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Interface Focus 2014 **4**, 20140020, published 22 August 2014

Supplementary data

["Data Supplement"](#)

</content/suppl/2014/08/19/rsfs.2014.0020.DC1.html>

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Cite this article: Aschbacher K, Rodriguez-Fernandez M, van Wietmarschen H, Tomiyama AJ, Jain S, Epel E, Doyle III FJ, van der Greef J. 2014 The hypothalamic–pituitary–adrenal–leptin axis and metabolic health: a systems approach to resilience, robustness and control. *Interface Focus* 4: 20140020.

<http://dx.doi.org/10.1098/rfs.2014.0020>

One contribution of 9 to a Theme Issue 'Towards a systems model of resilience'.

Subject Areas:

bioengineering, systems biology

Keywords:

psychological stress, obesity, metabolic syndrome, dynamic systems, stress-eating, robustness

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rfs.2014.0020> or via <http://rfs.royalsocietypublishing.org>.

The hypothalamic–pituitary–adrenal–leptin axis and metabolic health: a systems approach to resilience, robustness and control

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Glucocorticoids contribute to obesity and metabolic syndrome; however, the mechanisms are unclear, and prognostic measures are unavailable. A systems level understanding of the hypothalamic–pituitary–adrenal (HPA)–leptin axis may reveal novel insights. Eighteen obese premenopausal women provided blood samples every 10 min over 24 h, which were assayed for cortisol, adrenocorticotropic releasing hormone (ACTH) and leptin. A published personalized HPA systems model was extended to incorporate leptin, yielding three parameters: (i) cortisol inhibitory feedback signalling, (ii) ACTH–adrenal signalling, and (iii) leptin–cortisol antagonism. We investigated associations between these parameters and metabolic risk profiles: fat and lean body mass (LBM; using dual-energy X-ray absorptiometry), and insulin resistance. Decreased cortisol inhibitory feedback signalling was significantly associated with greater fat (kg ; $p = 0.01$) and insulin resistance ($p = 0.03$) but not LBM. Leptin significantly antagonized cortisol dynamics in eight women, who exhibited significantly lower 24 h mean leptin levels, LBM and higher ACTH–adrenal signalling nocturnally (all $p < 0.05$), compared with women without antagonism. Traditional neuroendocrine measures did not predict metabolic health, whereas a dynamic systems approach revealed that lower central inhibitory cortisol feedback signalling was significantly associated with greater metabolic risk. While exploratory, leptin–cortisol antagonism may reflect a 'neuroendocrine starvation' response.

1. Introduction

Psychological stress prospectively predicts weight gain and metabolic abnormalities among some individuals, whereas others are resilient to this risk [1,2]. The biological basis for this propensity is not well understood. Glucocorticoid hormones, which are produced by the hypothalamic–pituitary–adrenal (HPA) axis and are classic mediators of stress responses, have been linked with obesogenic changes in eating behaviours, body fat distribution and metabolism [3–5]. However, the clinical literature is troubled by inconsistent associations [6]; therefore, a novel approach may be needed. Long-term metabolic health requires a fine balance between energy input and output. Sophisticated brain–body feedback loops provide the control systems that maintain and restore this delicate balance following

perturbations. Specifically, the HPA, in concert with the adipose-derived hormone leptin, constitutes a key brain–body feedback loop, which enables the central nervous system to sense and regulate peripheral fat stores, energy homeostasis and feeding behaviours. Hence, we hypothesize that impaired HPA–leptin system control [7] may contribute to the development of metabolic risk, and test this in obese patients using an applied dynamic systems approach.

In patients with Cushing's syndrome, excess cortisol is produced, which contributes to rapid weight gain, visceral obesity and insulin resistance [8]. This phenomenon begs the question of whether *constant* high levels of cortisol are required, or, whether poorly controlled *dynamics* might be sufficient. Heightened HPA responses to acute stress have been associated with greater visceral adiposity in several human studies [9,10], suggesting that altered dynamics may be an independent risk factor. Further, in an animal model, high cortisol responses to adrenocorticotropin releasing hormone (ACTH) augmented risk for diet-induced obesity, owing to effects on muscle thermogenesis consistent with decreased energy expenditure [11]. However, the evidence linking various indices of cortisol and fat in humans is inconsistent overall [6], perhaps owing to the plethora of metrics (e.g. levels in the morning, evening, 24 h averages, diurnal slopes, etc.) [6]. Ultimately, the problem may be more profound than selecting the least meddlesome of these measures; it may require a paradigm shift to a systems level understanding, which could ultimately provide us a unifying framework for these indices.

Robustness theory, which emerged from the field of control systems engineering, provides the foundation for a new understanding of *stress-system resilience*. Robustness is defined as 'a property that allows a system to maintain its functions despite internal and external perturbations' [12,13]. The first step in quantifying robustness is to create a mathematical model that converts knowledge about a system's structure and mechanisms into equations that predict system behaviour. This is a particularly powerful framework because it explains how architectural features of the system (e.g. feedback loops) give rise to system function. Well-established methods, such as sensitivity analysis [14], can then be used to quantify robustness, by assessing how difficult it is for the modelled system to restore equilibrium when its components (e.g. parameters) are perturbed. There are important parallels between the concepts of robustness and *resilience*. For example, resilience has been variously defined as thriving in the face of adversity, or alternatively, the capacity to maintain homeostasis/allotaxis under adversity [15]. While allotaxis, defined as 'stability through change' [16], is conceptually similar to robustness, the corresponding index, 'allostatic load', aggregates biomarkers of resting state dysregulation across multiple systems (e.g. a weighted sum of normalized scores) [17,18]. By contrast, the proposed model predicts the dynamic responses of a particular system as a function of its components and their mechanistic and temporal interrelationships. Hence, the application of robustness theory to HPA–leptin dynamics in metabolic health is a fundamentally distinct and novel approach.

Control systems theory has established that 'negative feedback is the principal mode of control that enables robust response (or robust adaptations) to perturbations' [12]. A healthy metabolic profile, therefore, should be characterized by moderate negative feedback capacity, that is neither hyper- nor hyposensitive. The primary negative feedback mechanism of the HPA is generated by cortisol binding to

receptors in the hypothalamus and pituitary brain regions. Heretofore, the gold-standard method for assessing central glucocorticoid feedback signalling has been a pharmacologic challenge known as the dexamethasone suppression test (DST). Dexamethasone, a synthetic glucocorticoid, is administered in the evening before bedtime, cortisol levels are assessed the following morning and greater cortisol suppression is interpreted as an index of greater (more sensitive) feedback signalling [19]. Obesity appears to be associated with altered DST responses in some studies, but not consistently [9,20–22]. Potential measurement limitations of the DST include the fact that it differs from cortisol in terms of potency, receptor specificity and access to brain receptors [23,24].

Moreover, the DST does not permit differentiation between fast and slow feedback, which are likely regulated by different signalling mechanisms and brain receptors [25,26]. Hence, complementary methods may significantly enhance our understanding of the HPA's involvement in metabolic health. Several previous modelling approaches to the HPA have shown promise in this regard [27–29]. Whereas much research has focused on classical genomic glucocorticoid mechanisms, it is now becoming clear that rapid, non-transcriptional mechanisms may play a particularly important role in stress responses [30], negative feedback inhibition of the HPA, consolidation of aversive memories and food intake [26,31,32]. So-called rapid effects appear to be mediated via G protein-coupled receptors in hypothalamic neuroendocrine cells [26,33] and are primarily distinguished through temporal criteria—i.e. any effect occurring within 10 min precludes the time required for translocation, transcription and translation [34]. One recent study combining experimental cortisol infusion and neuroimaging techniques has confirmed that cortisol exerts rapid effects on neuronal activity; however, this technique does not yet provide an actual functional index of feedback signalling [30]. This study will provide the proof of concept for a systems-based index of rapid cortisol inhibitory feedback signalling.

Leptin, a hormone produced by adipose tissue in proportion to fat stores, provides an additional negative feedback signal to the brain to inhibit HPA activation [35,36] and decrease food intake [37]. Leptin has a dynamic circadian rhythm that is generally inverse to cortisol, suggesting counter-regulation or 'antagonism'¹ [36,38]. Leptin can inhibit glucocorticoid actions, both centrally in the paraventricular nucleus of the hypothalamus [35] and peripherally in the adrenal gland [39,40]. During caloric restriction, leptin levels decrease disproportionately relative to fat mass, which triggers counter-regulatory increases in cortisol, activating brain circuitry that drives motivation to eat [36]. Hence, the coordination of leptin and cortisol represents the intersection of neuroendocrine circuitry integrating the stress response and energy homeostasis. Moreover, weight loss-induced changes in key hormones (e.g. leptin and insulin), taken alone, do not consistently predict weight regain [41], suggesting a more complex approach may be needed. We hypothesized that insufficient leptin–cortisol antagonism may arise either from leptin resistance, or from intact leptin signalling in the presence of relatively low leptin levels.

This is the first study to investigate whether a clinically applied dynamic systems approach can reveal novel insights about the influence of HPA and leptin dynamics on a metabolic risk phenotype (high fat mass, low lean body mass (LBM) and insulin resistance) in a group of obese women. The primary goals of this study were to extend a

previously published applied HPA dynamic systems model [27] to incorporate leptin–HPA interactions and test associations with metabolic risk. We hypothesized that (i) decreased HPA control and robustness would be associated with greater metabolic risk and (ii) insufficient inhibition of cortisol by leptin (antagonism) would further exacerbate these effects. The systems-based approach is contrasted with more traditional measures, such as 24 h mean hormone levels and the diurnal cortisol slope, to establish what unique insights a systems approach provides.

2. Methods

2.1. Participants

Eighteen obese premenopausal women (mean BMI = 33, range: 30–41 kg m⁻²; mean age = 37.5, range: 22–51 years) participated in a rapid sampling study of circadian hormone cycles, which was approved by the Medical Ethics Committee of Leiden University [42]. Participants were recruited through local newspaper advertisements. Exclusion criteria included shift-work, recent trans-meridian flights, weight change of more than 5 kg in the three months preceding the study, depression (present or previous), use of oral contraceptives, irregular menstrual cycles, smoking, alcohol abuse or head trauma. Additionally, participants were required to be free of acute or chronic disease, as assessed by physician examination, medical history, standard clinical chemistry, haematology and urine testing. Blood was drawn during the early follicular phase of the menstrual cycle, as this cycle is known to affect cortisol levels and HPA responsiveness [43].

2.2. Blood draw procedure

The procedure has been described in detail in a previous publication [44]. Participants came into the Clinical Research Unit of the Department of Internal Medicine at 07.00, a cannula attached to a three-way stopcock was inserted, continuous 0.9% NaCl and heparin (1 U ml⁻¹) infusion was used to prevent obstruction, and blood samples were taken every 10 min for 24 h, starting and ending at 09.00. Cortisol and ACTH were assayed at every time point, whereas leptin was assayed every 20 min and the intervening time points were interpolated. No naps were allowed. Standardized meals were provided on a fixed schedule: breakfast at 09.30, lunch at 13.00 and dinner at 18.30. Participants were required to consume meals within a fixed time period, and alcohol and caffeine were prohibited. Lights were turned off at 23.00, and nocturnal blood draws were done with utmost care to avoid awakening the patient. Lights were turned on, and the patient was awakened at 07.30.

2.3. Hormone assays and metabolic measurements

Plasma ACTH was measured with an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The detection limit is 2 ng l⁻¹, and the intra-assay CV is between 2.8% and 7.5%. Plasma cortisol was measured with a radioimmunoassay (DiaSorin, Stillwater, MN). The detection limit is 25 nmol l⁻¹, and the intra-assay CV ranges between 2% and 4% [45]. Plasma leptin concentrations were determined by radioimmunoassay (Linco Research, St Charles, MO). The detection limit was 0.5 ng l⁻¹, and the interassay CV was 3.6–6.8%.

Fasting glucose levels were assessed in serum using an automated Modular P 800, and serum insulin was measured by immunoradiometric assay (Biosource Europe, Nivelles, Belgium). Insulin resistance was calculated using the homeostatic model assessment (HOMA) formula [46]. Fat mass and LBM were assessed in kilograms using dual-energy X-ray absorptiometry

(DEXA) scans. LBM was calculated as the sum of the lean mass of soft tissue and the total-body bone mineral. The fat percentage of the body is calculated as the fat mass of soft tissue divided by the DEXA weight, which is roughly equivalent to the sum of LBM and fat mass [47].

2.4. Model development

The model used herein is an extension of a previously published personalized dynamic systems model of the HPA axis [27]. We extended the model by adding leptin's influence on cortisol:

$$\frac{dC}{dt}(t) = \lambda_I - \lambda_C C(t) + \lambda_A A(t) - \lambda_L L(t),$$

where C denotes the cortisol concentration, A is the ACTH concentration and L is the leptin concentration. To determine the optimal time frame, a review of the literature was conducted, and analyses were performed assessing model fit (R^2) to the actual cortisol data using different lags in which leptin preceded cortisol by up to several hours. The R^2 was optimized by a lag of 0 min (i.e. no time lag). Parameter estimation was conducted for the original and extended model, and the fit of both models was compared using Akaike's information criterion [48].

The final model yielded four parameters per participant: (i) λ_I : external influences/the model intercept, (ii) λ_C : inhibitory cortisol feedback signalling (higher numbers indicate stronger HPA inhibition by cortisol), (iii) λ_A : ACTH–adrenal signalling (cortisol produced per unit ACTH), and (iv) λ_L : leptin–cortisol antagonism. We anticipated that greater metabolic risk would be associated with impaired HPA control, quantified as: higher ACTH–adrenal signalling, lower feedback signalling and insufficient leptin–cortisol antagonism (*either* leptin resistance indicated by a lack of antagonism *or* intact signalling in the presence of low leptin levels).

2.5. Parameter sensitivity analyses and robustness

Parameter sensitivity was conducted to analyse the robustness of cortisol dynamics to changes in model parameters (λ_I , λ_C , λ_A and λ_L) using the SensSB toolbox [49]. Higher sensitivity indices represent lower robustness and a smaller zone of high-performance system function. In this context, high-performance function indicates the capacity to maintain or restore cortisol to a desired (steady state) level after a perturbation that acutely alters cortisol secretion (e.g. stress). The relative sensitivity function for each of the parameters was computed as follows:

$$S_\lambda(t) = \frac{\lambda}{C(t)} \frac{\partial C(t)}{\partial \lambda}.$$

The average of the absolute value of S_λ evaluated at each sampling point was used as the sensitivity index (SI) [49].

2.6. Traditional metrics

In order to compare the dynamic systems parameters with more traditional metrics, we quantified means, slopes and cosinor analyses. Means were calculated as the average of each hormone level over a 24 h period. Consistent with our previous work [27], the diurnal cortisol slope was specified as the slope from 09.00 to 23.00, and the nocturnal slope was modelled from 01.00 to 09.00. Cortisol dynamics have been previously characterized using an indirect response model in which the cortisol secretion rate is a single cosine function [50]. This model yields three parameters: (i) mesor (average 24 h cortisol), (ii) amplitude (the height of the cortisol waveform), and (iii) acrophase (time at which the cortisol peak occurs). We approximated the cortisol awakening response (CAR) [51] by calculating it as the increase from 07.30 to 08.00, because the lights were turned on and patients awakened at 07.30.

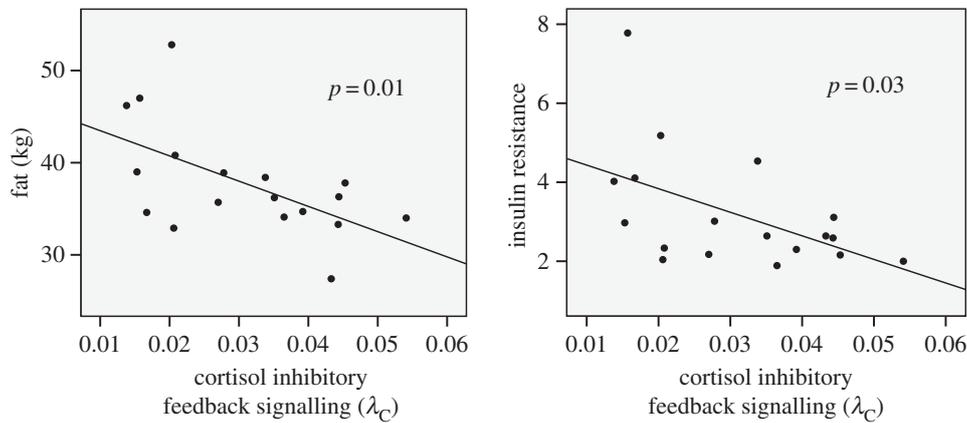


Figure 1. Lower central inhibitory cortisol feedback signalling (λ_C) is significantly associated with greater metabolic risk.

2.7. Statistical analyses

As previously stated, the applied dynamic systems model yielded four parameters per patient. Pearson correlations were used to determine the relations between all HPA–leptin indices (means, slopes, cosinor parameters and dynamic parameters) and metabolic risk markers, using a two-tailed critical alpha of 0.05. Prior to statistical analysis, all variables were visually inspected for normality.² As a point of interest, we noted that the distribution of feedback signalling (λ_C) was bimodal, which could indicate patient subgroups, conceivably arising from genetic or epigenetic factors.

3. Results

3.1. Traditional hypothalamic–pituitary–adrenal metrics and metabolic risk

As an important point of comparison, we explored whether standard metrics (24 h means and diurnal/nocturnal slopes) were associated with metabolic risk. No significant correlations were found between 24 h hormone levels of cortisol or ACTH, their diurnal/nocturnal linear slopes or our approximation of the CAR and the obese phenotype. Higher 24 h leptin levels were not significantly associated with fat mass or per cent in this sample; however, the effect size for fat mass was small to moderate and in the expected direction ($r = 0.36$, $p = 0.14$), and the range was restricted, because all participants were obese. The previously published cosinor model described in [50] was fitted to the cortisol data, but the fit was poor relative to the dynamic systems model presented here. Further, no significant associations between cosinor model parameters (mesor, amplitude, acrophase) and metabolic risk were found.

3.2. Hypothalamic–pituitary–adrenal dynamics and metabolic risk

We examined the associations between the parameters and indices of metabolic risk—fat mass, LBM and insulin resistance. Lower inhibitory cortisol feedback signalling (λ_C) was correlated with significantly greater fat mass ($r = -0.58$, $p = 0.01$), marginally greater fat per cent ($r = -0.45$, $p = 0.06$) and significantly higher insulin resistance ($r = -0.52$, $p = 0.03$), but was not associated with LBM ($r = -0.26$, $p = 0.30$; figure 1; electronic supplementary material, table S1). Because the bimodal distribution

of feedback signalling suggested that there might be two patient subgroups (high/low), we also conducted *t*-test comparisons using a median split variable for feedback signalling, which was also significantly related to fat mass, fat per cent and insulin resistance (all $ps < 0.05$). No associations were found with the other parameters. Further, the model parameters were not associated with traditional indices of cortisol, suggesting they capture a unique aspect of HPA control (electronic supplementary material, table S2).

To visualize how changes in feedback signalling impact model predictions of cortisol dynamics (assuming all other parameters are unchanged), we conducted an *in silico* test plotting predicted cortisol values for a representative participant with her actual λ_C value (moderate) against hypothetical λ_C values one standard deviation higher and lower (figure 2). (Also see the electronic supplementary material, figure S1 for visualization of λ_A .)

3.3. Parameter robustness and metabolic risk

To explore the hypothesis that metabolic risk and obesity are associated with decreased HPA system robustness, we applied parameter sensitivity analysis [49], yielding one SI for each HPA model parameter. Greater fat mass and insulin resistance were significantly associated with greater sensitivity (lower robustness) for all parameters (λ_I , λ_C , λ_L) except ACTH–adrenal signalling (λ_A ; $0.56 < rs < 0.71$, all $ps < 0.02$). Greater sensitivity of ACTH–adrenal signalling (λ_A) and leptin–cortisol antagonism (λ_L) was significantly associated with greater LBM ($0.57 < rs < 0.60$, all $ps < 0.02$). (Electronic supplementary material, figure S2 provides surface plots depicting robustness–performance relationships related to negative feedback parameters: λ_C , λ_L .)

3.4. Antagonism of leptin and cortisol dynamics

An inverse relationship between circadian variability of cortisol and leptin has been reported in a previous study of six healthy men [38]. However, we hypothesized that altered leptin levels or leptin resistance owing to obesity could result in a lack of antagonism. Hence, we explored antagonism for each patient using a regression-based difference equation that mirrors the differential equation, per previously established methods [27]. Hence, the regression predicts how leptin at time '*t*' predicts change in cortisol from time '*t*' to '*t* + 10 min', controlling for ACTH and cortisol at time '*t*'

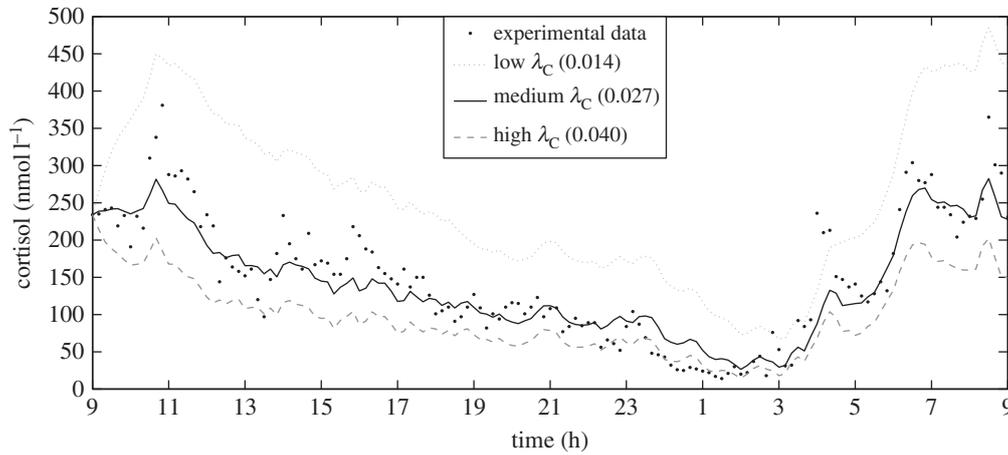


Figure 2. Simulating the impact of variations in central inhibitory cortisol feedback signalling (λ_C) on circadian cortisol patterns.

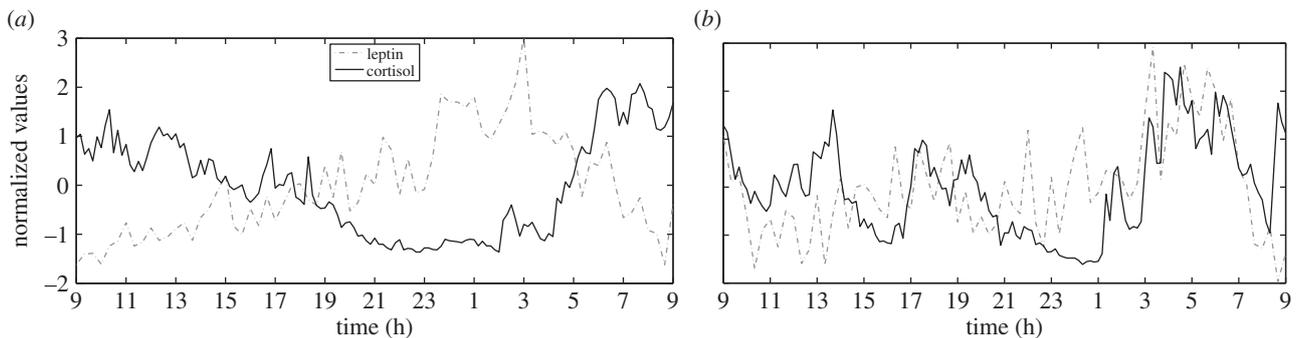


Figure 3. Actual cortisol and leptin circadian patterns in a patient with antagonism (a), compared with a patient with no antagonism (b). The black line depicts measured cortisol levels, while the dotted line depicts measured leptin levels.

and an intercept term (external influences/basal secretion). Interestingly, leptin was significantly inversely associated with the change in cortisol in only eight of the 18 patients, henceforth termed leptin–cortisol ‘antagonism’. In a ninth patient, there was a significant but positive leptin–cortisol association. The remaining nine patients had no significant relationship (‘uncorrelated’). Representative figures plotting the actual values of cortisol and leptin over time are provided for one patient exhibiting antagonism and another without antagonism (figure 3). We confirmed that the addition of leptin improved the fit of the dynamic systems model significantly per Akaike’s information criterion [48], but *only* among patients with antagonism, and had little or no impact on model fit among patients without association (electronic supplementary material, figure S3).

3.5. *Post hoc* investigations of leptin–cortisol antagonism

As *post hoc*, exploratory follow-up, we explored whether leptin–cortisol antagonism was consistent with either (i) a positive feature reflecting intact central leptin sensitivity or (ii) a negative feature reflecting relative leptin deficiency and a counter-regulatory ‘neuroendocrine starvation’ response [36]. We conducted uncorrected *post hoc t*-tests comparing the group with antagonism (ANT) with the group with no association (no-ANT) on the obese phenotype, average hormone levels and HPA parameters. ANT had significantly lower 24 h mean leptin levels ($p = 0.02$), no differences in 24 h mean cortisol ($p = 0.29$) and non-significantly lower 24 h mean

ACTH ($p = 0.10$; figure 4a). ANT had significantly lower LBM ($p = 0.02$; figure 4b), and no differences in fat mass, fat per cent or insulin resistance (all $ps = n.s.$). No differences were found in terms of 24 h HPA parameters, except, by definition, for leptin–cortisol antagonism (λ_L). However, we reasoned that food intake and sleep might result in differential system function at night versus during the day. Sleep is also the longest period of fasting in a typical day, and fasting is one hypothesized stimulus for changes in leptin–cortisol relationships. Previous work suggested that the ACTH–adrenal signalling (λ_A) may be most evident during the early-morning awakening response [27], which we previously tested by assessing diurnal and nocturnal parameters separately [27]. These *post hoc* analyses revealed that ANT had significantly higher nocturnal ACTH–adrenal signalling (λ_A) than no-ANT ($p = 0.03$; figure 4c), and no other significant parameter differences (all $ps = n.s.$, except, by definition, λ_L).

We considered whether a median split of λ_L might provide a better stratification method. Statistically, if the coefficient for λ_L is not significant, this indicates that the value for λ_L could also be zero, and hence interpretations on this basis could be unreliable. Therefore, we elected to split the sample based on the significance of λ_L . Nonetheless, a median split λ_L led to a very similar pattern of results in terms of LBM and leptin levels (only one participant was differentially classified than in ANT versus no-ANT). With a median split λ_L , greater antagonism was associated with borderline lower HOMA ($p = 0.06$) and lower mean ACTH ($p = 0.04$) but non-significant λ_A . Therefore, our final interpretation emphasizes the convergent findings from both approaches.

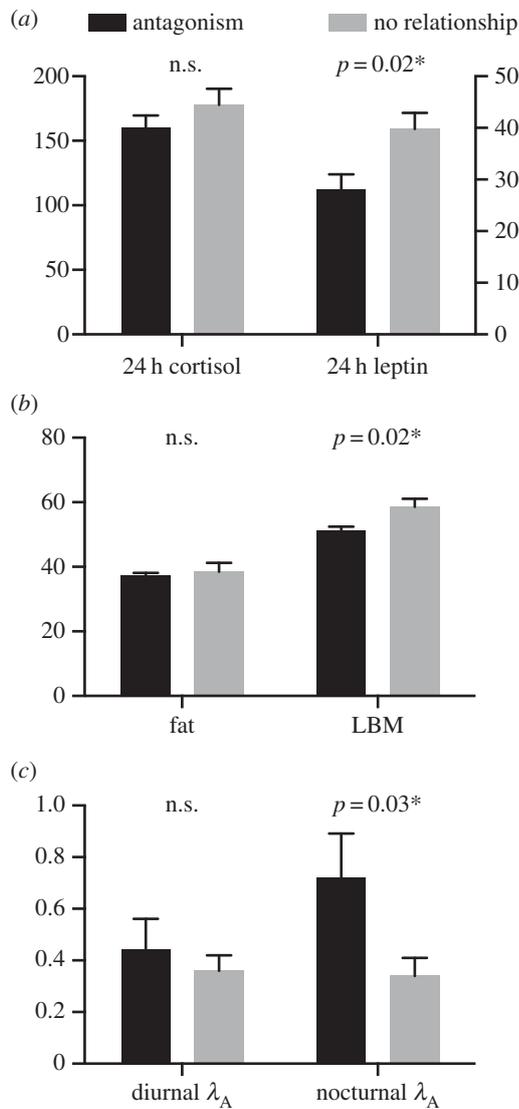


Figure 4. In *post hoc* exploratory analyses, women with leptin–cortisol antagonism significantly differed on: mean 24 h leptin levels (a), lean body mass (b) and nocturnal ACTH–adrenal signalling (c), compared with women with no antagonism. Units as follows: cortisol (nmol l^{-1}), leptin ($\mu\text{g l}^{-1}$), fat (kg), LBM (kg), λ_A (per the differential equation).

4. Discussion

Cortisol dysregulation, which can be brought about by chronic psychological stress [52], is a risk factor for weight gain, obesity and metabolic syndrome [27]. However, the field lacks a clear understanding of *how* and *for whom* psychological stress contributes to metabolic risk; hence, there are no clinically accepted biomarkers of vulnerability or resilience to guide treatment. The scientific literature investigating the link between cortisol with obesity and metabolic risk is fraught with inconsistencies [6], which may be improved through a systems understanding of the HPA–leptin axis. These data are consistent with the interpretation that impaired HPA feedback control in the central nervous system is associated with greater metabolic risk among obese premenopausal women. Further, these systems-based markers identify subgroups of patients with greater metabolic risk. Hence, these data invite the question of whether markers of HPA system control could help identify women at high risk for metabolic syndrome and weight gain, leading to novel approaches to prevention and treatment.

Cortisol provides a ‘brake’ (inhibitory feedback) for the HPA, which is critical to maintaining equilibrium in the face of adversity (robust HPA control). An exciting finding of this study was that lower inhibitory cortisol feedback signalling (i.e. a defective brake), quantified by dynamic systems analysis, was associated with greater fat and insulin resistance. Low inhibitory feedback signalling means that the HPA axis is less effectively shut off or dampened following a perturbation, which contributes to high cortisol levels. These data suggest that this mathematical model may provide a unique window into the central nervous system mechanisms underlying HPA feedback control. Future studies might investigate this possibility by looking at the correlation between this model parameter and other purported measures of feedback signalling, such as the DST or neuroimaging during cortisol injections [30]. However, the results of DST may differ from feedback regulation by endogenous cortisol owing to differences in potency, receptor binding affinity, specificity of rapid glucocorticoid receptor mechanisms, access to brain receptors and time scales of the underlying neural mechanisms [24,31,34]. Given this study’s time-sampling design, these inhibitory parameters may speak to ‘rapid feedback’ mechanisms, which play a key role in chronic stress-induced HPA plasticity [44,53,54] and its interaction with eating behaviour in relation to obesity and metabolism [35,54,55]. For future studies, multi-method approaches and challenge tests (acute psychological stress and pharmacological) may help triangulate upon a more specific and clinically feasible index.

Experimentally induced hypercortisolemia can contribute to metabolic syndrome [56]; however, hypercortisolemia is not generally found in obese humans [6]; hence, a gap exists between these experimental models and the naturalistic phenomenon. In animal models, elevated glucocorticoids promote gluconeogenesis, increasing vulnerability to hyperglycaemia provoked by stress or obesity [57]. Moreover, in humans, pharmacologically induced hypercortisolemia, in combination with bed rest, produced peripheral insulin insensitivity in otherwise healthy adults [56]. The current study reveals a significant association of higher insulin resistance with lower cortisol feedback signalling, but finds no association with other cortisol metrics including high 24 h levels, circadian or entropy measures. This suggests the possibility that HPA dynamics may be a unique marker of vulnerability to metabolic risk.

Cortisol is not the only brake on the HPA axis; the adipose-produced hormone leptin is reported to provide an important secondary source of inhibitory control [38]. In investigating the same-time relationship between leptin and cortisol dynamics, an intriguing pattern of ‘leptin–cortisol antagonism’ was revealed: leptin was inversely associated with cortisol in only eight of the 18 women. In those women, the addition of leptin significantly improved the ability to predict cortisol dynamics (model fit), whereas in the remainder of the sample, leptin neither enhanced nor detracted from fit. The fact that leptin–cortisol antagonism can become dissociated in obesity *in vivo* constitutes a novel contribution in view of previous literature [38]. One possibility worthy of future study is that leptin–cortisol antagonism may reflect endocannabinoid mediated effects of leptin on hypothalamic HPA control, a critical system for energy homeostasis that integrates stress and dietary inputs [35,58].

To determine, as a *post hoc* exploration, whether antagonism was associated with a differential metabolic risk profile, we compared the subgroup exhibiting leptin–cortisol

antagonism to the subgroup with no association. Women exhibiting antagonism had significantly lower 24 h leptin levels, lower LBM and greater nocturnal ACTH–adrenal signalling. In the whole sample, the average leptin levels reported in this study are similar to others previously reported in obese samples [59,60]. The presence of relatively low leptin levels in the antagonism subgroup suggests the possibility that this decrease in a negative feedback mechanism could be responsible for excess ACTH–adrenal signalling (i.e. as the brake is removed, the car speeds forward). Leptin has been shown to inhibit ACTH-induced cortisol production by the adrenal gland in two studies [39,40]. Formerly, obese individuals who achieve weight loss through behavioural strategies exhibit serum leptin levels that are lower than expected based on their body fat percentage [61,62]. These observations (low leptin and LBM combined with high cortisol reactivity) invite the possibility that leptin–cortisol antagonism could reflect the ‘neuroendocrine starvation’ response [36], activated, paradoxically, despite obesity. However, no behavioural data exist to explore whether the antagonistic subgroup recently lost weight, and fat mass did not differ. Moreover, it raises the possibility that a *lack* of antagonism might also reflect leptin resistance arising from higher leptin levels. It is unlikely that the antagonism subgroups are attributable to insulin resistance damping leptin [63], as the subgroups did not differ on insulin resistance. Given that antagonism related to LBM but not fat mass, it suggests the possibility of a preferential relationship to decreased activity levels and reduced energy expenditure [58,64], though this is highly speculative. Although these results are provocative, they are *post hoc*, uncorrected and based upon a small sample. We cannot establish whether antagonism was a trait or state characteristic with a single day of sampling.

Just as impaired glucose control is central to the disease process of diabetes, we hypothesized that impaired HPA–leptin system control may play a pathophysiological role in obesity. *High* overall cortisol *per se* may not as critical to understanding the influence of stress or cortisol on metabolic health as the capacity to regulate cortisol dynamics. Consistent with this hypothesis, these data demonstrate that decreased central inhibitory cortisol feedback signalling (a key regulator of HPA control) is associated with greater metabolic risk. Sensitivity analyses, a well-accepted tool in control systems engineering [49], show that low robustness of inhibitory feedback parameters in the model are associated with greater fat mass. Broadly, this suggests that women whose HPA systems have more difficulty regulating cortisol secretion in the face of challenges are at heightened risk for obesity. This conclusion is based on the hypothesis that the parameters for inhibitory feedback reflect hypothalamic neural mechanisms of rapid inhibition [54], which are known to exhibit plasticity in response to psychological stress [53,54]. Repeated and prolonged exposure to psychological stress can modulate the strength of rapid glucocorticoid inhibitory feedback governing HPA control [65]. Moreover, the plasticity of rapid feedback may influence the transition from an adaptive, acute stress response to a ‘toxic’ or chronic stress response [52,53]. Robustness provides a mathematical way to ask the question of this HPA model—how vulnerable is a given patient to lability of these inhibitory feedback processes? In principle, this approach illustrates how robustness provides a novel framework and toolbox to help understand stress-system resilience in relation to metabolism and obesity.

The clinical promise of these findings is limited by the high burden and cost of rapid sampling. HOMA, a measure of insulin resistance derived from simple fasting measures of insulin and glucose, began as a dynamic systems modelling approach that was eventually optimized through validation studies involving pharmacologic testing [66]. Hence, it may eventually be possible to apply a similar process with an HPA dynamic systems model in order to derive a low-burden index of central glucocorticoid resistance (or decreased feedback signalling). Key limitations of the study include the lack of a non-obese control group and the fact that all participants were women. Owing to the cross-sectional nature of the correlations, it is not clear whether poor HPA control causes metabolic dysregulation or vice versa. Further, the study lacked data on participants’ psychological status, dietary habits or activity levels, which will be an important direction for future research. For example, acute laboratory stress-induced cortisol responses can be integrated into future models [65], where acute psychological responses would influence the stress-input parameter [52,67]. Future models could also incorporate markers of local adipose glucocorticoid metabolism (e.g. 11 β -hydroxysteroid dehydrogenase activity or gene expression) [68] and explore the interactions of insulin with cortisol and/or leptin [63]. While the sample size was small in terms of participant number, given the majority of the variance in cortisol occurs within an individual over the course of a day [51], statistical power is substantially enhanced by the wealth of within-person data. Other mathematical models of the HPA have been proposed [29,50,65], some of which are more mechanistically detailed; however, many mechanisms of interest cannot be measured in clinical research with living humans. A primary advantage of this approach is that it can be more easily applied in clinical research because it provides patient-specific parameter values (i.e. the model is ‘personalized’ to each patient’s system).

The concepts of resilience and robustness are juxtaposed in this proof-of-concept study demonstrating that robust control of the HPA, a key system mediating response to psychological stress, is associated with better metabolic health. This study is founded in fundamental principles of control theory, which are well known in engineering and mathematics [12], but are little used clinically. This study provides a new tool, which, with further optimization, might be used to calibrate promising preventative treatments [69], targeting root psychosocial or environmental causes of HPA–leptin system imbalance. In addition, this type of personalized systems modelling approach may be a useful tool in clinical trials of pharmacological treatments targeting the HPA and cortisol metabolism [68]. Further, there are successful examples, such as the artificial pancreas, that have resulted from applying advanced control to biomedical problems (e.g. treatment of diabetes) [70,71]. Hence, one could envision a future in which a combination of behavioural, pharmacological and bioengineering strategies might be used to investigate whether restoring HPA balance will improve the prevention and treatment of metabolic disease and obesity.

Endnotes

¹Biological antagonism can, sometimes, refer to muscle groups that oppose one another, and our intended meaning is analogous in describing hormone levels that move inversely to one another. This term is *not* meant to imply that the two hormones competitively

bind to the same receptor, inhibiting one another's responses, as in pharmacological research.

²HOMA exhibited one high value with a z-score of 3.2 (3 is a standard cut-off for outliers). In order to avoid biasing the correlation coefficient, this point was winsorized to a value 2.5 standard deviations above the mean, which corresponds to a 95% confidence

interval (CI) and is consistent with an alpha of 0.05. The distribution of the winsorized HOMA variable did not significantly deviate from a normal distribution per the Kolmogorov–Smirnov test. We confirmed that the pattern of significance in the results reported was not affected by using the winsorized versus original HOMA.

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