# Cortisol, blood pressure and heart rate responses to food intake were independent of physical fitness levels in women.

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## Abstract

This research tested the hypothesis that women who had higher levels of physical fitness will have lower hypothalamo-pituitary adrenal (HPA) axis (cortisol) and sympatho-adrenal medullary (SAM) system (blood pressure and heart rate) responses to food intake compared with women who had low levels of physical fitness. Lower fitness (n=22; $VO_2$ max = 27.4±1.0 ml/kg*min) and higher fitness (n=22; $VO_2$ max = 41.9±1.6 ml/kg*min) women (aged 30-50 years; in the follicular phase of the menstrual cycle) who participated in levels of physical activity which met (Lower fitness = 2.7±0.5 h/per week) or considerably exceeded (Higher fitness = 7.1±1.4 h/per week) physical activity guidelines made their own lunch using standardised ingredients at 1200 h. Concentrations of cortisol were measured in blood samples collected every 15 min from 1145 h-1400 h. Blood pressures and heart rate were also measured every 15 min between 1145 h and 1400 h. The meal consumed by the participants consisted of 20% protein, 61% carbohydrates and 19% fat. There was a significant overall response to lunch in all of the parameters measured (time effect for all $p<0.01$). The cortisol response to lunch was not significantly different between the groups (time*treatment $p = 0.882$). Overall, both groups showed the same pattern of cortisol secretion (treatment $p = 0.839$). SBP, DBP, MAP or HR responses (time*treatment $p = 0.726$, 0.898, 0.713, 0.620, respectively) were also similar between higher and lower fitness women. Results suggest that the physiological response to food intake in women is quite resistant to modification by elevated physical fitness levels.


Key words: HPA axis, SAM system, women, fitness, food intake, physical activity

45 ***Introduction***

46 Food intake is a physiological challenge experienced by the human body several
47 times per day. We and others have shown that food intake is a challenge that can
48 activate both the sympatho-adrenal medullary (SAM) system (Chang et al. 2010;
49 Cozzolino et al. 2010; Jayasinghe et al. 2014; Kawaguchi et al. 2002; Sauder et al.
50 2012; Tentolouris et al. 2003) and the hypothalamo-pituitary adrenal (HPA) axis
51 (Gibson et al. 1999; Jayasinghe et al. 2014; Martens et al. 2010; Vicennati et al.
52 2002). Hyperactivity of these pathways is associated with the development of
53 numerous chronic diseases (Carroll et al. 2008; Chida and Hamer 2008; Hamer and
54 Steptoe 2011). Thus, there lies the possibility that excessive SAM system and HPA
55 axis responses to food intake may place individuals at increased risk of developing
56 stress-related chronic conditions. Therefore, investigation of acute physiological
57 responses of both SAM system and HPA axis to food intake is of utmost importance.

58

59 Physical fitness status (Rimmele et al. 2009) and adiposity (Epel et al. 2000) are
60 physiological conditions that can alter the activity of the stress pathways. Available
61 evidence suggests that increased adiposity can be associated with higher HPA axis
62 activity in response to food intake (Jayasinghe et al. 2014; Vicennati et al. 2002).
63 Nevertheless, the influence of physical fitness status on physiological responses
64 (both HPA axis and SAM system) to food intake has not been investigated before.
65 Exercise brings about many health benefits including lowering progression to chronic
66 disease by influencing heart rate, blood pressure and vascular endothelial
67 functioning in response to stress (Hamer 2012; Throne et al. 2000; Tsatsoulis and
68 Fountoulakis 2006). Moderating the HPA axis and SAM system responses to food

intake may well be another avenue by which exercise exerts its protective capabilities against the development of chronic disease.

Activity of the SAM system increases cardiovascular (heart rate and blood pressure) activity (Grassi and Esler 1999). Activity of the HPA axis results in the secretion of cortisol from the adrenal cortex (Tilbrook 2007). Therefore, all of the parameters mentioned above can be used to measures the activity of the HPA axis and the SAM system. It is often best to include a collection of measures in order to fully characterise the activity of the stress pathways.

The aims of this study were to measure HPA axis and SAM system responses to food intake in women (in the follicular phase of the menstrual cycle) who differed in their levels of physical fitness. Given the marked influence of sex steroids on the activity of the stress pathways (Kajantie and Phillips 2006; Lustyk et al. 2010), this study was conducted in women only so as not to confound the results by including both genders. Since the change in levels of sex steroids during the menstrual cycle can also influence the activity of the stress pathways (Lustyk et al. 2010), this study investigated women in the same phase of the menstrual cycle (follicular phase) at the time of testing. It was hypothesised that women who had higher levels of physical fitness will have lower HPA axis and SAM system responses to food intake.

## Materials and Methods

Women (n=44) aged 30-50 years were recruited using newspaper and online advertisements, emails, fliers in community centres and medical clinics. Exclusion criteria were 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. This information was self-reported by the participants via a telephone interview. Given the influence of sex steroids on activity of the stress pathways (Kajantie and Phillips 2006), post-menopausal women, peri-menopausal women and all women who were on any form of steroidal contraception (including oral contraceptives, steroidal implants and steroidal IUDs) were excluded from the study.

All participants provided written informed consent prior to participation in the study. All procedures were approved by the Human Research Ethics Committee of Deakin University (Project code: 2011-242) and conformed to the guidelines of the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007).

### *Experimental procedure*

Women reported to the laboratory on two separate days. The first visit was to obtain additional health information (details below), a fasting blood sample for the measurement of cardio-metabolic risk markers and to measure cardiorespiratory fitness (maximum oxygen consumption-$VO_2$ max). The stress pathway activation in

response to food intake (details below) was investigated on the second visit which occurred at least one week after the first visit.

***Day 1 testing***

Participants were given instructions to fast overnight (for at least 10 hours) prior to attending the laboratory. Day 1 testing was completed between 0600h – 1200h. Weight was recorded in kilograms to the nearest 0.1 kg with digital scales (TANITA, Wedderburn, Melbourne, Australia) on a firm surface. Height was measured to the nearest millimetre using a freestanding stadiometer (Measurement Concepts, North Bend, Australia). Participants were not wearing shoes in both measurements. BMI was calculated as weight (kg) divided by height (m) squared. Women whose BMI fell outside the range 18-30 ($kg/m^2$) were excluded from the study. Resting blood pressure was measured four times (Criticare systems Inc, Wisconsin, USA) at 2 min intervals and the average of the last three measurements were used to confirm whether resting blood pressure was within the required range (<160mmHg for systolic and <90mmHg for diastolic). This threshold for systolic was used since isolated systolic blood pressure of >160mmHg is considered by the Heart Foundation of Australia as the point at which anti-hypertensive medication should be recommended (Heart Foundation 2008). Hypertension is defined in the Australian Heart Foundation Guide to Management of Hypertension as >140/90mmHg. None of the women recruited for this study exceeded a resting blood pressure of 140/90mmHg.

All eligible participants were subsequently subjected to a single venipuncture in a vein of the antecubital fossa of the forearm using a sterile vacuette safety blood collection set (GreinerBio-One GmbH, Kremsmunster, Austria). Blood was collected into a 9ml serum separator tube (GreinerBio-One GmbH, Kremsmunster, Austria) and two 2ml plasma EDTA (GreinerBio-One GmbH, Kremsmunster, Austria) tubes. Serum was sent to a commercial pathology laboratory (Dorevitch, Melbourne, Australia) for analysis of lipid profile (total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides), fasting serum glucose, and C-reactive protein.

Participants were allowed to have a snack (a selection of foods from muesli bars, nuts, dried fruit and juice boxes were made available) after collection of blood. A Physical Activity Readiness Questionnaire (PAR-Q) was filled in at this time to assess if it was safe for each participant to undertake a $VO_2$ max test. Participants also filled out an International Physical Activity Questionnaire (IPAQ) (Bauman et al. 2009) to measure levels of high and moderate intensity physical activity, a State-Trait Anxiety Inventory (STAI) (Spielberger et al. 1983) to measure levels of anxiety and a Beck Depression Inventory (BDI-ii) (Beck et al. 2006) to measure levels of depressive symptoms. Water was available *ad libitum* to all participants throughout the testing session. This was immediately followed by the graded $VO_2$ max test on an electronically braked cycle ergometer (Lode N.V. Groningen, Netherlands). After ranking women by $VO_2$ max score, a median split was then used to allocate women evenly into a higher fitness group (n=22) and a lower fitness group (n=22).

161 ***Day 2 testing***

162 Participants were in their mid-follicular phase of the menstrual cycle at the time of
163 this testing session. Mid follicular phase was defined as Days 5-9 of the menstrual
164 cycle, inclusive, where Day 1 was the first day of menses onset (Lustyk et al. 2010).
165 Participants were asked to abstain from smoking, ingesting any caffeine containing
166 beverages (e.g. tea, coffee, cola), liquorice, alcohol or drugs (except for any regular
167 medications) and from strenuous physical activity during the 12 hours prior to Day 2
168 testing.

169 Participants were instructed to arrive at the laboratory at 1100h. Between 1100h-
170 1145h, measurements of waist and hip circumference and body fat (TANITA,
171 Wedderburn, Melbourne, Australia) were obtained and participants were asked to fill
172 in a background questionnaire about their alcohol consumption and physical activity
173 in the week preceding the testing day. Waist circumference was measured at the
174 midpoint between the last rib and the anterior superior iliac spine using a tape
175 measure and hip circumference was measured at the widest point of the gluteal area
176 (Dettwyler 1993). Waist to hip ratio was calculated by dividing waist circumference
177 by hip circumference. Also during this period, an intra-venous catheter (Smiths
178 Medical, Ohio, USA) was inserted into an antecubital vein of the forearm for
179 subsequent sampling of blood.

180

181 Participants were given a test meal (details below) at 1200h. They were allowed to
182 consume food between 1200h-1230h. Blood samples were collected every 15 min
183 between 1145h-1400h. Systolic blood pressure (SBP), diastolic blood pressure
184 (DBP), mean arterial pressure (MAP) and heart rate (HR) were also measured at 15

min intervals during this period using a clinical blood pressure monitor (Criticare systems Inc, Wisconsin, USA) to gauge the activity levels of the SAM system. Although not considered as direct measures, SBP, DBP, MAP and HR have been used as proxy measures of SAM system activity under different circumstances (de Geus et al. 1993; Grassi and Esler 1999; Webb et al. 2013). We have also previously shown that food intake can cause significant changes in heart rate and blood pressure (Jayasinghe et al. 2014). Participants were allowed a break to use the bathroom immediately after the 1330h blood and blood pressure/heart rate sampling.

Lithium Heparin tubes (GreinerBio-One GmbH, Kremsmunster, Austria) were used to collect blood samples (5ml) for cortisol assays. All tubes were spun at 3000 rpm for 6 min. Plasma was separated and stored at -80ºC until assay.

***Test meal***

The test meal consisted of lunch made by the participants from a choice of standardised ingredients including bread, margarine, processed meat (ham or chicken), tomato, cucumber, cheese, nuts, fruit bars and a fruit drink (juice box). Water was available *ad libitum.* The investigator took records of the foods consumed. Dietary intake was determined using household measures. Total energy, macronutrient and sodium intake was determined using FoodWorks professional edition (version 7; Xyris software, Brisbane, Queensland, Australia).

### *Plasma cortisol assays*

Plasma concentrations of cortisol were measured using a radio immunoassay (Demeditec Diagnostics, Kiel, Germany). Forty-four assays were conducted. The intra-assay coefficient of variation was 9.8% at 92 ng/mL and 9.4% at 193 ng/ml. The inter-assay coefficient of variation was 10.7% at 146 ng/ml and 10.2% at 137 ng/ml.

### *Statistical analysis*

### *Preliminary analysis*

**Pre- treatment** salivary cortisol was defined as the concentration of cortisol in the sample collected at 1200h. Pre- treatment SBP, DBP, MAP and HR were defined as the average of values recorded at 1145h and 1200h. **Peak height** for cortisol was defined as the highest value obtained for each individual between 1215h- 1400h, inclusive. Peak height for all cardiovascular parameters was defined as the highest value obtained between 1215h-1330h. Data from 1345h- 1400h were not used in this calculation because of the apparent effects on cardiovascular parameters of physical movements during the bathroom break. **Reactivity** was calculated by subtracting the pre-treatment value from the peak height for all parameters. **Area under the curve** (with respect to increase) was calculated for cortisol using all values between 1200h – 1400h and for SBP, DBP, MAP and HR using values between 1200h-1330h after the subtraction of the pre-treatment value from each data point. Area under the curve for all parameters was calculated using the

trapezoid rule utilising Sigmaplot 12.5 graphing software (Systat Software Inc., California, USA).

***Analysis***

Data were analysed using the Statistical Package for the Social Sciences software version 21.0 for Windows (SPSS. Inc, Chicago, USA). Kolmogorov-Smirnov and Shapiro –Wilk tests were conducted to test for normality. Tests for homogeneity of variance were conducted using Levene's test of equality of error variances. Descriptive characteristics were compared between groups using univariate analysis of variance. Plasma cortisol, blood pressure and heart rate were compared within and between subjects using repeated measures analysis of variance. The within subjects factor was time and the between subjects factor was treatment. Derived plasma cortisol and cardiovascular parameters (pre- treatment, peak height, reactivity and area under the curve) were compared between groups using univariate analysis of variance. $P<0.05$ was considered statistically significant.

We estimated that 32 participants in total were needed to find a difference between groups in salivary cortisol of the same magnitude as that found by Klaperski and colleagues (Klaperski et al. 2013) with a significance level of 0.05 and a power of 90%.

254 **Results**

255 ***Participants***

256 A total of 44 women completed the study. Women were ranked according to their
257 $VO_2$ max score and a median split was used to allocate women to two even groups
258 (lower fitness group; n = 22 and higher fitness group; n = 22). One woman in the
259 higher fitness group had to be excluded from the cortisol analyses due to a blocked
260 cannula which prevented the collection of several blood samples.

261

262 ***Participant characteristics***

263

264 The labels given to the groups in this study were relative terms used to differentiate
265 between the higher $VO_2$ max group and the lower $VO_2$ max group. According to the
266 American Heart Association cardiorespiratory fitness classification criteria (AHA
267 1972), women in our lower fitness group would be classified as “fair” to “average”
268 whereas women in our higher fitness group would be classified as “good” to “high”.
269 Women in the higher fitness group had significantly higher $VO_2$ max levels and
270 participated in a significantly higher number of hours of moderate and vigorous
271 intensity physical activity compared with the women in the lower fitness group
272 ($p<0.01$ for both; Table 1). The number of hours of moderate and vigorous intensity
273 physical activity undertaken by lower fitness women (2.7±0.5 h/per week) was
274 sufficient to meet the physical activity recommendations of the American Heart
275 Association (AHA) and the number of hours undertaken by higher fitness women
276 (7.1±1.3 h/per week) considerably exceeded these recommendations (Centers for
277 Disease Control and Prevention 2008). Percentage body fat and waist

278 circumference were significantly lower in the higher fitness group compared with the
279 lower fitness group ($p<0.05$ for both). Furthermore, the lower fitness group had
280 significantly more abdominal body fat compared with the higher fitness group as
281 indicated by the waist to hip ratio ($p=0.004$; Table 1). Higher fitness women had
282 significantly lower serum triglyceride levels, serum CHOL/HDL ratio, serum glucose
283 concentrations and HOMA-IR compared with lower fitness women ($p<0.05$ for all;
284 Table 2). There were no significant differences between the groups in serum C-
285 reactive protein levels, serum cholesterol levels, depression and state or trait anxiety
286 scores (Table 2).

287

288

289 ***Test meal***

290 Lower fitness and higher fitness women consumed similar amounts of total energy,
291 protein, carbohydrate, fat and sodium (Table 3). There were no significant
292 differences between the groups in these parameters. Overall, both groups
293 combined, the meal consumed by the participants consisted of 20% protein, 61%
294 carbohydrates and 19% fat.

295

296 ***Plasma cortisol***

297 Plasma concentrations of cortisol in lower fitness and higher fitness women are
298 shown in Figure 1 and Table 4. Repeated measures analysis of variance revealed
299 that there was a significant effect of time ($F(9, 33) = 2.657$, $p=0.05$; Figure 1).
300 Overall, with both groups combined, the peak height (174.4 ± 9.8 ng/mL) of cortisol

concentrations was significantly higher ($p<0.001$) than the pre-treatment (137.4 ± 10.4 ng/mL) cortisol concentrations. This represented a 27% increase from the pre-treatment cortisol concentrations.

In response to lunch, plasma concentrations of cortisol did not differ significantly between lower fitness and higher fitness women (time*treatment, $F(9, 33) = 0.488$, $p = 0.882$; Figure 1). Furthermore, there was no significant differences between groups in peak height, cortisol reactivity and area under the curve ($p>0.05$ for all; Table 4). There was also no significant between subjects effect indicating that overall, higher fitness women had similar cortisol levels compared with lower fitness women ($F(9, 33) = 0.042$, $p = 0.839$).

***Cardiovascular parameters***

Cardiovascular parameters in lower fitness and higher fitness women are presented in Figure 2 and Table 5.

***Systolic blood pressure***

There was a significant effect of time ($F(9, 34) = 5.450$, $p<0.001$) for systolic blood pressure (Figure 2a). Overall (both groups combined), the peak height of systolic blood pressure (120±3 mmHg) was significantly higher than the pre-treatment systolic blood pressure (108 ±2 mmHg) ($p<0.001$). This represents a 12% increase.

Systolic blood pressure in response to the lunch did not differ significantly between lower fitness and higher fitness women (time*treatment, $F(9, 34) = 0.961$, $p= 0.472$;

Figure 2a). This lack of difference of the response between groups was further illustrated by there being no difference in peak height, reactivity and area under the response curve for systolic blood pressure between the two groups ($p > 0.05$ for both; Table 5). There was also no significant between subjects effect indicating that overall, higher fitness women had similar systolic blood pressure compared with the lower fitness women (treatment effect, $F(9, 34) = 3.627$, $p = 0.064$).

***Diastolic blood pressure***

There was a significant effect of time ($F(9, 34) = 7.915$, $p<0.001$) and no treatment effect ($p=0.180$) for diastolic blood pressure (Figure 2b). Overall (both groups combined), the peak height of diastolic blood pressure (72±2 mmHg) was significantly higher than the pre-treatment diastolic blood pressure (63 ±1 mmHg) ($p<0.001$). This represents a 14% increase.

Diastolic blood pressure in response to the lunch did not differ significantly between lower fitness and higher fitness women (time*treatment, $F(9, 34) = 0.514$, $p= 0.864$; Figure 2b). Furthermore, diastolic blood pressure peak height, reactivity and area under the curve did not differ between the two groups (Table 5). There was also no significant between subjects effect indicating that overall, higher fitness women had similar diastolic blood pressure compared with the lower fitness women (treatment effect, $F(9, 34) = 1.862$, $p = 0.180$).

***Mean Arterial pressure***

There was a significant effect of time ($F(9, 34) = 7.657$, $p<0.001$) for mean arterial pressure (Figure 2c). Overall (both groups combined), the peak height of mean arterial pressure (88±2 mmHg) was significantly higher than the pre-treatment mean arterial pressure (80 ±2 mmHg) ($p<0.001$). This represents an 11% increase.

Mean arterial pressure in response to the lunch did not differ significantly between lower fitness and higher fitness women (time*treatment, $F(9, 34) = 0.590$, $p= 0.805$; Figure 2c). Peak height, reactivity and area under the response curve for mean arterial pressure were similar between the groups ($p > 0.05$ for both; Table 5). There was also no significant between subjects effect indicating that overall, higher fitness women had similar mean arterial pressures compared with the lower fitness group ($F(9, 34) = 3.205$, $p=0.081$).

***Heart rate***

There was a significant effect of time ($F(9, 34) = 4.933$, $p<0.001$) for heart rate (Figure 2d). Overall (both groups combined), the peak height of heart rate (75±2 mmHg) was significantly higher from the pre-treatment heart rate (66±2 mmHg) ($p<0.001$). This represents a 14% increase.

Heart rate in response to the lunch did not differ significantly between lower fitness and higher fitness women (time*treatment, $F(9, 34) = 1.319$, $p= 0.225$; Figure 2d). Nevertheless, higher fitness women had a significantly lower ($p=0.005$) peak height of the heart rate response compared with lower fitness women. Nevertheless, heart

rate reactivity was not different (p=0.084) between higher fitness and lower fitness women. Pre-treatment values and area under the curve did not differ between the two groups (Table 5). Overall, lower fitness women had significantly higher levels of heart rate compared with the higher fitness women as indicated by the significant treatment effect ($F(9, 34) = 7.703$, $p=0.008$).

## Discussion

Our hypothesis that women who had higher levels of physical fitness would have lower SAM system and HPA axis responses to the ingestion of a standardised lunch compared with women who had lower levels of fitness was not supported. While all of the parameters tested (plasma cortisol, blood pressure and heart rate) increased significantly in response to food intake, none of these responses differed between the groups. These results suggest that there is comparable SAM system and HPA axis activity in response to food intake in women with different physical fitness statuses. Indeed, it seems that HPA axis and SAM system responses to food intake are independent of physical fitness status in women.

In the present study, there was a substantial elevation of cortisol in response to lunch as indicated by the time effect and the significant increase in cortisol levels from baseline to the peak of the response (27%; both groups combined), despite there being no difference between lower fitness and higher fitness groups in this response. In an earlier experiment (Jayasinghe et al. 2014) we observed a significant HPA axis response (salivary cortisol) to food intake in overweight/obese men but no response in lean men. While the percentage increase of cortisol in overweight/obese men (86%) was substantially higher than the percentage increase of cortisol in women (27%; both groups combined), the men's study measured salivary cortisol whereas the current study measured plasma cortisol. Since salivary cortisol indicates the free fraction of cortisol (a small proportion of total cortisol), it is possible the differences in percentage increases that were observed may have been due to the differences in HPA axis activity measures (saliva vs plasma) that were

403 used. As such, it is not meaningful to make direct comparisons of the percentage
404 increases between the studies.

405

406 In the current experiment, all SAM system parameters in both groups were elevated
407 in response to lunch. These increases are in accordance with the reports of
408 Harthoorn et al who found increases in sympathetic nervous system (heart rate and
409 salivary alpha amylase) activity after ingestion of a standardised meal (15-20%
410 protein, 35-40% fat and 40-45% carbohydrate) in a group of healthy men and women
411 (Harthoorn & DransWeld, 2008). There is also evidence suggesting that ingestion of
412 food can cause significant changes in parameters of heart rate variability (high
413 frequency power, low frequency power and low to high frequency ratio) (Kawaguchi
414 et al. 2002). Previous reports also indicated that the rise in sympathetic activity
415 following a meal is dependent on the nutrient content of the foods ingested. For
416 instance, Tentolouris et al (2003) reported increases in sympathetic activity
417 (measured via plasma noradrenaline and heart rate variability) in lean healthy young
418 women, only after consuming a high (95%) carbohydrate meal (Tentolouris et al.
419 2003). Nevertheless, these elevations are indicative of the physiological demands
420 that food intake places on the sympathetic nervous system activity (Jager et al.,
421 1986). There was a reduction in systolic blood pressure, diastolic blood pressure
422 and mean arterial pressure in the postprandial period in both groups (i.e., 1230h-
423 1330h in the current experiment) which may indicate a reduction in resistance to
424 blood flow in the mesenteric vessels and perhaps even an indication that the satiety
425 hormones are having an inhibitory effect on the sympathetic nervous system during
426 this period (Burcelin, 2005; Fan et al., 2004). It has also been suggested that down
427 regulation of catecholamines (in particular noradrenaline) could be a possible

mechanism of this postprandial hypotensive effect (Kawaguchi et al. 2002). Nevertheless, the absence of any significant differences in SAM system parameters between higher fitness and lower fitness women in the current experiment suggests that, on the whole, the post prandial sympathetic activity is independent of physical fitness statuses in women.

Abdominal obesity can have a significant impact on the activity of the stress pathways (Epel et al., 2000; Katz et al., 2000). Vicennati and colleagues reported that high carbohydrate meals (89% carbohydrate, 11% protein, 0% fat) can result in a significant HPA axis response in women who predominantly had a visceral body fat distribution compared with women with peripheral body fat distribution and normal weight healthy controls (Vicennati et al., 2002). Despite there being no difference between the groups in BMI in the current study, waist to hip ratios and percent body fat levels indicate that the lower fitness women had significantly more abdominally based body fat compared with their fitter counterparts. However, it should be noted that the WHR of women who had visceral body fat in Vicennati and colleagues research, was much higher (0.92±0.01) than the WHR of lower fitness women (0.84±0.01) in the current experiment. Furthermore, it appears that obesity does not influence food intake related SAM system activity in the same way as it does influence food intake evoked HPA axis responses. For instance, Tentolouris and colleagues reported no increases in sympathetic activity (measured via plasma noradrenaline and heart rate variability) in obese young women after the consumption of a high (95%) carbohydrate meal (Tentolouris et al. 2003). Nevertheless, given that there were no significant differences between the groups in SAM and HPA axis activity in the current study, it suggests that having greater

quantities of abdominally based body fat may not accentuate SAM system and HPA axis responses to food intake in lower fitness women.

None of the previous experiments in the area had investigated the effects of physical fitness status (objectively measured via maximal oxygen consumption) on SAM system and HPA axis response to food intake. Higher fitness women in the current study had an average $VO_2$ max level of 41.9±1.6 ml/kg*min. Therefore, it is possible that women with even higher maximal oxygen consumption levels would be required to observe a different response pattern to food intake. This could be studied in future studies. Furthermore, since sex steroids can influence the activity of the stress systems (Lustyk et al. 2010), this study considered women in the follicular phase of the menstrual cycle in order to standardise the sex steroid milieu of the women. It is possible that physical fitness status may have an effect on SAM system and HPA axis responses to food intake of women in other phases of the menstrual cycle and this could be the focus of future research. Further to this, men could also be considered in testing whether physical fitness status (objectively measured via maximal oxygen consumption) can influence SAM system and HPA axis response to food intake. Ideally, future experiments would also include a longer lead in time prior to the administration of lunch and would include lunch made with the same food items for all the subjects with the same proportion of macronutrients calculated as a percentage of daily energy expenditure or resting metabolic rate.

This experiment showed that physical fitness status (objectively measured via maximal oxygen consumption) in women (in the follicular phase of the menstrual

477 cycle) did not have a significant influence on HPA axis and SAM system activity after
478 the ingestion of a meal consisting of 20% protein, 61% carbohydrates and 19% fat.
479 This suggests that ingesting a standardised meal does not result in excessive HPA
480 axis and SAM system activation in women of 30–50 years who have lower levels of
481 physical fitness compared with age matched women who have higher levels of
482 physical fitness.

483

484 **Acknowledgements**

485 Authors thank Mais Hussein for technical support throughout the experiment and all
486 the subjects for participating in this research.

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599 **Tables**

600 **Table 1**: Mean (±SEM) descriptive characteristics of lower and higher fitness women

| | Lower fitness (n= 22) | Higher fitness (n=22) | p value* |
|---|---|---|---|
| Age (years) | 40.4±1.4 | 38.1±1.3 | 0.233 |
| $VO_2$max (ml/kg*min) | 27.4±1.0 | 41.9±1.6 | <0.001 |
| Physical activity (hours) | 2.7±0.5 | 7.1±1.3 | 0.004 |
| Weight (kg) | 62.6±2.3 | 62.2±1.6 | 0.833 |
| BMI ($kg/m^2$) | 23.1±0.7 | 22.2±0.4 | 0.241 |
| % Fat | 30.3±1.4 | 25.5±1.2 | 0.013 |
| Waist circumference (cm) | 82.5±2.2 | 76.4±1.3 | 0.021 |
| Hip circumference (cm) | 97.5±1.6 | 96.1±1.2 | 0.481 |
| WHR | 0.84±0.01 | 0.80±0.01 | 0.004 |
| Resting HR (bpm) | 71±2 | 66±2 | 0.089 |
| Resting SBP (mmHg) | 115±3 | 114±2 | 0.926 |
| Resting DBP (mmHg) | 70±2 | 67±2 | 0.232 |

601 * univariate analysis of variance

602

603 **Table 2**: Mean (±SEM) of cardio-metabolic risk markers and mental health scores in
604 lower fitness and higher fitness women

| | Lower fitness (n=22) | Higher fitness (n=22) | p value* |
|---|---|---|---|
| CRP (mg/L) | 1.4±0.7 | 0.6±0.2 | 0.279 |
| Cholesterol (mmol/L) | 5.0±0.1 | 4.8±0.2 | 0.256 |
| Triglycerides (mmol/L) | 0.9±0.1 | 0.7±0.1 | 0.024 |
| CHOL/HDL ratio | 3.3±0.2 | 2.6±0.1 | 0.001 |
| Glucose (mmol/L) | 5.3±0.1 | 4.7±0.2 | 0.024 |
| BDI-ii score | 4.4±1.1 | 2.8±0.9 | 0.271 |
| STAI score (trait) | 31.9±1.5 | 31.7±1.9 | 0.469 |
| STAI score (state) | 32.4±1.2 | 30.7±2.1 | 0.475 |

605 * univariate analysis of variance

606

607 **Table 3**: Mean (± SEM) total energy, macronutrient and sodium intake in lower
608 fitness and higher fitness women.

| | Lower fitness (n=22) | Higher fitness (n=22) | p value* |
|---|---|---|---|
| Total energy (kJ) | 2047±162 | 2094±116 | 0.811 |
| Protein (g) | 19.7±1.8 | 19.3±1.6 | 0.847 |
| Carbohydrate (g) | 58.9±4.6 | 59.6±3.3 | 0.901 |
| Fat (g) | 17.5±2.3 | 19.2±2.0 | 0.592 |
| Sodium (mg) | 1047±115 | 860±72 | 0.176 |

609 * univariate analysis of variance

610

611 **Table 4.** Mean (±SEM) pre-treatment cortisol, peak height of cortisol, cortisol
612 reactivity and area under the curve for lower fitness and higher fitness women

| | Lower fitness (n=22) | Higher fitness (n=21) | p value* |
|---|---|---|---|
| Pre-treatment (ng/mL) | 138.7±16.7 | 136.1±12.7 | 0.900 |
| Peak height (ng/mL) | 172.9±13.8 | 175.9±14.2 | 0.881 |
| Reactivity (ng/mL) | 34.2±10.3 | 39.8±8.3 | 0.673 |
| AUC (ng*min/mL) | -433.1±1045 | -263.3±822 | 0.900 |

613 * Univariate analysis of variance

614 AUC = Area under the curve

615

616 **Table 5**: Mean (±SEM) pre-treatment, peak height, reactivity and area under the
617 curve for heart rate and blood pressure in lower fitness and higher fitness women

| | | Lower fitness (n=22) | Higher fitness (n=22) | p value* |
|---|---|---|---|---|
| **SBP** | Pre-treatment (mmHg) | 112±4 | 104±2 | 0.115 |
| | Peak height (mmHg) | 125±4 | 116±3 | 0.057 |
| | Reactivity (mmHg) | 14±3 | 12±3 | 0.642 |
| | AUC (mmHg*min) | 188±163 | 104±118 | 0.688 |
| **DBP** | Pre-treatment (mmHg) | 65±2 | 62±2 | 0.374 |
| | Peak height (mmHg) | 72±2 | 72±3 | 0.833 |
| | Reactivity (mmHg) | 8±2 | 10±3 | 0.607 |
| | AUC (mmHg*min) | -166±90 | -216±112 | 0.730 |
| **MAP** | Pre-treatment (mmHg) | 82±3 | 77±2 | 0.119 |
| | Peak height (mmHg) | 90±3 | 87±3 | 0.507 |
| | Reactivity (mmHg) | 7±2 | 10±3 | 0.503 |
| | AUC (mmHg*min) | -148±115 | -165±124 | 0.920 |
| **HR** | Pre-treatment (bpm) | 69±2 | 64±2 | 0.113 |
| | Peak height (bpm) | 80±2 | 71±2 | 0.005 |
| | Reactivity (bpm) | 11±2 | 7±1 | 0.084 |
| | AUC (bpm*min) | 239±129 | 54±69 | 0.212 |

618 *Univariate analysis of variance

619 SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial
620 pressure, HR = heart rate

621

## Figure captions

**Figure 1**: Mean (±SEM) plasma cortisol concentrations in lower and higher fitness women from 1145h-1400h. The box labelled “lunch” represents the timing of the lunch period and the hashed box represents the timing of the break to use the bathroom

**Figure 2**: Mean (±SEM) a) systolic, b) diastolic and c) mean arterial pressures and d) heart rate in lower fitness and higher fitness women from 1145h-1400h. The boxes labelled “lunch” represent the timing of the lunch period and the hashed boxes represent the timing of the break to use the bathroom.