Am J Physiol Regul Integr Comp Physiol 322: R144–R151, 2022.
First published December 22, 2021; doi:10.1152/ajpregu.00276.2021

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY

RESEARCH ARTICLE

Stress system dysfunction revealed by integrating reactivity of stress pathways to psychological stress in lean and overweight/obese men

Sisitha Udara Jayasinghe,[1,2] Sarah Janet Hall,[1] Susan Jane Torres,[1] and Anne Isabella Turner[1]

[1]*Institute for Physical Activity and Nutrition, Deakin University, Geelong, Victoria, Australia and* [2]*College of Health and Medicine, University of Tasmania, Launceston, Tasmania, Australia*

Abstract

Although the patterns of response within the sympathoadrenal medullary (SAM) system and hypothalamo-pituitary adrenal (HPA) axis are interesting and important in their own accord, the overall response to acute psychological stress involves reactivity of both pathways. We tested the hypothesis that consideration of the integrated response of these pathways may reveal dysregulation of the stress systems, which is not evident when considering either system alone. Age-matched lean and overweight/obese men were subjected to a Trier Social Stress Test and reactivity of the SAM system (salivary α-amylase, systolic blood pressure, diastolic blood pressure, and heart rate) and the HPA axis (salivary cortisol) were measured. Relative reactivity of SAM system and HPA axis was calculated as the ratio between the measures from each pathway. Although analysis of reactivity of individual stress pathways showed no evidence of dysfunction in overweight/obese compared with lean men, analysis of HPA/SAM reactivity revealed significantly lower cortisol over systolic blood pressure (CoSBP) and cortisol over diastolic blood pressure (CoDBP) reactivity in overweight/obese compared with lean men. Other measures of HPA/SAM reactivity and all measures of SAM/HPA reactivity were unaltered in overweight/obese compared with lean men. These findings suggest that the cortisol response per unit of blood pressure response is blunted in men with elevated adiposity. Furthermore, these findings support a notion of a coordinated overall approach to activation of the stress pathways with the degree of activation in one pathway being related to the degree of activation in the other.

blood pressure; cortisol; hypothalamo-pituitary adrenal axis; salivary α amylase; sympathoadrenal medullary system

INTRODUCTION

Reactivity of the sympathoadrenal medullary (SAM) and hypothalamo-pituitary adrenal (HPA) pathways to acute psychological stress that is high (exaggerated) and low (blunted) is related to a vast array of future adverse physical and mental health and disease outcomes, including adiposity measures and risk of obesity (1). Many studies have measured individual markers of SAM and HPA reactivity and found links to adverse health and disease outcomes at follow-up after one or more years (1). Commonly used measures of SAM reactivity are systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and concentrations of epinephrine, norepinephrine, and salivary α-amylase (sAA), whereas commonly used measures of HPA reactivity are salivary and plasma concentrations of cortisol. In a cross-sectional study in men, however, we found only limited evidence of links between body mass index (BMI) status (lean vs. overweight/obese) and reactivity of the stress pathways to psychological stress (Trier Social Stress Test; TSST). Although reactivity of SBP (measured by Finometer) differed between lean and overweight/obese men [blunted in overweight/obese men; Torres et al. (2)], there were no differences between groups in reactivity of DBP and HR [measured by Finometer; (2)], reactivity of HR (measured by electrocardiogram), salivary α-amylase, or salivary cortisol (3).

Evidence is now emerging that the pattern of SAM system response variables may be important in determining the link to health and disease outcomes (1). For example, a cluster analysis by Brindle et al. (4) in 55- to 60-yr-old males and females in the Dutch Famine Birth Cohort Study showed that a cluster with exaggerated blood pressure, but relatively small heart rate responses to acute psychological stress had the greatest risk of hypertension at 5.5-year follow-up. Furthermore, in 20- to 35-yr-old males and females in the CARDIA study, coronary artery calcification at 13-year follow-up was predicted by both exaggerated systolic blood pressure (SBP) reactivity and blunted heart rate (HR) reactivity at baseline (in blacks but not whites) (5). Interestingly, and importantly for the topic of this study, in 19-yr-old Norwegian males screened at military draft, both exaggerated norepinephrine and blunted epinephrine reactivity at baseline predicted higher waist circumference at 18-year

Correspondence: S. U. Jayasinghe (sisitha.jayasinghe@utas.edu.au).
Submitted 8 November 2021 / Revised 15 December 2021 / Accepted 21 December 2021

follow-up (6). Norepinephrine has a greater effect on peripheral vasoconstriction (via α-adrenergic receptors), whereas epinephrine has a greater effect on the heart (via β-adrenergic receptors) (7). Collectively, these three abovementioned studies (4–6) show a consistent pattern in which SAM system reactivity consisting of exaggerated peripheral vasoconstriction response (indicated by SBP and norepinephrine) and blunted cardiac response (indicated by HR and epinephrine) may confer the greatest risk of future adverse health and disease outcomes.Although the pattern of response within the SAM system is interesting and important, the overall response to acute psychological stress involves both the SAM system and the HPA axis. The studies described above have not included the role of the HPA axis in determining risk of future health and disease outcomes. There are bidirectional stimulatory connections between the SAM system control center in the brainstem and the HPA axis control center in the hypothalamus, such that activation of either one of these systems results in activation of the other (8). Indeed, there is thought to be an interaction between these stress pathways in response to acute psychological stress, whereby the magnitude of response of one pathway may be compensated for by the magnitude of response of the other pathway (9). Although many studies have focused on the role of either SAM system or HPA axis reactivity in predicting future health and disease outcomes (1), only two have considered both pathways within the same participants in the same study (10, 11). Neither study considered the interaction of these two pathways in response to stress. This appears to be a gap in this field to date, as the relative reactivity of these pathways may reveal more about the integrated response to psychological stress and its relationship to health and disease outcomes than testing each pathway alone.

There are different methods available for measuring the integrated response of these pathways (12). As an example, some work has considered the integrated response of the

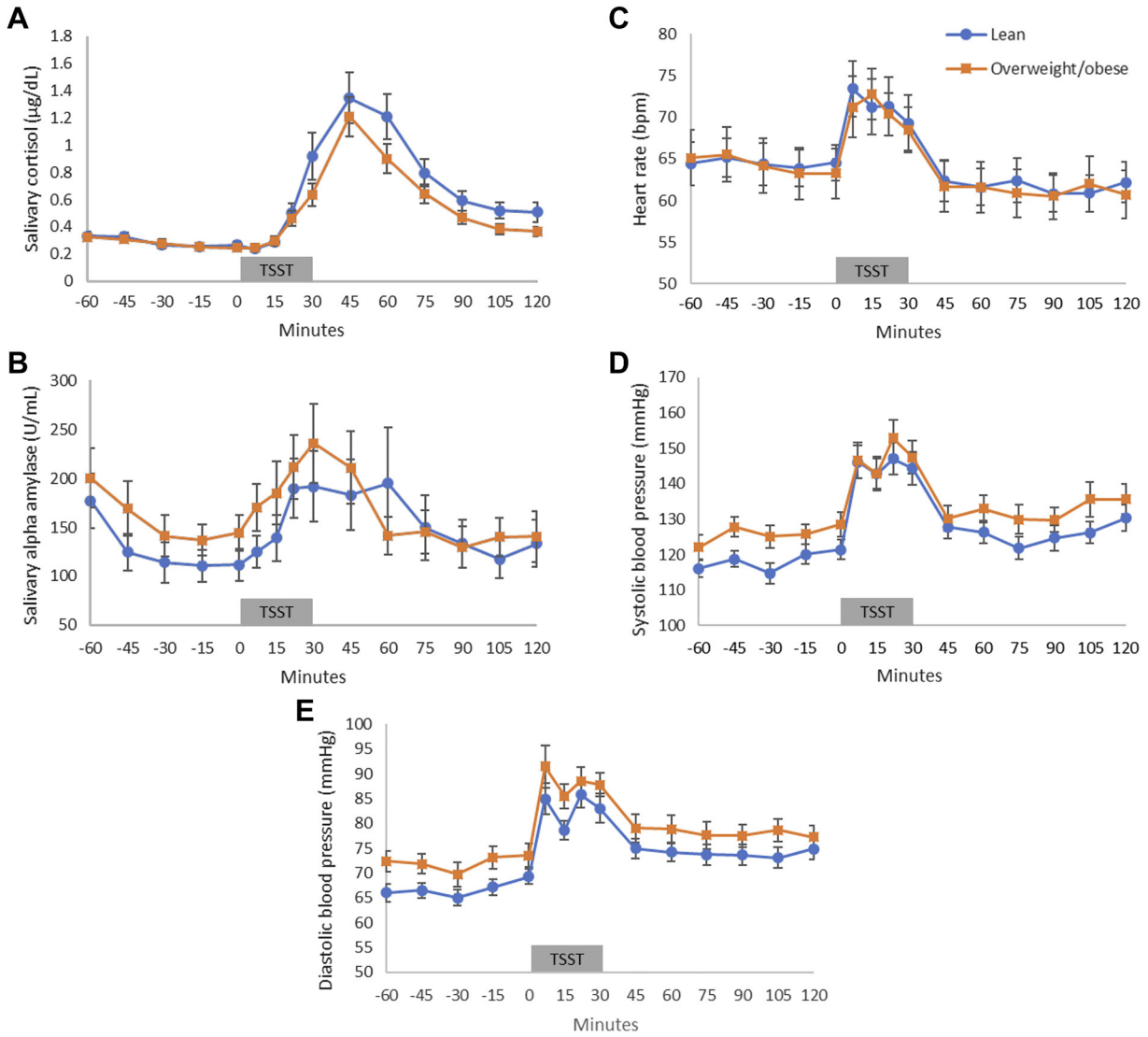

Figure 1. Means ± SE of (*A*) cortisol, (*B*) sAA, (*C*) heart rate, (*D*) systolic blood pressure, and (*E*) diastolic blood pressure in lean and overweight/obese men from 1400 h (−60 min) to 1700 h (120 min); TSST, Trier Social Stress Test; statistical method, repeated-measures analysis of variance, lean (n = 19 subjects); overweight/obese (n = 17 subjects); cortisol and sAA data are reproduced from endocrine connections from Jayasinghe et al. (25). sAA, salivary α-amylase.

SAM system and HPA axis by measuring the ratio of the response of these two pathways (13). In their study, Ali and Pruessner (13) found that self-reported levels of stress and anxiety and depressive systems were more strongly related to the ratio of sAA over cortisol (AoC) in response to stress than to the ratio of cortisol over sAA (CoA) or to either stress marker alone. In other words, AoC reactivity was a better indicator of stress pathway dysregulation than CoA reactivity or than sAA or cortisol reactivity alone.

In a further analysis from our earlier study in which we considered individual markers of SAM and HPA pathway reactivity (2, 3), the aim of this study is to consider the integrated reactivity of the SAM and HPA pathways in response to psychological stress in lean versus overweight/obese men. We hypothesize that consideration of the integrated response of these pathways may reveal dysregulation of the stress pathways, which is not evident when considering either pathway alone.

MATERIALS AND METHODS

Participants

A detailed description of the recruitment strategies and experimental procedures has been published elsewhere (3). Briefly, lean (BMI = 20–25 kg/m^2; n = 19) and overweight/obese (BMI = 27–35 kg/m^2; n = 17) men aged 50–70 yr, recruited from localities in Melbourne, Australia, participated in the study. Men were excluded if they had any prior diagnosis with Cushing's syndrome, any stress or anxiety disorder, depression, any diseases of the adrenal gland, type 2 diabetes, heart disease (including use of a pacemaker), high cholesterol, stroke, or cancer. Written informed consent was obtained from all participants before being enrolled in the study. All procedures were approved by the Human Research Ethics Committee of Deakin University (Project code: EC00213) and conformed to the guidelines of the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research 2007 (Updated 2018).

Table 1. *Pretreatment, peak height, reactivity, AUCi, and AUCg for cortisol, sAA, HR, SBP, and DBP in lean and overweight/obese men*

	Lean (n = 19)	Overweight/Obese (n = 17)	P Value*
Cortisol**			
Pretreatment, μg/dL	0.29 ± 0.02	0.28 ± 0.02	0.788
Peak height, μg/dL	1.52 ± 0.22	1.21 ± 0.15	0.254
Reactivity, μg/dL	1.23 ± 0.21	0.93 ± 0.15	0.263
Cortisol AUCi, μg/dL/min	55.3 ± 10.3	38.7 ± 7.7	0.118
Cortisol AUCg, μg/dL/min	107.3 ± 11.2	89.4 ± 7.6	0.204
sAA**			
Pretreatment, U/mL	112.1 ± 16.1	140.8 ± 16.5	0.224
Peak height, U/mL	267.3 ± 55.5	295.6 ± 41.2	0.690
Reactivity, U/mL	155.1 ± 51.2	154.9 ± 31.6	0.997
sAA AUCi, U/mL/min	5,221 ± 2,735	3,131 ± 1,525	0.523
sAA AUCg, U/mL/min	26,081 ± 4,206	29,310 ± 3,759	0.575
Pretreatment HR, beats/min	64 ± 2	64 ± 3	0.850
Peak height HR, beats/min	77 ± 4	76 ± 3	0.871
Reactivity HR, beats/min	13 ± 2	13 ± 2	0.986
HR AUCi, beats/min	32 ± 126	89 ± 88	0.720
HR AUCg, beats/min	11,600 ± 431	11,528 ± 523	0.916
Pretreatment SBP, mmHg	119 ± 3	127 ± 3	0.054
Peak height SBP, mmHg	154 ± 5	163 ± 5	0.220
Reactivity SBP, mmHg	36 ± 3	36 ± 4	0.877
SBP AUCi, mmHg/min	1,449 ± 183	1,148 ± 225	0.303
SBP AUCg, mmHg/min	22,827 ± 500	23,923 ± 572	0.157
Pretreatment DBP, mmHg	67 ± 1	72 ± 2	0.068
Peak height DBP, mmHg	94 ± 3	99 ± 3	0.318
Reactivity DBP, mmHg	27 ± 3	26 ± 3	0.904
DBP AUCi, mmHg/min	1,081 ± 117	1,043 ± 104	0.812
DBP AUCg, mmHg/min	13,166 ± 267	14,027 ± 384	0.070

Values are represented as means; n, number of subject ± SE. *Univariate analysis of variance, AUCi, area under the curve with respect to increase; AUCg, area under the curve with respect to ground; DBP, diastolic blood pressure; HR, heart rate; sAA, salivary α-amylase; SBP, systolic blood pressure; **cortisol and sAA data are reproduced with permission from endocrine connections from Jayasinghe et al. (3).

Experimental Procedure

The TSST is a well-characterized psychosocial stress protocol that includes a resting/preparation period, public speaking component, and a mental arithmetic exercise performed in sequence (14). A detailed description of the experimental procedure is published elsewhere (3). Briefly, lean, and overweight/obese men were subjected to prestress (1400 h–1500 h), stress (TSST, 1500 h–1530 h), and recovery (1530 h–1700 h) periods (Supplemental Fig. S1; see https://doi.org/10.5281/zenodo.5778084). Saliva samples were collected using Salivette sampling tubes (Sarstedt, Ingle Farm, SA, Australia) every 15 min during prestress and recovery periods. More frequent sample collection (1500, 1507, 1515, 1522, and 1530 h) was undertaken during the TSST to ascertain detailed profiling of how the stress parameters responded. Furthermore, to elicit maximum potency of the stressor, a relatively long prestress resting period (i.e., 60 min) was implemented and the TSST was imposed at 1500 h, during the diurnal cortisol nadir (15). Saliva samples were centrifuged at 3,000 rpm for 5 min at 4°C and then aliquots were stored at −80°C until assayed. Alongside each saliva sample, time-matched HR, SBP, and DBP measurements were also obtained, using a clinical blood pressure monitor (Criticare Systems, Inc., Waukesha, WI).

Hormone Assays

Saliva concentrations of cortisol and α-amylase were quantified using enzyme immuno and kinetic assays, respectively (Diagnostic Systems Laboratories, Webster, TX, and Salimetrics, Carlsbad, CA, respectively). For cortisol, 31 assays were conducted with a mean sensitivity of 0.035 μg/dL. The intra-assay coefficient of variation was 6.9% at 0.25 μg/dL and 8.2% at 2.0 μg/dL. The interassay coefficient of variation was 9.4% at 0.28 μg/dL and 7.7% at 1.8 μg/dL. For sAA, 36 assays were conducted with a mean sensitivity of 0.4 U/mL. The intra-assay coefficient of variation was 7.4% at 156.3 ± 4.1 U/mL. The interassay coefficient of variation was 7.4% at 20.7 U/mL and 7.0% at 257.3 U/mL.

Statistical Analysis

Preliminary analysis.

Pretreatment for cortisol was defined as the average of the five values from 1400 to 1500 h (1400, 1415, 1430, 1445, and 1500 h). Pretreatment sAA, HR, SBP, and DBP were defined as the average of the three values from 1430 to 1500 h (1430,

1445, and 1500 h). Peak height for all parameters was defined as the highest value that was obtained for each individual after the commencement of the stress. Reactivity was calculated by subtracting the pretreatment value from the peak height for all parameters. The area under the curve with respect to increase (AUCi) and with respect to ground (AUCg) was calculated using the trapezoid method for all parameters (16). Relative reactivity of SAM system and HPA axis for all parameters was calculated as the ratio between the SAM parameter of interest and the corresponding value of salivary cortisol concentration. The position of salivary cortisol concentration as the numerator or the denominator was changed depending on the ratio of interest (i.e., HPA/SAM or SAM/HPA).

Analysis.

Data were analyzed using the Statistical Package for the Social Sciences software version 26.0 for Windows (SPSS, Inc., Chicago, IL). Descriptive characteristics were compared between groups using univariate analysis of variance (ANOVA). Salivary cortisol, sAA, HR, SBP, and DBP were compared within and between groups using repeated-measures ANOVA. The within-subjects factor was time and the between-subjects factor was treatment. Similarly, all ratios (i.e., relative reactivity of the SAM system and HPA axis) were also compared using repeated-measures ANOVA. Derived parameters (pretreatment, peak height, reactivity, and AUC) for all variables were compared between groups using univariate ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Participants

The results from 19 lean and 17 overweight/obese men were included in the final analyses and there were no significant differences between groups in age (63.3 ± 1.1 vs. 61.1.0 ± 1.1 yr, respectively, $P = 0.166$). Overweight/obese men had ~30% higher body weight and BMI compared with lean men (93.8 ± 2.3 vs. 69.7 ± 1.6 kg and 30.6 ± 0.6 vs. 23.5 ± 0.3 kg/m^2, respectively, $P < 0.001$ for both). On average, overweight/obese men had 7.9% more body fat compared with lean men (28.1 ± 0.9 vs. 20.2 ± 1.1%, respectively, $P < 0.001$). Furthermore, compared with lean men, overweight/obese individuals had ~25%, 12%, and 11% larger waist circumferences (86.1 ± 1.5 vs. 106.9 ± 1.5 cm, $P < 0.001$), hip circumferences (97.5 ± 1.2 vs. 109.2 ± 1.3 cm, $P < 0.001$), and waist-to-hip ratios (0.88 ± 0.01 vs. 0.98 ± 0.01, $P < 0.001$).

Responses to TSST in Lean versus Overweight/Obese Men

This section considers whether adiposity influences SAM system and/or HPA axis reactivity in response to the TSST. Responses of cortisol, sAA, HR, SBP, and DBP to TSST in lean and overweight/obese men are shown in Fig. 1 and Table 1. In all instances, there was a significant effect of time ($P < 0.001$ for all) confirming the robustness of the stressor imposed. Both groups responded to the TSST with a substantial elevation in salivary cortisol (372%), sAA (123%), HR (22%), SBP (128%), and DBP (139%). Repeated-

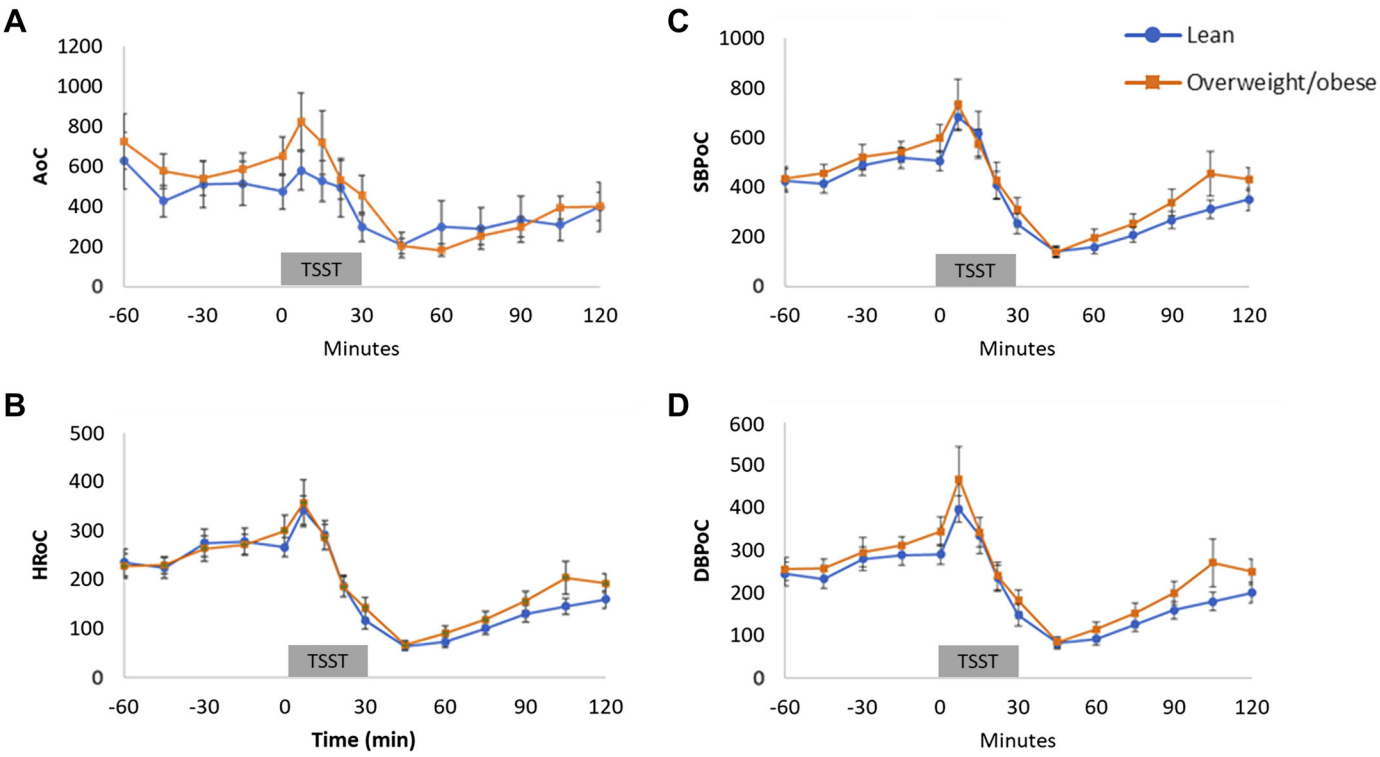

Figure 2. Ratios (means ± SE) of (*A*) amylase over cortisol (AoC), (*B*) heart rate over cortisol (HRoC), (*C*) systolic blood pressure over cortisol (SBPoC), and (*D*) diastolic blood pressure over cortisol (DBPoC) in lean and overweight/obese men from 1400 h (−60 min) to 1700 h (120 min); TSST, Trier Social Stress Test; statistical method, repeated-measures analysis of variance, lean ($n = 19$ subjects); overweight/obese ($n = 17$ subjects).

measures analysis of variance indicated that cortisol, sAA, HR, SBP, and DBP responses to TSST did not differ between lean and overweight/obese men (time × treatment $P = 0.187$, 0.288, 0.572, 0.990, and 0.999, respectively, Fig. 1, *A–E*). Furthermore, there were no overall differences between the groups for cortisol, sAA, and HR (between-subjects effect; $P = 0.210$, 0.332, and 0.196, respectively), although, SBP and DBP showed trends toward having an overall difference between the groups (between-subjects effect; $P = 0.063$ and 0.082, respectively). There were no differences between groups in pretreatment, peak height, reactivity, or area under the curve (Table 1), although there was a trend toward overweight/obese men having higher pretreatment SBP ($P = 0.054$), pretreatment DBP ($P = 0.068$), and DBP AUCg ($P = 0.070$) compared with lean men (Table 1).

SAM over HPA Ratio in Response to TSST in Lean versus Overweight/Obese Men

This section considers whether adiposity influences reactivity of the SAM system relative to reactivity of the HPA axis in response to the TSST. Ratios of SAM measures (sAA, HR, SBP, and DBP) over our HPA measure (cortisol) in lean and overweight/obese men are shown in Fig. 2, *A–D*. Repeated-measures analysis of variance revealed that there was a significant effect of time ($P < 0.001$ for all; Fig. 2, *A–D*). Nevertheless, AoC, heart rate over cortisol (HRoC), systolic blood pressure over cortisol (SBPoC), and diastolic blood pressure over cortisol (DBPoC) in response to the TSST did not statistically differ between lean and overweight/obese men (time × treatment, $P = 0.247$, 0.912, 0.882, and 0.910, respectively, Fig. 2, *A–D*). Furthermore, there was also no significant between-subjects effect, indicating that there were no significant overall differences between the groups (treatment effect, $P = 0.540$, 0.506, 0.358, and 0.243, respectively, Fig. 2, *A–D*). Accordingly, there were no significant differences between groups in pretreatment, peak height, reactivity, AUCi, or AUCg for AoC, HRoC, SBPoC, and DBPoC ($P > 0.1$ for all; data not shown).

HPA over SAM Ratio in Response to TSST in Lean versus Overweight/Obese Men

This section considers whether adiposity influences reactivity of the HPA axis relative to reactivity of the SAM system in response to the TSST. Ratios of our HPA axis measure (cortisol) over our SAM system measures (sAA, HR, SBP, and DBP) in lean and overweight/obese men are shown in Fig. 3, *A–D*, and Table 2. Repeated measures analysis of variance revealed that there was no significant effect of time for CoA ($P = 0.168$; Fig. 3*A*). However, significant effects of time were evident for cortisol over heart rate (CoHR), cortisol over systolic blood pressure (CoSBP), and cortisol over diastolic blood pressure (CoDBP) ($P < 0.001$ for all; Fig. 3, *B–D*). CoA and CoHR in response to the TSST did not statistically differ between lean and overweight/obese men (time × treatment, $P = 0.457$ and 0.365, respectively; Fig. 3, *A* and *B*). Significant time × treatment effects were evident for CoSBP and CoDBP ($P = 0.018$ and 0.022, respectively; Fig. 3, *C* and *D*, respectively) demonstrating a differential response pattern (lean > overweight/obese) in response to TSST when the activity of HPA axis (cortisol) is considered

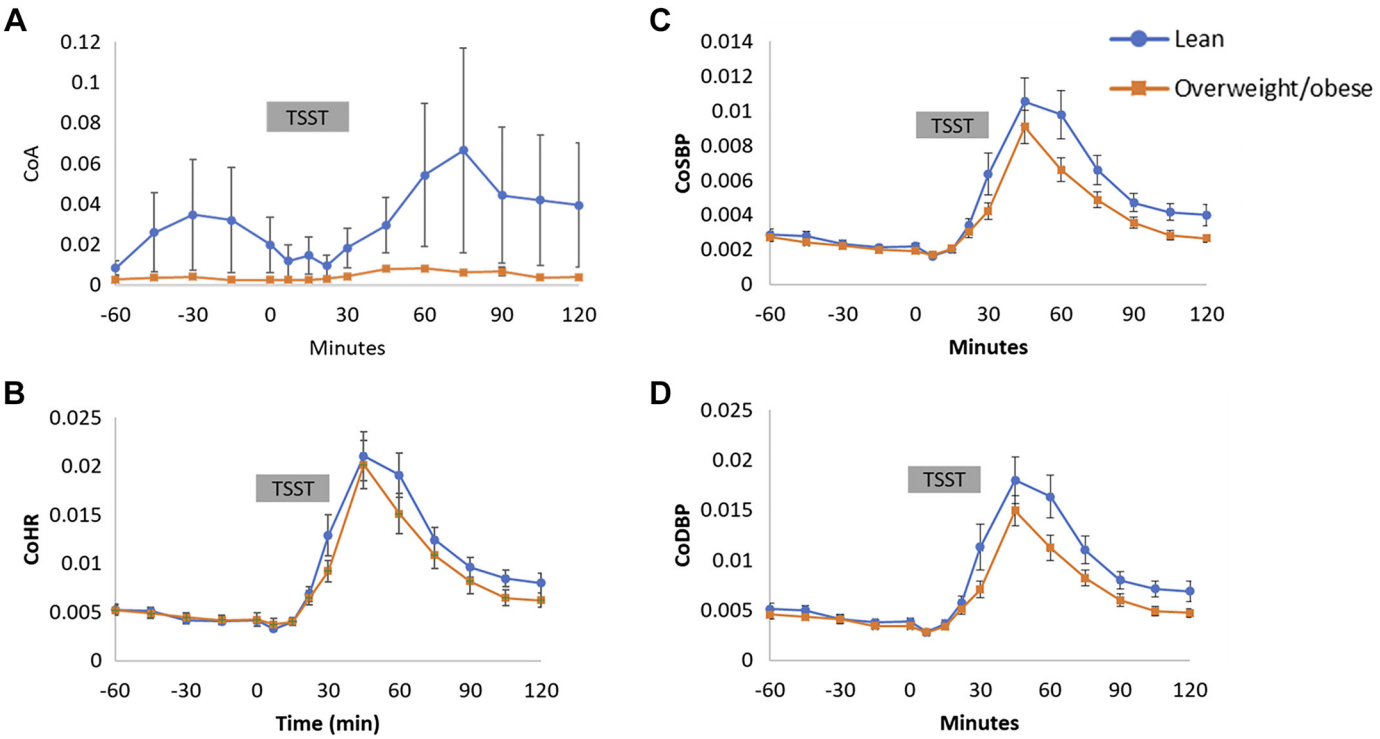

Figure 3. Ratios (means ± SE) of (*A*) cortisol over sAA (CoA), (*B*) cortisol over heart rate (CoHR), (*C*) cortisol over systolic blood pressure (CoSBP), and (*D*) cortisol over diastolic blood pressure (CoDBP) in lean and overweight/obese men from 1400 h (−60 min) to 1700 h (120 min); TSST, Trier Social Stress Test; statistical method, repeated-measures analysis of variance, lean ($n = 19$ subjects); overweight/obese ($n = 17$ subjects). Significant time × treatment effects were evident for CoSBP and CoDBP ($P = 0.018$ and 0.022, respectively; *C* and *D*, respectively).

relative to blood pressure activity (SBP and DBP). There were no significant between-subject effects for CoA and CoHR indicating that there were no significant overall differences between the groups (P = 0.241 and 0.346, respectively). However, there was a trend toward a between-subject effect for CoSBP and CoDBP (P = 0.084 and 0.066, respectively; Fig. 3, *C* and *D*, respectively).

No statistical differences between groups were found in pretreatment, peak height, reactivity, AUCi, or AUCg for CoA, CoHR, CoSBP, or CoDBP (Table 2), although there was a trend toward a difference for AUCi for CoHR (P = 0.057, Table 2), and for AUCg for CoSBP and CoDBP (P = 0.076 and 0.070, respectively, Table 2).

DISCUSSION

This study investigated the integrated reactivity of the SAM system and HPA axis in response to psychological stress in lean versus overweight/obese men. Our results support our hypothesis that consideration of the integrated response of these pathways may reveal dysregulation of the stress systems not seen when each pathway is studied alone. When each pathway was initially considered in isolation, both groups responded to the TSST with a substantial elevation in salivary cortisol, sAA, HR, SBP, and DBP. Nevertheless, these responses did not differ significantly between the groups (time × treatment; P = 0.187, 0.288, 0.572, 0.990, and 0.999, respectively, Fig. 1, *A*–*E*) providing confirmation for the limited potential of the siloed approach to stress pathway analysis, traditionally implemented in psychoneuroendocrinology research. Although consideration of SAM over HPA reactivity provided no further insight, analysis of HPA over SAM reactivity proved valuable in revealing significant stress system dysfunction not previously identified.

Table 2. *Pretreatment, peak height, reactivity, AUCi, and AUCg for CoA, CoHR, CoSBP, and CoDBP in lean and overweight/obese men*

	Lean (n = 19)	Overweight/Obese (n = 17)	P Value*
Pretreatment CoA	0.0245 ± 0.0177	0.0028 ± 0.0006	0.257
Peak height CoA	0.0242 ± 0.0118	0.0050 ± 0.0007	0.135
Reactivity CoA	0.0575 ± 0.0297	0.0108 ± 0.0029	0.149
CoA AUCi	0.0306 ± 0.0149	0.0318 ± 0.0244	0.966
CoA AUCg	0.0250 ± 0.0153	0.0039 ± 0.0005	0.202
Pretreatment CoHR	0.0045 ± 0.0003	0.0046 ± 0.0005	0.851
Peak height CoHR	0.0188 ± 0.0021	0.0157 ± 0.0017	0.267
Reactivity CoHR	0.1047 ± 0.0357	0.0670 ± 0.0331	0.448
CoHR AUCi	−0.2307 ± 0.2599	2.7456 ± 1.5702	0.057
CoHR AUCg	0.0091 ± 0.0008	0.0080 ± 0.0008	0.321
Pretreatment CoSBP	0.0025 ± 0.0002	0.0022 ± 0.0002	0.415
Peak height CoSBP	0.0099 ± 0.0014	0.0074 ± 0.0008	0.138
Reactivity CoSBP	0.0374 ± 0.0068	0.0301 ± 0.0049	0.401
CoSBP AUCi	0.0769 ± 0.1133	0.2296 ± 0.1429	0.404
CoSBP AUCg	0.0047 ± 0.0005	0.0037 ± 0.0003	0.076
Pretreatment CoDBP	0.0043 ± 0.0003	0.0040 ± 0.0003	0.409
Peak height CoDBP	0.0159 ± 0.0021	0.0123 ± 0.0014	0.187
Reactivity CoDBP	0.0484 ± 0.0087	0.0445 ± 0.0087	0.758
CoDBP AUCi	0.1541 ± 0.0378	0.1320 ± 0.0363	0.677
CoDBP AUCg	0.0081 ± 0.0008	0.0063 ± 0.0005	0.070

Values are represented as means ± SE. *Univariate analysis of variance; AUCg, area under the curve with respect to ground; AUCi, area under the curve with respect to increase; CoDBP, cortisol over diastolic blood pressure; CoHR, cortisol over heart rate; CoA, cortisol over sAA; CoSBP, cortisol over systolic blood pressure; sAA, salivary α-amylase.

Indeed, HPA/SAM ratio revealed some very interesting findings. Specifically, significant time × treatment effects were evident for CoSBP and CoDBP (P = 0.018 and 0.022, respectively; Fig. 3, *C* and *D*, respectively) suggesting a differential response pattern (lean > overweight/obese) in response to TSST. These findings suggest that, per unit of SBP and DBP response, cortisol response was blunted in overweight/obese men compared with lean men. Nevertheless, it is important to note that glucocorticoid synthesis is a complex process and that multiple other sources (de novo synthesis in extra-adrenal tissue or through activation of cortisone) could be contributing to this observed cortisol reactivity pattern (17). Furthermore, the emergence of significant findings, when considering the interaction of stress pathway reactivity (compared with analyzing each pathway alone), supports the notion that the body's overall response to stress may involve coordinated activation of the available pathways whereby the magnitude of activation of one pathway is related to (or compensated for by) the magnitude of activation of one or more other pathways (9). Interestingly though, our findings in relation to HPA/SAM reactivity were specific to cortisol in relation to blood pressure since significant findings were not seen for cortisol in relation to heart rate or sAA. Further research would be valuable to confirm these findings and determine the biological relevance of a relationship between overweight/obesity and the interaction between HPA axis and SAM system reactivity in response to psychological stress.

The SAM/HPA ratio on the other hand did not suggest an asymmetry or dysregulation in stress responsiveness between lean and overweight/obese men. This contrasts with previous findings in stress response patterns observed in individuals that were exposed to chronic stress via early life adversity (13) and posttraumatic stress (18). The findings of these earlier studies suggested that SAM/HPA is a better marker of stress pathway dysregulation than either system alone. The different characteristics of the cohorts considered in these earlier studies and the current cohort (i.e., chronically stressed individuals vs. men with different levels of adiposity with no chronic stress) are one possible explanation for the divergent pattern of results. Nonetheless, it is not prudent (rather it is premature) to definitively conclude whether SAM/HPA or HPA/SAM should be a preferred method of evaluating reciprocity between the main stress pathways, as more research is required to investigate the prevalence and biological meaning of such findings.

There is a growing body of evidence pertaining to the implementation of the "ratio method" to analyze time-dependent physiological system interaction in neuroendocrine research (12, 13, 19–22). From a historical perspective, it is apparent that there are two main methodologies used to perform this analysis: *1*) repeated computation of ratios for each time point of measurement; and *2*) calculation of a composite ratio score using AUC for time points of interest. The latter strategy may be particularly useful because the HPA axis has a temporal lag (compared with SAM system) in its reaction to the TSST. We implemented both strategies in this study to investigate

relative activity of the HPA axis and SAM system. It is noteworthy that the first method revealed significant outcomes (significant time × treatment effects were found for CoSBP and CoDBP; $P = 0.018$ and 0.022, respectively; Fig. 3, *C* and *D*, respectively), whereas the second method revealed trends only (AUCi for CoHR, $P = 0.057$ and AUCg for CoSBP and CoDBP, $P = 0.076$ and 0.070, respectively; Table 2). Consequently, there appears to be merit in using both approaches while this field is still in the development phase.

It must be noted that the statistical implications of compounding two biological measures into a single value have not yet been sufficiently explored. Neither has the complexity of interpretation of hormone ratios been successfully navigated to date. In endocrine research, for instance, there is no biological imperative for the validity of the choice of hormone (or any biological/physiological measurement) assignment to the numerator and the denominator of a ratio (12). The choice of numerator and denominator in a ratio can have profound effects on the interpretation of the outcomes (12). As such, we analyzed both inherent forms of the HPA axis-SAM system quotient (i.e., SAM/HPA and HPA/SAM ratios) to obtain a holistic view of the interaction of these mutually dependent stress pathways. This strategy enabled the examination of the response of each pathway after controlling for the variation of their counterpart. However, we acknowledge that there can be some mathematical limitations associated with this form of ratio analysis. For instance, previous research indicated that standardization of the numerator variable for variation in the denominator is only fully successful when both variables of interest are proportional to one another (23).

This study had strengths and limitations. A strength of this study is the robust nature of the underlying data set, which included sufficient sampling times to capture the profile of response for each variable and sufficient lead-in time before the start of sampling to ensure familiarity of participants with the procedures used. As indicated above, limitations include the mathematical and statistical complexities associated with the use of ratios. It is also possible that additional measures of subjective/emotional responses to TSST may aid in obtaining a holistic understanding of the interaction between stress pathway activity. Since obesity is not a unitary phenomenon, more direct indices of physiological obesity such as blood levels of various hormones associated with obesity characteristics (e.g., lipids, insulin, and leptin) could also be measured and scrutinized in future ratio analyses. Given the invasiveness of blood sampling and its potential impact on both SAM system and HPA axis reactivity, we did not collect blood samples from the current cohort. As such, blood measures such as adrenocorticotropic hormone (ACTH) were not considered in this investigation. Because of the reported differences in reactivity patterns of the stress systems between sexes in response to external stimuli (24), we limited our study to male participants only, which limits the generalizability of our findings.

Conclusions

Although analysis of reactivity of individual stress pathways showed no evidence of dysfunction in overweight/obese compared with lean men, analysis of HPA/SAM reactivity revealed significantly lower CoSBP and CoDBP reactivity in overweight/obese men. Other measures of HPA/SAM reactivity (CoA and CoHR) and all measures of SAM/HPA reactivity (AoC, HRoC, SBPoC, and DBPoC) were unaltered in overweight/obese compared with lean men. These findings suggest that the cortisol response per unit of blood pressure response is blunted in men with elevated adiposity.

Perspectives and Significance

These findings support the notion of a coordinated overall approach to activation of the stress pathways with the degree of activation in one pathway being related to the degree of activation of another. Consequently, it is important for researchers to measure multiple stress systems in stress reactivity research and to consider the integrated response, as there is increasing evidence that a siloed approach may lead to missed information. Nevertheless, further research is required to successfully circumvent some of the inherent statistical and interpretational complexities of the "ratio method."

SUPPLEMENTAL DATA

Supplemental Fig. S1: https://doi.org/10.5281/zenodo.5778084.

ACKNOWLEDGMENTS

The authors thank all the participants for being involved in this research.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.U.J., S.J.T., and A.I.T. conceived and designed research; S.U.J. performed experiments; S.U.J. analyzed data; S.U.J., S.J.H., S.J.T., and A.I.T. interpreted results of experiments; S.U.J. prepared figures; S.U.J. drafted manuscript; S.U.J., S.J.H., S.J.T., and A.I.T. edited and revised manuscript; S.U.J., S.J.H., S.J.T., and A.I.T. approved final version of manuscript.

REFERENCES

1. **Turner AI**, **Smyth N**, **Hall SJ**, **Torres SJ**, **Hussein M**, **Jayasinghe SU**, **Ball K**, **Clow AJ.** Psychological stress reactivity and future health and disease outcomes: a systematic review of prospective evidence. *Psychoneuroendocrinology* 114: 104599, 2020. doi:10.1016/j.psyneuen.2020.104599.
2. **Torres SJ**, **Turner AI**, **Jayasinghe SU**, **Reynolds J**, **Nowson CA.** The effect of overweight/obesity on cardiovascular responses to acute psychological stress in men aged 50-70 years. *Obes Facts* 7: 339–350, 2014. doi:10.1159/000369854.
3. **Jayasinghe SU**, **Torres SJ**, **Nowson CA**, **Tilbrook AJ**, **Turner AI.** Physiological responses to psychological stress: importance of adiposity in men aged 50–70 years. *Endocr Connect* 3: 110–119, 2014. doi:10.1530/EC-14-0042.
4. **Brindle RC**, **Ginty AT**, **Jones A**, **Phillips AC**, **Roseboom TJ**, **Carroll D**, **Painter RC**, **de Rooij SR.** Cardiovasular reactivity patterns and pathways to hypertension: a multivariate cluster analysis. *J Hum Hypertens* 30: 755–760, 2016. doi:10.1038/jhh.2016.35.
5. **Matthews KA**, **Zhu S**, **Tucker DC**, **Whooley MA.** Blood pressure reactivity to psychological stress and coronary calcification in the

Coronary Artery Risk Development in Young Adults Study. *Hypertension* 47: 391–395, 2006. doi:10.1161/01.HYP.0000200713.44895.38.

6. **Flaa A**, **Sandvik L**, **Kjeldsen SE**, **Eide IK**, **Rostrup M.** Does sympathoadrenal activity predict changes in body fat? An 18-y follow-up study. *Am J Clin Nutr* 87: 1596–1601, 2008. doi:10.1093/ajcn/87.6.1596.
7. **Brunton LLe**, **Knollmann BCe**, **Hilal-Dandan R.** *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, (13th ed.). New York, N.Y.: McGraw-Hill Education LLC, 2018. doi:10.1036/0071443436.
8. **Chrousos GP.** Stress and disorders of the stress system. *Nat Rev Endocrinol* 5: 374–381, 2009. doi:10.1038/nrendo.2009.106.
9. **Andrews J**, **Ali N**, **Pruessner JC.** Reflections on the interaction of psychogenic stress systems in humans: the stress coherence/compensation model. *Psychoneuroendocrinology* 38: 947–961, 2013. doi:10.1016/j.psyneuen.2013.02.010.
10. **Hamer M**, **Endrighi R**, **Venuraju SM**, **Lahiri A**, **Steptoe A.** Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. *PLoS One* 7: e31356, 2012. doi:10.1371/journal.pone.0031356.
11. **Phillips AC**, **Roseboom TJ**, **Carroll D**, **de Rooij SR.** Cardiovascular and cortisol reactions to acute psychological stress and adiposity: cross-sectional and prospective associations in the dutch famine birth cohort study. *Psychosom Med* 74: 699–710, 2012. doi:10.1097/PSY.0b013e31825e3b91.
12. **Sollberger S**, **Ehlert U.** How to use and interpret hormone ratios. *Psychoneuroendocrinology* 63: 385–397, 2016. doi:10.1016/j.psyneuen.2015.09.031.
13. **Ali N**, **Pruessner JC.** The salivary alpha amylase over cortisol ratio as a marker to assess dysregulations of the stress systems. *Physiol Behav* 106: 65–72, 2012. doi:10.1016/j.physbeh.2011.10.003.
14. **Kirschbaum C**, **Pirke KM**, **Hellhammer DH.** The 'Trier Social stress test' - a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28: 76–81, 1993. doi:10.1159/000119004.
15. **Miller R**, **Stalder T**, **Jarczok M**, **Almeida DM**, **Badrick E**, **Bartels M**, **Boomsma DI**, **Coe CL**, **Dekker MCJ**, **Donzella B**, **Fischer JE**, **Gunnar MR**, **Kumari M**, **Lederbogen F**, **Power C**, **Ryff CD**, **Subramanian SV**, **Tiemeier H**, **Watamura SE**, **Kirschbaum C.** The CIRCORT database: reference ranges and seasonal changes in diurnal salivary cortisol derived from a meta-dataset comprised of 15 field studies. *Psychoneuroendocrinology* 73: 16–23, 2016. doi:10.1016/j.psyneuen.2016.07.201.
16. **Pruessner JC**, **Kirschbaum C**, **Meinlschmid G**, **Hellhammer DH.** Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28: 916–931, 2003. doi:10.1016/s0306-4530(02)00108-7.
17. **Slominski RM**, **Tuckey RC**, **Manna PR**, **Jetten AM**, **Postlethwaite A**, **Raman C**, **Slominski AT.** Extra-adrenal glucocorticoid biosynthesis: implications for autoimmune and inflammatory disorders. *Genes Immun* 21: 150–168, 2020. doi:10.1038/s41435-020-0096-6.
18. **Mason JW**, **Giller EL**, **Kosten TR**, **Harkness L.** Elevation of urinary norepinephrine/cortisol ratio in posttraumatic stress disorder. *J Nerv Ment Dis* 176: 498–502, 1988. doi:10.1097/00005053-198808000-00008.
19. **Caufriez A**, **Leproult R**, **L'Hermite-Balériaux M**, **Kerkhofs M**, **Copinschi G.** Effects of a 3-week dehydroepiandrosterone administration on sleep, sex steroids and multiple 24-h hormonal profiles in postmenopausal women: a pilot study. *Clin Endocrinol (Oxf)* 79: 716–724, 2013. doi:10.1111/cen.12201.
20. **Gomes R**, **Moreira A**, **Lodo L**, **Nosaka K**, **Coutts A**, **Aoki M.** Monitoring training loads, stress, immune-endocrine responses and performance in tennis players. *Biol Sport* 30: 173–180, 2013. doi:10.5604/20831862.1059169.
21. **Choi JC**, **Min S**, **Kim YK**, **Choi J-H**, **Seo SM**, **Chang S-J.** Changes in pain perception and hormones pre-and post-kumdo competition. *Horm Behav* 64: 618–623, 2013. doi:10.1016/j.yhbeh.2013.08.013.
22. **Glenn AL**, **Raine A**, **Schug RA**, **Gao Y**, **Granger DA.** Increased testosterone-to-cortisol ratio in psychopathy. *J Abnorm Psychol* 120: 389–399, 2011. doi:10.1037/a0021407.
23. **Curran-Everett D.** Explorations in statistics: the analysis of ratios and normalized data. *Adv Physiol Educ* 37: 213–219, 2013. doi:10.1152/advan.00053.2013.
24. **Bale TL**, **Epperson CN.** Sex differences and stress across the lifespan. *Nat Neurosci* 18: 1413–1420, 2015. doi:10.1038/nn.4112.
25. **Jayasinghe SU**, **Torres SJ**, **Nowson CA**, **Tilbrook AJ**, **Turner AI.** Cortisol, alpha amylase, blood pressure and heart rate responses to food intake in men aged 50–70 years: importance of adiposity. *BMC Obesity* 1: 1–10, 2014. doi:10.1186/s40608-014-0014-4.