UNIVERSITY OF MURCIA

INTERNATIONAL SCHOOL OF DOCTORAL STUDIES OF THE UNIVERISTY OF MURCIA (EIDUM)

STUDY PROTOCOL

EFFECT OF PHYSIOTHERAPY ON THE PROMOTION OF BONE MINERALIZATION IN PRETERM INFANTS

RESEARCH TEAM
GALAAD TORRÓ FERRERO, FRANCISCO JAVIER FERNÁNDEZ REGO, JUAN JOSÉ AGÜERA ARENAS, JESICA PIÑERO PEÑALVER, MARIA DEL MAR SÁNCHEZ JOYA, ANTONIA GÓMEZ CONESA,

Contact: galaad.torro@um.es
University of Murcia

ClinicalTrials.gov ID: NCT04356807

MURCIA, JULY 2015

INDEX

1.- INTRODUCTION

1.1.- Background

According to the World Health Organization (WHO), a pretemr infant is considered to be any baby born alive before 37 weeks of gestational age (ga), regardless of birth weight[1].

Prematurity is the neonatal condition that occurs most frequently, carrying with it a high risk of disability[2]. For this reason, the WHO considers prevention and care of its complications as top priorities for premature babies[3]; because those who survive face a lifetime of significant disability in many cases, special care is required and are at increased risk for serious health problems[4].

In this sense, one of the characteristic pathologies in this population is osteopenia of prematurity, it is estimated that between 30% and 42% of premature children worldwide suffer osteopenia[5]. This entity is multifactorial, progressive and of variable severity, of a pathogenesis not yet well described, characterized mainly by a bone mineral deficiency. It appears in 30% of children under 1500 g and occurs in more than 50% of newborns weighing less than 1000 g[5]

One of the causes for which it occurs can be explained by the early termination of pregnancy. It is estimated that during the third trimester the mother contributes to the fetus about 80% of the total calcium[5]. In this way, premature birth deprives the baby from the supply of nutrients, proteins and minerals, essential for the formation of the bone matrix[5]. This is why infants born prematurely, even in the last trimester (30-36 + 6 ga), have smaller bones and a lower level of bone mineralization compared to infants born at term[6]. In this sense, it has been observed in different studies that in preterm infants, mineralization is much lower than expected intrauterine bone mineralization[7]. It has been observed that these rates of poor mineralization are maintained in children and young adults born prematurely[8,9], a situation that leads to a reduction in maximum bone mass, weaker bones, shorter height, and a higher rate of fractures in comparison with those born at term[10].

On the other hand, some of the reasons why an osteopenic condition may be aggravated in the premature, can be found in the nutritional conditions (prolonged parenteral nutrition); postnatal morbidity, such as bronchopulmonary dysplasia and necrotizing enterocolitis; by diuretic drugs; by steroid drugs and by caffeine (commonly used to treat apnea)[11,12]. Finally, other aspects that influence this are the baby's own inactivity caused by her lack of tone and weakness; in addition to the usual care in relation to promoting development established in Neonatal Intensive Care Units (NICUs), which include minimal management of the newborn, with nest containments that limit their movements and deprive them of both sensory and physical stimuli[13].

All these aspects cause immobility of the baby with an increase in bone resorption, decreased bone formation, and therefore a deficiency in mineral density that can lead to a picture of osteopenia[14].

Bone is a living, dynamic tissue that is continually creating and destroying itself. This dynamism is mediated by the action of osteoblasts, responsible for formation and osteoclasts, responsible for resorption (destruction). The amount of bone mass that an individual has at a given time will depend on the proportion between these activities, that is, on the rate of bone turnover[15]. Under normal conditions, bone metabolism is in balance to maintain the mass and microstructure of the skeleton. In this sense, quantitative changes in bone metabolism can be assessed by measuring biochemical markers of bone remodeling in serum or urine, resulting from activity in bone throughout the entire skeleton. In contrast, bone mass measurements and radiographs provide a static picture of a specific site of the skeleton.

1.2.- Justification

An estimated 15 million babies are born worldwide each year before 37 ga, that is, more than 10% of babies born are premature[16]. In almost all countries with reliable data, the rate of premature births is increasing. Of these countries, all but three have registered an increase in prematurity rates in the last 20 years. An estimated 8.6% of live births in developed countries are premature[17].

In Spain, we have gone from having in 1996 a 5.9% of preterm births to 6.5% in 2013, with a total of 27,015 preterm births, according to data from the National Institute of Statistics[18]. Of which, most are premature 32-36 sg

Despite the fact that the data on prematurity have been reduced since 2008, they are still a high number if we think about the cost of premature births. In the United States, the average medical cost of the first year of life for premature babies, including both hospital and outpatient care, was about 10 times higher for premature babies than for full-term newborns. In addition, hospital stay was also higher on average in the premature than in the full-term newborn (about six times higher on average). In total, the cost of hospital stay for premature babies reaches 5.8 billion dollars, which represents 47% of the cost of all infant hospitalizations and 27% of all pediatric hospitalizations[19]. On the other hand, the rates of large premature babies have not been reduced, but have been maintained over time.

Taking these data into account, it is necessary to develop therapeutic strategies that address the morbidity associated with prematurity.

As previously mentioned, osteopenia is one of the characteristic pathologies of the premature population. In this sense, mechanical stress has been shown to be one of the most stimulating factors in bone formation and growth, thus increasing bone mass in children, adolescents and adults[20,21]; while the inactivity facilitated by the usual management practices for the newborn favors bone resorption and a decrease in bone mineral density[22].

Currently, Physiotherapy has proven to be one of the therapeutic resources with which the best results have been obtained in the approach to osteopenia in the premature infant. The physiotherapy techniques used in the treatment of this clinical entity have been mainly

based on the use of passive mobilizations with joint pressure, described, obtaining favorable results.[23].

The results of some studies show that, for the treatment of osteopenia, active mobilizations will be more effective than passive ones[24], and to achieve the latter, we will need methods that do not require the subject's will to achieve active movement, due to the young age of the same. In this sense, reflex locomotion therapy (RLT)[25] may be a suitable method to achieve this goal, since it generates involuntary activity, developing an active-resisted movement in the population of preterm infants.

With the application of this technique, we achieve a stimulation of the central nervous system (CNS), and an activation of the locomotion patterns of the first year of life. These patterns are triggered reflexively, placing the baby in certain positions, and generating proprioceptive stimuli through the pressure exerted by our fingers on specific areas[25–27], thus producing a series of synergistic muscle contractions in an exactly defined coordination for all the skeletal muscles, also producing a specific active movement[25–27]. This is because through RLT, CNS activation takes place from the spinal level to the subcortical and cortical areas[27].

Knowing the effect of RLT, we think that it can be effective in the treatment of osteopenia, since by applying it, we would be causing an active-resisted muscular contraction of the entire body, which is what is intended in any treatment for osteoporosis. . Although these are reflexive and non-voluntary active mobilizations, they are perhaps more effective than passive mobilizations.

1.3.- Objective

To determine whether RLT is effective for the prevention of osteopenia in preterm infants and compare its effectiveness over other physiotherapeutic methods

2.- METHODS

2.1.- Design

Multicenter randomized clinical trial, consisting of three intervention groups, two treatment and one control; and three measures: pre, inter and post.

This project will be carried out at the Virgen de la Arrixaca University Hospital, the Torrecárdenas Hospital in Almería and the Hospital General Universitario in Elche. The Virgen de la Arrixaca University Hospital is the coordinating center of the study.

2.2.- Eligibility

Inclusion Criteria:

- Preterm infants
- 26 to 34 weeks of gestational age
- Admitted in neonates

- Hemodynamically stable
- Complete enteral nutrition
- Parents or guardians signed an informed consent authorizing the participation of the baby in this study.

Exclusion Criteria:

- Neurological disorders
- Mechanical ventilation
- Bronchopulmonary dysplasia
- Congenital malformations
- Metabolic diseases
- Genetic diseases
- Intraventricular hemorrhage III-IV,
- Diuretic medication or corticosteroids
- Bone fractures at the time of inclusion.

2.3.- Sample size

Sample size has been calculated considering the f^2 with Cohen's criteria, using a f^2 value between moderated and low 0.15, between 0,10 (low) and 0,25 (moderated). We will assume a 5% significance level, a 80% statistical power with three levels for each ones of the factors iner and intra group. This way it is projected a sample size of 93 participants, 31 per group. The software program was nQuery Advisor version 7.0[28,29]. We use this method because there are no previous studies with tree groups[30].

Assuming possible deviations from these premises, we opted for a sample of 33 patients per group. As the present study requires three treatment groups, the final sample size will be 99 participants who, distributed among the three hospitals, give us a sample of 33 babies per hospital. For its part, each hospital will have the same number of participants per treatment group, so 11 patients will be distributed by each group in each hospital.

2.4.- Origin and inclusion procedure

All subjets will be infants admitted to the Virgen de la Arrixaca University Hospital (HUVA), the Hospital Torrecárdenas of Almería Hospital and the Hospital General Universitario of Elche, dividing the population into groups of 33 patients for each hospital.

2.5.- Groups formation

The groups will be formed by simple randomization. The randomization procedure will consist 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.

2.6.- Variables

Those variables that we intend to measure in our study are bone mineralization, bone formation, bone resorption and anthropometry. The main variable being bone mineralization obtained with the tibial sound velocity.

In order to measure bone mineralization, the tibial speed of sound (Tibial-SOS) will be recorded using a quantitative ultrasound device (QUS) (*Sunlight Omnisense 7000*)[31–33]. It will be measured on the third lower part of the left tibia, keeping the knee bended to a 90 degree angle. The measurement point is made perpendicular to the direction of the bone. Three to five consecutive measurements are going to be made, and the average will be calculated to determine the Tibial-SOS (m/s). This variable will only be taken at the Virgen de la Arrixaca University Hospital.

To measure bone formation and resorption, we will use serum and urine biomarkers, respectively, to reveal analytical data on the speed of bone formation and its metabolism[34]. Specifically, in bone formation, we will use bone-specific phosphatase markers, and osteocalcin markers, since at present, they are the most sensitive formation markers[15]. Resorption biomarkers are of special interest to us, because it is observed that osteopenia is more caused by bone resorption[7], therefore studying this variable is of great interest. We will specifically use the N-telopeptides of collagen bonds (NTx) and Beta-cross Laps (BC) since they are the most sensitive and specific markers to measure this quality[15,35].

Tests of the urine biomarkers and the Tibial SOS will be carried out one day before starting treatment sessions, two weeks laterand at the end of treatment. The Tibial SOS, since it is probable that in the other hospitals they do not have this measurement system, it will only be carried out in the HUCVA.

Serum biomarker tests will be performed one day before starting sessions and at the end of treatment. The serum biomarkers, during the hospitalization period, will always be taken coinciding with blood collection guidelines already ordered by their doctor, in any case will blood samples be taken exclusively for the purposes of this study.

Then for anthropometry, measurements on weight, length and head circumference will be collected with a scale and a tape measure, these being the same for all babies and for all measurements. Anthropometric measures will be 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 will be carried out by such staff. For our analysis, we will use those that coincide with the day the Tibial-SOS and biomarkers are measured, or, failing that, the last measurement made before that day.

All the personnel in charge of carrying out the measurement tests are external to the study and will be masked to which intervention group the patients belonged to. Likewise, participants, family, and data analysts will be also masked. The physiotherapists in charge of performing the treatments will be masked against the objectives of the study.

The data will be collected in digital format on a computer, where it will be stored in external memory and where the name and data of the patients will be protected in code, to guarantee their confidentiality.

2.7.- Treatment program

The program will be applied the same in all centers, as described below, by different physical therapists (one in each hospital) with experience in the different treatment modalities. For a better standardization of the study in all hospitals, regarding the nutritional aspect, the recommendations proposed in the protocols of the Spanish Society of Neonatology (SENeo) will be followed, with the variations that may occur in terms of the specific characteristics of each patient.

The participants in this study will be divided into three groups that, along with the usual nursing care, will recieve different Physiotherapy treatments.

Control group (CG), will be 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 mineralization[36–41].

Experimental group (EGpmc), with passive movements with gentle compression (PMC), described by Moyer-Mileur, et al.[23] and with the adaptations of Vignochi, et al.[42] 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 authors[25–27], 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 will be 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 30º, 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[25].

For reflex creeping, the child is placed proned, 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[43].

2.8.- Statistical analysis

The qualitative baseline sex characteristics of the infants will be compared using a crosstab and a Chi-square test for its analysis. Quantitative variables (gestational age, birth weight, gestational age at baseline, weight, height, head circumference, Tibial-SOS at baseline and serum and urine biomarkers at baseline), are going to be analized performing a one-way analysis of variance. Repeated measures analysis of variance will be performed to compare the effect of the intervention on anthropometric, Tibial-SOS and biomarkers measures using the time of measurement as the intra-subject factor and the treatment group as the inter-subject factor. All analyzes will be performed using SPSS (Statistical Package for the Social Sciences) for Windows (v.22.0)[44]. Statistical analysis with intention to treat is going to be performed for all variables. Statistical significance is stipulated with $p < 0.05$. For the effect size, the partial eta square (η_p^2) will be 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[29,45,46]. Data will be presented as mean ± standard deviation.

2.9.- Ethical aspects

All parents or guardians of infants participating in the study must sign the informed consent

The project will follow the Declarations of the 1975 Helsinki World Medical Association and must be approved by the ethical committee of each hospital, that is, of the Virgen de la Arrixaca University Hospital, the Torrecárdenas Hospital in Almería and the Hospital General Universitario of Elche. All parents/guardians will be informed about the nature of the study, voluntary participation in it, the proposed objectives, as well as any possible adverse effects that may take place in its conduct. Before beginning the study, each parent or guardian will be asked to consent to participate in the study. The baby will leave the study at any time, if the parent or guardian so wishes.

Those patients whose parents or guardians do not sign the informed consent will not be able to participate in the study.

In the study, the ethical principles of beneficence, non-maleficence, justice and autonomy and those principles dictated by the Clinical Research Ethics Committee will be followed at all times.

3.- EXPECTED RESULTS

With the completion of this research, it is intended to demonstrate that children who receive Physiotherapy treatment with RLT have a better improvement in bone mineralization and resorption than children who receive other types of treatment and who have homogeneous characteristics.

4.- REFERENCES

1. World Health Organization. www.who.int. Published 2015.
2. Alonso C, Bértolo J de la C, López M. *Apoyo Al Desarrollo de Los Niños Nacidos Demasiado Pequeños, Demasiado Pronto*. 1st ed. Madrid: Centro Español de Documentación sobre Discapacidad del Real Patronato; 2000.
3. Fernando Althabe, Zulfiqar Bhutta, Hannah Blencowe, Venkatraman Chandra-Mouli DC, Anthony Costello, Simon Cousens, Ruth Davidge, Joseph de Graft Johnson, Sohni Dean CH, Ayesha Iman, Joanne Katz, Matthais Keller, Mary Kinney, Eve Lackritz, Zhora Lassi, Joy Lawn EM, Ramkumar Menon, Mario Merialdi, Ann-Beth Moller, Elizabeth Molyneaux, Mikael Oestergaard VP, Jennifer Requejo, Lale Say, Joel Segre S von X. *Born Too Soon: The Global Action Report on Preterm Birth*. (Christopher Howson, Mary Kinney JL, ed.). World Health Organization; 2012. www.who.int.
4. Behrman RE, Butler AS. *Preterm Birth: Causes, Consequences, and Prevention - PubMed - NCBI*. Washington (DC) doi:DOI: 10.17226/11622
5. Carsi-Bocanegra EE, Frausto-Cárdenas OY, Aguilar-Quiñones GC. Incidencia de osteopenia en pacientes menores de 34 semanas de gestación en una unidad de cuidados intensivos neonatales. *Perinatol y Reprod Humana*. 2014;28:193-197.
6. Specker BL, Johannsen N, Binkley T, Finn K. Total body bone mineral content and tibial cortical bone measures in preschool children. *J Bone Miner Res*. 2001;16(12):2298-2305. doi:10.1359/jbmr.2001.16.12.2298
7. Beyers N, Alheit B, Taljaard JF, Hall JM, Hough SF. High turnover osteopenia in preterm babies. *Bone*. 1994;15(1):5-13. doi:10.1016/8756-3282(94)90884-2
8. Fewtrell MS. Early nutritional predictors of long-term bone health in preterm infants. *Curr Opin Clin Nutr Metab Care*. 2011;14(3):297-301. doi:10.1097/MCO.0b013e328345361b
9. Fewtrell MS, Williams JE, Singhal A, Murgatroyd PR, Fuller N, Lucas A. Early diet and peak bone mass: 20 year follow-up of a randomized trial of early diet in infants born preterm. *Bone*. 2009;45(1):142-149. doi:10.1016/j.bone.2009.03.657
10. Chan GM, Armstrong C, Moyer-Mileur L, Hoff C. Growth and bone mineralization in children born prematurely. *J Perinatol*. 2008;28(9):619-623. doi:10.1038/jp.2008.59
11. Backström MC, Kuusela A-L, Mäki R. Metabolic Bone Disease of Prematurity. *Ann Med*. 1996;28(4):275-282. doi:10.3109/07853899608999080
12. Backström MC, Kouri T, Kuusela AL, et al. Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. *Acta Paediatr*. 2000;89(7):867-873.
13. Cabral LA, Velloso M. Comparing the effects of minimal handling protocols on the physiological parameters of preterm infants receiving exogenous surfactant therapy.

Brazilian J Phys Ther. 2014;18(2):152-164. doi:10.1590/s1413-35552012005000154

14. Miller ME. The bone disease of preterm birth: a biomechanical perspective. *Pediatr Res*. 2003;53(1):10-15. doi:10.1203/00006450-200301000-00005

15. Romero Barco CM, Manrique Arija S, Rodríguez Pérez M. Biochemical markers in osteoporosis: usefulness in clinical practice. *Reumatol Clin*. 2012;8(3):149-152. doi:10.1016/j.reuma.2011.05.010

16. Stacy Beck, Daniel Wojdyla, Lale Say, Ana Pilar Betran, Mario Merialdi, Jennifer Harris Requejo, Craig Rubens RM& PFVL. OMS | Incidencia mundial de parto prematuro: revisión sistemática de la morbilidad y mortalidad maternas. *Boletín la Organ Mund la salud*. 2010;88:1-80.

17. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet*. 2012;379(9832):2162-2172. doi:10.1016/S0140-6736(12)60820-4

18. Instituto Nacional de Estadística. www.ine.es. Published 2015.

19. Russell RB, Green NS, Steiner CA, et al. Cost of Hospitalization for Preterm and Low Birth Weight Infants in the United States. *Pediatrics*. 2007;120(1):e1-e9. doi:10.1542/peds.2006-2386

20. Eliakim A, Raisz L. Evidence for Increased Bone Formation Following a Brief Endurance-Type Training Intervention in Adolescent Males. *J Bone Miner Res*. 1997;12(10):1708-1713. doi:https://doi.org/10.1359/jbmr.1997.12.10.1708

21. Slemenda C, Miller J. Role of physical activity in the development of skeletal mass in children. *J Bone Miner Res*. 1991;6(11):1227-1233. doi:https://doi.org/10.1002/jbmr.5650061113

22. Mazess RB, Whedon GD. Immobilization and bone. *Calcif Tissue Int*. 1983;35(1):265-267. doi:10.1007/BF02405043

23. Moyer-Mileur LJ, Luetkemeier M, Boomer L, Chan GM. Effect of physical activity on bone mineralization in premature infants. *J Pediatr*. 1995;127(4):620-625.

24. Nikander R, Sievänen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. *BMC Med*. 2010;8:47. doi:10.1186/1741-7015-8-47

25. Giannantonio C, Papacci P, Ciarniello R, et al. Chest physiotherapy in preterm infants with lung diseases. *Ital J Pediatr*. 2010;36(1):65. doi:10.1186/1824-7288-36-65; 10.1186/1824-7288-36-65

26. El-shaarawy MK, Rahman SAA, Fakher M, El A, Salah WM. Effect of rolling on oxygen saturation and incubation period in preterm neonates with respiratory distress syndrome. *Int J Dev Res*. 2017;07(01):11319-11323.

27. Sanz-Esteban I, Calvo-Lobo C, Ríos-Lago M, Álvarez-Linera J, Muñoz-García D, Rodríguez-Sanz D. Mapping the human brain during a specific Vojta's tactile input: The ipsilateral putamen's role. *Med (United States)*. 2018;97(13):1-9. doi:10.1097/MD.0000000000010253

28. Elashoff J. nQuery Advisor. 2007.

29. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale NJ: Lawrence Earlbaum Associates; 1988.

30. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Academic Press; 1977.

31. Baroncelli GI. Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application. *Pediatr Res*. 2008;63(3):220-228. doi:10.1203/PDR.0b013e318163a286

32. Rubinacci A, Moro GE, Boehm G, De Terlizzi F, Moro GL, Cadossi R. Quantitative ultrasound for the assessment of osteopenia in preterm infants. *Eur J Endocrinol*. 2003;149(4):307-315.

33. Rack B, Lochmüller E-M, Janni W, et al. Ultrasound for the assessment of bone quality in preterm and term infants. *J Perinatol*. 2012;32(3):218-226. doi:10.1038/jp.2011.82

34. Molina FC. Marcadores Bioquímicos de Remodelado Óseo. *Rev Metab oseo y Miner*. 2003;1(Tabla 2):91-98.

35. Reynaga Montecinos B, Zeni SN. Marcadores bioquímicos del remodelamiento óseo: Utilidad clínica. *Acta bioquímica clínica Latinoam*. 43(2):177-193. http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S0325-29572009000200002&lng=es&nrm=iso&tlng=es. Accessed March 18, 2015.

36. Nemet D, Dolfin T, Litmanowitz I, Shainkin-Kestenbaum R, Lis M, Eliakim A. Evidence for exercise-induced bone formation in premature infants. *Int J Sports Med*. 2002;23(2):82-85. doi:10.1055/s-2002-20134

37. Eliakim A, Dolfin T, Weiss E, Shainkin-Kestenbaum R, Lis M, Nemet D. The effects of exercise on body weight and circulating leptin in premature infants. *J Perinatol*. 2002;22:550-554. doi:10.1038/sj.jp.7210788

38. Massaro AN, Hammad TA, Jazzo B, Aly H. Massage with kinesthetic stimulation improves weight gain in preterm infants. *J Perinatol*. 2009;29(5):352-357. doi:10.1038/jp.2008.230

39. Moyer-Mileur LJ, Ball SD, Brunstetter VL, Chan GM. Maternal-administered physical activity enhances bone mineral acquisition in premature very low birth weight infants. *J Perinatol*. 2008;28(6):432-437. doi:10.1038/jp.2008.17

40. Moyer-Mileur LJ, Brunstetter V, McNaught TP, Gill G, Chan GM. Daily physical activity program increases bone mineralization and growth in preterm very low birth weight infants. *Pediatrics*. 2000;106(5):1088-1092.

41. Litmanovitz I, Dolfin T, Friedland O, et al. Early Physical Activity Intervention Prevents Decrease of Bone Strength in Very Low Birth Weight Infants. *Pediatrics*. 2003;112(1):15-19. doi:10.1542/peds.112.1.15

42. Vignochi CM, Miura E, Canani LHS. Effects of motor physical therapy on bone mineralization in premature infants: a randomized controlled study. *J Perinatol*. 2008;28(9):624-631. doi:10.1038/jp.2008.60

43. Wu C, Peng X, Li X, Niu Q, Guo H, Huang H. Vojta and Bobath combined treatment for high risk infants with brain damage at early period. *Neural Regen Res*. 2007;2(2):121-125. doi:10.1016/S1673-5374(07)60027-9

44. IMB Corp. IBM SPSS Statistics for Windows. 2013.

45. Cárdenas Castro JM. Potencia estadística y cálculo del tamaño del efecto en G*Power: complementos a las pruebas de significación estadística y su aplicación en psicología. *Salud Soc*. 2014;5(2):210-244. doi:10.22199/s07187475.2014.0002.00006

46. Faul F, ErdFelder E, Lang A-G, Buchner A. G*Power 3.1 manual. *Behav Res Methods*. 2007;39(2):175-191. doi:10.3758/BF03193146