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M.A. Guillén-Sacoto[a,*], B. Barquiel[a], N. Hillman[a], M.A. Burgos[b], L. Herranz[a]
[a]*Division of Diabetes, Department of Endocrinology and Nutrition, Hospital Universitario La Paz, 261, P° de la Castellana, 28046 Madrid, Spain*
[b]*Department of Obstetrics and Gynecology, Hospital Universitario La Paz, 261, P° de la Castellana, 28046 Madrid, Spain*

*Corresponding author. Hospital Universitario de Móstoles, Calle Río Júcar S/N, 28935 Móstoles, Madrid, Spain
E-mail address: maugusta.guillen@salud.madrid.org (M.A. Guillén-Sacoto).

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Gestational diabetes mellitus and the ghrelin system

Introduction

Gestational diabetes mellitus (GDM) is associated with adverse pregnancy and perinatal outcomes. A variety of serum biomarkers (such as inflammatory cytokines, adipokines and other circulating proteins) have been explored in attempts to identify a reliable predictor in early pregnancy for the subsequent development of GDM, but so far, none has been found [1]. Thus, the pool of biochemical markers requires further exploration.

Ghrelin, a gastrointestinal peptide hormone, is the endogenous ligand for growth hormone secretagogue receptor (GHSR) type 1a. Total serum ghrelin levels are composed of acylated ghrelin (AG) and unacylated ghrelin (UAG). The enzyme ghrelin o-acyltransferase (GOAT) is required for acylation of ghrelin. Ghrelin appears to have a wide range of biological activities and has been implicated in the regulation of glucose homoeostasis [2].

Ghrelin or ghrelin mRNA is expressed in the human ovary, testis and placenta, suggesting a role in fertility and pregnancy [3]. The pathophysiological role of ghrelin in GDM remains unclear, however. It has been reported that ghrelin levels are lower in women with GDM, which may reflect the inhibitory effect of insulin on ghrelin secretion [4]. Other studies have found decreased ghrelin levels in pregnancy irrespective of glucose tolerance [5]. However, to date, most studies measured total ghrelin without differentiating between AG and UAG. Furthermore, single-antibody ghrelin assays recognize either the COOH-terminal (total ghrelin) or acylated NH_2-terminal part of the peptide (AG) and are therefore measuring full-length ghrelin as well as circulating fragments of ghrelin, which have unknown biological activities. Indeed, it has been estimated that 60% of the ghrelin measured using these assays is fragmented [6].

For this reason, the present study has used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), which measures full-length AG and UAG. Our hypothesis is that women with GDM have higher degrees of insulin resistance and hyperinsulinaemia and, consequently, lower ghrelin levels than women with normal glucose tolerance (NGT). Thus, the aim of the study was to compare AG and UAG levels, and AG/UAG ratios, between pregnant women with GDM and with NGT.

Methods and materials

Subjects

A total of 19 pregnant women with GDM and 19 women with NGT were enrolled in our study. Women were prospectively recruited from the gynaecology outpatients' clinic at Maasstad Hospital in Rotterdam, Netherlands. Women with a singleton pregnancy and aged $\geq$ 18 years at gestational ages 24–28 weeks and at high risk of GDM, according to the Dutch Society of Obstetrics and Gynaecology [7], were eligible for inclusion. Women were excluded if they met any of the following criteria:

- endocrine disorders such as acromegaly, preexisting type 2 diabetes mellitus or Cushing's syndrome;
- use of glucocorticoid medications;
- inflammatory diseases or active infections;
- and/or a history of gastrointestinal surgery or hormonal treatments before or during pregnancy, including insulin.

All subjects were screened for GDM at 24–28 weeks of gestation by means of a 75-g oral glucose tolerance test (OGTT) and GDM diagnosis was based on International Association of the Diabetes and Pregnancy Study Groups (IADPSG)/World Health Organization (WHO) 2013 diagnostic criteria. Blood samples were drawn during fasting and at 2 h post-glucose load. Patient demographics, such as age, body mass index (BMI; kg/m^2), first trimester glucose (mmol/L) and gestational age at the time of sampling (weeks), were obtained from electronic medical records. All patients gave their written informed consent before inclusion in the study, which was approved by the relevant medical ethics committee.

Materials

Vacutainers (catalogue # 367899, 6-mL K2 EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used, and 4-(2-aminoethyl) benzenesulphonyl fluoride hydrochloride (AEBSF; Pefabloc SC, catalogue # 11429876001) was purchased from Roche Applied Science (Penzberg, Germany). Aliquots of 200 mg/mL stock solutions of AEBSF were prepared with distilled water and stored at −80 °C for a maximum of 3 months. Human AG and UAG were determined by a double-antibody sandwich technique and the enzyme immunoassay (EIA) kits (A05106 and A05119, respectively) were obtained from Bertin Pharma (Montigny-le-Bretonneux, France).

Table 1
Clinical characteristics of pregnant women with gestational diabetes mellitus (GDM) or normal glucose tolerance (NGT).

Parameter	GDM ($n = 19$)	NGT ($n = 19$)	P
Age, years	35 (30–38)	34 (29–37)	0.320
Body mass index, kg/m^2	28.4 (25–35)	29.7 (25–35)	0.942
Prepregnancy body mass index, kg/m^2	28 (24–35)	29 (23.4–35)	0.827
First trimester glucose, mmol/L	5.1 (4.4–5.3)	4.7 (4.3–5.4)	0.428
Gestational age at blood collection, weeks	24 (24)	24 (24–25)	0.139
75-g OGTT: fasting glucose, mmol/L	5.3 (5.1–5.8)	4.6 (4.3–4.8)	0.001*
75-g OGTT: 2-h glucose, mmol/L	8.8 (7.1–9.9)	6.2 (5.5–7.0)	0.001*
HbA1c, mmol/mol	35 (33–38)	31 (29–32)	0.001*

*Statistically significant ($P < 0.05$); OGTT: oral glucose tolerance test; data are medians (interquartile range).

OGTT, sample collection and storage

After an overnight fast, the 75-g OGTT was performed. Baseline serum parameters were glucose (4-mL heparin tube), HbA1c (8.5-mL serum separating tube), AG and UAG (both 4-mL EDTA). At 2 h after glucose ingestion, the glucose, AG and UAG levels were assessed. Immediately after sample collection, AEBSF (1:100 dilution) was added to the AG and UAG blood samples to prevent des-acylation of AG. The tubes were carefully mixed by inversion and stored on ice (0 °C) until centrifugation at 2500 g at 4 °C for 5 min. Plasma samples were stored in 300-μL aliquots at −80 °C until assayed for AG and UAG. After slow thawing on ice, all plasma samples were briefly cleared by centrifugation before being transferred to assay plates. All samples were analyzed in duplicate (50 μL/well) [8]. Cubic polynomial fitting was used to determine concentrations from calibration curves, resulting in $r^2 > 0.995$ for all assays performed. Samples that were below the limit of detection (8.5% < 4 pg/mL) were set at 4 pg/mL. The intra-assay coefficient of variation (CV) is typically 2.7% for AG and 3.4% for UAG whereas, in this case, the CV was 13.2% and 15.0% for AG and UAG, respectively (manufacturer's suggested cut-off: 25%).

Statistical analysis

The comparative analysis between groups was calculated by Mann–Whitney U and Wilcoxon's signed-rank tests. All statistical analyses were performed using SPSS version 16 software for Windows (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.04 (GraphPad Software, La Jolla, CA, USA). The results were expressed as medians ± interquartile range (IQR), and P values < 0.05 were considered statistically significant.

Results

Our patients' characteristics are shown in Table 1. Clearly, pregnant women with GDM had significantly higher HbA1c, fasting and 2-h post-glucose load levels than pregnant women with NGT ($P = 0.001$).

Fasting median plasma AG levels in the GDM ($n = 19$) and NGT ($n = 19$) women were 11.5 (IQR: 5.4–16.8) pg/mL and 13.7 (IQR: 10–25.6) pg/mL, respectively ($P = 0.473$; AG reference levels ranged from 22.7 to 61.9 pg/mL) [8], while median 2-h post-OGTT plasma AG levels were 10.4 (IQR: 4–13.9) and 10.2 (IQR: 6.0–13.6) pg/mL, respectively ($P = 0.724$). Fasting median plasma UAG levels in GDM and NGT women were 44.6 (IQR: 37.8–84.2) pg/mL and 72.8 (IQR: 39–146) pg/mL, respectively ($P = 0.320$; UAG reference levels ranged from 23.3 to 33.4 pg/mL) [8], while median 2-h post-OGTT UAG levels were 34.4 (IQR: 26–49.3) and 46 (IQR: 26.4–99.6) pg/mL, respectively ($P = 0.293$). The fasting median AG/UAG ratio in GDM was 0.2 (IQR: 0.14–0.31), which was not significantly different from the ratio in NGT [0.19 (IQR: 0.17–0.29); $P = 0.626$]. The 2-h post-glucose load AG/UAG ratios in GDM and NGT were also similar at 0.25 (IQR: 0.15–0.29) and 0.19 (IQR: 0.15–0.28), respectively ($P = 0.539$).

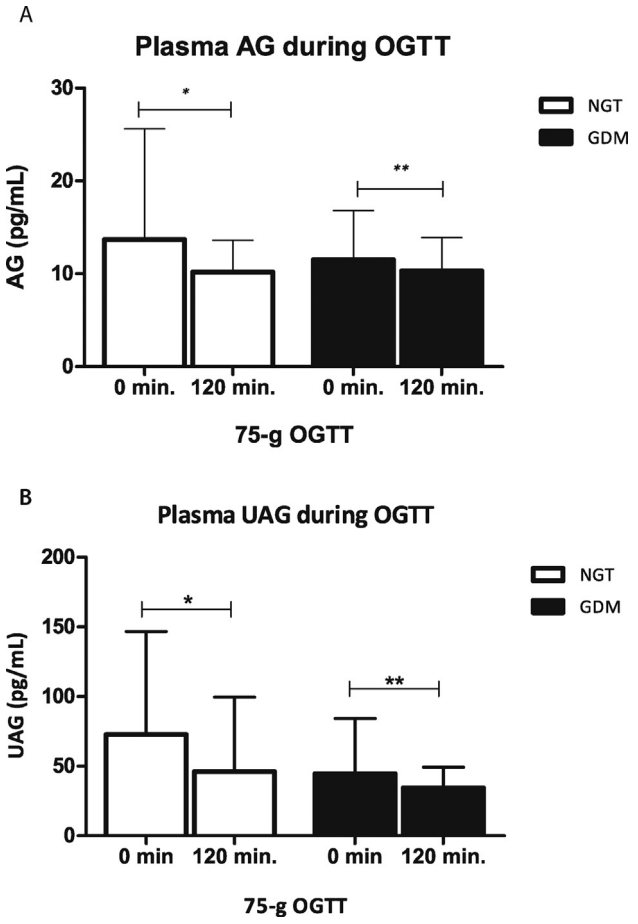

Fig. 1. Plasma (A) acylated ghrelin (AG) and (B) unacylated ghrelin (UAG) levels during oral glucose tolerance test (OGTT) in women with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM). Data are medians with interquartile ranges. (A) *$P = 0.002$, **$P = 0.001$; (B) *$P = 0.0002$, **$P = 0.0001$.

Plasma AG concentrations were significantly decreased at 2 h post-OGTT in GDM ($P = 0.001$) as well as in NGT ($P = 0.002$) women (Fig. 1A). Similarly, plasma UAG was significantly decreased at 2 h post-OGTT in both GDM ($P = 0.0001$) and NGT ($P = 0.0002$) groups (Fig. 1B).

Discussion

In contrast to our hypothesis, plasma ghrelin levels, as measured by a sensitive assay, were not lower in women with GDM compared with women with NGT. However, ghrelin levels decreased significantly at 2 h post-OGTT in both groups, which suggests that the negative physiological effects of oral glucose intakes on ghrelin levels were still intact. These results indicate that ghrelin is not a useful biomarker in GDM.

In addition, our present data are in agreement with the results of a study reported by Riedl et al. [5], which could find no association between fasting and post-load plasma ghrelin levels and, therefore, reported that ghrelin suppression in GDM is not the result of insulin resistance. Those authors also suggested that ghrelin suppression is essential for the physiological insulin

resistance necessary for the growth and nourishment of the fetus [5]. Also, a study by Telejko et al. [3] found reduced ghrelin levels during pregnancy irrespective of glucose tolerance status. However, other studies have, in contrast, reported lower ghrelin levels in GDM compared with healthy pregnant women. Palik et al. [9], for example, showed that serum AG levels, as measured by a different assay from ours, were significantly lower in women with GDM vs NGT during the third trimester of pregnancy.

In fact, ghrelin levels appeared to be suppressed during pregnancy in comparison to reference levels based on healthy non-pregnant women [8]. Likewise, Tham et al. [4] showed that ghrelin levels, using a similar assay, were suppressed in relation to postpartum levels. Furthermore, those authors demonstrated that AG levels recovered after pregnancy, which implies that the low AG/UAG ratio was a result of being pregnant.

Pregnancy is characterized by increased food intakes, maternal weight gain and progressive insulin resistance and the orexigenic effects of ghrelin may have contributed to the positive energy balance, whereas adipose tissue imposes negative feedback regulation on ghrelin production [9]. Ghrelin levels decrease with hyperglycaemia and hyperinsulinaemia, reflecting the inhibitory effect of insulin on ghrelin secretion [4]. This hypothesis is supported by the increased ghrelin levels present at mid-pregnancy and decreased levels during late gestation [10]. Nevertheless, whether low ghrelin levels are a risk factor or compensatory mechanism remains unknown.

The strength of our study was that both AG and UAG were measured by highly sensitive assays. However, there are also some limitations. First, comparing our results with other study outcomes is problematic because of the different assay techniques used. Second, a larger sample size might have improved the reliability of the outcome, although our data showed no suggestion of any association between ghrelin levels in early pregnancy and the development of GDM in the studied population.

In conclusion, both AG and UAG levels are low during pregnancy regardless of the level of glycaemic control and both decrease normally after an oral glucose load.

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Disclosure of interest

Aart Jan van der Lely is a cofounder and shareholder of Alizé Pharma, Ecully, France.

The other authors declare that they have no competing interest.

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H.S. Brink[a,*], A.J. van der Lely[b], P.J.D. Delhanty[b], M. Huisman[b], J. van der Linden[a]
[a]*Department of Internal Medicine, Maasstad Hospital, P.O. Box 9100, 3007, Rotterdam, CA, The Netherlands*
[b]*Department of Internal Medicine, Erasmus MC, P.O. Box 2040, 3000 CA, Rotterdam, The Netherlands*

[*]Corresponding author
E-mail address: BrinkH@maasstadziekenhuis.nl (H.S. Brink).

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Hypoglycaemic episodes and risk of diabetic peripheral neuropathy in patients with type 2 diabetes

1. Introduction

Hypoglycaemia is one of the most serious complications of diabetes therapy, as it increases the risk of injury and death. Many studies have investigated the effects of hypoglycaemia on the central nervous system and cardiovascular system, but very few reports have focused on the peripheral nervous system (PNS) [1]. Indeed, to date, no study has evaluated whether hypoglycaemia is associated with the risk of diabetic peripheral neuropathy (DPN) in patients with type 2 diabetes (T2D).

The objective of the present study was to determine whether previous hypoglycaemic events requiring hospitalization or emergency department (ED) visits are associated with an increased risk of DPN in a large cohort of adult patients with T2D.

2. Methods

2.1. Study design and cohort

This was a cross-sectional, hospital-based observational study. Eligible participants were patients with T2D aged > 20 years