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Effect of whole-body vibration training on bone mass in adolescents with and without Down syndrome: a randomized controlled trial

--Manuscript Draft--

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Abstract:	Introduction This study aims to observe the differences between adolescents with and without Down syndrome (DS) in the effects of 20 weeks of whole-body vibration (WBV) training, on bone mineal content (BMC), and density (BMD). Methods 26 adolescents (13 DS; 12-18 years) were measured with dual-energy x-ray absorptiometry before and after the intervention (3/week, 10 repetitions (30-60 seconds) and 1-min rest, frequency 25-30 Hz, and peak-to-peak displacement of 2 mm (peak acceleration 2.5-3.6 g)). Both, an intention-to-treat (ITT) analysis designed to assess the effects on bone mass, and a per-protocol analysis, designed to compare poor- and high-compliers, were performed. Results The ITT analysis revealed significant increases in all BMC and BMD parameters (dz = 0.66 to 1.64; all $p<0.05$) in the non-DS group, whilst DS group improved whole-body, subtotal (whole-body less head), upper limbs (ULIMBS), pelvis, lower limbs (LLIMBS) and spine BMC (dz = 0.75 to 1.76; all $p<0.05$) and subtotal, pelvis, LLIMBS, and spine BMD (dz = 0.73 to 1.28; all $p<0.05$). Significantly greater increases were evident in the absolute and percent changes of the non-DS group over DS group (d = 0.88 to 3.85; all $p<0.05$). ULIMBS BMD showed a tendency towards an interaction (f = 0.41 and p = 0.086) with higher increase for non-DS group. When a per-protocol analysis was considered, high-complier adolescents had 8.1 versus 5.3 % of gains in the spine BMC over poor-complier adolescents (d = 0.93; $p<0.05$). Conclusions 20 weeks of WBV training may improve BMC, and BMD in clinically relevant skeletal sites in both groups. Nevertheless, this type of training seems to provoke a lesser response in adolescents with DS than in those without DS.
Corresponding Author:	José Antonio Casajús, Ph.D., M.D. SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Ángel Matute-Llorente, M.Sc.
First Author Secondary Information:	
Order of Authors:	Ángel Matute-Llorente, M.Sc. Alejandro González-Agüero, Ph.D

	Alba Gómez-Cabello, Ph.D
	Julio Tous-Fajardo, Ph.D
	Germán Vicente-Rodríguez, Ph.D
	José Antonio Casajús, Ph.D., M.D.
Order of Authors Secondary Information:	
Author Comments:	Tuesday, June 16, 2015 Dear Editor, Please, find attached a revised version of our manuscript entitled "Effect of whole-body vibration training on bone mass in adolescents with and without Down syndrome: a randomized controlled trial" to be reconsidered for publication in your prestigious journal. We have taken into account all the comments made from the reviewer and a point-by-point response has been attached to our submission. In addition, we have highlighted in yellow the pertinent changes in the main manuscript for an easier revision. This manuscript represents original unpublished material; is not under consideration for publication elsewhere, and further, it will not be submitted for publication elsewhere until a decision is made regarding its acceptability for publication in your Journal. All authors have read and approved of this final version, are responsible for the reported research and have contributed significantly to the research of the present manuscript. There are no conflicts of interest and financial disclosures for any author of this manuscript. None of the authors have any financial interest. Sincerely yours, Jose A Casajús, M.D., Ph.D. Faculty of Health and Sport Sciences University of Zaragoza
Response to Reviewers:	Comments for the Author: Reviewer #1: Manuscript ID: OSIN-D-15-00365 The manuscript presented by Matute-Llorente and colleagues is clearly of interest to people working in all health related disciplines. The manuscript provides an interesting argument in the understanding of the relationship between Down syndrome changes in bone mass after vibration training. The structure of the document is well written and follows an appropriate structure that makes for an easy, detailed and informative read. Response: Thank you very much for your revision of our manuscript. All your comments have been addressed and explained below. Also, the changes have been highlighted in yellow in the manuscript in order to make easier the revision process. MINOR CONCERNS: P4L49 how will gender impact the changes in BMD between these two groups? R: Thanks for your comment, we have realised the excessive length and lack of clarity in that sentence. This sentence has been reworded in order to clarify that these studies were not comparing gender groups, but instead are completely different studies. The sentence states like this now: Results on WBV therapies are not entirely clear; some studies have shown increases in BMC at the lumbar spine [17], and areal BMD at the femur [18, 19] and the spine [19-21] in different disabled populations. On the other hand, changes on bone parameters were not present nor in osteopenic girls with adolescent idiopathic scoliosis [22] neither in children with Duchene muscular dystrophy [23] with a similar WBV therapy. P5L49 the authors first use of a table is "Table 2" ? Table 1 is not mentioned until later pages. R: thanks, that was a typo due to swap paragraphs after naming Tables. The paragraph involving WBV intervention has been moved above the one on the participants. Table 1 is now mentioned earlier than table 2.

P6L41 how was a 90 degree knee angle measured and maintained during the vibration intervention? how can the researchers be confident that this was the case for all subjects ? were the subjects allowed to hold onto a rail during the vibration intervention ?
R: The angle was not measured but it was observed and controlled by a specialist. There were no problems in squatting position working with non-DS group, all participants performed the exercise properly. The researchers are confident with exercise realization because all participants (with and without DS) were accompanied by a researcher in each training. The researcher was competent on the squatting technique due to the appropriate training, he provided verbal feedbacks when spotted a mistake. Participants were allowed to hold onto a strap during the vibration intervention. This has been added onto the manuscript.

P6L42 did the authors consider body composition and body position changing the dissipation of the vibration signal through the body and possibly impacting the changes to BMD/BMC ?
R: Yes we did. Authors were aware of the possible vibration dissipation throughout the body due to the squatting position; however, taking into account the special needs and learning difficulties of half of the participants, decide to use this easy and comfortable position. Also, being the first study with adolescents with DS we decided it would be safer to perform the therapy with a less aggressive protocol, compared with other for example standing on the platform.

P11L51 reword the sentence "For that reasons' …"
R: Reworded.
“It seems therefore realistic to affirm that young persons with DS may benefit from WBV interventions in order to improve their body composition, specifically their bone mass.”
References
Ref 38, 39, 42 incomplete and missing journal volume and page numbers
R: Ref 42 has been updated.
Refs 38 and 39 are still ahead of print.

Figures 2,3 these figures are repetitive as the data is available in the tables - not warranted and difficult to read.
R: Following the reviewer´s advice, figures 2,3 have been deleted.

Effect of whole-body vibration training on bone mass in adolescents with and without Down syndrome: a randomized controlled trial

A. Matute-Llorente[1,2], E-mail: amatute@unizar.es; A. González-Agüero[1,3], E-mail: alg28@aber.ac.uk; A. Gómez-Cabello[1,4], E-mail: agomez@unizar.es; J. Tous-Fajardo[5-6], E-mail: jtous17@yahoo.com; G. Vicente-Rodríguez[1,2], E-mail: gervicen@unizar.es; J.A. Casajús[1,2], E-mail: joseant@unizar.es

[1] GENUD (Growth, Exercise, Nutrition and Development) Research Group
University of Zaragoza C/Pedro Cerbuna nº 12, 50009, Zaragoza, Spain

[2] Faculty of Health and Sport Science (FCSD), Department of Physiatry and Nursing
Universidad de Zaragoza Ronda Misericordia 5, 22001-Huesca, Spain

[3] Department of Sport and Exercise Science
Aberystwyth University Ceredigion, UK

[4]Centro Universitario de la Defensa
Zaragoza, Spain

[5]Juventus Football Club
Turin, Italy

[6]Sports Performance Lab, Sport Sciences Research Group
INEFC Av. de l'Estadi 12, 08038 Barcelona, Spain

*Corresponding author: José Antonio Casajus, e-mail: joseant@unizar.es
GENUD Research Group
C/Pedro Cerbuna nº 12
50009 Zaragoza, Spain.
Phone: +34 876 533 755

Abstract

Summary

Whole body vibration training (WBV) attracts great interest as osteoporosis prevention strategy. Twenty-six adolescents with and without Down syndrome (13 DS; 12-18 years) performed 20 weeks of WBV. The results indicate that WBV seems to provoke a lesser response in adolescents with DS than in those without DS.

Introduction

This study aims to observe the differences between adolescents with and without Down syndrome (DS) in the effects of 20 weeks of whole-body vibration (WBV) training, on bone mineal content (BMC), and density (BMD).

Methods

26 adolescents (13 DS; 12-18 years) were measured with dual-energy x-ray absorptiometry before and after the intervention (3/week, 10 repetitions (30-60 seconds) and 1-min rest, frequency 25-30 Hz, and peak-to-peak displacement of 2 mm (peak acceleration 2.5-3.6 g)). Both, an intention-to-treat (ITT) analysis designed to assess the effects on bone mass, and a per-protocol analysis, designed to compare poor- and high-compliers, were performed.

Results

The ITT analysis revealed significant increases in all BMC and BMD parameters (dz = 0.66 to 1.64; all p<0.05) in the non-DS group, whilst DS group improved whole-body, subtotal (whole-body less head), upper limbs (ULIMBS), pelvis, lower limbs (LLIMBS) and spine BMC (dz = 0.75 to 1.76; all p<0.05) and subtotal, pelvis, LLIMBS, and spine BMD (dz = 0.73 to 1.28; all p<0.05). Significantly greater increases were evident in the absolute and percent changes of the non-DS group over DS group (d = 0.88 to 3.85; all p<0.05). ULIMBS BMD showed a tendency towards an interaction (f = 0.41 and p = 0.086) with higher increase for non-DS group. When a per-protocol analysis was considered, high-complier adolescents had

8.1 versus 5.3 % of gains in the spine BMC over poor-complier adolescents (d = 0.93; p<0.05).

Conclusions

20 weeks of WBV training may improve BMC, and BMD in clinically relevant skeletal sites in both groups. Nevertheless, this type of training seems to provoke a lesser response in adolescents with DS than in those without DS.

Keywords

Bone health

Down´s syndrome

Exercise

Osteoporosis

Vibration

Introduction

The increase in the life expectancy of individuals with Down syndrome (DS) [1] is fostering the appearance of some illnesses such as osteopenia or osteoporosis that previously were not diagnosed in this population. These diseases generate an increased risk of suffering from a bone fracture because of low bone density [2]. Also, taken into account that lower levels of bone mineral content (BMC) and bone mineral density (BMD) have been found in DS population [3-8], and that a low BMD was described by Lips et al. [9] as one of the main factors involved in fracture risk, it might be said that individuals with DS are a population at risk. Thus, special attention should be given to improve bone mass in DS population by implementing specific lifestyle interventions (i.e. exercise and nutrition). In fact, a recent study has questioned the use of antiresorptive therapy in DS population and focused attention on increasing bone mass by other interventions like weight-bearing training [10].

It has been well documented that physical exercise is one of the best non-pharmacological ways to improve several health aspects [11] including bone mass [12]. Low-amplitude high-frequency whole body vibration (WBV) training is recently receiving much attention for treating low levels of bone mass [13]. Nowadays, WBV has been applied mainly as a therapy method in children and adolescents with disabling conditions [14] and also in seniors [15, 16]. Results on WBV therapies are not entirely clear; some studies have shown increases in BMC at the lumbar spine [17], and areal BMD at the femur [18, 19] and the spine [19-21] in different disabled populations. On the other hand, changes on bone parameters were not present nor in osteopenic girls with adolescent idiopathic scoliosis [22] neither in children with Duchenne muscular dystrophy [23] with a similar WBV therapy. Nevertheless, the only common finding in almost every study was the lack of severe negative side effects [14, 20, 21, 23, 24] after the WBV interventions. For that reason, WBV has been defined as a well-tolerated training method which may be used as an intervention to increase bone health in

people with DS. Some efforts have been made on studying bone mass in DS populations [4-7], also focusing on the effects of some physical training interventions [25-28]. Despite this, information concerning body composition, specially bone mass, in youths with DS is scarce [29]. Furthermore, the fact that DS is a genetic condition, with possibly different expression in many genes, may have a direct influence in the adaptive response to WBV training. For this reason, it is also possible that musculoskeletal system of non-disabled populations may be or not as responsive to this type of training.

Therefore, the main aim of this study was to observe the differences between adolescents with and without DS in the effects of a WBV training program, on BMC and BMD. It was hypothesized that 20-weeks of WBV will show greater improvements in bone mass of adolescents with DS compared with those without.

Material and methods

The study design, protocol and consent forms were performed in accordance with the Helsinki Declaration of 1964 (revised in Fortaleza, 2013) and were reviewed and approved by the Research Ethics Committee of the Government of Aragon (CEICA, Spain) [C.I. PI10/026]. The research study was registered in a public database [NCT02380638]. The CONSORT 2010 Statement was used as a guideline for reporting a randomized controlled trial[30].

WBV intervention

The training protocol and the mechanical vibration device used in this study have been described in detail elsewhere[24]. In brief, the protocol consisted of three times per week, 10 repetitions (30 to 60 seconds) with a 1-min rest, a frequency 25-30 Hz and an a peak-to-peak displacement of 2 mm for 20 weeks. The detailed schedule of training is shown in Table 1. All participants exercised, with the same trainers in each session, on a vertical vibration platform (Power Plate® Pro5; PowerPlate, Amsterdam, The Netherlands) supervised by a

researcher during each session. The role of the researcher was to ensure safety and the correct performance (squat, bent knees at 90º) during all sets. The researcher was competent on the squatting technique due to the appropriate training, he provided verbal feedbacks when spotted a mistake. Participants were allowed to hold onto a strap during the vibration intervention. In addition to this, the researcher registered any mishap or problem throughout the intervention and kept track of the participants' attendance. Compliance was calculated as the percentage of actual time using the platform over the expected time during the 20-week treatment period.

Participants

A parallel randomized controlled trial was conducted with a total sample of 26 adolescents; 13 with DS (6 females) and 13 without DS (5 females), between 12 and 18 years (Table 2). Participants were recruited from three different schools and institutions of Aragón (Spain). Before the start of the study, an initial interview in each institution was conducted with the participants and their parents to inform about the aims and procedures of the study as well as the possible benefits and risks derived. A written informed consent from the parents of each participant and verbal assent from the participants were obtained. An experienced cardiologist examined the adolescents with DS and gave them permission to participate in the training. Adolescents without DS were healthy and were free of medication for at least 6 months prior to the beginning of the study.

The randomization process, generated by computer, divided the sample in 2 non-equal number groups due to possible withdrawals or removals for lack of attendance. Thus, both intervention groups (DS and non-DS) received WBV training for 20 weeks being asked to avoid any change in their day-to-day lifestyle during the course of the project. Groups were as explained with the Consort Flow Diagram (Fig. 1).

Anthropometric measures and puberty

Anthropometric parameters including height, measured with a stadiometer to the nearest 0.1 cm (SECA 225, SECA, Hamburg, Germany) and weight, measured with a scale to the nearest 0.1 kg (SECA 861, SECA, Hamburg, Germany) were measured without shoes and minimal clothing following the procedures by the International Society for the Advancement in Kinanthropometry (ISAK) [31]. Body mass index (BMI = weight (kg) / height^2 (m) was determined.

All participants underwent a physical examination to determine their stage of sexual development. An expert physician classified the participants by direct observation according to the stages proposed by Tanner and Whitehouse [32].

Determinations of bone

Details of dual energy X-ray absorptiometry (DXA) measurements carried out in our laboratory have been described in previous studies [5, 24, 33]. DXA equipment was calibrated daily with a lumbar spine phantom and step densities phantom following the Hologic guidelines. The *in vivo* coefficient of variation in measuring BMC (g), areal BMD (g/cm^2) and bone area of the whole body in our lab were 2.3, 1.3, and 2.6 %, respectively measured in 49 adolescents [33].

All subjects were assessed with the pediatric version of the QDR-Explorer software (Hologic Corp. Software version 12.4, Bedford, MA 01730) while the assessments in pre- and post-training moments were performed by the same technician who had been fully trained in the operation of the scanner, the positioning of subjects, and the analysis of results, according to the manufacturer's guidelines.

The primary outcomes were BMC and BMD measured for the whole-body (WBTOT), lumbar spine (L_1-L_4; SPINE) and proximal region of the femur (hip [HIP] and femoral neck [FNECK]). In addition, subtotal (total body less head; SUBTOT), upper and lower limbs (ULIMBS and LLIMBS) and pelvis (PELV) BMC, and BMD were also determined.

Calcium intake

Participants (parents in DS group) were specifically asked to give their current daily intake of dairy products to estimate calcium intake using a structured questionnaire. Afterwards, calcium calculations were made according to the *Centro de Enseñanza Superior de Nutrición y Dietética* (CESNID) tables of Spanish food composition [34].

Statistical analysis

Both, an intention-to-treat analysis which included all participants who began the protocol at baseline, and a per-protocol analysis designed to compare low- against high-compliers, were performed. The Statistical Package for the Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All values shown are presented as mean (standard deviation, SD), unless otherwise stated. The sample size was based on calculations for the longitudinal study to detect a 2 % change in whole body BMD, allowing for a between individual coefficient of variation in BMD of 5 %, with 95 % confidence and 90 % power. Kolmogorov-Smirnov tests showed normal distribution of the variables.

In the intention-to-treat analysis, Chi-square test was performed to evaluate differences in Tanner stage before and after the 20 weeks in both groups. Differences in physical characteristics between vibration groups (DS and non-DS) at baseline and after the training were compared with a two-independent-samples *t*-test.

Raw values for DXA measurements in pre- and post- intervention moments were compared with a two-independent-samples *t*-test and two-paired-samples *t*-test evaluated changes within each group over baseline. Thereafter, analyses of covariance (ANCOVA) were performed (adjusting by subtotal area, subtotal lean, height, calcium intake and Tanner stage for BMC and BMD parameters and by Tanner stage, weight and height for lean and fat masses) to obtain DXA values in pre- and post-training moments and calculate percentage of change.

Two-independent-samples Student´s *t* tests were used to compare both actual changes as well as the relative (percentage) changes over time for DS and non-DS groups.

Moreover, analysis of variance (ANOVA) for repeated measures 2 (condition) x 2 (time) were performed to determine the effects of the training on body composition parameters.

The per-protocol analysis was established to identify a threshold response. In this analysis participants were classified as poor compliers (attendance below 60 %) and high compliers (attendance equal or above 60 %). It was done following the results of previous research [19, 24, 35] showing that the some gains in bone mass after a WBV training were strongly correlated with a threshold of compliance.

Effect size were calculated for all the previous statistical tests according to the methods proposed by Cohen [36], and taking into account the cut-offs defined, the effect size can be small ($d \leq 0.2$ or $f \leq 0.1$), medium ($d > 0.2$ and < 0.8 or $f < 0.1$ and > 0.2), or large ($d \geq 0.8$ or $f \geq 0.4$).

Results

Six participants, three in each group (DS and non-DS), participated only in the first assessment and consequently, data from 13 adolescents with DS and 13 without DS were analysed for the primary outcomes. The Consort Flow Diagram is shown in Fig. 1.

Descriptive data

Descriptive data regarding age, weight, height, BMI, calcium intake and Tanner stage are shown in Table 2. Adolescents with DS were smaller than non-DS adolescents at pre- and post-training moments (d=1.21 and 1.28; p=0.01 and 0.05, respectively).

Intention-to-treat analysis

Table 3 summarizes the results with pre- and post-training DXA values for bone mass.

BMC at WBTOT, SUBTOT, PELV, LLIMBS, and FNECK; and BMD at PELV and HIP were significantly lower in DS group compared with non-DS group at pre- (d=0.88 to 1.24; all p<0.05) and post- training moments (d=0.88 to 1.31; all p<0.05).

BMD at WTOT and SUBTOT showed a tendency towards differences at pre-training (d=0.70 and 0.79; p=0.05 and 0.09, respectively) and differences after training (d=0.81 and 0.90; both p<0.05). For BMC ULIMBS and BMD LLIMBS a tendency towards differences at pre- and post-training moments were found (d=0.71 to 0.79; p=0.06 to 0.08).

In the case of HIP BMC, a significant difference was found at baseline (d=0.88 and p=0.03) but not after the training (d=0.80 and p=0.05), showing non-DS group higher values than DS group in both times.

After the 20 weeks of training, significant increases were found in all BMC and BMD parameters in the non-DS group (dz=0.66 to 1.64; all p<0.05), whilst DS group improved BMC at WBTOT, SUBTOT, ULIMBS, PELV, LLIMBS and SPINE (dz=0.75 to 1.76; all p<0.05) and BMD at SUBTOT, PELV, LLIMBS, and SPINE (dz=0.73 to 1.28; all p<0.05).

Table 4 presents the absolute and percentage-adjusted changes for bone DXA measures in each of the two groups. In BMC and BMD parameters, significantly greater increases were found in absolute and percentage changes of the non-DS group over DS group (d=0.88 to 3.85; all p<0.05). No significant condition (DS vs non-DS) by time interactions were found for any variable after the 20 weeks of training (all p>0.05). ULIMBS BMD showed a tendency towards an interaction (f=0.41 and p=0.086) with higher increase for non-DS group.

Per-protocol analysis

Table 5 summarizes the absolute and percentage changes measured from DXA divided in condition groups, comparing by compliance to the training. There were no significant differences between groups in the absolute changes for any of these DXA measures of bone

(all p>0.05). Highly compliant adolescents had 8.1 % versus 5.3 % of gains in the SPINE BMC over poorly compliant adolescents (d=0.93; p<0.05). No condition by time interactions were found for any variable (all p>0.05).

Discussion

The main finding of the present study is that 20 weeks of WBV training with the proposed protocol provoke a slightly different response in adolescents with and without DS.

To date, only four studies have examined the effects of a WBV intervention in adolescents with DS, looking at balance [37], balance and muscle strength [38], fat and lean masses [24], and one at bone health [39]. Whilst the previous studies compared the effects of WBV training against a DS control group, to our knowledge, this is the first attempt of performing WBV training in adolescents with and without DS analysing and comparing the effects of this type of training on body composition.

Firstly, González-Agüero et al. [24] reported that 20-week WBV training was not enough by itself for improving lean mass in adolescents with DS. With the same intervention protocol, Matute-Llorente et al. [39] pointed out that WBV training might be useful to improve different bone parameters in clinically relevant skeletal sites in adolescents with DS and Villarroya et al. [37] found positive effects in balance of DS adolescents although only under specific conditions, whilst there were no balance improvements of those adolescents without DS. In concordance, Eid [38] showed greater improvements in stability indices and muscle strength in children with DS after 6 months of WBV and receiving a physical therapy program, than those only receiving the physical therapy program. It seems therefore realistic to affirm that young persons with DS may benefit from WBV interventions in order to improve their body composition, specifically their bone mass.

The body composition values shown at pre- and post-training moments in this study by adolescents with DS are consistent with several previous studies [4, 6, 8, 29]. It has been well-documented that adolescents with DS have lower levels of muscle strength and poor bone-health, and so early interventions to stimulate muscle strength and bone accrual are of clinical importance in preventing osteoporosis [14]. Wysocki et al. [13] stated that the optimal target population for the WBV training has not been defined, while Slatkosvka et al. [40] pointed out in a meta-analysis that children and adolescents with compromised bones might be this target population. As previously stated, adolescents with DS have compromised bones so they could highly benefit from this type of intervention. This statement is also supported by a study carried out in mice with low BMD, where it was found that they were more sensitive to the mechanical stimulus than mice with normal BMD [41]; however, whether it happens in humans is yet to be elucidated.

Analysing our raw data, DS adolescents showed important increases for bone mass in several body sites such as the whole body, the limbs, the spine or the pelvis. The effects of WBV training on body composition and particularly in bone health have been also studied in other children with compromised bones such as cerebral palsy or idiopathic scoliosis [17, 22, 42]. Recently, Kilebrant et al. [42] demonstrated that WBV training had a modest effect on bone mass in children with severe motor disabilities after a 6-month intervention period. This is in accordance with another study carried out by Dalen et al. [17], who showed increased BMC at the lumbar spine as well as in both legs in children with cerebral palsy. Lam et al. [22] studied the effects of WBV training in adolescents with idiopathic scoliosis showing improvements in areal BMD at the FNECK of the dominant side and lumbar spine BMC. Despite different exercises, intensities and platforms have been used, the use of WBV training might be appropriate in disabled populations [14].

In the present study, adolescents without DS showed higher number of significant changes in BMC, and BMD than those with DS. Adjusting for appropriate covariates, non-DS group still showed higher absolute changes than DS group for all bone parameters. A recent study carried out by Ferry et al. [27] showed that 1 year of training with osteogenic activities increased BMC and BMD values at the lumbar spine (7 % and 4 % respectively) in children and adolescents with DS. In the present study, lower improvements were found for BMD at the whole body (3.6 % in the non-DS group and 0.9 % in the DS group), but it needs to be taken into account the training period was 20 weeks (5 months) against 12-months in Ferry's study. Besides, Kilebrant et al. [42] indicated that disabled children had a reduced capacity for bone accrual due to higher carboxy-terminal telopeptides of type I collagen and lower osteocalcin values. To our knowledge, the analysis and study of biochemical markers of bone formation and resorption in adolescents with DS remain unknown. Nevertheless, in a cohort of 30 community-dwelling DS adults, McKelvet et al. [10] stated that low BMD was correlated with a significant decrease in bone formation markers, compared to controls without DS, and pointed out that diminished osteoblastic bone formation and inadequate accrual of bone mass were responsible for the low bone mass in that particular population. It would be possible to hypothesize that the low BMD and BMC values in our study can be due to increased bone resorption and/or decreased bone formation. Further studies are needed to corroborate this hypothesis.

As with any training, it may be expected that the effectiveness of the intervention is related to the compliance. The per-protocol analysis revealed greater percentage of change in the high compliers, independently of the condition, than in the low compliers, achieving the differences statistical significance for BMC at the SPINE (5.3 vs. 8.1 %). Gilsanz et al. [19] found that women with low BMD who trained at least 2 minutes per day in a WBV platform, increased up to 3.9 % the trabecular bone of the spine and 2.9 % the cortical bone of the

femur. However, no additional benefits were obtained from training more than two minutes per day and the authors suggested that a biologic response was triggered rather than accumulated based on the study carried out by Rubin et al. [43]. In our study, a 60 % of attendance was established as cut-off between poor- and high-compliers. This cut-off would equal 4.2 minutes per day of WBV training, being higher than the 2-minute threshold proposed by Gilsanz et al. [19]. Important considerations should be taken into account, since Gilsanz et al. conducted a 12-month trial with the same vibration protocol (10 minutes, 30Hz, and 0.3g) which let them to establish a vibration threshold. In our study, WBV training was gradually applied increasing frequency and duration, and for that reason, further studies are needed to establish and appropriate vibration dose in DS population.

Adolescents with DS in this study attended special schools that incorporated physical activity programs over the last few years. This progress may have contributed to achieving some improvements in terms of health in the daily life of adolescents with DS but it could be interfering in our results. Despite of this, the present study suggests that WBV training has the potential to influence bone mass in reducing osteoporosis risk factors in adolescents with and without DS. In addition to this, our results might have also been influenced by the protocol used in the study. Higher intensities caused by higher peak-to-peak displacements and frequencies, the increase in the exercise intensity with unilateral exercises (one-leg Squat) or the addition of weights (i.e. 10 % of body weight in a backpack) could have generated greater increases; but the proposed 20-week WBV training was chosen following the study performed by Lam et al. [22], which indicated that WBV treatment might be more efficient when it is used in a structured way, two or three times per week for 10 minutes each time. Importantly, no negative side effects were found in our study as previously indicated [14, 17, 20, 23].

This study is not exempt of limitations; firstly, the absence of a control group who would have performed the same protocol (squat position for the same amount of time) with the

platform turned off, to observe the possible improvements due to the isometric exercise by itself. And finally, the lack of data about participants' physical activity levels could be masking some interactions with body composition. Further studies taking into account bone structure and bone metabolism markers might help to define whether an intervention of WBV alone is effective for improving body composition in population with and without DS. On the other hand, the main strength of this study is the inclusion of both genders and the use of an age-, pubertal status- and gender-matched non-DS group.

Conclusion

In conclusion, 20 weeks of WBV training with the proposed protocol may improve BMC, and BMD in clinically relevant skeletal sites in adolescents with and without DS. Nevertheless, this type of training seems to provoke a lesser response in adolescents with DS than in those without DS suggesting that specific training and adaptations should be studied. Identifying a threshold of response, the adolescents who trained over 60 % of compliance got higher increases than those under 60 %. Moreover, as no side effects or withdrawals were noticed during the intervention, WBV training could be defined as a safe and well-tolerated treatment in both groups.

Disclosures

Ángel Matute-Llorente, Alejandro González-Agüero, Alba Gómez-Cabello, Julio Tous-Fajardo, Germán Vicente-Rodríguez, and José Antonio Casajús declare that they have no conflict of interest.

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Fig. 1. Consort flow diagram of the follow-up of the participants

Table1
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Table 1 Protocol for WBV groups three times per week.

	Sessions	Frequency (Hz)	Peak-to-peak displacement (mm)	Duration (s)	Rest (s)	Repetitions	Vibration total time (min)	Training total time (min)	Peak acceleration (g)
Month 1	12	25	2	30	60	10	5	15	2.5
Month 2	12	28	2	30	60	10	5	15	3.2
Month 3	12	28	2	45	60	10	7.5	17.5	3.2
Month 4	12	28	2	45	60	10	7.5	17.5	3.2
Month 5	12	30	2	60	60	10	10	20	3.6

Table2
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Table 2 Pre-training physical characteristics of the participants.

	Pre-training						Post-training					
	DS (n = 13)		non-DS (n = 13)		Student´s t p	Cohen´s d	DS (n = 13)		non-DS (n = 13)		Student´s t p	Cohen´s d
	Mean	SD	Mean	SD			Mean	SD	Mean	SD		
Age (year)	15.0	2.0	14.0	2.2	0.245	0.47	15.5	2.0	14.4	2.2	0.191	0.52
Weight (kg)	48.4	9.2	56.6	16.1	0.127	0.62	49.3	9.3	58.8	15.6	0.071	0.73
Height (cm)	148.1	8.0	162.4	16.4	0.012	1.21	148.8	7.3	164.5	15.7	0.005	1.28
BMI (kg/m^2)	21.9	3.4	21.0	2.9	0.459	0.28	22.1	3.5	21.3	2.8	0.522	0.25
Calcium Intake (mg/day)	730.4	275.1	642.6	159.3	0.329	0.39	819.0	398.2	687.7	224.6	0.335	0.40
					Chi-square sig.						Chi-square sig.	
Tanner stage (I/II/III/IV/V)	0/1/2/3/7		1/2/4/0/6		0.279	-	0/1/2/2/8		1/1/4/1/6		0.683	-

DS Down syndrome group, non-DS non-Down syndrome group, BMI body mass index.
d=Effect size conventions, small (*d*≤0.2), medium (*d*>0.2 and <0.8), or large (*d*≥0.8).

Table3
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Table 3 Pre- and post-training bone values for both non-DS and DS groups (N=13 in each group).

		DS						non-DS						Independent *t* test			
		Pre-training		Post-training		Paired *t* test		Pre-training		Post-training		Paired *t* test		Pre-training		Post-training	
		Mean	SD	Mean	SD	*p*	*dz*	Mean	SD	Mean	SD	*p*	*dz*	*p*	*d*	*p*	*d*
BMC (g)	WBTOT	1453.87	272.36	1505.56	268.48	0.006	0.90	1983.40	756.38	2129.24	785.74	<0.001	1.63	0.031	0.93	0.016	1.06
	SUBTOT	1077.24	212.52	1131.06	201.89	<0.001	1.32	1533.58	632.17	1630.25	644.95	<0.001	1.48	0.026	0.96	0.018	1.04
	ULIMBS	185.14	42.11	193.71	36.13	0.019	0.75	238.80	97.51	252.69	98.34	0.006	0.91	0.087	0.71	0.060	0.79
	PELV	144.03	32.34	153.83	33.63	0.018	0.76	219.95	112.82	248.64	118.87	<0.001	1.64	0.035	0.91	0.015	1.08
	LLIMBS	494.92	105.98	521.66	102.56	<0.001	1.76	792.06	325.51	838.72	326.94	<0.001	1.30	0.007	1.22	0.005	1.30
	SPINE	41.52	8.96	44.04	8.50	0.005	0.94	47.27	18.89	50.52	18.94	<0.001	1.35	0.335	0.38	0.276	0.44
	FNECK	3.21	0.58	3.26	0.59	0.567	0.19	4.10	1.32	4.44	1.36	<0.001	1.49	0.042	0.87	0.011	1.12
	HIP	23.44	5.75	26.15	9.44	0.144	0.43	32.20	12.80	35.60	13.62	0.001	1.22	0.038	0.88	0.052	0.80
BMD (g/cm^2)	WBTOT	0.933	0.083	0.941	0.078	0.169	0.38	1.031	0.178	1.068	0.182	<0.001	1.43	0.091	0.70	0.035	0.90
	SUBTOT	0.803	0.071	0.819	0.066	0.001	1.28	0.905	0.167	0.931	0.165	0.002	1.10	0.059	0.79	0.037	0.81
	ULIMBS	1.290	0.128	1.304	0.118	0.176	0.41	1.321	0.182	1.350	0.169	0.009	0.85	0.630	0.19	0.433	0.31
	PELV	0.862	0.080	0.882	0.073	0.022	0.73	1.024	0.219	1.067	0.223	0.007	0.90	0.025	0.98	0.013	1.11
	LLIMBS	1.994	0.217	2.046	0.214	0.003	1.01	2.257	0.461	2.321	0.449	0.012	0.83	0.080	0.72	0.063	0.78
	SPINE	0.820	0.106	0.844	0.105	0.015	0.80	0.858	0.166	0.887	0.169	0.009	0.88	0.493	0.27	0.445	0.30
	FNECK	0.760	0.100	0.791	0.170	0.267	0.31	0.831	0.134	0.866	0.158	0.003	1.08	0.137	0.60	0.261	0.45
	HIP	0.812	0.090	0.846	0.129	0.062	0.57	0.922	0.141	0.970	0.150	0.001	1.21	0.027	0.92	0.033	0.88

DS Down syndrome group, non-DS non-Down syndrome group, BMC bone mineral content, WBTOT total whole-body, SUBTOT subtotal body (total body less head), ULIMBS upper limbs, PELV pelvis, LLIMBS lower limbs, SPINE lumbar spine, FNECK femoral neck, HIP total hip, BMD bone mineral density.

d=Effect size conventions, small (*d*≤0.2), medium (*d*>0.2 and <0.8), or large (*d*≥0.8).

Table4
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Table 4 Absolute and percent adjusted-change in bone DXA measures for both non-DS and DS groups (N=13 in each group).

		Absolute change						Percent change						Repeated measures Condition by time	
		DS		non-DS		Independent *t* test		DS		non-DS		Independent *t* test			
		Mean	SD	Mean	SD	*p*	*d*	Mean	SD	Mean	SD	*p*	*d*	*p*	*f*
BMC (g)	WBTOT	51.68	28.56	145.83	36.38	<0.001	2.87	3.69	2.35	7.92	2.31	<0.001	1.81	0.797	0.20
	SUBTOT	53.81	35.69	96.66	32.66	0.004	1.25	5.42	4.45	7.25	3.67	0.264	0.44	0.192	0.31
	ULIMBS	8.57	5.89	13.89	6.07	0.033	0.88	4.91	3.47	7.10	4.25	0.163	0.56	0.984	0.03
	PELV	9.80	5.88	28.69	9.09	<0.001	2.46	7.06	4.29	14.72	5.80	0.001	1.50	0.628	0.11
	LLIMBS	26.73	13.86	46.66	12.19	0.001	1.52	5.96	4.17	6.94	3.56	0.526	0.25	0.558	0.13
	SPINE	2.52	1.20	3.25	0.91	0.095	0.68	6.59	3.74	7.95	4.24	0.396	0.34	0.709	0.08
	FNECK	0.04	0.09	0.34	0.09	<0.001	3.33	1.46	3.01	9.02	3.96	<0.001	2.14	0.463	0.17
	HIP	2.70	2.18	3.39	1.75	0.380	0.34	11.95	8.63	10.98	4.82	0.727	0.13	0.711	0.08
BMD (g/cm^2)	WBTOT	0.008	0.008	0.037	0.007	<0.001	3.85	0.93	0.86	3.65	0.81	<0.001	3.25	0.264	0.26
	SUBTOT	0.016	0.011	0.025	0.010	0.029	0.85	2.05	1.62	3.00	1.44	0.129	0.61	0.458	0.17
	ULIMBS	0.013	0.007	0.029	0.021	0.029	1.02	1.06	0.60	2.41	1.89	0.027	0.96	0.086	0.41
	PELV	0.020	0.021	0.043	0.021	0.013	1.09	2.41	2.76	4.22	1.93	0.065	0.76	0.156	0.33
	LLIMBS	0.052	0.032	0.064	0.027	0.329	0.40	2.69	1.76	3.05	1.58	0.590	0.21	0.462	0.17
	SPINE	0.023	0.013	0.028	0.008	0.290	0.46	2.94	1.75	3.49	1.52	0.405	0.33	0.993	0.01
	FNECK	0.031	0.030	0.034	0.026	0.810	0.10	4.05	3.91	3.98	2.68	0.953	0.02	0.369	0.21
	HIP	0.033	0.018	0.047	0.019	0.069	0.75	4.11	2.21	5.11	1.75	0.216	0.50	0.815	0.05

DS Down syndrome group, non-DS non-Down syndrome group, BMC bone mineral content, WBTOT total whole-body, SUBTOT subtotal body (total body less head), ULIMBS upper limbs, PELV pelvis, LLIMBS lower limbs, SPINE lumbar spine, FNECK femoral neck, HIP total hip, BMD bone mineral density.

Repeated measures *p* values are obtained from the condition by time interactions adjusting by subtotal bone area, subtotal lean mass, height, calcium intake, and Tanner stage.

d and *f*= Effect size conventions, small ($d \leq 0.2$ or $f \leq 0.1$), medium ($d > 0.2$ and < 0.8 or $f < 0.1$ and > 0.2), or large ($d \geq 0.8$ or $f \geq 0.4$).

Table5
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Table 5 Using a per protocol analysis, the absolute and percent changes measured from DXA were compared between the poor compliers (lower than 60 % of compliance, N=8) and the high compliers (higher than 60 %, N=18).

		Absolute change						Percent change						Repeated measures Condition by time	
		Poor compliers		High compliers		Independent *t* test		Poor compliers		High compliers		Independent *t* test			
		Mean	SD	Mean	SD	*p*	*d*	Mean	SD	Mean	SD	*p*	*d*	*p*	*f*
BMC (g)	WBTOT	105.96	68.68	95.56	54.05	0.680	0.16	4.85	2.24	6.22	3.44	0.315	0.48	0.894	0.03
	SUBTOT	72.78	41.52	76.33	40.49	0.839	0.08	4.49	1.88	7.15	4.58	0.130	0.82	0.896	0.03
	ULIMBS	10.39	5.82	11.60	6.85	0.667	0.19	4.30	2.77	6.76	4.24	0.148	0.70	0.740	0.07
	PELV	20.10	16.92	18.86	10.04	0.852	0.09	8.01	5.65	12.17	6.35	0.125	0.69	0.509	0.15
	LLIMBS	35.26	15.13	37.33	17.22	0.772	0.12	4.75	1.49	7.20	4.32	0.135	0.84	0.989	0.03
	SPINE	2.79	0.96	2.93	1.19	0.771	0.13	5.37	1.41	8.12	4.47	0.028	0.93	0.188	0.31
	FNECK	0.16	0.20	0.20	0.16	0.654	0.22	3.52	4.64	6.00	5.33	0.267	0.49	0.277	0.25
	HIP	3.18	2.31	2.99	1.86	0.823	0.09	9.68	7.23	12.26	6.75	0.388	0.36	0.232	0.28
BMD (g/cm^2)	WBTOT	0.023	0.018	0.022	0.016	0.863	0.05	2.14	1.52	2.36	1.68	0.762	0.13	0.321	0.23
	SUBTOT	0.017	0.008	0.022	0.012	0.311	0.50	1.84	0.85	2.83	1.74	0.063	0.76	0.200	0.30
	ULIMBS	0.014	0.017	0.024	0.017	0.203	0.58	1.13	1.40	2.01	1.55	0.183	0.59	0.055	0.46
	PELV	0.032	0.027	0.031	0.023	0.917	0.04	2.94	2.21	3.49	2.67	0.621	0.22	0.914	0.03
	LLIMBS	0.046	0.022	0.063	0.031	0.174	0.65	2.01	0.99	3.26	1.76	0.077	0.91	0.836	0.04
	SPINE	0.022	0.007	0.027	0.012	0.373	0.55	2.54	0.89	3.51	1.81	0.165	0.71	0.188	0.31
	FNECK	0.039	0.026	0.029	0.028	0.429	0.37	4.70	3.11	3.71	3.40	0.493	0.30	0.529	0.14
	HIP	0.040	0.025	0.040	0.017	0.952	0.01	4.25	2.46	4.77	1.85	0.553	0.24	0.419	0.19

DS Down syndrome group, non-DS non-Down syndrome group, BMC bone mineral content, WBTOT total whole-body, SUBTOT subtotal body (total body less head), ULIMBS upper limbs, PELV pelvis, LLIMBS lower limbs, SPINE lumbar spine, FNECK femoral neck, HIP total hip, BMD bone mineral density.

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Figure 1
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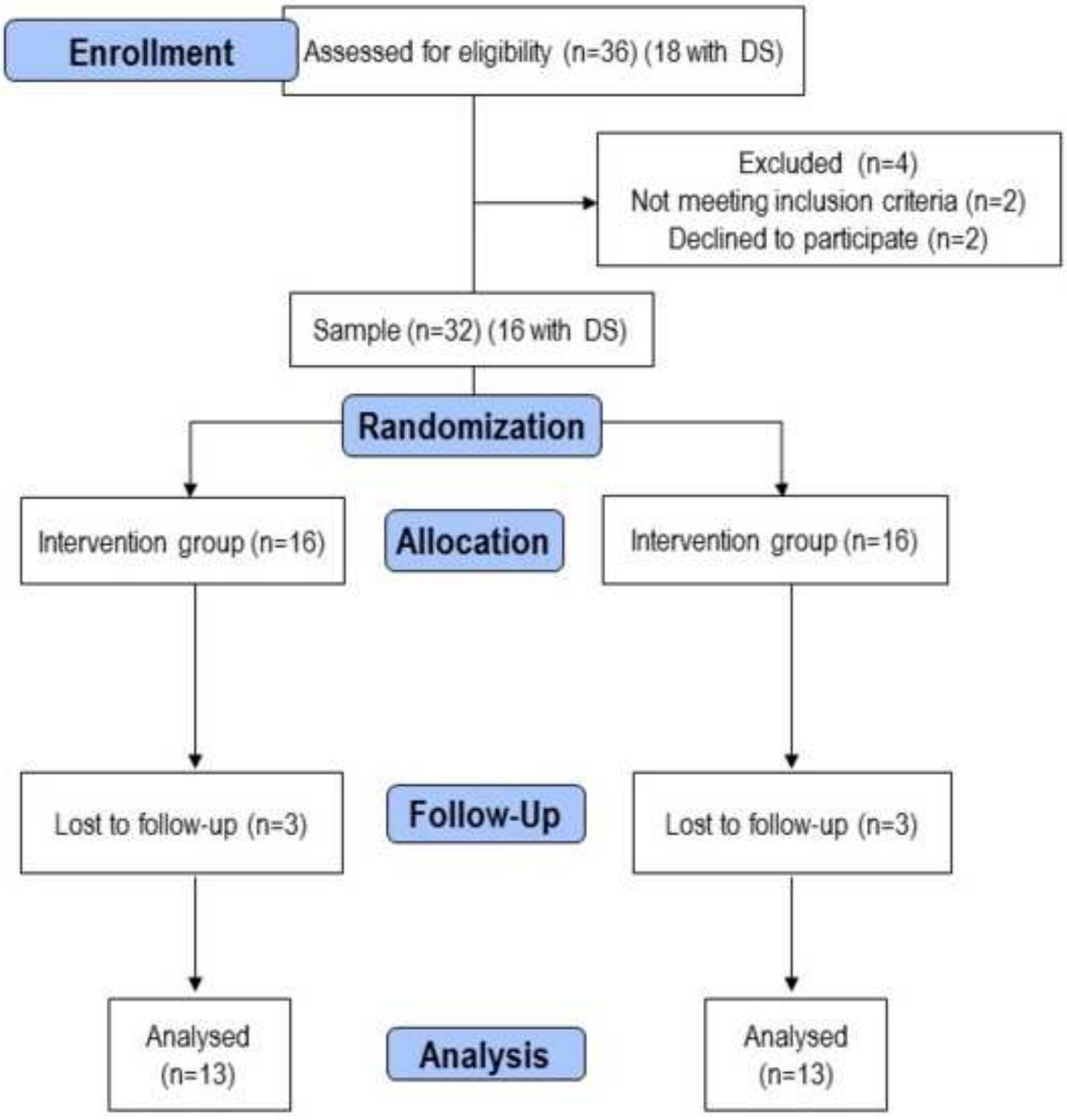

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First Author: Matute-Llorente Angel
E-mail: amatute@unizar.es

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