

Cholinergic regulation of ghrelin and PYY release may be impaired in obesity

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Objective: Ghrelin and PYY are both hormones derived from the gastrointestinal tract involved in appetite regulation. The cholinergic part of the vagal nerve is involved in the regulation of glucose and insulin. The aim of the study was to examine the effects of the cholinergic antagonist atropine on ghrelin, PYY, glucose and insulin under basal conditions and after meal ingestion in lean and obese subjects.

Research design and Methods: 8 lean and 8 obese subjects in a randomized, double-blind, placebo controlled crossover study design with four study days in randomized order: Atropine/placebo +/- breakfast. Plasma ghrelin, PYY, insulin and glucose were measured. Hunger and satiety feelings were rated on 10 cm visual analog scales.

Results: In lean individuals atropine led to a decrease in ghrelin concentrations comparable to and non-additive with breakfast ingestion and to a significant decrease in both basal and meal induced PYY concentrations. In obese subjects atropine did not significantly change ghrelin or PYY concentrations whereas it induced a comparable increase in heart rate and in meal induced glucose concentrations in the two study groups. Only lean, but not obese subjects experienced sustained feelings of satiety after breakfast.

Conclusions: The impaired cholinergic regulation of the postprandial drop in ghrelin concentrations and rise in PYY concentrations might be part of the deregulated food intake in obese subjects.

Energy homeostasis is a tightly regulated process involving hormone signaling from the periphery via vagal afferents to the hindbrain (namely the nucleus tractus solitarius, NTS) and the hypothalamus (especially the nucleus arcuatus, ARC) where these signals are integrated with informations from other brain regions and processed to convey information to the periphery via the sympathetic nervous system and the efferent part of the vagal nerve (1; 2). Peripheral organs sending and receiving information to and from the brain include the stomach and intestine, the pancreas, and the adipose tissue (3). The latter has been a main focus of interest over the last decade, driven by the discovery of leptin, and subsequently other adipokines (4).

A renewed interest in the regulation of appetite via a gut-brain interaction came with new findings about two hormones: ghrelin and peptide YY (PYY).

Ghrelin is the natural ligand of the GH secretagogue receptor (GHS-R) and is produced mainly in the stomach (5). Although initially characterized as a potent GH releasing hormone ghrelin very soon turned out to be also a potent orexigenic peptide (6). Ghrelin is involved in both short- (7; 8) and long-term appetite regulation (6; 9) and seems to exert its appetite-regulating effects mainly at the hypothalamic level (10). Two hypothalamic regions have been shown to be targeted by ghrelin, the arcuate nucleus and the lateral hypothalamus. Food intake induced by central administration of ghrelin has been shown to be mediated via activating Neuropeptide Y/Agouti-related protein (NPY/AGRP) neurons (11). Thus, ghrelin antagonizes the actions of leptin on the hypothalamus. On the other hand, ghrelin has also been shown to interact with the orexin pathway at the lateral hypothalamus (12).

Peptide YY or PYY, named for the two tyrosine residues on the C and N terminal

termini of its 36 amino acid structure, is produced by endocrine L cells mainly in the terminal ileum and colon and coexpressed in these cells with GLP-1(13). PYY levels rise after ingestion of a high caloric meal (but before nutrients reach the ileum) and remains elevated for at least 120 min(14). It has been characterized as an agent inhibiting gastrointestinal motility(15) but the anorexigenic effect of the active form PYY(3-36) has been shown only recently in humans(16). Upon infusion of PYY(3-36) subsequent food consumption was reduced in both lean and obese subjects (17). Although a subsequent study challenged these first results(18) and other data suggested that this effect can only be sustained by a carefully chosen intermittent infusion scheme to prevent compensatory hyperphagia(19) the fact that PYY is able to reduce caloric intake raised hopes that a new anti-obesity treatment could have been found. PYY levels are reported to be reduced(17) or unaltered(20) in obesity and increased in anorexia nervosa(20).

Only few data exist about the regulation of ghrelin and PYY release from the gut. One main factor influencing ghrelin plasma levels is food intake: shortly after oral glucose load ghrelin levels fall significantly(21), whereas neither gastric distension(6) nor rises in plasma glucose or insulin levels alone (22) can suppress ghrelin release. Ghrelin levels also rise anticipatory to meal initiation in both humans (23) and sheep(24) and it has been suggested that this rise is elicited centrally and mediated via the vagal nerve to the stomach mucosa(24). In vagotomized rats, baseline ghrelin levels and suppression of ghrelin levels by nutrient load were unaltered, but increase of ghrelin levels induced by 48h food deprivation was abolished completely and this result was mimicked by treatment with the unspecific cholinergic antagonist atropine(25). In a group of young healthy

human volunteers atropine promptly and significantly decreased ghrelin plasma concentrations after an overnight fast(26).

PYY, on the other hand, increases after meal intake with a maximum reached after 1 - 2 h that is dependent on the amount of calories(27), in particular fat(28), ingested. Recent data indicate that PYY increase in response to meals is impaired in obesity(27; 29). The time course of PYY release (before nutrients reach the colon) suggests neuronal control, and animal data showed an atropine-sensitive cholinergic pathway to be involved(30). To our knowledge the effect of atropine on PYY concentrations has not been tested in humans.

In the study presented here we addressed the following questions: Does atropine decrease plasma ghrelin concentrations to the same amount as eating a standard meal? Is this effect additive to meal induced ghrelin suppression? Is there any correlation between atropine effects on heart rate and ghrelin? How does atropine interact with PYY? Is there any association with subjective ratings of hunger and satiety? And are there any different results in obese subjects?

RESEARCH DESIGN AND METHODS

Subjects. 8 overweight (OB, BMI > 30 kg/m²) and 8 normal weight control subjects (CO, BMI < 25 kg/m²) were recruited from the obesity outpatients clinic, and from hospital staff. All of them were nonsmokers; none of the CO were taking any medication (with the exception of oral contraceptives); one of the OB subjects was on antidepressant and antihypertensive therapy. All subjects underwent prestudy screening and had normal findings in laboratory, ECG and physical examination. All subjects underwent a 3h oral glucose tolerance test (3h-oGTT, 75g glucose). The study protocol had been approved by the Ethics Committee of the Medical University of Vienna, and all

subjects had given informed consent before study entry.

Study design and schedule. The study was conducted as a prospective, randomized, single-blind, placebo-controlled crossover study. For each subject, four study days (A-D) were scheduled in randomized order with at least 3-day washout intervals.

On study days subjects arrived between 8.00 and 11.00 after an overnight fast. Studies were conducted in a quiet room with an ambient temperature of 22°C. Subjects abstained from alcohol and stimulating beverages containing caffeine 12 h before the study days.

A plastic cannula (Venflon[®]) was inserted into an antecubital vein at time point -45 min. Blood samples were drawn at time points -30, -15, 0, 15, 30, 60 and 90 min for measurements of plasma ghrelin, PYY, insulin and glucose. At the same time points (plus time points +45 and +75 min) subjects rated their hunger and satiety feelings on 10 cm visual analog scales.

At time point -30, 1 mg atropine (Atropinum sulfuricum "Nycomed"[®], Nycomed, study days A and B) or placebo (isotonic saline, study days C and D) was given intravenously over 30 sec.

At time point 0 subjects received a standard breakfast consisting of 2 rolls with 15 g butter and 250 ml milk with 10 g commercially available cocoa mix ("Benco"[®], Suchard), total calorie content 590 kcal, total fat content 23 g, total carbohydrate content 75 g, total protein content 18.5 g, on study days A and C only. Researchers and volunteers were blinded to atropine/placebo dosage, and, until time point 0, to breakfast/no breakfast.

Blood pressure and pulse rate was monitored during the study period using automated devices and recorded at time points -30, -29, -27, -25, -20, -15, 0, +15, +30, +45, +60, +75, and +90 min.

Laboratory monitoring. Samples for plasma hormone measurements were centrifuged

immediately at 4°C and the supernatants stored at -30°C until analysis.

Insulin levels (μU/ml) were assayed by a commercially available RIA (Pharmacia-Upjohn, Uppsala, Sweden). Blood glucose was determined according to standard laboratory procedures.

Plasma ghrelin (pg/mL) was measured with a commercial RIA (Peninsula Labs, San Carlos, CA) that uses I-125-labeled bioactive ghrelin as a tracer and polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. The inter- and intraassay variations were both less than 10.9 %. PYY (pg/mL) was measured using a commercial RIA (Linco Research, St. Charles, MO) with inter- and intraassay variations being 8.2% and 9% respectively.

Statistical analysis. Homeostatic model assessment (HOMA) model index was calculated using HOMA calculator 2.2 (www.dtu.ox.ac.uk). Oral glucose sensitivity index (OGIS) was calculated according to the formula published in (31) using OGIS calculator

(www.ladseb.pd.cnr.it/bioing/ogis/home.html). Hormone concentrations at single time points and Δ hormone levels, heart rate and VAS values at the four different study days were compared with one-way ANOVA followed by multiple t-tests with Bonferroni correction as post-hoc statistics, if appropriate. Baseline parameters and heart rate response between the two groups were compared with unpaired student's t-test. Linear regression analysis was performed to evaluate the association (or lack of) between parameters, as indicated. SPSS release 12.0.1 was used as statistical software. P < 0.05 was considered statistically significant. Results are presented as mean ± S.E.M.

RESULTS

Baseline characteristics of the subjects are shown in table 1. The two groups were well matched for age, gender distribution and

height. Apart from having a significantly higher BMI (group defining criterion) OB subjects had significantly higher fasting glucose and insulin levels and were significantly more insulin resistant according to both HOMA and OGIS indices. Three OB subjects had impaired glucose tolerance according to their 120min glucose concentrations (range 143-155 mg/dL).

Ghrelin. Ghrelin concentrations on the four study days are given in Fig. 1a (controls, CO) and 1b (obese, OB).

In CO both atropine and meal ingestion led to a decrease of ghrelin levels. There was a significant difference between ghrelin concentrations on the different study days at time points +30, +60, and +90 min (as compared by one-way ANOVA). When comparing differences between baseline and +90 min ghrelin concentrations (Δ ghrelin -30/+90) values of the three study days were significantly different from the placebo day without breakfast; the study days (A: atropine + breakfast, B: atropine alone, C: breakfast alone) did not differ significantly from each other (Fig. 1c).

In OB atropine had no effect on ghrelin concentrations. When compared with ANOVA ghrelin concentrations did not differ significantly at any single time point on the four different study days (Fig. 1b); when comparing Δ ghrelin -30/+90 values only breakfast had a significant effect on ghrelin concentrations (Fig. 1d).

PYY. PYY concentrations on the four study days are given in Fig. 2a (controls) and 2b (obese).

In the control group atropine (study day B) led to a significant decrease of PYY concentrations at timepoint +90 min as compared to breakfast alone (study day C). Using Δ-30/+90 values (differences between baseline and +90 min PYY concentrations) atropine led to a significant decrease of PYY concentrations as compared to all other study

days which did not differ significantly from each other (Fig. 2c).

In obese subjects, there were no significant differences between any of the study days at all, neither at single time points nor between the Δ -30/+90 values (Fig. 2b and 2d, respectively).

Heart rate, glucose and insulin. Heart rates on the four study days are given in Fig. 3a (CO) and Fig. 3b (OB).

Atropine led to a significant increase in heart rate in both obese and lean subjects (baseline values $72.0 \pm 3 \text{ min}^{-1}$, CO vs. $74.5 \pm 2 \text{ min}^{-1}$, OB, $p = 0.5$) with peak values at time point -25min (5 min after atropine application, $100.3 \pm 7 \text{ min}^{-1}$, CO vs. $97.8 \pm 2 \text{ min}^{-1}$, OB, $p = 0.7$). Thus, heart rate increase between baseline and time point -25min (Δ -30/-25) was comparable in lean and obese subjects ($23.25 \pm 1.8 \text{ min}^{-1}$ vs. $28.25 \pm 5.8 \text{ min}^{-1}$, $p = 0.42$, unpaired students t-test).

Plasma glucose and insulin concentrations are given in Fig. 4.

Atropine alone did not change plasma insulin or glucose concentrations as compared to placebo (study days C and D). Meal induced glucose increase was significantly affected by atropine only in OB. Plasma glucose in CO between study days A and B were not significantly different at any single time points as was glucose difference between baseline and timepoint +30 min (Δ -30/+30) ($+10.8 \pm 4.5 \text{ mg/dL}$ vs. $-1.8 \pm 1.2 \text{ mg/dL}$, $p = 0.178$). Insulin concentrations between study days A and B were significantly different at time points +60 and +90 min as was the difference between baseline and timepoint +30 min (Δ -30/+30) ($23.3 \pm 9.4 \text{ } \mu\text{U/mL}$ vs. $-2.2 \pm 0.9 \text{ } \mu\text{U/mL}$, $p = 0.003$).

In OB atropine significantly reduced meal induced blood glucose at time point +60 min ($p = 0.003$). Glucose difference between baseline and timepoint +30 min (Δ -30/30) was not significantly different between study days A and B (5.1 ± 1.7 vs. $-3.8 \pm 1.7 \text{ mg/dL}$, $p = 0.18$). Insulin levels were significantly

different between study days A and B at time point +60 min ($p = 0.001$). Δ -30/+30 values were different between study days A and B, but this difference did not reach statistical significance in the ANOVA analysis ($23.3 \pm 9.4 \text{ } \mu\text{U/mL}$ vs. $0 \pm 0.4 \text{ } \mu\text{U/mL}$, $p = 0.133$).

Visual analog scales. An overview of the time course of hunger and satiety visual analog scales (VAS) is given in fig. 5a and c (CO) and 5b and d (OB), respectively.

Meal ingestion alone and in combination with atropine led to sustained decreases in hunger ratings and increase in satiety ratings in lean individuals. Hunger scores tended to be lower and satiety scores were higher on atropine alone versus placebo days yet this difference did not reach statistical significance in the ANOVA analysis. When differences between baseline and timepoint +90 (Δ -30/+90) were compared (fig. 5e) both hunger and satiety Δ -30/+90 values for all breakfast days were significantly different from all days with no breakfast but atropine and placebo days did not significantly differ from each other.

In obese subjects hunger ratings tended to be higher and satiety ratings tended to be lower on atropine compared to placebo days (fig. 5b, again not statistically different for any single time point in the ANOVA analysis). Hunger scores on breakfast days started to slowly increase after reaching a nadir value directly after meal ingestion. Consequently there were no statistical differences between any of the study days in Δ -30/+90 values. When comparing Δ -30/+90 values for satiety VAS only study days 2 vs. 3 differed significantly from each other ($p = 0.012$), all other values were not statistically different from each other (fig. 5f).

Correlations. In lean subjects there was a significant relationship between Δ heart rate (-30/-25) and Δ ghrelin (-30/90) as revealed by linear regression analysis ($R^2 = 0.373$, $p = 0.0002$, Fig 6a). There was also a significant relationship between Δ heart rate (-30/-25) and

Δ PYY (-30/90) ($R^2 = 0.227$, $p = 0.0058$, fig. 6c). In obese subjects, there was no relationship between Δ heart rate (-30/-25) and Δ ghrelin (-30/90) ($R^2 = 0.042$, $p = 0.27$, fig. 6b) and a weak but significant relationship between Δ heart rate (-30/-25) and Δ PYY (-30/90) ($R^2 = 0.136$, $p = 0.041$, fig. 6d).

In lean subjects Δ ghrelin (-30/90) showed a significant correlation with Δ VAS hunger (-30/90) ($R^2 = 0.178$, $p = 0.016$, fig. 6e) and Δ PYY (-30/90) correlated significantly with Δ VAS satiety (-30/90) ($R^2 = 0.294$, $p = 0.002$, fig. 6g) while there was no significant correlation in obese subjects (fig. 6f and 6h, respectively).

Insulin resistance as quantified by HOMA and OGIS indices was not significantly correlated with ghrelin and PYY changes induced by atropine or breakfast (data not shown).

DISCUSSION

Several effects of ghrelin and PYY are conveyed by the cholinergic autonomous nervous system, and signaling from the gut to the brain is thought to be mediated in part by cholinergic fibers of the vagal nerve(32). We demonstrate in this study that in lean, healthy humans also ghrelin and PYY release from the gut is controlled by the cholinergic system. Ghrelin concentrations are suppressed by the unspecific muscarinic inhibitor atropine to the same amount as by meal ingestion in a non-additive manner, and PYY release is significantly reduced after atropine application as compared to placebo. This suppression of hormone release strongly correlates with the amount of heart rate increase induced by atropine.

In contrast, in obese subjects there was no clear evidence of an influence of the cholinergic system on ghrelin or PYY release. There were no significant differences in hormone concentrations at any single time points or in Δ -30/+90 values and only a weak association between PYY and heart rate

increase, while there was no correlation between heart rate and ghrelin differences, although all 4 study days were included in the regression analysis to enhance data spreading. Despite this lack of effect on gut hormone release atropine induced heart rate increase in obese subjects was comparable to lean subjects. The PYY data are somewhat limited by the higher variability of baseline levels between study days and the correlation data are not as clear as those for ghrelin. Nevertheless, our data show that not the response to muscarinic inhibition per se, but the cholinergic control of orexigenic ghrelin and (to a lesser extent) anorexic PYY release is disturbed in obesity.

These results are well in line with the large body of evidence showing adrenergic sympathetic dysregulation of gut hormone, in particular PYY release in obesity(33).

In parallel to the lack of cholinergic gut control there was also a disturbed sensing of hunger and satiety in response to meals in the obese subjects as compared to their lean counterparts. Visual analog scales (VAS) showed that the differences in hunger ratings between baseline and postprandial states were not statistically significantly different between any study days (although they tended to be lower on breakfast than on non-breakfast days), while lean subjects reported a sustained suppression of hunger feelings at the end of the study period (90 min after breakfast) on breakfast compared to non breakfast days. To a lesser extent, the same could be observed for ratings of satiety with statistically significant differences between all breakfast vs. non breakfast days in lean and ratings being only different between study days 2 (atropine alone) and 3 (breakfast alone) in obese subjects (although they also tended to be lower on the other breakfast day than on non-breakfast days). Moreover, in lean subjects there was a correlation between Δ (-30/90) values for hunger ratings and ghrelin, and satiety ratings and PYY,

respectively, (as would be expected when all four study days are included in the analysis), but surprisingly again a complete lack of association in obese subjects.

Actually only part of the circulating ghrelin seems to be associated to feelings of appetite, namely the active (acylated) ghrelin, which in the circulation becomes rapidly degraded and inactive. Thus, since we measured only total (acylated plus deacylated) ghrelin, our results relate mainly to changes in ghrelin release and the extension to feelings of hunger and satiety must be regarded with caution. It is possible that differences in ghrelin deacylation between lean and obese subjects rather than the differences in ghrelin release were responsible for the observed lack of correlation in obese subjects. Nonetheless, also ghrelin release – as shown by the changes in the surrogate parameter total ghrelin – seems to be remarkably different in lean and obese subjects.

It has been proposed that the blunted ghrelin response to meal ingestion could be responsible for the development of obesity in adolescents(34). While in the absence of prospective data it is impossible to decide whether this blunted hormone response is cause or consequence of the disturbed eating behavior in obese subjects it has been shown that improved sensing of meal induced hunger suppression is associated with increased postprandial ghrelin drop in subjects losing weight(35). Similarly it is impossible to know whether the disturbed cholinergic gut hormone regulation was present before the subjects studied here became obese or whether the deregulation of the system developed as a consequence of overeating. Even if cholinergic regulation was the primary factor in gut hormone release, feelings of hunger and satiety are of course not solely driven by changes in gut hormone levels alone: Although atropine caused a comparable drop in ghrelin levels and increase in PYY levels as breakfast ingestion,

there was no accompanying drop in hunger or increase in satiety ratings in lean subjects. Yet even if a disturbed hormone regulation would lead to only a slight impairment in sensing of hunger and satiety resulting in a small but daily additional calorie intake this would ultimately considerably contribute to weight gain in the long term.

To our knowledge there are no prospective data on the involvement of either gut hormone alterations or impairment of autonomic regulation in the etiology of obesity, and although it seems by now clear that the ample availability of energy dense food is part of the epidemic it is currently unclear why some people contract obesity while others seem to be resistant.

However, there are some data to support the hypothesis of cholinergic pathways being a crucial part of appetite regulation: M3 muscarinic receptor knockout mice are reported to be hypophagic and lean as compared to their wild type littermates(36). The M3 receptor is expressed in the lateral hypothalamus, and the MCH containing neurons of this area are apparently responsible for the M3 knockout phenotype. Interestingly, these same neurons also receive projections from AGRP/NPY neurons in the medial hypothalamus(37) (where ghrelin exerts its central effects on appetite).

The obese subjects of this study were also hyperinsulinemic and insulin resistant as compared to their lean counterparts. It has been shown that cholinergic regulation is an important part of meal induced insulin release(38). The M3 knockout mice mentioned above were reported to have improved glucose tolerance despite a blunted increase in serum insulin after oral glucose load that was only partly explained by their leanness; in vitro studies showed a lack of cholinergic stimulation of insulin release from pancreatic islets of these mice(39). A genetic variant of the M3 receptor has been associated

with an increased risk for developing type 2 diabetes in Pima Indians(40). Atropine has been shown repeatedly to alter meal induced glucose increase in humans(38; 41-43) as was the case in the subjects studied here. One of these studies (42) reported a greater postprandial attenuation of insulin in obese as compared to lean subjects. We report for the first time in this study that, while the alterations of meal induced insulin and glucose release by atropine were largely comparable in lean and obese subjects, their atropine induced regulation of gut hormone release was markedly different.

Insulin resistance as quantified by HOMA and OGIS indices was not significantly correlated with ghrelin and PYY changes induced by atropine or breakfast in this study sample. In line with our data it has been shown in lean but insulin resistant Pima Indians that postprandial early phase insulin release was inhibited by atropine but not to the same amount as that of pancreatic polypeptide and it was concluded that the

hyperinsulinemia in this population was not due to increased vagal input to the pancreatic beta cell(43). Prolonged glucose infusion in lean healthy humans resulted in vagally mediated compensatory increase in C peptide secretion, but not in alterations of hunger ratings or food intake(44). These data argue against insulin resistance being the driving force of the disturbed cholinergic control observed in this group of obese subjects.

Taken together the data presented here show that 1) in lean individuals the cholinergic system is involved in the regulation of gut hormone release 2) gut hormone release is associated with subjective ratings of hunger and satiety 3) this regulatory system is markedly impaired in obese subjects. At present, however, it is not clear whether this impaired regulation of gut hormone release actually contributes to the impaired sensing of hunger and satiety seen in these obese subjects and might actively have contributed to their weight gain and/or hinder them losing weight.

Table 1

Parameter	Lean	Obese	p
Age (years)	28.7 ± 2.4	29.1 ± 2.0	n.s.
Gender distribution (f/m)	6/2	6/2	n.s.
Height (cm)	173.6 ± 3.5	169.1 ± 4.0	n.s.
Weight (kg)	69.3 ± 4.2	113.1 ± 6.9	< 0.0001
BMI (kg/m ²)	22.9 ± 1.0	39.6 ± 2.2	< 0.0001
Waist/hip ratio	0.77 ± 0.01	0.89 ± 0.03	0.01
Fasting glucose (mg/dL)	83 ± 2.1	94.4 ± 2.6	0.005
Fasting insulin (μU/mL)	10.5 ± 1.5	20.3 ± 3.2	0.021
HOMA	2.58 ± 0.4	5.24 ± 0.3	< 0.0001
OGIS	454.3 ± 12.7	379.7 ± 26.1	0.028

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Fig. 1: The effect of atropine (1 mg i.v. at time point -30 min.) and breakfast (550 kcal at time point 0) on ghrelin plasma concentrations in lean (CO) and obese subjects (OB)

Fig. 1a and b: Solid lines = with breakfast, dotted lines = without breakfast; open symbols = with atropine, filled symbols = with placebo

Fig. 1 c and d: asterisks = study days significantly different from placebo day

Fig. 1a Plasma ghrelin concentrations in CO on the four study days

Fig. 1b Plasma ghrelin concentrations in OB on the four study days

Fig. 1c Differences in baseline and postprandial ghrelin concentrations (Δ -30/+90) on the four study days in CO

Fig. 1d Differences in baseline and postprandial ghrelin concentrations (Δ -30/+90) on the four study days in OB

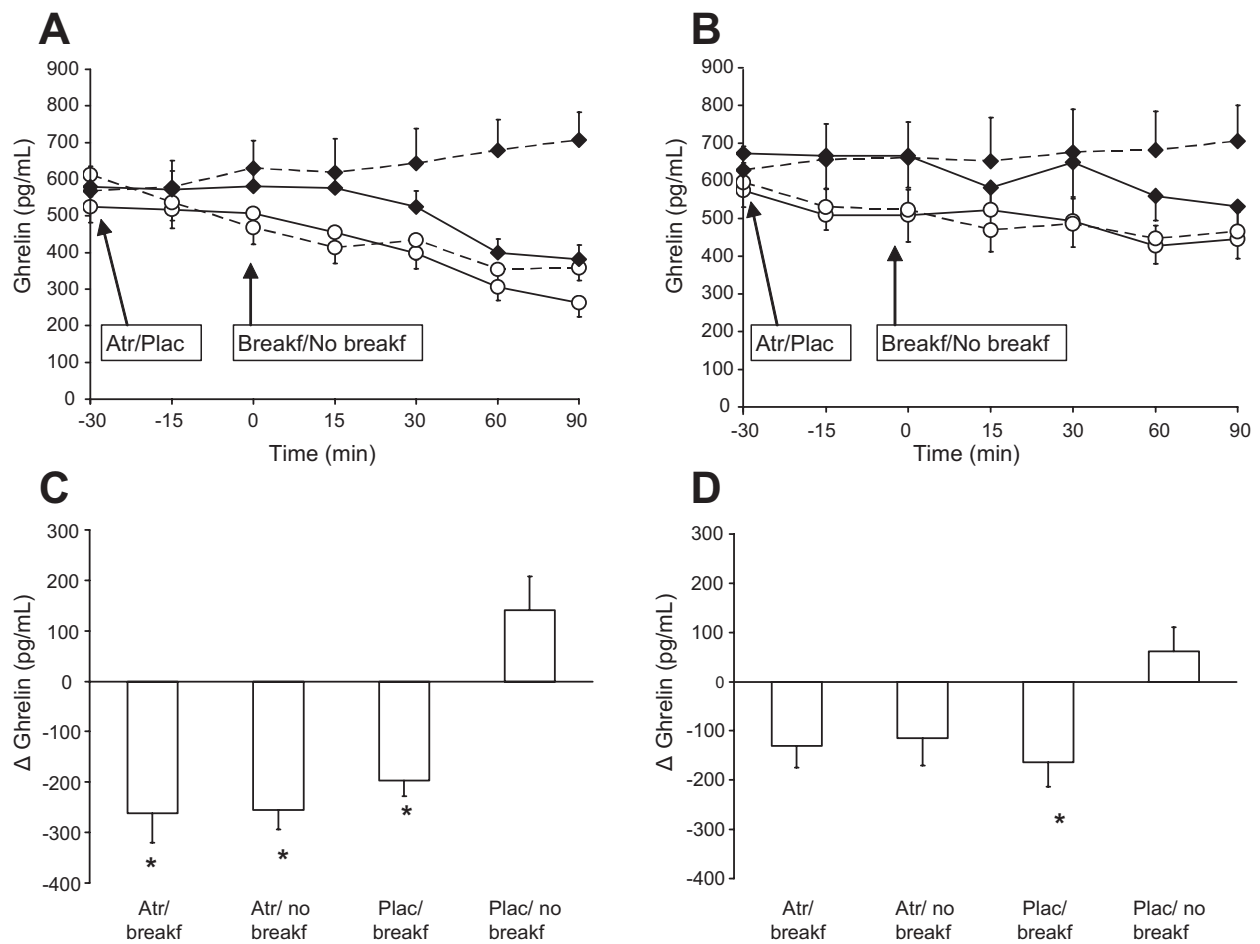


Fig. 2: The effect of atropine (1 mg i.v. at time point -30 min.) and breakfast (550 kcal at time point 0) on PYY plasma concentrations in lean (CO) and obese subjects (OB)

Fig. 2a and b: Solid lines = with breakfast, dotted lines = without breakfast; open symbols = with atropine, filled symbols = with placebo

Fig. 2 c and d: asterisks = study days significantly different from placebo day

Fig. 2a Plasma PYY concentrations in CO on the four study days

Fig. 2b Plasma PYY concentrations in OB on the four study days

Fig. 2c Differences in baseline and postprandial PYY concentrations (Δ -30/+90) on the four study days in CO

Fig. 2d Differences in baseline and postprandial PYY concentrations (Δ -30/+90) on the four study days in OB

Figure 2

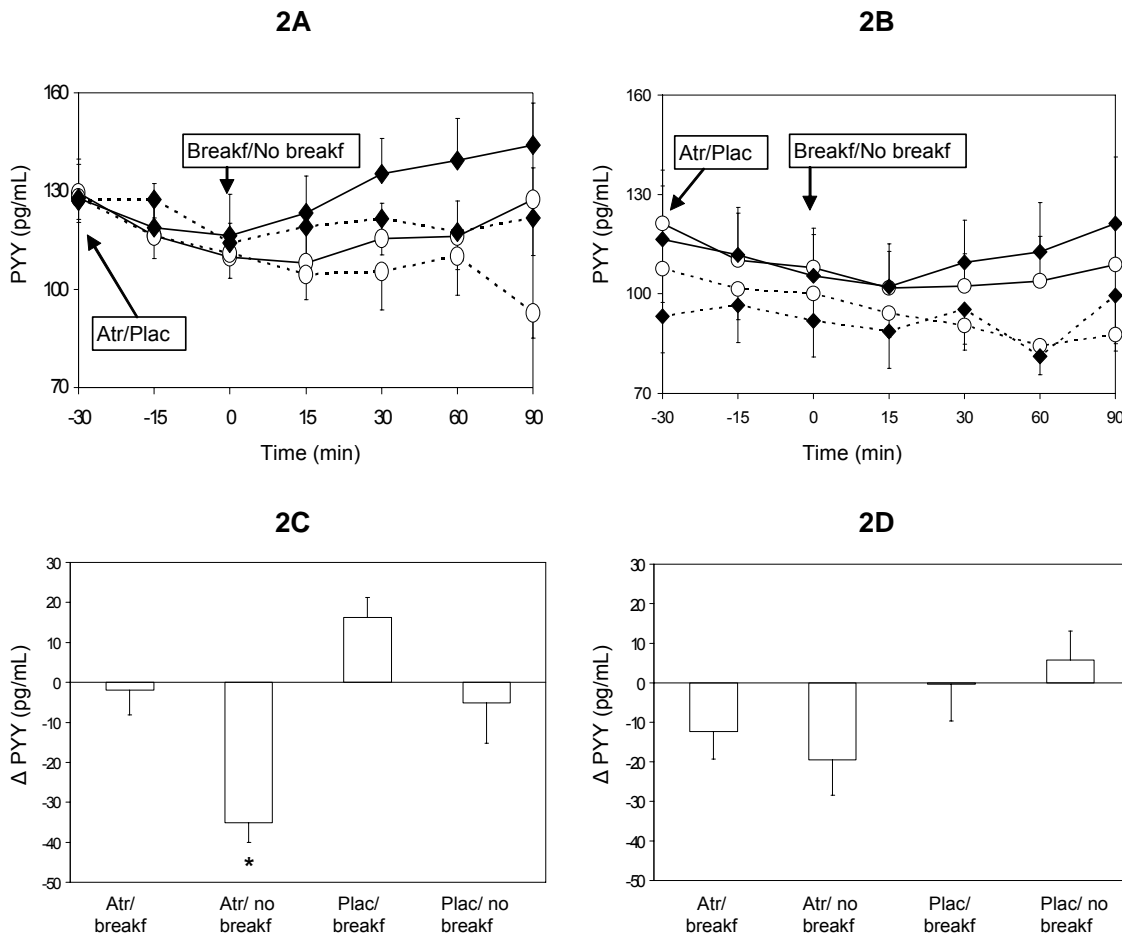


Fig. 3: The effect of atropine (1 mg i.v. at time point -30 min) and breakfast on heart rate in lean (CO) and obese (OB) subjects

Solid lines = with breakfast, dotted lines = without breakfast); open symbols = with atropine, filled symbols = with placebo

Fig. 3a Heart rates on the four study days in CO

Fig. 3b Heart rates on the four study days in OB

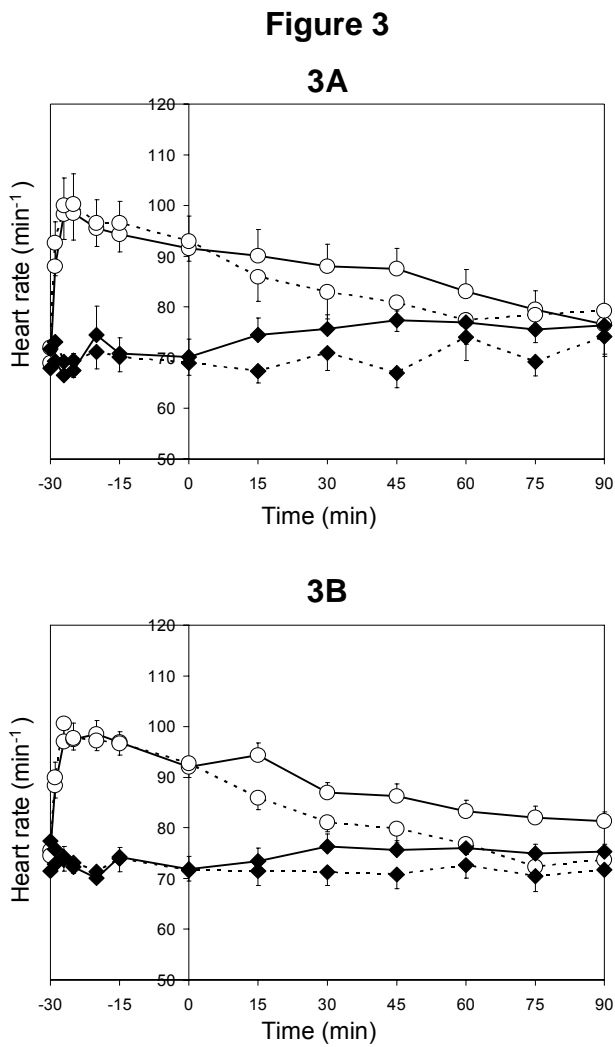


Fig. 4: The effect of atropine (1 mg i.v. at time point -30 min) and breakfast on plasma glucose and serum insulin in lean (CO) and obese (OB) subjects

Solid lines = with breakfast, dotted lines = without breakfast; open symbols = with atropine, filled symbols = with placebo

Fig. 4a Plasma glucose on the four study days in CO

Fig. 4b Plasma glucose on the four study days in OB

Fig. 4c Serum insulin on the four study days in CO

Fig. 4d Serum insulin on the four study days in OB

Figure 4

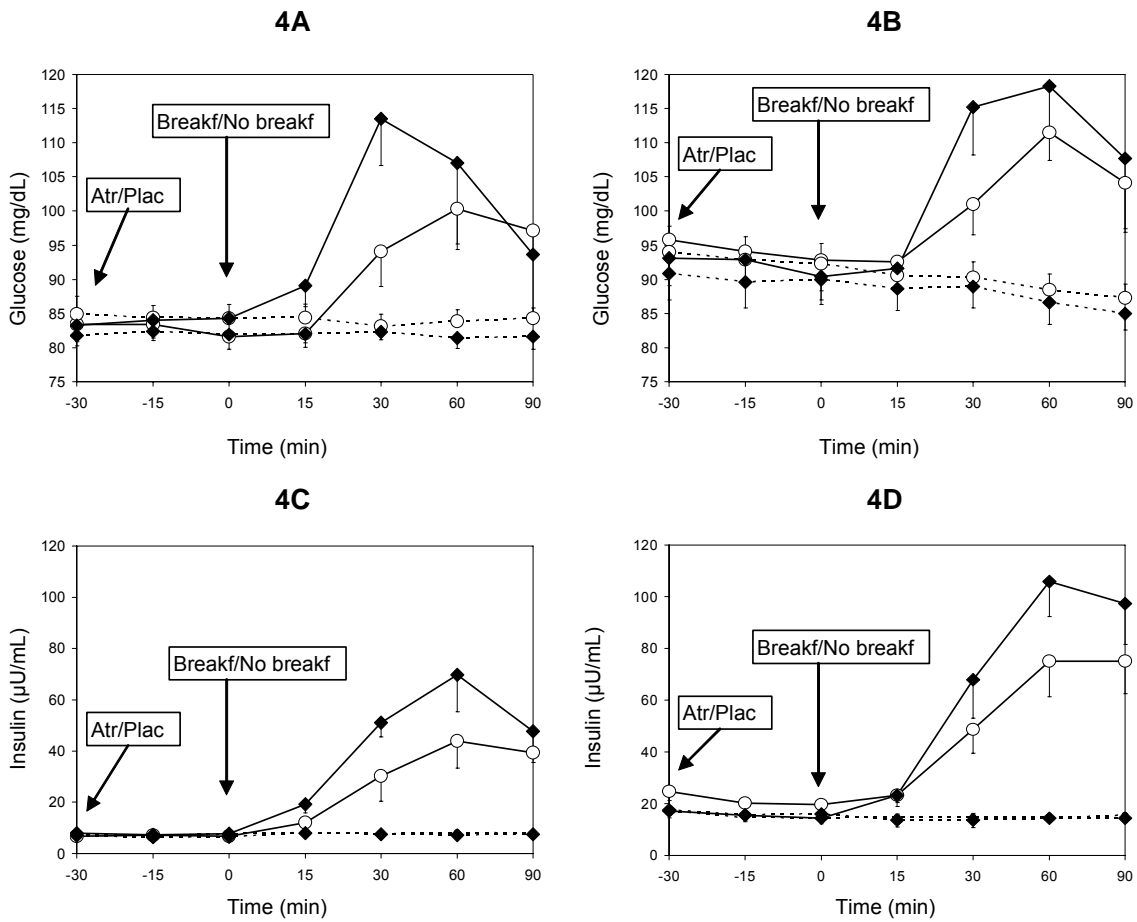


Fig. 5 The effect of atropine (1 mg i.v. at time point -30 min) and breakfast on hunger (fig. 5a,b) and satiety (fig. 5c,d) ratings from visual analog scales (VAS) in lean (CO) and obese (OB) subjects

Fig. 5a-d Solid lines = with breakfast, dotted lines = without breakfast; open symbols = with atropine, filled symbols = with placebo

Fig. 5e, f asterisks = study days significantly different from placebo; white bars = differences between baseline and timepoint +90 min (Δ -30/+90) in satiety VAS, shaded bars = Δ -30/+90 in hunger VAS

Fig. 5a Hunger VAS ratings on the four study days in CO

Fig. 5b Hunger VAS ratings on the four study days in OB

Fig. 5c Satiety VAS ratings on the four study days in CO

Fig. 5d Satiety VAS ratings on the four study days in OB

Fig. 5e Δ hunger and satiety VAS on the four study days in CO

Fig. 5f Δ hunger and satiety VAS on the four study days in OB

Figure 5

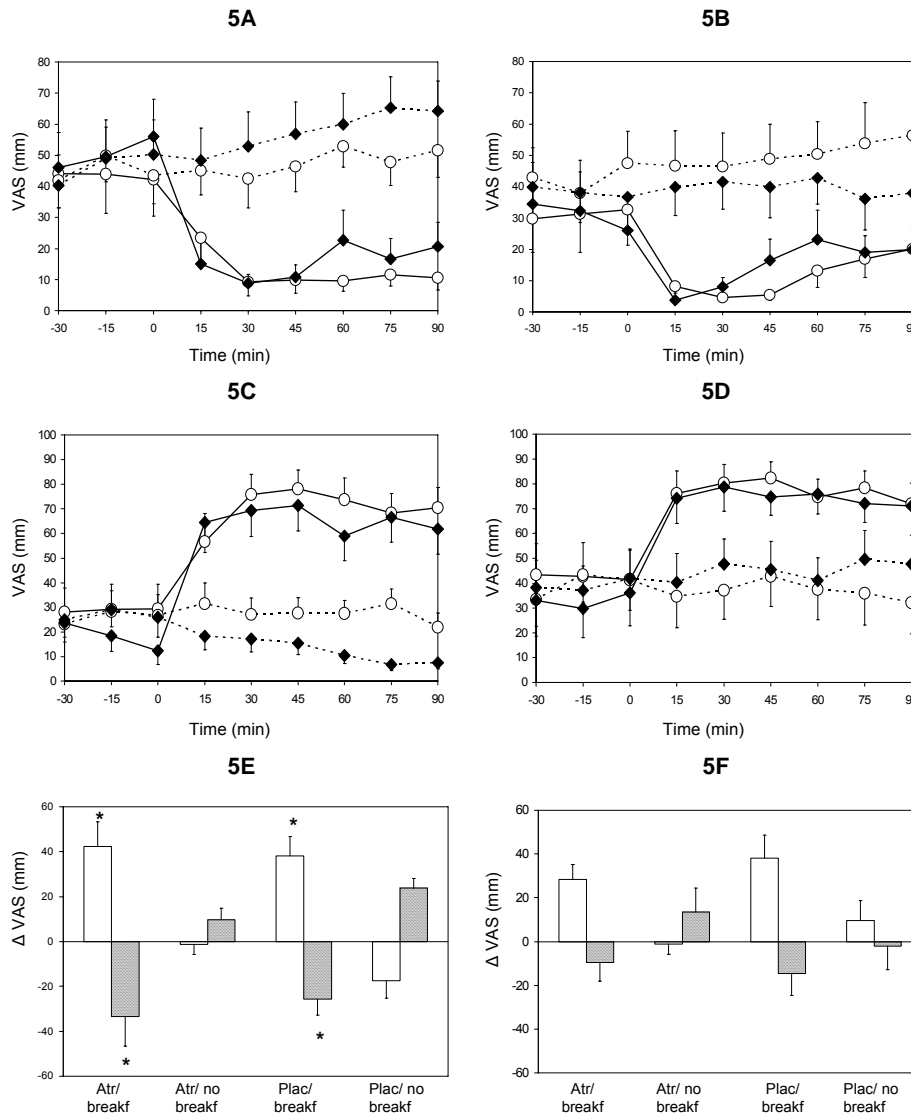


Fig. 6 Linear regression analysis of correlations between various parameters (all four study days grouped together)

- Fig. 6a Δ heart rate (-30/-25) vs. Δ ghrelin (-30/+90) in CO, $R^2 = 0.373$, $p = 0.0002$
- Fig. 6b Δ heart rate (-30/-25) vs. Δ ghrelin (-30/+90) in OB, n.s.
- Fig. 6c Δ heart rate (-30/-25) vs. Δ PYY (-30/+90) in CO, $R^2 = 0.227$, $p = 0.0058$
- Fig. 6d Δ heart rate (-30/-25) vs. Δ PYY (-30/+90) in OB, $R^2 = 0.227$, $p = 0.041$
- Fig. 6e Δ hunger VAS (-30/+90 vs. Δ ghrelin (-30/+90) in CO, $R^2 = 0.178$, $p = 0.016$
- Fig. 6f Δ hunger VAS (-30/+90 vs. Δ ghrelin (-30/+90) in OB, n.s.
- Fig. 6g Δ satiety VAS (-30/+90 vs. Δ ghrelin (-30/+90) in CO, $R^2 = 0.294$, $p = 0.002$
- Fig. 6h Δ satiety VAS (-30/+90 vs. Δ ghrelin (-30/+90) in OB, n.s.

Figure 6

