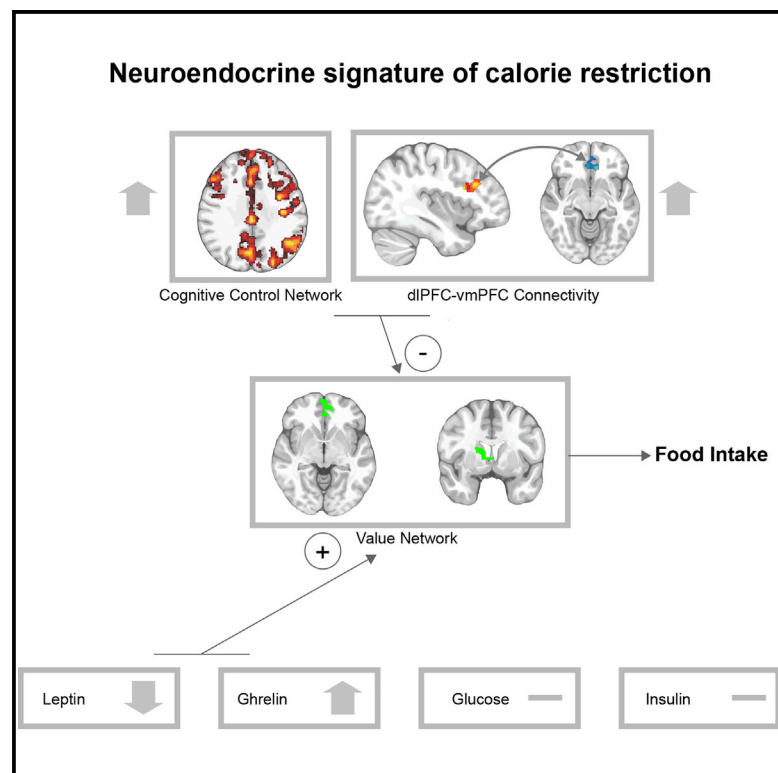


Neurocognitive and Hormonal Correlates of Voluntary Weight Loss in Humans

Graphical Abstract



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In Brief

Dagher et al. tested the hypothesis that hormonal adaptations during dieting override eating self-control in 24 overweight participants on a 1,200 kcal/day diet. They found that brain activity in cognitive control regions, rather than hormones associated with energy balance, plays a critical role in weight loss.

Highlights

- We performed functional MRI in individuals who undertook a weight-loss regimen
- Calorie restriction led to weight loss and leptin and ghrelin adaptations
- We uncovered a neural signature of successful weight loss
- The best predictor of success was activation in prefrontal cortex during the regime



Neurocognitive and Hormonal Correlates of Voluntary Weight Loss in Humans

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SUMMARY

Insufficient responses to hypocaloric diets have been attributed to hormonal adaptations that override self-control of food intake. We tested this hypothesis by measuring circulating energy-balance hormones and brain functional magnetic resonance imaging reactivity to food cues in 24 overweight/obese participants before, and 1 and 3 months after starting a calorie restriction diet. Increased activity and functional connectivity in prefrontal regions at month 1 correlated with weight loss at months 1 and 3. Weight loss was also correlated with increased plasma ghrelin and decreased leptin, and these changes were associated with food cue reactivity in reward-related brain regions. However, the reduction in leptin did not counteract weight loss; indeed, it was correlated with further weight loss at month 3. Activation in prefrontal regions associated with self-control could contribute to successful weight loss and maintenance. This work supports the role of higher-level cognitive brain function in body-weight regulation in humans.

INTRODUCTION

Weight loss can improve comorbidities and cardiometabolic risk factors associated with obesity. Two-thirds of the American population have undertaken reducing diets at least once (Gudzune et al., 2015). However, achieving and maintaining weight loss remain challenging (Anastasiou et al., 2015). Several studies indicate that high-order executive cognitive processes implicated in self-regulation play an important role in healthy food decisions and weight management (Gettens and Gorin, 2017; Michaud et al., 2017; Stoeckel et al., 2017). However, hormonal responses to negative energy balance during calorie restriction can modulate the activity of brain systems implicated in feeding in favor of increased calorie intake (Berthoud et al., 2012; Morton et al., 2014). In humans, it remains to be tested whether the changes

in energy-balance signals during calorie restriction can modulate the brain networks associated with food intake, override self-control, and oppose weight loss.

Brain circuitry underlying food decisions can be divided into three interacting systems: (1) a homeostatic system centered around the hypothalamus and hindbrain circuits; (2) a reward-related appetitive network including the striatum and the ventromedial prefrontal cortex (vmPFC) that encodes the subjective value of food cues; and (3) an executive control network that relies on the function of interconnected prefrontal regions including the anterior cingulate cortex, dorsolateral prefrontal cortex (dlPFC), inferior frontal gyrus (IFG), and posterior parietal cortex (Dagher, 2012; Ochner et al., 2013). Cognitive control, defined here as the ability to restrict calorie intake and to sustain weight maintenance, is thought to rely on these executive structures. It is proposed that cognitive control ability mediates the relationship between weight-loss intention and action (Gettens and Gorin, 2017). The dlPFC and IFG have been repeatedly implicated in dietary self-control studied with functional magnetic resonance imaging (fMRI). An increase in blood-oxygen-level-dependent (BOLD) signal in the dlPFC and IFG is seen when subjects are asked to voluntarily suppress the desire to eat in response to food cues (Batterink et al., 2010; Hollmann et al., 2012), and predicts subsequent reduced food intake outside the lab (Lopez et al., 2014, 2017). fMRI studies also support a model according to which dlPFC and IFG downregulate the activity of value-encoding regions (e.g., vmPFC) when participants choose healthy over unhealthy foods or regulate their food cravings (Hare et al., 2009, 2011). The relative balance of activity in regions associated with self-regulation over those associated with reward has been used to compute a brain-derived measure of self-regulation ability, which relates to healthier real-life food choices in dieters and non-dieters (Lopez et al., 2014, 2017). This has led to dual systems theories where behavioral outcomes depend on the balance between self-control and reactivity to reward. Although few studies have examined brain activity longitudinally in individuals undergoing calorie restriction, there is some support for the role of dlPFC in successful weight loss (Weygandt et al., 2013) and of ventral striatum activity in worse outcomes (Murdaugh et al., 2012).

According to the dual systems theory, the magnitude of weight loss during calorie restriction may be related to the following



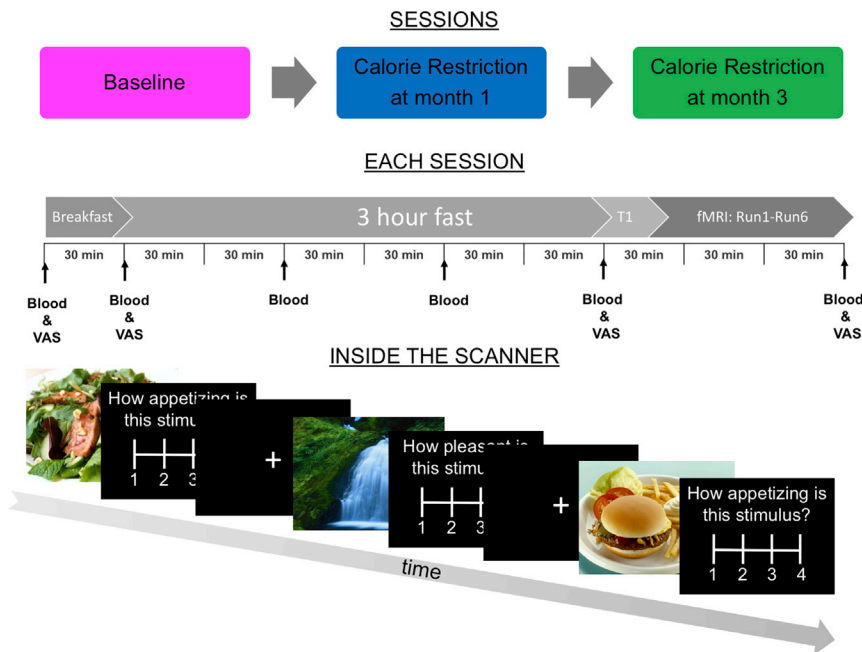


Figure 1. Experimental Design

Participants ($n = 24$) were tested three times: at baseline, then at 1 and 3 months during voluntary calorie restriction. On each scan day, participants ate a standard breakfast 3 hr prior to fMRI imaging. Venous blood and self-reported hunger level on a visual analog scale (VAS) were sampled at the times indicated. Anatomical scanning (T1) was followed by six 7-min functional runs. During fMRI participants viewed pictures of foods and scenery followed by a rating.

fMRI findings: (1) increased brain activity in regions associated with cognitive control, (2) increased functional connectivity of these areas to regions implicated in value processing (e.g., vmPFC and striatum), and (3) downregulation of activity in these value-related brain regions. However, CNS networks are also modulated by internal states, such as current energy-balance status (Neseliler et al., 2017; Rangel, 2013). During calorie restriction, ghrelin and leptin reflect changes in energy balance. Leptin plasma levels decline rapidly in response to calorie restriction, and more slowly with reduction of fat mass (Friedman and Mantzoros, 2015). Patients with leptin-deficient states show increased fMRI food cue reactivity in the striatum and orbitofrontal cortex (OFC) compared with controls (Aotani et al., 2012). Striatal response to food cues in the striatum is reduced by leptin administration in these patients (Aotani et al., 2012; Farooqi et al., 2007). In normoleptinemic participants, leptin levels negatively correlate with food cue reactivity in the striatum (Groschans et al., 2012) and leptin administration to weight-reduced subjects results in increased activity in regions associated with cognitive control (Rosenbaum et al., 2008). These studies suggest that reductions in leptin levels can result in increased activity in the mesolimbic reward system and, possibly, reduced activity in brain regions associated with cognitive control. Conversely, ghrelin, an orexigenic hormone secreted by the stomach, increases rapidly in response to calorie deficit (Borer et al., 2009). Post-translational modification converts ghrelin to acyl-ghrelin, its active form (Müller et al., 2015). Ghrelin can increase the neural response to food cues in regions associated with value and motivation and potentiate food intake (Goldstone et al., 2014; Malik et al., 2008; Müller et al., 2015). During weight loss, the fall in leptin and rise in ghrelin levels could modulate the activity of brain networks involved in reward signaling to shift the balance toward increased food intake (Morton et al., 2014).

We designed this study to test these two predictions on the role of the CNS in voluntary calorie restriction: (1) that activation

of cognitive control networks during fMRI would predict weight loss and (2) that early weight loss should lead to changes in energy-balance signaling (leptin and ghrelin), which would lead to increased activity in regions associated with reward and counteract self-control mechanisms. Twenty-four individuals underwent a 3-month calorie restriction program with measurement of fMRI and metabolic variables at baseline (before initiating the diet), and at months 1 and 3 (Figure 1). During fMRI they viewed 216 pictures of appetizing foods or scenery, and rated them on a 4-point scale. Our results support the first prediction: weight-loss success correlated with activity in cognitive control regions. However, while leptin and ghrelin levels changed in response to weight loss and were associated with the expected increases in activity in reward-related regions, these changes failed to predict subsequent weight gain. Indeed, activation in prefrontal regions associated with cognitive control continued to explain most of the individual variability in weight loss at subsequent time points.

RESULTS AND DISCUSSION

Calorie Restriction Resulted in Weight Loss

The 24 participants (1 male) had a mean age of 37.2 (SD = ± 8.4) years and a mean body mass index (BMI) at entry of 30.4 (SD = ± 3.2). The personality measures of the study population are listed in Table S1 (tab 1). We analyzed the changes in weight, physical activity, hunger levels, and energy-balance hormone levels at months 1 and 3 during calorie restriction, compared with baseline (Figure 2). All the analyses were conducted using linear mixed effect modeling. There were significant reductions in BMI across the three sessions of calorie restriction ($F(2,38.1) = 41.04$, $p = 3.14 \times 10^{-10}$). Pairwise comparisons showed that the reductions in BMI were significant from baseline to month 1 ($F(1,38.1) = 21.77$, $p = 0.0001$) and from month 1 to month 3 ($F(1,38.1) = 16.31$, $p = 0.0007$) (Figure 2A; Table S1, tab 2). Self-reported physical activity levels and hunger levels assessed by visual analog scale (VAS) did not show significant differences across the sessions (Table S1, tabs 2 and 3).

Activity in Cognitive Control Networks Correlated with Weight Loss

We hypothesized that weight loss would be related to activity in regions implicated in cognitive control. In line with this

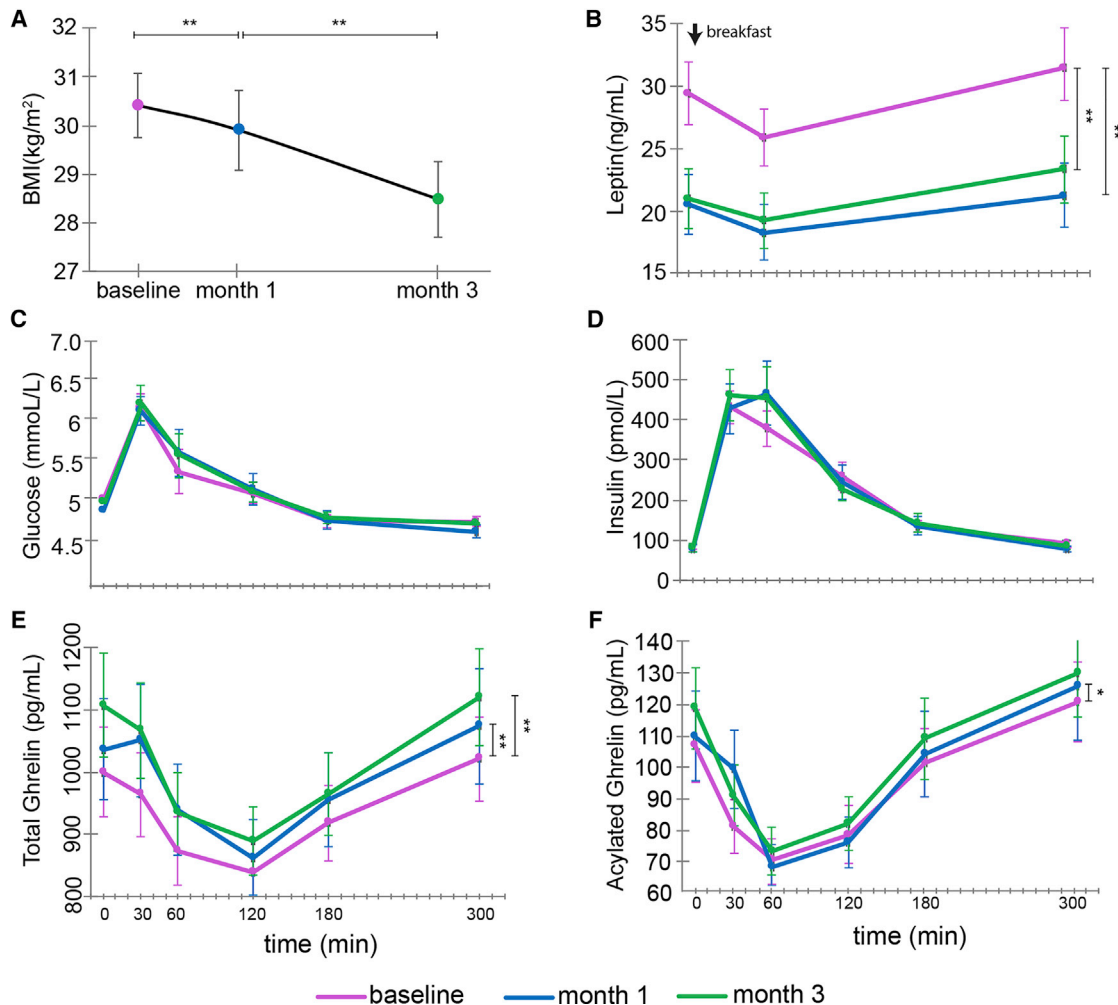


Figure 2. Effects of Voluntary Calorie Restriction

(A) BMI decreased significantly across the sessions.

(B–F) The plasma concentrations were measured throughout the experiment (0–300 min). 0 min refers to morning (pre-meal) levels, after which participants consumed the standard breakfast (shown as an arrow in B). In all panels, 30 min indicates the response after the breakfast. (B) Leptin decreased at month 1 during the diet compared with baseline, but was not significantly different at month 3 compared with month 1. There was a decrease from time 0 to 60 min after breakfast ($F(1,168.1) = 8.10$, $p = 0.015$), followed by an increase post scan ($F(1,168) = 19.04$, $p = 6.69 \times 10^{-5}$), to levels not significantly different from baseline ($F(1,168.07) = 2.30$, $p = 0.13$). Glucose (C) and insulin (D) did not show significant differences across sessions. Total ghrelin (E) levels were significantly higher at month 1 and month 3 compared with baseline. During the day, levels decreased to a nadir at 120 min after the breakfast ($F(1,360) = 89.5$, $p = 2.5 \times 10^{-18}$) then rising, the increase being significant from 120 to 180 min ($F(1,360) = 18.01$, $p = 1.67 \times 10^{-4}$), yet lower than at time 0 ($F(1,360) = 27.2$, $p = 1.87 \times 10^{-6}$). During the scan ghrelin increased further ($F(1,360) = 41.81$, $p = 1.9 \times 10^{-10}$), to a level not different from time 0 ($F(1,360) = 1.6$, $p = 0.21$). (F) Acylated ghrelin levels showed an increase at month 1, but were not significantly different at month 3 relative to baseline. Its levels decreased markedly by 60 min following breakfast ($F(1,360) = 82.2$, $p = 1.23 \times 10^{-17}$) then rose progressively thereafter. Levels at the beginning of scan (180 min) were not different than at time 0 ($F(1,360) = 2.2$, $p = 0.14$), but increased during the scan (180 versus 300 min) ($F(1,360.3) = 9.2$, $p = 0.02$).

Data are presented as mean \pm SEM. Statistics are derived from linear mixed models (MATLAB function fitline). * $p < 0.05$; ** $p < 0.01$. All data available in Table S1, tab 3.

hypothesis, initial weight loss at month 1 was correlated with an increase in BOLD (month 1 versus baseline) during the food minus scenery contrast in regions associated with cognitive control such as dlPFC, IFG, dACC, inferior parietal lobule, and caudate (Figures 3A and 3B; Table S1, tab 5). Food cue reactivity in the network of regions related to cognitive control at month 1 (Figure 3A) also correlated positively with subsequent weight loss from month 1 to month 3 ($r = 0.60$, $p = 0.026$; Figure 3C). Moreover, activity reductions in this network

at month 3 (i.e., a return toward baseline) correlated with weight regain 2 years later ($r = -0.64$, $p = 0.028$; Figure 3D) in the subset of patients for whom body-weight data were available ($n = 14$). These results were similar when only female subjects were analyzed.

We decomposed task fMRI data using independent component analysis. One component demonstrated positive task-related activity and mostly overlapped with the areas just described: the right frontoparietal network, thought to be

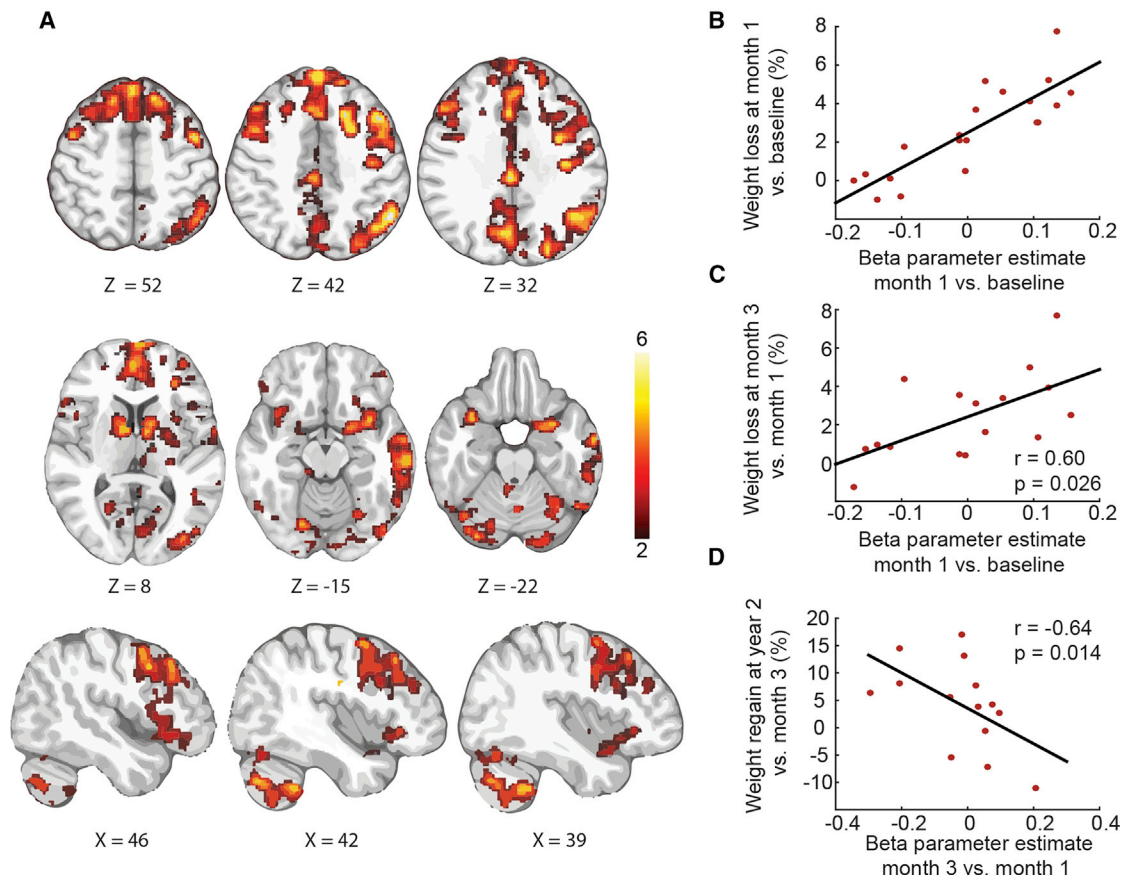


Figure 3. Weight Loss at Month 1 Correlated with Changes in BOLD in Regions Associated with Cognitive Control

(A) Activation to food cues compared with scenery cues at month 1 versus baseline correlated with weight loss ($p < 0.05$ FWER, $n = 20$; Table S1, tab 5; for female-only analysis, see Figure S3).

(B) Mean beta estimate of activation to food minus scenery at month 1 minus baseline derived from the significant cluster in (A) versus weight loss between month 1 and baseline.

(C) Mean beta estimate of activation to food minus scenery at month 1 minus baseline derived from the significant cluster in (A) versus weight loss from month 3 to month 1 ($n = 16$).

(D) Mean beta estimate of activation to food minus scenery at month 3 minus month 1 derived from the significant cluster in A versus weight regain at 2 years compared with month 3 ($n = 14$). The color scale presents t statistics derived from 5,000 permutations of the data. FWER, family-wise error rate. X and Z refer to the MNI coordinates in millimeters.

involved in cognitive control. We found that greater activity in this network to food cues compared with scenery cues at month 1 compared with baseline was correlated with weight loss at month 1 (Figure S1).

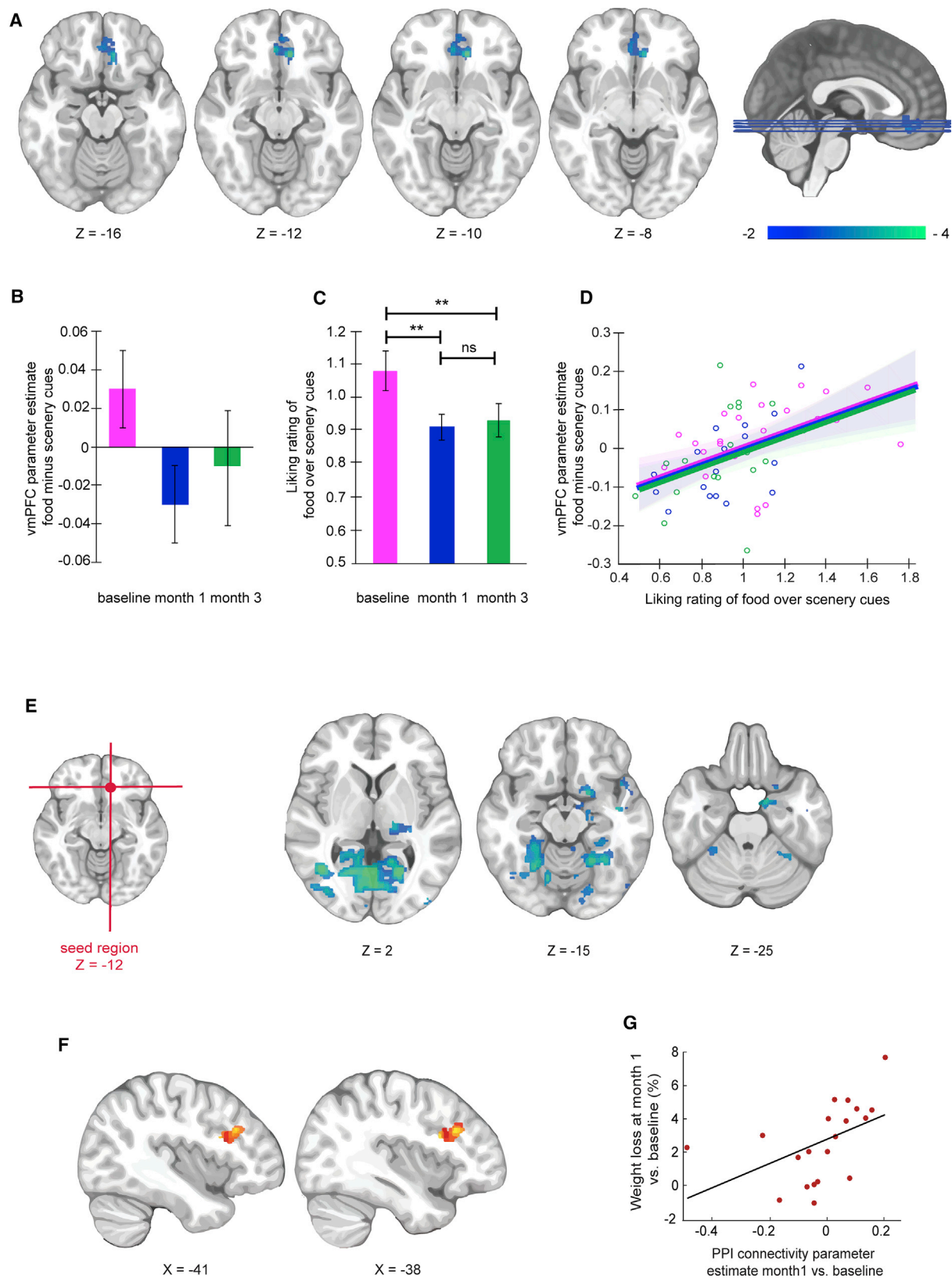
These results are in line with studies that show that cue-related dIPFC activity correlates with weight loss from dieting (Weygandt et al., 2013) or bariatric surgery (Goldman et al., 2013). Moreover, the mostly right lateralized frontoparietal network identified here was also found to be active when subjects deliberately reduced their craving for food cues in a recent meta-analysis (Han et al., 2018). In summary, engagement of prefrontal areas implicated in dietary self-control is correlated with initial weight loss at months 1 and 3 and weight-loss maintenance at 2 years.

To test whether activity in prefrontal regions was indeed related to cognitive-control ability, we used individual scores on the Binge Eating Scale (Gormally et al., 1982), which assesses symptoms associated with loss of control over eating over a

wide range (Vainik et al., 2015). Increases in activity in right IFG at month 1 compared with baseline were negatively correlated with baseline Binge Eating Scale score ($r = -0.52$, $p = 0.04$; Figure S2E). This is consistent with previous reports that link reduced IFG activity with binge-eating symptoms (Kessler et al., 2016). This result links brain activity to a behavioral correlate of cognitive control and suggests that increases in food cue reactivity in the right IFG during weight loss might reflect cognitive control of eating.

vmPFC BOLD Decreased during Calorie Restriction

Neurocomputational models of decision making assume that individuals make choices based on the subjective value assigned to options (Rangel, 2013). As an example, a subjective value signal for food stimuli might be computed by incorporating aspects of palatability and healthiness. Meta-analysis reveals that this stimulus value computation is reflected in the vmPFC (Bartra et al., 2013) and is influenced by its connectivity with



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regions implicated in reward processing, such as the striatum, and cognitive control, such as the dlPFC and IFG (Hare et al., 2009, 2011; Rangel, 2013). We tested two competing predictions regarding the change in vmPFC response to food cues during calorie restriction: (1) it could increase, reflecting greater valuation of food cues due to negative energy balance; (2) it could show a reduction, reflecting successful self-regulation (Hare et al., 2009). The second prediction was borne out: food cue reactivity was reduced in vmPFC at month 1 compared with baseline in a region of interest derived from a meta-analysis of subjective value (Bartra et al., 2013) (Figure 4A; Table S1, tab 6). Activity in vmPFC in the food minus scenery contrast remained lower than baseline at month 3 (Figure 4B) in line with continued weight loss. There was no difference in vmPFC activation (food minus scenery) at month 3 compared with month 1.

Food Liking Decreased during Calorie Restriction

Subjective liking ratings for food cues relative to scenery cues showed a session effect similar to the vmPFC signal ($F(2,41) = 9.50$, $p = 0.00041$): there was a significant reduction at month 1 ($F(1,40.8) = 15.35$, $p = 0.001$) that persisted at month 3 compared with baseline ($F(1,40.8) = 11.6$, $p = 0.0045$). Month 3 liking ratings were not significantly different from month 1 ($F(1,41.7) = 0.23$, $p = 0.63$) (Figure 4C). These results align with previous observations showing that food cravings subside during voluntary calorie restriction (Martin et al., 2006). Furthermore, liking for food versus scenery cues correlated with vmPFC food cue reactivity (linear mixed effects model $F(1,56.3) = 9.31$, $p = 0.0035$) across all sessions (Figure 4D), supporting a role for this region in value computation. In sum, both fMRI vmPFC signals and liking for food cues were reduced during voluntary calorie restriction, consistent with previous studies linking these to self-regulation of appetite (Hare et al., 2009; Wagner et al., 2013).

vmPFC Connectivity Changed during Calorie Restriction

The connectivity of the vmPFC (seed region Montreal Neurological Institute [MNI] coordinates: $x = -10$, $y = 34$, $z = -12$; Figure 4E) with regions associated with visual processing of food stimuli was reduced at month 1 versus baseline. These regions include the lingual gyrus and the lateral occipital cortex (Figure 4E; Table S1, tab 6). Focusing on the visual features of valued stimuli has been associated with BOLD response in these regions (van der Laan et al., 2011; Lim et al., 2011), which have been postulated to send processed visual information to the vmPFC, where an overall subjective value of the stimulus is

computed and utilized for decision making (Lim et al., 2013). Weight loss also correlated with increased vmPFC connectivity to left dlPFC regions previously associated with cognitive control (by using a mask generated from the search term “cognitive-control” in the meta-analytical tool NeuroSynth) (Figure 4F; Table S1, tab 6). These results support a model in which deliberate calorie restriction results from changes in value computations in vmPFC along with reduced connectivity with visual areas involved in computing attributes of stimuli (Lim et al., 2013) and increased connectivity with prefrontal areas implicated in self-control (Rangel, 2013). These results build on previous work showing that dlPFC activity and its connectivity to vmPFC correlate with self-regulation and reduced food consumption (Hare et al., 2009; Lopez et al., 2014).

IFG Activity Subsided from Month 1 to Month 3 during Calorie Restriction

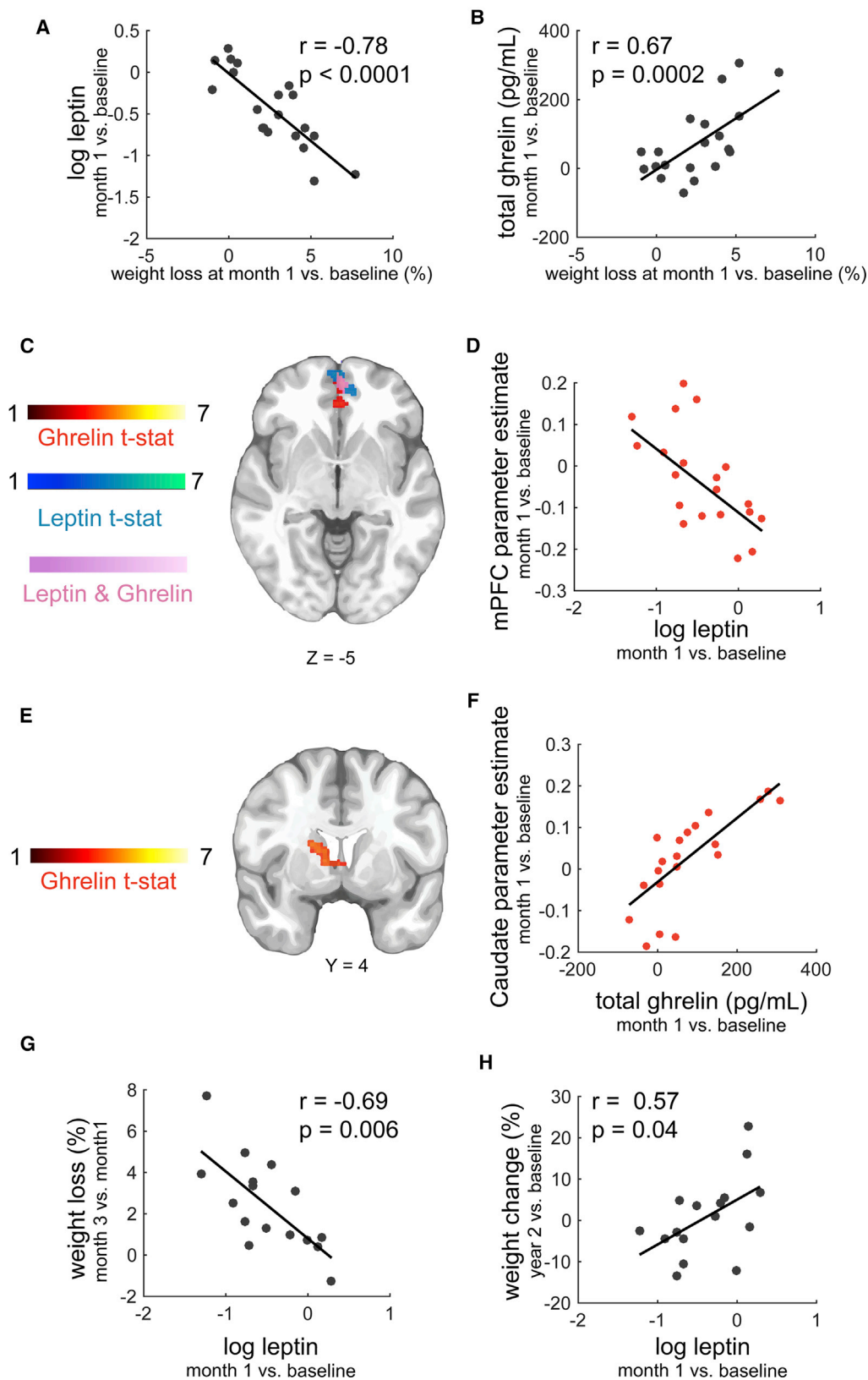
The IFG and vmPFC effects described above tended to subside from month 1 to month 3, when there was a reduction in activity in right IFG/frontal opercular cortex (Figure S2A; Table S1, tab 6), and reduced vmPFC connectivity to left dlPFC (Figure S2B; Table S1, tab 7). Participants who showed greater reduction in right IFG BOLD at month 3 versus month 1 showed greater weight regain at 2-year follow-up ($r = -0.61$, $p = 0.04$, $n = 14$; Figure S2B). Activation and connectivity changes in cognitive control networks may not be sustained, and this appears to correlate with subsequent weight regain (Figure 3D).

Leptin Decreased and Ghrelin Increased during Calorie Restriction

Leptin and ghrelin both showed adaptations to weight loss. Plasma leptin showed significant effects for both session ($F(2,168.8) = 66.47$, $p = 5.13 \times 10^{-22}$) and sampling time ($F(1,168.1) = 8.78$, $p = 0.0034$) (Figure 2B). Levels decreased from baseline at both month 1 ($F(1,168.6) = 100.52$, $p = 2.27 \times 10^{-18}$) and month 3 ($F(1,168.6) = 88.27$, $p = 1.19 \times 10^{-16}$), with no change between months 1 and 3 ($F(1,169.2) = 0.34$, $p = 0.56$). Total plasma ghrelin levels showed a main session effect with increase at month 1 ($F(1,360.4) = 28.2$, $p = 5.77 \times 10^{-7}$), remaining high at month 3 ($F(1,360.4) = 27.05$, $p = 9.96 \times 10^{-7}$), with no change between months 1 and 3 ($F(1,360.9) = 0.01$, $p = 0.92$) (Figure 2E). Plasma acyl-ghrelin (Figure 2F) showed an increase at month 1 ($F(1,360.9) = 5.92$, $p = 0.046$), but was not significantly different at month 3 relative to baseline ($F(1,360.9) = 5.02$, $p = 0.077$).

Figure 4. vmPFC Activity Following Calorie Restriction

- (A) Negative peaks: activation to food cues compared with scenery cues at month 1 of the diet compared with baseline ($p < 0.05$ FWER, SVC, $n = 20$; Table S1, tab 6; for females only, see also <https://neurovault.org/collections/XPQFOZLV>).
- (B) Changes in the vmPFC region beta estimate derived from the entire cluster in (A).
- (C) Liking for food cues relative to scenery cues at baseline, month 1, and month 3.
- (D) Mean vmPFC parameter estimate derived from the entire cluster to food minus scenery cues versus mean liking ratings of food cues relative to scenery cues ($F(1,56.3) = 9.31$, $p = 0.0035$). Shaded lines represent 95% confidence intervals derived from the linear mixed effect model (MATLAB fitme).
- (E) PPI analysis with the vmPFC seed revealed that left vmPFC connectivity is reduced with visual areas at month 1 compared with baseline (displayed $p < 0.001$, uncorrected, minimum voxel extent = 10 mm, $p < 0.05$ FWER corrected in Table S1, tab 6).
- (F) Left vmPFC connectivity to left dlPFC and left IFG at month 1 compared with baseline is positively correlated with weight loss (displayed $p < 0.001$, uncorrected, minimum voxel extent = 10 mm, $p < 0.05$ FWER corrected in Table S1, tab 6).
- (G) The mean beta estimate derived from the significant cluster (F) in month 1 compared with baseline versus weight loss between month 1 and baseline. Data are presented as mean \pm SEM and statistics are derived from linear mixed models. SVC, small volume correction; FWER, family-wise error rate; ns, not significant. ** $p < 0.01$. X and Z refer to MNI coordinates in millimeters.



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The magnitude of month 1 weight loss was correlated with changes in pre-meal log leptin and pre-meal ghrelin levels at month 1 compared with baseline. The association was negative for log leptin ($r = -0.78$, $p = 4.7 \times 10^{-5}$; Figure 5A) and positive for total ghrelin ($r = 0.67$, $p = 0.002$; Figure 5B), but insignificant for acyl-ghrelin ($r = 0.20$, $p = 0.38$). The effects remained at month 3 when weight loss at month 3 compared with baseline correlated inversely with changes in pre-meal leptin levels ($r = -0.86$, $p = 1.62 \times 10^{-5}$) and positively with changes in pre-meal total ghrelin levels ($r = 0.56$, $p = 0.048$), but not acyl-ghrelin ($r = 0.47$, $p = 0.14$) at month 3 versus baseline.

No significant effects of session on glucose or insulin levels were detected (Figures 2C and 2D). In addition, insulin sensitivity as assessed by homeostatic model assessment (HOMA) was not significantly different across sessions ($F(2, 39.6) = 0.61$, $p = 0.5$; Table S1 tab 2).

Leptin is produced by adipocytes. Its plasma levels fall quickly during negative energy balance, and more gradually with diminished fat mass (Friedman and Mantzoros, 2015). The orexigenic hormone ghrelin is increased in response to energy deficit (Borer et al., 2009; Müller et al., 2015). In the current study, leptin levels decreased while ghrelin levels increased at month 1, consistent with previous weight-loss studies (Wing et al., 1996). Despite continued weight loss, ghrelin and leptin levels did not change further at month 3, as described previously (Crujeiras et al., 2010; Sumithran et al., 2011). These results suggest that, on average, our participants were in negative energy balance at month 1 and month 3 compared with baseline.

Ghrelin and Leptin Changes Correlated with Cue Reactivity in Reward Regions

We tested the hypothesis that weight loss-induced changes in ghrelin and leptin would increase brain activation of reward-related areas while viewing food cues, and that this would promote overeating and diet failure. Previous studies found that leptin administration reduced food cue reactivity in leptin-deficient patients (Aotani et al., 2012; Farooqi et al., 2007), while ghrelin administration increased food cue reactivity (Malik et al., 2008) in regions associated with food value and motivation.

Consistent with previous studies (Goldstone et al., 2014; Kroemer et al., 2013; Malik et al., 2008), increased pre-meal ghrelin levels at month 1 correlated with increased activity at month 1 in mPFC, caudate, and visual cortex, all areas associated with appetitive processing (Figures 5C, 5E, and 5F; Table S1, tab 8). Weight loss-induced reductions in pre-meal leptin level at month 1 also correlated with increased activity in mPFC and visual cortex at month 1 compared with baseline (Figures 5C and 5D; Table S1,

tab 9). This suggests that metabolic adaptations at month 1 contributed to increased food cue reactivity at month 1 compared with baseline. This result is consistent with a previous positron emission tomography study in humans that linked reductions in leptin levels during weight loss with increased dopamine signaling (Dunn et al., 2017). This increase in dopaminergic tone can lead to increased food cue reactivity in the aforementioned regions that receive dopamine projections.

Ghrelin and Leptin Changes Did Not Oppose Continued Weight Loss

The next question we addressed was whether negative energy-balance signals could counteract participants' efforts to continue losing weight by increasing value-related food cue reactivity and food intake. Contrary to our hypothesis, leptin reductions at month 1 versus baseline correlated with further weight loss at month 3 versus month 1 ($r = -0.69$, $p = 0.064$; Figure 5G) and a lower weight at 2 years compared with baseline ($r = 0.57$, $p = 0.04$; Figure 5H). Increases in ghrelin levels did not significantly relate to weight change at 3 months ($r = 0.43$, $p = 0.19$), or at 2 years compared with baseline ($r = -0.34$, $p = 0.40$), although the direction of the effect was not consistent with ghrelin adaptations causing an increase in weight. These results suggest that leptin reductions and ghrelin increases did not oppose continued weight loss, even though they increased reward-related food cue reactivity. Indeed, leptin reductions predicted greater subsequent weight loss. This is likely because leptin reductions were greater in individuals with better appetite control, who lost more weight.

In this study, leptin and ghrelin adaptations to weight loss did not explain why diets are unsustainable in the long term. Our results suggest that the reduction of leptin at month 1 is a marker of successful weight loss, correlating with future weight loss and/or maintenance. Indeed, in the Look Ahead study, weight loss at month 1 predicted reduced weight up to 8 years later (Unick et al., 2015). Our results are not consistent with the suggestion that reduced leptin levels during energy restriction might promote increased food intake and contribute to the weight-loss plateau or weight regain (MacLean et al., 2015; Sumithran et al., 2011). This theory has been recently questioned. A recent meta-analytical review of voluntary weight-loss trials concluded that reduced leptin levels did not appear to predict weight regain (Strohacker et al., 2014). Furthermore, even though leptin pharmacotherapy for individuals undergoing calorie restriction changes energy expenditure and neural responses to food cues (Rosenbaum et al., 2005, 2008), it does not affect *ad libitum* food intake (Kissileff et al., 2012). Leptin administration also fails to increase weight loss during a calorie restriction diet (Hukshorn et al., 2000; Shetty

Figure 5. Correlations with Leptin and Ghrelin Levels

(A and B) Weight loss at month 1 versus baseline versus pre-meal plasma leptin (A) and total ghrelin at month 1 versus baseline (B). (C) Correlation of food minus scenery activation at month 1 versus baseline and the increase in pre-meal ghrelin levels (orange); and the reduction in pre-meal log leptin levels (blue) at month 1 versus baseline. Pink regions display areas significant in both analyses (displayed $p < 0.05$ FWER; Table S1, tabs 8 and 9; for female-only analysis, see Figure S4). (D) Depiction of the correlation from (C). The mean beta parameter estimate is derived from the orange cluster in (C) at month 1 versus baseline. (E) Correlation of food minus scenery activation at month 1 versus baseline and the increase in pre-meal ghrelin levels (orange) (displayed $p < 0.05$ FWER; Table S1, tab 8). (F–H) Depiction of the correlation from (E) is shown in (F). The mean beta parameter estimate was derived from the orange cluster in (E). Pre-meal log leptin levels at month 1 versus baseline correlated inversely with future weight loss at month 3 versus month 1 (G) and negatively with weight at 2 years (H) compared with baseline.

The color scales represent t statistics derived from 5,000 permutations of the data. FWER, family-wise error rate. Y and Z refer to MNI coordinates in millimeters.

et al., 2011; Zelissen et al., 2005; however, see Heymsfield et al., 1999) or after gastric bypass surgery (Korner et al., 2013). This may account for the disappointing results and relative paucity of publications of leptin pharmacotherapy for weight loss.

Limitations of Study

Although our small sample size is common for within-design neuroimaging studies, it limits our statistical power and the generalizability of our findings. In addition, we have a small cohort of participants with weight measures at 2 years, some of which are self-reported. This might have biased our results. Second, we only tested total ghrelin, acyl-ghrelin, leptin, glucose, and insulin levels. Therefore, we cannot generalize our results to other metabolic signaling molecules such as glucagon-like peptide-1. Third, the communication between the metabolic hormones and the brain might have changed as a function of weight status (Ravussin et al., 2014); for example, leptin resistance might have been present in our sample. We did not test for hormonal resistance, and therefore cannot determine the extent to which changes in peripheral signaling affected brain responses at different time points. Nonetheless, leptin and ghrelin did have the expected effects on cue reactivity in appetitive brain regions. As there was only one male participant in our group, our results are only applicable to females. The correlation analyses between variables of interest were repeated in only female participants, and the correlation coefficients did not differ by more than 0.03 compared with the all-subjects analyses (Table S1, tab 10). Another limitation is the lack of a control group. Further studies are justified, and could include larger cohorts, a control group, monitoring of daily food intake and activity, indirect calorimetry, more precise estimation of insulin sensitivity, and additional known modulators of food intake and activity.

Conclusions

Despite these limitations, our results are consistent with a model that places decision making and self-regulation at the center of weight control in humans (Berthoud, 2011; MacLean et al., 2011). Conversely, the hypothesis that homeostatic starvation signals from leptin, ghrelin, or insulin would counteract individuals' efforts to restrict calories was not supported here, although other hormones not measured here might have contributed. Moreover, there is another important caveat to consider. A repletion experiment similar to that in Zelissen et al. (2005), which showed that adjunctive leptin therapy failed to improve a calorie restriction weight-loss regimen, would have further supported our results. It is certainly possible that leptin administration could have led to even greater weight loss, even though this has not been borne out in clinical trials. Altogether, our results add to the pessimism of the existing literature regarding pharmacologically targeting leptin or ghrelin signaling as an adjunct target for treatment of obesity in conjunction with calorie restriction. We further suggest that strategies that target cognitive control have a role to play in weight loss.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and one table and can be found with this article online at <https://doi.org/10.1016/j.cmet.2018.09.024>.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.N., W.H., E.B.M., and A.D.; Methodology, S.N., W.H., K.L., M. Lamarche, E.B.M., M.D., Y.Z., and A.D.; Software, S.N., M.D., and K.L.; Investigation, S.N., W.H., M.Z., S.G.S., M. Lamarche, S.C.S., M. Larocque, and A.D.; Writing – Original Draft, S.N. and A.D.; Writing – Review & Editing, S.N., M.D., E.B.M., and A.D.; Funding Acquisition, E.B.M. and A.D.; Resources, E.B.M., M. Larocque, S.C.S., and A.D.; Supervision, E.B.M. and A.D.

DECLARATION OF INTERESTS

M. Larocque is the founder and owner of Clinique Motivation Minceur. He recruited the participants but did not fund the study. The other authors declare no conflict of interest.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
Total Ghrelin Radioimmunoassay	Millipore, Billerica, MA, USA	GHRT-89K
Acyl – Ghrelin Radioimmunoassay	Millipore, Billerica, MA, USA	GHRA-88HK
Leptin ELISA	R&D Systems, Minneapolis, MN, USA	DLP00
Glucose Glucose-oxidase(GM-9)	Analox Instruments, Lunenburg, MA, USA	N/A
Insulin Radioimmunoassay	Millipore, Billerica, MA, USA	HI-14K
Deposited Data		
Food cue reactivity fMRI data during CR	NeuroVault Repository	XPQFOZLV
Behavioral data	This paper	available upon request
Hormonal data	Mendeley data repository	https://doi.org/10.17632/3vf6wnhsds.1#file-3aade33a-4752-48e4-89f5-d5b6780038bf
Raw imaging data for all sessions	This paper	available upon request
Software and Algorithms		
MINCTOOLS	McConnell Brain Imaging Centre (BIC) of the Montreal Neurological Institute, McGill University, CANADA	http://www.bic.mni.mcgill.ca/Services/Software/MINC ; RRID: SCR_014138
FSL	Oxford Centre for Functional MRI of the Brain (FMRIB), Oxford University, UK	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki ; RRID: SCR_002823
MATLAB	Mathworks	https://www.mathworks.com/products/matlab.html ; RRID: SCR_001622
MRICron	Rorden Neuropsychology Labs at University of South Carolina	https://www.mccauslandcenter.sc.edu/crnl/mricron ; RRID: SCR_002403
Adobe Illustrator	Adobe Systems, San Jose, CA	https://www.adobe.com/ca/products/illustrator.html ; RRID: SCR_010279

CONTACT FOR REAGENT AND RESOURCE SHARING

Information and requests for data should be directed to and will be fulfilled by the Lead Contact, Alain Dagher (alain.dagher@mcgill.ca).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

29 right-handed participants [1 male, BMI = 30.94 (SD = ± 3.76); age = 37.28 (SD = ± 7.99)] were recruited in a private weight loss clinic (“Clinique Motivation Minceur”, Montréal QC Canada) before they started a prescribed weight-loss regimen. After the protocol was explained, those accepting to participate signed the consent form, underwent medical history, physical examinations and blood testing to look for comorbid conditions. Inclusion criteria were: healthy apart from obesity, and stable weight for at least three months. Exclusion criteria were diabetes, uncontrolled hypertension, currently smoking, substance abuse and current use of a central nervous system active medication, renal disease, non-dermatologic cancer in the previous 5 years and current or history of neurological, eating or psychiatric disorders. Candidates who could not undergo MRI due to claustrophobia, pregnancy, implanted metal, or BMI > 40 kg/m² were also excluded (n = 1). Each participant received an individualized non-ketogenic calorie-restricted diet program from the weight loss clinic. The prescribed diets contained 1100-1400 kcal/day, with 40% carbohydrate, 30% fat and 30% protein. Oral calcium and multivitamins were recommended, as was 30-45 min of brisk walking per day. Weekly visits included motivational counselling. The study was approved by the Montreal Neurological Institute Research Ethics Board. Volunteers received compensation for participation in the form of free nutritional supplements and reimbursement for travel.

Data from one participant was not included due the presence of uncorrectable fMRI brain artifacts (n = 1). Lack of button response to more than 25% of the stimulus ratings deemed a run incomplete as it indicated that the participant was not paying attention to the task. Any session with 50 % incomplete runs (3 out of 6 runs) was excluded. Three participants were inattentive during the fMRI scan during at all 3 sessions (n = 3). In addition, four did not successfully complete the fMRI at session 2 (leaving n=20) and four (others) at session 3 (leaving n = 20). This reduced the sample size for comparing baseline to month 1 to 20 participants, and the comparison of

month 1 to month 3 to 16 participants. Participants were contacted at two years following the experiment. Of 19 respondents, weights were reported by 10, and measured in 9. Of these 19 respondents, 14 people completed all three sessions of the fMRI successfully. Weight at two years is calculated as the percent change of weight from baseline to two years ($n = 19$); and weight regain as the percent change of weight from month 3 to two years ($n = 14$).

METHOD DETAILS

Experimental Design

Participants underwent fMRI on three occasions: first immediately prior to initiation of the diet program, then at month 1 and month 3 during calorie restriction. Women were always scanned during the luteal phase of their menstrual cycle. The scanning sessions started between 12:00 and 1:30 PM and kept the same schedule for the three sessions. On the scan days, participants presented themselves to the lab in the morning, having fasted from midnight the night before. All participants received the same standardized breakfast. This included $\frac{3}{4}$ cup of 2% milk, 1 slice of brown toast, 10 mL of peanut butter, one medium-sized hard-boiled egg, one small apple, a protein bar and 150 mL of black coffee or tea without sugar. It contained 550 kcal, 43% carbohydrate, 32% fat and 25% protein and was eaten over 10–15 minutes. Venous blood samples were drawn through a stopcock in an antecubital vein immediately prior to breakfast, then at 30, 60, 120 and 180 min (just prior to the fMRI scan) then just after the scan at 300 min (Figure 1) to measure hormone levels (see below). In addition, at each session, blood tests were conducted to measure C reactive protein, ketones, cholesterol, triglyceride, and glycated hemoglobin (HbA1c) levels (Table S1, tab 2). Participants were confirmed to have negative blood ketones at each session.

Psychological and Physical Activity Measures

Participants completed the Binge Eating Scale, the Dutch Eating Behavior (van Strien et al., 1986) and Power of Food Questionnaires (Lowe et al., 2009) to assess the eating styles. Sensitivity to reward and punishment was assessed using the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (Torrubia et al., 2001) and the BIS/BAS Scale (Carver and White, 1994), depression levels using the Beck Depression Inventory (Beck and Beck, 1972), and chronic stress during the previous month using the Perceived Stress Scale (PSS) (Cohen et al., 1983) (Table S1, tab 1). Participants reported their activity levels over the past week by answering two questions: “Using the past week as a reference, how much time have you given to the following activities? Jogging, cycling, swimming, cross-country skiing, aerobic dance or other similar activities.” and “Over the past week, how much time have you spent doing strenuous work, a physical activity or a sport other than those mentioned in the previous question?” The mean scores from these two questions were used to calculate changes in self-reported exercise (Table S1, tab 2). Hunger levels were assessed using Visual Analog Scales (VAS) four times throughout the day across the sessions (Figure 1). On the VAS, we asked the participants “On a scale from 0 to 10 how hungry do you feel now?” (Table S1, tab 3).

Hormone Measurements

The blood samples were centrifuged, and plasma and serum stored at -80°C until analysis. All samples from each participant were measured within the same assay. Plasma glucose was measured by the glucose-oxidase technique (GM-9, Analox Instruments, Lunenburg, MA, USA), and insulin using a specific radioimmunoassay (RIA) (Millipore, Billerica, MA, USA). We measured both total and acylated ghrelin, as the latter is considered “active”. All samples for acyl-ghrelin were centrifuged as soon as possible after collection, and the plasma obtained was preserved with freshly prepared phenylmethanesulfonyl fluoride protease inhibitor (Sigma-Aldrich, St-Louis, Missouri, USA) and acidified with HCl (as per assay requirements). Samples required acidification before storage to prevent degradation (Hosoda and Kangawa, 2012). Both acyl and total ghrelin were measured by RIA (Millipore, Billerica, MA, USA). Leptin was assayed using a solid phase ELISA (R&D Systems Inc, Minneapolis, MN, USA). The HOMA $-IR$ (insulin resistance) index was calculated as fasting (glucose \times insulin)/22.5.

fMRI Session

Neuroimaging was carried out with a Siemens Magnetom Trio 3T MRI scanner at the Montreal Neurological Institute (MNI). High-resolution T1-weighted anatomical images with voxel size = $1 \times 1 \times 1$ mm were obtained first. Functional data were acquired with an echo-planar T2*-weighted sequence for BOLD contrast (TR = 2 s; TE = 30 ms; flip angle, 90° ; FOV = 224 mm, voxel size = $3.5 \times 3.5 \times 3.5$ mm³, number of slices = 38).

For each session, participants underwent six 7-minute functional runs. During each run subjects viewed images of food or scenery, presented via a projector and a mirror placed on the head coil. The food and scenery images (examples in Figure 1) had been previously matched for visual appeal (Malik et al., 2008). Each run comprised 36 unique images, 12 each of high and low-calorie foods (e.g. brownies, vegetables) and scenery (Figure 1). The order of picture presentation was randomized across subjects and runs. Images were presented for 4.0 seconds and were followed by a rating of the stimulus on a 1–4 scale. For food pictures, participants rated “How appetizing is this stimulus?” and for scenery pictures participants rated “How pleasant is this stimulus?”. Rating was followed by a fixation cross with a jittered interstimulus interval (2.5–6.0 seconds). Stimulus presentation was done using E-Prime (Psychology Software Tools, Sharpsburg PA, USA). Ratings were entered by subjects via a MR-compatible button device.

QUANTIFICATION AND STATISTICAL ANALYSIS

Hormone and Behavioral Analysis

The values and descriptive statistics for the hormone measurements are listed in [Table S1](#) (tab 3). The statistical analysis of hormonal and psychological measures was conducted using MATLAB (Version R2015a, MathWorks, Natick, MA, USA). We log-transformed leptin and insulin values to correct for non-normality. The longitudinal analyses were run using linear mixed effects modelling (MATLAB function `fitlme`) with subject as a random effect. The Akaike information criterion was utilized to select the best model ([Table S1](#), tab 4). To investigate the effect of the session (coded as a categorical variable), we performed an F test using the function: `anova` on our model estimated by the `fitlme` function. To compare across sessions and across time points, and to calculate the F and p values, we conducted linear hypothesis testing on the linear mixed regression model coefficients using `CoeffTest` implemented in MATLAB. The denominator degrees of freedom (df) are computed using the Satterthwaite approximation. To perform pairwise comparisons for different time points, we further coded the time variable as a categorical factor. The graphs were produced using the raw data, but the statistical analyses reported were based on linear mixed effect models. For correlations, we conducted Spearman rank correlations when the distribution was not normal. All of the reported p-values are adjusted for multiple-comparisons using the Bonferroni method.

Imaging Data Analysis

The T1-weighted MRIs were submitted to brain extraction using BEaST, a nonlocal segmentation method applied to the images linearly registered to ICBM-MNI template ([Eskildsen et al., 2012](#)).

Pre-processing of the BOLD data was conducted using FEAT (fMRI Expert Analysis Tool, Version 6, part of FMRIB's Software, <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) and consisted of slice timing motion correction, spatial smoothing (6mm), and high-pass filtering with a cut-off frequency of 0.1 Hz ([Jenkinson et al., 2012](#)). Linear registration (6 parameters) to T1-weighted and non-linear registration to standard T1-weighted ICBM-MNI152 template brain (voxel size = 2x2x2 mm³) were completed using FLIRT ([Jenkinson et al., 2002](#)) and FNIRT ([Andersson et al., 2007](#)) respectively, prior to statistical analysis.

Imaging Statistics

For the first-level statistical analysis of the BOLD time-series, a general linear model (GLM) was implemented using FILM ([Woolrich et al., 2004](#)). The regressors for the GLM were the motion parameters, button presses for ratings, missed events, and the food and scenery image presentation events which were modeled by convolving the time course with a double-gamma hemodynamic response function (HRF) and applying temporal filtering. The resulting contrast images (i.e. food minus scenery) were then passed onto a second-level fixed-effects analysis for each subject, to obtain mean contrast estimates over runs within subjects by forcing random effects variance to zero in FLAME (FMRIB's Local Analysis of Mixed Effects) ([Beckmann et al., 2003](#); [Woolrich et al., 2004](#)). The t-stat maps for the contrast of interest (i.e., session1 vs. session2, session2 vs. session3, etc.) were combined across subjects for the third-level analysis.

In the third level analysis, the results at the group level were analyzed using non-parametric permutation tests (n = 5,000 permutations) with the threshold-free cluster enhancement (TFCE) algorithm using `randomize` in FSL ([Smith and Nichols, 2009](#)). In order to explore how the percent weight loss or changes in hormone levels affected the reactivity to food cues (i.e. food vs. scenery images), we included them as regressors in the GLM model. We utilized percent change in weight loss to account for baseline weight.

To analyze the effects of weight loss on value related activity for food items, we performed a region of interest analysis using a mask from a fMRI meta-analysis for subjective value at the time of decision (see Figure 6A from [Bartra et al., 2013](#)). In addition, we investigated how weight loss related to changes in BOLD in regions implicated in cognitive control. For this purpose, we restricted our analysis to a regional forward-inference mask associated with the term "cognitive control" in the NeuroSynth database of fMRI studies (<http://neurosynth.org/analyses/terms/cognitive%20control/>) ([Yarkoni et al., 2011](#)). Featquery from FSL was utilized to extract the percent change in mean beta parameter estimates in each ROI that was used for plotting.

Functional Connectivity

For functional connectivity analysis we utilized psychophysiological interaction (PPI) ([O'Reilly et al., 2012](#)). We selected a seed region based on the peak coordinate of food minus scenery at session 1 minus session 2 (MNI: x = -10, y = 34, z = -12). This peak was used to form a 4 mm diameter seed region of interest (ROI) in the vmPFC. We further transformed this ROI in standard space into each individual's functional space using the previously obtained linear transformations (from FLIRT). For each subject, we determined the peak voxel within the ROI, and used it to create a new 4mm subject-specific ROI used to extract the mean BOLD time-series signal. The mean time series of the seed ROI and the contrast of interest (i.e. food cues vs. scenery images) were multiplied to create the PPI regressor in the first level analysis. Higher-level analysis was repeated as stated above.

For all of the univariate results reported here, the significance of the clusters was determined by threshold-free cluster enhancement (TFCE) with a significance level of p < 0.05 family wise error rate (FWER). All coordinates are given in MNI space.

Independent Component Analysis

Baseline session fMRI were also analyzed utilizing spatial independent component analysis (ICA) with the Group ICA v3.0b fMRI Toolbox (GIFT, <http://mialab.mrn.org/software/gift/index.html>) ([Calhoun et al., 2001](#); [Correa et al., 2005](#)). ICA utilizes a data-driven

approach to obtain non-overlapping spatial maps with temporally coherent time courses and maximum spatial independence. We limited our analysis to 30 ICA components (IC) derived using the Infomax algorithm. After visual inspection, we selected five components that we hypothesized to be related to our task based on known resting state networks (Smith et al., 2009). The relationship between the ICs and the experimental paradigm was investigated by regressing the ICA time courses with the GLM design matrix (in SPM 12), which contained 4 regressors: high-calorie food, low-calorie food, scenery pictures and liking rating for the task. The regression revealed a set of beta-weights, which represent the fit between the IC and task time course. T-test on the beta estimates revealed that one component related to our task positively, and two components negatively, after False Discovery Rate (FDR) correction at $p < 0.05$. We visually identified the component that related to the task positively as the right frontoparietal network (rFP) and the components that related to the task negatively as default mode network components (Smith et al., 2009). The weighted spatial right frontoparietal component was then utilized as a mask in Featquery to extract the mean beta estimates for food minus scenery for each individual across the sessions.

DATA AND SOFTWARE AVAILABILITY

The statistical maps of the brain imaging analysis have been deposited in NeuroVault (<https://neurovault.org/collections/>) under the accession number XPQFOZLV. The hormonal blood measurements are available at <https://doi.org/10.17632/3vf6wnhsds.1#file-3aade33a-4752-48e4-89f5-d5b6780038bf>.