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Short-term administration of the GLP-1 analog liraglutide decreases circulating leptin and increases GIP levels and these changes are associated with alterations in CNS responses to food cues: A randomized, placebo-controlled, crossover study

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Abstract

Background—GLP-1 agonists, including liraglutide, have emerged as effective therapies for type 2 diabetes (DM) and obesity. Here, we attempted to delineate how liraglutide, at doses approved for DM, may impact circulating hormones influencing energy homeostasis in diabetics.

Basic Procedures—Using a randomized, placebo-controlled, double-blind, cross-over trial of 20 patients with type 2 diabetes, we examined the effects of liraglutide as compared to placebo on fasting levels of circulating hormones important to energy homeostasis, including leptin, ghrelin, PYY, and GIP. After 17 days (0.6 mg for 7 days, 1.2 mg for 7 days and 1.8 mg for 3 days) of treatment, we also studied changes in fMRI responses to food cues.

Main Findings—By design, to avoid any confounding by weight changes, subjects were studied for 17 days, i.e. before body weight changed. Participants on liraglutide had significantly increased GLP-1 levels ($p<0.001$), decreased percent change in leptin levels ($p<0.01$) and increased GIP levels ($p<0.03$) in comparison to placebo treated subjects. Whole brain regressions of functional activity in response to food cues reveal that increased GIP levels were associated with deactivation of the attention- and reward-related insula. Decreases in leptin levels were associated with activations in the reward-related midbrain, precuneus, and dorsolateral prefrontal cortex (DLPFC),

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Conflicts of Interest

CSM has served on the scientific advisory board and is a shareholder of Novo Nordisk. All other authors have nothing to declare.

Contributions

CSM designed the study. OMF, MAT, FD, AF, and CSM collected the data. OMF and BJK analyzed the data. OMF wrote the manuscript. All authors (OMF, MAT, GT, FD, AF, BJK, and CSM) reviewed and edited the manuscript.

and sensorimotor-related motor cortex and with deactivations in the attention-related parietal cortex and the cognitive control-related thalamus and pre-SMA.

Principal Conclusions—We demonstrate herein short-term changes to circulating levels of GIP and leptin in response to GLP-1 agonist liraglutide therapy. These findings suggest that liraglutide may alter the circulating levels of hormones important in energy homeostasis that, in turn, influence CNS perception of food cues. This could possibly lead to compensatory changes in energy homeostasis that would over time limit the efficacy of liraglutide to decrease body weight. These novel findings, which, pointing to the potential advantages of combination therapies, may have therapeutic implications, will need to be confirmed by larger and longer-term trials.

Obesity and its comorbidities, including type 2 diabetes, are rapidly increasing problems in need of effective therapies [1]. Multiple circulating hormones, including glucagon-like peptide-1 (GLP-1), that are secreted from the gut are known to convey information about nutritional status to the brain, regulating satiety and food intake, thus providing a crucial link between peripheral metabolic processes and the central nervous system. GLP-1 agonists, such as liraglutide, are becoming an increasingly attractive option for patients with type 2 diabetes whose symptoms would also be improved by weight loss and for whom glycated hemoglobin levels are moderately elevated [2]. Through mechanisms not fully understood, liraglutide in doses approved for type 2 diabetes (1.8 mg) is associated with a significant reduction in weight (treatment difference −1.4 to −3.5 kg when compared with placebo and/or other anti-diabetes medications such as the dipeptidyl-peptidase-4 inhibitor sitagliptin, insulin, or sulfonylureas such as glimepiride) [3].

Although GLP-1 slows gastric emptying, thereby promoting gastric distention and sensation of satiety [4], increasing evidence points to central mechanisms of action, demonstrating that GLP-1 acts in the brain of mice and limited number of human studies [5–7]. Enterohormonal signals may also mediate how the brain responds to food cues, providing an indirect mechanism for central actions of GLP-1 agonists [8–11]. We have recently shown decreased attention-related parietal cortex activations to highly desirable food cues during a fasting-state fMRI study with a short course of liraglutide, pointing to a central mechanism of action [12]. However, whether GLP-1 analog administration interacts with peripheral signals, i.e. alters levels of circulating hormones in the fasting state, and these changes lead, in turn, to altered functional brain activation, remains to be studied.

We sought to explore whether GLP-1 related weight loss in humans is linked to altered levels of hormones important in energy homeostasis using the maximum daily dose (1.8 mg) of liraglutide approved for diabetics in the context of a dose escalation, randomized, placebo-controlled, cross-over study for 17 days, i.e. a time period that allows for participants to escalate to the maximum dose to eliminate side effects but which does not allow for weight loss. We first studied how circulating biomarkers and hormones which are important in energy homeostasis and weight loss may be changed in response to treatment with liraglutide and thus mediate the effects of liraglutide in the short-term, i.e. before actual weight loss had occurred. We also analyzed how these hormonal changes in turn related to functional changes in the brain, thus determining whether liraglutide’s actions on peripheral cues may mediate a central (brain) response.

Research Design and Methods

As part of a larger study, twenty men and women with type 2 diabetes mellitus (DM; defined as fasting plasma glucose > 125 mg/dL and/or HbA1c > 6.5%) provided written informed consent to participate in a randomized, cross-over, placebo-controlled, double-blind study, approved by the Beth Israel Deaconess Medical Center (BIDMC) Institutional Review Board (full study details in [12]). Patients were being treated for their DM with metformin or with lifestyle modification (diet and exercise); patients receiving other treatments were excluded from the study.

Briefly, participants were randomized to receive liraglutide or placebo for their first phase; they then received the opposite for the second phase after a three week to three month wash-out period. Doses of liraglutide were escalated during the phase, which consisted of four visits. The first three visits were each a week apart where daily doses of 0.6mg and 1.2mg were begun at the first two and 1.8mg began at the third and continued for 3 days before participants returned for their 4th visit of the phase, which consisted of an overnight visit after which they underwent MRI (as previously described [12]). Detailed anthropometric data (e.g. dual energy x-ray absorptiometry or DEXA) and resting metabolic rate (RMR) were collected at the first and fourth visit of each phase.

Biochemical measurements and analysis

Fasting blood was drawn by venipuncture by a registered nurse between 8 and 10 am. Nurses took vital signs in the morning before fasting blood draws. Samples were immediately processed for plasma and serum isolation according to standard operating procedures and stored at −80°C until analysis as previously described [12–14]. All samples and standards were assayed in duplicate and only results with a CV <15% were used.

Amylin, gastric inhibitory peptide (GIP), and pancreatic polypeptide (PP) were measured in serum samples by commercially available enzyme-linked immunosorbent assay (ELISA; Millipore, Billerica, MA, USA). The GIP ELISA does not cross-react with GLP-1 or GLP-2. Fibroblast growth factor 21 (FGF-21) was measured by commercially available ELISA (R&D Systems, Inc. Minneapolis, MN). Irisin was measured by ELISA (Phoenix Pharmaceuticals. Burlingame, CA). GLP-1, leptin, ghrelin, peptide YY (PYY), and adiponectin in serum was measured by commercially available radioimmunoassay (Millipore Co. Billerica, MA USA). Fructosamine was analyzed in serum samples by the Roche cobas c311 clinical chemistry analyzer using a standardized kit (cat#04537939-190). All assays were performed as previously described [12–14].

Fasting serum glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), amylase, and lipase were measured by LabCorp (Raritan, NJ), a CLIA-certified laboratory. Fasting blood samples were sent directly to LabCorp by the BIDMC CRC as per standard protocols.

Data were analyzed using the Statistical Package for Social Sciences (SPSS), v.19 and were first summarized with descriptive statistics. For example, continuous variables are presented as mean ± standard error of the mean (SE). Data for categorical variables are presented as

numbers and/or percentages. Kolmogorov-Smirnov test and frequency histograms were used to check the normality of distribution of the continuous variables. We obtained the skew statistic and standard error of skew (using the descriptive command in SPSS) to identify variables that are non-normal. The treatment effect was assessed using a general linear mixed model. The variables of treatment, visit, and sequence were included in the model as fixed effects and patient-within-sequence was included as a random effect. Baseline values were included as a covariate. The values of anthropometric, clinical, and laboratory variables in visit 4 and visit 8 were compared and used as a dependent variables. The dependent variables which did not fulfill the normality assumptions were log-transformed before analysis. The difference in values between visit 4 and visit 1 and visit 8 and visit 5 as well as the percent change between each period [(value in visit 4 – value in visit 1)/value in visit 1*100 vs. (value in visit 8 – value in visit 5)/value in visit 5 *100] was also compared and assessed as dependent variables. Visual analog scale ratings were correlated using a Spearman correlation with hormones that showed a significant change with liraglutide, and those with P<.025 were considered significant.

fMRI protocol and analysis

MRI scanning occurred at the Center for Biomedical Imaging, Boston University School of Medicine, using a 3-T Philips Intera whole-body MRI (Philips Medical Systems, Best, The Netherlands), as previously described [12]. The fMRI protocol consisted of six runs during both the fasting and fed scans, during which subjects viewed blocks of images and provided responses on how much they like each image (on a 1–3 scale) using a fiberoptic response box held in their right hand.

BOLD data was preprocessed using the SPM8 (Statistical Parametric Mapping; The Wellcome Trust Centre of Neuroimaging; London, UK). First-level processing occurred as previously described [12]. The contrast images of the first-level analysis were used for the second-level group statistics. Whole brain regressions were run with hormone levels or change in hormone levels for fasting state images with the highly desirable- less desirable food cues contrast. Activations significant at p<.001, uncorrected and p<.025, Family-wise Error (FWE) corrected for voxel are reported. We chose the stringent criteria of p<.025 since we tested 2 hormones (leptin and GIP; 0.05/2=0.025).

Results

Metabolic Impacts of liraglutide

20 participants completed the study (11 males; aged 49.7±2.4 years). As previously reported, during the short course of liraglutide therapy, body weight and weight circumference did not change by design (i.e. duration of the study; Table 1) to avoid any confounding effects of weight loss. As previously reported, participants consumed less kcal per day while on 1.8mg liraglutide than while on placebo (placebo(averaged): 1782±127; 0.6mg: 1723±150; 1.2mg: 1640±93; 1.8mg: 1424±127; t(7)= 2.11; p<.07, two-tailed; p<.03, one-tailed).

There was a significant decrease in the percentage change of leptin while on liraglutide therapy (−10.4 ± 7.1%) compared to the placebo (16.6 ± 7.1%; p<.010), and this remained

significant when adjusted for BMI (p<.014). Furthermore, this percentage change in leptin while on liraglutide inversely correlates with feelings of fullness from visual analog scale (VAS) ratings (rho=-.544, p=0.016). There were no changes in adiponectin (p<.371), irisin (p<.630), ghrelin (p<.723), amylin (p<.448), or thyroid hormone levels. Gastric inhibitory peptide (GIP) showed a significant increase with liraglutide treatment (p<.030). However, many other gut and neural related hormones showed no significant changes (Table 1).

Neurocognitive impacts of liraglutide

There were no changes in brain activations to food cues during the fed state. Whole brain regressions of the fMRI scans in the fasting state with significant hormones after 17 days of liraglutide therapy, reveal that GIP levels inversely correlate with activation of the insula while participants viewed highly desirable as compared to less desirable food images (Figure 1). Additionally, for the same contrast and time point, the change in leptin levels correlates positively with activations in the thalamus, parietal cortex, and pre-SMA, while it correlates inversely with activations in the dorsolateral prefrontal cortex (DLPFC), motor cortex, precuneus, midbrain, and cerebellum (Figure 2).

Discussion

This study gives insights for the first time into how GLP-1 agonists influence hormones important to energy homeostasis in the short-term (17 days) and how these changes in turn influence central nervous system response to food cues in the fasting (but not the fed) state 17 days after initiation of therapy. These changes, observed using the ideal randomized, cross-over, double-blind design, if they persist, may influence weight loss outcomes indirectly and in a compensatory manner over time.

We have reported a significant decrease of caloric intake with liraglutide during the 17 days of treatment (using a one-tailed t-test consistent with our *a priori* hypothesis) [12]. In contrast, we do not observe any changes in resting energy expenditure. Of note, we did not measure 24-hour energy expenditure and thus cannot state whether activity/energy expenditure changed with liraglutide therapy. In another study, 24-hour energy expenditure, as measured in a respiratory chamber, was reduced after incremental doses of liraglutide (1.8 mg/d and 3.0 mg/d) for 5 weeks in apparently healthy, obese participants by 350kJ and 581kJ (~3–5%), respectively [15]. Longer-term studies have found no effects of liraglutide administration (0.6, 1.2 or 1.8 mg/day) either alone or in combination with metformin on energy expenditure and/or RQ acutely [16], after 4 weeks [17] or 1 year [18] compared to control and/or other medication in obese patients with type 2 diabetes, suggesting that falling leptin levels with prolonged therapy may be responsible for the discrepant results.

We then focused on peripheral hormones potentially mediating or otherwise modifying the weight loss induced by liraglutide. We observed changes in GIP and leptin levels. Leptin is the prototypical adipokine which signals energy homeostasis to the brain by circulating at levels proportional to the amount of body fat as well as to acute changes in caloric intake [19]. We observed acute changes in leptin without changes in fat mass, which remained significantly different after controlling for BMI. These changes may reflect either the effect of decreasing caloric intake and/or a direct effect of GLP-1 to decrease leptin levels. The

possibility that GLP-1 may decrease leptin production by adipocytes remains to be confirmed by dedicated molecular studies in humans, but this effect has already been observed in two studies of liraglutide with rodents [20, 21]. Other GLP-1 analogs have resulted in similar decreases of leptin levels in rodents [20–29]. However, other studies with rodents have not showed decreases in leptin with GLP-1 analogs [30–33]. Similarly, one study has shown that GLP-1 analogs decrease levels of leptin in humans [34], while most have shown no changes in leptin levels [35–40].Such a decrease of leptin levels could possibly be expected to be even more pronounced in response to long-term weight loss resulting in decreases of both main predictors of leptin levels i.e. energy intake and fat mass.

Greater liraglutide-induced decreases of fasting leptin levels result in increased activation of the reward-related midbrain, precuneus, and DLPFC and sensorimotor-related motor cortex (inverse correlation with the change in leptin levels) and less activation of the attention-related parietal cortex and cognitive control-related thalamus and pre-SMA (positive correlation with the change in leptin levels) to highly desirable as compared to less desirable food cues. Leptin receptors are expressed throughout the brain [41, 42], and thus may mediate the central response to GLP-1 analogs, leading to changes in energy intake and/or expenditure. These findings, if confirmed, could be interpreted as decreased leptin leading to altered brain activations to food cues and consequent weight changes.

Increased activity of the DLPFC, midbrain, and precuneus with decreased leptin may indicate altered control and reward-related circuitry induced by leptin in response to GLP-1 administration. Indeed, decreased control- and reward-related DLPFC activation after food consumption has been associated with obesity [43–45]. Additionally, the midbrain, including the ventral tegmental area, is well-known to be involved in the rewarding aspect of food [46]. The precuneus is more involved in attention and saliency processing but has also been shown to be impacted by rewarding stimuli, as these are highly salient [47–50]. The motor cortex activation encompasses a large area of sensorimotor cortex and may indicate greater sensory and motor processing of highly desirable food cues with greater decreases in leptin while on liraglutide, indicating that they may be more appetitive or salient [51]. Altogether, these results support the notion that lower leptin levels may be counteracting the effects of liraglutide, by increasing reward and salience related brain activations and decreasing cortical and control related activations to highly palatable food cues during the fasting state.

Furthermore, we observed greater changes in leptin levels with liraglutide correlating with decreased activity in the thalamus, parietal cortex, and pre-SMA while viewing highly desirable food cues in the fasting state. The thalamus and pre-SMA are involved in cognitive and motor-related control [52–54], while the parietal cortex is a part of the attention system and shows activation to salient stimuli [55–57]. This may suggest that these higher cortical attention- and cognitive-related systems are attempting to counteract the appetite-reducing effects of liraglutide in the brain.

Taken altogether, these results suggest a number of systems related to the control of eating are impacted in relation to leptin levels during liraglutide therapy. The role of leptin in feeding is known to be complex. One recent study has shown that communication between frontal and parietal regions plays a role in value-based choices [58]. The relationship of

leptin with pre-SMA, motor cortex, parietal cortex, and precuneus that we observed in this study could suggest a potential role for leptin in those complex neuronal circuits which evaluate food choices. The decreases in leptin before weight loss may be a compensatory mechanism which counteracts the appetite-reducing effects of liraglutide peripherally and centrally. Thus, a future study combining leptin in replacement doses with liraglutide therapy could be warranted since it could potentially offer additional weight loss.

GIP is secreted in response to food intake and regulates glucose postprandially by increasing the secretion of insulin, similar to GLP-1 [59]. It has been repeatedly observed that individuals with DM have a diminished insulin response to GIP [59]. Furthermore, GIP has been shown to increase GLP-1 levels in animal models [60] and DPP-4 inhibitors which increase GLP-1 also increase GIP [61]. Altogether, these data support the existence of a feedback loop between the incretins GLP-1 and GIP [59, 60], which may be activated by the GLP-1 analog, liraglutide, as it increases GIP in our study. In the past, others have found no effect of GLP-1 analogs given intravenously on GIP levels in humans [62–66]. In contrast, another group found that intravenous GLP-1 decreased GIP in type 1 diabetics and healthy participants [67], and another found that intravenous GLP-1 attenuated the breakfast-induced increase of GIP [68]. Confirming our findings, another study of intravenous GLP-1 found that although GIP decreased over the first hour of GLP-1 administration, it then increased [69]. Considering our blood samples are obtained more than an hour after liraglutide administration, we may be capturing this same effect. Regarding the potential liraglutide-mediating effects of GIP on the brain, we found an inverse correlation between GIP levels and insula activation while participants are on liraglutide, demonstrating that higher GIP levels observed with liraglutide decrease insular activation. Insula is known to be involved in reward and saliency processing [70–74], indicating the higher levels of GIP with liraglutide decrease the rewarding value and saliency of highly desirable food cues. In addition, GIP per se has been found to be expressed widely throughout the brain. A role for GIP has been identified in adult hippocampal progenitor cell proliferation, as well as one of neurotransmitter or neuromodulator [75, 76]. This may support a role for GIP in mediating and augmenting, in part, the activity of liraglutide on the central nervous system and consequently on weight loss.

In summary, an increase in GIP may promote the anorexigenic actions of liraglutide, whereas compensatory decreases of leptin may counteract the effects of liraglutide. Of note, these hormonal changes may reflect short-term effects in response to early liraglutide therapy and may be attenuated or altered with longer term therapy pointing to a need for more long term studies. Indeed, findings in longer-term studies demonstrate that there are long-term differences in hormone levels induced by liraglutide that are not captured in our study. For instance, in a 48-week trial with liraglutide, increases in glucagon levels were observed with liraglutide [77], while we do not observe any changes in glucagon in the 17 days of our trial. Future longitudinal studies are needed to determine short-term versus long-term changes with liraglutide therapy both at low-dose 1.8mg daily and high-dose 3.0mg daily administration. Additionally, a relatively small sample size might account for a lack of differences and larger studies should confirm our results. If our data are confirmed by future independent studies, combination therapies with leptin analogues would be warranted for additional weight loss effects.

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Abbreviations

DM	type 2 diabetes
GLP-1	glucagon-like peptide-1
fMRI	functional magnetic resonance imaging
CNS	central nervous system
DLPFC	dorsolateral prefrontal cortex
pre-SMA	pre-supplementary motor area
BMI	body mass index
BIDMC	Beth Israel Deaconess Medical Center
DEXA	dual energy x-ray absorptiometry
RMR	resting metabolic rate
PP	pancreatic polypeptide
GIP	gastric inhibitory peptide
FGF21	fibroblast growth factor 21
SBP	systolic blood pressure
DBP	diastolic blood pressure
HbA1c	hemoglobin A1c
HDL	high density lipoproteins
LDL	low density lipoproteins
TSH	thyroid-stimulating hormone
T3	triiodothyronine
T4	thyroxine
TBG	thyroxine-binding globulin
IGF-1	insulin-like growth factor-1

FFA	free fatty acids
PYY	peptide YY
SE	standard error
SPSS	statistical package for the social sciences
BOLD	blood oxygenation level dependent
FWE	family-wise error

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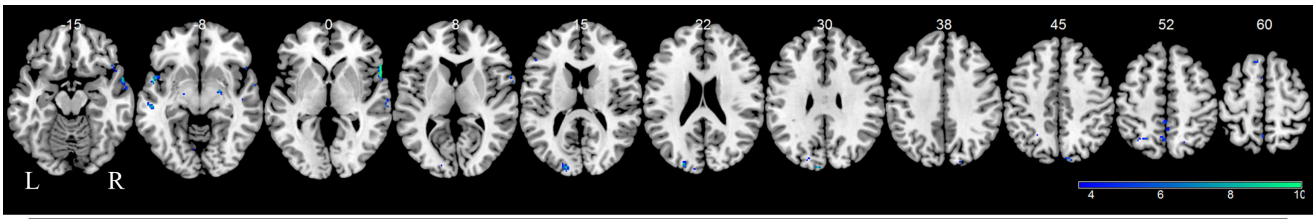
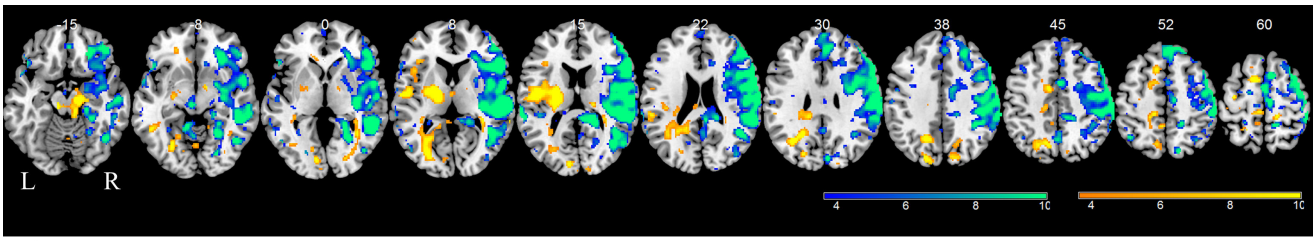


		MNI Coordinates (mm)					
Cluster Size (voxels)	Voxel Z Value	X	Y	Z	Side	Identified Region	General Brain Area
Inverse correlation with GIP levels							
105	5.34	60	14	4	R	insula	insula

Statistical threshold: p<0.001, uncorrected; p<.025, FWE-corrected for peak, Gyrus; S, Sulcus; L, left; R, right; C, center. *significant at p<.05, FWE corrected for cluster. Peaks shown for clusters are the most significant along the same identified region.

Figure 1.

Results of whole brain regressions while participants were on liraglutide with GIP levels at 17 days during the fasting state while viewing highly desirable as compared to less desirable food cues at p<.001. BOLD contrasts are superimposed on a T1 structural image in neurological orientation. The color bar represents voxel T value. Table shown below figure for activations significant at P<.025, FWE-corrected for peak.



		MNI Coordinates (mm)					
Cluster Size (voxels)	Voxel Z Value	X	Y	Z	Side	Identified Region	General Brain Area
Positive correlation with change in leptin levels (hot colors)							
3590*	6.19	-16	-10	10	L	thalamus	thalamus
	5.7	-20	-18	12	L	thalamus	thalamus
316*	6.12	12	-18	-18	R	thalamus	thalamus
113	5.5	-54	-34	26	L	inferior parietal G	parietal cortex
296*	5.26	-4	-30	64	C	superior parietal G	parietal cortex
444*	4.93	-8	4	60	C	superior frontal G	pre-SMA
Inverse correlation with change in leptin levels (cold colors)							
26513*	6.05	32	12	62	R	middle frontal G	DLPFC
	5.79	56	0	22	R	precentral G	motor cortex
	5.7	52	-4	16	R	precentral G	motor cortex
48	5.71	16	-74	54	R	precuneus	precuneus
224*	5.19	-4	-22	-8	C	midbrain	midbrain
287*	5.05	-10	-46	-6	L	cerebellum	cerebellum

Statistical threshold: p<0.001, uncorrected; p<.025, FWE-corrected for peak, Gyrus; S, Sulcus; L, left; R, right; C, center. *significant at p<.05, FWE corrected for cluster. Peaks shown for clusters are the most significant along the same identified region.

Figure 2.

Results of whole brain regressions while participants were on liraglutide with the change in leptin levels from baseline to 17 days during the fasting state while viewing highly desirable as compared to less desirable food cues at p<.001. BOLD contrasts are superimposed on a T1 structural image in neurological orientation. The color bar represents voxel T value. Table shown below figure for activations significant at P<.025, FWE-corrected for peak.

Table 1

Body Composition and Hormonal Measurements after liraglutide or placebo. Data shown as means ± standard error (SE) of the mean.

	Placebo (mean ± SE)			Liraglutide (mean ± SE)			*P*	*P*
	Visit 1	**Visit 4**	**percent change**	**Visit 1**	**Visit 4**	**percent change**	***mixed model***	***percent change***
Anthropometry								
BMI (kg/m^2)	31.2 ± 1.7	30.5 ± 1.4	−0.5 ± 0.9	31.9 ± 1.7	31.5 ± 1.7	−1.2 ± 0.9	0.95	0.59
Body weight (kg)	93.4 ± 4.8	91.1 ± 1.0	−0.4 ± 0.7	94.7 ± 4.7	91.0 ± 1.0	−1.2 ± 0.7	0.43	0.46
WC iliac (cm)	105.1 ± 3.5	104.1 ± 3.4	−0.5 ± 0.7	106.8 ± 3.5	105.3 ± 3.4	−1.3 ± 0.6	0.34	0.34
Fat body mass (kg)	30.9 ± 3.1	29.2 ± 1.1	−1.2 ± 1.1	31.9 ± 3.1	29.2 ± 1.1	−0.1 ± 1.1	0.70	0.51
Fat body mass (percentage)	32.3 ± 2.0	32.9 ± 2.0	−1.0 ± 1.1	32.8 ± 2.1	33.2 ± 2.0	1.3 ± 1.0	0.14	0.14
Bone mass density (g/cm^2)	1.27 ± 0.03	1.2 ± 1.0	−0.7 ± 0.7	1.26 ± 0.03	1.2 ± 1.0	0.0 ± 0.6	0.59	0.47
Lean body mass (kg)	62.9 ± 2.9	62.4 ± 2.6	0.3 ± 0.7	63.5 ± 2.8	61.6 ± 2.6	−1.9 ± 0.7	0.07	0.03
SBP (mmHg)	134.4 ± 2.4	130.8 ± 2.3	−2.4 ± 2.4	136.6 ± 3.8	132 ± 3.0	−2.4 ± 2.4	0.83	0.99
DBP (mmHg)	78.9 ± 2.6	78.8 ± 2.0	1.3 ± 3.1	84.6 ± 245	83.8 ± 2.6	−1.0 ± 3.0	0.44	0.55
VO_2 (L/min)	ND	0.23 ± 0.01	N/A	ND	0.24 ± 0.01	N/A	0.27	N/A
VO_2/kg (mL/kg/min)	ND	2.5 ± 1.0	N/A	ND	2.6 ± 1.0	N/A	0.22	N/A
VCO_2 (L/min)	ND	0.19 ± 0.01	N/A	ND	0.19 ± 0.01	N/A	0.87	N/A
Respiratory quotient	ND	0.84 ± 0.02	N/A	ND	0.81 ± 0.02	N/A	0.23	N/A
Resting energy expenditure (Kcal/day)	ND	1583.2 ± 52.9	N/A	ND	1622.6 ± 52.9	N/A	0.31	N/A
Predicted basal metabolic rate (Kcal/day)	ND	1746.3 ± 62.6	N/A	ND	1738.1 ± 63.5	N/A	0.72	N/A
Total energy intake (Kcal/day)	ND	1782±542	N/A	ND	1434±537	N/A	0.07	N/A
Metabolic Profile								
GLP-1 (pM)	49.8 ± 8.6	38.2 ± 1.2	50.4 ± 44.6	89.3 ± 12.1	912.3 ± 1.2	1674.2 ± 212.3	<0.001	<0.001
Glucose (mg/dL)	119 ± 6.3	122.3 ± 6.3	5.8 ± 5.4	124.7 ± 6.2	95.4 ± 4.1	−22.4 ± 5.4	<0.001	0.001
Fructosamine (umol/L)	296.8 ± 9.9	273.8 ± 7.9	−6.8 ± 2.5	300.3 ± 8.8	271.7 ± 7.9	−9.5 ± 2.4	0.36	0.35
HbA1c (g/dL)	ND	6.7 ± 1.2	N/A	ND	6.6 ± 1.2	N/A	0.82	N/A
Insulin (uIU/mL)	16.2 ± 2.8	11.5 ± 2.6	−19.4 ± 9.9	15.6 ± 2.3	11.2 ± 1.4	−10.8 ± 9.9	0.48	0.54
C-peptide (ng/mL)	2.1 ± 0.2	1.7 ± 0.1	−13.3 ± 9.7	2.4 ± 0.2	2.2 ± 0.2	−4.4 ± 9.3	0.48	0.51
Glucagon (pg/mL)	181.0 ± 27.6	97.4 ± 1.1	−19.3 ± 11.7	135.6 ± 18.2	84.9 ± 1.1	−12.5 ± 11.5	0.41	0.68

	Placebo (mean ± SE)			Liraglutide (mean ± SE)			*P*	*P*
	Visit 1	**Visit 4**	**percent change**	**Visit 1**	**Visit 4**	**percent change**	***mixed model***	***percent change***
Total cholesterol (mg/dL)	177.1 ± 5.7	167.9 ± 5.3	−4.5 ± 3.5	186.8 ± 7.5	170.6 ± 5.6	0.5 ± 3.7	0.18	0.34
Triglycerides (mg/dL)	115.6 ± 20	89.8 ± 12.6	−17.0 ± 11.8	118.1 ± 18.3	87.4 ± 12.6	−16.6 ± 12.6	0.23	0.06
HDL (mg/mL)	53.3 ± 2.7	52.4 ± 2.3	−0.8 ± 4.5	53.5 ± 2.6	50.8 ± 2.5	−7.3 ± 4.8	0.22	0.30
LDL (mg/mL)	100.8 ± 4.8	99.3 ± 4.7	1.2 ± 5.7	107.0 ± 5.4	100.8 ± 4.6	6.1 ± 6.2	0.22	0.56
Key Hormones in Energy Homeostasis								
Leptin (ng/mL)	19.8 ± 3.5	23.0 ± 4.4	16.6 ± 7.1	25.1 ± 4.3	20.8 ± 4.3	−10.4 ± 7.1	0.12	0.01
Adiponectin (ug/mL)	8.8 ± 1.3	6.7 ± 1.2	−18.4 ± 12.2	16.1 ± 8.7	8.4 ± 2.3	−9.3 ± 10.2	0.37	0.20
Irisin (ng/mL)	166.8 ± 14.9	164.1 ± 20.9	−1.7 ± 3.8	167.1 ± 15.3	168.3 ± 20.9	0.2 ± 3.8	0.63	0.73
PP (pg/mL)	277.0 ± 48.9	206.4 ± 43.2	−37.0 ± 11.6	230.2 ± 47.9	244.4 ± 45.7	12.9 ± 6.9	0.09	0.21
GIP (pg/mL)	72.6 ± 12.0	42.1 ± 15.6	−34.5 ± 24.6	62.6 ± 10.6	91.0 ± 14.8	53.2 ± 24.6	0.03	0.02
Amylin (pM)	11.4 ± 1.7	11.6 ± 1.9	−11.3 ± 8.6	11.6 ± 1.8	10.5 ± 1.8	−21.9 ± 9.3	0.45	0.92
Ghrelin (pg/mL)	868.6 ± 89.3	698.5 ± 90.6	−13.8 ± 11.3	696.3 ± 103.1	671.4 ± 91.6	−3.2 ± 3.8	0.72	0.16
TSH (uIU/mL)	2.3 ± 0.4	1.9 ± 0.4	−8.2 ± 16.0	2.4 ± 0.7	1.9 ± 0.4	−15.2 ± 15.3	0.81	0.75
Free T3 (pg/mL)	3.4 ± 0.3	2.9 ± 0.1	−10.3 ± 11.5	3.5 ± 0.1	2.9 ± 0.2	−15.5 ± 11.5	0.99	0.76
Free T4 (ng/dL)	1.1 ± 0.0	1.1 ± 0.0	−2.2 ± 3.4	1.1 ± 0.0	1.1 ± 0.0	1.7 ± 3.4	0.43	0.43
TBG (ug/mL)	18.7 ± 1.7	16.0 ± 1.2	−11.3 ± 3.0	18.0 ± 1.4	17.0 ± 1.3	−3.8 ± 3.0	0.18	0.09
FGF21 (pg/mL)	164.3 ± 24.8	88.5 ± 1.3	−7.4 ± 17.5	165.1 ± 36.5	97.6 ± 1.3	−1.0 ± 17.5	0.49	0.71
IGF-1 (ng/mL)	181.1 ± 16.3	192.6 ± 14.7	13.1 ± 10.5	201.4 ± 19.2	213.5.4 ± 15.8	10.7 ± 10.5	0.77	0.88
FFA (mEq/L)	1.0 ± 0.1	1.0 ± 1.1	42.9 ± 35.0	1.1 ± 0.1	1.0 ± 1.1	29.8 ± 33.6	0.57	0.79
PYY (pg/mL)	183.7 ± 21.5	177.6 ± 23.2	−4.8 ± 13.4	174.7 ± 22.9	146.9 ± 11.3	−6.3 ± 13.9	0.48	0.46

SE, standard error; ND, no data available; N/A, not applicable (due to missing visit 1 values); SBP, systolic blood pressure; DBP, diastolic blood pressure; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; HDL, high density lipoproteins; LDL, low density lipoproteins; PP, pancreatic polypeptide; GIP, gastric inhibitory peptide; TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine; TBG, thyroxine-binding globulin; FGF21, fibroblast growth factor 21; IGF-1, insulin-like growth factor-1; FFA, free fatty acids; PYY, peptide YY