Evidence for Exercise-Induced Bone Formation in Premature Infants

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Abstract

We assessed the effect of a four weeks exercise training intervention on bone turnover markers in premature infants. Twenty-four very low birth weight premature infants were matched for gestational age, birth weight, gender, as well as for corrected age and weight at initiation of the study. Then the subjects were randomly divided into an exercise (n = 12) and a control group (n = 12). Exercise consisted of passive range of motion exercise with gentle compression of both the upper and lower extremities lasting 5 - 10 minutes each day, 5 days per week for 4 weeks [18]. This protocol has been shown to increase bone mineral density in premature infants. Bone formation was assessed by measurements of circulating bone specific alkaline phosphatase (BSAP) and the C-terminal procollagen peptide (PICP). Bone resorption was determined by serum measurements of C- terminal cross-links telopeptide of type-I collagen (ICTP). Training led to a significant ($P < 0.05$) increase in weight gain (767 ± 49 versus 586 ± 24 gr in trained and control premature infants, respectively); and to a significant increase in BSAP (37.2 ± 14.6 versus 4.1 ± 8.4 % in trained and control premature infants, respectively) PICP increased also following exercise (34.6 ± 18.9 versus 5.4 ± 9.1 % in trained and control subjects, respectively), however this increase was not statistically significant. Exercise led to a significant decrease in ICTP (-24.7±3.1 versus -5.5 ± 5.4% in trained and control subjects, respectively). A relatively brief exercise intervention was associated with a biochemical evidence of bone formation in very low birth weight premature infants.

Key words
Premature · osteopenia · exercise · bone-turnover markers

Introduction

Osteopenia of prematurity is very common mainly because the period of the greatest bone accretion in pregnancy occurs during the last trimester [12,23] The rate of osteopenia is inversely related to birth weight and gestational age, and the prevalence of osteopenia is estimated as 50% in infants born at extreme low birth weight (ELBW), with a high fracture rate [3,13]. Severe morbidity during the neonatal period (e.g. bronchopulmonary dysplasia - BPD), and chronic drug therapy increase further the risk of bone demineralization [6].

Mechanical strain is one of the most powerful stimulators of bone formation and growth. Several studies have demonstrated that physical activity increases bone mass in children, adolescents and adults [8,19,22], while inactivity results in bone resorption, and decreased bone mineral density [17]. Therefore the prolonged hospitalization of premature infants in neonatal intensive care units (weeks or even months) without sensory and physical stimulation may cause bone demineralization.

Recently, Moyer-Mileur et al. [18] demonstrated that a passive range of motion exercise with gentle compression of both the upper and lower extremities in premature infants resulted in increased bone width, and bone mineral density (by single beam photon absorptiometry). However, the mechanism by which physical activity affects bone mineralization in premature infants is yet unknown.

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The development of new laboratory markers for biochemical analysis of bone turnover [5,9] may provide us with more information on the influence of different stimuli such as exercise on bone metabolism. In the present study we used the Moyer-Mileur et al. exercise protocol to determine the effect of exercise on circulating bone turnover markers in premature infants. Markers for bone formation included bone specific alkaline phosphatase (BSAP), and C-terminal procollagen peptide (PICP), which are released to the circulation during collagen synthesis [5,9]. Serum levels of the carboxy terminal cross-links telopeptide of type-I collagen (ICTP) was used as a marker of bone resorption reflecting osteoclastic activity [14]. We hypothesized that exercise training in premature infants will lead to increased bone formation.

Methods

Twenty-four premature infants hospitalized in the neonatal department and the neonatal intensive care unit at the Meir General Hospital, Sapir Medical Center, Israel participated in the study. Subjects were matched for gestational age, birth weight, gender, corrected age and weight at initiation of the exercise protocol, and the use of medication and the prevalence of BPD (Table **1**). Then the subjects were randomly divided into an exercise (n = 12) and a control group (n = 12). Premature infants with intra-uterine growth retardation, severe central nervous system disorders, neurological abnormalities, suspected bone and/or muscular diseases or subjects who developed sepsis during the follow-up were excluded from the study.

All premature infants were able to tolerate enteral feeding at 100 kcal/kg body weight/day. Subjects were fed either by fortified human milk (Enfamil Human milk fortifier, Ca 90 mg/100 cc; P 45 mg/100 cc), or by neonatal special formula (Similac Special Care 24, Ca 146 mg/100 cc; P 73 mg/100 cc). Mean energy, protein, calcium, phosphorus and vitamin D intake did not differ between the control and exercise groups during the study period. The study was approved by the Institutional Human Subject Review Board, and informed consent was obtained from the subject's parents.

Table **1** Characteristics of the study participants

	Control group (n = 12)	*Exercise group (n = 12)*
Birth weight (gr)	1007 ± 64	1079 ± 93
Gestational age (weeks)	28.4 ± 0.6	28.3 ± 0.6
Gender (males/females)	5/7	5/7
Corrected age at study entrance (weeks)	32.9 ± 0.5	32.9 ± 0.5
Weight at study entrance (gr)	1261 ± 39	1361 ± 47
BPD/diuretic use (# of subjects)	5/3	5/4

No significant differences were found between the groups. Data presented as mean ± SEM.

Exercise protocol

The physical activity program was based on the Moyer-Mileur et al. [18] protocol. Briefly, this protocol involves extension and flexion, range of motion exercise with passive resistance of both the upper and lower extremities. Both extension and flexion were performed five times at the wrist, elbow, shoulder, ankle, knee and the hip joints. The activity was performed five times per week for four weeks by the same trainer. The control subjects had a similar time of daily interactive periods of holding and stroking without range of motion activity, since tactile stimulation might influence bone growth and development.

Blood sampling protocol

Early morning venous blood samples for the evaluation of bone turnover markers were collected before and at the end of the intervention in both trained and control premature infants as a part of the routine follow up blood tests (i.e. routine chemistry panel and complete blood count are performed weekly for premature infants in our NICU).

Bone specific alkaline phosphatase (BSAP)

Circulating bone specific alkaline phosphatase levels were measured by immunoassay utilizing a monoclonal anti-BSAP (Metra Biosystems, Inc. Alkaphas-B kit, Mountain View, California). The enzyme activity of the captured BSAP is detected with a p-Nitrophenyl Phosphate substrate. Inter-assay c.v. was 5.0 – 7.6%, and intra-assay c.v. was 3.9 – 5.8%. Assay sensitivity was 0.7 U/L.

C-Terminal propeptide of type-I collagen (PICP)

PICP levels were measured by a sandwich immunoassay using the Metra Biosystems, Inc. Procolagen-C kit (Mountain View, California). Inter-assay c.v. was 5.0 – 7.2%, and intra-assay c.v. was 5.5 – 6.8%. Assay sensitivity was 0.2 mg/l.

C-Terminal cross-links telopeptide of type-I collagen (ICTP)

ICTP levels were determined by equilibrium radioimmunoassay with I^{125} serving as a tracer, using the Diasorin ICTP kit (Stillwater, Minnesota). Inter-assay c.v. was 4.1 – 7.9%, and intra-assay c.v. was 2.8 – 6.2%. Assay sensitivity was 0.5 mcg/L.

Statistical analysis

Unpaired t-test was used to determine differences in birth weight, gestational age, corrected age, body weight and bone turnover markers prior to the training intervention between the exercise and control subjects. A two-way repeated measure ANOVA was used to compare the effect of the intervention on body weight, bone formation and resorption markers with time serving as the within group, and exercise as the between group factor. Statistical significance was taken at $p < 0.05$. Data presented as mean ± standard error (SEM).

Results

No significant differences were found in gestational age, birth weight, gender, corrected age and body weight at the beginning of the intervention, as well as in the use of medications and the prevalence of BPD between the control and exercise training premature groups (Table **1**).

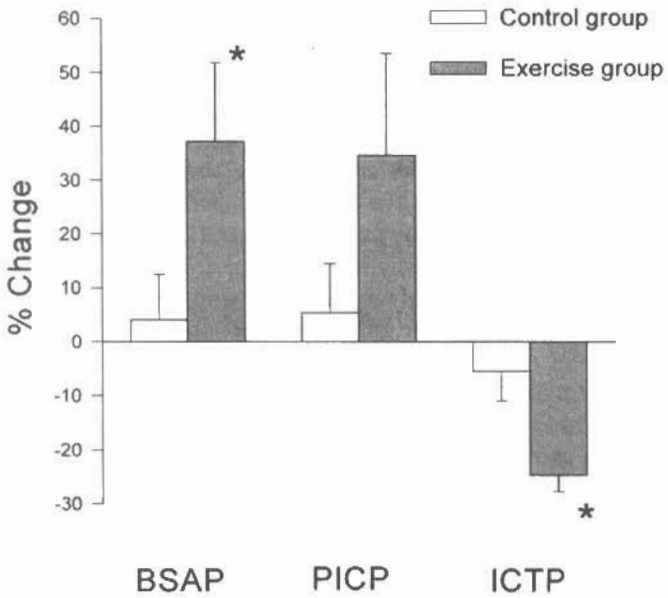

Fig. 1 The effect of passive range of motion exercise of both the upper and lower extremities on bone turnover markers in premature infants. Exercise led to an increase in bone formation markers, as well as to a significant decrease in bone resorption markers (*$p < 0.05$ for between group difference).

Table 2 The effect of exercise on weight gain and bone turnover markers in premature infants

	Control group (n = 12)		*Exercise group (n = 12)*	
	Pre	*Post*	*Pre*	*Post*
Weight (gr)	1261 ± 39	1847 ± 50	1361 ± 47	2129 ± 85*
BSAP (U/L)	216.9 ± 19.6	215.4 ± 21.8	202.9 ± 23.1	259.6 ± 27.0*
PICP (ng/ml)	3012 ± 295	2974 ± 207	3251 ± 289	3963 ± 325
ICTP (mcg/l)	130.9 ± 9.7	121.2 ± 10.4	129.7 ± 6.0	97.1 ± 5.2*

Data presented as mean ± SEM. *significant ($p < 0.05$) between group differences

The effect of the exercise intervention on weight gain and bone turnover markers is shown in Table **2** and Fig. **1**. No significant differences in these measures were noted between the groups prior to the intervention. Despite no significant differences in caloric and nutrient intake, exercise led to a significant increase in weight gain. Exercise was associated with a significant increase in BSAP, and with an increase in PICP; the latter, however, was not statistically significant. In addition, exercise was associated with a significant decrease in the bone resorption marker.

Discussion

Complete limb immobilization leads quickly to bone loss and decreased bone density, while bone formation increases dramatically when immobilized subjects resume exercise [1,15,16]. This led to the conclusion that physical activity stimulates bone formation and increases bone mineral density (BMD). This is particularly important for premature infant since during the prolonged hospitalization in the NICU (i.e. in small incubators and cradles) their standard care involves minimal sensory and/or tactile stimulation. Therefore, the limited physical activity of hospitalized premature infants increases the risk of developing bone demineralization and osteopenia of prematurity. So far, since the major cause of osteopenia of prematurity is inadequate calcium and phosphorus intake compared to the accretion of these minerals during the last trimester of pregnancy [3,13], most of the preventive efforts of osteopenia of prematurity have been focused on nutritional changes. However, despite the nutritional goal to provide optimal support for growth [4] (similar to the growth in utero during the last trimester), nutritional interventions were only partially successful in improving premature infants' bone mineralization. There have been no efforts, however, to change patterns of physical activity of premature infants.

Recently, Moyer-Mileur et al. [18] have demonstrated that a brief (4 weeks) passive range of motion exercise with gentle compression of both the upper and lower extremities in premature infants resulted in increased bone mineral density. They concluded that when premature infants perform daily physical activity in addition to the consumption of the recommended energy and nutrient intake, bone mass accretion is similar to the increase in bone indexes in utero during the last trimester. This enhancement of bone mass by physical activity was not accompanied by changes in circulating levels of calcium, phosphate, alkaline phosphatase, parathyroid hormone or vitamin D. Therefore, the mechanism for the exercise-induced increased bone density in premature infants was unknown. Using the Moyer-Mileur protocol we found in the present randomized, prospective study that exercise led to an increase in bone formation markers (i.e. BSAP), and to a significant decrease in the bone resorption marker (Fig. **1**). It is important to note that the postnatal period in premature infants is characterized by increases in bone formation markers [20,21], but we did not observe these changes in the control subjects over the study period. Therefore, our results support the hypothesis that the relatively brief exercise training intervention in premature infants stimulated new bone formation independently of the ongoing postnatal increase of these markers.

Circulating levels of PICP were also increased following exercise (712 ± 366 versus −38 ± 190 ng/ml in exercise and control subjects, respectively), however, this increase did not reach a between group statistical significance. It is important to note that PICP, while abundant in bone, is not solely limited to bones [10]. PICP is released from connective tissues as well. Therefore the reduced subcutaneous and connective tissue in preterm infants may have influenced the effects of exercise on PICP in this unique population. Studies with larger sample size will be needed to confirm these results and speculation.

The exercise training in premature infants led to changes in bone turnover markers in the range of 20 – 40 %. This is consistent with previous reports of increased bone formation following 5 weeks of endurance-type exercise training in adolescent males and females [7,8]. These results emphasize, perhaps, the important role of exercise training for bone formation during periods of rapid bone development.

The present study demonstrated that exercise training for premature infants resulted in a biochemical evidence for increased bone formation and decreased bone resorption. It is not known yet whether osteopenia of prematurity is a self-resolving condi-

tion or a disease with long-term consequences. Recently, however, Bowden et al. demonstrated reduced bone mineralization in 8 year-old children who were born prematurely [2]. Even if bone mineralization improves spontaneously in most infants, it does not imply that the period of demineralization is acceptable. While the long-term consequences are not clear, benefits of prevention and treatment include avoidance of fractures, and possibly, improved linear growth [6]. Whether this degree of exercise is optimal for premature infants is also not known. However, since this protocol was brief, easy to perform, and was not associated with "side effects", this approach might serve as a model for further investigations or programs designed to increase levels of physical activity and bone mass in premature infants, and to prevent osteopenia of prematurity.

Other possible beneficial effects of this exercise protocol may include an earlier discharge from the NICU (due to the greater increase in body weight). In addition, we recently demonstrated reduced physical ability in 5–8 year old healthy children who were born prematurely [11]. Early exercise interventions for preterm infants may increase muscle mass, may lead to an earlier maturation of the neuromuscular junction, and, if continued, may, perhaps, improve physical fitness later in life.

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