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Original article

Intravenous lipid infusion and total plasma fatty acids positively modulate plasma acylated ghrelin in vivo

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SUMMARY

Background & aims: Ghrelin is a gastric orexigenic hormone whose activating acylation plays a relevant role in the regulation of energy balance. Nutritional modulators of ghrelin acylation and plasma acylated ghrelin (AG) concentration remain however largely undefined. We aimed at investigating whether circulating free fatty acids (FFA) contribute to regulate plasma AG and its ratio (AG/TG) to total hormone (TG).

Methods: Plasma FFA, TG, AG and AG/TG were measured in a primary outpatient care setting in a community-based population cohort of 850 individuals (age 54 ± 10 years, M/F: 408/442) from the North-East Italy MoMa study. 150-min intravenous lipid infusions in rodents (10% lipids, 600 μl/h) were used to investigate the potential causal role of FFA in the regulation of plasma ghrelin profile.

Results: Plasma FFA were associated positively with AG and AG/TG while negatively with TG ($P < 0.01$). Associations between FFA, AG and AG/TG remained statistically significant ($P < 0.02$) in multiple regression analysis including HOMA insulin resistance and metabolic confounders, and both AG and AG/TG but not TG increased through plasma FFA quartiles ($P < 0.01$). Consistent with these findings, intravenous lipid infusion with plasma FFA elevation caused elevations of AG and AG/TG ($P < 0.05$) with no TG modifications.

Conclusions: The current findings demonstrate a novel role for circulating FFA availability to up-regulate plasma AG, which could involve FFA-induced stimulation of ghrelin acylation.

1. Introduction

Ghrelin is a gastric hormone whose activating acylation by ghrelin-o acyl transferase (GOAT) plays a relevant role in the regulation of energy balance [1–3]. The acylated form of ghrelin (AG) has orexigenic effects and has been reported to be associated with unfavorable cardiometabolic risk profile [1–5], while GOAT deletion and AG suppression in animal models may result in protection from diet-induced obesity and its complications [5]. Little is however known on potential modulation of ghrelin acylation by nutritional signals in vivo. Circulating free fatty acids (FFA) are influenced by food intake and nutritional status [6] and are therefore effective energy sensors that could contribute to ghrelin-mediated regulation of food intake and energy balance. Interactions between plasma fatty acids and plasma acylated ghrelin remain however poorly defined. Some [7,8] but not all studies [9] on acute modifications of circulating total FFA in humans reported a negative impact of increasing fatty acids on total circulating ghrelin (TG). Available reports are however limited in number and sample size, studies were performed in lean young volunteers and did not measure acylated hormone [7–9].

In the current investigation we aimed at assessing whether circulating free fatty acids (FFA) contribute to regulate plasma AG and its ratio (AG/TG) to total hormone (TG). To this aim, we measured TG, AG and AG/TG in a community-based population cohort participating in the North-East Italy MoMa epidemiological study [10]. In additional experiments, we further determined the potential causal role of enhanced plasma fatty acid availability by measuring plasma ghrelin profile following 150-min intravenous

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lipid infusion in a rodent model. Long-chain fatty acid (LCFA)-lipid emulsions were employed in rodent experiments since they represent the predominant component of dietary and circulating FFA in humans and rodents [6]. Finally, since appetite-modulating effects have been reported for long-chain n-3 polyunsaturated fatty acids (PUFA) with inconsistent results under different clinical conditions [11–13], lipid emulsions with or without n-3 PUFA enrichment were infused to determine the potential differential impact of n-3 PUFA per se on ghrelin acylation.

2. Materials and methods

2.1. MoMa epidemiological study

Study population – The study participants were recruited in the setting of the MoMa study, a Friuli-Venezia Giulia Region-supported project aimed at investigating the prevalence of metabolic syndrome in MOntereale Valcellina and MAniago, Pordenone, Italy [10]. The study was approved by the Pordenone Hospital Ethics Committee and each subject provided written consent to participation after receiving detailed oral and written information on aims and risks of the study. Exclusion criteria for the current investigation were known diagnosis as well as clinical or laboratory findings indicating liver failure or disease, renal failure (plasma creatinine concentration above 1.5 mg/dl), alcohol abuse or daily alcohol intake above 50 g. Smoking status was also assessed and defined as current smoker, non-smoker or ex-smoker after quitting for more than one year. In the whole study population, TG, AG, and AG/TG were comparable in the three subgroups and this variable was therefore not included in analyses (not shown).

Experimental protocol – On the morning of the study day, each participant was admitted to the General Medicine outpatient wards in Montereale Valcellina or Maniago under postabsorptive conditions. Detailed medical examinations were performed and medical history and blood samples were collected. Blood pressure was measured on both arms with a standard mercury sphygmomanometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (WC) was measured during midrespiration at the indentation between the 10th rib and iliac crest. All variables were measured in duplicate, and the average of two measures was recorded.

Plasma metabolic profile and ghrelin forms – Plasma glucose, triglycerides, total and high-density lipoprotein (HDL) cholesterol and insulin concentrations were measured using standard analytical methods at the Analysis Laboratory of Pordenone Hospital, Italy. Plasma FFA were determined by spectrophotometry (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan). TG (intraassay variation coefficient 4%; interassay variation coefficient 7.6%) and AG (intraassay variation coefficient 4.2%; interassay variation coefficient 8.4%) were measured using RIA (Linco, St. Charles, MO) [7]. Insulin sensitivity was estimated through the homeostasis model assessment (HOMA) index [4]. The following formula was used: HOMA = (FPG*FPI)/22.5, where FPG and FPI are fasting plasma glucose (mmol/L) and fasting plasma insulin (pmol/L), respectively.

2.2. Lipid infusion study

Animals and experimental protocol – The Committee for Animal Studies at Trieste University approved the experimental protocol for rat lipid infusion. 12-week-old male Wistar rats were purchased from Harlan-Italy (n = 28; San Pietro al Natisone, Udine, Italy) and maintained in the Animal Facility of the University of Trieste for 2 weeks in individual cages with a 12-h light–dark cycle (06:00/18:00 h). Intravenous infusions were performed as previously described [14]. 4 h tail-restraint periods were performed each day for three days before the study by securing the tail through a fitting hole in the cage to a horizontal Plexiglass support. Animals had free access to water and standard rat chow during these periods (Harlan 2018, 14.2 kJ/g, Harlan, San Pietro al Natisone, Italy). Identical restraining training and study protocols were applied to all animals in all study groups. On the morning of the study day, infusion catheters were inserted into one tail vein and one tail artery under local lidocaine anesthesia [14]. The tail was then secured as described above, and food was withdrawn and animals were kept in this position for 4 h. Before the start of infusions (T0), blood samples (250 μl) were drawn from the artery and stored at −80 °C for measurement of basal insulin and FFA. Rats were randomly assigned to one of the following 150-min treatments [1]: control, receiving saline infusion (n = 8) [2]; lipid emulsion (Intralipid 10%, Fresenius Kabi) at 600 μl/h with heparin (20 U/h; Epsoclar, Biologici Italia, Novate Milanese, Italia), aimed at elevating circulating FFA through a mixture with saturated, monounsaturated and n-6 PUFA long-chain fatty acids (n = 8) [3]; lipid emulsion (Omegaven 10%, Fresenius Kabi) at 600 μl/h with heparin, aimed at elevating circulating FFA through n-3 PUFA-enriched long-chain fatty acids (n = 8). Lipid emulsions with or without n-3 PUFA enrichment were infused to determine the potential physiological impact of n-3 PUFA per se on acylated ghrelin in vivo, since differential and partly controversial appetite-modulating effects have been reported for n-3 PUFA in different clinical settings [11–13]. Heparin infusion identical to that in the lipid infusion groups was performed in control group. At 150 min (T150), 250 μl blood samples were again taken and plasma was separated and stored at −80 °C.

2.3. Statistical analysis

Data distribution for continuous variables was assessed by Shapiro–Wilk test. Since several parameters including HOMA, FFA, TG, AG and AG/TG did not present a normal data distribution, associations between variables were evaluated by Spearman correlation and log-transformed values were used for further analyses. Statistically associated parameters from univariate analyses ($p < 0.05$) were included in models of stepwise multiple linear regression, to assess their potential involvement in the relationship between FFA and ghrelin forms in the presence of potential confounders. Multiple regression analyses were validated by assessing the normality of residuals. In quartile analyses differences were tested by ANOVA followed by post-hoc pairwise tests with Bonferroni correction and by trend linear regression analysis. Paired t-test was used to compare variables before and following lipid infusions in rodent studies. P values < 0.05 were considered statistically significant. The SPSS v.17 software (SPSS Inc., Chicago, IL) was used for all analyses.

3. Results

Associations between plasma ghrelin forms and anthropometric and metabolic profile (Tables 1 and 2) – Anthropometric and metabolic characteristics of the study population are reported in Table 1. Plasma TG was associated negatively with male gender, age, BMI, waist circumference, plasma triglycerides, glucose and insulin, HOMA index and arterial pressure. Prevalence of diabetes, hypertension and dyslipidemia were also negatively associated with TG, while a positive association was observed between TG and plasma HDL-cholesterol. Weaker negative associations were also observed between AG and male gender, BMI, waist circumference and HOMA index. In contrast, the AG/TG ratio was associated positively with age, BMI, waist circumference, plasma triglycerides, HOMA index, arterial pressure and presence of hypertension and dyslipidemia.

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