CLINICAL STUDY

Increased hypothalamic–pituitary–adrenal drive is associated with decreased appetite and hypoactivation of food-motivation neurocircuitry in anorexia nervosa

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Abstract

Objective: Corticotrophin-releasing hormone (CRH)-mediated hypercortisolemia has been demonstrated in anorexia nervosa (AN), a psychiatric disorder characterized by food restriction despite low body weight. While CRH is anorexigenic, downstream cortisol stimulates hunger. Using a food-related functional magnetic resonance imaging (fMRI) paradigm, we have demonstrated hypoactivation of brain regions involved in food motivation in women with AN, even after weight recovery. The relationship between hypothalamic–pituitary–adrenal (HPA) axis dysregulation and appetite and the association with food-motivation neurocircuitry hypoactivation are unknown in AN. We investigated the relationship between HPA activity, appetite, and food-motivation neurocircuitry hypoactivation in AN.

Design: Cross-sectional study of 36 women (13 AN, ten weight-recovered AN (ANWR), and 13 healthy controls (HC)).

Methods: Peripheral cortisol and ACTH levels were measured in a fasting state and 30, 60, and 120 min after a standardized mixed meal. The visual analog scale was used to assess homeostatic and hedonic appetite. fMRI was performed during visual processing of food and non-food stimuli to measure the brain activation pre- and post-meal.

Results: In each group, serum cortisol levels decreased following the meal. Mean fasting, 120 min post-meal, and nadir cortisol levels were high in AN vs HC. Mean postprandial ACTH levels were high in ANWR compared with HC and AN subjects. Cortisol levels were associated with lower fasting homeostatic and hedonic appetite, independent of BMI and depressive symptoms. Cortisol levels were also associated with between-group variance in activation in the food-motivation brain regions (e.g. hypothalamus, amygdala, hippocampus, orbitofrontal cortex, and insula).

Conclusions: HPA activation may contribute to the maintenance of AN by the suppression of appetitive drive.

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Introduction

Anorexia nervosa (AN) is a psychiatric disorder characterized by food restriction despite extremely low weight. Corticotrophin-releasing hormone (CRH)-mediated hypercortisolemia has been described in these patients, presumably due to the stress of chronic starvation (1, 2, 3, 4, 5, 6, 7). However, hypothalamic–pituitary–adrenal (HPA) dysregulation may persist in AN after weight gain, suggesting that this pathway may be involved in disease pathogenesis (8, 9). While CRH is anorexigenic, signaling satiety, cortisol excess, such as

in Cushing's disease or exogenous glucocorticoid exposure, results in increased appetite and weight gain (10, 11, 12). This may be related to a direct effect of cortisol on the appetite-regulating regions of the brain, or indirect effects through inhibition of the anorexigenic hypothalamic hormone CRH or modulation of other appetite-regulating hormones (11, 13, 14). We have previously shown that nocturnal cortisol levels are associated with the severity of disordered eating psychopathology in women across the weight spectrum, independent of BMI (15). Using a food-related functional magnetic resonance imaging (fMRI)

paradigm, we have also reported hypoactivation of food-motivation neurocircuitry in women with AN compared with healthy women, even after recovery (16). In the aforementioned study, women with AN reported lower subjective appetite levels than healthy women (16). Whether HPA dysregulation is associated with differences in appetite or hypoactivation of brain circuits involved in food motivation in AN is unknown.

In this study, we use a previously validated fMRI paradigm with food and non-food visual stimuli and endocrine assessment during fasting and following a standardized mixed meal to examine the link between HPA secretory abnormalities and appetite and brain circuitry deficits in AN (16). Comorbid psychiatric disorders, including depression, are common in AN, and there is overlap between brain regions (e.g. hypothalamus, amygdala, hippocampus, and insula) involving stress and food-motivation pathways. Importantly, modulation of appetite and feeding behavior by HPA hormones is specifically potentiated through dense expression of receptors (CRF1, CRF2, and GR) in these same limbic and paralimbic regions: hypothalamic nuclei (ventromedial hypothalamus; paraventricular nucleus), amygdala, hippocampus, nucleus accumbens, and insula (17, 18, 19, 20, 21). We hypothesized that the relationships between HPA hormone levels and measures of appetite and brain activation would be independent of depressive symptoms.

Subjects and methods

Subjects

We studied 36 women between 18 and 28 years of age: 13 with AN, ten who had recovered from AN (ANWR), and 13 normal weight healthy controls (HC). Subject demographics, validation of the fMRI paradigm, and cortisol levels have been previously published (16, 22, 23). In this paper, we investigated the relationship between HPA activation and appetite and food-motivation neurocircuitry. All study participants were recruited from the community through advertisements and were referrals from healthcare providers.

Subjects were excluded if they had any contraindication to MRI such as an implanted medical device, significant orthopedic hardware, or severe claustrophobia. Additional exclusion criteria included active abuse of drugs or alcohol, use of hormones or medications known to affect hormone levels (including estrogen) within 8 weeks of the study visit, use of depot medroxyprogesterone within 6 months, diabetes mellitus, history of gastrointestinal tract surgery, pregnancy or breastfeeding within 8 weeks of the study, and hematocrit <30% or hemoglobin <10 g/dl.

Subjects met the diagnostic criteria for AN with the Structured Clinical Interview for DSM Disorders-IV (SCID), including intense fear of gaining weight,

evidence of body image disturbance, significantly low body weight (operationalized as <85% of ideal body weight (IBW) as determined by the 1983 Metropolitan Life tables), and amenorrhea for at least 3 consecutive months (24). AN subjects who reported more than one binge and one purge episode per month in the 3 months preceding the study were excluded. Subjects with a history of psychosis by SCID were also excluded.

ANWR subjects were between 90 and 110% of IBW and were required to have regular menstrual cycles and stable weight for at least 6 months prior to the study. ANWR subjects met a diagnosis of AN by DSM-IV criteria other than amenorrhea, as assessed by SCID, in the past. The recovered subjects had not exercised more than 10 h/week and had not run more than 25 miles/week in the 3 months preceding the study.

HC were between 90 and 110% of IBW and reported regular menstrual cycles. HC had no history of amenorrhea, no acute or chronic illnesses, and no history of a psychiatric disorder (including an eating disorder) as assessed by SCID. HC were excluded if they had exercised more than 10 h/week or ran more than 25 miles/week in the 3 months preceding the study.

Methods

This study was approved by the Partners Human Research Committee. Written informed consent was obtained from all subjects prior to conducting any procedures. All subjects were admitted to the Massachusetts General Hospital (MGH) Clinical Research Center for an outpatient screening visit and to the MGH Clinical Research Center and Athinoula A Martinos Imaging Center for a morning, outpatient main visit.

At the screening visit, height, weight, and elbow breadth were measured by research dietitians, blood was drawn for screening laboratory tests, and a comprehensive history and physical exam was performed. Exercise patterns and alcohol intake were assessed. Percent IBW was calculated as above. BMI was obtained by dividing the weight in kilograms by the square of height in meters. Frame size was determined by comparing elbow breadth with race-specific norms derived from the US Health and Nutritional Examination Survey-I (25). The mood episode, psychotic and associated symptoms, mood disorder, anxiety, somatoform, substance abuse, and disordered eating modules of the structured clinical interview for DSM disorders-IV (SCID) were administered in person during the screening visit or over the telephone before the main visit by a trained psychiatric nurse practitioner or psychologist (24).

At the main visit, %IBW and BMI were reevaluated. A brief medical history was performed. HC and ANWR were presented during the follicular phase of the menstrual cycle (days 1–10). Subjects were asked to fast for 12 h prior to the visit. Subjects were given a 400 kcal mixed breakfast meal standardized for micro- and

macronutrient content (~20% calories from protein, 20% from fat, and 60% from carbohydrates) at 0900 h. The participants selected one of the following options of similar macro- and micronutrient content, provided by the Clinical Research Center bionutritionists: cereal, lowfat milk, yogurt, and wheat germ; or minibagel with peanut butter, nonfat lactaid milk, or raisins. Participants were asked to eat the entire meal over a 15 min interval. Upon completion of the meal, the bionutrition staff weighed the meal to determine exact caloric intake. Blood was drawn at four different time points throughout the morning for hormone levels: a fasting blood draw obtained immediately before the standardized mixed meal, and blood draws at 30, 60, and 120 min after the meal. Subjective appetite was assessed using the visual analog scale. Depressive symptoms were assessed using the Beck Depression Inventory 2 (BDI 2). fMRI using a food-related paradigm was performed before and after the meal (see below).

Biochemical analysis

Plasma samples were immediately placed on ice. Serum and plasma samples were stored at -80°C until analysis. Serum cortisol levels were measured using a chemiluminescent immunoassay from Beckman Coulter (Fullerton, CA, USA). The intra-assay coefficient of variation (CV) was 4.4–6.7%, the inter-assay CV was 6.4–7.9%, and the sensitivity was 0.4 $\mu\text{g/dl}$. Plasma ACTH levels were measured using an IRMA assay from DiaSorin, Inc. (Stillwater, MN, USA). The intra-assay CV was 3.5–4.8%, the inter-assay CV was 3.2–5.7%, and the lowest reportable value was 1.5 pg/ml . Area under the curve (AUC) was calculated using the trapezoidal method.

Assessment of appetite

Visual analog scales, a reliable and widely used method to assess appetite (26), were administered during the fasting state and following the mixed meal. Subjects were asked to answer questions about appetite by making a mark on a line with extremes on either end indicating how they felt at that moment. For example, in response to the question, 'How hungry are you?', they marked their degree of current hunger between the two extremes, 'I am not hungry at all', on the left and 'I have never been more hungry', on the right. Scores were calculated by measuring the distance from the left side of the line.

Statistical analysis

JMP Statistical Discoveries (version 9.0; SAS Institute, Inc., Cary, NC, USA) was used for statistical analyses. Hormone levels were not normally distributed and were log-transformed before analysis. Clinical characteristics,

hormone levels, and visual analog scores were compared using the overall ANOVA; variables that were significantly different were then compared by Fisher's least significant difference test. Within group comparisons of hormone levels and appetite at different time points were made using the two-sided paired *t*-test. Linear regression analyses were used to investigate the relationships between cortisol levels and subjective appetite measures. Multivariate least-square analyses were constructed to control for potential confounders. Statistical significance was defined as a two-tailed *P* value <0.05 . Data are reported as mean $\pm$ S.E.M.

fMRI procedures

fMRI procedures have been previously validated in this population (16). Briefly, fMRI scanning was performed while subjects viewed high-calorie food stimuli, low-calorie food stimuli, non-food stimuli, and low-level baseline stimuli in a block design, while participants underwent standard gradient-echo EPI imaging on a Siemens 3T Trio (Malvern, PA, USA).

fMRI data analysis

fMRI data were analyzed as previously described (16). Data were preprocessed using Statistical Parametric Mapping (SPM8; Wellcome Trust Centre for Neuroimaging at University College London, 2008) and custom routines in MATLAB (Mathworks, Inc., 2000, Natick, MA, USA). Standard preprocessing steps included realignment and geometric unwarping of EPI images using magnetic fieldmaps, correction for bulk-head motion, nonlinear volume-based spatial normalization using the standard brain template from Montreal Neurological Institute (Montreal, Canada), spatial smoothing with a Gaussian filter (6 mm full-width at half-maximum), and outlier detection and exclusion (27). Following preprocessing, statistical analysis was performed at the single-subject level. Specific comparisons of interest (high-calorie foods vs objects, separately for pre- and post-meal) were tested using linear contrasts, and SPM maps were created based on these contrasts. Results from the single-subject level were submitted to a second-level random effects analysis. Independent sample *t*-tests were used to compare the size of a particular effect between groups. Clusters were identified within our regions of interest (hypothalamus, amygdala, hippocampus, orbitofrontal cortex (OFC), and anterior insula) in between-group contrasts, significant at $P<0.05$ (uncorrected) and $P<0.1$ (corrected for multiple comparisons within the search volume using voxel-level family-wise error correction).

Anatomic overlays were used on each subject's statistical maps to acquire signal change values across the regions of interest. Values indicated the degree of change in magnetic resonance signal detected between the high-calorie food and object conditions. Average

percent signal change values (beta weights averaged across all voxels within an anatomical region) were obtained using the REX toolbox for SPM8 (17) and used for brain-hormone general linear model (GLM) analyses. Using PROC MIXED model approach in SAS (version 9.2; SAS Institute, Inc.), the effect of cortisol on the association between group status and brain activity was assessed. The percent change in the estimate for case status when the model was adjusted for the hormone was calculated as the estimate for case status in the univariate model (b1) minus the estimate for case status in the model adjusted for the hormone (b2) together over the univariate estimate (b1) $((b1 - b2)/b1)$. Owing to our interest in the mediating effect of cortisol on the case status effect on brain activity, decreases in percent change were of interest, indicating the percent of the case group's effect on brain activity accounted for by the hormone. To examine whether the effects of cortisol on between-group differences in brain activity were independent of depressive symptoms, BDI2 scores were entered into the model with cortisol. The percent change in the estimate for case status in the model adjusted for both cortisol and the potential confounder (BDI2) (b3) was compared with the estimate for the case status adjusted solely for the hormone (b2). Again, decreases in percent change ($b3 > b2$) were of interest, indicating the percent of the case group's effect on brain activity was not confounded by BDI2 scores.

Results

Subject characteristics

The mean age of subjects was 22.3 ± 0.4 years and did not differ between groups. As per study design, BMI and %IBW were lower in AN ($17.7 \pm 0.3 \text{ kg/m}^2$

and $80.6 \pm 1.3\%$ respectively) than ANWR ($21.9 \pm 0.7 \text{ kg/m}^2$ and $97.7 \pm 3.4\%$) and HC ($22.5 \pm 0.4 \text{ kg/m}^2$ and $97.2 \pm 1.7\%$) ($P < 0.0001$). Mean time since last menstrual period was 50.2 ± 11.1 months for AN. For ANWR, time since weight recovery was 41.5 ± 11.1 months, and time since restoration of menstrual cycles was 42.4 ± 15.4 months. All ANWR reported weight stability for at least 12 months and regular menstrual cycles for at least 14 months. Duration of illness did not significantly differ between groups (AN 52.7 ± 11.2 vs ANWR 43.2 ± 9.0 months). Three AN and four ANWR reported a remote (at least 14 months before the study) history of purging activity, but none were actively bingeing or purging. Five AN were taking psychotropic medications: two were taking venlafaxine, one was taking fluoxetine, one was taking a low dose of amphetamine/dextroamphetamine (5 mg 24 h before the scan), and one was taking escitalopram and aripiprazole. Two ANWR were taking psychotropic medications: one was taking fluoxetine and one was taking bupropion and lorazepam. BDI2 scores were higher in AN compared with ANWR and HC (16.8 ± 3.3 vs 7.7 ± 2.2 and 0.8 ± 0.4 , $P < 0.02$), indicating greater severity of depressive symptoms. No subjects smoked cigarettes in the morning of the study period or consumed caffeine within 12 h of the study period. Hours of sleep the prior night, time since last p.o. intake, and calories consumed at breakfast did not differ between the groups.

Hormone levels

Hormone levels are presented in Table 1. Mean fasting, 120 min post-meal, and nadir cortisol levels were higher in AN than HC. In contrast, mean ACTH levels were higher in ANWR than AN and HC at 60 min post-meal and HC at 120 min post-meal. In each group,

Table 1 Hormone levels.

	Mean $\pm$ S.E.M. (pg/ml)			P values			
	AN	ANWR	HC	AN vs ANWR	AN vs HC	ANWR vs HC	Overall
Serum cortisol							
T0 fasting	15.9 ± 1.4	12.9 ± 1.0	11.7 ± 1.1	NS	0.013	NS	0.041
T30	15.1 ± 1.6	$11.3 \pm 1.08^*$	11.7 ± 0.8	—	—	—	0.089
T60	$13.3 \pm 1.5^*$	11.5 ± 0.7	10.9 ± 0.8	—	—	—	NS
T120	$12.9 \pm 1.3^*$	11.1 ± 0.8	$8.8 \pm 0.7^*$	NS	0.006	0.067	0.019
Nadir ^a	$11.7 \pm 1.1^*$	$9.9 \pm 0.7^*$	$8.4 \pm 0.6^*$	NS	0.008	NS	0.028
AUC	1679 ± 159	1383 ± 83	1280 ± 73	—	—	—	0.084
Plasma ACTH							
T0 fasting	27.4 ± 2.3	26.0 ± 2.1	21.3 ± 1.3	—	—	—	NS
T30	23.6 ± 3.0	25.6 ± 1.9	22.4 ± 1.4	—	—	—	NS
T60	21.8 ± 1.7	29.8 ± 3.1	21.9 ± 1.2	0.017	NS	0.024	0.033
T120	23.2 ± 1.7	26.2 ± 1.7	19.6 ± 1.2	0.098	—	0.006	0.023
Nadir ^a	20.9 ± 1.9	24.2 ± 1.6	$19.0 \pm 1.2^*$	—	—	—	0.074
AUC	2689 ± 262	3286 ± 247	2563 ± 135	—	—	—	0.096

* $P < 0.05$ compared to T0. $P < 0.05$ are shown in bold.

^aNadir, minimum post-meal level.

Figure 1 Pre- and post-meal appetite scores. Premeal hunger ratings were lower in AN than the other groups, and desire to eat was lower in AN and ANWR than HC. All groups reported significant decreases in hunger, and all but AN reported a reduction in desire to eat after the meal. For post-meal, there were no significant between-group differences in hunger or desire to eat. * $P < 0.04$ compared with HC. † $P < 0.05$ vs premeal. Black, AN (active anorexia nervosa); striped, ANWR (weight-recovered anorexia nervosa); and grey, HC (healthy controls).

cortisol levels decreased after the meal. However, the post-meal decrease in ACTH levels was significant only in HC, not in AN or ANWR.

Fasting and post-prandial appetite ratings

Subjective ratings of homeostatic (i.e. hunger) and hedonic (i.e. desire to eat favorite food) appetite using the visual analog scale are reported in Fig. 1. AN reported less hunger and lower desire to eat favorite foods and ANWR reported lower desire to eat favorite foods than HC in the fasting state ($P < 0.04$). In all groups, subjective hunger decreased after the meal. In ANWR and HC, but not AN, desire to eat favorite foods decreased after the meal. Post-prandial appetite ratings did not differ between groups.

Relationship between HPA activation and appetite

Associations between cortisol levels and subjective appetite are shown in Fig. 2. Across groups, fasting serum cortisol and cortisol AUC were negatively associated with subjective assessment of homeostatic (i.e. hunger) and hedonic (i.e. desire to eat favorite food) appetite in the fasting state. After controlling for BMI and depressive symptoms as assessed by BDI2 scores, these relationships remained significant.

Relationship between cortisol levels and neurocircuitry involved in appetite and food motivation

Table 2 shows how much of the variance in group differences in signal changes in brain activity associated with our significant food-motivation brain regions is related to cortisol levels, as measured by T0 and AUC. We previously demonstrated premeal hypoactivation in AN (vs HC) in the hypothalamus, amygdala, hippocampus, OFC, and insula, and in ANWR (vs HC) in the hypothalamus, amygdala, and insula. We now demonstrate that cortisol levels, as assessed by T0 and AUC, are associated with variance in activation in the hypothalamus (16–26%), amygdala (24–45%), hippocampus (20–23%), OFC (19–46%), and insula (11–12%) in AN vs HC, and the hypothalamus (10–18%), amygdala (31–42%), and insula (10–12%) in ANWR vs HC. After the meal, we reported decreased activation in AN (vs HC) in the amygdala and insula. Cortisol was associated with 13–16% of the between-group difference in amygdala activation. Finally, we previously found that post-meal activation in AN (vs ANWR) was increased in the amygdala and decreased in the insula. Cortisol was associated with 9–12% of between-group differences in brain activation in the insula. Controlling for BDI2 did not alter the results for AN vs HC, indicating that cortisol–brain relationships for this between-group contrast are independent of depressive symptoms in the AN group.

Discussion

Using a novel approach combining neuroendocrine, subjective appetite, and brain imaging assessments during the fasting state and in response to food, we show for the first time that HPA dysregulation in women with active and weight-recovered AN is associated with altered subjective appetite and food-motivation brain circuits. Importantly, these findings are independent of depressive symptoms. These data suggest that abnormalities in HPA secretory patterns are associated with altered perception of appetite, increasing the maintenance of AN symptoms, and, given findings in weight-recovered cases, represent a trait of this illness.

We found that cortisol levels were increased in women with active AN compared with healthy women during the fasting state and in response to a meal. Although postprandial cortisol levels have been studied in healthy individuals, with mixed results of increased, unchanged, or decreased levels following food intake (28, 29, 30, 31), there are little data on cortisol and ACTH patterns following a meal in AN. Gastric infusion of liquid caloric mixtures resulted in an increase in ACTH and cortisol in 15 women with AN compared with no change in 15 healthy women (31). To our knowledge, however, the effect of eating a meal

Figure 2 Cortisol levels and appetite. Fasting and AUC cortisol levels are inversely associated with fasting assessments of hunger (A and B respectively) and desire to eat favorite foods (C and D respectively). Squares, active anorexia nervosa; triangles, weight-recovered anorexia nervosa; and diamonds, healthy controls.

on HPA hormones has not been reported in women with AN. In our study, cortisol levels decreased after the meal in all groups, while ACTH decreased in the healthy women only, suggesting excessive postprandial ACTH secretion in active NR and ANWR. The relatively higher cortisol levels that we report in women with active AN before and after a meal are consistent with the known HPA hyperactivation in this disorder (1, 2, 3, 4, 5, 6, 7). We also found evidence of increased HPA drive in weight-recovered women with AN, who had higher postprandial ACTH levels compared with women with active AN and healthy women despite comparable cortisol levels. This is in line with the prior reports of persistent dysregulated HPA function in AN following weight gain (8, 9).

Reports of appetite are abnormal in AN (32, 33). We report lower levels of subjective homeostatic (i.e. hunger) and hedonic (i.e. desire to eat favorite foods) appetite in women with active AN and lower levels of hedonic appetite in weight-recovered women with AN compared with healthy women. We now show that cortisol levels, as assessed by fasting cortisol or postprandial cortisol AUC, are negatively associated with self-reported homeostatic and hedonic appetite levels in AN, independent of BMI or depressive symptoms. Our data raise the question of whether HPA dysregulation, presumably driven by anorexigenic CRH, may promote altered perception of appetite in AN.

Our previous work demonstrated hypoactivation of numerous regions of the brain involved in food motivation, including the hypothalamus (a key control center for appetitive signaling), amygdala (a region important for learning satiety cues and assessing the reward value of food), hippocampus (implicated in processing food-related memories), OFC (involved in integration of emotion and reward expectation), and insula (integrates visceral, homeostatic, and emotional signals) in women with AN compared with healthy women (16). Recent human neuroimaging studies have offered additional insight into the role of these regions, particularly in the interaction between appetite and stress in healthy populations. For example, acute stress has been shown to elicit variable responses to rewarding food stimuli in the amygdala and hippocampus, depending on appetite level and BMI classification (34, 35), with a significant association between basal cortisol and amygdala activation in response to palatable food (35). Moreover, the OFC appears to modulate the long-term effects of this interaction, with activation positively related to BMI only during stress (35). These findings demonstrate the dynamic interplay between HPA activation and food intake in these regions, although the effect of chronic stress on these systems is yet to be investigated.

Using a food-related fMRI paradigm, we previously showed that in response to viewing high-calorie foods compared with objects, premeal brain activation was decreased in women with active AN compared with

Table 2 Mediation of group effects at selected brain regions by cortisol T0 and cortisol AUC.

Session	Group contrast	Region	Hemisphere	MNI coordi- nates (x, y, z)	Estimate	Estimate, adjusted for cortisol T0	%Change in estimate, adjusted for cortisol T0	Estimate, adjusted for cortisol AUC	%Change in estimate, adjusted for cortisol AUC
Premeal	AN vs HC	Hypothalamus	L	-3, -7, -5	-0.92	-0.69	25.5	-0.77	15.8
		Amygdala	L	-21, -10, -11	-0.61	-0.34	44.8	-0.47	23.8
		Hippocampus	L	-9, -40, 1	-2.27	-1.73	23.8	-1.80	20.5
		OFC	R	36, 23, -11	-1.12	-0.61	45.7	-0.90	19.5
	ANWR vs HC	Insula	R	33, 8, 4	-1.39	-1.23	11.5	-1.26	-
			R	-30, 17, 7	-1.16	-1.04	10.8	-1.13	-
		Hypothalamus	L	9, -7, -5	-0.66	-0.57	14.1	-0.59	10.2
			L	-6, -10, -5	-0.90	-0.79	11.8	-0.74	17.5
		Amygdala	L	-24, -10, -11	-0.68	-0.40	41.5	-0.47	31.4
		Insula	R	30, 26, -11	-0.80	-0.71	11.8	-0.71	11.5
Post-meal	AN vs HC		R	39, 26, -8	-1.14	-1.03	9.7	-1.11	-
		Amygdala	L	-24, -10, -14	-0.37	-0.32	13.2	-0.31	16.0
			L	-30, -1, -20	-0.67	-0.64	-	-0.64	-
		Insula	L	-33, 5, -5	-0.63	-0.76	-	-0.77	-
	AN vs ANWR		L	-39, -7, 4	-0.99	-1.11	-	-1.13	-
			R	15, -1, -17	0.61	0.66	-	0.64	-
		Amygdala	R	36, -10, 13	-0.99	-0.90	8.9	-1.06	-
		Insula	L	-39, -7, 4	-1.49	-1.31	12.0	-1.53	-

healthy women in the hypothalamus, amygdala, hippocampus, OFC, and insula (16). We now show that postprandial cortisol levels are associated with 16–46% of these between-group differences (HC vs AN) in activation in the hypothalamus, amygdala, hippocampus, OFC, and insula. Premeal brain activation was decreased in women with weight-recovered AN in the hypothalamus, amygdala, and insula (16). Cortisol levels are associated with 10–42% of between-group differences (HC vs ANWR) in activation in the hypothalamus, amygdala, and insula. After the meal, activation was decreased in women with active AN in the amygdala compared with healthy women and compared with healthy and weight-recovered women in the insula (16). Cortisol levels were associated with 4–16% of between-group differences in the activation of the amygdala and 9–12% of between-group differences in insular activation. Importantly in AN vs HC, these associations were independent of depressive symptoms as measured by the BDI2.

Overall, these results are consistent with the findings relating to acute HPA activation and response to food stimuli in the amygdala, hippocampus, and OFC. Importantly, they extend these previous results suggesting HPA disruption of appetitive signals to the effect of chronic stress and hypercortisolemia as a function of state (i.e. AN) as well as trait (ANWR). Given the density of CRF2 receptors, which (as opposed to CRF1 receptors) are particularly involved in appetitive signaling of CRF and downstream hormones (18), in these limbic and paralimbic regions (17), in combination with the evidence of disruption of HPA hormone levels in AN and ANWR, it is likely that the anorexigenic actions of CRH and orexigenic effects of cortisol influence activation of regions outside the hypothalamus and pituitary to influence hedonic and homeostatic perception of appetite in AN and ANWR.

Limitations of this study include small sample size, which may have reduced the power to detect between-group differences in endpoints, as well as correlations between HPA secretory patterns and appetite and brain activation. However, even with a relatively small sample size, our findings are robust. This is a cross-sectional study and causality cannot be established. Further research will be important to explore the effect of HPA dysregulation on appetite pathways in AN.

In summary, we provide evidence that dysregulation of HPA signaling in AN may be involved in disease pathogenesis. Fasting and postprandial cortisol levels are higher in women with active AN compared with healthy women, while postprandial ACTH levels are higher in women with weight-recovered AN compared with healthy women despite no significant difference in cortisol levels. Cortisol levels are negatively associated with homeostatic and hedonic measures of subjective appetite, independent of BMI and depressive symptoms. In addition, cortisol levels are associated with between-group differences in activation of brain regions

involved in food motivation, independent of depressive symptoms. Together, these data suggest that HPA dysregulation is associated with the maintenance of AN symptoms, particularly given the findings in weight-recovered cases, through altered perception of appetite.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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