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Association of leptin receptor expression in placenta and peripheral blood mononuclear cell with maternal weight in birth outcomes

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ABSTRACT

Introduction: The pregnancy period represents the most intense period of growth and development. Pre-pregnancy weight influences weight gain during pregnancy. Leptin is a hormone mainly derived from white adipose tissue, during pregnancy leptin is also produced by the placenta. It has been suggested that the effects of placental leptin on the mother may contribute to endocrine-mediated alterations in energy balance; a dysregulation in leptin levels or its receptors may lead to poor birth outcomes. Therefore, the main goal of the present study was to analyze the differences in birth outcomes by maternal weight with the expression level of leptin receptor in maternal peripheral blood mononuclear cell (PBMC) and placental tissue.
Methods: Women with full-term gestation and its offspring were enrolled. Total RNA from maternal PBMC and placenta was obtained to perform the analysis of expression of the leptin receptor (*LEPR*) gene trough real-time PCR technique. Data were analyzed using one-way ANOVA or Mann-Whitney u test when applicable. Pearson correlation coefficient was used to determine the relationship between continuous variables (Stata v.13); $p \leq 0.05$ was considered statistically significant.
Results: No statistically significant differences were found between *LEPR* expression level and the BMI studied groups in maternal PBMC and placental tissue. Interaction between gestational weight gain (GWG) and *LEPR* in maternal PBMC explain in a 32% the variability of the newborn weight.
Conclusions: LEPR expression level in maternal PBMC correlates with newborn measurements independent from sex. GWG can affect fetal development by increasing fetal birth weight.

1. Introduction

The pregnancy period represents the most intense period of growth and development [1,2]. Pre-pregnancy weight influences weight gain during pregnancy, the higher the pre-pregnancy the less the increase needed during pregnancy because a portion of the energy stores can be used to support fetal growth [3]. National Academies of Sciences, Engineering, and Medicine (NAS) guidelines recommend a total weight gain of 5.0–11.3 kg (11–25 lb.) for overweight women [4]; women with gestational weight gain (GWG) greater than recommended present an increased risk of complications during labor, fetal macrosomy, lactation failure, postpartum weight retention and subsequent development of obesity [5].

Leptin is a well-known hormone mainly derived from white adipose tissue, and principally regulates body weight homeostasis [6], during pregnancy leptin is also produced by the placenta, its effects are mediated by binding to leptin receptors (*LEPR*). Circulating leptin levels change with nutritional state and reflect adipose tissue size [7]. In normal pregnancy, leptin expression is increased compared to non-pregnant women. It has been suggested that the effects of placental leptin on the mother may contribute to endocrine-mediated alterations in energy balance, such as the mobilization of maternal fat, which occurs during the second half of pregnancy and support implantation, human chorionic gonadotrophin production, placental growth, amino acid

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uptake, and mitogenesis. Thus, a dysregulation in leptin levels or its receptors may indicate or lead to feto-maternal disease [7,8]. The aim of the present study was therefore to analyze the differences in birth outcomes by maternal weight with the expression level of leptin receptor in maternal PBMC and placental tissue.

2. Materials and methods

2.1. Patients

All patients in our study were recruited at the maternity care unit at two public hospitals in Sinaloa. A power analysis of the sample size was performed taking in consideration all maternal population in the collection period consisting from 2016 to 2018. The inclusion criteria were women with full-term gestation (>38 gestational weeks) in an age range of 18 to 35 years.

Two comparative groups were established based on the pre pregnancy BMI [9], being constituted as follows: 1) Normal weight group (NW), conformed by pregnant women with a pre pregnancy BMI from 18.0 to 24.99; 2) Overweight group (OW), formed by pregnant women with BMI >25 at the beginning of pregnancy or the one reported before the first 12 weeks of gestation. Patients with pre-existing metabolic diseases, were under pharmacological treatment or in whom it was not possible access to the placenta and the newborn, were excluded from the study.

2.2. Methodological design

A cross-sectional study was conducted over a period of two years, ethical approval was granted by local research ethics committees and Hospital's law and ethics committees. All procedures involving biological samples, as well as confidentiality of the data provided was maintained according to the guidelines of the Code of Ethics of the Ministry of Health and the Helsinki Declaration of 2013. At the time of delivery, written informed consent of the pregnant who agreed to participate in our study was obtained, an interview consisting of multiple sections as gynecological-obstetric history and clinical data of the mother and the newborn was applied.

2.3. Maternal anthropometric measurements

The pre pregnancy height and weight were obtained from clinical records; pre pregnancy BMI was computed as reported weight (kg) divided by square of measured height (m). Gestational weight gain was obtained with the differences between weight measured before delivery and weight before pregnancy. Since one objective of this paper was to measure the impact of quality of maternal weight, GWG was categorized into adequate or inadequate according to NAS, 2019 guidelines, inadequate GWG were subdivided in upper and lower than recommended.

2.4. Placenta measurements

After delivery the placentas were weighed and examined according to the method of Benirschke et al. [10], the thickest non-folding part of the placenta was measured perpendicularly. The maximal placental width was measured in the range of between both edges of placenta. The placental height was the distance from the level of the width measurement to the base of the placenta vertically.

2.5. Newborn anthropometric measurements

The gestational age of each newborn was calculated by the neonatologist using the Capurro method for term infants. The newborn measurements included birth weight, height, head, thoracic and abdominal circumferences. The nutritional status in relation to gestational age was classified in accordance to Battaglia and Lubchenco scales; Large, Adequate and Small for gestational age (LGA, AGA and SGA) births were defined when birth weights were over the 90th, 50th and below the 10th percentiles, respectively. The Clinical Assessment of Nutritional Status score (CANScore) proposed by Metcoff was applied to qualitatively assess fat deposits in skinfolds of the arms, chest, abdomen, back, buttocks, and legs, giving scores of 1 to 4, being 4 the higher value for nutritional quality, a score $\leq$ 24 was interpreted as fetal malnutrition developed in the third trimester even when the parameters of weight and height were within the appropriate limits for the gestational age.

2.6. Biological samples obtainment

Via venipuncture, a maternal peripheral blood sample of 5 ml was obtained in Vacutainer tubes with EDTA. PBMC of the mother were separated from the whole blood using centrifugation at 2200 for 30 min. The placenta was obtained after delivery, the amniotic and chorionic layer were separated manually, a 1 cm^2 biopsy was taken, subsequently, multiple washes with PBS at 4° C were performed until the rest of erythrocytes were removed.

2.7. LEPR gene expression analysis

Total ribonucleic acid (RNA) from PBMC and placental tissue was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. RNA was quantified on a NanoDrop 2000 spectrophotometer (Thermo Scientific, Barrington, IL, USA). Reverse transcription reaction was performed using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems™, Thermo Fisher Scientific Inc). Quantitative real time polymerase chain reaction (PCR) was run on the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, USA) using TaqMan probes (Life Technologies, USA) in accordance with the manufacturer's instructions, the relative *LEPR* (Hs00174497_m1) gene expression levels were compared with endogenous control, *GAPDH* (Hs03929097_g1). All experiments were performed in triplicate. The expression level of *LEPR* between the studied groups was compared using relative quantification analysis with the method published by Pfaffl [11].

2.8. Statistical analysis

Data was entered in excel data sheet and analyzed using statistical software Stata version 13 (StataCorp LLC, College Station, TX, USA). The sample size was calculated by the statistician based on an error margin no>5%, a confidence interval of 95%, and a standard deviation of 0.5. Quantitative data were expressed as mean ± sd. Qualitative data were expressed in frequency and percentages. Data were analyzed using one-way ANOVA with pre pregnancy BMI, newborn gender and CANScore category as factors. Bonferroni or t-Student test were used as post hoc tests when applicable. Mann-Whitney u test was used when the normality test or the equal variance test failed. Pearson correlation coefficient was used to determine the relationship between continuous variables; R^2 was calculated as a measure of the strength of the association. Statistical significance was determined as a p-value < 0.05.

3. Results

3.1. Maternal weight gain analysis

There were no differences in gestational weight gain between two groups (Normal weight: 12.67 + 4.65; Overweight: 10.22 + 7.65 kg.; $p > 0.05$). Only 46.11% of all women had an adequate gestational weight according to guidelines of Institute of Medicine. Inadequate gaining was upper in 72.09% and lower in 27.91% in overweight group patients, while in normal weight pregnant were 63.41% and 36.59% for upper and lower respectively ($p < 0.05$). Higher rates of caesarean birth were observed in overweight pregnant (57.38% vs. 41.18%). Higher levels of

glucose were observed in overweight pregnant (85.38 vs. 72.91 mg/dL; $p < 0.05$; Table 1). Blood pressure was also slightly higher in overweight pregnant; however, this measure was no statistically significant. Participants were asked about smoking and alcohol habits before and during pregnancy. All participants denied using alcohol and tobacco during pregnancy, no statistically significant differences were identified between alcohol and tobacco consumption and obstetric outcomes.

3.2. Effect of maternal weight on placenta measurements

Placentas from overweight pregnant were 40 g heavier than normal weight pregnant (556.1 vs. 513.9 g.); Differences in placental measurements were not statistically different between the studied BMI categories (Table 1). Placental weight was positively correlated with newborn weight ($r = 0.4905$; $p = 0.0000$). Pre pregnancy BMI and GWG did not altered weight, length, width, or number of cotyledons in placenta (Table 2).

Table 1
Characteristics of the study sample.

	All women	BMI Category		p-value
	Total (n = 232)	NW (n = 118)	OW (n = 114)	
Maternal				
Gravidity				0.2380[c]
Primigravid	139(59.91)	77(65.25)	62(54.39)	
Multigravid	93(40.09)	41(34.75)	52(45.61)	
Glucose (mg/dL)	79.94 ± 19.54	72.91 ± 18.57	85.38 ± 18.70	**0.0045[b]**
Blood pressure				
Systolic	141.6 ± 13.5	112.9 ± 13.2	116.1 ± 13.9	0.1132[c]
Diastolic	72.5 ± 11.9	71.5 ± 13.8	73.6 ± 9.7	0.1538[c]
Pre pregnancy weight (kg)	65.98 ± 14.03	56.48 ± 6.59	75.81 ± 12.88	**0.0001[c]**
Height (m)	1.59 ± 0.06	1.59 ± 0.06	1.59 ± 0.06	0.6972[b]
Pre pregnancy BMI	25.94 ± 4.96	22.28 ± 1.75	29.73 ± 4.33	**0.0001[c]**
GWG (kg)	11.36 ± 6.52	12.67 ± 4.65	10.22 ± 7.65	**0.0043[c]**
Adequate	60(40.27)	25(35.71)	35(44.30)	
Inadequate	89(59.73)	45(64.29)	44(55.70)	**0.0009[c]**
Upper	46(45.24)	15(36.59)	31(72.09)	
Lower	38(45.24)	26(63.41)	12(27.91)	
Pre pregnancy drinking	46(22.89)	23(21.30)	23(24.73)	0.5655[b]
Pre pregnancy smoking	19(9.18)	9(7.89)	10(10.75)	0.4811[b]
Placenta				
Weight (g)	532.92 ± 126.05	513.92 ± 105.08	556.09 ± 146.06	0.1623[b]
Length (cm)	17.74 ± 2.72	18.15 ± 1.70	17.25 ± 3.56	0.4098[c]
Width (cm)	2.49 ± 2.10	2.30 ± 0.64	2.72 ± 3.06	0.5402[c]
Cotyledons (n)	13.15 ± 3.99	13.87 ± 4.16	12.63 ± 3.88	0.3526[b]
Newborn				
Gestational age (weeks)	38.85 ± 1.73	38.93 ± 1.23	38.76 ± 2.17	0.7556[c]
Males	60(46.88)	29(43.28)	31(50.82)	0.3975[b]
Caesarean birth	63(48.84)	28(41.18)	35(57.38)	0.0669[b]
Weight (g)	3300.94 ± 569.08	3276.43 ± 585.95	3329.77 ± 551.49	0.2799[b]
Height (cm)	50.02 ± 3.42	49.27 ± 3.81	50.86 ± 2.71	**0.0003[c]**
Circumferences (cm)				
Head	34.40 ± 1.47	34.23 ± 1.55	34.59 ± 1.36	0.6605[b]
Thoracic	33.54 ± 2.07	33.25 ± 2.06	33.91 ± 2.05	0.2395[b]
Abdominal	31.84 ± 2.10	31.60 ± 2.10	32.14 ± 2.85	0.6428[b]
Percentiles				0.4336[c]
SGA	72(56.69)	36(52.94)	36(61.02)	
AGA	33(25.98)	19(27.94)	14(23.73)	
LGA	22(17.32)	13(19.11)	9(15.25)	
CANScore				0.5281[c]
Normal	103(81.10)	53(77.94)	50(84.75)	
Malnutrition	24(18.90)	15(22.06)	9(15.25)	

Some variables does not add up to 100% due to missing data; qualitative data presented as number (percentage); quantitative data presented as mean ± SD; b: *t*-test; c: Mann-Whitney *u* test; Values in **bold** are considered statistically significant at 5%. Abbreviations: BMI = Body Mass Index; GWG = Gestational weight gain; NW = Normal weight; OW = Overweight; SGA = Small for Gestational Age; AGA = Adequate for Gestational Age; LGA = Large for gestational Age

3.3. Effect of maternal weight on newborn measurements

Newborn from normal weight were smaller than newborn form overweight women (49.27 vs. 50.86 cm; $p < 0.05$). Maternal final weight was positively correlated with newborn weight in 5.48% ($p = 0.0045$)

The CANScore result shown that, newborn from malnutrition category were 250 g. lighter than newborn classified as normal for this test (2846.95 vs. 3100.00 g; $p > 0.05$), not statistically differences were identified between BMI categories and other newborn measurements (Table 1).

Pre pregnancy BMI and GWG did not alter height, percentiles or CANScore measurements. Gestational weight gain correlated positively with newborn weight ($r = 0.2716$; $p = 0.0020$), thoracic circumference ($r = 0.2668$; $p = 0.0029$) and with abdominal circumference ($r = 0.2963$; $p = 0.0009$) (Table 2).

3.4. Leptin receptor expression level

After normalization of the data with the reference GAPDH gene, relative gene expression of LEPR in maternal PBMC and placental tissue was evaluated between pre pregnancy BMI, newborn gender and CANScore result.

The relative amounts of copies of LEPR in maternal PBMC was 3.15 + 2.51 and 2.12 + 2.14 for normal weight and overweight respectively and expression level in placental tissue was 2.25 + 3.38 and 1.37 + 1.56 (Fig. 1); no statistically significant differences were found between the BMI studied groups.

The expression level of leptin receptor in maternal PBMC by newborn gender, did not shown statistically differences, 2.69 vs. 2.39 for females and males respectively and 1.75 vs. 1.87 for females and males respectively in placental tissue (Fig. 2).

The results of the analysis of leptin receptor expression level according to CANScore, are shown in Fig. 3; result in maternal PBMC was increase 1.5 fold change in the newborn categorized with malnutrition

Table 2
Correlation coefficients of pre pregnancy BMI and GWG with placental and offspring measurements.

	Pre pregnancy BMI		Gestational weight gain	
	Correlation	p-value	Correlation	p-value
Placental				
Weight (g)	0.1379	0.2515	0.2070	0.0856
Length (cm)	−0.1562	0.2103	0.0382	0.7627
Width (cm)	−0.0018	0.2341	−0.0276	0.9558
Cotyledons (n)	−0.1718	0.3243	0.0511	0.9640
Newborn				
Gestational age (weeks)	−0.0381	0.6682	0.1227	0.1678
Weight (g)	0.0466	0.6014	0.2716	**0.0020**
Height (cm)	0.1534	0.0838	0.1389	0.1193
Circumferences (cm)				
Head	0.0969	0.2823	0.1266	0.1612
Thoracic	0.0692	0.4450	0.2668	**0.0029**
Abdominal	0.0137	0.8802	0.2963	**0.0009**
Percentiles	−0.1299	0.1440	0.0357	0.6899
CANScore	0.0677	0.4550	0.1535	0.0902

Pearson correlation coefficients for the relation of maternal pre pregnancy BMI and GWG with placental and newborn measurements. Values in in **bold** are considered statistically significant at 5%. Abbreviations: BMI = Body Mass Index; GWG = Gestational weight gain; CANScore: Clinical Assessment of Nutritional Status Score.

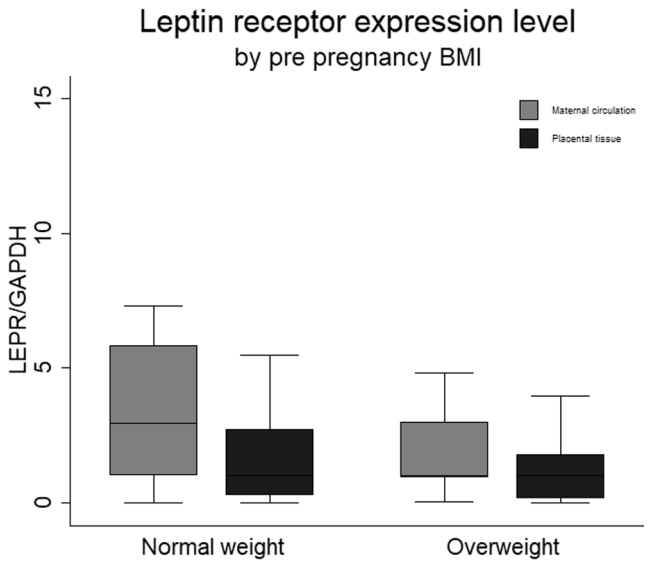

Fig. 1. Leptin receptor expression level by pre pregnancy BMI. Box presenting means ± SE are shown. There were no statistically significant differences between group means as determined by t-Student test in maternal circulation or placental tissue respectively (P = 0.2276; P = 0.3413).

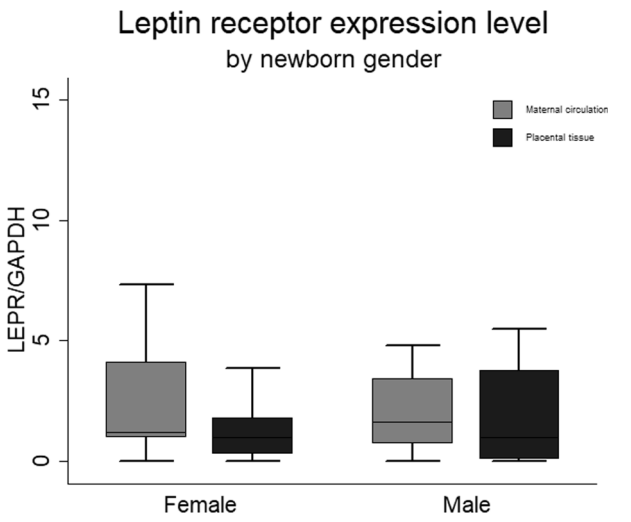

Fig. 2. Leptin receptor expression level by newborn gender. Box presenting means ± SE are shown. There were no statistically significant differences between group means as determined by t-Student test in maternal circulation or placental tissue respectively (P = 0.7409; P = 0.9053).

compared to those who obtained a normal result (3.84 vs. 2.39; p > 0.05). The expression level of leptin receptor according to CANScore result in placental tissue was increase one-fold change in the newborn categorized with malnutrition compared to those who obtained a normal result (2.52 vs. 1.54; p > 0.05).

3.5. Relationships among maternal and newborn measurements and leptin receptor expression level

Leptin receptor expression did not show a correlation with pre pregnancy BMI or GWG. The newborn weight correlated negatively with leptin receptor expression level in maternal PBMC (r = -0.4718; p = 0.0085). Head, thoracic, and abdominal circumferences correlated negatively with leptin receptor expression level in maternal PBMC (Table 3). The points obtained in CANScore decreased the leptin receptor expression level in maternal PBMC (r = -0.4775; p = 0.0076).

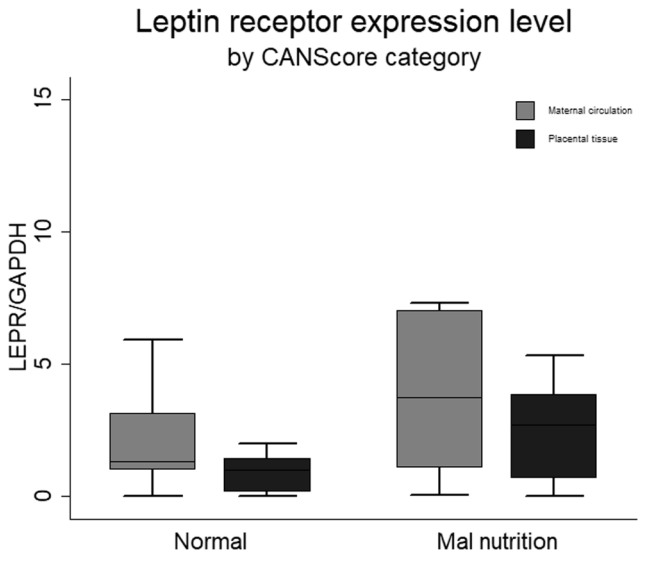

Fig. 3. Leptin receptor expression level by CANScore category. Box presenting means ± SE are shown. There were no statistically significant differences between group means as determined by t-Student test in maternal circulation or placental tissue respectively (P = 0.2149; P = 0.4481).

Table 3
Correlation coefficients of leptin receptor expression with maternal, and offspring measurements.

	Maternal PBMC		Placenta	
	Correlation	p-value	Correlation	p-value
Maternal				
Pre pregnancy BMI	−0.1371	0.4622	−0.0676	0.7180
GWG (kg)	0.0307	0.8698	−0.1656	0.3733
Newborn				
Gestational age (weeks)	−0.2775	0.1307	0.1573	0.3979
Weight (g)	−0.4718	**0.0085**	−0.1034	0.5887
Height (cm)	−0.1723	0.3626	0.1502	0.4282
Circumferences (cm)				
Head	−0.4980	**0.0051**	0.0695	0.7150
Thoracic	−0.6084	**0.0004**	−0.0282	0.8823
Abdominal	−0.6198	**0.0003**	−0.0868	0.6483
Percentiles	−0.1787	0.3449	−0.2069	0.2727
CANScore	−0.4775	**0.0076**	−0.2097	0.2661

Pearson correlation coefficients for the relation of leptin receptor expression in maternal PBMC and placental tissue wit maternal and newborn measurements. Values in in **bold** are considered statistically significant at 5%. Abbreviations: BMI = Body Mass Index; GWG = Gestational weight gain; CANScore: Clinical Assessment of Nutritional Status Score.

Leptin receptor expression level in placental tissue did not correlate with any of the measurements studied. The strongest association between maternal and newborn characteristics (R^2 = 32%) was seen for gestational weight gain and leptin receptor expression level in maternal PBMC (Fig. 4). Leptin receptor expression in placental tissue was mainly associated with leptin receptor expression in maternal PBMC (R^2 = 21%) (Fig. 5).

4. Discussion

The aim of the present study was to analyze the differences in birth outcomes by maternal weight with the expression level of leptin receptor in maternal PBMC and placental tissue. The main finding of the present study is that interaction between GWG and *LEPR* in maternal PBMC explain in a 32% the variability of the newborn weight.

More than half of the patients had inadequate GWG, nevertheless, patients in the normal weight group gained less weight than

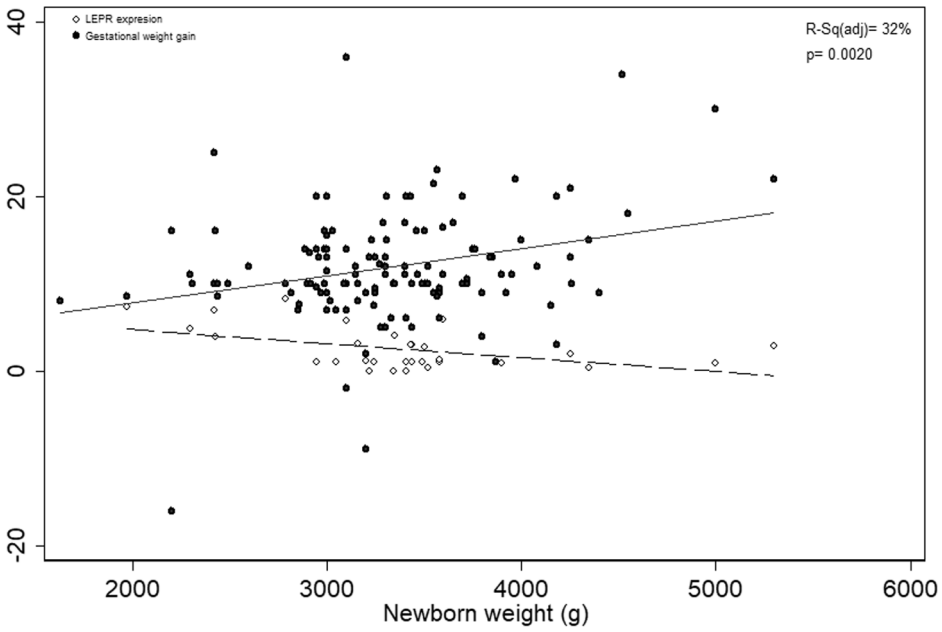

Fig. 4. Effect of leptin receptor in maternal circulation and gestational weight gain on newborn weight. Coefficient of determination adjusted is shown. The interaction between gestational weight gain and leptin receptor in maternal circulation explain in a 32% the variability of the newborn weight ($p = 0.0020$).

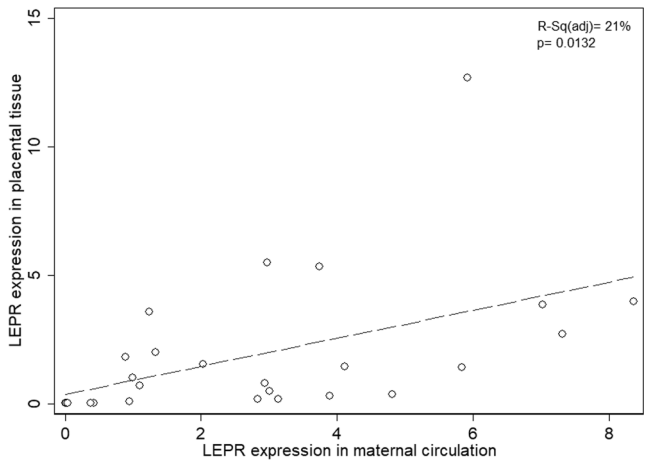

Fig. 5. Association between leptin receptor expression in maternal circulation and placental tissue. Coefficient of determination adjusted is shown. The interaction between the expression level of leptin receptor in maternal circulation and placental tissue is related in a 21% ($p = 0.0132$).

recommended, while patients in the overweight group exceeded the recommended GWG guidelines, our findings differ from those of Heude et al., who report that women who were overweight before pregnancy generally gained less weight and very few had an excessive weight gain [12].

WHO set an optimal rate of 10–15% for caesarean birth to optimize maternal and child health. There are still worldwide variations in caesarean birth, despite all the evidence regarding the risk and benefits of its practice.

Even when there's evidence indicating that maternal overweight has shown a significant positive association with caesarean birth rate [13] in our study we identified that 49% of newborn had a caesarean birth and the maternal overweight did not represent a factor for the increase in the caesarean births rate, this finding highlights the inequity in the use of caesarean birth.

Given the evidence that maternal life habits have an impact on fetal health, the effect of maternal pre-pregnancy drug addiction on weight in the newborn was evaluated; however, no statistically significant differences were observed between these variables evaluated. Although all the participants denied consuming alcohol or tobacco during pregnancy, however, we consider is important to evaluate in future studies in our group the effect of drug addiction on fetal development and growth and the behavior of the *LEPR* gene given that there is currently no consensus on the fact that infants from smokers with low maternal BMI could be at greater risk of reduced fetal growth than those from obese mothers [14]. Some studies establishes that endocrine pancreatic dysfunction does not appear to be a major contributing factor to nicotine-associated fetal growth restriction [15], regarding the behavior of *LEPR* gene, studies rendered contradictory results, some indicating that lower birth weight and maternal smoking are associated with decreased leptin concentration in cord blood, whereas others concluded there is no association [16].

There is solid evidence that placental weight is linked to maternal obesity [3] and that bigger babies generally have heavier placentas [4], our data showed that placental weight was only related to newborn weight without maternal weight or sex interaction, maybe because of missing data in this analysis. Maternal weight did not influence other placental measured, our findings are consisting with Barker et al., who found that more cotyledons does not reduce nutrient supply to the fetus but enhances it [4].

The investigation of the relationship between maternal characteristics and anthropometry of the newborn has been widely studied. There is no consensus as to which maternal characteristics during pregnancy are associated with newborn anthropometric measurements. One study reported that maternal size is independently associated with neonatal body composition [5]. Another study found that the risk of delivering an SGA infant, increased more with increasing BMI [6]. In our study, pre pregnancy BMI did not have an effect on newborn size, nevertheless, GWG correlated positively with newborn circumferences, which demonstrates the importance of nutritional control during pregnancy to ensure adequate GWG for each stage of pregnancy and to achieve an adequate fetal growth from the early stages of perinatal life. The GWG was found to associate with newborn weight only in a 7%. The present study, however, agrees well with Heude et al., who report that both pre pregnancy BMI and GWG are positively associated with birth weight, and the lowest risk of SGA concern obese women who gain more weight

[1].

The findings of Elshibly and Schmalisch study, also support the present results who mentioned that some birth outcomes are in part the result of an intergenerational growth process and that some outcomes are already established before a mother conceives or gives birth [7].

A limitation of this study was the impossibility to determine body fat with a Dual-energy X-ray absorptiometry (DXA), since this is a validated method for determining body fat, however, fat mass values determined by skinfold thickness, are an indirect measurement of adiposity that correlates with DXA measurements [3]. However, no differences in newborn adiposity were identified in the studied groups according to CANScore.

Fetal leptin level is believed to be independent from maternal and placental contributions and to correlate with fetal fat mass [8]. There is also evidence from human and animal studies that differential molecular expression according to the sex of the placenta may lead to different nutritional conditions for the fetus [3].

Our data suggest there is no correlation between maternal weight and leptin receptor expression in placenta or maternal PBMC, these findings are not consistent with the fact that leptin levels are proportional to the amount of adipose tissue [9,10], however, there is evidence that during pregnancy, placental leptin is largely delivered into maternal circulation, what may contribute to maternal circulating leptin levels during pregnancy, despite of maternal gain of fat mass [8,11]. Buhling et al., also support the present findings, who conducted a study to explore the relationship between leptin concentrations and blood pressure during pregnancy and conclude that leptin concentrations are correlated with heart rate independent of BMI [12].

One factor that could be influencing *LEPR* expression is maternal leptin insensitivity. Klein et al., explain that offspring of exposed to a high fat diet due to maternal leptin insensitivity still remain sensitive to maternal leptin [13], which supports the notion that maternal high fat diet exposure during pregnancy does not have a major impact in the expression of transcripts associated with leptin.

The expression of *LEPR* remained without variations according to maternal weight, this suggest that, either our current paradigm was not effective in the development of the circuits that set up newborn size, or that other factors not explored here are affected. The potential for the latter argument is high given the complexity of the factors implicated in the regulation of fat deposits, food intake and energy balance, as well as the developmental factors recruited during gestational period. Limitations of this study include the fact that placental measurements could not be compared with leptin receptor expression levels due to missing data in this analysis.

We acknowledge that the relationship between maternal health conditions and birth outcome is complex since especially maternal anthropometry is the consequence of many other genetic or environmental factors such as eating habits, exercise and social economic conditions that themselves can impact on pregnancy outcome [12].

5. Conclusions

There are no differences between *LEPR* expression level in placental tissue by maternal weight, however *LEPR* expression level in maternal PBMC correlates with newborn measurements independent from sex. GWG can affect fetal development by increasing fetal birth weight.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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