

Clinical Trials**High-frequency, low-intensity vibrations increase bone mass and muscle strength in upper limbs, improving autonomy in disabled children****Running title:** Vibrations: bone density, strength, quality of life, disabled children.**Authors:**

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Conflict of Interest Page

All authors have no conflicts of interest.

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Abstract

Background: Disuse osteoporosis in children is a progressive disease, which can affect quality of life. High-frequency, low-magnitude vibrations (HFLMV) act as anabolic signals for bone and muscle. **Methods:** A prospective, randomized double-blind and placebo controlled clinical trial to assess the efficacy and safety of regional HFLMV in disabled children was performed. Sixty-five children, 6 to 9 year-old, were randomized into three groups: placebo, 60 Hz and 90 Hz. In the two active groups, a 0.3 g mechanical vibration was delivered to the radii and femora for 5 minutes each day. Following the 6 months, the main outcome was bone mineral density (BMD) at the ultradistal radius (UDR), 33% radii (33%R) and femoral necks (FN). Secondary outcomes were area and BMC at the UDR, 33%R, and FN; grip force of the upper and lower limbs; motor function; and PedsQL evaluation. An intention-to-treat analysis was used. **Results:** Fifty-seven children (88%) completed the protocol. A significant increase was observed in the 60 Hz group relative to the other groups in BMD-UDR ($p=0.011$), grip force of the upper limbs ($p=0.035$), and “Daily activities item” ($p=0.035$). A mixed model to evaluate the response to intervention showed a stronger effect of 60 Hz, on patients with cerebral palsy, on UDR, and that between-subject variability significantly affected the response. There were no reported side effects of the intervention. **Discussion:** This work provides evidence that regional HFLMV is an effective and safe strategy to improve bone mass, muscle strength, and possibly independence in children with motor disabilities.

INTRODUCTION:

There is increasing evidence that high-frequency, low-magnitude vibration (HFLMV) serves as an anabolic signal to the musculoskeletal system, with clinical results showing improvements in bone mass, muscle strength and balance (1-4). Given the long-term complications of disuse osteoporosis in children (5,6), these non-invasive mechanical signals – if shown to be effective – could help combat this bone and muscle loss and ultimately may benefit quality of life in the disabled. Ward K. et al. reported promising results of HFLMV on bone mass in a pilot study, in which 20 children were stimulated with whole-body HFLMV (90 Hz @ 0.3 g, where 1 g is earth's gravitational field or 9.8 m/s^2) for ten minutes each day over a 6 month period. This study showed a net benefit to volumetric trabecular BMD in the proximal tibia, but neither diaphyseal bone nor muscle mass muscle parameters showed any response to the mechanical signals. However, this study also had several important weaknesses that may have influenced the outcomes; the heterogeneity in age and pubertal status of the subjects within the intervention and control groups and the low compliance, attributable to the degree of difficulty of standing on the platform (7). More recently, Ruch J et al. reported a small pilot study using whole-body vibration to treat children with cerebral palsy, with results indicating a *decrease* in bone quality in the treated group, but a small increase in walking speed. These investigators used mechanical vibration parameters that were distinct from those used in the Ward trial, with a protocol of high-magnitude (6 mm @ 18 Hz, delivering 7.8 g) vibration at relatively low frequencies (12 to 18 Hz) and with compliance compromised by complaints of fatigue and/or pain (8). Together, these studies indicate the potential benefits of a non-drug anabolic intervention for musculoskeletal complications of a disabled pediatric population but with full recognition

that there could be limitations to the use of the interventions. Therefore, the aims of this study were to assess the effectiveness and safety of the regional use of HFLMV in a randomized, double-blind, controlled trial in children 6 to 9 years old with motor disabilities.

METHODS:

Study protocol and subjects:

A randomized, double-blind, placebo-controlled study was conducted from January 2005 to June 2007. Sixty-five children with motor disabilities from the Telethon Rehabilitation Institute and Neurorehabilitation Unit of Catholic University were recruited to participate. This protocol was reviewed and approved by the Ethics Committee of our institution, and all legal guardians provided written informed consent.

Inclusion criteria:

Prepubertal boys and girls aged 6 to 9 years old and with motor disabilities caused by a first- or second-order neuron disease, myopathies, or peripheral nerve disease were recruited. To be included, the subject had to be able to sit and had to have an Intellectual Coefficient greater than 70. It was also required that the Z score of the bone mineral density of the FN was less than -1.5 in at least one limb, according to the values defined by Chilean healthy children reference values (9).

Exclusion criteria:

Subjects were excluded if they had any of the following: 1) malnutrition, 2) constitutional, endocrine or bone disease other than low BMD, 3) a requirement of

chronic medication (particularly anticonvulsant, glucocorticoid or bisphosphonate medications, 4) low calcium intake according to RDA, 5) 25 OH vitamin D < 15 ng/mL, 6) PTH > 45 pg/mL, 7) TSH higher than 4 UI/mL, 8) alkaline phosphatases > 300 UI/mL, and 9) joint contractures or involuntary movements (e.g., spasticity) that prevented adequate and reproducible positioning for DXA measurement.

Biochemical measurements:

At the start of the study, blood samples were drawn for 25 OH vitamin D, PTH, TSH, serum calcium, phosphate, total alkaline phosphatases, albumin, and transaminases measurements. The protocols have been published elsewhere (10,11).

Densitometry:

BMD, Bone Mineral Content (BMC), and bone areas at the UDR, 33%R and the FN were evaluated, at baseline and the end of the protocol, by DXA (Lunar Prodigy), using eENCORE Software 2004, version 845.006, Lunar Radiation, Madison, Corp. WI. For all regions of evaluation, the manufacturer's software was used with the low-intensity scan protocol. The BMC precisions determined using a 15-subject sample were 1.67% for UDR, 1.89% for 33%R and 2.52% for FN.

Muscle strength and motor function evaluation:

Three standardized and validated measures were used: the dynamometric muscular strength measure and two functional tests, the Gross Motor Function Measure (GMFM) (12-14), and the Motor Function Measure (MFM) (15,16). The GMFM assesses the gross motor abilities of children with CP and MFM of children with neuromuscular diseases. The scores of both scales were transformed into a percentage of the

maximum score, and these percentages were analyzed together as a representation of motor function. Functional testing and dynamometric evaluation were conducted by two physical therapists who had experience with the management and evaluation of patients with CP and neuromuscular diseases. A calibrated digital hand-held dynamometer (Nicholas MMT model 01160 and Baseline hand dynamometer) was used. Maximum isometric contraction values were measured in 11 different muscle groups (shoulder abductors and flexors, elbow flexors and extensors, wrist extensors, grip, hip flexors and abductors, knee extensors and flexors, foot dorsiflexors) by using the “break” technique (17,18), in which the examiner gradually overcomes the muscle force and stops at the moment the extremity gives way. The highest value of the three contractions with an interval of at least 30 s was recorded for each group. In our laboratory, interrater reliability (Interclass coefficient) was for all measurement more than 0.71; SEM 5.8% and test-retest reliability was more than 0.81; SEM 5.2%. Average values were calculated for the upper and lower limbs separately, which included proximal and distal muscle groups. This average was reported and used in the statistical analysis.

Quality of life evaluation:

At baseline and at the end of the protocol, the quality of life in the subjects was evaluated by PedsQL, using the cerebral palsy module for all children. This module includes the following items: Daily activities, School activities, Movement and balance, Pain and hurt, Fatigue, Eating activities, and Speech and communication (19). The questionnaire was validated for use in Chilean children.

Intervention:

To produce an axial propagation of vibration through the radii or femora, a high-frequency, low-intensity vibration device, active at either 60 or 90 Hz and with displacements of the actuator of 100 μm , was placed on both elbows and knees (limbs at an angle of 90°). The HFLMV device was pressed onto the limb and retained at the region of interest by wrapping with an elastic band and Velcro. The guardians of each subject were trained to use the device for 5 minutes on each limb, seven days per week, for 6 months. The devices were programmed to stop automatically following 5 minutes of use. The devices could be set up in the placebo or the active mode. In the placebo mode, the device produced a 500 Hz noise but did not vibrate. Devices were able to record the number of sessions, the duration, and the dates and times of use. This information was used to assess compliance. This device (Fig. 1) acts by acceleration and not by an action-reaction mechanism because this is not a weight bearing device. This device allows the displacement and frequency to be set independently; hence, when frequencies of 60 or 90 Hz were used, the amplitude always was 100 μm .

Safety:

Reporting of any minor adverse events was recorded by a monthly phone call request. Participants and their guardians were advised to report any serious adverse events immediately and to stop use of the device. The number and days of hospitalization and episodes of intercurrent disease were recorded monthly. The hospital review board was advised that, according to ISO-2631, Threshold Advisory for Human Exposure to Whole

Body Vibration, stated that vibration of the magnitude/frequency delivered by the HFLMV device was considered safe for up to four hours each day.

Statistics:

The primary outcome measures included the BMD at the UDR, 33%R and FN. Secondary outcome measures included the BMC and bone area at these sites, upper extremity muscle strength, and the quality of life score. Power calculations were based on data from a similar pilot study (7). A sample size of 54 (three groups of 18 subjects) was required to detect a 15% difference with 80% power at the $p=0.05$ level. We recruited 65 subjects, allowing for a 15% dropout. We analyzed data using an intention-to-treat approach, comprising all randomized subjects.

All variables were expressed as the median \pm range to account for non-normal distributions. Statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc, Chicago, USA), with differences assessed by the Kruskal-Wallis test. Right and left radii variables were analyzed separated; the same was done for the femora. So, the sample unit was the radii and femora. A variable that indicated that these two radii and femora belong to the same subject was included in the analysis. The effect of clinical variables on the response to HFLMV intervention was evaluated using a linear mixed model. The strength and direction of the linear relationship between two continuous variables was assessed with a Spearman correlation. A linear mixed model was performed to explain how the intervention affected the percentage of change in outcomes and the influence of clinical variables on this change. A p -value <0.05 was considered statistically significant.

RESULTS:

Baseline comparisons of the three groups are shown in Table 1. There were significant differences between groups with respect to gender distribution. In the mixed model, the effect of the HFLMV intervention was not influenced by gender, so no post-hoc adjustments were made. Databases from the two participating institutions were filtered by age, diagnosis, and QI. A total of 101 subjects were contacted. During the phone call, the eligibility criteria were evaluated. Eighty-three subjects met the inclusion criteria and were invited to participate. Of those who completed the protocol, the primary afflictions included the following: cerebral palsy (39); spinal muscular atrophy (3), Duchenne muscular dystrophy (9); congenital myopathies (3); and axonal polyneuropathy (3). Eleven of 57 children (19%) had sustained at least one fracture in the appendicular skeleton at least 6 months prior to enrollment.

Flow diagram of the study:

A flow diagram of the progress of screened subjects and randomization is shown in Fig. 2. Sixty-five children were enrolled in the study, and 61 met the biochemical and densitometric criteria. Fifty-seven children completed the six month protocol (93.4%). Two subjects in the 60 and 90 Hz groups withdrew because of the absence of any positive change, as observed by their parents.

Changes in bone mineral density:

As compared to baseline, the BMD-UDR value of the 60 Hz group increased $31.88 \pm 28.30\%$, which represented a significant increase above that in the placebo and 90 Hz

groups, ($p=0.011$; Table 2, Fig. 3). In contrast, the BMC-UDR in the 90 Hz group increased $6.42 \pm 14.32\%$, which also represented a significant increase above that in the placebo and 60 Hz groups ($p=0.035$). We did not observe any other significant differences in 33%R or FN.

In the 60 Hz group, the percentage changes in the BMD-UDR and 33%R were negatively correlated with the baseline values, $r= -0.148$ ($p=0.05$) and $r= -0.447$ ($p= 0.0001$), respectively. Additionally, the bone area at the UDR and 33%R correlated negatively with the initial values, $r=0.362$ ($p=0.0001$) and -0.311 ($p= 0.001$), respectively. We did not find any significant correlations for the 90 Hz group.

Changes in motor function and muscle strength:

Muscle forces measured at upper and lower limbs were evaluated independently. At six months, and compared to baseline, the average muscle forces measured for the upper limbs increased by $165.27 \pm 64.81\%$ in the 60 Hz group, whereas in the placebo and 90 Hz groups, the average muscle forces decreased by -19.98 ± 49.98 and -5.27 ± 94.26 , respectively, ($p=0.035$) (Fig. 3, Table 2). In contrast, the muscle strength at the lower limbs did not change significantly for any of the groups.

The percentage change in the motor function score in the 3 groups correlated positively with the following parameters: the percentage change in BMD-33%R ($r=0.693$, $p=0.004$), BMC-33%R ($r=0.679$, $p=0.005$), and area-UDR ($r=0.73$, $p=0.001$). The percentage change in the UDR-BMD correlated positively with percentage change in abduction and flexion strength at the upper limbs ($r=0.739$, $p=0.002$ and $r=0.674$, $p=0.006$, respectively). Additionally, the percentage change of the BMC-UR correlated with the percentage change of abduction strength of the upper limbs ($r=0.579$, $p=0.024$).

There were no significant correlations between muscle strengths and densitometric variables for the lower limbs.

Changes in quality of life assessment:

Changes in the PedsQL scores are shown in Table 3. The average score change was not significantly different, but the change in the “Daily activities item” score was significant. The “Daily activities item” included questions about how many problems the child had with the following: putting shoes on, buttoning a shirt, pulling a shirt on over the head, putting pants on, brushing their hair, getting into the bathroom to use the toilet, undressing to use the toilet, getting in and out of bathtub/shower, and brushing teeth. Thus, the “Daily activities item” assesses autonomy.

Compliance and adverse events:

Average compliances in terms of number of sessions prescribed were 34.92 ± 32.92 , 35.91 ± 28.36 and 42.13 ± 25.47 ($p=0.547$) for the placebo, 60 Hz and 90 Hz groups, respectively. The averages in terms of total time prescribed were $28.04 \pm 26.96\%$, $31.62 \pm 25.73\%$ and $34.22 \pm 23.31\%$ ($p=0.599$) for the placebo, 60 Hz and 90 Hz groups, respectively. Compliance did not affect the response to intervention, as evaluated by a mixed model. No side effects were reported in any of the three treatment groups. During the 6 month intervention, there were no significant differences in hospitalization days (placebo: 6/22, 60 Hz: 2/20, and 90 Hz: 7/16; $p=0.14$) or the number of intercurrent diseases (placebo: 19/22, 60 Hz: 2/20, and 90 Hz: 5/16; $p=0.13$) between the groups.

Effect of clinical variables on the response to intervention:

The results are shown in Table 4. Age, sex, weight, height, body mass index (BMI), vitamin D status did not affect the response to therapy. The baseline value significantly affected the response to the intervention; thus, the model was adjusted to take into account the initial values ($p < 0.0001$). The influence of the mechanical intervention on BMD and BMC was stronger with the 60 Hz signal than with the placebo or the 90 Hz signal (Estimation 34.18 %, $p < 0.0001$); in contrast, 60 Hz or 90 Hz were not different from placebo with respect to Area. The effect of the intervention on BMC, BMD, and BA was greater at the UDR than at the 33%R (estimation: 27.45%, $p < 0.0001$; estimation: 12.34, $p < 0.0001$; estimation: 3.5%, $p < 0.0012$, respectively). Additionally, the responses in BMD, BMC and BA were stronger in children with cerebral palsy than in children with other diseases (estimation: 26.51%, $p = 0.0001$; 9.84%: $p < 0.0001$, estimation: 4.24%, $p = 0.012$); the responses were also stronger for left side limbs for BMD and BMC (estimation: 0.52%, $p = 0.023$; estimation: 0.46%, $p = 0.01$, respectively). The between-subject variability significantly affected the response to the intervention with respect to BMD and BMC (estimation: 7.43%, $p = 0.011$; and estimation: 5.18%, $p = 0.013$, respectively). There was no a significant effect of therapy on FN.

DISCUSSION:

This study demonstrates that high-frequency, low-magnitude vibration can improve bone mineral density, muscle strength, and, ultimately, autonomy for children with disabling conditions. Importantly, this study emphasizes that these mechanical signals can be delivered to specific regions of the appendicular skeleton, rather than requiring exposure to whole-body vibration, which would limit use to those patients who are

ambulatory. We believe that these data provide evidence that this non-drug, non-invasive intervention is both safe and effective and could ultimately provide a means to strengthen the musculoskeletal system of those patients who are not mobile. Although the mechanism of action is not known, and compliance was far from ideal, these data support the hypothesis that mechanical signals need not be large to be effective, nor do they have to be endured for long periods of time. Because there were no reported complications of use, we are hopeful that these data provide supportive evidence for translating this biomechanical strategy into the clinical setting as a means of reducing the risk of fracture and building independence for children.

Moreover, this is the first study proposing regional rather than total-body stimulation. This kind of stimulation facilitates the use of this intervention on the upper limbs, which is not possible with total-body vibration.

The studies of Garman et al. (20) and Hwang et al. (21) showed that bone does not need to be loaded to be responsive to mechanical signals, and thus it may be that osteoblasts and their progenitors (mesenchymal stem cells) are sensitive to acceleration, thus facilitating the application of the HFLMV signal. Our study supports these findings in a clinical setting. We observed a more significant effect on the UDR than on the 33%R and FN scores, which suggests a stronger effect on trabecular bone than on cortical bone. However, not all, most studies in animals and humans are in agreement with a greater effect on trabecular bone (22-24).

Notably, a stronger effect in children with CP (first-order neuron disease) could indicate the importance of the indemnity of second neurons or peripheral innervations on the mechanical stimulation of osteogenesis. Sample et al. have reported that the

innervation of bone could be a critical mediator of Wolff's skeletal adaptation to mechanical signals. The authors demonstrated that an intense mechanical stimulus can translate to the contralateral control limb, and innervation is critical to the transformation of local loading into an adaptive event (25).

A stronger effect of HFLMV on the left side limbs could represent a confounder because the left side limbs showed lower baseline BMD and BMC values, and a negative correlation was found between the initial value and the percentage change.

We observed a stronger effect on bone and muscle strength in the 60 Hz group. Bone response, as evaluated by bone histomorphometry, are not linearly correlated with frequency. A review of animal studies showed that the optimal frequency appears to be around 50 Hz. Between 10 and 50 Hz, there is not significant difference in bone histomorphometry parameters. However, very low (1 Hz) and high (100 Hz) frequencies are significant less effective compared with the 10 to 50 Hz range. Thus, the stronger effect of 60 Hz stimulation in this work, is in agreement with the animal studies experience (26,27).

HFLMV did not have a significant effect on densitometric or strength parameters at the FN. In this protocol, evaluation at the FN may not be the best place to see the effect on mineralization. Evaluation of densitometric parameters at distal lateral femora, a site mostly composed of trabecular bone and a frequent site of fracture in these children, would be a better site to assess the effect of HFLMV (28). Another possible explanation is that we did not stimulate in the correct way. The signals may not have been transmitted or may have been damped out by sitting (29). Future studies could examine different frequencies, displacement, and assessment tools.

The work reported herein helps to emphasize that the frequency, amplitude, duration and transmission of vibration are all important variables that contribute to the anabolic response in muscle and bone (22,24). We demonstrated that subject variability significantly modified the response to HFLMV. In agreement, studies in the clinic (7) and laboratory (24) have emphasized that genetics and subject variability play critical roles in mechanosensitivity and the response to treatment. Of note, the benefit on muscle strength appears to be more consistent across studies (30-34). Xie et al. have shown that these mechanical signals are anabolic to both bone and muscle (35). However, a synergy has been demonstrated between muscle and bone mass and strength (36,37).

We observed a significant negative correlation between the percentage BMD change and the initial value. This finding has also been reported for antiresorptive therapy. HFLMV may be more effective in more osteoporotic children. Thus, even this small signal may be sufficient to mobilize the adaptive response in the bone.

A significant improvement in the “Daily activities item” of the PedsQL questionnaire was observed, although this study did not have sufficient power to assess this outcome. This improvement emphasizes the importance of bone mass on motor performance. Additionally, pamidronate has been shown to improve motor abilities in children with osteogenesis imperfecta (38). The improvement in independency observed in these children is in agreement with the strong increase in muscle strength at the upper limbs demonstrated in this study. Thus, enhanced mobility could contribute to bone mass and muscle strength in a virtuous cycle. An increase in autonomy is a primary clinical goal, which has a major impact on the life of these children.

The strengths of this study were as follows: 1. the homogeneity of subjects in terms of age, anthropometry and pubertal stage, 2. the strict eligibility criteria that controlled for most variables affecting growth and mineral accretion, 3. the new way of delivering mechanical signals, and 4. the compilation of muscle, bone and quality of life data to determine if this treatment could benefit autonomy.

The limitations of this study were as follows: 1. a sample size that was too small and a duration that was too short to study the effect of HFLMV on the fracture rate, 2. the patients were not evaluated by computed tomography, which could better evaluate the effect of HFLMV on true volumetric density and on bone geometrical and mechanical variables, and 3. the evaluation of the mechanical signal and how it was delivered (transmissibility) to the regions of interest could have been more comprehensive.

In summary, this work demonstrated that the use of HFLMV is an effective and safe therapy to improve bone mass and muscle strength as well as indices of autonomy in children with motor disabilities. The improvement in independency was likely possible due to the innovation of regional use.

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Table 1: Clinical characteristics of the subjects at baseline.

Item	Placebo n=21	60 Hz n=22	90 Hz n=18	p
Age (years)	9.14±1.42	8.46±2.05	8.37±1.11	0.270
Sex (F/M)	5/16	10/12	10/8	0.041
Weight (K)	31.89±8.95	24.86±6.49	27.25±7.90	0.678
Height (m)	129.43±9.76	121.76±11.34	125.83±8.95	0.967
BMI (K/m ²)	18.73±3.45	16.46±2.31	16.92±3.27	0.433
BMD-UDR (g/cm ²)	0.23±0.05	0.22±0.03	0.23±0.04	0.061
BMC-UDR (g)	0.58±0.24	0.55±0.17	0.59±0.17	0.077
A-UDR (cm ²)	2.75±0.49	2.52±0.530	2.52±0.52	0.296
BMD-33%R (g/cm ²)	0.41±0.07	0.37±0.07	0.40±0.05	0.072
BMC-33%R (g)	0.84±0.21	0.75±0.23	0.81±0.31	0.346
A-33%R (cm ²)	1.91±0.27	1.93±0.52	1.73±0.39	0.296
BMD-FN (g/cm ²)	0.652±0.153	0.593±0.114	0.643±0.157	0.372
BMC-FN (g)	1.45±0.81	1.00±1.38	1.26±0.46	0.141
A-FN (cm ²)	2.08±0.72	1.67±0.48	1.87±0.51	0.171

1 st neuron/2 nd neuron/ other disease (n)	18/1/2	19/1/2	14/2/2	0.457
Motor function (%)	69.99±23.88	45.90±21.79	63.12±28.55	0.558
Muscle strength (K) upper limbs	4.04±2.06	1.94±1.82	2.50±2.14	0.168
Muscle strength (K) lower limbs	4.88±2.04	2.16±2.51	3.48±2.94	0.162
Alkaline phosphatases (UI/L)	214.14±78.58	215.1±62.17	233.79±82.62	0.791
25 OH vitamin D ng/mL	19.61±4.07	21.39±7.83	20.54±8.00	0.091
PTH pg/mL	39.73±14.51	30.45±14.46	36.06±13.16	0.179
TSH uUI/L	2.31±0.92	2.73±1.17	2.91±1.42	0.445

Table 2: Percentage change from baseline in outcomes and comorbidity.

Item / % of change	Placebo n=21	60 Hz n=22	90 Hz n=18	p
BMD-UDR	0.90±19.05	31.88±28.30*	5.20±8.63	0.011
BMC-UDR	-1.35± 18.21	-0.01± 30.78	6.42±14.32 [#]	0.035
Area-UDR	-4.43±25.67	2.55±30.07	1.60±20.56	0.490
BMD-33%R	7.33±10.99	11.58±14.78	7.10±8.14	0.160
BMC-33%R	7.33±10.99	11.58±14.78	7.10±8.14	0.060
Area-33%R	-4.43±15.82	2.55±12.76	1.60±21.23	0.346
BMD-FN	1.66±31.26	1.59±10.34	2.42±36.73	0.703
BMC-FN	-6.49±21.21	-5.23±40.34	-11.18±38.78	0.177
Area-FN	-7.84±32.56	-2.20±50.85	-9.72±54.14	0.140
Motor function (%)	-3.25±4.80	1.41±6.10	1.03±13.78	0.078
Muscle strength upper limbs	-19.98±49.89	165.27±64.81*	-5.27±94.26	0.035
Muscle strength lower limbs	8.82±64.03	76.21±114.44	70.32±96.71	0.441
Hospitalization days	0.27±0.62	0.10±0.05	0.44±0.65	0.142
Intercurrent diseases (n)	0.86±0.57	0.10±0.06	0.31±0.51	0.138

Table 3: Effects of the intervention on percentage of change of BMD, BMC, and Area; and the influence of clinical variables on this change. Results of the linear mixed model.

VARIABLE	% Change of BMD		% Change of BMC		% Change of Area	
	Estimation	P	Estimation	P	Estimation	P
Treatment group						
Placebo	0*		0*		0*	
60 Hz	34.18	<0.0001	11.32	0.020	6.23	0.041
90 Hz	0.34	NS	0.000	<0.0001	-0.27	NS
Diagnosis group						
1 (1st neuron)	26.51	<0.0001	9.84	<0.0001	4.24	0.012
2 (others)	0*		0*		0*	
Radius site						
UDR	27.45	<0.0001	12.34	<0.0001	3.5	<0.012
33%R	0*		0*		0*	
Body side						
Right side	0*		0*			
Left	0.52	0.023	0.46	0.03	0.01	NS
Between-subject variability	7.43	0.011	5.18	0.013	1.25	NS

Table 4: Percentage change from baseline in PedsQL scores.

Item	Placebo % n=21	60 Hz % n=22	90 Hz % n=18	p
Daily activities	21.18±72.28	46.52±104.39*	-17.85±51.26	0.046
School activities	6.85± 12.55	1.17± 10.11	3.64±15.00	0.447
Movement and balance	-12.89±20.88	-5.96±22.62	2.56±12.42	0.083
Pain and hurt	3.40±11.40	6.43±13.77	4.03±14.25	0.832
Fatigue	6.07±12.51	11.58±14.78	7.10±8.14	0.278
Eating activities	6.92±11.68	4.30±7.43	2.72±11.32	0.506
Speech and communication	0.99±6.85	1.93±10.12	0.18±14.61	0.828
Total score	19.13±27.56	11.90±24.73	-2.20±29.83	0.234

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Figure legends:

Figure 1: A child undergoing stimulation of the radius (A) and femur (B) using the device (arrows).

Figure 2: Flow chart summarizing the experimental design and the distribution of the participants following screening and randomization.

Figure 3: A: Percentage change from baseline in BMD-URD; the 60 Hz group was significantly different from the other groups, $p= 0.011$. B: Percentage change from baseline in grip strength of the upper limb; the 60 Hz group was significantly different from the other groups, $p= 0.045$. C: Percentage change from baseline in the PedsQL “Daily activities” scores; the 60 Hz group was significantly different from the other groups, $p= 0.046$.

Table 2: * The 60 Hz group was significantly different from the placebo and 90 Hz groups. # The 90 Hz group was significantly different from the placebo and 60 Hz groups.

Table 3: Result of the linear mixed model. Estimation represents the percentage change of BMD, BMC and Area in response to the intervention. All variables were adjusted using the baseline values. 0* represents the reference variable, against which the other variables were compared. NS= not significant.

Table 4: * The 60 Hz group was significantly different from the placebo and 90 Hz groups.



Figure 1

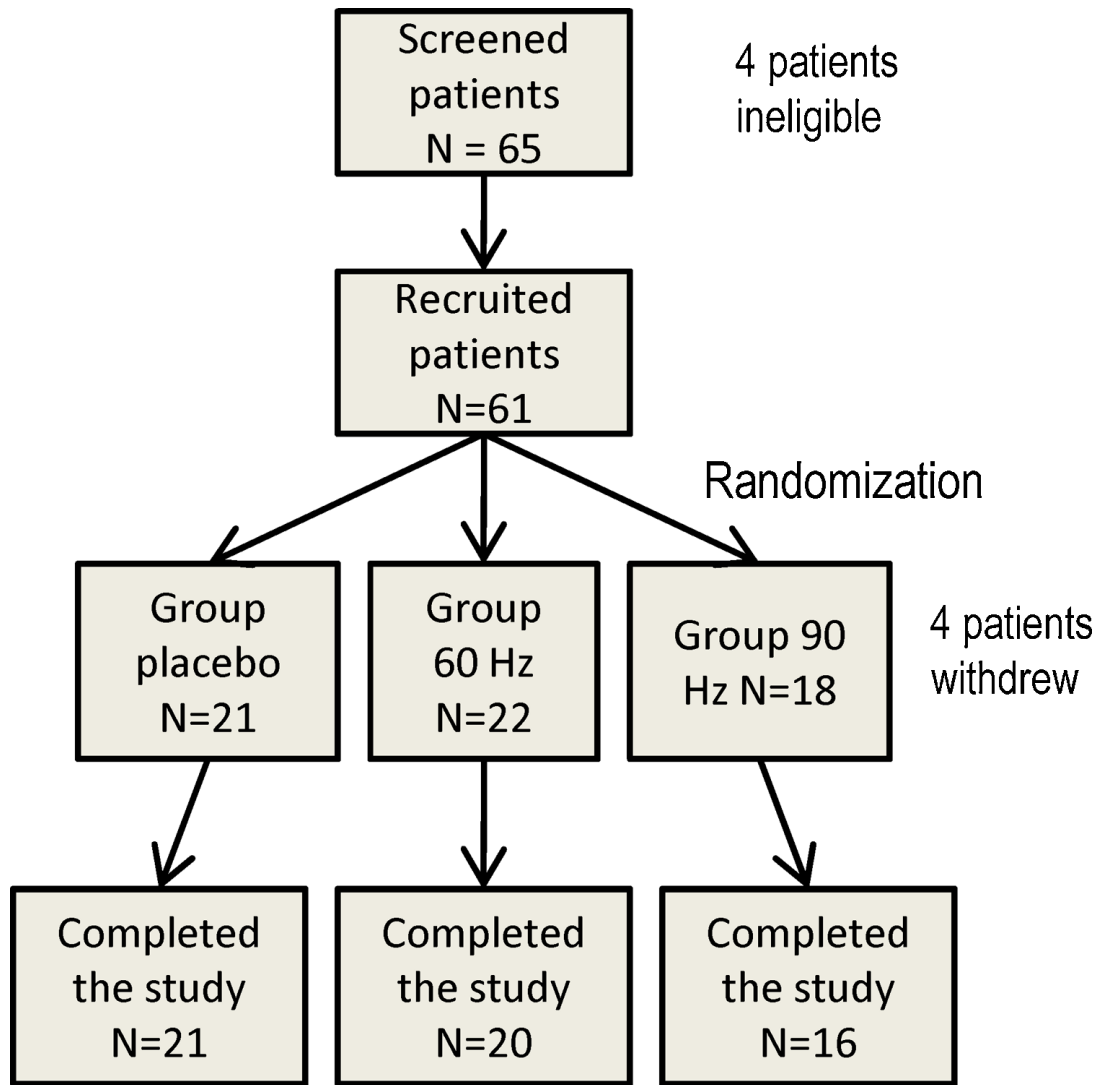


Figure 2

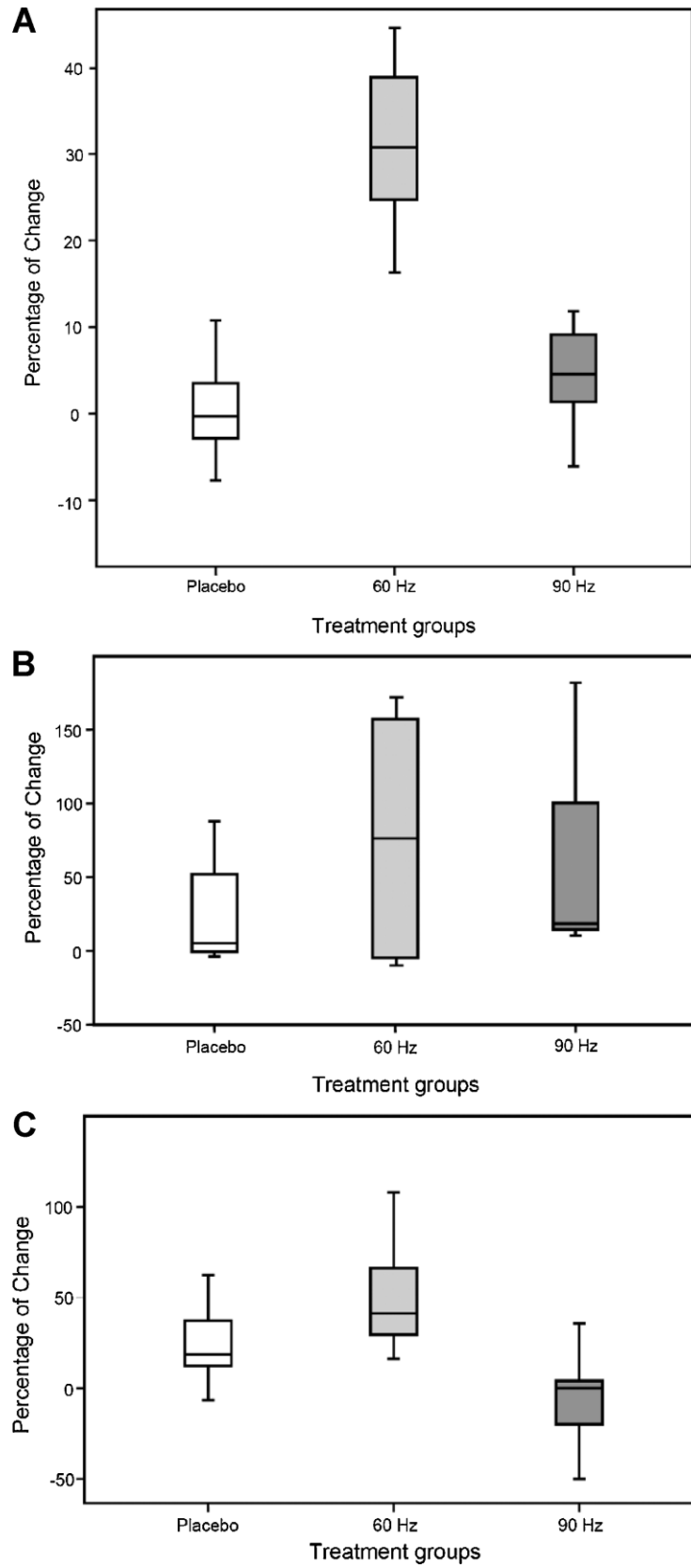


Figure 3