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Systemic effects of segmental vibration in an animal model of hand-arm vibration syndrome

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Abstract

Objective—Epidemiology suggests that occupational exposure to hand-transmitted (segmental) vibration has local and systemic effects. This study used an animal model of segmental vibration to characterize the systemic effects of vibration.

Methods—Male Sprague Dawley rats were exposed to tail vibration for 10 days. Genes indicative of inflammation, oxidative stress, and cell cycle, along were measured in the heart, kidney, prostate and liver.

Results—Vibration increased oxidative stress and pro-inflammatory gene expression, and decreased anti-oxidant enzymes in heart tissue. In the prostate and liver, vibration resulted in changes in the expression of pro-inflammatory factors and genes involved in cell cycle regulation.

Conclusions—These changes are consistent with epidemiological studies suggesting that segmental vibration has systemic effects. These effects may be mediated by changes in autonomic nervous system function, and/or inflammation and oxidative stress.

Introduction

Occupational exposure to hand-transmitted (or segmental) vibration has been associated with changes in the peripheral vascular and sensorineural systems. Symptoms include cold-induced vasospasms, finger blanching, reductions in tactile sensitivity and weakness in the fingers and hands (1–3). Collectively, these symptoms have been referred to as hand-arm vibration syndrome (HAVS). Although most symptoms of HAVS are attributed to the direct mechanical effects of vibration on the appendage being exposed, there are reports of indirect effects of vibration. For example, workers exposed to hand-transmitted vibration can show cold-induced blanching of the feet independent of finger blanching (4–7), hearing loss, that is hypothesized to be the result of vasoconstriction in the cochlea (8, 9), and changes in resting heart rate (9). It is believed that these symptoms are due to sensory information regarding the vibration stimulus feeding back onto the autonomic nervous system and altering homeostasis (9–12).

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Transmission of the vibration signal to the autonomic nervous system, in part occurs through sensory receptors located in the skin of the exposed region. Although a number of sensory receptors are activated by vibration, the primary receptor that appears to transmit information to the autonomic nervous system is the Pacinian corpuscle (13). Pacinian corpuscles are located in the mesentery, skin and joints of the body, and transmit information about vibration and pressure to the central nervous system (13–16). In addition, anatomical and physiological studies have shown that neurons from the sympathetic nervous system synapse on Pacinian corpuscles, making them more sensitive to incoming sensory signals from the periphery. In turn, the Pacinian corpuscles may also affect the activity of sympathetic neurons (14, 15, 17). Therefore, there is a feedback loop between the peripheral sensory and autonomic nervous system. In addition, the rat tail has guard hairs that are innervated by sensory receptors and are affected by vibration. Studies in other animals have shown that there is sympathetic innervation of guard hairs, and alterations in sympathetic activity can change the responsiveness of these receptors to stimulation (18, 19). Because the sympathetic nervous system plays a role in regulating homeostatic processes, including cardiovascular output, it is likely that sensory signals, acting through the Pacinian corpuscles, not only have an effect on sensation, but these signals may also affect homeostasis of the organism. The goal of this study was to use an animal model of segmental vibration-induced dysfunction to identify and characterize the systemic effects of this exposure.

The rat-tail has been used by a number of laboratories to model the effects of vibration exposure on peripheral vascular and sensorineural systems (18, 20–22). This model has been used because the physical responses to vibration are similar to those seen in the human finger. For example, the resonant frequencies of the human finger and rat tail are approximately in the same range (i.e., 100-300 Hz depending on the location of the measurement (23)). In addition, vibration induced changes in vascular and sensorineural function seen in human fingers are similar to those seen in the rat tail (21, 24–32). Published results using the rat tail model also suggest that there may be indirect effects of vibration exposure on other physiological symptoms, similar to the results seen in humans (28, 30, 33). Based on previous findings in humans showing the indirect effects of vibration exposure on vascular function in areas of the body that are not directly exposed to signal, we hypothesized that exposing the tail of rats to vibration would result in changes in markers of cardiovascular dysfunction. To test this hypothesis, this experiment used the rat-tail model described above (25, 34). Sensorineural function in rats was tested over the exposure period to determine if the effects of vibration on responses to pressure were similar to those seen in our previous study (26). Changes in cardiovascular biology were then measured by examining biomarker expression in the cardiac and renal systems. Because we previously demonstrated that there were changes in sensory function that occurred after 10 days of vibration exposure, we used these measures to demonstrate that the effects of the exposure were similar to those previously seen, and to correlate known changes that occur in response to direct exposure to segmental vibration with responses that occur distal from the exposure. We also looked at the effects of vibration on cell cycle in a number of abdominal organs because previous work suggested that exposure to vibration may result in changes in gene transcripts involved in regulating the cell cycle and cancer (35–38).

Methods

Animals

Male Sprague Dawley rats ([H1a: (SD) CVF, n = 8 rats/group, 6 weeks of age at arrival) were obtained from Hilltop Lab Animals, Inc., Scottdale, PA. All animals were free of viral pathogens, parasites, *mycoplasms*, *Heliobacter* and cilia-associated respiratory (CAR) bacillus. The rats were acclimated for 1 week after arrival and housed in cages ventilated with HEPA (high efficiency particulate air)-filtered under controlled temperature and humidity conditions and a 12-h light/12-h dark cycle. Food (Teklad 7913) and tap water were provided *ad libitum*. The animal facilities are specific pathogen-free, environmental controlled, and accredited by AAALAC International. 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.

Exposures

Prior to exposure, rats were acclimated to restraint in Broome style restrainers. Animals were placed in a restrainer, and were acclimated by gradually increasing restraint duration over 5 days until the rats could be held in the restrainers for 4 h. On day one of the experiment, rats were assigned to the restraint control (control) or vibration exposed (vibrated) group. The tails of rats in the vibrated group were exposed to sinusoidal vibration (250 Hz, 49 m/s^2 rms) for 4 h/day for 10 consecutive days. This frequency and amplitude was chosen because vibration with these characteristics generate resonance in the rat tail (23) Previous studies have shown that exposure at this frequency has the greatest effect on peripheral vascular and sensorineural function (26, 39), and this is the frequency at which the Pacinian corpuscles are most sensitive (13, 40). A description of the shaker and vibration control system was previously published (23). Briefly, prior to the exposure, rats were placed into Broome style restrainers, and their tails were placed through an enlarged opening in the lid of the restrainer. The enlarged opening in the lid was to reduce pressure on the ventral surface of the tail. Each rat's tail was secured to a platform that was attached to a shaker using four, 1 cm wide, elastic straps. The straps were placed over each animal's tail to prevent them from moving it off of the platform during the exposure, but very little pressure was applied to prevent force-induced damage to the tail. Control rats were treated in an identical manner except that their tails were secured to a platform attached to isolation blocks. Rats were exposed to vibration for 4 hrs between the times of 0600 and 1800 h for 10 consecutive days. Although previous data from our laboratory suggested that there were no effects of time of day on responses to vibration (when animals were exposed during the light phase of the cycle), control and vibrated rats were exposed in blocks so that differences between blocks of animals could be included in the statistical analysis. After each exposure, rats were returned to their home cages in the colony room.

Randall-Sellito pressure test

On days 1, 5 and 9 of the study, sensitivity to pressure applied to the tail was assessed using an automated Randall-Sellito analgesia meter (IITC Life Sciences, Woodland Hills, CA). This test uses a two millimeter probe and pressure is gradually applied to a specific area of

the tail. Once there is a response (e.g. tail flick or withdrawal) the force applied is recorded. If the applied pressure reaches 200 g, the test is stopped and 200 g is considered to be the pressure eliciting a response. However, in this study, most animals responded before 200 g of pressure was applied. The test was repeated two times during each test session with a 1 minute inter-test interval and the average of those tests was used as a measure of sensitivity to pressure. To assess pre-exposure sensitivity, measurements were taken on day 1 of the experiment, prior to the exposure. The region tested was marked so that repeated tests could be performed on the same area of the tail. To determine if there were acute changes in sensitivity to applied pressure, measurements were made following the first exposure and compared to pre-exposure measures. To assess long-term changes in tactile sensitivity in response to vibration, tests were also performed before the exposure on days 5 and 9 of the experiment.

Biological measures

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR)

The day following the last exposure (day 11 of the study) rats were anesthetized using an i.p. injection of pentobarbital (Fatal Plus; 100 mg/kg body weight), and exsanguinated via cardiac puncture. Heart, kidney, prostate, liver, and a section of skin from the C14-16 region were dissected, put into cryotubes, frozen on dry ice and stored at -80 °C until assayed. One portion of each tissue was used to examine vibration-induced changes in gene expression (see Table 1 for a list of transcripts and tissue examined). The transcripts chosen are associated with the development of cardiovascular disease and included pro-inflammatory, oxidative stress and cell cycle markers. RNA was isolated from tissues, and first-strand cDNA was synthesized from 1 μg total RNA using Reverse Transcription System (Invitrogen, Carlsbad, CA) as previously described (41). Control RNA from each tissue was run at 10X dilutions to determine the efficiency of the assay. Ribosomal 18s was used to determine if there were differences in RNA concentrations in samples. Fold changes in transcripts of interest were calculated by subtracting individual critical threshold (Ct) values for a specific transcript from the mean of the control Ct for that transcript. If samples did not show a single defined melt peak in the 80 °C range were not included in the dataset.

Reactive oxygen species (ROS)

To measure ROS, tissues were homogenized in 0.01 M phosphate buffer (pH 7.4) with protease inhibitors (cOmplete cocktail tablets, Sigma-Aldrich, St Louis MO), using a Biospec bead beater (Biospec, Barrisville, OK). Lysates were removed, centrifuged at 12,000 × g and then stored at -80°C until used for ROS or protein assays. Total protein concentrations in each sample were measured using a Nanodrop spectrophotometer (Thermofisher, Waltham, MA). Nitrous oxide concentrations and activity concentrations were measured in supernatants using the nitrate/nitrite assay (N_{ox}) from Caymen Chemicals (Ann Arbor, MI), following the manufacturer's instructions. General oxidative activity was also measured using the fluorescent compound, 2′7′-dichlorofluorescien diacetate (DCFH-DA; Sigma-Aldrich, St. Louis, MO). To measure general levels of reactive oxygen species (ROS), 10 μl of each sample was incubated at RT for 45 min with 50 μl of 1 mM DCFH-DA. To measure colormetric changes (N_{ox} ; 425-450 nM excitation range) and fluorescence

changes (ROS; 480-530 nM excitation rant), a Synergy H1 All in One microplate reader was used along with the Gen 5 Software package (Biotek; Winooski, VT). N_{ox} and ROS concentrations per μg protein were calculated and used for analyses. Although the fluorescence of DCFH-DA is used as an indicator of hydrogen peroxide activity, it is also affected by nitric oxide and peroxynitrite concentrations (42). Therefore, measurements made using this method provide information regarding overall oxidative activity, and are not indicative of the activity of a specific free radical unless some treatment known to induce a specific free radical is applied.

Analyses

Body weight data were analyzed using a repeated measures ANOVA. All other data were analyzed using one-way ANOVAs and *Jmp* 13.0.0 Software (SAS Institute; Cary, NC). Post-hoc tests were performed using Student's t-tests. Differences with $p \leq 0.05$ were considered significantly different.

Results

Body weight

Both groups of rats gained weight at a similar rate over the course of the study (Figure 1).

Effects of vibration on sensorineural function

Randall-Sellito test—Prior to beginning the experiment, sensitivity to applied pressure or force was similar in control and vibration-exposed rats. When pre-exposure measures of sensitivity were compared to post-exposure measures from the first day of the experiment, there was an increase in sensitivity (i.e., a reduction in the amount of force needed to elicit a response, in both control and vibration exposed rats (Figure 2A). To examine the effects of repeated exposures on sensitivity to pressure, pre-exposure values and those collected on days 5 and 9 were compared. On day 5, there was an increase in sensitivity to applied pressure in control rats (Figure 2B). In contrast, in vibrated rats, the amount of pressure needed to elicit a response was increased on 5 of the study (i.e., the rats were less sensitive to the stimulus Figure 2B; * different that day 1 values $p < 0.05$; ^ different than controls $p < 0.05$). There were no significant changes in sensitivity to pressure on day 9.

Transcription changes in skin—Interleukin 1-β (*Il1-β*) and tumor necrosis factor-α (*tnf*-α) was significantly increased in skin from vibrated animals, and there was a statistically insignificant increase in interleukin-6 (*Il-6;* Fig 2C, # $p < 0.06$). Other gene transcripts that were measured in the skin can are listed in Table 2.

Effects of vibration on markers of cardiovascular function

ROS measurements—Exposure to vibration resulted in an increase in both N_{ox} and ROS in heart tissue (Figures 3A and B). N_{ox} concentrations were not detectable in the kidney, and ROS concentrations were not affected by vibration exposure (Figure 3C).

Transcript levels in the heart and kidney—Transcripts measured by PCR in the heart are presented in Figure 4. In the heart, exposure to vibration increased neuronal nitric oxide

synthase (*nNOS*), *tnf-α* and runt-related transcription factor-1 (*runx-1*) (Fig 4B *p < 0.05 and Table 1). In the kidney, exposure to vibration resulted in a decrease in superoxide dismutase 2 (*sod2*) that was not statistically significant (p < 0.06). However, vibration did induce a significant increase in *Il1β* in kidney tissue (Table 1).

Vibration-induced changes in gene transcription in other organs

Transcript levels—Transcripts measured in prostate are shown in Figures 5A and B and Table 2. In the prostate, changes in the transcription of genes that have been identified as being part of breast/ovarian/prostate cancer-related BRCA1 pathway are presented in Figure 5A. *Brca1* expression was undetectable in the prostate of rats in both groups. However, *bard1* expression was increased, and *b-raf* expression decreased in the prostates of rats exposed to vibration (# p ≤ 0.06). Expression of additional genes altered by exposure to vibration are presented in Figure 5B. Expression of the cell cycle regulatory genes, *chk1*, *sfn* and *pmel* were increased in the prostate of rats exposed to vibration. Pro-inflammatory, oxidative stress, and cell-cycle associated genes, *Il-1β, Il-6*, endothelial-nitric oxide synthase (*eNOS*) and stratafin (*sfn*), were increased in the prostate of rats (Figure 5B; * p ≤ 0.05, # p ≤ 0.06).

In the liver (Table 1), exposure to vibration resulted in an increase in the pro-inflammatory factors, *Il1β* and *tnf-α* (*p ≤ 0.05). There was also a reduction in the circadian/cell cycle gene *per1* and the immediate early gene *rasd1* (# p ≤ 0.06).

Discussion

Numerous studies have demonstrated the effects of hand-transmitted vibration on peripheral vascular and sensorineural systems. In addition, there have been a number of studies that have suggested that there may be indirect effects that are due to vibration-induced changes in autonomic function. In this experiment we examined the direct effects of vibration (i.e., on sensorineural function) to demonstrate that the exposure used produced effects consistent with those reported in previous studies (26, 39). In addition, we also examined the indirect effects of segmental vibration that may be associated with changes in autonomic or inflammatory mechanisms. To determine if there were indirect effects of vibration on other systems in the body, we examined the effects of the vibration exposure on the transcription of biomarkers that have been associated with inflammation, oxidative stress or changes in cell cycle regulation. The results of this study suggests that repeated exposure to segmental vibration induces changes in protein and/or gene expression in the heart and kidney that are consistent with the development of cardiovascular disease. There were also changes in the expression of pro-inflammatory, anti-oxidant and cell cycle associated genes in the skin, liver and prostate.

Effects of vibration on sensorineural function

Previous studies have demonstrated that vibration can induce both acute and long-term changes in sensorineural function; the current experiment used the sensitivity to applied pressure to determine if the effects of this specific exposure (250 Hz 49 m/s^2) were similar to those of previous studies (18, 21, 26, 39, 43, 44). In this study, there was an increased

sensitivity to pressure application after an acute exposure to control and vibrated conditions (pre vs post day 1; Figure 2A). This is consistent with findings of previous work in our laboratory (43). When changes in responsiveness to applied pressure were analyzed across the study (pre-exposure day 1 vs days 5 and 9), vibrated rats displayed a reduced sensitivity to applied pressure (i.e., more was applied to induce a response) on day 5 of the study. In contrast, control rats showed an increased sensitivity to applied pressure, or possibly a learning effect (i.e., they remembered the application of the stimulus, and when experiencing it again, responded more quickly). By day 9 responses to applied pressure were similar to those seen on day 1. Although the pattern of the response is similar to that reported in previous studies (i.e., a reduction in tactile sensitivity followed by an increase in tactile sensitivity (20, 26, 45), there was no significant difference between control and vibrated rats on day 9. These differences may be due to the fact that a different method for assessing responsiveness to applied pressure was utilized in this experiment (i.e., filaments vs a forceps and meter). A previous study from that found that after 9 days of exposure to vibration at 250 Hz rats were more sensitive to pressure applied using a von Frey filament with a tensile strength of 60 g than control rats or rats exposed to vibration at 62.5 or 125 Hz (26). Application with the Randall-Sellito apparatus would have distributed the applied pressure over a larger area, possibly stimulating additional receptors and producing a different result that that seen with the filaments (width if the von Frey filament = 0.71 mm, width of the Randall-Sellitto = 1.97 mm). There is also pressure on the top of the tail when the Randall-Sellitto test is used. Both these factors may have contributed to the change in the response between studies. Finally, the differences between the results of this study and previous studies could also be due to normal variation in responses between different subjects; the subjects in this experiment may have responded at a slightly different rate than subjects in previous studies.

Changes in gene expression in the skin that may underlie changes in sensory function were also examined in this study. Expression of the pro-inflammatory cytokines, *Il1β*, *Il6* and *tnf-α* were increased. Previous studies have shown that there are a number of resident pro-inflammatory cells located in this skin. It is believed that these pro-inflammatory cells are located in the skin because the skin is the first line of defense against invading organisms, and if the skin barrier is broken, the resident immune cells will act to prevent the development and/or spread of infection. Because vibration can cause local inflammation and edema, and the migration of additional pro-inflammatory cells to the skin in case there is a break in the barrier, or the cells that are there may increase transcription of pro-inflammatory cytokines (46, 47).

Effects of vibration on markers of cardiovascular function

Exposure to vibration for 10 consecutive days was associated with an increase in measures of oxidative stress in the heart. These changes included increases in general measures of oxidative stress and concentrations of N_{ox}, along with increases in *nNOS*, *runx* and *tnf-α* transcription. Numerous studies have demonstrated that increases in oxidative stress and pro-inflammatory factors, along with reductions in the production of anti-oxidants, are associated with the development of heart disease. *nNOS* expression is found in both cardiovascular smooth muscle and in nerves innervating peripheral and cardiovascular

tissues (39, 45). Thus, changes in *nNOS* expression in the heart are consistent with other studies examining the effects of vibration on cardiovascular function. Although we did not measure electrocardiograms (EKG) in the current study, changes in both NOS, NO and ROS may lead to cardiovascular disease (48). It is possible that a longer term exposure, or the presentation to a cardiovascular challenge, may induce a change in the EKG in vibration exposed rats similar to the changes people with hand-arm vibration syndrome who have altered electrocardiograms (EKG's (9)).

Vibration also induced an increase in the transcription of pro-inflammatory factor, *tnf-α*, and the transcription factor *runx* in the heart. Increases in the expression of a number of pro-inflammatory factors, including *tnf-α*, have been associated with cardiovascular disease. Therefore, these data are consistent with the oxidative stress data, and suggest that exposure to segmental vibration may indirectly induce physiological and biological changes that lead to heart disease. There was also a vibration-induced increase in *runx* expression. Increases in *runx* in vascular tissue stimulates the growth of new blood vessels, which can help maintain vascular flow (39, 48). For example, in workers repeatedly exposed to vibration, there can be additional vascularization within the fingers, which helps maintain normal blood flow. However, overtime this increase in vascularization becomes aberrant in the periphery, tortuous vessels develop, and there is a reduction in blood flow in the fingers (49). There is evidence that tortuous type blood vessels also develop in the heart (50, 51), it is possible that vibration-induced increases in oxidative stress stimulate neovascularization in the heart in the short term to help reduce increases in blood pressure that may be due to vasoconstriction in the periphery. However, in the long-term, this neovascularization may be detrimental, reducing blood flow as it does in patients with hand-arm vibration syndrome. Because there is evidence that with other diseases, such as diabetes, there is aberrant vascularization in the heart, and that these anomalies in vascularization can have detrimental effects on cardiovascular function (50).

Although there were no measureable changes in oxidative stress in the kidney, there was a reduction in SOD_2 and *Il1β* was increased in kidneys from vibrated animals. The kidneys affect cardiovascular function by regulating electrolyte concentrations and blood volume. Changes in blood volume affect blood pressure, and changes in electrolyte levels in the blood can have significant effects on cardiac function. Although we did not measure blood pressure, electrolyte levels or markers of fluid regulation in the kidneys, exposure to vibration results in peripheral vasoconstriction (25, 39), and other studies suggest there is also central vasoconstriction, which in turn results in changes in blood pressure (9, 11, 52). Peripheral information about blood pressure and blood volume is transferred back to both the sympathetic and central nervous systems to bring heart rate and blood pressure back to normal and maintain homeostasis within the system. However, over time the sympathetic nervous system becomes more sensitive to these incoming signals, enhancing the autonomic nervous systems response to the incoming vibration signal, inducing a response that occurs more quickly and is maintained longer. These maintained physiological responses (e.g., vasoconstriction, increases in heart rate and blood pressure) induce the cellular and molecular responses that in the short-term are adaptive and help the system regulate it's response to vibration exposure, but in the long term are maladaptive and lead to vibration-white finger, muscle weakness, reductions in manual dexterity and pain (2, 53).

Vibration-induced changes in gene transcription in other organs

Previous studies demonstrated that after 10 days of exposure to tail vibration, there were changes in the transcription of cell cycle markers and genes that are part of the BRCA1 pathway (38). Although changes in cell cycle markers could contribute to the development of skin cancer, there is no evidence that exposure to vibration is associated with skin cancer. However, epidemiological studies have suggested that vibration exposure may serve as a risk factor for the development of prostate cancer (35–37, 54), and possibly cancer in other organs in the lower abdomen (38). Because transcription of genes that are part of the BRCA1 pathway may be affected by vibration (38), and changes in the transcription of genes in this pathway are associated with the development of prostate cancer (54), we examined the effects of vibration on gene transcription in the prostate. *Brca-1* gene transcription was undetectable in the prostate and other tissues examined. Although *brca1* is expressed in a number of tissues in primates (55), recent studies suggest that in rodents, it is expressed at detectable levels in developing mammary tissue and ovarian tissue, and in mammary tumors (56, 57). Exposure to vibration resulted in an increased expression of *bard*, and a reduction in the expression of *b-raf1*. Bard1 protein can dimerize with Brca1and have tumor suppressor function. However, in tissues where *bard1* is present, but *brca1* is not, *bard1* can have oncogenic effects (58). In other species it is located in numerous tissues throughout the body (55, 59). Because *bard1* is increased in the prostate in the absence of *brca1*, it is possible that the increase in the expression of this gene may increase the risk of developing cancer. On the other hand, *b-raf1* was reduced. Although this gene has been implicated in the *brca1* pathway, it has also been implicated in cellular pathways that play a role in the development of melanoma (60, 61). The expression of *b-raf1* is regulated by extracellular-receptor kinase activity. Factors that inhibit *b-raf1* transcription are associated with anti-tumorigenic activity (60). Interestingly, *pmel* was increased by vibration in the prostate. This gene has been shown to play a role in the development of melanomas, which can occur anywhere in the body (60, 62, 63). Therefore, there are two cellular pathways activated by vibration that may contribute to an increased risk of developing cancer. In addition, there were increases in *chk1*gene expression, which is involved in regulating cell cycle. An increase in this gene is consistent with the changes in the *brca1*- and *pmel*-related pathways, and suggests that vibration alters cell cycle regulation in the prostate.

One of the ways vibration may alter cell cycle is by affecting blood flow to tissue. In this study, vibration exposure induced an increase in *eNOS* and the pro-inflammatory factors, *Il1β* and *Il6*. Previous work suggests that acute increases in pro-inflammatory factors may act to stimulate *eNOS*, which in turn would result in vasodilation and an increase in blood flow to the affected tissue (45, 64, 65). However, it is also possible that the increases in oxidative factors along with inflammation may induce angiogenesis to support an increased rate of cell division (54, 55, 57, 66). Although the precise pathways by which vibration may be contributing to these changes needs additional examination, it is clear that vibration activates a number of pathways that may increase the risk of developing prostate cancer (Figure 6).

There were also some changes in the expression of pro-inflammatory factors and cell cycle/ circadian related genes in the liver. Previous studies have shown that the circadian clock

genes (e.g. *per1*), which help regulate rhythmic function of organs, and cell cycle, are altered by a number of different factors, including diet and food intake (67–69). Because vibration has been shown to alter circulating glucose and insulin levels (45), it is possible that the changes in gene expression seen in this study were associated with the effects of vibration exposure on metabolism.

The results of these studies indicate that exposure to segmental vibration does have effects on organs and physiological systems that are not associated with direct exposure to the vibration stimulus. These effects may occur through changes in activity of the autonomic nervous system, or through an increase in oxidative stress and systemic inflammation. Reducing the transmission of vibration to the body through the use anti-vibration gloves (70, 71) or through job rotation, where workers reduce their exposure by alternating jobs where they are exposed to vibration with jobs where there is no exposure, may help reduce the direct, and indirect consequences of vibration on worker health (1, 27).

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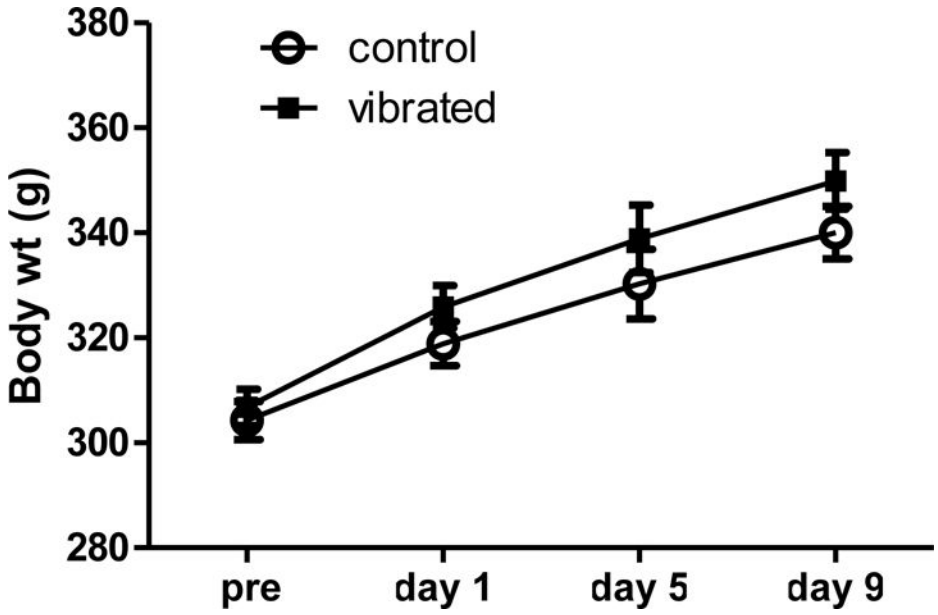

Figure 1.
Animals in both the control and vibrated conditions showed similar increases in weight gain (i.e., growth) during the experiment.

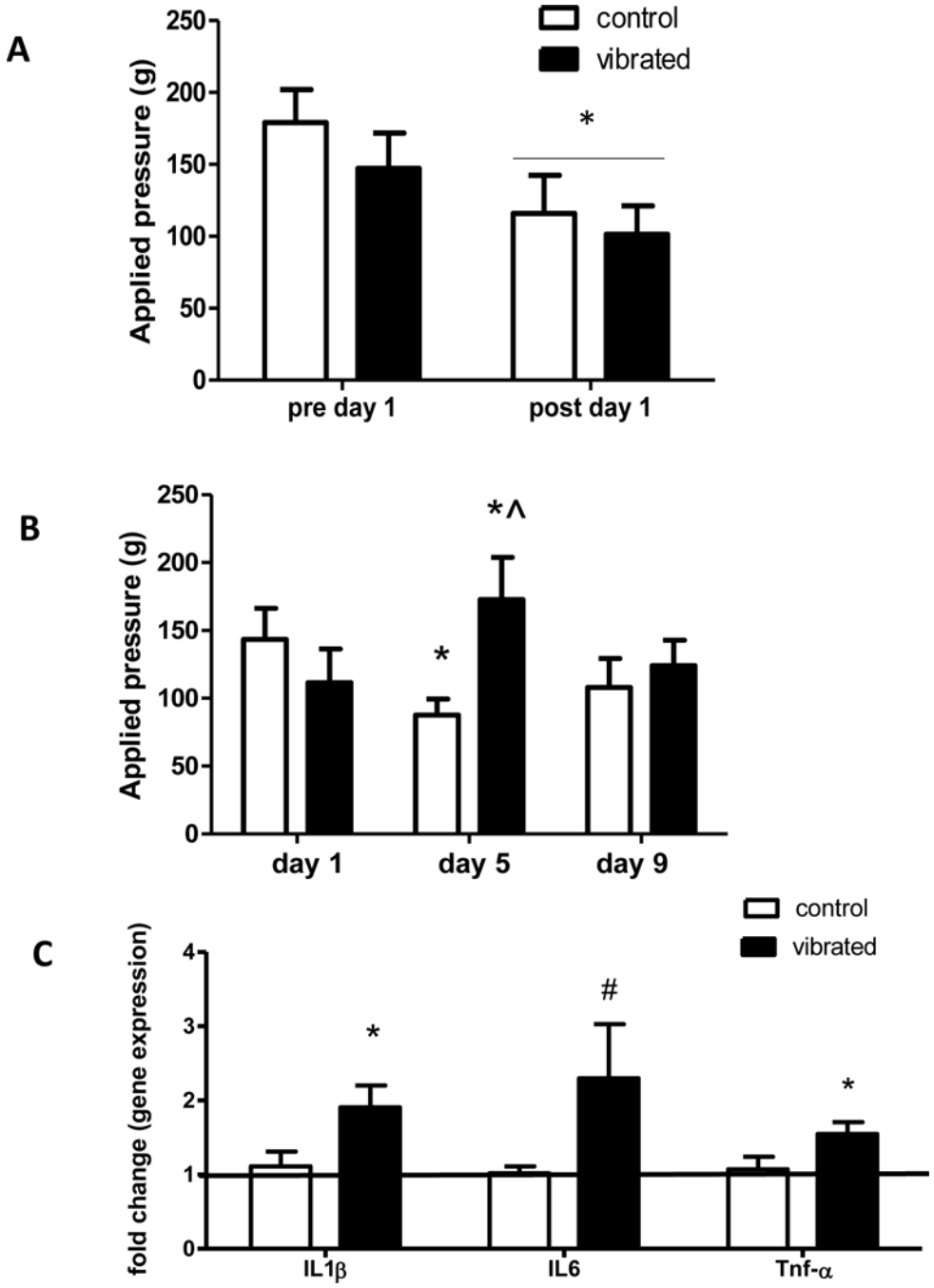

Figure 2.

Responses to the Randal-Sellito applied pressure test prior to the beginning of the experiment and following the exposure on day 1. On day 1 of the study, the pressure needed to induce a tail-flick or withdrawal was significantly lower (i.e., the animals were more sensitive) after the exposure than before the exposure in both groups of rats (* main effect of exposure, $p < 0.05$). Figure 2B show the pressure needed to generate a response on days 1, 5 and 9 of the study (pre-exposure measure). In control animals, the pressure needed to induce a response was lower on day 5 than on day 1, whereas in vibrated rats the pressure need to

induce a response on day 5 was greater than on day 1 (* different that day 1, $p < 0.05$). In addition, on day 5, the pressure needed to induce a response was greater in vibrated that control rats, suggesting that they were less sensitive to the stimulus (^ $p < 0.05$). By day 9, sensitivity to applied pressure was similar to that seen on day 1. Vibration-induced changes in gene transcription in the skin are presented in Figure 2C. Exposure to vibration resulted in increases in *Il1β*, *tnf-α* (*$p < 0.05$) and *Il6* (# $p < 0.06$). All data are expressed as the group mean ± SEM.

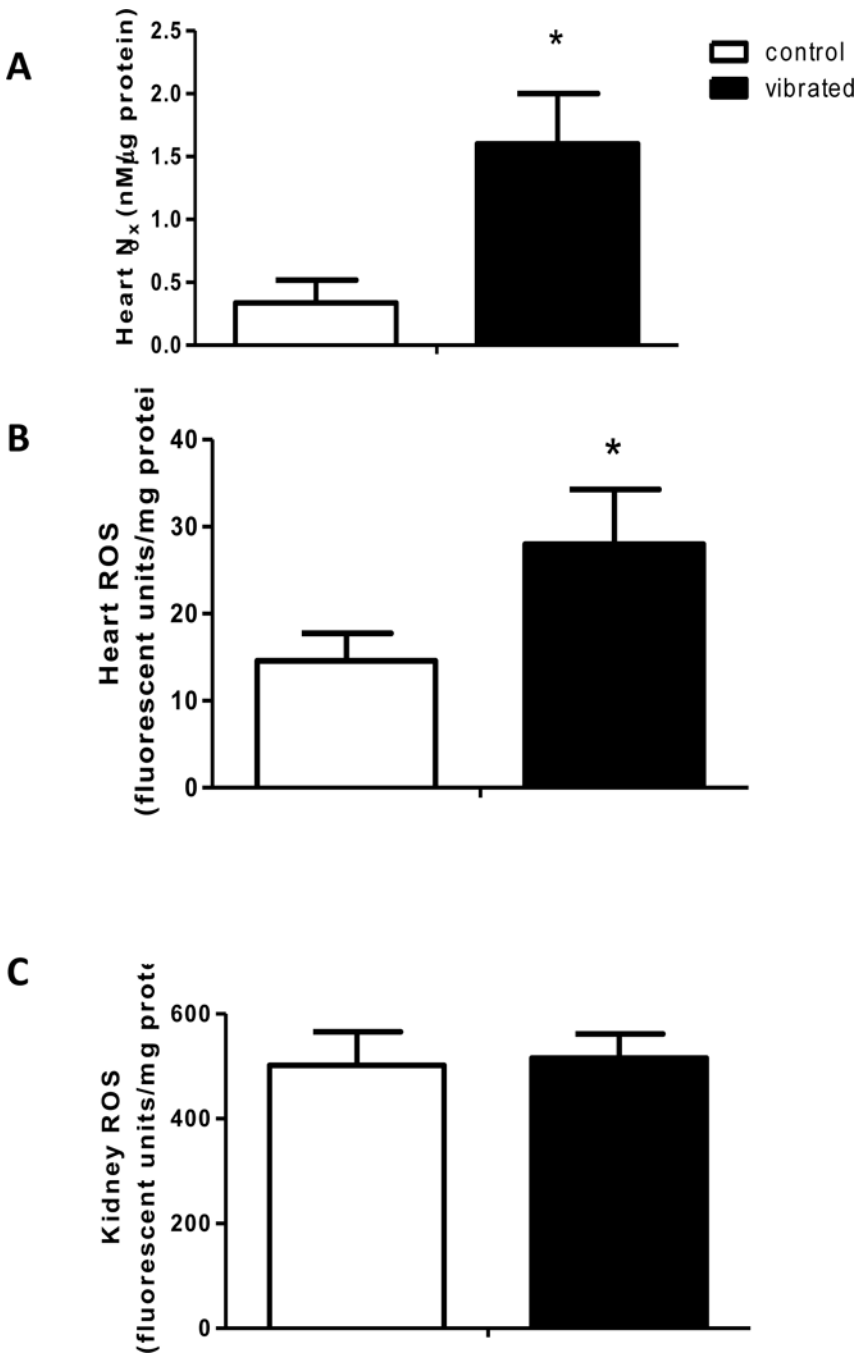

Figure 3.

Measures of oxidative stress in the heart (A and B) and kidney (C). Both N_{ox} and l ROS levels were increased in the heart of vibrated rats (A and B; *$p < 0.05$). N_{ox} concentrations were not measureable in the in the kidney, and there was no change in overall ROS levels (C).

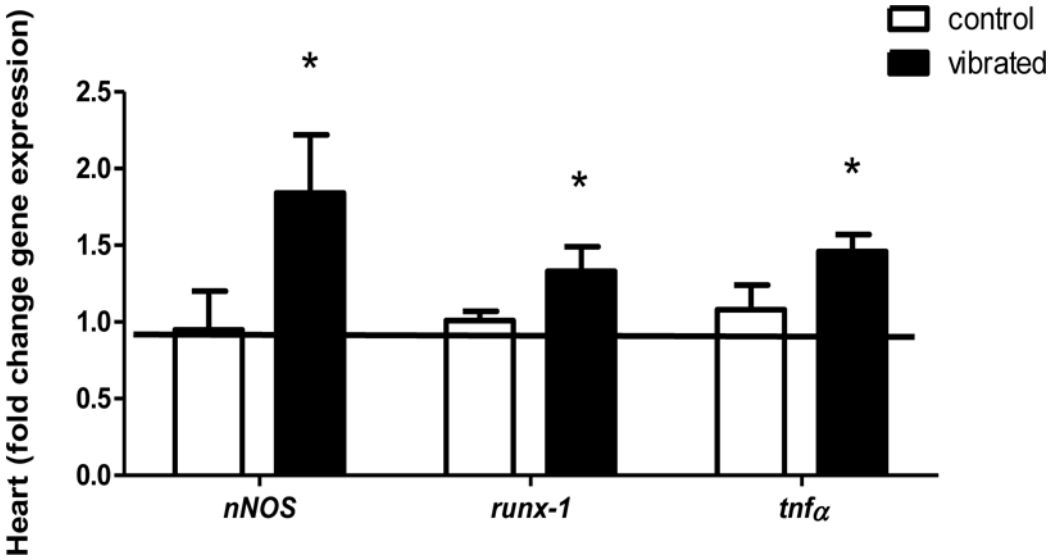

Figure 4.

Vibration exposure resulted in significant increases in the expression of *nNOS, runx-1 and tnf-α* in heart tissue (* $p < 0.05$).

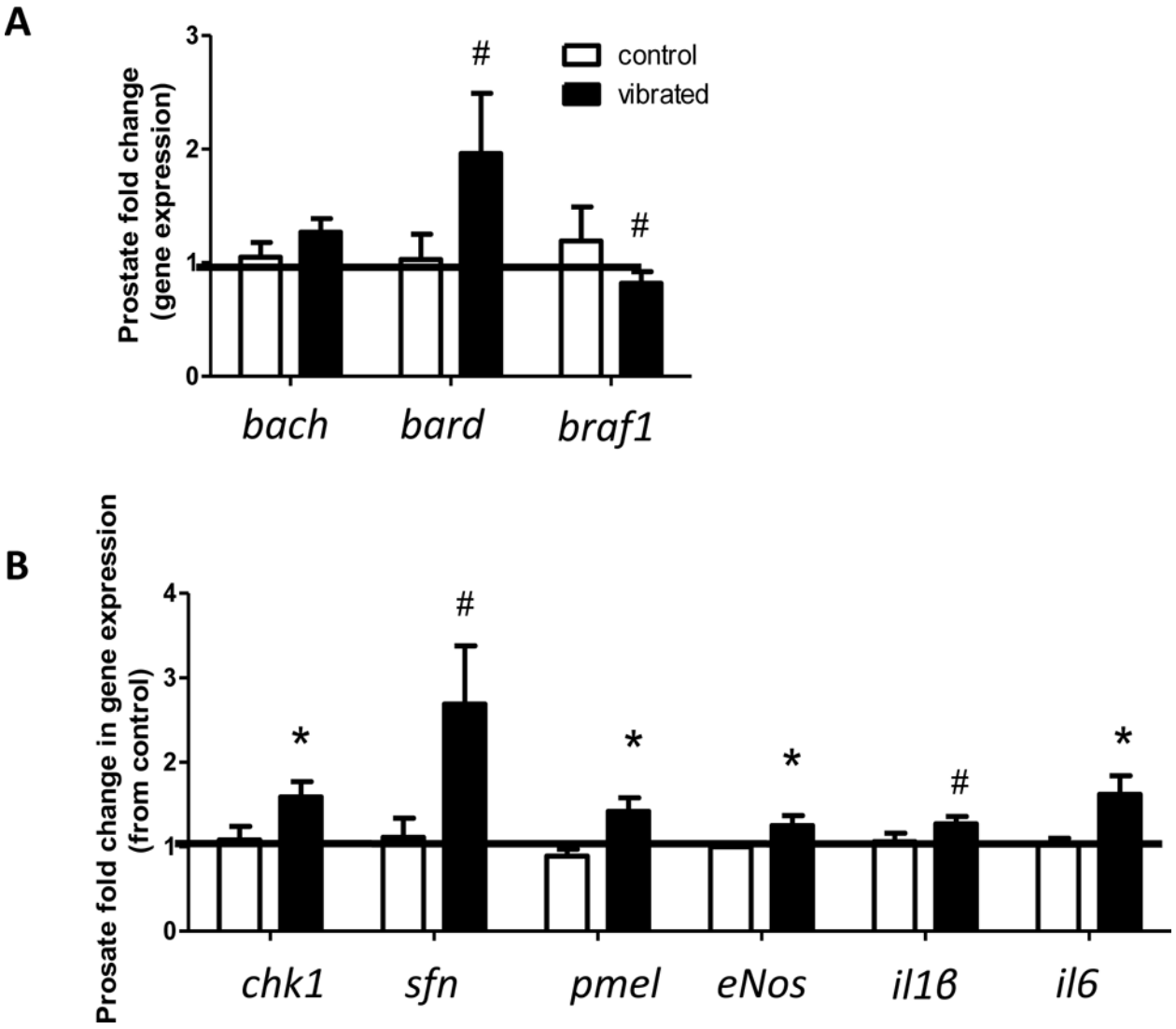

Figure 5.

Fig 5A shows mean (± SEM) fold changes in the prostate of transcripts that have been identified as being part of the BRCA1 pathway. There were insignificant changes in *bard* and *braf*. Fig 5B shows fold changes in other transcripts that are involved in regulating cell cycle or inflammation. For both figures, *$p < 0.05$ and # $p < 0.06$).

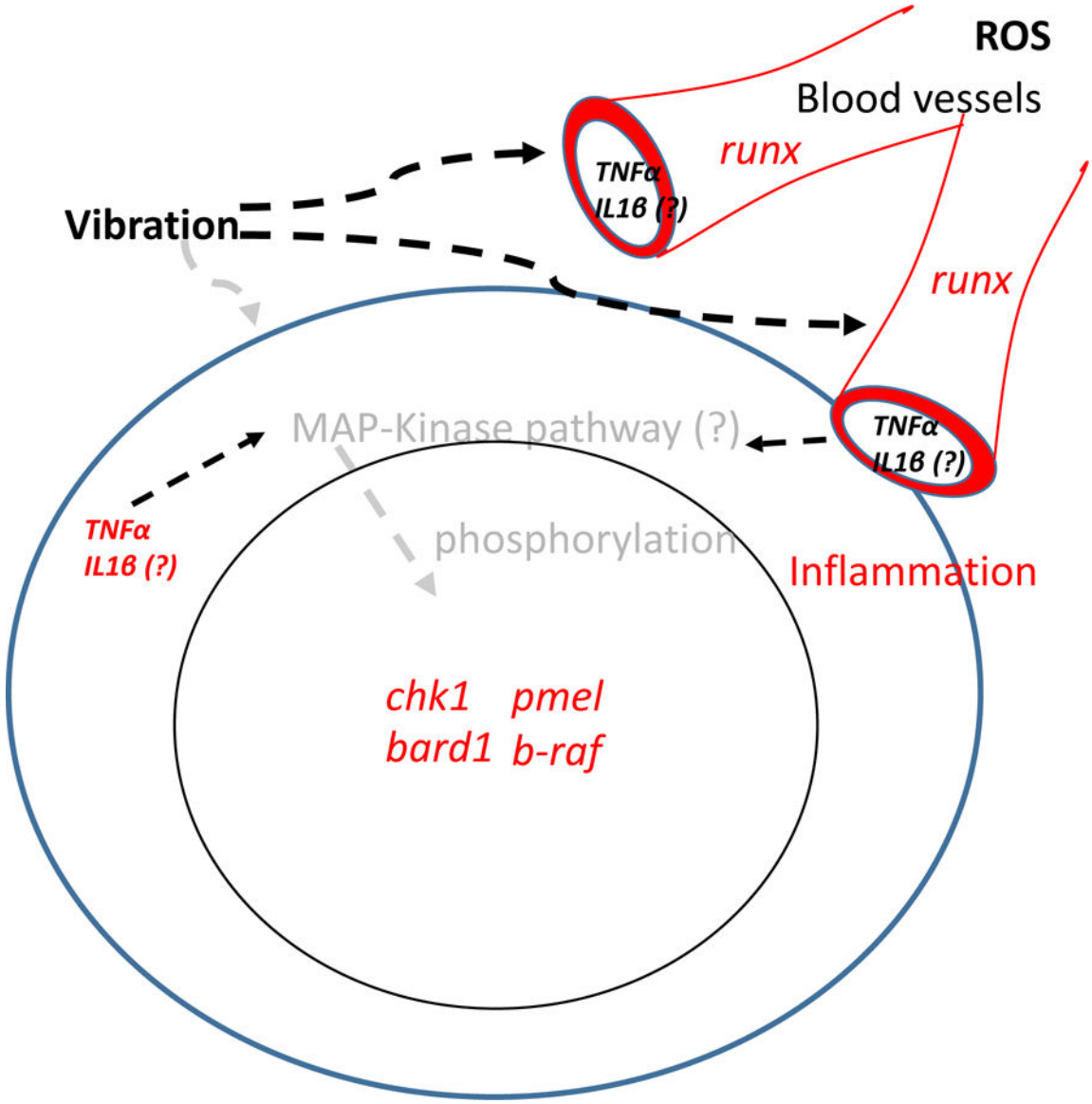

Figure 6.

A hypothetical pathway that may underlie vibration-induced changes in gene transcription in the prostate. The blue outer circle represents a cell in the prostate and the black, inner circle is the nucleus of the cell. Vibration-induced changes in reactive oxygen species (ROS, either locally or systemically), may result in an increase the transcription of pro-inflammatory factors in the circulation, or within the cells of the prostate itself. Vibration-induced increases in ROS may also increase the transcription of *runx*, which in turn can stimulate vascular remodeling. Increases in pro-inflammatory factors may activate the mitogen-activated protein-kinase (MAPK) cell signaling pathway, and through phosphorylation of other cell signaling enzymes, affect the transcription of factors that regulate cell cycle or carcinogenesis such as *chk1, pmel, bard1 and b-raf*. Transcripts in red were increased by vibration exposure in the prostate. Black dashed arrows are hypothetical pathways by which vibration or changes in cellular processes may affect transcription within the prostate.

Kinases (in grey) or grey dashed arrows are hypothetic pathways that may be activated and affect gene transcription, based upon the results of previous studies (60, 61).

Table 1

This table lists the gene transcripts that were examined (and the accession numbers), and the fold changes (from control) in specific tissues.

Gene name (accession number)	Heart (fold change mean ± sem)		kidney (fold change mean ± sem)	
	control	vibrated	control	vibrated
interleukin-1β (IL1β) NM_031512	1.08 ± 0.16	1.20 ± 0.2	**1.02 ± 0.09**	**1.47 ± 0.19***
interleukin 6 (IL6) NM_012589.1	1.25 ± 0.28	1.37 ± 0.5	1.06 ± 0.16	1.79 ± 0.44
glutathione peroxidase (gpx) AJ002278	1.33 ± 0.39	0.74 ± 0.1	1.09 ± 0.18	1.06 ± 0.28
neuronal nitric oxide synthase (nNOS) AC_000080.1	**0.95 ± 0.25**	**1.84 ± 0.38***	1.07 ± 0.16	1.31 ± 0.21
inducible nitric oxide synthase (iNos) NM_012611	1.09 ± 0.17	1.17 ± 0.12	1.15 ± 0.24	0.87 ±0.22
endothelial nitric oxide synthase (eNOS) NM_021838	0.97 ± 0.04	0.92 ± 0.12	1.02 ± 0.08	0.92 ±0.10
RAS dexamethasone-induced 1 (Rasd1) NM_001270954.1	N.D.	N.D	1.10 ± 0.18	1.12 ± 0.14
runt-related transcription factor-1 (runx) NM_017325.1	**1.01 ± 0.06**	**1.33 ± 0.16***	N.D	N.D
supraoxide drismutase-1 (SOD-1) NM_017050.1	1.01 ± 0.07	1.04 ± 0.04	1.05 ± 0.11	0.84 ± 0.10
supraoxide dismutase-2 (SOD-2) NM_017051	1.01 ± 0.07	1.03 ± 0.1	1.03 ± 0.1	0.79 ± 0.1
tissue inhibitor of metallopepetidase-1 (timp) NM_053819.1	1.15 ± 0.22	1.06 ± 0.42	N.D	N.D
tumor necrosis factor-α (TNFα) AJ002278	**1.08 ± 0.16**	**1.46 ± 0.11***	1.05 ± 0.13	1.22 ± 0.11
vascular endothelial growth factor (VEGF) AY702972	N.D	N.D	1.07 ± 0.07	0.99 ± 0.07

Genes that showed changes in transcription in response to vibration are highlighted (bold * $p < 0.05$, only highlighted $p < 0.06$). N.D. indicates not determined.

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Table 2

This table lists the gene transcripts that were examined (and the accession numbers), and the fold changes (from control) in specific tissues.

Gene and accession number	**Prostate (mean ± sem)**		**Liver (mean ± sem)**		**Skin (mean ± sem)**	
	control	**vibrated**	**control**	**vibrated**		
Adiponectin (Adipoq)	1.44 ± 0.64	0.89 ± 0.38				
Serine/threonine-protein kinase b-raf XM_231692.9			1.03 ± 0.11	0.93 ± 0.13	N.D	N.D
c-fos NM_022197.2	N.D	N.D	N.D	N.D	1.05 ± 0.35	0.76 ± 0.15
c-jun NM_021835.3	N.D	N.D	N.D	N.D	1.04 ± 0.11	0.87 ± 0.10
Check point 1 kinase (chk1) AF414135.1	**1.08 ± 0.16**	**1.59 ± 0.18***	N.D	N.D	N.D	N.D
interleukin-1β (IL1β) NM_031512	1.06 ± 0.10	1.27 ± 0.09	**1.04 ± 0.10**	**1.28 ± 0.10***	**1.11 ± 0.20**	**1.91 ± 0.29***
interleukin 6 (IL6) NM_031512	**1.02 ± 0.08**	**1.62 ± 0.22***	1.14 ± 0.22	1.54 ± 0.32	1.02 ± 0.09	2.30 ± 0.73
neuronal nitric oxide synthase (nNOS) AC_000080.1	1.05 ± 0.14	1.09 ± 0.19	1.08 ± 0.16	1.23 ± 0.10	1.04 ± 0.12	1.26 ± 0.18
endothelial nitric oxide synthase (eNOS) NM_021838	**1.00 ± 0.02**	**1.25 ± 0.12***	1.00 ± 0.02	1.08 ± 0.05	1.02 ± 0.09	1.19 ± 0.10
Mitogen-activated kinase-8 (Mapk8) XM_006240779.3	1.04 ± 0.13	0.95 ± 0.12	N.D	N.D	N.D N.D	N.D
Period-1 (Per1) NM_001270954.1	1.14 ± 0.22	1.08 ± 0.80	1.26 ± 0.34	0.66 ± 0.15	N.D	N.D
Pre-melanosome protein (Pmel) XM_006240779.3	**0.89 ± 0.08**	**1.42 ± 0.16***	1.04 ± 0.11	1.47 ± 0.29	1.09 ± 0.20	0.99 ± 0.20
RAS dexamethasone-induced 1 (Rasd1) NM_001270954.1	0.79 ± 0.17	0.99 ± 0.28	1.06 ± 0.17	0.73 ± 0.11	1.05 ± 0.14	1.38 ± 0.33
Signal transducer and activator of transcription-3 (Stat) NM_012747.2	N.D	N.D	N.D	N.D	1.04 ± 0.12	0.97 ± 0.08
stratafin (Sfn) XM_003750048.3	1.11 ± 0.23	2.69 ± 0.69	N.D	N.D	N.D	N.D
tumor necrosis factor-α (TNFα) AJ002278	1.04 ± 0.12	1.25 ± 0.12	**1.02 ± 0.09**	**1.48 ± 0.15***	**1.07 ± 0.17**	**1.55 ± 0.16***
vascular endothelial growth factor	0.98 ± 0.09	0.96 ± 0.18	N.D	N.D	N.D	N.D

Gene and accession number	Prostate (mean ± sem)		Liver (mean ± sem)		Skin (mean ± sem)	
	control	vibrated	control	vibrated		
(VEGF) AY702972						

Genes that showed changes in transcription in response to vibration are highlighted (bold * $p < 0.05$, only highlighted $p < 0.06$). N.D. indicates not determined.