THE EFFECTS OF WHOLE BODY VIBRATION AND EXERCISE ON FIBRINOLYSIS IN MEN

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BY

LERYN J. BOYLE

THESIS CHAIR-PAUL R. NAGELKIRK

BALL STATE UNIVERSITY

MUNCIE, IN

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ABSTRACT

THESIS: The effects of whole body vibration and exercise on fibrinolysis in men

STUDENT: Leryn J. Boyle

DEGREE: Master of Science

COLLEGE: Applied Sciences and Technology

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Purpose. The purpose of this study was to examine the fibrinolytic response to whole body vibration (WBV) and exercise in men. Methods. Twenty healthy males (23.8 ± 4.2 years, 80.8 ± 3.3 kg·m⁻²) participated in the study. Each subject performed 3 trials in randomized order separated by 1 week. The trials consisted of exercise (X), vibration (V) and vibration + exercise (VX). Exercise sessions consisted of 15 minutes of unloaded squatting at a rate of 20 per minute. Vibration sessions were conducted on a WBV platform vibrating at a frequency of 30 Hz and amplitude of 1.5mm for 15 minutes. Plasma concentrations of active tPA and PAI-1 samples were assessed at baseline and immediately after each session. Results. tPA activity change from pre to post trial was found to be significantly greater in the VX condition (0.87 ± 0.35 IU·ml⁻¹ to 3.21 ± 1.06 IU·ml⁻¹) compared to the X (0.71 ± 0.36 IU·ml⁻¹ to 2.37 ± 1.13 IU·ml⁻¹) or V (0.83 ± 0.25 IU·ml⁻¹ to 1.00 ± 0.37 IU·ml⁻¹) condition. tPA activity change from pre to post trial was found to be significantly greater in the X condition compared to the V condition. PAI-1
activity change from pre to post trial was found to be significantly decreased in the VX (6.54 ± 5.53 IU·ml⁻¹ to 4.89 ± 4.13 IU·ml⁻¹) and X (9.76 ± 8.19 IU·ml⁻¹ to 7.48 ± 7.11 IU·ml⁻¹) conditions compared to the V (5.68 ± 3.53 IU·ml⁻¹ to 5.84 ± 3.52 IU·ml⁻¹) condition. Heart rate change from pre to post exercise for the V condition (pre, 75 ± 8 bpm; post, 90 ± 7 bpm) was less than the change in the VX condition (pre, 77 ± 13 bpm; post, 148 ± 19 bpm) and X condition (pre, 71± 11 bpm; post, 139 ± 22 bpm). The change in heart rate was found to be similar in the X and VX conditions. Peak RPE was not significantly different between X and VX sessions. **Conclusions.** WBV does not stimulate increased fibrinolytic activity in young men. However, the significant increase in fibrinolytic potential observed during squatting exercise is enhanced by concurrent WBV.
Chapter I

Introduction

Hemostasis is the process of locally stopping bleeding in blood vessels. When a blood vessel becomes damaged, in a healthy individual, thrombi form to stop the bleeding. Once the bleeding has stopped, the clot is dissolved. However, if left unresolved occlusive thrombi may result in an ischemic event (60).

Fibrinolysis is the process in which fibrin clots are dissolved or lysed. This process is continuously occurring, and in a normal, physiologically healthy individual, is balanced with coagulation to prevent the formation of an unnecessary clot. Plasminogen, synthesized in the liver, is the inactive form of plasmin. Once activated, plasmin digests fibrin, breaking it into fibrin dimer proteins that can then be cleared by the liver. Two proteins that regulate fibrinolytic activity are tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPa) (11). tPA is the most abundant activator of fibrinolysis. tPA is synthesized, stored, and released from endothelial cells in the vasculature, sympathetic neurons and skeletal muscle (11). Factors known to stimulate the release of tPA include epinephrine, low blood glucose and thrombin (60).
Atherothrombosis is associated with the main causes of death worldwide (2). Plaque in the blood vessels may rupture, resulting in the creation of a blood clot, or thrombus, which may occlude an artery and cause an ischemic event (8, 24, 60). A low fibrinolytic potential has been shown to be a marker for increased risk of an adverse coronary event (26, 41). This makes fibrinolysis clinically significant to populations at risk for an ischemic event.

Acute exercise increases the risk of an adverse coronary event, in part, due to an increase in coagulation. However, acute exercise has also been shown to elicit an increase in fibrinolytic activity. (31). Immediately following an acute bout of both aerobic and resistance exercise, fibrinolytic activity is shown to increase as evidenced by an increase in the plasma concentration of active tPA and decrease in plasma PAI-1, resulting in the potential breakdown of clots (19, 61). This response is partially due to increased shear stress being placed on the endothelial cells (33) which leads to increased release of endothelial tPA. The increased fibrinolytic potential observed during exercise may offset a similar increase in coagulation activity, and may provide a protective mechanism through which clot-induced events may be avoided.

Whole-body vibration (WBV) is a novel modality in which subjects stand on a platform, in a static position or performing dynamic exercises, that moves at a predetermined frequency and amplitude, transmitting the vibration from the platform up through the feet and legs. Some studies involving WBV have shown increases in plasma epinephrine levels, as well as an increase in blood flow, indices of mechanical stress on the endothelial walls of blood vessels (29, 62). This catecholamine response and
endothelial stimulation is similar to the mechanisms through which aerobic exercise and resistance training are proposed to cause an increase in fibrinolytic potential leading to the speculation that WBV may also promote increased fibrinolysis. Thus, WBV may augment the exercise-induced fibrinolytic increase, thus enhancing the cardioprotective benefits of a therapeutic exercise regimen.

Studies have not examined the effects of whole body vibration and fibrinolysis in humans. However, enhanced endothelial function and fibrinolytic potential have been observed subsequent to use of other modalities that promote blood flow changes similar to those experienced during WBV. A recent study of supine piglets suggests fibrinolytic activity is increased during and after periodic acceleration, a pulsatile movement added in the spinal axis of the body that augments shear stress on vascular endothelial cells. Plasma levels of tPA were increased in the group that underwent the pulsatile stress compared to the control group which sat still on the motion platform (1). It is hypothesized that pulsatile stress causes increased laminar and shear stress on the endothelial cells of blood vessels resulting in the release of tPA. Results of a recent study suggested that the cardiovascular improvements associated with external counterpulsation treatment (ECPT) may be due to increased endothelial shear stress, potentially stimulating the release of vasodilatory and fibrinolytic agents. ECPT utilizes inflatable cuffs to increase blood flow through peripheral and coronary arteries. In doing so, ECPT therapy has been shown to promote positive hemodynamic adaptations such as reduced angina threshold and increased exercise tolerance in patients who were too disabled to participate in an exercise rehabilitation program (49). These studies suggest that
enhancing blood flow and vascular shear stress using mechanical means may induce a positive fibrinolytic response.

**Significance of the Study**

WBV may provide an additive effect of improved fibrinolytic activity in combination with exercise. This would be beneficial in improving the hemostatic response. Traditional exercise programs are generally effective in producing positive changes in risk factors such as blood pressure and HDL cholesterol, but may fail to affect any beneficial changes with regard to coagulation and fibrinolysis, especially in diseased populations that may be too disabled to exercise at intensities and durations that are sufficient to induce any beneficial adaptations (43). This study may enhance understanding of the cardiovascular responses to WBV, an area of study that is lacking. Furthermore, this study will add to the growing body of literature that describes the fibrinolytic responses to various types of acute exercise. Results of this study may yield valuable information regarding a potential modality to be used for the prevention and treatment of coronary artery disease (CAD).

**Statement of Purpose**

The primary purpose of this study was to examine the fibrinolytic response to exercise, WBV, and the combination of exercise and WBV in healthy men. It was hypothesized that tPA activity would increase and PAI-1 activity would decrease in the vibration (V), exercise (X) and vibration plus exercise (VX) conditions; and that the VX condition would produce a greater increase and decrease in tPA and PAI-1 activity,
respectively, than either the X or V conditions. It was also hypothesized that the X condition would produce a greater increase and decrease in tPA and PAI-1 activity, respectively, than the V condition.

**Limitations**

A limitation of this study was the population used. While it seems that women should have the same fibrinolytic response to exercise as men, it is unclear whether the results of this study can be applied to female populations (10). The population used in this study was free from CAD. Therefore, patients with CAD may experience a different response to WBV than their healthy counterparts.

**Delimitations**

Twenty males from Ball State University participated in this study. Only healthy, non medicated, non smokers, and free from musculoskeletal injury people were included. The study’s protocol included 15 minutes of WBV with and without unloaded squatting exercise, as well as 15 minutes of unloaded squatting with no vibration.

**Definitions**

Thrombus, thrombi: The final product of blood coagulation within a vessel. Blood clot, clots

Fibrinolysis: The process of dissolving a blood clot.

tPA: Tissue Plasminogen Activator- A protein involved in the breakdown of clots.
PaI-1: Plasminogen Activator Inhibitor-1 - A protein that is the primary inhibitor of tPA.
Chapter II

Literature Review

Fibrinolysis

Fibrinolysis is defined as the breakdown of a fibrin clot. This process is continuously occurring, and in a normal, physiologically healthy individual, is balanced with coagulation to breakdown any unnecessary clots. Plasminogen, synthesized in the liver, is the inactive protein of plasmin. When bound to tPA or urokinase plasminogen activator (uPa), two proteins that regulate fibrinolytic activity, plasminogen converts to plasmin (11). Once bound, plasmin binds to fibrin to cleave the clot.

tPA is synthesized, stored, and released from endothelial cells in the vasculature, sympathetic neurons and skeletal muscle (11). Factors known to stimulate the release of tPA include thrombin, histamine, bradykinin, adrenaline, acetylcholine, arginine vasopressin, gonadotropins, exercise, venous occlusion, and shear stress. The half life of tPA in the blood is short at 5 minutes (11).
PAI-1 is the most rapid inhibitor of fibrinolysis (11). PAI-1 is released from endothelial cells, monocytes, macrophages, hepatocytes, adipocytes, and platelets. When released, usually due to stimulation of cytokines, growth factors or lipoproteins, PAI-1 binds to tPA to inactivate it (11, 51). The inactivated form of tPA cannot bind to plasminogen thereby inhibiting fibrinolysis. tPA and PAI-1 can be quantified in two ways in the body. tPA activity and PAI-1 activity are a measure of unbound tPA and unbound PAI-1. tPA antigen and PAI-1 antigen are a measure of unbound tPA and PAI-1, as well as tPA and PAI-1 that are bound together. Increased tPA antigen, and PAI-1 activity and antigen are associated with reduced fibrinolytic activity. Increased tPA activity is associated with increased fibrinolytic activity. AU/ml, IU/ml, and U/ml are all synonymous measurements of tPA and PAI-1 activity. The variety of measurements is due to various differences in measurement techniques.

**Clinical Relevance of Fibrinolysis**

One in every 2.8 deaths in the United States is related to cardiovascular disease (2). Myocardial infarctions (MI) and strokes are typically a result of unresolved blood clot formation. Thus, fibrinolysis may be clinically important with regard to preventing adverse coronary events (60).

Patients with cardiovascular disease (CVD) have impaired fibrinolytic activity (17, 37, 47, 50). This is defined as either increased plasma levels of PAI-1 or an impaired ability to release tPA (59). Elevated levels of PAI-1 increase the risk of recurrent atherothrombotic events and promote the progression of vascular disease. Multiple studies have found that increased levels of PAI-1 activity are a risk for a future
recurrent myocardial infarction. Wiman et al (59) compared the PAI levels of 1212 men and women (45-65 years) in the Stockholm Heart Epidemiology Program (SHEEP) study who had a recurrent myocardial infarction to an equal number of age-matched subjects who did not have a recurrent MI as well as an equal number of aged matched healthy controls. Those who had a recurrent MI had significantly higher levels of PAI-1 than those without a recurrent MI and the control group (males, 22.1±17.5, 18.2 ± 16.5, 15.7 ± 11.7 U·ml⁻¹; females, 15.4 ± 13.6, 17.8 ± 12.4, 15.4 ± 11.0 U·ml⁻¹, respectively).

Hamsten et al (30) examined 109 men with a previous MI before the age of 45 years. In the 3 year follow up period patients were seen by one physician on a regular basis at undescribed time points. Sixteen of the patients were found to have had a recurrent myocardial infarction by the end of the follow up period. High levels of PAI-1 were significantly related to recurrent MI in those patients, as determined by PAI-1 activity levels in the 90th percentile of age and sex matched controls average PAI-1 activity levels (17.5 AU·c).

High levels of tPA antigen are also related to increased incidence of an MI. Ridker et al (46) studied 231 men (aged 41-84 years old) and compared them to their age-matched controls. Those who had a previous MI had significantly higher levels of tPA antigen than their healthy aged matched controls (10.4 vs. 9.2 ng·ml⁻¹, respectively).

Thogersen et al. (54) found significantly higher levels of tPA in 78 subjects who developed a first acute myocardial infarction (men, 12.2 µg·L⁻¹; women, 13.3 µg·L⁻¹) compared to 156 aged and gender matched control (men, 9.3 µg·L⁻¹; women, 8.3 µg·L⁻¹); this increase was shown to be independent of other risk CVD factors.
The amount of fibrinolytic impairment may be a predictor of the extent of CVD progression. A study conducted by Killewich et al. (37) examined 80 men with a mean age 69 years. Eighteen of those subjects had mild intermittent claudication, 51 had severe claudication, and the other 11 served as controls that were free of peripheral artery disease (PAD). The levels of tPA and PAI-1 activity were measured. tPA activity was significantly lower in the group with severe claudication (1.32 ± 0.11 IU·ml⁻¹) when compared to mild claudication (1.5 ± 0.21 IU·ml⁻¹) or normal, healthy subjects (1.74 ± 0.25 IU·ml⁻¹). The levels of PAI-1 were not different between groups. Results of this study suggest that the degree of CVD, at least in PAD patients, is closely linked to fibrinolytic activity.

**Acute Exercise and Fibrinolysis**

Fibrinolysis has been shown to be affected by acute exercise. Multiple studies have shown that after a bout of acute exercise tPA levels in the plasma have increased; while a decrease in PAI-1 levels have been shown. The increase in tPA during exercise is associated with an increased catecholamine response and mechanisms that stimulate tPA release during exercise.

Epinephrine, a catecholamine released during exercise, has been studied to increase fibrinolytic activity. In a study conducted by van der Poll et al (57), 17 healthy men, mean age of 28 years old, were continuously infused with epinephrine (30 ng/kg/min), or a normal saline solution. Measurements of tPA and PAI-1 were taken at baseline and hours 1, 2, 4, and 8. The researchers of this study found that those infused
with epinephrine had modestly increased levels of tPA while PAI-1 levels did not change when compared to the control group. This study, while supporting the finding that epinephrine injections are associated with an increased release of tPA, does not provide support that the levels of epinephrine secreted into plasma during exercise have any effect on tPA release. Chandler et al (14) however, examined this relationship of tPA activity and plasma epinephrine levels during exercise. He examined whether exercise induced increases of tPA were associated with plasma epinephrine concentrations during exercise. The tPA levels of 14 healthy men, 24-62 years old, were observed during two different trials. The first trial was based upon epinephrine infusions at 10, 25, and 50 ng/kg per minute. The second trial consisted of a graded cycle ergometer test until exhaustion. During the epinephrine infusions, tPA levels increased linearly with plasma epinephrine concentration levels (slope [± SEM] of 0.062 ± 0.003). In the exercise trial tPA did not increase until plasma epinephrine levels increased. At this point tPA levels increased linearly with plasma epinephrine levels but at twice the rate observed with the plasma epinephrine infusion trial (0.131 ± 0.005). tPA was directly proportional to epinephrine concentrations in both trials, suggesting that epinephrine release may in part be responsible for increases in tPA during cycle exercise to exhaustion. While it would seem that any aerobic exercise would elicit a similar tPA response to exercise, the present study utilized a squatting exercise that was time limited (instead of exhaustion limited). Therefore it is not possible to determine (unless by direct measurement) if the protocol of the present study will elicit the same responses.
Plasma bradykinin levels have been shown to increase during acute exercise in healthy adults, and well controlled type II diabetics (53). Bradykinin, an active vasodilator, has been shown to stimulate the release of tPA. In a study conducted by Brown et al. (7), 10 subjects (32.5 ± 2.8 years; BMI, 25.1 ± 1.1 kg·m⁻²) were infused, using an intravenous catheter inserted into the antecubital vein, with graded doses (100, 200, and 400 ng·m⁻²) of bradykinin. Before the infusion of bradykinin, subjects sat for 30 minutes. The authors of the study saw a significant increase in tPA release when infusion of bradykinin took place (50.6 ± 13.3 at the highest dose versus 0.9 ± 0.4 ng·100 mL⁻¹·min⁻¹ at baseline). This data suggest that bradykinin stimulates the endothelium of vessels to release tPA. This study utilized the same ELISA kit for tPA determination as well as the discard of the first 3 mL of blood before the rest of the sample was drawn into the same acidified citrate solution as the present study used. This makes comparison of tPA levels in each study easier to be generalized to each other.

Acute exercise causes an increase in blood pressure. An increase in blood pressure is thought to increase shear stress placed on endothelial walls of blood vessels. In a study performed by Diamond et al. (21) cultured endothelial cells were exposed to fluid shear stress (15 and 25 dynes·cm⁻²) for 24 hours using a parallel plate-flow chamber system with re-circulating medium driven by a constant hydrostatic head. tPA levels were found to be 2.1 and 3.0 times greater, respectively, than basal tPA secretion rate. This suggests that shear stress increases tPA. It is unknown if amount of shear stress placed on the endothelial cells in the previous study is of similar amount shown during exercise.
Many researchers have looked at the effects of various modes of acute exercise on fibrinolytic activity in both healthy and non-healthy populations. The most prevalent form of exercise studied is the effects of aerobic exercise on fibrinolysis. Cooper et al (16) evaluated eight healthy males during a graded treadmill exercise test. Baseline samples of tPA and PAI-1 activity were taken, followed by samples taken at minutes 1, 2, 4, 6, 8, and 10 post exercise. A significant increase in tPA activity and antigen and a significant decrease in PAI-1 activity were observed from pre exercise to post exercise. Although it is unlikely to cause any difference, the use of a catheter in the previous study may have affected the fibrinolytic proteins differently than a venepuncture taken with a butterfly needle as used in the present study, although no study has examined this. Similar results in increases in tPA after an acute bout of aerobic exercise has been shown by Syzmanski et al (52). Fourteen physically inactive men (mean age, 34.7 ± 4.0 years; mean VO2 max, 38.6 ± 5.6 ml·kg⁻¹·min⁻¹) were compared to 12 regularly active men (mean age, 34.8 ± 4.0 years; mean VO2 max 51.4 ± 3.9 ml·kg⁻¹·min⁻¹) during two different treadmill exercise sessions at 50% of subject’s VO2 max for 30 minutes. Each exercise session was performed on separate days and separate time points. One session was performed in the morning and the other in the evening. tPA activity was measured at baseline and immediately after. Regardless of time of day, tPA activity was shown to increase by about 60% post exercise. The blood analysis of the previous study mentioned utilized a chromogenic assay (13), therefore there may be some discrepancies between the values of tPA activity in this study compared to the tPA values in the present study. Increases in tPA activity during exercise have also been shown in unhealthy populations.
with known cardiovascular disease. A study conducted by Ivey et al (31) examined 18 untrained, stroke patients during a single bout of aerobic exercise. Subjects walked on the treadmill for 20 minutes at 60% of their maximal heart rate reserve. tPA and PAI-1 activity were measured at baseline, immediately after, and 60 minutes after exercise. tPA activity was significantly elevated immediately after, and 1 hour post exercise when compared to baseline. PAI-1 activity was found to be significantly decreased immediately after (79% increase) and 1 hour post (43% increase) exercise when compared to baseline. The results of this study suggest a favorable fibrinolytic response to a single bout of aerobic exercise, even after 1 hour after termination.

These results have been shown in acute resistance type exercises as well. In a study conducted by DeJong et al. (19) 14 men (mean age, 57.6 ± 9 years; body mass index, 26.7 ± 4.0 kg·m⁻²) with known cardiovascular disease were recruited for the study. These men participated in 8 resistance type exercises, performing 1 set of 10 repetitions to volitional fatigue while resting 1 minute between each exercise. Significant changes in tPA and PAI-1 levels were shown by increasing and decreasing, respectively. This study showed that even in populations with known cardiovascular disease, acute resistance exercises can increase fibrinolytic activity. The intensity of the exercise can play a role on increasing fibrinolytic activity. This has been shown primarily in aerobic exercise.

The fibrinolytic response to exercise at various aerobic exercise intensities has been described in previous literature. It has been shown that as exercise intensity increases so does fibrinolysis in healthy populations (42). Moderate intensity exercise (68% VO₂ max) has been shown to provoke an increase in fibrinolytic activity without the
Concomitant increase in coagulation shown at high intensity levels (83% VO2 max) (58).

Womack et al (61) examined 15 healthy males who performed a cycle ergometer test above and below lactate threshold on two different days. tPA activity increased significantly in the above lactate threshold group (pre-exercise, 1.57 +/- 0.44 IU · ml⁻¹, post-exercise, 3.85 ± 4.72 IU · ml⁻¹) but not in the below lactate threshold group (pre-exercise, 1.51 ± 0.36 IU · ml⁻¹, post-exercise, 1.94 ± 1.00 IU · ml⁻¹). PAI-1 activity decreased significantly in both the above (pre-exercise, 15.0 ± 2.73 AU · ml⁻¹, post-exercise, 10.1 ± 2.90 AU · ml⁻¹) and below (pre-exercise, 14.5 ± 2.80 AU · ml⁻¹, post-exercise, 12.6 ± 2.20 AU · ml⁻¹) lactate threshold intensities. PAI-1 activity was found to be significantly less in the above lactate threshold exercise test than in the below lactate threshold bout. These results indicate that exercise at higher intensities stimulates the fibrinolytic system more than exercise at lower intensities. This study utilized the same ELISA kit to determine tPA and PAI-1 activity as in the present study, which makes results of this study easy to generalize to the present one. An increase in tPA and decrease in PAI-1 activity during aerobic exercise was also shown in a study by Rankinen et al. (45). Nine healthy males were assessed during two separate cycle ergometer tests at 50% and 78% of VO2 max. Subjects cycled for 30 minutes, and blood samples were taken at baseline, immediately post exercise and 24 hours post exercise. tPA activity was shown to increase from baseline to immediately post exercise in both exercise sessions. However, the greatest increase was shown in the 78% VO2 max test. PAI-1 activity was found to decrease significantly in the 78% VO2 max exercise session. The length of the exercise session (30 minutes) as well as the collection of gases in the previous study is
much different than the protocol used in the present study. This is something that will affect the tPA and PAI-1 activity levels in the blood and therefore needs to be considered when comparing the plasma levels of the proteins between the two studies.

Volume has been shown to play a role in resistance exercises to increase fibrinolytic activity. A study conducted by El-Sayed et al (23) found significant changes in fibrinolytic activity in both low and high volume acute bouts of resistance exercises. Seven healthy men were recruited for this study and participated in three groups: control, high volume resistance exercises, and low volume resistance exercises. Both exercise groups consisted of 5 sets of 9 exercises. However, the high volume trial consisted of 10 repetitions in each set, while the low volume group only did 4. This study showed an increase and decrease in tPA activity and PAI-1 activity, respectively, after an acute bout of resistance exercises. However, the high volume resistance training exercises were shown to have a greater fibrinolytic response than the low volume resistance exercises.

Acute exercise has been shown to increase fibrinolysis. This is due to increased release of epinephrine and bradykinin, as well as increased blood pressure and muscle activation. All as previously mentioned have been shown to be stimulators of tPA release (the primary stimulator of fibrinolysis). Aerobic exercise has been the primary type of exercise effects on fibrinolysis studied in the literature. While few data exist, resistance training has been shown to increase fibrinolytic activity as well. These studies have primarily examined the effects of different intensities of exercise on fibrinolysis.
Whole Body Vibration

Repeated, long term exposure to WBV, such as in the workplace, has been shown to have detrimental effects on the body (48). However, it has been suggested that short term exposure to WBV may have beneficial effects on the body, in both men and women. WBV has been shown to improve many performance measures. Through increased neural activation, improved blood flow, and catecholamine responses performance has been enhanced.

Use of WBV has been shown to produce athletic benefits. A study conducted by Torvinen et al. examined the changes in vertical jump over a 4 month training study using WBV. In this study, 56 men and women (ages 19-38 years) were recruited and randomized into a control or vibration group. The vibration group participated in 4 minutes of vibration at 26 Hz per session 3 to 5 days a week for 4 weeks. At the end of the fourth month jump height was assessed and compared to the pre training measure. Jump height was significantly increased (8.5%) in the vibration group (55). Improved athletic performance has also been shown in short term exercise training after only a few days. Bosco et al. (6) showed increased jump height after only 10 days of WBV training. In this study he recruited 14 male subjects to participate in a short term training study. The study protocol required the subjects to stand on the WBV plate for 5 sets of 90 seconds of whole body vibration followed by 40 seconds of rest. The frequency for this study was set at 26 Hz. Following vibration, the subjects were then asked to perform countermovement jumps. Jump height was shown to be significantly higher (1.6% increase) as well as power output of best jump (3.3%). These results support the notion
that WBV has favorable outcomes on neuromuscular adaptations. Bosco et al. speculated that the increased jump height and power is due to improved synchronization activity of motor units as well as an increased inhibition of antagonist muscles (5). This suggests that WBV may have neuromuscular effects.

**Whole Body Vibration Relevance to Fibrinolysis**

WBV may also affect changes to the cardiovascular system, such as increased blood flow. Kerschan-Schindel et al. (36) examined the relationship between WBV and lower leg blood flow. Twenty healthy subjects (12 males mean age, 32.7 ± 3.3 years; BMI, 24.1 kg·m⁻²; 8 females mean age, 28.5 ± 2.2 years; BMI, 19.9 kg·m⁻²) were recruited for this study. Subjects were required to stand on the WBV platform for 9 minutes while vibrating at a frequency of 26 Hz. Blood flow was measured through the popliteal artery before and after vibration by the Doppler method. Vibration led to a significantly increased amount of blood flow to the calf when compared to rest (6.5 to 13.0 cm·s⁻¹). Maloney et al. also found similar results when they compared various frequencies of forearm vibration. In this study 18 subjects were recruited (mean age 20.3 years) and randomly assigned to one of three groups: 30 Hz, 50 Hz, or both 30 and 50 Hz vibration. Only the forearm was exposed to the vibration platform. Skin blood flow at the forearm (using the Doppler method) was examined during vibration, which lasted 10 minutes, and then into a 15 minute recovery without vibration. Blood flow was found to be significantly increased for both frequencies within the first 4 minutes of exercise (30 Hz= 293%, 50 Hz = 513%) with peak blood flow reaching its maximum at 5 minutes (30 Hz= 517%, 50 Hz= 764%). During recovery, skin blood flow was lower than at baseline.
when vibrated at 30 Hz, while skin blood flow when vibrated at the 50 Hz remained above baseline. Increased blood flow due to vibration may have some clinical importance, especially in diabetic populations who may have circulatory problems (40). The increased blood flow shown with vibration also corresponds with an increase in certain blood hormones (i.e. testosterone, epinephrine) (5, 28, 29) after bouts of WBV.

Studies have not examined the effects of WBV and fibrinolysis in humans. However, other modalities that promote blood flow changes similar to those experienced during WBV have been reported to enhance endothelial function as well as fibrinolytic potential. A recent study by Adams et al. (1) examined the effects of periodic acceleration, a pulsatile movement added in the spinal axis of the body, on 12 anesthetized piglets lying in the supine position. The platform moved the piglets in a head to foot motion at 0.4 g, 180 cpm for 60 minutes. Blood samples were taken at baseline, immediately post exercise and 180 minutes post exercise. Plasma levels of tPA activity were increased in the group that underwent the pulsatile stress (0.59 ± 0.26 AU·ml⁻¹ at baseline to 2.6 ± 1.0 AU·ml⁻¹ post exercise) compared to the control group which sat still on the motion platform immediately after exercise. This value returned back towards baseline by 180 minutes post exercise (0.32 ± 6.4 AU·ml⁻¹). The increased tPA was attributed to increased shear stress on the endothelial cells of the blood vessels caused by the pulsatile motion. Pulsatile stress placed on the body causes increased laminar and shear stress on the endothelial cells of blood vessels resulting in the release of tPA. Results of a recent study by Schetcher et al (49) suggested that the cardiovascular improvements associated with external counterpulsation treatment (ECPT)
therapy may be due to increased endothelial shear stress potentially stimulating the release of vasodilatory and fibrinolytic agents. ECPT utilizes inflatable cuffs to stimulate blood flow through peripheral and coronary arteries. In this study 20 patients (15 males, 5 females), mean age 68 ± 11 years, with coronary artery disease (CAD) and untreatable angina pectoris were evaluated before and after a session of ECPT. Baseline and immediately post exercise endothelium dependent brachial artery flow mediated dilation (FMD) and endothelium independent nitroglycerin mediated vasodilation (NTG) was assessed via ultrasound. Significant improvements in FMD post ECPT were shown (8.2 ± 2.1% compared with controls 3.1± 2.2%); however, there was no significant effect of treatment on NTG when the treatment group was compared to controls (10.7 ± 2.8% vs. 10.2 ± 2.4%, respectively. ECPT therapy improves vascular endothelial function in patients with untreatable angina pectoris, and may promote positive hemodynamic adaptations such as decreased chest pain threshold and increased exercise tolerance in patients who were too disabled to participate in an exercise rehabilitation program (49). Both of the studies show promising results using mechanical stimulation of blood flow and endothelial shear stress to induce a fibrinolytic response, which is much the same way that acute exercise uses mechanical stimulation to promote increased fibrinolytic activity.

As mentioned in previous sections of this literature review, acute exercise causes the release of specific hormones. These hormones have been associated, in part, with an increase in fibrinolytic activity (7, 14, 53, 57). Thus, leading to the speculation that if WBV results in similar hormonal changes, fibrinolytic activity may increase as well.
Increases in plasma hormones have been observed during WBV. Bosco et al. (5) examined the effects of whole body vibration on blood hormone concentrations. 14 males (mean (SD) age, 25 ± 4.6 years; BMI, 25.5 kg·m⁻²), were recruited to undergo 10 minutes of non continuous vibration at a frequency of 30 Hz. Multiple hormones were measured including testosterone, growth hormone, and cortisol. Testosterone and growth hormone significantly increased from pre vibration to post vibration (22.7 ± 6.6 nmol·l⁻¹, 24.3 ± 6.6 nmol·l⁻¹ respectively) while cortisol was found to be significantly decreased (682 ± 255 nmol·l⁻¹, 464 ± 257 nmol·l⁻¹). Two studies have examined the effects of testosterone on fibrinolytic activities. One study by Glueck et al. (28) examined the effects of testosterone on basal fibrinolytic activity in 55 hyperlipidemic men. Testosterone correlated positively with tPA (r=0.30) and inversely with PAI-1(r=-0.33). The other study by Fearnley et al. (25) examined 6 male subjects, 4 with occlusive arterial disease, 1 with breast cancer, and 1 healthy. Between 25-100 mg of testosterone was administered intramuscularly between 10-11:00 am. Fibrinolytic activity was found to increase in all subjects after injection of testosterone. Thus, testosterone is positively correlated with an increase in fibrinolytic activity (28). Kvorning et al. also examined the effects of WBV on testosterone. Twenty-eight young men were randomized into 3 groups: squats (mean age 24 ± 1.7 years), squats plus vibration (23± 0.6 years), and vibration alone (23 ± 0.7 years). The squatting consisted of 6 sets of 8 repetitions with a corresponding 8 repetition maximum load with a 2 minute break in between each set. Kvorning and his colleagues found that after a single bout of vibration testosterone was increased in the squat and squat plus vibration group. All of these results suggest that
vibration when combined exercise provides to be the additional stimulus to cause a release of testosterone (38). The results of these studies suggest that WBV may lead to acute hormonal responses that correlate positively with an increase in fibrinolytic activity.

Di Loreto (20) et al. examined the effects of WBV on the human endocrine system. He recruited 10 healthy male subjects (age 39 ± 3, BMI of 23.5 ± 0.5 kg·m⁻², mean ± SEM) for this study. Each subject was examined twice during the study. The subjects were required to stand for 25 minutes with and without vibration at a frequency of 30 Hz. Vibration was found to increase plasma norepinephrine when compared to the control trial (1.29 ± 0.18, 1.01 ± 0.07 nM, p=0.038 respectively). Goto et al. examined 8 healthy, male students (mean ± SE: age 23.4 ± 0.9 years; BMI 24.6 ± 2.2 kg·m⁻²) after a bout of WBV and after a control trial without vibration. Blood samples, to determine plasma epinephrine and norepinephrine levels, were drawn pre and post vibration. During the trials which consisted of ten sets of 60 seconds on the vibration plate with 60 seconds off, subjects were required to stand at a half squat (knees bent to 120 degree angle). The frequency was set at 26 Hz with amplitude of 2.5 mm. Increased concentrations of plasma epinephrine (Epi, 26.7 ± 5.4 pg·ml to 38.0 ± 5.0 pg·ml) and norepinephrine (NE, 288.0 ± 38.7 pg·ml to 456.4 ± 89.8 pg·ml) levels were shown with the vibration session. As described earlier high levels of epinephrine and norepinephrine have been associated with an increase in fibrinolytic activity (14, 32, 57). Therefore, it is thought that WBV, by means of increasing catecholamines, may increase fibrinolytic activity.
To conclude, while long term exposure has shown detrimental effects, short term exposure to WBV has been reported to be beneficial. This has been shown in studies that have examined the effects of WBV on neuromuscular adaptations, which have lead to increases in performance, as well as a release of various endocrine hormones, and an increase in blood flow (29, 62). This catecholamine and mechanical stimulation is similar to the way aerobic exercise and resistance training causes an increase in the fibrinolytic response leading to the speculation that WBV may also result in an increased fibrinolytic response.

Summary

CVD is the leading cause of death in the United States. Heart attacks and strokes are typically a result of unresolved blood clot formation. Thus, fibrinolysis, the breakdown of a fibrin blood clot, may be clinically important with regard to reducing adverse coronary events (60). Increasing fibrinolytic activity has the potential to reduce the risk of clot-related events (31). Mechanical stimulation of blood flow increases the release of endothelial fibrinolytic activators (1). WBV is an emerging modality that is known to promote increased blood flow and catecholamine response (29, 36). The chemical response and the mechanical stimulation that promotes increased blood flow may also induce a fibrinolytic response and, thus, be a valuable addition to a therapeutic exercise intervention designed to reduce cardiovascular risk.
Chapter III.

METHODS

Prior to commencement of this investigation, all study procedures were approved by the Institutional Review Board at Ball State University (BSU). Each participant provided signed informed consent prior to participation.

Subjects

20 men (23.8 ± 0.9 years; 25.6 ± 0.2 kg·m⁻²) completed this study. Subjects were not taking any medications, non-smokers, and were free from any musculoskeletal injury or illness that could be exacerbated by participation in the study.

Study Design

Subjects completed 3 treatment sessions, conducted in random order and separated by not less than 1 week. Only data from subjects who completed each of the 3 conditions were included in the final analyses (tPA and PAI-1 analysis, 19 subjects; heart rate, 18 subjects; rating of perceived exertion, 20 subjects). Subjects came to the
laboratory after fasting for 12 hours and were instructed to abstain from exercise, alcohol, and caffeine for 24 hours prior to testing. Upon arrival to the laboratory, the 3 protocols were explained to the subjects, who were then asked to sign the informed consent form. All testing took place between 6:00AM and 10:00AM to control for diurnal changes in fibrinolysis. Subjects were asked to fill out a health history questionnaire at visit 1, which was used to screen for conditions that may compromise the integrity of the data or the safety of the subject during participation in the study, and verbally instructed to the procedures. Measures of height and weight were then obtained using standard procedures. Subjects were instructed to remove their shoes, stand with their feet flat, together, and against the wall. Legs were straight and arms were straight hanging at the side of the subject. Shoulders were level. The stadiometer arm was then placed directly on top of the subjects head. Height was taken to the nearest 0.1 cm. Weight was determined as well, by having the subjects remove their shoes, subjects then stepped on the middle of the scale, making sure that no part of their feet were hanging off the platform. Weight was taken to the nearest 1.1 kg. Subjects assumed a seated position for 20 minutes prior to a baseline blood draw. Immediately after the blood sample, the subjects performed 1 of 3 treatment protocols: exercise (X), vibration (V), or exercise + vibration (VX). Heart rates (Polar Electro, USA) were obtained at rest and minute 15, and Borg’s Ratings of Perceived Exertion (RPE) (3) were obtained at minute 15 of each condition, with the exception of RPE in the vibration condition. A second blood sample was taken immediately following the treatment.
Experimental Procedures

Each session was completed with the subject standing on a WBV platform, with socks on, feet positioned shoulder width apart (Pineapple Pro, Hollywood, CA). All squatting conditions consisted of subjects squatting until knees were bent at approximately a 90° angle, then returning to the standing position and repeating.

Treatment Protocols.

Protocol (X): Subjects performed unloaded squats at a rate of 20 per minute for 15 minutes. Squatting was paced using an audio and visual signal from a metronome.

Protocol (VX): Subjects performed the same exercise as described for Protocol #1 with the WBV platform vibrating at a frequency of 30 Hz, amplitude 1.5 mm.

Protocol (V): While standing on the Pineapple Pro (Hollywood, CA) subjects stood for 15 minutes with knees slightly bent, as determined subjectively by the investigator, on the WBV platform vibrating at a frequency of 30 Hz, amplitude 1.5 mm,

Blood Sampling/ Assays

Blood samples were drawn from an antecubital vein using minimal stasis with a 21.5 gauge butterfly needle. 5 ml of blood were first drawn and discarded. Then a second 5 ml sample was then drawn into a tube with acidified citrate solution (Biopool Stabilyte). Whole blood, from the acidified citrate solution, was drawn by inserting the
micro-hematocrit capillary tubes into the acidified citrate solution (Fisher Scientific, Pittsburgh, PA), which were sealed with wax and centrifuged for 5 minutes for hematocrit determination. Hematocrits were measured in duplicate using a micro-capillary reader (DAMON/IEC Division, Needham Hts, MA). The average value of the two measurements was used in statistical analysis. Citrated blood samples were immediately centrifuged in the acidified citrate solution tube for 20 minutes at 1,500xg and 4° C to obtain platelet-poor plasma. Platelet-poor plasma was then pipetted and placed in another tube to be centrifuged for 5 minutes at 1,500xg and 4° C. Plasma was separated into 0.4 ml aliquots and stored at -80° C until assayed. Determination of plasma concentration of active tPA and PAI-1 for each time interval was done using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Chromolyze tPA, PAI-1, Biopool International; Ventura, CA) according to manufacturer instructions. In the ELISA assay an antigen is bound to the surface of a microtiter plate. An antibody is then used to wash the plate to allow the antigen to bind to the antibody; the bound molecules are then quantified by an enzyme. This enzyme allows the antigen/antibody complexes to fluorescence, allowing the amount of the antigen in the sample to be determined by the magnitude of fluorescence. All samples were assayed in duplicate and the average of the two values was used in statistical analyses. Due to known intravariability for fibrinolytic proteins (18) no outlier criteria was set. Post exercise values were corrected for plasma volume changes using the van Beaumont equation (56).
Statistical Analysis

Subject characteristics were depicted using descriptive statistics. Plasma concentrations of tPA and PAI-1 were analyzed using two-way repeated analyses of variance (condition x time). Due to loss of one subject’s plasma samples for a single trial, tPA and PAI-1 were analyzed in 19 subjects. Heart rate was analyzed using a two-way repeated analysis of variance (condition x time). Heart rate was obtained in only 18 subjects due to malfunction of the Polar heart rate monitor. Post-hoc pairwise comparisons were done using Fishers LSD method. Statistical significance for all analyses was set at $\alpha = 0.05$. Peak RPE in the X and VX conditions were analyzed using a paired samples test. Statistics on RPE were not done on the V condition.
Chapter IV.

RESEARCH MANUSCRIPT

Journal Format: Medicine and Science in Sport and Exercise
The Effects of Whole Body Vibration and Exercise on Fibrinolysis in Men

Abstract

Purpose. The purpose of this study was to examine the fibrinolytic response to whole body vibration (WBV) and exercise in men. Methods. Twenty healthy males (23.8 ± 4.2 years, 80.8 ± 3.3 kg·m⁻²) participated in the study. Each subject performed 3 trials in randomized order separated by 1 week. The trials consisted of exercise (X), vibration (V) and vibration + exercise (VX). Exercise sessions consisted of 15 minutes of unloaded squatting at a rate of 20 per minute. Vibration sessions were conducted on a WBV platform vibrating at a frequency of 30 Hz and amplitude of 1.5mm for 15 minutes. Plasma concentrations of active tPA and PAI-1 samples were assessed at baseline and immediately after each session. Results. tPA activity change from pre to post trial was found to be significantly greater in the VX condition (0.87 ± 0.35 IU·ml⁻¹ to 3.21 ± 1.06 IU·ml⁻¹) compared to the X (0.71 ± 0.36 IU·ml⁻¹ to 2.37 ± 1.13 IU·ml⁻¹) or V (0.83 ± 0.25 IU·ml⁻¹ to 1.00 ± 0.37 IU·ml⁻¹) condition. tPA activity change from pre to post trial was found to be significantly greater in the X condition compared to the V condition. PAI-1 activity change from pre to post trial was found to be significantly decreased in the VX (6.54 ± 5.53 IU·ml⁻¹ to 4.89 ± 4.13 IU·ml⁻¹) and X (9.76 ± 8.19 IU·ml⁻¹ to 7.48 ± 7.11 IU·ml⁻¹) conditions compared to the V (5.68 ± 3.53 IU·ml⁻¹ to 5.84 ± 3.52 IU·ml⁻¹) condition. Heart rate change from pre to post exercise for the V condition (pre, 75 ± 8 bpm; post, 90 ± 7 bpm) was less than the change in the VX condition (pre, 77 ± 13 bpm; post, 148 ± 19 bpm) and X condition (pre, 71 ± 11 bpm; post, 139 ± 22 bpm). The change in heart rate was found to be similar in the X and VX conditions. Peak RPE was not significantly different between X and VX sessions. Conclusions. WBV does not stimulate increased fibrinolytic activity in young men. However, the significant increase in fibrinolytic potential observed during squatting exercise is enhanced by concurrent WBV.
Introduction

Fibrinolysis is the process by which fibrin clots are broken down or lysed. The primary regulators of fibrinolysis in humans are tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1). tPA is the primary stimulator of fibrinolysis, while PAI-1 is the primary inhibitor. Fibrinolytic activity is stimulated by a number of factors including epinephrine, low blood glucose, shear stress placed on endothelial cells of blood vessels (15) and exercise (8, 12, 26). Low fibrinolytic potential is associated with an increased risk for an ischemic event (18, 26).

Fibrinolytic activity increases during acute bouts of various types of exercise (8, 27). The fibrinolytic response is intensity and duration dependent (11). It may not be practical or safe for some individuals to engage in physical activity of sufficient intensity or duration to elicit beneficial hemostatic adaptations, such as those with peripheral arterial disease which makes exercise very painful to perform. Therefore it would be beneficial to identify therapeutic modalities, such as whole body vibration (WBV) that may enhance the cardioprotective effects of exercise.

WBV is a novel modality in which subjects stand on a platform, in a static position or performing dynamic exercises. The platform vibrates at a predetermined frequency and amplitude, transmitting the vibration from the platform up through the feet and legs. Studies of WBV show neuromuscular and athletic performance improvements (9, 23). Furthermore, WBV increases various factors that regulate fibrinolytic activity such as plasma catecholamine level. Increases in plasma epinephrine levels in healthy
men (mean ± SE: age 23.4 ± 0.9 years; BMI 24.6 ± 2.2 kg·m⁻²) have been documented after acute bouts of WBV (13). Increased blood flow, which may result in increased stress on endothelial walls of blood vessels, has also been shown with WBV (17); leading to the speculation that WBV may also result in an increased fibrinolytic response. Thus, WBV may affect fibrinolytic activity and enhance the cardioprotective benefits of a therapeutic exercise regimen.

The primary purpose of this study was to examine the fibrinolytic response to exercise, WBV, and the combination of WBV and exercise in healthy men. It was hypothesized that plasma concentrations of active tPA would increase and plasma PAI-1 would decrease in response to WBV and in response to exercise. It was further hypothesized that exercise with concurrent WBV would result in greater changes in plasma tPA and PAI than either WBV or exercise alone.

Methods

Prior to commencement of this investigation, the study protocol was approved by the Institutional Review Board at Ball State University (BSU). Each participant provided signed informed consent prior to participation.

Subjects. 20 men (23.8 ± 0.9 years; BMI 25.6 ± 0.2 kg·m⁻²) volunteered for this study however, only plasma samples from 19 subjects were used due to issues in plasma storage of one sample. Subjects were not taking any medications, were non-smokers, and were free from any musculoskeletal injury or illness that could be exacerbated by participation in the study.
Study Protocol. This study was conducted over 3 separate days each separated by 1 week. All trials were chosen in random order. Testing took place between 6:00AM and 10:00AM to control for diurnal changes in fibrinolysis. Subjects arrived to the lab after fasting for 12 hours and were instructed to abstain from exercise, alcohol, and caffeine for 24 hours prior to testing. On the first visit subjects signed an informed consent and were asked to fill out a health screening questionnaire. Measures of height and weight were then obtained using standard procedures. Subjects were instructed to remove their shoes, stand with their feet flat, together, and against the wall. Legs were straight and arms were straight hanging at the side of the subject with shoulders level. The stadiometer arm was then placed directly on top of the subjects head. Height was taken to the nearest 0.1 cm. Weight was determined by having the subjects remove their shoes, and step on the middle of the scale, making sure that no part of their feet were hanging off the platform. Weight was taken to the nearest 1.1 kg. Subjects assumed a seated position for 20 minutes prior to a baseline blood draw from the antecubital vein. After the blood sample, the subjects performed 1 of the 3 conditions protocols: exercise (X), vibration (V), or vibration + exercise (VX). Heart rate was obtained at minutes 0 and 15 for all conditions, and ratings of perceived exertion (RPE) were obtained at minutes 5 and 15 of the 2 exercise conditions. Due to lack of physical exertion during the vibration trial, RPE was not taken during the V condition. Heart rate was determined using a telemetric monitor (Polar Electro, Kempele, Finland). The Borg 6-20 category scale standardized instructions were used to assess RPE (1). A second blood sample was taken immediately following the treatment.
Treatment Protocols.

Vibration Protocol (V): While standing on the Pineapple Pro (Hollywood, CA) subjects stood for 15 minutes with knees slightly bent on the WBV platform vibrating at a frequency of 30 Hz, amplitude 1.5 mm.

Exercise Protocol (X): Subjects performed unloaded squats at a rate of 20 per minute for 15 minutes. Squatting was paced using an audio and visual signal from a metronome.

Vibration + Exercise Protocol (VX): Subjects performed the same exercise as described for Protocol X with the WBV platform vibrating at a frequency of 30 Hz, amplitude 1.5 mm.

Blood Sampling/Assays. At each time point blood was drawn from an antecubital vein with minimal stasis using sterile procedures. Blood was drawn from the EDTA-treated vacutainer tubes into micro-hematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA), which were sealed and centrifuged for five minutes. Hematocrit was measured in duplicate using a micro-capillary reader (DAMON/IEC Division, Needham Hts, MA). Citrated blood samples were immediately centrifuged for 20 minutes at 1,500xg and 4°C to obtain platelet-poor plasma. Platelet-poor plasma was then pipetted and placed in another tube to be centrifuged for five minutes at 1,500xg and 4°C. Plasma was separated into 0.4 ml aliquots and stored at -80°C until assayed. Determination of plasma concentration of active tPA and PAI-1 for each time interval was done using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Chromolyze tPA, PAI-1, Biopool International; Ventura, CA) according to
manufacturer instructions. All samples were assayed in duplicate and the average of the two values was used in statistical analyses. Post exercise values of tPA and PAI-1 were corrected for plasma volume changes using the van Beaumont equation (24).

Statistical Analysis. The change in HR and plasma concentrations of tPA and PAI-1 were analyzed using two way analyses of variance (treatment x time). Post-hoc pairwise comparisons were done using Fishers LSD method. Only 19 subjects were used in the determination of tPA and PAI-1 activity due to loss of plasma samples in 1 subject. Only 18 subjects’ peak heart rates were analyzed due to malfunction of the heart rate monitor during two trials. Statistical significance for all analyses was set at $\alpha = 0.05$. Differences in peak RPE for the V and VX conditions were analyzed using a one-way ANOVA.

Results

A main effect of time was observed for the change in heart rate, with peak HR being significantly greater than baseline HR. A main effect of condition was also observed. Heart rate was highest in the VX condition (148 ± 20 bpm) compared to the X (139 ± 22 bpm) and V (90 ± 7 bpm) conditions. Heart rate in the X condition was also higher than the V condition. A significant time x condition interaction for heart rate indicated that the change in heart rate in the V condition was found to be smaller than in the X and VX conditions. The heart rate increases in the X and VX conditions were not different from one another.

Main effects of time and condition were observed for tPA activity. tPA activity was higher post condition than pre condition, and was higher in the VX condition than in
either of the other two trials, and higher in the X condition compared to the V condition. A significant time x condition interaction for tPA activity was also observed. The increase in tPA activity was greatest in the VX trial compared to the other two conditions, and higher in the X condition compared to the V condition. tPA activity increased from $0.71 \pm 0.36 \text{IU} \cdot \text{ml}^{-1}$ to $2.37 \pm 1.13 \text{IU} \cdot \text{ml}^{-1}$ in the X condition; $0.83 \pm 0.25 \text{IU} \cdot \text{ml}^{-1}$ to $1.00 \pm 0.37 \text{IU} \cdot \text{ml}^{-1}$ in the V condition; and $0.87 \pm 0.35 \text{IU} \cdot \text{ml}^{-1}$ to $3.21 \pm 1.06 \text{IU} \cdot \text{ml}^{-1}$ in the VX condition (Figure 1).

A main effect of time was observed for PAI-1 activity. PAI-1 activity was significantly decreased post condition in the X and VX conditions. There was no effect of condition for PAI-1 activity. A significant time x group interaction indicates that the decrease in PAI-1 activity was greater in the VX and X conditions compared to the V condition, but no difference was observed between the VX and X trials. PAI-1 activity changed from $9.76 \pm 8.19 \text{IU} \cdot \text{ml}^{-1}$ to $7.48 \pm 7.11 \text{IU} \cdot \text{ml}^{-1}$ in the X condition; $5.68 \pm 3.53 \text{IU} \cdot \text{ml}^{-1}$ to $5.84 \pm 3.52 \text{IU} \cdot \text{ml}^{-1}$ in the V condition; and $6.54 \pm 5.53 \text{IU} \cdot \text{ml}^{-1}$ to $4.89 \pm 4.13 \text{IU} \cdot \text{ml}^{-1}$ in the VX condition (Figure 2).

A paired samples t-test revealed no significant changes in peak RPE from the X or VX conditions ($13.7 \pm 0.56 \text{IU} \cdot \text{ml}^{-1}$, $13.7 \pm 0.57 \text{IU} \cdot \text{ml}^{-1}$, respectively).

Repeated measures ANOVA revealed no main effect of time for percent plasma volume change. Plasma volume change for the V, X, and VX conditions decreased ($6.00 \pm 7.80\%$, $11.25 \pm 7.50\%$, and $8.30 \pm 9.10\%$, respectively).
Discussion

The purpose of this study was to examine the effects of WBV and exercise on fibrinolytic activity in healthy men. The primary findings of this study were that the addition of WBV to moderate intensity exercise increased fibrinolytic activity more than exercise alone, as determined by the greater increase in tPA activity in the VX condition compared to the X condition.

The mechanism underlying the fibrinolytic responses reported in the present study may be related to vascular shear stress. WBV increased blood flow in healthy males and females (12 men mean age, 32.7 ± 3.3 years; BMI, 24.2 kg·m⁻²; 8 women mean age, 28.5 ± 2.2 years; BMI, 24.0 kg·m⁻²) vibrating for 9 minutes at a frequency of 26 Hz (17). This increase in blood flow may result in an increase in shear stress on blood vessels. Increased vascular shear stress has been shown to increase fibrinolytic activity in cultured endothelial cells (10). However, in the present study, the speculated increase in blood flow in the V alone condition did not provide enough of a stimulus to increase fibrinolytic activity to the same extent as in the X and VX conditions.

WBV has been shown to cause muscle activation. Skeletal muscle is known to be a release site of tPA, suggesting that skeletal muscle activation in WBV may stimulate the muscle to release tPA. Cardinale et al. (4) examined muscle activation in the vastus lateralis in 16 professional women volleyball players (age, 23.9 ± 3.6 years; BMI, 23.8 kg·m⁻²). EMG root mean square (rms) was recorded for 60 seconds in each condition (control, 30 Hz, 40 Hz, 50 Hz vibration). The condition order was randomized, and the
subjects had a 60 second rest period with no vibration in between each of the 4 conditions. EMG rms was significantly elevated in all groups compared to baseline. The greatest change in EMG rms was found at the 30 Hz group (34%) compared to the 40 Hz (10%) and 50 Hz (20%). Skeletal muscle activation may have been a stimulator in the present study for the increased tPA activity shown in both the X and VX conditions. Also, since both exercise and WBV cause muscle activation, an additive effect of both may explain the greater increase in the VX condition compared to the X condition.

Specific catecholamines (i.e. epinephrine and norepinephrine) have been shown to increase fibrinolytic activity. Both WBV and exercise have been reported to result in a significant increase in catecholamine concentration (3, 6). Goto et al (13) examined 8 healthy, male students (mean ± SE: age 23.4 ± 0.9 years; BMI 24.6 ± 2.2 kg·m⁻²) after a bout of WBV and after a control trial without vibration. Blood samples, to determine plasma epinephrine and norepinephrine levels, were drawn pre and post vibration. During the trials which consisted of 10 sets of 60 seconds on the vibration plate with 60 seconds off, subjects were required to stand at a half squat (knees bent to 120° angle). The frequency was set at 26 Hz with amplitude of 2.5 mm. Goto et al. found increased concentrations of plasma epinephrine (26.7 ± 5.4 pg·ml⁻¹ to 38.0 ± 5.0 pg·ml⁻¹) and norepinephrine (288.0 ± 38.7 pg·ml⁻¹ to 456.4 ± 89.8 pg·ml⁻¹). Although catecholamines were not measured in the present study it can be speculated that increases in catecholamines may have contributed to the increase in fibrinolytic activity in the X and VX conditions.
Moderate intensity aerobic exercise (60% heart rate reserve) decreases PAI-1 activity (14). Therefore, the PAI-1 decrease in the X and VX conditions of the present study was expected. However, it is unclear why the VX condition did not have a more pronounced PAI-1 change than the X condition, as was observed for tPA activity. During exercise plasma concentration of active PAI-1 decreases primarily by PAI-1 binding to tPA, forming a tPA/PAI-1 complex (5). Since tPA activity increased more during the VX condition than the X condition, it would be expected that increased tPA/PAI-1 binding would result in a measureable difference in PAI-1 concentration. One possible explanation for this observation is that secretion of PAI-1 may be accelerated during WBV. WBV has also shown to stimulate platelet aggregation. Kent et al. (16) examined 12 subjects (8 males, mean age 41 years; 4 females, mean age 39 years) after 1 minute of hand vibration. Platelet aggregation, as assessed by β-thromboglobulin, increased significantly from a median (interquartile range) of 35.5 (22-47) to 47.5 (27-52) ng·ml⁻¹. These results suggest that platelet aggregation occurs as a result of vibration. Since platelets are known to stimulate the release of much of the PAI-1 measured in the plasma, it can be inferred that WBV increasing platelet aggregation will also increase the release of PAI-1. Therefore, this ‘extra’ PAI-1 bound to the ‘extra’ tPA in the VX condition allowing the measurable levels of PAI-1 to remain the same. This potential mechanism is purely speculative, and should be addressed in future research efforts.

Additional findings of this study included heart rate change, which was significantly smaller in the V condition than the X and VX conditions. However, the change in heart rates between the X and VX conditions was found to be similar. This is
supported by previous literature which shows no changes in heart rate during WBV sessions (17). No study has examined the heart rate response to exercise with and without vibration. The lack of difference in heart rate change between the X and VX conditions coincides with no change in RPE between these conditions. According to Borg et al., heart rate is linearly related to RPE (2). Since the heart rate change in the present study was the same between the two exercise trials, it would not be expected that RPE would be different.

A limitation of this study was that physical fitness level was not controlled for. The literature is quite divided on whether or not long term exercise affects fibrinolytic activity. This is due to a wide array of training programs and populations studied. However there is a potential for a training effect on fibrinolysis (11). This study only examined one side of the hemostasis equation. It is unknown if coagulation is affected by WBV. Future research might focus on using different frequencies and amplitudes than the one used in this study to determine if greater effects of WBV on fibrinolysis may occur with varying frequencies. Also, future research would benefit from focusing on a diversified subject pool. Women, older populations, and subjects of different ethnicities would allow for further conclusions on the effect of WBV on fibrinolysis that are unable to be made at this time. Additionally, since implications of this study are related to cardiovascular risk, studying people with normal healthy physiology may have yielded different results than if patients with CVD were examined. Finally, it is recommended that future studies should examine the fibrinolytic profile intervariability and intravariability between subjects. Although fibrinolytic variability between subjects has
been examined in a previous study by de Maat et al. (7), the mechanisms behind this variability are unknown.

To summarize, the present study suggests that 15 minutes of moderate-intensity squatting exercise with and without vibration at a frequency of 30 HZ and amplitude of 1.5mm increases fibrinolytic activity in healthy male subjects. Furthermore, fibrinolytic activity, as shown by an increase in tPA activity, was found to be significantly greater during the sessions that combined exercise with WBV compared to the exercise alone, which may have implications for maintaining good cardiovascular health.


Table 1. Heart rate (HR) and rating of perceived exertion (RPE) at rest and peak condition

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<td>Resting HR (bpm)</td>
<td>75 ± 8</td>
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<td>Peak HR (bpm)</td>
<td>90 ± 7</td>
<td>139 ± 22*</td>
<td>148 ± 20*</td>
</tr>
<tr>
<td>Peak RPE (bpm)</td>
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<td>13.70 ± 2.54</td>
<td>13.70 ± 2.58</td>
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</tbody>
</table>

X: Exercise trial
VX: Exercise plus whole body vibration trial
V: Whole body vibration trial

Values are displayed as means ± SD
RPE was not taken during the V sessions
Heart rates were obtained in only 16 subjects for the X and V sessions due to malfunction of the heart rate monitor.

*Change in rest to peak heart rate in the V condition compared to the X and VX conditions (p < 0.05)
Figure 1. tPA Activity Pre and Post Trial
Figure 2. PAI-1 Activity Pre and Post Trial
Legends:

Figure 1. tPA activity pre and post trial (n=19)
tPA X: tissue plasminogen activator activity in the exercise trial
tPA VX: tissue plasminogen activator activity in the whole body vibration plus exercise trial
tPA V: tissue plasminogen activator activity in the whole body vibration trial
Values are displayed as means ± SE
A significant main effect of condition revealed that tPA activity was VX>X>V conditions.
A significant main effect of time revealed that tPA activity was greater post condition in all groups.
A significant time by condition interaction revealed that the change in tPA activity was VX>X>V conditions.

Figure 2. PAI-1 activity pre and post trial (n=19) PAI-1 X: plasminogen activator inhibitor-1 activity in the exercise trial
PAI-1 VX: plasminogen activator inhibitor-1 activity in the whole body vibration plus exercise trial
PAI-1 V: plasminogen activator inhibitor-1 activity in the whole body vibration trial
Values are displayed as means ± SE
A significant main effect of time revealed that PAI-1 activity decreased pre to post condition in the VX and X conditions.
A significant time by condition interaction revealed that the change in PAI-1 activity was greater in the VX and X conditions compared to the V condition.
The primary findings of this study were that the addition of whole body vibration (WBV) to moderate intensity exercise appeared to stimulate fibrinolysis more than exercise alone. The change in tPA activity was significantly greater in the VX condition compared to the X condition. PAI-1 activity decreased similarly in the VX condition compared to the X condition. Vibration alone did not appear to stimulate fibrinolytic activity.

This study represents the first time the effects of WBV on fibrinolytic activity have been examined. There are several factors that play a role in regulating fibrinolytic activity. Some of these factors are discussed in the following paragraphs as possible explanations to the findings of this study.

Skeletal muscle is known to be a release site of tPA, suggesting that skeletal muscle activation in WBV will stimulate the muscle to release tPA. Cardinale et al. (9) examined muscle activation in the vastus lateralis in 16 professional women volleyball players (age, 23.9 years; BMI, 23.8 kg·m$^{-2}$). EMG root mean square (rms) was recorded...
for 60 seconds in each condition (control, 30 Hz, 40 Hz, 50 Hz vibration). EMG rms was significantly elevated in all groups compared to baseline. The greatest change in EMG rms was found at the 30 Hz group (34%) compared to the 40 Hz (10%) and 50 Hz (20%). These mechanisms may have been a stimulator in the present study for the increased tPA activity shown in both the X and VX conditions. Also, since both exercise and WBV cause muscle activation, an additive effect of both may explain the greater increase in the VX condition compared to the X condition.

WBV and exercise independently increase blood flow and shear stress (36, 39, 40), and increased shear stress is associated with increased levels of fibrinolytic activity (36, 40). WBV alone in healthy males and females (12 men mean age, 32.7 years; BMI, 24.1 kg·m⁻²; 8 females mean age, 28.5 years; BMI, 19.9 kg·m⁻²) vibrating for 9 minutes at a frequency of 26 Hz has been shown to increase blood flow (36, 39, 40). Although blood flow was not measured in the present study, it can be assumed that blood flow increased, resulting in increased vascular shear stress, which when combined with exercise was enough of a stimulus to increase in fibrinolytic activity in the VX condition more so than in the X condition. Since fibrinolysis is known to be intensity dependent (22) it appears that vibration alone was not enough of a stimulus to cause an increase in fibrinolytic activity in the V condition.

Specific catecholamines (i.e. epinephrine and norepinephrine) have been shown to increase the fibrinolytic activity. Studies of WBV and exercise alone have been shown to produce a significant increase in these catecholamines (5, 15). Goto et al.(29) examined 8 healthy, male students (mean ± SE: age 23.4 ± 0.9 years; BMI 24.6 ± 2.2) after a bout
of whole body vibration and after a control trial without vibration. Blood samples, to determine plasma epinephrine and norepinephrine levels, were drawn pre and post vibration. During the trials which consisted of 10 sets of 60 seconds on the vibration plate with 60 seconds off, subjects were required to stand at a half squat (knees bent to 120 degree angle). The frequency was set at 26 Hz with amplitude of 2.5 mm. Increased concentrations of plasma epinephrine (Epi, 26.7 ± 5.4 pg·ml⁻¹ to 38.0 ± 5.0 pg·ml⁻¹) and norepinephrine (NE, 288.0 ± 38.7 pg·ml⁻¹ to 456.4 ± 89.8 pg·ml⁻¹) were shown vibration. Although catecholamines were not measured in the present study it can be speculated that increases in catecholamines may have contributed to the increase in fibrinolytic activity in the X and VX conditions.

Like the increases in shear stress with WBV, the potential release of catecholamines during the squatting exercise in the present study, due to both WBV and exercise, may have provided an additional stimulus to increase fibrinolytic activity more in the VX than in X trial. The combination of factors such as shear stress and catecholamine release may have played a role in increasing fibrinolytic activity (34, 44).

Moderate intensity exercise decreases PAI-1 activity (31). Therefore, it was expected, that since the X and VX trials intensities in the current study were of moderate intensity as indicated by heart rate and RPE (4), PAI-1 activity would be decreased. The decrease between the two exercise trials was found to be similar. It is unclear why the PAI-1 change in the VX trial was not different than the X trial, similar to the change in tPA activity. During exercise plasma concentration of active PAI-1 decreases primarily by PAI-1 binding to tPA, forming a tPA/PAI-1 complex (12). Since tPA activity
increased more during the VX trial than the X condition, it would be expected that increased tPA/PAI-1 binding would result in a measureable difference in PAI-1 concentration. One possible explanation for this observation is that secretion of PAI-1 may be accelerated during WBV. PAI-1 is released from endothelial cells as well as by platelets (11, 27). Adding a WBV stimulus to the exercise session may have augmented the exercise induced increase in platelet count, aggregation and activation. Kent et al. (35) examined 12 subjects after 1 minute of hand vibration. Platelet aggregation, as assessed by β-thromboglobulin, increased significantly from a median (interquartile range) of 35 (22-47) to 47 (27-52) ng·ml⁻¹. These results suggest that platelet aggregation occurs as a result of vibration. Since platelets are known to stimulate the release of much of the PAI-1 measured in the plasma, it can be inferred that WBV increasing platelet aggregation will also increase the release of PAI-1. Therefore, this ‘extra’ PAI-1 bound to the ‘extra’ tPA in the VX condition allowing the measurable levels of PAI-1 to remain the same. It is also possible that the vibration stimulus impacted the ability of tPA to bind with PAI-1, although this seems much less likely than the previously described theory. Each of these potential mechanisms is entirely speculative and must be elucidated through additional research.

Additional findings of this study included the following: heart rate change was lowest in the V condition and found to be significantly different from the X and VX conditions. However, the change in heart rate between the X and VX conditions was found to be no different. This is supported by previous literature which show no changes in heart rate during WBV sessions (36). No study has examined the heart rate response
to exercise with and without vibration. The lack of increase in heart rate between the X and VX conditions explains why no change in RPE between these conditions were reported. According to Borg et al. as heart rate increases so will RPE (4). Since the heart rate in the present study was the same between each trial it would not be expected that RPE would be different.

Increased fibrinolytic activity, as found in the current study strengthens the present knowledge that fibrinolytic activity is regulated by several physiological factors. WBV has been shown to increase these factors, however it is evident by the lack of significant increase in the V trial that the frequency and amplitude chosen in the present study was not enough of a stimulus to produce a significant change in fibrinolytic activity without the additive effects of exercise.

**Recommendations for further study**

There are opportunities for further research in the area of the effects of WBV on fibrinolysis. The mechanisms to an increase in heart rate and a decrease in PAI-1 activity, during the VX trial compared to the X trial, are unknown. Therefore it would be interesting to research these mechanisms to determine physiologically what is occurring. Also, examining the within subject and between subject variability of fibrinolytic activity proteins to determine the cause of the variability shown in the present study would be beneficial. Future studies should also focus on increasing the frequency and amplitudes of vibration to produce a greater stimulus, as the present study may have lacked a sufficient stimulus to produce significant changes in the V condition.
A second recommendation would be to recruit a more diverse population. This study only recruited healthy males; therefore no study has examined the effects of WBV plus exercise on fibrinolysis in women or CVD patients. This area of research is lacking and warrants some attention. If a beneficial fibrinolytic response was shown in CVD patients, future cardiac rehabilitation programs could benefit by implementing WBV into their exercise programs.

Lastly, this study recruited a small number of subjects. Using a similar protocol, with greater intensities and/or frequencies of vibration, along with a larger subject pool may show significant differences in fibrinolytic activity in all trials, including the V trial.

In conclusion, the present study indicates that 15 minutes of moderate-intensity squatting exercise with and without vibration at a frequency of 30 HZ and an amplitude of 1.5mm increases tPA activity in healthy male subjects. The change in tPA activity was greater in the condition that combined exercise with vibration compared to the X condition, which may have implications for populations with CVD and exercise-based cardiac rehabilitation interventions. Further research into the risks and benefits of using WBV as a means of enhancing the cardioprotective effects of exercise must be explored before definitive recommendations can be made.


APPENDIX A

INFORMED CONSENT
Consent Form: Fibrinolytic Responses to Whole-Body Vibration.

The purpose of this study is to assess the fibrinolytic responses to whole-body vibration (WBV) as well as during exercise with and without simultaneous WBV.

Most adverse cardiovascular events such as heart attack and stroke are caused by a blood clot that interferes with blood flow to the heart or brain. One of the cardiovascular benefits of exercise is that the ability to dissolve blood clots (a.k.a. “fibrinolysis”) increases during exertion. This exercise-induced fibrinolytic increase is thought to be a result of augmented blood flow that stimulates the cells that produce pro-fibrinolytic enzymes. Whole-body vibration also appears to stimulate these cells, and has previously been shown to enhance blood flow much like exercise does. It is not known if WBV also causes an increase in fibrinolytic activity. We are studying how WBV, with and without concurrent low to moderate-intensity exercise, affects fibrinolysis. Results of this study may be used to improve the cardiovascular benefit of a regular exercise program, which is particularly important for people who are most at risk of developing a blood clot (e.g. people with coronary artery disease).

Apparently healthy men who are 18-45 yrs of age may be eligible to participate in this study, which will be done in three separate visits to the Biomechanics Laboratory. During each visit, you will stand on a WBV platform for fifteen minutes during which you will (a) do deep-knee bends (b) do deep-knee bends with vibration (c) stand quietly with vibration. These sessions will be done: once while the WBV platform is vibrating (30 Hz). The three sessions will be separated by a minimum of 7 days and conducted in random order. A 5-ml (1 tsp) blood sample will be obtained from a vein in your arm before and immediately after each session. Your total time commitment to this study is expected to be approximately 2 hours (45 min per session).

There is a risk that the low to moderate-intensity exercise sessions may cause muscle or joint soreness, and a slight risk of more serious muscular or cardiovascular injury. Very long-term exposure to vibration such as is experienced in certain occupational settings may result in neurological damage or chronic pain. There are no known risks associated with WBV for the brief durations that are used in the present study. Risks of exercise include acute and/or delayed muscle soreness or discomfort associated with squatting. Risks of blood drawing may include discomfort, bruising, and, in rare instances, infection, lightheadedness, and fainting. We will use sterile procedures and trained personnel to ensure that there is minimal discomfort with obtaining the blood samples. Emergency medical treatment is available in the event of injury. You will assume responsibility for the costs of medical care that is provided. In the unlikely event of injury or illness of any kind as a result of participation in this research project, Ball State
University, its agents and employees will assume whatever responsibility is required by law.

The results of this study may indicate that WBV stimulation produces hemostatic responses that are cardioprotective. Exercise programs, particularly for patients with coronary disease, might benefit from the inclusion of WBV technology.

Your participation in this study is completely voluntary and you are free to withdraw from the study at any time for any reason without penalty or prejudice from the investigators. Please feel free to ask any questions of the investigator before signing this Informed Consent form and beginning the study, and at any time during the study. All study materials will be maintained as confidential. Data will be stored in a locked filing cabinet in the researcher’s office. Electronic files, which will be password protected, will be stored on the principal investigator’s computer and preferred backup medium.

For one’s rights as a research subject, contact the Coordinator of Research Compliance, Office of Academic Research and Sponsored Programs, Ball State University, Muncie, IN 47306, (765) 285-5070.

**********

I, _______________________________, agree to participate in this research project entitled, “Fibrinolytic Responses to Whole-Body Vibration.” I have had the study explained to me and my questions have been answered to my satisfaction. I have read the description of this project and give my consent to participate. I understand that I will receive a copy of this informed consent form to keep for future reference.

_____________________________    _______________
Participant’s Signature            Date

_____________________________
Investigator’s Signature

Paul R. Nagelkirk, Ph.D., Assistant Professor
School of Physical Education, Sport & Exercise Science
Ball State University
Muncie, IN 47306
(765) 285-1472; prnagelkirk@bsu.edu
APPENDIX B

HEALTH HISTORY QUESTIONNAIRE
Health History Questionnaire:

FIBRINOLYTIC RESPONSES TO WHOLE BODY VIBRATION

Please read the questions carefully and answer each one honestly: check YES or NO. Investigators may ask further questions regarding your answers.

YES  NO

☐  ☐ Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

☐  ☐ Do you feel pain in your chest when you do physical activity?

☐  ☐ Do you lose balance because of dizziness or do you ever lose consciousness?

☐  ☐ Do you have a bone or joint problem (e.g. ankle, knee or hip) that could be made worse by the exercise involved in this study?

☐  ☐ Do you know of any other reason why you should not do physical activity?

☐  ☐ Do you smoke or use smokeless tobacco?

☐  ☐ Do you have diabetes or liver disease (e.g. hepatitis)?

☐  ☐ Have you been diagnosed with a chronic pain condition (e.g. fibromyalgia, low back pain, etc.)?

☐  ☐ Are you taking any medications, including over-the-counter drugs?

Please list medications on the lines provided

_________________________  __________________________

_________________________  __________________________

_________________________  __________________________

_________________________  __________________________

_________________________  __________________________
APPENDIX C

BORG’S 6-20 RATING OF PERCEIVED EXERTION SCALE
**Perceived Exertion Scale:** Perceived Exertion and Pain Related to Resistance Training

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</table>

**Instructions:** During the exercise session we want you to pay close attention to how hard you feel the exercise work rate is. This feeling should reflect your total amount of exertion and fatigue, combining all sensations and feelings of physical stress, effort and fatigue. Don’t concern yourself with any one factor such as muscle pain, shortness of breath or exercise intensity, but try to concentrate on your total, inner feeling of exertion. Try not to underestimate or overestimate your feelings of exertion; be as accurate as you can.

APPENDIX D

SUBJECT DATA
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Table D 2 Resting and Peak Heart Rates and Peak Rating of Perceived Exertion

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<th>Peak HR (bpm) VX</th>
<th>Peak RPE VX</th>
<th>Resting HR (bpm) V</th>
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Due to heart rate monitor malfunction, heart rates were not obtained in all subjects.
Table D 3 PAI-1 activity (IU·ml⁻¹) Resting and Post Condition Values

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<th>PaI-1 Post X_cor</th>
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<th>PaI-1 Pre V</th>
<th>PaI-1 Post V_cor</th>
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