Original article

Plasma total and unacylated ghrelin predict 5-year changes in insulin resistance

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SUMMARY

Background & aims: Ghrelin is a gastric hormone circulating in acylated (AG) and unacylated (UG) forms, and higher plasma total ghrelin (TG) and UG may be cross-sectionally associated with lower insulin resistance in metabolic syndrome patients. The potential value of ghrelin forms in predicting insulin resistance and its time-related changes in community-based population cohorts remains unknown.
Methods: We measured TG, AG and calculated UG (TG-AG) in 716 individuals from the North-East-Italy MoMa study (age: 55 ± 9 years, BMI: 29 ± 5 kg/m^2, M/F:349/367) to test the hypothesis that circulating TG and UG, but not AG are negatively associated with insulin resistance (HOMA). We further hypothesized that baseline TG and UG negatively predict 5-year HOMA changes in a 350-individual subgroup.
Results: Baseline TG and UG were associated negatively with HOMA after adjusting for gender and body mass index (BMI). Baseline gender- and BMI-adjusted TG and UG were also negatively associated with HOMA at 5-year follow-up (n = 350), and changes in TG and UG were negatively associated with changes in HOMA (P < 0.05) after adjustment for anthropometric and metabolic confounders. No statistically significant correlations were observed between AG and baseline or 5-year HOMA.
Conclusions: In a North-East Italy community-based population cohort, plasma TG and UG but not AG are negatively associated with HOMA. TG and UG and their changes also independently predict 5-year HOMA changes. TG and UG are therefore novel potential modulators of insulin resistance and may contribute to predict its time-related changes in humans.

1. Introduction

Ghrelin is a gastric hormone whose total plasma concentration (TG) may be low in obesity and insulin resistant states [1–3]. Total plasma ghrelin however includes both acylated (AG) and unacylated (UG) hormones [1], and differential metabolic effects are emerging for each ghrelin form [4]. AG is a well-established orexigenic regulator of appetite, and its sustained administration may increase body weight and circulating glucose by increasing food intake, hepatic gluconeogenesis and fat deposition in experimental models [4–6]. In recent years, more favorable metabolic effects have been reported for the unacylated ghrelin form, and UG co-administration may counteract glucogenic effects of AG and AG-induced hyperglycemia [5]. Consistent with these observations, negative associations have been reported for circulating UG with body mass index (BMI) and insulin resistance markers in metabolic syndrome patients [7]. No information is however available on potential differential associations between ghrelin forms and insulin resistance in cross-sectional and prospective studies of community-based population cohorts.

We therefore measured plasma ghrelin profile (TG, AG and calculated UG) and insulin resistance as reflected by the commonly-used HOMA index in a 716-individual community-based cohort from the North-East Italy MoMa epidemiological study [8]. We hypothesized that TG and UG, but not AG, are negatively associated with insulin resistance, and that baseline TG and UG but not AG are independent predictors of insulin resistance and its time-related changes in individuals undergoing 5-year follow-up evaluation.

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2. Materials and methods

2.1. Experimental protocol and study population

2.1.1. Basal

The study was performed in the setting of the MoMa study, a Friuli-Venezia Giulia Region-supported project aimed at investigating the prevalence of metabolic syndrome in the municipalities of MOntereale Valcellina and MAniago, Pordenone, Italy. The study was approved by the Pordenone Hospital Ethics Committee, and each subject received extensive oral and written information on study aims and risks before giving written consent to participate. Exclusion criteria for the current investigation were history or clinical or laboratory evidence of chronic disease including liver failure, renal failure (plasma creatinine > 1.5 mg/dl), thyroid disease, cancer. History of alcohol abuse or self-reported alcohol intake above 50 g/day were also exclusion criteria. Smoking status was also assessed and defined as current smoker, non-smoker or ex-smoker after quitting for more than one year. In all study population, TG, AG, and UG were comparable in the three subgroups and this variable was therefore not included in analyses (not shown).

For data and plasma sample collection, participants were admitted to the outpatient General Medicine wards in Montereale Valcellina or Maniago. A blood sample was collected under postabsorptive conditions after a 10-h overnight fast for measurement of biochemical parameters for diagnosis of metabolic syndrome. A detailed medical examination was also performed, and medical history was collected. Blood pressure was measured using a standard mercury sphygmomanometer, and DBP + 1/3 Diff-BP was defined as mean arterial pressure (MAP), with DBP and Diff-BP as diastolic blood pressure and differential blood pressure, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was measured at the natural indentation between the 10th rib and iliac crest to the nearest 0.5 cm. General characteristics of the whole MoMa population sample have been previously reported [8]. For measurement of plasma ghrelin profile in the current study, individual samples were randomly selected from the lean, overweight and obese population subgroups in proportions of 1:2:2 respectively. Presence of diabetes mellitus, hypertension or dyslipidemia were defined based on clinical history, medications or, respectively, by fasting plasma glucose >126 mg/dl, systolic or diastolic blood pressure >140 or 90 mmHg, plasma triglycerides >150 mg/dl or plasma HDL cholesterol <35 mg/dl in males and 40 mg/dl in females.

2.2. Follow-up

The follow-up study was aimed at assessing changes in hormonal and metabolic profiles in a subgroup of individuals with or without metabolic syndrome. The follow-up group was selected by randomly inviting 200 individuals with and 200 without metabolic syndrome for a 5-year evaluation. 350 individuals (184 with and 166 without metabolic syndrome) responded positively and participated in this study.

2.3. Plasma metabolic profile and ghrelin forms

Plasma glucose, triglycerides, total and high-density lipoprotein (HDL) cholesterol and insulin concentrations were measured at the Analysis Laboratory of Pordenone Hospital, Italy. TG and AG were measured using RIA (Linco, St. Charles, MO) [7]. Intraassay co-efficients of variation were 4% and 4.2% while interassay co-efficients of variation were 7.6% and 8.4%, respectively. Plasma UG

was calculated by subtracting AG from TG. Insulin sensitivity was estimated through the homeostasis model assessment (HOMA) index [7]. The following formula was used: HOMA = (FPG * FPI)/22.5, with FPG and FPI as fasting plasma glucose (mmol) and fasting plasma insulin (μU/ml), respectively.

2.4. Statistical analysis

Data distribution for continuous variables was determined by Shapiro−Wilk test. Statistical similarity between the whole study population and subjects randomly selected for follow-up screening was tested for continuous variables using Student's t test or Mann−Whitney u test as appropriate. Similarity for percentage expressed data was checked by χ-square test. For most parameters including HOMA and BMI and their transformations data distribution showed to be asymmetrical, therefore associations between variables were evaluated by Spearman correlation. In the presence of statistically significant associations ($p < 0.05$), parameters were included in further stepwise multiple linear regression analyses, in order to assess their potential role in the relationships between HOMA, BMI and ghrelin forms. Multiple linear regression analyses were validated by assessing the normality of residuals. Regression coefficients for ghrelin forms, which appear very low in absolute values due to the impact of the different order of magnitude among the variables tested, are presented multiplied by a 1000× factor. P values < 0.05 were considered statistically significant. All analyses were performed using the SPSS v.17 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Basal

Anthropometric and metabolic parameters and plasma ghrelin forms (Table 1) − Anthropometric and metabolic parameters in the whole study population, as well as those of the subgroup undergoing 5-year follow-up evaluation are reported in Table 1. No statistically significant differences between the two groups were observed for any variable.

Associations between HOMA index and plasma ghrelin forms (Tables 2 and 3) − In all subjects, HOMA index was associated positively with BMI, waist circumference, plasma triglycerides and systolic and diastolic blood pressure, while negative associations were observed between BMI and plasma HDL cholesterol (Table 2). HOMA was also associated negatively with plasma TG and UG (Table 2), and these associations remained statistically significant after adjusting for sex, BMI, metabolic syndrome parameters and pharmacological treatments in multiple regression analysis (Table 3). In contrast, no statistically significant associations were observed between HOMA and AG (Table 2).

Associations between BMI and plasma ghrelin forms (Tables 4 and 5) − Relationships between ghrelin forms and anthropometric parameters were also analyzed, in order to further determine potential changes in circulating ghrelin forms with increasing BMI (or waist circumference) and their potential role in BMI-related insulin resistance. In all subjects, BMI was expectedly associated positively with HOMA index and metabolic syndrome parameters (Table 4). Negative correlations were observed between BMI and sex-adjusted plasma TG and UG (r = −0.324, −0.320; Table 4), and these associations were also independent of metabolic syndrome parameters and pharmacological treatments for type 2 diabetes, hypertension or hyperlipidemia in multiple regression analyses (Table 5). Similar associations were also observed for waist circumference (not shown). A weaker (r = − 0.144; Table 4) but statistically significant negative association between BMI and AG