Effect of Physiotherapy on the Promotion of Bone Mineralization in Preterm Infants. A Randomized Controlled Trial.

Galaad Torró-Ferrero (✉ galaad.torro@um.es)

University of Murcia

Francisco Javier Fernández-Rego

University of Murcia

Juan Jose Agüera-Arenas

Hospital Universitario Virgen de la Arrixaca

Antonia Gómez-Conesa

University of Murcia

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Abstract

Preterm infants have a low level of bone mineralization compared to those born at term. The purpose of the present study was to investigate the effect of reflex locomotion therapy (RLT) on bone mineralization and growth in preterm infants and compare its effect against other physiotherapy procedures. 46 preterm infants born at 29-34 weeks were randomized to three groups: One group received RLT (n=17); other group received passive movements with gentle joint compression (n=14); and control group received massage (n=15). All treatments were carried out at the neonatal unit lasting one month. The main outcome measure was the bone mineralization measured with Tibial Speed of Sound (Tibial-SOS). All groups were similar in terms of gestational age (31.8 ± 1.18), birth weight ($1,583.41 \pm 311.9$), and Tibia-SOS ($1,604.7 \pm 27.9$), at the beginning of the intervention. At the end of the study, significant differences were found among groups in Tibial-SOS [$F(4,86)=2.77$, $p=0.049$, $\eta_p^2 = 0.114$] in benefit of RLT group. In conclusion, RLT has been effective in the improvement of Tibial-SOS, and has been more effective than other physical therapy modalities; therefore it could be considered as one of the most effective physiotherapeutic modalities for the prevention and treatment of osteopenia of prematurity.

Introduction

Osteopenia of prematurity is characterized by a reduction on the bone mineral content. It is multifactorial, progressive and has variable severity. It appears in 30% of children under 1,500 g and occurs in more than 50% of newborns weighing less than 1,000 g¹.

Postnatal bone mineralization in preterm babies is significantly behind of the expected intrauterine bone mineralization²; in addition, poor mineralization rate is maintained in children and young adults born prematurely^{3,4}, this situation leads to a reduction in peak bone mass, weaker bones, shorter height, and higher fracture rate compared to term infants⁵.

Mechanical stress is one of the most stimulating factors for bone formation and growth; and while physical activity increases bone mass in children, adolescents and adults⁶⁻⁸, inactivity leads to bone resorption and a decrease in bone mineral density^{8,9}.

Physiotherapy modalities have shown effectiveness in the treatment of osteopenia in preterm infants, obtaining favourable results when applying passive movements with gentle compression¹⁰⁻¹⁴.

Based on data from the adult population, active versus passive mobilizations are more effective for the prevention of osteopenia¹⁵, however, in order to develop an active movement in preterm infants, methods that do not require their will are necessary to achieve it. In this sense, reflex locomotion therapy (RLT)¹⁶ may be a suitable method of choice.

The RLT consists in a stimulation of the central nervous system (CNS) by proprioceptive stimulation, which activates innate locomotion patterns. These stimulation provokes a reaction that involves the whole body, producing synergistic muscle contractions, which leads to specific active and involuntary movement patterns¹⁶⁻¹⁹. This is because through the RLT, CNS activation takes place from the spinal level to the subcortical and cortical areas^{18,19}.

Taking all this into account, we set out to investigate the effect of active-resisted mobilizations that are triggered by proprioceptive stimulation from RLT, on bone mineralization and growth in preterm infants and to compare their effect against another passive modality of Physiotherapy, that has previously demonstrated its effectiveness¹⁰⁻¹⁴.

Results

52 preterm infants admitted to HUCVA were selected to be included in this study (Figure 1) from February 2016 to July 2020. Of them, 6 were discarded because they met an exclusion criteria prior to randomization: one of them developed intraventricular hemorrhage grade IV, two developed necrotizing enterocolitis that required surgical intervention, and the remaining maintained mechanical ventilation at the time of acquiring the full enteral nutrition. The 46 infants were randomized to three groups (EGrlt: 17, EGpmc: 14, and CG: 15) Of the 46 preterm infants who started the study, 11 (23.91%) could not be performed the last measurement (4 from the EGrlt, 4 from the EGpmc and 3 from the CG), as they were discharged prior the end of the 4 weeks of scheduled treatment. (Figure 1)

The three groups were similar in terms of GA (M=31.8; SD=1.18; p=0.75), birth weight (M=1,583.41; SD=311.9; p=0.247) and birth weight Z score (M=-0.29; SD=0.93; p=0.084), being a group of very preterm infants with low birth weight. The groups were also similar in terms of age (M=33.5; SD=1.24; p=0.689) and Tibial-SOS (M=1604.7; SD=27.9; p=0.706) at the beginning of the intervention; however, significant differences were found regarding gender (p=0.000; female in EGrlt=58.82%; EGpmc=50%; CG=40%) (Table 1).

Table 1
Baseline data

	EGrlt (n = 17)	EGpmc (n = 14)	CG (n = 15)
Gestational age (weeks)	31.76±1.41	31.99±1.36	31.66±0.65
Birth weight (g)	1,508.24±321.5	1,695±404.65	1,564.47±151.06
Birth weight Z score	-1.33±0.23	-0.49±0.26	-0.78±0.25
Gender (female)*	10 (58%)	7 (50%)	6 (40%)
Gestational age at the beginning of the intervention (weeks)	33.45±1.33	33.74±1.47	33.34±0.89
Weight at the beginning of the intervention (g)*	1,588.59±247.23	1,965.14±449.32*	1,653.6±138.23
Height at the beginning of the intervention (cm)	41.37±1.99	43.96±2.99	42.9±1.20
Head circumference at the beginning of the intervention (cm)	29.06±1.22	30.71±1.73	29.6±1.14
Tibial speed of sound at the beginning of the intervention (m/s)	1,608.06±29.73	1,599.64±29.3	1,605.6±25.62
* <i>p</i> <0.05 for differences among groups. CG = Control Group. EGpmc = Experimental group treated with passive movements with gentle compression. EGrlt = Experimental group treated with reflex locomotion therapy. Data is presented as Mean±SD.			

No adverse effects were detected in the participants as a consequence of the different types of treatments.

Tibial speed of sound

Regarding Tibial-SOS, significant differences were observed among the different types of interventions regarding the different moments of measurement $F(2,86) = 40.42$, $p < 0.001$, $\eta^2 = 0.223$], the values obtained by the different groups [$F(2,43) = 3.64$, $p = 0.034$, $\eta^2 = 0.105$] and the interaction of the variable in the different groups [$F(4,86) = 2.77$, $p = 0.049$, $\eta^2 = 0.038$]

The results show that there are differences among the groups, regarding how they evolve at the different moments of measurement in the Tibial-SOS, and if we look at Figure 2, we can see that the group with the best evolution is the EGrlt; and the group with the worst evolution is the CG.

Results among the groups and the different moments of measurement show significant differences in the Tibial-SOS between the infants who received RLT compared to those who received massage at two weeks after starting treatment, being better the results of EGrlt ($p=0.016$; $CI95\%=6.607$ to 36.01). At the end of the treatment, EGrlt showed significantly better Tibial-SOS values compared to EGpmc ($p=0.015$; $CI95\%=5.399$ to 33.8) and CG ($p=0.001$; $CI95\%=12.859$ to 40.47). There were no meaningful differences in the Tibial-SOS values between the group treated with PMC and the one treated with massage both in the second evaluation ($p=0.729$; $CI95\%=-6.891$ to 21.15) and in the third ($P=0.666$; $CI95\%=-5.452$ to 19.58) (Table 2, Figure 2).

Table 2: Effect of the different interventions in the Tibial speed of sound (m/s) and in the anthropometric measures during the study period

Finally, we can observe that all groups significantly lose bone mineralization between the first and third measurement (EGrlt: $p = 0.043$, $95\% CI = 0.355$ to 30.704 ; EGpmc: $p = 0.001$, $95\% CI = 9.993$ to 43.436 ; CG: $p < 0.001$, $95\% CI = 23.579$ to 55.888), but this loss is less pronounced in the EGrlt, since in this group there are no significant differences between the first and second measurements ($p = 0.069$; $95\% CI = -0.815$ to 28.581), neither between the second and the third ($p = 0.950$; $95\% CI = -6.816$ to 10.11); on the contrary, in the other two groups, we did observe these significant losses between the first and second measurements (EGpmc: $p = 0.013$, $95\% CI = 3.446$ to 35.84 ; CG: $p < 0.001$, $95\% CI = 17.085$ to 48.381).

Anthropometric measurements

Regarding weight, significant differences were observed among the different moments of measurement [$F(2,10.535) = 197.30$, $p < 0.001$, $\eta^2 = 0.628$] and regarding the groups [$F(2,9.057) = 8.617$, $p = 0.008$, $\eta^2 = 0.346$], but not for the variable interaction [$F(4,8.81) = 1.601$, $p = 1.6$, $\eta^2 = 0.046$]. With regards to height, differences are found among the measurement moments [$F(2,11.394) = 82.073$, $p < 0.001$, $\eta^2 = 0.402$], but not among the groups [$F(2,8.556) = 1.985$, $p = 0.196$, $\eta^2 = 0.173$], neither regarding the interaction of the variable [$F(4,9.359) = 1.753$, $p = 0.22$, $\eta^2 = 0.035$]. Finally, regarding head circumference, differences are found for the moments of measurement [$F(2,34) = 148.027$, $p < 0.001$, $\eta^2 = 0.543$], but not regarding the group [$F(2,17) = 1.605$, $p = 0.23$, $\eta^2 = 0.14$] nor the interaction [$F(4,34) = 0.827$, $p = 0.517$, $\eta^2 = 0.013$] (Table 2, Figure 3).

Regarding the Z scores, no differences are observed in terms of the interaction of these variables on the group with respect to weight [$F(4,86) = 1.541$, $p = 0.215$, $\eta^2 = 0.067$], height [$F(4,86) = 1.068$, $p = 0.354$, $\eta^2 = 0.047$] and head circumference [$F(4,86) = 0.482$, $p = 0.697$, $\eta^2 = 0.022$].

When analyzing the results among the different groups and the different moments of measurement in relation to weight, height and head circumference, we found that all groups showed statistically significant improvements in weight, height and head circumference, after each

		EGrlt (Mean±SD)	EGpmc (Mean±SD)	CG (Mean±SD)	Pairwise comparison	Mean Difference	95% CI	
							Lower Bound	Upper Bound
Tibial-SOS (m/s)	Pre- treatment	1,608.06±29.73	1,599.64±29.3	1,605.6±25.62	EGrlt=EGpmc	8.42	-13.39	30.22
					EGrlt=CG	2.46	-17.72	22.63
					EGpmc=CG	-5.96	-26.89	14.97
	2 weeks	1,594.18±23.47	1,580±20.67	1,572.87±15.99	EGrlt=Egpmc	14.18	-22.51	30.60
					EGrlt>CG*	21.31	6.61	36.01
					EGpmc=CG	7.13	-6.89	21.16
	Post- treatment	1,592.53±21.2	1,572.93±16.51	1,565.87±16.32	EGrlt>EGpmc*	19,60	5.40	33.80
					EGrlt>CG*	26,66	12.86	40.47
					EGpmc=CG	7.06	-5.45	19.58
Weight (g)	Pre- treatment	1,588.59±247.23	1,965.14±449.32	1,653.6±138.23	EGrlt<EGpmc*	-348.32	-752.00	-80.00
					EGrlt=CG	-150.00	-260.00	-20.00
					EGpmc>CG*	230.94	-75.00	570.00
	2 weeks	2,007.82±286.97	2,431.79±501.62	2,256.73±229.87	EGrlt<EGpmc*	-485.34	-1030.00	-50.00
					EGrlt=CG	-296.45	-490.00	-130.00
					EGpmc=CG	201.53	-270.00	710.00
	Post- treatment	2,302.94±331.11	2,726.07±510.05	2,598.07±396.25	EGrlt<EGpmc*	-635.11	-1029.40	-240.80
					EGrlt=CG	-552.75	-851.50	-254.00
					EGpmc=CG	82.36	-355.10	519.80
Height (cm)	Pre- treatment	41.37±1.99	43.96±2.99	42.9±1.2	EGrlt<EGpmc*	-5.00	-7.00	-0.50
					EGrlt=CG	-2.00	-3.50	0.00
					EGpmc=CG	2.00	-1.00	5.00
	2 weeks	44.59±3.29	46.04±2.48	45.73±1.96	EGrlt=Egpmc	-1.63	-4.72	1.47
					EGrlt=CG	-0.88	-3.97	2.22
					EGpmc=CG	0.75	-1.64	3.14
	Post- treatment	45.85±2.69	47.18±2.84	46.53±2.05	EGrlt=Egpmc	-2.08	-4.31	0.16
					EGrlt=CG	-0.45	-2.56	1.66
					EGpmc=CG	1.63	-0.90	4.14
Head circumference (cm)	Pre- treatment	29.06±1.22	30.71±1.73	29.6±1.14	EGrlt<EGpmc*	-1.76	-3.90	0.40
					EGrlt=CG	-0.53	-1.94	0.89
					EGpmc=CG	1.23	-0.51	2.98
	2 weeks	30.88±1.36	32.36±1.97	30.93±2.43	EGrlt=Egpmc	-1.11	-3.78	1.55
					EGrlt=CG	-0.34	-2.19	1.51
					EGpmc=CG	0.78	-1.30	2.86
	Post- treatment	32.06±1.34	33.43±1.79	32.1±2.87	EGrlt=Egpmc	-1.67	-3.58	0.24
					EGrlt=CG	-1.10	-2.96	0.76
					EGpmc=CG	0.57	-1.02	2.16
<i>*p<0.05 for differences among groups. CG = Control Group. EGpmc = Experimental group treated with passive movements with gentle compression. EGrlt = Experimental group treated with reflex locomotion therapy. 95% CI = 95% Confidence Interval. Data is presented as Mean±SD.</i>								

measurement compared to the previous one. Although if we observe the graphs (Figure 3), the group that evolves the best in weight gain is the CG, in height the EGrIt and regarding head circumference all evolve in a similar way.

At the beginning of the study, the weight in the EGpmc was significantly higher than the weight observed in the EGrIt ($p = 0.034$; 95% CI = -752 to -80). Regarding the second measurement, carried out two weeks after treatment was started, there were no longer differences in weight between the groups treated with PMC and RLT ($p = 0.091$; 95% CI = -1030 to -50), but there were differences between the CG and EGrIt ($p = 0.02$, 95% CI = -490 to -130). At the end of the treatment, we found differences in weight in favor of EGpmc ($p = 0.0025$; 95% CI = -1029.4 to -240.8) and CG ($p = 0.011$; 95% CI = -355.1 to 519.8) with respect to EGrIt.

Despite these differences, as no differences were found for the interaction of the variables, we can conclude that all groups evolve equally in terms of weight gain, height and head circumferences.

Harms

There were no adverse events reported due to intervention in any of the three groups.

Discussion

The expected sample size was 66 preterm infants, 22 for each group; however, due to sepsis affecting the hospital's NICU, the unit was forced to close. No more patients were admitted and ongoing studies had to be postponed. This situation led us to reach the scheduled closing date, so the study had to end. Therefore, the total sample size was 69.69% of the total sample size (46 newborns distributed in three study groups), showing a significant difference in relation to the intervention studied.

The main objective of this research is to verify the effect of RLT, understood as active-resisted mobilizations, on bone mineralization and growth in preterm infants and to compare its effect against other passive Physiotherapy modalities.

In this term, the outcome of this study indicates that RLT has a positive effect on the bone mineralization measured with QUS; consisting on a reduction of the drop in bone mineralization characteristic of preterm infants that can lead to osteopenia.

When comparing the three treatments performed, the modality with RLT has shown better results in the Tibial-SOS values with respect to the group treated with PMC and to the massage group (control) showing a small effect size; however, no significant differences were found between the group treated with PMC and the control group, contrasting with the results obtained by other authors, which highlight the positive effect of PMC compared to control groups where placebo was not applied^{26,36,37}, although in one of them, the times and frequency of PMC treatment were greater³⁷. In our study, in line with other authors²²⁻²⁵, the results show that massage has no impact on bone mineralization.

Despite the energy expenditure that RLT treatment could entail for the baby, considering that it performs active-resisted work, there has been no negative effect in relation to measures of weight, height and head circumference, since as the data showed, all groups evolved equally in terms of anthropometric variables. Anthropometric measurements have also not been diminished in children in the EGpmc and CG groups, same as other studies carried out with low birth weight babies treated with PMC compared with control without treatment or placebo with caresses^{23,25,26,36}, in which, as in our study, no significant differences were found among the study groups in weight, height, and head circumference. These results are supported by those obtained by the Z scores, which show no differences among the groups.

In the present study, the EGrIt group started from a point of significant disadvantage regarding weight, height and head circumference compared to the EGpmc (Table 2). Although infants treated with RLT showed a lower weight than the group treated with PMC, the EGrIt significantly increased their weight in each measure, showing a similar gain to the rest of the groups, since no differences were observed in terms of interaction (Figure 3). An explanatory hypothesis of this result may be due to the consideration of RLT as an active exercise, taking into account the results of different meta-analyses that relate active physical exercise and weight control, which although are carried out in pediatric population, could be extrapolated to the neonatal population^{38,39}.

The weight gain in the CG of our study is in line with other authors findings, who show weight gain in preterm infants who receive massage⁴⁰⁻⁴³, and in contrast to Eliakim, et al. 2002²² that found weight improvements in favor of PMC compared to caresses and tactile stimulation.

Some limitations of our trial should be noted. Heterogeneity of the preterm infants risk factors could influence the results obtained. Another limitation is the lack of long-term follow-up to determine the effect of the interventions on the Tibial-SOS.

It would be advisable to carry out multicenter clinical trials with a large sample of preterm infants that guarantees observation of the intervention effect at different gestational ages. It would also be convenient to carry out studies with long-term follow-ups, where the evolution of these children in early childhood and adolescence could be observed.

We can conclude that RLT has been effective in the improvement of Tibial-SOS values, which may have a positive effect in the prevention of osteopenia in preterm infants; furthermore, RLT has proved to be more effective than other physical therapy modalities such as PMC and massage in improving Tibial-SOS.

Due to the characteristics of the sample on which the intervention has been carried out, the results indicate that treatment with RLT is effective in improving bone mineralization in healthy preterm infants; and it could be considered as one of the best treatments to prevent osteopenia in this population.

Methods

Trial design

This prospective randomized clinical trial was carried out in the neonatal service of the Virgen de la Arrixaca Clinical University Hospital (HUCVA); from February 2016 to July 2020; 46 preterm infants participated on it, and were randomized into three intervention groups, two treatment groups and a control one. This study was approved by the HUCVA ethical committee for clinical research, carrying out all the procedures stipulated in the Declaration of Helsinki²⁰. This study has been registered at ClinicalTrials.gov with identification number: NCT04356807

Participants

The participants in this study were preterm infants born between the 29th and the 34th +2 weeks of gestational age (GA), admitted in the neonatal unit, with hemodynamical stability and full enteral nutrition, whose parents or guardians signed the consent form authorizing the participation of their infant in this study. Newborns with neurological disorders, mechanical ventilation, bronchopulmonary dysplasia, congenital malformations, metabolic diseases, genetic diseases, intraventricular hemorrhage III-IV, those taking diuretic or corticosteroid medication, and those with bone fractures at the time of randomization were excluded.

Interventions

The participants in this study were divided into three groups that, along with the standard nursing care, received different Physiotherapy treatments.

Control group (CG), was given limb and core massage, with gentle deep pressures and caresses; lasting 15 minutes a day in a single Physiotherapy session, 5 days per week, for 4 weeks; considering it a placebo since this intervention has no influence on bone mineralization²¹⁻²⁶.

Experimental group (EGpmc), with passive movements with gentle compression (PMC), described by Moyer-Mileur, et al.¹² and with the adaptations of Vignochi, et al.¹⁰ in a 15 minutes Physiotherapy session, 5 days per week for 4 weeks. These mobilizations consist of flexion and extension movements in all the joints of both the upper and lower extremities and ending with chest movements following the baby's respiratory pace.

Experimental group (EGrlt), with RLT according to the procedures used by other authors¹⁶⁻¹⁹, for 16 minutes divided into two Physiotherapy sessions of 8 minutes each, 5 days per week for 4 weeks. The exercises corresponding to the motor complexes of the 1st phase of reflex rolling and the reflex creeping were performed, dedicating one minute to each side and performing two repetitions in each session.

For the 1st phase of reflex rolling, the child is placed in dorsal decubitus, with the head turned to one side at an angle of 30°, the spine as aligned as possible, and the limbs relaxed. The physiotherapist makes gentle pressure with his thumb, at the point of intersection of the mammillary line with the diaphragm, between the 6th-7th intercostal space, in the hemithorax on the side towards which the head rotates, with a dorsal-medial-cranial direction, while resisting with the other hand the turning of the head towards the other side^{16,17,19}.

For reflex creeping, the child is placed prone, passively bringing the head to axial neck extension and 30 degrees of rotation. The upper limb, on the side which the head is turned, is placed in a position of shoulder flexion between 120 degrees and 135 degrees, with 30 degrees of abduction, leaving the epitrochlea supported; the wrist is aligned with the shoulder, the forearm rests on the palmar face, and the longitudinal axis of the humerus points towards the vertex of the lumbosacral hinge. The opposite arm is placed relaxed parallel towards the longitudinal axis of the body.

The leg on the side where the child's head is turned, is supported extended and relaxed. The other leg is placed with the hip in external rotation and abduction, leaving the support on the internal condyle of the femur, the knee slightly flexed, and the heel aligned with the ischium. The stimulation is carried out, with the index finger of one hand, on the lateral tuberosity of the calcaneus, in the ventral-cranial-medial direction of the leg opposite to the turn of the head, and with the index finger of the other hand, on the epitrochlea of the arm towards which the head is turned, in a dorsal-medial-cranial direction^{18,19}.

All the treatments were applied by the same physiotherapist with more than 5 years of experience and the infants of the 3 groups were evaluated under the same conditions.

Outcomes

Bone mineralization data and anthropometric measurements of weight, height, and head circumference were collected.

In order to measure bone mineralization, the tibial speed of sound (Tibial-SOS) was recorded using a quantitative ultrasound device (QUS) (*Sunlight Omnisense 7000*)^{27–29}. It was measured on the third lower part of the left tibia, keeping the knee bended to a 90-degree angle. The measurement point was made perpendicular to the direction of the bone. Three to five consecutive measurements were made, and the average was calculated to determine the Tibial-SOS (m/s).

Measurements of weight, height and head circumference were also collected. Body weight was measured by placing the baby naked on a digital scale (SECA), height was measured as the distance from head to heel with a non-elastic tape, and head circumference was measured at its widest part between the eyebrows and the occiput, also using a non-elastic tape.

Tibial-SOS measurements were carried out by a neonatologist three times: one day before starting treatment sessions, after two weeks of treatment and at the end of it. Inter-rater reliability and intra-rater reliability were high, with intraclass correlation coefficients (ICC) of 0.852 and 0.861 respectively.

Anthropometric measures were taken from one day before starting the treatment to one day after finishing it, collecting them in alternating days, according to the nursing protocol, and were carried out by such staff. For our analysis, we used those that coincided with the day the Tibial-SOS was measured, or, failing that, the last measurement made before that day.

The Z score was calculated for birth weight and for weight, height and head circumference at the different measurement moments, following the 2013 Fenton growth charts for this purpose³⁰.

Sample size calculation

Sample size was determined considering a 5% significance level, a 80% statistical power and a high magnitude effect size according to Cohen's criteria³¹. The software program nQuery Advisor version 7.0^{31,32}, gave us a previous sample size of 66 preterm infants (22 for each study group). However, due to a sepsis that affected the hospital's neonatal intensive care unit (NICU), this unit was forced to close. No more patients were being admitted and the studies that were being carried out had to be postponed. This situation caused us to reach the scheduled date of closure, so the study had to finish. Therefore, the total sample size was 46 newborns distributed in three study groups. The statistical analysis was performed with 70% of the total sample size and a significant difference was observed in relation to the studied intervention.

Randomization

The groups were formed by simple randomization. The randomization procedure consisted of sealed envelope labels containing a number for each group. A non-research person drew a random number from the envelope each time a new patient was proposed for treatment and made the assignment. For ethical reasons, twins and triplets were assigned to the same group with the same number.

Blinding

All the personnel who carried out the measurement tests are external to the study and were blinded to which intervention group the patients belonged to. Likewise, participants, family, and data analysts were also blinded. The physiotherapist who carried out the treatments was blinded against the objectives of the study.

Statistical methods

The qualitative baseline sex characteristics of the infants were compared using a crosstab and a Chi-square test was performed for its analysis. Quantitative variables (gestational age, birth weight, birth weight Z score, gestational age at baseline, weight, height, head circumference, anthropometric Z scores, and Tibial-SOS at baseline), were analysed performing a one-way analysis of variance. A mixed repeated measures analysis of variance was performed to compare the effect of the intervention on anthropometric and Tibial-SOS measures and on anthropometric Z scores, using the time of measurement as the intra-subject factor and the treatment group as the inter-subject factor. In those cases where the homoscedasticity assumption was not met, a robust mixed repeated measures analysis of variance was performed. The 95% confidence intervals (95% CI) were adjusted by Bonferroni. Statistical analysis with intention to treat was performed for all variables using SPSS (Statistical Package for the Social Sciences) for Windows (v.22.0)³³. Statistical significance was stipulated with $p < 0.05$. For the effect size, the eta square (η^2) was calculated, considering a value > 0.14 as high; moderate with values between 0.14 and 0.06; and small values between 0.06 and 0.01^{31,34,35}. Data are presented as mean $\pm$ standard deviation.

Declarations

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Author contributions statement

GTF, FJFR and AGC conceived and designed the study, carried out the experiment, supervised the findings of this work, interpreted the results and wrote the manuscript. JJAA provided patients for the study, monitored patients' medical conditions, helped in the design and interpretation of data, and critically reviewed the work. All authors reviewed and approved the final manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Additional information

Ethics approval and consent to participate

The study was approved by the HUCVA ethical committee for clinical research. Reference number: 07/15

Consent for publication

Not applicable.

Availability of data and materials

The full trial protocol can be found at ClinicalTrials.gov.

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

The pre-print version of this article is present on <https://www.researchsquare.com/article/rs-1037228/v1>. This article is not published nor is under publication elsewhere."

Competing interests

The authors declare that they have no competing interests

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Registration

Trial registered at ClinicalTrials.gov. First posted date 22/04/2020. Registration number: NCT04356807.

URL:<https://clinicaltrials.gov/ct2/show/NCT04356807?cond=Physical+Therapy+to+Prevent+Osteopenia+in+Preterm+Infants&draw=2&rank=1>

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Figures

Figure 1

Participant flow diagram

Figure 2

Boxplot of the Tibial speed of sound changes along the study period

Figure 3

Effect of the intervention in the anthropometric measures of weight (a), height (b) and head circumference (c)