Early Physical Activity Intervention Prevents Decrease of Bone Strength in Very Low Birth Weight Infants

Ita Litmanovitz, MD*; Tzipora Dolfin, MD*; Orit Friedland, MD‡; Shmuel Arnon, MD*; Rivka Regev, MD*;
Ruth Shainkin-Kestenbaum, PhD§; Monika Lis, MSc§; and Alon Eliakim, MD‡

ABSTRACT. *Objective.* **To evaluate the effect of early range-of-motion intervention on bone strength and bone turnover in very low birth weight infants.**

Methods. **Twenty-four infants (mean birth weight: 1135 ± 247 g; mean gestational age: 28.5 ± 2.3 weeks) were matched for gestational age and birth weight and then randomly assigned into exercise ($n = 12$) and control ($n = 12$) groups. Exercise protocol started at the first week of life and involved daily extension and flexion range of motion against passive resistance of the upper and lower extremities (5 minutes per day, 5 days per week, 4 weeks). Growth parameters, bone strength, and biochemical markers of bone homeostasis were measured at enrollment and after 4 weeks. Bone strength was determined using quantitative ultrasound measurement of bone speed of sound (SOS) at the middle left tibial shaft.**

Results. **Bone SOS decreased significantly in the control group during the study period (from 2892 ± 30 m/sec to 2799 ± 26), whereas bone SOS of the exercise group remained stable (2825 ± 32 m/sec and 2827 ± 26 m/sec at baseline and 4 weeks, respectively). This significant difference in bone SOS was not expressed in the biochemical markers of bone homeostasis.**

Conclusions. **There is a significant postnatal decrease in the bone SOS of very low birth weight infants. A brief range-of-motion exercise attenuates the decrease in bone strength and may decrease the risk of osteopenia.** *Pediatrics* **2003;112:15–19;** ***premature, osteopenia, exercise, quantitative ultrasound, speed of sound.***

ABBREVIATIONS. VLBW, very low birth weight; DEXA, dual-energy x-ray absorptiometry; QUS, quantitative ultrasound; SOS, speed of sound; BSAP, bone-specific alkaline phosphate; ICTP, carboxy terminal cross-links telopeptide of type-I collagen; CV, coefficient of variation.

Very low birth weight (VLBW) infants have an increased risk of osteopenia because of limited accretion of bone mass in utero and a greater need for bone nutrients.[1,2] The rate of osteopenia is inversely related to birth weight and gestational age and correlates with postnatal morbidity (eg, bronchopulmonary dysplasia, necrotizing enterocolitis).[3–6]

Currently, the diagnosis of osteopenia is based on clinical and radiologic signs and measurements of biochemical markers, such as serum alkaline phosphatase.[4] Advanced techniques, such as dual-energy x-ray absorptiometry (DEXA) for the evaluation of bone mineral content are used, rarely, to determine less severe forms of bone demineralization.[7–9] More recently, quantitative ultrasound (QUS) measurement of bone speed of sound (SOS) was developed. This method measures, in addition to bone density, other bone properties, such as cortical thickness, elasticity, and microarchitecture, thus providing a more complete picture of bone strength.[10–13] In a previous study, Nemet et al[14] demonstrated that QUS successfully assesses bone strength in premature infants. In addition, the relatively new development of assays for circulating biochemical markers of bone turnover[15–17] allows us to gain greater insight into the mechanistic effects of prematurity on bone development and may be useful in the diagnosis of osteopenia of prematurity.[18]

Mechanical strain is a powerful stimulator of bone formation and growth. Several studies have demonstrated that physical activity increases bone density in children, adolescents, and adults,[19–21] whereas inactivity results in bone resorption and decreased bone mineral density.[22,23] Therefore, the prolonged period of hospitalization of premature infants without physical stimulation may contribute to bone demineralization.

Recently, Moyer-Mileur et al[24] demonstrated that a passive range-of-motion exercise of both the upper and lower extremities resulted in increased bone mineral density (determined by single-photon absorptiometry and DEXA[25]) in premature infants. In this prospective, randomized study, we used Moyer-Miller's protocol to evaluate the effect of early exercise training (ie, first week of life) on bone strength and turnover in VLBW premature infants. Bone strength was assessed by QUS, and bone turnover was determined by measurements of circulating bone formation and bone resorption markers.

METHODS

Twenty-four VLBW infants from the neonatal intensive care unit at Meir General Hospital, Kfar Saba, Israel, participated in the study. Infants were eligible for study when they met the following criteria: birth weight of <1500 g, body size appropriate for gestational age, postnatal age of <1 week, and informed parental consent received. Premature infants with intrauterine growth retardation, severe central nervous system disorder, or major congenital anomalies were excluded. After matching for gestational age and birth weight, infants were randomly assigned into exercise ($n = 12$) and control ($n = 12$) groups.

From the Departments of *Neonatology, ‡Pediatrics, and §Biochemistry, Meir General Hospital, Sapir Medical Center, Kfar Saba, Israel.

Received for publication May 14, 2002; accepted Oct 14, 2002.

Reprint requests to (I.L.) Department of Neonatology, Meir Medical Center, Kfar Saba, Israel. E-mail: litmani@clalit.org.il

PEDIATRICS (ISSN 0031 4005).

Nutritional Management

All preterm infants received intravenous glucose 5% to 10% supplemented with calcium gluconate 10% (300 mg/100 mL) in the first 24 hours. Total parental nutrition was initiated at 24 hours of age (Primine, 10% [Baxter, Deerfield, IL]; protein, 2 g/100 mL; calcium, 200–400 mg/100 mL; phosphor, 0.76 mmol/100 mL). Enteral feeding was introduced gradually according to the attending physician decision (days 1–9). Subjects were fed either by fortified human milk (Similac Human Milk Fortifier [Ross, Abbott Laboratories, Abbott Park, IL]; calcium, 117 mg/100 mL; Phosphor, 67 mg/100 mL) or by preterm special formula (Similac Special Care [Ross]; calcium, 146 mg/100 mL; phosphor, 73 mg/100 mL). Total parental nutrition was stopped when enteral feeding reached 100 mL/kg/d. Enteral feeding was targeted to 120 kcal/kg. Oral vitamin D 200 IU/d was added to all premature infants (control and exercise) who received enteral feeding at 2 weeks of age.

Exercise Protocol

The physical activity program was based on the Moyer-Mileur et al protocol[24] and started after initial cardiorespiratory stabilization (days 4–7). Briefly, this protocol involves extension and flexion range-of-motion exercise against passive resistance of both the upper and lower extremities. Both extension and flexion were performed 5 times at the wrist, elbow, shoulder, ankle, knee, and hip joints (~5 minutes for each session). The same person performed this activity 5 times per week for 4 weeks. The control subjects had a similar time (5 min/d) of daily interactive periods of holding and stroking without range-of-motion activity, because it is possible that tactile stimulation might influence bone growth and development.

QUS Measurements of Bone SOS

The left tibial SOS was measured by QUS (Sunlight Omnisense Premier), a method designed to measure SOS at multiple skeletal sites by an axial transmission. Concisely, the SOS measurement is based on the fact that ultrasound waves propagate faster through bone than through soft tissue. The device consists of a desktop main unit and a number of small probes, designed to measure SOS at different sites. The probe was moved across the mid-tibial plane, searching for the site with maximal reading. The measurement site was defined as the midpoint between the apex of the medial malleolus and the distal patellar apex. The mean of 3 measurements of tibial SOS was selected for data analysis. The same technician, who was blinded to the group assignment, performed all measurements. The instrumental accuracy is 0.25% to 0.5%, and the precision is 0.4% to 0.8%. The precision for the present study population (based on 2 separate measurements of 35 preterm infants) was 0.32% (9.1 m/sec). Measurements were done on the enrollment day and the day after the last exercise session.

Blood Sampling Protocol

Early-morning venous blood samples for the evaluation of bone turnover markers were collected before and at the end of the program in both the control and exercise groups as a part of the routine follow-up blood tests (ie, routine chemistry panel and complete blood count that are performed weekly in our neonatal intensive care unit). Bone osteoblastic activity was assessed by measurements of circulating bone-specific alkaline phosphates (BSAPs).[15] Bone resorption was assessed by measurements of serum levels of the carboxy terminal cross-links telopeptide of type-I collagen (ICTP), which reflect osteoclastic activity.[26] All serum samples were kept frozen at −20°C until analyzed. All specimens from each individual were analyzed in the same batch by the same laboratory worker, who was blinded to the subject's group and to the order of the samples.

BSAP

Circulating BSAP levels were measured by enzyme-linked immunosorbent assay, using a monoclonal anti-BSAP (Alkaphas-B kit; Metra Biosystems, Inc, Mountain View, CA). The enzyme activity of the captured BSAP is detected with a p-nitrophenyl phosphate substrate. Interassay coefficient of variation (CV) was 5.0% to 7.6%, and intra-assay CV was 3.9% to 5.8%. Assay sensitivity was 0.7 U/L.

ICTP

ICTP levels were determined by equilibrium radioimmunoassay with I^{125} serving as a tracer, using the Diasorin ICTP kit (Stillwater, MN). Interassay CV was 4.1% to 7.9%, and intra-assay CV was 2.8% to 6.2%. Assay sensitivity was 0.5 μg/L.

Statistical Analysis

Unpaired *t* test was used to determine differences in birth weight, gestational age, initial and full day of enteral feeding, age at enrollment, bone SOS, and bone turnover markers between the exercise and control subjects before the training intervention. A 2-way repeated measure analysis of variance was used to compare the effect of the intervention on body weight, bone SOS, and bone turnover markers, using time as the within-group and exercise as the between-group factors. $P < .05$ was considered to be statistically significant. Data are presented as mean ± standard error.

RESULTS

Birth weight, gestational age, gender, ethnicity, morbidity (respiratory distress syndrome, bronchopulmonary dysplasia, sepsis), enrollment day, and day of initial and full oral feeding in the control and exercise group subjects are presented in Table 1. No significant difference was found in any of these parameters between the control and exercise group subjects.

Mean body weight, body length, head circumference, and tibial bone SOS before and after the intervention in both the control and exercise groups are shown in Table 2. No significant differences in these measures were noted between the groups before the intervention. There was a significant increase in body weight, body length, and head circumference in both

TABLE 1. Characteristics of the Study Participants

	Control (n = 12)	Exercise (n = 12)
Gestational age (wk)	28.4 ± 0.6	28.7 ± 0.7
Birth weight (g)	1118 ± 65.5	1153.0 ± 73.4
Gender (female/male)	6/6	5/7
Ethnicity (Jewish/Arab)	7/5	8/4
Respiratory distress syndrome	6	5
Oxygen at 28 d	4	4
Sepsis	2	2
Feeding (fortified human milk/preterm formula)	5/7	6/6
Initial enteral nutrition (d)	2.6 ± 0.3	3.7 ± 0.7
Full enteral nutrition (d)	11.6 ± 1.5	12.3 ± 2.3
Enrollment (d)	5.5 ± 1.4	5.6 ± 1.2

Data are presented as mean ± standard error of the mean (SEM). There were no statistically significant differences in any of the parameters between the groups.

TABLE 2. Changes in Anthropometric Measures and Bone SOS During the Study Period in the Control and Trained Subjects

	Control Group (n = 12)		Exercise Group (n = 12)	
	Pre	Post	Pre	Post
Body weight (g)	1117.6 ± 65.7	1484.0 ± 111.5*	1149.1 ± 75.6	1502.1 ± 112.4*
Body length (cm)	38.5 ± 3.4	41.4 ± 2.8*	36.9 ± 2.8	40.0 ± 3.0*
Head circumference (cm)	25.5 ± 2.6	28.6 ± 2.2*	25.6 ± 1.9	29.1 ± 2.3*
Bone SOS (m/sec)	2892.3 ± 29.5	2799.5 ± 25.5	2825.0 ± 32.2	2827.0 ± 26.0†

Data are presented as mean ± SEM.
* $P < .05$ for within-group difference.
† $P < .006$ for between-group difference (analysis of variance).

groups, but no significant difference was observed between the groups. Tibial SOS of the control group subjects decreased significantly, whereas tibial SOS of the exercise group subjects remained stable during the 4-week study period (significant difference between groups, $P < .006$; Fig 1).

Mean circulating bone turnover markers level in the control and exercise group subjects are shown in Table 3. No significant differences in these markers were noted between the groups before the intervention. In both groups, there was a significant increase in the bone formation marker (BSAP) and a significant decrease in the bone resorption marker (ICTP) without a significant between-group differences.

DISCUSSION

This study demonstrates that bone SOS decreases during the first 4 postnatal weeks in VLBW premature infants, despite major advances in neonatal intensive standard care and improvement of nutritional supply. This is consistent with our previous cross-sectional report showing that tibial SOS in VLBW infants correlates positively with gestational age but inversely with postnatal age.[14] The major finding of this study was that relatively brief, daily, passive range-of-motion physical activity attenuated the decrease in bone SOS in this unique population.

Bone mineralization is modulated by genetic, nutritional, hormonal, and mechanical factors.[27] However, because the major cause of osteopenia and osteomalacia in premature infants is inadequate postnatal calcium and phosphorus intake, compared with the accretion of these minerals during the last trimester of pregnancy,[3,4] most therapeutic efforts to prevent osteopenia of prematurity have focused on nutritional changes. However, despite the nutritional goal of providing optimal support for growth among premature infants (similar to the growth in utero during the last trimester),[28] nutritional interventions have been only partially successful in improving their bone mineralization.[4]

The importance of mechanical stimulation for bone development in preterm infants has long been neglected. Recently Moyer-Mileur et al[24,25] used single-photon absorptiometry and DEXA to demonstrate that daily passive range-of-motion exercise increased bone mineral content and density in VLBW infants. Our finding that physical activity attenuated the decrease in bone SOS (using QUS measurements) is consistent with these findings and suggests that

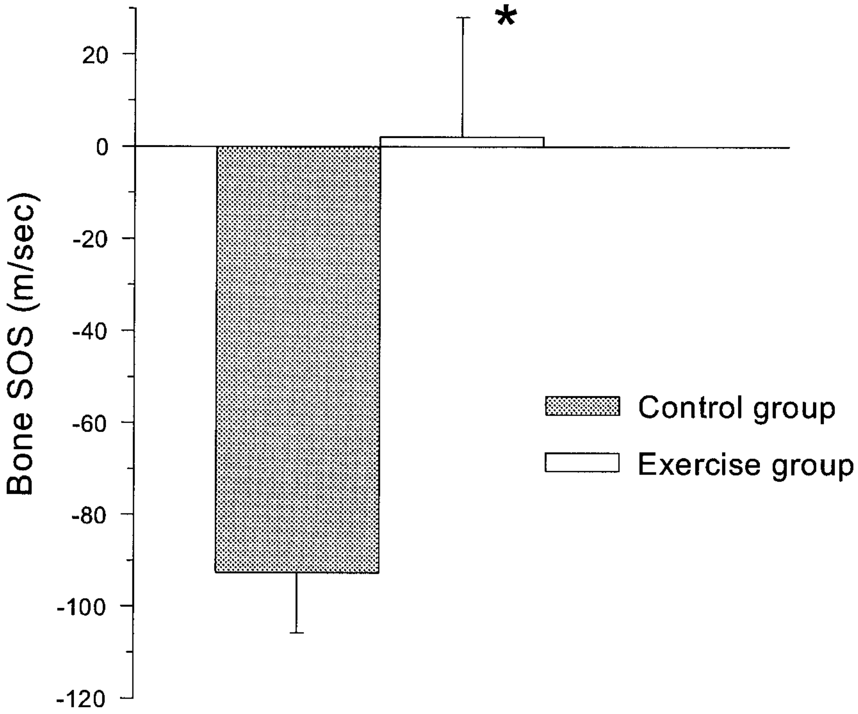

Fig 1. Changes in bone SOS in VLBW infants during the study period. Bone SOS decreased significantly in the control subjects but remained unchanged in the exercise group (*$P < .006$).

TABLE 3. Changes in Circulating Bone Turnover Marker During the Study Period

	Control Group (n = 12)		Exercise Group (n = 12)	
	Pre	Post	Pre	Post
BSAP (U/L)	104.6 ± 12.1 (63–243)	117.3 ± 11.9* (80–280)	119.0 ± 18.4 (63–243)	136.9 ± 18.4* (80–280)
ICTP (ng/mL)	141.2 ± 7.8 (61–205)	100.0 ± 2.4* (45–185)	138.4 ± 10.6 (61–205)	114.6 ± 5.2* (45–185)

Data are presented as mean ± SEM. Normal values for the first (pre) and fourth (post) week of life in premature infants are in parentheses.[18] No significance between group differences was found.
* $P < .05$ for within-group differences.

exercise has an important role in bone development during the neonatal period and may contribute to the prevention of osteopenia of prematurity.

It is still unclear why the same exercise protocol resulted in increased mineralization in the Moyer-Mileur studies[24,25] but only attenuated the decrease in bone SOS in our study. The answer is probably related to the difference between the methods used. Single-photon absorptiometry and DEXA, as used by Moyer-Mileur et al, measure mainly quantitative aspects of bone such as mineral density, whereas QUS, in addition to the quantitative measures (bone mineralization), also assesses qualitative factors that contribute to bone strength, such as bone elasticity, microarchitecture, and fatigue damage.[10–13,29] Increase in bone mass is not always accompanied by an increase in bone strength, which is functionally the most important property of the bone.[27] We believe that the combination of reduced qualitative and quantitative bone properties contributes to the postnatal decrease in tibial SOS in VLBW premature infants. Exercise was able only to prevent this decline.

Severe morbidity during the neonatal period (eg, bronchopulmonary dysplasia) also increases the risk of bone demineralization in premature infants.[3] However, there were no differences in morbidity between the control and exercise group subjects in the present study (Table 1).

It is important to note that QUS assessment of tibial SOS was performed successfully in all of the premature infants and was able to determine the changes in tibial SOS after the intervention. Therefore, we believe that this method can serve as a useful tool to estimate bone strength in premature infants and that QUS may also prove to be effective in the evaluation and follow-up of osteopenia in premature infants.

Despite the favorable effects of exercise on bone SOS, physical activity was not accompanied by significant changes in both bone formation (BSAP) and resorption markers (ICTP). Previous studies by Moyer-Mileur et al[25] also did not find any increase in bone formation markers in premature infants who started their exercise intervention relatively early after birth (2 weeks). In contrast, we have previously reported an exercise-induced increase in bone formation markers (BSAP and C-terminal Procollagen) and a decrease in bone resorption markers (ICTP) in VLBW premature infants who started their exercise intervention at a postnatal age of 4 to 5 weeks.[30] We speculate that the different results in the present study are attributable to a high bone turnover state that occurs in the first 3 postnatal weeks, as described by Shiff et al.[18] In the current study, the exercise protocol was initiated very early (days 4–7 of life). Therefore, it is possible that the marked early postnatal increase in these markers may have masked more subtle physical activity-associated effects.

Finally, the same exercise intervention, when started at a postnatal age of 4 to 5 weeks, was associated with a greater increase in weight gain.[24,30] The different result in this study suggests that the accelerated weight gain occurs only when exercise training is introduced later in the neonatal course (4–5 weeks vs first week). This observation is in agreement with Moyer-Miller et al,[25] who also found that the increase in weight gain was significant only when the premature infants reached body weight of 1.8 to 2 kg. Because early range-of-motion intervention induced positive bone effects without differences in weight gain, it may suggest that the positive bone effects were not related to the increased weight gain.

Using QUS, we have successfully shown that an early onset of a daily range-of-motion exercise program (only 5 min/d) prevented the postnatal decline of bone SOS in VLBW infants. Our data are consistent with previous reports showing different beneficial bone effects in VLBW premature infants by the same exercise protocol,[24,25,30] suggesting, therefore, that exercise has an important role in bone metabolism in this unique population. It is still unclear whether the positive bone effects are related just to the 5 minutes of range-of-motion intervention or to longer metabolic changes after the brief exercise. Moreover, whether this degree of exercise is the optimal intervention for bone development in premature infants still needs to be determined.

REFERENCES

1. Forbes GB. Calcium accumulated by the fetus. *Pediatrics.* 1976;57:976–977
2. Ziegler EE, O'Donnel AM, Nelson SE, Fomon SJ. Body composition of the reference fetus. *Growth.* 1976;40:329–341
3. Callenbach JC, Sheehan MB, Abramson SJ, Hall RT. Etiologic factor in rickets of very low birth-weight infants. *J Pediatr.* 1981;98:976–986
4. James JR, Condon PJ, Truscott J, Horsman A, Arthur R. Osteopenia of prematurity. *Arch Dis Child.* 1986;61:871–876
5. Bishop N. Bone disease in preterm infant. *Arch Dis Child.* 1989;64:403–409
6. Kulkarni PB, Hall RT, Rhodes PG, et al. Rickets in very low-birth-weight infants. *J Pediatr.* 1980;96:249–252
7. Rigo J, De Curtis M, Pieltain C, Picaud JC, Salle BL, Senterre J. Bone mineral metabolism in the micropremie. *Clin Perinatol.* 2000;27:147–170
8. Lyon AJ, Hawkes DJ, Duran M, McInntosh N, Chan F. Bone mineralization in preterm infants measured by dual energy radiographic densitometry. *Arch Dis Child.* 1989;64:919–923

9. Koo WWK, Mason LR, Walthers J. Validation of accuracy and precision of dual energy radiograph absorptiometry for infants. *J Bone Miner Res.* 1995;10:1111–1115
10. Foldes AJ, Rimon A, Keinan DD, Popovitzer MM. Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. *Bone.* 1995;17:363–377
11. Kang C, Speller R. Comparison of ultrasound and dual energy X-ray absorptiometry measurements in the calcaneus. *Br J Radiol.* 1998;56: 861–867
12. Prins SH, Jorgensen HL, Hassager C. The role of quantitative ultrasound in the assessment of bone: a review. *Clin Physiol.* 1998;18:3–17
13. Pearce S, Hurtig MB, Runciman J, Dickey J. Effect of age, anatomic site and soft tissue on quantitative ultrasound. *J Bone Miner Res.* 2000;15: S407
14. Nemet D, Dolfin T, Wolach B, Eliakim A. Quantitative ultrasound measurements of bone speed of sound in premature infants. *Eur J Pediatr.* 2001;160:737–740
15. Delmas PD. Biochemical markers of bone turnover. *Acta Orthop Scand.* 1995;266(suppl):176–182
16. Charles P, Hasling C, Risteli L, Risteli J, Mosekilde L, Eriksen EF. Assessment of bone formation by biochemical markers in metabolic bone disease: separation between osteoblastic activity at the cell and tissue level. *Calcif Tissue Int.* 1992;51:406–411
17. Eriksen EF, Charles P, Melsen F, Mosekilde L, Risteli L, Risteli J. Serum markers of type I collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. *J Bone Miner Res.* 1994;9:347–354
18. Shiff Y, Eliakim A, Sheinkin-Kestenbaum R, Arnon S, Lis M, Dolfin T. Measurements of bone turnover markers in premature infants. *J Pediatr Endocrinol Metab.* 2001;14:389–395
19. Eliakim A, Raisz LG, Brasel JA, Cooper DM. Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. *J Bone Miner Res.* 1993;8:127–132
20. Myburgh KH. Exercise and peak bone mass: an update. *S Afr J Sport Med.* 1998;5:3–9
21. Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnson CC. Role of physical activity in the development of skeletal mass in children. *J Bone Miner Res.* 1991;6:1227–1233
22. Mazess RB, Whedon GD. Immobilization and bone. *Calcif Tissue Int.* 1983;35:265–267
23. Rodriguez JI, Garcia-Alix A, Palacios J, Paniagua R. Changes in the long bones due to fetal immobility caused by neuromuscular disease: a radiographic and histological study. *J Bone Joint Surg Am.* 1988;70: 1052–1060
24. Moyer-Mileur L, Luetkemeler M, Boomer L, Chan GM. Effect of physical activity on bone mineralization in premature infants. *J Pediatr.* 1995;127:620–625
25. Moyer-Mileur L, 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;106:1088–1092
26. Kent NG. Markers of bone turnover. *J Int Fed Clin Chem.* 1997;9:31–35
27. Rauch F, Schoenau E. The developing bone: slave or master of its cells and molecules? *Pediatr Res.* 2001;50:309–314
28. American Academy of Pediatrics, Committee on Nutrition. Nutritional needs of very low birth-weight infants. *Pediatrics.* 1985;75:976–989
29. Abendschein W, Hyatt GW. Ultrasonic and selected physical properties of bone. *Clin Orthop.* 1970;69:294–301
30. Nemet D, Dolfin T, Litmanovitz I, Shainkin-Kestenbaum R, Lis M, Eliakim A. Evidence for exercise-induced bone formation in premature infants. *Int J Sports Med.* 2002;23:82–85

THERAPEUTIC MISCONCEPTION

"When doctors experiment at the frontiers of medicine, no one can say at the outset how the research will end. Doctors may warn patients of debilitating side effects and even death, but nonetheless, experts say, patients often fall prey to 'the therapeutic misconception,' the idea that if a doctor offers a treatment, it must have therapeutic value. 'These people get to the point where they are willing to try anything,' said Dr Judith P. Swazey, a medical historian who has done extensive research on the artificial heart. 'When they are in that position, I'm not sure how informed informed consent can be.'"

Stolberg SG. On medicine's frontier. *New York Times*. October 8, 2002

Submitted by Student

Early Physical Activity Intervention Prevents Decrease of Bone Strength in Very Low Birth Weight Infants

Ita Litmanovitz, Tzipora Dolfin, Orit Friedland, Shmuel Arnon, Rivka Regev, Ruth Shainkin-Kestenbaum, Monika Lis and Alon Eliakim

Pediatrics 2003;112;15-19
DOI: 10.1542/peds.112.1.15