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ORIGINAL ARTICLE

Factors associated with prospective leptin concentrations throughout pregnancy in pregestational normal weight, overweight and obese women

Ana Beatriz Franco-Sena*,†, Livia Costa de Oliveira*,†, Thatiana de Jesus Pereira Pinto*,†, Dayana Rodrigues Farias*,†, Juliana dos Santos Vaz*,‡ and Gilberto Kac*

*Nutritional Epidemiology Observatory, Department of Social and Applied Nutrition, Institute of Nutrition Josué de Castro, Rio de Janeiro Federal University, †Graduate Program in Nutrition, Institute of Nutrition Josué de Castro, Rio de Janeiro Federal University, Rio de Janeiro, and ‡Faculty of Nutrition, Pelotas Federal University, Pelotas, RS, Brazil

Summary

Background Leptin concentrations increase throughout pregnancy but little is known about factors that influence this physiological change and whether they differ according to pregestational body mass index (BMI).

Objective To assess whether longitudinal trends of leptin in pregnancy are influenced by biochemical, anthropometric and lifestyle factors in women with normal weight (NW), overweight (OW) or obese (OB) pregestational BMI.

Design and methods Prospective cohort of 232 pregnant women followed at 5–13th, 20–26th and 30–36th gestational weeks. The effect of selected variables on longitudinal behaviour of plasma leptin concentrations, stratifying for NW (18·5–24·9 kg/m^2), OW (25–29·9 kg/m^2) and OB (≥30·0 kg/m^2) pregestational BMI was assessed through longitudinal linear mixed-effects models.

Results The multiple regression model for women with NW revealed associations of maternal body weight (β = 0·714, CI = 0·491 to 0·937), serum HDL-cholesterol (β = 0·239, CI = 0·089 to 0·388) and C-reactive protein (CRP) (β = −0·138, CI = −0·272 to −0·004) with plasma leptin concentrations. Maternal body weight (β = −0·871, CI = 0·475 to 1·267) and serum HDL-cholesterol concentrations (β = 0·315, CI = −0·022 to 0·651) were also associated with leptin in OW women. In OB women, serum HDL-cholesterol (β = 0·722, CI = 0·219 to 1·226), maternal body weight (β = 0·666, CI = 0·187 to 1·145), triglycerides concentrations (β = −0·130, CI = −0·241 to −0·020) and dietary carbohydrate (β = 0·075, CI = 0·023 to 0·126) were significantly associated with plasma leptin.

Conclusion Maternal body weight and serum concentrations of HDL-cholesterol were associated with leptin changes independent of pregestational BMI. Serum CRP concentrations were associated with leptin only in NW women and serum triglycerides concentrations and dietary carbohydrate only in OB. These results indicate that factors that influence leptin concentrations differ according to pregestational BMI.

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Correspondence: Professor Gilberto Kac, Nutritional Epidemiology Observatory, Institute of Nutrition Josué de Castro, Rio de Janeiro Federal University, Avenida Carlos Chagas Filho, 373, CCS, Bloco J, 2° andar, Cidade Universitária – Ilha do Fundão, CEP: 21941-902, Rio de Janeiro – RJ, Brasil. Tel.: (+55) 21 25626595; Fax: (+55) 21 22808343; E-mail: gilberto.kac@gmail.com

Introduction

Leptin is a peptide hormone synthesized and released predominantly in adipose tissue.[1] During pregnancy, this process also occurs in the placenta.[2] Leptin transports information about the body's energy reserves to the brain and subsequently controls food intake.[1,3] Several authors have shown that leptin concentrations increase throughout gestation[4–7] with more significant increases in the second trimester.[7] Studies have also shown an association between leptin, weight and body mass index (BMI) during pregnancy,[8,9] as well as body adiposity.[1,8]

Misra & Trudeau[10] observed that the rate at which leptin increases during pregnancy was significantly lower in overweight or obese women, which is due in part to lower gestational weight gain. However, when evaluating changes in leptin per kg, it increased in normal-weight women and decreased in overweight women. These results indicate that factors other than weight gain influence the variation in leptin concentrations during pregnancy, and they can differ based on the pregestational BMI.

Our hypothesis is that biochemical variables produced by the adipose tissue or known to be related to body fat, as well as dietary variables may have an effect on glucose metabolism and thus be associated with leptin concentrations. Thus, the aim of the present study was to evaluate whether biochemical, dietary and lifestyle variables are associated with leptin plasma

concentrations during pregnancy in women with normal weight (NW), overweight (OW) or obese (OB) pregestational BMI.

Methods

Study design and participants eligibility criteria

This was a prospective cohort of pregnant women followed at a municipal health centre in Rio de Janeiro, Brazil. Participants were recruited for 24 months between 2009 and 2011. The women were assessed at the following stages of pregnancy: 5–13, 20–24 and 30–36 weeks. In total, 299 women were recruited. Women were between 20 and 40 years of age, between 5 and 13 weeks of gestation and did not have any known chronic noncommunicable diseases other than obesity. After baseline clinical evaluation, women were excluded if they had a confirmed pregestational diagnosis of chronic noncommunicable diseases ($n = 9$), infectious or parasitic diseases ($n = 9$), were expecting twins ($n = 4$), suffered a miscarriage prior to the first evaluation ($n = 3$), did not have blood samples collected prior to week 13 of gestation ($n = 13$) or did not have leptin measurements ($n = 4$). Additionally, women were excluded if they did not know their pregestational weight ($n = 12$) or if they had a pregestational BMI (based on self-reported weight) classified as underweight [$<18{\cdot}5$ kg/m^2] ($n = 13$). Thus, the baseline sample consisted of 232 women.

Measurements

The individual evaluations included anthropometric measurements, blood draws, and structured interviews.

Blood was collected during each follow-up visit following a 12-h fast. The samples were centrifuged, and plasma and serum were separated and stored at −80 °C for subsequent analysis. Adiponectin (U/ml), insulin (U/ml) and leptin (ng/dl) plasma concentrations were determined using commercial ELISA kits (Millipore, St. Charles, MO, USA) with sensitivities of 0·78 ng/ml, 2 μU/ml and 0·5 ng/dl, respectively. C-reactive protein (CRP) serum concentrations were measured using the immunoturbidimetric method with ultra-sensitive commercial kits (CRP U-hs FS*; sensitivity of 0·05 mg/dl). Triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl) and high-density lipoprotein cholesterol (HDL-cholesterol; mg/dl) were measured using enzymatic colorimetric methods with commercial kits (Labtest Diagnóstica, Lagoa Santa, Minas Gerais, Brazil). Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated using the formula reported by Friedewald *et al.*[11] Maternal blood glucose was measured using the oxidase-peroxidase glucose enzymatic colorimetric method with a Wiener Lab kit (Rosario, Argentina). The homeostasis model assessment for insulin resistance (HOMA-IR) [insulin (mU/ml) × [glucose (mg/dl) × 0·055]/22·5] and the quantitative insulin sensitivity check (QUICKI) [1/[log of insulin (mU/ml) + log of glucose (mg/dl)]] indexes were calculated and used as indicators of insulin sensitivity.[12]

Gestational age was calculated based on data from the first ultrasonography (USG) examination performed on each pregnant woman ($n = 200$, 86%) or the date of the last menstrual period ($n = 32$) if the first USG was not performed prior to week 26 of gestation.

Socioeconomic, demographic, reproductive and lifestyle variables were obtained via interviews with structured questionnaires administered during the first follow-up visit (5–13 weeks of gestation). Maternal body weight was measured in all three follow-up visits using a digital scale (Filizzola PL 150, Filizzola Ltda, Brazil), and height was measured only in the first wave of follow-up using a portable stadiometer attached to the wall (Seca Ltda., Hamburg, Germany). Pregestational BMI (weight [kg]/height [m²]) was calculated based on self-reported pregestational weight obtained during the first follow-up visit and was classified as NW (18·5–24·9 kg/m^2), OW (25·0–29·9 kg/m^2) or OB (≥30·0 kg/m^2), according to the World Health Organizaton criteria.[13] Dietary variables were obtained through a semi-quantitative food frequency questionnaire, which has been validated using the doubly labelled water method to estimate energy intake.[14] The Brazilian Food Composition Table (*Tabela Brasileira de Composição de Alimentos* – TACO)[15] database was used to determine the composition of foods, and the American table proposed by the 'United States Department of Agriculture'[16] was used for foods that did not have available information in the TACO. Total energy intake and intake of macronutrients (grams) were evaluated.

Statistical analyses

General characteristics of the subjects were described using means and 95% confidence intervals (CI) for symmetric variables and median and interquartile ranges (IQR) for asymmetric variables, all of which were stratified by pregestational BMI categories. Comparisons between groups were performed using ANOVA or Kruskal-Wallis tests for symmetric and asymmetric variables, respectively. Scatterplots were constructed using a longitudinal linear function to visually represent the evolution of leptin concentrations during pregnancy and differences based on the three pregestational BMI categories. Longitudinal linear mixed-effects regression models for leptin with the inclusion of variables related to time only were performed. The gestational age and quadratic gestational age were both included because longitudinal changes in leptin concentrations during pregnancy resemble a parabola, that is, a situation where there is a rise and subsequently a drop in the outcome variable, or the inverse. These models allow us to determine whether there was a significant change in leptin concentrations throughout pregnancy. They also account for the dependency between observations of the same individual and for the exact gestational age of blood collection, not grouping women by gestational trimester.

Longitudinal linear regression models were also used to investigate the association between selected variables and plasma leptin concentrations during pregnancy. A model was generated for each pregestational BMI category (NW/OW/OB). The regression coefficient (β) and standard error (SE) generated by the model

provided a combined estimate of the effect between individuals (with respect to the time association between the independent variables and leptin concentrations) and within individuals (representing the effect of independent variable variation on changes in leptin concentrations during pregnancy). This model accommodates time-dependent and independent covariates and allows for the inclusion of data from non-equidistant repeated measures.[17] All independent variables included on the final model are time-dependent, that is, varied over time. This allows taking into account their longitudinal behaviour throughout pregnancy. Gestational age and quadratic gestational age were included in all models as variables indicative of time. Gestational age was considered a random effect variable to allow for individual variation in leptin concentration over time. All other variables were considered as fixed effects.

The covariates tested were selected based on their biological plausibility of having an association with leptin concentrations as well as with the mechanism of weight gain/fat accumulation during pregnancy. Associations between sociodemographic (such as parity, education and per-capita family income) and lifestyle (such as alcohol consumption and smoking) variables, as well of maternal body weight were assessed. Maternal body weight was incorporated into the models to take into account the effect of weight gain on the evolution of leptin concentrations during pregnancy. Plasma concentrations of glucose, insulin and adiponectin, serum concentrations of CRP, TG, TC and cholesterol fractions as well as the HOMA-IR and QUICKI indexes were also evaluated as time-dependent variables.

Variables with *P*-values <0·20 in the longitudinal bivariate regression models were included in the full models. A backward selection from the full model was employed, in which all variables were included and then removed, one by one, from the model when the *P*-value was >0·05. We opted to adjust all final models for the same variables, that is, if a certain variable was significantly associated in any of the bivariate models it was included in all final models, independently of its statistical significance. This approach allows to inform the variables that did not associate with leptin and also to make comparisons between the effect-size of such variables according to BMI categories.

Statistical analyses were performed using STATA version 12.0. A *P*-value <0·05 was considered statistically significant.

The present study was approved by the Research Ethics Committees (REC) of the Maternity Hospital of Rio de Janeiro Federal University.

Results

No significant differences in height, education, parity, per-capita family income, blood glucose and HDL-cholesterol were observed between BMI groups. All other variables evaluated were significantly different between NW, OW and OB women. Baseline leptin concentrations were significantly higher in OW and OB women. OW and OB women had higher baseline insulin concentrations, HOMA-IR values and lower QUICKI values, although no difference was observed in their blood glucose concentrations (Table 1).

The pregestational BMI values varied from 18·7 to 45·5 kg/m^2. From the 232 women in the sample, 27·6% (n = 64) were classified as OW and 14·2% (n = 33) as OB. Maternal weight gain rates (MWGR) throughout pregnancy for NW, OW and OB women were 0·429 (CI = 0·394 to 0·465), 0·374 (CI = 0·324 to 0·423) and 0·297 (CI = 0·234 to 0·360) kg/week, respectively (data not shown in tables). Mean MWGR was significantly different among BMI categories.

In NW women, there was an increase in leptin concentrations from the first to the second trimester (β = 1·996, CI = 1·364 to 2·583, $P < 0·001$) and a decrease from the second to the third trimester (β = −0·036, CI = −0·050 to −0·020, $P < 0·001$). The same trend was observed for OW women, but the association was not statistically significant. In OB women, there was a decrease in leptin concentrations from the first to the second trimester with a subsequent increase until the third trimester, but the association was not statistically significant (Fig. 1). The leptin trend throughout pregnancy is visually different among BMI categories, but not statistically significant, taking in comparison the CIs regression coefficients (β), as there is an overlap between them.

The variables maternal body weight throughout pregnancy, adiponectin and HDL-cholesterol concentrations were selected to compose the three full models based on the bivariate longitudinal analyses. CRP, QUICKI, TG and dietary carbohydrates were the other variables included in the full models that differed according to BMI categories (Table 2).

Maternal body weight throughout pregnancy (NW: β = 0·714, CI = 0·491 to 0·937, $P < 0·001$; OW: β = 0·871, CI = 0·475 to 1·267, $P < 0·001$; OB: β = 0·666, CI = 0·187 to 1·145, P = 0·001) and HDL-cholesterol concentrations (NW: β = 0·239, CI = 0·089 to 0·388, P = 0·002; OW: β = 0·315, CI = −0·022 to 0·651, P = 0·067; OB: β = 0·722, CI = 0·219 to 1·226, P = 0·005) were positively associated with leptin concentrations in all BMI categories. Additionally, CRP concentrations were negatively associated with leptin concentrations in NW women (β = −0·138, CI = −0·272 to −0·004, P = 0·044) and TG concentrations were negatively (β = −0·130, CI = −0·241 to −0·020, P = 0·021) and dietary carbohydrates positively (β = 0·075, CI = 0·023 to 0·126, P = 0·004) associated with leptin in OB women (Table 3).

Discussion

The present study has two main results. First, this study confirms the significant increase in plasma leptin concentrations from the first to the second trimester, with a subsequent decrease until the third trimester, but only in NW women. Second, this study provides further evidence that factors associated with the evolution of leptin concentrations during pregnancy differ between pregestational BMI categories. Maternal body weight throughout pregnancy and HDL-cholesterol were positively associated with leptin concentrations in all BMI groups. CRP concentrations were negatively associated with leptin only in pregestational NW women. In contrast, TG concentrations and dietary carbohydrate only showed association with leptin in pregestational obese women.

Table 1. Baseline characteristics stratifying for pregestational BMI categories of the pregnant women followed at a public health centre in Rio de Janeiro, Brazil, 2009–2012

	Pregestational BMI (kg/m^2)								
	18·5–24·9			25·0–29·9			≥30·0		
Symmetric variables	*N*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
Pregestational BMI (kg/m²)*	135	22·56	22·22–22·90	64	27·31	26·88–27·75	33	34·44	33·16–35·72
Total cholesterol (mg/dl)*	135	157·65	153·11–162·20	64	166·92	158·87–174·98	33	167·55	159·14–175·95
LDL-cholesterol (mg/dl)*	135	94·69	91·44–97·94	64	101·92	95·72–108·12	33	102·44	95·60–109·23
Assymetric variables	N	Median	IQR	*n*	Median	IQR	*n*	Median	IQR
Age (years)*	135	25·00	21·00–31·00	64	27·00	24·00–32·00	33	25·00	22·00–28·00
Body weight (kg)*	135	57·10	52·80–61·30	64	70·05	65·15–74·15	33	85·70	79·10–97·80
Height (cm)	135	159·40	155·50–163·20	64	160·00	154·00–165·05	33	159·10	156·00–162·20
Education (years)	135	10·00	7·00–11·00	64	9·00	7·50–11·00	33	9·00	7·00–11·0
Parity (number of parturition)	135	1·00	0·00–1·00	64	1·00	0·00–2·00	33	1·00	0·00–1·00
Monthly per-capita family income (USD $)	128	295·32	191·81–428·64	64	273·10	150·58–385·26	33	194·54	136·61–330·60
Adiponectin (ng/ml)*	135	11·92	7·73–14·94	64	8·92	6·44–12·95	33	7·70	5·87–11·58
Insulin (μU/ml)*	135	3·88	2·90–5·75	64	5·63	3·92–7·70	33	7·95	5·42–11·12
Leptin (ng/dl)*	135	12·58	8·32–19·14	64	22·20	17·80–33·38	33	33·69	22·73–44·54
Fasting glucose (mg/dl)	134	83·00	78·00–88·00	64	84·00	78·00–89·50	32	85·00	80·00–90·50
HOMA-IR*	134	0·82	0·57–1·23	64	1·14	0·83–1·73	32	1·78	1·06–2·47
QUICKI*	134	0·17	0·16–0·18	64	0·16	0·15–0·17	32	0·15	0·14–0·16
CRP (mg/L)*	125	2·99	1·43–6·12	58	5·28	3·00–11·04	29	9·28	5·35–14·85
Triglycerídes (mg/dl)*	135	69·00	54·00–87·00	64	74·00	61·50–106·00	33	88·00	73·00–117·00
HDL-cholesterol (mg/dl)	135	49·00	42·00–54·00	64	48·50	43·00–52·00	33	47·00	41·00–51·00

Baseline, between 5th and 13th gestational week; *n*, number of observations; CI, confidence interval; BMI, body mass index; LDL, low-density lipoprotein; IQR, interquartile range; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; CRP, C-reactive protein; HDL, high-density lipoprotein.
**P*-value <0·05 – *P*-value refers to ANOVA and Kruskal-Wallis tests, that were performed to compare symmetric and asymmetric variables, respectively.

Plasma concentrations of leptin increased from the first to the second trimester and decreased from the second to the third trimester of pregnancy in NW and OW women, but this pattern did not present statistical significance in OW women. This pattern was significant when we evaluated the overall sample (data not shown). We believe that the well-defined pattern in NW women may have skewed the results for the total sample by increasing the power of the analyses and suggesting that this pattern occurs across the entire group. Yang[9] evaluated serum samples from 114 women, and they reported that concentrations increased progressively throughout pregnancy with a statistically significant decline in the last month. However, Anin-Nyame *et al.*[5] evaluated the plasma concentrations of leptin from 13 women at seven different stages of pregnancy and, although they reported that the concentrations gradually increased up to 32 weeks, the decrease at the end of pregnancy was not statistically significant. This was likely due to the small sample size and the lack of stratification by BMI categories.

In the present study, leptin concentrations were significantly higher in OW and OB women, which is consistent with previous reports by several authors.[9,10,18] Misra & Trudeau[10] evaluated 143 women at 4 different points during pregnancy and reported that OW/OB women had significantly higher leptin concentrations than NW women at each time point evaluated. In 2005, Yang[9] reported that leptin concentrations correlated with weight and BMI in the three trimesters of pregnancy. Schubring *et al.*[18] also reported a correlation between serum concentrations of leptin at 5 time points during pregnancy and maternal weight and BMI.

Highman *et al.*[19] tested correlations between leptin concentrations and body fat percentage and reported significant positive values before pregnancy ($r = 0{\cdot}90$), from 12 to 14 weeks ($r = 0{\cdot}91$) and from 34 to 36 weeks ($r = 0{\cdot}87$) of pregnancy. However, longitudinal analyses of the data showed that, during the period in which there was a more significant increase in leptin concentrations (from the pregestational period to the first trimester), there was an average 3% decrease in fat percentage. Thus, the result of a significant increase in circulating leptin concentrations, concomitant with little or no increase in fat mass during early pregnancy, corroborates the hypothesis that other factors also contribute to the increase in leptin concentrations during the first trimester.[19] One possible pathway for leptin concentrations increase may be through placental production.[20] It was already reported that placental leptin expression patterns coincide with maternal serum leptin concentrations patterns throughout pregnancy, evidencing the

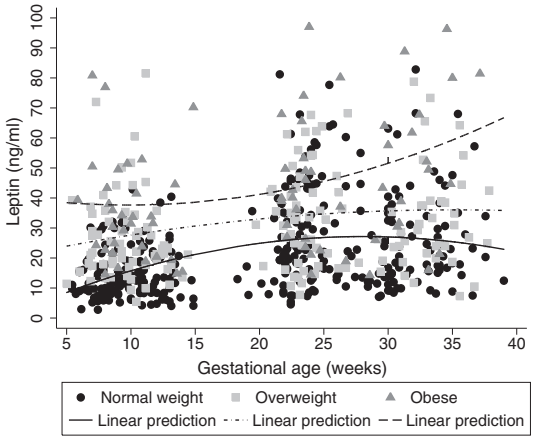

Fig. 1 Changes in plasmatic leptin concentrations stratifying for pregestational BMI categories in women followed at a public health centre in Rio de Janeiro, Brazil, 2009–2012.

Normal weight (18·5 ≤ BMI <25·0): Mean gestational age (95% confidence interval) at pregnancy trimesters: 1st = 9·67 (9·28–10·06); 2nd = 23·49 (23·11–23·87); 3rd = 32·20 (31·67–32·73). Median leptin plasmatic concentrations (interquartile range) at pregnancy trimesters: 1st (n = 135): 12·58 (8·32–19·14); 2nd (n = 109): 22·7 (14·93–37·42); 3rd (n = 87): 20·55 (14·29–34·18). Longitudinal linear regression coefficients (95% confidence intervals): 1·996 (1·364 to 2·583; P-value <0·001) and −0·036 (−0·050 to −0·020; P-value <0·001) for gestational age and quadratic gestational age, respectively. Overweight (25·0 ≤ BMI <30·0): Mean gestational age (95% confidence interval) at pregnancy trimesters: 1st= 9·68 (9·13–10·23); 2nd = 23·46 (23·01–23·91); 3rd = 33·18 (32·43–33·92). Median leptin plasmatic concentrations (interquartile range) at pregnancy trimesters: 1st (n = 64): 22·20 (17·80–33·38); 2nd (n = 51): 33·59 (18·30–47·13); 3rd (n = 41): 28·61 (21·55–38·87). Longitudinal linear regression coefficients (95% confidence intervals): 0·935 (−0·359 to 2·229; P-value = 0·157) and −0·013 (−0·044 to 0·018; P-value = 0·400) for gestational age and quadratic gestational age, respectively. Obese (BMI ≥30·0): Mean gestational age (95% confidence interval) at pregnancy trimesters: 1st = 10·00 (9·18–10·83); 2nd = 23·87 (23·09–24·64); 3rd = 32·31 (31·24–33·38). Median leptin plasmatic concentrations (interquartile range) at pregnancy trimesters: 1st (n = 33): 33·69 (22·73–44·54); 2nd (n = 26): 40·08 (26·45–53·24); 3rd (n = 19): 52·15 (31·64–80·05). Longitudinal linear regression coefficients (95% confidence intervals): −0·670 (−3·017 to 1·676; P-value = 0·575) and 0·034 (−0·023 to 0·092; P-value = 0·244) for gestational age and quadratic gestational age, respectively.

contribution of placental leptin in the determination of maternal plasma concentrations.[21]

In our final linear regression model, maternal body weight throughout pregnancy was associated with leptin concentrations, indicating that, even if other factors are involved, maternal body weight throughout pregnancy is still directly associated with the evolution of leptin concentrations. As we did not adjust our analyses for body fat content, maternal body weight throughout pregnancy may be reflecting body fat in the association with leptin. Although maternal obesity and associated hyperleptinaemia appear to have a negative impact on placental amino-acid transport and other functions, it was already reported that placental leptin expression is comparable between OW/OB and NW women.[22] Thus, higher leptin concentrations in OW/OB women are presumably due to increased adiposity.[21] We suggest that even in the same BMI classification group, maternal body weight may act as a proxy for body fat in a situation where the higher the adiposity, the higher are the leptin concentrations.

Serum HDL-cholesterol concentrations were positively associated with plasma leptin concentrations in women in all BMI categories. However, TG concentrations remained negatively associated in the final model only for OB women. Some authors have reported an association between serum lipids and leptin concentrations in adult women.[23,24] Couilard *et al.*[23] reported significant correlations between TG (r = 0·41) and HDL-cholesterol (r = −0·36) with plasma leptin concentrations in non-smoking women. These correlations lost statistical significance after adjustment for body fat mass. However, after this adjustment, the directions of the correlations changed (r = −0·07 and 0·08 for TG and HDL-cholesterol concentrations, respectively), being similar to the ones found in our study. We need to emphasize that the sample in Couilard's study is small (n = 48), which diminishes statistical power. It was already reported that attenuation of leptin sensitivity in the brain leads to excess TG accumulation in adipose tissue,[25] probably with lesser serum release. We suggest this is one possible explanation to the inverse association between serum TG and leptin because OB individuals are more likely to present resistance to leptin's action.[26]

The QUICKI index was negatively associated with leptin concentrations during pregnancy in NW women in the nonadjusted model. However, this variable lost statistical significance after adjustment by TG and dietary carbohydrate. We opted to maintain this variable in the models considering its high β values, borderline statistical significance in NW and OB adjusted final models, and clinical relevance. Vähämiko *et al.*[27] reported a positive correlation between third-trimester leptin concentrations with insulin concentrations and HOMA-IR values and a negative association with the QUICKI index, which were independent of the pregestational BMI category. This result suggests that leptin acts as regulator of glucose metabolism during pregnancy. In a study on women between 26 and 36 weeks of gestation by Yilmaz *et al.*,[28] leptin concentrations were negatively correlated with the QUICKI index (r = −0·384). An inverse association between leptin concentrations and QUICKI index was expected as leptin may present insulin-sensitizing actions.[29] Thus, the more sensitive to insulin the woman is, the smaller is the need for leptin.

CRP concentrations were negatively associated with leptin concentrations in NW women. However, there was no association with OW nor OB women. Molvarec *et al.*[30] analysed 60 healthy women and reported that there were associations between leptin plasma concentrations, BMI and CRP (β = 0·43 and 0·45, respectively). However, the direction of the association with CRP was opposite of that found in the present study.

Dietary carbohydrate was positively associated with leptin concentrations only in OB women. Studies on animal models indicate that chronic exposure to dietary components – such as fructose, saccharose and fat – may lead to leptin resistance,[31,32]

Table 2. Bivariate longitudinal regression model for leptin concentrations throughout pregnancy stratifying for pregestational BMI categories in women followed at a public health centre in Rio de Janeiro, Brazil, 2009–2012

	18·5 ≤ BMI <25·0			25·0 ≤ BMI ≥29·9			BMI ≥30·0		
Variables	β	95% CI	*P*-value*	β	95% CI	*P*-value*	β	95% CI	*P*-value*
Gestational age (weeks)	1·974	1·364 to 2·583	<0·001	0·935	−0·359 to −2·229	0·157	−0·670	−3·017 to 1·676	0·575
Quadratic gestational age (weeks†)	−0·035	−0·050 to −0·020	<0·001	−0·013	−0·044 to 0·018	0·400	0·034	−0·023 to 0·092	0·244
Maternal weight throughout pregnancy (kg)	0·795	0·581 to 1·008	<0·001	0·774	0·396 to 1·153	<0·001	0·391	−0·113 to 0·894	0·128
Adiponectin (ng/ml)	−0·052	−0·125 to 0·020	0·155	0·001	−0·197 to 0·198	<0·001	0·346	−0·018 to 0·711	0·062
Insulin (μU/ml)	0·175	−0·008 to 0·358	0·062	−0·012	−0·528 to 0·503	0·963	−0·131	−0·883 to 0·622	0·734
Fasting glucose (mg/dl)	0·035	−0·097 to 0·168	0·601	−0·038	−0·320 to 0·245	0·793	−0·470	−0·952 to 0·012	0·056
HOMA-IR	0·707	−0·005 to 1·419	0·052	−0·054	−2·187 to 2·078	0·960	−1·258	−4·590 to 2·074	0·459
QUICKI	−87·749	−150·641 to −24·854	0·006	−14·166	−187·352 to 159·39	0·873	213·612	−113·541 to 540·764	0·201
CRP (mg/L)	−0·116	−0·256 to 0·024	0·105	0·269	−0·136 to 0·674	0·194	−0·029	−0·369 to 0·310	0·866
Triglycerides (mg/dl)	0·026	−0·016 to 0·068	0·225	−0·045	−0·109 to 0·019	0·166	−0·122	−0·242 to −0·003	0·045
Total cholesterol (mg/dl)	0·054	0·006 to 0·103	0·029	−0·021	−0·114 to 0·071	0·651	0·095	−0·072 to 0·261	0·265
HDL-cholesterol (mg/dl)	0·221	0·070 to 0·372	0·004	0·388	0·042 to 0·734	0·028	0·599	0·040 to 1·157	0·036
LDL-cholesterol (mg/dl)	0·040	−0·025 to 0·105	0·225	−0·041	−0·151 to 0·068	0·461	0·142	−0·068 to 0·353	0·186
Total caloric intake (kcal)	−0·001	−0·004 to 0·003	0·936	−0·001	−0·007 to 0·004	0·634	0·001	−0·011 to 0·014	0·822
Dietary carbohydrates (grams)	0·005	−0·010 to 0·020	0·532	0·007	−0·043 to 0·029	0·703	0·057	0·001 to 0·114	0·048
Alcohol consumption (no/yes)†	−2·538	−5·760 to 0·684	0·123	0·668	−6·097 to 7·433	0·846	0·391	−10·996 to 11·778	0·946
Smoking (no/yes)†	−4·495	−10·655 to 1·665	0·153	−5·395	−17·540 to 6·745	0·384	10·806	−5·125 to 26·738	0·184

BMI, body mass index; CI, confidence interval; β, longitudinal linear regression coefficient for leptin; SE, standard error; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

**P*-values refer to restricted maximum likelihood estimator.

†Categorical variables for which the first category is the reference and the second is the exposure.

Table 3. Multiple longitudinal regression model for leptin concentrations throughout pregnancy stratifying for pregestational BMI categories in women followed at a public health centre in Rio de Janeiro, Brazil, 2009–2012

	18·5 ≤ BMI <25·0*,†			25·0 ≤ BMI ≥29·9†,‡			BMI ≥30·0†,§		
Fixed effects	β	95% CI	*P*-value¶	β	95% CI	*P*-value¶	β	95% CI	*P*-value¶
Intercept	−42·562	−62·350 to −22·774	<0·001	−65·230	−114·065 to −16·395	0·009	−106·312	−189·269 to −23·352	0·012
Maternal weight throughout pregnancy (kg)	0·714	0·491 to 0·937	<0·001	0·871	0·475 to 1·267	<0·001	0·666	0·187 to 1·145	0·006
HDL-cholesterol (mg/dl)	0·239	0·089 to 0·388	0·002	0·315	−0·022 to 0·651	0·067	0·722	0·219 to 1·226	0·005
CRP (mg/l)	−0·138	−0·272 to −0·004	0·044	0·195	−0·204 to 0·593	0·339	−0·220	−0·526 to 0·085	0·158
QUICKI	−53·585	−114·670 to 7·500	0·086	63·479	−113·058 to 240·017	0·481	287·925	−32·923 to 608·768	0·079
Triglycerides (mg/dl)	0·016	−0·025 to 0·058	0·440	−0·022	−0·088 to 0·043	0·505	−0·130	−0·241 to −0·020	0·021
Dietary carbohydrates (grams)	0·009	−0·005 to 0·023	0·440	0·002	−0·031 to 0·35	0·903	0·075	0·023 to 0·126	0·004
Gestational age (weeks)	1·446	0·751 to 2·142	<0·001	0·552	−0·947 to 2·051	0·470	−0·209	−2·989 to 2·570	0·883
Quadratic gestational age (weeks†)	−0·033	−0·049 to −0·016	<0·001	−0·011	−0·046 to 0·023	0·530	0·024	−0·041 to 0·089	0·471
Restricted Maximum Likelihood	−1109·824			−578·695			−283·280		
AIC	2243·6448			1181·39			590·561		

Random effects	β (95% CI)	β (95% CI)	β (95% CI)
σ Intercept	0·223 (0·156–0·319)	0·186 (0·082–0·418)	0·219 (0·070–0·687)
σ Gestational age	−0·223 (−0·303 to −0·144)	−0·186 (−0·337 to −0·035)	0·219 (−0·031 to 0·469)
σ Residual	64·887 (52·783–79·768)	180·493 (133·368–244·270)	230·328 (148·308–357·7)

BMI, body mass index; β, longitudinal linear regression coefficient for leptin; SE, standard error; HDL, high-density lipoprotein; CRP, C-reactive protein; QUICKI, quantitative insulin sensitivity check index; CI, confidence interval; AIC, Akaike Information Criterion.

*Number of observations = 292; Number of groups = 132; Average of 2·2 observations per group.

†The number of individuals (number of groups) in each model does not necessarily match the number of individuals with baseline leptin measurements because an individual must have information at least at one time-point for all variables included in the model. If a woman presents missing data for the three time points in one independent variable, she is excluded from the model.

‡Number of observations = 139; Number of groups = 64; Average of 2·2 observations per group.

§Number of observations = 66; Number of groups = 33; Average of 2·0 observations per group.

¶*P*-values refer to restricted maximum likelihood estimator.

what may result in greater leptin concentrations. We did not find studies that longitudinally assessed associations between food intake and leptin in pregnancy. However, in a sample of 103 pregnant women, Vähämiko *et al.*[27] observed that third-trimester leptin concentrations in OW/OB women ($n = 42$) were positively correlated to saccharose intake, and this correlation was not observed in NW women.

This is the first exploratory longitudinal study of factors associated with the evolution of leptin concentrations during pregnancy. Thus, comparisons between our data and the available literature are limited. This study opens up additional avenues for experimental studies, as it raises the hypothesis that there are associations between biochemical variables, food intake and the behaviour of leptin concentrations during pregnancy. It is important to emphasize that although we tried to minimize the effect of different patterns of gestational weight gain among BMI categories including maternal body weight throughout pregnancy as a covariate in all three models, potential interactions between biochemical variables and BMI may have interfered with our results.

One potential limitation of this study is patient loss during follow-up, which is inherent to longitudinal studies. Of the 232 women evaluated, 68 did not have complete information for the three follow-up waves. Additionally, it was already reported that the use of USG to estimate gestational age may be less accurate in OB women.[33] To increase the robustness of the data, we also performed all the analysis using gestational age calculated based on the date of last menstrual period (LMP) instead of the one based on USG. Indeed, the results barely changed, meaning that the employment of gestational age either based on USG or LMP did not affect the current results.

A strength of this study is the number of women included because previous studies describing leptin concentrations during pregnancy have had much smaller sample sizes. The statistical analyses also strengthen the results, given that most other studies have only performed basic descriptive analyses to compare means and correlations. In addition, this is the first study to evaluate the effect of biological markers, such as hormones and lipids, on leptin concentrations throughout pregnancy, stratifying by pregestational BMI categories.

In summary, maternal body weight throughout pregnancy and serum concentrations of HDL-cholesterol were associated with plasma concentrations of leptin during pregnancy independently of pregestational BMI. However, serum concentrations of CRP were only associated in women with a NW pregestational BMI, and serum concentrations of TG and the amount of dietary carbohydrates were associated with leptin concentrations only in women with pregestational obesity. These results indicate that the factors that influence leptin concentrations during pregnancy are different according to pregestational BMI.

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Conflict of interest

The authors declare no conflict of interests.

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