January 2014

The Effects Of Whole Body Vibrations On Salivary Cortisol In Young Females In Squat Position

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THE EFFECTS OF WHOLE BODY VIBRATIONS ON SALIVARY CORTISOL IN YOUNG FEMALES IN SQUAT POSITION

By

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A Thesis
Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota
May
2014

This thesis, submitted by Rohini Gawande in partial fulfillment of the requirements for the Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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Department	Kinesiology
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ACKNOWLEDGMENTS

I would like to express my apprecation to Dr. Joshua Guggenheimer for the academic guidance, and tremendous encouragement throughout my academic and research work. I would also like to express my deepest gratitude to Dr. Dennis Caine and Dr. Jim Whitehead for their direction, and support. Special thanks to Dr. Caine for processing funding for my study without which it wasn't possible for me to finish my study. I thank to my committee members for the valuable comments, suggestions and considerate review for my thesis.

I would like to acknowledge my research study participants for this thesis, I would like to specially thank all the voluntarily participant who participated in this study under the tight time schedule.

I also thank to all students and staffs at Kinesiology and Public Health for sharing their knowledge and friendship.

Thank you to my parents: Dr. Sukhadeorao Gawande and Mrs. Pramila Gawande for their endless support and encouragement. I am also grateful to my husband Jivan for his encouragement, and motivation in every step of my thesis work, and his support and patience.

ABSTRACT

Purpose: Despite the catabolic effect of cortisol on bone metabolism, and the potentially counteracting anabolic effect of whole body vibration (WBV) on bone, only a few studies have explored the effects of WBV on cortisol, the findings of which have been inconclusive. Therefore, the purpose of this study was to determine the effect of an acute bout of WBV on salivary cortisol levels in women. Method: This study utilized a randomized cross-over design, consisting of two individual treatment days: the vibration intervention (V) and the non-vibration control (NV), with a washout period of 2-3 days between the two. Participants consisted of a convergence sample 12 women (19 to 30 yrs. old) with varying levels of physical activity. WBV consisted of a 30-sec bout of isometric squatting with 1100 knee flexion followed by 30-sec rest. WBV parameters were set to a frequency of 30 HZ and an amplitude of 4 mm. Saliva was sampled both before and immediately after exposure to each of the conditions. Differences between pre- and post-test values for each condition were compared using a paired samples t-test. Significance was set at $p < 0.05$; with a 95% confidence interval.

Results: paired-samples t-tests for both the experimental and the control group showed no significant difference between pre- and post-test data ($t(13) = 0.154$, $p = 0.88$, and $t(13) = -11.314$, $p = 0.451$). Discussion: The main finding of this study was that an acute bout of WBV did not appear to have a significant influence on salivary cortisol. It may have been that the chosen volume of WBV was inadequate to provide a significant stimulus to result in an endocrine response. Future WBV research should be focused on

determining the optimal workload needed to induce hormonal changes in both women and men.

CHAPTER 1

INTRODUCTION

The advent of whole body vibration (WBV) in the field of physical activity has attracted researchers and lay persons alike. This attraction has derived from its use in various fields as an effective non-pharmacologic, user-friendly therapeutic intervention known to influence several physiological systems [9]. The initial application of vibration as a supplement to exercise was conducted by Russian scientists, who found that vibration was effective at enhancing strength in well-trained subjects in a microgravity environment [11]. Since then, WBV has been used to manipulate multiple physiological parameters, such as bone density, skeletal muscle function, obesity, metabolic rate, and tissue perfusion [5, 14, 19, 28, 41]. Since its introduction, the use of WBV has been supported for its effectiveness in a variety of fields including sports, space travel, and rehabilitation after injury, although the extent of its effectiveness depends upon the prescriptions, which has yet to be conclusively agreed upon [24].

The mechanism of action for WBV on skeletal muscle mass appears to be linked to direct mechanical stimulation or an increase in tissue blood supply at the working muscle. Additionally, it has been stated that vibration-induced fluctuations in hormones may be capable of modulating bone cell activity, which may also be responsible for WBV-induced skeletal muscle changes [33]. Of the many stated mechanisms behind the

effects of WBV, understanding the physiological responses of the endocrine system during acute and chronic vibratory protocols may be imperative to deciphering the mechanisms involved in enhanced muscle mass and bone remodeling and also in in determining an individual's response to vibration exercise [16, 33]. Accordingly, several investigations have examined and reported hormonal fluctuations in growth hormone, cortisol, and testosterone following bouts of WBV, suggesting that the endocrine system may be affected by WBV [3, 16, 24]. Considering the above findings, it seems clear that WBV is a potential means of enhancing many physiological parameters including the endocrine system.

Cortisol is the primary catabolic hormone in human metabolism. As such, it is responsible for increasing protein degradation and decreasing protein synthesis [13, 20]. Furthermore, cortisol also increases bone resorption, corresponding to dramatic decreases in bone formation [29]. Evidence also confirms that changes in cortisol concentrations can modulate neuromuscular performance through various short-term mechanisms such as secondary messengers, lipid/protein pathways, neuronal activity, behavior, cognition, motor-system function, muscle properties, and energy metabolism [13]. It is therefore clear that cortisol also acts at the molecular level, and correspondingly may have significant influence on neuromuscular events effecting physiological performance.

Despite the catabolic effect of cortisol on bone metabolism, and the potentially counteracting anabolic effect of WBV on bone, only a few studies have explored the effects of WBV on cortisol, the findings of which were hardly conclusive [3, 5, 8, 16, 17, 23, 24]. Considering the conflicting findings taken from these studies, there is an

apparent need for more investigation regarding WBV and the impact it may have on hormonal profile [16]. Furthermore, based upon a current review of the literature, only one study could be found exploring the effects of WBV on cortisol level in females, while all others were done with a male population. Specific sex differences in basal hormone levels, hormone production and hormone metabolism reinforce the apparent need for endocrine related research on the sex-specific populations [12]. Despite the initial attention given approximately 30 years ago to female exercise endocrinology, the topic is still far from being completely understood. Therefore the purpose of this study is to examine the effects of WBV on the salivary cortisol in younger females. It is hypothesized that salivary cortisol level will decrease following exposure to WBV. This study will provide data regarding female hormonal salivary cortisol response to WBV exposure that can then be compared to existing data on male cortisol responses to WBV exposure. The goal of this research is to help prescribe appropriate exercise regimens in order to optimize adaptations for both female and male populations. The primary goal of this study is to find out whether WBV has any role to play as a noninvasive tool in manipulating cortisol related pathological conditions such as bone loss, muscle loss and depression.

CHAPTER 2

LITERATURE REVIEW

2.1 Whole Body Vibration

When used in a controlled setting, WBV is delivered in the form of sinusoidal vibrations, which provide a mechanical stimulus characterized by an oscillatory motion [10]. This mechanical stimulus consists of three components: frequency, amplitude, and acceleration [33]. The repetition rate of the cycles of oscillation determines the frequency of the vibration and is measured in Hz, or cycles per second. The extent of the oscillatory motion determines the amplitude (peak to peak displacement in mm) of the vibration. Both amplitude and frequency can be seen below in Figure 2.1.

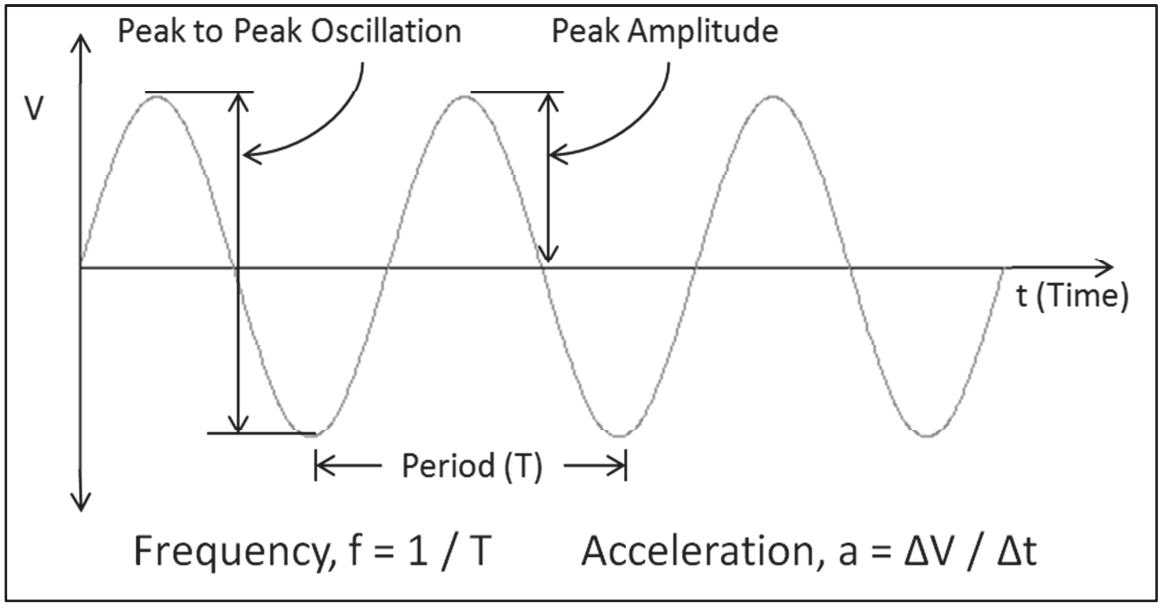

Figure 2.1 : Cycles of WBV Oscillation

When the velocity of any object changes it is said to be accelerating, which is the rate of change in velocity over time and is typically represented relative to earth's gravity (g). Due to the wide range of possible frequency and amplitude combinations, there are many whole-body vibration protocols to choose from [10]. Typically, WBV amplitudes range from <1 to 10 mm and frequencies of oscillations ranging from 15 to 60

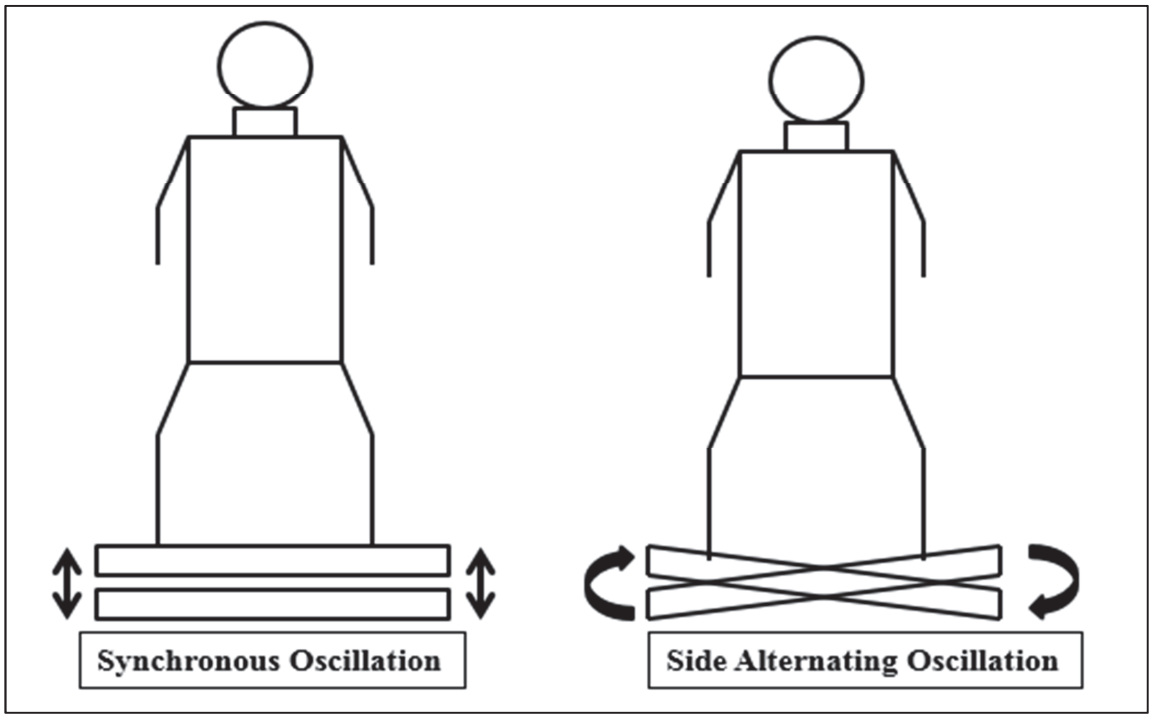

Figure 2.2 : Types of WBV devices (a) synchronous oscillation device where both legs oscillated at the same time. (b) Side alternating oscillation devise where each leg exposed to WBV in alternating time, so that one leg act as a agonist and other leg acts an antagonist for that leg.

Devices used to deliver vibrations differ not only with regard to frequency, peak-to-peak displacement, and acceleration, but also in the method of delivery. Some models apply vibrations separately to the right and left foot in a side alternating way, whereas others apply vibrations to both legs at the same time (see

Figure **2.2**) [34]. It has been argued that side-alternating vibration would evoke rotational movements around the hip and lumbo-sacral joints, therefore reducing the overall vibration transmission to the trunk whereas in synchronous mode both legs extend and stretch at the same time, and a purely linear acceleration is directed to the trunk [36].

2.2 Effects of WBV

WBV has been studied for its effects on different physiological parameters such as muscle and tendon mechanics, neurophysiological responses, energy metabolism, and its effects upon bone [36]. In order to explore the effects of WBV on bone mineral density (BMD) a 12 month study of young women with low BMD was conducted [19]. It was found that 10 minutes per day of WBV at 30 Hz and 0.3 g enhanced cancellous bone in the spine and cortical bone in the femur [19]. Similarly, a meta-analysis with 13 randomized trials (18 studies) investigated the effects of WBV on BMD and leg muscle strength in older adult women (50 years of age or older) [25]. The authors [25] examined a variety of WBV parameters with frequencies ranging from 10-54 Hz, amplitudes between 0.05-8 mm, and accelerations from 0.05-32.2 g. The conclusion of the meta-analysis was that WBV had no overall treatment effect on BMD in older women. However, the findings of this review suggested that WBV was beneficial for enhancing leg muscle strength among older adults [25]. Another study [5] explored the effects of WBV on skeletal muscle in six female volleyball players who performed dynamic leg press exercises on a slide machine with loads of 70, 90, 110, and 130 kg. Exposure to WBV was performed at 26 Hz, with a displacement of 10 mm, and accelerations equaling 5.5 g. During the test only one leg was exposed to WBV, while the other leg was used as

a control [5]. The authors [5] reported finding a significant improvement in average velocity, average force, and average power in the skeletal muscle of the leg exposed to the WBV [5]. From the results of the three studies mentioned above, one can conclude that certain physiological factors such as age, previous bone mineral density, and muscle strength, along with methodological components like WBV frequency, amplitude, acceleration, and duration need to be considered carefully in order to interpret results accurately.

In searching for the mechanism by which WBV affects BMD and muscle strength, it is useful to consider Reynolds et al.'s [35] study, in which authors looked for endogenous factors affecting the rate of bone loss in healthy elderly men and women. It was found that in men, elevated peak plasma cortisol levels were associated with accelerated loss of mineral density in the lumbar spine, and in women, elevated peak plasma cortisol levels were associated with lower baseline BMD and also a higher bone loss rate at the femoral neck [35]. In similar studies examining Cushing's syndrome and corticosteroid therapy, high cortisol levels have been found to be associated with muscle weakness, osteoporosis, hypertension, diabetes mellitus and susceptibility to infections [22]. Conversely, WBV has been shown to reduce circulating cortisol in some cases [3, 5, 17, 23]; it is therefore plausible that it may correspondingly reduce bone resorption as well. Moreover, it is also possible that the reduced cortisol levels after WBV exposure might be the mechanism behind the increase in muscle power and the increase in BMD observed in other studies [5, 19, 25].

Researchers have also studied the effects of WBV on visceral adipose tissue [41, 42]. The first of two reviewed studies combined WBV with the caloric restrictions and compared it to other exercise protocols including walking, running, cycling, and stepping in with no caloric restriction in obese adults [41]. Vissers et al. [41] concluded that WBV could possibly be a meaningful addition to future weight loss programs combined with aerobic exercise and caloric restriction [41]. A second study was done to determine the effects of WBV on neuromuscular performance and body composition in post-menopausal women [42]. Participants were randomly assigned to three groups: (1) a training group (TG), which performed 60 minutes of aerobic and strength exercises twice weekly, (2) a training group with the addition of WBV (VTG), and (3) a wellness control group (CG), which performed a low-intensity ‘‘wellness’’ program [42]. Von Stengel et al. [42] found that in the TG, lean body mass, total body fat, and abdominal fat was favorably affected, but no additive effects were generated by the vibration stimulus received by the VTG. In summary, Von Stengel et al., [42] concluded that WBV embedded in a multipurpose exercise program showed only minor additive effects on body composition and neuromuscular performance [42]. It is already well established that body weight and energy expenditure are inversely correlated [27], so it would be advantageous to elucidate the effects of WBV on energy expenditure. Regarding the effects of WBV on energy expenditure, Da Silva, et al., [14] has found that when half-squat strength training was combined with WBV, total energy expenditure (EE_{tot}) was higher compared to half-squat strength training without WBV [14]. Moreover, the increase in VO2 and EE_{tot} was proportional to the speed of the squatting. Therefore,

squatting velocity must be considered when vibration exercise is prescribed. The findings of the above three studies imply that it may be beneficial to introduce vibration exercises into regular training programs, particularly those whose key objective is muscle hypertrophy along with fat reduction [18].

When searching for the mechanism through which WBV affects body composition and energy expenditure, it is useful to consider the process of glucose breakdown into energy and the factors affecting it. In an attempt to better understand this process, Adam et al., [1] observed overweight Latino youths and found that there was increased serum cortisol, decreased acute insulin release and beta-cell functions in association with increased fasting glucose concentration [1]. Similar findings of very high cortisol levels were found to cause major alterations in glucose utilization ranging from insulin-resistance to overt and complicated diabetes [21]. Considering these observations, there is a possibility that the decrease in cortisol levels found after exposure to WBV [3, 5, 17, and 23] may be one of the mechanisms responsible for reducing visceral body fat and increasing glucose utilization by enhancing the release of insulin. However, the conclusion of the aforementioned studies raises questions to what extent WBV alone can act as a potential tool for weight loss, and whether or not we should consider developing a weight loss program that includes WBV along with other more traditional means for weight loss, such as caloric restriction and aerobic exercises.

2.3 WBV and Cortisol

Depending on the variable examined (e.g., neuromuscular performance, body composition, energy expenditure, bone density), there may be different mechanisms

underlying the effects of WBV on physiological performance. Among these different mechanisms, change in hormone levels is arguably one of the important factors. Furthermore, among the different hormone level changes caused by WBV, cortisol is thought to be an influential hormone, because it plays a vital role in modulating various physiological processes, including stress responses, growth, metabolism and immune responses [31]. Of the eight studies reviewed below, five reported decreases in subject's cortisol levels following WBV.

In evaluating the acute responses of blood hormone concentrations, Bosco et al. [4] found significant increases in the plasma concentration of testosterone and growth hormone and a corresponding decrease in cortisol following a WBV treatment among 14 men. Participants also exhibited enhancement in mechanical power output of the leg extensor muscles and increased jump performance for the counter-movement jump. Bosco et al., [4] proposed the possibility of inhibitory influences on hypothalamic neuro-secretory centers from the hippocampal serotonergic structures as a reason behind the decreased cortisol levels [3, 23]. Moreover, Bosco et al. [4] argued that because of the decreased cortisol levels, the hormonal response to vibration is likely not typical of a general emotional stress reaction, nor it is a response typical of high intensity exercise. Bosco et al.'s comments are supported by the notion that a hallmark of the emotional stress response is the activation of the autonomic nervous system and hypothalamo-pituitary-adrenal (HPA) axis (see Figure 3), stimulating the production of glucocorticoid, which is the major "stress hormone" by the adrenal cortex [15, 30]. Bosco et al., [4] concluded that WBV treatment could lead to acute changes in hormone levels and

neuromuscular effectiveness that may enhance neuromuscular performance. Moreover, Bosco et al. [4] reported that since the hormonal responses and the increase in neuromuscular effectiveness were simultaneous but independent, the two phenomena might have common underlying mechanisms [15].

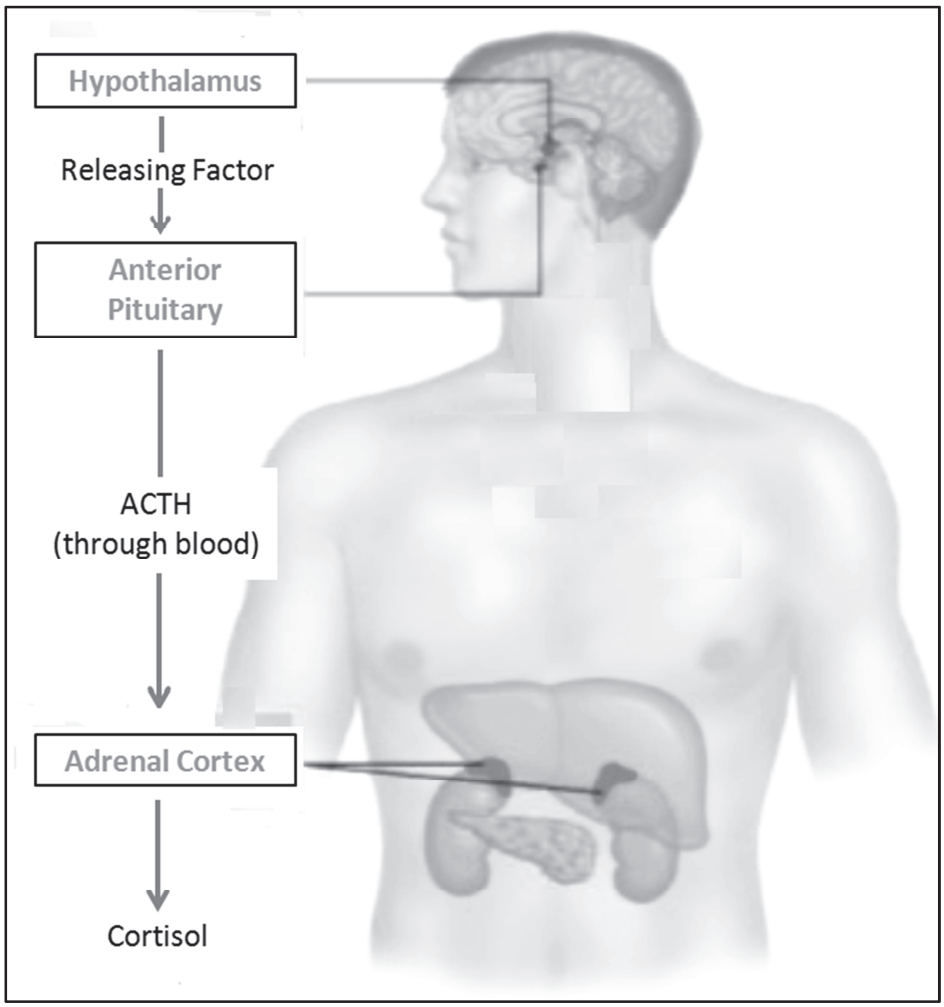

Figure 2.3 : Hypothalamo Pituitary Adrenal Axis

Bosco et al. [5], had earlier examined the effects of WBV on both hormone profile and jumping performance, and found a decrease in testosterone, cortisol and jumping height after 7 one-minute bouts of WBV. The authors concluded that the effects

of seven bouts of WBV on neuromuscular behavior were similar to effects exhibited after a single session of classic heavy resistance training, which has been shown to induce "neuromuscular fatigue" in well-trained athletes after a single training session. Specifically, the authors concluded that reductions in testosterone were due to WBV-induced suppression of activity in the pituitary testicular system and the reduction in cortisol was due to suppressed activity in the pituitary adrenocortical system [5]. Similarly, a study with twenty healthy voluntary participants (10 women) was done to investigate the effects of side-alternating WBV (26 Hz; amplitude, 1 mm; acceleration, 2.7 g) on hormone secretion and metabolism [17]. The complete treatment session consisted of 2 sets of 5 60-second exposures to WBV with a break of 60 seconds between episodes. The 2 series were interrupted by a break of 6 minutes. [17]. Fricke et al. [17] found decreased levels of adrenocorticotropic hormone, cortisol, glucose and thyroid-stimulating hormone after WBV exposure in both men and women. To explain the reasons for the decreased cortisol levels, Fricke et al. [17] raised the possibility of inhibitory influences of serotonergic structures on the cortisol-releasing centers present in the hippocampus (Figure 3), which was supported by the similar findings of Bosco et al. [4].

When we consider the above studies we can conclude that WBV has a promising capacity to influence the endocrine system in healthy populations; however, there appears to be little consensus on the extent of such hormonal responses, and the necessary prescription needed to optimize them.

In order to examine the acute and long-term effects of local high frequency vibrations (LHV) (Frequency = 300Hz) on the endocrine system and muscle strength, 18 subjects were studied in two sessions, either with or without LHV [23]. Hormonal levels were measured before, immediately after and 1 hour following the treatment. A significant reduction in cortisol levels was noted among the participants exposed to LHV, immediately after and 1 hour after the training when compared to starting values [23]. The control group also exhibited some decrease in cortisol levels, although the reduction was not statistically significant. As [23] study was performed using only local LHV, the authors concluded that vibration-induced changes in cortisol levels were not related to gravitational load or posture but rather the frequency and location of the vibration [23].

Regarding the aforementioned factors, Sousa e Silva et al. [39] found that prolonged physical exercise induced adaptive alterations in the hypothalamic-pituitary axis, increasing cortisol metabolism, and reducing cortisol synthesis and glucocorticoid sensitivity. The mechanisms responsible for this relative glucocorticoid resistance remain unknown but may involve a reduction in the expression of genes encoding glucocorticoid receptors (GR), and/or a reduction in the number of pathways and cytokines that signal inflammatory molecules of nuclear factor kappa B1 (NFkB1) [39]. A decrease in glucocorticoid sensitivity and the mRNA levels of the GR gene, combined with decreased mRNA levels of genes related to the NFkB1 pathway, might be the cause of reduced cortisol synthesis following prolonged exercise [39].

Only one study could be found specifically examining changes in cortisol levels after WBV exposure in females, in which the authors [3] examined the acute effects of

WBV on interlukin-10 and cortisol responses in athlete and non-athlete girls [3]. The study found a significant reduction in serum cortisol levels in the athletic girls immediately after training, but those reduced cortisol levels returned to baseline 2 hours after the training was complete. The non-athletic girls also showed a significant decline in serum cortisol levels immediately after training and those levels remained significant 2 hours after the training. The authors [3] concluded that insignificant stress due to vibration training might be a reason behind the decreased cortisol found immediately after WBV training. The above data suggest that there is a deficiency of information revealing the effects of WBV on hormones in female population and there is a need of further studies in these directions.

In a study examining the effects of WBV and resistance training on neuromuscular and hormone measures, Kvorning et al. [24] found that the combination of WBV along with conventional resistance training (CRT) induced a higher increase in neuromuscular and hormonal measures when compared with CRT or WBV alone [24]. Kvorning et al. [24] randomly assigned three groups: a squat group (S), a squat and vibration group (S+V), which performed squats with a weight-loaded bar on a vibrating platform, and a strictly vibration group (V) [24]. All three groups trained for a total of 9 weeks, and had their cortisol levels assessed prior to starting, after two weeks, and at again at the completion of testing after 9 weeks. The results indicated that cortisol was only increased in S+V whereas it decreased in the V group after 2 weeks of training, which was consistent with Iodice et al.'s [23] study. But there were no significant changes in pre-values for cortisol for any group after 9 weeks of training sessions.

Kvorning et al., [24] postulated that the increase in cortisol in the S+V group might have been an indication that a larger training stimulus was present and that a certain amount of physical stress was needed to trigger a cortisol response [24]. Kvorning et al. [24] initially suggested that this increase in cortisol in the S+V group was a reaction in response to the training and would reduce the muscles' ability to hypertrophy because of the catabolic effects of cortisol [24]. The authors further speculated that perhaps the S group adapted more positively to the training period compared to the S+V group in terms of mechanical performance, as there was no increase of cortisol in the S group [24]. However, considering the relatively short duration changes in cortisol level, the author postulated that the acute increase in cortisol might have instead reflected the metabolic demands of the training session rather than a catabolic phase [24].

In a randomized cross-over study in two separate sessions at 2-week intervals where older adults (70 ± years) performed isometric squats, Cardinale et al. [8] found no significant difference between the experimental and control group in short-term cortisol levels following 5 minutes of exposure to WBV at 30 Hz and 4 mm [8]. However, both groups did show increases in cortisol levels immediately post-WBV, and decreases in cortisol levels 1 and 2 hours post-treatment. These findings suggest that WBV exercise could represent an effective stimulation for the neuroendocrine system [8].

Erskine et al. [16] studied neuromuscular and hormonal responses to an acute bout of isometric half-squat exercise with and without WBV. Seven healthy men (23.3 ± 2.7 years) performed 10 sets of half squat isometric exercise for 1 min with 1 min rest between sets. Salivary concentration of testosterone and cortisol were measured and

maximal isometric unilateral knee extensions (MVC) were completed before, immediately after, and 1, 2 and 24 hours after WBV exposure. The researchers found that a 10-minute session of intermittent WBV produced an acute reduction in MVC in healthy individuals, and this observed MVC reduction returned to baseline after 24 hrs. No significant changes were identified in salivary concentrations of testosterone or cortisol, suggesting that WBV with a frequency of 30 Hz, with a peak-to-peak displacement of 4 mm and a magnitude of 3.5 g did not represent a significantly stressful stimulus for the neuro-endocrine system. Erskine et al. [16] suggested that the WBV parameters stated above were perhaps not markedly stressful for young healthy males, arguing that the magnitude of vibration was perhaps too low. Kvorning et al. [24] has also suggested that in young healthy individuals WBV should be superimposed onto high levels of muscle tension in order to elicit a marked hormonal response.

2.4 Cortisol and Gender

In order to examine the differences between men and women in HPA-axis response to stress, Seeman et al., [38] examined changes in salivary "free" cortisol in young and old subjects of both sexes [38]. Comparisons of maximum increases in cortisol during the challenge session across age and gender groupings revealed that among the younger subjects, men exhibited a greater relative percentage increase than with women. The reverse pattern was seen in the older group, where women exhibited a greater relative increase compared with men. For baseline values there was no significant gender difference among the younger subjects. However among the older subjects, the men had significantly higher baseline cortisol levels than the women. This evidence shows that

there may be a difference in normal baseline levels of cortisol depending upon one's age and gender, and that such cortisol levels change in response to stress. The authors emphasized the need for further research to extend understanding of the underlying mechanisms for gender difference in age-related trends towards increased HPA-axis reactivity. They further emphasized the importance of these finding by stating that "If confirmed, this increased HPA -axis reactivity to challenge at an older age in women may represent one underlying mechanism for the relative decline in postmenopausal women's protection against major sources of morbidity and mortality such as cardiovascular disease, osteoporosis and cancer" [38].

Similarly Laughlin et al., [26] conducted a cross-sectional study examining age and gender differences in plasma levels of cortisol in adults from 50-89 years of age. For their study the researchers [26] assayed plasma hormone levels in samples obtained between 0730 h and 1100 h from men and non-estrogen-using, postmenopausal women. Hormone levels were stratified by 10-yr age groups and compared by two-factor (gender and age) ANOVA. Cortisol levels were shown to be 10% higher in women than men. Furthermore, there appeared to be an overall increase of 20% in cortisol levels from the 50s to the 80s in both genders [26]. In summary, among older adults cortisol levels were higher in women than men. The increase in cortisol may also play a role in the metabolic shift to a catabolic state during aging and has been speculated to be a causal link to increase in several physical diseases and psychiatric disorders among older people [26]. Furthermore, Laughlin et al., [26] suggested that "These findings may have important

implications for a host of age-related processes including cardiovascular disease, brain function, and bone metabolism" [26].

Another study was conducted to investigate sex differences in HPA axis response to corticotropin-releasing hormone (CRH) infused during puberty in healthy children (41% girls), aged 6-16 [40]. Pubertal maturation levels were determined by Tanner staging, which is used to determine sexual maturity levels in boys and girls. After allowing 24 hrs for participants to adjust to the CRH infusion, plasma cortisol samples were collected. The authors found subtle changes in cortisol responses to CRH infusion during puberty in girls but not in boys. Girls showed increases in total cortisol response to CRH infusion during puberty, while boys showed little change in total cortisol response to CRH infusion. Through the study the author emphasized that "given known potent effects of glucocorticoids and increased plasticity of the adolescent brain, even subtle increases in cortisol output may have implications for influencing girl's future brain, behavioral, and endocrine response to CRH infusion". Furthermore, the observed sex differences in HPA regulation may be amplified in high-risk girls, which could possibly explain the greater rates of depression during puberty for girls [40].

Based on these studies, it appears that there is a physiological difference in baseline cortisol levels between males and females regardless of age [40]. It also appears as though males and females of various ages showed different cortisol changes when exposed to various stressors and challenges. Despite the above findings it is clear that most of the research has focused on the effects of WBV and cortisol in males and there is an obvious dearth of studies with females. As already discussed in this review, cortisol is

a key modulator of physiological processes, including stress response, growth, metabolism and immune response [31]. Moreover, changes in cortisol levels are likely linked to such things as cardiovascular disease, brain function, and bone metabolism [26]. At the same time, WBV exposure has been shown to influence fluctuations in cortisol levels [4, 5, 8, 16, 17, 23, 24], hence the need to further examine the influence of WBV exercise in a young female population.

CHAPETR 3

METHOD

3.1 Study Design

A randomized cross-over study design was used for this study, which consisted of two individual treatment days separated by a washout period of 2-3 days to ensure that any residual effects of a WBV treatment and any delayed onset muscle soreness disappeared by the next treatment [2, 6, 37]. The exercise treatments under investigation were: non-vibration (control group) and vibration (experimental group). The order in which the subjects received the treatments (control, or experimental) was assigned in a counterbalanced fashion. Approval for this study was received from the University of North Dakota Institutional Review Board.

3.2 Participants

Approximately 12 young female participants were required for this study. The required sample size was determined by doing a t-test power analysis using the G power software with a correlation between serum and salivary concentrations of cortisol in response to resistance exercise [7]. The power analysis used an effect size = 0.80 with a beta value of 0.9 and alpha value of 0.05. A total sample size of 12 was determined to be adequate in order to detect significant differences between various cortisol levels within the young adult population.

All the participants with varying levels of physical activity were recruited from various departments of the University of North Dakota with an age range of 19 to 30 years old. For the recruitment of participants, oral presentations were given by the investigator in several undergraduate and graduate classes. Along with the oral presentations, recruitment posters were posted in the UND campus building. Both forms of communication provided the respective inclusion and exclusion criteria necessary for participation in the test, in addition to explaining the special requirements for the study. All participants were screened for specific exclusion criteria including a history of any diseases affecting cortisol levels (e.g., Cushing syndrome), bone pain, recent fractures, irregular menstrual pattern and oral drug use (e.g., contraceptive pills, steroids).

Informed written consent was acquired from each accepted participant. The consent form was used to inform participants about the study protocol, risk factors, and benefits from the study (see appendix). Height and weight measurements were taken separately by using stadiometer and scale (detecto) in a standing position for each recruited participant. For the height measurements, reading was considered correct at the level of the head-plate touching the head of the participants but making sure that the participant was step off the stadiometer without ducking their head. Height measurements were ranged from 150 to 165 cm. Weight measurements were ranged from 94 to 160 pounds. Detailed menstrual history was collected from each participant, in order to account for the effects of the menstrual cycle on cortisol levels [32]. Furthermore, all of the participants were underwent treatments between the 14^{th} day and following their menstruation date. Participants were asked not to eat for 8 hours prior to the study and to

remain refrain from consuming alcohol within 12 hours of their participation in the study. To facilitate the above stipulations, all data were collected between 8:00 AM and 11:00 AM. In order to account for diurnal fluctuations of cortisol, the participants were asked to come in at the same time for each day of data collection. Participants were also inquired about their stress levels before taking any readings by asking them to grade their stress levels. Any participant with very high stress was not allowed to participate in the study.

3.3 Study Protocol

As can be seen in Figure 4, all participants underwent two separate treatments using a randomized counterbalanced cross-over design with 2-3 days gap between the protocols. Participants were initially made familiarized with the study protocol and were given an opportunity to observe demonstrations of the saliva collection method. On the first treatment day, both control and experimental participants were assigned a designation number in order to keep a confidential record, of which sample belongs to which participant.

The participants were asked to rinse their mouths with water, and after 10 minutes the pre-test saliva sample (baseline) was collected. Next, they were asked to get on the WBV plate (PneuMex PneuVibe Pro, Sandpoint Idaho) with bare feet directly in contact with the vibration plate and in a squat position with a knee angle of 110 degrees for 30 seconds followed by a rest of 30 seconds. During the rest time, participants were asked to sit quietly. The 30-second isometric squat followed by a rest of 30 seconds was considered one full cycle and was repeated 10 times. The post-test saliva samples were then collected immediately after the 10^{th} cycle was completed.

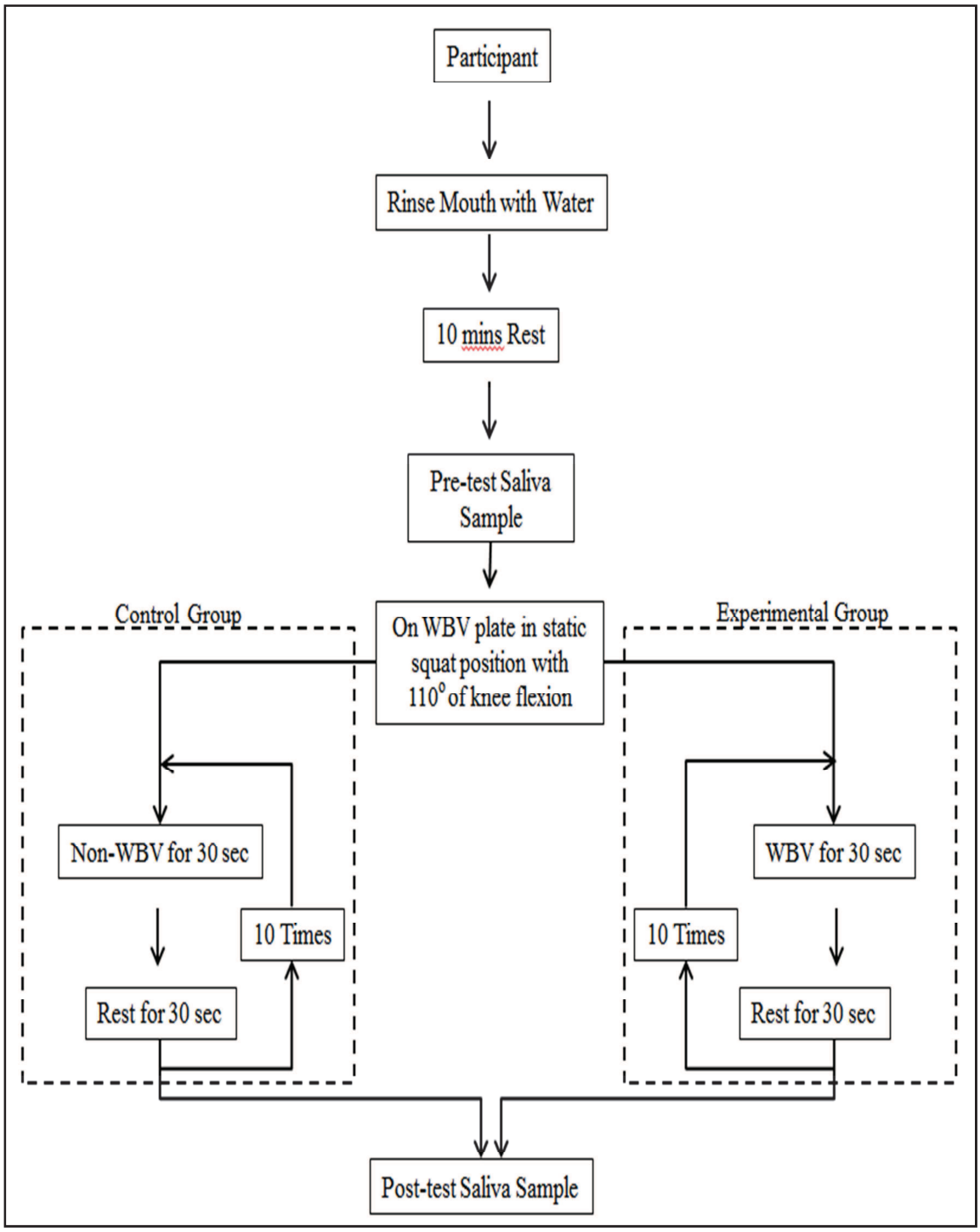

Figure 3.1 : Study Protocol

3.4 Experimental Condition

The participants in the experimental group were exposed to WBV at a frequency of 30 Hz and peak-to-peak displacements of 4 mm, totaling a gravitational magnitude of 3.5 g. These dimensions were chosen due to the fact that they have been shown to be optimal in producing the greatest magnitude of response in electromyography (EMG) activity of the vastus lateralis muscle in an isometric half squat position [9, 11,16]. Control group participants were asked to perform the exact same activities as described above, with the only difference being that the plate was not vibrating during the static squat exercises.

3.5 Instruments

Both pre-test and post-test samples were collected according to the manufacturer's recommendations, starting with washing and thorough drying of the hands before collecting the sample. The Salimetrics research Center's (State College, PA) recommended saliva collection method of passive drool was used. In this sample collection method, participants were instructed to allow saliva to pool in their mouths. Once a sufficient amount of saliva had accumulated, participants were asked to tilt their head forward and allow saliva to drool into a collecting vial. The amount of sample collected was based on the instructions provided with the test kit. Then each sample was properly labeled with date, time and a designation number. All the samples were refrigerated below-20ºC after collection, and sent to the Salimetrics Research Center (State College, PA) for testing.

3.6 Statistical Analysis

SPSS (version 14) was used to analyze the results from the saliva test. The analysis helped to determine the influence of the independent variable of WBV on the dependent variable salivary cortisol by using a paired sample *t*-test. This influence was examined by first calculating the differences between pre- and post-test values and then compared between protocols using a paired samples *t*-test [7]. Significance was set at $p < 0.05$; a 95% confidence interval was set for all tests.

3.7 Data Analysis

The purpose of this experimental study was to find out effects of whole body vibrations (WBV) on the salivary cortisol in female. The effects of WBV on the dependent variable (cortisol levels) were analyzed using paired-samples t-tests. For this SPSS (version 14) statistical software was used. Confidence intervals were set up at 95%.

CHAPTER 4

RESULTS

Overall, no significant differences were found within the paired-sample t tests for the levels of cortisol in between control and experimental groups. A paired-samples t test for the experimental group showed no significant difference (t (13) = 0.154, P = 0.88). There was also no significant difference found between pretest and post- test cortisol levels (t (13) = -11.314, P =0.451) in control group.

Table 1 Cortisol Level between the Two Groups

Cortisol levels (mg/dl)	**Experimental Group**		**Control group**	
	Pre WBV	Post WBV	Pre No WBV	Post No WBV
M	0.252	0.250	0.260	0.306
SD	0.126	0.133	0.114	0.299
P	0.88		0.451	

M= Mean, *SD*= Standard Deviation

CHAPTER 5

DISCUSSION

The main purpose of this study was to examine the effects of WBV on the salivary cortisol in younger females. It appeared that WBV had no impact on cortisol levels, as indicated by the lack of significant difference in the cortisol levels between the experimental group who performed squat exercise with WBV and the control group who performed squat exercise without WBV. This study was done to provide data regarding female hormonal salivary cortisol response to WBV exposure that can then be compared to existing data on male cortisol responses to WBV exposure.

In a similar crossover study, Erskine et al. [16] asked their participants, which consisted of seven healthy men (23.3 ± 2.7 years), to perform 10 sets of half squat isometric exercise for 1 min with 1min rest between sets. This was done in order to examine the neuromuscular and hormonal responses to an acute bout of isometric half-squat exercise with and without WBV. Salivary concentration of testosterone and cortisol were measured and maximal isometric unilateral knee extensions were completed before, immediately after, and 1, 2, and 24 hours after WBV exposure. No significant changes were identified in salivary concentrations of testosterone or cortisol. Erskine et al.'s findings suggested that WBV parameters similar to those used in the current study (30

Hz, a peak-to-peak displacement of 4 mm and a magnitude of 3.5 g) did not represent a significantly stressful stimulus for the neuro-endocrine system. Similar to the findings of Erskine et al., participants from the current study demonstrated no changes in cortisol after WBV exposure. These findings suggest that WBV with the above parameters were not an effectively stressful stimulus for the young healthy females to decrease cortisol levels, implying that the magnitude of vibrations was perhaps too low.

Kvorning et al. [22] also examined the effects of extra load on salivary cortisol levels after WBV exposure, by randomly assigning participants to one of three groups: a squat group (S), a squat and vibration group (S+V), which performed squats with a weight-loaded bar on a vibrating platform, and a strictly vibration group (V). All three groups trained for a total of 9 weeks and had their cortisol levels assessed prior to starting, after two weeks, and again at the completion of testing after 9 weeks. The results indicated that cortisol was only increased in S+V, whereas it decreased in the V group after 2 weeks of training. Their findings did not indicate an increase in cortisol immediately after vibration exposure in the V group. However, they did find that the combination of WBV along with conventional resistance training (CRT) induced a higher increase in neuromuscular and hormonal measures when compared with CRT or WBV alone [22]. The findings from the current study failed to indicate any differences in cortisol levels after exposure to WBV, perhaps due to a lack of adequate stimulus which is similar to that found in immediately after vibration exposure in the V group but differing from S+ V in Kvorning et al.'s study. Differing WBV stimuli may be one reason for the different findings reported in the current study when compared to those

reported by Kvorning et al. [22]; such conflicting results illustrate the importance of discerning an optimal stimulus necessary for desired cortisol level changes. It may be postulated that under more aggressive WBV parameters the current study would have produced changes in cortisol levels similar to those found by Kvorning et al. [22].

Examination of a similar preliminary study from Cardinal et al. [9] examining the acute effects of different WBV amplitudes on the serum testosterone (T) and insulin growth factor-1 (IGF-1) in young men demonstrated that a single session of WBV exposure with a frequency of 30 Hz and amplitudes of 1.5 and 3 mm did not noticeably alter serum T and IGF-1 levels. However, the researchers [9] were attempting to find out the effects of WBV on T and IGF-1 levels and not cortisol levels which were examined in the current study. The authors [9] proposed that the lack of significant findings could be explained by the relatively low level of neuromuscular stimulation generated by the low amplitudes used in their study [9] which was very similar to postulations made to support current study. Furthermore, Cardinale et al. [9] emphasized that muscle activation while squatting on a vibrating plate without added weight, along with small vibration amplitudes was unlikely to result in intense muscle activation for well-trained and young individuals. The above study was not done to examine the effects of WBV on cortisol specifically, but rather to illustrate the effects of WBV on the endocrine system in general.

It has been stated that vibration-induced fluctuations in hormones may be capable of modulating bone cell activity, which may also be responsible for WBV-induced skeletal muscle changes [33]. Accordingly, several investigations have examined and

reported hormonal fluctuations in growth hormone, cortisol, and testosterone following bouts of WBV, suggesting that the endocrine system may indeed be affected by WBV [3, 16, 24]. Hence Cardinal et al's [9] study is important to consider as their findings corroborate those found in the current study.

When the above studies are considered, it can be concluded that in healthy individuals higher levels of WBV parameters should be used in order to elicit a marked hormonal response. Cardinale and Wakeling [10] also supported the notion of higher levels of WBV in order to activate target muscles and optimize hormonal release into the system, further emphasizing the notion by suggesting inadequate stimulation of target muscles from WBV as one of the reasons behind their studies failure to show any positive effect.

Cardinale et al's. [8] study, consisted of two separate sessions at 2-week intervals where older adults (70 +/- years) performed isometric squats. Cardinale et al. [8] found no significant difference between the experimental and control group in short-term cortisol levels following 5 minutes of exposure to WBV at 30 Hz and 4 mm [8]. However, both groups did show increases in cortisol levels immediately post-WBV, and decreases in cortisol levels 1 and 2 hours post-treatment. Based on the Cardinale et al. [8] study, there may be a possibility of delay between the changes in the serum and salivary cortisol concentrations. Therefore, it is plausible that in the current study, the time frame which was utilized was not adequate for detection of long-term changes in salivary cortisol concentrations, as we chose to examine cortisol only immediately after the exercise bout, differentiating it from the aforementioned study [8]. These findings

suggest that WBV exercise could represent an effective stimulation for the neuroendocrine system [8]; however, a sufficient amount of time may need to elapse before cortisol levels are significantly changed to the extent where they can be detected in the saliva. More studies are needed on this topic in order to see the time-response of salivary cortisol following WBV.

5.1 Limitations:

One limitation of this study was the sample size, as participants were excluded if they were taking any form of contraception, which is fairly common among college-aged women. Recruitment was further hindered by the fact that participants were asked to engage in data collection 14 days prior to menstruation in order to control for cortisol fluctuations throughout the menstrual cycle, thus limiting student interest due to the rigid scheduling requirements. Another limitation was that the current experiment required costly instruments for analysis of the cortisol levels. As this study was not funded, sampling frequency was limited to pre- and post-WBV due to the cost of sample analysis.

5.2 Delimitations:

The main delimitation of this study was that this study was done with females, so we cannot apply any of the results to the male population. Furthermore, participants were recruited from the UND campus and were of varying levels of physical activity, and therefore, the results cannot be applied to a non-collegiate population.

Based on the above findings, it is clear that there is a need for more studies in order to determine adequate and optimum WBV parameters necessary for eliciting changes in hormone concentrations. There appears to be little consensus on the extent of

such hormonal responses, and the necessary prescriptions needed to optimize them. This study was done to explore the effects of WBV on the salivary cortisol in younger females. It was hypothesized that salivary cortisol level would decrease following exposure to WBV. However, it appeared that WBV had no impact on cortisol levels, as indicated by the lack of a significant difference in the cortisol levels between the experimental group who performed squat exercise with WBV and the control group who performed squat exercise without WBV. Despite these findings, the current study will help to establish that the WBV parameters used to establish changes in cortisol levels in collegiate females were not enough to decrease the cortisol levels. When considering participant selection, researchers should consider the contraceptive, steroid and other drugs usage that may alter the cortisol levels in the participants. Also, it is necessary to control the menstrual cycle phase by asking all participants to participate in the specific phase of menstruation phase. In conclusion, there is a need for more studies, especially the studies directly comparing the effects of WBV on cortisol levels in males and females in the same settings, to discover the effective ways to see the effects of WBV on males and females.

CHAPTER 6

CONCLUSION

The purpose of this study was to determine the effect of an acute bout of WBV on salivary cortisol levels in women. Our hypotheses were that salivary cortisol level would decrease following exposure to WBV. Our hypothesis failed to prove and the main finding of this study was that an acute bout of WBV did not appear to have a significant influence on salivary cortisol. It may have been that the chosen volume of WBV was inadequate to provide a significant stimulus to result in an endocrine response. However, the current study provides data that parameters used to establish changes in cortisol levels in collegiate females were not enough to decrease the cortisol levels. Future WBV research should be focused on determining the optimal workload needed to induce hormonal changes in both women and men.

CHAPTER 7

APPENDICES

APPENDIX A

INFORMED CONSENT

TITLE: *Effects of WBV on salivary cortisol in young females*

PROJECT DIRECTOR: *Rohini Gawande*

PHONE #: *701-330-2663*

DEPARTMENT: *Physical Education, Exercise Science, and Wellness*

STATEMENT OF RESEARCH

A person who is to participate in the research must give his or her informed consent to such participation. This consent must be based on an understanding of the nature and risks of the research. This document provides information that is important for this understanding. Research projects include only subjects who choose to take part. Please take your time in making your decision as to whether or not to participate. If you have questions at any time, please ask.

WHAT IS THE PURPOSE OF THIS STUDY?

You are invited to be in a research study about the effects of whole body vibration (WBV) on salivary cortisol in young females because you are a female aged approximately 21 +/- 2 years and regularly participate in lower body resistance training like squats, lunges and leg presses. You are not consuming any drugs like birth control pills; steroids etc. You also don't have Cushing disease, Addison's disease, depression, any oral disease, previous fracture, and irregular menstrual pattern.
The purpose of this research study is to examine the effects of WBV on the salivary cortisol in the young females as a potential means of treatment of cortisol related diseases and establish salivary cortisol as a non-invasive tool for cortisol measurement. It is hypothesized that salivary cortisol level will decrease following exposure to WBV. This study will provide data regarding female hormonal salivary cortisol response to WBV exposure that can then be compared to existing data on male cortisol responses to WBV exposure. This will help prescribe appropriate exercise regimens in order to optimize adaptations for both female and male populations.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 14 young females will take part in this study at the University of North Dakota.

HOW LONG WILL I BE IN THIS STUDY?

Your participation in the study will last for 3 days. You will need to visit the Human Performance laboratory of the PXW department, at UND 3 times. Each visit will take about 30 to 40 minutes.

WHAT WILL HAPPEN DURING THIS STUDY?

You will have to undergo two separate treatments with approximately 3 days in between the experimental and control protocols. All the data collection will be done in between the 2nd and 14th day following their menstruation phase. In order to account for diurnal fluctuations of cortisol, you will be asked to come in at the same time for each day of data collection and all the data will be collected between 7:00 AM and 10:00AM.
You will initially be familiarized with the study protocol and will be given an opportunity to observe demonstrations of the saliva collection method and exercise protocol. Your height and weight measurements will be taken on the familiarization day. Detailed menstrual history will be collected from you, in order to account for the effects of the menstrual cycle on cortisol levels. You will be asked not to eat for 60 minutes prior to the study and to refrain from alcohol consumption within 12 hours of participating in the study.
On the first treatment day, you will be given a designation number for keeping a confidential record of which sample belongs to which participant. Then you will be asked to rinse your mouth with water, and after 10 min the pre-test saliva sample (baseline) will be collected. Next, you will be asked to get on the WBV plate (PneuMex PneuVibe Pro, Sandpoint Idaho) with bare feet directly in contact with the vibration plate and will be positioned in a squat position with 110 degree knee flexion for 30 seconds followed by a rest of 30 seconds. During the rest time, a relaxed sitting posture will be maintained. The 30 second isometric squat followed by a rest of 30 seconds will be considered one cycle and will be repeated 10 times. The post-test saliva samples will then be collected immediately after the 10th cycle is completed.
When you will be in the experimental group, you will be exposed to WBV at a frequency of 30 Hz and peak-to-peak displacements of 4 mm, totaling a gravitational magnitude of 3.5 g. When you will be in the control group you will be asked to perform the exact same activities as described above, with the only difference being that the plate will not be vibrating during the static squat exercises.

WHAT ARE THE RISKS OF THE STUDY?

There may be some risk from being in this study. The risks associated with this type of study are minimal with no injury being reported in previous similar studies. There may be some discomfort experienced such as fatigue and/or muscle soreness after the

WBV exposure, but this typically dissipates within a day or two. In order to minimize these risks, we are recruiting experienced participants that are trained and accustomed to performing lower body resistance training.

WHAT ARE THE BENEFITS OF THIS STUDY?

You may not benefit personally from being in this study. However, we hope that, in the future, other people might benefit from this study because this study will provide data regarding female hormonal salivary cortisol response to WBV exposure that can then be compared to existing data on male cortisol responses to WBV exposure. This will help prescribe appropriate exercise regimens in order to optimize adaptations for both female and male populations. This will help us to establish potential of WBV exercise as an effective therapeutic tool for various diseases with changes in cortisol level like Cushing disease and chronic stress syndrome etc.

ALTERNATIVES TO PARTICIPATING IN THIS STUDY

Instructors will be asked to provide extra credit for your participation in this research study based upon the activities you will do for this research study and how much time you are going to spend for this study. If you choose not to participate in this study, you may earn extra credit in your course in other ways. Please ask your instructor, who will provide you with comparable assignments that you may choose to complete (e.g. writing assignments, participation in other research experiments etc.).

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You will not have any costs for being in this research study.

WILL I BE PAID FOR PARTICIPATING?

You will not be paid for being in this research study**.**

WHO IS FUNDING THE STUDY?

The University of North Dakota and the research team are receiving no payments from other agencies, organizations, or companies to conduct this research study.

CONFIDENTIALITY

The records of this study will be kept private to the extent permitted by law. In any report about this study that might be published, you will not be identified. Your study record may be reviewed by Government agencies, the UND Research Development and Compliance office, and the University of North Dakota Institutional Review Board.

Any information that is obtained in this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. Confidentiality will be maintained by means of coding the data to a master list and the list will be kept separate from the consent form and the subject's personal information. The data will be kept in locked laboratory and in restricted access computer. This data will not be assessable to anyone other than the PI and the involved PI-advisor.
If we write a report or article about this study, we will describe the study results in a summarized manner so that you cannot be identified.

IS THIS STUDY VOLUNTARY?

Your participation is voluntary. You may choose not to participate or you may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled. Your decision whether or not to participate will not affect your current or future relations with the University of North Dakota.

If you decide to leave the study early, we ask that you inform the project director by calling or via email. The contact information is provided in this form. There will be no consequences of your withdrawal from the study at any time.

CONTACTS AND QUESTIONS?

The researchers conducting this study are Rohini Gawande and Dr. Joshua Guggenheimer. You may ask any questions you have now. If you later have questions, concerns, or complaints about the research please contact Rohini Gawande at 701-330-2663 during the day and at 701-777-9520 after hours or you can also contact Dr. Joshua Guggenheimer at 701-777-2988.
If you have questions regarding your rights as a research subject, or if you have any concerns or complaints about the research, you may contact the University of North Dakota Institutional Review Board at (701) 777-4279. Please call this number if you cannot reach research staff, or you wish to talk with someone else.
Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Subjects Name: _____

_____ _____
Signature of Subject Date

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative.

_____ _____
Signature of Person Who Obtained Consent Date

APPENDIX B

WBV and Salivary Cortisol Study

1. Name : _____
2. Age : _____
3. Stress level: 1-2-3 Mild 4-5-6 Moderate 7-8-9-10 Severe
4. Designated No: _____
5. Contact info: _____
6. Height: _____
7. Weight: _____
8. H/O injuries and drugs: _____
9. H/O Diseases: _____
10. First day of last menstrual period: _____
11. 1st day of visit :_____
12. 2nd day of visit:_____

CHAPTER 8

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