Cortisol Responses and Energy Expenditure at Different Times of Day in Obese Vs. Lean Men

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Abstract: The effect of time of day on cortisol responses and energy expenditure to exercise in obese vs. lean men was studied. Six obese (VO$_{2\text{max}}$ 35.45±3.9 ml/min/kg; BMI 31.08±1.8 kg/m$^2$) and six lean (VO$_{2\text{max}}$ 45.86±3 ml/min/kg; BMI 18.2±1.3 kg/m$^2$) sedentary men was studied. Subjects exercised at a constant velocity on a treadmill for 30 min of 65% maximal oxygen consumption beginning at 08:00 and 18:00. Student’s $t$-test and one-way ANOVA with repeated measure was used to evaluate cortisol, EE, VO$_2$ and RER between groups. Results showed that during baseline, there were no differences between lean and obese groups for salivary cortisol in morning and evening. In contrast, immediately, 15-min and 30-min after exercise obese group had significantly higher cortisol concentration than lean group (p<0.05). There were no significant differences between cortisol responses to 30-min exercise in lean and obese groups. Mean energy expenditure and oxygen consumption during exercise in evening were significantly higher than morning (p<0.05). In conclusion body fat percent affect on cortisol response and energy expenditure, obese men had higher EE and cortisol level than lean men. Also, time of day can modulate cortisol responses and EE in obese and lean men.

Key words: Cortisol, Energy expenditure, Lean vs Obese.

INTRODUCTION

Cortisol is the primary glucocorticoid secreted by the adrenal cortex and an important regulatory hormone for blood glucose homeostasis [1]. Exercise of appropriate intensity is a potent stimulus for cortisol secretion. The cortisol responses to exercise is dependent on the relative exercise workload, but other factors modulate these hormonal responses, including the mode and duration of exercise, anaerobic vs. aerobic exercise, prior meal ingestion, body fat mass and fitness level of the subject [2-5]. Underlying pulsatile and circadian/diurnal rhythms of this hormone [cortisol] potentially may modulate the exercise response observed [4, 6]. Cortisol secretion is pulsatile and is highly dependent on time of day, with a morning maximum, declining levels throughout the afternoon and evening, a quiescent phase centered around midnight, followed by an abrupt rise toward morning levels [7, 8].

Few studies have assessed the impact of time of day on cortisol responses to exercise. Three studies have reported that the cortisol responses to exercise are similar in the morning and evening [3, 9, 10]. In contrast, Scheen and colleague [1998] employed a low exercise intensity that elicited a cortisol response in the afternoon, but no response in the morning or evening [11]. Kanaley et al. [2001] reported that both baseline and peak cortisol concentrations were significantly higher at 07:00 h than at 19:00 or 24:00 h [12]. When young men ate to increase body fat mass, cortisol production rates were increased [2], indicating that slight to moderate obesity induced by over nutrition is followed by elevated cortisol secretion [2]. Although resting cortisol level in the obese may be higher compared with those in lean individual [13, 14], its response to exercise is not well documented. Some have documented that there was no significant difference between lean and obese groups in fasting salivary cortisol concentration in different times of day [15-17]. Whereas others [18] observed a significantly greater cortisol responses in the obese compared with the lean subjects. Contradictory findings may be due to differences in prescribed exercise duration and intensity [1, 17]. Therefore additional investigations are needed to clarify the cortisol responses to exercise.
More recently, salivary cortisol has been confirmed to be a valid, reliable, stress-free and noninvasive indicator of the biological active free fraction of serum cortisol levels. Correlation ranging from r=0.70-0.99 between salivary and serum cortisol have been reported in adults at rest, as well as exercise [10, 19, 20]. Finally, saliva is easy to collect, less risky to handle than blood and due to its noninvasive nature, it may be more appealing to the subject than the collecting of blood [1, 20, 21].

Studies showed that obesity has risen to epidemic proportions [22] and in adults it is associated with development of type 2 diabetes and other chronic disease such as coronary heart disease [CHD], hypertension, osteoarthritis, pulmonary dysfunction and some form of cancer [22-24]. Some studies reported that the degree of adiposity, as calculated from the sum of triceps and medial calf skinfolds, contributes to the weight-relative energy cost of walking and running [25, 26]. Energy expenditure during weight-bearing exercise is higher in obese children and adolescents, when compared with nonobese controls [27-30]. There are a lot of studies about measuring energy expenditure during rest and exercise in adolescents [25, 27-30], but to our knowledge there is no any study that investigates effect of different times of day on energy expenditure in obese and lean men.

Although effect of time of day on components of sport performance have been all documented [11], it is not known whether metabolic and hormonal responses to exercise vary according to time of day, because almost all detailed studies have been performed in the morning [31], therefore the present study wanted to examin the influence of time of day on cortisol responses and energy expenditure to exercise in obese vs. lean men.

**MATERIAL AND METHODS**

Twelve Healthy, untrained \( VO_{2\max} <50 \text{ ml/kg/min} \) and less than three sessions per week of physical activity] men between the ages of 20 and 25 yr agreed to participate in this study. Six obese subjects [BMI >30] and six lean controls [BMI <20] were studied. The characteristics of the subjects are listed in table 1. Self-administrated questionnaires established participants to be nonsmoking, free from diabetes, hypertension and coronary heart disease and they were not taking medications known to influence metabolism and cortisol secretion. All subjects were weight stable (±3 kg) at least 3 months before our study. Subjects were instructed not to engage in any strenuous exercise on the day preceding an experimental test and they participated in 3 separate trials spaced between 5 to 7 days from each other. The order of trials was randomized and followed a counterbalanced format. An institutional ethics review board at University of Guilan-Iran approved this study and all volunteers provided written informed consent before participation.

| Table 1: Characteristics of obese and lean subjects. |
|-------------|-------------|-------------|
| **Obese (n=6)** | **Lean (n=6)** |
| Age (yr) | 22.83 ± 7 | 21.17 ± 0.4 |
| Height (cm) | 177.67 ± 2.8 | 178.25 ± 7 |
| Weight (kg) | 98 ± 4.2 | 58.08 ± 1.6 |
| BMI (kg/m²) | 31.08 ± 7 | 18.2 ± 5 |
| Fat mass (kg) | 28.22 ± 1.8 | 8.45 ± 7 |
| FFM (kg) | 69.76 ± 8.3 | 49.55 ± 2.9 |
| Body fat (%) | 28.25 ± 9 | 10.25 ± 5 |
| \( VO_{2\max} \) (liter/min) | 3.4 ± 0.2 | 2.6 ± 0.1 |
| \( VO_{max} \) (ml/kg/min) | 35.45 ± 1.6 | 45.86 ± 1.2 |

Values are means ± SD. FFM: Fat Free Mass; BMI: Body Mass Index.

\(^{\text{a}}\) Significantly different from lean (P< 0.05).

**Measurement and Protocols:** Body composition and aerobic exercise capacity [maximal oxygen consumption \( VO_{2\max} \)] were determined approximately 1 week before the first admission. Maximal oxygen consumption was determined using an incremental treadmill protocol. The treadmill (COSMED ITALY MED150) velocity was initially set at 3 km/h at 0% grade and every 1 min the velocity was increased by 1 km/h. Subjects were verbally encouraged throughout the test and the test was terminated when the subject reached volitional fatigue. \( VO_{max} \) was considered to be attained if any two of the following criteria were met: 1) volitional exhaustion; 2) RER greater than 1.1; 3) maximum age-predicted heart rate (220 - age) is attained; and 4) peak and plateau in \( VO_{2} \) (<100 ml/min) despite increased work load [17]. Percent body fat was assessed using skinfold caliper (Laffayette Caliper, Model 01127, USA) to measure subcutaneous fat, with three sites method on the right side of the body [32]. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

**Energy Intake:** To standardized metabolic conditions among subjects during the test period, all subjects were given a standard meal that contained 16 Cal/kg BW (50% carbohydrate, 30% fat and 20% protein) [12], 8 h before the exercise test in morning and 5 h before the exercise test in evening [10]. After the standard meal, subjects fasted until the study was completed.
Testing Protocol: On the second session of reporting to the laboratory, each subject changed into exercise clothes and rested in a sitting position for 20 min and then baseline saliva sampling collected at 08:00 in morning session. Exercise began at 08:00 with a warm-up period of 5 min at 40% and 5 min at 50% $\text{VO}_2\text{max}$. After this, 65% $\text{VO}_2\text{max}$ exercise was began and continued for 30 min. An intensity level set at 65% $\text{VO}_2\text{max}$ was selected because key hormonal responses are elicited at higher intensity exercise (>60% $\text{VO}_2\text{max}$ and/or >20 min) [17].

Oxygen consumption was measured continuously using standard open circuit method (COSMED, Quark $\text{b}^2$, s.r.l. Rome, Italy) during warm-up and exercise period. Calibration of the gas analyzer was performed before and immediately after each test. As a result of the familiarization sessions, minimal adjustments were required during the experiment. Saliva samples were obtained immediately, 15-min and 30-min after exercise.

On the third session of reporting to the laboratory, each subject rested in a sitting position for 20 min and then baseline saliva sampling collected at 18:00 in evening session. Exercise began at 18:00 with a warm-up period of 5 min at 40% and 5 min at 50% $\text{VO}_2\text{max}$. After this, 65% $\text{VO}_2\text{max}$ exercise was began and continued for 30 min. Saliva samples were obtained immediately, 15-min and 30-min after exercise.

Saliva samples were refrigerated until the end of the session and then frozen to solidify mucus and other contaminants. Samples were then thawed and centrifuged and the supernatant removed, refrozen at -20°C and stored until all sessions for each subject were completed. Salivary cortisol concentrations were measured in duplicate by using a commercially prepared ELISA kit (Diagnostics Biochem Canada, Inc) with modified procedures suggested by manufacturer. A lower limit of detection for saliva cortisol was 1.0 ng/dl.

Statistical Analysis: Results are reported as the mean±SD. Student’s $t$-test was used to compare subject characteristics and $\text{VO}_2$, RER, EE and cortisol concentration for OG and LG. A two-way ANOVA [obese vs. lean] with one factor repeated measure [time] was used to evaluate $\text{VO}_2$, RER, EE and cortisol between groups during the baseline, exercise and 15-min and 30-min after exercise periods. For all statistical procedures, the significance level was set at $p<0.05$.

RESULTS

Characteristics of the obese and lean subjects are reported in table 1. Results demonstrated that higher body fat percent did not increase baseline cortisol in OG compared with LG and salivary cortisol level before exercise at 08:00 and at 18:00 were similar in obese and lean subjects. Also, during baseline protocol there were no significant differences detected between groups for $\text{VO}_2$, RER and EE in the morning and in the evening.

Results for cortisol response to 30-min exercise at 65% $\text{VO}_2\text{max}$ are presented in figure 2. Salivary cortisol level immediately, 15-min and 30-min post exercise in the OG were greater than those in the LG ($p<0.05$). Peak cortisol concentrations in response to exercise were noted at time 30 and were significantly greater at 08:00 than at 18:00 in both groups ($p<0.05$). Circadian rhythms did not have a significant effect on the magnitude of the cortisol responses to 30 min exercise and there were no significant differences for cortisol responses between groups in the morning and in the evening. The statistical analysis indicated that exercise significantly elevated salivary cortisol levels immediately, 15-min and 30-min post exercise compared with resting values for both groups in the morning and in the evening ($p<0.05$). (Figure 1 and 2).

| Table 2: Mean EE, RER and $\text{VO}_2$ in lean and obese group in the morning and in the evening |
|---------------------------------------------------------------|----------|---------------|----------------|
|                                                                | Obese    | lean          |
|                                                                | 08:00    | 18:00         | 08:00          | 18:00         |
| **EE** (Kcal/min)                                              | 10.4 ± .5| 12.5 ± .8     | 8.4± 0.3       | 9.1 ± 0.3     |
| **EE** (Kcal/min/FFM)                                          | 0.181± 0.003 | 0.205 ± 0.009 | ± 0.003       | ±0.007       |
| **RER**                                                       | 0.96± 0.01 | 0.90± 0.01    | 0.95± 0.01    | 0.93 ± 0.04  |
| **$\text{VO}_2$** (ml/min/kg)                                  | 23.04± 1.0 | 25.29±.85    | 29.8±8         | 31.33 ± .7   |

Values are means ± SD. FFM: Fat Free Mass
** Significantly different from lean ($p < 0.05$).
* Significantly different from morning ($p < 0.05$).
Results for mean EE, RER and VO\textsubscript{2} in 30-min exercise at 65\% VO\textsubscript{2}\text{max} are presented in table 2. Our results revealed a significant effect of time of day on RER, VO\textsubscript{2} and EE in both group. During exercise mean RER in lean and obese subjects were significantly lower at 18:00 than at 08:00 (p<0.05) and mean EE and VO\textsubscript{2} during 30-min exercise in both groups were significantly higher at 18:00 than at 08:00 (p<0.05). Also in OG mean EE and VO\textsubscript{2} during 30-min exercise were significantly higher than LG in the morning and in the evening (p<0.05).

**DISCUSSION**

The purpose of this study was to investigate the influence of time of day on cortisol responses and energy expenditure to exercise in obese vs. lean men. During the baseline condition, there were no detectable differences between the lean and obese groups. Resting RER, VO\textsubscript{2} EE and concentrations of cortisol were similar between LG and OG, agreeing with the findings of others [19, 17]. Studies have suggested that the hypothalamus pituitary adrenal (HPA) axis is hypersensitive in obesity with predominance of central, visceral adipose tissue depots [13]. The consequences of this will be a frequent over stimulation of the HPA axis, with elevated secretion of cortisol concentrations [2, 33]. Finding from our study showed that cortisol concentration immediately, 15-min and 30-min post exercise in OG were significantly higher than those in the LG in the morning and in the evening and these have been documented by others [17, 34, 35]. In contrast, Garlaschi et al. reported that physical exertion caused no change in cortisol levels in the groups [14]. Studies show that cortisol response to exercise is dependent on the relative exercise workload, mode and duration of exercise and body fat mass [2-5]. Therefore comparing data from different studies is difficult, because the subjects varied in age, BMI and metabolic condition [i.e. some were diabetic].

The cortisol level increased during exercise, possibly explained as the end product of HPA axis activity stimulated in turn by the intensity and duration of the running test. Previous studies have shown that exercise at more than 60\% of VO\textsubscript{2}\text{max} increase the cortisol levels [10, 14, 17]. However, after 15-min and 30-min post-exercise cortisol level decreased in OG and LG in the morning and evening, as has been shown elsewhere [1]. This can be explained by negative feedback regulation [19]. However, the large inter subject variability in cortisol levels makes interpretation difficult. This suggests it as unsuitable as a monitoring variable for groups of subjects, although it may prove useful for monitoring individuals [19, 36]. Circadian rhythms did not have a significant effect on the magnitude of the cortisol response to 30-min at 65\% VO\textsubscript{2}\text{max} treadmill running, which is in line with other studies [9, 10, 12]. This indicates that exercise induced cortisol response is independent of variations throughout the day.

Dimitriou et al. [2002] found that the swimming exercise induced cortisol response was greater (76.1\%) in the evening than the morning (21.1\%), suggesting that the exercise induced response of salivary cortisol is primarily dependent on the baseline levels measured before exercise, which in turn are mainly regulated by circadian rhythms [19].

Results showed that mean EE and VO\textsubscript{2} during 30-min exercise at 65\% VO\textsubscript{2}\text{max} in lean and obese subjects were significantly higher at 18:00 than at 08:00. Also, during
exercise RER in both groups were significantly lower in the evening than in the morning. The body temperature and VO₂ displays a circadian rhythm and decreases to a minimum during sleep at around 04:00 and being to increase before wakefulness [37]. This increase usually continues until the acrophase of the rhythm are reached at around 18:00 [37]. Plasma levels of epinephrine and norepinephrine also are peak in the afternoon [36]. A possible interpretation of our findings is that the increase in catecholamine and body temperature may be able to increase EE, VO₂ and also fat metabolism and lead to lower RER in the evening compared to the morning. We showed that mean energy expenditure (kcal/min or kcal/min/FFM) and VO₂ during 30-min treadmill exercise in OG were significantly higher than those in the LG and these have been documented by others [27, 29, 38, 39]. Total body mass is a major determinant of the gross energy cost during weight-bearing activities such as walking and running [27]. The authors suggested that differences in EE between groups might reflect mechanical differences in gate style, which reduce economy of exercise in OG. Also, it is possible, thought that the difference in metabolic cost results from differences in the cost of ventilation. The obese subjects had a lower tidal volume and a higher respiratory rate [27]. Whipp and Davies [1984] found that the energy cost of breathing is excessive in obese adults, especially at high ventilatory levels [40]. Probably the differences in mean EE between groups may due to higher body weight and total body mass in OG than LG and increase cost of ventilation and mechanical differences in gate style, which reduce economy of exercise in obese subjects.

CONCLUSION

It can be concluded that body fat percent affect on cortisol responses and energy expenditure and after 30-min of exercise at 65% of VO₂max, energy expenditure and cortisol level in OG were significantly higher than LG. Also time of day can affect on metabolic condition and mean EE and VO₂ during 30-min exercise in obese and lean men were significantly higher at 18:00 than at 08:00. During exercise RER in both group were significantly lower in the evening than in the morning. Thus we suggest that the optimal time for training and weight loss purposes—that is, the time of day with the least immunosuppressive effect—is the evening, because basal and post exercise cortisol levels are low [19] and total energy expenditure is higher. Although the current study include relatively small numbers of participants (n=12), we suggest further studies on large samples of the populations to realize the effect of time of day on cortisol responses and energy expenditure.

REFERENCES


