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CLINICAL STUDY

Daily timing of salivary cortisol responses and aerobic performance in lean and obese active females

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Abstract: The main aim of the present study was to study the effects of morning and afternoon physical activities on cortisol responses in obese and lean women. Twenty women volunteered to participate in this study. Subjects were divided into an obese group (BMI =29.1 kg/m^2) and a lean group (BMI =19 kg/m^2). All subjects participated in an exercise program consisting of treadmill running at 65±2 % VO_{2max} until exhaustion. In order to study effects of circadian rhythm, exercise was performed at a similar intensity and in similar environmental conditions at both 8:00 AM and 4:00 PM. Saliva specimens were collected at rest 20 minutes before activity and then immediately after the exercise in both morning and afternoon sessions. Morning and afternoon exercise resulted in a significant increase in salivary cortisol concentrations compared to basal levels in both lean and obese women (p<0.05), though the change in cortisol concentrations were higher in lean. The aerobic function of lean and obese women in the morning and afternoon showed a significant increase of 13.8 % and 5.9 %; respectively, with lean being consistently higher than obese. In conclusion, the stress response to exercise is related to circadian rhythm and individual's body weight. Based on the results of this study, it is suggested that overweight women perform exercises in the afternoon to minimize the stress response for the exercise volume performed *(Tab. 1, Fig. 3, Ref. 39)*. Full Text in free PDF *www.bmj.sk.*
Key words: salivary cortisol, circadian rhythm, aerobic function, women.

Cortisol is released from adrenal glands and is among the stress hormones excreted in response to physical and psychological stress (Miller and O'Callaghan, 2002). Cortisol excretion allows a routine course and the range and amount of excreted is modulated by circadian rhythm (Raff et al, 1998). It has been demonstrated that before waking up in the morning, plasma concentration of this hormone is relatively high, but decreases gradually during the day reaching a nadir in the early evening (Edwards et al, 2001).

Studies carried out on the cortisol response to physical activity are contradictory. One study has shown that exercise at high or moderate-intensity (90 % and 70 % VO_2max) produces a similar response in the morning and evening (Galliven et a., 1997), yet others have shown that baseline (before exercise) and peak cortisol concentrations were significantly higher at 07.00 than at 19.00 or 24.00 (Kanaley et al, 2001). In agreement with the latter, Miles and coworkers (2008) observed decreased cortisol in the afternoon compared to a morning exercise session. Clearly, more thorough investigation of how time of day impacts on the stress hormone response to exercise is required.

In addition, only a few studies have distinguished between the cortisol concentrations in obese and lean humans and again these results are somewhat contradictory. Some research shows that the resting level of cortisol is higher in obese than lean individuals (Garlasch, 1975; Bjorntorp, 1996) (Putignano et al, 2001), yet others show no difference (Wong and Harber, 2006). A comparison of cortisol levels between these two body types in response to exercise is less well documented. Similarity in response between non-obese and obese subjects has been shown by Gustafson et al. (1990), whereas others (Jungmann et al, 1991) observed a significantly greater cortisol response to exercise in the obese compared with lean subjects (Wong and Harber, 2006). Contradictory findings may reflect differences in prescribed exercise duration and intensity (Duclos et al, 1997).

Although there are many published studies on alterations in the stress hormone response, as indicated by salivary cortisol levels, to aerobic activity, existing information is not directed to diurnal effects or a comparison of obese and lean women. Thus, the purpose of this study was to describe the effects of diurnal rhythm on the salivary cortisol response to exercise and how this may differ in obese and lean women.

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Material and methods

Subjects

Twenty sedentary women volunteered to participate in this study. After giving oral and written information about the study's plans and procedures, subjects signed an informed consent. Subjects were divided into two groups (obese and lean) according to their body mass index (BMI). Those with a BMI equal or greater than 29.1 kg/m^2 were allocated to the obese group and those with BMI less or equal to 19 kg/m^2 were allocated to the lean group. The interview and examination by physicians showed that subjects were in good state of health. All subjects were non-smokers, were not under medical treatment, not on a diet, and did not engaged in a regular exercise (Tab. 1).

Anthropometric and performance measurements

The degree of fatness was assessed using body mass index [weight (kg)/height (m^2)]. Height and body mass were measured using a digital scale, with the subject wearing a light gown. Five days before the first exercise test, each subject was familiarized with the procedures for running on the treadmill (Techno Jim HL 4200). For the first test subjects were asked to attend the laboratory in the morning (08.00) after a 9±2 hour fast for an ergo metric treadmill test to measure aerobic fitness. Maximal oxygen consumption (VO_2max) was estimated from time to exhaustion using a modified Bruce protocol (McInnis et al, 1994) and an electronic treadmill.

Daily timing of physical exercise

After estimation of VO_{2max}, subjects were asked to again attend the physiology laboratory two days later at 07.00 am after 10 hours of rest and following a light breakfast. Saliva samples were collected and then each subject ran on the treadmill at a speed estimated to elicit 65±2 % VO_{2max} until exhaustion (Fig. 1). Four days later subjects came back to the laboratory to perform the same protocol at 4 pm. In order to obtain the net effects of the circadian rhythm, similar conditions to the morning session (environmental conditions, intensity and duration of exercise and fluid intake before and during running) were maintained.

Saliva sampling

For measurement of salivary cortisol before exercise, subjects sat down and rested for 25 minutes before a saliva sample was collected. The saliva samples were collected 20 minutes before activity (pre exercise) and immediately after physical exercises (post exercise) in both morning and afternoon as follows. Before starting the exercise and during rest, each subject drank one glass of water (200 ml) to prevent dehydration and to wash their mouth. After few minutes subjects put four milliliters of non-stimulated saliva inside collection tubes. A salivary sample was again collected after 30 minutes of exercise following a similar procedure. Both samples were put on ice and sent immediately to the laboratory for freezing at –20 degrees centigrade for subsequent testing. In addition, to prevent any environmental influences apart from time of day, samples were taken from all subjects in similar conditions.

Tab. 1. Demographic and anthropometric data (means ± SD) for the subjects at the start of the study.

	Obese	Lean
Age (yr)	24.7±2.9	24.6±2.0
Body weight (kg)	82.35±6.07	49.90±3.73
Height (m)	162.5±4.86	166.60±4.33
BMI (kg/m^2)	31.2±1.6	17.9±0.5
Vo_{2max} (ml.kg^{-1}.min^{-1})	32.84±1.59	29.4±1.6

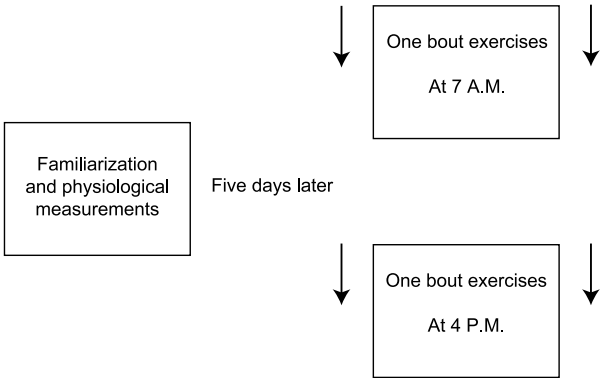

Fig. 1. Study design. ↓ indicating salivary sampling.

Hormonal assay

Salivary cortisol concentration was measured by enzyme linked immunosorbant assay (ELISA). The kit for cortisol was obtained from RADIM (Pomezia, Italy) with sensitivity 6 pg/ml and standard range 10–5000 pg/ml. All assays were carried out in duplicate. Quality controls were included in all series of determination. All samples from the same individual participants were tested in the same series to avoid any variations between assays.

Statistical methods

The results were expressed as the mean ± SD. Data for the pre- and post-exercise trials at 8 am and 4 pm were compared using a two-tailed, paired *t*-test in each group. Unpaired *t*-test was used to compare the statistical differences in lean and obese groups. Differences among means were considered statistically significant by the criterion of probability value =0.05.

Results

An acute bout of exercise in the morning significantly enhanced salivary cortisol concentration in both obese (p=0.001) and lean (p=0.001) subjects but this enhancement was greater in lean group than obese. Similarly, exercise in the afternoon enhanced salivary cortisol concentrations in both groups and again enhancement was greater in the lean (p=0.001) group than the obese (p=0.001). Comparison of cortisol concentrations in the

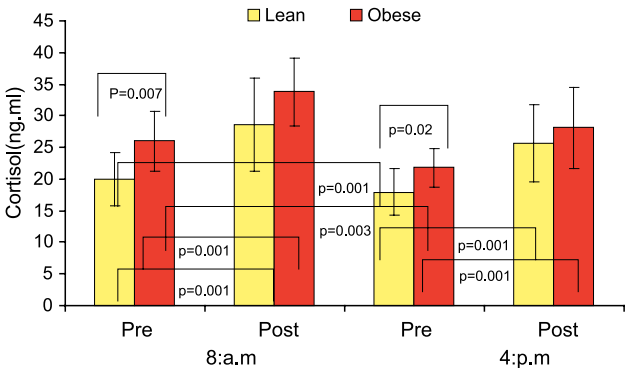

Fig. 2. Mean levels of salivary cortisol for each group before and after exercise at 8 am and 4 pm. (Error bars indicate S.D.)

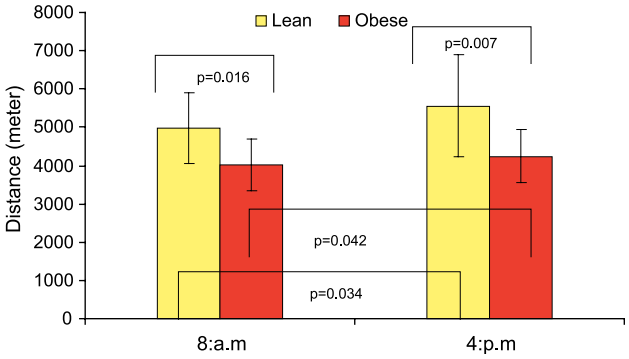

Fig. 3. Running distance for each group at 8 am and 4 pm (Error bars indicate SD).

morning and afternoon indicated that the level of this hormone was greater in the morning than the afternoon (p=0.001). Comparison between resting state of saliva cortisol indicated that both in the morning (p=0.007) and afternoon (p=0.02) obese subjects (or individuals) have greater absolute values than the lean participants (Fig. 2).

Aerobic performance in both groups significantly increased in the afternoon compared to the morning (obese group: p=0.042, lean group: p=0.034). Comparing the aerobic performance of obese and lean women indicated that both in morning (p=0.016) and afternoon (p=0.007), it was relatively less in obese women (Fig. 3).

Discussion

The first finding of this study was that one session of exercise either in the morning or afternoon resulted in a significant increase in salivary cortisol concentration in both obese and lean women. This finding is in agreement with the results of previously published studies (Shinakai et al, 1996; Kern et al, 1995; McMarray et al, 1995).

Stimulation of the hypothalamus – pituitary – adrenal axis (HPA) and increase in ACTH excretion from pituitary is the most important factor in cortisol excretion (Lutoslawska et al, 1991). During physical activity, the HPA is activated, the outcome being an increase in secretory rate from the adrenal glands. Circulating cortisol concentrations rise as decreased metabolic clearance by liver during exercise appears insignificant (Kuoppasulmi et al, 1980; Lac et al, 1997).

The intensity of exercise is one of the important factors to which the HPA axis. responds In order to activate this process, exercise needs to be performed it an intensity near the anaerobic threshold (Snegovskaya and Viro, 1993; Väänänen et al, 2002). The products synthesized through anaerobic metabolism such as lactate accumulation, fall of pH and hypoxia are among the specific stimuli in HPA axis via stimulation of ACTH excretion (Filaire et al, 1996). Previous studies have demonstrated that running on treadmill for 8 to 20 minutes increases ACTH concentration up to ten-fold from rest and in this condition, the cortisol concentration increases in parallel to ACTH (Buono et al, 1986).

In the present study, the subjects ran on treadmill until exhaustion at a workload calculated to elicit approximately 65%VO2max. Although ACTH concentration was not measured it is likely that this intensity and duration of exercise would cause a significant increase in ACTH concentration and thus cortisol concentration. Another mechanism which increases cortisol concentration, especially free cortisol, is an increase in body core temperature (Deligiannis et al, 1993; Lac et al, 1997). Both factors facilitate release of the hormone from protein carriers and increase the 'free' cortisol concentration in the blood, which is then reflected in salivary concentrations (Mcmurray and Hackney, 2004). In the present study, since there is only free cortisol in saliva, the increase in saliva cortisol could be attributed to an increase in cortisol release from carrier proteins and/or changes in the carrier proteins.

Circadian rhythm is another important effective factor in cortisol excretion in normal conditions and following exercises. The synthesis and excretion of this hormone follows the circadian rhythm (Cauter et al, 1996;, Kanaley et al, 2001). Previous studies have demonstrated that cortisol concentration is in its highest level prior to waking up from sleep due to increase in the range and amount of excretion; however, its excretion decreases gradually during the day and thus its concentration lowers in both blood and saliva and reaches its nadir at night (Davidson et al, 1991; Thuma et al, 1995).

In present study the cortisol concentration before the exercise both in obese and lean subjects in the morning was greater than in the afternoon confirming that cortisol excretion follows a circadian rhythm regardless of body composition. Another interesting point was that, although the intensity of exercise was the same in the morning and afternoon, the amount of cortisol in the afternoon was less than morning, showing that respond of cortisol to exercise is affected by the circadian rhythm.

Adiposity is another potential factor in circulating cortisol concentrations. Results of previous studies show that cortisol concentrations in obese people are higher than lean individuals (Garlaschi et al, 1975; Rosmond et al, 1998); however, other

studies did not confirm this finding and reported that there was no significant differences in the basal levels of cortisol with same resting levels between obese and lean people (Pasquali et al, 1998; Paulet al, 1999; Wong and Harber, 2006). Findings of present study are in agreement with the thesis that cortisol excretion in obese people is higher than in lean individuals. Indeed the basal levels of cortisol were higher in obese human than lean human in all conditions (morning and afternoon) both before and after exercise suggesting that the increase in adiposity increases the sensitivity of HPA axis. The relationship is such that in people suffering from Cushings syndrome or those with serious depression, cortisol excretion is high and fatness is common. In this group of patients, hyptercortisolisms is quite common and their basal cortisol value is higher than normal people.

The results of other studies showed that the proportion of waist to hip, as one of the indexes for obesity status, being also related to the amount of cortisol secretion during physical activities (Elissa et al, 2000; Westerbacka et al, 2003). In the present study, although the basal levels of cortisol was the same in obese and lean individuals, there was significant differences in concentration of cortisol after exercise and the level of increase was higher in obese subjects ((Pasquali et al, 1998; Wong and Harber, 2006). However, this was associated with a relatively decreased exercise endurance in the obese group, suggesting that even for the same relative exercise intensity, obese women are under a greater hormonal stress. This is perhaps not surprising considering that the increase in BMI in the heavier group was likely fat mass and not recruitable muscle.

An interesting finding of this study was the difference between exercise capacity in morning and afternoon, with subjects running further in the afternoon than morning. This observation is in agreement with others (Hill et al, 1992; Hill, 1996; Souissi et al, 2007). The probable mechanism of this phenomena is presently not clear.

Conclusion

The results of this study showed that one bout exercise leads to significant changes in the salivary cortisol concentration in obese and lean women in both mornings and afternoons. However, the stress response to exercise was greater in obese women even when their exercise capacity was relatively lower. Furthermore, in both groups the cortisol response was less in the morning than the afternoon. An important effect of cortisol secretion is to increase hepatic glucose release. For obese subjects, who are likely to also suffer from hyperglycaemia, minimizing the stress response will be useful for glycaemic control. Our data suggest that a more acceptable and effective exercise prescription in obese women may be to prescribe exercise in the morning than that afternoon.

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