

Differential Regulation of Plasma Obestatin and Ghrelin by Meal Intake and the Cholinergic System in Lean, But Not Obese Individuals

Christina Maier, Michaela Riedl, Greisa Vila, Michael Wolzt, Martin Clodi, Bernhard Ludvik, and Anton Luger

Department of Clinical Endocrinology and Metabolism, University Clinics of Medicine III (C.M., M.R., G.V., B.L., A.L.), and Department of Clinical Pharmacology (M.W.), Medical University of Vienna, 1090 Vienna, Austria; and First Medical Department (M.C.), Kaiser-Franz-Joseph Hospital, 1100 Vienna, Austria

Context: Obestatin is cosecreted with and stemming from the same precursor as ghrelin and is apparently involved in energy metabolism. Relatively little is known about the regulation of obestatin release.

Objective: The regulation of obestatin release and obestatin-to-ghrelin ratios by meal intake and the cholinergic system were studied in lean and obese subjects.

Design, Participants, and Setting: We conducted a randomized, double-blind, placebo-controlled, crossover study with 4 study days in eight obese (body mass index >30 kg/m²) and eight matched lean (body mass index <25 kg/m²) healthy subjects (two males and six females per group) at a University Clinical Research Unit.

Interventions: Atropine (1 mg iv) was administered alone and in combination with breakfast (550 kcal) intake, or placebo (isotonic saline) alone and in combination with breakfast.

Main Outcome Measures: We measured plasma obestatin and obestatin/ghrelin ratios.

Results: Both obestatin and ghrelin/obestatin ratios decreased significantly from baseline by either atropine or meal intake in lean individuals, with the two effects adding up on the combined atropine/breakfast day. In contrast, there were no statistically significant differences in obese subjects, who also showed significantly greater association between ghrelin and obestatin values than their lean counterparts.

Conclusions: Obestatin and ghrelin release is differentially regulated by meal intake and the cholinergic system in lean individuals. This regulation is impaired in obesity. (*J Clin Endocrinol Metab* 95: 0000–0000, 2010)

Ghrelin, the octanoylated peptide produced mainly by the stomach and natural ligand to the GH secretagogue-receptor, is now well established as an orexigenic hormone mediating increased food intake resulting in a positive energy balance (1). Obestatin, an amidated cleavage product of the preproghrelin gene, was initially described as opposing the functions of ghrelin on appetite,

resulting in decreased gastric food intake and weight gain in rodents, by binding to its cognate receptor, an (until then) orphan receptor termed g protein coupled receptor 39 (2). These results have been partially confirmed, but also called into question (see Ref. 3 and the references therein).

Obestatin does bind to membranes of pancreatic cells and cell lines with high affinity and promotes survival of

pancreatic β -cells (4), and recently it has been reported to inhibit cerulean-induced acute pancreatitis (5). Functional interplay between ghrelin and obestatin is suggested by data showing that obestatin interacted with ghrelin binding sites on β -cells—albeit with low affinity (4).

Obestatin has also been reported to have anxiolytic effects and to improve memory performance (6) and sleep (7). Obestatin plasma values were associated with elevated blood pressure in the third trimester of pregnancy (8) and were found to be elevated in spontaneously hypertensive rats (9).

Remarkably, obestatin release seems to be regulated differently from ghrelin. Although obestatin plasma concentrations in obese individuals were lower compared with lean controls, just as ghrelin levels, an increased ghrelin to obestatin ratio has been described in obese subjects (10, 11).

We recently found a role of the cholinergic system in the control of the two gut-derived hormones, ghrelin and peptide YY (PYY) (12). In this study, the effects of the unspecific cholinergic antagonist atropine and breakfast ingestion on appetite were studied, as well as ghrelin and PYY plasma concentrations. In lean subjects, atropine was found to suppress ghrelin plasma concentrations to the same amount as breakfast ingestion. There was a significant correlation between plasma ghrelin concentrations and subjective feelings of hunger, and between PYY plasma concentrations and feelings of satiety. All of these effects were impaired in obese subjects.

The aim of this study was to investigate the influence of meal intake and atropine on obestatin plasma concentrations and to study possible short-time changes in ghrelin/obestatin ratio (as an indicator of differential regulation) in lean and obese individuals.

Subjects and Methods

Details of the study protocol have been published elsewhere (12). Briefly, eight obese (body mass index >30 kg/m², mean, 39.6 kg/m²) and eight age- and sex-matched (two male and six female subjects per group) normal-weight (body mass index <25 kg/m²; mean, 22.9 kg/m²) subjects were included. Obese subjects showed significantly higher fasting glucose (94.4 vs. 83 mg/dl) and insulin concentrations (20.3 vs. 10.5 μ U/ml) than their lean counterparts.

The study protocol was approved by the Ethics Committee of the Medical University of Vienna, and written informed consent was obtained from all subjects before study entry.

In a double-blind, randomized, crossover design study, after a 12-h overnight fast, subjects received 1 mg atropine (Atropinum sulfuricum; Nycomed, Vienna, Austria) iv at time point -30 min and a standard 550-kcal breakfast at time point 0 (study day A); atropine and no breakfast (study day B); placebo and breakfast (study day C); or placebo and no breakfast (study day D). Blood samples were drawn at baseline and at timed intervals for measurements of plasma ghrelin and obestatin. Blood was centrifuged at $2500 \times g$, and the supernatant was

separated and stored at -20 C until batch analysis. Plasma ghrelin and obestatin were measured with commercial RIAs (Peninsula Labs, San Carlos, CA; and Phoenix Peptides, Karlsruhe, Germany, respectively). The lower limit of quantification was 5 pg/ml for ghrelin and 2.5 pg/ml for obestatin, with an intraassay coefficient of variation of less than 6%.

Ghrelin/obestatin ratios and differences (Δ -30/90) were calculated

Ghrelin and obestatin plasma concentrations and ghrelin/obestatin ratios were compared using one-way ANOVA, followed by multiple *t* tests with Bonferroni corrections as *post hoc* tests, if appropriate.

Linear regression analysis was performed to study the amount of correlation between obestatin and ghrelin plasma concentrations over the 4 study days together (all data points). The χ^2 test was then used to compare R² values between lean and obese subjects. A *P* value of <0.05 was set as significant. Data are presented as mean \pm SEM, unless stated otherwise.

Results

In lean individuals, obestatin plasma concentrations remained relatively stable from baseline to the end of the study on the placebo day (study day D, time point $+90$ min) (Δ -30/90 = 10.0 ± 9.4 pg/ml); decreased by 35.0 ± 9.8 and 38.75 ± 6.9 pg/ml after atropine administration (study day B) and meal ingestion (study day C), respectively; and decreased by 91.63 ± 10.2 pg/ml on the combined atropine and breakfast day (study day A; Fig. 1A). All differences in Δ -30/90 values, apart from study day B vs. C, were statistically significant (A vs. B, *P* = 0.002; A vs. C, *P* = 0.001; A vs. D, *P* < 0.001 ; B vs. D, *P* = 0.005; C vs. D, *P* = 0.01, multiple *t* tests with Bonferroni correction; Fig. 2A).

In obese individuals, obestatin plasma concentrations remained stable from baseline to the end of the placebo day (study day D, Δ -30/90 = -2.75 ± 6.7 pg/ml) and decreased by 35.57 ± 10.0 and 35.0 ± 15.0 pg/ml after meal ingestion (study day C) and atropine administration (study day B), respectively, and by 33.13 ± 6.24 pg/ml after combined atropine and breakfast (study day D; Fig. 1D). There was a statistically significant difference between the 4 study days, but no difference between any of the single study days (*P* = 0.043, one-way ANOVA; *post hoc* tests, not significant; Fig. 2B).

Obestatin plasma concentrations were not significantly different between the 4 study days at any single time points in either lean or obese subjects, as compared by one-way ANOVA.

Ghrelin plasma concentrations of lean and obese subjects have been published elsewhere (12), and an overview of the time course of these values is given in Fig. 1, B and E, for the sake of comparison. Briefly, there was a signif-

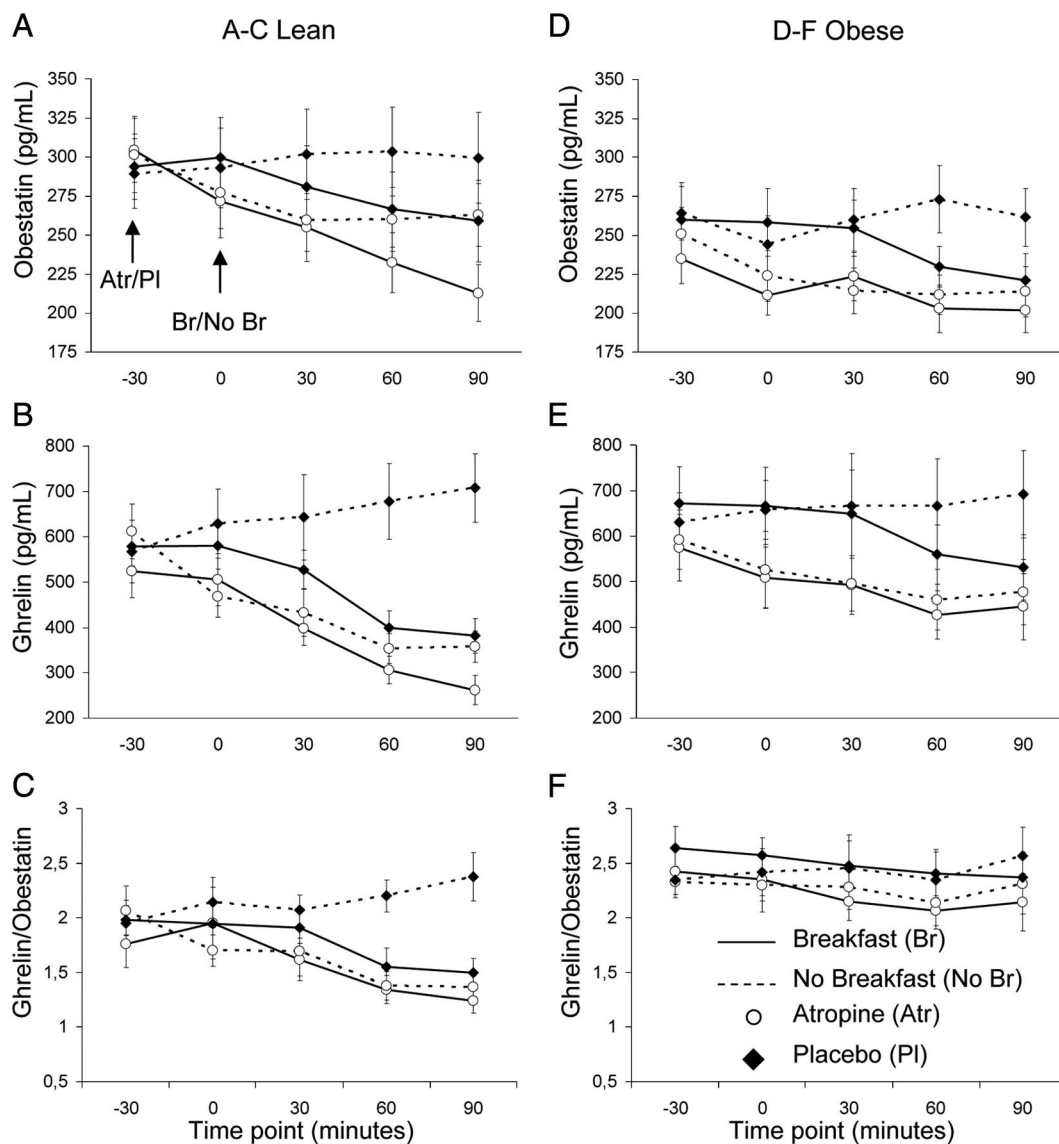


FIG. 1. Obestatin (A and D), ghrelin (B and E) and ghrelin/obestatin ratios (C and F) in lean (A–C) and obese (D–F) individuals on the 4 study days. ○, Atropine at time point –30 min; ◆, placebo; solid lines, breakfast at time point 0; dashed lines, no breakfast.

icant decrease of ghrelin plasma concentrations on all 3 study days compared with placebo day—with no significant difference between the intervention days—in lean individuals. In contrast, in the obese individuals, only Δ -30/90 values differed significantly between study days C (breakfast) and D (placebo).

Ghrelin/obestatin ratios in lean individuals decreased continually on all intervention days (Fig. 1C), resulting in statistically significant differences from placebo at time points +60 and +90 min (+60 min, $P = 0.001$; study day A vs. D, $P = 0.001$; B vs. D, $P = 0.002$; C vs. D, $P = 0.022$; and +90 min, $P = 0.001$; study day A vs. D, $P < 0.001$; B vs. D, $P < 0.001$; C vs. D, $P = 0.002$; one-way ANOVA, followed by multiple t tests with Bonferroni correction).

In obese individuals, ghrelin/obestatin ratios remained stable on all 4 study days (Fig. 1F); there were no statis-

tically significant differences between the 4 study days at any time point.

When comparing relative changes in ghrelin/obestatin ratios throughout the study days (Δ -30/90) there was a significant difference between the study days in the lean individuals (Fig. 2A, $P = 0.001$, one-way ANOVA), and all three intervention days were significantly different from the placebo day (study day A vs. D, $P = 0.005$; B vs. D, $P = 0.001$; C vs. D, $P = 0.007$, multiple t tests with Bonferroni correction).

There were no significant differences in obese individuals (Fig. 2B).

Regression analysis showed a significant correlation between ghrelin and obestatin values in both lean and obese individuals ($R^2 = 0.680$, obese, $P < 0.001$; $R^2 = 0.361$, lean, $P < 0.001$), with significantly more variability

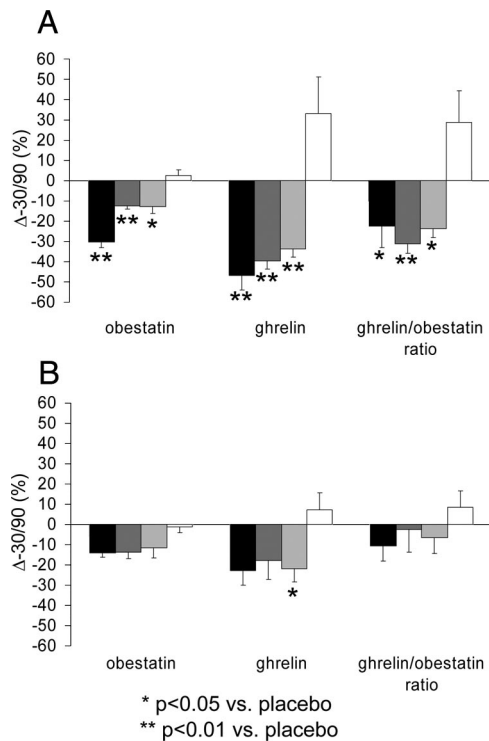


FIG. 2. Differences between baseline values and values at time point +90 min (= Δ -30/90) for obestatin, ghrelin, and ghrelin/obestatin ratio in lean (A) and obese (B) subjects on the 4 study days. *Black*, Atropine (at time point -30 min) + breakfast (at time point 0); *dark gray*, atropine, no breakfast; *light gray*, placebo + breakfast; *white*, placebo, no breakfast. Statistics were calculated for absolute values, but values are presented as relative (percentage) changes for the sake of comparison. **, $P < 0.01$; *, $P < 0.05$.

of obestatin values explained by ghrelin variability in obese than in lean individuals (obese *vs.* lean, $P < 0.001$, χ^2 test).

In both lean and obese subjects, atropine administration led to a significant increase in heart rate that was not significantly different between study groups (see Ref. 12 for details).

Discussion

In this study, the plasma changes of obestatin and ghrelin/obestatin ratio were used to identify differential patterns of regulation by cholinergic inhibition and/or meal intake in obese and lean subjects. In the data presented here, obestatin plasma concentrations in lean subjects showed a significant decrease from baseline to time point +90 min on all 3 study days compared with the placebo day. Interestingly, obestatin levels dropped to a significantly greater amount in response to the combination of atropine and breakfast than to any of the two treatments alone. Thus, the effect of atropine and breakfast on obestatin plasma concentrations seems to be additive, indicating independent regulation by nutrients and the cholinergic sys-

tem. In contrast, ghrelin plasma concentrations were suppressed by atropine, breakfast, and the combination of both substances to the same amount, in a nonadditive manner (Figs. 1, A and B, and 2A) (12).

Both ghrelin and obestatin plasma concentrations decreased after meal ingestion and/or atropine administration, but the ghrelin/obestatin ratio also showed a significant decline; these data are in agreement with a recent paper showing a significant decline of ghrelin/obestatin ratio in fasted rats refed with a chow diet (13).

These changes were completely absent in obese individuals, with no significant differences or changes in either obestatin concentrations or ghrelin/obestatin ratios (Figs. 1, D and F, and 2B). Moreover, there was a significantly stronger correlation between ghrelin and obestatin plasma concentrations in obese than in lean individuals, again pointing in the direction of a possible loss of independent regulation in obesity.

Obestatin plasma concentrations and ghrelin/obestatin ratio have been studied in the context of obesity (11, 14, 15) and weight loss (10, 11, 15, 16) as well as states of cachexia (17), anorexia (18, 19), and bulimia (19). Obestatin plasma concentrations were reported to be stable after Roux-en-Y gastric bypass surgery with significantly reduced ghrelin/obestatin ratio (16), and obestatin was shown to increase in obese children upon weight loss (15). On the other hand, both obestatin and ghrelin/obestatin ratio were increased in anorexia nervosa, but not bulimia nervosa (19).

A few studies also examined the effect of food intake on plasma obestatin concentrations; whereas the initial paper (2) reported no changes around a fixed meal, in two other papers (11, 20), both obestatin and ghrelin/obestatin ratios were reduced after meal intake.

Together with the data presented here, these results show that obestatin, although cosecreted with and derived from the same precursor as ghrelin, is regulated in its own way. Whether this regulation is carried out at the production, release, or clearance level cannot be differentiated with the study design used here, yet the finding that—like ghrelin and PYY—obestatin is apparently regulated by the cholinergic system is another indicator of its possible role in appetite regulation.

In summary, we found evidence for differential regulation of ghrelin and obestatin plasma concentrations by meal intake and atropine administration in lean individuals, which was disturbed in obesity.

Acknowledgments

Address all correspondence and requests for reprints to: Dr. Christina Maier, University Clinics of Medicine III, Clinical Department of Endocrinology and Metabolism, Medical University

of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
E-mail: Christina.maier@meduniwien.ac.at.

Disclosure Summary: The authors have nothing to disclose.

References

- van der Lely AJ, Tschöp M, Heiman ML, Ghigo E 2004 Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev* 25:426–457
- Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ 2005 Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 310:996–999
- Annemie VD, Debby VD, Valentijn V, Bart de S, Walter L, Liliane S, Peter Paul de D 2009 Central administration of obestatin fails to show inhibitory effects on food and water intake in mice. *Regul Pept* 156:77–82
- Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, Nano R, Annunziata M, Campiglia P, Arnoletti E, Ghè C, Volante M, Papotti M, Muccioli G, Ghigo E 2008 Obestatin promotes survival of pancreatic β -cells and human islets and induces expression of genes involved in the regulation of β -cell mass and function. *Diabetes* 57:967–979
- Ceranowicz P, Warzecha Z, Dembinski A, Cieszkowski J, Dembinski M, Sendur R, Kusnierz-Cabala B, Tomaszewska R, Kuwahara A, Kato I 2009 Pretreatment with obestatin inhibits the development of cerulein-induced pancreatitis. *J Physiol Pharmacol* 60:95–101
- Carlini VP, Schiöth HB, Debarioglio SR 2007 Obestatin improves memory performance and causes anxiolytic effects in rats. *Biochem Biophys Res Commun* 352:907–912
- Szentirmai E, Krueger JM 2006 Obestatin alters sleep in rats. *Neurosci Lett* 404:222–226
- Ren AJ, He Q, Shi JS, Guo ZF, Zheng X, Lin L, Wang YK, Xia SY, Sun LL, Du X, Sun Y, Zhang LM, Yuan WJ 2009 Association of obestatin with blood pressure in the third trimester of pregnancy. *Peptides* 30:1742–1745
- Li ZF, Guo ZF, Cao J, Hu JQ, Zhao XX, Xu RL, Huang XM, Qin YW, Zheng X 2010 Plasma ghrelin and obestatin levels are increased in spontaneously hypertensive rats. *Peptides* 31:297–300
- Gao XY, Kuang HY, Liu XM, Wang XY, Pan YH, Ma XX 2008 Decreased obestatin in plasma in metabolically obese, normal-weight men with normal glucose tolerance. *Diabetes Res Clin Pract* 79:e5–e6
- Guo ZF, Zheng X, Qin YW, Hu JQ, Chen SP, Zhang Z 2007 Circulating preprandial ghrelin to obestatin ratio is increased in human obesity. *J Clin Endocrinol Metab* 92:1875–1880
- Maier C, Riedl M, Vila G, Nowotny P, Wolzt M, Clodi M, Ludvik B, Luger A 2008 Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity. *Diabetes* 57:2332–2340
- Guo ZF, Ren AJ, Zheng X, Qin YW, Cheng F, Zhang J, Wu H, Yuan WJ, Zou L 2008 Different responses of circulating ghrelin, obestatin levels to fasting, re-feeding and different food compositions, and their local expressions in rats. *Peptides* 29:1247–1254
- Vicennati V, Genghini S, De Iasio R, Pasqui F, Pagotto U, Pasquali R 2007 Circulating obestatin levels and the ghrelin/obestatin ratio in obese women. *Eur J Endocrinol* 157:295–301
- Reinehr T, de Sousa G, Roth CL 2008 Obestatin and ghrelin levels in obese children and adolescents before and after reduction of overweight. *Clin Endocrinol (Oxf)* 68:304–310
- Roth CL, Reinehr T, Scherthaner GH, Kopp HP, Kriwanek S, Scherthaner G 2009 Ghrelin and obestatin levels in severely obese women before and after weight loss after Roux-en-Y gastric bypass surgery. *Obes Surg* 19:29–35
- Xin X, Ren AJ, Zheng X, Qin YW, Zhao XX, Yuan WJ, Guo ZF 2009 Disturbance of circulating ghrelin and obestatin in chronic heart failure patients especially in those with cachexia. *Peptides* 30:2281–2285
- Germain N, Galusca B, Grouselle D, Frere D, Tolle V, Zizzari P, Lang F, Epelbaum J, Estour B 2009 Ghrelin/obestatin ratio in two populations with low bodyweight: constitutional thinness and anorexia nervosa. *Psychoneuroendocrinology* 34:413–419
- Monteleone P, Serritella C, Martiadis V, Scognamiglio P, Maj M 2008 Plasma obestatin, ghrelin, and ghrelin/obestatin ratio are increased in underweight patients with anorexia nervosa but not in symptomatic patients with bulimia nervosa. *J Clin Endocrinol Metab* 93:4418–4421
- Sedlackova D, Dostalova I, Hainer V, Beranova L, Kvasnickova H, Hill M, Haluzik M, Nedvidkova J 2008 Simultaneous decrease of plasma obestatin and ghrelin levels after a high-carbohydrate breakfast in healthy women. *Physiol Res* 57(Suppl 1):S29–S37