Article:
Harrison, R., Ward, K., Lee, E. orcid.org/0000-0003-4529-7410 et al. (3 more authors) (2015) Acute bone response to whole body vibration in healthy pre-pubertal boys. Journal of Musculoskeletal and Neuronal Interactions (JMNI), 15 (2). pp. 112-122. ISSN 1108-7161

J Musculoskelet Neuronal Interact 2015; 15(2):112-122

Original Article

Hylonome

Acute bone response to whole body vibration in healthy pre-pubertal boys

R. Harrison[1], K. Ward[2], E. Lee[3], H. Razaghi[4], C. Horne[4], N.J. Bishop[1]

[1]Academic Unit of Child Health, Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH, UK; [2]Nutrition and Bone Health, Medical Research Council Human Nutrition Research Unit, Elsie Widdowson Laboratory, 120 Fulbourn Road, Cambridge CB1 9NL UK; [3]School of Health and Related Research (ScHARR), University of Sheffield, Regent Court, 30 Regent Street, Sheffield, S1 4DA, UK; [4]Materials and Engineering Research Institute, Sheffield Hallam University, City Campus, Howard Street, Sheffield S1 1WB, UK

Abstract

The skeleton responds to mechanical stimulation. We wished to ascertain the magnitude and speed of the growing skeleton's response to a standardised form of mechanical stimulation, vibration. 36 prepubertal boys stood for 10 minutes in total on one of two vibrating platforms (high (>2 g) or low (<1 g) magnitude vibration) on either 1, 3 or 5 successive days (n=12 for each duration); 15 control subjects stood on an inactive platform. Blood samples were taken at intervals before and after vibration to measure bone formation (P1NP, osteocalcin) and resorption (CTx) markers as well as osteoprotegerin and sclerostin. There were no significant differences between platform and control groups in bone turnover markers immediately after vibration on days 1, 3 and 5. Combining platform groups, at day 8 P1NP increased by 25.1% (CI 12.3 to 38.0; paired t-test p=0.005) and bone resorption increased by 10.9% (CI 3.6 to 18.2; paired t-test p=0.009) compared to baseline. Osteocalcin, osteoprotogerin and sclerostin did not change significantly. The growing skeleton can respond quickly to vibration of either high or low magnitude. Further work is needed to determine the utility of such "stimulation-testing" in clinical practice.

Keywords: Child, Bone, Vibration, Bone Turnover Markers, Stimulation Test

Introduction

Children in the UK suffer 100,000 fractures annually accounting for 10% of attendances at accident and emergency departments[1]. The incidence of childhood fractures has increased in the last 30 years by approximately 40%[2] and 20-30% of children who fracture are likely to fracture again[3-5]. Evidence suggests that children with narrower, i.e. more slender bones and a low bone mass are more likely to fracture than children with larger, wider bones[6,7]. Studies have shown that mechanical loading in childhood can increase bone size and mass[8-10], and that these gains can persist into adult life[11-13], potentially improving skeletal health across all ages.

Whole body vibration (WBV) delivered via vibrating platforms has been investigated as a technique to load bone, with the desired outcome of increasing bone size and strength. The vibratory stimulus provided differs between platforms. One type is designed to mimic low level postural strains that form a dominant component of the skeleton's 24 hour strain history[14], low magnitude, high frequency synchronous (vertical) vibration. The second type is designed to fatigue muscle and stimulate increases in loading to bone via the muscle by applying high magnitude, high frequency vibration that can be delivered in a synchronous or side-alternating motion. The majority of animal investigations have employed low magnitude high frequency WBV, and demonstrated increased bone density, size, formation, and strength[14-22]. Clinical studies over 6-12 month periods of children with disabling conditions such as cerebral palsy, young women with low bone mass and women with post-menopausal osteoporosis have also shown increased bone mass at the tibia, femur, and spine using synchronous, low magnitude, high frequency WBV[23-25] and at the femoral neck and hip using asynchronous high magnitude, high frequency WBV[26,27]. However findings across the studies are not conclusive, either in terms of the size of the effect or

The authors have no conflict of interest.

Corresponding author: Nick Bishop, Academic Unit of Child Health, Sheffield Children's Hospital, Western Bank, Sheffield, S10 2TH, UK
E-mail: n.j.bishop@sheffield.ac.uk

Edited by: J. Rittweger
Accepted 15 April 2015

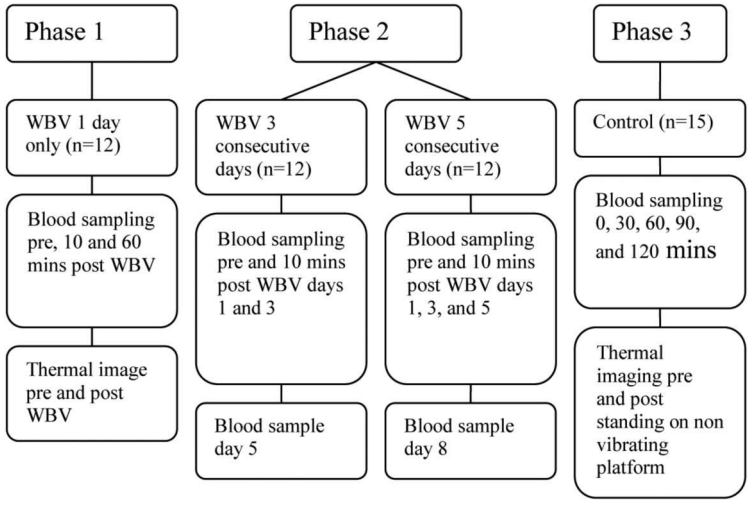


Figure 1. Study design showing number of days of whole body vibration, blood sampling regime, and thermal imaging by phase of the study.

its site specificity, and a number of studies have failed to show any skeletal benefits at all[28-31].

Adult studies suggest that WBV has little or no effect on bone outcomes in apparently healthy subjects when given 2-7 times a week for up to 12 months[29,30,32,33]. We know from exercise studies that the response to loading is greater in children than in adults however, suggesting that children may also have a greater capacity for a bone response to vibration. There are no published data on the acute effect of vibration on the apparently healthy growing skeleton. It is not known if the bone response to vibration in such children would be similar to that seen for children with reduced bone mass.

An alternative use for vibration would be as part of a stimulatory test to assess the skeleton's responsiveness to mechanical loading, similar to cardiac stress testing using a treadmill. There could be advantages to such a form of testing if acute changes in bone formation or resportion were predictive of longer term response to either pharmacological or mechanical interventions. The response to mechanical loading before, during and after intervention could be assessed in a variety of disease states both within individuals and between groups of individuals. Such a test could also be used in assessing outcomes for phase II studies of bone-targeted compounds to reassure investigators that their intervention had not abrogated responses to mechanical loading.

We wanted to determine the extent and timing of any changes in bone turnover markers following very short periods of WBV and whether this was influenced by the type of vibration stimulus, either high magnitude or low magnitude, used. This is a pragmatic approach, reflecting the fact that these two types of machine, providing either high magnitude, side-alternating (signal intensity >2.0 g) or low magnitude, vertical (signal intensity <1.0 g) vibration are widely available, but nothing is known as to their short term effects on bone metabolism. We undertook thermal imaging of calf muscles as a proxy for muscle activation[34] to determine if this was similar between the high and low magnitude vibration groups. The overall aim of this study therefore was to assess the acute response of bone to low and high magnitude WBV in the growing skeleton.

Materials and methods

The study was approved by the South Humber Local Research Ethics Committee and was carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from the parents, and written assent from the study participants.

Study participants

Healthy white Caucasian pre-pubertal boys (Tanner stage 1) aged 9-12 years were recruited to the study by invitation letters which were handed out in local schools and through staff at local university hospitals. Boys with a history of bone disease or other chronic illness, those currently taking medication known to affect bone and those who had previously fractured were excluded from the study. In total 1138 letters were sent out; the parents of 166 boys expressed an interest in the study (14%), and 64 (38%) signed a consent form.

Study design

The study was conducted in 3 phases as illustrated in Figure 1. Each boy participated in one phase only. In phase 1 we assessed the immediate response of bone biomarkers to vibration; in phase 2, we assessed the biomarker response to repeated episodes of vibration; and in phase 3 the variation in biomarkers immediately after standing on a non-vibrating platform. Gradually increasing overall periods of vibration were used in phase 2 because of uncertainty as to what duration of exposure (i.e. mechanical stimulation dose) would be

needed in order to generate a response in biomarkers.

In phase 1 (immediate response), boys were randomly allocated to platform type, either Juvent (low, <1.0 g magnitude) or Galileo (high, >2.0 g magnitude). Blood samples were collected immediately before and 10 and 60 minutes after vibration.

In phase 2 (response after repeated exposure), boys were randomly allocated to platform type, and duration of intervention. Half the boys received three cycles of vibration, the remainder five cycles.

In those receiving three cycles, blood samples were taken immediately before and 10 minutes after vibration on days 1 and 3, with a further sample being taken 2 days later.

In those receiving five cycles, blood samples were taken immediately before and 10 minutes after vibration on days 1, 3 and 5 with a further sample being taken 3 days later.

The variation in the later sample collection timings reflected both the need to fit with boys' school and family commitments (sample collection was timed to allow normal school attendance and avoid weekend attendance) and the uncertainty as to whether a biomarker response would occur either immediately or in a more delayed manner.

In phase 3, boys stood on a non-vibrating platform and samples were taken at 30 minute intervals over a 2 hour period, similar to the timing of samples in those exposed to vibration, in order for us to allow for any underlying time-related changes.

A thermal image was recorded pre and post vibration of the legs below the knee in the phase 1 boys exposed to a single 10 minute period of vibration and at 30 minute intervals (the same time points as for blood sampling) in the control group.

Recruitment to each phase of the study followed completion of the previous phase. Boys were randomly assigned to vibration platform (phase 1 and 2) and number of consecutive days of intervention (phase 2 only) by the successive opening of opaque envelopes containing the randomisation code; the randomisation to duration and platform was balanced. Neither the participants nor the researcher could be blinded to the intervention groups due to the difference in shape and size of platforms and the mode of vibration. Study visits occurred in the mornings; for the boys in phase 1 and the control group they took place in the Clinical Research Facility at Sheffield Children's Hospital. Phase 2 visits took place in the child's home or school for participant convenience. All visits were carried out by the same researcher (RH).

Loading regime

Participants stood either on the Galileo Advanced platform (Novotec Medical GmbH, Pforzheim, Germany) which delivers a high frequency high magnitude side-alternating signal, or the Juvent MDT 1000 platform (Marodyne, Lakeland, Florida, USA) which delivers a high frequency low magnitude synchronous (vertical) signal. The Galileo platform was set at a frequency of 20 Hz with the boy's feet positioned on the foot plate to give a peak to peak displacement of 4 mm, providing a peak to peak acceleration of approximately 6.4 g (earth's gravity; 1 g= 9.81 m/s^2), although the acceleration experience by the head is never >1 g. The Juvent delivers 32-37 Hz, displacement 0.085 mm, at an acceleration of approximately 0.3 g. All participants were asked to stand barefoot on the platforms facing forward with their knees slightly bent for 4 cycles of 2.5 minutes on the platform and 30 seconds off, totalling 10 minutes vibration. Insertion of a rest period between cycles of loading is thought to enhance the anabolic response of bone[35-38]. A training period to familiarise participants to the platforms was not possible as the intervention was conducted over such a short time scale. The rest periods therefore also helped participants to tolerate the full 10 minutes of vibration from day 1.

Outcome measures/blood sampling

Blood samples were collected to measure the bone formation markers pro-collagen type 1 N-terminal propeptide (P1NP; Elecsys, Cobas E411, Roche Diagnostics, UK; intraassay %CV <1.7%) and osteocalcin (OCN; Cobas E411, Roche Diagnostics, Germany; intraassay %CV 1.4-3.3%), and the bone resorption marker C-terminal cross-linked telopeptide of type 1 collagen (CTx; Elecsys β-CrossLaps/serum kit, Cobas E411, Roche Diagnostics, UK; intraassay %CV 2.8-8.4%). Factors affecting bone resorption - osteoprotegerin (OPG; ELISA, BioVendor, Czech Republic; intraassay %CV 2.5-4.9%) - and bone formation - sclerostin (SCL; Enzyme Immunoassay, Biomedica Gruppe, Germany; intraassay %CV <7%) - were also measured. Sampling was undertaken as per Figure 1. All samples were collected in the mornings starting at approximately 08:00 following an overnight fast. Samples were centrifuged, separated and stored at -80°C within 2 hours of collection. Serum samples were assayed in duplicate. P1NP, CTx OPG and SCL were assayed for each sample; OCN only for the baseline and day 8 samples in those receiving 5 days of vibration (insufficient sample for intermediate measures).

Thermal imaging

Thermal images of the legs below the knee were obtained pre- and post vibration on boys exposed to 1 day only of WBV (n=12) and on half of the boys (randomly selected) in the control group (n=7) to measure skin surface temperature. Images were recorded with the participants standing on the platform prior to commencement of WBV and immediately upon completion of the 4 cycles of vibration. Boys in the control group were asked to stand on and off the Juvent platform at the same timing as for the boys exposed to WBV, with the platform turned off. Images were captured using a hand held Land Guide M4 thermal imaging camera (Asten Instruments Ltd, Market Rasen, UK) with a temperature range of -20°C to 250°C and a sensitivity of 0.12°C, CV 2%, and were analysed using the manufacturer's software. All images were recorded by one of two of the researchers (CH or HR) in the same room with windows and doors kept closed and blinds drawn to block out sunlight and/or draughts. As the region of interest was the surface area over the gastrocnemius and soleus muscles, boys were asked to wear shorts or to roll their trouser legs up above their knees. Thermal images were recorded as a non-invasive surrogate measure of blood flow to the skin over the calf mus-

	Low magnitude platform n=18			High magnitude platform n=18			Control n=15		
	Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Age (years)	10.4	0.8	18	10.4	0.9	18	10.8	0.6	15
Height (cm)	141.8	6.5	18	145.4	8.5	18	143.2	7.0	15
Weight (kg)	34.3	4.0	18	37.1	6.8	18	38.6	10.9	15
Body Mass Index ($kg.m^{-2}$)	17.1	1.7	18	17.4	1.8	18	18.6	4.1	15
Activity score (METS units)	79	25	18	94	33	18	67	26	15
P1NP Day 1Pre (ng/ml)	671.5	281.1	18	794.5	204.6	17	679.5	186.5	14
OCN Day 1Pre (ng/ml)	86.4	15.5	5	88.3	32.2	5	-	-	-
CTx Day 1Pre (ng/ml)	1.85	0.55	18	2.00	0.48	17	1.91	0.40	14
OPG Day 1Pre (pmol/L)	3.67	0.53	16	3.71	0.97	17	3.07	0.46	14
SCL Day 1 Pre (pmol/L)	25.54	6.25	16	29.13	6.22	17	24.25	7.11	14
Time between samples day 1 (minutes)	33.06	17.01	17	29.76	5.21	17	31.07	2.27	14

SD= standard deviation, n= number of participants

METS: metabolic equivalents
P1NP: pro-collagen type1 N-terminal propeptide
OCN: osteocalcin
CTx: C-terminal cross-linked telopeptide of type 1 collagen
OPG: osteoprotegerin
SCL: sclerostin

Table 1. Baseline characteristics of participants by intervention group.

cles following WBV to detect if there was a difference in blood flow due to muscular activity in boys exposed to the low or high magnitude WBV.

Exercise and anthropometry

Participants were asked about the amount of exercise and sport activity they had taken part in over the 7 days prior to their first study visit using the validated Godin-Shepard Leisure-Time questionnaire[39]. The frequency and intensity of the types of exercise given in answer to these questions were multiplied by the anticipated metabolic equivalents (METs) of nine, five, and three for strenuous, moderate, and mild exercise respectively, to provide an activity score for comparison. Boys were not asked to alter their normal activities for the duration of the study. Weight was measured to the nearest 0.1 kg with the child wearing light clothing using electronic balance scales (Seca GmbH & Co, Hamburg, Germany). Height was measured to the nearest 0.1 cm without shoes using a portable stadiometer (Leicester height measure, Invicta, Leicester, UK). Pubertal status (Tanner stage) was determined during interview by self-assessment using a gender appropriate validated pictorial scale depicting the different stages of puberty[40]. Boys who indicated that they were at Tanner stage 2 or above were excluded from the study.

Statistical analysis

No sample size calculation was performed as this was a pilot study to determine the rate and range of acute responses in markers of bone turnover and bone-derived factors to WBV. Statistical analysis was performed using SPSS version 19 (IBM, New York, USA). Statistical significance testing of baseline characteristics was not performed[41].

Within group change; pre to post-vibration

A repeated measure ANOVA was used to assess within group changes in phase 1 up to time 60 minutes post-vibration. Day 1 data from phase 1 and phase 2 boys were combined to assess the immediate bone biomarker response to vibration (i.e. comparing pre-vibration vs immediately post-vibration). Paired t-tests were used to test for change within a group from pre-vibration to immediately post-vibration on each day (day 1, day 3 and day 5). The within group differences are reported as percentage changes as this allows the reader an understanding of the magnitude of change for the individual subjects.

Between group differences

Changes in bone markers (P1NP, CTx) and bone cell-derived factors (OPG, SCL) at day 1 pre to immediately post-vibration were compared between high and low magnitude WBV and control groups using ANOVA and ANCOVA (adjusting for baseline bone turnover markers, number of days of vibration, age, activity score). Activity score was included as a covariate to adjust for observed baseline imbalances. Adjustment for days of vibration was included to account for the recruitment of groups of subjects in successive phases (day 1 data is combined from phase 1 and 2). This adjusts for potential differences between the boys recruited in the different phases.

Changes in bone markers (P1NP, CTx) and bone cell-derived factors (OPG, SCL) pre to 60 minutes post-vibration on

		Control mean (95% CI)	n	low magnitude platform mean (95% CI)	n	high magnitude platform mean (95% CI)	n	p value ANOVA
Day 1 Mean change (unadjusted)								
P1NP	10 min post	-61.3 (-103.9 to -18.8)	14	-67.6 (-108.7 to -26.5)	17	-71.3 (-164.5 to 21.8)	16	0.97
ng/ml	60 min post	-83.7 (-135.1 to -32.2)	14	-58.5 (-160.1 to 43.0)	6	-16.5 (-172.4 to 139.4)	5	0.43
CTx	10 min post	-0.11 (-0.21 to 0.05)	14	-0.17 (-0.25 to -0.09)	17	-0.08 (-0.21 to 0.05)	16	0.36
ng/ml	60 min post	-0.14 (-0.27 to -0.02)	14	-0.17 (-0.37 to 0.03)	6	-0.07 (-0.15 to 0.01)	5	0.64
Day 1 Adjusted mean change (adjusted*)								
								ANCOVA
P1NP	10 min post	-71.6 (-160.4 to 17.3)	14	-70.7 (-122.6 to -18.9)	17	-64.8 (-121.6 to -8.0)	16	0.91
ng/ml	60 min post	-76.6 (-131.4 to -21.7)	14	-75.6 (-155.4 to 4.1)	6	-15.8 (-123.4 to 91.7)	5	0.17
CTx	10 min post	-0.11 (-0.27 to 0.05)	14	-0.17 (-0.26 to -0.07)	17	-0.09 (-0.19 to 0.01)	16	0.14
ng/ml	60 min post	-0.15 (-0.26 to -0.03)	14	-0.20 (-0.37 to -0.03)	6	-0.02 (-0.25 to -0.21)	5	0.59

**Adjusted for baseline CTx/P1NP, length of treatment, age, activity score, time between samples*
CI= confidence interval

Table 2. Change in serum P1NP and CTx values from baseline at 10 and 60 minutes post WBV by platform group.

		Low magnitude platform				High magnitude platform				Control			
		N	Mean	SD	p value	N	Mean	SD	p value	N	Mean	SD	p value
P1NP ng/ml	Day 1 pre	17	669.8	289.7	**0.003**	16	786.8	208.8	0.12	14	697.5	186.5	**0.008**
	Day 1 10 mins post		602.2	245.5			715.4	191.6			618.2	141.7	
	Day 1 pre*	6	600.6	409.7	0.2	5	830.1	287.3	0.78	14	697.5	186.5	**0.04**
	Day 1 60 mins post		542.1	332.7			813.6	249.4			595.8	194.5	
	Day 3 pre	11	703.2	184.4	**<0.001**	10	749.8	122	**0.004**		-	-	
	Day 3 post		575.6	139.3			646.8	103.8			-	-	
	Day 5 pre	5	712.6	212.7	**0.008**	4	763.7	169.4	**0.05**		-	-	
	Day 5 post		608.9	225			681	145			-	-	
CTx ng/ml	Day 1 pre	17	1.83	0.56	**<0.001**	16	2.00	0.50	0.2	14	1.91	0.40	**0.04**
	Day 1 10 mins post		1.67	0.49			1.93	0.50			1.80	0.45	
	Day 1 pre	6	1.71	0.73	0.08	5	2.40	0.59	0.08	14	1.91	0.40	**0.03**
	Day 1 60 mins post		1.54	0.65			2.34	0.56			1.76	0.39	
	Day 3 pre	11	1.89	0.51	**0.007**	10	1.93	0.30	0.2		-	-	
	Day 3 post		1.76	0.47			1.86	0.28			-	-	
	Day 5 pre	5	1.91	0.66	**0.004**	5	1.99	0.16	0.9		-	-	
	Day 5 post		1.76	0.62			2.01	0.33			-	-	

Mean pre and post WBV P1NP and CTx per intervention group for samples included in the paired t-test analysis of pre and post values on each day of sample collection.
SD - standard deviation, N - number of participant samples included in the analysis.
**Only participants allocated to 1 day of WBV or control group had blood collected at 60 minutes post WBV (or equivalent time in the control group); these 6 boys are a subgroup of the 17 measured on day 1.*

Table 3. Mean (SD) bone turnover marker (P1NP, CTx) values Pre and post WBV on days 1, 3, 5.

day 1, and pre to immediately post-vibration on day 3 and day 5 were compared between groups using ANOVA and ANCOVA (adjusting for baseline bone turnover marker). No other covariates were included due to lack of power. After day 1 data was only collected in boys exposed to the vibration (high and low magnitude WBV groups, not the control group); the comparison for day 3 and day 5 are between WBV groups only.

Combined change at day 8

Change in bone markers (P1NP, CTx, OCN) and bone cell-derived factors (OPG, SCL) after 5 days of vibration were combined across the WBV groups due to limited data. Paired t-tests were used to test for change from baseline using day 1 (pre-test) and day 8 measurements.

To account for camera temperature drift between the recorded images, the post-image temperature was adjusted and within participant temperature change pre to post WBV reported as the outcome. For each participants' pre- and post image a reference area not expected to change in temperature over the short time lapse between the images (i.e. where the camera was pointed at the wall) was determined with the region temperature recording used to adjust for any camera drift.

Results

In total 64 boys consented to study participation. Of these 8 were excluded on the basis of pubertal stage greater than Tanner 1, 2 withdrew consent prior to data collection and commencement of the intervention, and 3 withdrew due to difficulties in obtaining blood samples on day 1. Data was collected and analysed on 51 boys in total; 12 boys in phase 1 (1 day/10 minutes only of WBV), 24 boys in phase 2 (3 or 5 days of WBV, 12 subjects in each group), and 15 boys in the control group (no WBV); see Figure 1. The baseline characteristics of each group are shown in Table 1. No formal statistical testing was undertaken. Age, height, body mass index (BMI), and weight were similar between the groups. The activity scores may appear to be different across the intervention groups with the high magnitude group scoring higher but it should be noted that these scores have a large standard deviation (SD). Baseline P1NP, OCN, CTx, OPG, and SCL values were similar between the groups. The time taken between the pre and post vibration samples on day 1 was slightly longer in the Juvent group. One boy in this group felt faint after cannulation and rested prior to standing on the vibration platform, accounting for the greater time lag and larger SD in this group.

Bone turnover markers – changes across individual cycles of vibration

Within control group (n=14)

P1NP decreased by 7.8% (CI -13.4 to -2.2; p=0.008, paired t-test) at 10 minutes, and by 12.0% (CI -19.3 to -4.7; p=0.04) at 60 minutes compared to baseline. Osteocalcin was not measured. CTx decreased by 12.0% (CI -19.3 to -4.7; p=0.04) at 10 minutes and by 7.0% (CI -13.7 to-0.4; p=0.03) at 60 minutes (actual values, Table 2).

Within low magnitude group, first cycle of vibration (n=17)

P1NP decreased by 7.9% (CI -14.0 to -1.9; p=0.003, paired t-test) at 10 minutes, and by 0.18% (CI -20.6 to 21.0; p=0.20) at 60 minutes (n=5). CTx decreased by 6.2% (CI -12.2 to -0.2; p=0.04) at 10 minutes post WBV and by 9.4% (CI -21.1 to 2.2; p=0.08, statistically not significant) at 60 minutes post vibration (actual values Table 2).

Within high magnitude group, first cycle of vibration (n=16)

No change was seen in P1NP (decreased by 6.8% CI -18.4 to 4.9; p=0.1) at 10 minutes, and by 6.04% (CI -9.2 to 21.3 p=0.78) at 60 minutes (n=6). Neither was there a change in CTx at 10 minutes (n=16; 3.6% decrease observed, CI -9.9 to 2.8; p=0.2) or 60 minutes (2.6% decrease; CI -5.4 to 0.1; p=0.08; actual values Table 2).

There were no changes in either osteoprotegerin or sclerostin over the period from baseline to 60 minutes post WBV for either low or high magnitude WBV.

Changes across individual vibration cycles; Day 3 and Day 5

On day 3 P1NP decreased pre to post vibration in the low magnitude group (n=11) by 17.5% (CI -22.6 to -12.5; p<0.001) and in the high magnitude group (n=10) by 13.3% (CI -20.2 to -6.3; p=0.004). CTx decreased following WBV in the low magnitude group by 6.2% (CI -10.4 to -2.1; p=0.007; n=11), but did not change in the high magnitude group (day 3: 3.4% decrease observed, CI -8.1 to 1.3 p=0.2; n=10; actual values Table 3).

Day 5 showed a decrease following WBV in P1NP in the low magnitude group (n=5) of 15.9% (CI -20.2 to -6.3; p=0.008) and in the high magnitude group (n=4) of 10.6% (CI -20.2 to -0.9; p=0.05; Table 3). CTx decreased following WBV in the low magnitude group by 8.1% (CI -10.3 to -5.7; p=0.004; n=5) and was unchanged in the high magnitude group (0.5% increase observed, CI -16.5 to 17.4; p=0.9; n=4; actual values Table 3).

Differences in bone marker responses between platforms

There were no differences between the control and platform groups in the day 1 P1NP and CTx response to vibration (ANOVA and adjusted ANCOVA; Table 2). There was also no difference between the platform groups in response to WBV on days 3 and 5 from immediately pre-vibration to 10 minutes after vibration (no control data collected) though as on day 1 within group changes were detected, as shown above.

Changes from baseline after 5 days of vibration

Bone turnover markers – P1NP, OCN, CTx

In contrast to the decrease shown in the immediate pre to post WBV time period, boys exposed to 5 consecutive days of WBV (platform groups combined, n=11, measurements on day 8 vs baseline measurements) had a significant increase in P1NP of 25.1% (CI 12.3 to 38.0; paired t-test p=0.005; Figure 2). No significant change was detected in the formation marker OCN (measured at day 1 and day 8 in the 5 day subjects only n=11; change +11.5% CI -8.3 to 31.2; p=0.2; Figure 2). At day 8, CTx was greater in the boys exposed to 5 days of WBV on both of the platforms than at baseline with an increase of 10.9% (CI 3.6 to 18.2; paired t-test p=0.009; Figure 2).

OPG and sclerostin

OPG and sclerostin were analysed at each time point. OPG showed a trend for an increase of 7.2% (CI -1.4 to 15.8; p=0.08) on day 8 compared to baseline (n=11; Figure 3). No difference was detected at any other time point within or between groups. Additionally no change within or between

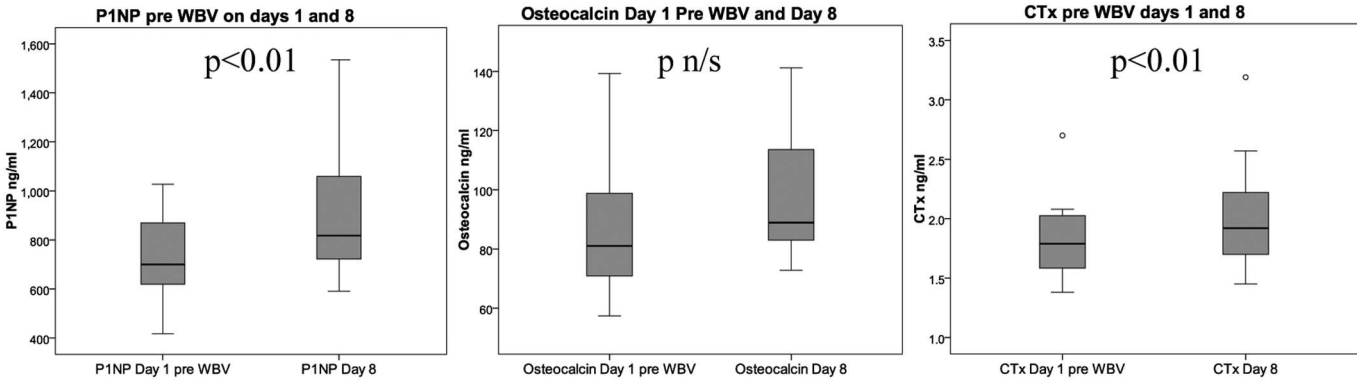


Figure 2. Boxplots illustrating the absolute values of a) P1NP, b) Osteocalcin and c) CTx at baseline and day 8 for boys exposed to 5 consecutive days of WBV.

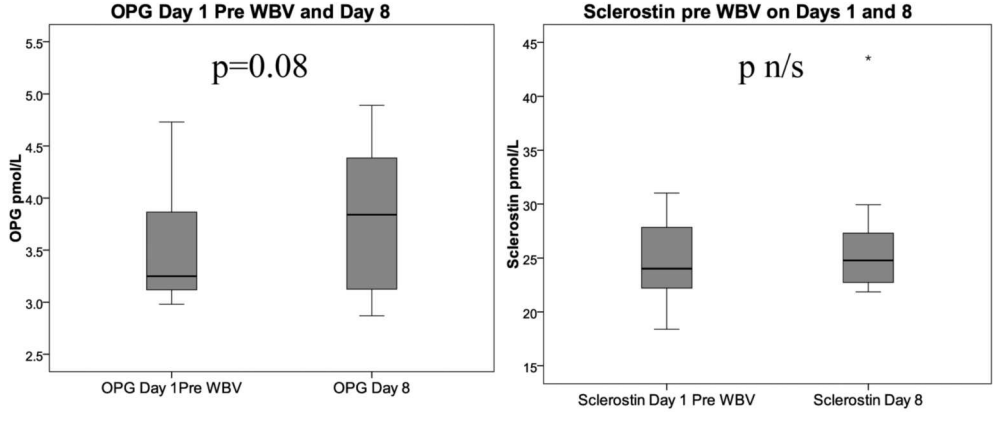


Figure 3. Boxplots illustrating the absolute values of a) OPG and b) Sclerostin at baseline and day 8 for boys exposed to 5 consecutive days of WBV.

groups was detected at any time point for sclerostin (change of +7.2% CI -7.45 to 21.73; p=0.3).

Thermal imaging

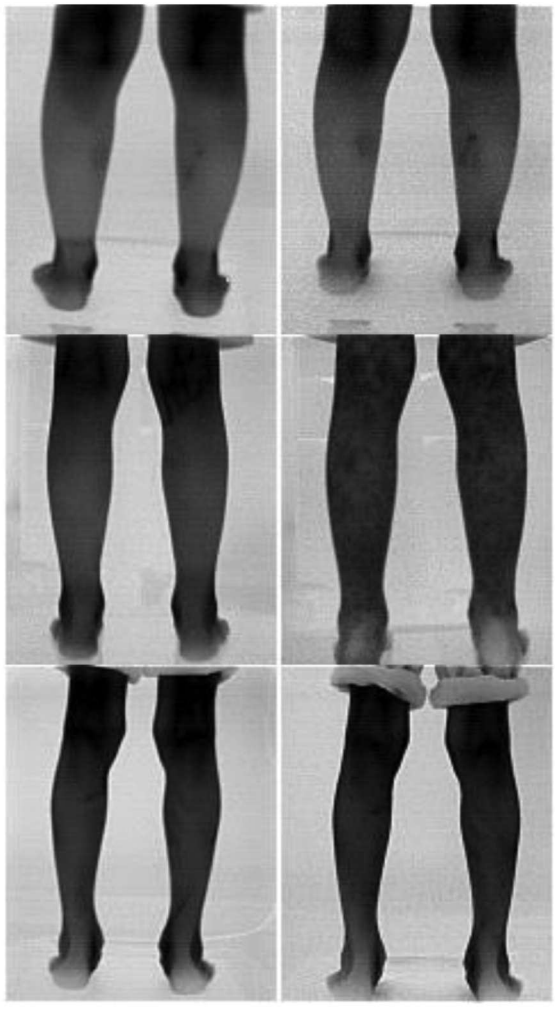
Thermal images were captured on 7 boys in the control group and on all boys exposed to 1 day only of vibration (n=7). However it was not possible to compare images in 2 of the boys and these were excluded from the analysis. Images therefore were analysed on 5 boys exposed to each of the vibrating platforms and 7 boys from the control group. The change in skin surface temperature pre- to post vibration ranged from -1°C to 1.6°C (mean 0.3°C, CI -0.4 to 1.1) in the control group, 1.4°C to 4.2°C (mean 2.9°C, CI 1.5 to 4.4) in the high magnitude group, and 0.2°C to 2.8°C (mean 0.9°C, CI -0.4 to 2.3) in the low magnitude group. There was a significant difference in the response of the boys in the high magnitude group compared to the control (p=0.002) and low magnitude groups (p=0.02; ANOVA, bonferroni post hoc test). In addition, when visually assessing the pre and post images, a difference in the temperature distribution was seen in the boys exposed to the high magnitude platform that was not seen in the control or low magnitude groups (Figure 4).

Adverse events

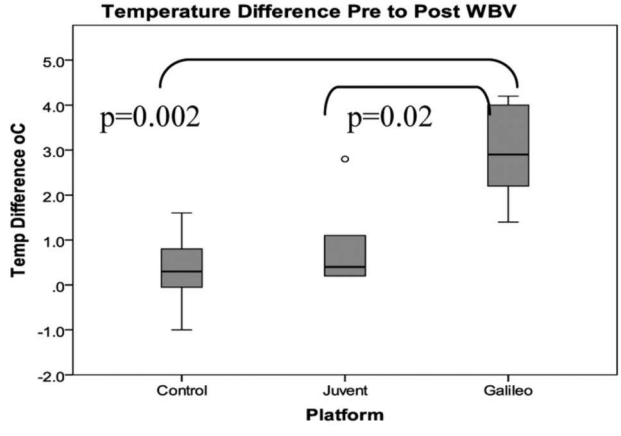
WBV was well tolerated by the study participants. Minimal side effects were reported; itching or "weird feeling" in the calves or legs (high magnitude), tickling sensation in the feet (low magnitude) and anxiety in relation to cannulation. In all cases these resolved on or shortly after completion of the WBV.

Discussion

We observed a fall in both P1NP and CTx ten minutes after vibration using the low magnitude platform on days 1, 3 and 5 and on days 3 and 5 following vibration using the high magnitude platform. Similar reductions were seen, however, in boys who stood on a platform that did not vibrate over the same time period. There were no differences between the groups in the changes in P1NP or CTx across the individual cycles of vibration exposure.



a)
Top left: Control pre WBV, top right: Control post WBV
Middle left: Galileo pre WBV, middle right: Galileo post WBV
Bottom left: Juvent pre WBV, bottom right: Juvent post WBV



b)

Figure 4. a) Thermal images taken immediately before and after vibration. b) Boxplots showing comparison of the pre to post vibration temperature change between the groups.

By contrast, five consecutive days of WBV (both groups combined) increased the bone formation marker P1NP by 25.1% and bone resorption marker CTx by 10.9% between the pre-vibration baseline and the day 8 measurement. The second bone formation marker measured, osteocalcin, did not change over the period of observation. This may be because osteocalcin is produced later in the process of endochondral ossification, when mineralisation is taking place. By contrast, P1NP is produced early in the bone formation process, when the osteoid matrix is being formed and deposited.

The greater increase over the 8 day period in the formation marker as opposed to the resorption marker suggests that there is an uncoupling of bone turnover in favour of formation in response to vibration. These changes are consistent with the reported effects of increased activation of the canonical wnt-signalling pathway through LRP5/6 where bone formation is increased and there is increased osteoblastic expression of osteoprotegerin. Our data indicated this with a trend towards an increase in OPG. However increased expression of OPG should also be associated with reduced bone resorption which is not confirmed by our CTx results.

Sclerostin is widely recognised to be a key inhibitor of bone formation by the canonical wnt-signalling pathway through LRP5/6[42] and is an important factor in bone response to mechanical loading[43]. However no significant change in serum sclerostin was detected either over time or between groups and could not therefore explain our observations of increased P1NP. Animal studies have shown down-regulation of sclerostin production at sites of new bone formation following even short periods of mechanical loading[43]. It may be the case that changes in serum sclerostin do not reflect accurately or quickly on changes occurring at a tissue level. An alternative explanation is that the rapid changes observed in bone formation and resorption here were the result of activity either in other pathways, or in other regulators of the wnt-signalling pathway through LRP5/6. Additional inhibitors of the pathway are well described, including DKK1 and WISE, both secreted by osteocytes. PTH inhibits the production of both sclerostin and DKK1 and also interacts via the PTH-PTHR1 complex with LRP6, initiating signalling in the absence of Wnt-ligands[44]. It will be important in future studies to include such factors to understand the underlying physiological basis of acute responses to mechanical loading.

Other studies that have looked at bone turnover marker response to WBV have been conducted in adult populations. Corrie et al[45] found an increase in P1NP of 17.5% in adults aged 64 years and over exposed to either synchronous or side-alternating vibration for 12 weeks, but no change in CTx. This suggests, as shown in our paediatric population, that bone marker response is not dependent on the method of vibration. In contrast to the increase we saw in resorption, Turner et al[46] showed a 34% decrease in urinary NTx following 6 weeks of WBV in postmenopausal women, a third of whom had osteoporosis. Following one episode of vibration and exercise Sherk et al[47] also found a decrease in CTx in healthy young women exposed to one episode of high magnitude synchronous WBV. The de-

crease was greater at 30 minutes post exercise when the women were exposed to WBV immediately prior to resistance exercise than following resistance exercise only (-12.5% vs -1.3%).These studies demonstrate a change in bone turnover markers that suggests an uncoupling of bone turnover in favour of formation as we found in our paediatric population. However, Rubin et al[24] found only a slight decrease of 3% in the resorption marker hydroxyproline in their active vibration group with a much greater decrease in the placebo group of 16%. In accord with our data others have failed to demonstrate a change in the formation marker osteocalcin following WBV[24,27,30].

Our focus on children and the response of bone in the growing skeleton rather than on a fully developed skeleton may explain some differences seen between our results and those of others. The increase in resorption and greater increase in formation in our group, not typically seen in the adult populations, may reflect the enhanced response to loading that has been well reported in the growing as opposed to adult skeleton. Five separate papers[24,27-30] found no change in bone markers following 6-12 months WBV in healthy young adults and postmenopausal women, but this may reflect the adaptation of bone structure and bone remodelling to the continuing stimulus over a prolonged period.

The mechanism of the bone response to WBV is thought to be due to a direct response within the bone tissue to loading or via muscle; either by contractions loading the bone or due to increased muscle mass and/ or force increasing load to bone[48]. Increased muscle activity and blood flow as a result of WBV have been reported in a number of studies[49-55]. To determine if muscle activation following WBV was different between the low and high magnitude groups our study recorded skin surface temperature of the lower leg, as a surrogate measure, pre and post vibration in a sub group of boys. Although we found no difference between groups in bone turnover marker response in the immediate pre to post vibration period, thermal imaging detected a 31% greater increase in skin surface temperature in boys exposed to the high magnitude versus the low magnitude platform. Skin surface temperature has been shown to be increased in passive vibration[56] as well as weight bearing vibration[57] suggesting that some degree of muscle activation occurs regardless of the magnitude or method of the stimulation. Nevertheless, our results suggest muscle activation is not a prerequisite for bone to respond to mechanical stimulation.

In terms of possible future application, we speculate that this approach might have utility in identifying subjects more likely to benefit from vibration exercise-based interventions to increase bone mass; to identify potential adverse "damping" effects from pharmacological therapies targeting bone; and to assess the responsiveness of the skeleton to vibration in different disease states.

Limitations

This study was designed as a pilot study to determine the size and variability of the response of bone turnover markers to vibration and was therefore not powered to detect any specific degree of change. The intervention and control groups had relatively small numbers and so the study findings must be regarded as preliminary. Whilst the study has met its aim of identifying the rate and range of response of bone turnover markers in pre-pubertal boys to WBV it is not clear if the same response would be detected in pre-pubertal girls, or during other stages of growth.

The instructions regarding stance (stand with knees slightly bent) were the same for the subjects irrespective of platform; whilst appropriate for the high magnitude device, this stance may have abrogated the skeletal response to low magnitude stimulation.

The length of time between the last period of vibration and collection of the final samples was different between the boys exposed to 3 or 5 days of vibration, 48 and 72 hours respectively, due to logistical issues. The thermal imaging produced interesting data, however there were some limitations in our methods. A drift in the temperature reading of the camera was observed during the study and the distance of the camera to the point of interest (the boys leg's) was not standardised between participants. Our findings could however be confirmed in future studies using more robust techniques and methods such as use of a thermal imaging suite.

Summary

Five consecutive days of WBV increased the bone formation marker P1NP by 25.1% and the resorption marker CTx by 10.9%, demonstrating an alteration in bone turnover that is in favour of bone formation. The direct mechanism of this acute response is not clear as sclerostin was unchanged by vibration in this study and OPG increased but so did bone resorption. Studies in healthy adult populations have been unable to demonstrate an anabolic effect of WBV on bone over longer periods, but we have shown here that bone in a healthy growing skeleton does have the capacity to respond quickly to WBV irrespective of the magnitude of that vibration. The possible broader application of this approach in other settings requires careful evaluation, but these initial data are encouraging in terms of the size and consistency of response across different types of vibration platforms.

Acknowledgements

Funding for this study was provided by The Children's Hospital Charity. The authors would like to thank all the participants for taking part in the study. They would also like to thank Chisombili Omabu for her assistance with thermal imaging and Fatma Gossiel who performed all the biochemical assays. KAW is supported by the core programme of the MRC Nutrition and Bone Health group at MRC Human Nutrition Research, funded by the UK Medical Research Council (Grant code U10590371).

References

1. Cooper C, Dennison EM, Leufkens HG, Bishop N, van Staa TP. Epidemiology of childhood fractures in Britain: a study using the general practice research database. J Bone Miner Res 2004;19:1976-81.

2. Khosla S, Melton LJ, 3rd, Dekutoski MB, Achenbach SJ, Oberg AL, Riggs BL. Incidence of childhood distal forearm fractures over 30 years: a population-based study. JAMA 2003;290:1479-85.
3. Goulding A, Jones IE, Williams SM, et al. First fracture is associated with increased risk of new fractures during growth. J Pediatr 2005;146:286-8.
4. Valerio G, Galle F, Mancusi C, et al. Pattern of fractures across pediatric age groups: analysis of individual and lifestyle factors. BMC Public Health 2010;10:656.
5. Yeh FJ, Grant AM, Williams SM, Goulding A. Children who experience their first fracture at a young age have high rates of fracture. Osteoporos Int 2006;17:267-72.
6. Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. J Bone Miner Res 2006;21:1489-95.
7. Manias K, McCabe D, Bishop N. Fractures and recurrent fractures in children; varying effects of environmental factors as well as bone size and mass. Bone 2006;39:652-7.
8. Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J Bone Miner Res 1999;14:1672-9.
9. Ducher G, Bass SL, Saxon L, Daly RM. Effects of repetitive loading on the growth-induced changes in bone mass and cortical bone geometry: a 12-month study in pre/peri- and postmenarcheal tennis players. J Bone Miner Res 2011;26:1321-9.
10. Ducher G, Daly RM, Bass SL. Effects of repetitive loading on bone mass and geometry in young male tennis players: a quantitative study using MRI. J Bone Miner Res 2009;24:1686-92.
11. Bass S, Pearce G, Bradney M, et al. Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. J Bone Miner Res 1998;13:500-7.
12. Nilsson M, Ohlsson C, Mellstrom D, Lorentzon M. Previous sport activity during childhood and adolescence is associated with increased cortical bone size in young adult men. J Bone Miner Res 2009;24:125-33.
13. Erlandson MC, Kontulainen SA, Chilibeck PD, Arnold CM, Faulkner RA, Baxter-Jones AD. Higher premenarcheal bone mass in elite gymnasts is maintained into young adulthood after long-term retirement from sport: a 14-year follow-up. J Bone Miner Res 2012;27:104-10.
14. Rubin C, Xu G, Judex S. The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli. FASEB J 2001;15:2225-9.
15. Flieger J, Karachalios T, Khaldi L, Raptou P, Lyritis G. Mechanical stimulation in the form of vibration prevents postmenopausal bone loss in ovariectomized rats. Calcif Tissue Int 1998;63:510-4.
16. Judex S, Lei X, Han D, Rubin C. Low-magnitude mechanical signals that stimulate bone formation in the ovariectomized rat are dependent on the applied frequency but not on the strain magnitude. J Biomech 2007;40:1333-9.
17. Oxlund BS, Ortoft G, Andreassen TT, Oxlund H. Low-intensity, high-frequency vibration appears to prevent the decrease in strength of the femur and tibia associated with ovariectomy of adult rats. Bone 2003;32:69-77.
18. Rubin C, Turner AS, Muller R, et al. Quantity and quality of trabecular bone in the femur are enhanced by a strongly anabolic, noninvasive mechanical intervention. J Bone Miner Res 2002;17:349-57.
19. Rubin C, Turner AS, Mallinckrodt C, Jerome C, McLeod K, Bain S. Mechanical strain, induced noninvasively in the high-frequency domain, is anabolic to cancellous bone, but not cortical bone. Bone 2002;30:445-52.
20. Rubinacci A, Marenzana M, Cavani F, et al. Ovariectomy sensitizes rat cortical bone to whole-body vibration. Calcif Tissue Int 2008;82:316-26.
21. Sehmisch S, Galal R, Kolios L, et al. Effects of low-magnitude, high-frequency mechanical stimulation in the rat osteopenia model. Osteoporos Int 2009;20:1999-2008.
22. Xie L, Jacobson JM, Choi ES, et al. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. Bone 2006;39:1059-66.
23. Gilsanz V, Wren TA, Sanchez M, Dorey F, Judex S, Rubin C. Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. J Bone Miner Res 2006;21:1464-74.
24. Rubin C, Recker R, Cullen D, Ryaby J, McCabe J, McLeod K. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: a clinical trial assessing compliance, efficacy, and safety. J Bone Miner Res 2004;19:343-51.
25. Ward K, Alsop C, Caulton J, Rubin C, Adams J, Mughal Z. Low magnitude mechanical loading is osteogenic in children with disabling conditions. J Bone Miner Res 2004;19:360-9.
26. Gusi N, Raimundo A, Leal A. Low-frequency vibratory exercise reduces the risk of bone fracture more than walking: a randomized controlled trial. BMC Musculoskelet Disord 2006;7:92.
27. Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. J Bone Miner Res 2004; 19:352-9.
28. Bemben DA, Palmer IJ, Bemben MG, Knehans AW. Effects of combined whole-body vibration and resistance training on muscular strength and bone metabolism in postmenopausal women. Bone 2010;47:650-6.
29. Russo CR, Lauretani F, Bandinelli S, et al. High-frequency vibration training increases muscle power in postmenopausal women. Arch Phys Med Rehabil 2003; 84:1854-7.
30. Torvinen S, Kannus P, Sievanen H, et al. Effect of 8-

month vertical whole body vibration on bone, muscle performance, and body balance: a randomized controlled study. J Bone Miner Res 2003;18:876-84.
31. Ruck J, Chabot G, Rauch F. Vibration treatment in cerebral palsy: A randomized controlled pilot study. J Musculoskelet Neuronal Interact 2010;10:77-83.
32. Humphries B, Fenning A, Dugan E, Guinane J, MacRae K. Whole-body vibration effects on bone mineral density in women with or without resistance training. Aviat Space Environ Med 2009;80:1025-31.
33. Slatkovska L, Alibhai SM, Beyene J, Hu H, Demaras A, Cheung AM. Effect of 12 months of whole-body vibration therapy on bone density and structure in postmenopausal women: a randomized trial. Ann Intern Med 2011;155:668-79, W205.
34. Formenti D, Ludwig N, Gargano M, et al. Thermal imaging of exercise-associated skin temperature changes in trained and untrained female subjects. Ann Biomed Eng 2013;41:863-71.
35. Burr DB, Robling AG, Turner CH. Effects of biomechanical stress on bones in animals. Bone 2002;30:781-6.
36. Gross TS, Poliachik SL, Ausk BJ, Sanford DA, Becker BA, Srinivasan S. Why rest stimulates bone formation: a hypothesis based on complex adaptive phenomenon. Exerc Sport Sci Rev 2004;32:9-13.
37. LaMothe JM, Zernicke RF. Rest insertion combined with high-frequency loading enhances osteogenesis. J Appl Physiol (1985) 2004;96:1788-93.
38. Srinivasan S, Ausk BJ, Poliachik SL, Warner SE, Richardson TS, Gross TS. Rest-inserted loading rapidly amplifies the response of bone to small increases in strain and load cycles. J Appl Physiol (1985) 2007;102:1945-52.
39. Godin G, Shephard, R J. A simple method to assess exercise behaviour in the community. Canadian Journal of Applied Sport Sciences 1985;10:141-6.
40. Lissauer T, Clayden G. The Illustrated Textbook of Paediatrics. London: Mosby; 1999.
41. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 2010; 340:c869.
42. Li X, Zhang Y, Kang H, et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem 2005;280:19883-7.
43. Tu X, Rhee Y, Condon KW, et al. Sost downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. Bone 2012;50:209-17.
44. Canalis E. Wnt signalling in osteoporosis: mechanisms and novel therapeutic approaches. Nat Rev Endocrinol 2013;9:575-83.
45. Corrie H, Brooke-Wavell K, MAnsfield N, et al. Effect of whole body vibration on bone formation and resorption in older patients: A randomised controlled trial. Osteoporosis International 2007;18 (Supplement 3):S285.
46. Turner S, Torode M, Climstein M, et al. A randomized controlled trial of whole body vibration exposure on markers of bone turnover in postmenopausal women. J Osteoporos 2011;2011:710387.
47. Sherk VD, Chrisman C, Smith J, et al. Acute bone marker responses to whole-body vibration and resistance exercise in young women. J Clin Densitom 2013;16:104-9.
48. Judex S, Rubin CT. Is bone formation induced by high-frequency mechanical signals modulated by muscle activity? J Musculoskelet Neuronal Interact 2010;10:3-11.
49. Cochrane DJ, Loram ID, Stannard SR, Rittweger J. Changes in joint angle, muscle-tendon complex length, muscle contractile tissue displacement, and modulation of EMG activity during acute whole-body vibration. Muscle Nerve 2009;40:420-9.
50. Torvinen S, Kannu P, Sievanen H, et al. Effect of a vibration exposure on muscular performance and body balance. Randomized cross-over study. Clin Physiol Funct Imaging 2002;22:145-52.
51. Pollock RD, Woledge RC, Mills KR, Martin FC, Newham DJ. Muscle activity and acceleration during whole body vibration: effect of frequency and amplitude. Clin Biomech (Bristol, Avon) 2010;25:840-6.
52. Kerschan-Schindl K, Grampp S, Henk C, et al. Whole-body vibration exercise leads to alterations in muscle blood volume. Clin Physiol 2001;21:377-82.
53. Lohman EB, 3rd, Petrofsky JS, Maloney-Hinds C, Betts-Schwab H, Thorpe D. The effect of whole body vibration on lower extremity skin blood flow in normal subjects. Med Sci Monit 2007;13:CR71-6.
54. Lythgo N, Eser P, de Groot P, Galea M. Whole-body vibration dosage alters leg blood flow. Clin Physiol Funct Imaging 2009;29:53-9.
55. Maloney-Hinds C, Petrofsky JS, Zimmerman G. The effect of 30 Hz vs. 50 Hz passive vibration and duration of vibration on skin blood flow in the arm. Med Sci Monit 2008;14:CR112-6.
56. Oliveri DJ, Lynn K, Hong CZ. Increased skin temperature after vibratory stimulation. Am J Phys Med Rehabil 1989;68:81-5.
57. Hazell TJ, Thomas GW, Deguire JR, Lemon PW. Vertical whole-body vibration does not increase cardiovascular stress to static semi-squat exercise. Eur J Appl Physiol 2008;104:903-8.