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

Running head: Stress pathway interaction

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Abstract

While the patterns of response within the sympatho-adrenal 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 that 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 alpha 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. While 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. Further, 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 of the other.

Key words:

sympatho-adrenal medullary system; hypothalamo-pituitary adrenal axis; cortisol; salivary alpha amylase; heart rate; systolic blood pressure; diastolic blood pressure

Introduction

Reactivity of the sympatho-adrenal 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 adrenaline, noradrenaline and salivary alpha amylase (sAA), while 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 BMI status (lean vs overweight/obese) and reactivity of the stress pathways to psychological stress (Trier Social Stress Test; TSST). While 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 alpha 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 and colleagues (4) in 55-60 year 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 greatest risk of hypertension at 5.5-year follow-up. Furthermore, in 20-35 year 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 year old Norwegian males screened at military draft, both exaggerated noradrenaline and blunted adrenaline reactivity at baseline predicted higher waist circumference at 18-year follow-up (6). Noradrenaline has a greater effect on peripheral vasoconstriction (via alpha-adrenergic

receptors), whereas adrenaline has a greater effect on the heart (via beta-adrenergic receptors)(7). Collectively, these three studies above (4-6) show a consistent pattern in which SAM system reactivity consisting of exaggerated peripheral vasoconstriction response (indicated by SBP and noradrenaline) and blunted cardiac response (indicated by HR and adrenaline) may confer the greatest risk of future adverse health and disease outcomes.

While 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 centre in the brainstem and the HPA axis control centre 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 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). While many studies have focussed 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 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 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 vs overweight/obese men. We hypothesise that consideration of the integrated response of these pathways may reveal dysregulation of the stress pathways that 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 years, 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 prior to 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).

Experimental procedure

The TSST is a well-characterised psychosocial stress protocol which 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 pre-stress (1400h – 1500h), stress (TSST, 1500h- 1530h) and recovery (1530h- 1700h) periods (Supplementary Figure 1 10.5281/zenodo.5778084). Saliva samples were collected using Salivette sampling tubes (Sarstedt, Ingle Farm, SA, Australia) every 15 min during pre-stress and recovery periods. More frequent sample collection (1500, 1507, 1515, 1522 and

1530h) was undertaken during the TSST to ascertain detailed profiling of how the stress parameters responded. Further, to elicit maximum potency of the stressor, a relatively long pre-stress resting period (i.e., 60 minutes) was implemented and the TSST was imposed at 1500h, during the diurnal cortisol nadir (15). Saliva samples were centrifuged at 3000rpm 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, USA).

Hormone assays

Saliva concentrations of cortisol and alpha amylase were quantified using enzyme immuno and kinetic assays, respectively (Diagnostic Systems Laboratories, Webster, TX, USA and Salimetrics, Carlsbad, CA, USA, respectively). For cortisol, 31 assays were conducted with a mean sensitivity of 0.035 µg/d. The intra-assay coefficient of variation was 6.9% at 0.25 µg/dl and 8.2% at 2.0 µg/dl. The inter-assay 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 inter-assay coefficient of variation was 7.4% at 20.7 U/ml and 7.0% at 257.3 U/ml.

Statistical analysis

Preliminary analysis

Pre-treatment for cortisol was defined as the average of the five values from 1400 to 1500 h (1400, 1415, 1430, 1445 and 1500 h). Pre-treatment sAA, HR, SBP and DBP was 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 pre-treatment value from the peak height for all parameters. Area under the curve with respect to increase (AUCi) and with respect to ground (AUCg) were 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 analysed using the Statistical Package for the Social Sciences software version 26.0 for Windows (SPSS, Inc., Chicago, IL, USA). 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 (pre-treatment, 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 years, respectively, $p = 0.166$). Overweight/obese men had ~30% higher body weight and BMI compared to 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 approximately 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 vs 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 Figure 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 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, 0.999, respectively, Figure 1a-e). Furthermore, there were no overall differences between the groups for cortisol, sAA and HR (between-subjects effect $p=0.210$, 0.332, 0.196, respectively), although, SBP and DBP showed trends towards having an overall difference between the groups (between-subjects effect $p=0.063$ and 0.082, respectively). There were no differences between groups in pre-treatment, peak height, reactivity, or area under the curve (Table 1), although there was a trend towards overweight/obese men having higher pre-treatment SBP ($p=0.054$), pre-treatment DBP ($p=0.068$) and DBP AUCg ($p=0.070$) compared with lean men (Table 1).

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195 **SAM over HPA ratio in response to TSST in lean vs overweight/ obese men**

196 This section considers whether adiposity influences reactivity of the SAM system relative to reactivity
197 of the HPA axis in response to the TSST. Ratios of SAM measures (sAA, HR, SBP and DBP) over
198 our HPA measure (cortisol) in lean and overweight/obese men are shown in Figure 2a-d. Repeated
199 measures analysis of variance revealed that there was a significant effect of time ($p<0.001$ for all;
200 Figure 2a-d). Nevertheless, AoC, heart rate over cortisol (HRoC), systolic blood pressure over cortisol
201 (SBPoC) and diastolic blood pressure over cortisol (DBPoC) in response to the TSST did not
202 statistically differ between lean and overweight/obese men (time*treatment, $p=0.247$, 0.912, 0.882
203 and 0.910, respectively, Figure 2a-d). Further, there was also no significant between-subjects effect,
204 indicating that there were no significant overall differences between the groups (treatment effect,
205 $p=0.540$, 0.506, 0.358 and 0.243, respectively, Figure 2a-d). Accordingly, there were no significant
206 differences between groups in pre-treatment, peak height, reactivity, AUCi or AUCg for AoC, HRoC,
207 SBPoC and DBPoC ($p>0.1$ for all; data not shown).

208

209 **HPA over SAM ratio in response to TSST in lean vs overweight/obese men**

210 This section considers whether adiposity influences reactivity of the HPA axis relative to reactivity of
211 the SAM system in response to the TSST. Ratios of our HPA axis measure (cortisol) over our SAM
212 system measures (sAA, HR, SBP, DBP) in lean and overweight/obese men are shown in Figure 3a-d
213 and Table 2. Repeated measures analysis of variance revealed that there was no significant effect of
214 time for CoA ($p=0.168$; Figure 3a). However, significant effects of time were evident for cortisol over
215 heart rate (CoHR), cortisol over systolic blood pressure (CoSBP) and cortisol over diastolic blood
216 pressure (CoDBP) ($p<0.001$ for all; Figure 3b-d). CoA and CoHR in response to the TSST did not
217 statistically differ between lean and overweight/obese men (time*treatment, $p = 0.457$ and 0.365,
218 respectively; Figure 3a and b). Significant time* treatment effects were evident for CoSBP and
219 CoDBP ($p= 0.018$ and 0.022, respectively; Figure 3c and 3d, respectively) demonstrating a
220 differential response pattern (lean>overweight/obese) in response to TSST when the activity of HPA

axis (cortisol) is considered relative to blood pressure activity (SBP and DBP). There were no significant between-subjects 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 towards a between-subjects effect for CoSBP and CoDBP (p=0.084 and 0.066, respectively; Figure 3c and 3d, respectively).

No statistical differences between groups were found in pre-treatment, peak height, reactivity, AUCi or AUCg for CoA, CoHR, CoSBP or CoDBP (Table 2), although there was a trend towards 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 vs 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, 0.999, respectively, Figure 1a-e) providing confirmation for the limited potential of the siloed approach to stress pathway analysis, traditionally implemented in psychoneuroendocrinology research. While 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.

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; Figure 3c and 3d, 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 this observed cortisol reactivity pattern (17). Furthermore, the emergence of significant findings, when considering the interaction of stress pathway reactivity (compared to analysing 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 post-traumatic 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) is 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 analyse 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; 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

283 time* treatment effects were found for CoSBP and CoDBP; p= 0.018 and 0.022, respectively; Figure
284 3c and 3d, respectively), while the second method revealed trends only (AUCi for CoHR, p=0.057
285 and AUCg for CoSBP and CoDBP, p=0.076 and 0.070, respectively; Table 2). Consequently, there
286 appears to be merit in using both approaches while this field is still in a development phase.

287

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

301

302 This study had strengths and limitations. A strength of this study is the robust nature of the underlying
303 data set, which included sufficient sampling times to capture the profile of response for each variable
304 and sufficient lead-in time before the start of sampling to ensure familiarity of participants with the
305 procedures used. As indicated above, limitations include the mathematical and statistical complexities
306 associated with the use of ratios. It is also possible that additional measures of subjective/emotional
307 responses to TSST may aid in obtaining a holistic understanding of the interaction between stress
308 pathway activity. Since obesity is not a unitary phenomenon, more direct indices of physiologic

obesity such as blood levels of various hormones associated with obesity characteristics (e.g. lipids, insulin and leptin) could also be measured and scrutinised 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 generalisability of our findings.

Conclusion

While 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’.

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407 **Table 1.** Mean (±SEM) pre-treatment, peak height, reactivity, AUCi and AUCg for cortisol, sAA, HR,
408 SBP and DBP in lean and overweight/obese men.

	Lean (n=19)	Overweight/Obese (n=17)	p value*
Cortisol**			
Pre-treatment (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 per min)	55.3±10.3	38.7±7.7	0.118
Cortisol AUCg (μg/dl per min)	107.3±11.2	89.4±7.6	0.204
sAA**			
Pre-treatment (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 per min)	5221±2735	3131±1525	0.523
sAA AUCg (U/ml per min)	26081±4206	29310±3759	0.575
Pre-treatment HR (bpm)	64±2	64±3	0.850
Peak height HR (bpm)	77±4	76±3	0.871
Reactivity HR (bpm)	13±2	13±2	0.986
HR AUCi (bpm per min)	32±126	89±88	0.720
HR AUCg (bpm per min)	11600±431	11528±523	0.916
Pre-treatment 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 per min)	1449±183	1148±225	0.303
SBP AUCg (mmHg per min)	22827±500	23923±572	0.157
Pre-treatment 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 per min)	1081±117	1043±104	0.812
DBP AUCg (mmHg per min)	13166±267	14027±384	0.070

409 * Univariate Analysis of Variance, AUCi, area under the curve with respect to increase; AUCg, area
410 under the curve with respect to ground; sAA, salivary alpha amylase; HR, heart rate; SBP, systolic
411 blood pressure; DBP, diastolic blood pressure; ** Cortisol and sAA data are reproduced with
412 permission from Endocrine Connections from Jayasinghe, Torres, Nowson, Tilbrook and Turner (3)

413

414 **Table 2.** Mean (±SEM) pre-treatment, peak height, reactivity, AUCi and AUCg for CoA, CoHR,
415 CoSBP and CoDBP in lean and overweight/obese men.

	Lean (n=19)	Overweight/Obese (n=17)	p value*
Pre-treatment 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
Pre-treatment 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
Pre-treatment 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
Pre-treatment 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

* Univariate Analysis of Variance; AUCi, Area under the curve with respect to increase; AUCg, Area under the curve with respect to ground; CoA, cortisol over sAA; CoHR, cortisol over heart rate; CoSBP, cortisol over systolic blood pressure; CoDBP, cortisol over diastolic blood pressure.

Figure Captions

Figure 1: Mean (±SEM) of (a) cortisol, (b) sAA, (c) heart rate, (d) systolic blood pressure and (e) diastolic blood pressure in lean and overweight/obese men from 1400h (-60 min) to 1700h (120 min); TSST, Trier Social Stress Test; Statistical method, Repeated Measures Analysis of Variance, Lean (n=19); Overweight/ obese (n=17); Cortisol and sAA data are reproduced with permissions from Endocrine Connections from Jayasinghe et al 2014 (25).

Figure 2: Ratios (±SEM) 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 1400h (-60 min) to 1700h (120 min); TSST, Trier Social Stress Test; Statistical method, Repeated Measures Analysis of Variance, Lean (n=19); Overweight/ obese (n=17).

Figure 3: Ratios (±SEM) 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 1400h (-60 min) to 1700h (120 min); TSST, Trier Social Stress Test; Statistical method, Repeated Measures Analysis of Variance, Lean (n=19); Overweight/ obese (n=17). Significant time* treatment effects were evident for CoSBP and CoDBP (p= 0.018 and 0.022, respectively; Figure 3c and 3d, respectively).

Data supplements can be found here:

https://doi.org/10.5281/zenodo.5778084

Figure 1

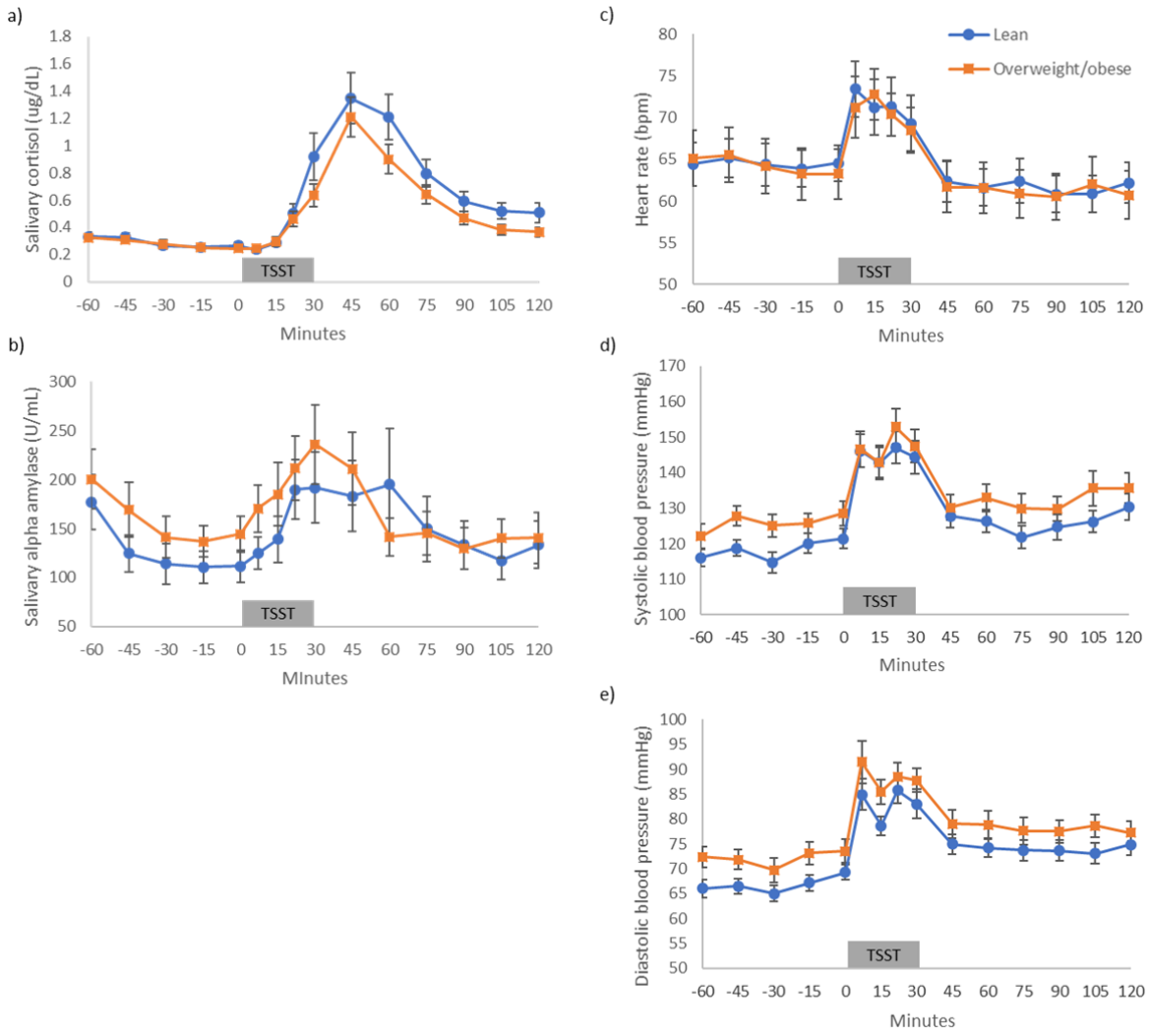

Figure 2

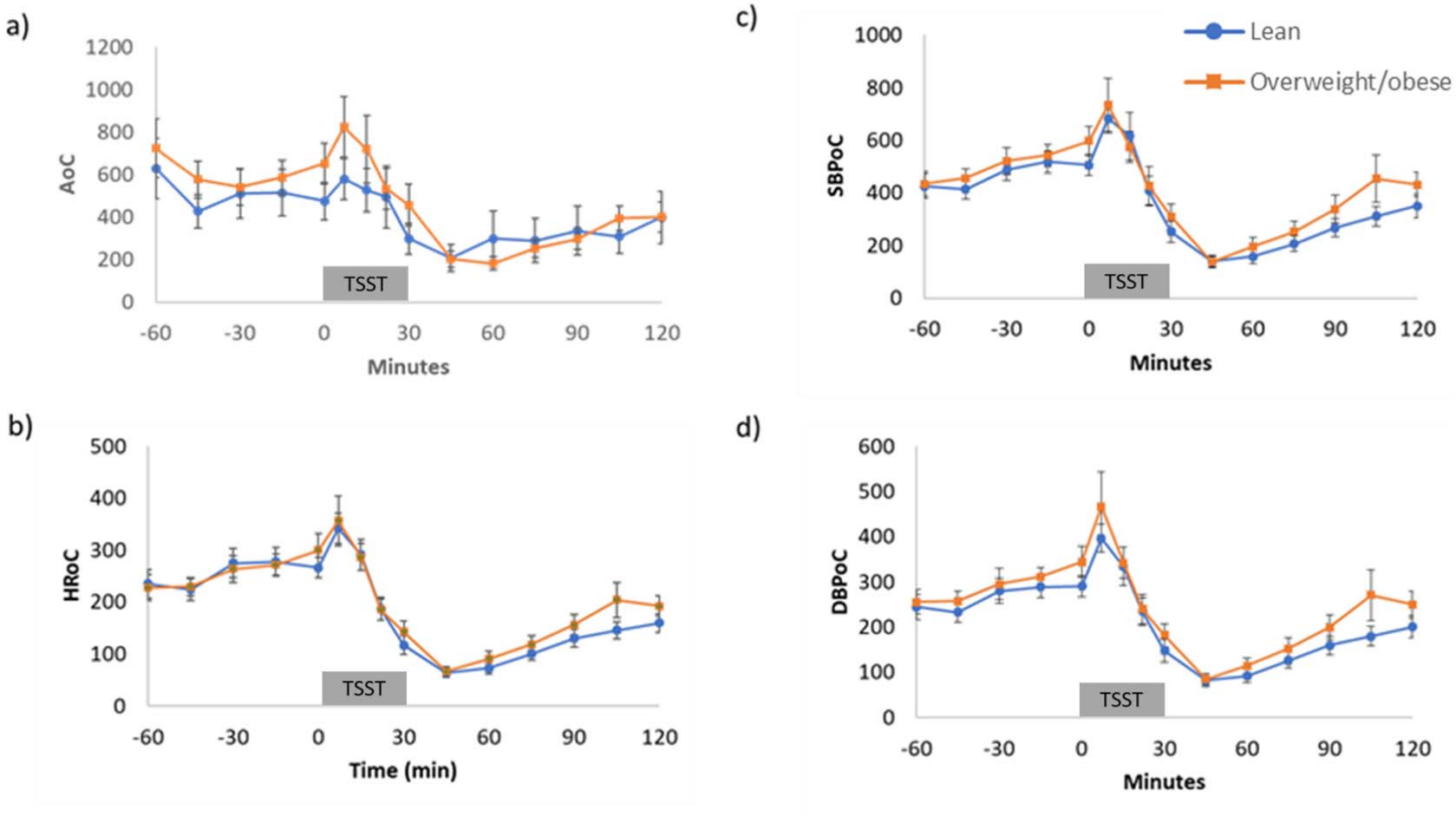

Figure 3