The Influence of Overweight and Obesity on Longitudinal Trends in Maternal Serum Leptin Levels During Pregnancy

Vinod K. Misra[1] and Sheri Trudeau[1]

Maternal obesity influences a number of metabolic factors that can affect the course of pregnancy. Among these factors, leptin plays an important role in energy metabolism and fetal development during pregnancy. Our objective was to estimate the influence of maternal overweight/obesity on variation in the maternal serum leptin profile during pregnancy. In a prospective cohort of 143 adult gravidas with singleton pregnancies presenting for general prenatal care, we measured serum leptin levels at 6–10, 10–14, 16–20, 22–26, and 32–36 weeks' gestation. The longitudinal effects of maternal prepregnancy BMI, categorized as nonoverweight (≤26.0 kg/m^2) and overweight/obese (>26.0 kg/m^2), on serum leptin concentration were analyzed using linear mixed models. Overweight/obese women had significantly higher serum leptin concentrations than their nonoverweight counterparts throughout pregnancy ($P < 0.01$). Although these concentrations increased significantly across gestation for both groups, the rate of increase was significantly smaller for overweight/obese women ($P < 0.05$). To investigate whether these differences merely reflected differences in weight-gain patterns between the two groups, we examined an index of leptin concentration per unit body weight (leptin (ng/ml)/weight (kg)). Overweight/obese women had a significantly higher index throughout pregnancy ($P < 0.01$). However, although this index increased significantly across pregnancy for nonoverweight women, it actually decreased significantly for overweight/obese women ($P < 0.01$). Our results suggest that factors other than fat mass alone influence leptin concentrations in overweight/obese women compared to normal-weight women during pregnancy. Such factors may contribute to differences in the intrauterine environment and its influence on pregnancy outcomes in the two groups.

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INTRODUCTION

Maternal obesity influences a number of metabolic and physiological factors that can affect the course of pregnancy (1–3). Many of the effects of obesity may be associated with metabolic dysregulation characterized by adipose tissue dysfunction and disruption of the normal feedback systems that maintain metabolic homeostasis (4–6). Pregnancy is itself a unique metabolic state associated with a complex set of physiological changes that optimize energy reserve for the mother and substrate provision for the fetus (7). There are few descriptions of how the metabolic dysregulation associated with obesity may influence the physiological changes of pregnancy. As one example, it has recently been shown that insulin may have different metabolic effects in obese compared to nonobese pregnant women (8).

Metabolic changes that arise from adipocyte and adipose tissue dysfunction in obesity influence a number of physiological factors (5) that may affect the course of pregnancy. Among these factors, leptin plays a particularly important role in the regulation of maternal energy metabolism during pregnancy (9–11). Serum leptin levels are generally thought to be related to adipose tissue mass and are correlated with body fat mass and BMI in both nonpregnant (12) and pregnant adults (13). However, the regulation of maternal leptin during pregnancy is complex (11). Serum leptin concentrations nearly doubled during the course of a normal pregnancy (13–15), though factors other than fat mass alone, such as placental production, are responsible for this increase (11,16,17). Such changes are thought to be involved in optimizing the availability of substrates necessary for fetal growth, particularly by mobilizing maternal fat stores (11). Metabolic disturbances arising from adipocyte and adipose tissue dysfunction in obese women may alter the regulation of leptin during pregnancy. However, prior studies have not adequately documented how the longitudinal trends in maternal plasma leptin levels during pregnancy may differ in obese women compared to nonobese women.

[1]Division of Medical Genetics, Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan, USA.
Correspondence: Vinod K. Misra (vmisra@umich.edu)

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Our goal was to characterize longitudinal trends in maternal serum leptin levels during pregnancy in overweight and obese women, compared to their nonoverweight counterparts. We specifically examined the relationship of maternal prepregnancy BMI with gestational age- and maternal weight–dependent changes in leptin levels in pregnant women enrolled in a prospective cohort study. These analyses are a preliminary step toward defining how the metabolic dysregulation associated with maternal overweight and obesity may influence human pregnancy.

METHODS AND PROCEDURES

Study sample

Data were collected as part of a prospective cohort study of pregnant women who presented for prenatal care in early first trimester to the University of Michigan Health System. The Institutional Review Board of the University of Michigan Medical School approved the study protocols. At the time of enrollment, participants were 18–45 years of age, between 6 and 10 weeks' gestation with a singleton pregnancy, and intended to deliver at the study hospital. Informed consent was obtained at the initial visit. Participants were seen at four additional study visits at 10–14, 16–20, 22–26, and 32–36 weeks' gestation. Pregnancies complicated by major structural fetal abnormalities or prenatally diagnosed aneuploidy were excluded from the analysis. We analyzed data obtained at each visit from a brief interview, maternal anthropometric measurements, and fetal ultrasound measurements. Less than 1% of data were missing for any variable. Data analyses were carried out on a cohort of 143 women who delivered a live infant and were measured for all relevant variables.

Data collection and study variables

Baseline maternal characteristics were collected by questionnaire upon entry into the study as well as subsequent review of medical records. These data included age and self-report of race and ethnicity, years of education completed, income, marital status, date of last menstrual period, prior and current pregnancy histories, maternal chronic illness, medications, alcohol use, and exposure to tobacco smoke. Standing height was measured at the baseline visit using a wall-mounted stadiometer. Weight was measured in light street clothes, without shoes, on a calibrated electronic scale (Scale-Tronix, White Plains, NY). Maternal prepregnancy weight was collected by self-report at the baseline visit. Prepregnancy BMI was calculated using height and prepregnancy weight (BMI = weight/height^2), and categorized into two levels using the Institute for Medicine's classification (18) as nonoverweight (≤26.0 kg/m^2) and overweight/obese (>26.0 kg/m^2).

Gestational age was estimated from participant's recall of the first day of the last menstrual period prior to 8 weeks' gestation and corroborated by three independent ultrasonographic measurements of crown-rump length between 6 and 10 weeks' gestation. If the discrepancy with dates was >6 days, the ultrasound estimate of gestational age was used. Although early first-trimester ultrasound-based dating is generally thought to be highly accurate (19), it may be systematically biased in overweight and obese gravidas (20,21). As such, our approach was meant to provide a best estimate of the gestational age using ultrasound data to reduce potential errors in participant reporting of the first day of the last menstrual period (22).

At each visit, maternal serum was collected using a standard serum separator tube (BD, Franklin Lakes, NJ), aliquoted, and stored at −80 °C for analysis. Serum leptin concentration was measured using a standard commercial radioimmunoassay kit (Linco Research, St Charles, MO). This kit is a double-antibody radioimmunoassay using an ^{125}I-human leptin tracer, a rabbit antihuman leptin serum as the first antibody, and a goat anti-rabbit gamma globulin–polyethylene glycol complex as the second antibody. A purified recombinant human leptin is used as standard. The limit of sensitivity for the assay is 0.5 ng/ml. The interassay coefficient of variation is 6.4% at 3.5 ng/ml and 6.0% at 23.5 ng/ml. These assays were performed in the Chemistry Laboratory of the Michigan Diabetes Research and Training Center.

Statistical analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Univariate analyses were used to describe the demographic characteristics of the study sample.

The Fisher exact test was used to assess statistical significance of categorical variables; the Student's *t*-test was used for continuous variables. A P value of <0.05 was considered statistically significant.

Our analysis includes repeated leptin measurements over the course of pregnancy. To account for the correlations between repeated measures of leptin over gestation, we used a mixed linear regression model as implemented in the MIXED module in SAS (23). Unlike conventional linear regression models, mixed linear regression models allow the data to exhibit correlation and nonconstant variability by including both fixed effect and covariance parameters. It is possible to model the covariance structure of the repeated measurements to increase efficiency so that the estimates and standard errors can be efficiently generated. In addition, the mixed linear modeling procedure used here implements a likelihood-based estimation method so that all available data are used in the analysis without excluding subjects with missing data. For these analyses, repeated measures of leptin across pregnancy were analyzed using a model in which BMI stratum was a fixed factor that varied between subjects; gestational age at each leptin measurement was a repeated factor that varied within subjects; and an interaction term between BMI stratum and time (gestational age) was included to estimate differences in the rate of change of the measurements over the course of gestation for both BMI groups.

RESULTS

Table 1 presents the sociodemographic and health characteristics of the 143 participants in our sample. Our sample is quite homogeneous with regard to measures of socioeconomic status and race. However, as shown in **Figure 1**, there was considerable variation in prepregnancy BMI. As shown in **Table 1**, there were significant differences in the characteristics of women with nonoverweight BMIs compared to those with overweight/obese BMIs. **Figure 2** presents the maternal weight and weight-gain characteristics of our sample. As expected, we found that the maternal weight measured at each study visit increased steadily from 6 to 36 weeks' gestation. However, the rate at which the maternal weight increased across gestation was significantly lower for women with overweight/obese prepregnancy BMIs compared to women with nonoverweight BMIs ($P < 0.05$).

We then analyzed the relationship between maternal serum leptin and gestational age in nonoverweight and overweight/obese women. **Figure 3** contains plots of leptin levels over the course of pregnancy, stratified on maternal prepregnancy BMI. Starting in early pregnancy, we found that leptin concentration significantly increased with advancing gestation in both strata. Women with overweight/obese BMIs had significantly higher serum leptin concentrations than their nonoverweight counterparts. However, the rate at which leptin levels increased across gestation was significantly lower for women with overweight/obese BMIs ($P < 0.01$).

To investigate whether differences in the leptin profile merely reflected differences in weight and gestational weight

Table 1 A description of participant characteristics in the study sample

	All participants *N* (%)	Nonoverweight BMI ≤26.0 kg/m²* *N* (%)	Overweight/ obese BMI >26.0 kg/m²** *N* (%)
Sample size	143	85 (59.4)	58 (40.6)
*Race**			
White	115 (80.4)	72 (84.7)	43 (74.1)
African-American	11 (7.7)	3 (3.5)	8 (13.8)
Asian	7 (4.9)	6 (7.1)	1 (1.7)
Other	10 (7.0)	4 (4.7)	6 (10.3)
*Ethnicity**			
Non-Hispanic	137 (95.8)	84 (98.8)	53 (91.4)
Hispanic	6 (4.2)	1 (1.2)	5 (8.6)
*Maternal age***			
≤30	79 (55.2)	39 (45.9)	40 (69.0)
>30	64 (44.8)	46 (54.1)	18 (31.0)
Parity			
Nulliparous	54 (37.8)	33 (38.8)	21 (36.2)
Multiparous	89 (62.2)	52 (61.2)	37 (63.8)
Marital status			
Married	118 (82.5)	74 (87.1)	44 (75.9)
Not married	25 (17.5)	11 (12.9)	14 (24.1)
*Highest educational level completed***			
College or less	85 (59.4)	41 (48.2)	44 (75.9)
Post graduate	58 (40.6)	44 (51.8)	14 (24.1)
*Income***			
≤$80,000 per year	72 (50.3)	33 (38.8)	39 (67.3)
>$80,000 per year	70 (49.0)	52 (61.2)	18 (31.0)
Missing	1 (0.7)	0 (—)	1 (1.7)
Insurance			
Private insurance	125 (87.4)	77 (90.6)	48 (82.8)
Medicaid/ Medicare	18 (12.6)	8 (9.4)	10 (17.2)
Smoking			
Not during pregnancy	132 (92.3)	78 (91.8)	54 (93.1)
During pregnancy	11 (7.7)	7 (8.2)	4 (6.9)

*$P < 0.05$; statistical significance of difference between low and high BMI groups.
**$P < 0.01$; statistical significance of difference between low and high BMI groups.

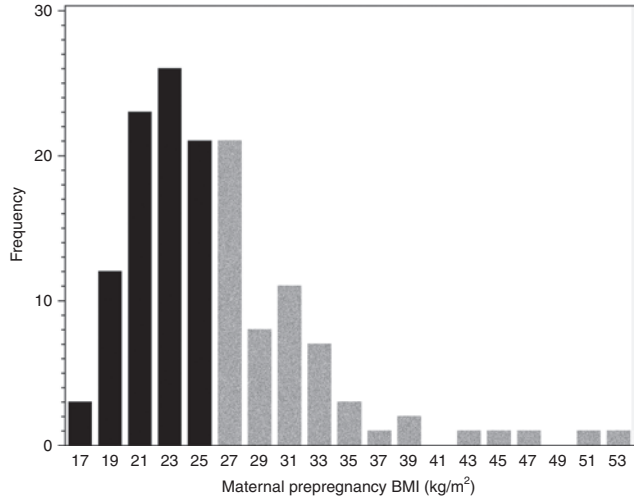

Figure 1 The distribution of maternal prepregnancy BMI for our sample. The BMI values for nonoverweight women (≤26.0 kg/m²) are shown in black and the values for overweight/obese women (>26.0 kg/m²) are shown in gray.

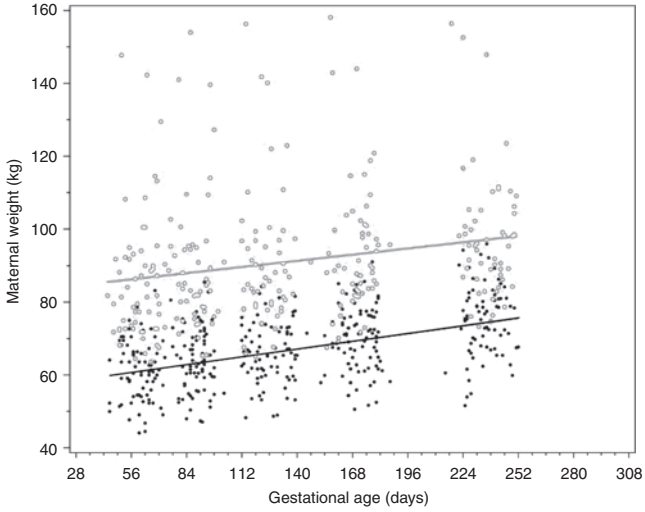

Figure 2 The relationship of maternal weight (kg) to gestational age for women with nonoverweight prepregnancy BMI (β = 0.530 kg/week, 95% confidence interval (CI): 0.545, 0.515) and overweight/obese prepregnancy BMI (β = 0.424, 95% CI: 0.454, 0.394). Data for nonoverweight prepregnancy BMI are shown in black; and overweight/obese prepregnancy BMI are shown in gray.

gain between the two groups, we subsequently examined an index of leptin concentration per unit body weight (leptin (ng/ml)/weight (kg)). Figure 4 contains plots of this index across pregnancy. Women with overweight/obese BMIs had significantly higher serum leptin per unit body weight than their nonoverweight counterparts throughout pregnancy ($P < 0.01$); in early first trimester, the value of this index was 0.24 ng/ml/kg for nonoverweight weight women and 0.42 ng/ml/kg for overweight/obese women. Notably, although the leptin per body weight index increased significantly across pregnancy for nonoverweight women, it actually decreased significantly for overweight/obese women (Figure 4); by third trimester, the index had increased to nearly 0.30 ng/ml/kg in nonoverweight women, but had decreased to about 0.37 ng/ml/kg in overweight/obese women. As a result, although the average circulating leptin concentration per kilogram of body mass

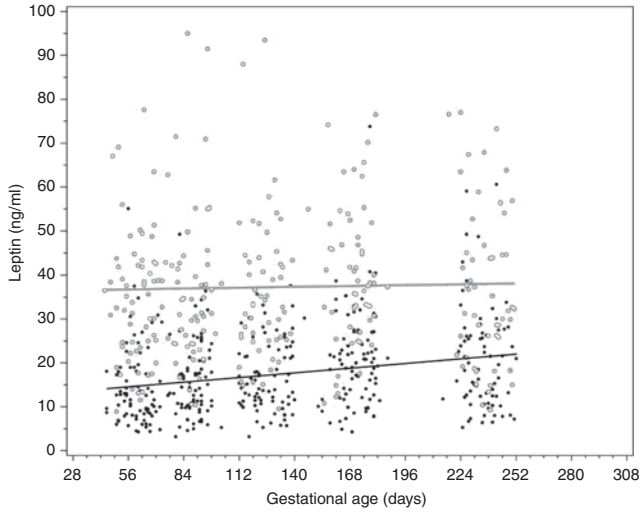

Figure 3 The relationship of maternal serum concentrations of leptin (ng/ml) to gestational age (days) for women with nonoverweight prepregnancy BMI (β = 0.038, 95% confidence interval (CI): 0.044, 0.032) and overweight/obese prepregnancy BMI (β = 0.007, 95% CI: 0.015, −0.001). Data for nonoverweight prepregnancy BMI are shown in black; and overweight/obese prepregnancy BMI are shown in gray.

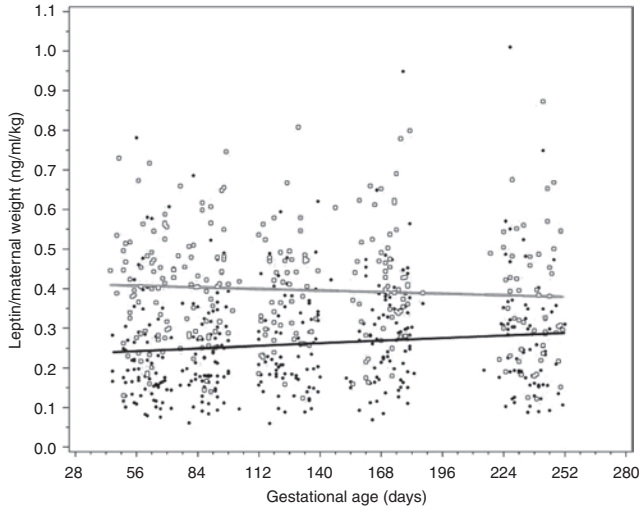

Figure 4 The relationship of maternal serum concentrations of leptin per unit body weight (ng/ml/kg) to gestational age (days) for women with nonoverweight prepregnancy BMI (β = 0.00025, 95% confidence interval (CI): 0.00034, 0.00017) and overweight/obese prepregnancy BMI (β = −0.00020, 95% CI: −0.00009, −0.00027). Data for nonoverweight prepregnancy BMI are shown in black; and overweight/obese prepregnancy BMI are shown in gray.

was 1.8 times higher in obese/overweight women at the beginning of pregnancy, it was only 1.2 times higher at the end of pregnancy ($P < 0.01$).

DISCUSSION

This work is the first to clearly demonstrate that overweight/obese (prepregnancy BMI >26.0 kg/m^2) women have qualitatively different leptin profiles across pregnancy than nonoverweight (prepregnancy BMIs ≤26.0 kg/m^2) women. Serum leptin concentrations are closely correlated with the percentage of body fat in both nonpregnant (12) and pregnant adults (13). Accordingly, we found that maternal serum leptin concentrations were proportional to maternal body mass and weight gain from the start of pregnancy. In addition, consistent with several prior studies on smaller cohorts of women (13–15), we observed that maternal serum leptin concentration significantly increased with advancing gestation in both strata. At every time point, women with overweight/obese prepregnancy BMIs had significantly higher serum leptin concentrations than their nonoverweight counterparts. However, we observed that the rate at which leptin levels increased across gestation was significantly lower for overweight/obese women, in part, due to their lower weight gain. These differences are conventionally attributed to differences in the weight-gain characteristics of the two groups, particularly because studies of body composition during pregnancy suggest that the gain in fat mass per unit body weight is less for overweight/obese compared to nonoverweight women (24).

However, the differences we observe in the index of leptin to body weight (**Figure 4**) suggest that factors other than fat mass alone must contribute due the differences in leptin concentrations between the two groups. In nonpregnant women, leptin release per gram of adipose tissue is approximately two times greater in obese than in lean subjects (25). This is consistent with the differences we observe in the index of leptin to body weight at the beginning of pregnancy (**Figure 3**). However, although the index of leptin to body weight increased significantly for nonoverweight women, it actually decreased significantly in overweight/obese women. In other words, overweight/obese gravidas do not show the progressive increases in leptin production per unit of body mass that are seen in nonoverweight women. As a result, the average circulating leptin concentration per kilogram of body mass was only 1.2 times higher for overweight/obese than nonoverweight women at the end of pregnancy.

The observed differences in the leptin/body weight index suggest that the factors regulating leptin levels during pregnancy differ between overweight/obese and normal-weight women. For example, our findings may reflect a lower production of leptin per unit mass of adipose or placental tissue in overweight/obese women as pregnancy progresses. Such differences may be due to progressively lower adipocyte *ob* gene expression in these tissues. Variation in *ob* gene expression in adipocytes is an important cellular mechanism responsible for regulating circulating leptin levels in nonpregnant obese women (25). In pregnant women, differences in leptin production in nonadipose tissue, such as the placenta (11,17), may also contribute to our findings. Our study was not designed to differentiate between these mechanisms.

Differences in gene expression may be a consequence of more general metabolic diferences between the two groups. For example, both plasma insulin and glucocorticoids are associated with expression of leptin mRNA and the leptin secretion rate (26). However, in obese individuals, prolonged exposure to the combination of insulin and dexamethasone

(but neither hormone alone) simply maintains initial rates of *ob* gene expression and leptin secretion, whereas in lean individuals, leptin expression and secretion actually increase in the adipose tissue (26). Moreover, changes in insulin response may have different physiological implications for obese women than for normal-weight women during pregnancy (8). We speculate that the observed differences in circulating leptin levels may also be influenced by differences in leptin bioavailability or clearance, although little is known about these pathways.

These findings corroborate recent data suggesting that the regulation of metabolic pathways may be substantially different in overweight and obese individuals compared to their normal-weight counterparts (4–6). Although few studies have investigated how such metabolic dysregulation may influence the physiological changes of pregnancy, one recent study demonstrated that obesity status early in pregnancy modified the association between insulin homeostasis and gestational weight gain (8). There have been no comparable studies documenting how other obesity-related factors, such as adipokine profiles, may differ between overweight/obese and normal-weight pregnant women. Our findings are among the first to describe the dynamic course of leptin levels from the earliest stages of pregnancy and emphasize the substantial differences in the trajectories between these groups.

Although our statistical analysis was somewhat limited by our relatively modest sample size, this work represents one of the largest prospective, longitudinal studies of leptin concentrations during pregnancy reported in the literature; similar studies are limited to one or two time points during gestation, or include far fewer women. In addition, we were able to obtain multiple measures of maternal serum leptin concentrations on each participant starting in early first trimester and at multiple time points across pregnancy. Few studies have used appropriate longitudinal statistical methods to analyze such data. Our analytical approach used linear mixed models that accounted for the longitudinal data structure to describe gestational age–dependent changes in leptin levels during pregnancy. As a result, we were able to map a detailed trajectory of leptin levels in a population-based cohort of pregnant women. This study design and analytical plan specifically enabled us to discern effects of maternal overweight/obesity on these trajectories.

Despite our sample size and multiple measures of our covariates, we still lack statistical power for meaningful multivariable models to investigate the interrelationships among covariates. The effects of psychosocial, nutritional, and other metabolic variables on the relationship between maternal prepregnancy BMI and leptin trajectory remain to be determined. However, we hypothesize that the effects of such distal obesity-related factors may act through a set of proximal metabolic and physiological factors, including adipokine pathways, that affect the course of pregnancy.

Our study design also inherently limits study participation to a group of women who present for early prenatal care and are able to attend multiple study visits. Women without prenatal care or with late, interrupted, or sporadic care are less likely to have been included. As a result, maternal sociodemographic covariates show limited variation. Although our sample is homogeneous with regard to measures of socioeconomic status and race, there is considerable variation in prepregnancy BMI, our exposure of interest. By focusing on this population, many potential confounding factors, both measured and unmeasured, are accounted for by our sampling, allowing us to focus primarily on the effects of maternal overweight/obesity.

Many metabolic and physiological systems contribute to the "obese intrauterine environment." The differences we observe in serum leptin profiles between overweight and nonoverweight women may represent one factor that contributes to this environment. We speculate that fetal exposure to such differences in the obese intrauterine environment may "program" a set of physiological responses that predisposes offspring to metabolic and cardiovascular disease later in life (1–3). Our study is a first step toward understanding the effects of maternal obesity on changes in maternal metabolic and physiological factors that influence the intrauterine environment and pregnancy outcomes. Such an understanding may have implications for the design of interventions to reduce obesity-related pregnancy complications and adverse pregnancy outcomes. If the consequences of maternal obesity result from lasting dysregulation of metabolic homeostasis, then efforts to change health behaviors and nutrition without correcting the underlying metabolic abnormalities may not be effective. As such, future studies are needed to evaluate how the pathogenic effects of obesity may be associated with metabolic dysregulation and disruption of the normal feedback systems that maintain metabolic homeostasis during pregnancy.

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DISCLOSURE

The authors declared no conflict of interest.

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