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Maternal Pre-Gravid Weight is the Primary Determinant of Serum Leptin and its Metabolic Associations in Pregnancy, Irrespective of Gestational Glucose Tolerance Status

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ABSTRACT

CONTEXT: Several previous studies have investigated circulating levels of the adipokine leptin in relation to gestational diabetes mellitus (GDM). However, these studies have yielded markedly conflicting results, including increased, decreased and unchanged leptin levels in women with GDM as compared to their peers.

OBJECTIVE: We sought to evaluate the metabolic determinants of serum leptin in a well-characterized cohort reflecting the full spectrum of glucose intolerance in pregnancy.

DESIGN/SETTING/PARTICIPANTS: Metabolic characterization, including oral glucose tolerance test (OGTT) and measurement of serum leptin, insulin, lipids, adiponectin, and C-reactive protein (CRP), was performed in 817 pregnant women. The OGTT identified 198 women with GDM, 142 with gestational impaired glucose tolerance (GIGT) and 477 with normal glucose tolerance (NGT).

RESULTS: Median leptin (ng/ml) did not differ between the NGT (33.7), GIGT (36.3), and GDM (36.4) groups ($p=0.085$). On univariate correlation analysis, leptin was most strongly associated with pre-pregnancy BMI ($r=0.54$, $p<0.0001$), fasting insulin ($r=0.60$, $p<0.0001$), and CRP ($r=0.38$, $p<0.0001$), but only weakly associated with area-under-the-glucose-curve ($AUC_{glucose}$) on the OGTT ($r=0.10$, $p=0.0066$). On multiple linear regression analysis, the strongest independent determinant of leptin was pre-pregnancy BMI ($t=11.55$, $p<0.0001$), while $AUC_{glucose}$ was not a significant predictor ($t=-0.95$, $p=0.34$). Furthermore, while its respective associations with fasting insulin, triglycerides and adiponectin varied across tertiles of pre-pregnancy BMI, leptin was not significantly associated with $AUC_{glucose}$ in any BMI tertile.

CONCLUSIONS: Pre-gravid BMI, rather than gestational glucose tolerance, is the primary determinant of serum leptin concentration in pregnancy.

INTRODUCTION

Leptin is an adipocyte-secreted hormone (adipokine) with pleiotropic bio-activity, including effects on regulation of body weight, energy homeostasis, reproduction, and immune function [1, 2]. Though it has been proposed as a biomarker of diabetic risk, studies of leptin as a predictor of type 2 diabetes have yielded variable results [3-8]. **The relationship between leptin and gestational diabetes mellitus (GDM) remains similarly unclear because, although several previous studies have investigated leptin levels in GDM, they have yielded markedly conflicting results. Indeed, whereas many studies have reported antepartum leptin levels to be higher in women with GDM than in their peers [9-15], others have found no difference [16-18]. Moreover, some studies have even reported lower leptin concentrations in women with GDM as compared to their peers [19, 20]. Importantly, it should be noted that these studies have been limited by (i) modest numbers of women with GDM, (ii) heterogeneity in the glucose tolerance status of comparators, and (iii) varying degrees of adjustment for potential confounders. Thus, hypothesizing that these limitations have contributed to this inconclusive literature, our objective was to evaluate the metabolic determinants of serum leptin in a large cohort of women reflecting the full spectrum of glucose intolerance in pregnancy, with adjustment for a broad array of potential covariates.**

MATERIALS AND METHODS

Study Population

This analysis was conducted as part of an ongoing cohort study in which pregnant women are recruited at the time of antepartum screening for GDM [21]. The study protocol was approved by the Mount Sinai Hospital Research Ethics Board and has been described in detail previously [21, 22]. In brief, all participants undergo a 3-hour 100g oral glucose tolerance test (OGTT) in late 2^{nd} / early 3^{rd} trimester and simultaneous metabolic characterization consisting of interviewer-administered questionnaires, physical examination,

and assessment of cardio-metabolic risk factors. As previously described, the OGTT enables stratification of participants into the following three gestational glucose tolerance categories: (i) GDM (defined by exceeding $\geq$ 2 glycemic thresholds on the OGTT, as defined by National Diabetes Data Group (NDDG) criteria); (ii) gestational impaired glucose tolerance (GIGT, defined by exceeding 1 NDDG glycemic threshold); or (iii) normal glucose tolerance (NGT). All participants provided written informed consent. The current analysis was restricted to women with singleton pregnancies (n=817).

Laboratory Measurements

All OGTTs were performed in the morning after an overnight fast. During the OGTT, venous blood samples were drawn for measurement of glucose and insulin at fasting and at 30, 60, 120 and 180 minutes after ingestion of the glucose load. The area-under-the-glucose-curve during the OGTT ($AUC_{glucose}$) was calculated by trapezoidal rule, thereby providing a continuous measure of glycemia to complement the categorical glucose tolerance status defined above. Specific insulin was measured using the Roche Modular system and the electrochemiluminescence immunoassay kit (Roche Diagnostics, Laval, Quebec, Canada). Fasting lipids (total cholesterol, HDL cholesterol and triglycerides) were measured using the Roche Cobas 6000 c 501 analyzer (Roche Diagnostics, Laval, Canada), with LDL cholesterol determined by Friedewald formula. Fasting serum leptin was measured by enzyme-linked immunosorbent assay (ELISA) #EZHL-80SK (Millipore, Linco Research, St Charles, Missouri, USA). Total adiponectin was measured by ELISA (Millipore, Linco Research, St. Charles, Missouri, USA). High-sensitivity CRP was measured by endpoint nephelometry using the Dade-Behring BN Prospec and the N high-sensitivity CRP reagent (Dade-Behring, Mississauga, Ontario, Canada).

Statistical Analyses

All analyses were conducted using the Statistical Analysis System (SAS, Version 9.2, SAS Institute, Cary, North Carolina, USA). In Table 1, data are presented as mean (standard

deviation) for normally-distributed continuous variables and as median (interquartile range) for skewed variables. Natural log transformations were used in subsequent analyses of skewed variables, where necessary. Chi-square tests (categorical variables) and analysis of variance (ANOVA) (continuous variables) were used to determine differences across the three glucose tolerance categories in pregnancy. Univariate correlations between leptin and continuous variables were assessed by Spearman correlation analysis (Table 2). Multiple linear regression analysis was performed to determine independent relationships between antepartum characteristics and dependent variable leptin (Table 3). Covariates included in this analysis consisted of demographic/clinical variables (age, ethnicity, family history of diabetes), reproductive factors (parity, previous GDM, weeks gestation at OGTT), and metabolic factors (pre-pregnancy BMI, gestational weight gain up to the OGTT, $AUC_{glucose}$, fasting insulin, LDL, HDL, triglycerides, CRP, adiponectin). Owing to the significant interactions of metabolic variables ($AUC_{glucose}$, fasting insulin, adiponectin) with pre-pregnancy BMI, the multiple linear regression analyses were repeated after stratification of the study population into tertiles of pre-gravid BMI (Table 4).

RESULTS

Table 1 shows the characteristics of the study population stratified into the following three gestational glucose tolerance groups: NGT (n=477), GIGT (n=142), and GDM (n=198). There were no significant differences between the groups in age or parity. The antepartum OGTT was performed slightly earlier in women with GDM (median 29 weeks compared to 30 weeks in the other 2 groups; overall p=0.014). As expected, there were significant differences between the three glucose tolerance groups in GDM risk factors (ethnicity, family history of diabetes, previous GDM, pre-pregnancy BMI) and metabolic variables ($AUC_{glucose}$, fasting insulin, HDL cholesterol, triglycerides, CRP, adiponectin). However, there was no significant difference in leptin concentration across the gestational glucose tolerance groups (p=0.085).

On Spearman univariate correlation analysis (Table 2), leptin was most strongly associated with pre-pregnancy BMI ($r=0.54$, $p<0.0001$), fasting insulin ($r=0.60$, $p<0.0001$), CRP ($r=0.38$, $p<0.0001$), adiponectin ($r=-0.24$, $p<0.0001$), and gestational weight gain up to the OGTT ($r=0.15$, $p<0.0001$). However, leptin was only weakly associated with $AUC_{glucose}$ ($r=0.10$, $p=0.0066$). Weak inverse associations with LDL ($r=-0.09$, $p=0.0088$) and age ($r=-0.07$, $p=0.042$) were also noted.

On multiple linear regression analysis (Table 3), a model consisting of age, weeks gestation, ethnicity, family history of diabetes, parity, previous GDM, pre-pregnancy BMI, gestational weight gain, $AUC_{glucose}$, fasting insulin, LDL, HDL, triglycerides, CRP and adiponectin explained 51.4% of the variance associated with dependent variable leptin. This analysis revealed that the strongest independent determinant of leptin was pre-pregnancy BMI ($t=11.55$, $p<0.0001$). Other significant covariates were fasting insulin ($t=9.97$, $p<0.0001$), gestational weight gain up to the OGTT ($t=9.30$, $p<0.0001$), CRP ($t=6.48$, $p<0.0001$), parity ≥ 1 ($t=-4.98$, $p<0.0001$), ethnicity other than Caucasian or Asian ($t=3.43$, $p=0.0006$), and triglycerides ($t=-2.68$, $p=0.0076$). Repeating this multiple linear regression analysis with categorical glucose tolerance status (NGT, GIGT, GDM) as a covariate in place of $AUC_{glucose}$ yielded the very same significant predictors, with neither GDM ($t=-1.23$, $p=0.22$) nor GIGT ($t=-0.81$, $p=0.42$) emerging as independent covariates of leptin (data not shown).

On testing the model in Table 3 for interactions between covariates, we found significant interactions between pre-pregnancy BMI and each of the following variables: $AUC_{glucose}$ (interaction $p=0.0004$), fasting insulin (interaction $p<0.0001$), and adiponectin (interaction $p<0.0001$). In light of these findings, we stratified the study population into tertiles of pre-pregnancy BMI and repeated the multiple linear regression models within each of these 3 groups (Table 4). These analyses revealed that pre-pregnancy BMI, gestational weight gain, fasting insulin, CRP, and parity were independently associated with leptin within each BMI tertile. In contrast, the significant associations between leptin and ethnicity, triglycerides and adiponectin, respectively, were found in only certain tertiles.

Figure 1 shows examples of different patterns of effect modification by pre-pregnancy BMI on the metabolic correlates of antepartum leptin. First, as shown in Panel A, $AUC_{glucose}$ was not significantly associated with leptin in any BMI tertile, though may be trending towards a weak inverse relationship in the heaviest women (third tertile). In contrast, though fasting insulin was significantly correlated with leptin in all three tertiles (Panel B), the magnitude of this association was far greater in the leanest women (beta=0.53) than in the middle or heaviest tertiles (beta=0.33 and beta=0.21, respectively). Third, triglyceride level (Panel C) was inversely associated with leptin in the leanest women (beta=-0.17, p=0.001) but not at all related in the middle (beta=-0.04, p=0.35) and highest (beta=-0.06, p=0.19) tertiles. Lastly, adiponectin (Panel D) was significantly correlated with leptin in only the middle tertile of pre-pregnancy BMI (beta=0.03, p=0.035).

As a supplemental analysis, the multiple linear regression models of leptin were repeated after stratifying the study population into the following 3 groups based on pre-pregnancy BMI: (i) BMI < 25 kg/m^2 (n=512); (ii) BMI between 25.0 and 29.99 kg/m^2 inclusive (n=183); and (iii) BMI $\geq$ 30.0 kg/m^2 (n=122). These analyses showed that, as with the tertile analysis in Table 4, pre-pregnancy BMI and gestational weight gain up to the OGTT were positive independent predictors of leptin in each of the three groups, while $AUC_{glucose}$ was not associated with leptin in any group, and other variables had group-specific associations that varied according to the pre-pregnancy BMI group (Online Table).

DISCUSSION

Previous reports of antepartum serum leptin in GDM have been conflicting: several studies have reported higher leptin levels in GDM than in NGT controls [9-13]; while others have reported lower leptin levels in GDM than controls, after adjusting or matching for BMI [19, 20]; and finally, other studies have reported no difference in leptin concentration between participants with and without GDM [16-18], including after adjustment for BMI [23].

However, there are important limitations of this literature that are likely contributing to these conflicting results. First, these studies have had relatively modest numbers of women with GDM (generally between 50-60), with the largest reporting only 63 such patients. Second, the gestational glucose tolerance status of the comparator women (i.e. the group against which GDM has been compared) has varied. Specifically, some studies compared the levels of leptin in GDM against those in women with confirmed normal glucose tolerance (NGT), whereas other studies defined the comparator group by the absence of GDM (i.e. which could include gestational glucose intolerance). Third, the previous studies have varied in the extent of their adjustment for covariates, an important issue given the relationships between leptin and numerous metabolic variables. Thus, in light of these limitations, our study aimed to reconcile the previously-reported conflicting data through the following design features: (i) assessment of more than three times as many participants with GDM than any of the previous studies; (ii) evaluation across the full range of gestational glucose tolerance status; and (iii) adjustment for a broad array of metabolic variables.

Bolstered by these strengths in study design, our data demonstrate that there is no significant independent relationship between glucose tolerance status in pregnancy and maternal serum leptin levels. The robustness of this finding is supported by the fact that the regression models explained a high proportion of the variance in leptin concentration (eg. model adjusted R^2=51.4% in Table 3) and identified several independent determinants thereof, but neither $AUC_{glucose}$ nor GDM was one of them. Instead, we found that pre-pregnancy BMI is the primary determinant of leptin levels in pregnancy. Leptin is produced by adipocytes, is elevated in obese women, and correlates with BMI and body fat percentage in the setting of pregnancy and GDM [9, 23-25]. Although placental production likely contributes to the rise in circulating leptin levels that takes place in pregnancy, it has recently been suggested that the overall impact of this placental contribution is modest [26]. Indeed, consistent with this suggestion, we found that pre-gravid BMI was a much stronger determinant of antepartum leptin concentration than was gestational weight gain and that weeks gestation was not a significant predictor (Tables 2 and 3). Thus, placental contribution

notwithstanding, the current data emphasize the dominant role of pre-pregnancy BMI in determining the absolute levels of serum leptin in late pregnancy.

Furthermore, we have also demonstrated that pre-pregnancy BMI modifies the associations of leptin with various metabolic factors. As demonstrated in Figure 1, the impact of pre-pregnancy BMI on the relationships between leptin and cardiometabolic risk factors is complex and varied. Indeed, triglycerides were inversely associated with leptin in only the lowest tertile of pre-gravid BMI, while adiponectin was positively correlated in only the middle tertile. Conversely, though the significance of their associations with leptin did not change between tertiles, both fasting insulin and $AUC_{glucose}$ exhibited significant interactions with pre-pregnancy BMI. Specifically, the magnitude of the association beween leptin and fasting insulin decreased as pre-gravid BMI rose, while $AUC_{glucose}$ showed a non-significant trend towards an inverse relationship with leptin in the heaviest tertile that was not at all apparent in the other two tertiles. Importantly, our findings only emerge after BMI stratification and covariate adjustment in a very large sample size across a broad range of glucose tolerance in pregnancy. This previously unrecognised aspect of leptin physiology in pregnancy could have also contributed to the earlier conflicting findings in the literature and again underscores the central importance of pre-pregnancy BMI.

Other independent covariates in the multiple regression analysis of maternal leptin in the current study included ethnicity other than Asian or Caucasian, parity $\geq$1, and CRP. Although there was no dominant ethnic group within the "other" category, ethnic differences in leptin levels have been previously reported [27]. The basis for the inverse association between parity $\geq$1 and leptin is not clear, but potentially may relate to the physiologic effects of leptin on reproductive function [28]. **Finally, it has previously been demonstrated that physiologic concentrations of serum leptin can stimulate hepatocyte expression of CRP and that CRP can bind to leptin (as a serum leptin-interacting protein (SLIP)) [29]. In doing so, CRP can block the binding of leptin to its receptors and thereby induce target tissue insensitivity to the biologic effects of leptin (i.e. leptin resistance) [29].** In this

context, the current study confirms that an independent association exists between leptin and CRP in pregnancy and is consistent across BMI categories.

While the current study demonstrates that maternal adiposity (rather than glucose intolerance) is the primary determinant of the circulating leptin concentration in pregnancy, the physiologic implications of this relationship remain to be determined. **Interestingly, we and others have demonstrated that, after adjustment for BMI, maternal serum leptin is inversely associated with infant birthweight [30, 31]. These data raise the possibility that, in pregnancy, leptin may play a role in attenuating the pro-macrosomic effects of maternal obesity.** Ultimately, further longitudinal study is needed to evaluate the long-term implications of antepartum leptin on outcomes for both mother and child. With respect to the effects on the offspring, it is of interest that maternal glycemia has recently been associated with epigenetic modification of the leptin gene on the fetal side of the placenta [26]. Thus, while our study demonstrates that gestational dysglycemia does not affect serum leptin levels in pregnancy, it still may be relevant to long-term leptin physiology in the offspring.

This study is limited by its observational nature and thus cannot examine causal relationships between leptin, maternal BMI and cardiometabolic risk factors. **Secondly, this cross-sectional analysis cannot provide insight on the relationships between these variables over the course of pregnancy.** Thirdly, the mechanisms underlying the apparent modifying effect of pre-pregnancy BMI are unclear, though leptin resistance is a recognized phenomenon in other settings [29, 32]. Neverthelesss, through its evaluation of the complex relationships between leptin and an array of metabolic factors in a large cohort of women across the full spectrum of glucose tolerance in pregnancy, the current study has provided a potential reconciliation of the existing conflicting literature on leptin in GDM that should lead to further studies.

In conclusion, we have reported that maternal antepartum leptin levels do not differ significantly across categories of gestational glucose tolerance. Instead, pre-pregnancy BMI is a key driver of maternal leptin levels, and modifies the relationships between leptin and several key cardiometabolic risk factors. With this understanding, longitudinal studies are

now required to assess the role of leptin in pregnancy and its implications for long-term metabolic risk of mother and offspring.

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Figure Legends

Figure 1: Adjusted relationships between log leptin and (Panel A) $AUC_{glucose}$, (Panel B) log fasting insulin, (Panel C) triglycerides, and (Panel D) adiponectin, within each tertile of pre-pregnancy BMI. Each relationship is adjusted for the following covariates: age, weeks gestation, ethnicity, family history of diabetes, parity, previous GDM, pre-pregnancy BMI, gestational weight gain up to the OGTT, $AUC_{glucose}$, fasting insulin, LDL, HDL, triglycerides, CRP and leptin**.**

Table 1: Participant characteristics by glucose tolerance status in pregnancy

Characteristic	**NGT (n=477)**	**GIGT (n=142)**	**GDM (n=198)**	**p value**
Age (years)	34.0 ± 4.2	33.9 ± 4.3	34.6 ± 4.5	0.1796
Weeks gestation at OGTT	30 ± 2.9	30 ± 3.0	29 ± 2.9	0.0144
Ethnicity:				
White (%)	350 (73.4)	96 (67.6)	123 (62.1)	0.0217
Asian (%)	48 (10.1)	23 (16.2)	35 (17.7)	
Other (%)	79 (16.6)	23 (16.2)	40 (20.2)	
Family history of DM	251 (52.6)	84 (59.2)	136 (68.7)	0.0006
Parity:				
Nulliparous	255 (53.5)	80 (56.3)	110 (55.6)	0.7825
≥1	222 (46.6)	62 (43.7)	88 (44.4)	
Previous GDM[a]	18 (3.8)	14 (9.9)	17 (8.6)	0.0058
Pre-pregnancy BMI (kg/m^2)	23.2 (21.1-26.5)	23.2 (21.3-26.6)	24.6 (22.1-28.9)	0.0002
Gestational weight gain up to OGTT (kg)	11.1 ± 5.4	11.0 ± 5.0	10.1 ± 5.3	0.0799
$AUC_{glucose}$ on OGTT	20.6 ± 2.4	24.7 ± 2.5	28.0 ± 2.5	<0.0001
Fasting insulin (pmol/l)	57 (40-79)	71.5 (52-99)	79 (48-115)	<0.0001
LDL cholesterol (mmol/L)	3.7 + 1.1	3.6 ± 1.2	3.5 ± 1.2	0.1637
HDL cholesterol (mmol/L)	1.7 ± 0.4	1.7 ± 0.3	1.6 ± 0.4	0.0001
Triglycerides (mmol/L)	2.4 ± 0.8	2.5 ± 0.9	2.7 ± 0.8	0.0002
CRP (mg/L)	4.1 (2.4-7.0)	4.0 (2.0-7.5)	5.6 (3.2-9.6)	<0.0001
Adiponectin (ug/ml)	8.4 ± 3.0	7.4 ± 2.6	7.0 ± 2.4	<0.0001
Leptin (ng/ml)	33.7 (23.1-48.6)	36.3 (23.6-48.1)	36.4 (26.2-50.5)	0.0852

Continuous data are presented as mean ± standard deviation, with the exception of the skewed variables pre-pregnancy BMI, fasting insulin, CRP and leptin (which are presented as median followed by interquartile range in parentheses). Categorical data are presented n (%). p values refer to overall differences across groups using analysis of variance for continuous variables or $\chi 2$ test for categorical variables.

[a]Previous GDM or delivery of a macrosomic infant

Table 2: Spearman correlations of maternal metabolic variables with leptin

Variable	**Correlation Coefficient**	**p value**
Age	-0.07	0.0423
Weeks gestation	0.04	0.2991
Pre-pregnancy BMI	0.54	<0.0001
Gestational weight gain up to OGTT	0.15	<0.0001
$AUC_{glucose}$	0.10	0.0066
Fasting insulin	0.60	<0.0001
LDL cholesterol	-0.09	0.0088
HDL cholesterol	-0.04	0.2540
Triglycerides	0.04	0.2945
CRP	0.38	<0.0001
Adiponectin	-0.24	<0.0001

Table 3: Multiple linear regression analysis of (dependent variable) leptin (model adjusted R^2 = 51.4%)

Variable	**Beta Coefficient**	**Standard Error**	**T**	**p**
Age	-0.0010	0.0038	-0.26	0.7938
Weeks gestation	-0.0056	0.0059	-0.96	0.3384
Ethnicity:				
Asian	-0.0269	0.0532	-0.50	0.6140
Other	0.1493	0.0436	3.43	0.0006
Family history DM	-0.0012	0.0317	-0.04	0.9706
Parity ≥1	-0.1635	0.0328	-4.98	<0.0001
Previous GDM[a]	0.0472	0.0691	0.68	0.4949
Pre-pregnancy BMI	0.0466	0.0040	11.55	<0.0001
Gestational weight gain up to OGTT	0.0308	0.0033	9.30	<0.0001
$AUC_{glucose}$	-0.0040	0.0042	-0.95	0.3416
Fasting insulin[b]	0.3566	0.0358	9.97	<0.0001
LDL cholesterol	0.0186	0.0143	1.26	0.2073
HDL cholesterol	0.0720	0.0471	1.53	0.1264
Triglycerides	-0.0694	0.0259	-2.68	0.0076
CRP[b]	0.1256	0.01937	6.48	<0.0001
Adiponectin	0.0023	0.0065	0.36	0.7217

Reference group for ethnicity is Caucasian.
[a]Previous GDM or macrosomic infant
[b]log transformed

Table 4: Multiple linear regression analyses of (dependent variable) leptin within each tertile of pre-pregnancy BMI

	1st BMI Tertile (BMI range: 16.6 – 22.0 kg/m^2) Model adjusted R^2 = 41.3%		**2nd BMI Tertile** (BMI range: 22.0 - 25.5 kg/m^2) Model adjusted R^2 = 39.7%		**3rd BMI Tertile** (BMI range: 25.5 – 56.9 kg/m^2) Model adjusted R^2 = 36.0%	
Variable	**Beta Coefficient (SE)**	**p**	**Beta Coefficient (SE)**	**p**	**Beta Coefficient (SE)**	**p**
Age	-0.0048 (0.0076)	0.5267	-0.005 (0.0065)	0.9442	-0.0058 (0.0056)	0.3009
Weeks gestation	-0.0163 (0.0115)	0.1554	-0.0106 (0.0113)	0.3471	0.0074 (0.0080)	0.3543
Ethnicity: Asian	-0.0494 (0.0903)	0.5850	-0.0484 (0.0924)	0.6007	0.0344 (0.0973)	0.7241
Other	0.1260 (0.0864)	0.1460	0.2325 (0.0763)	**0.0026**	0.1316 (0.0640)	**0.0410**
Family history DM	0.0575 (0.0590)	0.3307	0.0114 (0.0531)	0.8302	-0.0440 (0.0509)	0.3882
Parity ≥1	-0.1275 (0.0623)	**0.0419**	-0.1879 (0.0569)	**0.0011**	-0.1194 (0.0505)	**0.0189**
Previous GDM[a]	0.0196 (0.1424)	0.8908	0.0581 (0.1194)	0.6271	0.0818 (0.0977)	0.4031
Pre-pregnancy BMI	0.0863 (0.0264)	**0.0012**	0.0897 (0.0267)	**0.0009**	0.0361 (0.0060)	**<0.0001**
Gestational weight gain up to OGTT	0.0386 (0.0075)	**<0.0001**	0.0321 (0.0059)	**<0.0001**	0.0219 (0.0044)	**<0.0001**
$AUC_{glucose}$	0.0025 (0.0080)	0.7574	-0.0001 (0.0074)	0.9884	-0.0110 (0.0063)	0.0831
Fasting insulin[b]	0.5282 (0.0724)	**<0.0001**	0.3319 (0.0596)	**<0.0001**	0.2115 (0.0559)	**0.0002**
LDL cholesterol	0.0107 (0.0282)	0.7057	0.0105 (0.0247)	0.6715	0.0067 (0.0230)	0.7704
HDL cholesterol	-0.0417 (0.0880)	0.6356	0.0900 (0.0824)	0.2755	0.1325 (0.0710)	0.0631
Triglycerides	-0.1715 (0.0522)	**0.0012**	-0.0387 (0.0410)	0.3469	-0.0552 (0.0419)	0.1897

CRP[b]	0.1082 (0.0343)	**0.0018**	0.1470 (0.033)	**<0.0001**	0.1001 (0.0329)	**0.0027**
Adiponectin	-0.0109 (0.0114)	0.3434	0.0250 (0.0118)	**0.0347**	0.0086 (0.0104)	0.4114

SE, standard error
Bold indicates $p<0.05$
Reference group for ethnicity is Caucasian.
[a]Previous GDM or macrosomic infant
[b]log transformed