Published in final edited form as:

Metabolism. 2010 June ; 59(6): 841–847. doi:10.1016/j.metabol.2009.09.033.

Effects of Glucose and Insulin on Acyl ghrelin and Desacyl ghrelin, Leptin and Adiponectin in Pregnant Women with Diabetes

WT Gibson^{1,2}, J Liu³, B Gaylinn³, M Thorner³, GS Meneilly⁴, SL Babich^{1,2}, D Thompson⁴, and JP Chanoine^{2,5}

¹Department of Medical Genetics, University of British Columbia, Vancouver, Canada

²Child and Family Research Institute, University of British Columbia, Vancouver, Canada

³Department of Medicine, Division of Endocrinology, University of Virginia Health System, Charlottesville, United States

⁴Department of Medicine, University of British Columbia, Vancouver, Canada

⁵Endocrinology and Diabetes Unit, British Columbia Children's Hospital, Vancouver, Canada

Structured Summary

Aim—To compare the regulation of ghrelin, leptin and adiponectin by insulin and glucose during the 2nd and 3rd trimester of pregnancy in women with diabetes.

Methods—We studied 9 pregnant women with diabetes. All women were treated with insulin and omitted the morning dose on the day of the test. Following collection of baseline fasting samples, we performed 3 successive glucose clamps: 2 euglycemic clamps (glucose 5mmol/L, insulin infusion at 20 and 40 mU m⁻² min⁻¹) and 1 hyperglycemic clamp (glucose 10 mmol/L, insulin infusion at 40 mU m⁻² min⁻¹). We determined concentrations of acyl- and desacyl ghrelin (using a double antibody sandwich assay that recognizes the full length molecule), leptin and adiponectin.

Results—Fasting desacyl ghrelin concentrations decreased while insulin and leptin concentrations increased between the 2nd and 3rd trimesters of pregnancy ($P \leq 0.011$). During the clamp studies, desacyl ghrelin concentrations decreased by 33% (2nd trimester, $P = 0.004$) and 27% (3rd trimester, $P = 0.09$) with increasing glucose and insulin concentrations while acyl ghrelin, leptin and adiponectin concentrations were unaffected.

Conclusions—Glucose and insulin regulate desacyl ghrelin concentrations in pregnant women with diabetes. Impaired desacyl ghrelin regulation may affect energy metabolism in pregnant women with poorly controlled diabetes.

Keywords

Acyl ghrelin; Desacyl ghrelin; Type 2 Diabetes; Pregnancy; Clamping Studies

Address correspondence and reprint requests to William T. Gibson, MD, PhD, FRCPC, FCCMG, Assistant Professor, Dept. of Medical Genetics, UBC, Child and Family Research, Institute, 950 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4, Tel: (604) 875-2000 ext. 6783, Fax: (604) 875-2373, wgibson@cw.bc.ca.

Conflict-of-interest/Financial Disclosure Statement: The Authors have no competing interests to declare.

Declaration of Competing Interests: Nothing to declare.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Ghrelin is a peptide hormone mainly secreted by the stomach. It circulates as acyl ghrelin (AG) and desacyl ghrelin (DG). The presence of an acyl chain (octanoate residue) added by the enzyme ghrelin-O-acyl transferase to the serine 3 moiety of ghrelin [1,2] is required for binding to the GH secretagogue receptor (GHSR), which mediates many of the actions ascribed to ghrelin [3]. Once in the plasma, AG is rapidly deacylated into DG by cholinesterase [4,5].

Both AG and DG potentially play a role in energy balance. Exogenous administration of AG stimulates GH secretion [6] and has orexigenic effects [7] in humans. Though the role of DG in control of food intake is controversial [8,9], in rats both AG and DG promote adipogenesis via GHSR-independent pathways that remain to be characterized [10]. Recent in vitro evidence suggests this may also be true in humans [11]. Ghrelin concentrations increase with fasting and decrease after caloric intake [12-14], suggesting a role in hunger and meal initiation. Ghrelin concentrations are also closely linked to glucose metabolism. Hyperglycemia and insulin resistance [15,16] are independently associated with a decrease in ghrelin concentrations [17].

In humans, we recently characterized ghrelin homeostasis in pregnant women with and without gestational diabetes [5]. We found markedly decreased AG concentrations during pregnancy (the significance of which remains unclear), whereas DG concentrations were similar during pregnancy and after delivery. The expected decrease in both AG and DG following ingestion of a meal was preserved during pregnancy. Surprisingly, despite higher glucose concentrations, gestational diabetes did not affect AG concentrations. Glucose tolerance was only mildly affected in these women, whose gestational diabetes was successfully controlled with diet alone. In contrast, DG concentrations were almost doubled in subjects with gestational diabetes compared to control subjects, not only during pregnancy but also during the postpartum period.

We hypothesized that pregnant subjects with preexisting diabetes may be more insulin resistant, and that acute changes in glucose and insulin concentrations would affect AG and DG concentrations. The objective of the current study was to compare AG and DG concentrations at two time points during diabetic pregnancy using a series of glucose and insulin clamps. We also investigated the relationship between ghrelin, leptin and adiponectin under these experimental conditions.

Methods

Nine pregnant women with diabetes were recruited from the Diabetes and Pregnancy Clinic at Children's and Women's Health Centre (D.T.). The subjects were part of a research protocol investigating the role of glucose and insulin on carotid artery relaxation. Eight had been diagnosed with Type 2 diabetes prior to pregnancy according to the Canadian Diabetes Association criteria [18] and one had mature onset diabetes of the young (MODY) due to a confirmed glucokinase missense mutation (L324P, patient 10 in [19]). Inclusion criteria were as follows: age > 18 years, singleton pregnancy, no pre-eclampsia. Before pregnancy, treatments included diet (n=1), metformin alone (n=4), metformin + glyburide (n=2) or insulin (n=2).

During pregnancy, all subjects were receiving two daily injections of NPH ("Neutral Protamine Hagedorn") insulin and regular insulin (before breakfast and before supper). The subjects' diabetes was well-controlled on the twice-daily regime. Detailed daily recordings of blood glucose were available for 8 subjects (one subject, who also did not return for the second clamp study, failed to provide records). These records showed a mean glucose ($\pm$ SE) before meals of 5.11 ± 0.08 ; at bedtime, the mean $\pm$ SE glucose was 5.38 ± 0.16 (N=8). The mean average

number of weeks prior to delivery for which detailed recordings were available was 30.5 (median 30.5, range 25-36).

Each subject underwent 2 glycemic clamp studies, once during the 2nd trimester of pregnancy (12-18 weeks) and once during the 3rd trimester of pregnancy (30-36 weeks). Each study was composed of a succession of 3 clamps (performed over a 6 hour period) that differed by their glycemic target and their rate of insulin infusion (see below). At delivery, birthweight and gestational age were recorded and birthweight Z-score was calculated [20]. Baseline characteristics, gestational age at delivery and birth weight are presented in Table 1. The study protocol was approved by the University of British Columbia Clinical Research Ethics Board, and by the institutional review boards of the Vancouver Coastal Health Authority and the Children's and Women's Health Centre of British Columbia. After detailed explanation of the study, all patients gave free, written informed consent to the procedures, which were conducted in accordance with Declaration of Helsinki principles.

Clamp Studies

Subjects were admitted to the testing room between 0700 and 0800 after an overnight fast, and omitted their morning insulin injection. Weight (kg) and height (m) were used to calculate body surface area ($[\text{kg} \cdot \text{m}/36]^{1/2}$) and BMI (kg/m^2). An arm vein was cannulated for infusion of potassium phosphate, insulin and dextrose, and a contralateral hand vein was cannulated in a retrograde fashion for sampling, with the hand placed in a heated box (70°C) to “arterialize” venous blood. Baseline insulin and glucose concentrations were determined at -10 and 0 min prior to insulin and glucose infusions. We then began infusing insulin at a rate of 20 mU/m² per minute for 120 min while clamping serum glucose at 5 mmol/L. From 120 to 240 min, we infused insulin at a rate of 40 mU/m² per min while clamping serum glucose at 5 mmol/L. Finally, between 240 and 360 min, we infused insulin at a rate of 40 mU/m² per min while clamping glucose at 10 mmol/L (Tables 2 and 3). Glucose was measured immediately at the bedside at 5 min intervals, with adjustment of the infusion rate to maintain plasma glucose at the appropriate level. Circulating insulin levels were measured every 20 min. At 100, 220 and 340 min, blood was collected for the determination of AG, DG, leptin and adiponectin.

Sample Collection and Assays

Plasma glucose was measured using a YSI glucose analyzer (YSI Life Sciences, Yellow Springs, OH). All other blood samples (collected in tubes containing 1.25mg sodium EDTA per mL whole blood) were kept on ice and centrifuged within 20 min at 1,500 g and 4°C for 15 min. Plasma ghrelin was detected using a two-site sandwich assay that specifically detects full-length forms of both AG and DG. The sensitivity and intra-assay coefficient of variation (CV) for AG and DG have been published [5]. Due to AG instability, special precautions were taken during sample collection to protect against plasma esterases [21]. After centrifugation, 10 µL of phenylmethanesulfonyl fluoride (PMSF, 10 mg/mL solution in ethanol) and 50 µL of 1 M HCl were added per mL plasma. Insulin was measured in duplicate by radioimmunoassay (HI-14K, Linco, St. Charles, MO). The M/I ratio was defined as the ratio of the steady state glucose disposal rate ($\text{M: mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) over the steady state concentration of insulin (I: pmol/L) and was calculated at the end of the euglycemic (between 100-120 min) and hyperinsulinemic clamps (between 220-240 min). Steady state of insulin level or glucose infusion rate was calculated as the average of the values obtained during the last 20 minutes of each 120 min interval. Leptin and total adiponectin concentrations were measured using commercially available kits. According to the manufacturer's data (Alpco diagnostics, Salem, NH), the inter-assay coefficient of variation (CV) is 6.8%, and the intra-assay CV is 5.5% for the leptin ELISA kit. For the adiponectin ELISA kit, the inter-assay CV is 5.0%, and the intra-assay CV is 5.4%.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. We used nonparametric testing for related samples to examine the effect of time (fasting and each of the 3 clamps) in each trimester (Friedman test) and the effect of pregnancy trimester (2nd vs 3rd trimester, Wilcoxon). A P value <0.05 was considered significant. A sample size of 9 subjects provided 80% power to detect a shift in the mean value by 0.75 standard deviation units at an alpha-level of 0.05, based on samples paired within subjects. We used Spearman's rank correlation coefficient (r_s) to examine the relationships among leptin and adiponectin, as well as between birthweight SDS and ghrelin. Data were analyzed with SPSS version 17.0 (SPSS inc., Chicago IL, 2008).

Results

Characteristics of the subjects are presented in Table 1. Tables 2 (2nd trimester) and 3 (3rd trimester) show fasting glucose and hormonal concentrations alongside those during the steady state period of each of the three successive clamps. Two subjects were excluded from analysis of the second clamp study; one because she failed to return and one because of technical difficulties with the infusion rate that failed to cause an increase in insulin concentrations.

Glucose and Insulin concentrations

Glucose concentrations were very close to target values during all 6 clamps, and insulin progressively increased during the 3 consecutive phases of the clamps ($P<0.001$). Overall, insulin concentrations increased from the 2nd to the 3rd trimester of pregnancy ($P=0.011$). Insulin sensitivity was not significantly different between the 2nd and 3rd trimesters of pregnancy. The M/I ratio measured at the end of the euglycemic normoinsulinemic clamp (20 mU insulin $m^{-2} min^{-1}$) was 0.0036 (0.0010) $mg\ kg^{-1}\ min^{-1}$ of glucose infused per pmol/L of plasma insulin during the 2nd trimester [95% CI: 0.0015-0.0057] and 0.0027 (0.0011) [95% CI: 0.0002-0.0053] during the 3rd trimester. These values were 0.0056 (0.0014) [95% CI: 0.0025-0.0087] and 0.0045 (0.0012) [95% CI: 0.0018-0.0072] for the euglycemic hyperinsulinemic clamp (40 mU insulin $m^{-2} min^{-1}$).

Full length Acyl ghrelin (AG) and Desacyl ghrelin (DG) Concentrations

Baseline desacyl ghrelin concentrations decreased from the 2nd to the 3rd trimester of pregnancy ($P=0.04$). Desacyl ghrelin concentrations also decreased with increasing glucose and insulin concentrations ($P=0.004$ during the 2nd trimester $N=9$, $P=0.09$ during the 3rd trimester $N=7$) although the decrease only reached statistical significance in the 2nd trimester. Overall, there was a 27% (3rd trimester) to 33% (2nd trimester) decrease in DG between the fasting state and the hyperglycemic-hyperinsulinemic clamp, while glucose concentrations doubled (from 5 to 10 mmol/L) and insulin concentrations almost tripled (Tables 1 and 2). AG concentrations were relatively low, and were similar during the 2nd and 3rd trimester of pregnancy and were not significantly different in the fasting state or during the clamps. Individual subject responses for AG and DG are presented in Fig.1.

Leptin and Adiponectin

Fasting leptin concentrations increased significantly between the 2nd and 3rd trimester of pregnancy ($P=0.018$) while adiponectin concentrations remained similar. Leptin and adiponectin were not affected by the changes in glucose and insulin concentrations.

Correlations

There was a positive correlation between fasting leptin and adiponectin during the 2nd ($r_s^2 = 0.34$, $P=0.09$) and 3rd trimester ($r_s^2 = 0.69$, $P=0.01$) that only reached significance during the 3rd trimester. There was also a correlation between birthweight Z-score and the percent of total

ghrelin that was acylated during the 3rd trimester of pregnancy ($r_s^2 = 0.73$, $P = 0.007$). There were no significant correlations between BMI and hormonal parameters, before or during pregnancy.

Discussion

Using a ghrelin assay that recognizes both full-length AG and DG concentrations, we clarify the role of insulin and glucose on ghrelin concentrations in pregnant women affected with diabetes. We demonstrate that glucose and insulin cause a 27-33 % decrease in circulating DG concentrations, without affecting AG concentrations. DG has an emerging role in energy balance regulation, though whether its primary effect is in the control of caloric intake [9], nutrient partitioning [11] glucose homeostasis [22] or energy expenditure remains to be determined.

Ghrelin is also emerging as a potentially important player during pregnancy. Animal studies have shown that AG administration to the pregnant dam caused an increase in birth weight, suggesting that maternal ghrelin could play an important role in perinatal growth. The increase in birth weight was thought to be due to transplacental transfer of AG, was independent of maternal food intake and was not observed when DG was administered to the dam [23]. Whether this is also true in humans is unknown, but our finding of a positive correlation between the percent AG and birth weight SDS is consistent with this hypothesis. Interestingly, we have also observed a positive correlation between birth weight and percent AG during the 3rd trimester of pregnancy in another study investigating pregnant subjects with and without gestational diabetes ($r=0.43$, $P=0.034$, unpublished data) [5].

The low absolute concentrations of AG in the present study are similar to our data in pregnant women with and without gestational diabetes [5]. To better study the relationship between glucose homeostasis and ghrelin, we investigated a population characterized by impaired insulin secretion and sensitivity due to the additive effect of pregnancy and diabetes. Our design resulted in a progressive increase in insulin and glucose from the fasting state to the hyperglycemic-hyperinsulinemic clamp. However, despite a 2 fold increase in glucose concentrations and a 2-3 fold increase in insulin concentrations, AG concentrations remained unchanged throughout the study. In contrast, we observed a 27-33% decrease in DG concentrations as insulin and glucose concentrations progressively increased. Visual analysis of Figure 1 (and of Tables 2 and 3) shows that the greatest decrease in DG occurs between fasting and the first clamp, and is associated with stable glucose concentrations in the face of a 60% increase in insulin concentrations, suggesting that insulin partly modulates these changes.

Several authors have used the glucose clamp to investigate the role of glucose and insulin on ghrelin concentrations, but this is the first such study performed in pregnant women. In addition, the hormone assays used in most published studies did not differentiate between AG and DG. Flanagan *et al* [24] observed a 18% decrease in total (AG + DG) ghrelin concentrations that was mostly the result of increased insulin concentrations and was independent of glycemia. Similar results were observed in women with and without polycystic ovarian syndrome [25] and in subjects with uncomplicated obesity [26] during a euglycemic-hyperinsulinemic clamp. A decrease in circulating total ghrelin levels was also seen by Anderwald *et al.* in subjects with Type 2 diabetes, and was found to be smaller than in subjects without Type 2 diabetes [27]. In a study measuring both AG and DG concentrations (using a single antibody method), St-Pierre *et al.* observed a decrease in DG concentrations in insulin-sensitive and insulin-resistant, nondiabetic obese subjects during a euglycemic hyperinsulinemic clamp [28]. Acyl ghrelin concentrations were found to be decreased only in insulin-sensitive obese subjects. Taken

together, these data suggest that insulin causes a decrease in ghrelin concentrations that may be blunted by insulin resistance.

Our results also show that insulin inhibits DG during pregnancy. However, in the absence of a control group of non diabetic pregnant women, we cannot determine whether insulin resistance reduces this response. Interestingly, AG was unaffected by changes in insulin and glucose concentrations during the clamps. This may reflect the presence of obesity and insulin resistance in our patients, two situations that have recently been reported to be associated with increased AG concentrations in non pregnant subjects [11,28]. However, this could also be explained by the specific effect of pregnancy on AG concentrations. We have recently shown that circulating AG concentrations were lower in pregnant compared to non-pregnant subjects [5], a finding that may be due to decreased acylation of ghrelin in the stomach, at least in rodents (Chanoine, unpublished results). Finally, because AG concentrations are low during pregnancy and close to the limit of detection of our assay, we cannot rule out a small, unrecognized decrease in AG.

As expected during pregnancy [29], leptin levels increased significantly between the 2nd and 3rd trimesters. Fasting leptin concentrations were markedly higher in our study (mean (SD) 86 (29) ng/mL) than those reported by Butte et al. [30] in healthy nondiabetic women (30 (17) ng/mL) during the 3rd trimester of pregnancy. This likely reflected the greater BMI (and presumably fat mass) of our subjects (38.7 (5.9) vs. 28.1 (3.8) kg/m²). Interestingly, leptin concentrations were not affected by increasing insulin and glucose concentrations. *In vitro* data show that insulin stimulates leptin secretion by the adipocyte [31], but neither insulin or glucose acutely stimulate leptin secretion by placenta explants [32]. *In vivo*, clamp studies performed in nonpregnant subjects have generally reported a stimulatory effect of insulin on circulating leptin [33], consistent with the *in vitro* effect mentioned above. However, this effect of insulin on leptin secretion is abolished by insulin resistance [33,34]. Taken together, the lack of a significant effect of insulin on leptin concentrations in our subjects likely reflects synergistic insulin resistance secondary to pregnancy as well as to diabetes.

Our small sample size reflects the difficulty of recruiting pregnant women for these studies, and of performing clamp studies at two occasions during pregnancy. As a consequence, we did not have the power to detect small differences in ghrelin concentrations between the various phases of the clamp, nor to differentiate between the respective effects of insulin and glucose. In addition, several AG measurements were close to the detection limit of the assay, which would further limit our ability to demonstrate insulin-induced suppression of AG. We did not study a control group of non diabetic pregnant women, as recruitment of normal pregnant women for these procedures was deemed unethical. Nonetheless, our study presents several strengths. First, we achieved three different combinations of glucose and insulin steady-state concentrations, providing a wide range of glucose and insulin concentrations. Second, the subjects were investigated in two different trimesters of pregnancy.

In conclusion, we show that acute increases in insulin and glucose in pregnant subjects with diabetes are associated with a 30% decrease in DG concentrations, without changes in their already suppressed AG. Emerging data show that DG affects energy expenditure. We speculate that changes in DG concentrations may contribute to impaired energy balance in pregnant women with poor control of diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to thank the nurses from the gestational diabetes clinic for their help in recruiting the patients, C Lockhart for expert technical assistance and Drs. S Stafford and R Khurana for help with data collection. WTG receives salary support from the CIHR Clinician Scientist Phase II program and from the CFRI Clinician Scientist program. The study was funded in part by grant #1637 from the Canadian Diabetes Association (CDA) (to J.P.C.), by grant OOP-79614 from the CIHR-INMD (to W.T.G.), and by NIH grants MO1 RR 00847 (to the GCRC at the University of Virginia) and RO1 DK076037 (to M.T.).

References

1. Gutierrez JA, Solenberg PJ, Perkins DR, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A* 2008;105:6320–6325. [PubMed: 18443287]
2. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 2008;132:387–396. [PubMed: 18267071]
3. van der Lely AJ, Tschop M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev* 2004;25:426–457. [PubMed: 15180951]
4. De Vriese C, Gregoire F, Lema-Kisoka R, Waelbroeck M, Robberecht P, Delpote C. Ghrelin degradation by serum and tissue homogenates: Identification of the cleavage sites. *Endocrinology* 2004;145:4997–5005. [PubMed: 15256494]
5. Tham E, Liu J, Innis S, et al. Acylated ghrelin concentrations are markedly decreased during pregnancy in mothers with and without gestational diabetes: Relationship with cholinesterase. *Am J Physiol Endocrinol Metab* 2009;296:E1093–1100. [PubMed: 19240252]
6. Takaya K, Ariyasu H, Kanamoto N, et al. Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* 2000;85:4908–4911. [PubMed: 11134161]
7. Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001;86:5992. [PubMed: 11739476]
8. Toshinai K, Yamaguchi H, Sun Y, et al. Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. *Endocrinology* 2006;147:2306–2314. [PubMed: 16484324]
9. Inhoff T, Monnikes H, Noetzel S, et al. Desacyl ghrelin inhibits the orexigenic effect of peripherally injected ghrelin in rats. *Peptides* 2008;29:2159–2168. [PubMed: 18938204]
10. Thompson NM, Gill DA, Davies R, et al. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. *Endocrinology* 2004;145:234–242. [PubMed: 14551228]
11. Rodriguez A, Gomez-Ambrosi J, Catalan V, et al. Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes. *Int J Obes (Lond)* 2009;33:541–552. [PubMed: 19238155]
12. Tschop M, Wawarta R, Riepl RL, et al. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* 2001;24:RC19–21. [PubMed: 11434675]
13. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001;50:1714–1719. [PubMed: 11473029]
14. Ariyasu H, Takaya K, Tagami T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001;86:4753–4758. [PubMed: 11600536]
15. Saad MF, Bernaba B, Hwu CM, et al. Insulin regulates plasma ghrelin concentration. *J Clin Endocrinol Metab* 2002;87:3997–4000. [PubMed: 12161550]
16. Schofl C, Horn R, Schill T, Schlosser HW, Muller MJ, Brabant G. Circulating ghrelin levels in patients with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2002;87:4607–4610. [PubMed: 12364442]
17. Nakagawa E, Nagaya N, Okumura H, et al. Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: Responses to the intravenous and oral administration of glucose. *Clin Sci (Lond)* 2002;103:325–328. [PubMed: 12193159]

18. Committee CDACPGE. Canadian diabetes association 2008 clinical practice guidelines for the prevention and management of diabetes in canada. Toronto: Canadian Diabetes Association; 2008.
19. McKinney JL, Cao H, Robinson JF, et al. Spectrum of hnf1a and gck mutations in Canadian families with maturity-onset diabetes of the young (mody). Clin Invest Med 2004;27:135–141. [PubMed: 15305805]
20. Kramer MS, Platt RW, Wen SW, et al. A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics 2001;108:E35. [PubMed: 11483845]
21. Liu J, Prudom CE, Nass R, et al. Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. J Clin Endocrinol Metab 2008;93:1980–1987. [PubMed: 18349056]
22. Zhang W, Chai B, Li JY, Wang H, Mulholland MW. Effect of des-acyl ghrelin on adiposity and glucose metabolism. Endocrinology 2008;149:4710–4716. [PubMed: 18535105]
23. Nakahara K, Nakagawa M, Baba Y, et al. Maternal ghrelin plays an important role in rat fetal development during pregnancy. Endocrinology 2006;147:1333–1342. [PubMed: 16339208]
24. Flanagan DE, Evans ML, Monsod TP, et al. The influence of insulin on circulating ghrelin. Am J Physiol Endocrinol Metab 2003;284:E313–316. [PubMed: 12531744]
25. Micic D, Sumarac-Dumanovic M, Kendereski A, et al. Total ghrelin levels during acute insulin infusion in patients with polycystic ovary syndrome. J Endocrinol Invest 2007;30:820–827. [PubMed: 18075283]
26. Leonetti F, Iacobellis G, Ribaudo MC, et al. Acute insulin infusion decreases plasma ghrelin levels in uncomplicated obesity. Regul Pept 2004;122:179–183. [PubMed: 15491789]
27. Anderwald C, Brabant G, Bernroider E, et al. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. Diabetes 2003;52:1792–1798. [PubMed: 12829648]
28. St-Pierre DH, Karelis AD, Coderre L, et al. Association of acylated and nonacylated ghrelin with insulin sensitivity in overweight and obese postmenopausal women. J Clin Endocrinol Metab 2007;92:264–269. [PubMed: 17062757]
29. Masuzaki H, Ogawa Y, Sagawa N, et al. Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. Nat Med 1997;3:1029–1033. [PubMed: 9288733]
30. Butte NF, Hopkinson JM, Nicolson MA. Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. J Clin Endocrinol Metab 1997;82:585–589. [PubMed: 9024259]
31. Seufert J. Leptin effects on pancreatic beta-cell gene expression and function. Diabetes 2004;53:1512–1518. [PubMed: 14749281]
32. Lappas M, Yee K, Permezel M, Rice GE. Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies. J Endocrinol 2005;186:457–465. [PubMed: 16135665]
33. Ludwig AK, Weiss JM, Tauchert S, et al. Influence of hypo- and hyperglycaemia on plasma leptin concentrations in healthy women and in women with polycystic ovary syndrome. Hum Reprod 2007;22:1555–1561. [PubMed: 17395684]
34. Fruehwald-Schultes B, Oltmanns KM, Kern W, Born J, Fehm HL, Peters A. The effect of experimentally induced insulin resistance on the leptin response to hyperinsulinaemia. Int J Obes Relat Metab Disord 2002;26:510–516. [PubMed: 12075578]

Abbreviations

AG	acyl ghrelin
BMI	body-mass index
DG	desacyl ghrelin
GH	growth hormone
GHSR	growth hormone secretagogue receptor

M/I ratio steady state glucose disposal rate (M: mg of glucose infused per kilogram body weight per minute) divided by the concentration of insulin circulating at steady state for each clamp (I: pmol/L)

Figure 1.

Individual responses of acyl ghrelin (A and B) and desacyl ghrelin (C and D) during the 2nd trimester (A and C, N=9) and 3rd (B and D, N=7) trimesters of pregnancy in subjects with diabetes. The MODY patient is indicated with filled squares and asterisks, and was not an outlier. Baseline desacyl ghrelin concentrations decreased from the 2nd to the 3rd trimester of pregnancy (P=0.04). Desacyl ghrelin concentrations decreased with increasing glucose and insulin concentrations (P=0.004 during the 2nd trimester N=9, P=0.09 during the 3rd trimester N=7). There was a 27% (3rd trimester) to 33% (2nd trimester) decrease in DG between the fasting state and the hyperglycemic-hyperinsulinemic clamp. AG concentrations were relatively low, and were similar during the 2nd and 3rd trimester of pregnancy and were not significantly different in the fasting state or during the clamps.

Conversion:

acyl ghrelin: to convert pg/mL to pmol/L, divide by 3.371

desacyl ghrelin : to convert pg/mL to pmol/L, divide by 3.189

Table 1

Baseline characteristics of the subjects (N=9)

	Mean (SE)	Median	Range	95% CI
Age at enrollment (years)	33.1 (1.5)	32	26 - 39	29.6-36.6
Duration of diabetes (years)	7.1 (1.6)	6	1 - 14	3.3-10.9
Pre-pregnancy hemoglobin A1c (%)	7.5 (0.7)	6.7	5.8 - 10.9	6.0-9.0
Pre-pregnancy BMI (kg/m ²)	33.6 (1.9)	33.8	23.6 - 42.5	29.1-38.0
BMI 2 nd trimester (kg/m ²)	35.8 (1.8)	36.9	28.2 - 46.4	31.7-40.0
BMI 3 rd trimester (kg/m ²)	38.8 (2.1)	38.7	30.6 - 48.1	35.0-44.8
Gestational age at delivery (weeks)	37.5 (0.5)	38.0	35.0 - 40.1	36.4-38.7
Peripartum hemoglobin A1c (%)	6.0 (0.3)	6.2	4.7-7.5	5.4-6.7
Birth weight (g)	3384 (247)	3345	2420 - 5035	2863-4092
Birth weight (Z-Score)	0.49 (0.59)	0.31	-2.0 - +3.8	-0.88-1.86

Table 2

Glucose and hormonal concentrations in the fasting state and during 3 consecutive clamps in pregnant subjects with diabetes (2nd trimester of pregnancy). The M/I ratio measured at the end of the euglycemic normoinsulinemic clamp (20 mU insulin m⁻² min⁻¹) was 0.0036 (SE 0.0010) mg kg⁻¹ min⁻¹ of glucose infused per pmol/L of plasma insulin [95% CI: 0.0015-0.0057]. The M/I ratio was 0.0056 (SE 0.0014) [95% CI: 0.0025-0.0087] for the euglycemic hyperinsulinemic clamp (40 mU insulin m⁻² min⁻¹). Desacyl ghrelin concentrations decreased with increasing glucose and insulin concentrations (P=0.004, N=9). There was a 33% decrease in DG between the fasting state and the hyperglycemic-hyperinsulinemic clamp. AG concentrations were not significantly different between the fasting state and the clamps. The correlation between fasting leptin and adiponectin did not reach statistical significance ($r_s^2 = 0.34$, P = 0.09).

	Fasting	Clamp 1: 0-120 min Insulin infusion: 20 mU kg⁻¹ min⁻¹ Glycemia clamped at 5 mmol/L	Clamp 2: 120-240 min Insulin infusion: 40 mU kg⁻¹ min⁻¹ Glycemia clamped at 5 mmol/L	Clamp 3: 240-360 min Insulin infusion: 40 mU kg⁻¹ min⁻¹ Glycemia clamped at 10 mmol/L
Glucose (mmol/L)	5.0 (0.33) [4.3-5.8]	5.03 (0.04) [4.9-5.1]	4.85 (0.05) [4.7-5.0]	10.0 (0.01) [9.9-10.0]
Insulin (pmol/L)	269 (46) [163-376]	438 (59) [318-558]	570 (53) [450-692]	728 (87) [526-929]
Acyl ghrelin (pg/mL)	25.1 (5.2) [13.3-37.0]	27.4 (4.8) [16.4-38.4]	31.0 (6.9) [15.2-46.8]	26.0 (5.3) [13.8-38.1]
Desacyl ghrelin (pg/mL)	249 (35) [168-330]	190 (22) [138-241]	192 (23) [140-244]	168 (22) [116-219]
Leptin (ng/mL)	71 (7) [56-87]	66 (6) [52-80]	65 (6) [51-78]	64 (5) [52-76]
Adiponectin (µg/mL)	6.6 (1.5) [3.1-10.0]	6.1 (1.4) [2.9-9.2]	5.8 (1.2) [3.1-8.6]	5.8 (1.4) [2.7-9.0]

N=9, Mean (SE) [95% CI]

Conversion factors:

glucose: to convert mmol/L to mg/dL, multiply by 18

insulin: to convert pmol/L to microU/mL, divide by 6

acyl ghrelin: to convert pg/mL to pmol/L, divide by 3.371

desacyl ghrelin: to convert pg/mL to pmol/L, divide by 3.189

Table 3

Glucose and hormonal concentrations in the fasting state and during 3 consecutive clamps in pregnant subjects with diabetes (3rd trimester of pregnancy). The M/I ratio measured at the end of the euglycemic normoinsulinemic clamp (20 mU insulin m⁻² min⁻¹) was 0.0027 (SE 0.0011) [95% CI: 0.0002-0.0053] during the 3rd trimester. The M/I ratio was 0.0045 (SE 0.0012) [95% CI: 0.0018-0.0072] for the euglycemic hyperinsulinemic clamp (40 mU insulin m⁻² min⁻¹). Baseline desacyl ghrelin concentrations decreased from the 2nd to the 3rd trimester of pregnancy (P=0.04). Desacyl ghrelin concentrations also decreased with increasing glucose and insulin concentrations (P=0.09, N=7) although the decrease only reached statistical significance in the 2nd trimester. Overall, there was a 27% decrease in DG between the fasting state and the hyperglycemic-hyperinsulinemic clamp. AG concentrations were not significantly different in the fasting state or during the clamps. Fasting leptin concentrations increased significantly between the 2nd and 3rd trimester of pregnancy (P=0.018) while adiponectin concentrations remained similar. There was a positive correlation between fasting leptin and adiponectin ($r_s^2 = 0.69$, P = 0.01), but leptin and adiponectin were not affected by the changes in glucose and insulin concentrations. There was also a correlation between birthweight Z-score and the percent of fasting AG that was acylated during the 3rd trimester ($r_s^2 = 0.73$, P = 0.007).

	Fasting	Clamp 1: 0-120 min Insulin infusion: 20mU kg ⁻¹ min ⁻¹ Glycemia clamped at 5 mmol/L	Clamp 2: 120-240 min Insulin infusion: 40mU kg ⁻¹ min ⁻¹ Glycemia clamped at 5 mmol/L	Clamp 3: 240-360 min Insulin infusion: 40mU kg ⁻¹ min ⁻¹ Glycemia clamped at 10 mmol/L
Glucose (mmol/L)	4.80 (0.47) [3.7-5.9]	5.26 (0.20) [4.8-5.7]	5.04 (0.13) [4.7-5.3]	10.08 (0.05) [9.9-10.2]
Insulin (pmol/L)	392 (97) [163-622]	612 (115) [340-883]	700 (117) [422-977]	987 (186) [548-1427]
Acyl ghrelin (pg/mL)	32.4 (12.4) [3.2-61.6]	31.8 (13.6) [0-64]	35.7 (14.1) [2.4-69.0]	32.7 (12.3) [3.7-61.8]
Desacyl ghrelin (pg/mL)	178 (33) [99-257]	134 (21) [84-183]	134 (17) [94-174]	123 (18) [80-166]
Leptin (ng/mL)	86 (10) [61-110]	78 (11) [52-105]	74 (11) [49-100]	69 (10) [45-93]
Adiponectin (μg/mL)	6.0 (1.2) [3.1-8.8]	5.6 (1.1) [3.0-8.1]	5.4 (1.1) [2.7-8.0]	5.4 (1.2) [2.6-8.1]

N=8 for fasting values, 7 for Clamped values, Mean (SE) [95% CI]

Conversion factors:

glucose: to convert mmol/L to mg/dL, multiply by 18

insulin: to convert pmol/L to microU/mL, divide by 6

acyl ghrelin: to convert pg/mL to pmol/L, divide by 3.371

desacyl ghrelin: to convert pg/mL to pmol/L, divide by 3.189