Effects of moderate exercise and dissonant music on salivary cortisol fluctuations

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Thesis Abstract

The influences of moderate exercise and dissonant music on Hypothalamus-Pituitary-Adrenal (HPA) axis activity was studied in 14 healthy individuals. Both moderate exercise and music are potent stimuli that influence HPA axis activity. Exercise and music have been used in clinical settings to help alleviate pain and improve quality of life among patients. The effects of music on hormonal release and cortisol levels depend on the type of music being used. The aim of this study was to better understand how cortisol levels were affected by dissonant music, which had not been studied as extensively as consonant music. To reduce the subjectivity aspect of music, a dissonant piece of music that was unfamiliar to participants was selected.

To gain a better understanding of the effects of moderate exercise and dissonant music on HPA axis activity, cortisol (an end product of the HPA axis) from the saliva samples of 14 participants was measured after random exposure to the following activities:

1. Moderate exercise (stationary cycling at 60% of $VO_{2\ max}$)
2. Listening to dissonant music
3. Listening to dissonant music during moderate exercise (stationary cycling at 60% of $VO_{2\ max}$)
4. Resting on stationary bike without pedaling and without listening to music

Saliva samples were collected prior to and at specific time intervals after the intervention. Salivary cortisol was measured using enzyme-linked immunosorbent assay (ELISA). A two-way repeated measures ANOVA was used to investigate the effects of the four variable activities and variable time. Both variables, activity and time, were within-subject factors. The potential significance of the interaction of the two variables, activity and time was also studied. The effect of variable activity was not statistically significant, as repeated F-tests uniformly displayed p-values well above the significance level of 0.05. However, a significant main effect of time was observed. Salivary cortisol levels changed across time points. Salivary cortisol levels decreased over time in each group. Time had a significant effect on the salivary cortisol level, with $F(2, 26) = 9.216$ and p-value=.001, according to the F-test assuming sphericity. Music was not statistically significant, with $F(1,12) = 0.162$ and p-value = 0.695.These findings suggest that moderate exercise and pre-selected musical stimuli had no effect on HPA axis activity as indicated by cortisol levels in young, healthy adults.

Keywords: music, dissonant, moderate exercise, HPA axis, salivary cortisol

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Introduction

Music and exercise interventions have been used in clinical settings to promote pain alleviation and relieve the emotional burden of health issues (Sa, 2014; Schmid and Aldridge, 2004). As an auditory stimulus, music affects the activity of the Hypothalamus-Pituitary-Adrenal HPA axis (Thoma et al., 2013). The HPA axis is part of the neuro-endocrine system, and cortisol is one of the end products of the HPA axis and is considered to be a marker of its activation (Kirschbaum and Hellhammer, 1999). The kind of effect that music has on cortisol levels depends on the type of music (Koelsch et al., 2011). For instance, pleasant music composed of consonant intervals and predictable beats and tempo, such as classical music, lowers cortisol levels. Patients who listened to happy and pleasant instrumental music during surgery experienced lower cortisol levels compared to patients who did not listen to music during the same surgery (Khalfa et al., 2003; Koelsch et al., 2011). One problem when studying music is that a person's familiarity with the music affects their experience (Pereira et al., 2011). To overcome this, dissonant music can be studied. Unlike consonant sound, dissonant music has no consistent rhythm or note structure and causes tension in listeners because intervals are not resolved as predicted (McDermott and Hauser, 2005; Blood et al., 1999). Across cultures, dissonant music is known to evoke unpleasant emotions (Chanda and Levitin, 2013). Dissonant sound has a beating or roughness quality due to the simultaneous playing of two tones that are close but not identical in frequency (Plantinga and Trehub, 2013).

The familiarity aspect of a piece of music is an important factor that influences physiological responses (Pereira et al., 2011). According to Khalfa et al. (2003), listening to self-selected music for 30 minutes resulted in decreased cortisol levels. On the contrary, listening to relaxing music selected by researchers did not lower cortisol levels after stressful tasks, such as public speaking and solving arithmetic problems (Thoma et al., 2013). Musical preference is subjective, and researchers often choose dissonant and pre-selected music in order to reduce the subjectivity of music when studying its effects on physiological markers, such as hormonal changes. Dissonant music is studied because years of research show that the preference for consonance is innate (Plantinga and Trehub, 2014).

Another HPA axis stimulus is physical activity. Variables such as exercise intensity, duration and the physical state of a person influence the level of hormonal response and cortisol levels (Hill et al., 2008; Karkoulias et al., 20008). In response to an exercise stimulus, the HPA axis secretes cortisol (Kraemer and Ratamess, 2005). An acute bout of resistance exercise has been shown to be sufficient to enhance cortisol release. For example, eight sets of 10RM (Repetition Maximum– the maximum number of weights that can be lifted for 10 repetitions) leg press exercise with one-minute rest intervals lead to significant acute cortisol response (Kraemer and Ratamess, 2005).

Dynamic exercise such as continuous cycling and running also stimulate the HPA axis, thereby affecting cortisol release (Hill et al., 2008). Moderate exercise, such as 30 minutes of stationary cycling at 60% $VO_{2\ max}$, triggers the HPA axis, this value is right at the threshold required to elicit significant cortisol release. The cortisol change at 60% $VO_{2\ max}$ is not always detectable, or it is a lower magnitude change than the same exercise mode at a higher intensity (e.g., 80% $VO_{2\ max}$)(Brownlee et al., 2005; Hill et al., 2008; VanBruggen et al., 2011). Trained individuals show a lower cortisol response to the same exercise program compared to sedentary individuals (Tremblay et al., 2005).

To study the effects of music and exercise on HPA axis activity, cortisol was chosen as a biomarker of HPA activity. Cortisol is synthesized and released in response to activation of the HPA axis by various stimuli. The hypothalamus releases corticotrophin-releasing hormone (CRH), which travels to the anterior pituitary gland and signals cells to secrete adrenocorticotropic hormone (ACTH) into the blood. ACTH travels to the adrenal gland and signals the adrenal cortex to secrete cortisol into the circulation (Kirschbaum and Hellhammer, 1999). Cortisol levels follow a daily rhythm, with levels peaking in the morning upon awakening, decreasing throughout the day, and reaching a nadir within a few hours of sleep onset (Papacosta and Nassis, 2011). Due to the stability of cortisol in saliva even at room temperature, cortisol remains stable in saliva at room temperature for one week, which allows for repeated measures (Eller et al., 2006; Aardal and Holm, 1995). This is why saliva is used in many studies to measure cortisol levels. Salivary cortisol correlates closely with the free cortisol fraction in serum and saliva, with a coefficient correlation ranging from r= 0.71 to r= 0.96 (VanBruggen et al., 2011). Salivary cortisol provides an index of measurement of biologically active cortisol. Unlike plasma cortisol, which is largely bound to carrier proteins, only free and unbound cortisol appears in saliva (Kirschbaum and Hellhammer, 1994). Patterns of change in blood and saliva cortisol levels are similar, and the levels of unbound (free) cortisol in blood and saliva are comparable, with $r > 0.90$. The strong correlation is due to the passive diffusion of cortisol into the oral cavity, which is independent of the flow rate of saliva (Aardal and Holm, 1995; Kirschbaum and Hellhammer, 1999).

Furthermore, measuring salivary cortisol is a non-invasive method compared to measuring serum cortisol, which requires blood drawing, which can result in stress responses and raised cortisol levels in many participants, thereby introducing a confounding variable (Kirschbaum and Hellhammer, 1999). With regards to the timing of salivary cortisol, it usually takes 5 to 30 minutes for cortisol to peak in saliva. For example, salivary cortisol reaches its maximum concentration 30 minutes after the cessation of marathon running and cycle ergometer (Kirschbaum and Hellhammer, 1994). After an intravenous injection of cortisol, the transfer of cortisol from plasma to saliva requires only 2 to 3 minutes to observe peak levels of cortisol in saliva. Salivary cortisol is also a valid measure of plasma cortisol in response to exercise, as both increase linearly in response to exercise intensity (Chiharro et al., 1998). The salivary cortisol response to 30 minutes of acute stationary cycling at 75% of VO_{2max} correlates with the plasma cortisol response after 30 minutes of recovery ($r = 0.90$) and 15 minutes of recovery ($r = 0.93$)(O'Conner and Corrigan, 1987). Therefore, saliva is an attractive medium for researchers and clinicians who study hormonal levels.

The aim of this study is to examine salivary cortisol levels following 30 minutes of moderate cycling, 30 minutes of listening to dissonant and unfamiliar music, and 30 minutes of moderate cycling while listening to dissonant and unfamiliar music. For this study, stationary cycling for 30 minutes at 60% $VO_{2\,max}$ in untrained healthy individuals was selected to examine the salivary cortisol response. Moderate exercise was chosen because one of the potential applications of this research is to perform exercise interventions in chronically diseased populations with limited physical abilities, which will be discussed in detail in the Future Directions section.

Hypotheses

1. Moderate exercise will not significantly change the level of salivary cortisol.

2. Listening to dissonant music will increase salivary cortisol levels.

3. Listening to dissonant music while exercising will increase salivary cortisol levels in a synergistic fashion.

Rationale

I chose to study the effects of 30 minutes of moderate exercise in healthy people in order to establish methodology and to determine if the chosen activities were enough to induce cortisol secretions. These parameters were chosen because people with disabilities can have trouble running on a treadmill, sustaining more than 30 minutes of exercise, or reaching above 60% of their $VO_{2\ max}$ (Learmonth et al., 2014). Being on a stationary bicycle is more applicable to a disabled population, as are the reduction in time and intensity. Choosing 60% $VO_{2\ max}$ of aerobic exercise is not optimal for causing a cortisol secretion (Usui et al., 2011). This is why I chose to add psychological input (i.e., agitating music). Agitating music, such as techno music, increases cortisol levels in people (Gerra et al., 2011), and people who exercise while being agitated show greater elevation in cortisol compared to people who exercise without additional agitation (Raymond, 1972). The concept was to raise cortisol in disabled people by having a synergy between moderate exercise and dissonant music. To begin to address this I did a pilot study on healthy subjects.

Methods

Participants. Twenty two participants were recruited via social media, flyers offering monetary compensation, and from the Department of Exercise Science at Concordia University, and they qualified and consented to the study. Each participant from the department was rewarded a 3% course credit. In an effort to attract older participants, individuals between the ages of 20 and 25 years old who were not enrolled in courses at the Department of Exercise Science were paid $20.00 and participants aged 25 and over were paid $60.00. Certification of ethical acceptability for research involving human subjects (Certificate 30001940) was obtained from the Concordia University Research Ethics Committee. Five participants did not complete all four activities due to conflicts of schedule, and their data was not used in the final analyses. Three participants completed the study, however, not enough saliva was obtained for complete analysis. In terms of age, sex, and other relevant factors, there were no differences between the people who were incomplete, and the 14 subjects who completed the study.

Of the 14 participants included in the study, 11 were women and 3 were men (mean age = 22 years old; age range = 20-30). Participants were healthy, medication-free, and non-smokers. Exclusion criteria included chronic or acute diseases (e.g., heart disease and autoimmune disease) and/or prior or current use of immuno-modulatory medications. No one was excluded due to these criteria. According to participants' self-reported activity, they had all participated in moderate exercise sessions on a weekly basis (mean weekly exercise = 1.2 ±0.8 hours) prior to the first day of the study. Moderate activity was defined as an activity that requires moderate physical effort and causes a small increase in breathing and/or heart rate.

Smoking and alcohol are important factors that affect HPA axis activity and cortisol levels. Both chronic and acute smoking can stimulate the HPA axis and induce cortisol release (Fukudo and Morimoto, 2001). Heavy drinking can potentially attenuate HPA axis activity and cortisol response (King et al., 2006). In order to control for effects of these stimuli, only non-smokers were recruited for this study, and they were asked to abstain from alcohol consumption for at least 24 hours prior to their visits.

Testing protocol. The subject was consented, and they answered a standard PAR-Q questionnaire designed to assess participants' exercise readiness by obtaining information about their current health status. They then completed the YMCA test in order to estimate their VO_{2max}. Participants then completed four activities in a random order on four different days. Each activity commenced at 10:00 am (or as close to that time as possible) to minimize the confounding factor of diurnal rhythms. Each activity day was separated by a minimum of two intervening days, with a maximum of seven intervening days. The four activities were Exercise, music, exercise and music, or resting. Participants were instructed to abstain from sexual activity and alcohol for 24 hours prior to the study day and to refrain from caffeine and food intake for 2 hours before their scheduled session. All subjects adhered to these rules. The details of each activity are found in the next sections.

YMCA testing protocol. A submaximal test was used to determine the heat rate (HR) requirement for the desired $VO_{2\ max}$; 60% of $VO_{2\ max}$ (Golding, 1989). Subjects must not have caffeine or nicotine products for 24 hours prior to the test. The test is conducted on a stationary exercise bike that stresses the subject's cardiovascular system to a safe, predetermined intensity. It is completed when the subject's heart rate reaches 85% of HR_{max} (the theoretical upper limit of beats per minute that the subject can achieve through exercise stress without experiencing potential health problems). A general equation to determine age related HR_{max} is to subtract the subject's age from 220. The subject's HR is recorded at regular intervals as the difficulty of the exercise increases. The test is terminated when the subject's HR reaches 85% of HR_{max}, when he/she begins to show symptoms of fatigue or loss of consciousness, or if the subject requests to stop. The HR measures are then extrapolated to 100% of HR_{max} in order to determine the subject's $VO_{2\ max}$. These values are used to categorize the health status of the participant and to determine the level of exercise required for 60% capacity.

Music stimulus. "Grinder in the Sky" by SCHNEIDER TM was chosen, it is an instrumental track of random noises that sounds similar to construction noises. This stimulus was selected because non-relaxing music has the potential to induce cortisol (Gerra et al.,1998). A single music stimulus was selected in order to eliminate potential influences of memory or subjective association with music chosen by the participants (Chanda and Levitin, 2013; Pereira et al., 2011). After listening to the music for the first time, participants rated the music on a 10-point Likert scale (i.e., 10 = "very agitating" and 1 = "not at all agitating"). To listen to music, the subject sat on the exercise bike without pedaling and listened to Grinder in the Sky for 30 minutes with headphones and an MP3 player. The subject was allowed to choose the volume based on instructions that it should not be too loud as to hurt their ears, but loud enough so that they cannot hear anyone talking. Participants had been asked to rate the music on a scale of 1-10 (where 1= like and 10 = dislike) after listening to it for the first time. For instance, if participants marked between 1-5 for statements such as "this music was agitating," they were categorized in the "like" group. On the other hand, if they answered between 6-10 for the same question, they were categorized in the "dislike" group. Seven out of 14 participants disliked the music, and the other half liked the music.

Exercise stimulus. The exercise was conducted on a stationary bike for 30 minutes at 60% of their estimated $VO_{2\ max}$, as determined beforehand by the YMCA testing. The room temperature was kept at 20°C to minimize overheating. To prevent saliva dilution, participants were not allowed to drink water for 10 minutes prior to sample collection. A researcher observed the exercise session to insure that the subject did not drop below the 60% level and to provide consistent motivation to the subject if they dropped below the 60% level. For the exercise with music group, participants engaged in the same type of exercise while listening to the music. The resting group sat on the stationary bike for 30 minutes without pedaling or listening to music.

The combined music and exercise activity involved pedaling at 60% of their $VO_{2\ max}$ while listening to Grinder in the Sky. The resting group sat on the exercise bike without pedaling for 30 minutes.

Saliva collection. Saliva was collected at three time points: pre-activity, 5 minutes post-activity, and 30 minutes after each activity. Each time, the subject filled a small micro centrifuge tube with saliva (~1.5mL) using a sterile plastic transfer pipette. Tissues were on hand in case of spillage. The tubes were sealed in a plastic bag, placed in a box containing absorbent paper, and transported from the metabolic assessment lab (where the exercise took place) to the clinical analysis lab down the hall in the PERFORM Centre. The samples were opened, vortexed for 10 seconds, and centrifuged at 2000 rpm for 10 minutes as per the recommendation of the enzyme linked immunosorbant assay ELISA kit for salivary cortisol. The supernatants were removed carefully without disturbing the pellet at the bottom of the tube, and the fractions of supernatants were divided into several appropriately labeled cryotubes. This was done in the biosafety cabinet to minimize risk of aerosol release exposure. Each aliquoted tube was filled with ~70 ml of saliva, which is enough to run samples in duplicate for a single ELISA. Tubes were labeled with a unique code that protects the subject's identity and provides a blinding for the lab personnel responsible for analysis. The tubes were then placed in a cryobox and stored at -80ºC until being used for analysis. Tape labels designed towithstand-80ºC were used for coding, and the coding of subjects was recorded on paper files that were secured in a locked drawer. Subjects' identities remained undisclosed for the duration of the study. At the end of the study (i.e., upon publication), the saliva samples will be disposed of in the biohazard waste container, which is autoclaved.

Hormone analysis. Levels of cortisol were determined using enzyme-linked immunosorbant assay (ELISA), which is a competitive, colorimetric, non-radioactive method. For this study, the ELISA Abnova Kit 1885was used. This test kit is based on competitive ELISA, where hormone conjugate and the cortisol in the sample (saliva) compete for a limited number of binding sites on the antibody-coated plate. First, the standard solution and saliva samples are added to the coated plate. Then, the hormone conjugate is added and the mixture is kept at room temperature for 60 minutes. During this time, the competition for binding sites occurs. The plate is washed to remove unbounded material. To detect the hormone (cortisol) bound to the antibody, a substrate is added that produces an optimal colour within 30 minutes. To obtain quantitative test results, samples are measured and compared based on absorbance of the samples with the standards using an ELISA plate reader (BioTek Gen5) at 450 nm. The amount of cortisol in the sample is inversely proportional to the colour intensity, wherein a darker colour correlates to a lower cortisol concentration in the sample. This procedure has an inter-assay CV of 8.33% and an intra-assay CV of 8.27%, according to the manufacturer. All of the samples were run in duplicate, and all samples of the same subject were analyzed in the same run to reduce error variance due to imprecision of intra assay. The expected normal value for salivary cortisol levels for midday samplings is between 0.3-5.7 ng/ml (Abnova Salivary ELISA kit 1885).

Statistics and data analysis. A repeated measure analysis was used for this study. 14 participants performed all four different activities. For each activity, saliva was collected prior to activity, 5 minutes post activity, and 30 minutes post activity. In order to minimize the carry over effect, participants performed the activities in random order. Independent variables (IV) were dissonant music and moderate cycling, and the dependent variable (DV) was the salivary cortisol level. Repeated measure ANOVA was used to analyze the main effect of group (i.e., differences between activities), main effect of time (i.e., difference between time points), and the interaction effect between group and time. Contrast post-hoc comparison provided assessment of main and interactive effects. To investigate any possible interaction between music preference and salivary cortisol levels, participants were categorized into "liked music" or "disliked music" groups based on the music questionnaire answers. For all analyses, results were considered significant at $p \leq 0.05$. Results were shown as mean ± SD, unless otherwise indicated. Data analyses were performed using SPSS (22.0) software. The potential significance of the interaction of Activity and Time was also studied using Mauchly's sphericity test (appendix table 1).

Results

The effect of dissonant music and moderate exercise on salivary cortisol levels was investigated in 14 healthy young adults. A two-way repeated measures ANOVA was used to investigate the effects of the different activities (variable Activity) and the effect of time (variable Time) on the level of cortisol measured in the saliva samples. Both Activity and Time were within-subject factors. The data was analyzed to examine the effects of each activity on salivary cortisol levels. Salivary cortisol levels did not significantly change as a result of any of four activities (Table 2).Variable activity was not statistically significant, with various repeated measures F-tests uniformly displaying p-values well above the significance level of 0.05. In particular, the optimal in this situation F-test assuming sphericity has the p-value of 0.211. Thus, there were no differences in cortisol between the different activities.

The data was also analyzed to examine the effects of time on salivary cortisol levels. A significant main effect of time was observed. Salivary cortisol levels decreased over time in each group when comparing the 5 minute post to the pre- value, or when comparing the 30 minute post to the pre-value (Table 3 and Table 4). Therefore, time had a significant effect on the salivary cortisol level, with $F(2, 26) = 9.216$, (p=.001), according to the F-test assuming sphericity.

In order to evaluate potential effects of time and music preferences, participants were classified into two groups: “like” and “dislike”. A two-way ANOVA with one within-subject factor (Time) and one between-subjects factor (Music) was performed to evaluate the interaction of time and music preference on salivary cortisol levels. Results showed that music preference and time interaction did not have a significant effect, with $F(1, 12) = 0.162$ and a p-value = 0.695 (Table 5).

	N	Age	VO_{2max} (mL/Kg/min)	Body weight (lb)
Female participants	11	21.6	36.4	137.5
Male participants	3	21.3	43.8	169
Range	Female Male	22-23 21-22	30-55 39-49	102-177 134-188
SD	Female Male	0.88 0.47	8 4.2	20.3 24.7

Table 1.Characteristics of 14 participants. Results are shown as means.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Activity	Sphericity Assumed	16.326	3	5.442	1.573	.211	.108
	Greenhouse-Geisser	16.326	2.433	6.711	1.573	.220	.108
	Huynh-Feldt	16.326	3.000	5.442	1.573	.211	.108
	Lower-bound	16.326	1.000	16.326	1.573	.232	.108

Table 2.Repeated measure analyses revealed variable activity. No significant effect on salivary cortisol levels were found.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	17.917	2	8.958	9.216	.001	.415
	Greenhouse-Geisser	17.917	1.713	10.460	9.216	.002	.415
	Huynh-Feldt	17.917	1.947	9.201	9.216	.001	.415
	Lower-bound	17.917	1.000	17.917	9.216	.010	.415
Error(Time)	Sphericity Assumed	25.275	26	.972			
	Greenhouse-Geisser	25.275	22.267	1.135			
	Huynh-Feldt	25.275	25.315	.998			
	Lower-bound	25.275	13.000	1.944			

Table 3.Tests of Within-Subject Effects shows that time had a significant effect on the salivary cortisol level, $F(2, 26) = 9.216$, (p=.001).

	EXERCISE			MUSIC			MUSIC+EXERCISE			RESTING		
	A	B	C	A	B	C	A	B	C	A	B	C
Mean	3.36	2.99	2.72	3.14	3.01	2.69	3.51	3.24	3.00	4.51	3.81	2.91
Standard Error	0.40	0.48	0.43	0.51	0.54	0.48	0.33	0.44	0.44	0.52	0.42	0.35
Standard Deviation	1.54	1.88	1.67	1.97	2.11	1.85	1.30	1.70	1.71	2.02	1.64	1.35

Table 4.Salivary cortisol decreased linearly across time points for all the activities. Cortisol was measured by ELISA from saliva samples (A) before, (B) 5 minutes after, and (C) 30 minutes after the indicated activity. The table shows the mean value of cortisol levels in the saliva in the unit of ng/mL. The data is expressed as a mean value of 14 participants with standard error and standard deviation. There was a notable linear trend with respect to time using the F-test for the corresponding contrast, $F(2, 26) = 9.216$, p=.001.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	588.036	1	588.036	81.757	.000
Like/dislike Music	1.163	1	1.163	.162	.695
Error	86.310	12	7.193		

Table 5. Tests of main effect of like/dislike within-subject contrasts showed that music preference multiplied by time interaction had no significant effect on salivary cortisol levels, $F(1, 12) = 0.162$ and p- value = 0.695.

Discussion

This study was conducted to examine the effects of moderate exercise and dissonant music on in healthy individuals.

Effects of moderate exercise and stationary cycling on salivary cortisol levels. The first hypothesis stated that, when compared to baseline values, salivary cortisol levels would not be significantly higher during the recovery period following exercise.

Studies have shown that a "threshold intensity" of ~60% of $VO_{2\ max}$ can induce an increase in circulating cortisol (Hill et al., 2008; Duclos et al., 1997). Hill and colleagues examined the plasma cortisol levels that were collected before and after each exercise session at three different intensities (i.e., 40, 60, and 80% $VO_{2\ max}$) in 12 moderately trained men (18-30 years old) who trained for three days per week (~60 minutes per session) for 12 months leading up to their participation in the study. Salivary cortisol levels significantly increased at post-exercise sampling, which took place immediately after the exercise session (only at 60% and 80% intensities) (Hill et al., 2008).

Although 60% has been proposed as the "threshold intensity" for eliciting cortisol elevation, other factors, such as the duration of the type of exercise and the physical state of the individual, also play key roles in determining the level of hormones such as cortisol. A study by Duclos et al., (1997) concluded that both the intensity and duration of an exercise session contribute to changes in hormonal levels. Cortisol levels in plasma were examined in both sedentary males and male marathon runners (mean age of 24 years). Plasma cortisol levels significantly increased only after prolonged and intense running (120 minutes of 80% HR_{max}) for both groups with similar cortisol level values. In three other settings: short/intense (20 min, 80%HR_{max}), short/light (20 min, 50%HR_{max}) and prolonged/light (120 min, 80%HR_{max}), plasma cortisol levels only fluctuated during exercise sessions and remained unchanged post exercise (Duclos et al., 1997).

Although saliva and blood samples are often collected only before and after an exercise session, it is important to acknowledge any possible hormonal modulation that occurs during an exercise session. This study did not measure cortisol levels during the four activities. It can be argued that salivary cortisol may have been elevated during moderate stationary cycling because all participants reached 60% intensity, which is believed to be the minimum level that induces cortisol release.

The current study found that stationary cycling at 60% intensity decreased cortisol levels since a significant main effect of time was observed. However, the 'resting' control activity also decreased cortisol since a significant main effect of time was observed. Moderate exercise did not appear to reduce cortisol to a greater extent than just resting. This indicates that moderate exercise had no effect on cortisol fluctuations, which is consistent with Usui et al who found that 60% intensity of aerobic exercise is sub-optimal for causing cortisol secretion in healthy individuals (Usui et al., 2011). In a sample of both trained and sedentary participants (N=20), comprised of healthy male university students, salivary cortisol levels remained unchanged after a bout of moderate exercise (walking on a treadmill at 60-70% VO_{2max} for 30 minutes) (Zschucke et al., 2015). We had decided to study exercise at 60% intensity as it might be the maximum level of intensity a person with a disability is able to undertake, and such intensity may be sufficient to activate HPA axis activity and elicit cortisol release.

Another factor that may alter an HPA axis response to exercise is the training factor. Compared to a sedentary population, trained individuals have a higher intensity threshold for inducing hormonal or cortisol elevation (Viru et al., 2004; Bloom et al., 1976).Due to the fitness level of the participants, it is possible that cycling at 60% VO_{2max} was not sufficient to reach the intensity threshold and, therefore, did not provoke a significant increase in salivary cortisol. Participants were young, healthy adults who, according to the self-report questionnaires, engaged in moderate exercise sessions on a weekly basis at a local or school gym (mean weekly exercise = 1.2 ±0.8 hours, range = 0.5-3 hours). On the questionnaire, moderate activity was defined as an activity that requires moderate physical effort and causes a small increase in breathing and/or heart rate. The average mean for females was VO_{2max} was 36.4 ml/kg/min (range= 26-55 ml/kg/min) and, for male participants, the mean VO_{2max} was 43.8 ml/kg/min (range=39-49 ml/kg/min). According to the normative data table, both male and female participants' VO_{2max} average values fall in the "good" category (Hayward, 1998). This suggests that although non-athletes, these participants were able to exercise more intensely compared to similar age/sex groups whose fitness levels are lower according to the VO_{2max} normative values.

Music effect on cortisol levels. The second hypothesis of this study was that listening to dissonant and unfamiliar music would raise salivary cortisol levels. Surprisingly, it was found that listening to music did not raise saliva cortisol levels. In their study on the effects of fast-tempo techno music on plasma cortisol in 16 healthy adults (8 female and 8 men with a mean age of 18 years old), Gerra et al. (2011) established that a sense of tension and anguish induced cortisol release.

It was expected that dissonant music would increase salivary cortisol levels because, using imaging technology, researchers have found that dissonant music is unpleasant to listeners (Blood and Zatorre, 2001). In consonant sound, the intervals between musical notes are resolved as expected; however, in dissonant sound, the intervals are often delayed or not resolved as expected, creating a sense of tension (Plantinga andTrehub, 2013). "The Grinder in the Sky" was chosen as the stimulus for this study because it is composed of the atonal and random noises that characterize dissonant music. In addition, according to the self-report questionnaires, this piece of music was unfamiliar to all participants, thereby ensuring that their responses were not influenced by any pre-existing memories or associations with the music, as decades of music research have established that listening to familiar music cues involuntary emotional responses in listeners (Pereira et al., 2011).

Half of the participants reported that they disliked the music, and half reported that they liked the music. Participants were asked to rate the music on a scale of 1-10 (1= like; 10 = dislike) and categorized into two groups depending on their responses. If participants marked 1-5 in response to, "this music was agitating," they were categorized as "like." On the other hand, if they marked 6-10, they were categorized as "dislike."

Although the musical stimulus was perceived as unpleasant by half of the participants, listening to the music for 30 minutes did not increase overall cortisol levels. Of the seven participants who reported that they did not like the music, two experienced a transient increase in cortisol levels, and this spike was not seen in participants who reported liking the music, although contrast post-hoc analyses showed that there was no significant interaction between time and music preferences. In other words, there was no significant change in salivary cortisol levels in either group of participants.

Despite being unfamiliar and generally disliked, perhaps the music needed to be even more unpleasant in order to induce a hormonal or cortisol response. To make the music more unpleasant, sound characteristics such as tempo and volume could be modified, both of which contribute to the musical experience of listeners (Clifton, 1983). Listening to high tempo music, such as techno, results in tension and anguish in listeners; however, listening to slower tempo music, such as classical music, elicits serene and calm feelings (Gerra et al., 2011). Although dissonant, “The Grinder in the Sky” is composed primarily of slow tempo sound. Also, in the current study, participants were allowed to adjust the volume of their headsets to a comfortable level. Future studies should set the volume at around 70Db for all participants in order to limit the potential confounding effects of volume (Thoma et al., 2011).

The combination of music and exercise. No significant changes in salivary cortisol release were observed when moderate cycling and listening to music were combined. It was expected that the two stimuli would each raise cortisol on their own, and when the two were combined together there would be a synergistic effect. One possible explanation is that dissonant sounds may attenuate cortisol. In Thoma et al, the sound of rippling water, which was considered dissonant, lowered cortisol responses from a subsequent social stress test. (Thoma et al., 2013). In our results, neither exercise nor music altered cortisol (compared to the resting group), thus it is not surprising that the combination of the two activities did not alter cortisol.

Resting condition. This study included a control "resting" activity in which the subjects sat on the bike but did not pedal for 30 minutes. When subjects rested on the bike, cortisol levels lowered over time. The resting activity served as our control group to account for potential effects of the laboratory environment and the time of day. Across time points in all activities (i.e., moderate cycling, listening to dissonant music, cycling while listening to music and resting), there was a significant linear decrease in salivary cortisol (Table 4). Statistical analyses showed that there was a significant main effect of time on the level of cortisol in all of the treatment groups (p=0.01). It might be reasoned that the linear decrease of salivary cortisol across time points was due to the natural daily pattern of cortisol levels (Wolfram et al., 2013) since it also occurred in the control group. Cortisol levels are known to spike in the early morning, about 30 minutes after awakening, and then decline throughout the day, with an episodic increase after lunch (Papacosta and Nassis, 2011). For each participant, all four activities were conducted at the same time of day (10:00 am -13:00pm), when cortisol is expected to be on the decline. In order to control for episodic increases in cortisol levels due to food and/or drink intake, all participants were required to abstain from food and caffeine intake for at least two hours prior to their arrival time.

In a study by Zschucke et al. (2015), participants in the placebo group, who only did light stretch exercises such as ankle rotation for 30 minutes, showed a significant decrease of salivary cortisol, while participants who engaged in moderate exercise (i.e., walking on a treadmill at 60-70% VO_{2max} for 30 minutes) experienced no significant change in salivary cortisol levels. Researchers believed that the decline in cortisol levels in the placebo group was due to the normal diurnal pattern of salivary cortisol (Zschucke et al., 2015).

In the current study, the slope of cortisol decline appeared to be steeper for the control group compared to all other groups, suggesting that the activities may have slowed the normal decline of cortisol; however, this trend association was not found to be statistically valid. In the resting group, we noticed that the average pre-activity cortisol value was higher than the other three groups; however, this apparent difference was not statistically significant. Anticipation of the activity could not explain these differences since subjects were not told in advance what the activity would be, and the order of activities for each subject was randomized.

Another explanation for why cortisol did not increase as predicted is that environmental factors may have altered HPA axis activity and cancelled out the cortisol fluctuations. For example, the novelty of the place (i.e., location where the study is conducted), low sense of control, and being evaluated by research staff are factors that affect HPA axis activation, and hence cortisol release (Muehlhan et al., 2011). Being in a room with research staff can be perceived as a stressful event for some people, which can influence the response of cortisol levels to other later stimuli (e.g., exercise or music) (Zschucke et al., 2015; Muehlhan et al., 2011). I speculate that, in accordance with the negative feedback loop theory, the activation of the HPA axis in response to previous stimuli (i.e., environmental factors) inhibited the cortisol response to the subsequent stressors (i.e., exercise and music). A study by Zschucke et al. (2015) showed that a bout of moderate exercise (walking on treadmill at 60-70% $VO_{2\ max}$ for 30 minutes) 90 minutes prior to undergoing a social stressor task, such as solving arithmetic problems, in a group of male participants (including both trained and sedentary people, n=20) had an inhibitory effect on salivary cortisol response. For participants of the placebo group (n=20) who did not participate in the aforementioned exercise session, elevation of salivary cortisol levels was observed after a social stress task (i.e., solving math problems with a social evaluation component) (Zschucke et al., 2015; Brandenberger et al., 1982). In the study by Zschucke et al. (2015), fMRI analyses revealed that that participants in the exercise group experienced sustained brain activity for about 90 minutes after the exercise sessions in the hippocampus, a region of the brain that is positively correlated with cortisol levels (Zschucke et al., 2015). In the current study, when possible effects of environmental factors on HPA axis activation were still present, participants performed an activity upon arrival.

This finding suggests that moderate exercise and pre-selected musical stimulus have no differential effect on HPA axis activity as measured by cortisol levels in young healthy adults.

Limitations of the study

The aim of this study was to better understand the effects of moderate exercise and dissonant music on HPA axis activity. There are number of limitations with regard to the results.

The results of this pilot study were obtained from a small sample of participants. With a larger sample size, it would be possible to consider the order of activities in the statistical analyses, and it would be possible to investigate the contribution of sex. Since there were only 3 men and 11 women an analysis of sex was not possible.

Most previous studies examined the effects of cortisol response to exercise in only male participants. The current study, however, included both female and male participants, which is an important step towards a better comprehension of cortisol response to different stimuli in both male and female. Future studies should account for female participants' menstrual cycles and daily time of awakening for all participants, as some studies suggest that the cortisol awakening response increases during ovulation due to secretion of steroid hormones (Wolfram et al., 2010). On the other hand, other studies suggest that the cortisol awakening response is only influenced by the time of awakening and not by the menstrual cycle (Kudielka and Kirschbaum, 2003).

Future studies should also use both subject-selected music and pre-selected music (i.e., preferred vs. non-preferred music) and different genres of music (i.e., both consonant and dissonant sound) to investigate the effects of such music on hormonal levels and cortisol fluctuations. A better understanding of the effects of different types of music on our bodies (i.e., hormonal response) will improve the clinical application of music listening. Also, future studies should collect saliva samples during each activity. Frequent sampling provides a precise picture of salivary cortisol fluctuations in response to different activities. In order to evaluate the effects of environmental factors, such as the location of the experiment, future studies should provide appropriate questionnaires to participants upon each visit. This will help to determine if and how environmental factors affect participants’ moods and stress levels, and if such effects diminish in later visits.

Assessment of Quality of Methodology

Cortisol was measured from saliva samples using a commercially available ELISA kit purchased from Salimetrics, an established company that specializes in saliva analysis. The standard curve (as described in the materials and methods section) had a correlation coefficient R^2 of no less than 0.997 when fit to a sigmoidal curve, as expected per the manufacturer's data. The analytical sensitivity of this ELISA is 0.024ng/mL as calculated by subtracting 2 standard deviations from the means of 20 replicates. The assay dynamic range is 0 - 30ng/mL.

The levels and ranges of cortisol we obtained were similar to published results. The normal range of free cortisol in saliva from both male and female adults is presented in Table 6; these values were presented in the Salimetrics ELISA kit used for this study.

Aardal and Holm (1995) established references for salivary cortisol levels. Table 7 shows the results of their study for 20- to 30-year-old participants. In the current study, the average salivary cortisol from all four activities across all time points (as measured using ELISA) ranged from 2.69 ng/mL to 4.51 ng/mL (Table 4). In a study by Rosmalen et al. (2005), levels of salivary cortisol of 1768 participants , 10-12 year old children ranged between 4.17 ng/mL at 7:30 AM to 0.70 ng/mL at 10:00 PM (Rosmalen et al., 2005).

Time of Day	Range (ng/mL)	N
Morning	1-11.3	234
Mid-day	0.3-5.7	427
Evening	0.2-2.7	419
Midnight	< 1.0	26

Table 6. Salimetrics ELISA Kit provides the salivary cortisol levels in healthy male and female participants at different times of day (Abnova ELISA Kit, 1885).

Age	Sex	N	AM salivary cortisol (ng/mL)	PM salivary cortisol (ng/mL)
21-30	Male	26	3.62	0.79
21-30	Female	20	5.2	0.39

Table 7. Cortisol in saliva - reference ranges in both male and female were established in a study by Aardal and Holm (1995).

Also, salivary cortisol values across time points in all four activities are similar to those from other studies that also measured salivary cortisol in response to moderate exercise and/or music. In the study by Zschucke et al. (2015), the average salivary cortisol levels before and after moderate exercise (walking on treadmill at 60-70% $VO_{2\,max}$ for 30 minutes) were reported as 3.62 ng/mL and 3.55 ng/mL, respectively (N=20) (Zschucke et al., 2015). In our study, salivary cortisol levels before, 5 minutes after and 30 minutes after moderate cycling at 60% VO_{2max}were 3.36

ng/mL, 2.99ng/mL and 2.72 ng/mL, respectively (N=14) (Table 4). In the above-mentioned study, the placebo group (N=20) within the same time frame as the exercise group (30 minutes) had the following values for salivary cortisol levels: 3.2 ng/mL (pre) and 1.81 ng/mL (post 30 minutes). In the current study, salivary cortisol levels before, 5 minutes after and 30 minutes after resting were 4.51 ng/mL, 3.81 ng/mL and 2.91 ng/mL, respectively (Table 4).

In the study conducted by Thoma et al. (2013), the average salivary cortisol levels before and after listening to the sound of rippling water in 60 healthy female participants (20 - 30 years old) were ~1.8 ng/mL and ~1.5 ng/mL. Results of the current study revealed the following values for salivary cortisol levels before, 5 minutes after, and 30 minutes after listening to dissonant music: 3.14 ng/mL, 3.01ng/mL, and 2.69 ng/mL (Table 4). Therefore, salivary cortisol values obtained from this study were comparable to similar published studies.

As for the distribution of salivary cortisol levels, a normal distribution (i.e., Gaussian distribution) was observed. Within groups, the cortisol levels showed relatively little deviation from the mean values. Some papers indicate a non-Gaussian distribution of cortisol in a given population (Mentzel and Wiedemann, 1993), while others have shown more Gaussian distributions similar to what we observed (Rosmalen et al., 2004). Why might some studies show a greater range of cortisol? In the current study, many aspects that could affect cortisol (for example, age, and time of day, medications, diseases, smoking, and stressful events that could alter participants' cortisol) were controlled for. Moreover, the ELISA kit used has been highly standardized by the manufacturer, and each plate that we analyzed contained an internal standard curve that reduces the inter-assay variability.

Relevance

The anti-inflammatory effects of exercise are being explored as a possible treatment for a variety of inflammatory disorders and diseases (Ploeger et al., 2009; Schultz et al., 2004). Exercise can induce the secretion of cortisol, which is the primary reason why exercise is regarded as an anti-inflammatory (Kraemer and Ratamess, 2005). Cortisol has potent anti-inflammatory properties and also promotes tissue repair, which is why it has been the primary focus of ample research on the effects of exercise on inflammation (Elenkov and Chrousos, 2002).

Future Direction

Moderate exercise was chosen because one of the potential applications of this research is to perform exercise interventions in chronically diseased populations with limited physical abilities. For instance, moderate exercise might result in HPA axis activity in patients with multiple sclerosis (MS). This speculation, which will be examined in future studies, is based on the finding that MS patients with longer disease duration have HPA axis impairments that may result in exacerbation of their symptoms (Kern et al., 2014). Therefore, moderate exercise might act as a potent stimulus to activate HPA axis activity in this population, thereby improving the functionality of the HPA axis. Furthermore, moderate exercise has the potential to encourage MS patients to participate in appropriate and regular exercise programs, which may aide in energy preservation and reducing feelings of frustration and loss of control, thereby contributing to enhanced well-being (Petajan et al., 1996). The results of this study will help us to design a better protocol for future studies that will examine long-term effects of moderate exercise and listening to music on MS patients' HPA axis activity.

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Appendix 1.

Within-Subject Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Activity	.684	4.444	5	.489	.811	1.000	.333
Time	.832	2.202	2	.333	.856	.974	.500
Activity * Time	.098	25.037	20	.218	.598	.855	.167

Appendix Table 1. Mauchly's Test of Sphericity[a] shows that our data does not violate the assumption of sphericity, and the F-tests assuming sphericity are valid. Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix. The potential significance of the interaction of activity and time was also studied. Mauchly's sphericity tests were run on the data. The p-values for activity, time and the interaction of activity and time were 0.489, 0.333, and 0.218, respectively. All p-values were well above the significance level of 0.05. Therefore, the tests showed that our data does not violate the assumption of sphericity, and there is no need to modify the degree of freedom. The F-tests assuming sphericity are valid.

a. Design: Intercept

Within-Subject Design: Activity + Time + Activity * Time

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subject Effects table.

Appendix 2.

CONSENT TO PARTICIPATE IN A STUDY

Title: "Treatment of Autoimmune Disease with Exercise and Music (TADEM) Study" conducted by Dr. Peter Darlington of the Department of Exercise Science, Concordia University, PERFORM Centre.

Purpose: I understand that the purpose of this research is to use combined exercise and music therapy to raise the body's natural anti-inflammatory hormone cortisol.

Procedure: I understand this is a five day study at the PERFORM Centre. First, I sign this consent form and fill out questionnaires to determine my health status and music preferences. I will do a YMCA test to determine my exercise capacity. This is a moderate exercise routine where my heart rate is monitored on a stationary bicycle. On subsequent days, I will do one of four things: 1) listen to music, 2) exercise, 3) listen to music while exercising, or 4) just relax. The exercise is moderate: only 60-70% of my maximum exercise capacity for 30 minutes on a stationary bike. I will provide saliva samples before and after, which are stored in coded tubes in a freezer and then analyzed for cortisol and stress hormones.

Risk and Benefits: This experiment involves minimal risks to me. The exercise is moderate and will be done under supervision of students and staff. The saliva is collected and stored in a clean and safe manner using sealable tubes. The research will help develop new therapies for treating patients without immune diseases such as multiple sclerosis.

Conditions of Participation: I understand that through signing this form that all my personal information will be kept private and secure. They may use my anonymous data in their research study. I will disclose any medical conditions or relevant health issues on the PAR-Q data questionnaire. I cannot participate if I have chronic or acute heart disease or autoimmune disease, physical limitations that prevents bicycle exercise, or I am taking immuno-modulatory medicine. During the test period I will not exercise on my own time or do any strenuous activity, and I will abstain from drinking alcohol, smoking, using recreational drugs or any stimulant including caffeine. At any point I may withdraw from the study. If I withdraw from the study, any data and samples collected will be kept and analyzed. At the end of the study all samples will be discarded.

Signatures: I HAVE CAREFULLY READ THE ABOVE AND UNDERSTAND THIS AGREEMENT. I FREELY CONSENT AND VOLUNTARILY AGREE TO PARTICIPATE.

NAME (please print) ____________________________________

SIGNATURE ___________________________________ DATE (mm/dd/yy)__________________

If you have questions please contact the study leader Dr. Peter Darlington, 514-848-2424 ext. 3306. If you have questions about your rights as a participant, please contact the Research Ethics and Compliance Advisor, Concordia University, 514-848-2424 ext. 7481.F. **Statement of person conducting informed consent** I have discussed this document with the participant; in my opinion they understand the risks, benefits, and procedures involved.

NAME (please print) ____________________________________

SIGNATURE ___________________________________ DATE(mm/dd/yy)__________________

Appendix 3.

Music Assessment

Please check of the appropriate box for how you felt about the music you listened to.

	1 (Not at all)	2	3	4	5	6	7	8	9	10 (Very much)
The music was annoying										
The music was agitating										
The music was stimulating										
The music was calming										
The music was rhythmic										