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Enhancement of the adolescent murine musculoskeletal system using low-level mechanical vibrations

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Xie L, Rubin C, Judex S. Enhancement of the adolescent murine musculoskeletal system using low-level mechanical vibrations. *J Appl Physiol* 104: 1056–1062, 2008. First Published February 7, 2008; doi:10.1152/japplphysiol.00764.2007.—Mechanical signals are recognized as anabolic to both bone and muscle, but the specific parameters that are critical to this stimulus remain unknown. Here we examined the potential of extremely low-magnitude, high-frequency mechanical stimuli to enhance the quality of the adolescent musculoskeletal system. Eight-week-old female BALB/cByJ mice were divided into three groups: baseline controls (BC, $n = 8$), age-matched controls (AC, $n = 12$), and whole body vibration (WBV, $n = 12$) at 45 Hz (0.3 g) for 15 min/day. Following 6 wk of WBV, bone mineralizing surfaces of trabeculae in the proximal metaphysis of the tibia were 75% greater ($P < 0.05$) than AC, while osteoclast activity was not significantly different. The tibial metaphysis of WBV mice had 14% greater trabecular bone volume ($P < 0.05$) than AC, while periosteal bone area, bone marrow area, cortical bone area, and the moments of inertia of this region were all significantly greater (up to 29%, $P < 0.05$). The soleus muscle also realized gains by WBV, with total cross-sectional area as well as type I and type II fiber area as much as 29% greater ($P < 0.05$) in mice that received the vibratory mechanical stimulus. The small magnitude and brief application of the noninvasive intervention emphasize that the mechanosensitive elements of the musculoskeletal system are not necessarily dependent on strenuous, long-term activity to initiate a structurally relevant response in the adolescent musculoskeletal system. If maintained into adulthood, the beneficial structural changes in trabecular bone, cortical bone, and muscle may serve to decrease the incidence of osteoporotic fractures and sarcopenia later in life.

high-frequency mechanical stimuli; whole body vibration; peak bone mass; muscle morphology; trabecular bone; cortical bone; sarcopenia; osteopenia

USING EXERCISE TO STRENGTHEN the musculoskeletal system during adolescence and early adulthood has the potential to reduce the incidence of skeletal fractures later in life as the immature skeleton may be more responsive to physical stimuli than the adult skeleton (18, 26, 30). Direct skeletal benefits from exercise during growth include increases in bone formation (24), peak bone mass (18), and bone strength (40) as well as reduced levels of bone resorption (31). Bone fractures, however, are not exclusively caused by a decrease in bone strength but are often associated with an increased propensity of falling (25). Not surprisingly, exercise that improves muscle mass (11), strength (20), coordination, and balance (20) can also decrease an individual's susceptibility to falls and bone fracture (19). Thus prophylactic or therapeutic treatments that aim to decrease fracture rates, ideally address the aggregate of the intervention's impact on the musculoskeletal system, to include benefits to both bone and muscle.

Despite the important benefits of exercise to musculoskeletal health, the time committed to physical activity by children and adolescents is decreasing, and alternatives need to be identified that can harness bone and muscle's sensitivity to mechanical signals. Whole body vibrations (WBV), applied at acceleration magnitudes several times greater than the acceleration of the Earth, can enhance muscle mass, muscle strength, muscular performance, and balance (4, 7, 36). However, large magnitude vibratory signals used in these studies may exceed International Safety Organization advisories for human tolerance (ISO-2631) and, particularly in children and adolescents, may also elevate the risks of impact loading on soft tissues (1, 6). In contrast to high-magnitude accelerations, there is increasing evidence that extremely low-magnitude mechanical signals, if induced at a relatively high frequency, can be anabolic to bone tissue. Indeed, 30- to 90-Hz signals applied at <0.5 g, while considered by ISO to be safe for up to 4 h/day, have been demonstrated as anabolic to the bone tissue of children with disabling conditions (39), as well as young (16–20 yr) women with osteopenia (15).

Animal models have been developed to aid in the identification of the physical and biological mechanisms by which such extremely low-level mechanical signals can be perceived by cells and tissues (9, 21). In female adolescent BALB/cByJ mice, we found that a 0.3 g WBV regimen, applied for 15 min/day, can reduce trabecular bone resorption, site specifically attenuate the declining levels of bone formation, and maintain a high level of matrix quality (42). While the low-level WBV signal altered the cellular activity in bone, perhaps due to the brevity of the relatively short 3-wk protocol, it did not produce structural adaptations in either trabecular or cortical bone. Furthermore, the impact of the signal on muscle was not examined. Here, in an effort to determine if such low-magnitude signals can enhance the musculoskeletal system when given a longer period to adapt, the experimental duration was doubled from 3 to 6 wk. It was hypothesized that the anabolic nature of the low-level vibratory signal would ultimately enhance the structural quality of trabecular and cortical tissue in the weight-bearing skeleton and that a similar benefit would be realized in the adjacent musculature.

METHODS

Experimental design. All experimental procedures were reviewed and approved by Stony Brook's Institutional Animal Care and Use Committee. Thirty-two 8-wk-old female BALB/cByJ (BALB) mice

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were obtained from The Jackson Laboratory (Bar Harbor, ME) and randomly divided into three groups: *1*) baseline control (BC, $n = 8$), *2*) age-matched control (AC, $n = 12$), and *3*) mice subjected to WBV at 45 Hz, 0.3 g for 15 min once per day, 5 day/wk (WBV, $n = 12$). Data from the BC group have been published previously (42). At 8 wk of age, mice are reproductively mature but have not reached peak bone mass, which in many mouse strains is reached at ~16 wk of age. All mice were single housed and were given free access to a standard rodent chow and water.

Once a day, mice from both the AC and WBV groups were transferred to a plastic container, similar in size to a regular mouse cage but without any bedding to prevent dampening of the mechanical signal. Containers holding WBV mice were placed on the vertically oscillating plate for 15 min/day (42), effectively superimposing the vibratory mechanical signal onto normal cage activities. The total vertical displacement of the vibrated cage was ~74 μm, barely perceptible to human touch. Containers with AC mice were placed on an inactive plate for 15 min/day. AC and WBV mice were allowed to freely roam the cages during these 15 min. No qualitative differences in behavior or activity patterns were observed between the two groups of mice.

To enable measurement of dynamic indexes of bone formation, mice were injected (ip) with calcein (15 mg/kg) on *days 35* and *40* of the experimental protocol. After the 6-wk experimental duration, the right tibia was harvested and submerged in 70% ethanol for micro-computed tomography (microCT) and histomorphometry. The left tibia was fixed overnight in 10% neutral buffered formalin for staining of osteoclastic resorptive by-products via tartrate resistant acid phosphatase (TRAP). The length of the right tibia was measured with digital calipers. Morphological and cellular bone analyses focused on the proximal tibia because anabolic and anti-catabolic effects of a 3-wk WBV regimen were observed in this region (42). The right soleus muscle was physically fixed onto small wooden applicators to approximately retain its original in vivo length. The pinned muscle specimens were embedded in TBS tissue freezing medium (Triangle Biomedical Sciences, Durham, NC), snap frozen in liquid nitrogen cooled isopentane, and stored in an isopentane-filled Eppendorf tube at −80°C until sectioning.

MicroCT. Trabecular and cortical bone morphology of the right proximal tibia was reconstructed via microCT at a voxel size of 12 μm in all mice (MicroCT 40, Scanco Medical, SUI). The epiphysis (240 μm in length) and metaphysis (720 μm in length) were defined according to precise landmarks (22). For trabecular regions, bone volume fraction (BV/TV), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), trabecular number (Tb.N), and connectivity density (Conn.D) were determined. Cortical bone was analyzed from the metaphysis (surrounding the trabecular volume of interest). Cortical bone area (Ct.Ar), areas of the endocortical (Ec.En or bone marrow area) and periosteal (Ps.En or periosteal area) envelopes, cortical bone thickness (Ct.Th), principal and polar moments of inertia (I_{max}, I_{min}, and I_p) were calculated using Image Processing Language (IPL) as provided by the microCT manufacturer.

Histomorphometry. Following tomographic scanning, the right proximal and diaphyseal tibia were embedded in methyl methacrylate resin (MMA) using a standard protocol (42). The proximal specimens were sectioned longitudinally in the center with a microtome to yield 5 μm frontal sections (RM 2165 microtome, Leica). The evaluated regions in the epiphysis (trabecular bone) and metaphysis (trabecular and cortical bone) were similar to the regions scanned by microCT and spanned 200 μm in length in the epiphysis and 800 μm in length in the secondary spongiosa of the metaphysis. Because of a lack of consistent double labels at the periosteal surface of the metaphysis, indexes of cortical bone formation were only quantified at the endocortical surface of the metaphysis (Osteomeasure, OsteoMetrics, Atlanta, GA). Mineralizing surface with bone surface as referent (MS/BS, %), an indicator of the fraction of bone surfaces that are undergoing formation, was obtained by adding the ratio of double-labeled surface to bone surface (dLS/BS) to half of the single-labeled surfaces with bone surface as referent (sLS/BS, %) (32). Mineral apposition rate (MAR, μm/day), an indicator of the mean activity level at a given bone-forming surface, was calculated as the distance between double labels divided by the labeling interval (32). A standard measure of bone formation rates (BFR/BS, μm/yr) was calculated, with one year as referent (32).

Assessment of osteoclastic activity. Sections of the left proximal tibia were stained in situ for TRAP, an indicator of osteoclastic by-products, by previously verified methods (29). On overnight fixation in 10% neutral buffered formalin, tibiae were decalcified in 2.5% formic acid (pH 4.2) for 4 days and dehydrated. Samples were embedded in glycol methacrylate (GMA) according to the manual of the JB-4 embedding kit (Polysciences, Warrington, PA). Frontal sections were cut at 7 μm thickness and stained for TRAP activity. Hexazotization was achieved by mixing equal amounts of 4% $NaNO_2$ and 4% pararosaniline solution. Naphthol-ASTR-phosphate (Sigma, St. Louis, MO) was used as a substrate and the enzyme reaction was carried out in the presence of tartrate (10 mM) to demonstrate TRAP activity (pH 5 in 0.1 M acetate buffer). Sections were counterstained with methyl green to improve contrast (42). The ratio of osteoclast surface (Oc.S) to bone surface (BS) was determined for trabecular and cortical bone surfaces in the metaphysis and epiphysis using commercially available histology software (Osteomeasure). The epiphyseal and metaphyseal regions that were analyzed matched those of the histomorphometric analyses described above.

Muscle analysis. Prior to sectioning, each sample was re-embedded and snap frozen in Tissue-Tek OCT (Sakura Finetek, Torrance, CA). Multiple frozen transverse sections (8 μm) were cut from the mid-belly region using a cryostat at −20°C (CM3050S microtome, Leica, Germany). To identify myofibers, myocyte myosin ATPase activity was stained histochemically by standard methods that classify fibers into slow twitch and fast twitch. The preincubation (pH 10.4) inactivated the myosin-ATPase enzyme in fiber type I (slow), and the remaining active ATPase enzyme in fiber type II (fast) produced a black insoluble compound. Cross-sectional images of the soleus were taken under a light microscope with a ×4 objective (Axiovert 200M, Zeiss, Germany). Type I and type II fiber number and cross-sectional area per fiber were determined with Image J (NIH, Bethesda, MD). Total fiber area was calculated as the product of fiber number and cross-sectional area per fiber.

Statistical analysis. As primary comparisons (testing the hypotheses of this study), unpaired two-tailed *t*-tests contrasted the vibrated group (WBV) with the age-matched control group (AC). Secondary comparisons (aiding in the interpretation of the data), also via unpaired two-tailed *t*-tests, entailed age-related differences in musculoskeletal variables between the 8-wk-old baseline (BC) and 14-wk-old age-matched (AC) control groups. Changes in body mass during the 6-wk protocol were assessed by paired *t*-tests. Statistical significance was set at 5% (SPSS 13.0, Chicago, IL). All data were expressed as means ± SD.

RESULTS

Effect of WBV on body mass and indexes of skeletal growth. The mean body mass of the three groups was ~19.6 g at the beginning of the study. Each of the two experimental groups (AC, WBV) gained similar amounts of body mass (13–14%, $P < 0.01$) to reach 22.3 g at the end of the 6-wk experimental period. Tibial length at completion of the experimental period was greater than at baseline (15.7 ± 0.6 mm) but no significant differences were detected between age-matched control (16.6 ± 0.5 mm) and WBV (16.8 ± 0.6 mm) mice.

Bone formation and resorption. Compared with 8-wk-old baseline controls, indexes of bone formation, including mineralizing surface (MS/BS), mineral apposition (MAR), and bone

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formation rates (BFR/BS), were up to 85% lower ($P < 0.05$ each) in the tibial trabecular metaphysis and epiphysis of 14-wk-old age-matched control animals (Table 1), indicating a slowing of growth over the 6-wk experimental period. Similarly, MAR and BFR/BS in the tibial cortical metaphysis decreased by 70%, from 8.21 μm/day and 2,695 μm/yr to 2.56 μm/day and 870 μm/yr, during the experimental protocol ($P < 0.01$). Mechanical vibrations retained, at least in part, population levels of active osteoblasts in the trabecular metaphysis in which mineralizing surfaces (MS/BS) were 75% greater ($P < 0.01$) in WBV mice compared with age-matched controls (Table 1).

Normal growth over the 6-wk experimental period, as reflected by the difference between AC and BC, also decreased the activity of osteoclasts (Oc.S/BS) in trabecular bone by 25% ($P < 0.01$) in the tibial epiphysis and metaphysis. No significant differences in Oc.S/BS were observed between AC and WBV groups in any of the trabecular or cortical regions of interest (Table 1).

Trabecular bone morphology. In both the metaphysis and epiphysis of the tibia, normal growth over the 6-wk period significantly increased tissue volume (TV, bone marrow volume), trabecular bone volume (BV), trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular separation by up to 44% ($P < 0.05$), whereas trabecular number was significantly decreased ($P < 0.001$) (Table 1 and Fig. 1).

In addition to the age-related increase, superimposition of short-duration, 0.3 g, 45-Hz vibrations further increased bone marrow volume (TV) by 10.5% (1.33 ± 0.13 vs. 1.20 ± 0.10 mm^3, $P < 0.05$) and trabecular BV by 13.7% (0.28 ± 0.04 vs. 0.24 ± 0.02 mm^3, $P < 0.05$) in the tibial metaphysis (Fig. 1). The relative ratio of these two parameters (BV/TV), however, was not different between AC and WBV mice (0.21 ± 0.04 vs. 0.20 ± 0.01). WBV also had no significant influence on trabecular number, trabecular thickness, and trabecular separation in the metaphysis, or any morphological index of the epiphysis.

Table 1. *Indexes of bone formation, resorption, and bone morphology in the proximal tibia*

Index	BC ($n = 8$)	AC ($n = 12$)	WBV ($n = 12$)
Trabecular metaphysis			
MS/BS, %	27.8±5.3	10.1±3.0†	17.6±6.5‡
MAR, μm/day	2.44±0.26	0.96±0.37†	0.76±0.29
BFR/BS, μm/yr	236±39	35.1±16.0†	48.9±25.1
Oc.S/BS, %	31.1±14.3	23.2±7.3*	24.6±8.6
Tb.Th, μm	35.2±2.6	50.6±1.6†	51.9±4.2
Tb.Sp, μm	143±12	187±7.8†	187±20
Tb.N, mm^{-1}	7.2±0.6	5.2±0.2†	5.2±0.5
Conn.D	660±114	185±19†	188±30
Trabecular epiphysis			
MS/BS, %	32.7±17.6	7.6±2.2†	8.5±3.3
MAR, μm/day	1.61±0.35	0.68±0.19†	0.82±0.41
BFR/BS, μm/yr	208±111	18.9±7.8†	24.7±11.3
Oc.S/BS, %	34.9±11.4	22.3±6.9*	23.1±5.6
BV/TV, %	27.6±4.7	33.3±1.9†	33.8±1.6
Tb.Th, μm	45.9±5.2	54.8±3.0†	56.4±1.8
Tb.Sp, μm	894±52	1,069±44†	1062±38
Tb.N, mm^{-1}	15.7±1.4	12.0±0.7†	12.1±0.6
Conn.D	266±68	221±30	207±35

Values are means ± SD. MS, mineralizing surface; BS, bone surface; MAR, mineral apposition rate; BFR, bone formation rate; Oc.S, osteoclast surface; Tb.Th, trabecular thickness; Tp.Sp, trabecular separation; Tb.N, trabecular number; BV, bone volume; TV, tissue volume; Conn.D, connectivity density. *$P < 0.05$ between age-matched controls (AC) and baseline controls (BC); †$P < 0.01$ between AC and BC; ‡$P < 0.01$ between whole body vibration (WBV) and AC.

Cortical bone morphology. In the metaphyseal cortical bone of the proximal tibia, normal growth over the 6-wk period significantly increased cortical bone area ($P < 0.05$), bone marrow area ($P < 0.05$), periosteal area ($P < 0.01$), the maximum moment of inertia ($P < 0.01$), the minimal moment of inertia (0.51 ± 0.07 vs. 0.44 ± 0.05 mm^4, $P < 0.01$), and the polar moment of inertia (1.08 ± 0.15 vs. 0.85 ± 0.10 mm^4, $P < 0.01$) by up to 29% (Fig. 2). Cortical thickness was not significantly different between 8 and 14 wk of age (0.26 ± 0.03 vs. 0.26 ± 0.02 mm).

As contrasted to 14-wk age-matched control mice, the application of WBV further increased cortical bone area by 11% ($P < 0.05$), bone marrow area by 12% ($P < 0.05$), periosteal area by 12% ($P < 0.01$, Fig. 2), the polar moment of inertia by 25% ($P < 0.05$), and the maximum moment of inertia by 29% ($P < 0.01$, Fig. 2). WBV did not have a significant influence on the minimal moment of inertia (0.56 ± 0.06 mm^4) or cortical thickness (0.27 ± 0.03 mm).

Muscle area and fiber type. Compared with 8-wk-old baseline control mice, 14-wk-old age-matched control mice had a 41% greater ($P < 0.01$) soleus total cross-sectional area, a 60% greater ($P < 0.01$) total type I fiber area, and a 35% greater ($P < 0.05$) area per type I fiber (Table 2 and Fig. 3). Type II fiber characteristics, type I or type II myofiber number, and the ratio of either fiber type number to the total number of fibers did not significantly change during growth.

Compared with age-matched controls (AC), WBV mice had a 27% greater ($P < 0.05$) total cross-sectional area (1.04 ± 0.23 vs. 0.84 ± 0.14, Fig. 3), a 24% greater ($P < 0.05$) type I fiber area, and a 29% greater ($P < 0.05$) type II fiber area (Table 2). WBV did not significantly alter myofiber number, area per fiber of either type I or type II fibers, or the ratio of type I to type II fibers in the soleus muscle over AC (Table 2).

DISCUSSION

Data from these studies indicate that brief, low-magnitude, high-frequency mechanical signals, if delivered over a 6-wk period, can leverage the cellular response observed at 3 wk to improve the structural quality of bone. More specifically, superimposing a 15-min bout of WBV onto daily activities of growing, sexually mature mice increased trabecular bone volume and enhanced cortical moments of inertia in the metaphysis, both attributes critical to a stronger skeleton. As importantly, these signals stimulated an anabolic response in the soleus, resulting in a greater cross-sectional area of this muscle and indicating an overall improvement in the quantity and quality of the musculoskeletal system. Considering the critical importance of both muscle and bone in the prevention of falls and skeletal fractures, this early data suggest that low-level, high-frequency mechanical signals may provide benefits beyond direct effects on the skeleton and can enhance the health of the musculoskeletal system. Furthermore, these data offer some insight into small clinical trials in which low-level vibrations enhanced not only bone, but also muscle (15), and may ultimately serve as a means to improving our understand-

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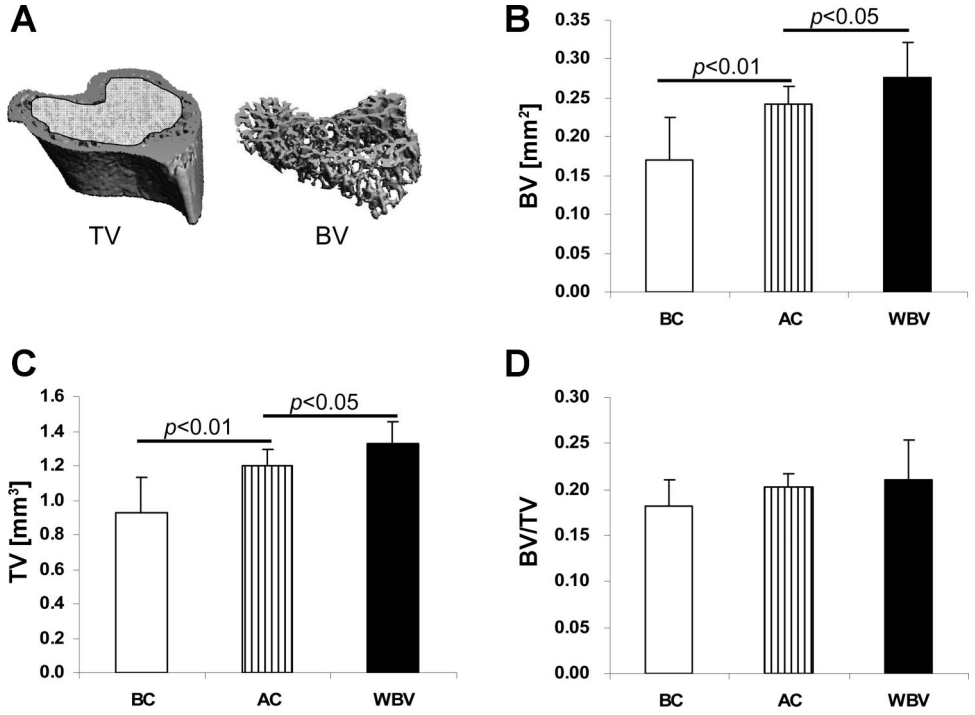

Fig. 1. Metaphyseal trabecular bone parameters of the proximal tibia in baseline controls (BC), age-matched controls (AC), and whole body vibrated mice (WBV). *A*: microcomputed tomography (microCT) image of bone marrow volume (TV) and trabecular bone volume (BV) of the tibial metaphysis. *B*: metaphyseal trabecular bone volume. *C*: metaphyseal bone marrow volume. *D*: metaphyseal trabecular bone volume fraction (BV/TV).

ing of the mechanisms by which vibratory stimuli are sensed by bone and muscle tissue.

Altered cellular activity in a previous 3-wk study (42) translated into significant morphological changes in this 6-wk study, resulting in enhanced cortical bone geometry and moments of inertia. In the absence of changes in material properties (42), greater moments of inertia are associated with greater bone strength and resistance to bending, showing that this low-level mechanical intervention can produce a stiffer and stronger bone that will be less prone to fracture. In contrast to the adult skeleton in which changes in bone morphology in response to vibrations appear to be focused on trabecular compartments (23, 33), these data suggest that growth and development is the most opportune phase for the skeleton to gain structural benefits from the application of low-level mechanical signals. While it is important that structural adaptations in trabecular and cortical bone were realized by doubling the experimental duration, even 6 wk is still a relatively short duration. Despite some clinical evidence that enhanced bone morphology acquired during adolescence, in particular for

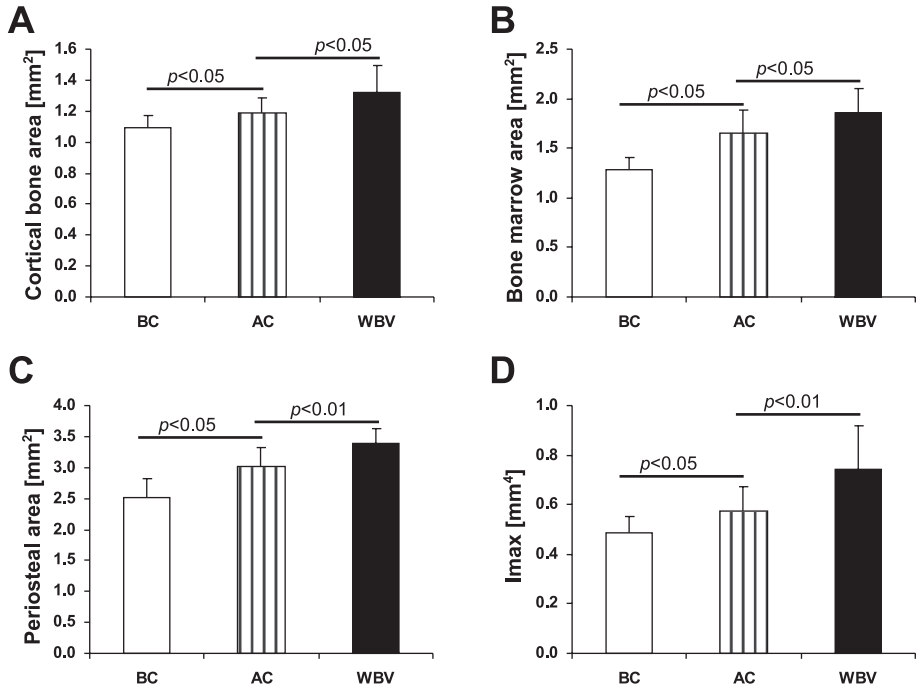

Fig. 2. Metaphyseal cortical bone parameters of the proximal tibia in BC, AC, and WBV. *A*: cortical bone area; *B*: bone marrow area; *C*: periosteal area; *D*: maximum moment of inertia (I_{max}).

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Table 2. *Fiber number, area, and relative percent of myofibers in the soleus of control and vibrated group*

Index	BC (n = 8)	AC (n = 12)	WBV (n = 12)
Total type I fiber area, mm^2	0.22±0.08	0.36±0.04†	0.45±0.12‡
Total Type II fiber area, mm^2	0.28±0.12	0.38±0.10	0.50±0.10‡
Type I fiber number	157±43	199±46	209±44
Type II fiber number	357±127	384±57	453±87
Area per type I fiber, μm^2	1,384±376	1,860±438*	2,185±472
Area per type II fiber, μm^2	781±253	993±195	1,114±238
Type I fiber number/total number, %	31.7±4.2	33.9±4.3	31.6±3.1
Type II fiber number/total number, %	68.3±4.2	66.1±4.3	68.4±3.1
Type I fiber number/type II fiber number, %	46.8±9.9	51.9±10.4	46.4±7.1

Values are means ± SD. *$P < 0.05$ between AC and BC; †$P < 0.01$ between AC and BC; ‡$P < 0.05$ between WBV and AC.

cortical bone, can be preserved into adulthood (3, 27), it will be critical to demonstrate in future studies that the skeletal attributes gained over this period of time will be retained to benefit during older age.

In addition to the anabolic response measured in the tibia, 15 min/day of extremely low-level mechanical signals superimposed onto normal locomotory activity stimulated a significant increase in cross-sectional area of the soleus muscle, thus providing benefits to the musculoskeletal *system*. The main function of the soleus is to support weight bearing and stabilization, and this muscle is activated ~90% of the time during standing or walking (16). Together with a recent study in young osteopenic women who, when exposed to short bouts of low-level WBV, accrued significantly greater amounts of bone and muscle than controls (15), these data indicate that g-forces at least an order of magnitude below those that have been previously associated with increased muscle strength and power (4, 7, 36) can be sensed by muscle. Future studies with more detailed assays and greater statistical power will be required to define the molecular, cellular, and morphological changes induced by low-level vibrations and to identify the mechanisms by which they occur.

The precise mechanisms by which musculoskeletal tissues sense and respond to mechanical vibrations have not been identified. Muscle spindle, golgi organ tendons, or somatosensory receptors are known to respond to oscillatory stimuli (10, 12). If the excitation frequency of the stimulus is close to the natural frequency of the particular soft tissue (8), muscle activity along with vibration damping and power dissipation may increase (38). Here, the frequency of the applied signal was in the range of the natural frequencies for muscle (10–50 Hz) (37), perhaps increasing muscle activity to dampen the applied vibration (37). The extremely small magnitude of the vibratory signal, however, may suggest that the mechanisms by which muscle and bone cells sensed the stimulus did not rely on the production of forces and deformations per se (9, 23). Cells, or the nuclei of cells, can sense high-frequency mechanical signals directly without experiencing significant deformations (2, 13, 14) and may provide an alternative pathway by which the signals were perceived. Considering that mice in this study were still growing, it is also possible that the mechanical signals influenced cell precursors within the bone marrow toward differentiation of bone and muscle cells (34). Whether the signal was modulated by independent sensory systems in bone and muscle or whether adaptation occurred interdependently (35), for instance by increased contact stresses at the tendon-bone interface (5), also remains unknown. Experimental testing of the above hypotheses will be critical toward identifying the underlying physical mechanisms and accelerate the optimization and translation of this very low-level mechanical intervention to the clinic.

The similarity in the response of the human and murine musculoskeletal to low-magnitude WBV not only indicates that the enhanced muscle cross-sectional area in young women (15) may have included both type I and type II fibers, but also that this animal model may serve to investigate the physical, cellular, and molecular basis of anabolic low-level mechanical

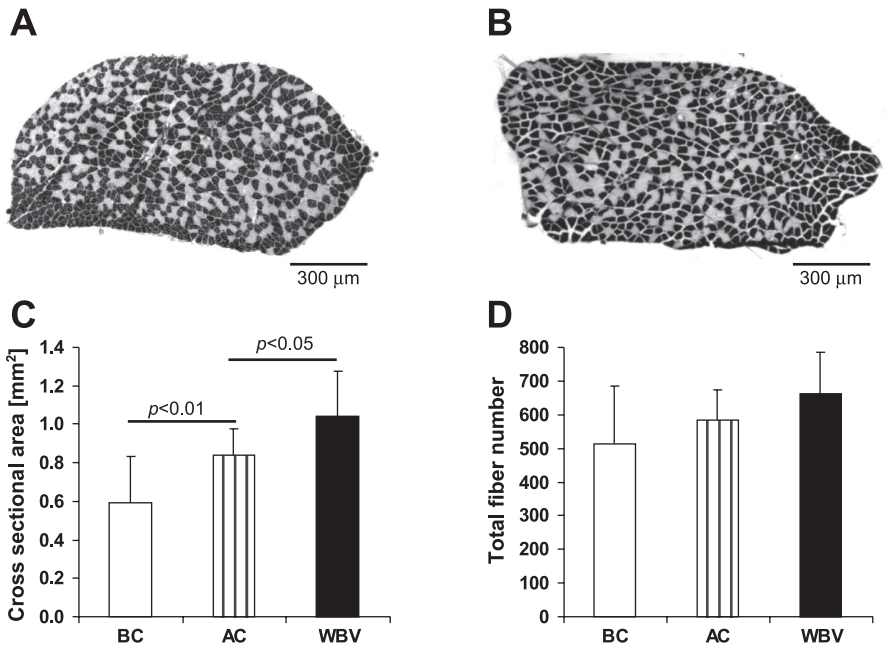

Fig. 3. Effect of short durations of whole body vibrations on the soleus muscle. The photomicrographs show an ATPase-stained cross section of the soleus from a mouse in the AC group (*A*) and WBV group (*B*). The white color presents type I muscle fiber, and the dark color presents type II muscle fiber. *C*: cross-sectional area of the soleus. *D*: total fiber number of the soleus.

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signals in the future. Previous data on the changes in fiber type in response to high-frequency physical stimuli are ambiguous, suggesting both a shift from type I to type II (17) and the reverse trend (41). In our study, no fiber type transformation but an increase in both fiber types was observed. In particular, the preferential loss of type II fibers during aging has been associated with an increasing instability and a greater tendency of falling (28), emphasizing the potential of this intervention to decrease the risk of falling (and fracture). Furthermore, in contrast to the studies in children with cerebral palsy and young women with low bone mineral density (15, 39), the musculoskeletal system of mice used here was not compromised by systemic stimuli and, thus, a pathologic musculoskeletal state is not a prerequisite for these mechanical signals to become effective.

In summary, this 6-wk study demonstrates that short daily periods of extremely low-magnitude, high-frequency mechanical signals have the ability to increase trabecular bone volume and cortical bone geometry in the tibial metaphysis, as well as enhance type I and type II muscle fiber areas in the soleus of adolescent mice. As the risk for osteoporotic fractures later in life may ultimately be reduced by maximizing the rates of muscle and bone accretion during puberty and early adulthood, stimulating the growing musculoskeletal system to increase bone quality and muscle quantity may present a unique opportunity to confer long-term health benefits (40). This study suggests that noninvasive and nonpharmacological low-level mechanical stimuli can be effective in producing beneficial structural changes in the adolescent musculoskeletal system, which, if maintained during adulthood, may serve to decrease the incidence of osteoporotic fractures and sarcopenia later in life.

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