The role of maternal gut hormones in normal pregnancy: fasting plasma active glucagon-like peptide 1 level is a negative predictor of fetal abdomen circumference and maternal weight change

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Abstract

Objective: Maternal weight in pregnancy contributes to a glycemic environment that affects fetal growth. Gut peptides (glucagon-like peptide 1 (GLP1), glucose-dependent insulinotropic peptide (GIP), ghrelin, and peptide YY (PYY)) have been related to insulin sensitivity and secretion, weight control, and adipose tissue metabolism. This study aimed at examining the associations of gut hormones during pregnancy with maternal glucose homeostasis, maternal weight, and fetal growth.

Methods: A total of 55 pregnant nonobese, nondiabetic Caucasian women were examined during the three trimesters of pregnancy, and anthropometric measurements, evaluation of fasting maternal plasma GLP1, GHREL (active), total PYY, total GIP, and a 75-g oral glucose tolerance test were done in them. Homeostasis model assessment (HOMA-R), insulin sensitivity index (ISI), and indices of insulin secretion were calculated. Fetal growth was estimated by ultrasound.

Results: Fasting GLP1 increased significantly from the second to the third trimester (P<0.05). Fasting GLP1 correlated positively with high-density lipoprotein cholesterol (r=0.52, P=0.04). At the second trimester, fasting GLP1 levels correlated negatively with fetal abdomen circumference (r=-0.55, P=0.034), birth weight (r=-0.50, P=0.040), HOMA-R (r=-0.65, P=0.001), insulin secretion, and triglycerides. At the first trimester, fasting ghrelin levels correlated negatively with HOMA-R and insulin secretion, and positively with ISI. In backward multiple regression analysis, the first trimester GLP1 levels were the best negative predictors of the second trimester fetal abdomen circumference (β=-0.96, P=0.009). In longitudinal regression model, maternal fat and HOMA-R were the positive predictors of maternal weight change during pregnancy, and fasting GLP1 levels were the negative predictors of maternal weight change during pregnancy.

Conclusions: During pregnancy, maternal GLP1 might be involved in mechanisms that compensate for the pregnancy-related increase in glycemia and insulin resistance, suggesting a role of this peptide in maternal metabolism and weight and fetal growth.

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Introduction

During pregnancy, there is an increase in insulin resistance from the second to the third trimester (1). The prevalence of prepregnancy maternal obesity also increases. The negative role of excess maternal weight gain in maternal carbohydrate metabolism during pregnancy as well as in fetal growth is well established (2–4). Reports from Scandinavia (5) and North America (6) show an increase in large for gestational age births.

Endometrial environment via its role in fetal growth is important for programming of future metabolic diseases during adult life (3, 7).

The role of a new class of gut-derived peptides such as glucagon-like peptide 1 (GLP1), glucose-dependent insulinotropic peptide (GIP), ghrelin, and peptide YY (PYY) in insulin sensitivity and secretion, weight control, and adipose tissue metabolism has been recently stressed. GLP1, an incretin, stimulates insulin and suppresses glucagon secretion, inhibits gastric
emptying, and reduces appetite and food intake. In insulin-resistant states (i.e. type 2 diabetes mellitus (T2DM) and obesity), GLP1 secretion is decreased (8, 9). The administration of GLP1 receptor agonists in T2DM improves glucose homeostasis, while it is followed by a dose-dependent weight loss compared with placebo (10–12). Furthermore, GIP; another incretin, has been associated with a decrease in resting energy expenditure in healthy humans (13).

Ghrelin, discovered as a natural ligand of the GH secretagogue receptor type 1a, is a gut–brain peptide with somatotropic, food intake-increasing and adipogenic effects. In subjects with T2DM as well as in those with abdominal obesity and/or metabolic syndrome, ghrelin levels are low (14, 15). The gut hormone PYY reduces feeding in obese humans and shows Y2-receptor-mediated anorectic effects, modulating neuronal activity within hypothalamic, brainstem, and brain regions involved in reward processing. Fasting circulating PYY concentrations correlate negatively with body mass index (BMI) and waist circumference in humans (16, 17). In morbidly obese individuals, GLP1 and PYY levels increase, while ghrelin levels decrease after Roux-en-Y gastric bypass surgery more than after laparoscopic adjustable gastric banding surgery. These changes have been associated with the importance of weight loss and the improvement in insulin sensitivity (18, 19).

The aim of this study was to examine the possible physiological associations of gut hormone levels in the same cohort of nonobese, nondiabetic pregnant women during the three trimesters of pregnancy with maternal glucose homeostasis and body weight as well as with fetal growth. The exclusion of obese or diabetic pregnant women from this study aimed at eliminating obvious confounding factors from the study of the direct interplay among fasting gut hormones, maternal insulin resistance, and fetal growth. Further studies targeting these specific populations should be undertaken.

Materials and methods

Patients

Sixty healthy pregnant Caucasian women aged 29.5 ± 4.5 (mean ± s.d.) years with a BMI of 24.7 ± 2.2 kg/m² before pregnancy, with no history of T2DM or other endocrine diseases, were recruited from the Obstetrics and Gynecology outpatient clinic of a university hospital during the first trimester of pregnancy after exclusion of women with BMI (before pregnancy) over 30 kg/m². The study was approved by the local ethics committee. Written informed consent was obtained from all subjects.

Protocol

The recruited pregnant women were seen in the outpatient clinic once during each of the three trimesters of their pregnancy at weeks 10–12, 24–26, and 34–36 respectively. Five of the above women developed gestational diabetes during pregnancy, and were subsequently excluded. At each visit, they were submitted to anthropometric measurements, a blood sampling for the measurement of gut hormones (active GLP1, total GIP, active ghrelin, and total PYY) and lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), and triglycerides) at fasting state, and an oral glucose tolerance test (OGTT). An ultrasound of the fetus was performed during the second (weeks 24–26) and third trimesters (weeks 34–36).

Anthropometric measurements All measurements were carried out by a single observer. Weight of all the subjects without shoes and light clothing was measured in kilograms to the nearest 0.1 kg on a beam balance. Height in meters was measured to the nearest millimeter using a stadiometer, and the BMI in kilograms per square meter was calculated. Maximum hip circumference in centimeters was measured in duplicate with a 6-mm-wide flexible tape at the widest part of the trochanters at a horizontal position with feet kept 20–30 cm apart. Skinfold thicknesses were measured on the left side of the body with a Harpenden skinfold caliper (Assist Creative Resources Ltd, Wrexham, UK) in triplicate to the nearest 0.2 mm. Biceps and triceps thicknesses were measured at the midpoint of the upper arm, between the acromion process and the tip of the bent elbow. Subscapular skinfold thickness was measured at the natural fold about 2–3 mm below the shoulder blade at an oblique angle. Suprailiac skinfold was pinned at 2–3 cm above the iliac crest on the lateral side and mid-axillary line. The sum of all the skinfold measurements made at four locations was estimated, and percent body fat was determined using charts interpolating for age based on the Durnin and Womersly data (20). Supine blood pressure was recorded with a mercury sphygmomanometer using the mean of three measurements.

Blood sampling for gut hormone collection For the measurement of gut hormones, blood samples were collected in tubes with EDTA as an anticogulant. After blood collection, DPPIV inhibitor for GLP1 measurement and serine protease inhibitor for active ghrelin measurement were added. Tubes were inverted several times to mix the contents and then they were centrifuged, and the plasma that was collected after centrifugation was aliquoted and stored at −70 °C until assay.
**Fasting active GLP1 in normal pregnancy**

**Oral glucose tolerance test** After an overnight fast, a 75-g OGTT was performed. Glucose and insulin levels were determined in blood samples drawn at 0, 5, 15, 30, 60, 90, and 120 min time points. Blood samples were stored immediately at $-70^\circ$C.

**Fetal ultrasound measurements** At each ultrasound visit, fetal measurements (estimated weight, abdomen circumference, femur length, head circumference, and biparietal diameter) were carried out by a single observer. Ultrasound was performed by employing a Philips HD11 ultrasonographer.

**Blood chemistry and hormone assays**

Blood chemistry included measurements of glucose, serum total cholesterol, HDL-C, LDL-C, and triglycerides, which were done using the Siemens Advia 1800 Clinical Chemistry system, while insulin was determined using the Medgenic immunoenzymetric assay (Biosource, Nivelles, Belgium).

Active GLP1, total GIP, active ghrelin, and total PYY were measured in plasma simultaneously using a multiplex assay kit manufactured by Millipore and the Human Gut Hormone LINCOplex kit purchased from Linco Research Inc., St Charles, MO, USA. Intraassay and interassay coefficient of variation values were $<11$ and $<19\%$ respectively according to the manufacturer.

**Mathematical transformations**

**Indices of carbohydrate metabolism** Carbohydrate metabolism index derived from fasting values. Insulin resistance was estimated by the homeostasis model assessment (HOMA-R; insulin at baseline (pmol/l) $\times$ glucose at baseline (mmol/l))/135 (21).

Carbohydrate metabolism indices derived from OGTT values. Insulin sensitivity was estimated using the insulin sensitivity index (ISI) $=0.226-(0.0032$ $\times$ BMI $)-(0.0000645$ $\times$ insulin at 120 min (pmol/l)) $-(0.00375$ $\times$ glucose at 90 min (mmol/l)) (22). $\beta$-cell secretion of insulin was estimated using the following indices (22): predicted index of the first phase of insulin secretion (first PHIS) $=1.283+(1.289$ $\times$ insulin at 30 min (pmol/l)) $-(138.7$ $\times$ glucose at 30 min (mmol/l)) $+(3.772$ $\times$ insulin at baseline (pmol/l)).

**Statistical analysis**

Data are described as mean $\pm$ S.D. (median and interquartile range for data that are not normally distributed). To test the change in each variable during pregnancy, the one-way repeated-measures ANOVA was used in the case of normally distributed variables, and the nonparametric Friedman ANOVA was used in the case of nonnormally distributed variables. To test whether changes between different time points were different, we performed a paired $t$-test. To test the associations between different variables and to test the correlations between differences, the Spearman correlation analysis was performed. Backward regression analysis was performed to define the early predictive variables of the second trimester. To find predictors for maternal weight during the whole study time, we used a longitudinal regression model. A $P$ value $<0.05$ was considered to be significant. The SPSS statistical software was used for statistical analysis (SPSS Inc., Chicago, IL, USA).

**Results**

**Changes in anthropometric variables, ultrasound measurements, hormones, and carbohydrate metabolism parameters during pregnancy**

Maternal weight and percent of total body fat increased significantly in an incremental way from the first to the second and to the third trimester of pregnancy ($P<0.05$). Ultrasound fetal measurements of estimated weight, abdomen circumference, head circumference, biparietal diameter, and femur increased significantly from the second to the third trimester of pregnancy ($P<0.05$; Table 1).

Plasma GLP1 increased significantly from the second to the third trimester ($P<0.05$). There was no statistically significant change in plasma total GIP, active ghrelin, and total PYY during pregnancy ($P>0.05$).

Fasting insulin levels and HOMA-R index were significantly higher in the third trimester compared with the first and the second trimester ($P<0.05$), while the ISI decreased significantly in the third trimester compared with the first and the second trimester ($P<0.05$). At the second and the third trimester, first PHIS and second PHIS were significantly higher than those at the first trimester ($P<0.05$).

**Correlations among anthropometric variables, ultrasound measurements, hormones, and carbohydrate and lipid metabolism parameters during pregnancy**

**Anthropometric variables, ultrasound measurements, and maternal hormones** At the second trimester, fasting plasma active GLP1 correlated negatively with U/S-measured fetal abdomen circumference ($P=0.034$, $r=-0.55$; Fig. 1) and birth weight.
At the third trimester, fasting plasma active ghrelin levels correlated positively with fasting plasma total GIP levels ($r = 0.58$, $P < 0.001$). Correlations among hormones, carbohydrate metabolism parameters, and lipids.

**Correlations among hormones** At the first trimester, fasting plasma active ghrelin levels correlated positively with HOMA-R ($P = 0.027$, $r = 0.50$), first PHIS ($P = 0.03$, $r = 0.49$), and second PHIS ($P = 0.04$, $r = 0.41$), and positively with ISI ($P = 0.009$, $r = 0.77$). Fasting plasma GLP1 correlated positively with HDL-C ($P = 0.04$, $r = 0.52$). At the second trimester, fasting plasma active GLP1 levels correlated negatively with first PHIS ($P = 0.03$, $r = -0.54$), second PHIS ($P = 0.04$, $r = -0.50$), HOMA-R ($P = 0.001$, $r = -0.65$), and triglycerides ($P = 0.02$, $r = -0.55$). There was no other correlation between hormones, carbohydrate metabolism parameters, and lipids.

**Correlations between changes (delta, Δ) in the measured variables among the three trimesters of pregnancy**

Between the first and the second trimester, Δ maternal weight correlated positively with Δ first PHIS ($P = 0.02$, $r = 0.63$), Δ second PHIS ($P = 0.04$, $r = 0.59$), and Δ percent maternal fat ($P = 0.008$, $r = 0.51$). Between the second and the third trimester, fasting active Δ GLP1 correlated negatively and positively with Δ maternal weight ($P = 0.001$, $r = -0.97$) and fasting active Δ ghrelin ($P = 0.02$, $r = 0.69$) respectively. Delta maternal weight correlated positively with Δ maternal fat change ($P = 0.04$, $r = 0.45$).

**First trimester predictor of second trimester fetal abdomen circumference**

Backward multiple regression analysis revealed that among maternal fat, plasma levels of active ghrelin, and total GIP and total PYY levels of the first trimester, fasting plasma active GLP1 levels were the best negative predictors ($P = 0.009$, $\beta = -0.96$) of U/S-measured fetal abdomen circumference of the second trimester.
Predictors of maternal weight change during all trimesters of the pregnancy

The longitudinal regression model revealed that the best predictors of maternal weight change during the three trimesters of pregnancy were maternal fat ($P = 0.005$, $t$ value $= 3.12$) and HOMA-R ($P = 0.013$, $t$ value $= 2.79$) as positive predictors and fasting plasma active GLP1 levels ($P = 0.015$, $t$ value $= -2.71$) as negative predictors among the first PHIS, second PHIS, plasma total GIP, active ghrelin, and total PYY.

Discussion

We investigated the fasting circulating levels of maternal gut hormones (active GLP1, active ghrelin, total PYY, and total GIP) during each of the three trimesters of normal pregnancy in the same group of nonobese, nondiabetic women and their correlations with maternal weight, adipose tissue (total percent fat), and lipid and carbohydrate metabolism. The latter was evaluated from OGTT-based mathematically derived indices (23). GLP1 affects mostly postprandial glycemia, and it presents in two forms, the total reflecting stimulation of glucose-dependent insulin secretion (24–26) such as glucagon secretion, suppression of hepatic glucose output, and decrease in gastric emptying rate (8, 26). We found that during the second trimester, fasting plasma active GLP1 levels correlated negatively with HOMA-R. In the past, Cypryk et al. have shown a positive correlation of fasting total GLP1 with HOMA-R in a mixed population of 13 controls and 13 pregnancies with gestational diabetes mellitus (GDM) at the second trimester (27). This discrepancy might be due to the facts that in our study active GLP1 and not total GLP1 was measured, and active GLP1 was measured in a non-GDM population of women with normal pregnancies. In insulin-resistant states such as T2DM, GLP1 levels are significantly decreased, whereas in obesity, there is attenuated GLP1 secretion (8, 9). In addition, in this study, during the second trimester, fasting plasma active GLP1 levels correlated negatively with insulin secretion parameters. It is well accepted that most cases of increased insulin resistance are followed by increased insulin secretion (28, 29). Furthermore, we found that fasting plasma active GLP1 increased significantly from the second to the third trimester. This increase in fasting GLP1 might be part of the mechanisms involved in the compensation of the pregnancy-related increase in glycemia and the development of insulin resistance.

Delta fasting plasma active GLP1 levels from the second to the third trimester correlated negatively with Δ maternal weight. Treatment of nonpregnant diabetic or obese subjects with GLP1 receptor agonists resulted in weight loss (11, 30). An anorexigenic effect has been attributed to GLP1 exerted possibly via direct and/or indirect activation of CNS (31). In addition, in this study, fasting plasma active GLP1 levels were the negative predictors of maternal weight change in the longitudinal regression model applied from the first to the third trimester, whereas maternal total percent fat and HOMA-R were the positive predictors. Maternal total percent fat measured using the sum of skinfold caliper measurements is an approximation of the maternal fat content, and does not reflect central fat deposition. It is the only ethically available method that can be used for maternal fat content estimation during pregnancy. It is possible that GLP1 might play an important role in keeping maternal weight within normal parameters during pregnancy, thus limiting in an indirect way the development of insulin resistance.

Moreover, maternal fasting active GLP1 levels at the first trimester were the best negative predictors of fetal abdomen circumference at the second trimester. Early in pregnancy, the most important contributor for fetal growth is the genetic potential. Maternal metabolic response to alterations of the equilibrium is also based on the maternal genetic potential which, in part, is inherited by the fetus. For example, in the past, we have shown that the mothers of babies with intrauterine growth restriction share a common metabolic feature with their offspring, i.e. increased circulating leptin levels, compared with mothers of babies of normal growth (32).

At the second trimester, maternal fasting active GLP1 levels correlated negatively with fetal abdomen circumference as well as with birth weight. These anthropometric indices are positively associated with the development of hyperglycemia during pregnancy (7). During pregnancy, maternal glucose, amino acids, lipids, and vitamins cross the placenta, and are employed to sustain fetal growth. Glucose is quantitatively the most important nutrient. The fetus does not synthesize
glucose, and employs maternal glucose as its primary energy source for maintenance of its basal metabolism energy requirements for growth and storage. A maternal–fetal glucose gradient facilitates placental transfer of glucose, and its rate is related to maternal glucose levels (33). The concentration of most amino acids in fetus is higher than that found in the mother. Thus, placental transfer of amino acids is carried out by an active process using selective transporters and energy (34). Lipids cross the placenta with difficulty, and are taken up by placental receptors or as fatty acids. Long-chain polyunsaturated fatty acids are needed for fetal growth and development (35). Micronutrient (iron, iodine, calcium, zinc, etc.), vitamin (i.e. vitamins A, C, and E), and folate deficiencies are known to contribute to abnormal prenatal development (36–39).

Furthermore, in this paper, maternal fasting plasma active GLP1 levels correlated positively and negatively with HDL-C during the first trimester of pregnancy and with triglycerides during the second trimester of pregnancy respectively. This is in accordance with its negative correlation with maternal weight. Studies in ob/ob mice showed that GLP1 or the combination of a DPP-4 inhibitor and pioglitazone improves lipid profile (40, 41). The origin and regulation of the maternal fasting GLP1 secretion during pregnancy remain to be unraveled. Maternal fasting total GIP, fasting active ghrelin, and total PYY levels did not change throughout pregnancy, and they did not correlate with maternal weight and total percent fat in this nonobese, nondiabetic pregnant population. In the past, basal acylated ghrelin and PYY 3–36 were found to be increased and decreased respectively only in morbidly obese pregnant women compared with overweight or lean pregnant women (42). Others have reported that acylated ghrelin decreases markedly during pregnancy in mothers with and without gestational diabetes (43). In addition, ghrelin levels have been reported to be low in obesity, hyperinsulinemic, and insulin-resistant states (44, 45). Interestingly, in this study, Δ fasting active ghrelin correlated positively with Δ fasting active GLP1 from the second to the third trimester, and fasting active ghrelin levels correlated positively with fasting active GLP1 levels at the third trimester, suggesting a possible parallelism of their secretion during normal pregnancy. During the first trimester, active ghrelin correlated negatively with HOMA-R as well as with insulin secretion parameters, and positively with ISI. These correlations disappeared during the second and third trimesters of pregnancy, indicating that the maternal active ghrelin involvement in maternal carbohydrate metabolism at this point might be less significant.

This study shows the strong negative association of maternal fasting active GLP1 levels, among the rest of the gut peptides studied, with maternal weight change throughout pregnancy and the significance of the first trimester levels as an early negative predictor of fetal abdominal circumference at the second trimester, a period where fetal growth and the development of maternal insulin resistance accelerate. This is further supported by the negative correlation of maternal fasting active GLP1 levels with insulin resistance parameters during the second trimester, when active GLP1 levels increase gradually till the third trimester. Thus, fasting active GLP1 during pregnancy might be involved in physiological mechanisms of compensation of the pregnancy-related hyperglycemia and insulin resistance, suggesting a role of this peptide in maternal metabolism, maternal weight gain, and fetal abdomen circumference. Further physiological and interventional studies are needed involving obese and diabetic pregnancies to establish the role of active GLP1 in maternal weight in pregnancy and its effects on fetal growth.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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