

Original Article

Physical Activity Combined with Massage Improves Bone Mineralization in Premature Infants: A Randomized Trial

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BACKGROUND:

Osteopenia of prematurity is a known source for morbidity in preterm infants. Premature infants have shown favorable outcomes in response to massage and physical activity. Whether such intervention can stimulate bone formation or decrease bone resorption is yet to be determined.

OBJECTIVE:

To test the hypothesis that massage combined with physical activity can stimulate bone formation and ameliorate bone resorption in premature infants.

DESIGN/METHODS:

A prospective double-blinded randomized trial was conducted at the Neonatal Intensive Care Unit of Ain Shams University in Cairo, Egypt. Thirty preterm infants (28 to 35 weeks' gestation) were randomly assigned to either control group (Group I, $n = 15$) or intervention group (Group II, $n = 15$). Infants in the intervention group received a daily protocol of combined massage and physical activity. Serum type I collagen C-terminal propeptide (PICP) and urinary pyridinoline crosslinks of collagen (Pyd) were used as indices for bone formation and resorption, respectively. PICP and Pyd were measured at enrollment and at discharge for all subjects. *t*-Test, ANOVA and linear regression analysis were used for statistical analyses.

RESULTS:

There was no difference between groups I and II in gestational age (32.1 ± 1.8 vs 31.5 ± 1.4 weeks) or birth weight (1.429 ± 0.148 vs 1.467 ± 0.132 g). In the control group, serum PICP decreased over time

from 82.3 ± 8.5 to 68.78 ± 14.6 ($p < 0.01$), while urinary Pyd increased from 447.7 ± 282.8 to 744.9 ± 373.6 ($p < 0.01$) indicating decreased bone formation and increased bone resorption, respectively. In the intervention group, serum PICP increased over time from 62.5 ± 13.8 to 73.84 ± 12.9 ($p < 0.01$). Urinary Pyd also increased over time from 445.7 ± 266.5 to 716.8 ± 301.8 ($p < 0.01$). In a linear regression model including gestational age and intervention, serum PICP increased significantly in the intervention group (regression coefficient 18.8 ± 4.6 , $p = 0.0001$) while urinary Pyd did not differ between groups (regression coefficient = 5.6 ± 114.3 , $p = 0.961$).

CONCLUSIONS:

A combined massage and physical activity protocol improved bone formation (PICP) but did not affect bone resorption (Pyd). Pyd increased over time in both groups, possibly due to continuous bone resorption and Ca mobilization.

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BACKGROUND

Despite recent advances in neonatal intensive care practices and clearly defined nutritional goals for premature low-birth-weight infants, osteopenia of prematurity remains a well-identified source of morbidity in preterm infants.^{1,2} Bone mass in these infants does not approach normal ranges until after the first year of life and may continue to be suboptimal into childhood.³ Nutritional intervention alone did not produce the desired positive effect on postnatal bone mineralization in these infants.^{1,3} Bone formation and growth, however, can be stimulated by physical activity with mechanical loading on bone and joints.⁴ In previous studies, massage therapy increased weight gain and enabled earlier discharge of premature infants.^{5,6} Recent studies have also demonstrated the ability of exercise therapy to attenuate the decrease in bone strength associated with osteopenia of prematurity and to promote growth and bone mineral density in this population.^{7–9} Whether massage therapy combined with physical activity stimulates bone mineralization in premature infants and prevents osteopenia of prematurity is yet to be determined.

Skeletal development involves a delicate balance between two different bone activities. Osteoblastic activity constructs the organic bone matrix upon which mineral content is later incorporated. Osteoclastic activity is responsible for bone resorption and

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remodeling.¹⁰ Several biochemical markers that correlate to these two activities have been recently identified. C-terminal procollagen peptide or propeptide of type I collagen (PICP) correlates with collagen turnover and bone formation in premature infants.^{11,12} Urine pyridinoline crosslinks of collagen (Pyd) is a marker for bone resorption.^{12,13} These two markers provide a useful, noninvasive tool to monitor bone formation in premature infants. The goal of this trial is to examine serum biochemical markers of bone mineralization in preterm infants exposed to massage and physical activity, and to test the hypothesis that this intervention can stimulate bone formation in preterm infants.

PATIENTS AND METHODS

This prospective double-blinded randomized trial was conducted at the neonatal intensive care unit of Ain Shams University Children's Hospital in Cairo, Egypt. The institutional review board of the university approved the study and consents were obtained before infants' enrolment. Infants who were included in the study had a gestational age of 35 weeks or less, a postnatal age of less than 2 weeks, were tolerating full enteral feeds, and were not receiving any medications except for the recommended vitamin supplementation. Infants were excluded from the study if they had a history of congenital malformations, asphyxia, or musculoskeletal, liver or renal diseases. Assessment of maturity by date of last menstrual period, antenatal ultrasound when available and the new Ballard scoring system was performed for all neonates.¹⁴

Randomization

Infants were randomized to either the control group (group I) or the intervention group (group II) by selecting a closed envelope containing a group code.

Intervention

Infants in the intervention group received both physical activity and massage. Physical activity was composed of daily range of motion, with gentle compression and extension/flexion to both upper and lower extremities. Five repetitions of each movement were performed at both wrists, elbows, shoulders, ankles, knees and hips. Massage protocol was composed of gentle, slow stroking of each part of the body in turn. The infant was placed in a prone position and stroked for a 1 minute period (12 strokes at approximately 5 seconds per stroking motion) over each region in the following sequence: (1) from the infant's head and face to the neck; (2) from the neck across the shoulders; (3) from the upper back to the waist; (4) from the thigh to the foot on both legs; and (5) from the shoulder to the hand to the shoulder on both arms. Physical activity was performed daily by the same physician until the infant reached 1.8 kg. Vital signs and oxygen saturations were constantly monitored 15 minutes before, during and 15 minutes after each daily intervention.

Infants in the control group did not receive any of the above measures. For both groups, type of feeding was recorded and the amount of calories, calcium, phosphorus and protein given per day was calculated for all studied neonates. Body weight was recorded daily at a standard time each day, before feeding, with digital electronic scale (Universal Weight Enterprise, Inc., Taiwan) and was approximated to the nearest gram.

Laboratory measurements

Biomarkers of bone formation (serum PICP) and resorption (urine Pyd), as well as serum calcium, alkaline phosphatase and parathyroid hormone (PTH) were measured at study entry and at 1.8 kg. of body weight.

Serum level of calcium and alkaline phosphatase Sample was withdrawn without stasis (tourniquet), and immediately analyzed for calcium and alkaline phosphatase using the Hitachi 917[®] analyzer. The remainder of the serum was immediately stored at -20°C until use for assessment of other markers.

Serum PICP The procollagen-C assay is a sandwich immunoassay in a microtiter plate format utilizing a monoclonal anti-PICP antibody coated on the plate, a rabbit anti-PICP antiserum, a goat anti-rabbit alkaline phosphatase conjugate and a pNPP substrate to quantify PICP in human serum (Metra Biosystems, Inc., San Diego, CA, USA).

Urine Pyd This is competitive enzyme immunoassay in a microtiter strip format utilizing a monoclonal antipyridinoline antibody to measure Pyd and Dpd in urine. The Pyd and Dpd in the sample compete for the antibody with Pyd coated on the strip. The reaction is detected with a pNPP substrate. Ppyrlinks results are corrected for urinary concentration by correlating to creatinine (Metra Biosystems, Inc., San Diego, CA, USA).

PTH PTH hormone was measured by enzyme-linked immuno assay (ELISA) technique using PTH-EASIA, which is a solid phase enzyme amplified sensitivity immunoassay performed on a microtiter plate. It allows the determination of the intact human PTH in serum or plasma. Sample binds to the affinity chromatography purified antibodies (Pabs, goat anti-1-34 PTH fragment) coated on the inner surface of the microwell.

Statistical Analysis

Data were analyzed using the SAS System[®] Version 6.12.¹⁵ Demographic data were analyzed using *t*-test, Fisher's exact test and χ^2 -test. For the five-biochemical markers of bone metabolism (calcium, alkaline phosphatase, PICP, Pyd and parathyroid hormone), the absolute change over the study period was determined for each variable. Percent changes in variables were analyzed and compared between groups. The Kruskal–Wallis Test

(χ^2 approximation) was used to determine significant differences between the activity and control groups. A regression analysis was also performed in order to control for gestational age since previous reports have suggested its correlation with PICP levels.¹⁶

Power analysis We hypothesized that our intervention would increase the group difference in PICP by three fold. When we used the PICP baseline value from a previous study⁸ and assumed a correlation coefficient ($r = 0.7$) between pre- and postintervention observations, a sample of 30 subjects (15 in each group) would detect that difference with $\sigma = 80$, $\beta = 20$ and $\alpha = 0.05$ level of significance.

RESULTS

A total of 30 infants were enrolled in the study. They were randomized blindly into control and activity groups, with 15 infants assigned to each group. The groups were similar in gestational age, birth weight and gender distribution. They also had no significant differences in caloric, calcium or protein intake over the study period (Table 1). Laboratory values at enrolment and at conclusion of the study for both groups are shown in Table 2.

Serum calcium Mean serum calcium at study entry was 9.1 ± 1.6 mg/dl. for Group I and 8.5 ± 1.4 mg/dl for Group II ($p = 0.3$). Both groups had an increase in serum calcium levels over the course of the study. The relative percent increase was

higher in the activity group (19.5%) than in the control group (4.3%), $p = 0.002$.

Serum alkaline phosphatase Serum alkaline phosphatase was also similar for both groups at study entry ($P = 0.665$). These levels did not change significantly for either group over the course of the study.

Serum PICP Mean PICP concentration was higher in Group I (82.3 ± 8.5 ng/ml) than in Group II (62.5 ± 13.8 ng/ml) at the start of the study ($p < 0.001$). In the control group PICP levels decreased during the study period while they increased in the activity group (Figure 1). The percent change was significantly different for the two groups ($p < 0.001$).

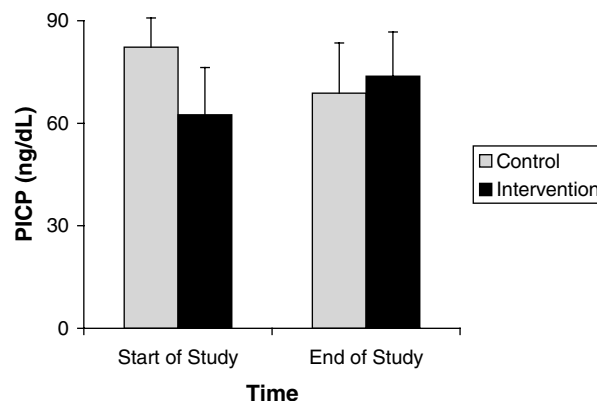


Figure 1. Serum level of PICP in both groups at the start and at the end of the trial.

	Control ($n = 15$)	Intervention ($n = 15$)	p -value
Gestational age (weeks)	32.13 ± 1.85	31.53 ± 1.46	0.328
Birth weight (grams)	1429.33 ± 148.51	1465.67 ± 132.83	0.430
Sex (% male)	53.3	53.3	0.984
Caloric intake (kcal/kg/day)	144.77 ± 19.24	135.00 ± 29.30	0.290
Calcium intake (mg/kg/day)	186.00 ± 17.12	169.60 ± 52.57	0.260
Protein intake (g/kg/day)	3.035 ± 0.892	3.650 ± 0.970	0.081

	Control		Intervention	
	Start	End	Start	End
Serum calcium (mg/dl)	9.1 ± 1.6	9.4 ± 1.0	8.5 ± 1.4	10.1 ± 1.2
Alkaline phosphatase (U/l)	395.9 ± 102.6	449.3 ± 140.1	410.2 ± 74.4	377.6 ± 95.2
PICP (ng/ml)	82.3 ± 8.5	68.8 ± 14.7	62.5 ± 13.8	73.8 ± 12.9
Pyd (ng/ml)	447.7 ± 282.8	744.9 ± 373.6	445.7 ± 266.6	716.8 ± 301.8
PTH	33.0 ± 15.8	25.7 ± 20.6	8.3 ± 3.3	15.1 ± 3.5

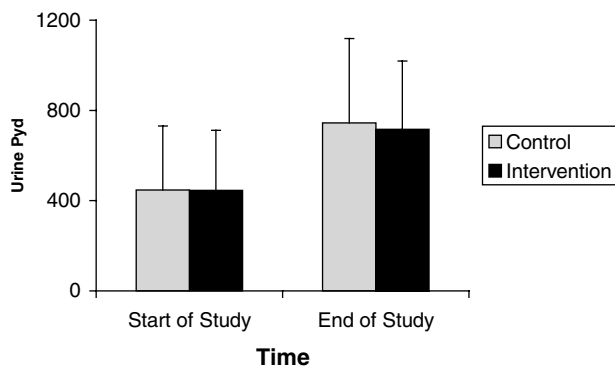


Figure 2. Urinary Pyd in both groups at the start and at the end of the trial.

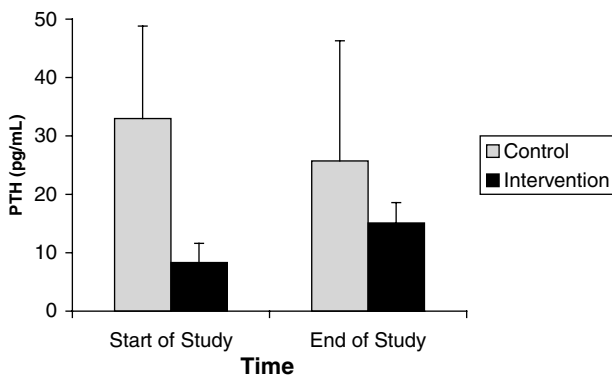


Figure 3. Serum PTH level in both groups at the start and at the end of the trial.

Urine Pyd Pyd concentration was similar between the two groups at study entry ($p = 0.984$). In both groups, Pyd levels increased over the study period (Figure 2). There was no difference between the two groups in the amount of percent increase.

PTH PTH levels were higher in Group I (33.0 ± 15.8 pg/ml) than in Group II (8.3 ± 3.3 pg/ml) at the start of the study ($p < 0.001$). Serum PTH decreased in the control group while it increased in the activity group (Figure 3). The percent change was significantly different between the two groups ($p < 0.001$). Differences between groups remained significant after controlling for gestational age using a regression analysis model.

DISCUSSION

This study showed that bone mineralization improved when premature infants experienced a combined protocol for physical activity and massage. Bone growth and metabolism is a complex process that involves deposition of bone matrix with incorporation of mineral content, which is balanced with enzymatic resorption and remodeling. Considering the fact that it enhances metabolic

efficiency and increases growth hormone levels in humans, it is plausible to expect physical activity to push the balance in favor of bone formation.^{17,18} Tactile stimulation, provided by infant massage, can also stimulate growth even without physical activity.^{5,6} Lack of stimulation due to maternal deprivation can decrease growth hormone levels and impair metabolic efficiency. Markers of tissue growth such as ornithine decarboxylase are decreased with maternal deprivation.^{19,20}

Osteopenia of prematurity occurs as a direct result of insufficient bone deposition or increased resorption of organic bone matrix. Several factors can produce such imbalance of bone homeostasis in the ill premature infant. Medications often used, such as diuretics and corticosteroids, adversely contribute to this imbalance. Other behavioral and environmental practices in the nursery such as swaddling and immobilization can certainly deprive premature infants from mechanical stimulation. Had they continued their term in the uterus, persistent kicking against the uterine wall would have provided a form of exercise to these fetuses.

Several methods have been used to evaluate bone growth and mineralization. Critiques developed to the precision and clinical applicability of each individual method. For example, markers of bone mineralization such as PICP and Pyd are currently reported with a considerably wide distribution that can indeed jeopardize claims of their precision. Anthropomorphic measurements, such as forearm length, can generally vary when different individuals perform the measurement. Recent studies have looked at bone speed of sound, single photon absorptiometry (SPA) and peripheral dual-energy X-ray (pDEXA) as measurements of bone strength. While these methods may provide useful information, they can be affected not only by physical density but also by bone size and geometry.^{7,9} For instance, in the first six months of life, total bone density of the femoral shaft decreases by 30% due to changes in bone geometry, whereas bone strength increases by threefold and absolute mineral content also increases during this time period.¹⁰

Serum PICP concentration decreased in the control group. Current standard NICU practices such as swaddling and immobilization contribute to the decreased bone formation observed in the hospitalized infants. This finding was previously reported by Moyer-Mileur et al.^{7,8} in two separate studies. In both of their reports, infants in the control group had decreased PICP concentration, while infants in the physical activity group maintained constant levels of PICP. Nevertheless, PICP concentration in our study increased in the intervention group. Of note, infants of the intervention group in our study received a combined massage and physical activity. We speculate that a strategy of combined physical activity and massage could produce a synergic effect on bone mineralization. This may explain why massage therapy or physical activity individually could not increase PICP concentration, while when combined together a significant increase was observed. We are currently conducting a randomized

controlled clinical trial in order to evaluate the effects of massage therapy alone and in combination with physical activity.

We were surprised to notice that PICP levels were different at the start of this randomized double-blinded trial. Gestational age is known to correlate with PICP levels.¹⁶ The difference among the two groups was maintained even after controlling for gestational age using a logistic regression model. Further studies are recommended to explore other factors that affect PICP levels and could have explained the differences observed between the two groups of this study. Urinary Pyd did not differ among groups, suggesting that bone resorption and remodeling are active processes that occur equally in all infants.

It is of interest that PTH levels increased significantly in infants in the intervention group. Intuitively, this may suggest a higher rate of bone resorption since PTH acts to mobilize calcium from bone in response to low serum calcium levels. However, PTH has been shown to enhance both the process of bone resorption and bone formation, with more stimulation of the latter. In fact, PTH administration has shown promise in the treatment of osteoporosis and is associated with increased bone mass.²¹ The increase in PTH levels may be a normal physiologic response to activity since PTH activity increases with physical activity in adults.²² It is difficult to separate this increase in PTH level from the increase in serum calcium since higher PTH activity works to increase serum calcium. The increase in serum calcium after activity may also be attributable to lactic acidosis and/or reduction in plasma volume.²³

It is important to recognize that infants in this study were provided with optimal nutritional intake. Growth and bone formation are, of course, linked to adequate calories, protein and calcium in the diet of the preterm neonate. Otherwise, physical activity in the absence of adequate calcium intake decreases bone mineral content.²⁴ Thus, it is essential to provide sufficient nutritional intake in order for physical activity to produce favorable effects on bone growth.

CONCLUSION

Physical activity combined with infant massage stimulates bone formation in premature infants as evidenced by an increase in PICP, a biochemical marker of bone formation, and an increase in PTH activity, which may further stimulate bone growth and mineralization.

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