunsaturated fatty acids) circulate in maternal plasma mainly in their esterified form associated with different lipoproteins, with a minor proportion being in the form of nonesterified

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received March 9, 2013. Accepted May 29, 2013.

Abbreviations: ANGPTL, angiopoietin-like; BMI, body mass index; GDM, gestational diabetes mellitus; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; TAG, triacylglycerol; VLDL, very low-density lipoprotein.

<zjs;> • <zjss;4,6,11,14>
<zshorttitle>ANGPTL4 in Maternal and Cord Serum

fatty acids (NEFAs) (3). The fatty acids of these maternal lipoproteins are released by extracellular endothelial triacylglycerol (TAG) hydrolases located in the placenta before uptake by the tissue. Lipoprotein lipase (LPL) is one of the TAG hydrolases responsible for transplacental transfer of TAG-derived fatty acids from maternal lipoproteins (4, 5). In addition, LPL is expressed in vessels of adipose tissue, in which it hydrolyzes very low-density lipoprotein (VLDL)-TAG to supply NEFAs and glycerol for resynthesis of TAG in adipocytes (6, 7); thus, adipose tissue is considered a major site for clearance of TAG-rich lipoproteins from blood.

Some studies support the hypothesis that alterations in placental LPL activity contributes to changes in the transport of the fatty acids to the fetus and consequently may affect its growth. For example, an increased placental LPL activity in placentas of women with type 1 diabetes mellitus has been described (8). The same has been observed in type 1 diabetic pregnancies that resulted in large-for-gestational-age neonates (9); conversely, there was a reduction of LPL activity in pregnancies complicated by intrauterine growth restriction (8), which is normally characterized by reduced fetal fat depots (10). However, there was no effect of maternal diabetes on the placental expression of mRNA encoding LPL and no correlation between LPL gene expression and infant birth weight or gestational age (8, 11), indicating that altered activity of LPL in type 1 diabetes is due to modulation of its activity at the posttranscriptional level. Recently it has been reported that the activity of some lipases is regulated by members of the angiopoietin-like (ANGPTL) protein family (12–14). These proteins irreversibly inhibit LPL activity, which is at least partially accounted for by promoting the irreversible conversion of active LPL dimers into inactive monomers (15, 16). This is the case with ANGPTL4, which is secreted into serum from adipose tissue, liver, and placenta (17). Overexpression in mice or treatment with ANGPTL4 led to an increase in TAG, whereas in the ANGPTL4 knockout mice or those treated with anti-ANGPTL4 antibody (18, 19), the concentration of plasma TAG was low.

The results concerning ANGPTL4 quoted above were obtained in animal models and tests in vitro; no studies have been carried out in any species during pregnancy, when hypertriacylglycerolemia and tissue-specific changes in LPL activity are routinely found (20). To understand the potential relationship of maternal and fetal plasma ANGPTL4 concentrations to newborn growth, fat mass, and serum TAG in humans, the present study was designed to determine these variables in mothers with gestational diabetes mellitus (GDM) and their offspring and to compare them with nondiabetic mothers and their offspring. Because neonatal fat mass may be increased in GDM, despite the absence significant differences in birth weights (21, 22), we decided to stratify the subjects according to their fat mass to establish whether the differences in the ANGPTL4 concentrations could be a factor in the effect.

Research Design and Methods

Study subjects

The study included 170 women and their neonates that were born at the Department of Obstetrics of the Vivantes Medical Center in Berlin. This is the same cohort used in our previous studies designed to evaluate metabolic variables in pregnancies complicated with GDM and their implications in intrauterine development (22–24). Diagnosis of GDM was established by a 75-g oral glucose tolerance test at 26 weeks of gestation interpreted according to the criteria of Carpenter and Coustan (95/180/155 mg/dL) (25). The accurate gestational age was confirmed by ultrasound examination before 20 weeks. Exclusion criteria were delivery before 34 weeks of gestation, preeclampsia identified fetal anomalies, nonsingleton pregnancies, and delivery by cesarean section. Thus, only term offspring born by vaginal delivery and their mothers were included in the study. In term offspring, birth weight and height were obtained shortly after vaginal delivery, and neonatal skin-fold thickness at the flank was measured within 48 hours to calculate fat mass by a formula derived from Catalano et al (26). All participating mothers gave informed written consent after having received verbal and written information on the study. The study protocol was approved by the Vivantes Medical Center of Berlin Ethics Committee.

AQ: 3 Please, replace with Carpenter and Coustan reference (view page 8)

Control (n = 90) and GDM (n = 80) subjects were separately allocated to subgroups according to neonatal fat mass. The neonatal fat mass showed a normal distribution without significant differences in the fat mass between the infants of diabetic mothers and those of control mothers (Table 1). The 25th percentile was at 327 g of fat and the 75th percentile was at 541 g, these being the cutoff points used here to establish the 3 subgroups of the study. Thus, subgroup 1 included pregnant women and their infants with neonatal fat mass below 327 g, subgroup 2 included those with neonatal fat mass between 327 and 541 g, and subgroup 3 included those with neonatal fat mass higher than 541 g.

Blood samples

Fasting maternal blood samples were drawn at the last visit to the obstetric clinic, no longer than 1 week before delivery. The umbilical cord blood was doubly clamped immediately after delivery and blood samples taken from one of the umbilical arteries from a segment of the cord. Maternal and cord blood samples were centrifuged (1500 × *g* at 4°C for 25 min) and aliquots of serum were immediately stored at −80°C until analysis. None of the samples used in the study showed hemolysis.

Analytical determinations

Serum glucose (Abbott Diagnostics, Lake Forest, Illinois), TAG (Menarini Diagnostics, Florence, Italy), and NEFA (Wako Chemicals GmbH, Neuss, Germany) were determined enzymat-

AQ: 4 OK

doi: 10.1210/jc.2013-1614

Table 1. Characteristics of Mothers and Their Offspring.

(n = CTR/GDM)		Subgroup 1, Mean ± SEM (n = 23/20)	Subgroup 2, Mean ± SEM (n = 45/40)	Subgroup 3, Mean ± SEM (n = 22/20)
Maternal characteristics				
Age, y	CTR	29.7 ± 1.1	28.9 ± 0.8	28.4 ± 1.3
	GDM	29.1 ± 1.1	30.6 ± 0.7	31.4 ± 0.9
Prepregnancy BMI, kg/m^2	CTR	24.5 ± 1.1	25.8 ± 0.8	26.6 ± 1.3
	GDM	26.0 ± 1.1	27.5 ± 0.8	28.4 ± 1.0
Gestational age, wks	CTR	38.8 ± 0.2^{a}	39.7 ± 0.2^{b}	40.2 ± 0.3^{b}
	GDM	38.8 ± 0.2^{A}	39.5 ± 0.1^{B}	40.0 ± 0.2^{B}
Neonatal characteristics				
Birth weight, kg	CTR	2.91 ± 0.06^{a}	3.67 ± 0.04^{b}	4.17 ± 0.07^{c}
	GDM	2.82 ± 0.05^{A}	3.41 ± 0.04^{B,d}	3.91 ± 0.05^{C,e}
Fat mass, g	CTR	235 ± 15^{a}	428 ± 11^{b}	631 ± 17^{c}
	GDM	228 ± 13^{A}	436 ± 9^{B}	625 ± 12^{C}
Fat mass to birth weight ratio, g per 100 g	CTR	7.94 ± 0.32^{a}	11.6 ± 0.2^{b}	15.1 ± 0.4^{c}
	GDM	8.00 ± 0.31^{A}	12.8 ± 0.2^{B,d}	16.0 ± 0.1^{C,f}
Placental weight, g	CTR	574 ± 23^{a}	663 ± 18^{b}	729 ± 27^{c}
	GDM	492 ± 24^{A,e}	548 ± 16^{B,d}	677 ± 21^{C}
Females sex, % (n)	CTR	48% (11)	42% (19)	73% (16)
	GDM	50% (10)	50% (20)	45% (9)

Abbreviation: CTR, control pregnancies. Subgroup 1 includes pregnant women and their infants, the neonatal fat mass of whom was below 327 g, corresponding to the 25th percentile; subgroup 2 includes pregnant women and their infants, the neonatal fat mass of whom was between 327 and 541 g, the 25th and 75th percentiles; and subgroup 3 includes pregnant women and their infants, the neonatal fat mass of whom was higher than 541 g, the 75th percentile.

A, B, C, Significant differences between subgroups ($P < .05$), comparisons in the GDM group.

a, b, c, Significant differences between subgroups ($P < .05$), comparisons in the control group.

[d] $P < .001$, significant difference between control and GDM groups.

[e] $P < .01$, significant difference between control and GDM groups.

[f] $P < .05$, significant difference between control and GDM groups.

ically using commercial kits. Serum insulin (Mercodia AB, Uppsala, Sweden) and ANGPTL4 (USCN Life Science, Wuhan, China) were determined using sandwich ELISA kits.

Statistics

Results are expressed as means ± SEM. The statistical difference between groups was determined by ANOVA after adjustment for possible confounding factors [ie, maternal prepregnancy body mass index (BMI), gestational age, and gender of neonate]; when differences were statistically significant, multiple comparisons were performed using the Tukey post hoc test. Given their skewed distributions, concentrations of TAG, NEFA, insulin, and ANGPTL4 were log transformed before statistical comparison. Correlations were tested with Pearson's method using the log-transformed data as indicated. All statistical analyses were performed using a computer software package (Statgraphics Centurion XV, version 15.2.06; Statistical Graphics Corp, Princeton, New Jersey).

AQ: 5
OK

Results

GDM and control subjects were separated into 3 subgroups on the basis of the fat mass of their neonates. Age and pregestational BMI did not differ between women with GDM and the controls in any of the subgroups (Table 1). Neonates of control and GDM women were born at similar gestational age, but values in those that had the lowest fat mass (ie, < 25th percentile) were born consistently earlier than in the other subgroups. Although there were no differences in the body weight of neonates between control and GDM in the total studied population (3.49 ± 0.05 and 3.80 ± 0.04 kg, respectively, $P > .05$), the body weight of the neonates increased in both control and GDM subjects in parallel to their fat mass, although in those from subgroups 2 and 3 (ie, having fat masses above the 25th percentile), body weights were lower in the GDM group than in controls. Therefore, although neonatal fat mass did not differ between the controls and GDM groups, its value as a proportion of body weight progressively increased as the fat mass percentile increased and was significantly higher in GDM than in controls in the subgroups 2 and 3. Placental weight progressively increased in parallel to the neonatal body weight and was

lower in the GDM group than in controls in the 2 subgroups at the lower fat mass percentiles (subgroups 1 and 2).

The concentration of several metabolic parameters (adjusted for prepregnancy BMI and gestational weeks at birth) in maternal serum close to delivery is shown in Table 2. Maternal serum glucose did not differ between controls and GDM groups, and values were independent of neonatal fat mass. Maternal serum insulin showed a tendency to be higher in the GDM subgroups than in controls, although the difference between them was significant only in those with neonates at the lowest fat mass percentiles (subgroup 1). However, the insulin to glucose ratio was higher in GDM women than in controls in all 3 subgroups ($P < .05$). Serum concentrations of either TAG or NEFA did not differ between control and GDM pregnancies, although in the latter, values of the 2 variables were highest in those having delivered the newborn with the highest fat mass (subgroup 3 as compared with subgroups 1 and 2). Overall ANGPTL4 concentrations in maternal serum did not differ between GDM and control (39.0 ± 4.1 vs 42.3 ± 3.0 ng/mL, respectively, $P > .05$), and as shown in Figure 1A, maternal serum ANGPTL4 concentrations showed no difference between control and GDM women, but they were significantly lower in both groups when newborns had the highest fat mass (subgroup 3) than in the other subgroups.

In cord blood serum, glucose values in the total studied population were higher from GDM than from control pregnancies (89.0 ± 2.4 vs 76.3 ± 2.6 mg/dL, respectively, $P < .001$); however, when looking at the subgroups, that difference was significant only in those newborns with the lowest fat mass (subgroup 1; see Table 3). Serum insulin was consistently higher in cord blood from GDM pregnancies than from controls, and whereas values progressively increased as the fat mass of the newborn increased, the change was statistically significant and more pronounced in GDM subjects than in controls. The insulin to glucose ratio followed a similar trend to insulin, although values in the newborns with the lowest fat mass did not differ between the 2 groups. Cord serum TAG remained very similar in controls of the 3 subgroups. In GDM pregnancies, however, values of cord serum TAG progressively decreased as the fat mass of the newborn increased, the difference from the controls becoming significant in those with the highest fat mass. Within the groups there was no difference in the NEFA values recorded in cord blood serum from the 3 subgroups, but values in the GDM group were significantly higher than those of the control group from the newborns from the 2 subgroups at the lower percentiles of the fat mass distribution (subgroups 1 and 2). Overall serum ANGPTL4 concentrations in cord serum were higher in those from GDM than from control pregnancies (41.4 ± 2.8 vs 27.8 ± 2.9 ng/mL, $P < .001$), and as shown in Figure 1B, the ANGPTL4 concentrations in cord blood serum were independent of fat mass in either control or GDM pregnancies. Nevertheless, in subgroups 1 and 2, the values of ANGPTL4 concentrations in cord blood serum were higher in newborns of GDM mothers than in controls (control vs GDM: 25.8 ± 3.5 vs 40.5 ± 7.0 ng/mL, $P < .05$, subgroup 1; 27.2 ± 2.7 vs 37.0 ± 4.9 ng/mL, $P < .001$, subgroup 2; 33.1 ± 4.6 vs 39.4 ± 5.9 ng/mL, $P > .05$, subgroups 3).

Discussion

The metabolic impact of gestational diabetes in fetal development, especially in women with well-controlled

Table 2. Metabolic Parameters in Maternal Serum Close to Delivery From Control and GDM Pregnant Women, Classified by Percentile of Neonatal Fat Mass

Percentile of neonatal FM (n = CTR/GDM)		Subgroup 1, Mean ± SEM (n = 23/20)	Subgroup 2, Mean ± SEM (n = 45/40)	Subgroup 3, Mean ± SEM (n = 22/20)
Glucose, mg/dL	CTR	81.9 ± 5.0	90.0 ± 3.5	94.4 ± 5.9
	GDM	81.0 ± 5.0	80.3 ± 3.0	83.0 ± 3.4
Insulin, μU/mL[a]	CTR	15.4 ± 4.3	17.5 ± 2.8	14.2 ± 4.8
	GDM	30.1 ± 5.1[b]	21.5 ± 3.1	22.7 ± 4.0
Insulin/Glucose, μU/mg[a]	CTR	16.4 ± 4.3	20.0 ± 2.8	16.6 ± 4.9
	GDM	31.6 ± 6.5[b]	29.8 ± 3.9[b]	28.2 ± 4.5[b]
Triacylglycerol, mg/dL[a]	CTR	239 ± 18	256 ± 12	266 ± 20
	GDM	235 ± 19[A]	248 ± 12[A]	295 ± 16[B]
NEFA, mg/dL[a]	CTR	11.6 ± 1.7	8.54 ± 1.18	10.4 ± 2.0
	GDM	6.19 ± 0.69[A]	6.93 ± 0.43[A]	8.75 ± 0.56[B]

Abbreviation: CTR, control pregnancies. CTR, GDM, and subgroups 1, 2, and 3 are described in the legend to Table 1. Data were adjusted by prepregnancy BMI and gestational age.

[a] Log transformed skewed data were used for statistical comparisons.

[b] $P < .05$, significant difference between control and GDM groups.

A, B Significant differences between subgroups, $P < .05$.

doi: 10.1210/jc.2013-1614

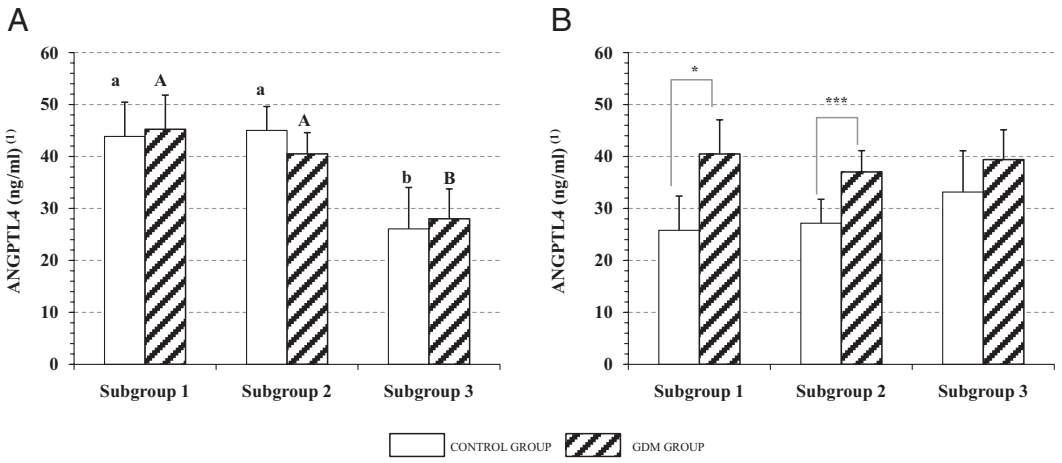

Figure 1. Maternal (A) and cord serum (B) concentrations of ANGPTL4 in control and GDM pregnant women and their neonates classified by percentile of neonatal fat mass. Maternal parameters were adjusted by prepregnancy BMI and gestational age; cord serum parameters were adjusted by prepregnancy BMI, gestational age, and sex of the neonate. Asterisks indicate significant differences between control and GDM groups (*, $P < .05$; **, $P < .01$; ***, $P < .001$). Different superscripted upper-case letters indicate significant differences between cord and maternal serum in the control group ($P < .001$); different superscripted lower-case letters indicate significant differences between the cord and maternal serum in the GDM group ($P < .001$). Open bars, Data from control subjects; filled bars, data from GDM subjects. All values are mean ± SEM. (1), log transformed for statistical comparisons.

lower
upper
different subgroups
different subgroups
data

Table 3. Metabolic Parameters in Cord Serum From Control and GDM Pregnant Women, Classified by Percentile of Neonatal Fat Mass

Percentile of Neonatal FM (n = CTR/GDM)		Subgroup 1, Mean ± SEM (n = 23/20)	Subgroup 2, Mean ± SEM (n = 45/40)	Subgroup 3, Mean ± SEM (n = 22/20)
Glucose, mg/dL	CTR	69.5 ± 5.8	79.2 ± 4.2	79.4 ± 7.1
	GDM	92.9 ± 4.6[a]	87.8 ± 3.2	87.2 ± 4.0
Insulin, μU/mL[b]	CTR	3.46 ± 0.87	4.94 ± 0.64	5.49 ± 1.09
	GDM	4.97 ± 1.22[c,A]	8.8 ± 0.84[B,d]	11.4 ± 1.1[C,d]
Insulin to glucose ratio, μU/mg[b]	CTR	6.15 ± 1.33	6.76 ± 0.97	7.58 ± 1.67
	GDM	5.55 ± 1.48[A]	10.2 ± 1.0[B,d]	13.3 ± 1.3[C,d]
Triacylglycerol, mg/dL[b]	CTR	39.1 ± 2.6	37.2 ± 1.9	37.5 ± 3.2
	GDM	51.6 ± 3.9[A]	40.1 ± 2.7[B]	29.6 ± 3.6[c,C]
NEFA, mg/dL[b]	CTR	2.62 ± 0.35	3.10 ± 0.26	3.03 ± 0.42
	GDM	3.86 ± 0.51[c]	4.36 ± 0.33[a]	3.72 ± 0.43

Abbreviation: CTR, control pregnancies. CTR, GDM, and subgroups 1, 2, and 3 are described in the legend to Table 1. Data were adjusted by prepregnancy BMI, gestational age, and gender of neonate.

[a] $P < .01$, significant differences between control and GDM groups.

[b] Log-transformed skewed data were used for statistical comparisons.

[c] $P < .05$, significant differences between control and GDM groups.

A, B, C Significant differences between subgroups ($P < .05$), comparisons in the GDM group.

[d] $P < .001$, significant differences between control and GDM groups.

GDM, can be masked by a normal birth weight. This is the case with the population in this study, in which there were no overall differences in birth weight or in neonatal fat mass between the infants of pregnant women with GDM and those of the control women (24), probably due to apparently good glucose control in mothers who had developed gestational diabetes. However, a more detailed analysis reveals that in a population of newborns of normal or higher fat mass (ie, above the 25th percentile), those of GDM mothers with normal serum glucose values, had a higher fat mass as a proportion of body weight than did controls. This observation is important because, normally, women with well-controlled GDM have been found to have newborns with normal body weight, and little attention has been paid to their body composition. The higher fat mass to birth weight ratio found here in neonates of GDM mothers suggests that the metabolic disturbances resulting from the development of GDM in pregnant women primarily affects the growth of fetal adipose tissue.

In our study the differences between the accretions of neonatal fat mass were not explained by differences in the

concentration of glucose or insulin in the serum of pregnant women. The exception is that higher insulin concentrations were found in the serum of the GDM mothers of the smallest newborn infants (subgroup 1), suggesting a role of their poorly controlled diabetic condition in the impairment of normal fetal growth; otherwise, there were no differences observed in maternal glucose and insulin concentrations between control and GDM mothers in the population under study.

We also observed that in the serum of both control and GDM pregnant women, close to delivery, the concentration of an extracellular inhibitor of LPL activity, ANGPTL4, decreased significantly in women whose neonates had a fat mass above the 75th percentile of the total studied population. In the case of the GDM women but not of controls, these variations were accompanied by a significant increase in maternal concentrations of TAG and NEFA. These results might seem contradictory because, although not previously studied in humans, a decrease in the concentration of ANGPTL4 has been associated in mice with increased LPL activity and with consequent increased VLDL catabolism, which would cause a decrease in the concentration of serum TAG (18, 19, 27). In humans, however, during late pregnancy LPL activity in adipose tissue is already very low for several other reasons (including insulin resistance, placental hormones, etc) (28), resulting in the possibility that in these circumstances such low concentrations of ANGPTL4 would have little effect on the already low activity of adipose LPL. It is possible that the reduction of ANGPTL4 could be a way of increasing the transfer of lipids to the fetus. The increase in maternal TAG takes place during the third trimester of pregnancy (29, 30), and the concomitant increase in placental LPL (9) facilitates the use of maternal lipoprotein TAG as a source of fatty acids for the growing fetus. This is plausible because LPL activity is regulated throughout pregnancy in a tissue-specific manner according to metabolic demands to ensure the supply of the appropriate amounts of fatty acids to the fetus (20).

It is possible that the enhanced activity of LPL in the placenta at this late stage of pregnancy is facilitated by the reduction of ANGPTL4 in maternal circulation found here. Together with the increased concentrations of NEFA and TAG in maternal circulation at this stage, LPL activity actively contributes to the enhanced availability of fatty acids in the fetus. This view is consistent with the reported positive correlation of placental LPL activity with fetal size or fat depots in the fetus in pregnancies complicated with either type 1 diabetes or intrauterine growth restriction (9). It must be pointed out that the expression of ANGPTL4 is relatively ubiquitous (31), the liver having been suggested to be the main source of plasma ANGPTL4 (31, 32). However, ANGPTL4 has also been shown to be an endocrine factor (33), and therefore, it is proposed that the changes in its concentrations in maternal serum described here may result in the effects on placental LPL activity reported here.

When analyzing cord serum, we found that, in infants with the most body fat, the concentration of TAG in cord serum was significantly lower than in those with less body fat but only when their mothers had GDM. These findings would be consistent with an increase in the catabolism of fetal VLDL by a higher LPL activity in adipose tissue in these fetuses. However, cord serum concentrations of ANGPTL4 were similar in the 3 subgroups of GDM neonates studied, and no correlation between ANGPTL4 concentrations and circulating TAG concentrations was found. It is therefore suggested that ANGPTL4 did not have a major impact on the overall accumulation of TAG in fetal adipose tissue and that fetal adipose tissue LPL activity probably would be controlled mostly by circulating insulin, the concentration of which was especially elevated in GDM mothers with infants having the highest fat depots (ie, subgroups 2 and 3).

This is the first time that the concentrations of ANGPTL4 in maternal and cord serum in control and GDM pregnancies have been reported. In humans, ANGPTL4 is highly expressed in liver, but detectable amounts of its transcripts are also present in adipose tissue and in placenta (13, 17). However, the contribution of the latter tissues to the ANGPTL4 pool present in the maternal or fetal circulation is probably not significant because the heaviest placentas in our study were in those mothers of newborns with the highest body weight and fat mass, in which the maternal circulating ANGPTL4 concentrations were the lowest and the cord blood serum values were unchanged. Moreover, the concentration of ANGPTL4 in cord blood serum in the 2 subgroups of neonates with the lowest fat masses was higher in the GDM group than in controls despite the fact that the weight of the placenta being lower in the GDM group. The lower placental weights in the GDM women could be the result of their higher insulin resistance because a negative correlation between placental weight and insulin resistance has been reported previously in GDM pregnancies (34). Thus, either the liver or adipose tissue seems to be responsible for the changes found in serum ANGPTL4 concentrations in mothers and their newborns, although the relative contributions of those tissues may differ.

Paradoxically, the higher proportion of fat mass in GDM newborns was associated with higher concentrations of ANGPTL4 in cord blood, the change being significant in subgroups 1 and 2. This finding contrasts with the reported increases of this protein in serum associated

with decreased LPL activity (19). However, the physiological roles of angiopoietin-like proteins in humans have been inferred largely from studies in mice, and results found in animals may not apply directly to the human situation. In rodents fat mass is very low at birth, and maturation of this tissue occurs during the postnatal period (2). In human fetuses fat deposition increases exponentially over the final third of gestation, reaching the highest rate of accretion just before term (35). Increases in umbilical cord insulin concentrations have been found in fetuses of obese pregnant women whose offspring had normal birth weights but increased neonatal fat mass (21). In our study, neonates of GDM mothers who had an augmented fat mass as a proportion of birth weight were also hyperinsulinemic; it is proposed that the anabolic effect of insulin contributed to their increased fat mass proportions and overrode the potential effects of their higher ANGPTL4 concentrations.

In summary, the present findings indicate that decreased concentrations of the LPL inhibitor ANGPTL4, found at term in both nondiabetic and well-controlled GDM women whose newborn infants had high fat mass, could be related to an increased placental transfer of lipids. Increases in maternal TAG concentrations in GDM women whose newborns had the highest fat mass corresponded to the lowest TAG concentrations in cord serum. This steeper maternofetal TAG gradient could also have been facilitating a greater fatty acid placental transfer that would contribute to the higher fetal fat accumulation. Newborns of GDM mothers have the lowest TAG concentrations, the highest fat mass to body weight ratio, and the highest concentrations of insulin. Because these changes in newborns from GDM mothers appeared in the absence of any differences in ANGPTL4 concentrations, it is proposed that the potential inhibitory effect of this protein on their adipose tissue LPL activity was overcome by their hyperinsulinemia.

The funding for this manuscript is SAF2012-39273

AQ: 7

Acknowledgments

We thank Milagros Morante (Universidad CEU San Pablo) for her excellent technical assistance; the midwives and obstetricians, especially Matthias Schmitter and Alfredo Gonzales (of the Vivantes Medical Center), for their support in recruiting patients and collecting the samples; and to pp-science-editing.com for editing and linguistic revision of the manuscript.

AQ: 8
URL is OK

Address all correspondence and requests for reprints to: Henar Ortega-Senovilla, PhD, Faculties of Pharmacy and Medicine, University CEU San Pablo, E-28668, Madrid, Spain. E-mail: henar@ceu.es.

This work was supported by the Spanish Ministry of Science and Innovation (Grant SAF2012-39273), Universidad CEU San Pablo (Grant USP-BSCH-05/08), and Fundación Ramón Areces (Grant CIVP16A1835).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Jansson T, Cetin I, Powell TL, Desoye G, Radaelli T, Ericsson A, Sibley CP.** Placental transport and metabolism in fetal overgrowth: a workshop report. *Placenta*. 2006;27:S109–S113.
2. **Widdowson EM.** Growth and composition of the fetus and newborn. In: ANS ed. *Biology of Gestation*. New York, NY: Academic Press; 1968:1–49.
3. **Herrera E.** Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine*. 2002;19:43–55.
4. **Bonet B, Brunzell JD, Gown AM, Knopp RH.** Metabolism of very-low-density lipoprotein triglyceride by human placental cells: the role of lipoprotein lipase. *Metabolism*. 1992;41:596–603.
5. **Waterman IJ, Emmison N, Dutta-Roy AK.** Characterisation of triacylglycerol hydrolase activities in human placenta. *Biochim Biophys Acta*. 1998;1394:169–176.
6. **Lasuncion MA, Herrera E.** "In vitro" utilization of labelled esterified fatty acids and glyceride glycerol from triglyceride-rich lipoproteins in rat adipose tissue. *Horm Metab Res*. 1981;13:335–339.
7. **Lasuncion MA, Herrera E.** Changes with starvation in the rat of the lipoprotein lipase activity and hydrolysis of triacylglycerols from triacylglycerol-rich lipoproteins in adipose tissue preparations. *Biochem J*. 1983;210:639–643.
8. **Magnusson AL, Waterman IJ, Wennergren M, Jansson T, Powell TL.** Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. *J Clin Endocrinol Metab*. 2004;89:4607–4614.
9. **Magnusson-Olsson AL, Hamark B, Ericsson A, Wennergren M, Jansson T, Powell TL.** Gestational and hormonal regulation of human placental lipoprotein lipase. *J Lipid Res*. 2006;47:2551–2561.
10. **Petersen S, Gotfredsen A, Knudsen FU.** Lean body mass in small for gestational age and appropriate for gestational age infants. *J Pediatr*. 1988;113:886–889.
11. **Lindegaard ML, Damm P, Mathiesen ER, Nielsen LB.** Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression. *J Lipid Res*. 2006;47:2581–2588.
12. **Kersten S.** Regulation of lipid metabolism via angiopoietin-like proteins. *Biochem Soc Trans*. 2005;33:1059–1062.
13. **Hato T, Tabata M, Oike Y.** The role of angiopoietin-like proteins in angiogenesis and metabolism. *Trends Cardiovasc Med*. 2008;18:6–14.
14. **Miida T, Hirayama S.** Impacts of angiopoietin-like proteins on lipoprotein metabolism and cardiovascular events. *Curr Opin Lipidol*. 2010;21:70–75.
15. **Shan L, Yu XC, Liu Z, et al.** The angiopoietin-like proteins ANGPTL3 and ANGPTL4 inhibit lipoprotein lipase activity through distinct mechanisms. *J Biol Chem*. 2009;284:1419–1424.
16. **Sukonina V, Lookene A, Olivecrona T, Olivecrona G.** Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci USA*. 2006;103:17450–17455.
17. **Romeo S, Yin W, Kozlitina J, et al.** Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. *J Clin Invest*. 2009;119:70–79.
18. **Desai U, Lee EC, Chung K, et al.** Lipid-lowering effects of anti-angiopoietin-like 4 antibody recapitulate the lipid phenotype found in angiopoietin-like 4 knockout mice. *Proc Natl Acad Sci USA*. 2007;104:11766–11771.
19. **Koster A, Chao YB, Mosior M, et al.** Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and

AQ: 9
Editor is Assali NS

angptl3: regulation of triglyceride metabolism. *Endocrinology*. 2005;146:4943–4950.

20. **Herrera E, Lasuncion MA, Gomez-Coronado D, Aranda P, Lopez-Luna P, Maier I.** Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am J Obstet Gynecol*. 1988;158:1575–1583.
21. **Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S.** Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care*. 2009;32:1076–1080.
22. **Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, et al.** Differences in the implications of maternal lipids on fetal metabolism and growth between gestational diabetes mellitus and control pregnancies. *Diabet Med*. 2011;28:1053–1059.
23. **Schaefer-Graf UM, Graf K, Kulbacka I, et al.** Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. *Diabetes Care*. 2008;31:1858–1863.
24. **Ortega-Senovilla H, Schaefer-Graf U, Meitzner K, et al.** Gestational diabetes mellitus causes changes in the concentrations of adipocyte fatty acid-binding protein and other adipocytokines in cord blood. *Diabetes Care*. 2011;34:2061–2066.
25. **American Diabetes Association.** Gestational diabetes mellitus. *Diabetes Care*. 2000;23(suppl 1):S77–S79.
26. **Catalano PM, Tyzbir ED, Allen SR, McBean JH, McAuliffe TL.** Evaluation of fetal growth by estimation of neonatal body composition. *Obstet Gynecol*. 1992;79:46–50.
27. **Yoshida K, Shimizugawa T, Ono M, Furukawa H.** Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. *J Lipid Res*. 2002;43:1770–1772.
28. **Herrera E, Ortega-Senovilla H.** Maternal lipid metabolism during normal pregnancy and its implications to fetal development. *Clin Lipidol*. 2010;5:899–911.
29. **Montelongo A, Lasuncion MA, Pallardo LF, Herrera E.** Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. *Diabetes*. 1992;41:1651–1659.
30. **Herrera E, Ortega H, Alvino G, Giovannini N, Amusquivar E, Cetin I.** Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. *Eur J Clin Nutr*. 2004;58:1231–1238.
31. **Kersten S, Lichtenstein L, Steenbergen E, et al.** Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. *Arterioscler Thromb Vasc Biol*. 2009;29:969–974.
32. **Jonker JT, Smit JW, Hammer S, et al.** Dietary modulation of plasma angiopoietin-like protein 4 concentrations in healthy volunteers and in patients with type 2 diabetes. *Am J Clin Nutr*. 2013;97:255–260.
33. **Kim I, Kim HG, Kim H, et al.** Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. *Biochem J*. 2000;346:603–610.
34. **Taricco E, Radaelli T, Nobile de Santis MS, Cetin I.** Foetal and placental weights in relation to maternal characteristics in gestational diabetes. *Placenta*. 2003;24:343–347.
35. **van Aerde JE, Wilke MS, Feldman M, Clandinin MT.** Accretion of lipid in the fetus and newborn. In: Polin RA, Fox WW, Abman SH, eds. *Fetal and Neonatal Physiology*. Philadelphia: Elsevier; 2011: 454–470.

Please in Ref 25, replace "American Diabetes Association. Gestational diabetes mellitus. Diabetes Care. 2000;23(suppl1):S77-S79" for "Carpenter MW, Coustan DR. Criteria for screnning tests for gestational diabetes. Am J Obstet Gynecol. 1982; 144: 768-773".