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GLP-1 Receptor Activation Modulates Appetite- and Reward-Related Brain Areas in Humans

OBESITY STUDIES

Gut-derived hormones, such as GLP-1, have been proposed to relay information to the brain to regulate appetite. GLP-1 receptor agonists, currently used for the treatment of type 2 diabetes (T2DM), improve glycemic control and stimulate satiety, leading to decreases in food intake and body weight. We hypothesized that food intake reduction after GLP-1 receptor activation is mediated through appetite- and reward-related brain areas. Obese T2DM patients and normoglycemic obese and lean individuals (n = 48) were studied in a randomized, crossover, placebo-controlled trial. Using functional MRI, we determined the acute effects of intravenous administration of the GLP-1 receptor agonist exenatide, with or without prior GLP-1 receptor blockade using exendin 9-39, on brain responses to food pictures during a somatostatin pancreatic-pituitary clamp. Obese T2DM patients and normoglycemic obese versus lean subjects showed increased brain responses to food pictures in appetite- and reward-related brain regions (insula and amygdala). Exenatide versus placebo decreased food intake and food-related brain responses in T2DM patients and obese subjects (in insula, amygdala, putamen, and orbitofrontal cortex). These effects were largely blocked by prior GLP-1 receptor blockade using exendin 9-39. Our findings provide novel insights into the mechanisms by which GLP-1 regulates food intake and how GLP-1 receptor agonists cause weight loss.

The global rise in obesity and type 2 diabetes (T2DM) prevalence is a major public health problem (1,2). It has been hypothesized that excessive eating due to changes in central nervous system (CNS) satiety and reward responses to food underlies the development of obesity and T2DM, comparable to the role for altered CNS responses in drug addiction (3). Several studies in obese individuals have demonstrated increased CNS responses to visual food cues in areas involved in appetite and reward processing (insula, amygdala, orbitofrontal cortex [OFC], and striatum) (4–6), and this increased CNS food-cue responsiveness predicts future weight gain (7). The mechanisms underlying these alterations in CNS responses to food cues are not clear, but multiple metabolic and hormonal factors seem to be involved (8–10).

Food ingestion activates the secretion of several gut-derived mediators, including the incretin hormone GLP-1. GLP-1 stimulates meal-related insulin secretion, inhibits glucagon release, and delays gastric emptying, mechanisms that all contribute to its glucometabolic effects (11). In addition, several observations suggest that GLP-1 has a role in the regulation of food intake. Systemic administration of GLP-1 reduced food intake in rodent and human studies (12), and blocking the GLP-1 receptor with

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exendin 9-39 resulted in hyperphagia and attenuated satiety after a meal in rodents (13).

GLP-1–based therapies, including GLP-1 receptor agonists such as exenatide, are currently successfully used in the treatment of patients with T2DM. GLP-1 receptor agonists improve glycemic control and stimulate satiety, leading to reductions in food intake and body weight (14). The mechanisms of the latter effects are as yet not fully understood, but GLP-1 receptor agonist actions on the brain may mediate satiety and weight effects in humans. In rodents, GLP-1 receptors are present in brain areas controlling feeding behavior and energy balance, such as the hypothalamus, nucleus tractus solitarii, area postrema, dorsal striatum, and nucleus accumbens (15). Recent additional data in rodents showed that central GLP-1 receptors are involved in the anorectic effects of GLP-1 receptor agonists (16). In humans, using positron emission tomography, a statistical association was shown between postprandial endogenous GLP-1 response and changes in neuronal activity of brain areas implicated in satiation and food intake regulation (dorsolateral prefrontal cortex and hypothalamus) (17). However, interventional studies determining the central effects of GLP-1 receptor activation, independent of glucometabolic changes, have not been performed.

We hypothesized that GLP-1 receptor agonists reduce food intake by affecting brain areas regulating appetite and reward, and that these effects are GLP-1 receptor mediated and independent of other hormonal and metabolic changes. Therefore, we assessed the acute effects of intravenous exenatide, with or without prior GLP-1 receptor blockade with intravenous exendin 9-39, on food intake and CNS responses to visual food cues using functional MRI (fMRI) in obese T2DM patients and normoglycemic obese and lean individuals. In order to study the effects of GLP-1 receptor activation per se, i.e., independent of hormonal or metabolic changes induced by GLP-1 receptor activation, all measurements were performed during a somatostatin pancreatic-pituitary clamp.

RESEARCH DESIGN AND METHODS

Participants

After screening 79 individuals, we included 16 obese T2DM patients and 16 obese normoglycemic and 16 healthy lean individuals, matched for sex and age. Inclusion criteria were age range 40–70 years, Caucasian ethnicity, right handedness, and stable body weight (<5% reported change during the previous 3 months). Women had to be postmenopausal (as ascertained by serum follicle-stimulating hormone >40 units/L) in order to avoid variations related to the menstrual cycle. Other inclusion criteria included BMI >30 kg/m^2 for obese individuals and T2DM patients, BMI <25 kg/m^2 for lean controls, and normoglycemia for obese individuals and lean controls as defined by fasting plasma glucose <5.6 mmol/L and 2-h glucose <7.8 mmol/L after a 75-g oral glucose tolerance test. For T2DM patients, HbA$_{1c}$ had to be 6.0–8.5% (42–69 mmol/mol) during treatment with metformin and/or sulfonylurea

derivative. Exclusion criteria for all participants were a history of cardiovascular, renal, and liver disease; malignancies; neurological or psychiatric disorders including eating disorders (assessed by the Eating Disorder Inventory II) (18) and depression (assessed by Center for Epidemiologic Studies Depression scale) (19); inability to undergo MRI scanning; past exposure to incretin-based therapy; substance abuse; and the use of oral glucocorticoids or any centrally acting agent. Twelve T2DM patients and 3 obese participants used antihypertensive medication, and 13 T2DM patients and 1 obese participant used statins. In the T2DM group, eight participants were treated with metformin monotherapy and eight used metformin in combination with a sulfonylurea. The lean controls did not use any medication. Reasons for screen failure were as follows: impaired glucose tolerance ($n = 9$), HbA$_{1c}$ not between 6.0 and 8.5% for patients with T2DM ($n = 3$), inability to undergo MRI scanning ($n = 6$), depressive symptoms ($n = 1$), incidental findings ($n = 4$), microalbuminuria ($n = 5$), no accessible veins ($n = 2$), and no stable body weight ($n = 1$).

The study (NCT01281228) was approved by the Medical Ethics Committee of the VU University Medical Center and was performed in accordance with the Helsinki Declaration. All participants provided written informed consent before participation.

Experimental Design

The study was a randomized, placebo-controlled, crossover study. Each participant underwent three fMRI sessions at separate visits (with at least 1 week between visits) in random order with intravenous infusion of 1) exenatide, 2) exenatide together with the GLP-1 receptor antagonist exendin 9-39, or 3) placebo (Fig. 1A). The participants were blinded to the type of infusions. All visits commenced at 8:30 A.M. after an overnight fast, and participants did not exercise or drink alcohol for 24 h before the sessions. Sulfonylureas were discontinued 3 days prior to examination, and metformin was discontinued on the day of examination. In order to study the effects of GLP-1 receptor activation per se, i.e., independent of hormonal or metabolic changes induced by GLP-1 receptor activation, all measurements were performed during a somatostatin pancreatic-pituitary clamp according to principles previously described (20). In short, somatostatin (Somatostatin; Eumedica) was infused at a rate of 60 ng/kg/min to suppress endogenous insulin, glucagon, growth hormone, and GLP-1 production. Human glucagon (0.6 ng/kg/min) (Glucagen; Novo Nordisk), growth hormone (2 ng/kg/min) (Genotropin; Pfizer), and insulin (0.6 mU/kg/min) (Actrapid; Novo Nordisk) were infused at constant rates to achieve stable levels. Glucose (200 g/L) was infused at a variable rate to clamp plasma glucose at 5.0 mmol/L. Intravenous exendin 9-39 (Bachem; Clinalfa Products, Bubendorf, Switzerland) or placebo was started 30 min after the start of the clamp at an infusion rate of 600 pmol/kg/min (21). Intravenous exenatide (Byetta; Eli Lilly and Company) or placebo infusion was started
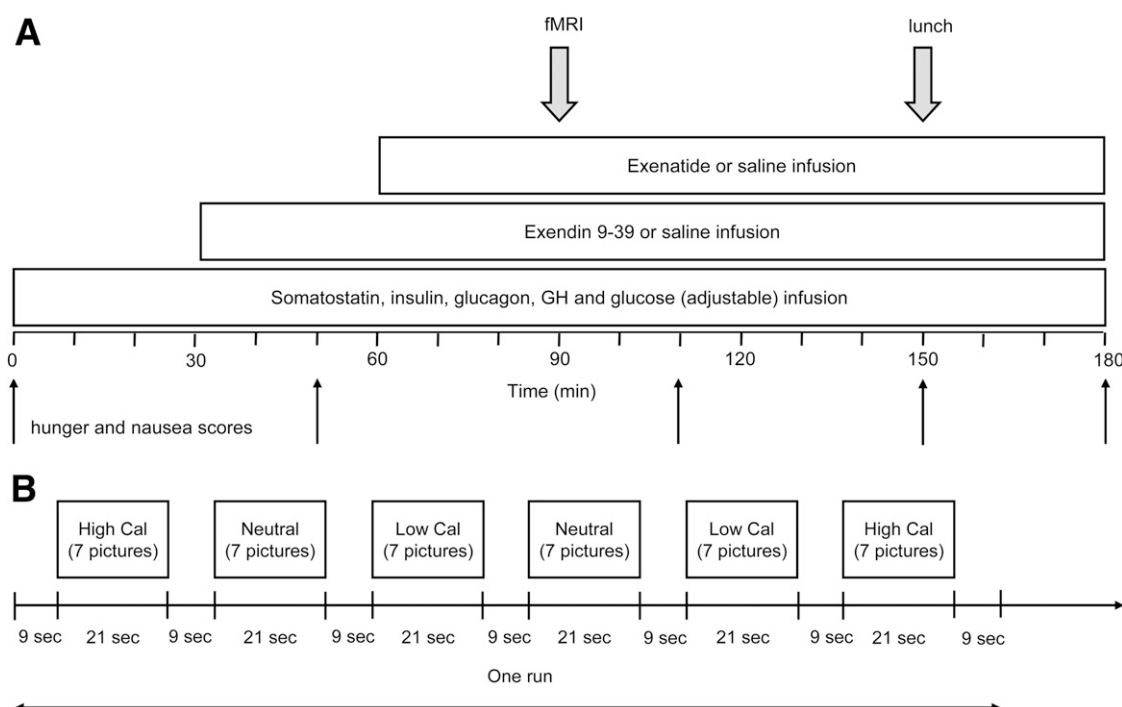
Figure 1—Study protocol. *A*: Study design. Obese T2DM patients (*n* = 16) and obese normoglycemic (*n* = 16) and lean (*n* = 16) subjects were studied in a randomized, placebo-controlled, crossover study. The acute effects of either intravenous exenatide or exenatide together with the GLP-1 receptor antagonist exendin 9-39 on CNS appetite and reward responses to visual food-related stimuli were measured, using fMRI, during a somatostatin pituitary-pancreatic clamp (glucose 5 mmol/L) after an overnight fast. After the fMRI, subjects were presented a lunch buffet to assess energy intake. Hunger and nausea scores were taken at *t* = 0, 50, 110, 150, and 180 min. *B*: fMRI paradigm. One of the three runs within one fMRI session. Each run consisted of six blocks of pictures, two each of high-calorie foods, low-calorie foods, and neutral pictures. The blocks were separated by 9 s of gray blank screen with a fixation cross. The order of blocks was randomized with the constraint that a given picture category was not followed by the same category. Cal, calorie; GH, growth hormone.

60 min after the start of the clamp at an infusion rate of 50 ng/min for 30 min and was decreased to 25 ng/min for the remaining time of the clamp procedure (22). Each peptide was diluted in saline containing 0.5% human serum albumin and was infused using a separate MRI-compatible infusion pump (MRidium 3850 MRI IV pump; Iradimed, Winter Park, FL). Participants assumed the reclining position and a catheter was inserted into a cubital vein for infusion of glucose and clamp hormones, and for the infusion of exenatide and/or exendin 9-39 or saline. A second catheter was inserted into a contralateral cubital vein for blood sampling. This arm was kept in a heated box (50°C) throughout the experiment to arterialize the venous blood. During the MRI session, a MRI-compatible heated box was used. Plasma glucose was measured every 10 min. Blood for measuring insulin, glucagon, and exenatide levels was drawn every 30 min. Blood for measuring growth hormone, free fatty acids, and cortisol was drawn at *t* = 0 min (fasting) and *t* = 120 min (during the fMRI session).

Questionnaires

At five time points (beginning of the experiment, before and after the MRI scan, and before and after the ad libitum lunch), participants were asked to rate their hunger, fullness, appetite, prospective food consumption, desire to eat, and feelings of nausea on a 10-point Likert scale (23). Participants also filled out the shortened version of the profile of mood state (POMS) at the beginning of the experiment and after the MRI scan (24).

fMRI Paradigm

The fMRI task consisted of 126 pictures within three categories: *1*) high-calorie food, *2*) low-calorie food, and *3*) nonfood, adapted from previous studies (6,25). High-calorie food pictures consisted of sweet and savory foods including ice cream, chocolate chip cookies, cheesecake, french fries, hamburgers, and pizza. Low-calorie food pictures consisted of fruit and vegetables including fruit salads, apples, strawberries, garden salads, cucumber, and tomatoes. Nonfood pictures consisted of rocks, shrubs, bricks, trees, and flowers. All pictures were presented via Eprime 1.2 (Psychology Software Tools, Inc., Pittsburg, PA). Pictures were presented in a block design format, with a total of three runs. Each run consisted of six blocks of pictures: two blocks of high-calorie foods, two blocks of low-calorie foods, and two blocks of nonfood pictures (Fig. 1*B*). Within each block of 21 s, seven individual pictures were presented for 2.5 s each followed by a 0.5-s gap each. The blocks were separated by 9 s of gray blank screen with a fixation cross. The order of blocks was randomized from run to run with the

constraint that a given picture category was not followed by the same category. Pictures were matched across blocks and sessions for shape and color. Since each participant was scanned three times, three versions with different pictures but otherwise identical design were created. The sequences of blocks were randomized across study visits. Participants were instructed to watch all pictures and to try to remember them for a recognition test. After the measurements, participants were given a recognition test consisting of 20 laminated color food and nonfood pictures (10 pictures of the MRI task randomly intermixed with 10 novel pictures).

Image Acquisition and Analysis

MRI data were acquired on a 3.0 Tesla GE Sigma HDxt scanner (General Electric, Milwaukee, WI). A 3-D structural MRI was obtained using a T1-weighted sequence. fMRI data were acquired using an echo planar imaging T2* blood oxygen level–dependent (BOLD) pulse sequence (repetition time = 2,160 ms, echo time = 30 ms, matrix 64×64, 211 mm^2 field of view, and flip angle = 80) with 40 ascending slices per volume (3 mm thickness, 0 mm gap), which gave whole-brain coverage.

Functional images were analyzed with SPM8 software (Wellcome Trust Centre for Neuroimaging, London, U.K.). The origin of each magnetic resonance volume was aligned to the anterior commissure. Series were corrected for differences in slice acquisition times and were realigned to the first volume. T1-coregistered volumes were normalized to Montreal Neurological Institute (MNI) space, resliced to $3 \times 3 \times 3$ mm voxels and spatially smoothed using an 8-mm full width at half maximum Gaussian kernel. After high-pass filtering (cutoff 128 s) to remove low-frequency noise, functional scans were analyzed in the context of the general linear model. At the first level, each block of high-calorie food, low-calorie food, and nonfood was modeled using boxcar functions convolved with the canonical hemodynamic response function. For each subject and for each condition, contrast images were computed (all food pictures vs. nonfood pictures; high-calorie food pictures vs. nonfood pictures). These first-level contrast images were entered into two separate second-level, random-effects ANOVAs to assess between- and within-group differences. To determine if changes in neuronal response (placebo vs. exenatide) were related to changes in caloric intake (placebo vs. exenatide), we performed a regression analysis in SPM using the change in caloric intake as a covariate of interest. A priori regions of interest were determined based on previous studies (i.e., insula, striatum, amygdala, and OFC) (4–6). Only significant brain activations that survived family-wise error (FWE) correction for multiple comparisons on the voxel level within the regions of interest using a small volume correction, or across the entire brain for regions not a priori of interest, are reported. Small volume correction was performed using 5-mm (for amygdala) or 10-mm (for insula, putamen, and OFC) radius spheres (26,27).

Ad Libitum Lunch Buffet

After the MRI session, while all infusions continued, participants were presented a varied choice buffet to assess energy intake (28). Participants were advised to eat as much as they wanted. They were not aware that their choices and food intake were being monitored. After 30 min, the buffet was taken away and the total kilocalories consumed and the percentages of kilocalories derived from fat, carbohydrates, and protein were being calculated.

Assays

Blood glucose was measured immediately after sampling using the glucose dehydrogenase method (Glucose Analyzer; HemoCue, Ängelholm, Sweden). All other blood samples were stored at −80°C until assay. Insulin levels were determined using an immunometric assay (ADVIA Centaur; Siemens Medical Solutions Diagnostics). Growth hormone levels were determined using a chemiluminescence immunoassay (Liaison Diasorin S.p.A., Saluggia, Italy). Nonesterified fatty acid (NEFA) levels were measured with an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). Cortisol levels were determined using a competitive assay (ADVIA Centaur). Glucagon levels were measured using an immunoassay (Lilly Research Laboratories, Indianapolis, IN). Exenatide levels were determined by an immunoenzymatic assay (Tandem Laboratories, San Diego, CA), with a detection limit of 20 pg/mL.

Statistical Analyses

Clinical group data are expressed as mean ± SEM (unless otherwise stated) and were analyzed with the Statistical Package for the Social Sciences (SPSS) version 20. Between-group differences in the placebo condition were analyzed with ANOVA or, in case of more than one time point, with repeated measures ANOVA using time (minutes) as within-subject factor and group as between-subject factor. Within-group differences were analyzed using repeated measures ANOVA using treatment and time (minutes) as within-subject factor. In case of a significant result, a post hoc Bonferroni multiple comparisons correction was used. In case of skewed data, Kruskal-Wallis test was used for between-group differences and Friedman test for within-group differences. In case of a significant result, Mann-Whitney U test for between-group analysis or Wilcoxon signed rank for within-group analysis with post hoc Bonferroni correction was performed. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

We included 16 obese T2DM patients and 16 normoglycemic obese and 16 lean subjects; 47 subjects completed all three test days. One subject only completed the exenatide and placebo test day. Subjects in the three groups were age and sex matched, and T2DM patients and normoglycemic obese subjects were also BMI matched (Table 1).

Plasma Levels of Hormones and Metabolites During the Pancreatic-Pituitary Clamp

During the fMRI session and ad libitum lunch (t = 90 until t = 180 min) (Fig. 1A), blood glucose was successfully clamped at ~5.0 mmol/L, with no statistically significant differences in glucose levels between groups and between sessions within each group (Supplementary Table 1 and Supplementary Fig. 1). As expected, the lean subjects needed higher glucose infusion rates in comparison with obese subjects and T2DM patients ($P < 0.05$), but infusion rates were not different between sessions within each group (Supplementary Table 1 and Supplementary Fig. 1). During the clamp, insulin levels were higher in T2DM patients and obese in comparison with lean subjects ($P < 0.001$); glucagon levels were lower in obese in comparison with lean subjects ($P < 0.05$); and NEFA levels were higher in T2DM patients as compared with lean and obese subjects ($P < 0.006$) (Supplementary Table 1). Due to the pancreatic clamp, there were no significant differences between sessions within each group in circulating hormones and metabolites (insulin, glucagon, growth hormone, and NEFA) (Supplementary Table 1 and Supplementary Fig. 2). There was a trend toward higher cortisol levels in the lean subjects during exenatide infusion in comparison with exenatide in combination with exendin 9-39 ($P = 0.12$). The infusion of exenatide resulted in pharmacologically relevant plasma concentrations that were similar in the lean, obese, and T2DM groups (181 ± 13, 154 ± 13, and 153 ± 13 pg/mL, respectively; P for between-group difference = 0.25) (29,30).

Brain Responses to Food Pictures in T2DM, Obese, and Lean Subjects

We investigated two main contrasts of interest, one to assess general food-related responses (all food pictures vs. nonfood pictures) and a second to assess reward-related responses (high-calorie food pictures vs. nonfood pictures). Effects of these contrasts across all subjects are reported in Supplementary Table 2. Obese versus lean subjects showed increased brain activation in bilateral insula (all food vs. nonfood and high calorie vs. nonfood) and right amygdala (all food vs. nonfood) (Fig. 2A and Supplementary Table 3). T2DM patients versus lean subjects showed increased brain activation in left insula (all food vs. nonfood) (Fig. 2B and Supplementary Table 3). Obese subjects versus T2DM patients showed increased activation in right insula (all food vs. nonfood and high calorie vs. nonfood).

GLP-1 Receptor Activation and Brain Responses to Food Pictures

Exenatide versus placebo decreased brain activations in obese subjects in right amygdala and insula (all food vs. nonfood) in right insula and left OFC (high calorie vs. nonfood) and in obese T2DM patients in bilateral insula, left putamen, and right OFC (all food vs. nonfood and high calorie vs. nonfood) (Fig. 3 and Table 2). Exenatide also decreased brain activations (all food vs. nonfood)

in the right putamen in the obese group and in the right amygdala in the T2DM group, but these effects were not statistically significant (P = 0.13 and 0.16, respectively; data not shown). In the lean subjects, we found no statistically significant effects of exenatide versus placebo on brain activations (all food vs. nonfood), although there was a trend toward attenuated activation in right insula, OFC, and putamen (P = 0.18, 0.09, 0.22, respectively). Pretreatment with the GLP-1 receptor antagonist exendin 9-39 largely blocked the exenatide-induced effects on brain activations, i.e., in obese subjects in right insula, amygdala, and left OFC and in T2DM patients in bilateral insula, left putamen, and right OFC (Table 2).

A recognition test was performed after all measurements to ensure that the subjects watched the pictures attentively during the fMRI session. There were no statistically significant differences in recognition test scores between groups and between sessions (data not shown).

GLP-1 Receptor Activation, Ad Libitum Food Intake, and Hunger Scores

There was a significant difference between sessions in caloric intake in each group ($P < 0.05$), with reductions in intake during exenatide versus saline infusion of 23 ± 8, 23 ± 10, and 14 ± 5% in the lean, obese, and T2DM groups, respectively. These effects of exenatide were greatly reduced by concomitant infusion with the GLP-1 receptor antagonist exendin 9-39 (Fig. 4). There were no significant differences between sessions in each group in the percentages of kilocalories derived from fat, carbohydrates, and proteins.

We found a positive correlation between changes in caloric intake (placebo vs. exenatide) and changes in CNS responses (placebo vs. exenatide, for the food vs. nonfood contrast) in T2DM patients in bilateral insula and right caudate nucleus, and in obese subjects in bilateral OFC, bilateral insula, and right caudate nucleus (Supplementary Table 4). As expected, no significant correlation was found in lean subjects, since there were no significant effects of GLP-1 receptor activation on CNS responses to food pictures in this group.

We found no statistically significant differences between groups and between conditions in hunger, fullness, appetite, prospective food consumption, desire to eat, and feelings of nausea. In addition, no between-group or within-group differences in mood state, as assessed by the POMS, were found (data not shown). Although there were no statistically significant differences in nausea scores before and directly after the lunch ($P > 0.19$), three subjects in the lean group and two in the obese group experienced nausea and vomiting on the exenatide test day after termination of all infusions and all measurements.

DISCUSSION

In the current study, we confirmed that normoglycemic obese versus lean subjects have increased activation in appetite- and reward-related brain areas in response to

Table 1—Baseline characteristics

	Lean (n = 16)	Obese (n = 16)	T2DM (n = 16)	ANOVA P value
Age (years)	57.8 ± 1.9	58.0 ± 2.1	61.4 ± 1.5	0.3
Sex, male/female (n)	8/8	8/8	8/8	—
Weight (kg)	71.1 ± 2.7	100.6 ± 2.8*	97.9 ± 3.0*	<0.001
BMI (kg/m^2)	23.2 ± 0.4	32.6 ± 0.7*	34.0 ± 0.9*	<0.001
Waist circumference (cm)	85.5 ± 1.9	112.7 ± 2.1*	115.7 ± 1.8*	<0.001
Systolic blood pressure (mmHg)	120 ± 4	127 ± 3	141 ± 3†	<0.001
Diastolic blood pressure (mmHg)	75 ± 2	79 ± 2	83 ± 2*	0.03
Fasting plasma glucose (mmol/L)	5.3 ± 0.1	5.3 ± 0.1	8.4 ± 0.5†	<0.001
Glucose 2 h after OGTT (mmol/L)	5.1 ± 0.3	5.5 ± 0.3	—	0.2
HbA$_{1c}$ (%)	5.5 ± 0.03	5.5 ± 0.07	6.9 ± 0.22†	<0.001
HbA$_{1c}$ (mmol/mol)	37.4 ± 0.3	37.5 ± 0.08	51.6 ± 2.4 †	<0.001
Total cholesterol (mmol/L)	5.6 ± 0.2	5.7 ± 0.2	4.5 ± 0.3†	0.001
LDL cholesterol (mmol/L)	3.3 ± 0.15	3.5 ± 0.18	2.3 ± 0.2†	<0.001
HDL cholesterol (mmol/L)	1.9 ± 0.1	1.4 ± 0.1*	1.3 ± 0.1*	<0.001
Triglycerides (mmol/L)	0.9 ± 0.1	1.8 ± 0.3	1.8 ± 0.3	0.03
Fasting NEFA (mmol/L)	0.46 ± 0.04	0.46 ± 0.03	0.64 ± 0.04†	0.001
Fasting insulin (pmol/L)	35 ± 2.6	83 ± 12*	117 ± 17*	<0.001
Fasting glucagon (pmol/L)	8.5 ± 0.9	7.7 ± 0.6	11.9 ± 1.5‡	0.016
Fasting growth hormone (mU/L)	2.4 ± 0.6	1.1 ± 0.4	1.4 ± 0.6	0.3
Fasting cortisol (nmol/L)	368 ± 15	305 ± 14*	330 ± 18	0.024
Diabetes duration (years)	—	—	7.0 [4.25, 10.75]	—

Data are means ± SEM or median [interquartile range]. OGTT, oral glucose tolerance test. *Statistically significant different from lean (post hoc Bonferroni corrected, $P < 0.05$). †Statistically significant different from lean and obese (post hoc Bonferroni corrected, $P < 0.05$). ‡Statistically significant different from obese (post hoc Bonferroni corrected, $P < 0.05$).

viewing food cues (4,6,25), and we expanded these observations by showing that obese T2DM patients have increased activation in the insula in comparison with lean subjects. Most importantly, using intravenous exenatide, we found that GLP-1 receptor activation reduced brain responses to food cues in normoglycemic obese subjects and obese T2DM patients in appetite- and reward-related brain areas, correlating with reductions in food intake. The exenatide-induced effects were GLP-1 receptor mediated as these could be inhibited by infusion of the GLP-1 receptor antagonist exendin 9-39, and occurred independently of circulating metabolic and hormonal factors.

Our study provides novel insights into the mechanisms by which GLP-1 receptor agonists reduce food intake. In obese and T2DM subjects, we observed effects of GLP-1 receptor activation on brain responses to food cues in the insula, amygdala, OFC, and putamen. These brain areas are assumed to be involved in the regulation of appetite and reward. The insula is involved in the processing of food cues and craving for food (31) and has been implicated in the devaluation of food cues when eating to satiety (32). The amygdala plays a pivotal role in emotional learning and in the association of cues with reward (33) by encoding the value of the reward predicted by the conditioned stimuli (34). The OFC and putamen are also implicated in reward processing (35), and the OFC is assumed to be involved in

decision making (36). Our findings are of interest since they are expanding findings from previous fMRI studies showing effects of leptin and the gut-derived hormones PYY and ghrelin on brain responses to food cues in similar brain areas (8,9,37,38). In the search for therapeutic targets for the treatment of obesity, all of these hormones have been explored, but currently to no avail (39). Interestingly, GLP-1 receptor agonists have shown modest but consistent weight loss in humans (14).

In the lean subjects, we also observed effects of GLP-1 receptor activation on food intake and brain responses to food cues in several brain areas (insula, OFC, and putamen), but the latter were not statistically significant. The lower brain activation in response to food cues in lean individuals per se may have reduced the likelihood to find significant effects of GLP-1 receptor activation on these brain activations. A previous study in lean subjects also failed to observe significant effects of intravenous administration of GLP-1 alone on brain responses to food cues, but coinfusion with the postprandial anorectic hormone PYY resulted in significant effects on appetite centers (38). It should be pointed out that these measurements may have been influenced by GLP-1– or PYY-induced changes in circulating hormones and metabolites, such as glucose, insulin, and glucagon. Since insulin and glucose modulate brain activity in areas controlling feeding
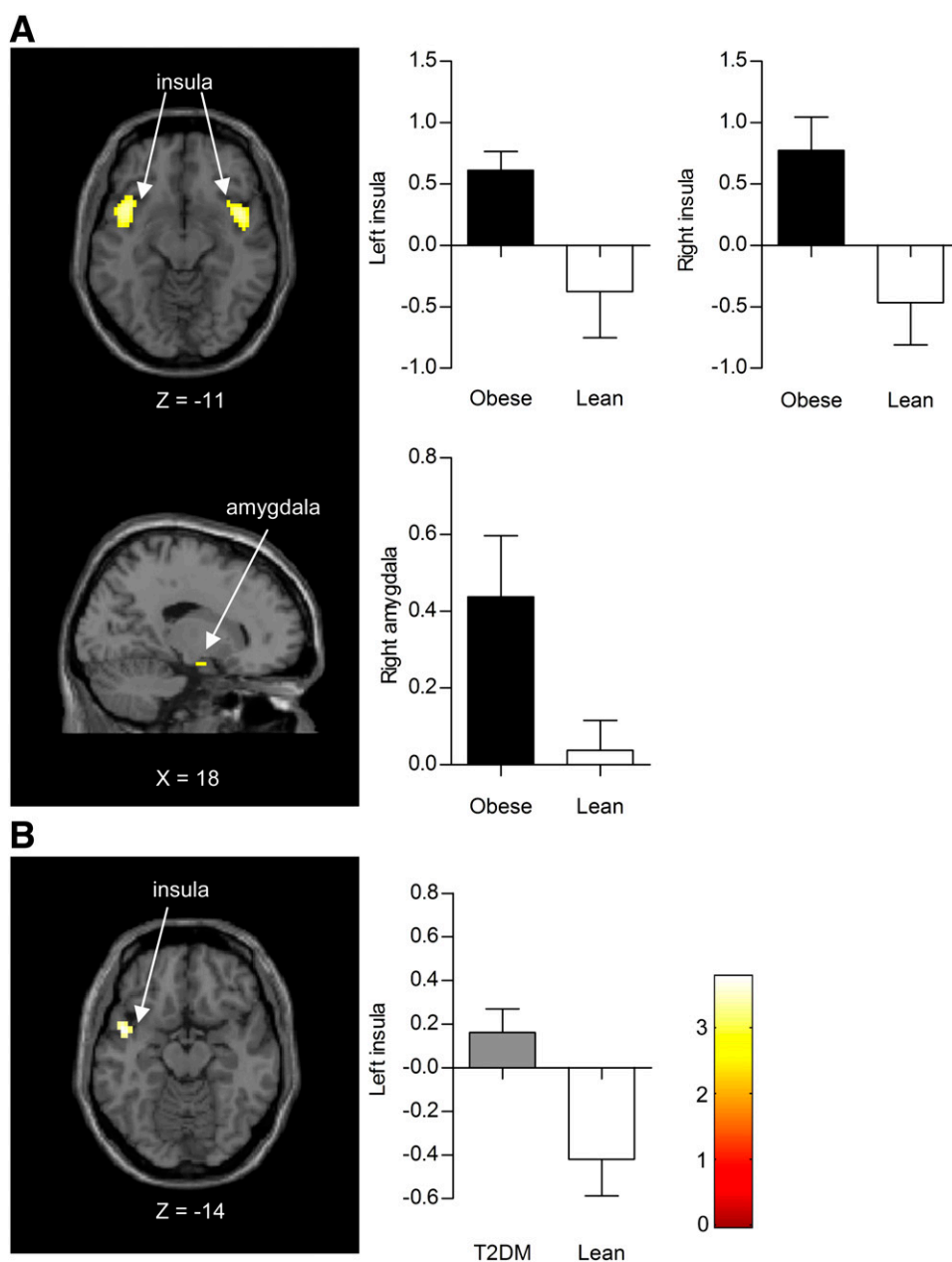
Figure 2—Between-group differences in CNS responses to food pictures. Axial and sagittal slices showing brain regions where obese vs. lean subjects (*A*) and T2DM patients vs. lean subjects (*B*) exhibited increased brain activation while viewing food pictures (all food vs. nonfood). Left side of the axial slices is the left side of the brain. Z is the MNI space Z coordinate of the axial slice and X is the MNI space X coordinate of the sagittal slice. The color scale reflects the T value of the functional activity. Results are presented at a threshold of $P < 0.05$, FWE corrected on the basis of cluster extent. In the graphs on the right, the BOLD signal intensity (effect size) for each group is plotted (arbitrary units), mean and SEM.

behavior (10), this may have confounded the measurements. In our study, all measurements were performed during a somatostatin pancreatic-pituitary clamp and were therefore independent of changes in circulating levels of glucose, insulin, glucagon, and growth hormone.

We found that the effects of exenatide on food intake and brain responses to food cues were largely inhibited by exendin 9-39, suggesting that the effects of exenatide are GLP-1 receptor mediated. Whether the CNS effects of exenatide are mediated via central or peripheral GLP-1 receptors cannot be determined from our study. In rodents, it was shown that exendin-4 (a peptide closely resembling exenatide structurally) is able to cross the blood-brain barrier (40) and to reduce the rewarding value of food-mediated via mesolimbic GLP-1 receptors (41), pointing toward direct effects on the CNS. However, other studies in rodents have shown that the effects of GLP-1 on the CNS may be partly mediated via indirect
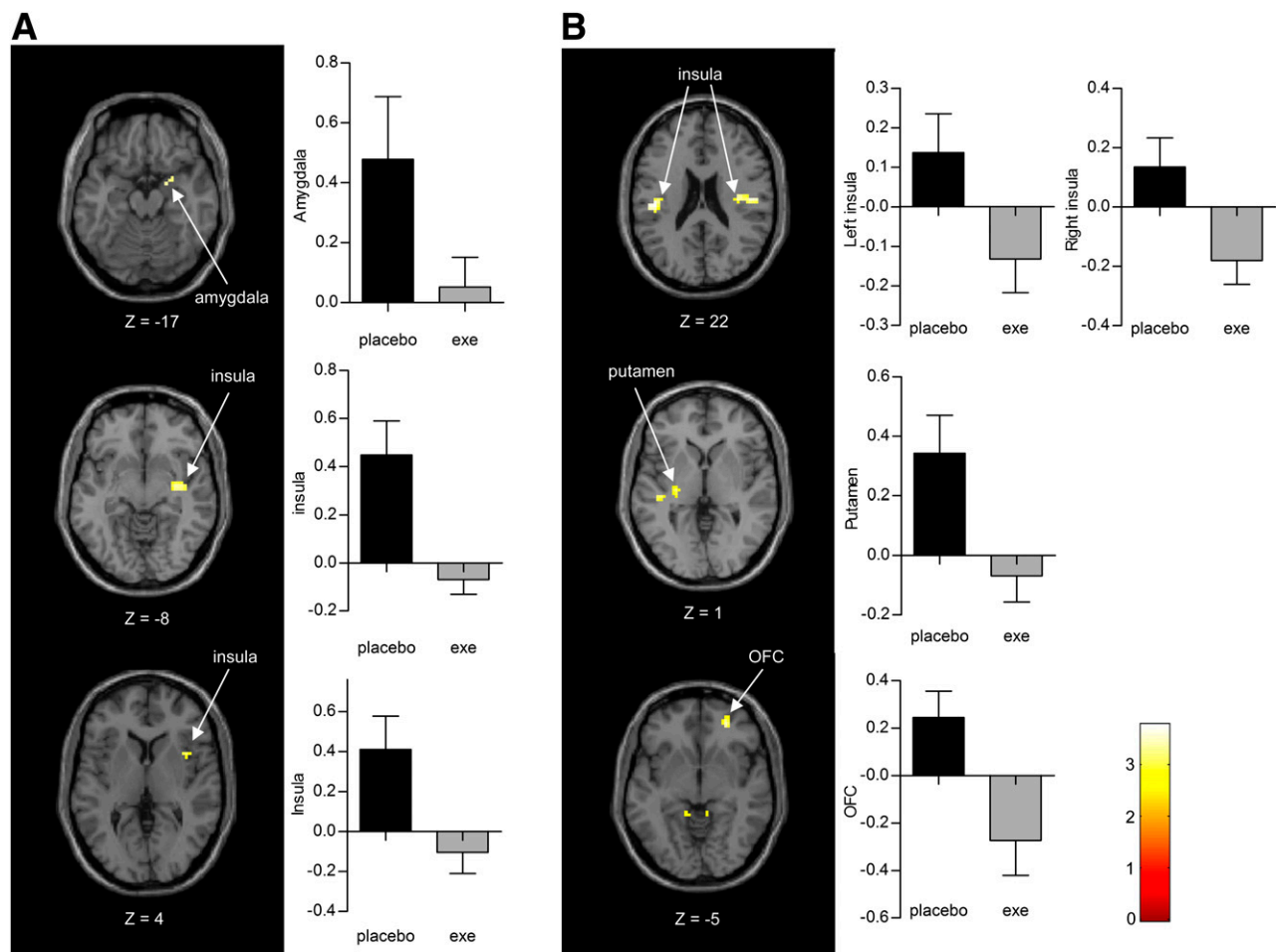
Figure 3—Effects of GLP-1 receptor activation on CNS responses to food pictures. Axial slices showing brain regions where exenatide vs. placebo reduced brain activation in obese subjects (*A*) and T2DM patients (*B*) while viewing food pictures (all food vs. nonfood). Left side of the axial slices is the left side of the brain. Z is the MNI space Z coordinate of the axial slice. The color scale reflects the T value of the functional activity. Results are presented at a threshold of *P* < 0.05, FWE corrected on the basis of cluster extent. In the graphs, BOLD signal intensity (effect size) for the different test days is plotted (arbitrary units), mean and SEM.

routes of action i.e., via GLP-1 receptors on vagal fibers signaling to the CNS (15). Vagotomy attenuated the effects of peripheral administered GLP-1 on food intake and neuronal activation in central food intake–regulating areas (42). Furthermore, peripheral administration of the GLP-1 albumin fusion protein (Albugon), which is unable to cross the blood-brain barrier, reduced food intake and induced neuronal activation, but these effects were less robust in comparison with exendin-4 (43). Since in our study exendin 9-39 largely blocked the CNS effects of exenatide, and in rodents the uptake of exendin 9-39 in the brain is low (44), this may imply that the observed CNS effects are mostly mediated via peripheral GLP-1 receptors. However, additional information regarding the contribution of peripheral and central GLP-1 receptors in the effects of GLP-1 (receptor agonists) on the CNS is needed in future studies.

GLP-1 has been shown to delay gastric emptying (45) and can induce nausea, which may also contribute to the satiety-inducing effects. However, reductions in appetite after GLP-1 administration are present in fasting subjects (with an empty stomach) (46). In the current study, subjects were also fasted during all measurements, and relevant effects on appetite- and reward-related brain areas were observed. Unfortunately, after the lunch when all infusions were terminated, some subjects experienced nausea and vomiting, which may have been caused by exenatide-induced effects on gastric emptying. However, there were no significant effects of exenatide on nausea scores during the fMRI measurements and before and directly after the lunch. There are several other reasons to assume that delayed gastric emptying and nausea are not the only cause of reductions in food intake and body weight during GLP-1 receptor agonist treatment. The weight reduction seen during GLP-1 receptor agonist treatment is also observed in the absence of nausea (47). Furthermore, the inhibitory effect of GLP-1 on gastric emptying is subject to tachyphylaxis (rapid desensitization)

Table 2—Effects of GLP-1 receptor activation on CNS responses to food pictures

	Comparison	Region	Side	Cluster	T	P_{FWE}	MNI (x, y, z)
Lean							
Food >nonfood	Placebo >EXE	NA					
	Placebo >EX9-39 + EXE	NA					
HC >nonfood	Placebo >EXE	NA					
	Placebo >EX9-39 + EXE	NA					
Obese							
Food >nonfood	Placebo >EXE	Amygdala	R	16	3.21	0.007	27, 2, −20
		Insula	R	24	3.06	0.025	39, −10, −8
		Insula	R	9	2.93	0.048	39, 11, 4
	Placebo >EX9-39 + EXE	Insula	L	8	3.26	0.021	−30, 14, −17
HC >nonfood	Placebo >EXE	Insula	R	53	3.66	0.007	39, −10, −8
		Insula	R	28	3.02	0.039	42, 2, 10
		OFC (BA 47)	L	6	3.10	0.032	−48, 20, −11
	Placebo >EX9-39 + EXE	NA					
T2DM							
Food >nonfood	Placebo >EXE	Insula	L	140	2.64	0.010	−39, −16, 22
		Insula (BA 13)	L	21	3.77	0.005	−42, −43, 19
		Insula	R	70	3.19	0.026	36, −16, 22
		Putamen	L	37	2.92	0.032	−30, −19, 1
		OFC (BA 11)	R	17	3.43	0.009	27, 47, −5
	Placebo >EX9-39 + EXE	NA					
HC >nonfood	Placebo >EXE	Insula (BA 13)	R	108	3.74	0.005	36, −13, 22
		Putamen	L	45	3.38	0.015	−30, −19, 4
		OFC (BA 11)	R	17	3.51	0.011	27, 47, −5
		Insula	L	140	3.54	0.010	−45, −22, 22
	Placebo >EX9-39 + EXE	Insula (BA 13)	R	40	3.08	0.034	36, 23, 10

MNI coordinates are in mm. BA, Brodmann area; EX9-39, exendin 9-39; EXE, exenatide; L, left; P_{FWE}, P value FWE corrected for multiple comparisons on the basis of cluster extent; R, right; T, t value.

(48), whereas the weight-lowering effects persist over periods up to 3 years (49).

In contrast to previous studies (50), we found no statistically significant effects of GLP-1 receptor activation on scores of hunger, fullness, appetite, prospective food consumption, and desire to eat. In subjects eating their normal diets in their normal environment, appetite scores have been shown to correlate with, but not reliably predict, energy intake to the extent that they could be used as a proxy of energy intake (51). Under experimental conditions, such as those in our complex experimental fMRI study, appetite scores may be less sensitive (51).

We demonstrated the acute effects of GLP-1 receptor activation on CNS responses to food cues and food intake during a somatostatin pancreatic-pituitary clamp. As a consequence, our measurements were performed under nonphysiological circumstances. Therefore it may be difficult to directly translate our results to, for example, clinical use of GLP-1 receptor agonists. However, the observed food intake–suppressive effect of exenatide in our study is in line with studies using intravenous GLP-1 in a more physiological setting (12) and with observed weight loss during chronic treatment with GLP-1 receptor agonists (14). Long-term studies using fMRI measurements of food-related CNS responses during chronic treatment with GLP-1 receptor agonists are of interest.

In summary, we found that the GLP-1 receptor agonist exenatide decreases hyperactivation in appetite- and reward-related brain regions, elicited by viewing food cues in obese T2DM and obese normoglycemic subjects, thus restoring an activation pattern that more closely resembles that of lean individuals. We found that these effects are GLP-1 receptor mediated and independent of circulating metabolic and hormonal factors. Our findings provide novel insights into the mechanisms by which GLP-1 regulates food intake and how GLP-1 receptor
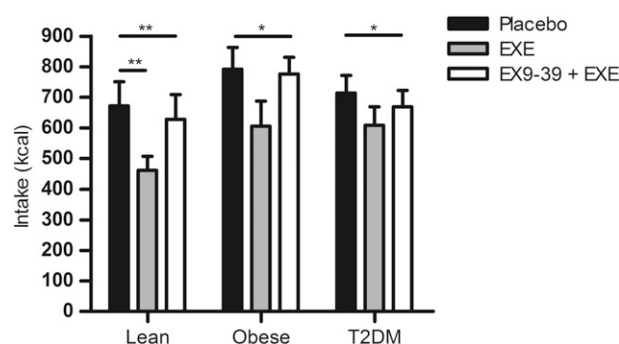

Figure 4—Effects of GLP-1 receptor activation on caloric intake. Bar chart with caloric intake in three groups during different infusions. Within-group differences were tested with repeated measures ANOVA (indicated by top line). Post hoc tests were performed by two-sample Student t tests with Bonferroni correction for multiple testing. Data are presented as mean and SEM. *P < 0.05; **P < 0.005. EX9-39, exendin 9-39; EXE, exenatide.

agonists cause weight loss. Further insights into the central regulation of food intake may help to develop new treatment strategies for obesity and T2DM.

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