Industrial Health 2009, **47**, 533–542 **Original Article**

Vibration Disrupts Vascular Function in a Model of Metabolic Syndrome

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Received March 16, 2009 and accepted June 9, 2009

Abstract: Vibration-induced white finger (VWF) is a disorder seen in workers exposed to hand-transmitted vibration, and is characterized by cold-induced vasospasms and finger blanching. Because overweight people with metabolic syndrome are pre-disposed to developing peripheral vascular disorders, it has been suggested that they also may be at greater risk of developing VWF if exposed to occupational vibration. We used an animal model of metabolic syndrome, the obese Zucker rat, to determine if metabolic syndrome alters vascular responses to vibration. Tails of lean and obese Zucker rats were exposed to vibration (125 Hz, 49 m/s^2 r.m.s.) or control conditions for 4 h/d for 10 d. Ventral tail arteries were collected and assessed for changes in gene expression, levels of reactive oxygen species (ROS) and for responsiveness to vasomodulating factors. Vibration exposure generally reduced the sensitivity of arteries to acetylcholine (ACh)-induced vasodilation. This decrease in sensitivity was most apparent in obese rats. Vibration also induced reductions in vascular nitric oxide concentrations and increases in vascular concentrations of ROS in obese rats. These results indicate that vibration interferes with endothelial-mediated vasodilation, and that metabolic syndrome exacerbates these effects. These findings are consistent with idea that workers with metabolic syndrome have an increased risk of developing VWF.

Key words: Vibration-induced white finger, Zucker rats, Obesity, Endothelial function, Reactive oxygen species, Nitric oxide

Introduction

Metabolic syndrome, a disorder characterized by central adiposity, affects 10–40% of adults worldwide, depending on geographical location[1)]. Besides central adiposity, which is determined by waist circumference, people with metabolic syndrome also have at least 2 of the following symptoms as identified by the National Cholesterol Education Programs Adult Treatment Panel III report: elevated blood pressure, elevated triglycerides and/or reduced HDL cholesterol levels, insulin resistance with or without glucose intolerance[2)]. Numerous studies have demonstrated that having metabolic syndrome puts individuals at risk of developing cardio- and peripheral vascular diseases and type II diabetes mellitus[3–5)]. Metabolic syndrome and overweight may also increase the risk of developing certain occupationally-induced diseases or disorders, including musculoskeletal disorders[6)]. For example, because workers with metabolic disorders, including metabolic syndrome, diabetes and thyroid disorders, are more likely to develop vascular disease, it has been hypothesized that these workers may also be at an increased risk of developing the peripheral vascular dysfunction that is one of the hallmark symptoms of hand-arm vibration syndrome (HAVS).

HAVS is a multifocal disorder that is seen in workers exposed to hand-transmitted vibration through the use of powered and pneumatic hand tools. The hallmark symp-

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tom of HAVS is cold-induced vasospasms of the peripheral vasculature that result in finger blanching and is referred to as vibration-induced white finger (VWF[7]). Other symptoms of HAVS include reductions in tactile sensation, pain and paresthesia, along with reductions in grip strength and manual dexterity[8]. Although international standards (ISO 5349 standard[9]) suggest that workers who are pre-disposed to cardiovascular and metabolic disorders may have an increased risk of developing HAVS, workers with these disorders are usually excluded from epidemiological and experimental studies, so the risk associated with exposure, and responses to vibration, have not been described in this population.

To begin to understand if the physiological and metabolic changes associated with metabolic syndrome affect vascular responses to vibration, we assessed the responses of the peripheral vascular system to vibration in an animal model of metabolic syndrome, obese Zucker (*fa/fa*) rats. Obese Zucker rats have an autosomal recessive mutation of the leptin receptor gene that disrupts leptin signaling and results in hyperphagia and weight gain throughout the life of the animal. These rats are overweight, display hyperinsulinemia, increased triglyceride levels and develop hypertension as they get older[10]. Obese rats also display changes in cardiovascular function that are similar to those seen in humans with metabolic syndrome[11].

Studies in humans and obese Zucker rats have demonstrated that the symptoms of metabolic syndrome are associated with an increase in oxidative activity and inflammation in the vascular system and alterations in vascular responsiveness to factors that mediate vasoconstriction and endothelial-induced vasodilation[12, 13]. Using a rat-tail model of vibration-induced injury, our laboratory has demonstrated that vibration also alters vascular responsiveness to α2C-adrenoreceptor-mediated vasoconstriction and nitric oxide-mediated vasodilation, and that these effects are most likely the result of an increase in oxidative stress[14–16]. Based on these findings we hypothesized that repeated exposures to tail vibration would induce a greater increase in oxidative stress in obese rats than in their lean siblings, and that this vibration-induced increase in oxidative activity would be associated with alterations in responsiveness to vasomodulating factors. We specifically predicted that vibration would enhance α2C-adrenoreceptor-mediated vasoconstriction[14] and attenuate endothelial-mediated vasodilation[16].

Methods

Animals

Male obese Zucker (n=16) and lean Zucker rats (n=16) [Crl: ZUC-*Lepr*^{fa}] were obtained at 6 wk of age (Charles River, Wilmington, MA) and were on average 12 wk of age when used in the study. Rats were maintained in a colony room with a 12:12 reversed light: dark cycle (lights off 0700 h) with Teklad 2918 rodent diet and tap water available *ad libitum*, at the National Institute for Occupational Safety and Health (NIOSH) facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All procedures were approved by the NIOSH Animal Care and Use Committee and were in compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.

Vibration exposures

The equipment and protocol for exposing animals to vibration has been previously described[14]. Briefly, after one week of acclimation to restraint in Broome style restrainers, rats were randomly assigned to the vibrated or restraint-control (control) group. During exposures, vibrated rats had their tails secured to a vibrating platform and were exposed to 4 h bouts of tail vibration (125 Hz, unweighted acceleration of 49 m/s^2 r.m.s.) between 0900 and 1300 h for 10 consecutive days. We chose this frequency and acceleration because many tools, including grinders, cement saws and chipping hammers, display vibration characteristics at or above this frequency. We also chose these vibration parameters because previous work in our laboratory has demonstrated that the biodynamic response of the rat tail is similar to the response of the human fingers at this frequency and acceleration[17]. Control rats were treated in an identical manner except that their tails were secured to a non-vibrating platform mounted onto isolation blocks.

Cutaneous temperature

Tail skin temperatures were measured using an infrared camera (IRRIS 256ST, Cincinnati Electronics, Cincinnati, OH, USA). The camera was computer controlled and thermal images of the tail were digitized, saved, and analyzed using Thermosoft II software (Version 1.2; EIC, Jenison, MI, USA). Cutaneous skin temperature and background temperatures were collected immediately before and after exposures on days 1, 5 and 9 of the study.

Tissue samples

One hour after the completion of the day 10 exposure, rats were deeply anesthetized using pentobarbital (100 mg/kg) and exsanguinated by cardiac puncture. Blood glucose levels were immediately measured using a ReliOn® Ultima Blood Glucose Monitoring System (Solartek Products Inc, Alameda, CA, USA). Serum was isolated from the remaining blood and stored at –80˚C.

Table 1. Transcripts examined by qPCR and primer sequences

Transcript		Primer sequence
α2A-adrenoreceptor	Forward	5'-GTGTGTTGGTTCCCGTTCTT-3'
	Reverse	5'-CGGAAGTCGTGGTTGAAAAT-3'
α2C-adrenoreceptor	Forward	5'-GGGTTTCCTCATCGTTTTCA-3'
	Reverse	5'-GAAAAGGGCATGACCAGTGT-3'
CGRP	Forward	5'CCCAGAAGAGATCCTGCAAC-3'
	Reverse	5'GTGGGCACAAAGTTGTCCTT-3'
HIF-1a	Forward	5'-AAGCACTAGACAAAGCTCACCTG-3'
	Reverse	5'-CCATATCGCTGTCCACATCA-3'
MCP-1	Forward	5'AGCATCCACGTGCTGTCTC-3'
	Reverse	5'GATCATCTTGCCAGTGAATGAG-3'
NOS-1	Forward	5'GATGAGGCACCCCAACTCT-3'
	Reverse	5'GGAAAGAAACGCAAGGGTTC-3'
NOS-2	Forward	5'-CCTGTGTTCCACCAGGAGAT-3'
	Reverse	5'-CGCTTTCACCAAGACTGTGA-3'
NOS-3	Forward	5'-TGACCCTCACCGATACAACA-3'
	Reverse	5'-CTGTACAGCACAGCCACGTT-3'
TNF-α	Forward	5'-ATGTGGAACTGGCAGAGGAG-3'
	Reverse	5'CAATCACCCCGAAGTTCAGT-3'
TRPV1	Forward	5'GGTGTGCCTGCACCTAGC-3'
	Reverse	5'CTCTTGGGGTGGGGACTC-3'
TRPV4	Forward	5'TGTCCCTCAGCAGTTCGTTA-3'
	Reverse	5'CTGGTTTACAACAGCAAGATCG-3'

Ventral tail arteries were dissected from the C7–9 and C12–15 regions of the tail, frozen in cryovials and stored at –80˚C. The remaining, distal portion of the tail was also collected, placed into cold Dulbeccos modified Eagle's medium (DMEM; Invitrogen; Carlsbad, CA, USA), and stored at 4˚C for *in vitro* analyses of vascular function as described below. Arteries were dissected from these specific regions of the tail because the physical stress and strain of vibration is greatest in regions between the strap restraints[17] and these regions display altered vascular responses after exposure to a single bout of vibration[14].

Quantitative RT-PCR

qRT-PCR was performed on arteries from the C7–9 region of the tail using previously described methods[18]. We assessed changes in transcript levels for pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1), hypoxia-induced factor-1 (HIF-1), and other factors involved in vasomodulation, such as calcitonin gene-related peptide (CGRP), nitric oxide synthases 1–3, the transient receptor potential cation channels sub-family-V (TRPV)1 and 4, and the α2C adrenoreceptor. Ribosomal 18s was used as a loading control. Specific transcripts and the primer sequences are presented in Table 1. Briefly, RNA was isolated and purified using previously described methods[18] and first strand cDNA was synthesized from 1 μg of total RNA using Invitrogen's Reverse Transcription System (Invitrogen). Control RNA from heart or brain was run at 10X dilutions for each transcript to establish a standard curve of relative transcript levels, and relative RNA levels were calculated using this curve. Samples that did not show a single defined melt peak in the 80˚C range were not included in the data set.

Ventral artery physiology

Ventral tail arteries were dissected from the C18–21 region of the tail and mounted in a microvessel chamber (Living Systems, Burlington, VT, USA). All vasoconstricting and vasodilating factors were obtained from Sigma Chemicals (St Louis, MO USA) unless otherwise noted. Vessels were maintained at 37˚C in HEPES bicarbonate solution and held at a constant pressure of 80 mmHg. Preliminary studies examining changes in vascular diameter in response to increasing pressure did not reveal any significant differences in vascular tone in the tail arteries of lean and obese rats. To assess the effects

of vibration on α2C-adrenoreceptor mediated vasoconstriction, dose-response curves to the agonist UK14304 were generated by applying the agonist in half-log increments (from 10^{-9} to 10^{-5}). Vessels were then rinsed and allowed to recover to baseline diameters. To assess the effects of vasodilating factors arteries were first constricted to 50–60% of their resting diameter using the α-1 adrenoreceptor agonist, phenylephrine. Dose-response curves to the NO donor, S-nitroso-N-acetylpenicillamine, (SNAP) and acetylcholine (ACh) were generated by increasing the concentrations of these agonists in half-log increments (from 10^{-9} to $10^{-5.0}$ M for ACh, and $10^{-8.5}$ to $10^{-5.0}$ M for SNAP). Changes in the internal diameter of arteries were continuously measured during treatments using a XC-ST30 video camera mounted onto a Nikon T1-SM inverted microscope, a video dimension analyzer (Living systems) and Data-Q Instruments software (Akron, OH USA).

Nitrate/nitrite (NO_x) and hydrogen peroxide (H_2O_2) assays

Artery samples from the C12–C15 region were homogenized in 200 μl lysis buffer (10 mM Tris base, 1 mM EDTA, 1 mM EGTA, 150 mM sodium chloride, 1% Triton-X 100) and NO_x and hydrogen peroxide concentrations were measured using the NO_x colormetric Assay (Caymen Chemical Company; Ann Arbor, MI USA) and the Fluoro H_2O_2™ assay (Cell Technology Inc., Mountain View, CA USA) and the manufacturer's protocol. Protein concentrations were analyzed using the BCA assay (Pierce, Rockford, IL USA).

ELISAs

Circulating insulin concentrations were measured in duplicate serum samples using a rat insulin ELISA (Crystal Chemical Inc., Downers Grove, IL USA). The assay was performed using the manufacturer's protocols.

Analyses

Tail temperatures collected on days 1, 5 and 9 were analyzed using a 4-way (phenotype × treatment × pre vs. post exposure × day of exposure) mixed model ANOVA with animal as a random variable. ELISA and qPCR data were analyzed using 2-way ANOVAs (phenotype × treatment). For the *in vitro* vascular studies, a non-linear regression model (Prism GraphPad, San Diego, CA USA) was used to plot the dose-dependent vasoconstriction and vasodilation that occurred in response to UK14304, ACh, or SNAP. The effects of vibration and restraint on vasoconstriction and dilation were analyzed using 3-way (phenotype × treatment × dose) repeated-measure ANOVAs. Pairwise comparisons for all analyses were performed using Student's *t*-tests. For all analyses, probability values with $p<0.05$ were considered significant. Analyses were performed using JMP version 5.0.1 (SAS Institute, Cary, NC USA) unless otherwise noted.

Results

Animals

Body weights, blood glucose and serum insulin concentrations are presented in Table 2. Analyses of body weights demonstrated that body weights were similar in vibrated and control rats. However, obese rats weighed more than lean rats, regardless of condition ($F(1, 28)=129.19$, $p<0.0001$). Blood glucose levels and serum insulin concentrations were also significantly higher in obese than lean rats ($F(1, 28)=25.42$, $p<0.001$; and $F(1, 28)=175.93$, $p<0.0001$, respectively), regardless of their exposure condition. Although blood lipids were not directly measured, it was noted that a thick lipid layer formed on the top of all blood samples from obese rats, but none of the samples from lean rats.

Cutaneous temperatures

Tail skin temperatures are presented in Fig. 1. The analyses resulted in a significant phenotype × pre-post exposure × day of exposure interaction ($F(2, 136)=3.094$, $p<0.05$). Additional analyses of this interaction demonstrated that tail temperatures were generally lower immediately after vibration exposure than prior to exposure in obese rats ($p<0.01$). On day 5, obese rats in the control

Table 2. Body weights, blood glucose and serum insulin concentrations in obese and lean Zucker rats exposed to control or vibration conditions

	Body wt (g)	Blood glucose (mg/dl)	Serum insulin (ng/ml)
Lean control n=8	322.40 ± 25.45*	122.25 ± 9.36*	0.47 ± 0.31*
Lean vibrated n=8	311.44 ± 22.00*	156.63 ± 8.69*	0.39 ± 0.18*
Obese control n=8	529.41 ± 44.49	207.13 ± 21.53	10.12 ± 1.05
Obese vibrated n=8	532.70 ± 39.73	207.35 ± 9.84	8.46 ± 0.76

Data represent the means ± sem. Analyses of these data demonstrated that all measures were significantly lower in lean than obese Zucker rats regardless of condition (*less than obese rats, $p<0.0001$).

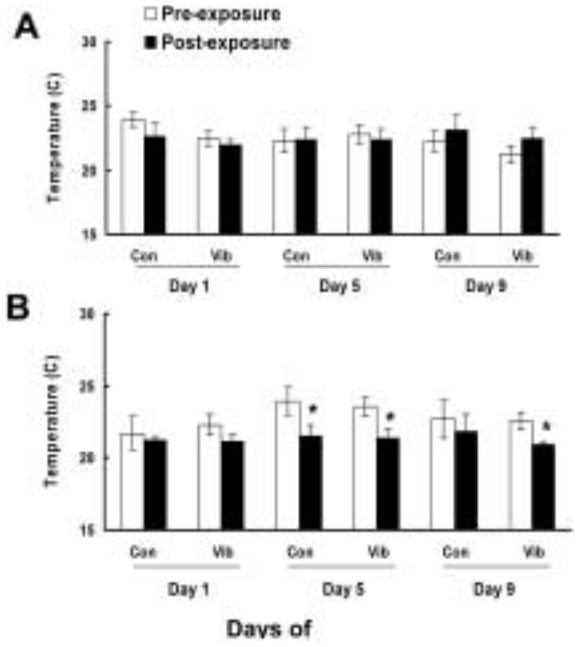

Fig. 1. Cutaneous tail temperatures in lean (A) and obese (B) Zucker rats before and after exposure to control conditions or tail vibration on days 1, 5 and 9 of the study.
Exposure to vibration did not affect tail temperatures in lean rats. However, in obese rats, vibration resulted in a post-exposure reduction in tail temperatures. On day 5, obese rats exposed to control conditions also showed a reduction in tail temperatures after exposure. (*less than pre-exposure temperatures, $p<0.05$, n=8 rats/group).

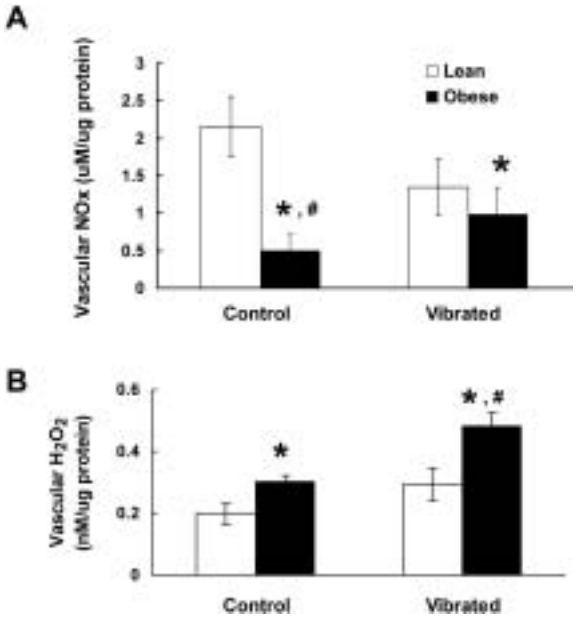

Fig. 2. Vascular NO_x (A) and H_2O_2 (B) concentrations in arteries from lean and obese rats exposed to vibration or control conditions (data presented are mean ± sem, n=8 rats/group).
Vascular NO_x concentrations were lower in obese rats than in lean rats (*main effect of phenotype, $p<0.05$). Vibration appeared to induce a reduction in No_x concentrations in lean rats, but this difference was not significant ($p<0.09$). In contrast, vascular H_2O_2 concentrations were higher in obese rats than in lean rats (*main effect of phenotype, $p<0.05$). Vibration exposure resulted in an additional increase in vascular H_2O_2 in obese rat (#different than obese controls), but not in lean rats.

condition also showed a post-exposure reduction in tail temperature ($p<0.05$). There were no significant differences in tail temperatures in lean rats.

Reactive oxygen species

Vascular concentrations of NO_x were significantly lower in obese rats than in lean rats ($F(1, 29)=4.10$, $p=0.05$; Fig. 2). This difference was primarily due to lower vascular concentrations of No_x in non-vibrated rats ($p<0.04$). No_x levels were also marginally lower in lean vibrated than lean control rats ($p<0.09$). Analysis of vascular H_2O_2 concentrations uncovered a significant main effect of treatment, where H_2O_2 concentrations were higher in vibrated than control rats. Additional analyses of these data revealed that this treatment effect was primarily the result of significant changes in obese rats ($p<0.02$).

Gene expression

Fold changes in transcript levels (relative to lean control rats) are presented in Fig. 3. Transcript levels for NOS-2 (inducible NOS or iNOS) were higher in arteries of obese rats than lean rats ($F(1, 30)=5.21$, $p<0.04$). NOS-2 transcript levels were also higher in vibrated than control rats ($F(1, 30)=4.92$, $p<0.04$). This increase in NOS-2 expression was more pronounced in obese rats; NOS-2 expression in arteries of obese rats exposed to vibration was approximately 2-fold higher than expression in arteries from lean rats exposed to vibration. Expression of the pro-inflammatory factor, MCP-1, and of NOS-3 (endothelial NOS or eNOS) transcripts was greater in arteries from obese than lean ($F(1, 30)=27.12$, $p<0.0001$ and $F(1, 30)=20.81$, $p<0.0001$, respectively). There were no significant phenotype- or treatment- related changes in gene expression for any other transcripts measured.

In vitro microvessel analyses

Baseline internal diameters of ventral arteries were not different between the groups at the beginning of the study (average internal diameters in μm ± sem; lean control: 383.75 ± 17.19, lean vibrated: 386.40 ± 15.87, obese control: 420 ± 15.38, obese vibrated: 388.14 ± 12.99). Dose-response curves to the α2C-adrenoreceptor agonist, UK14304, are presented in Fig. 4. Analyses of these data revealed a significant phenotype × treatment × dose interaction ($F(24, 208)=2.46$, $p<0.003$). In lean rats, animals

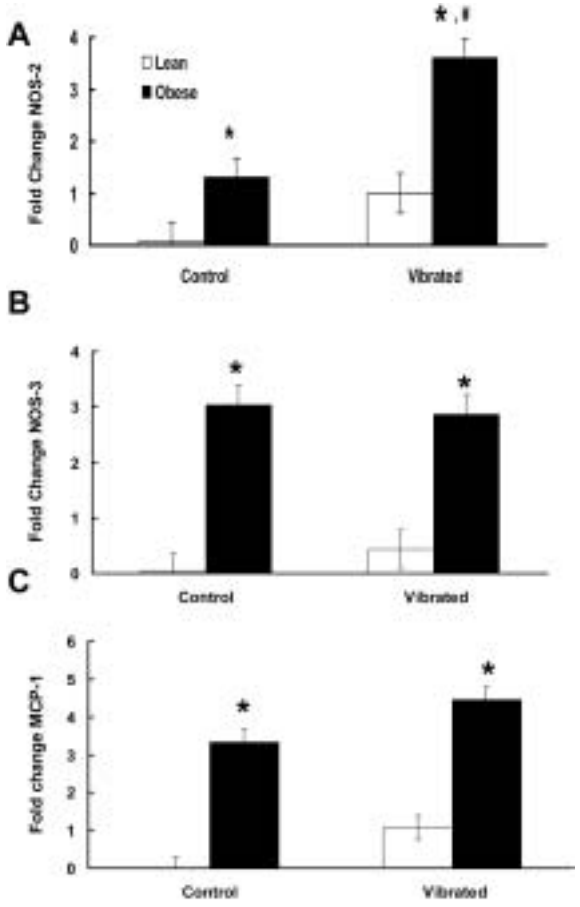

Fig. 3. Changes in vascular transcript levels for NOS-2 (A), NOS-3 (B) and MCP-1 (C).

Transcript levels for NOS-2, NOS-3 and MCP-1 were higher in ventral arteries from obese rats than in lean rats (*main effect of phenotype, $p<0.05$). Vibration resulted in an additional increase in NOS-2 transcript levels in arteries from obese rats (#different from obese controls, $p<0.05$). Data are presented as the mean fold change (± sem) in transcript levels compared to lean cage controls (n=8 rats/group).

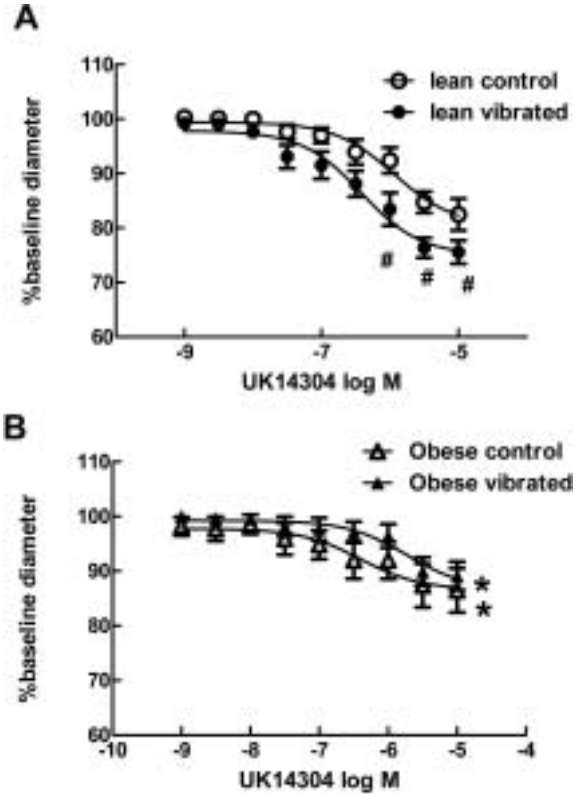

Fig. 4. Dose-dependent vasoconstriction in response to the α2C-adrenoreceptor agonist, UK14304 in lean (A) and obese (B) Zucker rats.

Obese rats were less sensitive to α2C-adrenoreceptor-mediated vasoconstriction than lean rats (*main effect of phenotype, different than lean rats, $p<0.05$). However, in lean rats, vibration resulted in an increased sensitivity to α2C-adrenoreceptor-induced constriction, especially at higher doses of the agonist (#different than lean controls, $p<0.05$). The data are presented as the reduction in diameter from baseline (mean % change ± sem, n=8/group) after treatment with varying doses of UK14304. Lines were fitted to the dose-response data using a non-linear regression model.

exposed to vibration were generally more sensitive to UK14304-induced vasoconstriction than obese rats ($p<0.04$). In addition, the dose of UK14304 inducing a 15% constriction from baseline was lower in lean vibrated than lean control rat ($p<0.05$; mean log dose ± sem; vibrated rats $10^{-6.65 \pm 0.19}$ M, control rats $10^{-5.63 \pm 0.18}$ M). In contrast, arteries from obese rats were generally less sensitive to UK14304-induced vasoconstriction than arteries from lean rats ($F(3, 208)=3.29$, $p<0.04$), but vibration did not have any additional effects of vascular responsiveness to this agent.

Analyses of the dose-dependent vasodilation in response to ACh also resulted in a significant 3-way interaction ($F(24, 208)=2.59$, $p<0.003$); Fig. 5). Arteries from obese rats were generally less sensitive to ACh-induced vasodilation than arteries from lean rats ($p<0.05$). In addition, arteries from vibrated rats were generally less responsive to ACh-induced vasodilation than arteries from control rats ($p<0.05$), with EC50 values being significantly lower in lean control than lean vibrated rats ($10^{-7.06 \pm 0.26}$ M vs. $10^{-5.92 \pm 0.28}$ M respectively, $p<0.05$) and in obese control than obese vibrated rats ($10^{-6.16 \pm 0.24}$ M vs. $10^{-5.28 \pm 0.26}$ M; respectively, $p<0.05$). Neither vibration nor phenotype significantly affected vasodilation in response to the NO-donor, SNAP (Fig. 6).

Discussion

Metabolic syndrome serves as a major risk factor in the development of cardio- and peripheral vascular disorders[3]. Because people with metabolic syndrome are pre-

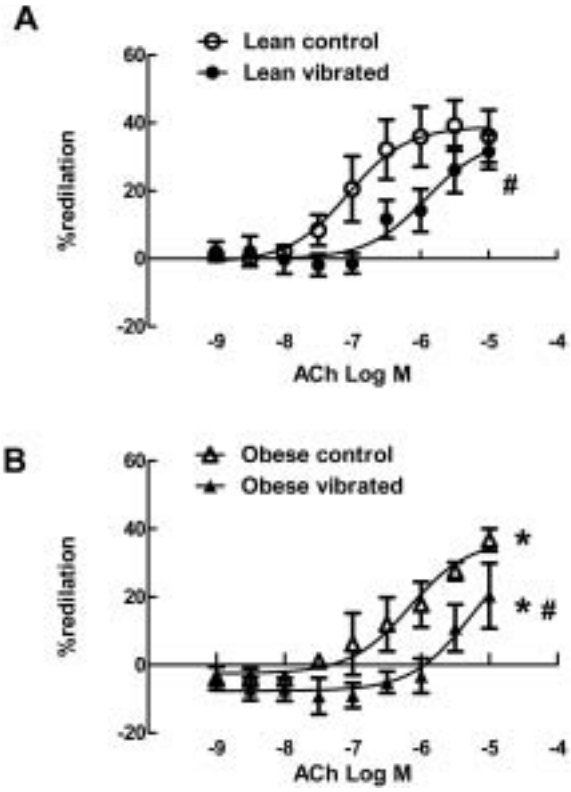

Fig. 5. Dose-dependent vasodilation to ACh in lean (A) and obese (B) Zucker rats.

Obese rats were generally less sensitive to ACh-induced vasodilation than lean rats (*main effect of phenotype, $p<0.05$). Exposure to vibration also resulted in a reduced sensitivity to ACh-induced vasodilation in lean and obese rats (#main effect of treatment, $p<0.05$). However, the effect of vibration was more pronounced in obese rats. Arteries were constricted to 50–60% of the baseline diameter with phenylephrine and redilation in response to ACH was measured. The data presented are the increase diameter (mean % change ± sem, n=8/group) after treatment with varying doses of ACh. Lines were fitted to the dose-response data using a non-linear regression model.

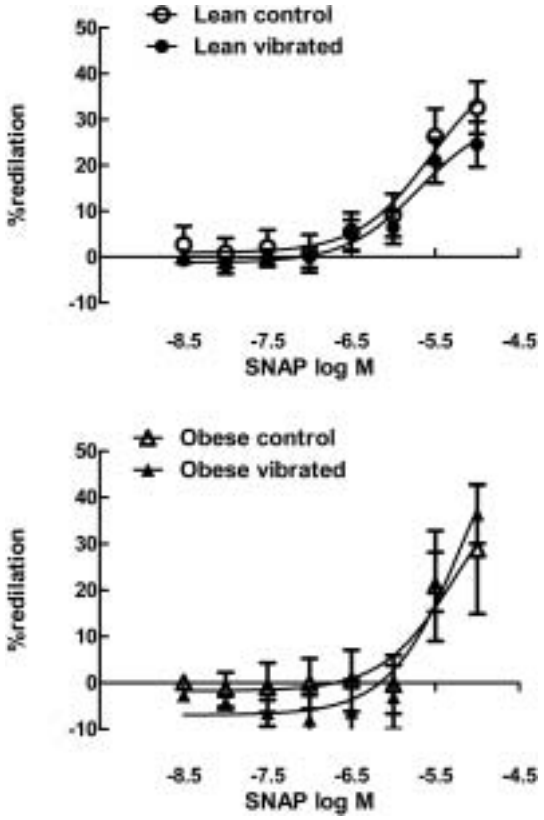

Fig. 6. Dose-dependent vasodilation to the NO donor, SNAP in lean (A) and obese Zucker rats.

Neither phenotype nor treatment had a significant effect on SNAP-induced vasodilation. Arteries were constricted to 50–60% of the baseline diameter with phenylephrine and redilation in response to SNAP was measured. The data presented are the increase diameter (mean % change ± sem, n=8/group) after treatment with varying doses of SNAP. Lines were fitted to the dose-response data using a non-linear regression model.

disposed to vascular dysfunction, they may also be at greater risk of developing VWF if exposed to hand-transmitted vibration at work. In this study, we used an animal model of metabolic syndrome, obese Zucker rats, to determine if vascular responses to vibration were different in obese rats than in their lean siblings. The major findings of this study were that after 10 d of exposure to vibration, oxidative stress was increased and there was a reduction in vascular sensitivity to ACh-induced vasodilation in ventral tail arteries from both lean and obese rats. However, these vibration-induced changes were more pronounced in obese rats. These findings are consistent with the hypothesis that workers with metabolic syndrome or diabetes are a greater risk of developing VWF.

This study did not directly assess the effects of vibration on blood flow *in vivo*. However, vibration-induced changes in peripheral vascular flow were indirectly assessed by measuring cutaneous tail temperatures before and after vibration exposure. Reductions in tail-temperature are indicative of decreases in tail blood flow and have been associated with constriction of the ventral tail artery in Sprague Dawley rats exposed to a single bout of vibration[14, 19]. Neither control nor vibration exposures affected tail temperatures in lean rats. However, in obese rats, vibration generally resulted in a decrease in cutaneous tail temperatures. Although control conditions did not affect temperatures on days 1 and 9 of the study, obese control rats did display a reduction in tail temperature on day 5 after the exposure. Restraint stress alone can induce a reduction in blood flow to the tail and a reduction in tail temperature[19, 20], and thus it is possible that the exposure-induced reduction in tail temperatures seen in control rats was due to the stress of being restrained.

However, this only occurred on day 5 of the study. In contrast, tail temperatures in obese rats were consistently lower after exposure to vibration. Thus, even if restraint alone did result in a decrease in temperature in obese rats, vibration exacerbated that effect and consistently induced a significant reduction in tail temperature. Acute exposure to vibration also results in a transient vasoconstriction and reductions in finger blood flow in humans[21]. Bouts of vasoconstriction induced by single exposures to vibration have been associated with vacuole formation and disruption of the endothelial cell layer in animals[22]. However, it is unclear if these acute bouts of vasoconstriction and morphological changes in peripheral arteries contribute to the vascular dysfunction associated with VWF.

Vibration-induced vasoconstriction may be the result of an increase in the release of, or sensitivity to vasoconstricting factors. Because previous work in our laboratory suggested that vibration resulted in an increased sensitivity of vascular smooth muscle to α2C-adrenoreceptor-mediated vasoconstriction in Sprague Dawley rats[14], we examined the effects of vibration on this system in Zucker rats. Consistent with our previous findings, vibration did induce an increase in the responsiveness of the tail artery to the α2C-adrenoreceptor agonist, UK14304 in lean rats. However, obese rats were generally less responsive to α2C-mediated vasoconstriction than lean rats, and vibration did not affect α2C-adrenoreceptor-mediated constriction in obese rats. This was unexpected because other studies examining the responsiveness of resistance arteries in skeletal muscle have demonstrated that obese rats are generally more sensitive to adrenoreceptor-mediated vasoconstriction than lean rats[13]. However, these studies were primarily looking at α-1 adrenoreceptor mediated constriction[11, 13]. Other studies have demonstrated that mesenteric arteries of obese rats are less sensitive to adrenoreceptor-mediated vasoconstriction, and that this decrease in sensitivity may occur to compensate for the reductions in NO-mediated vasodilation, particularly in younger rats (i.e., rats less than 18 wk of age[23]). In addition, we have recently demonstrated that vibration reduces vascular responsiveness to α1- adrenoreceptor- and 5-hydroxytryptamine-induced vasoconstriction in resistance arteries from rat paws, and that this reduced responsiveness appears to be the result of endothelial-generated reactive oxygen species interfering with the ability of vascular smooth muscle to respond to vasoconstricting factors[16]. These data suggest that although an increased sensitivity to adrenoreceptor-mediated vasoconstriction may contribute to vibration-induced vascular dysfunction, other factors may play a prominent role in mediating the vascular response to vibration.

Exposure to vibration could also induce a repeated or prolonged vasoconstriction by interfering with the activity of factors that induce vasodilation. In this study, arteries from vibrated rats were generally less sensitive to ACh-mediated vasodilation than arteries in control rats. However, this reduced sensitivity was particularly apparent in obese rats. ACh induces dilation primarily by stimulating NO release from endothelial cells. Reductions in ACh-mediated vasodilation can be the result of a reduction in NO bioavailability or due to a reduced sensitivity of the vascular smooth muscle to NO-induced relaxation. Vascular responsiveness to the NO donor, SNAP, was not affected by vibration, indicating that reduced responsiveness to ACh-induced vasodilation was not due to changes in the response of vascular smooth muscle to NO. Thus, vibration-induced changes in NO release or availability most likely were responsible for the reduced responsiveness to ACh, and these changes most likely play a role in mediating vibration-induced changes in vascular function.

We assessed bioavailable NO by measuring NO_x concentrations in ventral tail arteries. NO_x concentrations were lower in obese rats than in lean rats. Vibration also resulted in a marginal reduction in vascular NO_x concentrations in lean rats, but did not affect concentrations in obese rats. These results are consistent with studies demonstrating that reduced responsiveness to endothelial-mediated vasodilation is in part the result of a reduced availability of NO in obese rats[24]. The findings in lean rats are also consistent with studies demonstrating that exposure to a single bout of vibration reduces vascular NO concentrations in both tail[25] and paw arteries[16]. The failure to see a vibration-induced reduction in NO in obese rats may have been due to the fact that NO levels were already suppressed in obese rats, and vibration may not have been able to induce an additional reduction. Humans with metabolic syndrome also display a reduced responsiveness to endothelial-mediated vasodilation that is believed to be caused by a reduction in NO bioavailability[12]. Additional evidence suggests that the reduction in NO bioavailability in obese Zucker rats and people with metabolic syndrome is the result of an increase in vascular oxidative stress[11].

Reductions in NO concentrations were also associated with an increase in vascular concentrations of the reactive oxygen species (ROS), H_2O_2, in vibrated and obese rats. Obese rats had generally higher concentrations of ROS than lean rats, and the vibration-induced increase in H_2O_2 was greater in obese than lean rats. ROS can interfere with NO bioavailability by interfering with NO synthesis[26] or by scavenging available NO[13, 24]. Analyses of NOS-2 and 3 transcript levels demonstrated that expression of these factors was higher in obese than lean rats, and that vibration exposure resulted in an addition-

al increase in NOS-2 expression only in obese animals. Thus, oxidative stress does not appear to have affected NO synthesis by reducing NOS transcript levels. However, studies have demonstrated that ROS can interfere with NOS activity by inducing oxidation of tetrahydrobiopterin (BH_4), which can cause an uncoupling of the NOS enzyme so that ROS other than NO are preferentially generated[27, 28]. Thus, the increase in ROS seen in obese rats may have suppressed NOS activity resulting in a decrease in vascular NO concentrations. In addition, studies in Zucker rats have demonstrated that treating obese animals with antioxidants improves endothelial-mediated vasodilation[13], suggesting that scavenging of NO by free radicals also is in part responsible for a reduction in NO bioavailability and the reduced sensitivity of obese rats to ACh-mediated vasodilation.

Vibration can affect ACh-mediated vasodilation by altering oxidative activity in the artery. However, vibration-induced stress and strain on peripheral vessels could also have a direct effect on vascular function by altering NO concentrations. For example, previous studies have demonstrated that flow-induced shear stress on endothelial cells stimulates NOS-3 levels and NOS-3-mediated NO production[29–31]. In this study, the shear stress induced by vibration resulted in an increase in NOS-2 and NOS-3 expression in arteries of obese, but not vibrated rats. These results suggest that the effects of vibration on the peripheral vascular system are in part due to an organism's physiological response to vibration, and are not solely a result of the direct effects of vibration on the tissue. However, data collected in rats[17, 32, 33] and in humans[21, 34, 35] have demonstrated that both the shear and bending stresses of soft tissues, and the responses of the vascular system to vibration, are frequency and amplitude dependent. These data indicate that vibration also has some direct effects on vascular function. Thus, mechanisms underlying vibration-induced changes in vascular function are complex and are most likely dependent upon the physiological and physical response of the exposed tissues to vibration.

In conclusion, the results of this study support other studies that have demonstrated that vibration can increase α2C-adrenoreceptor-mediated vasoconstriction in some instances[14, 36]. However, vibration also consistently induces an increase in oxidative stress and reduction in endothelial-mediated vasodilation[14, 16, 37], and these effects were more pronounced in obese than in lean rats. Because obese rats display many of the symptoms seen in humans with metabolic syndrome[11], we believe that these results suggest that pre-existing vascular dysfunction caused by metabolic syndrome may make workers more susceptible to vibration-induced vascular injury, and put them at greater risk of developing VWF.

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