NeuroImage: Clinical 36 (2022) 103202

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Stress-level glucocorticoids increase fasting hunger and decrease cerebral blood flow in regions regulating eating

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ARTICLE INFO

Keywords:
fMRI
Glucocorticoids
Cortisol
Stress
Hunger

ABSTRACT

Context: The neural regulation of appetite and energy homeostasis significantly overlaps with the neurobiology of stress. Frequent exposure to repeated acute stressors may cause increased allostatic load and subsequent dysregulation of the cortico-limbic striatal system leading to inefficient integration of postprandial homeostatic and hedonic signals. It is therefore important to understand the neural mechanisms by which stress generates alterations in appetite that may drive weight gain.
Objective: To determine glucocorticoid effects on metabolic, neural and behavioral factors that may underlie the association between glucocorticoids, appetite and obesity risk.
Methods: A randomized double-blind cross-over design of overnight infusion of hydrocortisone or saline followed by a fasting morning perfusion magnetic resonance imaging to assess regional cerebral blood flow (CBF) was completed. Visual Analog Scale (VAS) hunger, cortisol and metabolic hormones were also measured.
Results: Hydrocortisone relative to saline significantly decreased whole brain voxel based CBF responses in the hypothalamus and related cortico-striatal-limbic regions. Hydrocortisone significantly increased hunger VAS pre-scan, insulin, glucose and leptin, but not other metabolic hormones versus saline CBF groups. Hydrocortisone related increases in hunger were predicted by less reduction of CBF (hydrocortisone minus saline) in the medial OFC, medial brainstem and thalamus, left primary sensory cortex and right superior and medial temporal gyrus. Hunger ratings were also positively associated with plasma insulin on hydrocortisone but not saline day.
Conclusions: Increased glucocorticoids at levels akin to those experienced during psychological stress, result in increased fasting hunger and decreased regional cerebral blood flow in a distinct brain network of prefrontal, emotional, reward, motivation, sensory and homeostatic regions that underlie control of food intake.

1. Introduction

Nearly 50 % of Americans are predicted to have obesity by 2030. (Ward et al., 2019) The neural regulation of appetite and energy homeostasis significantly overlaps with the neurobiology of stress. (Sinha and Jastreboff, 2013) Frequent exposure to repeated acute stressors may cause increased allostatic load and subsequent dysregulation of the cortico-limbic striatal system leading to inefficient integration of postprandial homeostatic and hedonic signals. (Sinha and Jastreboff, 2013; McEwen, 2007; Dallman, 2010) In addition to the canonical hypothalamic–pituitaryadrenal (HPA)-axis of stress regulation, it is understood that the hippocampus, amygdala and prefrontal cortex (PFC) are

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https://doi.org/10.1016/j.nicl.2022.103202
Received 29 April 2022; Received in revised form 24 August 2022; Accepted 16 September 2022
Available online 16 September 2022

involved in the neurobiology of stress regulation (McEwen, 2007; Chattarji et al., 2015) These regions, regulating stress response throughout the brain, are also involved in processing and relaying information about the homeostatic and hedonic aspects of feeding behavior such as putamen/caudate (reward/motivation), hypothalamus (homeostasis), OFC/insula (taste/flavor perception), amygdala/hippocampus (emotion/memory), and PFC (executive function). (Gluck et al., 2017; Sominsky and Spencer, 2014; Rolls, 2016) It is therefore important to understand the neural mechanisms by which stress generates alterations in appetite that may drive weight gain.

There are several pathways where GC may interact with endocrine hormones. GC may affect hunger by altering the secretion of appetite-regulating hormones. GC increase the secretion of orexigenic peptides such as neuropeptide Y (NPY), and satiety hormones such as insulin and leptin, while increasing plasma glucose. (Tataranni et al., 1996; Konno, 2008) GC can promote systemic leptin and insulin resistance, which prevents both hormones from exerting an effective satiety signal. (Savontaus et al., 2002) GC and their associated hormonal changes may also contribute to a hypermetabolic state due to increased protein breakdown in skeletal muscle and increased hepatic glucose production. (Adam and Epel, 2007; Tempel and Leibowitz, 1994).

Increased plasma insulin alone has not been predictive of future weight gain; (Silver et al., 2006) however, previous research has shown that in individuals with obesity, over a 6-month follow-up period, higher plasma cortisol, insulin and reported stress levels together were predictive of weight gain, (Chao et al., 2017) associating stress hormone and insulin dysregulation with appetite and weight. Overall, there remains limited information regarding the role of endocrine hormones (e.g., insulin), GC-induced food intake, and brain regions involved in control of appetite and food intake. Recent investigations into this relationship in the human brain have used acute GC administration just prior to functional magnetic resonance imaging (fMRI), because of its fast data acquisition time and high temporal and spatial resolution. These studies have reported reduced brain activity in limbic regions, which are implicated in regulating appetite. (Montoya et al., 2014; Lovallo et al., 2010) Similarly, studies in rats (Endo et al., 1997) and dogs, (Yamazaki, 2021) utilized perfusion MRI to show that long-term administration of GCs reduced cerebral blood flow (CBF) in limbic regions. However, no previous work has examined the relationship between GC-associated CBF changes, endocrine hormones, and hunger.

The current study examined CBF in brain regions involved in controlling appetite, while concurrently measuring subjective hunger ratings and metabolic hormones. We hypothesized that an exogenous GC infusion that mimics physiological cortisol responses of acute life stressors such as hospitalization and invasive surgery, (Widmer et al., 2005) will increase hunger ratings and decrease cerebral blood flow in the hypothalamus and related cortico-striatal limbic regions involved in control of appetite and food intake, relative to a saline infusion. Further, we hypothesized that GC-related hunger will be predictive of GC-induced regional changes in CBF. To assess the regional CBF changes in response to GC, we used pulsed arterial spin labeling perfusion fMRI and repeated plasma and saliva sampling during each of the hydrocortisone and saline MRI scans.

2. Methods

2.1. Research subjects

Sixteen (10 M/6F) healthy lean individuals (Body Mass Index (BMI); Mean ± SD: 22.4 ± 2.2 kg/m^2) ages 18–45 (Mean ± SD: 26.4 ± 6.7 y) were recruited via web-based advertising and flyers for study participation. The study was approved by the Yale University Human Investigation Committee (HIC #: 1510016716) and Institutional Review Board and all individuals signed written informed consent. Individuals were screened and excluded for drug or alcohol use disorder, pregnancy, contraceptive use, endocrinopathies, psychiatric disorders and night shift workers or others with erratic sleep habits. As emotion and reward related behaviors can affect food intake and hunger and response to steroids, validated psychological questionnaires were administered to those that consented to assess emotion and reward related behaviors either at the visit, or in the form of a link sent via email to the participant. These questionnaires included the Eating Disorder Examination-Questionnaire and an Eating Behavior Inventory (EDE-Q and EBI).

2.2. Overnight hydrocortisone or saline infusion paradigm

Randomized crossover double-blind procedures were utilized by which investigators, study nurses, and participants were blind to the study condition and the order of the study condition was randomized and counterbalanced amongst the individual participants. The Yale Investigative Drug Pharmacy conducted the double-blind crossover procedures for subjects. On two separate days (mean ± SD 46 ± 45 days between scans; range (14–189 days), participants arrived to the Yale Hospital Research Unit (HRU) at approximately 7:30 pm the night before the scan. Participants were randomized to receive either an overnight infusion of hydrocortisone at a rate of 5 mg/m^2 of body surface area per hour (intervention) or saline (control), with the order of hydrocortisone or saline counterbalanced across subjects. Hydrocortisone and saline infusions were matched for both rate of infusion and mass per volume (1 mg/mL). At approximately 8:30 pm, an IV bolus of either saline or hydrocortisone (10 mg) followed by an infusion at 5 mg/m^2 body surface area per hour was initiated (Fig. 1A). (Askari et al., 2005) Hydrocortisone was chosen due to its bio-similarity to cortisol, including similar metabolism as cortisol. A bolus infusion paradigm allowed for a steady-state equilibrium to be reached sooner. The subsequent slow intravenous infusion allowed ample time for GC-associated hormonal changes to occur. (Askari et al., 2000; Shamoon et al., 1980) Individuals then stayed overnight in the HRU. A nurse monitored individuals for sleep disturbances overnight and collected venous blood samples for serum cortisol (Fig. 1B). No subjective differences between saline and hydrocortisone sessions were reported by subjects. Salivary cortisol was also measured at baseline and periodically throughout the study day to demonstrate a difference in levels between the saline and intervention (hydrocortisone) groups (Fig. 1C). The infusion was continued through the night and during the MRI study the following morning and ended after the completion of the MRI study, targeting a total dose of approximately 100–150 mg, which was designed to closely resemble endogenous cortisol levels during a moderate stress response. All testing completed on the day of the MRI was done in the fasting state.

On the morning of the MRI at 7:30 AM, blood was drawn to measure baseline serum total cortisol, glucose, insulin, glucagon, leptin, NPY, glucagon-like peptide 1 (GLP-1), adrenocorticotropic hormone (ACTH) in each participant. A salivary swab was completed for assessment of free cortisol periodically throughout the MRI scan. Participants also completed a Visual Analog Scale 1 (VAS1) hunger rating prior to the MRI scan at approximately 7:30am. Following this, the MRI component of the study was performed. Images were obtained in the Yale Magnetic Resonance Research Center using a 3-T Siemens Trio MRI system (Siemens Medical Systems, Erlangen, Germany) with a 64-channel coil. Initially, structural scans were obtained followed by cerebral blood flow measurements (see Imaging Parameters section).

2.3. fMRI imaging and acquisition parameters

Participants were positioned in the head coil with foam pillows to minimize head movements during image acquisition. A three-plane localizer was first acquired followed by a high-resolution whole-brain T1-weighted three-dimensional magnetization-prepared rapid gradient echo (MPRAGE) volume scan (Field-of-view (FOV): 256 × 256 mm^2, Phase oversampling: 0 %, Slice oversampling: 45 %, Slice per slab: 176, Slice thickness: 1.0 mm, repetition time (TR): 2530 ms, echo time (TE): 2.44 ms, Flip Angle (FA): 9°, TI: 900 ms, bandwidth: 240 Hz/Pixel, voxel

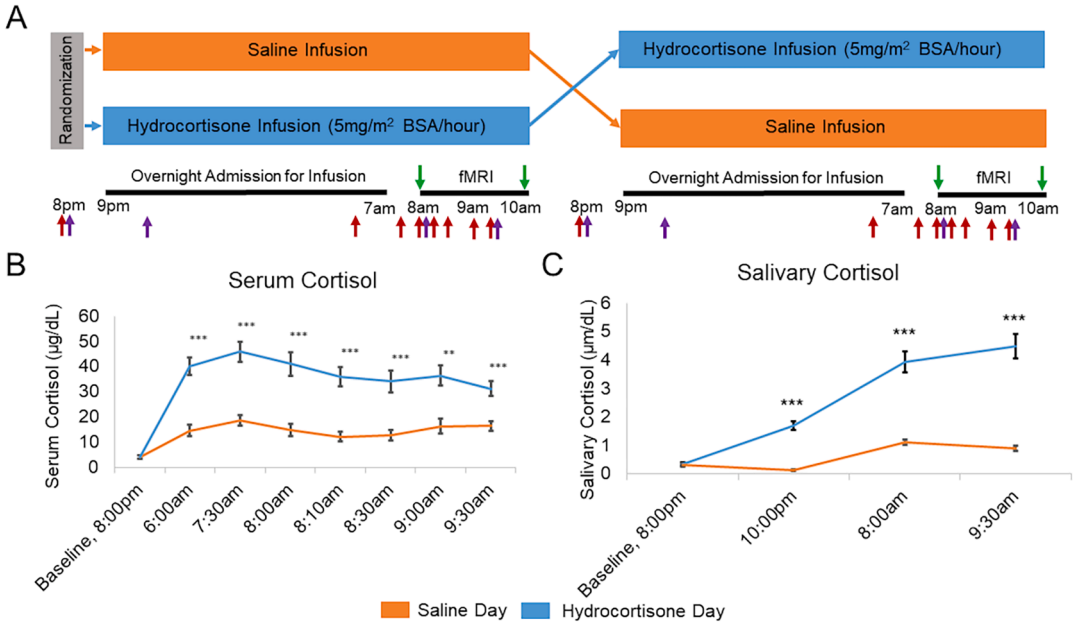

Fig. 1. Study design and measured cortisol levels on study days. A) Study design: randomized double-blind cross-over design of overnight infusion of hydrocortisone at a rate of 5 mg/m^2 of body surface area (intervention) or saline (control), and order counterbalanced across subjects. Red arrows correspond time of blood draws for serum cortisol measurements (8:00 pm, 6:00am, 7:30am, 8:00am, 8:10am, 8:30am 9:00am, and 9:30am). Purple arrows correspond to time of salivary cortisol measurements (8:00 pm, 10:00 pm, 8:00 (pre-scan swab) and 9:30am (post-scan swab). Green arrows represent the start and end of the MRI session. B) Serum cortisol values (Mean ± SEM) were significantly increased at all time points after baseline between hydrocortisone (H) vs saline (S) infusion days, including at the beginning of the scan at 8:00am (H: 41.0 ± 4.6 µg/dL; S: 15.0 ± 2.5 µg/dL, $p < 0.001$), and C) Salivary cortisol measurements that corroborated significant increases at all time points after baseline measurement and at the beginning of the scan (H: 4.0 ± 0.4 µg/dL; S: 1.1 ± 0.1 µg/dL; $p = 0.001$). $^{**}p < 0.01$, $^{***}p < 0.001$. Blood draws at baseline and during scans correspond to the times on the x-axis of Fig. 2B. VAS1 hunger ratings were performed just prior to fMRI scan, approximately 7:30am. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

size: 1.0x1.0x1.0 mm^3). Cerebral blood flow (CBF) throughout the brain was measured by PASL using the EPISTAR QUIPSS PASL MRI technique as previously (Page, 2011). The PASL acquisition parameters were as follows: time 2 × 5:12 min, FOV 256 × 256 mm^2, matrix 64 × 64, bandwidth 2,440 Hz/pixel, slice thickness 4.8 mm, TR 3000 ms, TE 20 ms, FA 90°. Proton density weighted images were collected using the same perfusion sequence, except for the following changes: TR was set to 10 s; the delay time TD was set to 0 ms; and the inversion time TI was set to maximum to allow a full longitudinal magnetization recovery. The proton density weighted image acquisition parameters were as follows: TR: 10000, TE: 20, bandwidth: 2440, FA: 90°, slice thickness: 4.8, FOV: 256 mmˆ2 matrix: 64x64.

2.4. CBF perfusion image processing

Images were corrected for small subject motions (using SPM8) and high-pass filtered to remove baseline drift. Maximum displacements over 1.5 mm and maximum rotations above 2 degrees were considered high motion (7 of 64 runs had motion above these criteria, between 15 and 60 frames were removed from each of the 7 runs to remove that motion), no run exceeded 0.15 mm frame-to-frame displacement. After correction of the linear global drift on a per-voxel basis and in-plane low-pass filtering with a Gaussian kernel of σ = [6,6] mm, time series of the perfusion-weighted images were obtained by pair-wise "surround" subtraction between interleaved label and control pairs, resulting in a temporal resolution of 2TR. (Wong et al., 1997; Aguirre et al., 2002; Wang, 2003) The mean difference map (ΔM) was calculated by averaging all the difference images in the time series for each of the two CBF runs. The same low-pass filtering was applied to the proton-density image (M_0*). Given ΔM and M_0* maps, the absolute CBF (f) (ml/100 g/minute) map was calculated using the Bloch Equation incorporating cerebral tissue perfusion terms, assuming $T_{1a} = 1490$ ms, $\lambda = 0.9$ ml / g, $\alpha_\pi = 0.95$, TI1 = 700 ms, TI2 = 1400 ms, and the post-labeling delay time of each slice. (Luh et al., 1999) To bring all single subject CBF average images into reference space, the Yale BioImage Suite software package (https://www.bioimagesuite.org/) was used to calculate two linear and one non-linear registration. These three registrations were concatenated and applied as one registration to bring the data into a common reference brain space. The Colin27 Brain in the Montreal Neurological Institute (MNI) space was used as the reference brain. For group level data analysis, linear effects modeling using AFNI 3dLME (https://afni.nimh.nih.gov) was implemented with a 2 session (Hydrocortisone, Saline) design. In this design, session was treated as a within-subject fixed-effect factor and subject as the random-effect factor. We also normalized individual CBF on each scan day (Hydrocortisone or Saline) by the mean CBF of either Hydrocortisone or saline day, creating a session centered mean CBF, which was used as a covariate in post-hoc follow-up analyses. To correct for multiple comparisons, we used family-wise errors (FWE) correction determined by Monte Carlo simulation using the AFNI 3dClustSim version (16.3.05, October 2016) program. Whole brain voxel based CBF results were conducted with an initial p threshold of $p < 0.001$ and an additional cluster correction at alpha $p < 0.05$.

2.5. Hormone analytical Methods

Throughout the study period, serum was collected for cortisol, glucose, insulin, glucagon, NPY, leptin, ACTH, and GLP-1. In addition, saliva was collected to measure free cortisol. Analyses were performed according to the manufacturer specifications using radioimmunoassay for Cortisol (MP Biomedical, #06B256440; RRID:AB_2801525), Insulin (EMD Millipore, #HI-14 K; RRID:AB_2801577), Glucagon (EMD Millipore, #GL-32 K; RRID:AB_2757819), Leptin (EMD Millipore, HL-81 K; RRID:AB_2756879) and ACTH (MP Biomedical, #07–106102; RRID: AB_2783719) or ELISA for NPY (Millipore, EZHNPY-25 K; RRID: AB_2909594) and GLP-1 Total and Active (43-GPTHU-E01; RRID:

AB_2801400). Glucose was measured at point of care (YSI).

2.6. Statistical considerations

Primary Endpoint: Change in cerebral blood flow after hydrocortisone versus saline administration. *Secondary Endpoint*: Change in appetite-regulating hormones and hunger ratings. *Statistical analysis:* Hormonal analysis and subject characteristics including age, gender, BMI, weight, and race were performed. Summary statistics including mean and standard deviation (or standard error of the mean, median, interquartile range when appropriate) for continuous variables, and n (percentage) for categorical variables are presented. Paired sample *t*-test or Wilcoxon rank sum test for continuous variables, and Chi-square or Fisher test for categorical variables were used for comparing subjects. Hunger was correlated with neural activity using multiple linear regression analysis and Pearson's correlations, setting the Bonferroni corrected threshold of $p < 0.001$ ($p = 0.05/45$ regions) for neural regions associated with hunger.

3. Results

Participant demographics such as sex, age, BMI, race/ethnicity and EDE-Q and EBI can be found in Table 1.

3.1. Metabolic and hunger response to hydrocortisone infusion

Mean ± SEM total hydrocortisone dose for all 16 participants from the hydrocortisone infusion was 109 ± 13 mg before scan start at 8am (Fig. 1A) and total dose at the end of the MRI scan protocol (10am) was 122 ± 13 mg. Mean ± SEM total saline infusions were well matched to hydrocortisone infusion days at the start of scan at 8am (107 ± 14 mg) and total dose at the end of the MRI protocol at 10am (121 ± 16 mg). Serum cortisol levels (mean ± SEM) were significantly increased at the beginning of the scan on hydrocortisone (H) vs saline (S) infusion days (H: 41.0 ± 4.6 μg/dL; S: 15.0 ± 2.5 μg/dL, $p < 0.001$) (Fig. 1B). Salivary cortisol measurements showed a similar increase at the beginning of the scan on hydrocortisone versus saline infusion days (H: 4.0 ± 0.4 μg/dL; S: 1.1 ± 0.1 μg/dL; $p = 0.001$) (Fig. 1C). Increases in serum and salivary cortisol levels compared to saline infusion days were sustained through the MRI scanning protocol (Fig. 1B/C). Pre-scan VAS hunger was significantly higher on the hydrocortisone versus saline days (H:5.3 ± 0.6; S 3.4 ± 0.6, $p = 0.04$, Fig. 2A). Average hormone levels of glucose (Fig. 2B), insulin (Fig. 2C), and leptin were significantly greater on hydrocortisone versus saline infusion days, while ACTH was significantly lower (Table 2). There were no significant differences in GLP-1, glucagon, or NPY between scan days. There were no correlations between plasma glucose or insulin with plasma or salivary (free) cortisol levels on either scan days. Change in fasting hunger was negatively correlated with change in free cortisol (hydrocortisone minus saline days; $R^2 = 0.31$, $p = 0.03$, Fig. 2D) and a negative but not significant correlation with plasma cortisol (hydrocortisone minus saline days; $R^2 = 0.17$, $p = 0.11$). Hunger was positively correlated with plasma insulin on hydrocortisone ($R^2 = 0.45$, $p = 0.004$, Fig. 2E) but not saline day ($R^2 = 0.02$, $p = 0.61$). There were no correlations between plasma glucose and hunger on either scan day. There were no other significant correlations between hormone measurements (Table 2) and fasting hunger on either saline or hydrocortisone scan days.

Table 1
Participant Demographics (N = 16).

Sex (M/F)	10/6
Age (years)	26.4 ± 6.7
BMI (kg/m^2)	22.1 ± 2.2
Education (years)	17.1 ± 2.8
Race/Ethnicity (%)	
Caucasian	63 %
Hispanic	6 %
African-American	19 %
Asian	12 %
EDEQ (screen for eating disorders)	0.53 ± 0.57
Eating Behavior Inventory	68.1 ± 6.5

M/F: Male/Female; EDEQ: eating disorder examination questionnaire.

3.2. Brain response to hydrocortisone versus saline infusion

Whole brain voxel-based CBF contrasting hydrocortisone (H) relative to saline (S) infusion showed decreased CBF in the anterior cingulate cortex (ACC), caudate, dorsolateral prefrontal cortex (DLPFC), hippocampus, hypothalamus, inferior frontal gyrus (IFG), insula, medial temporal gyrus (MTG), orbitofrontal cortex (OFC) extending into the ventromedial PFC (vmPFC), putamen, and thalamus (Fig. 3A). Quantitative changes in functional regional CBF response were calculated for all regions above (Fig. 3B). Mean percent difference of CBF were significant in the DLPFC (Brodmann Area 9) (–23 %, H: 62.2 v S: 85.0 ml/g/min; $p < 0.001$), MTG (–21 %, H: 49.2 v S: 70.0 ml/g/min; $p < 0.001$), ACC (–17 %, H: 49.2 v S: 70.0 ml/g/min; $p < 0.001$), IFG (–17 %, H: 49.2 v S: 70.0 ml/g/min; $p < 0.001$), hippocampus (–15 %, H: 50.5 v S: 65.5 ml/g/min; $p < 0.001$), thalamus (-13 %, H: 57.8 v S: 71.2 ml/g/min; $p = 0.008$), insula (-10 %, H: 58.7 v S: 68.7 ml/g/min; $p < 0.001$), OFC (-9%, H: 52.1 v S: 60.9 ml/g/min; $p < 0.001$), putamen (-5%, H: 63.3 v S: 68.8 ml/g/min; $p = 0.015$) and hypothalamus (-5%, H: 41.0 v S: 45.9 ml/g/min; $p = 0.015$). A non-significant difference in mean percent difference in caudate CBF on hydrocortisone day was −5% versus saline day (39.5 ± 3.0 v. 44.2 ± 3.5 ml/g/min, $p = 0.07$). Including both time between visits and order of hydrocortisone or saline infusion first in the model did not change the outcome. Session centered mean CBF, when used as a covariate in the model, produced very similar whole brain voxel-based CBF contrast of hydrocortisone (H) relative to saline (S) infusion (Supplemental Fig. 1). CBF on saline or cortisol day only did not correlate with salivary (free) or plasma cortisol on saline or cortisol day only.

Reduction in CBF (hydrocortisone minus saline) correlated with change in hunger (hydrocortisone minus saline) in the medial OFC ($R^2 = 0.58$, $p < 0.001$), medial brainstem and thalamus ($R^2 = 0.53$, $p = 0.001$), left primary sensory cortex ($R^2 = 0.63$, $p < 0.001$) and superior/medial temporal gyrus (STG/MTG) (Brodmann area 21 and 22) ($R^2 = 0.64$, $p < 0.001$) (Fig. 4).

4. Discussion

Our study is the first to demonstrate that single acute overnight administration of GC at physiological levels akin to acute life stress, (e.g., motor vehicle accident or emergency room visit), (Widmer et al., 2005) results in increased fasting hunger and decreased perfusion in regions of the brain which regulate reward-motivation (putamen/caudate), homeostasis (hypothalamus), taste and flavor perception/interoception (OFC/insula), emotion-memory (hippocampus), and executive function (ACC, DLPFC, OFC and VmPFC). GC-related increases in fasting hunger were predictive of an attenuated reduction in CBF in the OFC, brainstem, thalamus, primary sensory cortex and STG/MTG. Finally, hunger ratings positively correlated with insulin levels while the difference in hunger between GC and saline scan days was negatively correlated with the difference in free cortisol across both days.

Our data indicate that GC increased hunger with associated central effects (reduced CBF) that was not observed with saline infusion, supporting its role in altering appetite. The presence of glucocorticoid receptors (GR) and the cortisol-activating enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) throughout the brain indicates that GC serve important functions within the central nervous system. (Kilgour, 2015; Reul and Kloet, 1985) The density of GR and 11β-HSD1 is particularly high in areas of the cortico-limbic striatal system, although recent positron emission tomography imaging studies have demonstrated distribution throughout the brain. (Dallman, 2010; Kilgour, 2015; Mcewen et al., 1968; Harris et al., 2013; Bhatt et al., 2020; Bini,

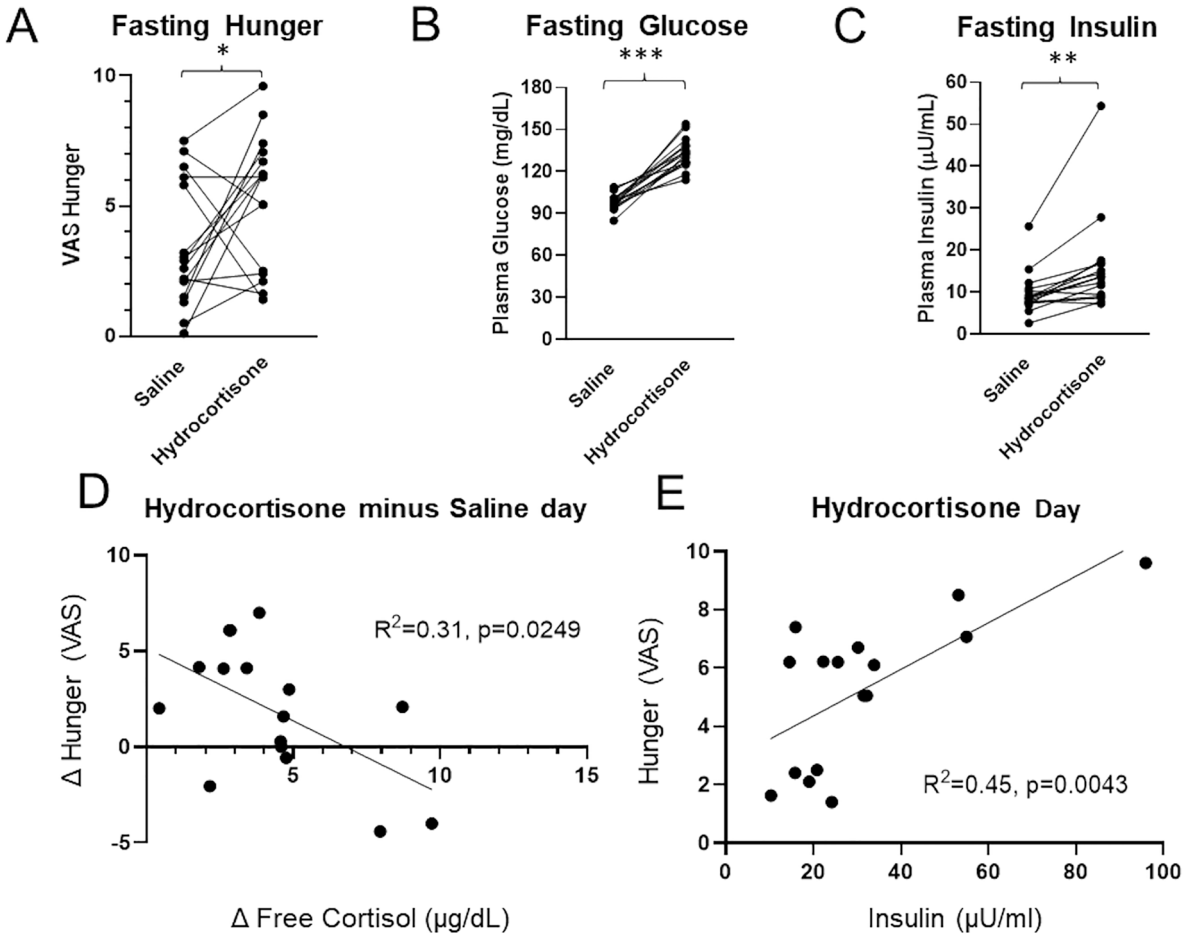

Fig. 2. Fasting hunger, glucose, and insulin and correlations with hunger. Fasting measurements (upon waking ~ 6:45am) on saline and hydrocortisone days of A) hunger (Visual Analog Scale (VAS): 0–10) and serum B) glucose and C) insulin. Pearson's correlations of D) changes in hunger and salivary (free) cortisol (hydrocortisone minus saline day) and E) hunger (VAS) and insulin on hydrocortisone infusion day. Mean ± SEM, *p < 0.05 **p < 0.01 ***p < 0.001.

Table 2
Average Fasting (7am on scan day) Hormone Levels on respective MRI scan days, except for ACTH which was only available from the 90 min post scan start blood sample. Mean ± SEM (n = 16) *p < 0.05.

	Saline	Hydrocortisone
Glucose (mg/dL)	97.9 ± 1.4	132.9 ± 2.7*
Insulin (µU/mL)	9.8 ± 1.3	16.1 ± 2.9*
GLP-1	8.5 ± 2.5	8.4 ± 2.4
Glucagon	61.8 ± 5.3	71.7 ± 5.1
NPY	16.1 ± 1.5	16.5 ± 1.9
Leptin (ng/mL)	7.2 ± 2.0	10.0 ± 2.2*
ACTH	90.1 ± 8.4	56.7 ± 8.1*

GLP-1: Glucagon-like peptide 1: NPY = Neuropeptide Y: ATCH = adrenocorticotropic hormone.

2020) The cortico-limbic striatal system is central to the regulation of emotion, memory, reward, and interacts with prefrontal regions involved in control of food intake. Notably, CBF in the OFC, which is a critical region for the reward value of food, taste, and flavor, was most significantly associated with GC-related hunger in the current study. (Sinha and Jastreboff, 2013; Rolls, 2016; Rolls, 2004; Rolls, 2005).

It has been hypothesized that in response to increasing stress, neural responses in self-control and regulatory prefrontal regions may be blunted, while signaling in motivation and limbic regions may be enhanced. (Sinha and Jastreboff, 2013) The current study showed reduced CBF in all regions from hydrocortisone relative to saline administration; however, reward regions such as the caudate and putamen were the least sensitive to changes in CBF compared to regions of executive function (ACC, DLPFC, and VmPFC). It remains to be seen if this acute stress paradigm similarly reflects changes caused by chronic stress, where a more prolonged elevation in cortisol may sustain the CBF changes seen in our study.

Few fMRI studies have investigated the effects of exogenous GCs in human (Montoya et al., 2014; Lovallo et al., 2010) and animal studies. (Endo et al., 1997; Yamazaki, 2021) Acute oral GC administration resulted in reduced BOLD fMRI activity in the striatum and amygdala during a monetary reward-inducing task, (Montoya et al., 2014) while intravenous administration of GC resulted in reduced activity in the hippocampus and amygdala, but no change in the thalamus. (Lovallo et al., 2010) In preclinical studies, 3-weeks of prednisolone administration to dogs reduced CBF in the thalamus and hippocampus; (Yamazaki, 2021) while 3-months of GC in rats reduced CBF in the hippocampus with concomitant histological damage in the CA1 and CA3 regions of the hippocampus. (Endo et al., 1997) The process of transition from acute GC reductions in CBF to possible histological damage of the hippocampus which has been seen in preclinical studies remains to be fully understood. Our findings are consistent with this previous work indicating that acute GC rises reduces blood flow in emotion/limbic, reward and executive control regions. However, individual variation in this reduced CBF response was inversely correlated with hunger. That is, those who did not show such dynamic responses to GC also reported greater hunger ratings. This suggests that blunted GC signaling centrally (reduced CBF) is associated with greater hunger ratings. It is possible that individuals who had such blunted reduction in CBF (hydrocortisone

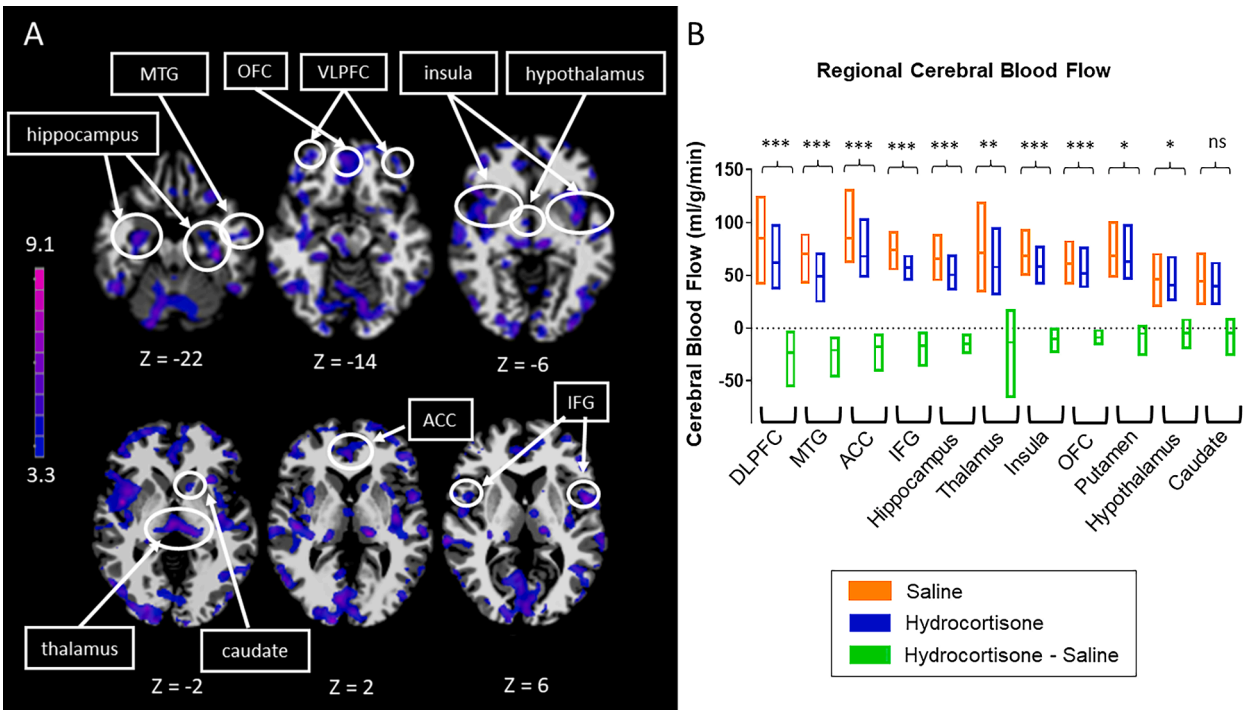

Fig. 3. Whole brain voxel-based contrasts and quantitative changes in regional cerebral blood flow (CBF) perfusion response of hydrocortisone vs saline days A) CBF hydrocortisone minus saline infusions p = 0.001 and cluster corrected at alpha = 0.05B) Mean (minimum and maximum) box plots for regional CBF values for saline (orange boxes) and Hydrocortisone (blue boxes) sessions in addition to hydrocortisone minus saline values (green boxes). *p < 0.05 **p < 0.01 ***p < 0.001 ACC: Anterior cingulate cortex; IFG: Inferior frontal gyrus; MTG: Medial temporal gyrus; OFC/VmPFC: Orbitofrontal cortex, extending into the ventromedial PFC; VLPFC: ventrolateral prefrontal cortex. Scale bar reflects reduction in CBF. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

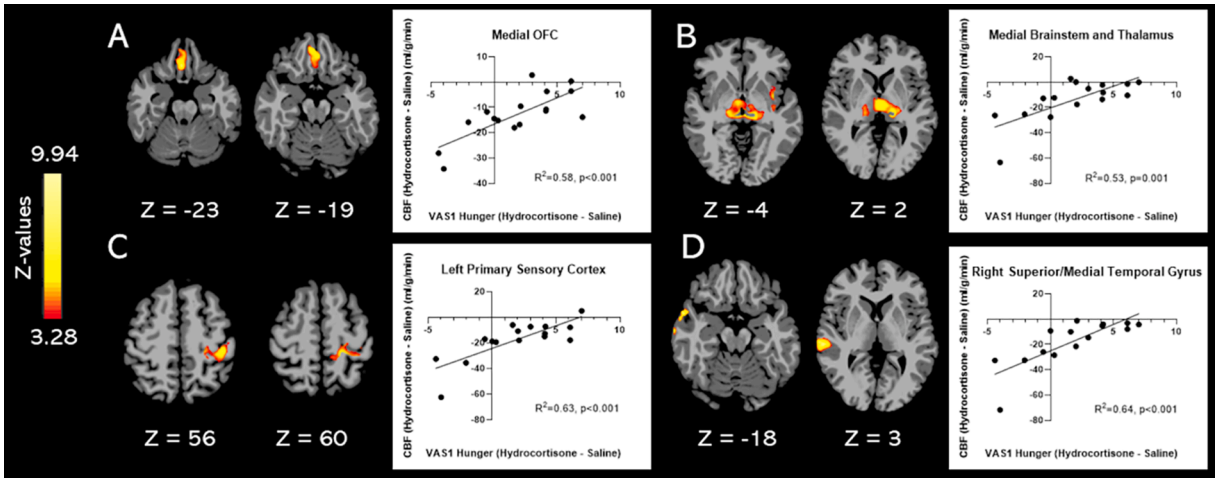

Fig. 4. Effect of session cerebral blood flow (CBF) (hydrocortisone minus saline, covarying for age, gender and fasting hunger, $p = 0.001$) and correlations with changes in fasting hunger A) orbitofrontal cortex (OFC) B) medial brainstem and thalamus C) left primary sensory cortex D) right superior and medial temporal gyrus (Brodmann area 21 and 22). MNI Z-coordinates are listed under each axial brain slice. Linear regressions correlating change in CBF (hydrocortisone minus saline day) versus change in fasting hunger (hydrocortisone minus saline day), significance set at p < 0.001, are displayed for each respective region A-D. VAS1 = Visual Analog Scale of Hunger.

minus saline) are 'stress habituators' with an increased allostatic load. (McEwen, 2007; Peters and Mcewen, 2015) Such individuals have a reduced central response to repeated stressors and could therefore be at risk for increased appetite, body weight gain and metabolic dysregulation – a process mediated by the prefrontal cortex. (Peters and Mcewen, 2015) It is possible that individuals with the least reduction of CBF in OFC and highest hunger (Fig. 4A) could be primed for further dysregulation of the stress response. Habituation or desensitization to repeated stress exposures (allostatic load) may also desensitize these individuals to food signals (reward, taste, flavor) that are integrated in the OFC and distributed to other brain regions (e.g., PFC, hypothalamus, amygdala, cingulate) causing increased appetite and possibly weight-gain and obesity.

In our current study, we found GC-related increases in fasting hunger as hypothesized, and such increases were predictive of less reduction in GC-related CBF (hydrocortisone minus saline) (Fig. 4) in the medial

OFC, medial brainstem and thalamus, left primary sensory cortex and STG/MTG. The thalamus participates in the integration pathway of taste, somatosensory and olfactory inputs of feeding and satiety signals (Rolls, 2005; Small, 2012) and the OFC has been implicated in the determination of reward salience and the flexible control of motivated behaviors such as eating. (Sinha and Jastreboff, 2013; Rolls, 2016; Rolls, 2004; Rolls, 2005; McEwen et al., 2016) Thus, reduction of CBF in the OFC and the thalamus may represent a homeostatic response to stress-level increments of peripheral GC that contributes to increased hunger and food intake. Food intake itself causes a postprandial rise in cortisol, (Stimson, et al., 2014) and it is possible that frequent food intake resulting from an increased appetite could exacerbate the risk of an acute adaptive mechanism transitioning into a long-term dysregulation of homeostatic integration. It remains to be seen whether these regional CBF changes associated with short-term (overnight) administration of exogenous GCs are similarly downregulated in subjects with long-term elevation of GCs or individuals with obesity.

Understanding the impact of GC on insulin is important to understanding the mechanisms driving appetite and weight gain. Insulin is an anorexogenic hormone in the CNS with effects in the hypothalamus, thalamus, insular cortex, anterior cingulate gyrus, and orbitofrontal cortex (Pliquett, 2006) – all regions that demonstrated reduced CBF during hydrocortisone infusion compared to saline in our study. The positive correlation of plasma insulin with hunger on hydrocortisone but not saline day (Fig. 2E) suggests that appetite-stimulating effects of acute stress-level GC may override the satiety signals of circulating plasma insulin levels, thereby driving hunger. Although one might expect change in free cortisol would be positively correlated with change in hunger, individuals with larger increases in hunger had smaller changes in salivary (free) cortisol (Fig. 2D), much like the smaller changes in CBF (Fig. 4). There was no relationship between change in insulin and change in hunger ($R^2 = 0.002$, data not shown), where one might expect a negative correlation given the anorexogenic nature of insulin. No correlations of plasma glucose with cortisol or hunger on both scan days were found. This suggests a discordance between insulin and cortisol signaling with respect to hunger and satiety. It is possible that the rise in GC may drive the rise in insulin, (Dallman et al., 2007) but the lack of CBF response could represent a lack of integration of these signals centrally which leads to greater hunger (Fig. 4). Future studies should explore the incremental role and interactions of both insulin and cortisol on CBF and hunger.

There are some limitations or differences within our study compared to others that deserve discussion. Our total hydrocortisone infusion is 4–12 times higher than previous studies (mean dose: 122 mg) making direct comparisons to previous studies difficult; however, our serum cortisol levels replicate physiological cortisol responses during stressful life events, such as hospitalization and invasive surgery, (Widmer et al., 2005) perhaps making our results more reflective of cerebral blood flow changes during physiologically acute stress. The current study has a relatively small sample size giving limited power to extrapolate these findings and therefore additional studies are necessary, including investigation of neural and hunger responses in individuals with obesity and those predisposed to developing obesity. Furthermore, given the current findings, it will be important to consider how chronic exogenous GC therapy (e.g., rheumatoid arthritis and chronic obstructive pulmonary disease) may impact hunger and weight gain, as well as examining additional hormones such as ghrelin, and PYY. Despite these limitations, our study demonstrates that in response to physiologically relevant stress-level steroids, individuals without obesity experience changes in desire to eat (hunger) and neural responses in brain regions which work in concert to regulate eating behavior. Finally, using exogenous GC as a stress probe, this study underscores the need to consider the body's response to stress as a critical contributor to mechanisms which alter eating behavior and may ultimately lead to weight gain.

Disclosure

AMJ serves as consultant for Novo Nordisk, Eli Lilly, Boehringer Ingelheim, Intellihealth, Scholar Rock, Pfizer, Rhythm Pharmaceuticals, and WW. All other authors have nothing to disclose.

CRediT author statement

Conceptualization (LP, RTC, RSS, RS, AMJ, DS, RBA, JJH), data curation (LP, CL, SS, SBR, KL, MH, RS, AMJ), formal analysis (JB, LP, CL, RSS, RS, MH, AMJ), writing – original draft writing the manuscript (JB, LP, CL, JJH, SS, SBR, DS, KL, MH, RBA, RTC, RSS, RS, AMJ), writing - reviewing & editing writing the manuscript (JB, LP, CL, JJH, SS, SBR, DS, KL, MH, RBA, RTC, RSS, RS, AMJ).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank the staff of the Yale Magnetic Resonance Research Center for their expert assistance. This work was supported by mentored training grants from the NIDDK (K01DK118005 (JB) and K23DK098286 (RBDA)), R01DK099039 (RS/AMJ) and R01DK117651 (RS/AMJ).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2022.103202.

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