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ORIGINAL ARTICLE

Maternal adiposity is associated with inflammatory gene expression in leukocytes at term human pregnancy: A pilot study

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Abstract

Background: Human labor is associated with an inflammatory process that takes place at the maternal–fetal interface, where leukocytes infiltrate and contribute to the local production of effector molecules such as cytokines, chemokines, MMPs, etc. This process may be altered by a low-grade chronic inflammation, characteristic of obesity, resulting in adverse pregnancy outcomes. In this cross-sectional pilot study, we analyzed the relationship between maternal adiposity and inflammation-related gene expression in leukocytes from six healthy women with term pregnancies without labor.

Methods: We estimated maternal adiposity and examined the relative expression of 211 inflammation-related genes in maternal peripheral blood leukocytes (MAT), placental intervillous blood leukocytes (PLA), and choriodecidual leukocytes (CHD) by real-time qPCR. Finally, we analyzed the correlation between maternal adiposity and gene expression.

Results: Participants' adiposity ranged from 27.6% to 61.1% (n = 6). The expression of 23 genes significantly differed ($p < 0.05$) in MAT, PLA, and CHD leukocytes, most of which code for chemokines and proinflammatory cytokines. Importantly, increasing maternal adiposity correlated ($r > 0.7$) mostly positively with the expression of genes related to activation, migration, infiltration, and proinflammation in MAT (36 genes) and PLA (31 genes). In contrast, in CHD leukocytes maternal adiposity correlated only negatively with seven genes, involved in migration and infiltration.

Conclusion: Our findings suggest that during term pregnancy, increased maternal adiposity may enhance the priming of peripheral leukocytes, while in choriodecidua it may alter leukocyte recruitment and proinflammatory activity. Maternal adiposity must be considered an important variable in further studies that analyze inflammation-related gene expression in pregnant women.

KEYWORDS

adiposity, choriodecidua, gene expression, human pregnancy, inflammation, leukocytes, maternal–fetal interface

1 | INTRODUCTION

Obesity, considered a world health problem and a mortality risk factor, is highly prevalent among women of reproductive age worldwide (World Health Organization 2020; WHO & 10 Facts on Obesity, 2017; World Obesity Federation, Data,n.d.). High maternal adiposity is associated with numerous short and long-term complications before the time of conception and during the perinatal period for both mother and child. These complications include gestational diabetes, hypertensive disorders, high-risk labor, hemorrhage, miscarriage, and preterm birth (Huda et al., 2010; McDonald et al., 2010).

Increased adiposity, characteristic of obesity, can alter several physiological conditions including inflammatory processes in which cytokine expression and secretion by leukocytes are modified (Martí et al., 2001; Schäffler et al., 2006). Such an inflammatory process is usually present during human term pregnancies, when leukocytes infiltrate the maternal–fetal interface in the absence of infection. There, they participate in the local inflammatory process that will lead to labor by producing several effector molecules like cytokines, chemokines, cell adhesion molecules, and proteolytic enzymes (Gomez-Lopez et al., 2010).

The chronic inflammatory environment characteristic of obesity may exacerbate the inflammation associated with term pregnancy (Challier et al., 2008; Challis et al., 2009; Madan et al., 2009) and result in adverse perinatal outcomes (Djelantik et al., 2012; Huda et al., 2010; Lashen et al., 2004). In a previous study, we showed that higher maternal adiposity is associated with lower circulatory anti-inflammatory markers (Vega-Sanchez, Barajas-Vega, et al., 2010).

In the present pilot study, we investigated if there is a relationship between maternal adiposity and the expression of 211 inflammation-related genes in leukocytes during healthy term pregnancy. We analyzed this relationship in leukocytes in three distinct anatomical compartments: maternal peripheral blood, placental blood, and choriodecidua; as there is much evidence that these compartments differentially display localized inflammatory environments related to the termination of pregnancy (Castillo-Castrejon et al., 2014; Gomez-Lopez et al., 2009; Vega-Sánchez et al., 2010; Vega-Sanchez, Gomez-Lopez, et al., 2010; Young et al., 2002). To our knowledge, this is the first study to explore the association between maternal adiposity and the expression of inflammation-related genes in leukocytes at human term pregnancy.

2 | METHODS

2.1 | Ethical compliance

This study was conducted according to the guidelines stated in the Declaration of Helsinki and all procedures involving participants were approved by the Internal Ethics and Research Committees (register number 212250-02191). Written informed consent was obtained from all participating women.

2.2 | Participating women

The present pilot study was carried out at the National Institute of Perinatology in Mexico City, a tertiary referral hospital for women with high-risk pregnancies. We included a convenience sample of six adult women with healthy term pregnancies, selected to cover a wide range of adiposity values. We chose women based on their pregestational body mass index (pgBMI), calculated from self-reported pregestational weight and measured height. We aimed to select participants that comprised every BMI category (low weight / normal weight / overweight / obesity, as defined by the World Health Organization (World Health Organization 2020), assuming this would yield a wide range of adiposity values.

Participants had singleton pregnancies and delivered by elective cesarean section without clinical signs of labor, such as regular uterine activity, cervical dilation, and/or rupture of fetal membranes. Delivery by cesarean section was indicated due to the personal history of pregnancy-related complications according to institutional policies. These complications included being over 35 years old, presenting cephalopelvic disproportion, and/or developing preeclampsia in a previous pregnancy. However, participants did not present any complications, infection, metabolic or autoimmune pathologies during their current gestation.

2.3 | Estimation of maternal adiposity

To estimate maternal adiposity we used the equation developed and validated by Villar et al., in a group of Guatemalan pregnant women (Villar et al., 1992). Although ethnicity may be regarded as a major source of genetic variation, we considered that Guatemalan-Mestizo pregnant women would better resemble Mexican-Mestizo women than other populations for which body composition equations have been developed. For example, equations by Paxton et al., include a mixture of "black, white, and Hispanic women in New York City" (Paxton et al., 1998).

All of the women in our study were of Mestizo origin, from the Mexico City metropolitan area, located in the high plateau basin of central Mexico. The equation we used, developed by Villar et al., is the following:

Total body fat (kg) = −0.59 + (0.87 * weight) – (26.9 * body surface area) + (0.099 * subscapular skinfold) + (0.076 * thigh skinfold) + (0.0108 * resistance)

We measured the participants' body weight (BWB-800 Tanita scale, Japan), height (Seca stadiometer, Germany),

mid-thigh and subscapular skinfolds (Lange skin caliper, U.S.A.), and resistance (RJL System, U.S.A.) the evening before the participants' programmed cesarean section was performed the next day. A single person (K.MD-R) performed all measurements using the same standardized anthropometric technique (Lohman et al., 1998). The anthropometric measurements were validated by averaging two consecutive measurements. The average intra-subject coefficients of variation were 3.3% for subscapular skinfold, 2.1% for thigh skinfold, 2.4% for reactance, and 0.1% for weight.

Body surface area (BSA) was calculated using the equation: BSA (m^2) = $weight^{(0.424)}$* $height^{(0.725)}$* 0.007184 (Du Bois & Du Bois, 1916)

From the total body fat calculated with the Villar et al., equation, we then estimated maternal adiposity as the percentage of total body fat to body weight. As mentioned above, for this pilot study we selected participants based on their adiposity values. We included a group of women who presented a wide range of adiposity, which allowed us to obtain a brief look at the association between this variable and leukocyte gene expression.

2.4 | Biological samples

The following day, immediately after delivery (approximately 12–16 hours after anthropometric measurements were obtained), we collected maternal peripheral blood by venipuncture of the forearm, placental intervillous blood by manually draining the cotyledons and the fetal membranes cut from the placenta. Leukocytes from maternal peripheral blood (MAT) and placental intervillous blood (PLA) were isolated by density gradient using Polymorphprep (Axis-Shield, Norway) and following the manufacturer's protocol. Choriodecidual leukocytes (CHD) were isolated from the chorionic layer of the fetal membranes by enzymatic digestion following a previously described method (MacDonald-Ramos et al., 2018).

Total cell count was obtained by a cell counter (AcT5diff, Beckman Coulter) and 10×10^6 leukocytes were stored in 1 ml of RNAlater (Ambion, U.S.A.) or 1 ml of TRIzol Reagent (Invitrogen Life Technologies, U.S.A.) at −70°C until further processing.

2.5 | RNA isolation, purification and quantification, and cDNA synthesis

We isolated total RNA from MAT, PLA, and CHD leukocytes using TRIzol Reagent according to the manufacturer's instructions. RNA was treated with DNAse to eliminate any possible residual DNA contamination using RNAqueous-4PCR kit (Ambion, U.S.A.) technique and reagents according to the manufacturer's instructions. RNA concentration was determined by spectrophotometry (NanoDrop 2000, Thermo Scientific, U.S.A.) using 1 µl per sample. Integrity of RNA samples was verified using 1% agarose gel electrophoresis, making sure that the two bands that correspond to the 28 s and 18 s ribosomal subunits were clearly visible and defined.

We then synthesized complementary DNA (cDNA) from 100 ng of total RNA using RT^2 First Strand Kit (Qiagen) following the manufacturer's protocol in a GeneAmp 9700 PCR System (Applied Biosystems, Singapore).

2.6 | Real-time qPCR

We analyzed the expression of 211 inflammation-related genes using pre-designed PCR arrays for Inflammatory Cytokines & Receptors, Chemokines & Receptors, and Extracellular Matrix & Adhesion Molecules and RT2 SYBR Green ROX qPCR Mastermix, all from SABiosciences (Qiagen, U.S.A).We analyzed samples individually using one array per sample, not as pools.

PCR arrays were analyzed in a ViiA 7 Real-Time PCR System (Applied Biosystems, Singapore), starting with one cycle at 95°C for 10 min, followed by 40 amplification cycles, each one 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. The quality control of the PCR runs was verified with software. RT^2 Profiler PCR Array Data Analysis version 3.5. Reactions that presented more than one peak on the melting curve or without an amplification signal were discarded.

Relative expression of each gene was calculated by normalizing raw expression data with the geometric mean of three housekeeping genes included in the arrays: *ACTB* (OMIM: 102630, GenBank: NM_001101), *B2M* (OMIM: 109700, GenBank: NM_004048), and *GAPDH* (OMIM: 138400, GenBank: NM_002046). We have previously shown that the expression of these genes does not change between anatomical compartments (MAT, PLA, and CHD) and are, therefore, a suitable reference (R. Vega-Sanchez et al., 2015).

2.7 | Statistical analyses

We evaluated the normality in the distribution of relative expression data for each gene by performing Kolmogorov–Smirnov and Shapiro–Wilk tests. Since many variables did not show a normal distribution, we used Kruskal–Wallis test to evaluate differences in gene expression between anatomical compartments, and Spearman correlation tests to analyze the association between maternal adiposity and gene expression.

Due to the small sample size, we considered only those correlations that were strong and significant ($r \geq 0.7$ and

$p \leq 0.05$) for the Results and Discussion sections. All statistical analyses were performed with software SPSS 20.

3 | RESULTS

3.1 | Characteristics of participating women

The characteristics of participating women are shown in Table 1. All participants had term pregnancies between 38 and 40 weeks of gestation with maternal age ranging from 22 to 41 years. Consistent with our selection method, women comprised all pgBMI categories (Table 1) and therefore presented a wide range of adiposity values, varying from 27.6% to 61.1%, which allowed us to observe the association between increasing adiposity and gene expression.

3.2 | Peripheral blood leukocytes, placental intervillous blood leukocytes, and choriodecidual leukocytes show different expression profiles

All 211 analyzed genes were expressed by MAT, PLA, and CHD leukocytes, although with distinct expression profiles. We observed differences in expression that were statistically significant in 23 of the analyzed genes between anatomical compartments (Figure 1). Interestingly, most of these genes (18/23) are involved in leukocyte migration and infiltration processes, and code for chemokines and their receptors, cell adhesion molecules or extracellular matrix components. The rest of the differentially expressed genes code for inflammatory cytokines.

A complete list of expression values for each analyzed gene in all three compartments can be found in Supplementary Table S1.

TABLE 1 Characteristics of participating women

Characteristics	Median (min - max) n = 6
Maternal age (years)	25 (18–41)
Gestational age (weeks)	39 (38–40)
Number of pregnancies	2(1–4)
Adiposity (%)	36.2 (27.6–61.1)
Pregestational body mass index (pgBMI)[a]	26.2 (16.7–34.9)
Newborn's sex	5 female, 1 male
Mode of delivery (cesarean section / vaginal)	6/0
Labor (absent / present)	6/0

[a]Maternal pgBMI was calculated from self-reported pregestational weight and measured height.

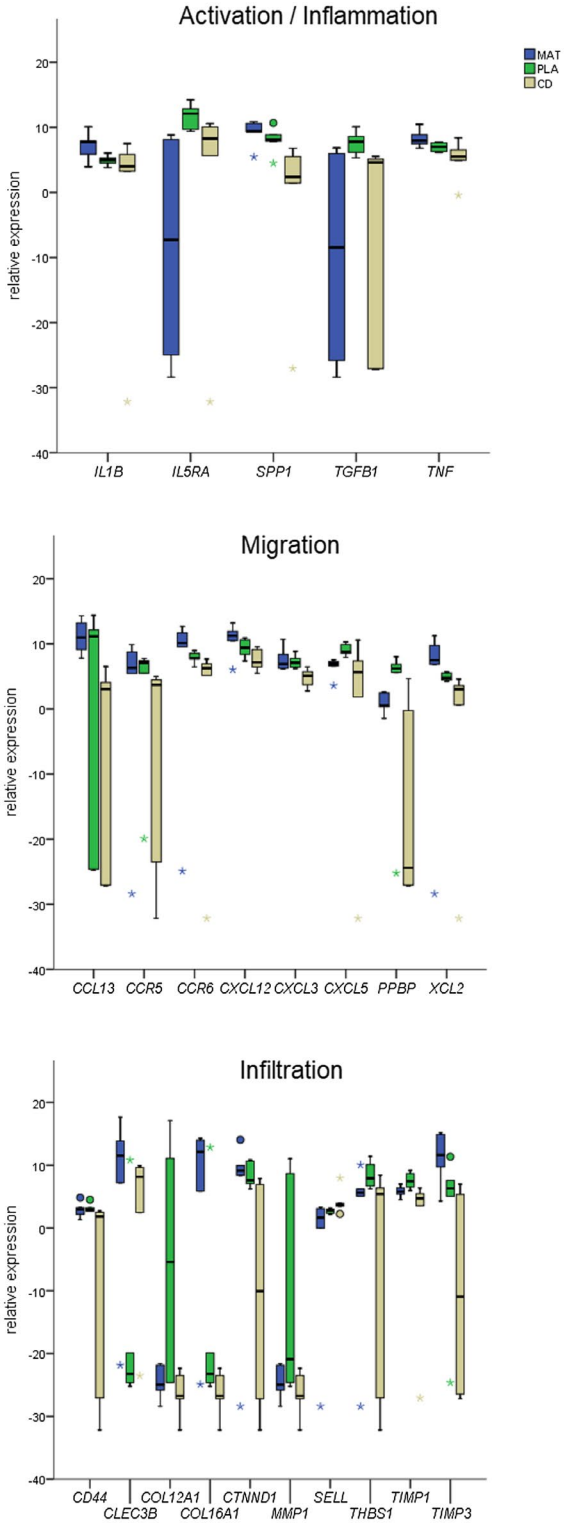

FIGURE 1 Genes with differential expression between MAT, PLA, and CHD leukocytes. Figure shows the relative expression of the 23 genes that showed significant differences ($p < 0.05$, n = 6) between anatomical compartments. Genes are grouped functionally into Activation / Inflammation, Migration and Infiltration. Graphs represent median values (black lines) with interquartile ranges (boxes) and outliers (circles and asterisks). Each gene's individual expression was normalized with the geometric mean of housekeeping genes *ACTB*, *B2M*, and *GAPDH*

3.3 | Maternal adiposity is associated with leukocyte gene expression

Gene expression was clearly associated with maternal adiposity in an anatomical compartment-dependent manner. Although several correlations were found, we only show those that were the strongest, that is, those showing a correlation coefficient $r > 0.7$ (Table 2). The majority of the correlations we observed in MAT and PLA leukocytes were positive, composed of genes involved in activation, migration, and infiltration processes. Conversely, in CHD leukocytes, maternal adiposity only correlated negatively with seven genes.

4 | DISCUSSION

Human term pregnancy has been characterized as a local inflammatory response modulated by invading leukocytes that infiltrate gestational tissues and the maternal–fetal interface (Gomez-Lopez et al., 2010), where inflammatory and other effector molecules are released (Gomez-Lopez et al., 2009). In this study, our goal was to evaluate if maternal adiposity is related to changes in leukocyte inflammatory-gene expression at term gestation.

In this pilot study, we were able to make two major observations. First, peripheral (MAT), placental (PLA) and choriodecidual leukocytes (CHD) show distinct gene expression profiles, with significant differences among the three anatomical compartments, with respect to the expression of 23 genes. These genes code for chemokines and their receptors, proinflammatory cytokines, and cell adhesion molecules, most of which were greatly expressed in MAT. Such differential expression profiles are likely to result from both phenotypical and functional differences between anatomical compartments. Indeed, peripheral blood, placental blood, and choriodecidua have distinct proportions of leukocyte subpopulations (Castillo-Castrejon et al., 2014; Vega-Sanchez, Gomez-Lopez, et al., 2010; Yuan et al., 2009). Our current findings support previously reported evidence that distinct localized inflammatory micro-environments occur within fetal membranes, maternal peripheral blood, and placental blood, where each anatomical compartment contributes differentially to the inflammatory process in human term gestation (Arenas-Hernandez et al., 2019; Gomez-Lopez et al., 2011; Vega-Sanchez, Gomez-Lopez, et al., 2010).

As seen in Figure 1, some genes show great expression variability, not only between anatomical compartments, but also between individuals. This may simply be the result of natural variability among individuals, but may also resemble differences in the extent of preparation for labor within individuals. None of the participants showed any clinical signs of labor; however, since all of the pregnancies were carried to term, it is possible that some of the molecular and cellular processes that lead to labor had begun.

Our second observation concerns the relationship between maternal adiposity and inflammatory gene expression in leukocytes. It has been proposed that under physiological conditions at term pregnancy, maternal leukocytes are primed in the periphery, developing the capability of responding to recruitment and homing signals that originate in the choriodecidua (Gomez-Lopez et al., 2009; Yuan et al., 2009). Once infiltrated to the maternal–fetal interface, they modulate and promote a local inflammatory process necessary for labor to occur (Arenas-Hernandez et al., 2019; Gomez-Lopez et al., 2013). It is unknown, however, how this process may be influenced by the presence of greater maternal adiposity. Although maternal adiposity has undoubtedly always been a component of human pregnancy, it has rarely been regarded as an influencing factor of leukocyte function at term gestation. To our knowledge, only one other report has evaluated the influence of maternal adiposity on leukocyte function, showing that it alters the cytokine production of *ex vivo* stimulated CD4+ T cells (Ozias et al., 2015). In the present study, we examined for the first time the relationship between maternal adiposity and the expression of a wide set of inflammation-related genes, not only in peripheral blood leukocytes but also in those present at the maternal–fetal interface, namely placental blood and choriodecidua.

Therein, the most important observation in our study is that inflammation-related gene expression in leukocytes is associated with maternal adiposity in an anatomical compartment-specific manner. Furthermore, such an association was all the more evident in peripheral blood and placental blood leukocytes, than in choriodecidual leukocytes. Increased exposure to adipokines in the former two, from the peripheral circulation, but much less so (if at all) in the choriodecidua, may offer a possible explanation for this observation. Some of these adipokines may include leptin and adiponectin, well known to up- and down-regulate proinflammatory cytokines, respectively (Martí et al., 2001; Ouchi & Walsh, 2007), but may also include other adipose tissue endocrine products whose characterization requires further research.

As mentioned above, it has been proposed that at term pregnancy, leukocytes are primed in the periphery to later carry out their function in the maternal–fetal interface. Priming as such is understood as a significant increase in leukocyte activation, migration and infiltration capacity, reactive oxygen species production, and inflammatory cytokine production (Yuan et al., 2009). Our results show that in women with higher adiposity the expression of many genes in MAT leukocytes is also higher; such genes are directly involved with activation, migration, infiltration, and other processes. This suggests that peripheral leukocyte priming may be enhanced in maternal peripheral blood with increasing maternal adiposity. As the design of our study did not include

TABLE 2 Correlations between maternal adiposity and expression of genes involved in various stages of the inflammatory process

MAT			PLA			CHD		
Gene	**r**	**p**	**Gene**	**r**	**p**	**Gene**	**r**	**p**
			ACTIVATION / INFLAMMATION					
IFNA2 (147562)	0.943	0.005 *	*IL10RA (146933)*	0.886	0.019 *	*IL7 (146660)*	−0.771	0.072
TNFSF11 (602642)	0.943	0.005 *	*IL15 (600554)*	0.829	0.042 *	*IL13 (147683)*	−0.771	0.072
CSF1 (120420)	0.886	0.019 *	*TNFRSF11B (602643)*	0.714	0.111	*TNFSF4 (603594)*	−0.829	0.042 *
CTGF (121009)	0.886	0.019 *	*TNFSF13 (604472)*	0.714	0.111			
IL3 (147740)	0.886	0.019 *	*TNFSF4 (603594)*	0.714	0.111			
IL15 (600545)	0.886	0.019 *						
IL1R1 (147810)	0.886	0.019 *						
IL5RA (147851)	0.829	0.042 *						
TNFSF4 (603594)	0.829	0.042 *						
IL33 (608678)	0.771	0.072						
TNFSF13 (604472)	0.771	0.072						
IL9 (146931)	0.714	0.111						
FASLG (134638)	0.714	0.111						
IL10RA (146933)	- 0.714	0.111						
HIF1A (603348)	- 0.771	0.072						
			MIGRATION					

(Continues)

TABLE 2 (Continued)

MAT			PLA			CHD		
Gene	**r**	**p**	**Gene**	**r**	**p**	**Gene**	**r**	**p**
C5 (120900)	0.886	0.019 *	*ACKR4 (606065)*	0.943	0.005 *	*CCR4 (604836)*	−0.714	0.111
CCL24 (602495)	0.886	0.019 *	*CXCR2 (146928)*	0.943	0.005 *	*CCL19 (602227)*	−0.771	0.072
CCL27 (604833)	0.886	0.0.019 *	*CCL17 (601520)*	0.829	0.042 *	*CXCL16 (605398)*	−0.829	0.042 *
CCRL2 (608379)	0.886	0.019 *	*CCR10 (600240)*	0.829	0.042 *			
CXCL14 (604186)	0.886	0.019 *	*CXCR1 (146929)*	0.771	0.072			
CXCR3 (300574)	0.886	0.019 *	*XCL1 (600250)*	0.771	0.072			
SLIT2 (603746)	0.886	0.019 *	*CCL24 (602495)*	0.771	0.072			
CX3CR1 (601470)	0.829	0.042 *	*CCL11 (601156)*	0.714	0.111			
CCL1 (182281)	0.771	0.072	*CCL7 (158106)*	0.714	0.111			
CCL4 (182284)	0.771	0.072	*CCR1 (601159)*	0.714	0.111			
CXCL11 (604852)	0.714	0.111	*CCR9 (604738)*	0.714	0.111			
			PPBP (121010)	0.714	0.111			
			CCR4 (608951)	−0.714	0.111			
			CCL18 (603757)	−0.771	0.072			
			CCL3 (182283)	−0.771	0.072			
			CXCR3 (300574)	−0.829 *	0.042 *			
			INFILTRATION					

(Continues)

TABLE 2 (Continued)

MAT			PLA			CHD		
Gene	**r**	**p**	**Gene**	**r**	**p**	**Gene**	**r**	**p**
ADAMTS13 (604134)	0.886	0.019 *	*MMP12 (601046)*	0.943	0.005 *	*COL11A1 (120280)*	−0.714	0.111
LAMA1 (150320)	0.886	0.019 *	*MMP13 (600108)*	0.829	0.042 *			
LAMB1 (150240)	0.886	0.019 *	*LAMB1 (150240)*	0.771	0.072			
THBS2 (188061)	0.886	0.019 *	*VTN (193190)*	0.771	0.072			
COL7A1 (120120)	0.771	0.072	*MMP14 (600754)*	0.771	0.072			
COL11A1 (120280)	0.771	0.072	*ITGB5 (147561)*	0.714	0.111			
LAMA3 (600805)	0.771	0.072	*PECAM1 (173445)*	−0.714	0.111			
LAMB3 (150310)	0.771	0.072	*CTNNB1 (116806)*	−0.771	0.072			
MMP13 (600108)	0.771	0.072	*ADAMTS8 (605175)*	−0.771	0.072			
THBS3 (188062)	0.714	0.111	*LAMA1 (150320)*	−0.829	0.042 *			

The OMIM accession number for each gene is included in parenthesis below the gene symbol. Columns show Spearman's correlation coefficients (r) and p values. Asterisks show significant correlations ($p < 0.05$).

a direct evaluation of migratory capacity and reactive oxygen species production, we could not show a functional increase in these activities. Nonetheless, as they are part of the priming process, we infer these may have also been present.

Similarly, in PLA leukocytes, gene expression was also positively associated with maternal adiposity mostly in genes involved in leukocyte migration and infiltration during an inflammatory process. Placental blood leukocytes have been shown to have distinct phenotypes and functional properties compared to peripheral leukocytes during term pregnancy, including a higher proportion of CD14+ monocytes/macrophages and different proteomic profiles (Vega-Sanchez, Gomez-Lopez, et al., 2010). These cells may be involved in pregnancy maintenance by keeping an M2 phenotype throughout gestation and later differentiating to DC-like cells with an immunostimulatory capability during term pregnancy (Wang et al., 2016). Furthermore, mucosal-associated invariant T (MAIT) cells, that display an effector memory phenotype and a more cytotoxic response against bacterial stimulation, are found abundantly in placental blood. This suggests a role for PLA leukocytes for the detection and combat of possible intrauterine infection (Solders et al., 2017). The degree to which adiposity-associated gene expression may influence these and other unknown functions of PLA leukocytes during pregnancy and particularly at labor deserves further research.

In contrast, expression in CHD leukocytes was much less associated with maternal adiposity. Moreover, every case involved a negative association, with lower expression in women with higher adiposity. Leukocytes infiltrate the maternal–fetal interface during term pregnancy as a result of a selective and highly regulated recruitment process (Gomez-Lopez et al., 2009, 2013). Infiltrating leukocytes include memory-like CD4+ T lymphocytes (Gomez-Lopez et al., 2013) and M2 macrophages that undergo an M1-like polarization during labor (Xu et al., 2016). Once infiltrated, choriodecidual leukocytes show a distinct expression profile (Arenas-Hernandez et al., 2019) and produce cytokines, chemokines, proteolytic enzymes, and other effector molecules, and thus contribute to the local inflammatory process that will lead to labor (Gomez-Lopez et al., 2010). Our results suggest that the local inflammatory process that involves infiltrated leukocytes may be altered with higher maternal adiposity, since the latter correlated with lower expression of genes related to migration and inflammation on CHD leukocytes.

Altogether, our results suggest that if leukocyte priming is enhanced in peripheral blood and placental blood, and recruitment is altered in choriodecidua as maternal adiposity increases, we might expect an overall modification of the process that leads to normal labor. How these adiposity-induced modifications of the normal inflammatory processes relate to adverse pregnancy outcomes, such as preterm labor, remains to be investigated.

Regarding our assessment of maternal adiposity, pregestational body mass index (pgBMI) is the most common measurement obtained when evaluating the influence of maternal overweight/obesity on various physiological processes. However, while pgBMI may somewhat parallel the amount of fat mass, it does not necessarily resemble the immunoendocrine functions of the maternal adipose tissue, particularly adipokine production. Moreover, such immunoendocrine functions are likely to change continuously during pregnancy due to the influence of a myriad of factors that may range from perceived stress to social support (Coussons-Read et al., 2007; Shapiro et al., 2013). Therefore, we did not regard pregestational weight (i.e., pgBMI) as an adequate parameter to gauge immunoendocrine events that take place many months later, such as leukocyte gene expression at term pregnancy. Consequently, we considered maternal adiposity, estimated within hours of sample collection, as a much more adequate parameter to correlate with inflammatory-gene expression.

Our study presents some limitations worth noting. The first and more important is the sample size, which limits the extent to which conclusions can be drawn from the present results. As mentioned earlier, the current work was designed as a pilot study. As such, our goal was to obtain the largest possible range of maternal adiposity with a few samples in order to explore if maternal adiposity was associated with leukocyte gene expression. In light of the present results, we are currently undergoing a cohort study to verify our findings in a much larger set of samples.

A second limitation is the method we used to estimate maternal adiposity. As mentioned in the Methods section, we used an equation that was developed and validated by Villar et al., in a group of Guatemalan pregnant women (Villar et al., 1992). To this day, no similar equations have been developed specifically for Mexican women and so we selected the Villar et al., equation, assuming that Guatemalan women would most closely resemble our population with respect to ethnicity. We are currently undergoing a large multi-centric cohort study for developing equations to estimate body composition in Mexican pregnant women. Other methods to estimate maternal body composition (e.g., bioelectric impedance, densitometry, air displacement plethysmography, hydrometry or dual-energy X-ray absorptiometry) are either unsuitable for use during pregnancy or require special apparatus and/or reagents that were not available for our study.

Finally, since all of our samples were obtained from women that gave birth by elective cesarean section at term pregnancy, we were not able to observe whether the adiposity-related differences in gene expression would be ultimately related to differences in pregnancy outcomes such as gestational age. Also, since our Institution is a referral hospital for high-risk pregnancies, we did not have access to women without a history of pregnancy complications for this pilot study. However, since the gestational background

is a variable that may affect gene expression, a "healthy" group of participants (without such a history) should be included in further studies.

In conclusion, with these preliminary results, we have shown that leukocyte gene expression is associated with maternal adiposity during term pregnancy. Therefore, we suggest that this variable be considered in future studies involving the analysis of inflammation-related gene expression in leukocytes. By including maternal adiposity as an influencing factor of gene expression, a more systemic way of analysis and interpretation of results may be achieved. Further studies are required to clarify the interaction between maternal adipose tissue and leukocytes at human term pregnancy.

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CONFLICT OF INTEREST

None.

AUTHORS CONTRIBUTIONS

K.M.R. carried out the study, analyzed data, wrote and edited the manuscript. R.V. S. formulated the research question, designed the study, analyzed data, and edited the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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