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

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2 **independent of physical fitness levels in women.**

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20

21 Abstract

22 This research tested the hypothesis that women who had higher levels of physical
23 fitness will have lower hypothalamo-pituitary adrenal (HPA) axis (cortisol) and
24 sympatho-adrenal medullary (SAM) system (blood pressure and heart rate)
25 responses to food intake compared with women who had low levels of physical
26 fitness. Lower fitness ($n=22$; $\text{VO}_2 \text{ max} = 27.4 \pm 1.0 \text{ ml/kg*min}$) and higher fitness
27 ($n=22$; $\text{VO}_2 \text{ max} = 41.9 \pm 1.6 \text{ ml/kg*min}$) women (aged 30-50 years; in the follicular
28 phase of the menstrual cycle) who participated in levels of physical activity which
29 met (Lower fitness = $2.7 \pm 0.5 \text{ h/per week}$) or considerably exceeded (Higher fitness =
30 $7.1 \pm 1.4 \text{ h/per week}$) physical activity guidelines made their own lunch using
31 standardised ingredients at 1200 h. Concentrations of cortisol were measured in
32 blood samples collected every 15 min from 1145 h-1400 h. Blood pressures and
33 heart rate were also measured every 15 min between 1145 h and 1400 h. The meal
34 consumed by the participants consisted of 20% protein, 61% carbohydrates and
35 19% fat. There was a significant overall response to lunch in all of the parameters
36 measured (time effect for all $p < 0.01$). The cortisol response to lunch was not
37 significantly different between the groups (time*treatment $p = 0.882$). Overall, both
38 groups showed the same pattern of cortisol secretion (treatment $p = 0.839$). SBP,
39 DBP, MAP or HR responses (time*treatment $p = 0.726, 0.898, 0.713, 0.620$,
40 respectively) were also similar between higher and lower fitness women. Results
41 suggest that the physiological response to food intake in women is quite resistant to
42 modification by elevated physical fitness levels.

43
44 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 Stephoe 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.

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

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

71

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

78

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

89

90 **Materials and Methods**

91 Women (n=44) aged 30-50 years were recruited using newspaper and online
92 advertisements, emails, fliers in community centres and medical clinics. Exclusion
93 criteria were prior diagnosis with Cushing's syndrome, any stress or anxiety disorder,
94 depression, any diseases of the adrenal gland, type 2 diabetes, heart disease
95 (including use of a pacemaker), high cholesterol, stroke or cancer. This information
96 was self-reported by the participants via a telephone interview. Given the influence
97 of sex steroids on activity of the stress pathways (Kajantie and Phillips 2006), post-
98 menopausal women, peri-menopausal women and all women who were on any form
99 of steroidal contraception (including oral contraceptives, steroidal implants and
100 steroidal IUDs) were excluded from the study.

101
102 All participants provided written informed consent prior to participation in the study.
103 All procedures were approved by the Human Research Ethics Committee of Deakin
104 University (Project code: 2011-242) and conformed to the guidelines of the National
105 Health and Medical Research Council's National Statement on Ethical Conduct in
106 Human Research (2007).

107
108
109 ***Experimental procedure***

110 Women reported to the laboratory on two separate days. The first visit was to obtain
111 additional health information (details below), a fasting blood sample for the
112 measurement of cardio-metabolic risk markers and to measure cardiorespiratory
113 fitness (maximum oxygen consumption-VO₂ 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 ($<160\text{mmHg}$ for systolic and $<90\text{mmHg}$ for diastolic). This threshold for systolic was used since isolated systolic blood pressure of $>160\text{mmHg}$ 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 $>140/90\text{mmHg}$. None of the women recruited for this study exceeded a resting blood pressure of $140/90\text{mmHg}$.

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

145

146 Participants were allowed to have a snack (a selection of foods from muesli bars,
147 nuts, dried fruit and juice boxes were made available) after collection of blood. A
148 Physical Activity Readiness Questionnaire (PAR-Q) was filled in at this time to
149 assess if it was safe for each participant to undertake a VO_2 max test. Participants
150 also filled out an International Physical Activity Questionnaire (IPAQ) (Bauman et al.
151 2009) to measure levels of high and moderate intensity physical activity, a State-
152 Trait Anxiety Inventory (STAI) (Spielberger et al. 1983) to measure levels of anxiety
153 and a Beck Depression Inventory (BDI-ii) (Beck et al. 2006) to measure levels of
154 depressive symptoms. Water was available *ad libitum* to all participants throughout
155 the testing session. This was immediately followed by the graded VO_2 max test on
156 an electronically braked cycle ergometer (Lode N.V. Groningen, Netherlands). After
157 ranking women by VO_2 max score, a median split was then used to allocate women
158 evenly into a higher fitness group (n=22) and a lower fitness group (n=22).

159

160

Day 2 testing

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

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

Participants were given a test meal (details below) at 1200h. They were allowed to consume food between 1200h-1230h. Blood samples were collected every 15 min between 1145h-1400h. Systolic blood pressure (SBP), diastolic blood pressure (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 -80°C 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).

209

210 ***Plasma cortisol assays***

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

216

217 ***Statistical analysis***218 ***Preliminary analysis***

219 **Pre- treatment** salivary cortisol was defined as the concentration of cortisol in the
220 sample collected at 1200h. Pre- treatment SBP, DBP, MAP and HR were defined as
221 the average of values recorded at 1145h and 1200h. **Peak height** for cortisol was
222 defined as the highest value obtained for each individual between 1215h- 1400h,
223 inclusive. Peak height for all cardiovascular parameters was defined as the highest
224 value obtained between 1215h-1330h. Data from 1345h- 1400h were not used in
225 this calculation because of the apparent effects on cardiovascular parameters of
226 physical movements during the bathroom break. **Reactivity** was calculated by
227 subtracting the pre-treatment value from the peak height for all parameters. **Area**
228 **under the curve** (with respect to increase) was calculated for cortisol using all
229 values between 1200h – 1400h and for SBP, DBP, MAP and HR using values
230 between 1200h-1330h after the subtraction of the pre-treatment value from each
231 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%.

Results

Participants

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

Participant characteristics

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

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

Test meal

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

Plasma cortisol

Plasma concentrations of cortisol in lower fitness and higher fitness women are shown in Figure 1 and Table 4. Repeated measures analysis of variance revealed that there was a significant effect of time ($F(9, 33) = 2.657$, $p = 0.05$; Figure 1). 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

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

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

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

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599 **Tables**600 **Table 1:** Mean ($\pm$ SEM) descriptive characteristics of lower and higher fitness women

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

601 * univariate analysis of variance

602

Table 2: Mean ($\pm$ SEM) of cardio-metabolic risk markers and mental health scores in lower fitness and higher fitness women

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

* univariate analysis of variance

607 **Table 3:** Mean ($\pm$ 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 $\pm$ 162	2094 $\pm$ 116	0.811
Protein (g)	19.7 $\pm$ 1.8	19.3 $\pm$ 1.6	0.847
Carbohydrate (g)	58.9 $\pm$ 4.6	59.6 $\pm$ 3.3	0.901
Fat (g)	17.5 $\pm$ 2.3	19.2 $\pm$ 2.0	0.592
Sodium (mg)	1047 $\pm$ 115	860 $\pm$ 72	0.176

609 * univariate analysis of variance

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Table 4. Mean ($\pm$ SEM) pre-treatment cortisol, peak height of cortisol, cortisol 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 $\pm$ 16.7	136.1 $\pm$ 12.7	0.900
Peak height (ng/mL)	172.9 $\pm$ 13.8	175.9 $\pm$ 14.2	0.881
Reactivity (ng/mL)	34.2 $\pm$ 10.3	39.8 $\pm$ 8.3	0.673
AUC (ng*min/mL)	-433.1 $\pm$ 1045	-263.3 $\pm$ 822	0.900

* Univariate analysis of variance

AUC = Area under the curve

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Table 5: Mean ($\pm$ SEM) pre-treatment, peak height, reactivity and area under the 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 $\pm$ 4	104 $\pm$ 2	0.115
	Peak height (mmHg)	125 $\pm$ 4	116 $\pm$ 3	0.057
	Reactivity (mmHg)	14 $\pm$ 3	12 $\pm$ 3	0.642
	AUC (mmHg*min)	188 $\pm$ 163	104 $\pm$ 118	0.688
DBP	Pre-treatment (mmHg)	65 $\pm$ 2	62 $\pm$ 2	0.374
	Peak height (mmHg)	72 $\pm$ 2	72 $\pm$ 3	0.833
	Reactivity (mmHg)	8 $\pm$ 2	10 $\pm$ 3	0.607
	AUC (mmHg*min)	-166 $\pm$ 90	-216 $\pm$ 112	0.730
MAP	Pre-treatment (mmHg)	82 $\pm$ 3	77 $\pm$ 2	0.119
	Peak height (mmHg)	90 $\pm$ 3	87 $\pm$ 3	0.507
	Reactivity (mmHg)	7 $\pm$ 2	10 $\pm$ 3	0.503
	AUC (mmHg*min)	-148 $\pm$ 115	-165 $\pm$ 124	0.920
HR	Pre-treatment (bpm)	69 $\pm$ 2	64 $\pm$ 2	0.113
	Peak height (bpm)	80 $\pm$ 2	71 $\pm$ 2	0.005
	Reactivity (bpm)	11 $\pm$ 2	7 $\pm$ 1	0.084
	AUC (bpm*min)	239 $\pm$ 129	54 $\pm$ 69	0.212

*Univariate analysis of variance

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

Figure captions

Figure 1: Mean ($\pm$ 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 ($\pm$ 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.

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