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Paula K. Johnson

Brigham Young University - Provo

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The Effect of Whole Body Vibration on Skin Blood Flow and Nitric Oxide Production

Paula K. Johnson

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Ulrike Hildegard Mitchell, Chair
Jeffrey Brent Feland
Aaron W. Johnson
Gary W. Mack

Department of Exercise Sciences

Brigham Young University

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ABSTRACT

The Effect of Whole Body Vibration on Skin Blood Flow and Nitric Oxide Production

Paula K. Johnson
Department of Exercise Sciences, BYU
Master of Science

Background: Vascular dysfunction due to hyperglycemia in individuals with diabetes is a factor contributing to distal symmetric polyneuropathy (DSP). Reactive oxygen species (ROS) reduce the bioavailability of nitric oxide (NO), a powerful vasodilator, resulting in reduced circulation and nerve ischemia. Increases in blood NO concentrations and circulation have been attributed to whole body vibration (WBV). The purpose of this study was to determine the effects of low frequency, low amplitude WBV on whole blood NO concentration and skin blood flow (SBF) in individuals with symptoms of DSP.

Research Design and Methods: Ten subjects with diabetes and impaired sensory perception in the lower limbs participated in this cross-over study. Each submitted to two treatment conditions, WBV and sham, with a one week washout period between. Blood draws for NO analysis and Doppler laser image scans of SBF were performed before, immediately after and following a 5 minute recovery of each the treatments.

Results: Low frequency, low amplitude WBV vibration significantly increased skin blood flow compared to the sham condition ($F_{2,18}=5.82, p=0.0115$). Whole blood nitric oxide concentrations did not differ between the WBV and sham condition immediately or 5 minutes post-treatment ($F_{2,18}=1.88, p=0.1813$)

Conclusions: These findings demonstrate that subjects with diabetes respond to whole body vibration with increased skin blood flow compared to sham condition. The implication is that WBV is a potential non-pharmacological therapy for neurovascular complications of diabetes.

Keywords: diabetes, neuropathy, nitric oxide, skin blood flow, whole body vibration

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Table of Contents

Title Page.....	i
Abstract	ii
Acknowledgements.....	iii
Table of Contents.....	iv
List of Tables	v
List of Figures.....	vi
Introduction	1
Methods.....	3
Study Design.....	3
Subjects.....	3
Instruments	4
Outcome Measures.....	5
Procedure	7
Statistical Analysis	8
Results	8
Discussion.....	9
Conclusion.....	13
References	15
Appendix A.....	22
Appendix B.....	23
Appendix C.....	24

List of Tables

Table 1: Participant Demographics.....	18
Table 2: Participant Sensory Profile	19

List of Figures

Figure 1: Mean Skin Blood Flow.....	20
Figure 2: Mean Whole Blood Nitric Oxide Concentration.....	21

Introduction

Neuropathies are among the most common complications of diabetes mellitus¹, and is the cause of more than 60% of all non-traumatic amputations in the United States². Neuropathy is not a single entity but a set of syndromes, each with a wide range of clinical and subclinical manifestations, the most common of which is distal symmetric polyneuropathy (DSP).^{3,4} DSP occurs in both Type I and Type II diabetics and the symptoms range considerably. Some patients experience no symptoms but show deficits during neurological exams, while others experience negative symptoms such as loss of thermal and tactile sensation especially in the lower limbs.¹ Still others may experience dysesthesia, a painful prickling or electric-shock-like sensation in the legs and/or feet, especially at night.⁵

There is currently no effective long-term treatment for diabetic neuropathy, primarily because the underlying mechanism is not clearly understood.¹ Years of intensive experimental studies have led to the current conclusion that the etiology is multifactorial, and the diversity of pharmacological therapies support that conclusion.¹ A common factor in each of the proposed underlying mechanisms appears to be reactive oxygen species (ROS) which are the products of metabolic dysfunctions that occur as a result of hyperglycemia.⁶⁻⁸ Oxidative stress from these free radicals is implicated in vascular dysfunction⁸, including a change in the expression of endothelial nitric oxide synthase⁹, resulting in the reduced bioavailability of nitric oxide (NO). Nitric oxide is a highly reactive, short-lived radical with a broad spectrum of metabolic functions, including vasodilation¹⁰, regulation of blood pressure¹⁰, and inhibition of platelet adhesion.¹⁰⁻¹² Reduced bioavailability of NO may be a factor in nerve ischemia¹³; therefore, therapies to increase NO may result in increased blood flow and a decrease in symptoms of DSP.

While the most effective intervention for preventing and retarding the progression of DSP is glycemic control¹⁴⁻¹⁷, other approaches involve pharmacological therapies.¹⁸ Part of the mechanism of these pharmaceuticals often involves increasing NO levels. For example, antioxidants attempt to attenuate oxidative stress¹, which is responsible for the lack of availability of NO⁹; protein kinase C (PKC) inhibitors prohibit the alteration of nitric oxide synthase and vascular endothelial growth factor¹⁹; and angiotensin-converting enzyme (ACE) inhibitors improve nerve function by, among other things, increasing the release of NO.^{20,21} Nitric oxide production is also induced by laminar shear stress resulting from the frictional forces between the vascular endothelium and the moving blood.^{18,32} An example of this type of shear stress can be found when blood flows in the vessels during moderate exercise.^{22,23} Externally applied low frequency vibration may also result in endothelial shear stress^{24,25} sufficient to produce nitric oxide^{26,27} and improve blood flow.^{28,29} This type of vibration is typically applied to the whole body by having the user stand on a platform that vibrates.

Prior research has shown that WBV improves lower leg skin blood flow (SBF)²⁹ and increases NO²⁶ when applied to healthy individuals. Maloney-Hinds et al.²⁷ showed that NO production and SBF also increases in individuals with diabetes. However, in their study the vibration was locally applied only to the arm by having it rest on the platform at 50 Hz and 5-6 mm amplitude. It is unclear if WBV produced sufficient shear stress to increase NO bioavailability to be considered an effective therapy for DSP. The purpose of this investigation was to determine if WBV augments whole blood NO concentration and skin blood flow in the feet of individuals with diabetic peripheral neuropathy. If so, then WBV may be a non-invasive, non-pharmaceutical means to address the one of the underlying complications of diabetes and alleviate the symptoms associated with diabetic neuropathy.

Methods

Study Design

This was a repeated measures crossover study consisting of two treatment conditions, WBV and sham. The dependent variables were skin blood flow and whole blood nitric oxide concentration, taken at three times (before treatment, immediately after treatment, and following a 5-minute recovery).

Subjects

Twelve individuals, four males and eight females with physician diagnosed diabetes and/or peripheral neuropathy, were recruited for this study. All candidates were screened for prescription medication that included NO donors, known cardiovascular disease, indications of deep vein thrombosis (DVT), or other health issues that may preclude them from safely participating in vibration treatments, such as orthopedic impairments. One candidate was excluded for failing the DVT screen. In addition to a self-reported loss of sensation, all research participants also submitted to a Quantitative Sensory Analysis (QST) to confirm that thermal sensory thresholds were outside of age-matched normative data. All research participants underwent two treatment conditions, a sham time control and a WBV treatment in a randomized block order. Subjects were required to make two visits, one for each treatment, with a one week wash out period between each visit. One participant was unable to return for a second visit; as a result his data was excluded. In all, ten older participants, (71 ± 8.27 year), three males and seven females diagnosed with diabetes and/or loss of sensation completed this research study (Table 1). The Institutional Review Board of Brigham Young University approved all procedures and all subjects provided written informed consent (Appendix C).

Instruments

Pre-participation Questionnaire

To screen for cardiovascular disease, lower limb injuries, and NO releasing medications, participants completed a pre-participation questionnaire (Appendix A).

Pre-test for Probability of Deep Vein Thrombosis and Deep Vein Ultrasound

To screen for the possibility of deep vein thrombosis (DVT) and rule out the threat of dislodging a clot during WBV, subjects completed a 10-question verbal “Clinical Model for Predicting the Pretest Probability for Deep Vein Thrombosis” questionnaire (Appendix B). The questionnaire resulted in a Wells score.³⁰ Subjects who scored below a two on the questionnaire, submitted to an ultrasound examination. During the ultrasound evaluation the compressibility of the common femoral vein in the upper inner thigh and the popliteal vein behind the knee was tested using a 9L transducer (LOGIQ P5 ultrasound system, GE Healthcare, Hertfordshire, UK). Unobstructed vessel walls will appose when the examiner places pressure on them with the transducer. Tests for compressibility of these vessels in these regions have a sensitivity for DVT of 97%.³¹

Quantitative Sensory Testing

Quantitative Sensory Testing (QST) for heat and cold sensation thresholds was employed to support to the diagnosis of neuropathy in small A-delta and C nerve fibers. Thermal sensory thresholds were quantified using a TSAII Neurosensory Analyzer (Medoc Ltd. Israel), which induces a measurable temperature stimulus in a Peltier thermode, applied to the dorsum of the foot along the L5 dermatone. Thermal thresholds were determined using the method of limits procedure.³² Individual results are reported in Table 2.

Whole Body Vibration

WBV was performed on a Galileo 2000 (Orthometrix, White Plains, NY), which is an alternating or reciprocating vibration platform. The frequency was set at 26 Hz. The amplitude, which was approximately 2 mm, was determined by the width of the subjects' stance on the platform. The participants stood in a semi-squat position with knees flexed 30°–40° in order to attenuate the vibration above the pelvis. The treatment duration and rest interval times were based on Bautman's investigation³³ conducted to determine if a population similar to ours, older individuals (mean age 77.5 year) and naïve to WBV, could and would participate in a WBV exercise program, and if the WBV program improved strength and balance. This group started their program with 30-second bouts and 60-second rest intervals. Their starting frequency and amplitude was 30 Hz and 2 mm respectively. In the sham condition, the subjects completed the same protocol as in the WBV, however, vibration was not imposed.

Outcome Measures

There were two dependent outcome measurements in this investigation; changes in dorsal foot SBF and whole blood nitric oxide (NO) concentrations. Measurements were taken at three different times: before the WBV or sham treatment, immediately after, and five minutes after the end of the treatment.

Skin Blood Flow Measurements

Skin blood flow was measured using a laser Doppler imager (LDI) (Moor Instruments, Inc., Oxford, England). The LDI enables rapid non-invasive analysis of changes in blood flow in skin and correlates well with other methods for measuring skin perfusion.^{34, 35}

When performing the LDI scan, the laser source was placed 35 cm from the middle of the scan area on the foot. The scan area was set at 5 cm x 5 cm and the resolution of the scan was set

at 100 x 100 pixels. The scan rate was 4 ms/pixel and a complete scan required 1 minute and 15 seconds. Markers were placed on the skin just outside of the scan area to allow for repeat measurements in the same region of interest (ROI). The resulting images were evaluated in processing software (MoorLDI Processing V3.1, Moor Instruments Inc., Oxford, England). The average flux was determined for a 15 cm² area centered over the ROI.

Blood NO Measurements

Three 2 ml samples of venous blood were drawn from the brachial vein at the cubital fossa. The blood drawn was placed in a tube containing 5 µl of 1000 U/ml heparin, and immediately treated with nitrite preservation solution in a 4:1 ratio (vol/vol; whole blood/preservative solution). To prepare the preservative solution 1320 mg ferricyanide was fully dissolved with 65 mg N-ethylmaleimide in 4.5 ml of ultrapure water for a final concentration of 0.8 M ferricyanide and 0.1 M N-ethylmaleimide. Then 500 µL of IGEPAL®CA-630 (equaling 10:1 vol/vol; total solution/IGEPAL®CA-630) was added and gently mixed. After mixing, each sample was placed in dry ice, and then stored at -80° C until analysis.³⁶

Whole blood nitrite concentration was determined using an ozone-based chemiluminescence detector (CDL) (Sievers NO Analyzer model 280i, GE Analytical Instruments, Boulder, CO). A glass purge vessel connected to the CDL contained an acidified triiodide solution consisting of 50 mg of potassium iodide, 2 ml of ultrapure water, and 5 mL of glacial acetic acid. A dilute solution of antifoaming agent (100 µl) was also added to the purge vessel. 100 µL nitrite-containing blood samples were injected with a Hamilton syringe (Hamilton Co., Reno, NV) into the purge vessel to undergo conversion to NO and subsequent chemiluminescent reaction with ozone. The emitted light was detected by a photomultiplier tube in the CLD and recorded as a voltage signal (Liquid Program, version 3.21). We determined the

area under the voltage peaks for each injection and standard solution to determine the NO concentration in each sample.

Procedure

Upon meeting the inclusion criteria and randomly determining the order of treatment (WBV or sham), the research participant was seated in a dental chair with knees bent at 90° and bare feet resting on an adjustable platform. Using a 5 cm x 5 cm square piece of heavy cardstock and a yellow highlighter marker, a 25 cm² ROI was marked on the center of the dorsum of the subject's right foot. The mark was used to consistently align the LDI scanner. After a 10-15 minute acclimatization period, the first LDI scan was performed. After the scan, a venous catheter was inserted into a large antecubital vein and secured to the arm. A 2 ml sample of blood was drawn, transferred to a heparin-laced vacutainer, mixed with nitrite preservative solution, and then held on dry ice until it could be stored at -80°C.

After putting shoes on, the subject was escorted to the vibration platform (5 feet from the chair) and then underwent the randomly assigned WBV or sham treatment. The subject stood on the vibration platform with knees flexed 30° to 40°, and their weight centered over the middle of each foot. Ten 30-second bouts of WBV were performed with a one minute rest between each bout. Following the last bout, the subject immediately returned to the chair, removed the right shoe and sock, and a second LDI scan was performed followed by a second blood draw. The subject remained seated for an additional five minutes from the end of the vibration treatment, after which the third LDI scan was performed and the final blood sample drawn. One week later the subject completed the protocol for the second treatment condition.

Upon completion all data collection, all the blood samples were processed for nitrite concentration analysis. The blood was thawed in a 37°C water bath, deproteinated with 100%

methanol (1:1 vol:vol), centrifuged for three minutes at 15,000 rpm, and the supernatant fraction was transferred to a clean tube. The centrifuge and transfer processes were repeated twice to remove any residual precipitate.

Immediately following the final separation process, 100 μ L aliquots of the blood samples were injected into the purge vessel filled with acidified tri-iodide as described in the previous section. Duplicate aliquots were injected from each sample, and the reducing agent was changed after every 4-6 injections. With each injection a voltage signal from the CDL was generated by data acquisition software. After all the samples had been injected, the baseline and peaks of the voltage signals were identified using selection tools in the data analysis program. Finally, the peak tracings were integrated, generating average values for the NO concentrations in each sample.

The LDI scans were evaluated for average flux in image processing software. We defined a 15 cm² ROI and applied it to the same location of each scanned image.

Statistical Analysis

To determine differences in SBF and NO production between treatment conditions, we used a 2 x 3 mixed model ANCOVA with baseline values as the covariate. We further used Tukey-Kramer post-hoc tests to determine difference between the conditions at each time point. We used JMP 9Pro (SAS Inc, Cary, NC) for all statistical analysis with an alpha level of $p \leq 0.05$.

Results

The WBV and sham conditions had similar SBF ($p=.999$) and whole blood NO concentrations ($p=1.0$) at baseline. Immediately following WBV treatment SBF significantly increased 15% compared to the sham condition ($F_{2,18}=5.82$, $p=0.01$). However there was no difference 5 minutes post-treatment between the conditions ($p=0.25$) (Figure 1). Whole blood

nitric oxide concentrations did not differ between the WBV and sham condition immediately or 5 minutes post-treatment ($F_{2,18}=1.88, p=0.18$) (Figure 2).

Discussion

The purpose of this study was to evaluate the effect of whole body vibration on SBF and blood NO concentrations in individuals with DSP. The results of this study indicate that low frequency, low amplitude WBV increases SBF in the feet of individuals with diabetic neuropathy. These results are consistent with findings from other groups^{27,29} who measured the effects of WBV on SBF.

While no other WBV studies connect increases in SBF with improvements in sensory nerve impairment, there is evidence that WBV improves sensory symptoms of neuropathy. In several case studies on patients with diabetic peripheral neuropathy, vibration treatments reduced neuropathic pain as well as improved balance and gait patterns. In one case the patient was seated in a chair with his feet on the vibration platform, which was set at 30 Hz and amplitude of 5 mm.³⁷ The vibration treatment consisted of three 2-minute bouts, with a one minute rest, five days a week for 8 weeks. In another case, the patient stood on the platform in a semi-squat and received four 3-minute bouts at 20 Hz.³⁸ In both cases there was a reduction in pain rating from above 6 (of 10) to below 2 on a visual analog scale (VAS) that lasted at least 3 hours after treatment, and by the end of the second week overall pain in the feet and legs was reduced. Similarly, Yoosefinejad³⁹ reported a case in which a following a six-week regimen of WBV, the patient's pain score for each foot had been reduced from 6 to 2 on a VAS, and her Michigan Diabetic Neuropathy Score was reduced from 13 (of 13) to 2. Guzman et al.⁴⁰ also reported significant decreases in numeric pain rating and Brief Pain Inventory Short Form scores following a 12-week WBV treatment program in 21 participants diagnosed with diabetic

neuropathy. Hong³⁸ attributed the reduction of pain to modulation of pain pathways in the CNS due to vibration, whereas Guzman et al.⁴⁰ speculated that increases in lower extremity strength and general physical function might be related to pain reduction. No consideration was given to the theory of increased skin blood flow.

While no WBV studies link increases in SBF to peripheral sensory changes, research of other therapeutic modalities that address DSP have shown an association. For example, in a study to test the effectiveness of the PKC- β inhibitor ruboxistaurin, Casellini et al.⁴¹ showed that after daily intake of the medication for six months SBF at the distal calf significantly increased and results on both the Neuropathy Total Symptoms Score (NTSS-6) questionnaire and the Norfolk Quality of Life Questionnaire - Diabetic Neuropathy (QOL-DN) improved. Rendell and Bamisedun⁴² showed that after six months of treatment with the antioxidant pentoxifylline, patients with neuropathy exhibited concurrent increases in lower extremity SBF, lower threshold current perception, and improvement in neuropathic symptoms. Ono et al.⁴³ not only showed that sensory nerve improvements accompanied increases in SBF, but that the degree of vibration thresholds in the lower extremities was positively correlated with the degree of SBF in the toes of patients with Type II diabetes after a four week daily dosage of KDI-792, a thromboxane A₂ antagonist and prostaglandin I₂ promoter. In a non-pharmacological study, twelve 30-minute treatments of monochromatic near-infrared light (NIR) therapy on the lower leg and foot improved the sensitivity in the feet of patients with diabetes.⁴⁴ The authors attributed the improvement to NO-induced increases in SBF. Each of these modalities is a very different path to the same end result of increased blood flow and improved symptoms of DSP.

Based on evidence from other studies,^{27, 44} we hypothesized that increases in SBF during WBV could be attributed to the action of NO on vascular tone. However, there were no

significant differences in blood NO concentrations between the sham and WBV conditions following the treatment. There are several possible explanations for why there was an increase in SBF without accompanying increases in blood NO concentration.

The increase in SBF that we measured could have been due to a local effect of NO induced by shear stress in the feet. This is supported by studies where NO increases were found concurrently with increases in SBF after vibration, and the blood for the NO sample was drawn at the same location as applied vibration.^{27, 45} However in our study, blood samples for NO concentration measurements were drawn from the antecubital fossa due to inherent problems associated with lower extremity wounds in diabetics, whereas the SBF measurements were made at the dorsum of the foot. In addition, the subject was in a squatting position to attenuate vibration, therefore shear stress-induced NO production may not have occurred in the arm. An additional study on healthy individuals wherein the blood samples are collected in the lower limb following WBV will provide a good test of whether NO is acting locally to increase SBF.

Another consideration is that factors other than NO may have caused the increase in SBF. For example, skin blood flow may have increased as part of a histamine response to the WBV. Indeed, in addition to the increase in blood flow, two of the subjects observed additional warmth and itchiness in their feet and legs following WBV. However, three of the subjects who indicated that they were taking an antihistamine showed an increase in SBF after WBV. An increase in blood pressure is another consideration. However, Otsuki et al.⁴⁶ found that there was no change in systolic and diastolic blood pressures in healthy subjects following ten 60-second bouts of WBV at a frequency and amplitude of 26 Hz and 2-4 mm respectively while standing in a squat. Similarly, Kerschman-Schindl et al.⁴⁷ observed no change in systolic and diastolic blood pressures

between baseline and vibration conditions, where each of the 20 healthy volunteers stood for three consecutive 3-minute bouts, each in a different standing position, including a squat.

While the mechanism by which skin blood flow increases with WBV is not certain, the implication remains that WBV has potential to be a non-pharmacological alternative to address DSP. This being the case, investigators need to determine the optimal dosage of WBV in order to produce a therapeutic effect. The smallest dosage reported with a significant decrease in neuropathic symptoms was in a four week case study in which the patient performed four 3-minute bouts (12 minutes) five days a week at 20 Hz.³⁸ In a larger study of 21 participants, four 3-minute bouts (12 minutes) of WBV at a frequency between 30-50 Hz three days a week significantly decreased indices of pain by the tenth week of a 12-week research period.⁴⁰ These studies indicate that a total of 12 minutes of WBV at a frequency between 20-50 Hz three to five days a week may be a sufficient dosage for pain reduction.

Another way to determine dosage might be in terms of percent increase in SBF. Reduction in painful symptoms of neuropathy were associated with a 32% increase in SBF due to pentoxifylline⁴², a 50% increase in flow due to KDI-792⁴³, and a 78.2% increase due to ruboxistaurin.⁴¹ Based on these pharmacological studies, 30% may of serve as a minimum measure of increased SBF sufficient to induce therapeutic effects. In our study, we measured an increase of 15% in SBF from baseline after 10 one-minute bouts. However changing the duration, frequency and/or amplitude of WBV can increase peripheral blood flow.²⁸ Maloney-Hinds²⁷ measured an increase in SBF of 223% from baseline in research participants with diabetes with the application of local vibration on the arm at a frequency of 50 Hz and 5-6 cm vertical displacement for five minutes. It would be useful to determine the changes in SBF following 12 minutes (four 3-minute bouts) of WBV at 30 Hz and 2 mm amplitude for four to 10

weeks, parameters similar to those used in the WBV studies reporting significantly reduced neuropathic pain.^{38, 40} Further investigations involving WBV and SBF should also include sensory tests such as QST to confirm that this increase is sufficient to produce symptom improvements.

A limitation in this study was the need for the research participants to wear their shoes and socks during the treatment period to protect their vulnerable feet. The warmth from the shoe and sock is a potential source of increased blood flow; however the compression from the shoe and sock and/or the change in temperature after exposure to the environment may decrease skin blood flow. It is likely that the footwear affected each participant in the same way, so their relative changes in SBF are not different than had no footwear been worn. Future investigations may control for this limitation with footwear that does not cover the ROI.

Conclusion

In summary, immediately following 10 one-minute bouts of WBV at a frequency of 26 Hz and amplitude of 2 mm, SBF on the dorsum of the foot was significantly elevated, but decreased towards baseline by 5 minutes into the recovery time. Blood NO concentration at the antecubital fossa is not affected, perhaps due to attenuation of vibration at that location, or perhaps because increase in NO due to shear stress is only a local affect. The relationship between increased SBF and reduced neuropathic pain is yet to be clearly established, but pharmacological and NIR light studies indicate an association implying that decreased pain may be attributed to increased SBF, and increased SBF may be attributed to NO-induced vessel dilation. The most effective dosage of WBV remains to be determined, but based on case studies^{37, 38} and a small trial reported by Guzman et al.⁴⁰, four 3-minute bouts at a frequency of

30 Hz and amplitude of 2 mm for 3-5 days/per week may result in reduced neuropathic pain as early as two weeks, with significant results occurring as early as four weeks into the treatment.

References

1. Boulton, A.J., A.I. Vinik, J.C. Arezzo, V. Bril, E.L. Feldman, R. Freeman, R.A. Malik, R.E. Maser, J.M. Sosenko and D. Ziegler: Diabetic neuropathies: A statement by the american diabetes association. *Diabetes Care*, 2005. 28: 956-962.
2. Li, Y.F., N. Burrows, E. Gregg and L. Geiss: Declining trends in hospitalizations for non-traumatic lower extremity amputation in the diabetic population-united states, 1988-2006. *Diabetes*, 2010. 59: A50-A50.
3. Argoff, C.E., B.E. Cole, D.A. Fishbain and G.A. Irving: Diabetic peripheral neuropathic pain: Clinical and quality-of-life issues. *Mayo Clin Proc*, 2006. 81: S3-11.
4. Vinik, A.I.: Diagnosis and management of diabetic neuropathy. *Clinics in Geriatric Medicine*, 1999. 15: 293-+.
5. Boulton, A.J., R.A. Malik, J.C. Arezzo and J.M. Sosenko: Diabetic somatic neuropathies. *Diabetes Care*, 2004. 27: 1458-1486.
6. Brownlee, M.: Biochemistry and molecular cell biology of diabetic complications. *Nature*, 2001. 414: 813-820.
7. Cameron, N.E., S.E.M. Eaton, M.A. Cotter and S. Tesfaye: Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia*, 2001. 44: 1973-1988.
8. Dickinson, P.J., A.L. Carrington, G.S. Frost and A.J.M. Boulton: Neurovascular disease, antioxidants and glycation in diabetes. *Diabetes-Metabolism Research and Reviews*, 2002. 18: 260-272.
9. Zou, M.H., C. Shi and R.A. Cohen: Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest*, 2002. 109: 817-826.
10. Schmidt, H.H. and U. Walter: No at work. *Cell*, 1994. 78: 919-925.
11. Michel, T. and O. Feron: Nitric oxide synthases: Which, where, how, and why? *J Clin Invest*, 1997. 100: 2146-2152.
12. Riddell, D.R. and J.S. Owen: Nitric oxide and platelet aggregation. *Vitam Horm*, 1999. 57: 25-48.
13. Cameron, N.E. and M.A. Cotter: The relationship of vascular changes to metabolic factors in diabetes-mellitus and their role in the development of peripheral-nerve complications. *Diabetes-Metabolism Reviews*, 1994. 10: 189-224.
14. Effect of intensive therapy on the development and progression of diabetic nephropathy in the diabetes control and complications trial. The diabetes control and complications (dcct) research group. *Kidney Int*, 1995. 47: 1703-1720.
15. Reichard, P., M. Pihl, U. Rosenqvist and J. Sule: Complications in iddm are caused by elevated blood glucose level: The stockholm diabetes intervention study (sdis) at 10-year follow up. *Diabetologia*, 1996. 39: 1483-1488.
16. Amthor, K.F., K. Dahl-Jorgensen, T.J. Berg, M.S. Heier, L. Sandvik, O. Aagenaes and K.F. Hanssen: The effect of 8 years of strict glycaemic control on peripheral nerve function in iddm patients: The oslo study. *Diabetologia*, 1994. 37: 579-584.

17. Ohkubo, Y., H. Kishikawa, E. Araki, T. Miyata, S. Isami, S. Motoyoshi, Y. Kojima, N. Furuyoshi and M. Shichiri: Intensive insulin therapy prevents the progression of diabetic microvascular complications in japanese patients with non-insulin-dependent diabetes mellitus: A randomized prospective 6-year study. *Diabetes Res Clin Pract*, 1995. 28: 103-117.
18. Boulton, A.J., F.A. Gries and J.A. Jervell: Guidelines for the diagnosis and outpatient management of diabetic peripheral neuropathy. *Diabet Med*, 1998. 15: 508-514.
19. Vinik, A.: The protein kinase c-beta inhibitor, ruboxistaurin, for the treatment of diabetic microvascular complications. *Expert Opin Investig Drugs*, 2005. 14: 1547-1559.
20. Malik, R.A., S. Williamson, C. Abbott, A.L. Carrington, J. Iqbal, W. Schady and A.J. Boulton: Effect of angiotensin-converting-enzyme (ace) inhibitor trandolapril on human diabetic neuropathy: Randomised double-blind controlled trial. *Lancet*, 1998. 352: 1978-1981.
21. Vanhoutte, P.M.: Endothelium-dependent responses and inhibition of angiotensin-converting enzyme. *Clin Exp Pharmacol Physiol*, 1996. 23: S23-29.
22. Sackner, M.A., E. Gummels and J.A. Adams: Effect of moderate-intensity exercise, whole-body periodic acceleration, and passive cycling on nitric oxide release into circulation. *Chest*, 2005. 128: 2794-2803.
23. Gielen, S., M. Sandri, S. Erbs and V. Adams: Exercise-induced modulation of endothelial nitric oxide production. *Curr Pharm Biotechnol*, 2011. 12: 1375-1384.
24. Yue, Z. and J. Mester: On the cardiovascular effects of whole-body vibration - part i. Longitudinal effects: Hydrodynamic analysis. *Studies in Applied Mathematics*, 2007. 119: 95-109.
25. Mester, J., H. Kleinoder and Z. Yue: Vibration training: Benefits and risks. *J Biomech*, 2006. 39: 1056-1065.
26. Sackner, M.A., E. Gummels and J.A. Adams: Nitric oxide is released into circulation with whole-body, periodic acceleration. *Chest*, 2005. 127: 30-39.
27. Maloney-Hinds, C., J.S. Petrofsky, G. Zimmerman and D.A. Hessinger: The role of nitric oxide in skin blood flow increases due to vibration in healthy adults and adults with type 2 diabetes. *Diabetes Technol Ther*, 2009. 11: 39-43.
28. Lythgo, N., P. Eser, P. de Groot and M. Galea: Whole-body vibration dosage alters leg blood flow. *Clin Physiol Funct Imaging*, 2009. 29: 53-59.
29. Lohman, E.B., 3rd, J.S. Petrofsky, C. Maloney-Hinds, H. Betts-Schwab and D. Thorpe: The effect of whole body vibration on lower extremity skin blood flow in normal subjects. *Med Sci Monit*, 2007. 13: CR71-76.
30. Wells, P.S., J. Hirsh, D.R. Anderson, A.W.A. Lensing, G. Foster, C. Kearon, J. Weitz, R. D'Ovidio, A. Cogo, P. Prandoni, A. Girolami and J.S. Ginsberg: A simple clinical model for the diagnosis of deep-vein thrombosis combined with impedance plethysmography: Potential for an improvement in the diagnostic process. *Journal of Internal Medicine*, 1998. 243: 15-23.
31. Scarvelis, D. and P.S. Wells: Diagnosis and treatment of deep-vein thrombosis. *CMAJ*, 2006. 175: 1087-1092.
32. Amer Assoc Electrodiagnost, M.: Technology literature review: Quantitative sensory testing. *Muscle & Nerve*, 2004. 29: 734-747.

33. Bautmans, I., E. Van Hees, J.C. Lemper and T. Mets: The feasibility of whole body vibration in institutionalised elderly persons and its influence on muscle performance, balance and mobility: A randomised controlled trial [isrctn62535013]. *BMC Geriatr*, 2005. 5: 17.
34. Millet, C., M. Roustit, S. Blaise and J.L. Cracowski: Comparison between laser speckle contrast imaging and laser doppler imaging to assess skin blood flow in humans. *Microvascular Research*, 2011. 82: 147-151.
35. Essex, T.J. and P.O. Byrne: A laser doppler scanner for imaging blood flow in skin. *J Biomed Eng*, 1991. 13: 189-194.
36. Pelletier, M.M., P. Kleinbongard, L. Ringwood, R. Hito, C.J. Hunter, A.N. Schechter, M.T. Gladwin and A. Dejam: The measurement of blood and plasma nitrite by chemiluminescence: Pitfalls and solutions. *Free Radic Biol Med*, 2006. 41: 541-548.
37. Hong, J.: Whole body vibration therapy for diabetic peripheral neuropathic pain: A case report. *Health Science Journal*, 2011. 5: 66-71.
38. Hong, J., M. Barnes and N. Kessler: Case study: Use of vibration therapy in the treatment of diabetic peripheral small fiber neuropathy. *J Bodyw Mov Ther*, 2013. 17: 235-238.
39. Yoosefinejad, A.K., A. Shadmehr, G. Olyaei, S. Talebian, H. Bagheri and M.R. Mohajeri-Tehrani: Effects of whole-body vibration on a diabetic type 2 patient with peripheral neuropathy. *Health Science Journal*, 2012. 6: 576-583.
40. Guzman, R.J., *Effecto of whole-body vibration on painful diabetic peripheral neuropathy*, in *Health and Human Science* 2012, Oregon State University: Corvallis, OR. p. 50.
41. Casellini, C.M., P.M. Barlow, A.L. Rice, M. Casey, K. Simmons, G. Pittenger, E.J. Bastyr, 3rd, A.M. Wolka and A.I. Vinik: A 6-month, randomized, double-masked, placebo-controlled study evaluating the effects of the protein kinase c-beta inhibitor ruboxistaurin on skin microvascular blood flow and other measures of diabetic peripheral neuropathy. *Diabetes Care*, 2007. 30: 896-902.
42. Rendell, M. and O. Bamisedun: Skin blood flow and current perception in pentoxifylline-treated diabetic neuropathy. *Angiology*, 1992. 43: 843-851.
43. Ono, Y., M. Katoh, A. Hirayama and T. Koike: Improvement in blood flow and diabetic neuropathy by thromboxane a2 dual blocker kdi-792. *Prostaglandins Leukot Essent Fatty Acids*, 1995. 53: 139-145.
44. Kochman, A.B., D.H. Carnegie and T.J. Burke: Symptomatic reversal of peripheral neuropathy in patients with diabetes. *Journal of the American Podiatric Medical Association*, 2002. 92: 125-130.
45. Ichioka, S., H. Yokogawa, G. Nakagami, N. Sekiya and H. Sanada: In vivo analysis of skin microcirculation and the role of nitric oxide during vibration (vol 57, pg 40, 2011). *Ostomy Wound Management*, 2011. 57: 28-28.
46. Otsuki, T., Y. Takanami, W. Aoi, Y. Kawai, H. Ichikawa and T. Yoshikawa: Arterial stiffness acutely decreases after whole-body vibration in humans. *Acta Physiol (Oxf)*, 2008. 194: 189-194.
47. Kerschman-Schindl, K., S. Grampp, C. Henk, H. Resch, E. Preisinger, V. Fialka-Moser and H. Imhof: Whole-body vibration exercise leads to alterations in muscle blood volume. *Clin Physiol*, 2001. 21: 377-382.

Table 1: Participant Demographics

Characteristic	
Gender	3 males; 7 females
Age (yr)	71 ± 8.27
Body Mass Index	30.1 ± 5.12
Duration of Diabetes (yr)	12.5 ± 6.9

Table 2: Participant Sensory Profile

Subject #	Gender	Age (yrs)	Self-Reported Loss of Sensation	QST-Cold Threshold (°C)	Cold Threshold within norm	QST-Hot Threshold (°C)	Hot Threshold within norm
1	M	65	Y	28.3	N	45.2	N
2	M	83	Y	3.4	N*	48.8	N*
3	M	73	Y	8.8	N	48.2	N
4	F	69	Y	10.6	N	45.8	N
5	F	77	Y	0	N	50	N
6	F	80	Y	24.8	N*	46.6	N*
7	F	79	Y	12.3	N	49.7	N
8	F	66	Y	20.6	N	46.9	N
9	F	58	Y	22.3	N	46	N
10	F	60	Y	28.8	Y	37.5	Y

QST=Quantitative Sensory Test; Y=Yes; N=No

*Normative data not available for this age and gender, so the range for one age group younger was used for comparison.

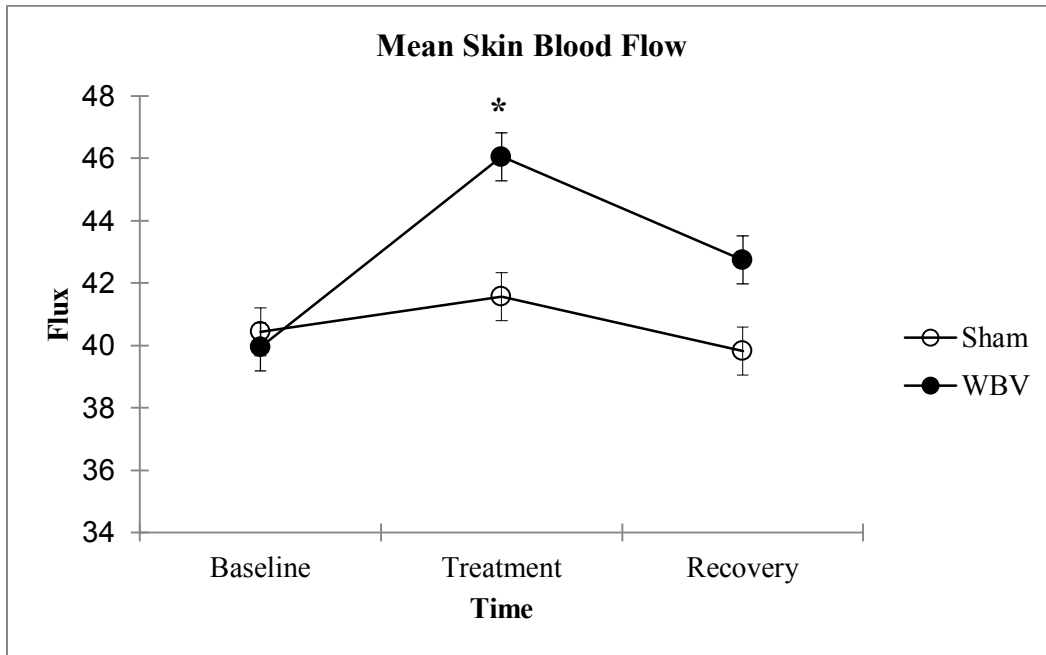


Figure 1: Mean skin blood flow and SE for each group (n=10) at baseline, immediately after the treatment condition, and following 5 min of recovery. * p<0.05 different from Sham.

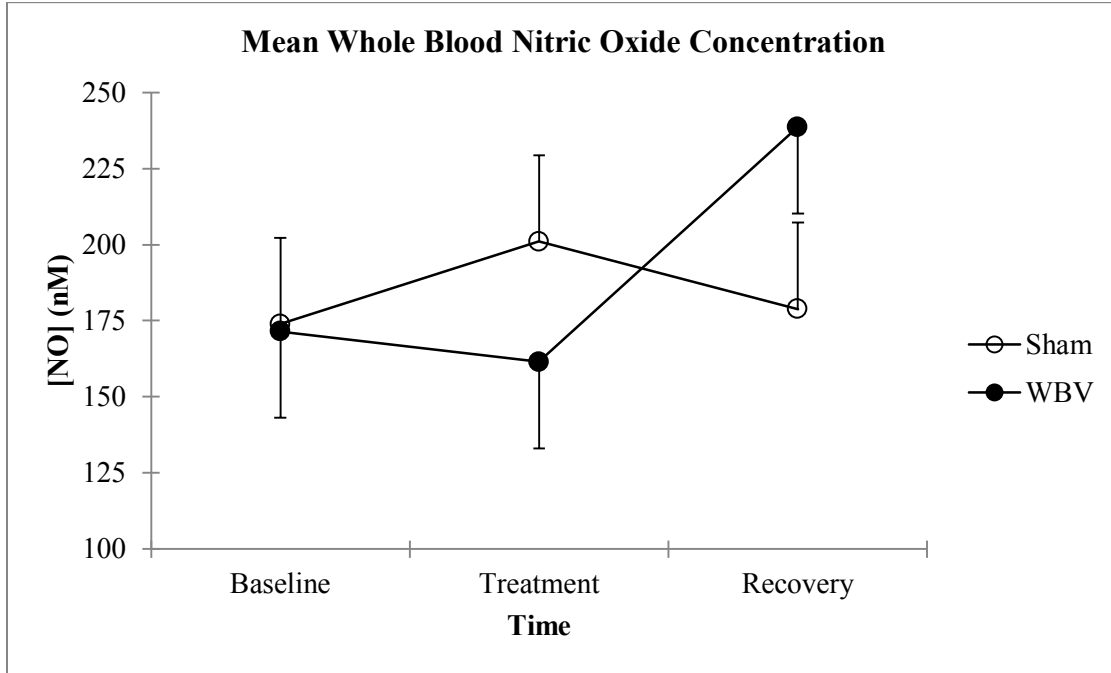


Figure 2: Mean whole blood nitric oxide concentration and SE for each group (n=10) at baseline, immediately following the treatment condition, and after 5 min of recovery.

Appendix A

Subject Pre-Participation Questionnaire

Name: _____ Phone number: _____

Email: _____

Age: _____ Weight: _____ Height: _____ Gender _____

How long ago were you diagnosed with diabetes? _____

Please answer the following questions to the best of your knowledge. Please place a check in the appropriate box. All information from this questionnaire will be kept confidential.

Please indicate the most appropriate answer to the following questions	YES	NO
1. Do you have any known lower extremity peripheral vascular disorders?		
2. Do you have any loss of sensation?		
3. Are you currently taking prescription medication for a cardiovascular disorder?		
4. Are you currently taking any blood pressure or cholesterol medication?		
5. Are you currently taking any nitric-oxide releasing medications?		
6. Are you currently taking a diuretic?		
7. Are you currently taking an antihistamine (i.e. for allergies)		
8. Have you had any surgery or injury to your lower extremity within the last three months?		
9. Do you smoke?		
10. Have you had any caffeinated drinks within the last 8 hours?		

Should you become ill and/or incapable of finishing the study, alert the investigator immediately and first aid will be administered. You may withdraw without prejudice.

Appendix B

Screen for Deep Vein Thrombosis (DVT) and Neuropathy (QST)

To be administered verbally by an investigator. All information from this questionnaire will be kept confidential.

Clinical Model for Predicting the Pretest Probability for Deep Vein Thrombosis

Score 1 point for affirmative responses	Score
1. Active cancer (treatment or palliation within 6 months)	
2. Paralysis, paresis, or recent (4 weeks) plaster immobilization of lower extremities	
3. Recently bedridden for > 3 days and/or major surgery within 4 weeks	
4. Localized tenderness along the distribution of the deep venous system	
5. Entire leg swollen	
6. Calf swelling >3 cm compared to the other leg	
7. Pitting edema, greater in the symptomatic leg	
8. Collateral dilated superficial veins (nonvaricose)	
9. Previously documented DVT	
10. Alternative diagnosis as or more likely than deep vein thrombosis. Score minus 2	
Total Score	

Calculate the clinical probability as follows: high ≥ 3 points, moderate 1 or 2 points, low < 0 points

QST results:

Cold sensation threshold _____

Warm sensation threshold _____

Within Norm? Y N

Within Norm? Y N

Compression Ultrasonography results:

Compressible Veins? Y N ("N" is an indication of DVT)

Appendix C

Consent to be a Research Subject

Introduction

This study is being conducted by Paula Johnson, a graduate student, and Dr. Ulrike Mitchell, her faculty mentor and an assistant professor in the Department of Exercise Sciences, Brigham Young University. The purpose is to investigate blood flow changes to the top of the foot and changes in nitric oxide concentrations in the blood in response to low frequency whole-body vibration.

Procedures

You will have to commit to come to the research lab two times. Your first visit will require about 80 minutes and your second visit will be about 60 minutes, for a total time commitment of less than two and a half hours. During the first 15-20 minutes on your first visit you will be screened for neuropathy by having the sensation of the top of your foot tested, and for deep vein thrombosis by having an ultrasound performed on the large veins in your inner thighs and the back of your lower legs. During the remainder of the time, and on your second visit you will be escorted to an environmental chamber and sit comfortably on an examining table for 10-15 minutes to stabilize your body temperature. After that time, a trained and licensed phlebotomist will insert a catheter in your arm and draw the first of three 3 ml blood samples (less than 1 teaspoon). The catheter will be secured to your arm with medical tape. Also, the top of your right foot will be scanned with a laser Doppler imager. The laser emits no heat. Following those procedures, you will stand with knees flexed on a whole-body vibration platform for 30 seconds while the platform vibrates with very small, fast, up and down movements. You will do this ten times with a one minute rest between each bout of vibration. Immediately after the last bout, the phlebotomist will take the second blood sample, and another scan of your foot will be performed. Five minutes after the last bout, the phlebotomist will take the last blood sample and the final foot scan will be performed.

Risk/Discomforts

You may experience slight discomfort, bruising or infection in the arm where a catheter will be inserted for the blood draw. This risk will be minimized by using a trained phlebotomist to perform the blood draws. You may also experience lower extremity skin irritation due to vibration stimulus. If, for any reason, you are unable to complete the full treatment and/or assessments, you may withdraw from the study.

Benefits

There are no anticipated long term benefits to you for your participation in this study. You may experience a temporary increase in blood flow to your legs. If so, it may or may not improve your sensation and decrease your pain. Nevertheless, your participation in this study will enable us to pursue further research looking at the relationship of nitric oxide on peripheral blood flow and neuropathy. This research, if successful, will enable us to show how blood flow is affected by whole-body vibration therapy in the diabetic patient. Such information should lead to new and improved treatment options for diabetics.

Confidentiality

All information provided will remain confidential and will only be reported as group data with no identifying information. All data including the pre-participation questionnaire will be kept secured and only those involved in the study will have access to this information. Following the investigation, the questionnaires will be destroyed.

Compensation

You will be compensated a total of \$40.00 for your participation. Upon completion of your first visit you will receive \$15.00, and upon completion of your second visit you will receive \$25.00.

Participation

Participation in this research study is voluntary. You have the right to withdraw at any time.

Questions about the Research

If you have questions regarding this study, you may contact:

Paula Johnson, Graduate Student: (916) 521-2125, paula.v.johnson@byu.edu

Dr. Ulrike Mitchell, Faculty Mentor/Assistant Professor: (801) 422-3344,
rike_mitchell@byu.edu

Questions about your Rights as Research participants

If you have questions you do not feel comfortable asking the researcher, you may contact:

IRB administrator
A 285 ASB
Brigham Young University
Provo, UT 84602
(801) 422-1461
irb@byu.edu

I have read, understood, and received a copy of the above consent and desire of my own free will to participate in this study.

Signature: _____

Date: _____