

**Association of Exercise Induced Salivary Cortisol Levels
To Exertional Percpetion and Affect**

by

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University of Pittsburgh, 2008

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The purpose of this research study was to examine the association of cortisol levels to exertional perception and exercise induced changes in affect during a bout of exercise. It was expected that increased levels of salivary cortisol would be associated with a negative shift in affect in response to a submaximal and maximal treadmill test. It was also hypothesized that increased levels of salivary cortisol levels would be associated with an increased perception of exertion during a submaximal and maximal treadmill test. Males and females between 27 and 35 years of age (n=33) were recruited to participate in this study. Salivary cortisol and affect were assessed at baseline, 5 minutes after the submaximal treadmill test, 5 minutes after the maximal treadmill test, and 30 minutes after the maximal treadmill test. Affect was measured using the Total Mood Disturbance (TMD) score of the Profile of Mood Scale (POMS). Perceived exertion (RPE) was measured using the OMNI Scale of Perceived Exertion. Final RPE for legs, chest and breathing, and overall body was assessed during the final minute of the submaximal treadmill test, and immediately at the end of the maximal treadmill test. Session RPE was assessed 5 minutes after the cessation of both the submaximal and maximal treadmill test. Partial correlations were conducted to examine the association between cortisol values, affect, and RPE. Results indicated there was a positive correlation between cortisol values and negative affect. This association was found only in males and the correlation was strongest in both the change in cortisol levels from baseline to 5 minutes and 30 minutes after the maximal treadmill test and the change in affect from baseline to 5 minutes after the maximal treadmill test. Final RPE assessed for the maximal treadmill test was positively correlated with cortisol levels 30 minutes after the maximal treadmill

test in females. There appears to be evidence that increased cortisol levels post exercise are associated with a negative mood shift following a maximal treadmill test and higher perceived exertion during a maximal treadmill test. Future examination of the association among cortisol levels, affect, and perceived exertion during exercise is warranted.

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1.0 INTRODUCTION

The purpose of this research is to examine the association of cortisol levels to exertional perception and affect during and after a bout of exercise. This chapter is composed of the following sections: (1.1) Rationale, (1.2) Significance, (1.3) Specific Aims, and (1.4) Research Hypotheses.

1.1 RATIONALE

Physical activity can improve both physical and psychological health in people of all ages. Many studies have shown that physical activity can reduce the risk of certain diseases such as obesity, hypertension, and heart disease (43). Regular physical activity has also been shown to increase an individual's feelings of well-being and to help relieve depression, anxiety, and stress (43). Yet despite all these benefits, many Americans remain sedentary in their leisure time or have less than recommended levels of physical activity. Thus, it is imperative to gain an understanding of the physiological and psychological mechanisms that influence physical activity behavior.

Many studies have examined the psychological mechanisms that may affect physical activity behavior. Results indicate that mediators such as social support, self-efficacy,

depression, and perceived stress may influence decisions to refrain from or engage in physical activity (6). However, studies have noted that some of these psychological mediators are correlated with physiological mechanisms (21). For example, catecholamine secretions have been found to be associated with psychological stress and depression (21). The stress hormone cortisol has been speculated to be a physiological response that leads to a psychological influence on physical activity. Cortisol is a glucocorticoid that is secreted from the adrenal cortex. It is also the key regulator of the physiological stress response in humans. While a certain amount of this hormone is essential, and many situations such as physical activity can normally increase cortisol levels, abnormally elevated or prolonged cortisol secretion can be detrimental to one's health. Studies have shown that prolonged secretion of cortisol is associated with major depression and anxiety (21). There is evidence that elevated cortisol levels are positively related to situations that simulate feelings of fear, anxiety, helplessness, and loss of control (14, 15).

Overall, most research has found that high intensity physical activity increases cortisol levels. The greater the intensity, the greater the rise in cortisol (39). However, this rise may be more apparent in certain individuals. A few studies have indicated that individuals who are less active have significantly greater cortisol responses to physical activity than those who are regularly active (16, 35, 39). In addition, studies have shown that participation in a regular physical activity program may decrease the cortisol response to exercise over time (21).

The link between negative affect and elevated cortisol levels has been examined during physical activity. Previous research has found a positive association between negative affect and exercise induced rises in cortisol levels (35). There may also be a physiological link between cortisol levels and ratings of perceived exertion (RPE). Previous studies found a positive relationship between cortisol levels and RPE during physical activity (2, 35, 42). For example, one study examined resistance exercise and found that final ratings of RPE were correlated with cortisol levels post exercise (10). This study suggests that higher perceived effort is associated with greater stress from physical activity which could stimulate the hypothalamic-pituitary-adrenal (HPA) axis which results in an increase in cortisol levels (11). It has been suggested that perhaps a greater perception of effort and negative affect in combination with higher levels of cortisol during physical activity may contribute to why some individuals have difficulty adhering to an exercise program or engaging in physical activity on a regular basis (35).

Studies have examined RPE and cortisol levels and affect and cortisol levels during physical activity. However, few studies have examined the relationship of exercise induced cortisol levels to both RPE and negative affect. The studies that have looked at all three variables have found mixed results (23, 35) One study (35) found a weak association among cortisol levels, affect, and RPE during a bout of exercise while another study (23) that examined these three variables among trained athletes did not find an association. Therefore, further examination of this relationship between exercise induced cortisol levels and perceived exertion and negative affect is needed.

1.2 SIGNIFICANCE

There are several implications of this research. People who exhibit higher levels of cortisol prior to a bout of physical activity may either view physical activity as a stress or they may naturally have abnormally high levels of cortisol. High levels of cortisol have been linked to a variety of adverse health effects, so perhaps these individuals may benefit from less intense exercises that have been shown to directly lower cortisol levels. Also, the predicted association of cortisol to RPE and mood can help us understand how cortisol may influence exercise adaptation and adherence. Regular physical activity has been shown to have many positive health effects, but if individuals feel negativity and greater perception of effort in the beginning of their activity, they may drop out of the program and never obtain the many benefits of physical activity. By understanding the psychological and physiological effects of physical activity on cortisol levels, interventions could be implemented to directly target this area. Studies have shown that low intensity exercise, such as yoga or Tai Chi, decrease cortisol levels (12). This type of activity may be a more suitable choice for an individual who is just beginning to engage in physical activity and may be having negative experiences during and after physical activity.

1.3 SPECIFIC AIMS

The purpose of this study will be to examine the association of cortisol levels to exertional perception and exercise induced changes in affect during a bout of exercise.

1.4 RESEARCH HYPOTHESES

The research hypotheses of this study are:

1. It is hypothesized that increased levels of salivary cortisol will be associated with an increased perception of exertion during a submaximal and maximal treadmill test.
2. It is hypothesized that increased levels of salivary cortisol will be associated with a negative shift in affect in response to a submaximal and maximal treadmill test.

2.0 REVIEW OF RELATED LITERATURE

The following literature review discusses the various studies that have examined cortisol in many different settings. The first section will first examine the role of cortisol in the body. The next section will look at the importance and quality of the measurement of salivary cortisol, as well as its advantages and disadvantages. Then, the influence of physical activity on cortisol levels will be examined. The following sections will discuss the relationship between cortisol and affect, as well as the relationship between cortisol and perceived exertion.

2.1 ROLE OF CORTISOL

Cortisol is a steroid hormone that is released in the body in response to physical and psychological stress. Due to circadian rhythm, daily cortisol profiles are characterized by a morning peak, which decreases in late morning and afternoon, followed by low levels at night (15). Cortisol is produced in the cortex of the adrenal glands located on top of each kidney. Two major functions of cortisol are energy regulation and mobilization. Cortisol regulates energy by selecting the amount and type of energy substrate that is needed by the body for a particular situation. Cortisol

mobilizes energy by delivering triglycerides to active muscles. Under stressful conditions, cortisol can provide the body with protein for energy production through gluconeogenesis, which is the process of converting amino acids into glucose in the liver. Cortisol also supports the growth of adipocytes into mature fat cells. Finally, cortisol may act as an anti-inflammatory agent by suppressing the immune system during times of physical and psychological stress. A certain amount of cortisol is necessary for life. However, an excess amount of cortisol can have adverse effects. Some of these negative effects include loss of muscle tone, immune system suppression, depression and anxiety, and loss of bone mass. Therefore, it is necessary for individuals to maintain normal levels of cortisol. It is important to have valid and reliable instruments to measure cortisol levels (14, 15, 17).

2.2 ASSESSMENT OF CORTISOL

Cortisol levels can be measured in plasma, urine, and saliva. Urinary sampling is not accurate in assessing short-term changes in endocrine activity, and therefore, is not widely used (31). Much attention has been given to the assessment of cortisol in saliva, due to the easy, stress free, and non-invasive nature of saliva collection (10, 14, 15, 46). However, measurements of salivary cortisol will only be of value if they reflect the plasma level of cortisol. Many studies have examined the relationship between salivary and plasma cortisol levels (10, 14, 15, 27, 41, 46).

Peters et al. (27) conducted a study that compared the behavior of cortisol concentrations in saliva with those in plasma. Peters found that salivary cortisol concentrations reacted similarly in terms of increasing and decreasing with plasma unbound cortisol during various situations. Umeda et al. (41) looked at morning levels of salivary cortisol and serum unbound cortisol and, similarly, found that the values were almost identical with a correlation coefficient of 0.893. In saliva, cortisol values were $0.99 \pm 0.42 \mu\text{g}/100\text{ml}$ and in serum, cortisol levels were $1.56 \pm 0.54 \mu\text{g}/100\text{ml}$. Other studies have found that salivary cortisol levels are lower than serum total cortisol concentrations (10, 14, 15). This is because only unbound cortisol reaches the saliva and elicits glucocorticoid effects (14, 15, 41). Umeda et al. (41) reported that changes in cortisol levels in response to stimulation are seen more accurately in salivary cortisol than serum total cortisol. Therefore, the monitoring of salivary cortisol may be superior to that of serum total cortisol in various clinical situations. Peters et al. (27) also reported that salivary cortisol and plasma free cortisol may provide information of greater diagnostic significance than total plasma concentrations because total concentrations may be affected by individual differences in plasma binding capacity that may not accurately reflect the true cortisol response. This belief is shared by numerous other researchers that have compared salivary and plasma cortisol (10, 14, 15, 31, 41). Therefore, cortisol levels in saliva have shown to accurately reflect the values of plasma unbound cortisol and can act as a tool in providing diagnostic information. Table 1 summarizes the findings of several studies that compared salivary cortisol to serum unbound cortisol.

Table 1: Comparison of salivary and serum unbound cortisol

Reference	Subjects	Results/Comments
Peters et al. 1982 (27)	n = 20 (males), 22 – 32 years	Salivary cortisol was nearly identical to plasma free cortisol. $r = 0.97$
Gallagher et al. 2006 (9)	n = 26 (males), 18 – 32 years	Salivary cortisol correlated highly with Plasma levels. $r > 0.85$
Umeda et al. 1981 (41)	n = 10 (males), 24 – 33 males	Salivary cortisol correlated highly with Serum unbound cortisol. $r = 0.893$
Gozansky et al. 2005 (10)	n = 12, 10 females, 2 males 23 – 65 years	Salivary cortisol was significantly Correlated with plasma free cortisol. $r = 0.89$

2.2.1 Advantages and Disadvantages of Salivary Cortisol

The assessment of cortisol in saliva has become a widely used method for several reasons. Kirschbaum and Hellhammer (14) noted that due to its stress-free sampling, laboratory independence, and lower costs, saliva cortisol assessment has several advantages over plasma cortisol analyses. While Vining and McGinley (46) noted these advantages, they also mention a few disadvantages of salivary collection. Sensitive assays are required to assess saliva levels and sometimes subjects have difficulty with “spitting” to collect saliva. However, this disappears when the use of an aid to salivation is implemented (46). Gallagher et al. (9) argued that using aids to stimulate saliva production would compromise the accuracy of the results. This study compared cortisol

levels in plasma, saliva, and saliva collected using a salivette device. A salivette device is a tube that contains a cotton swab for participants to chew on, stimulating saliva flow. The results showed that cortisol levels from unstimulated saliva and saliva collected using the salivette device correlated highly with plasma levels. Gozansky et al. (10) reported that a big advantage of monitoring salivary cortisol rather than total serum cortisol is that it eliminates the need to account for within-subjects changes or between-subject differences in cortisol binding globulin. Overall, many studies are now implementing the assessment of salivary cortisol over plasma assessment (10, 14, 15, 31, 41, 46).

2.3 CORTISOL AND PHYSICAL ACTIVITY

2.3.1 Intensity

Salivary cortisol has been shown to increase in response to various types of physical activities and exercise. It is important to note that this response to physical activity does not appear to differ between males and females (15). The level of intensity appears to play a major role in this elevation. In a review of several studies examining the role of glucocorticoids and exercise, Tharp (39) reported that in light to moderate physical activity, studies found mixed results on whether cortisol levels increased, decreased, or did not change significantly. In moderate to exhaustive physical activity,

nearly all the studies reported significant elevations in cortisol. It appears that higher amounts of cortisol are needed by the body during heavier bouts of physical activity (39).

Duclos et al. (7) examined cortisol reactions to strenuous exercise in a sample of eight endurance trained athletes. Subjects ran between 65 to 75% of VO_2 max for two hours. Cortisol was collected before and after exercise. Salivary cortisol values were significantly higher at the end of two hours of exercise than before exercise values (11.5 ± 1.3 vs. $6.5 \pm 0.8 \text{ nmol.l}^{-1}$, $p < 0.001$). O'Connor and Corrigan (22) examined the response of salivary cortisol levels to a submaximal and maximal bicycle ergometer test. For the submaximal test, the subjects exercised until they reached 75% of their VO_2 max. After both exercise tests, significant elevations in cortisol were seen in comparison to their pre-exercise levels immediately after exercise and 15 minutes after exercise. This is in agreement with other studies that reported that a given workload of greater than 60% of VO_2 max will cause an increase in cortisol levels (14, 15). However, not all studies have seen these results. For example, Ben-Aryeh et al. (3) examined salivary levels in 34 healthy adults during a submaximal ergometric test and a Wingate anaerobic test. Cortisol levels were measured 90 minutes before exercise and immediately after exercise. There was no significant increase in cortisol levels (10.76 ± 4.04 vs. $11.60 \pm 4.10 \text{ } \mu\text{g/dl}$). In contrast to this finding, Hellhammer and Kirschbaum (14) advise that investigators should obtain more than one post-exercise sample and that, if possible, a sample should be obtained about 30 minutes after exercise. Hellhammer and Kirschbaum have found that subjects show increasing salivary cortisol levels throughout short term physical

activity of an adequate workload ($>70\text{VO}_2\text{ max}$) with peak concentrations approximately 20 – 30 minutes after cessation of activity (14).

While many studies reported an increase in cortisol levels in workloads greater than 60% of $\text{VO}_2\text{ max}$, other studies reported a drop in cortisol levels in workloads that are below 50% of $\text{VO}_2\text{ max}$. Jin (12) examined cortisol levels in participants engaging in Tai Chi and found that cortisol levels were lower than baseline measures as well as values obtained at the same time under normal conditions.

Different intensities appear to influence the cortisol response to resistance exercise as well. McGuigan et al. (19) measured the salivary cortisol response to both high and low intensity resistance training protocols. There was a significant increase in the level of salivary cortisol immediately following the high intensity exercise session whereas the low intensity exercise session did not result in any significant changes in cortisol levels. The authors concluded that salivary cortisol can be used to delineate between high and low intensity exercise programs.

2.3.2 Physically Fit vs. Sedentary

Several studies have suggested that cortisol levels respond differently in active and sedentary individuals during exercise. Mathur, Toriola, and Dada (17) examined the short term exhaustive exercise on the serum levels in well trained distance runners and nonathletes. All the subjects participated in a maximal bicycle exercise test. Cortisol was assessed pre-exercise, post-exercise, and after one hour of recovery. Results showed

that the pre-exercise levels of cortisol in both groups were within normal limits. However, cortisol levels increased by 36.9% after exercise in the distance runners, but after one hour of recovery, the increase dropped to 18.6%. Cortisol levels in the nonathletes increased by 161.3% immediately after exercise, and after one hour of recovery, had rose to 173.9% from pre-exercise levels. These data suggest that there is a smaller increase in cortisol levels immediately after exercise in trained athletes, as well as a higher rate of dissipation in cortisol levels after exercise. Traustadottir et al. (40) tested the hypothesis that aerobic fitness would result in a blunted stress response during exercise in older women. They found that older fit women had significantly lower cortisol responses to stressors than older unfit women.

These studies are in partial agreement with Rudolph and McAuley (35) who looked at salivary cortisol levels during acute exercise in runners and non-runners. Though not significant, there was a trend toward higher cortisol levels in non-runners over runners during exercise. However, the reason that their findings were not significant may have been because they had subjects exercise at 60% of their VO_2 max which is at the lower end of the suggested intensity needed to produce significant rises in cortisol levels. Also, they reported that cortisol responses in the runners dissipated more quickly after exercise than in the non-runners. Rudolph and McAuley (34) conducted another study that examined self-efficacy and salivary cortisol responses in physically active and sedentary adults. Similar to the prior studies, cortisol levels increased in both groups during exercise, however, cortisol responses in the less active participants remained elevated 10 and 30 minutes postexercise and the cortisol responses in the active

participants decreased 10 and 30 minutes post exercise. In fact, the cortisol responses dropped below baseline at 30 minutes after exercise in the active participants. Table 2 summarizes the results of the studies examining cortisol and physical activity.

Table 2: Relationship between cortisol and physical activity.

Reference	Subjects	Exercise	Measurements	Results
Duclos et al. 1998 (7)	n = 8 (males) Mean – 41 years	120 min run 65 – 76% VO ₂ max	Salivary and serum cortisol pre and post exercise	Cortisol levels in both saliva and serum sig. increased post run. p < .001
O'Connor & Corrigan 1991 (22)	n = 8 (males) Mean - 22.9 years	Bicycle ergometer 75% VO ₂ max	Salivary cortisol at 15 min intervals before, during, after exercise	Sig. increase in cortisol post exercise and 15 min after. p < 0.05
Ben-Aryeh et al. 1989 (3)	n = 34 (males) Mean – 22 years	Ergometric test 85% VO ₂ max, Wingate Anaerobic test	Salivary and serum pre and post exercise	No significant increase.
Jin 1989 (12)	n = 66, 36 males, 30 females 16 – 75 years	60 min of Tai Chi < 50% VO ₂ max	Salivary cortisol before, during, and after exercise	Sig. decrease in cortisol during and after exercise. p < 0.01
McGuigan et al. 2004 (19)	n = 17, 8 males, 9 females 18 – 25 years	High intensity & low intensity resistance exercise	Salivary cortisol before, after, & 30 min after exercise	Sig. increase in cortisol levels for high intensity after exercise. p < 0.05
Mathur et al. 1986 (17)	n = 12 (males) 24 – 28 years	Maximal cycle test	Pre, post, & 1 hour post exercise	Cortisol values remained elevated post and 1 hour after exercise in non athletes. p < 0.001
Rudolph & McAuley et al. 1998 (35)	n = 26 (males) Runners – avg age – 19.8 Non runners – 21	30 min treadmill run at 60% VO ₂ max	Salivary cortisol pre, during, post exercise	Trend for elevated cortisol levels in non runners. p < 0.10
Rudolph & McAuley et al. 1995 (34)	n = 60 26 active males, 4 active females, 24 inactive males, 6 inactive females	30 min treadmill run at 60% VO ₂ max	Salivary cortisol pre, during, post exercise	Inactive subjects had sig. higher cortisol post exercise. p < .05

2.4 AFFECT AND CORTISOL

Studies have reported that several steroid hormones are affected by stress and mood, however, cortisol is still considered a major indicator of altered physiological states in response to stress and negative affect (15). Affect can be defined as the feelings an individual has that reflects a level of engagement with the environment. The feelings may be brief, longer lasting, or more stable (26). Clinical depression has been linked to hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis and changes at different levels of the HPA system are thought to contribute to the elevation in cortisol levels. Major depressive disorder is characterized by high negative affect and low positive affect (25). Pruessner et al. (30) examined the association of self-reported depressive symptoms with early morning free cortisol levels. Results showed that there was a positive association between self-reported depressive symptoms and morning cortisol levels.

In the study by Jin (12) that looked at low intensity Tai Chi and found that individuals showed a significant reduction in cortisol during and after 60 minutes of Tai Chi, affect was also measured using the profile of mood states (POMS). The results of Jin's study showed that negative affect, which was defined as ratings of tension, depression, anger, fatigue, and confusion were significantly lower during and after Tai Chi than beforehand. Vigor, which is a state of positive affect, was rated higher during and after Tai Chi than beforehand. Rudolph and McAuley (35) also looked at affective states and cortisol during a bout of physical activity. As stated in the previous section, Rudolph and McAuley saw elevated cortisol levels in subjects which were inversely

related to positive affect post exercise. The other study conducted by Rudolph and McAuley (34) that examined self-efficacy and cortisol levels during acute exercise found that self-efficacy was inversely related to cortisol responses 30 minutes after exercise, and pre-exercise efficacy cognitions significantly predicted cortisol levels during exercise. Though self-efficacy is not an actual mood state, it does have a positive effect on negative mood.

Polk et al. (28) examined 334 healthy adults to determine if cortisol level was related to the trait and state components of both negative and positive affect. They found that, in general, positive affect was associated with lower concentrations of cortisol and negative affect was associated with higher concentrations. Buchanan, al' Absi, and Lovallo (5) found similar results when they examined cortisol activity before and after a public speaking stressor and a positively activating and humorous video. They found that negative affect and cortisol increased only during the speech. After watching the video, cortisol levels decreased significantly, while positive affect increased. Smyth et al. (37) also looked at the association of affect and salivary cortisol levels and found that higher cortisol levels were also associated with higher negative affect and lower cortisol levels were associated with higher positive affect. Higher levels of negative affect were related to 0.81 nmol/l higher average cortisol levels (12% increase) and higher levels of positive affect were associated with 0.72 nmol/l lower average cortisol levels (10% reduction). These previous studies all found similar results in that higher cortisol levels were associated with negative affect while lower cortisol levels were associated with positive affect. However, two studies found slightly contrasting results. Van Eck et al. (44)

looked at the effects of perceived stress, mood states, and stressful daily events on cortisol. Similar to the previous studies, Van Eck reported that negative affect and agitation were associated with higher cortisol levels, but found that positive affect was associated with no change in cortisol levels. In agreement with Van Eck's study, Peeters et al. (25) examined the relationship between affect and cortisol and found that higher cortisol levels were associated with increases in negative affect, but positive affect was not associated with cortisol levels.

While there appears to be some disagreement over the relationship between positive affect and cortisol levels, most studies are in agreement that high cortisol levels are associated with more negative affect (5, 28, 30, 37, 44). Table 3 summarizes the results of the various studies that have examined the relationship between affect and cortisol levels.

Table 3: Relationship between cortisol and affect

Reference	Subjects	Measurements	Results
Smyth et al. 1998 (37)	n = 120, 35 males, 85 females Mean age – 36.70	Salivary cortisol, Affect scale	Higher cortisol levels were associated with negative affect. $p < .01$
Vedhara et al. 2000 (45)	n = 60, 36 males, 24 females Mean age - 22	Salivary cortisol, Perceived Stress Scale	Increased levels of stress were associated with lower cortisol levels. $p = .036$
Pruessner et al. 2003 (30)	n = 40, (males) 18 – 35 years	Salivary cortisol, Hamilton Depression Inventory	Higher levels of depressive symptoms were associated with greater cortisol levels. $p < .01$
Van Eck et al. 1996 (44)	n = 87, (males) 27 – 57 years	Salivary cortisol, Positive Affect, Negative Affect, Agitation	Both Negative Affect and Agitation were positively associated with cortisol. $p < .01$ for both
Buchanan et al. 1999 (5)	n = 30, (males) 20 – 30 years	Salivary cortisol, Positive Affectivity/Negative Affectivity	Negative affect increased during a speech stressor. $p < .001$ Cortisol increased during speech as well. $p < .0001$
Polk et al. 2005 (28)	n = 334, 175 females, 159 males 18 – 54 years	Salivary cortisol, Affect questionnaires	Negative affect was associated with higher cortisol levels. $p < .01$
Rudolph & McAuley 1997 (35)	n = 26 (males)	Salivary cortisol, Positive Affect	Positive affect was inversely related to cortisol level post exercise. $r = -.29$, $p < .05$
Peeters et al. 2002 (25)	n = 39, 16 males, 23 females, Mean age - 44	Salivary cortisol, Momentary Mood Assessment	Negative affect was associated with higher cortisol levels. $p < .01$

2.5 CORTISOL AND RATINGS OF PERCEIVED EXERTION (RPE)

Ratings of perceived exertion, or how a person feels, during physical activity are important because they provide a better understanding of subjective symptoms and how they may correlate to objective assessments. An individual's perceived exertion has been shown to be related to several physiological variables such as heart rate, blood pressure, and hormonal changes (4).

Perceptual responses during exercise are affected by various exertional symptoms such as strain, fatigue, and pain. The effort continua model, developed by Borg, explains the relation between physiological demands of exercise performance and the perception of exertion associated with that performance (33). Hormonal regulation is considered to be one of the underlying physiological events that mediate exertional perception (33).

Several studies have reported a link between perceptions of exertion and cortisol levels. Cortisol is secreted in response to emotional and psychological stress, as well as unpleasant sensations that accompany high-intensity exercise (43). Backhouse et al. (2) examined the influence of carbohydrate beverages on perception of exertion during prolonged cycle exercise. Results indicated that cortisol and perceived exertion were positively correlated, while cortisol levels were inversely related to greater feelings of pleasure during the cycling test. Similarly, Rudolph and McAuley (35) compared cortisol levels, perceived exertion, and affective responses in runners and non-runners. Non-runners showed a trend toward higher cortisol levels during exercise and had elevated levels of cortisol after exercise. Non-runners also reported greater perception of effort

and negative affect than the runners. Utter et al. (43) showed similar results dealing with ratings of perceived exertion in a study that determined the influence of exercise mode and 6% carbohydrate versus placebo beverage ingestion on perceived exertion and hormonal regulation during 2.5 hours of high-intensity running and cycling by ten triathletes. Results indicated that lower ratings of perceived exertion were associated with lower plasma cortisol and growth hormone levels. These studies provided support for a physiological link between ratings of perceived exertion and hormonal regulation during exercise.

Hollander et al. (11) compared perceptual and endocrine responses with concentric and eccentric resistance exercise protocols using the same workload. Results revealed that concentric exercises elicited a greater perceptual response and cortisol response than eccentric exercises. A study by Strachan et al. (38) found similar results when they compared the effects of amino acid supplements on perceived exertion and hormone responses during prolonged exercise. While the supplements appeared to have no effect on either perceived exertion or cortisol, both reacted similarly (increased) during each exercise trial. These studies provide evidence that ratings of perceived exertion can be a marker of physiological stress and are related to the same physiological cues of cortisol (11, 38). However, a study by O'Connor et al (21) found contrasting results. O'Connor examined salivary cortisol and perceived exertion in 18 female and 22 male swimmers over three days of increased training. They found that the elevated training was associated with greater perceptions of effort, however, cortisol levels remained

unchanged. Table 4 summarizes the various studies that have examined the relationship between cortisol and ratings of perceived exertion.

Table 4: Relationship Between Cortisol and Ratings of Perceived Exertion

Reference	Subjects	Exercise	Results
Utter et al. 2004 (42)	n = 16, 12 males, 4 females 38 – 58 years	180 min treadmill run, 70 % VO ₂ max	Lower RPE was associated with lower cortisol values
Hollander et al. 2003 (11)	n = 8 (males) 18 – 30 years	Concentric & eccentric resistance training	There was a significant correlation between the last rating of RPE and post exercise cortisol. $r = 0.51$, $p < .05$
Backhouse et al. 2005 (2)	n = 9 (males) Mean age – 25	120 min cycle ergometer 70 – 80% VO ₂ max Placebo & CHO	In the PLA trial, cortisol levels were higher, $p < .05$, affect was less positive, $p < .05$, and RPE was higher, $p < .05$
O'Connor et al. 1991 (23)	n = 40, 18 females, 22 males	3 days of increased swimming trials	Cortisol levels were not associated with RPE.
Strachan et al. 2004 (38)	n = 8 (males), Mean age – 38	3 cycle rides to exhaustion	Both serum cortisol and RPE increased over time.
Rudolph & McAuley 1997 (35)	n = 26 (males)	30 min treadmill run	RPE was positively related to cortisol levels post exercise. $p < .05$
Utter et al. 1999 (43)	n = 10, 8 males, 2 females 25 – 50 years	150 min run or cycle ~ 75% VO ₂ max	Lower RPE was associated with lower levels of cortisol in cycling.

2.6 SUMMARY OF REVIEW OF LITERATURE

Numerous studies have begun using saliva as an alternative method to plasma and urine in the assessment of cortisol due to its non-invasive technique, low cost, and stress free sampling. Studies that have compared the measures of salivary and plasma cortisol have found that salivary cortisol and serum unbound cortisol have nearly identical values. Also, there is substantial evidence that salivary cortisol may provide a more accurate measurement in response to psychological and physiological stimuli.

Physical activity appears to have an acute effect on cortisol levels. Level of intensity and the physical fitness of the individual appear to influence this effect. Studies report that at an intensity level greater than 60% of VO_2 max, cortisol levels will increase while at an intensity level lower than 50% of VO_2 max, the levels will decrease. The literature also indicates that sedentary individuals will elicit greater cortisol responses to physical activity than aerobically fit individuals. Over time, participation in a physical activity program may blunt the increase in cortisol levels during and after a bout of physical activity.

Many studies have reported a positive relationship between cortisol and negative mood. Interestingly, the results relating to the association of lower cortisol levels and positive mood have varied. Ratings of perceived exertion have also been shown to be positively correlated with levels of cortisol. Past studies report that cortisol is secreted in response to emotional stress and negative sensations which may be associated with perceived exertion.

Few studies have examined the association among cortisol, perceived exertion, and negative affect, and the results of these studies have been mixed. However, there appears to be evidence of both a physiological and psychological link among these three variables. Therefore, it is important to further investigate this relationship and examine whether the association among these three variables may help influence an individual's physical activity behavior.

3.0 METHODS

The purpose of this research study was to examine the relationship between cortisol levels, exertional perception, and exercise induced mood changes.

3.1 PARTICIPANTS

This study was incorporated into an existing longitudinal study that aims to examine the psycho-physiological mechanisms that explain level of physical activity participation and spontaneous change in physical activity over a two-year period in a cohort of 848 young adults. Only participants who are part of this cohort were eligible to participate in this study.

The sample was comprised of healthy males and females, ages 27-35 years, that have a racial/ethnic distribution representative of Allegheny County, Pennsylvania and is 81% white, 17% African American, and 2% other. No exclusion criteria were based on race, ethnicity, gender, or HIV status. A total of 30 subjects were recruited to participate in this project.

Subjects were scheduled for a clinic appointment for the ongoing longitudinal study. In order for the subjects to be eligible to participate in this study, their clinic appointment must have been scheduled between 8am and 10am in the morning so as to avoid any confounding effects due to changes in circadian rhythm. In addition, subjects had to be able to complete the two treadmill tests that are part of the clinic protocol. At the clinic appointment, the subjects were given the opportunity to participate in this study. If the subject agreed to participate, he/she were presented with an informed consent document describing the cortisol study which the subject were asked to sign. Subjects who were eligible for the study and agreed to participate would receive a payment of \$10.

3.2 MEASUREMENTS

3.2.1 Treadmill Protocols

Submaximal Treadmill Protocol

The submaximal treadmill test was a modified Balke protocol. The speed of the treadmill was held constant at 3.0 mph with the initial grade set at 0% and progressing by 2.5% at every stage. Each stage was three minutes and the subject straddled the treadmill belt for one minute in between stages. Termination of the test would occur when subjects reached 85% of their maximal heart rate (defined as $220 - \text{subject's age}$). Heart rate,

ratings of perceived exertion and pain, and oxygen consumption (VO_2), were assessed at each stage.

Maximal Treadmill Protocol

The maximal treadmill test was a Bruce protocol. The protocol consisted of three minute stages with no rest in between. The three minute stages were as follows: Stage 1- 1.7 mph at a 10% grade; Stage 2 – 2.5 mph at a 12% grade; Stage 3 – 3.4 mph at a 14% grade; Stage 4 – 4.2 mph at a 16% grade; Stage 5 – 5.5 mph at a 18% grade. Heart rate, ratings of perceived exertion and pain, and oxygen consumption were assessed at each stage. The exercise test was ended by volitional termination due to exhaustion. At the end of the test, subjects were given a cooldown at 2.0 mph and 0% grade for two minutes.

3.2.2 Salivary Cortisol Assessment

The cortisol assessments were only be taken during the morning (8am – 10am) clinic sessions. A total of four salivary cortisol samples were obtained; baseline, after the submaximal test, after the maximal test, and 30 minutes post exercise.

Before the baseline sample was taken, subjects were instructed to rinse their mouth out with water in order to clear away any particles that may alter cortisol levels. The saliva was collected in plastic tubes called salivettes, which contained cotton swabs inside of them. For all sample collections, a lab technician handed the subject a cotton swab that was inside the salivette. The subject placed the swab in his/her mouth and

chewed lightly for 30 – 60 seconds and then handed the swab back to the technician who placed it in the plastic tube. The technician wore gloves for this procedure.

The first saliva sample was taken prior to any of the testing, while the participant was sitting quietly in the interview room. The second saliva sample was taken five minutes after completion of the submaximal test. The lab technicians removed the headgear from the participant and led him/her to a chair in the room to sit down. This procedure was repeated after the maximal exercise test. The final saliva sample was taken 30 minutes after the completion of the maximal test. The participant was seated in a quiet room until the technician came to collect the final sample. After collection, the technician placed the four salivettes in a plastic bag and stored them in a refrigerator until they were taken to the Biobehavioral Oncology and Psychoneuroimmunology Laboratory at the Hillman Cancer Center in Pittsburgh to be processed. These tubes were numbered and labeled with the subject's ID number so as to maintain confidentiality. These samples were frozen and analyzed by radioimmunoassay using an ELISA kit purchased from Salumetrics in State College, PA.

Saliva Processing

On the day of assay, the frozen samples were thawed completely and then placed into a centrifuge at 3000 rpm for 15 minutes. A microtitre plate was coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competed with cortisol linked to horseradish peroxidase for the antibody binding sites. Bound cortisol peroxidase was measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produced a blue color. A yellow color was formed after stopping the reaction with sulfuric acid. Optical density was read on a standard plate read at 450nm. The amount of cortisol peroxidase

detected was inversely proportional to the amount of cortisol present. Special software capable of logistics were used to determine cortisol levels. Once the levels were calculated, they were entered into a Microsoft Excel spreadsheet.

3.2.3 Rating of Perceived Exertion (RPE)

RPE was assessed using the OMNI Scale of Perceived Exertion (32) to measure the participants' feelings of effort and fatigue during the treadmill tests. Prior to the exercise test, participants were oriented to the scale by a clinician who read instructions on how to give their rating of exertion, as well as to define the meaning of perceived exertion. After the orientation, participants were asked to rate their perceived exertion for several different physical activity situations. Participants rated their perception of exertion on a scale ranging from 0, "no effort" to 10, "maximum effort." The participants rated their exertion during each stage of the submaximal and maximal tests. For each stage, the participants rated the exertion they feel in their legs, chest and breathing, and overall body. Five minutes after the end of each test, participants gave an overall rating of their exertion for the entire test (session RPE). This study utilized both the session RPE and the final RPE of the submaximal and maximal exercise tests to examine the relationship to cortisol levels.

3.2.4 POMS (Profile of Mood State)

The parent study was currently using the POMS Brief questionnaire to measure affect. The POMS had 30 items that are rated on a five point scale ranging from 0, "Not at all" to 4,

“Extremely.” There were six subscales that are measured as well as an overall mood disturbance. The discrete affective states that are measured are: tension-anxiety, depression-dejection, anger-hostility, vigor, fatigue, and confusion-bewilderment. The six subscales are each made up of five items that are scored separately. Total mood disturbance was scored by adding the sums of five of the subscales and then subtracting the measure of vigor. The POMS was administered four times: before exercise testing, after the submaximal test, after the maximal test, and 30 minutes after the maximal test.

3.2.5 Past Year Physical Activity Questionnaire

Physical activity was assessed using an interviewer administered past-year questionnaire. This questionnaire consists of a number of leisure time and competitive activities. Participants selected the activities that they have engaged in at least ten times over the past year. Amount of times per week and number of minutes per day were assessed for each activity the participants selected. From these selections, a total number of minutes per week of physical activity were calculated.

3.3 STATISTICAL ANALYSIS

The objective of this study was to examine the association of cortisol levels to exertional perception and affect during and after a bout of physical activity. Specifically, the study was designed to test the hypothesis that an increase in cortisol levels during and after a submaximal and maximal treadmill test was associated with negative affect and increased perception of effort. In this study, there was a total of 33 participants. The correlation effect was expected to be about .5 (21, 27). Based on a priori power analysis, a total of 29 participants were required in order to achieve a power of .80 to detect a correlation of .5. The main variables of interest to be measured were cortisol levels, affect, and RPE. The change in cortisol was measured by subtracting the baseline cortisol values from cortisol levels after the submaximal exercise test, baseline cortisol values from cortisol levels after the maximal exercise test, and baseline cortisol values from cortisol levels 30 minutes after the maximal test. Affect was measured by the change in the Total Mood Disturbance score of the POMS. RPE was measured by the OMNI scale of Perceived Exertion. There was one measure for each of the two timepoints (5 minutes post submaximal exercise test, 5 minutes post maximal exercise test).

Cortisol values, the POMS, and RPE were analyzed as continuous variables. Descriptive statistics were calculated and the distributions were examined for normalcy. Partial correlations were used to examine the relationship between actual cortisol levels and the Total Mood Disturbance (TMD) score of the POMS. Partial correlations were also used to examine the association among the change in cortisol levels at three points (5 minutes post submaximal test, 5 minutes post maximal test, 30 minutes post maximal test), the change in scores on the POMS, and both session and final RPE after the submaximal and maximal test. Partial correlations were used to examine the association between TMD scores and both session and final RPE after the

submaximal and the maximal exercise test. Previous research suggested that past physical activity and aerobic fitness may influence cortisol levels. Past physical activity and maximal oxygen uptake were controlled for in the analysis.

4.0 RESULTS

The purpose of this investigation was to examine the association of cortisol levels to exertional perception and exercise induced changes in affect during a bout of exercise. It was hypothesized that increased levels of salivary cortisol would be associated with an increased perception of exertion during a submaximal and maximal treadmill test. It was also hypothesized that increased levels of salivary cortisol would be associated with a negative shift in affect in response to a submaximal and maximal treadmill test.

Subjects who volunteered for this study were part of a larger cohort participating in an ongoing longitudinal investigation examining psychophysiological influences on physical activity. As part of the established exercise tests protocol, cortisol sampling took place at baseline prior to any exercise tests (Timepoint 1), 5 minutes after the submaximal treadmill test (Timepoint 2), 5 minutes after the maximal treadmill test (Timepoint 3), and 30 minutes after the maximal treadmill test (Timepoint 4). Affect was measured by the POMS, using the Total Mood Disturbance Score (TMD). Measures were taken at: baseline (Timepoint 1); 5 minutes after the submaximal treadmill test (Timepoint 2); 5 minutes after the maximal treadmill test (Timepoint 3); and 30 minutes after the maximal treadmill test. Perceived exertion (RPE) was measured using both final RPE (legs, chest and breathing, overall), and session RPE. Final RPE was taken at the last stage of the submaximal treadmill test and immediately upon termination of the

maximal treadmill test. Session RPE was taken 5 minutes after termination of both the submaximal and maximal treadmill tests.

4.1 SUBJECT CHARACTERISTICS

Thirty three subjects participated in this investigation (14 males, 19 females) with a mean age of 30.52 ± 1.20 years. One participant was excluded from the analysis due to his outlying data. The sample included 78.8% Caucasian and 21.2% African American participants. Almost half (48.4%) of the participants had earned at least a 4 year college degree. Participants had a mean BMI of 26.52 ± 4.80 . The majority of the participants (88.2%) were non-smokers (Table 5).

Table 5: Subject demographics

Variable	Percent (%)	Mean	SD
Females	58		
Caucasion	78.8		
≥ 4 yr degree	48.4		
Non-smoker	88.2		
Age (yrs)		30.52	1.20
BMI (kg/m^2)		26.52	4.80

4.2 CORTISOL VALUES

A Friedman test was conducted to compare cortisol values at Timepoint 1 (baseline), Timepoint 2 (5 minutes after submaximal exercise test), Timepoint 3 (5 minutes after maximal exercise test), and Timepoint 4 (30 minutes after the maximal exercise test). Descriptive characteristics are presented in Table 6. There was a significant difference among median cortisol values ($p < .05$).

Table 6: Actual cortisol values (ug/dl) at each timepoint (n=33)

Time Period	Mean	Median	SD	Skewness	Kurtosis
Timepoint 1	.21	.16	.17	2.2	7.3
Timepoint 2	.16	.10*	.16	2.1	4.8
Timepoint 3	.14	.12*	.21	1.0	.60
Timepoint 4	.26	.21	.21	2.0	5.6

* $p < .05$

Cortisol values by gender were also examined to determine if any significant gender differences occurred. Figure 1 illustrates the actual cortisol values at each timepoint by gender. No significant gender effects were observed across time points.

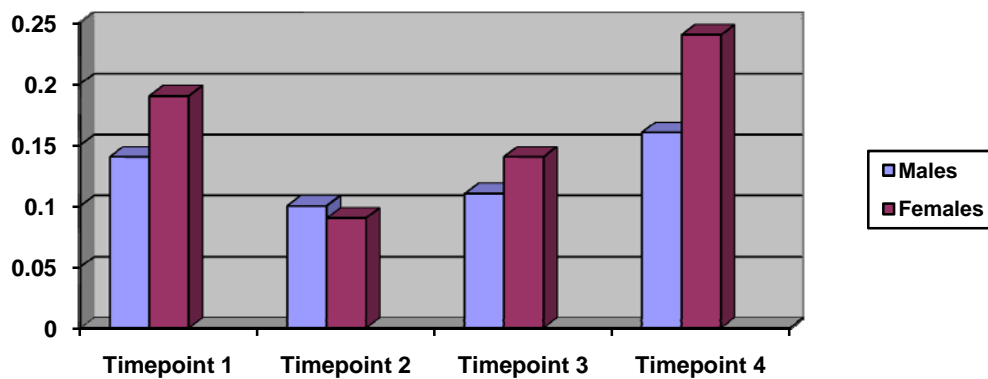


Figure 1: Cortisol values by gender

The change in cortisol values from baseline was also examined as well (Table 7). A Friedman test was conducted to compare the change in cortisol values from baseline to 5 minutes post submaximal treadmill test, 5 minutes post maximal treadmill test, and 30 minutes post maximal treadmill test. There was a significant difference among the change in cortisol values across timepoints ($p < .05$).

Table 7: Change in absolute cortisol values from baseline

	N	Mean	Median	SD
Change 1	33	-.04	-.05	.19
Change 2	33	-.07	-.05	.16
Change 3	33	.06	.00*	.25

*p < .05

Change 1 = change in cortisol values from baseline to 5 minutes post submaximal test,

Change 2 = change in cortisol values from baseline to 5 minutes post maximal test,

Change 3 = change in cortisol values from baseline to 30 minutes post maximal test

4.3 TOTAL MOOD DISTURBANCE SCORES

A one-way repeated measures ANOVA was conducted to compare Total Mood Disturbance (TMD) scores on the POMS at Timepoint 1 (Baseline), Timepoint 2 (5 minutes after the submaximal exercise test), Timepoint 3 (5 minutes after the maximal exercise test), and Timepoint 4 (30 minutes after the maximal exercise test). Descriptive characteristics are provided in Table 8. A significant time effect occurred in Timepoints 3 and 4 (*p<.05).

Table 8: TMD scores at each timepoint (n=33)

Time Period	Mean	Median	SD	Skewness	Kurtosis
Timepoint 1	-1.06	-3.00	7.12	.82	.94
Timepoint 2	-1.70	-2.00	7.16	.86	1.0
Timepoint 3	2.55*	2.00	7.64	.09	1.3
Timepoint 4	-5.07*	-4.00	6.32	.15	-.70

* $p < 0.05$

A mixed between-within subjects ANOVA was used to examine TMD scores by gender to determine if a significant difference occurred in scores between males and females (Figure 2). There were no significant differences in TMD scores at Timepoint 1 ($p = .22$), Timepoint 2 ($p = .18$), and Timepoint 3 ($p = .31$) between males and females. Males had significantly higher TMD scores at Timepoint 4 ($p < .01$) than females.

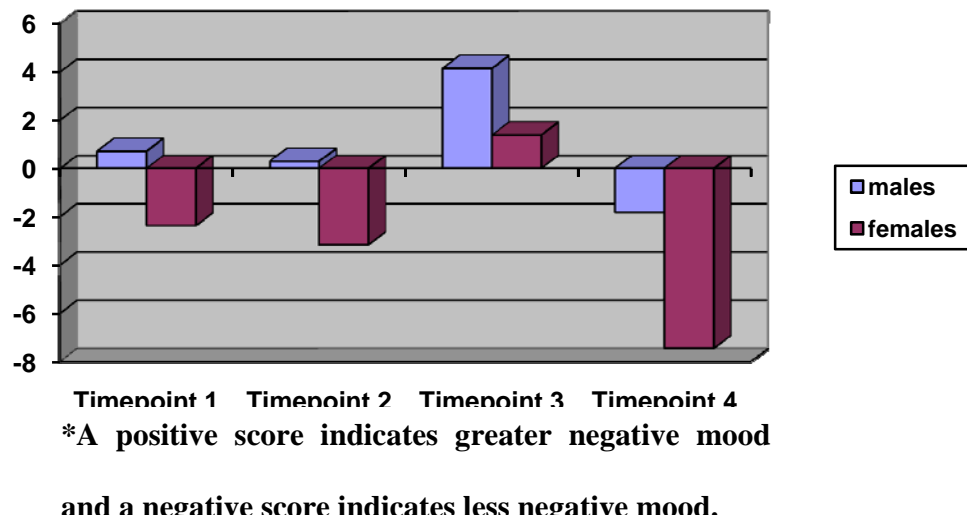


Figure 2: TMD scores by gender

A one-way repeated measures ANOVA was used to examine the change in TMD scores from baseline was calculated as well (Table 9). Change values were significantly different among all three timepoints ($p < .01$).

Table 9: Change in TMD scores (units) from baseline

	N	Mean	Median	SD
TMD Change 1	33	-.64*	.00	7.91
TMD Change 2	33	3.61*	2.00	7.00
TMD Change 3	33	-4.01*	-4.00	6.29

TMD Change 1 = change in TMD values from baseline to 5 minutes post submaximal test,

TMD Change 2 = change in TMD values from baseline to 5 minutes post maximal test,

TMD Change 3 = change in TMD values from baseline to 30 minutes post maximal test

4.4 RATINGS OF PERCEIVED EXERTION (RPE)

RPE was measured during both the submaximal and the maximal treadmill test. Session RPE was taken 5 minutes after the cessation of each test and described the overall feelings of exertion for each test. Final ratings of RPE for legs, chest and breathing, and overall body were also recorded. Final RPE measured the perceived exertion the participant was feeling at that moment, and was obtained during the final minute of the submaximal treadmill test and immediately upon termination of the maximal treadmill test.

4.4.1 Submaximal treadmill test RPE

Post Session RPE

Post session RPE measures were obtained 5 minutes after the cessation of the submaximal treadmill test. For the 33 participants, the mean post session RPE for the submaximal treadmill test was 5.63 ± 2.4 . For males, the mean post session RPE was 5.96 ± 2.3 and for females, the mean post session RPE was 5.39 ± 2.5 . An independent-samples t-test was conducted to compare males and females. There was no significant difference in submaximal post session RPE between males and females ($p=.49$).

Final RPE

Final RPE was taken at the last stage of the submaximal treadmill test. Table 10 presents the final RPE mean for legs, chest and breathing, and overall body for the entire combined sample, males, and females. An independent-samples t-test revealed there were no significant differences in final RPE for legs ($p=.86$), chest and breathing ($p=.88$), and overall body ($p=.69$) for males and females.

Table 10: Mean and SD for final RPE during submaximal test

	Entire Sample	Males	Females
Legs	6.42 ± 2.1	6.5 ± 1.8	6.4 ± 2.4
Chest and Breathing	6.06 ± 2.6	6.14 ± 2.5	6.0 ± 2.7
Overall Body	6.15 ± 2.5	6.36 ± 2.3	6.0 ± 2.7

4.4.2 Maximal treadmill test RPE

Post Session RPE

Post session RPE was taken five minutes after cessation of the maximal treadmill test. For the 33 participants, the mean post session RPE was 8.64 ± 1.24 . For males, the mean post session RPE was 8.71 ± 1.3 and for females, the mean post session RPE was 8.58 ± 1.3 . An independent-samples t-test revealed there was no significant difference in maximal post session RPE scores between males and females ($p=.76$).

Final RPE

Final RPE was taken immediately after cessation of the maximal treadmill test. Table 11 presents the final RPE mean for legs, chest and breathing, and overall body for the combined sample, males, and females. An independent-samples t-test revealed there were no significant differences in RPE for legs ($p=.24$) and chest and breathing ($p=.47$) for males and females. However, females had significantly higher final rating of perceived exertion for overall body ($p<.05$).

Table 11: Mean and SD for final RPE during maximal test

	Entire Sample	Males	Females
Legs	7.94 ± 1.9	7.46 ± 2.0	8.26 ± 1.8
Chest and Breathing	7.88 ± 2.1	7.54 ± 2.2	8.11 ± 2.1
Overall Body	7.63 ± 2.0	6.77 ± 1.9	8.21 ± 2.0

4.5 PHYSICAL ACTIVITY AND MAXIMAL OXYGEN UPTAKE (VO_{2MAX})

Total physical activity (hrs/wk) and aerobic fitness (VO_{2max}) were controlled for their possible influence on cortisol levels in this analysis. For the entire sample, median hrs/wk was 5.16 ± 4.1 and mean VO_{2max} was 35.47 ± 7.6 ml·kg·min⁻¹. For males, median total physical activity were 6.41 ± 4.6 hrs/wk and mean VO_{2max} was 35.51 ± 8.6 ml·kg·min⁻¹. For females, median total physical activity was 3.47 ± 3.3 hrs/wk and mean VO_{2max} was 35.44 ± 7.0

ml·kg·min⁻¹. There was no significant difference in VO_{2max} between males and females. However, males had significantly higher total physical activity than females (p<.05).

4.6 RESULTS OF PARTIAL CORRELATIONS

To examine the relationship among cortisol levels, affect, and perceived exertion, partial correlations were conducted, controlling for total physical activity and maximal oxygen uptake.

4.6.1 Correlation of cortisol values and TMD scores

Partial correlations were used to examine actual cortisol values and TMD scores at the 4 timepoints (baseline, 5 min post submaximal test, 5 min post maximal test, 30 min post maximal test). The r values of this analysis are shown in Table 12. The only significant correlation identified was between cortisol values 5 min post submaximal test and TMD scores 30 min post maximal test (TMD 4) (r=.50, p<.01).

Table 12: Correlations for actual values and TMD scores

	Cortisol 1	Cortisol 2	Cortisol 3	Cortisol 4
TMD 1	.29	.26	.20	-.06
TMD 2	-.18	.27	.29	-.13
TMD 3	-.12	.19	.30	.21
TMD 4	.21	.50**	.34	-.03

** p<.01

The correlations between cortisol values and TMD scores by gender were also examined. Tables 13 and 14 show the r-value for males and females. In males, there were significant positive correlations between cortisol values 5 min post submaximal test and TMD scores 30 min post maximal test ($r=.67$, $p<.01$) and cortisol values 5 min post maximal test and TMD scores 30 min post maximal test ($r=.62$, $p<.01$). In females, no significant correlations existed between cortisol values and TMD scores at any of the four timepoints.

Table 13: Correlations for cortisol values and TMD scores for males

	Cortisol 1	Cortisol 2	Cortisol 3	Cortisol 4
TMD 1	.50	.23	.33	-.12
TMD 2	-.17	.38	.45	-.37
TMD 3	-.23	.10	.40	.07
TMD 4	.31	.67**	.62**	.21

**p<.01

Table 14: Correlations for cortisol values and TMD scores for females

	Cortisol 1	Cortisol 2	Cortisol 3	Cortisol 4
TMD 1	.10	.12	.15	-.05
TMD 2	-.31	.05	.25	-.10
TMD 3	-.21	.07	.32	.26
TMD 4	.15	.26	.29	-.20

4.6.2 Changes in cortisol values and TMD scores

Partial correlations were used to examine the relationship between changes in cortisol values and TMD scores from baseline (Timepoint 1). The r-value is shown in Table 15. The change in cortisol values from baseline to 5 min post submaximal test was positively correlated with the change in TMD scores from baseline to 5 min post submaximal test ($r=.38$, $p<.05$). Also, the change in cortisol values from baseline to 5 min post maximal test was positively correlated with the change in TMD scores from baseline to 5 min post submaximal test ($r=.47$, $p<.01$) and the change in TMD scores from baseline to 5 min post maximal test ($r=.49$, $p<.01$). The change in cortisol values from baseline to 30 min post maximal test was positively correlated with changes in TMD scores from baseline to 5 min post maximal test ($r=.52$, $p<.01$).

Table 15: Correlation matrix for cortisol and TMD change scores

	C1	C2	C3	T1	T2	T3
C1	-	.83**	.49**	.38*	.33	.30
C2	.83**	-	.66**	.47**	.49**	.19
C3	.49**	.66**	-	.23	.52**	.11
T1	.38*	.47**	.23	-	.61**	.70**
T2	.33	.49**	.52**	.61**	-	.43*
T3	.30	.19	.11	.70**	.43*	-

*p < .05 level, ** p < .01

C1 = change in cortisol values from baseline to 5 min post submaximal test,

C2 = change in cortisol values from baseline to 5 min post maximal test,

C3 = change in cortisol values from baseline to 30 min post maximal test,

T1 = change in TMD scores from baseline to 5 min post submaximal test,

T2 = change in TMD scores from baseline to 5 min post maximal test,

T3 = change in TMD scores from baseline to 30 min post maximal test

The correlations among changes in cortisol values and TMD scores from baseline were also examined by gender. Table 16 and Table 17 present the correlation matrix for males and females, respectively. In males, there was a positive correlation between the change in cortisol values from baseline to 5 min post maximal test and the changes in TMD scores from baseline to 5 min post submaximal test ($r=.60$, $p<.05$) and 5 min post maximal test ($r=.68$, $p<.05$). In males, the change in cortisol values from baseline to 30 min post maximal test was positively correlated with changes in TMD scores from baseline to 5 min post maximal test ($r=.77$, $p<.01$). For

females, none of the changes in cortisol values from baseline were significantly correlated with changes in TMD scores from baseline.

Table 16: Correlation matrix for cortisol and TMD scores in males

	C1	C2	C3	T1	T2	T3
C1	-	.86**	.73**	.55	.45	.41
C2	.86**	-	.82**	.60*	.68*	.30
C3	.73**	.82**	-	.45	.77**	.39
T1	.55	.60*	.45	-	.58*	.68*
T2	.45	.68*	.77**	.58*	-	.28
T3	.41	.30	.39	.68*	.28	-

*p<.05, **p<.01

Table 17: Correlation matrix for cortisol and TMD scores in females

	C1	C2	C3	T1	T2	T3
C1	-	.84**	.54*	.33	.29	.09
C2	.84**	-	.75**	.41	.43	.04
C3	.54*	.75**	-	.12	.39	-.11
T1	.33	.41*	.12	-	.65**	.75**
T2	.29	.43	.39	.65**	-	.53*
T3	.09	.04	-.11	.75**	.53*	-

*p<.05, **p<.01

4.6.3 Correlations of RPE and cortisol

Partial correlations were used to examine the relationship between RPE and cortisol values. Session RPE and final RPE for legs, chest and breathing, and overall body were obtained to examine the relationship with actual cortisol values and change in cortisol values from baseline. Final RPE was taken at the final stage of the submaximal treadmill test and immediately upon termination of the maximal treadmill test. Session RPE was taken 5 minutes after the termination of both the submaximal and the maximal treadmill test and represented the perceived exertion for the entire test.

RPE for submaximal treadmill test

The association between RPE for the submaximal treadmill test and cortisol values 5 minutes post submaximal treadmill test (Cortisol 2) was examined for the overall sample, males, and females (Table 18). There were no significant correlations for the overall sample, males, or females ($p>.05$).

Table 18: Correlation of RPE and cortisol for submaximal test

	Cortisol 2 (Entire Sample)	Cortisol 2 (Males)	Cortisol 2 (Females)
Session RPE	-.18	-.44	.10
Final RPE – L	-.13	-.40	.27
Final RPE – C	-.14	-.29	.13
Final RPE - O	-.05	-.15	.17

RPE for maximal treadmill test

The association between RPE for the maximal treadmill test and cortisol values 5 minutes (Cortisol 3) and 30 minutes (Cortisol 4) post maximal treadmill test were examined (Table 19). There was a positive correlation between final overall RPE and cortisol values 30 minutes post maximal treadmill test ($r=.36$, $p<.05$). Table 20 shows the correlation for males and females. For males, there were no significant correlations between RPE and cortisol values at either of the two timepoints ($p>.05$). For females, there was a strong, positive correlation for final RPE in

legs and cortisol values 30 minutes post maximal treadmill test ($r=.60$, $p<.01$). There were positive correlations between final RPE in chest and breathing ($r=.53$, $p<.05$) and overall body ($r=.54$, $p<.05$) and cortisol values 30 minutes post maximal treadmill test.

Table 19: Correlation of RPE and cortisol for maximal test

	Cortisol 3	Cortisol 4
Session RPE	-.02	-.33
Final RPE – L	.12	.32
Final RPE – C	.08	.26
Final RPE - O	.18	.36*

* $p<.05$

Table 20: Correlation of maximal RPE and cortisol in males (M) and females (F)

	Cortisol 3		Cortisol 4	
	M	F	M	F
Session RPE	-.14	.23	-.52	-.08
Final RPE – L	-.01	.31	.15	.60**
Final RPE – C	.01	.17	-.04	.53*
Final RPE - O	.18	.20	.10	.54*

* $p<.05$, ** $p<.01$

4.6.4 Correlation of RPE and cortisol change scores

RPE for submaximal treadmill test

The association of post session and final RPE scores during the submaximal treadmill test and the change in cortisol scores from baseline to 5 minutes post submaximal treadmill test were examined. There were no significant correlations in the sample as a whole, or for either males or females ($p > .05$).

RPE for maximal treadmill test

The association of post session and final RPE during the maximal treadmill test and the change in cortisol values from baseline to 5 minutes (Cortisol Change 2) and 30 minutes (Cortisol Change 3) post maximal treadmill test was examined (Table 21). There were positive correlations between both final RPE in chest and breathing ($r=.37$, $p<.05$) and overall RPE ($r=.43$, $p<.05$) and the change in cortisol values from baseline to 30 minutes post maximal treadmill test. Table 22 presents the r-values for the correlations for both males and females. There were no significant correlations found for males ($p>.05$). For females, there was a strong, positive correlation between final RPE for chest and breathing and the change in cortisol values from baseline to 30 minutes post maximal treadmill test ($r=.58$, $p<.01$). There was a positive correlation between final RPE in legs ($r=.53$, $p<.05$) and overall body ($r=.56$, $p<.05$) and the change in cortisol values from baseline to 30 minutes post maximal treadmill test.

Table 21: Correlation of RPE and cortisol change scores for maximal test

	Cortisol Change 2	Cortisol Change 3
Session RPE	.08	-.23
Final RPE – L	.04	.26
Final RPE – C	.27	.37*
Final RPE - O	.28	.42*

*p<.05

Table 22: Correlation of maximal RPE and cortisol change scores by gender

	Cortisol Change 2		Cortisol Change 3	
	M	F	M	F
Session RPE	.06	.08	-.20	-.10
Final RPE – L	-.09	.28	.01	.53*
Final RPE – C	.21	.43	.18	.58**
Final RPE - O	.29	.39	.26	.56*

* p<.05, ** p<.01

4.6.5 Correlation of RPE and TMD scores

RPE for submaximal treadmill test

The association of TMD scores 5 minutes post submaximal treadmill test and post session and final RPE during the submaximal treadmill test was examined. There were no significant correlations between TMD scores 5 minutes post submaximal treadmill test and RPE for the combined overall sample, males, or females ($p > .05$).

RPE for maximal treadmill test

The association of TMD scores 5 minutes and 30 minutes after the maximal treadmill test and post session and final RPE during the maximal treadmill test was examined. There were no significant correlations between RPE during the maximal treadmill test and TMD scores at either of the two timepoints for the overall sample, males, or females ($p > .05$).

4.6.6 Correlation of RPE and TMD change scores

RPE for submaximal treadmill test

The association of post session and final RPE during the submaximal treadmill test and the change in TMD scores from baseline to 5 minutes post submaximal treadmill test was examined. Table 23 presents the r -values. There was a positive correlation between session RPE and change in TMD scores from baseline to 5 minutes post submaximal treadmill test

($p < .05$). There were no significant correlations found for males. For females, there was a positive correlation between post session RPE and change in TMD scores from baseline to 5 minutes post submaximal test ($p < .05$).

Table 23: RPE and TMD change scores for submaximal test

	TMD Change 1 (Entire Sample)	TMD Change 1 (Males)	TMD Change 1 (Females)
Session RPE	.40*	.00	.57*
Final RPE – L	-.01	.16	-.14
Final RPE – C	.02	.28	-.17
Final RPE - O	.02	.19	-.11

RPE for maximal treadmill test

The association of post session and final RPE during the maximal treadmill test and the change in TMD scores from baseline to 5 minutes post maximal treadmill test and 30 minutes post maximal treadmill test was examined. There were no significant correlations for the entire sample, males, or females.

4.7 SUMMARY OF KEY RESULTS

4.7.1 Cortisol Values

Median cortisol values 5 minutes after the submaximal treadmill test and 5 minutes after the maximal treadmill test were significantly lower than baseline values ($p < .05$). There was an increase in cortisol values 30 minutes post maximal treadmill test from baseline, but the results were not significant. There was a significant difference in the change in cortisol values from baseline to 30 minutes post maximal test and the change in cortisol values from baseline to both 5 minutes post submaximal and maximal treadmill test ($p < .05$).

4.7.2 TMD Scores

Mean TMD scores significantly increased 5 minutes post maximal treadmill test from baseline ($p < .05$) and significantly decreased 30 minutes post maximal treadmill test ($p < .05$). Males had significantly higher TMD scores than females 30 minutes post

maximal treadmill test ($p<.05$). The change values for all three TMD timepoints were significantly different ($p<.01$).

4.7.3 RPE

There were no significant differences in session RPE or final RPE (legs, chest and breathing, overall body) for the submaximal treadmill test between males and females. Females had significantly higher final RPE for overall body for the maximal treadmill test ($p<.05$).

4.7.4 Correlation of cortisol and TMD scores

Overall Sample

Change in cortisol values from baseline to 5 minutes post submaximal treadmill test was positively correlated with change in TMD scores from baseline to 5 minutes post submaximal test ($r=.38$, $p<.05$). Change in cortisol values from baseline to 5 minutes post maximal treadmill test was positively correlated with change in TMD scores from baseline to 5 minutes post maximal treadmill test ($r=.49$, $p<.01$). Change in cortisol values from baseline to 30 minutes post maximal treadmill test was positively correlated with change in TMD scores from baseline to 5 minutes post maximal treadmill test ($r=.52$, $p<.01$).

Males

There was a significant, positive correlation between cortisol values 5 minutes post maximal treadmill test and TMD scores 30 minutes post maximal treadmill test ($r=.62$, $p<.05$). Change in cortisol values from baseline to 5 minutes post maximal treadmill test was positively correlated with change in TMD scores from baseline to 5 minutes post maximal treadmill test ($r=.68$, $p<.05$). Change in cortisol values from baseline to 30 minutes post maximal treadmill test was correlated with change in TMD scores from baseline to 5 minutes post maximal treadmill test ($r=.77$, $p<.01$).

Females

There were no significant correlations between cortisol and TMD scores for either the submaximal or maximal treadmill test.

4.7.4 Correlation of cortisol and RPE

Overall Sample

No significant correlations existed between cortisol or change in cortisol values and RPE for the submaximal treadmill test. There was a positive correlation between final RPE for overall body for the maximal treadmill test and cortisol values 30 minutes post maximal treadmill test ($r=.36$, $p<.05$). There was a positive correlation between final RPE for chest and breathing ($r=.37$, $p<.05$) and overall body ($r=.42$, $p<.05$) for the maximal treadmill test and change in cortisol values from baseline to 30 minutes post maximal test.

Males

There were no significant correlations between cortisol and RPE for either the submaximal or maximal treadmill test.

Females

There was a strong, positive correlation between final RPE in legs ($r=.60$, $p<.01$), chest and breathing ($r=.53$, $p<.05$) and overall body ($r=.54$, $p<.05$) for the maximal treadmill test and cortisol values 30 minutes post maximal treadmill test. There was a strong, positive correlation between final RPE for legs ($r=.53$, $p<.05$), chest and breathing ($r=.58$, $p<.01$), and overall body ($r=.56$, $p<.05$) for the maximal treadmill test and change in cortisol values from baseline to 30 minutes post maximal treadmill test.

4.7.6 Correlation of TMD scores and RPE

Overall Sample

There was a positive correlation between session RPE for the submaximal treadmill test and change in TMD scores from baseline to 5 minutes post submaximal treadmill test ($r=.37$, $p<.05$).

Males

There were no significant correlations between TMD scores and RPE for either the submaximal or maximal treadmill test.

Females

There was a positive correlation between session RPE for the submaximal treadmill test and change in TMD scores from baseline to 5 minutes post submaximal treadmill test ($r=.57$, $p<.05$).

5.0 DISCUSSION

The purpose of this study was to examine the association of cortisol levels to exertional perception and exercise induced changes in affect during a bout of exercise. It was expected that increased levels of salivary cortisol would be associated with a negative shift in affect in response to a submaximal and maximal treadmill test. It was also hypothesized that increased levels of salivary cortisol would be associated with an increased perception of exertion during a submaximal and maximal treadmill test.

5.1 DISCUSSION OF RESULTS

5.1.1 Cortisol Values

Previous studies examining cortisol levels during and after moderate to exhaustive exercise have shown that cortisol levels increase significantly post exercise (35). Acevedo et al. found that cortisol values increased significantly during an incremental exercise test, suggesting that activity of the HPA axis increases with exercise. The release of cortisol facilitates gluconeogenesis, acts as an anti-inflammatory agent, and may aid in the repair and recovery process. These responses may serve to help the body meet the physical and mental demands of

the stress of exercise, and to support steady state adaptations and the return to homeostasis (1). Acevedo found that cortisol values increased significantly during an incremental exercise test (1).

In contrast, the present study found that cortisol values significantly decreased 5 minutes post submaximal and 5 minutes post maximal treadmill test from baseline values. Only cortisol levels 30 minutes post maximal treadmill test increased from baseline, though the results were not significant. Ben-Arah et al. provides partial support for the current findings. This study examined cortisol levels during a submaximal ergometric test and found no significant increase in cortisol levels (3). Although not significant, increased salivary cortisol levels 30 minutes post exercise in the present study is supported by several other studies that found an increase in cortisol levels 30 minutes to 1 hour after exercise in less physically active individuals. Mathur, Toriola, and Dada found that salivary cortisol levels significantly increased from baseline levels one hour after exercise in sedentary individuals (17). A possible reason for the lack of increased cortisol secretion may have been associated with time of day when the testing occurred. Similar to the present study, when Utter and colleagues tested individuals in the morning, a significant increase in cortisol was not found (43). Utter et al. suggested that afternoon testing may help control for the natural morning surge and sharp drop in cortisol levels (43).

Another possible reason why the present study did not find a significant increase in cortisol levels may have been the intensity of the exercise. The submaximal treadmill test may not have provided the intensity needed to elicit an increased cortisol response. Likewise, if individuals did not exert themselves to exhaustion during the maximal treadmill test, the intensity or duration may not have been great enough to elicit increase cortisol secretion.

5.1.2 Correlation of Cortisol and TMD scores

Previous studies that have examined cortisol levels and affect during exercise have found a positive relationship between the two variables (20). Frankenhauser et al. proposed that activation of the HPA axis may be indicative of distress or negative mood (8). Acevedo et al. suggested that if the exercise is perceived as aversive, especially as exercise intensity increases, then there will be an increase in negative mood as well as an increase in cortisol secretion (1). The current study found that cortisol levels 5 minutes and 30 minutes post exercise were associated with negative mood 5 minutes post exercise. There was also a small, but significant correlation between the change in cortisol levels from baseline to 5 minutes post submaximal treadmill test and the change in TMD scores 5 minutes post submaximal test. These findings were partially supported by Acevedo et al. This study found that both cortisol and negative affect were positively correlated during an incremental treadmill test. However, this study did not examine mood after the treadmill test or examine if cortisol levels post exercise were correlated with negative affect (1). This finding was also partially supported by Rudolph and McAuley (35) who found that negative affect during exercise was correlated with cortisol levels 30 minutes post exercise. In contrast to the current findings, Rudolph and McAuley (34) also found that increased negative affect 30 minutes post exercise was positively correlated with increased salivary cortisol 30 minutes post exercise. The present study found a significant decrease in negative mood 30 minutes post exercise, particularly in females. Few studies examined mood after a significant amount of time passed post exercise. Rudolph and McAuley found mood improved, though they implemented an instrument that was different from the POMS to measure affect. The current study used the TMD score, but it may be beneficial to

examine each of the subscales of the POMS to determine if certain subscales are better correlated with cortisol levels. For example, increased levels of salivary cortisol have been shown to be positively correlated with depression and anxiety (20, 29, 43). Therefore, the depression-dejection subscale or the tension-anxiety subscale may be more strongly correlated than any of the other subscales.

5.1.3 Correlation of Cortisol and RPE

Previous studies have shown an association between RPE and cortisol values during exercise. Utter et al. found that lower RPE was associated with lower cortisol values during a 3 hour treadmill run (42). Strachen et al also found that perceived exertion and cortisol responses were positively correlated during exercise (38). These studies provide evidence that ratings of perceived exertion may be indicative of physiological stress and may be related to the same physiological cues of cortisol. The present study examined both localized RPE at the final stage of both a submaximal and maximal treadmill test and session RPE (5 minutes after both treadmill tests). A significant correlation between final RPE was found for the maximal treadmill test and cortisol levels 30 minutes after the maximal treadmill test. These findings were similar to a study by Rudolph and McAuley (35) demonstrating that RPE in the last minute of a 30 minutes treadmill run was associated with cortisol levels 30 minutes post exercise. The findings of this study were also supported by Hollander et al. (11) who found that the last rating of perceived exertion during exercise was correlated with post exercise cortisol levels. Interestingly, the present study found this correlation between RPE and cortisol only in females.

There were no significant correlations in males. The studies mentioned above that provide support for this finding only examined males, suggesting that further investigation of a gender interaction is warranted.

It is interesting that cortisol levels 30 minutes post maximal treadmill test were correlated with RPE and affect 5 minutes post maximal treadmill test. It has been suggested that cortisol may help in repair and recovery from exercise, and that the more physically demanding an exercise is on the body (intensity and duration), the greater and longer lasting the elevation of cortisol levels will be (1). Therefore, it is plausible that strenuous physical exercise would produce a high level of perceived exertion, as well as a greater secretion of cortisol to aid and repair the body during recovery.

5.1.4 Correlation of RPE and TMD Scores

Though this finding was not part of the hypothesis, there appeared to be a correlation between session RPE for the submaximal treadmill test and TMD scores 5 minutes post submaximal test. An increased perception of effort was associated with more negative mood. This finding is in contrast to a study by Backhouse et al. who found that after a two hour cycle ride, affect and RPE were not significantly correlated with each other (2).

5.2 LIMITATIONS

There were several limitations to this study which will be discussed briefly.

5.2.1 Sample Size

The sample size of the current study (n=33) was a significant limitation. Most other studies that examined cortisol during exercise also has small sample sizes (between 8 – 28 participants) and reported this to be a limitation. A larger sample size is needed in order to better understand the relationship among cortisol, affect, and RPE during and after exercise.

5.2.2 Time of Day

Only participants that came in to the clinic between 8am and 10am were eligible to participate in this study. This method was employed to account for the natural circadian rhythm of cortisol levels. However, it appears the early morning hour was a time of peak cortisol secretion which began to significantly decrease throughout the morning. This natural surge and drop in levels may have confounded the effect exercise had on cortisol levels. It is recommended that participants exercise later in the day when natural cortisol activity is low.

Another limitation is time of awakening. Highest cortisol values occur 30 – 90 minutes after awakening. The time of the cortisol peak is not dependent upon absolute time and is not

influenced by daytime. It is completely dependent upon wake-up timing of each individual. In the present study, the awake time of participants was unknown.

5.2.3 Oral Contraceptives and the Menstrual Cycle

The current study did not account for birth control medication or current phase of the menstrual cycle in women which may have a significant effect on cortisol levels. According to Kirschbaum et al., the use of oral contraceptives has been shown to blunt salivary cortisol responses to physical and psychological stress (16). Also, Kirschbaum et al. found that women in the luteal phase of their menstrual cycle had greater salivary cortisol responses than women in their follicular phase (16).

5.2.4 Medication

This study did not control for certain medications which may affect cortisol levels. Medications such as prednisone, dexamethasone, and other steroids have been shown to affect cortisol levels. Use of prednisone tends to result in falsely high cortisol levels, while dexamethasone will significantly suppress cortisol secretion (36).

5.2.5 Individual Biorhythm

This study did not control for unique individual cortisol patterns. Individuals have a set cortisol rhythm profile. The majority of healthy individuals have a morning peak in cortisol

levels 30 – 90 minutes after waking which then swiftly drops throughout the morning hours. However, there are “flat cyclers” who do not show this normal pattern and do not have a morning rise or decline in levels (36). Although this lack of rise may be indicative of dysfunction, this may not be true of all cases. Also, it has been shown that baseline and response levels of cortisol are genetically determined which helps explain interindividual differences of cortisol responses (36).

5.2.6 Treadmill Protocol

Participants that came into our clinic completed both a submaximal and maximal treadmill test with a 5 minute rest in between the tests. Depending on the intensity and duration of the submaximal treadmill test, there may be a residual effect from the submaximal treadmill test that could impair performance on the maximal treadmill test.

5.2.7 Salivette Device

In the present study, Salivettes were used to collect the saliva. Until recently, they were the preferred saliva collection device. Now, it is recommended that use of a special ultra-pure polypropylene sampling device called a SaliCap be used. It has been found that the material used in the Salivettes may interfere with salivary analytes which then may show falsely lowered cortisol levels (36). Future studies should invest in SaliCaps for more accurate cortisol results (36).

5.3 RECOMMENDATION FOR THE FUTURE

The findings of the current study suggest several recommendations for further studies examining the relationship among cortisol levels, affect, and perceived exertion during exercise.

Gender Differences

There appears to be a strong correlation between increased cortisol levels and negative mood in males and a strong correlation between increased cortisol and increased exertional perception in females. In contrast to this study, almost all previous studies examined only one gender group. Further examination of these gender differences is warranted.

Examination of Other Physiological Measures

Along with cortisol, there are several other physiological measures that may be examined to better understand the connection between psychology and physiology during exercise. Measures that may be examined include norepinephrine, epinephrine, and lactate. It may be important to examine the responses of these physiological measures during exercise and to examine the effect they may have on mood and perceive exertion.

Influence of Physical Activity

There has been evidence that past and current physical activity plays a role in cortisol response to exercise. Due to the limited sample size, the current study was unable to divide the participants into categories based on physical activity. Future studies should examine the influence of past and current physical activity on cortisol response, affect, and perceived exertion during exercise.

Examination of Affect During Exercise

The current study examined affect immediately after the treadmill tests, but future studies should examine affect during the treadmill tests as well to better capture mood during exercise. The Feeling Scale, utilized by Rudolph and McAuley (35), has been shown to be a valid measurement of affect both during and after exercise.

5.4 CONCLUSIONS

The purpose of this study was to examine the association of exercise induced cortisol levels to exertional perception and exercise induced changes in affect during a bout of exercise. It was hypothesized that increased levels of salivary cortisol would be associated with a negative shift in affect in response to a submaximal and maximal treadmill test. It was also hypothesized that increased levels of salivary cortisol would be associated with an increased perception of exertion during a submaximal and maximal treadmill test. The hypotheses in the present study were partially supported. After adjusting for total physical activity and maximal oxygen uptake, an association was found between cortisol levels and affect. This association was more apparent in males and the correlation was strongest in both the change in cortisol levels from baseline to 5 minutes and 30 minutes post exercise and mood 5 minutes after exercise. As salivary cortisol levels increased 5 minutes after the maximal test, mood became more negative. Also, the increase in salivary cortisol levels 30 minutes after exercise was positively associated with an increased negative mood 5 minutes after the maximal treadmill test. Perceived exertion during the last stage of the maximal treadmill test was also associated with cortisol levels 30 minutes

after the maximal treadmill test, though this was found primarily in females. There appears to be evidence that increased cortisol levels post exercise are associated with a negative mood shift following a maximal treadmill test and higher perceived exertion during a maximal treadmill test. Future examination of the association among cortisol levels, affect, and perceived exertion during exercise is warranted. Examining the physiological and psychological responses to exercise may assist with the development of better strategies that will help increase exercise adaptation and adherence.

APPENDIX A

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Association of Exercise-Induced Salivary Cortisol Levels to Exertional Perception and Affect

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Why is this research being done?

Being physically active is an important component of health and has been found to decrease the risk of certain diseases such as heart disease and diabetes. Yet with so many benefits, many people do not choose to engage in regular physical activity. Studies have shown that changes in cortisol levels during exercise may play a role in physical activity behavior. Cortisol is a stress hormone that has been associated with a number of adverse health affects. This study will look at how cortisol levels may influence how a person feels while they are exercising. By examining how cortisol may influence exercise adaptation and adherence, interventions could be implemented to directly target this area. A total of 100 subjects will be enrolled in this study.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you have been participating in a study to examine changes in physical activity from adolescence to young adulthood. This research study is an additional project that will be incorporated into a larger study that you are also currently participating in at this time: Psycho-physiological Influences on Physical Activity.

What procedures will be performed for research purposes?

If you decide to take part in this research project, in addition to the procedures that you have agreed upon in the Psycho-physiological Influences on Physical Activity consent form, you will be asked to provide a saliva sample four times during the clinic visit. The procedure involves chewing on a cotton swab for 30-60 seconds. You will be asked to remain at the clinic for an additional 30 minutes after the exercise tests are complete in order to provide us with a final saliva sample and to complete the mood assessment questionnaire.

What are the possible risks, side effects, and discomforts of this research study?

There are no known risks associated with the procedures of this research protocol.

What are possible benefits from taking part in the study?

You will likely receive no direct benefit from taking part in this research study. A benefit of the study is obtaining knowledge on a factor that may influence exercise adaptation and adherence.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course if the study?

You will be promptly notified if, during the conduct of this research study, any new information develops which may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedure performed as part of the research study?

Neither you, nor your insurance provider, will be charged for the costs of any procedures performed for the purpose of this research study.

Will I be paid for this research study?

You will be paid an additional \$10 for providing 4 saliva samples and completing the mood assessment questionnaire.

Who will pay if I am injured as a result of taking part in this study?

University of Pittsburgh researchers and their associates who provide services at UPMC recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of the UPMC. It is possible that the UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the cost of this follow-up unless otherwise specifically stated below. You will not receive any monetary payment for, or associated with, any injury that you suffer in relation to this research.

Who will know about my participation in this research?

Any information about you obtained from this research will be kept confident (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Will this research study involve the use of disclosure of my identifiable medial information?

This research study will not involve the use or disclosure of any identifiable medical information.

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:

- Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable medical information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.
- In unusual cases, the investigators may be required to release identifiable information related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.
- Authorized people sponsoring this research study, NIH, because they need to make sure that the information collected is correct, accurate and complete, and to determine the results of this research study.
- Authorized representatives of the UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for

the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participations, (2) addressing correct payment for tests and procedures ordered by the investigators, and/or (3) for internal hospital operations (i.e. quality assurance).

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?

The investigators may continue to use and disclose, for the purposes described above, identifiable information related to your participation in this research study for a minimum of five years after final reporting or publication of a project.

Is my participation in this research study voluntary?

Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed, in general, to participate in the research study). Whether or not you provide your consent for participation in this research study will have no affect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no

affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. Any identifiable research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no affect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no affect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to take part in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers to protect your safety if you are unable or unwilling to complete the research protocol.

VOLUNTARY CONSENT:

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encourage to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

Participant's signature

Date

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions that the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise.

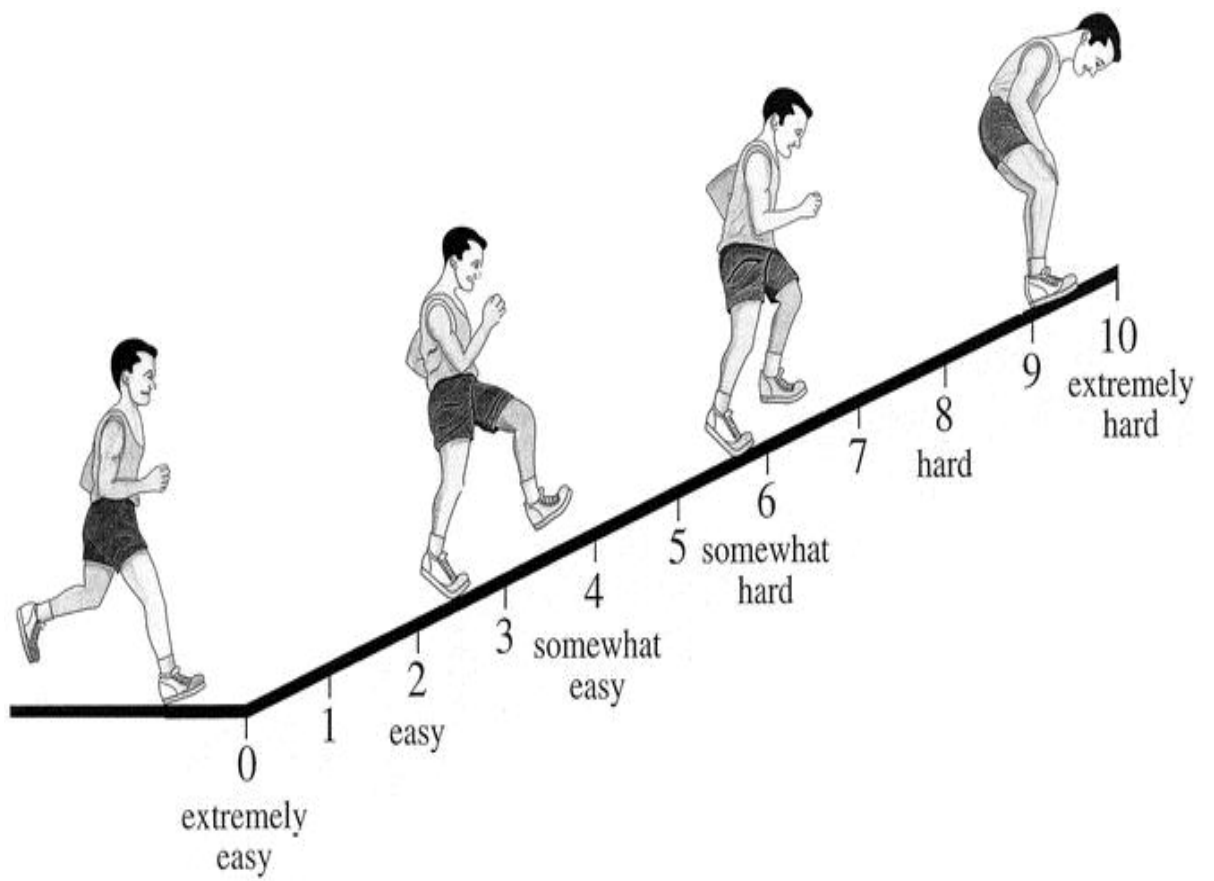
Printed Name of Person Obtaining Consent

Role in Research Study

Signature of Person Obtaining Consent

Date

APPENDIX B



APPENDIX C

POMS™ Brief Form 1

Below is a list of words that describe feelings that people have. Please read each word carefully. Then choose the letter that best describes how you feel right now. Fill in the appropriate circle on the answer sheet.

A = Not at all B = A little C = Moderately D = Quite a bit E =
Extremely

- 1) Tense
- 2) Angry
- 3) Worn out
- 4) Lively
- 5) Confused
- 6) Shaky
- 7) Sad
- 8) Active
- 9) Grouchy
- 10) Energetic
- 11) Unworthy
- 12) Uneasy
- 13) Fatigued
- 14) Annoyed
- 15) Discouraged

- 16) Nervous
- 17) Lonely
- 18) Muddled
- 19) Exhausted
- 20) Anxious
- 21) Gloomy
- 22) Sluggish
- 23) Weary
- 24) Bewildered
- 25) Furious
- 26) Efficient
- 27) Full of pep
- 28) Bad-tempered
- 29) Forgetful
- 30) Vigorous

BIBLIOGRAPHY

1. Acevedo EO, Kraemer RR, Kamimori GH, Durand RJ, Johnson LG, Castracane VD. Stress hormones, effort sense, and perceptions of stress during incremental exercise: an exploratory investigation. *Journal of Strength and Conditioning Research*. 2007; 21(1): 283-288.
2. Backhouse SH, Bishop NC, Biddle SJ, Williams C. Effect of carbohydrate and prolonged exercise on affect and perceived exertion. *Medicine & Science In Sports & Exercise*. 2005; 37(10): 1768-1773.
3. Ben-Aryeh H, Roll N, Lahav M, Dlin R, Hanne-Paparo N, Szargel R, Shein-Orr C, Laufer D. Effect of exercise on salivary composition and cortisol in serum and saliva in man. *Journal of Dental Research*. 1989; 68(11): 1495-1497.
4. Borg GA. Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise*. 1982; 14(5):377-381.
5. Buchanan TW, al'Absi M, Lovallo WR. Cortisol fluctuates with increases and decreases in negative affect. *Psychoneuroendocrinology*. 1999; 24: 227-241.
6. Cody M, Klesges LM, Garrison RJ, Johnson KC, O'Toole M, Morris GS. Health Opportunities with Physical Exercise (HOPE): social contextual interventions to reduce sedentary behavior in urban settings. *Health Education Research*. 2002;17(5): 637-647.
7. Duclos M, Corcuff JB, Arsac L, Moreau-Gaudry F, Rashedi M, Roger P, Tabarin A, Manier G. Corticotroph axis sensitivity after exercise in endurance-trained athletes. *Clinical Endocrinology*. 1998; 48, 493-501
8. Frankenhaeuser MB, Post B, Nordheden B, Sjoeborg H. Physiological and subjective reactions to different physical work loads. *Perceptual Motor Skills*. 1969; 28: 343-349.
9. Gallagher P, Leitch MM, Massey A, McAllister-Williams, Young AH. Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method. *Journal of Psychopharmacology*. 2006; 20(5): 643-649.

10. Gozansky WS, Lynn JS, Laudenslagert ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. *Clinical Endocrinology*. 2005; 63: 336-341.
11. Hollander DB, Durand RJ, Trynicki JL, Larock D, Castracane VD, Hebert EP, Kraemer RR. RPE, pain, and physiological adjustment to concentric and eccentric contractions. *Medicine & Science In Sports & Exercise*. 2003; 35(6), 1017-1025.
12. Jin P. Changes in heart rate, noradrenaline, cortisol and mood during Tai Chi. *Journal of Psychomatic Research*. 1989; 33(2): 197-206.
13. Kahn J, Rubinow DR, Davis CL, Kling M, Post RM. Salivary cortisol: A practical method for evaluation of adrenal function. *Biological Psychiatry*. 1988; 23: 335-349.
14. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology*. 1989; 22: 150-169.
15. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*. 1994; 19(4): 313-333.
16. Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. 1999; 61(2): 154-162.
17. Mathur DN, Toriola AL, Dada OA. Serum cortisol and testosterone levels in conditioned male distance runners and nonathletes after maximal exercise. *Journal of Sports Medicine*. 1986; 26: 245-250.
18. McEwen BS. Protective and damaging effects of stress mediators. *The New England Journal of Medicine*. 1998; 338(3): 171-179.
19. McGuigan MR, Egan AD, and Foster C. Salivary cortisol responses and perceived exertion during high intensity and low intensity bouts of resistance exercise. *Journal of Sports and Science and Medicine*. 2004; 3: 8-15.
20. McNair D, Lorr M, Dropplemen L. Profile of Mood States. Educational and Industrial Testing Service, San Diego, CA.
21. Nabkasorn C, Miyai N, Sootmongkol A, Junprasert S, Yamamoto H, Arita M, Miyashita K. Effects of physical exercise on depression, neuroendocrine stress hormones and physiological fitness in adolescent females with depressive symptoms.

- 2005; 16(2): 179-184.
22. O'Connor PJ, Corrigan DL. Influence of short-term cycling on salivary cortisol levels. *Med Sci Sports Exerc.* 1987; 19(3): 224-228.
 23. O'Connor PJ, Morgan WP, Raglin JS. Psychobiologic effects of 3 d of increased training in female and male swimmers. *Medicine & Science In Sports And Exercise.* 1991; 23(9): 1055-1061.
 24. O'Connor PJ, Morgan WP, Raglin JS, Barksdale CM, Kalin NH. Mood state and salivary cortisol levels following overtraining in female swimmers. *Psychoendocrinology.* 1989; 14(4): 303-310.
 25. Peeters F, Nicholson NA, Berkhof J. Cortisol responses to daily events in major depressive disorder. *Psychosomatic Medicine.* 2003; 65: 836-841.
 26. Peeters F, Nicholson NA, Berkhof J, Delespaul P, deVries M. Effects of daily events on mood states in major depressive disorder. *Journal of Abnormal Psychology.* 2003; 112(2): 203-211.
 27. Peters JR, Walker F, Riad-Fahmy D, Hall R. Salivary cortisol assays for assessing pituitary-adrenal reserve. *Clinical Endocrinology.* 1982; 17: 583-592.
 28. Polk DE, Cohen S, Doyle WJ, Skoner DP, Kirschbaum C. State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology.* 2005; 30: 261-272.
 29. Pressman SD, Cohen S. Does positive affect influence health? *Psychological Bulletin.* 2005; 131(6): 925-931.
 30. Pruessner M, Hellhammer DH, Pruessner JC, Luphen SJ. Self-reported depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. *Psychosomatic Medicine.* 2003; 65: 92-99.
 31. Riad-Fahmy D, Read GF, Walker RF. Salivary steroid assays for assessing variation in endocrine activity. *Journal of Steroid Biochemistry.* 1983; 19(1): 265-272.
 32. Robertson RJ. *Perceived Exertion for Practitioners.* Champaign, IL: Human Kinetics. 2004.
 33. Robertson RJ, Noble BJ. Perception of physical exertion: Methods, mediators, and applications. *Exercise and Sports Science Reviews.* 1997; 25: 407-452.
 34. Rudolph DL, McAuley E. Self-efficacy and salivary cortisol responses to acute

- exercise in physically active and less active adults. *Journal of Sport & Exercise Psychology*. 1995; 17: 206-213.
35. Rudolph DL, McAuley E. Cortisol and affective responses to exercise. *Journal of Sports Sciences*. 1998; 16: 121-128.
 36. Salivary Diagnostics: A discussion of steroid hormone assessment in saliva samples. 2006; version 1.2.
 37. Smyth J, Ockenfels MC, Porter L, Kirschbaum C, Helhammer DH, Stone AA. Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology*. 1998; 23(4): 353-370.
 38. Strachan AT, Leiper JB, Maughan RJ. Paroxetine administration to influence human exercise capacity, perceived effort or hormone responses during prolonged exercise in a warm environment. *Experimental Physiology*. 2004; 89(6): 657-664.
 39. Tharp GD. The role of glucocorticoids in exercise. *Medicine and Science in Sports*. 1975; 7(1): 6-11.
 40. Traustadottir T, Bosch PR, and Matt KS. The HPA axis response to stress in women: effects of aging and fitness. *Psychoneuroendocrinology*. 2005; 30(4): 392-402.
 41. Umeda T, Hiramatsu R, Iwaoka T, Shimada T, Miura F, Sato T. Use of saliva for monitoring unbound free cortisol levels in serum. *Clinica Chimica Acta*. 1981; 110: 245-253.
 42. Utter AC, Kang J, Nieman DC, Dumke CL, McAnulty SR, Vinci DM, McAnulty LS. Carbohydrate supplementation and perceived exertion during prolonged running. *Medicine & Science In Sports & Exercise*. 2004; 36(6): 1036-1041.
 43. Utter AC, Kang J, Nieman DC, Williams F, Robertson RJ, Henson DA, Davis JM, Butterworth DE. Effect of carbohydrate ingestion and hormonal responses on ratings of perceived exertion during prolonged cycling and running. *European Journal of Applied Physiology*. 1999; 80:92-99.
 44. Van Eck M, Berkhof H, Nicolson N, Sulon J. The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. *Psychosomatic Medicine*. 1996; 58(5): 447-458.
 45. Vedhara K, Hyde J, Gilchrist ID, Tytherleigh M, Plummer S. Acute stress, memory, attention and cortisol. *Psychoneuroendocrinology*. 2000; 25: 535-549.

46. Vining RF, McGinley RA. The measurement of hormones in saliva: Possibilities and pitfalls. *Journal of Steroid Biochemistry*. 1987; 27: 81-94.
47. Wifley D, Kuncce J. Differential physical and psychological effects of exercise. *Journal of Counseling Psychology*. 1986; 33(3): 337-342.