Cholinergic Regulation of Ghrelin and Peptide YY Release May Be Impaired in Obesity

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OBJECTIVE—Ghrelin and peptide YY (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 this 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.

REASEARCH DESIGN AND METHODS—Eight lean and eight obese subjects were included in a randomized, double-blind, placebo-controlled crossover study with 4 study days in randomized order (atropine/placebo ± breakfast). Plasma ghrelin, PYY, insulin, and glucose were measured. Hunger and satiety feelings were rated on a 10-cm visual analog scale.

RESULTS—In lean individuals, atropine led to a decrease in ghrelin concentrations comparable and nonadditive with breakfast ingestion and 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 meal-induced glucose concentrations in the two study groups. Only lean, 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. ***Diabetes*** **57:2332–2340, 2008**

Energy homeostasis is a tightly regulated process involving hormone signaling from the periphery via vagal afferents to the hindbrain (namely the nucleus tractus solitarius) and the hypothalamus (especially the nucleus arcuatus), where these signals are integrated with information 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 growth hormone secretagogue receptor and is produced mainly in the stomach (5). Although initially characterized as a potent growth hormone, ghrelin was quickly discovered to also be a potent orexigenic peptide (6). Ghrelin is involved in both short- (7,8) and long-term (6,9) appetite regulation 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 (NPY)/Agouti-related protein (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).

PYY, named for the two tyrosine residues on the C- and NH_2-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 glucagone-like peptide-1 (13). PYY levels rise after ingestion of a high-caloric meal (but before nutrients reach the ileum) and remain 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 of 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 initial 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 antiobesity treatment could have been found. PYY levels are reported to be reduced (17) or unaltered (20) in obesity and increased in anorexia nervosa (20).

Few data exist for the regulation of ghrelin and PYY release from the gut. A 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 an increase of ghrelin levels induced by 48-h food deprivation was abolished

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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).

On the other hand, PYY increases after meal intake, with a maximum reached after 1–2 h, dependent upon the amount of calories ingested (27), particularly via fat (28). Recent data indicate that increases in PYY 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 show that an atropine-sensitive cholinergic pathway is 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

Eight obese (BMI >30 kg/m^2) and eight normal-weight control subjects (BMI <25 kg/m^2) were recruited from the obesity outpatient clinic and hospital staff. All participants were nonsmokers, none of the control subjects were taking any medication (with the exception of oral contraceptives), and one of the obese subjects was on antidepressant and antihypertensive therapy. All subjects underwent prestudy screening and had normal findings on laboratory measurements, electrocardiogram, and physical examination. All subjects underwent a 3-h oral glucose tolerance test (75 g glucose). The study protocol was approved by the ethics committee of the Medical University of Vienna, and all subjects gave informed consent before study entry.

The study was conducted with a prospective, randomized, single-blind, placebo-controlled crossover design. For each subject, 4 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 A.M. after an overnight fast. Studies were conducted in a quiet room with an ambient temperature of 22°C. Subjects abstained from alcohol and 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 a 10-cm visual analog scale (VAS).

At time point −30, 1 mg atropine (atropimum sulfuricum, Nycomed; study days A and B) or placebo (isotonic saline; study days C and D) was given intravenously over 30 s. At time point 0, subjects received a standard breakfast consisting of two 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, and 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 were 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 were stored at −30°C until analysis. Insulin levels (in micro units per milliliter) were assayed by a commercially available RIA (Pharmacia-Upjohn, Uppsala, Sweden). Blood glucose was determined according to standard laboratory procedures.

Plasma ghrelin (in pico grams per milliliter) 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 COOH-terminal end of human ghrelin. The inter- and intra-assay variations were both <10.9%. PYY (in pico grams per milliliter) was measured using a commercial RIA (Linco Research, St .Charles, MO) with inter- and intra-assay variations of 8.2 and 9%, respectively.

TABLE 1
Baseline characteristics of study subjects

	Lean	Obese	*P*
Age (years)	28.7 ± 2.4	29.1 ± 2.0	NS
Sex distribution (F/M)	6/2	6/2	NS
Height (cm)	173.6 ± 3.5	169.1 ± 4.0	NS
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-to-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

Data are means ± SEM.

Statistical analysis. Homeostasis 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 a previously published formula (31) using the OGIS calculator (www.ladseb.pd.cnr.it/bioing/ogis/home.html). Hormone concentrations at single time points and Δhormone levels, Δheart rate, and ΔVAS values at the 4 different study days were compared with one-way ANOVA followed by multiple *t* tests with Bonferroni correction as post hoc statistics, where 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 statistical software release 12.0.1 was used. $P < 0.05$ was considered statistically significant. Results are presented as means ± SEM.

RESULTS

Baseline characteristics of the subjects are shown in Table 1. The two groups were well matched for age, sex, and height. Apart from having a significantly higher BMI (group-defining criterion), obese subjects had significantly higher fasting glucose and insulin levels and were significantly more insulin resistant according to both HOMA and OGIS indexes. Three obese subjects had impaired glucose tolerance according to their 120-min glucose concentrations (range 143–155 mg/dl).

Ghrelin. Ghrelin concentrations on the 4 study days are given in Fig. 1. In control subjects, both atropine and meal ingestion led to a decrease in 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 using one-way ANOVA). When comparing differences between baseline and +90 min ghrelin concentrations (Δghrelin −30/+90), values of the 3 study days were significantly different from the placebo day without breakfast; the study days (A: atropine + breakfast, B: atropine alone, and C: breakfast alone) did not differ significantly from each other (Fig. 1*C*).

In obese subjects, atropine had no effect on ghrelin concentrations. When compared by ANOVA, ghrelin concentrations did not differ significantly at any single time point on the 4 different study days (Fig. 1*B*); when comparing Δghrelin −30/+90 values, only breakfast had a significant effect on ghrelin concentrations (Fig. 1*D*).

PYY. PYY concentrations on the 4 study days are given in Fig. 2. In the control group, atropine (study day B) led to a significant decrease of PYY concentrations at time point +90 min compared with breakfast alone (study day C). Using Δ−30/+90 values (differences between baseline and +90 min PYY concentrations) atropine lead to a significant decrease of PYY concentrations compared with all other study days, which did not differ significantly from each other (Fig 2*C*). In obese subjects, there were no significant

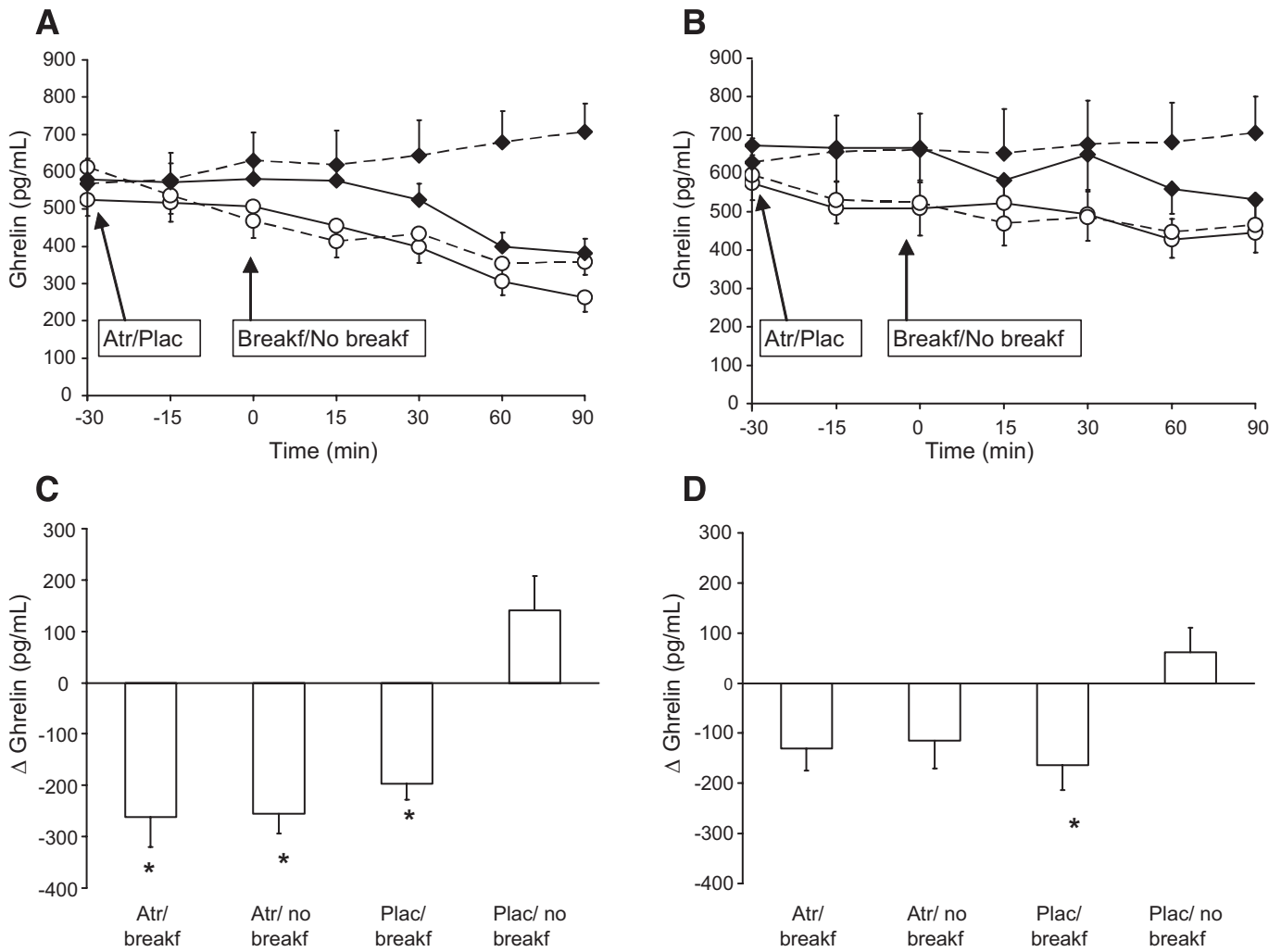

FIG. 1. Effect of atropine (Atr) (1 mg i.v. at time point −30 min) and breakfast (550 kcal at time point 0) on ghrelin plasma concentrations in lean (control) and obese subjects. *A* and *B*: Plasma ghrelin concentrations in control (*A*) and obese (*B*) subjects on the 4 study days. Solid lines, with breakfast; dotted lines, without breakfast; ○, with atropine; ♦, with placebo. *C* and *D*: Differences in baseline and postprandial ghrelin concentrations (Δ−30/+90) on the 4 study days in control (*C*) and obese (*D*) subjects. *Study days significantly different from placebo day.

differences between any of the study days, at single time points and between the Δ−30/+90 values (Fig. 2*B* and *D*, respectively).

Heart rate, glucose, and insulin. Heart rates on the 4 study days are given in Fig. 3. Atropine led to a significant increase in heart rate in both obese and lean subjects (baseline values, 72.0 ± 3 min^{-1} control vs. 74.5 ± 2 min^{-1} obese, $P = 0.5$) with peak values at time point −25 min (5 min after atropine application, 100.3 ± 7 min^{-1} control vs. 97.8 ± 2 min^{-1} obese, $P = 0.7$). Thus, heart rate increase between baseline and time point −25 min (Δ−30/−25) was comparable in lean and obese subjects (23.25 ± 1.8 vs. 28.25 ± 5.8 min^{-1}, $P = 0.42$, by unpaired Student's *t* test).

Plasma glucose and insulin concentrations are given in Fig. 4. Atropine alone did not change plasma insulin or glucose concentrations compared with placebo (study days C and D). Meal-induced glucose increase was significantly affected by atropine only in obese subjects. Plasma glucose in control subjects between study days A and B were not significantly different at any single time points, as was the glucose difference between baseline and time point +30 min (Δ−30/+30) (+10.8 ± 4.5 vs. −1.8 ± 1.2 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 time point +30 min (Δ−30/+30) (23.3 ± 9.4 vs. −2.2 ± 0.9 μU/ml, $P = 0.003$).

In obese subjects, atropine significantly reduced meal-induced blood glucose at time point +60 min ($P = 0.003$). Glucose difference between baseline and time point +30 min (Δ−30/30) was not significantly different between study days A and B (5.1 ± 1.7 vs. −3.8 ± 1.7 mg/dl, $P = 0.18$). Insulin levels were significantly different between study days A and B at time point +60 min ($P = 0.001$). The 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 ± 9.4 vs. 0 ± 0.4 μU/ml, $P = 0.133$).

VAS. An overview of the time course of hunger and satiety VAS is given in Fig. 5. Meal ingestion alone and in combination with atropine led to sustained decreases in hunger ratings and increases in satiety ratings in lean individuals. Hunger scores tended to be lower and satiety scores higher on atropine alone versus placebo days, yet this difference did not reach statistical significance in the ANOVA analysis. When differences between baseline and time point +90 (Δ−30/+90) were compared (Fig. 5*E*), both hunger and satiety Δ−30/+90 values for all breakfast days were significantly different from all days with no

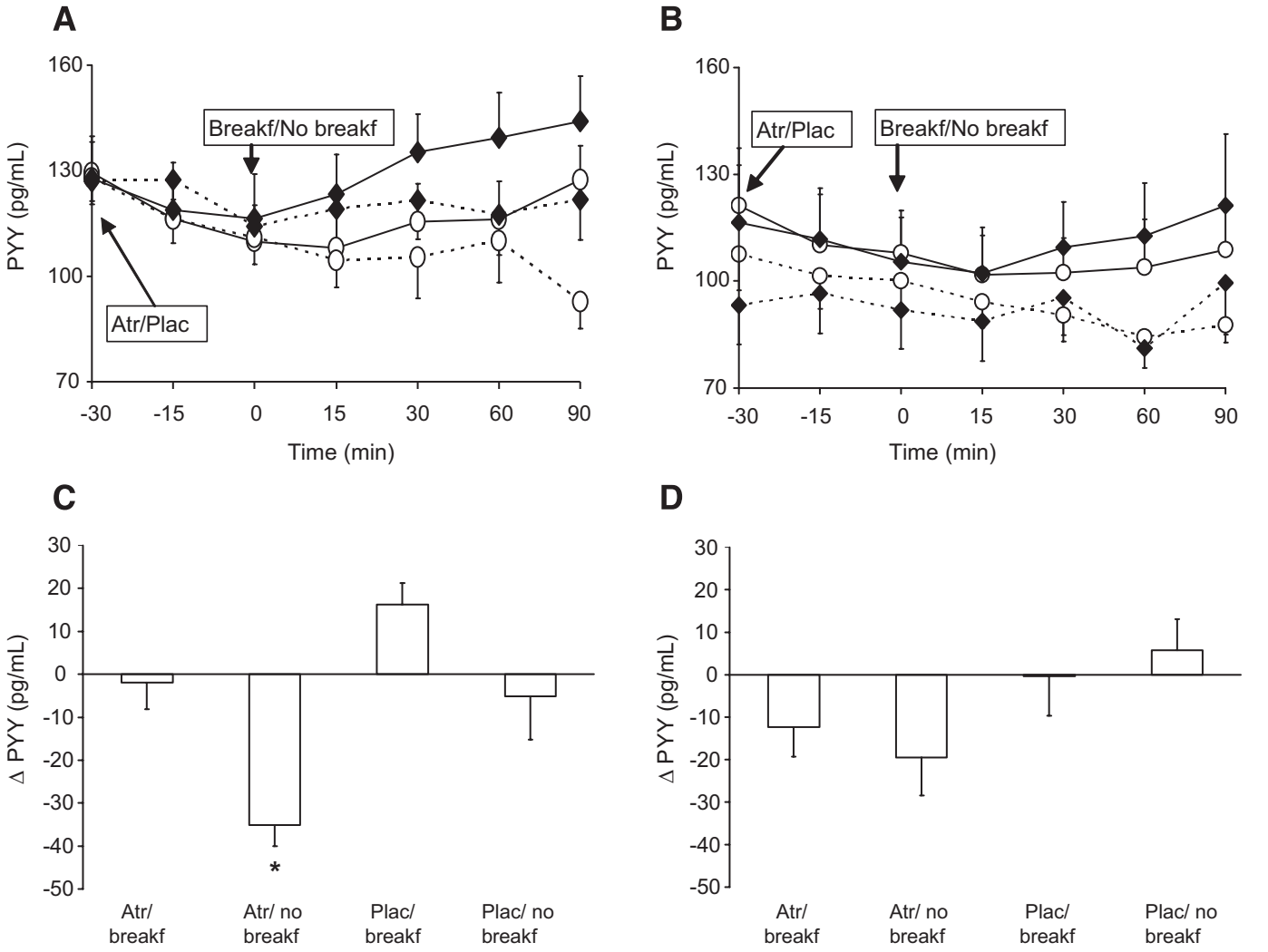

FIG. 2. Effect of atropine (Atr) (1 mg i.v. at time point −30 min) and breakfast (550 kcal at time point 0) on PYY plasma concentrations in lean (control) and obese subjects. *A* and *B*: Plasma PYY concentrations in control (*A*) and obese (*B*) subjects on the 4 study days. Solid lines, with breakfast; dotted lines, without breakfast; ○, with atropine; ♦, with placebo. *C* and *D*: Differences in baseline and postprandial PYY concentrations (Δ−30/+90) on the 4 study days in control (*C*) and obese (*D*) subjects. *Study days significantly different from placebo day.

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 lower on atropine compared with placebo days (Fig. 5*B*, 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 B and C differed significantly from each other ($P = 0.012$), all other values were not statistically different from each other (Fig. 5*F*).

Correlations. In lean subjects, there was a significant relationship between Δheart rate (−30/−25) and Δghrelin (−30/90), as revealed by linear regression analysis ($R^2 = 0.373$, $P = 0.0002$, Fig. 6*A*). There was also a significant relationship between Δheart rate (−30/−25) and ΔPYY (−30/90) ($R^2 = 0.227$, $P = 0.0058$, Fig. 6*C*). In obese subjects, there was no relationship between Δheart rate (−30/−25) and Δghrelin (−30/90) ($R^2 = 0.042$, $P = 0.27$, Fig. 6*B*) and a weak but significant relationship between Δheart rate (−30/−25) and ΔPYY (−30/90) ($R^2 = 0.136$, $P = 0.041$, Fig. 6*D*).

In lean subjects, Δghrelin (−30/90) showed a significant correlation with ΔVAS hunger (−30/90) ($R^2 = 0.178$, $P = 0.016$, Fig. 6*E*), and ΔPYY (−30/90) correlated significantly with ΔVAS satiety (−30/90) ($R^2 = 0.294$, $P = 0.002$, Fig. 6*G*), whereas there was no significant correlation in obese subjects (Fig. 6*F* and *H*, respectively). Insulin resistance as quantified by HOMA and OGIS indexes 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, ghrelin and PYY release from the gut is also 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 nonadditive manner, and PYY release is significantly reduced after atropine appli-

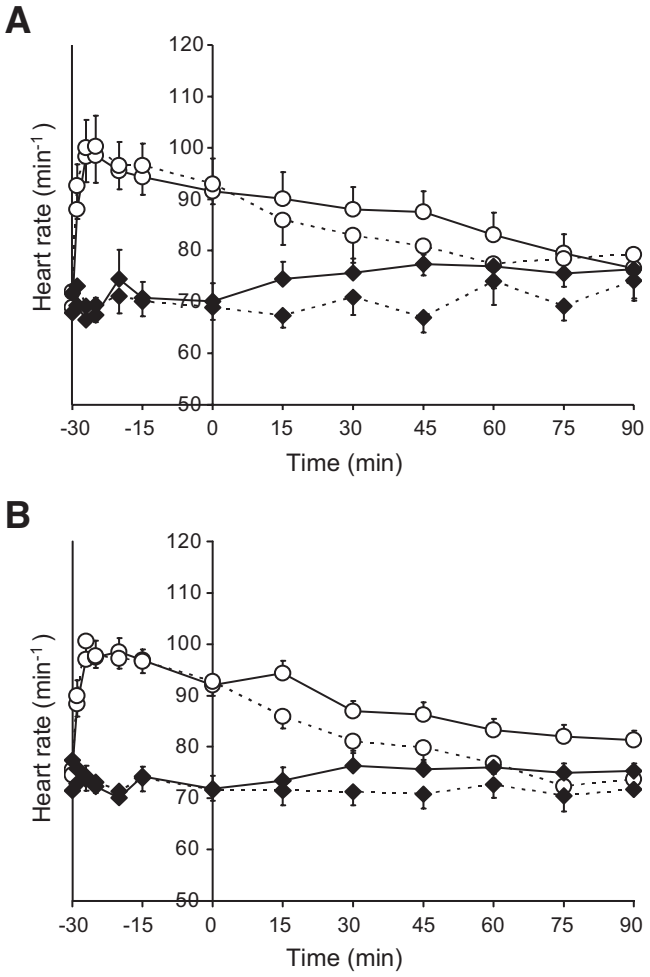

FIG. 3. The effect of atropine (1 mg i.v. at time point −30 min) and breakfast on heart rate in lean (control) and obese subjects. *A* and *B*: Heart rates on the 4 study days in control (*A*) and obese (*B*) subjects. Solid lines, with breakfast; dotted lines, without breakfast; ○, with atropine; ♦, with placebo.

cation compared with 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, whereas 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 increases in obese subjects was comparable with those in 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 rather 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).

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 compared with their lean counterparts. 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 nonbreakfast days), whereas lean subjects reported a sustained suppression of hunger feelings at the end of the study period (90 min after breakfast) on breakfast compared with nonbreakfast days. To a lesser extent, the same could be observed for ratings of satiety, with statistically significant differences between all breakfast versus nonbreakfast days in lean and ratings being only different between study days B (atropine alone) and C (breakfast alone) in obese subjects (although they also tended to be lower on the other breakfast day than on nonbreakfast 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 4 study days are included in the analysis), but surprisingly again there was a complete lack of association in obese subjects.

Actually, only part of the circulating ghrelin seems to be associated with feelings of appetite, namely the active (acylated) ghrelin, which in 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 differences in ghrelin release were responsible for the observed lack of correlation in obese subjects. Nonetheless, 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). Whereas in the absence of prospective data it is impossible to determine 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 an 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 clear by now that the ample availability of energy dense food is part of the epidemic, it is currently unclear why some people contract obesity

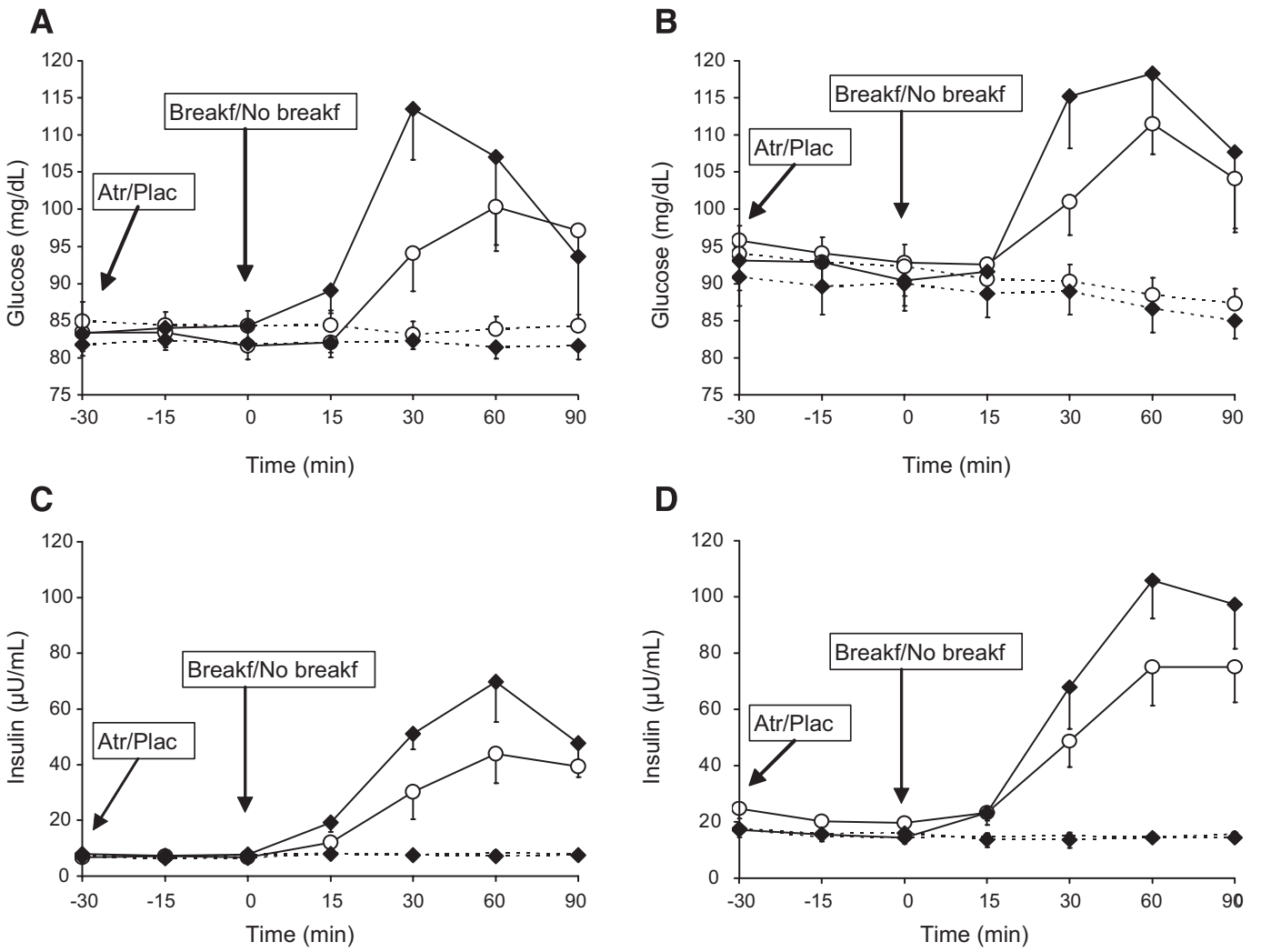

FIG. 4. The effect of atropine (Atr) (1 mg i.v. at time point −30 min) and breakfast on plasma glucose and serum insulin in lean (control) and obese subjects. *A* and *B*: Plasma glucose on the 4 study days in control (*A*) and obese (*B*) subjects. *C* and *D*: Serum insulin on the 4 study days in control (*C*) and obese (*D*) subjects. Solid lines, with breakfast; dotted lines, without breakfast; ○, with atropine; ♦, with placebo.

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 compared with their wild-type littermates (36). The M3 receptor is expressed in the lateral hypothalamus, and the melanin-concentrating hormone (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 compared with 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 compared with 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 indexes, 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 is inhibited by atropine but not to the same extent as by pancreatic polypeptide, and it was concluded that the hyperinsulinemia in this population was not due to increased vagal input to the pancreatic β-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.

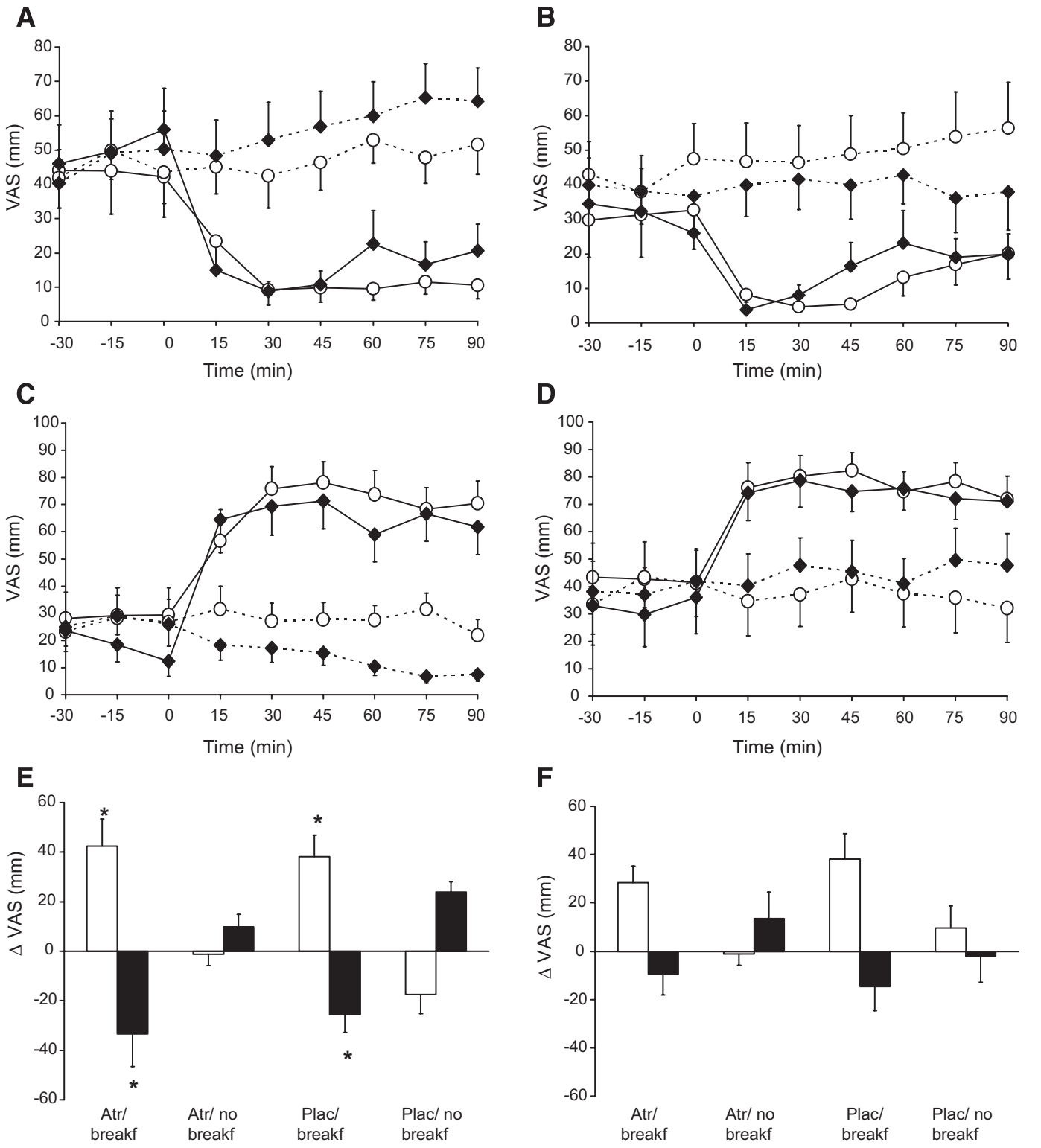

FIG. 5. Effect of atropine (1 mg i.v. at time point −30 min) and breakfast on hunger (*A* [control] and *B* [obese]) and satiety (*C* [control] and *D* [obese]) ratings from VAS in lean (control) and obese subjects. Solid lines, with breakfast; dotted lines, without breakfast; ○, with atropine; ♦, with placebo. *E* and *F*: ΔHunger and satiety VAS on the 4 study days in control (*E*) and obese (*F*) subjects. *Study days significantly different from placebo. Open bars, differences between baseline and time point +90 min (Δ−30/+90) in satiety VAS; shaded bars, Δ−30/+90 in hunger VAS.

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, and *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 may actively contribute to weight gain and/or hinder weight loss.

REFERENCES

1. Murphy KG, Bloom SR: Gut hormones and the regulation of energy homeostasis. *Nature* 444:854–859, 2006

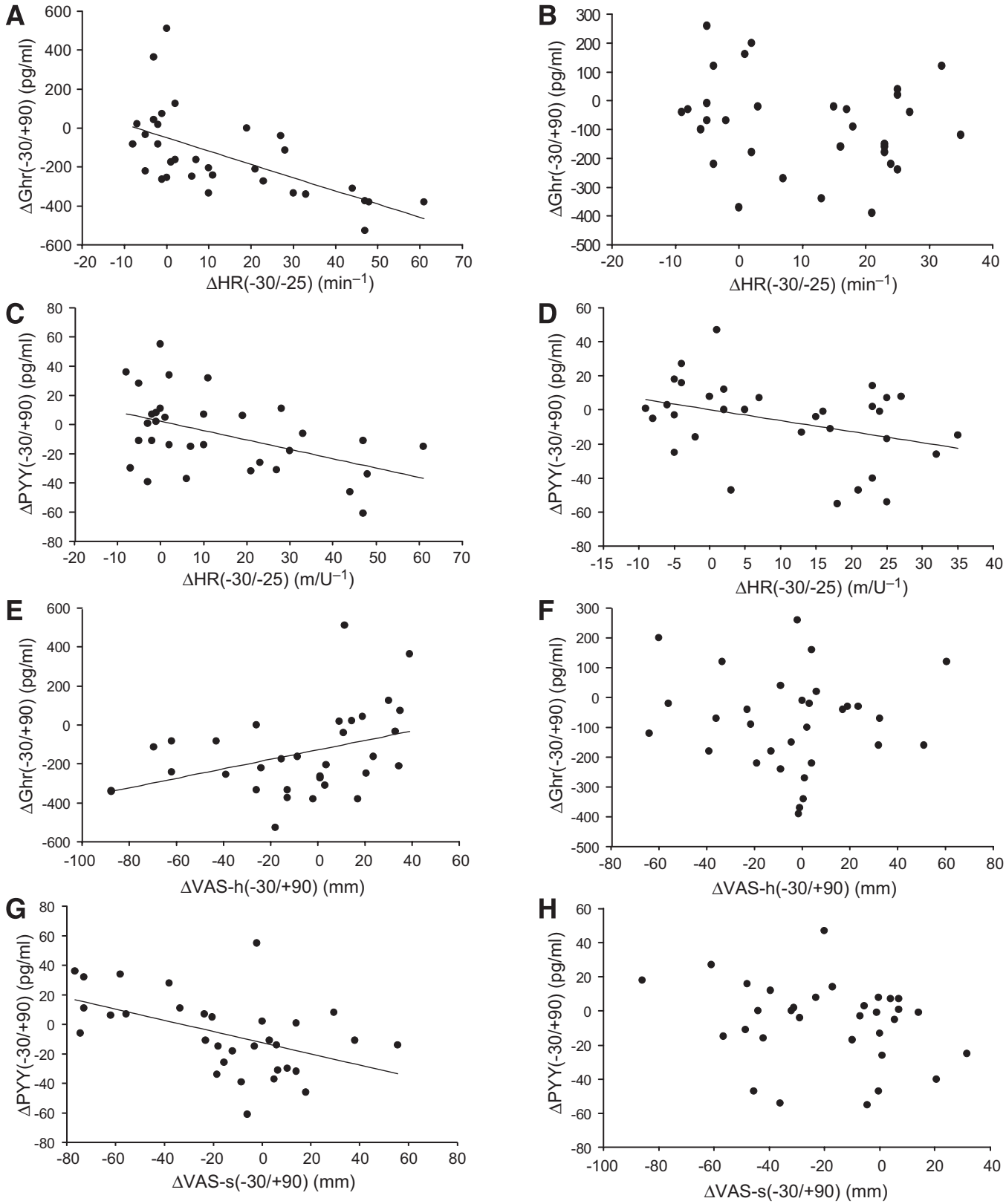

FIG. 6. Linear regression analysis of correlations between various parameters (all 4 study days grouped together). *A*: ΔHeart rate (−30/−25) vs. Δghrelin (−30/+90) in control subjects: $R^2 = 0.373$, $P = 0.0002$. *B*: ΔHeart rate (−30/−25) vs. Δghrelin (−30/+90) in obese subjects: NS. *C*: ΔHeart rate (−30/−25) vs. ΔPYY (−30/+90) in control subjects: $R^2 = 0.227$, $P = 0.0058$. *D*: ΔHeart rate (−30/−25) vs. ΔPYY (−30/+90) in obese subjects: $R^2 = 0.227$, $P = 0.041$. *E*: ΔHunger VAS (−30/+90 vs. Δghrelin (−30/+90) in control subjects: $R^2 = 0.178$, $P = 0.016$. *F*: ΔHunger VAS (−30/+90 vs. Δghrelin (−30/+90) in OB: NS. *G*: ΔSatiety VAS (−30/+90 vs. Δghrelin (−30/+90) in control subjects: $R^2 = 0.294$, $P = 0.002$. *H*: Six-hour Δsatiety VAS (−30/+90 vs. Δghrelin (−30/+90) in obese subjects: NS.

2. Berthoud HR: Multiple neural systems controlling food intake and body weight. *Neurosci Biobehav Rev* 26:393–428, 2002
3. Wren AM, Bloom SR: Gut hormones and appetite control. *Gastroenterology* 132:2116–2130, 2007
4. Havel PJ: Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* 53 (Suppl. 1):S143–S151, 2004
5. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402:656–660, 1999
6. Tschop M, Smiley DL, Heiman ML: Ghrelin induces adiposity in rodents. *Nature* 407:908–913, 2000
7. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M: Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 120:337–345, 2001
8. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR: Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 86:5992, 2001
9. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S: A role for ghrelin in the central regulation of feeding. *Nature* 409:194–198, 2001
10. Horvath TL, Diano S, Tschop M: Ghrelin in hypothalamic regulation of energy balance. *Curr Top Med Chem* 3:921–927, 2003
11. Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL: The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37:649–661, 2003
12. Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K, Nakazato M: Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 144:1506–1512, 2003
13. Wynne K, Bloom SR: The role of oxyntomodulin and peptide tyrosine-tyrosine (PYY) in appetite control. *Nat Clin Pract Endocrinol Metab* 2:612–620, 2006
14. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR: Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 89:1070–1077, 1985
15. Lundberg JM, Tatemoto K, Terenius L, Hellstrom PM, Mutt V, Hokfelt T, Hamberger B: Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. *Proc Natl Acad Sci U S A* 79:4471–4475, 1982
16. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR: Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature* 418:650–654, 2002
17. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR: Inhibition of food intake in obese subjects by peptide YY3–36. *N Engl J Med* 349:941–948, 2003
18. Tschop M, Castaneda TR, Joost HG, Thone-Reineke C, Ortmann S, Klaus S, Hagan MM, Chandler PC, Oswald KD, Benoit SC, Seeley RJ, Kinzig KP, Moran TH, Beck-sickinger AG, Koglin N, Rodgers RJ, Blundell JE, Ishii Y, Beattie AH, Holch P, Allison DB, Raun K, Madsen K, Wulff BS, Stidsen CE, Birringer M, Kreuzer OJ, Schindler M, Arndt K, Rudolf K, Mark M, Deng XY, Whitcomb DC, Halem H, Taylor J, Dong J, Datta R, Culler M, Craney S, Flora D, Smiley D, Heiman ML: Physiology: does gut hormone PYY3–36 decrease food intake in rodents? *Nature* 430:1038, 2004
19. Chelikani PK, Haver AC, Reidelberger RD: Intermittent intraperitoneal infusion of peptide YY(3–36) reduces daily food intake and adiposity in obese rats. *Am J Physiol Regul Integr Comp Physiol* 293:R39–R46, 2007
20. Pfluger PT, Kampe J, Castaneda TR, Vahl T, D'Alessio DA, Kruthaupt T, Benoit SC, Cuntz U, Rochlitz HJ, Moehlig M, Pfeiffer AF, Koebnick C, Weickert MO, Otto B, Spranger J, Tschop MH: Effect of human body weight changes on circulating levels of peptide YY and peptide YY3–36. *J Clin Endocrinol Metab* 92:583–588, 2007
21. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S: Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 87:240–244, 2002
22. Schaller G, Schmidt A, Pleiner J, Woloszczuk W, Wolzt M, Luger A: Plasma ghrelin concentrations are not regulated by glucose or insulin: a double-blind, placebo-controlled crossover clamp study. *Diabetes* 52:16–20, 2003
23. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS: A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50:1714–1719, 2001
24. Sugino T, Yamaura J, Yamagishi M, Kurose Y, Kojima M, Kangawa K, Hasegawa Y, Terashima Y: Involvement of cholinergic neurons in the regulation of the ghrelin secretory response to feeding in sheep. *Biochem Biophys Res Commun* 304:308–312, 2003
25. Williams DL, Grill HJ, Cummings DE, Kaplan JM: Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology* 144: 5184–5187, 2003
26. Maier C, Schaller G, Buranyi B, Nowotny P, Geyer G, Wolzt M, Luger A: The cholinergic system controls ghrelin release and ghrelin-induced growth hormone release in humans. *J Clin Endocrinol Metab* 89:4729–4733, 2004
27. Stock S, Leichner P, Wong AC, Ghatei MA, Kieffer TJ, Bloom SR, Chanoine JP: Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. *J Clin Endocrinol Metab* 90:2161–2168, 2005
28. McFadden DW, Rudnicki M, Nussbaum MS, Balasubramaniam A, Fischer JE: Independent release of peptide YY (PYY) into the circulation and ileal lumen of the awake dog. *J Surg Res* 46:380–385, 1989
29. le Roux CW, Batterham RL, Aylwin SJ, Patterson M, Borg CM, Wynne KJ, Kent A, Vincent RP, Gardiner J, Ghatei MA, Bloom SR: Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology* 147:3–8, 2006
30. Lin HC, Taylor IL: Release of peptide YY by fat in the proximal but not distal gut depends on an atropine-sensitive cholinergic pathway. *Regul Pept* 117:73–76, 2004
31. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ: A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* 24:539–548, 2001
32. Cummings DE, Overduin J: Gastrointestinal regulation of food intake. *J Clin Invest* 117:13–23, 2007
33. Lin HC, Neevel C, Chen PS, Suh G, Chen JH: Slowing of intestinal transit by fat or peptide YY depends on beta-adrenergic pathway. *Am J Physiol Gastrointest Liver Physiol* 285:G1310–G316, 2003
34. Chanoine JP: Editorial: Individual differences in the hormonal control of appetite: a step toward a (more) successful treatment of childhood overweight? *J Clin Endocrinol Metab* 91:2864–2866, 2006
35. Moran LJ, Luscombe-Marsh ND, Noakes M, Wittert GA, Keogh JB, Clifton PM: The satiating effect of dietary protein is unrelated to postprandial ghrelin secretion. *J Clin Endocrinol Metab* 90:5205–5211, 2005
36. Yamada M, Miyakawa T, Duttaroy A, Yamanaka A, Moriguchi T, Makita R, Ogawa M, Chou CJ, Xia B, Crawley JN, Felder CC, Deng CX, Wess J: Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean. *Nature* 410:207–212, 2001
37. Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS, Sakurai T, Yanagisawa M, Elmquist JK: Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol* 402:442–459, 1998
38. Ahren B, Holst JJ: The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. *Diabetes* 50:1030–1038, 2001
39. Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J: Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. *Diabetes* 53:1714–1720, 2004
40. Guo Y, Traurig M, Ma L, Kobes S, Harper I, Infante AM, Bogardus C, Baier LJ, Prochazka M: CHRM3 gene variation is associated with decreased acute insulin secretion and increased risk for early-onset type 2 diabetes in Pima Indians. *Diabetes* 55:3625–3629, 2006
41. D'Alessio DA, Kieffer TJ, Taborsky GJ, Jr, Havel PJ: Activation of the parasympathetic nervous system is necessary for normal meal-induced insulin secretion in rhesus macaques. *J Clin Endocrinol Metab* 86:1253–1259, 2001
42. Teff KL, Townsend RR: Early phase insulin infusion and muscarinic blockade in obese and lean subjects. *Am J Physiol* 277:R198–R208, 1999
43. Vozarova de Courten B, Weyer C, Stefan N, Horton M, DelParigi A, Havel P, Bogardus C, Tataranni PA: Parasympathetic blockade attenuates augmented pancreatic polypeptide but not insulin secretion in Pima Indians. *Diabetes* 53:663–671, 2004
44. Teff KL, Townsend RR: Prolonged mild hyperglycemia induces vagally mediated compensatory increase in C-Peptide secretion in humans. *J Clin Endocrinol Metab* 89:5606–5613, 2004