Acylated ghrelin concentrations are markedly decreased during pregnancy in mothers with and without gestational diabetes: relationship with cholinesterase activity

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Submitted 23 October 2008; accepted in final form 16 February 2009

Acylated ghrelin concentrations are markedly decreased during pregnancy in mothers with and without gestational diabetes: relationship with cholinesterase activity (705). During pregnancy compared with the postpartum period. In conclusion, acylated ghrelin and growth hormone were positively correlated to energy balance, but is lower in women with gestational diabetes (n = 14/group) during pregnancy and after delivery. We examined whether changes in ghrelin during a test meal were correlated with changes in pituitary growth hormone [assessed through calculation of the area under the curve (AUC) during the test meal]. In postpartum subjects, the percent of total ghrelin that is acylated was four to five times higher than previously observed using single antibody assays. During pregnancy, acylated ghrelin concentrations (mean ± SE) were lower compared with the postpartum period throughout the meal (AUC 1.2 ± 0.2 vs. 10.2 ± 1.9 ng·ml⁻¹·90 min⁻¹, P < 0.001). In the postpartum, acylated ghrelin and growth hormone were positively correlated (r = 0.50, P = 0.007). Desacyl (but not acylated) ghrelin was increased in subjects with gestational diabetes during and after pregnancy (AUC 15.4 ± 1.9 vs. 8.6 ± 1.2 ng·ml⁻¹·90 min⁻¹, P = 0.005). In a second group of subjects (n = 13), acylated ghrelin was similarly suppressed during pregnancy. Circulating octanoate concentrations (3.1 ± 0.5 vs. 4.5 ± 0.6 μg/ml, P = 0.029) and cholinesterase activity (705 ± 33 vs. 1,013 ± 56 U/ml, P < 0.001) were lower during pregnancy compared with the postpartum period. In conclusion, acylated ghrelin markedly decreases during pregnancy, likely because of a decrease in the acylation process. Desacyl ghrelin increases in gestational diabetes, possibly reflecting resistance to the inhibitory effect of insulin on ghrelin secretion.

desacyl ghrelin; cholinesterase; octanoate; pituitary growth hormone; insulin

GHRELIN IS A PEPTIDE HORMONE mainly secreted by the stomach. It results from the processing of preproghrelin and circulates as acylated (AG) and desacyl (DG) ghrelin. The presence of an acyl chain [octanoate residue added by the enzyme ghrelin O-acyltransferase on the Ser³ of ghrelin (15, 42)] is required for binding to the growth hormone (GH) secretagogue receptor that mediates many of the actions ascribed to ghrelin (reviewed in Ref. 39). In vitro studies suggest that, in humans, circulating ghrelin is deacylated rapidly, at least in part by the enzyme cholinesterase (also known as pseudocholinesterase or butyrylcholinesterase, EC 3.1.1.8) (9).

Both AG and DG could potentially play a physiological role in energy balance. Exogenous administration of AG, but not DG, stimulates GH secretion (35) and has orexigenic effects (41) in humans. AG injection also causes hyperglycemia and a decrease in circulating insulin (2). In addition, in rodents, administration of both AG and DG promotes adipogenesis through GH secretagogue receptor-independent pathways that remain to be characterized (38). The physiological role of endogenous ghrelin remains however, a matter of debate. Ghrelin concentrations increase with short-term fasting and decrease after meal ingestion (7, 8), suggesting that it may play a role in meal initiation (5).

Pregnancy is associated with physiological changes that include an increase in food intake, a major accumulation in fat mass (20), and a progressive increase in insulin resistance (44). Whether ghrelin plays a physiological role in energy balance during pregnancy is unclear. Inconclusive data have been reported on the pregnancy-induced changes in ghrelin. During the second trimester, AG (31) and total ghrelin (14) concentrations were found to be increased compared with nonpregnant subjects, contrasting with Riedl’s findings of lower total ghrelin concentrations during the same period (33). During the third trimester of pregnancy, AG (31) and total ghrelin (14, 26) concentrations were found to be lower compared with the postpartum period. Gestational diabetes (GD) seemed to have little effect on ghrelin during pregnancy (33). However, in three out of four studies, ghrelin concentrations were measured in assays that did not distinguish between AG and DG, making it impossible to distinguish between the specific effects of pregnancy on AG and DG. Furthermore, all studies used single-antibody ghrelin assays that recognize the COOH-terminal (total ghrelin) or the acylated NH₂-terminal part of the peptide (AG). These assays therefore measure full-length ghrelin as well as circulating fragments of ghrelin with unknown biological activity.

In our study, we used a novel, in-house, two-site sandwich enzyme-linked immunosorbent assay (ELISA) assay that measures full-length AG and DG (24). We hypothesized that, if ghrelin plays a role in the positive energy balance observed...
during pregnancy. AG concentrations should increase with advancing gestational age. They should be lower in pregnant women with GD, reflecting the greater insulin resistance and hyperinsulinism expected in these subjects. We also predicted that we would observe a positive correlation between AG and GH, reflecting stimulation of GH by endogenous AG. The objectives of our study were to compare the changes in ghrelin and in GH concentrations during pregnancy and after delivery in control subjects and in subjects with GD. To further investigate ghrelin metabolism, we assessed in a second group of subjects the relationship between AG concentrations and plasma cholinesterase activity or octanoate concentrations in normal pregnant women before and after delivery.

**MATERIALS AND METHODS**

**Subjects.** The first study group consisted of a prospective investigation of 28 pregnant women with normal glucose homeostasis (control) or with GD (n = 14/group). Control subjects were evaluated during the second trimester (22–25 wk gestational age), the third trimester (32–38 wk), and 8–13 wk postpartum. Because GD is not commonly detected before 28 wk of gestation, GD subjects were only evaluated during the third trimester and during postpartum. A mixed-meal challenge was performed at each of the visits, and cord blood was collected at delivery. All subjects were recruited from the GD clinic and from the family practice unit at British Columbia’s Children’s Hospital. Inclusion criteria were as follows: age > 18 yr; stable weight in the 3 mo preceding pregnancy; no previous gastrointestinal surgery; singleton pregnancy; no known glucose intolerance or type 2 diabetes before pregnancy; and absence of hormonal treatment before or during pregnancy (including exogenous insulin). All women with GD had either a diagnostic 50-g oral glucose tolerance test (1 h blood glucose > 10.3 mmol/l) (37) or a diagnostic 100-g oral glucose tolerance test [preceded or not by an abnormal 50-gm test with a blood glucose between 7.8 and 8.3 mmol/l (1)]. All were managed with diet alone. To confirm our results from the first study group and, in addition, to evaluate the relationship between ghrelin and cholinesterase activity or octanoate concentrations, we prospectively investigated a second group of 13 pregnant women with normal glucose homeostasis during the third trimester of pregnancy and after delivery. The study protocols were approved by the University of British Columbia Clinical Ethics Review Board. Written informed consent was received from all subjects.

**Study protocol and procedures.** For the first group, subjects were admitted to the testing room after a 10-h overnight fast. Weight and height were recorded, and an intravenous catheter was inserted in the arm. Following collection of fasting blood samples [between 0800 and 0900 (19)], subjects were asked to consume a mixed meal containing 25% of the estimated daily caloric intake. The meal consisted of whole wheat bread, scrambled eggs, cheese, and a sweetened drink; contained 20% protein-25% fat-55% carbohydrates (25% solid food, 30% glucose syrup); and was consumed over 15 min. The caloric content of the meal was calculated based on age, gestational age, height, weight, and lactation (12). A homemade, mixed meal rather than a commercial liquid meal was chosen to avoid the presence of fructose, a common component of commercial liquid meals known to decrease the postprandial suppression of ghrelin (36). Blood samples were drawn at baseline (time 0) and 15, 30, 60, and 90 min after completion of the meal for determination of blood glucose and of concentrations of plasma adiponectin and leptin (baseline only), insulin, AG, DG, and GH (all time points). For the second group of subjects, blood samples were collected after a 10-h overnight fast for the determination of cholinesterase activity and of AG, DG, and octanoate concentrations.

**Sample collection and additives.** Blood glucose was measured with each blood sample using the One-Touch Ultra glucose meter (Life Scan Canada, Burnaby, BC, Canada). Serum (for cholinesterase) or plasma (1.25 mg EDTA-2 Na/ml whole blood, all other determinations) was collected. All samples were centrifuged at 1,500 g for 15 min at 4°C. Full-length AG and DG were measured using an in-house two-site sandwich ELISA assay in unextracted EDTA, acidified plasma in 384-well plates. Detailed characterization of this assay and its correlation with physiological endpoints have been published previously (13, 24, 28). These two site assays allow improved specificity and do not respond to degraded peptide fragments. Because of AG instability, special precautions must be taken during sample collection to protect against plasma esteras (24). To this effect, for the first group of subjects, each EDTA tube contained 500 units aprotinin (10,000 kallikrein inhibitor units/ml Trasytol; Bayer, Toronto, Canada), and 100 µl of 1 M HCl were added per milliliter plasma before storage at −80°C. For the second group of subjects, each EDTA tube contained 4–(2-aminoethoxy)benzenesulfonyl fluoride hydrochloride (final concentration 4 mM; Sigma-Aldrich, Oakville, ON, Canada), and 200 µl of 1 M HCl were added per milliliter plasma. The AG assay sensitivity was 6.7 pg/ml with an intra-assay coefficient of variation (CV) of 9.1%, CV 30 pg/ml 12.6% at 100 pg/ml, and 16.8% at 300 pg/ml. The interassay CV was 17.8% at 50 pg/ml. The DG assay sensitivity was 4.6 pg/ml with an intra-assay CV of 12.5% at 50 pg/ml, 10.7% at 150 pg/ml, and 18.0% at 500 pg/ml. The interassay CV was 20.8% at 30 pg/ml. There was no significant cross-reactivity with ghrelin fragments or nonspecific peptides, but the DG assay did show a cross-reactivity of < 3% with AG. Plasma pituitary GH concentrations were measured in duplicate by fluoroimmunometric assay that does not recognize placental GH (Immulite 2000 analyzer; DPC, Flanders, NJ). The assay sensitivity was 0.01 µg/l, with an intra-assay CV < 3.4% and an interassay CV < 3.8%. Data collection and quality control validation were performed by the General Clinical Research Center Core Laboratory at the University of Virginia. Plasma insulin was determined in singlicate by ultrasensitive chemiluminescent immunosay (combined intra- and interassay CV < 5.6%; Beckman Coulter, Fullerton, CA). The homeostasis model assessment index [fasting plasma glucose (mmol/l) × fasting insulin (µU/ml)/22.5] was used to evaluate insulin sensitivity (27). Plasma adiponectin (no. HADP-61HK, intra-assay CV < 6.2%) and leptin (no. HL-81K, intra-assay CV < 8.3%) were measured by RIA (Linco, St. Charles, MO). Serum cholinesterase was determined according to Kalow and Lindsay (17) using benzoyl choline as a substrate. Octanoate (8:0) was quantified as its butyl ester by capillary gas-liquid chromatography, using 9:0, 13:0, and 17:0 as internal standards added to the plasma samples before analysis. The methodology is based on procedures for quantitative analysis of medium-chain fatty acids in human milk, designed to avoid the inevitable losses of short- and medium-chain fatty acids that occur in aqueous-organic solvent phase extractions (16, 23), but using butanol rather than methanol and a sample volume of 25 µl plasma with a SpeedVac (Savant SpeedVac Concentrator; Thermo Electron, Milford, MA) to evaporate the butanol from the butylated samples. Efficiency of recovery and standard curves for analyses were conducted with authentic 8:0 added to plasma and aqueous standard mixtures. Gas-liquid chromatography analysis of derivatized samples was prompt; butyl esters cannot be frozen until analyzed. Because authentic butyl ester standards are not readily available commercially, fatty acid retention times in gas-liquid chromatography were obtained by butylating common saturated and unsaturated vegetable oils, and authentic individual fatty acid standards, including 8:0, 10:0, and 12:0.

**Statistical analysis.** Data were expressed as means ± SE. To address objective 1 (comparison between control and GD subjects), the number of subjects in each group (n = 14) was calculated to detect a 20% difference in fasting ghrelin concentrations (α = 0.05, β = 0.80) between the control and GD groups. For the control group, we examined main effects (period [2nd or 3rd trimester and postpartum] and time [fasting (premeal) and 15, 30, 60, and 90 min postmeal])
using repeated-measures ANOVA. To compare the results between control and GD subjects, we examined main effects [period (3rd trimester and postpartum) and time] adjusted for group (control vs. GD) using repeated-measures ANOVA. A P value < 0.05 was considered significant. To address objective 2 (cholinesterase and octanoate), a convenience sample of 13 subjects was used. The area under the curve (AUC) was calculated using the trapezoidal method on non-log-transformed values. We used linear regression analysis to examine the relationships among hormonal and octanoate concentrations and/or enzyme activities. Using Bonferroni correction to account for multiple testing, a P value < 0.017 was considered significant. Plasma concentrations of ghrelin and GH and their AUCs were log transformed. Data were analyzed with SPSS version 16.0 (2007, Chicago, IL).

RESULTS

Characteristics of the subjects. The characteristics of the mothers in the first group are described in Table 1. One of the mothers in the control group could not be reached after delivery and missed the postpartum mixed-meal test. Maternal age at the time of delivery was 3.3 yr younger in the control group during both the third trimester of pregnancy and 8.3 (range 3.9–20.0) wk after delivery. The duration of pregnancy was 1.2 wk shorter in the GD group.

Glucose homeostasis, leptin, and adiponectin. Glucose and insulin concentrations during the mixed meal are shown in Fig. 1. Glucose concentrations (P < 0.001) and glycosylated hemoglobin (P = 0.004) were higher in the GD compared with the control group during both the third trimester of pregnancy and the postpartum period (Table 1). In contrast, insulin concentrations were similar in the control and GD groups at both the third trimester and postpartum visits. The duration of pregnancy was 1.2 wk shorter in the GD group. The second group of subjects consisted of 13 healthy women at 35 (33–38) wk of gestation and 8.3 (range 3.9–20.0) wk after delivery.

Pituitary GH. In control subjects, GH concentrations were lower at 35 wk compared with 22 wk of gestation and with 9 wk postpartum (ANOVA: P = 0.033, Fig. 4). The test meal caused a decrease in GH concentrations at 22 wk in control (data not available in GD) and at 9 wk postpartum (GD and control) between 0 and 90 min (P < 0.001), but not at 35 wk of gestation. At 9 wk postpartum (but not during pregnancy),

Table 1. Characteristics of the mothers in the control and GD groups

<table>
<thead>
<tr>
<th></th>
<th>Second Trimester</th>
<th>Third Trimester</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>GD</td>
<td>Control</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>ND</td>
<td>14</td>
</tr>
<tr>
<td>Gestational age/postpartum time, wk</td>
<td>23.2±0.2</td>
<td>ND</td>
<td>34.8±0.3</td>
</tr>
<tr>
<td>Meal, kcal</td>
<td>563±11</td>
<td>ND</td>
<td>600±11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8±1.2</td>
<td>ND</td>
<td>27.9±1.2</td>
</tr>
<tr>
<td>Glucose homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>92±2</td>
<td>ND</td>
<td>90±2</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>8.6±0.9</td>
<td>ND</td>
<td>13.4±1.4</td>
</tr>
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<td>HOMA</td>
<td>2.2±0.3</td>
<td>ND</td>
<td>2.9±0.4</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>4.9±0.1</td>
<td>ND</td>
<td>5.1±0.7</td>
</tr>
<tr>
<td>Fasting hormonal concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acylated ghrelin, pg/ml</td>
<td>17.7±3.6</td>
<td>ND</td>
<td>16.7±4.2</td>
</tr>
<tr>
<td>Desacylated ghrelin, pg/ml</td>
<td>225±41</td>
<td>ND</td>
<td>153±44</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>22.4±2.5</td>
<td>ND</td>
<td>22.7±3.1</td>
</tr>
<tr>
<td>Adiponectin, ng/ml</td>
<td>5.4±0.5</td>
<td>ND</td>
<td>4.9±0.6</td>
</tr>
<tr>
<td>Growth hormone, ng/ml</td>
<td>1.7±0.5</td>
<td>ND</td>
<td>0.19±0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. GD, gestational diabetes mellitus; BMI, body mass index; HOMA, homeostasis model assessment; Hb, hemoglobin. Conversions: glucose: mg/dl × 0.056 = mmol/l; insulin: μU/ml × 6.9 = pmol/l; ghrelin: pg/ml × 0.296 = pmol/l. ND, not done.

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there was a significant correlation between AUC for GH and AUC for AG (after log transformation, $r = 0.50$, $P = 0.007$). DG was not significantly associated with GH concentrations.

**Ghrelin, cholinesterase, and octanoate before and after delivery.** To clarify the role of cholinesterase and of octanoate availability in the deacylation and acylation, respectively, of AG during pregnancy, we measured AG, DG, and octanoate concentrations or plasma cholinesterase activity in a second group of subjects. Similar to the first study group, the percent full-length AG that was acylated was markedly lower in pregnant (16 ± 1%) compared with postpartum women (64 ± 3%, $P < 0.001$). Cholinesterase activity in these 13 healthy

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**Fig. 1.** Glucose (*top*) and insulin (*bottom*) concentration at 22 and 35 wk of gestation and 9 wk after delivery in control (C) subjects and in subjects with gestational diabetes (GD) during a meal test (0, 15, 30, 60, and 90 min). Glucose concentrations were higher ($P < 0.001$) in the GD compared with the control group during the third trimester of pregnancy and the postpartum period. In contrast, insulin concentrations were only significantly different between control and GD subjects 60 and 90 min after meal ingestion during the postpartum period (group $\times$ period $\times$ time interaction: $P = 0.038$). Means ± SE. Glucose: mg/dl $\times 0.056 = \text{mmol/l}$. Insulin: $\mu$ U/ml $\times 6.9 = \text{pmol/l}$.

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**Fig. 2.** Acylated ghrelin (AG) concentrations and percent of total ghrelin that is acylated in control subjects (open bars, 22 and 35 wk of gestation and 9 wk postpartum) and in GD subjects (gray bars, 35 wk of gestation and 9 wk postpartum) during a meal test (0, 15, 30, 60, and 90 min). Values are means ± SE. AG concentrations and the percent of total ghrelin that is acylated were lower during pregnancy compared with the postpartum period (ANOVA, $P < 0.001$). AG concentrations decreased significantly over the course of the meal during pregnancy and the postpartum period (ANOVA $P \leq 0.018$). ND, not done. Ghrelin: pg/ml $\times 0.296 = \text{pmol/l}$.

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women were lower during pregnancy (705 ± 33) compared with the postpartum period (1,013 ± 56 U/ml) (P < 0.001). After delivery (but not during pregnancy), cholinesterase activity and AG tended to be negatively correlated after controlling for the number of postpartum weeks (r = −0.57, P = 0.052, Fig. 5, A and B). DG concentrations and the percent of AG were not significantly associated with cholinesterase activity during pregnancy or after delivery. Plasma octanoate concentrations were lower during pregnancy (3.1 ± 0.5) compared with the postpartum period (4.5 ± 0.6 μg/ml, P = 0.029). During pregnancy, there was a trend toward a negative correlation between the percent of ghrelin that was acylated and octanoate, after controlling for maternal body mass index (r = −0.60, P = 0.04) (Fig. 5C).

**DISCUSSION**

Taking advantage of a novel ghrelin assay that measures full-length AG and DG (24), we clarify several key aspects of ghrelin homeostasis during pregnancy and the perinatal period. First, we showed in postpartum subjects that the percent of total ghrelin that is acylated is four to five times higher than previously observed using conventional, single antibody assays (25). In these subjects, consistent with the postulated physiological role of AG in GH stimulation, we observed significant associations between AG and GH. There was also a trend toward a negative relationship between AG and cholinesterase activity and AG that is acylated is four to five times higher than previously observed using conventional, single antibody assays (25). In these subjects, consistent with the postulated physiological role of AG in GH stimulation, we observed significant changes in the metabolism of AG and, to a lesser extent, a decrease in the overall production of ghrelin, as shown by the lower total ghrelin without statistically significant changes in DG concentrations during pregnancy compared with the postpartum period. The mechanisms underlying these effects are unclear. The absence of a significant relationship between AG and leptin (increased during pregnancy) or adiponectin (affected by pregnancy) in our study does not support a role for these hormones in the regulation of AG during pregnancy. Leptin administration does not affect total ghrelin concentrations in humans (4). Both positive (in lean women) and negative (in overweight women) correlations have been reported between leptin and AG (21). Using a single-antibody

![Fig. 3. Desacyl (DG) and total (DG + AG) ghrelin concentrations in control subjects (open bars, 22 and 35 wk of gestation and 9 wk postpartum) and in GD subjects (gray bars, 35 wk of gestation and 9 wk postpartum) during a meal test (0, 15, 30, 60, and 90 min). Values are means ± SE. In the control group, total ghrelin concentrations were lower at 22 and 35 wk of pregnancy compared with the postpartum period (ANOVA, P = 0.015). DG and total ghrelin concentrations decreased significantly over the course of the meal during pregnancy and the postpartum period (ANOVA, P ≤ 0.001). Ghrelin: pg/ml × 0.296 = pmol/l.](image1)

![Fig. 4. Pituitary growth hormone (GH) concentrations in control subjects (open bars, 22 and 35 wk of gestation and 9 wk postpartum) and in GD subjects (gray bars, 35 wk of gestation and 9 wk postpartum) during a meal test (0, 15, 30, 60, and 90 min). In control subjects, GH concentrations were lower at 35 wk compared with 22 wk of gestation and with 9 wk postpartum (ANOVA, P = 0.033). GH concentrations decreased during the test meal at 22 wk in control and at 9 wk postpartum (GD and control) (P < 0.001). Values are means ± SE.](image2)
ghrelin assay, Palik et al. (31) observed a positive correlation between adiponectin and AG during pregnancy but not in nonpregnant subjects. The low AG concentrations during pregnancy could conceptually be the result of increased deacylation or decreased acylation. Our observations of lower plasma cholinesterase activities (40) and of the absence of a significant correlation between plasma cholinesterase and AG concentrations during pregnancy make increased deacylation by cholinesterase an unlikely explanation. Because the availability of octanoate is known to affect ghrelin acylation (30), we tested the hypothesis that circulating octanoate availability may be limiting during pregnancy. However, while we showed for the first time that octanoate concentrations are 31% lower during pregnancy compared with the postpartum period, we feel that the 8–10 times decrease in circulating AG we observed during pregnancy is more likely to be because of a decrease or inhibition in the acylation of ghrelin. The enzyme that acylates ghrelin has been recently identified as a specific ghrelin O-acyltransferase (15, 42) and is present in ghrelin-producing tissues. Its activity is inhibited by specific ghrelin fragments (43). We speculate that pregnancy is associated with an increase in circulating ghrelin fragments that inhibit the activity of the acyltransferase. In support of our hypothesis, we suggest that the discrepancy between the results obtained by Palik et al. (31) (modest difference in AG concentrations between pregnancy and postpartum using single-antibody assays) and our results (marked decrease in full-length AG concentrations during pregnancy using double-antibody assays) may reflect an increased presence of circulating ghrelin fragments during pregnancy.

Exogenous administration of AG (which binds to the GHSR1a receptor) consistently causes a marked increase in plasma GH concentrations in humans (32). The role of endogenous AG in the physiology of GH secretion, while suspected, had not been convincingly demonstrated (10). Recently, using the same two-site sandwich assay that recognizes full-length AG, Nass et al. (28) observed in eight young, lean male subjects a significant correlation between the amplitude of GH pulses and the concentrations of AG, supporting the hypothesis that endogenous ghrelin modulates GH secretion. Our finding of a significant correlation between AG (but not DG) and GH concentrations in 27 young, postpartum female subjects further supports this hypothesis. In contrast, during pregnancy, there was no significant association between AG and pituitary GH concentrations. Furthermore, while AG concentrations were similarly low during the second and third trimesters of pregnancy, pituitary GH concentrations were higher during the second trimester (and similar to the concentrations observed in nonpregnant subjects) compared with the third trimester of pregnancy [likely reflecting the inhibition of pituitary GH by increasing concentrations of placental GH and/or insulin-like growth factor-I (3, 31)]. Taken together, these data suggest that

Fig. 5. Relationship between plasma cholinesterase activity and AG concentrations in the mother during the third trimester of gestation (A) and the postpartum period (B), and between plasma octanoate concentrations and the percent of ghrelin that was acylated during the third trimester of gestation (C) and the postpartum period (D). Ghrelin: pg/ml × 0.296 = pmol/l.
pituitary GH is also an unlikely explanation for the low levels of AG observed throughout pregnancy.

We further characterize the regulation of ghrelin by comparing the response with a meal during pregnancy and the postpartum period. Caloric intake was associated with the expected decrease in both AG and DG concentrations during the 90 min following meal ingestion. This decrease was smaller but present for AG during pregnancy, suggesting that nutritional regulation of AG concentrations is maintained during pregnancy despite much lower AG concentrations.

In contrast to our hypothesis, GD did not affect AG concentrations and was actually associated with an increase in DG and in total ghrelin throughout the meal test during the third trimester of pregnancy and the postpartum period. The relationship between ghrelin and glucose metabolism is complex. Insulin decreases ghrelin secretion by the stomach in vitro (18) and is associated with a decrease in ghrelin concentrations in vivo (34). In contrast, type 2 diabetes is characterized by a decrease in ghrelin suppression by insulin (1). In our study, subjects with GD were insulin resistant (as shown by the lower adiponectin concentrations) but were not (during pregnancy) or minimally (during postpartum) hyperinsulinemic, reflecting the presence of moderate GD. We speculate that the greater levels of circulating DG ghrelin we observed in GD in the absence of hyperinsulinism reflect insulin resistance at the stomach level. We cannot rule out that more severe GD with marked hyperinsulinism would have different effects on circulating ghrelin.

Plasma leptin concentrations were 32% lower in GD compared with control subjects at 35 wk of gestation. Previous studies have observed similar results in women with mild GD that did not require insulin therapy (11) and may reflect a change in the balance between leptin production by the placenta [decreased in GD (22)] and by adipose tissue and skeletal muscle [increased in GD, and in particular with insulin treatment (22)], secondary to insulin resistance.

A limitation of our study is that we used women in early gestation as controls, and it is conceivable that metabolic characteristics associated with this period could affect ghrelin concentrations. To our knowledge, there are no studies comparing AG concentrations in nonpregnant women and during pregnancy. However, cholinesterase activity is known to return to nonpregnant values a few weeks following delivery (40), and the AG-to-DG ratio observed in our postpartum women is similar to data recently reported by Liu et al. (24) in normal older subjects using the same ghrelin assay. Another limitation is that different collection methods were used for the first and second part of the study. To prevent ghrelin deacetylation, samples were systematically kept on ice until centrifugation at 4°C within 15 min of blood collection. However, in the second group of subjects, we added a serine protease and esterase inhibitor to the blood and increased the concentration of HCl in the plasma. These differences in sample handling are because of changing recommendations over time but did not affect our results, as shown by the similar absolute and relative AG values in pre- and postdelivery samples in both parts of the study.

In summary, pregnancy is characterized by a marked decrease in plasma AG concentrations compared with the postpartum period. This decrease is unlikely to be related to increased deacetylation or to decreased availability of circulating octanoate, and we speculate that pregnancy affects the activity of ghrelin O-acetyltransferase. Decreased acylation of ghrelin may facilitate the anabolism of pregnancy by decreasing the inhibition of maternal insulin release and ultimately be beneficial to the fetus. Women with GD have increased DG concentrations both during pregnancy and after delivery. We hypothesize that these changes are secondary to insulin resistance and may contribute to decreased energy expenditure and weight gain in these subjects.

ACKNOWLEDGMENTS

We are indebted to the nurses from the gestational diabetes clinic and from the family practice unit for help in recruiting the patients. We thank Dr. C Chow for measuring the cholinesterase activity and Life Scan for providing the One Touch Ultra meters as well as the glucose strips. Present addresses: E. Tham: Women’s and Children’s Hospital, North Adelaide, Australia; and R. Bogarin, National Children’s Hospital, San Jose, Costa Rica.

GRANTS

E. Tham was supported by a postdoctoral fellowship from the Patrick and Beryl Campbell Endowment for Pediatric Endocrinology. The study was funded by Grant No. 1637 from the Canadian Diabetes Association and (in part) by National Institutes of Health Grants MO1 RR-00847 (to the General Clinical Research Center at the University of Virginia) and RO1 DK-076037 (to M. Thormer).

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