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Fetal macrosomia in a Hispanic/Latinx predominant cohort and altered expressions of genes related to placental lipid transport and metabolism

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Abstract

Fetal overgrowth, termed fetal macrosomia when birth weight is greater than 4000 grams, is the major concern in the treatment of gestational diabetes mellitus (GDM). However, to date, the underlying mechanisms of fetal macrosomia have not been understood completely. Placental lipid metabolism is emerging as a critical player in fetal growth. In this study, we hypothesized that fatty acid transport and metabolism in the placental tissue was impaired in GDM women, dependent on fetal sex. To test this hypothesis, we analyzed the incidence of GDM, fetal macrosomia, and obesity in a large cohort consisting of 17995 pregnant subjects and majority of subjects being Hispanic/Latinx, and investigated expression of genes related to lipid transport and metabolism in placenta from obese women with or without GDM, and with or without fetal macrosomia. The main findings include: 1) There is a higher incidence of GDM and obesity in Hispanic subjects compared to non-Hispanic subjects, but not fetal macrosomia; 2) Expressions of most of genes related to placental lipid transport and metabolism are not altered by the presence of GDM, fetal macrosomia, or fetal sex; 3) Expression of *FABP4* is increased in obese women with GDM and fetal macrosomia, and this occurred in male placentas; 4) Expression of *LPL* is decreased in obese women with GDM despite fetal macrosomia, and this occurred in male

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CONFLICT OF INTEREST

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placentas; 5) Expression of *ANGPTL3* is decreased in obese women with GDM and fetal macrosomia, but is not altered when fetal sex is included in the analysis. This study indicates that there is race disparity in GDM with higher incidence of GDM in obese Hispanic women, although fetal macrosomia disparity is not present. Moreover, altered placental lipid transport may contribute to fetal overgrowth in obese women with GDM.

INTRODUCTION

Gestational diabetes mellitus (GDM), one of the major pregnancy-related complications, affects 10-18% of pregnant women in the United States and its prevalence is expected to increase with the worldwide epidemic of obesity (1). One of the major concerns in the treatment of GDM is fetal overgrowth, or fetal macrosomia (FM) when birth weight is greater than 4000 grams in humans. This condition increases risk of caesarean delivery, birth trauma, and other maternal and fetal outcomes and thereby, necessity of additional care (2). Currently the main goal of GDM treatments (dietary manipulation and medications) is to control maternal blood glucose levels – a goal based on the hypothesis emphasizing the excess glucose supply for fetal growth. However, the efficacy of these treatments is unsatisfactory as FM is still present in the glycemia well-controlled patients(3). More importantly, exposure to GDM predisposes metabolic and cardiovascular diseases in the offspring (4, 5) and the mother (6, 7), thus amplifying the severity of this disease and costs for future disease prevention, intervention, and treatments. Therefore, the underlying mechanism of GDM was warranted to be explored in order to develop effective prevention and treatments for GDM.

Unlike the extensive studies on glucose, lipid metabolism and its role in the development of GDM and fetal overgrowth has largely been ignored (8). Emerging evidence indicates that altered lipid metabolism during pregnancy is a potential trigger in the development of GDM. 1) GDM is positively associated with pre-pregnancy obesity and/or excess gestational weight gain which are primarily due to the fat accretion (9, 10); 2) Maternal triglyceride (TG), free fatty acids (FFA), and cholesterols are now understood to serve as important substrates for fetal fat accretion(9); 3) Higher maternal plasma TG and lower high-density lipoprotein (HDL)-cholesterol concentrations in early and mid-pregnancy were significantly associated with a greater risk of GDM(8, 10); 4) Maternal adipose tissue communicates with the placenta via classical endocrine signaling(8, 11) and emerging mechanisms such as exosomes(12); 5) There is excess accumulation of lipid droplets and reduced lipid oxidation in the placenta from women with GDM(13, 14); 6) Fetal plasma lipid profile is altered by GDM, demonstrating increased TG, phospholipids, and FFAs(8, 15). As a result, the altered lipid metabolism in women with GDM provide overload of lipid substrates for fetal growth.

The placenta nurtures fetal growth by providing nutrients from maternal circulation. Lipids are essential nutrients for fetal development which completely relies on maternal supply when the fetus has limited capacity of lipogenesis. How the placenta transport lipids from maternal to fetal circulation has not been understood completely. The knowledge of placental transport and metabolism have been reviewed extensively elsewhere(15). Briefly, the esterified form of fatty acids including triglyceride, LDL, HDL and VLDL, is de-

esterified by the two main types of lipases in the human placenta, lipolipase (LPL) and endothelial lipase (EL), then non-esterified fatty acids are taken up by placental syncytiotrophoblast cells via passive transport. Fatty acids in the trophoblast cells will be allotted to fatty acid oxidation or transported to fetal circulation (reviewed in (16)). Thus, the defects in lipid metabolism could be a cause of GDM, although this cause-effect relationship remains to be determined.

Besides excess macronutrient availability and transport, many other factors such as races and fetal sex may contribute to GDM associated FM. The prevalence of GDM demonstrates obvious race/ethical disparity and indicates higher incidence in East and Southeast Asians, other Pacific Island populations, Hispanics, and African Americans with high body mass index (BMI, weight in kilograms divided by the square of height in meters) but lower incidence in Caucasian populations(17-20). In addition, a few studies suggest that pregnant women carrying a boy have higher relative risk of GDM than those carrying a girl (21), due to poorer β-cell function and higher glucose intolerance(22). Furthermore, the incidence of FM is higher in male compared to female fetuses (23, 24). To date, the underlying mechanisms related to genetic background and fetal sex have not been well understood.

Restrained by the lack of reliable animal models of GDM, we initiated research on the role of placenta in the development of GDM by making use of human placental tissues from PeriBank Repository, Baylor College of Medicine, Houston, Texas. This cohort of pregnant women is unique in that Hispanic subjects predominate over other races, thus demonstrating potential to develop into a valuable research pool for mechanistic studies of GDM and eradication of minority health disparity. In this study we hypothesize that fatty acid metabolism in the placenta was impaired in women with GDM, and that the alteration is sex specific. To test this hypothesis, we conducted meta-analysis on a patient cohort recorded by PeriBank Repository to investigate the incidence of FM with the consideration of races, pre-pregnancy obesity and fetal sex. Furthermore, we investigated the mRNA expression of genes related to fatty acid metabolism in placental tissues from subjects with or without pre-pregnancy obesity and GDM. In both objectives, the effects of sex of fetus and GDM were assessed.

MATERIALS AND METHODS

Patients and clinical data analysis

In this retrospective study, a total of 26000 subjects receiving prenatal and perinatal cares in the Clinics of Baylor College of Medicine, Houston, Texas from August 1, 2011 to July 28, 2017, were screened using the PeriBank repository. Subjects with multiple births and/or unrecorded BMI were excluded in the analysis. To the end, 17995 subjects were included in our statistical analysis in which the confounding variables of ethnicity, pre-pregnancy BMI (body mass index prior to pregnancy), fetal sex, and birth weight were assessed. GDM was determined by the standard clinical diagnosis (American Diabetes Association 2011). Macrosomia was defined by a birth weight of ≥4000g. Obesity was defined by a pre-pregnancy BMI ≥30.0-39.9. Two race groups, Hispanic and non-Hispanic, were considered in the analysis of incidence of GDM and FM.

Subject data and specimens were obtained following full and informed subject consent with the generous support from the Departments of Obstetrics and Gynecology and Pathology and Laboratory Medicine at Texas Children's Hospital and Baylor College of Medicine on the PeriBank protocol (IRB H-26364, Dr. Kjersti Aagaard PI). Additional research was approved on the protocol IRB H-28617. Briefly, PeriBank is a biobank focusing on specimens collected during the perinatal period, which encompasses state-of-the-science processing and storage of specimens according to Best Practices recommended by International Society for Biological and Environmental Repositories. Recruitment, specimen processing, storage and retrieval systems were developed by a multi-disciplinary consortium of obstetrician-gynecologists and maternal-fetal medicine specialists, pathologists, nurses, and laboratory staff. Maternal, paternal, and cord blood along with placental tissue were collected from all consenting subjects admitted to Ben Taub General Hospital and Texas Children's Pavilion for Women (Houston, TX). Quality assurance of specimens in storage, including chemistry and hormonal assays and nucleic acid isolation, was implemented, as described previously(25).

Placental tissue collection

Three groups of obese pregnant women were selected in PeriBank repository, 1) with both GDM and FM (abbreviated as wGwM); 2) with GDM and without FM (wGnM); 3) without both GDM and FM (nGnM; taken as the control group) (n=5 placentas per group per sex of fetuses). Among GDM subjects, only those treated with insulin and/or glyburide (termed A2GDM) were selected for placental tissue requisition. Placental tissues in all 3 groups were collected between 37.71- and 40.57-week's gestation from singleton pregnancies. The pre-pregnancy BMI and gestational age (the length of pregnancy) were matched in all groups, while fetal weight in wGwM group was higher than other groups ($P < 0.001$)(Table 1). The standard procedure for tissue collection and storage was described previously(25). Briefly, placental samples were collected immediately after delivery. In each case a 1-2 cm^3 piece of tissue was removed from the center of the placenta, rinsed in normal saline, and dissected to remove chorionic membrane and blood vessels prior to being stored in RNAlater solution (Cat. AM7021; Ambion, Austin, TX) at −80°C.

RNA extraction, cDNA preparation and q-PCR analysis

Total mRNAs were extracted from around 100 mg placental tissue using MiRNeasy Mini kit (Cat. 217004; Qiagen Inc., Valencia, CA), followed by DNA cleanup with RNase free DNase I (Cat.79254; Qiagen) and reverse transcription with miScript II RT Kit (Cat. 218160; Qiagen), according to the manufacturers' instructions. Expression of genes related to fatty acid transport/uptake (*LIPG*, *LIPE*, *LPL*, *ATGL*, *ANGPTL3*, *4* and *8*, *CD36*, *GOT2*, *FABP4*), fatty acid oxidation (*CPT1B*, *PPKAA1*, *PPARA*), fatty acid accumulation/esterification (*ACC*, *FASN*, *MCAD*, *SCD*, *DGAT1*, *PLIN1*, *PLIN2*, *PPARG*, *SREBF1*) was measured by q-PCR. The primer sequences and product information are shown in Table 2. Q-PCR detection was performed on a CFX96Real-Time PCR Detection System (Cat.184-5096; Bio-Rad, Hercules, California). iTaq™ Universal Probes Supermix (Cat. 1725135; Bio-Rad) was used for amplification. The reaction mixture was incubated at 95°C for 10 min and cycled according to the following parameters: 95°C for 30 seconds and 60°C for 1 min for a total of 40 cycles. Negative control without cDNA was performed to test

primer specificity. The relative gene expression was calculated by use of the threshold cycle (CT) TBP /CT target genes.

Statistical analysis

The incidence of obesity, GDM, and macrosomia in any two groups of subjects was compared by Chi-square test. Data on gene expression was analyzed for the effects and interactions of FM and fetal sex, using least-squares analysis of variance (ANOVA) and the general linear model procedures of the Statistical Analysis System (Version 9.4., SAS Institute, Cary, NC). Log transformation of variables was performed when the variance of data was not homogenous among groups, as assessed by the Levene's test. A P-value ≤ 0.05 was considered significant. Data were presented as least-squares means (LSMs) with overall standard errors (SE).

RESULTS

The incidence of obesity, GDM and FM in the study cohort

Among total of 17995 subjects in our cohort, the proportion of Hispanic was significantly higher than that of non-Hispanic (9896 vs. 8099; 54.99 vs 45.01%; $P < 0.05$). 4822 subjects were obese, accounting for 26.80% of the total; 1326 were diagnosed with GDM, accounting for 7.37% of the total; 1425 delivered a newborn with macrosomia, accounting for 7.92% of the total. 3155 of Hispanic subjects were obese, accounting for 65.43% of the all obese subjects in this cohort; 920 of Hispanic subjects were GDM, accounting for 69.38% of all GDM subjects in this cohort; 750 of Hispanic subjects delivered a newborn with macrosomia, accounting for 52.63% of all patients with FM. Hispanic subjects had higher incidence of obesity (31.88 vs. 20.58%, $P < 0.01$), GDM (9.30 vs. 5.01%, $P < 0.01$), compared to that of non-Hispanic subjects, respectively. However, the incidence of FM in Hispanic subjects had a trend to be lower than that of non-Hispanic subjects (7.58 vs. 8.33%, P=0.062) (Table 3).

As there was no difference in the incidence of FM between Hispanic and non-Hispanic subjects, we further analyzed the interaction between GDM and pre-pregnancy obesity or FM and sex without considering race as a factor. The number of GDM and non-GDM subjects in Obese Group is 605 and 4218, respectively, while that in Non-obesity Group is 721 and 12452, respectively. The incidence of GDM in Obesity Group (12.55%) is much higher than the incidence of GDM in Non-obesity Group (12.55 vs. 5.47%; $P < 0.01$). The number of FM and non-FM in GDM subjects is 139 and 1187, respectively, and that in non-GDM subjects are 1286 and 15383, respectively. The incidence of FM in GDM subjects was higher than that of non-GDM subjects (10.48 vs. 7.71%; $P<0.01$). The number of female and male fetuses with macrosomia is 518 and 907, respectively, while that in fetuses without macrosomia is 8312 and 8258, respectively. There was higher incidence of macrosomia in males compared to female fetuses (63.65 vs. 36.35%; $P<0.01$).

Fatty acid transport and metabolism related gene expression in placental tissue

Considering pre-pregnancy obesity is a confounding factor for the development of GDM, this study was to investigate whether the placenta lipid transport and metabolism related

gene expressions are affected in obese GDM subjects compared to obese subjects without GDM and whether there is the effect of FM and fetal sex on these gene expressions, aiming at exploring potentially transcriptional mechanisms related to placental lipid transport and metabolism. We found that most of genes investigated in this study (Table 1), except *FABP4*, *LPL* and *ANGPTL3*, were not affected by GDM, FM, nor sex. mRNA levels of *FABP4* were 2.42-, and 1.67- fold higher ($P < 0.01$, < 0.05) in wGwM group compared to wGnM and nGnM group, respectively (Fig. 1A), and this occurred in placentas with male fetuses (Fig. 1B). In contrast, mRNA levels of *LPL* were 2.73- and 7.83-fold lower ($P < 0.05$) in wGwM and wGnM groups compared to nGnW group, respectively, and this occurred in placentas with male fetuses (Fig. 2B). Similarly, mRNA levels of *ANGPTL3* were 1.72-fold higher ($P < 0.05$) in wGwM group compared to wGnM group (Fig. 3A), but there was no statistical difference among subgroups when fetal sex was included in the analysis (Fig. 3B).

DISCUSSION

FM is one of the major concerns in the prevention and treatment of GDM, but to date, the current glycemic-centered treatments are not effective enough to overcome this problem (26, 27). Recently we have taken initiatives to tackle this problem by starting from exploring a cohort of pregnant women which demonstrates higher rates of GDM and Hispanic predominance. Based on this large cohort of subjects, we found that the Hispanic population, albeit higher incidence of GDM, do not have significantly higher incidence of FM as compared to non-Hispanic. The incidence of FM is significantly higher in obese women as compared to non-obese women. Strikingly, the incidence of FM is higher in male compared to female fetuses, which coincides with the elevated expression of FABP4 in the male placental tissue. The present study, a pilot research to address the cause of FM, suggests placental lipid metabolism may contribute to fetal overgrowth, although the dogma believes the elevated blood glucose levels in maternal circulation in GDM subjects cause fetal overgrowth (28).

Hispanic, non-Hispanic white, and non-Hispanic black are the three largest race groups in the United States (29), and Hispanic is the largest minority. Hispanic populations in general have less access to medical services due to socioeconomic limitations, thus there is a necessity to make a closer study focusing on Hispanic population. Thanks to the relatively concentrated Hispanic population in Houston’s urban area, PeriBank, a tissue repository run by Baylor College of Medicine provides a unique opportunity to analyze the causes of GDM and macrosomia through genetical, dietary and socioeconomic studies in an accessible large cohort of patients with a defined genetic similarity. Epidemiological studies and public data resources have indicated that Hispanic women have the highest incidence of GDM in the United States compared to other races (18, 19) . Our study confirms the higher incidence of GDM in Hispanic subjects (Table 3). Coincidently, the incidence of pre-pregnancy obesity in Hispanic subjects are much higher as compared to that in non-Hispanic subjects (Table 3). In literature, the association of GDM and pre-pregnancy obesity has been demonstrated despite race and ethnic differences. Overweight/obesity was the most important GDM risk factor for non-Hispanic whites, Hispanics, Asian Indians, and Filipinos when the WHO/ADA cut-off points were applied (30). However, obese Hispanic women are more prone to developing GDM as compared with non-Hispanic black and whites (31). Therefore, the coincidence of

elevated incidences of GDM and pre-pregnancy obesity in our present study supports that obesity is a confounding factor of GDM, and thus at least in the Hispanic population, reduction in pre-pregnancy body weight could be beneficial to prevent the development of GDM.

In contrast to the higher incidence of GDM and obesity in Hispanic women, our study demonstrates that there is no difference of FM between Hispanic and non-Hispanic women (Table 3). The National Center for Health Statistics reported that the incidence of FM in the US was 7.81% and Hispanic, non-Hispanic white and black accounted for 6.98%, 9.57% and 4.34% of the total FM, respectively (32). Currently, we do not know what prevents FM in the Hispanic women with GDM, however, logistic regression analyses showed that risk of macrosomia was positively associated with maternal age, pre-pregnancy BMI, gravidity, parity, maternal height, gestational weight gain, GDM, and male fetal sex (24). In fact, epidemiological studies indicate the body weight gain in early pregnancy is also associated with the occurrence of GDM (30, 33). Therefore, it is of great importance to differentiate pre-pregnancy obesity and gestational weight gain to explore the effects of fat accrual in the development of GDM and associated FM (3). In future, gestational weight gain and related genetic factors warrant to be investigated to elucidate the cause of FM in the settings of GDM.

Sex disparity in FM is confirmed in our cohort of subjects. Male neonates demonstrated higher incidence of macrosomia compared to female neonates (63.65 vs. 36.35%). Few studies reported that there is higher incidence of FM in male fetuses in the Chinese population (23, 24), and consequently male fetuses have higher neonatal risks and adverse outcomes(23). The rates of preterm birth (7.3% for males, 6.5% for females) and FM (8.3% for males, 5.1% for females) were higher for male newborns, whereas fetal growth restriction (8.0% for females, 5.4% for males) and malpresentation (4.3% for females, 3.6% for males) were more frequent among female-bearing mothers(23). However, the reason for this sex disparity remains mysterious to date. In addition to obvious sex differences in nutrition, growth, and metabolism in preterm infants(34), the placenta – transitory but critical fetal tissues to nurturing fetal development and growth – demonstrates sex dimorphism in many aspects including global gene expression(35), steroid hormone production(36), antioxidant buffering capacity (37), mitochondrial macronutrient metabolism(38), responses to adverse maternal milieu such as obesity, inflammation, hypoxia, GDM, preeclampsia, and other pregnancy related disorders(39, 40). In the present study, we tried to associate the alteration of placental gene expression with macrosomia bias in males and found that increased expression of FABP4 is coincident with the higher incidence of macrosomia in male fetuses (Fig. 1B). Although it could be risky to extrapolate this change of FABP4 expression as the increased placental lipid transport into fetal blood circulation, our study narrows down the direction and range of potential candidates in exploring underlying mechanisms of fetal overgrowth and male bias.

Placental lipid metabolism is emerging as a critical player in the development of GDM and FM, however, to date, we have not understood it completely. The complexity of the processes, interaction between multiple maternal organs and the placenta, and the unclear role of fetal originated factors make placental lipid metabolism a black box. The expression

of FABP4 is highly elevated in the male placenta in obese women with GDM (Fig.1). FABP4 is one of intracellular lipid chaperones that regulate lipid trafficking and responses in multiple types of cells(41). Accumulating evidence supports that FABP4 plays a critical role in the development of metabolic and cardiovascular diseases(42), primarily by insulin resistance enhancement, metabolically-driven low-grade and chronic inflammation, and atherosclerosis (43). Considering that FABP4 expression is primarily regulated at the transcriptional levels (42, 44), we expect that similar to its alteration in mRNA levels, the elevated FABP4 proteins levels may be present in the GDM placenta. Supportive to our notion, recent studies reported that FABP4 levels in maternal plasma are higher in GDM patients(45) and FABP4 levels in the umbilical cord serum are higher in the GDM offspring and also positively associated with the maternal serum FABP4 (46). In addition, FABP4 expression in human trophoblast cells is induced by fatty acids (47), which plasma levels are highly elevated in GDM patients. Furthermore, FABP4 stimulates human trophoblast cells proliferation, migration and invasion(48), which is parallel to the excess placental villi growth in GDM placenta(13, 49). More importantly, the cellular function of FABP4 is to promote lipolysis (50, 51), thus the increased FABP4 in trophoblast cells in GDM placenta could facilitate the transport of lipids into fetal capillary by increasing the gradient of fatty acids between trophoblast cells and neighboring fetal endothelial cells – the driving force of lipid transport.

In contrast to the elevated expression of FABP4, placental LPL, a key player in the uptake of LDL from maternal circulation into the trophoblast cells, and its endogenous inhibitors, angiopoietin-like proteins, are reduced in the GDM placenta; however, they are not affected by the presence of FM (Fig.2). LPL and ANGPTL determine the rate of the uptake of lipid from maternal blood circulation. Unlike FABP4, LPL expression is not regulated at the transcriptional level, but rather posttranslational level(52). Placental LPL activity measured in tissue culture may be affected by a variety of metabolites (triglyceride, free fatty acid, glucose) and hormones (insulin, cortisol, IGF-1, and estradiol) (52, 53). However, to date, the underlying regulatory mechanisms have not been clarified, possibly due to the limitations of ex vivo functional assay and the complex nature of lipid metabolism. In both obese and GDM subjects, the placental LPL activity was increased as compared to that of normal-weight subjects(54). Supportive to this finding, a recent study reported that placental LPL activity is a determinant in the LPL function and positively associated with fetal fat accretion(55). It is noteworthy that LPL activity together with other types of lipases, is inhibited by angiopoietin-like proteins in fat tissues and skeletal muscles. ANGPTL3 can increase the storage of adipocyte in the liver, stimulate lipolysis in adipocytes, and modulate plasma lipids especially in triglyceride-rich lipoproteins mainly by inhibiting the activity of LPL. Additionally, ANGPTL3 has a broader action on apoB and apoA-I-containing lipoprotein, as well as on free fatty acids and adipose tissue metabolism(56, 57). To date, the role of ANGPTL3 in the placenta remains unclear. We found ANGPTL3 expression is increased in GDM subjects with FM (Fig.3) while the expression of ANGPTL 4 and 8 was not affected by any factors considered in this study. Considering that all our GDM subjects were treated with insulin or glyburide for a prolonged period, the decreased LPL expression and enhanced expression of ANGPTL3 may represent a positive adaptation to increased

maternal plasma VLDL, or a result of administration of medicine to enhance the insulin levels.

This study has limitations, because it aimed to investigate the incidence of obesity, GDM, and FM and to screen the potential alteration of gene expressions which are related to lipid transport and metabolism in placental tissues. The detailed analyses on the correlation among a variety of GDM could be conducted using this unique cohort of subjects in the future. In addition, we will confirm whether the same pattern of changes occurs in protein levels as well as mRNA levels, and thus convincing the regulation of gene expression as a modulatory mechanism in lipid metabolism and a potential mechanism responsible for GDM. Furthermore, it could be valuable to investigate placental lipid transport and metabolism using the recently developed fluorescent labelled fatty acids(58) and ex vivo placental perfusion system (59), although these techniques are currently still challenging in many aspects.

In summary, this study initiated a study of GDM associated FM, starting by defining a predominately Hispanic cohort in Houston, Texas and exploring potential mechanisms of FM related to placental lipid transport and metabolism. Although there is significantly higher incidence of GDM in Hispanic subjects, the incidence of FM is not elevated accordingly. In addition, this study demonstrated that different genes were altered in response to GDM, obesity, FM and/or fetal sex, suggesting that these factors may affect placental lipid transport and metabolism through different unknown ways. Overall, our study supports the notion that variations in racial groups should be considered when we devise effective strategies to prevent large-for-gestational-age deliveries (60).

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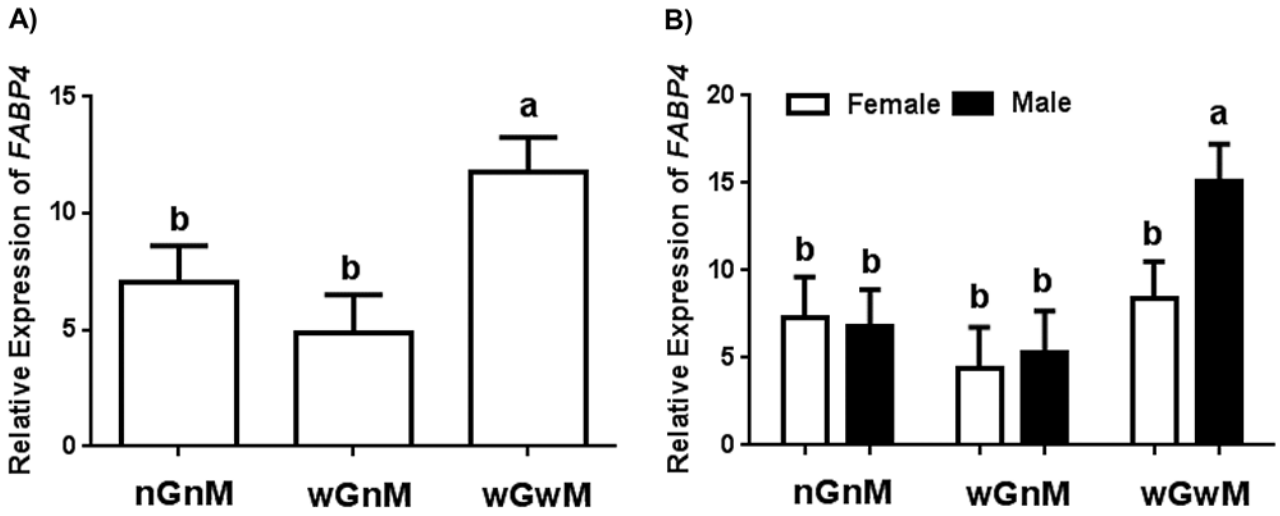

Figure 1.

Quantitative real-time PCR analysis of *FABP4* in placental tissues from obese women with or without gestational diabetes mellitus (GDM) and with or without fetal macrosomia. A) The effect of gestational diabetes mellitus and fetal macrosomia on relative expression of *FABP4*. (B) The effect of gestational diabetes mellitus, fetal macrosomia and fetal sex on relative expression of a *FABP4*. nGnM: without both GDM and fetal macrosomia; wGnM: with GDM without fetal macrosomia; wGwM: with both GDM and fetal macrosomia. The error bar represents the mean ± SEM expressed as relative units of mRNA standardized against *TBP* (n = 5 per group defined by GDM, fetal macrosomia and sex). The different letters indicate the statistically significance among groups.

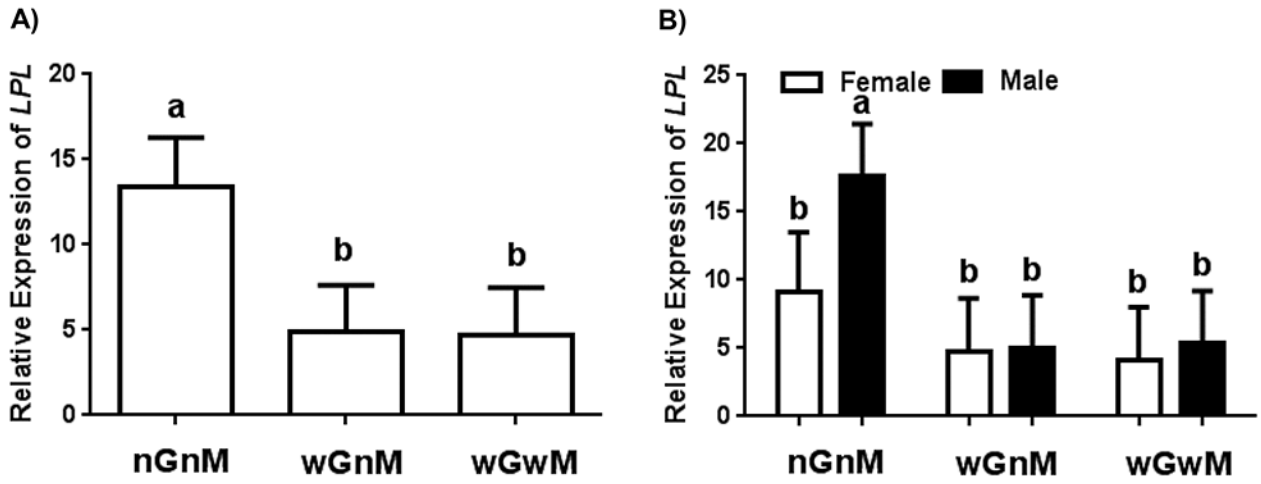

Figure 2.

Quantitative real-time PCR analysis of *LPL* in placental tissues from obese women with or without gestational diabetes mellitus (GDM) and with or without fetal macrosomia. A) The effect of gestational diabetes mellitus and fetal macrosomia on relative expression of *LPL*. (B) The effect of gestational diabetes mellitus, fetal macrosomia and fetal sex on relative expression of *LPL*. nGnM: without both GDM and fetal macrosomia; wGnM: with GDM without fetal macrosomia; wGwM: with both GDM and fetal macrosomia. The error bar represents the mean ± SEM expressed as relative units of mRNA standardized against *TBP* (n = 5 per group defined by GDM, fetal macrosomia and sex). The different letters indicate the statistically significance among groups.

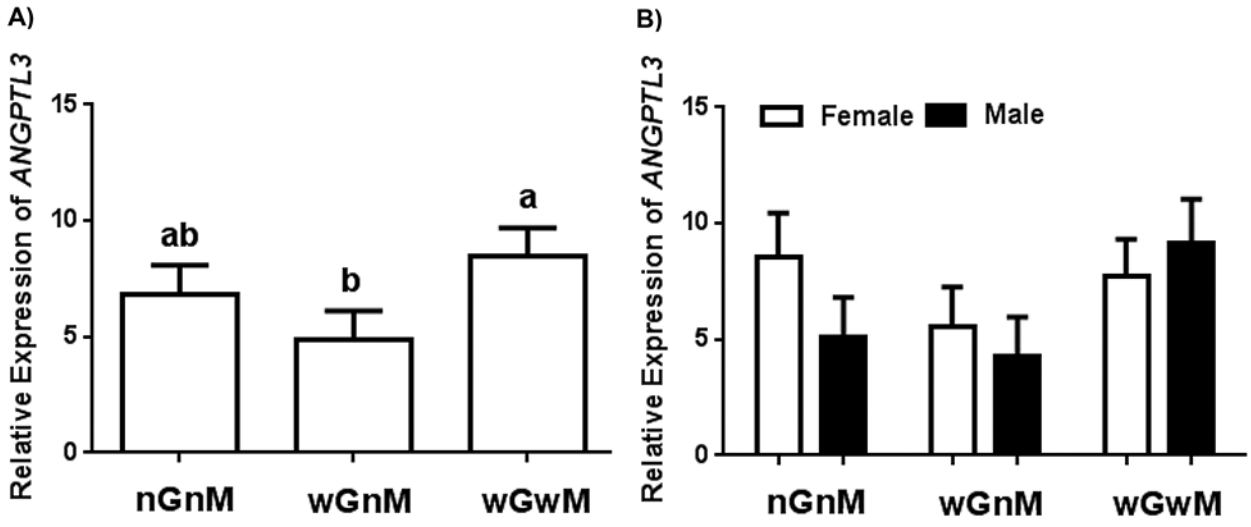

Figure 3.

Quantitative real-time PCR analysis of *ANGPTAL3* in placental tissues from obese women with or without gestational diabetes mellitus (GDM) and with or without fetal macrosomia. A) The effect of gestational diabetes mellitus and fetal macrosomia on relative expression of *ANGPTAL3*. (B) The effect of gestational diabetes mellitus, fetal macrosomia and fetal sex on relative expression of *ANGPTAL3*. nGnM: without both GDM and fetal macrosomia; wGnM: with GDM without fetal macrosomia; wGwM: with both GDM and fetal macrosomia. The error bar represents the mean ± SEM expressed as relative units of mRNA standardized against *TBP* (n = 5 per group defined by GDM, fetal macrosomia and sex). The different letters indicate the statistically significance among groups.

Table 1.

Pre-pregnancy body mass index, gestational age and fetal weight of pregnant women selected for placental tissue acquisition

Subject Group	Sex	Pre-pregnancy Body Mass Index	Gestational Age (weeks)	Fetal Weight (grams)
wGnM	Female	32.32±0.34	39.03±0.42	3513.20±101.98
	Male	32.48±0.93	37.88±0.52	3316.00±115.21
wGwM	Female	34.16±1.33	38.8±0.15	4111.40±61.78 *
	Male	34.30±0.65	38.66±0.19	4505.00±165.15 *
nGnM	Female	33.74±0.75	38.94±0.19	3384.80±64.67
	Male	33.26±0.83	39.63±0.13	3313.00±82.58

wGnM: subjects with gestational diabetes mellitus and without fetal macrosomia; 2) wGwM: subjects with both gestational diabetes mellitus and fetal macrosomia; 3) nGnM: subjects without both gestational diabetes mellitus and fetal macrosomia. n=5 per subject group

* $P < 0.001$; fetal weight in the wGwM group compared to the nGnM group.

Table 2.

Quantitative real-time PCR primers for lipid transport and metabolism related genes and house-keeping gene *TBP*

Gene Function	Gene Symbol	Forward Primer (5' →3')	Reverse Primer (5'→3')	GenBank Accession No.	Product Size (bp)
Fatty acid transport/uptake	*LIPG*	*AGCTCTGGTTTCGCAAGTGT*	*CTCCACAGTGGGACTGGTTT*	NM_006033.3	65
	LIPE	*GCACTACAAACGCAACGAGA*	*TGTGATCCGCTCAAACTCAG*	NM_005357.4	112
	LPL	*GTCCGTGGCTACCTGTCATT*	*TGGCACCCAACTCTCATACA*	M15856.1	94
	PNPLA2	*GCAGTTTCCTGCTGAAGGTC*	*GCTCGTCCTTGGAGTTGAAG*	AY894804.1	129
	ANGPTL3	*ATTTTAGCCAATGGCCTCCT*	*CACTGGTTTGCAGCGATAGA*	NM_014495.3	139
	ANGPTL4	*GCAGGATCCAGCAACTCTTC*	*AAACTGGCTTTGCAGATGCT*	NM-139314.2	92
	ANGPTL8	*AGCAGAGCCACATCCTATGG*	*CGCTGTGTGGAGTCTCTCCT*	NM_018687.6	110
	CD36	*AGATGCAGCCTCATTTCCAC*	*GCCTTGGATGGAAGAACAAA*	NM_001001548.2	150
	GOT2	*ATCCGTCCCATGTATTCCAA*	*TTCACTTCTTGCAGCCATTG*	NM_002080.3	101
	FABP4	*AACCTTAGATGGGGGTGTCC*	*GTGGAAGTGACGCCTTTCAT*	NM_001442.2	123
Fatty acid oxidation	*CPT1B*	*GCCAAAGAATTCCAGGACAA*	*TTGCTGTTCACCATGAGAGG*	NM_004377.3	143
	PRKAA1	*TGCACACATGAATGCAAAGA*	*TTCTGGTGCAGCATAGTTGG*	NM_206907.3	106
	PPARA	*ACGATTCGACTCAAGCTGGT*	*GTTGTGTGACATCCCGACAG*	NM_005036.4	123
Fatty acid accumulation/ esterification	*ACC*	*ACCACCAATGCCAAAGTAGC*	*CTGCAGGTTCTCAATGCAAA*	U19822.1	150
	FASN	*CACAGGGACAACCTGGAGTT*	*ACTCCACAGGTGGGAACAAG*	U26644.1	97
	ACADM	*TTGAGTTCACCGAACAGCAG*	*AGGGGGACTGGATATTCACC*	NM_000016.5	115
	SCD	*TGTTCGTTGCCACTTTCTTG*	*GGGGGCTAATGTTCTTGTCA*	NM_005063.4	111
	DGAT1	*GTTATTGCGGCCAATGTCTT*	*GCTGGGAAACACAGAATGGT*	NM_012079.5	131
	PLIN1	*GGAGGAGGAGGAAGAATTGG*	*AGGGCTGCTACCTCACTGAA*	NM_002666.4	57
	PLIN2	*TGGGATCCCTGTCTACCAAG*	*CCTGGCAAATTCAATCAGGT*	NM_001122.3	134
	PPARG	*ATCAAAGTGGAGCCTGCATC*	*CGACATTCAATTGCCATGAG*	NM_138712.3	104
	SREBF1	*CTGCTGTCCACAAAAGCAAA*	*GGTCAGTGTGTCCTCCACCT*	NM_001005291.2	113
	TBP	*CACCACAGCTCTTCCACTCA*	*GGGGAGGGATACAGTGGAGT*	NM_003194.4	73

Table 3.

Incidence of obesity, GDM and fetal macrosomia in Hispanic and non-Hispanic subjects

Disorders	Hispanic Group		non-Hispanic Group		P Value
	No.	Percentage (%)	No.	Percentage (%)	
Obesity	3155	31.88	1667	20.58	<0.01
GDM	920	9.30	406	5.01	<0.01
Macrosomia	750	7.58	675	8.33	0.062