Original Article

The Effects of Exercise on Body Weight and Circulating Leptin in Premature Infants

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OBJECTIVE:
To assess the effect of daily movements on weight gain, serum leptin, and insulin-like growth factor I (IGF-I) in premature infants.

STUDY DESIGN:
Twenty very-low-birth-weight premature infants were matched and randomized to a daily movement ($n=10$) and control groups ($n=10$). Daily movement consisted of passive range of motion with gentle compression of both the upper and lower extremities 5 days per week for 4 weeks.

RESULTS:
Daily movements led to a significant increase in weight gain (784 ± 51 vs 608 ± 26 g in movements and controls, respectively, $p<0.02$), and to a significant increase in leptin (0.60 ± 0.19 vs 0.13 ± 0.06 ng/ml in movements and controls, respectively, $p<0.05$). Changes in body weight correlated with changes in serum leptin ($r=0.48$, $p<0.03$). IGF-I also increased following daily movements (18.8 ± 4.1 vs 9.2 ± 4.1 ng/ml in movements and controls, respectively); however, this increase was not statistically significant.

CONCLUSION:
A relatively brief range of motion daily movement intervention was associated with greater weight gain and increased leptin levels in very-low-birth-weight premature infants. This may suggest that at least part of the daily movements associated with increase in body weight resulted from an increase in adipose tissue.
Journal of Perinatology (2002) **22,** 550–554 doi:10.1038/sj.jp.7210788

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INTRODUCTION

Leptin is produced and secreted mainly from the adipose tissue.[1] Recent studies suggest, however, that leptin is secreted from the placenta, stomach, muscle, chondrocytes, and osteoblasts as well.[2] Increased leptin level in obese children and adults suggests that leptin plays an important role in the regulation of appetite, food intake, energy expenditure, and as a consequence body fat and body weight.[3,4]

Several studies have demonstrated that elevated level of cord blood leptin is correlated with birth weight.[5,6] Leptin concentrations are increased in large-for-gestational-age neonates compared to appropriate and small-for-gestational-age neonates,[7] and decreased in premature infants.[8] Serum leptin levels decline rapidly in the first week of life, and increase gradually thereafter.[9,10] However, this increase does not correspond with the rapid weight gain.[11,12] These changes might reflect changes in hormonal and nutritional state during the perinatal period, and suggest that leptin plays an important role in the regulation of weight changes even in early infancy.

Recently, Moyer-Mileur et al.[13,14] demonstrated that a passive range of motion daily movements with gentle compression of both the upper and lower extremities in premature infants resulted in increased weight gain, bone width, and bone mineral density (assessed by single beam photon absorptiometry and DXA). Using the same protocol, we demonstrated[15] that these daily movements were associated with an increase in bone formation marker and a decrease in bone resorption marker, suggesting new bone formation. Interestingly, recent studies demonstrated that leptin is secreted from human osteoblasts and promotes bone mineralization.[16,17]

Therefore, in the present study, we used the Moyer-Mileur et al. daily movement protocol in order to determine whether the daily movement–induced increase in bone mineral density and weight

Table 1 Characteristics of the Study Participants

	Control group ($n=10$)	Exercise group ($n=10$)
Birth weight (g)	1057 ± 62	1047 ± 96
Gestational age (weeks)	28.9 ± 0.7	28.2 ± 0.5
Gender (males/females)	4/6	4/6
Corrected age at study entrance (weeks)	33.0 ± 0.5	33.1 ± 0.5
Weight at study entrance (g)	1263 ± 39	1361 ± 51
Fortified human milk/preterm formula (number of subjects)	5/5	5/5
BPD/diuretic use (number of subjects)	4/2	4/3

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

gain is associated with changes in leptin level. In addition, we determined the effect of exercise on insulin-like growth factor I (IGF-I), an anabolic hormone known to correlate with fetal growth,[18] and to increase in response to exercise in children and adults.[19,20] We hypothesized that daily movement in premature infants will be associated with increases in both leptin and IGF-I levels.

METHODS

Twenty premature infants hospitalized in the neonatal intensive care unit (NICU) 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 (Table 1). Because the presence of chronic lung disease and its treatment (e.g., systemic steroids and diuretics) may attenuate growth in premature infants, subjects were also matched for the prevalence of bronchopulmonary dysplasia (BPD) and for the use of medication. The mean duration of mechanical ventilation among the BPD patients was 20.6 ± 3.8 days, and the mean duration of oxygen supplementation was 55.3 ± 3.4 days.

Subjects were randomized to a daily movement ($n=10$) and control groups ($n=10$). Premature infants with intrauterine 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 per day and did not receive additional parenteral nutrition. Subjects were fed either fortified human milk (Enfamil Human Milk Fortifier, Ca^{2+} 90 mg/100 ml; P-45 mg/100 ml) or neonatal special formula (Similac Special Care 24, Ca^{2+} 146 mg/100 ml; P-73 mg/100 ml). Because leptin is present in preterm human milk, but not in preterm formulas,[21] subjects were also matched for the use of fortified human milk and preterm formula (Table 1). Mean energy, protein, fat, calcium, phosphorus, and vitamin D intake did not differ between the control and daily movements groups during the study period (Table 2). The study was approved by the Institutional Human Subject Review Board, and informed consent was obtained from the subject's parents.

Daily Movements Protocol

The physical activity program was based on the Moyer-Mileur et al.[13] protocol. Briefly, this protocol involves extension and flexion, and range of motion daily movements 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

Table 2 Changes in Dietary Intake of the Study Participants During the Intervention

		Week 1	Week 2	Week 3	Week 4
Caloric intake (kcal/kg per day)	Control	123.2 ± 1.6	129.0 ± 1.5	135.2 ± 1.8	132.8 ± 2.1
	Daily movements	123.5 ± 1.4	128.3 ± 1.3	136.1 ± 2.0	132.5 ± 2.3
Protein (kcal/kg per day)	Control	13.2 ± 0.1	13.9 ± 0.1	13.9 ± 0.1	14.0 ± 0.2
	Daily movements	13.3 ± 0.1	13.8 ± 0.1	14.0 ± 0.1	14.0 ± 0.2
Fat (kcal/kg per day)	Control	60.8 ± 1.3	63.6 ± 1.2	68.8 ± 2.0	66.4 ± 1.9
	Daily movements	61.0 ± 1.1	63.4 ± 1.1	69.3 ± 1.9	66.1 ± 1.8
Calcium (mg/kg per day)	Control	214.9 ± 1.7	223.9 ± 1.7	224.5 ± 1.3	223.9 ± 2.2
	Daily movements	215.2 ± 1.8	223.4 ± 1.6	225.3 ± 1.1	223.3 ± 2.4
Phosphorus (mg/kg per day)	Control	107.3 ± 1.0	111.9 ± 0.7	112.2 ± 0.7	111.9 ± 1.4
	Daily movements	107.6 ± 0.9	111.7 ± 0.8	112.6 ± 0.6	111.6 ± 1.2

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

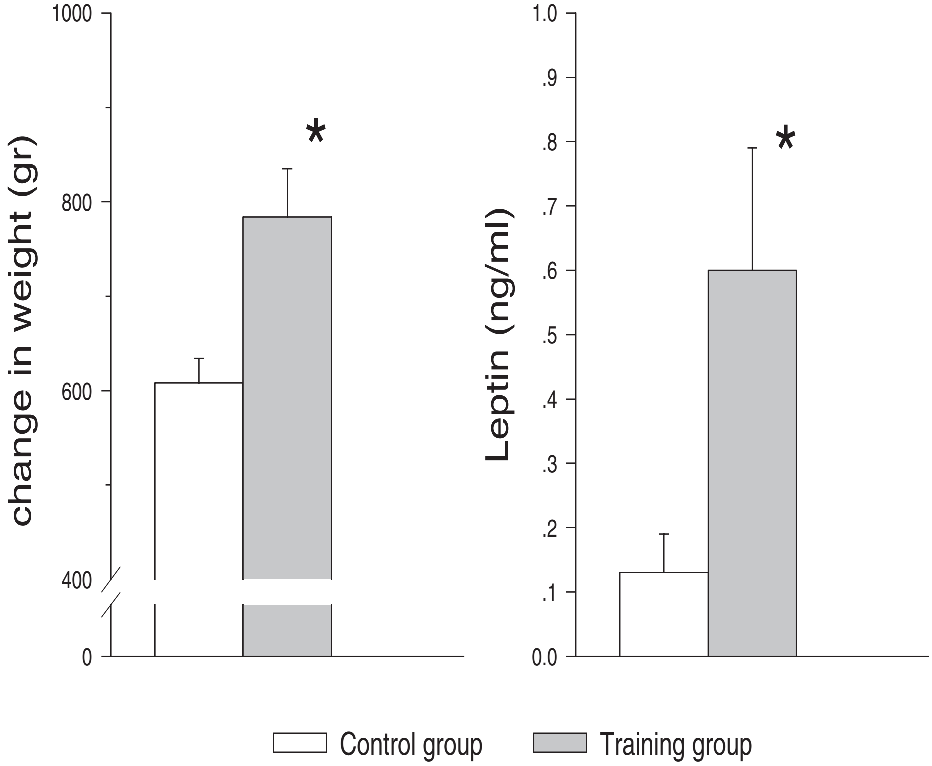

Figure 1. The effect of passive range of motion daily movements of both the upper and lower extremities on *changes* in body weight and circulating leptin level in premature infants. Daily movements led to a significant greater weight gain and leptin increase (*$p < 0.05$ for between-group difference).

the hip joints. The daily movements were performed five times per week for 4 weeks by the same trainer. The control subjects had a similar time of daily interactive periods of holding and stroking without range of motion movements, because tactile stimulation might influence bone growth and development.

Blood Sampling Protocol

Early morning (7–8 AM) venous blood samples for the evaluation of circulating leptin and IGF-I were collected before and at the end of the intervention in both daily movements and control premature infants. Samples were collected as a part of the routine follow up blood tests (i.e., routine chemistry panel and complete blood count are performed weekly in premature infants in our NICU). Premature infants in our NICU receive bolus enteral feeding every 3 hours. Therefore, in all subjects, both pre- and postdaily movements blood samples were collected after 3 hours of fasting.

Leptin. Serum leptin concentrations were determined by a two-site immunoradiometric assay (IRMA) using the DSL-23100 kit (Diagnostic System Laboratories, Webster, TX). Leptin interassay CV was 3.7% to 6.6% and intraassay CV was 2.6% to 4.9%. Assay sensitivity was 0.1 ng/ml.

IGF-I. IGF was extracted from IGFBPs using the acid–ethanol extraction method. Serum IGF-I concentrations were determined by a two-site IRMA using the DSL-5600 Active kit (Diagnostic System Laboratories). IGF-I interassay CV was 3.7% to 8.2% and intraassay CV was 1.5% to 3.4%. Assay sensitivity was 0.5 ng/ml.

Statistical Analysis

Two-sample t-tests were used to check for possible baseline differences in birth weight, gestational age, corrected age, weight, leptin, and IGF-I between the intervention and control subjects. A two-way repeated-measures ANOVA was used to compare the effect of

Table 3 The Effect of Daily Movements on Weight Gain, Circulating Leptin, and Serum IGF-I in Premature Infants With BPD

	Controls ($n = 4$)		Daily movements ($n = 4$)	
	Pre	Post	Pre	Post
Body weight (g)	1171 ± 84	1681 ± 101	1331 ± 80	2206 ± 118*
Leptin (ng/ml)	0.16 ± 0.05	0.37 ± 0.05	0.25 ± 0.08	1.01 ± 0.3*
IGF-I (ng/ml)	2.0 ± 1.1	2.0 ± 1.2	3.2 ± 1.4	17.2 ± 3.5*

*$p < 0.05$ for between-group differences. Data presented as mean ± SEM.

the intervention on body weight, leptin, and IGF-I, with time serving as the within-subjects factor and daily movements as the between-group factor. The test of interest was the interaction between the grouping factor and time. Because of possible "outliers," we repeated these analyses using nonparametric (Mann–Whitney) tests. Statistical significance was taken at $p<0.05$. Data are presented as mean ± standard error of the mean (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 fortified human milk, medication, and the prevalence of BPD between the control and daily movements premature groups (Table 1). Body weight increased significantly in both groups (608 ± 25 g [17.3 ± 0.7 g/kg per day] and 784 ± 50 g [20.5 ± 1.1 g/kg per day] in control and daily movements subjects, respectively). However, the increase in weight gain for the daily movements subjects was significantly greater ($p<0.02$, Figure 1).

No significant difference was found in circulating leptin level between the daily movements and control subjects prior to the intervention. Serum leptin increased significantly in both groups (from 0.23 ± 0.06 to 0.36 ± 0.05 ng/ml, and from 0.18 ± 0.04 to 0.78 ± 0.20 ng/ml in control and daily movements subjects, respectively). However, the increase in leptin in the daily movements subjects was significantly greater ($p<0.05$, Figure 1). In addition, the increase in body weight (for all subjects) correlated with changes in serum leptin ($r=0.48$, $p<0.03$).

No significant difference was found in circulating IGF-I level between the daily movements and control subjects prior to the intervention. Circulating IGF-I increased significantly in both groups (from 4.3 ± 1.4 to 13.6 ± 3.8 ng/ml, and from 1.5 ± 0.8 to 20.3 ± 4.2 ng/ml in control and daily movements subjects, respectively). The IGF-I increase in the daily movements subjects (18.8 ± 4.1 ng/ml) was greater than the increase in the control subjects (9.2 ± 4.1 ng/ml). However, this difference was not statistically significant ($p<0.09$).

Interestingly, when only premature infants with BPD were compared, the increase in weight gain, circulating leptin, and serum IGF-I in the daily movements group was significantly greater than controls (Table 3).

DISCUSSION

We found that a brief (4 weeks) passive range of motion daily movements with gentle compression of both the upper and lower extremities in premature infants resulted in increased weight gain, and with increases in circulating leptin levels. Moreover, changes in leptin correlated with the changes in body weight.

To our knowledge, this is the first study to examine the effect of exercise on growth and leptin levels in premature infants. Several studies examined the effect of exercise training on circulating leptin levels in adults. The majority of these studies included obese subjects, and were conducted as part of weight reduction programs. These studies showed that exercise training resulted in decreased leptin concentrations.[22–26] However, these decreases were related mainly to negative energy balance and/or to the loss of adipose tissue, and suggested that exercise training does not have an independent effect on circulating leptin. In the present study, we used daily movements protocol that was designed originally to increase bone mineralization in premature infants[13,14] and resulted in increased weight gain. The significant increase in circulating leptin and the correlation between the changes in leptin and the changes in body weight may suggest that at least part of the increase in body weight was due to an increase in adipose tissue.

The mechanism for the exercise-induced weight gain in premature infants is not clearly understood. Moyer-Mileur et al.[13,14] suggested that part of the exercise-induced weight gain is related to increased bone mineralization. Interestingly, several studies[16,17] indicate that leptin is secreted from human osteoblasts and promotes bone mineralization. Therefore, our finding of increase in circulating leptin using the same daily movements protocol may also explain the exercise-induced increase in bone mineral density in premature infants.

Another possible explanation for the increase in serum leptin in premature infants could be the presence of leptin in preterm human breast milk, and not in preterm formulas.[21] However, in the present study, there was no between-group difference in the number of fortified human milk– versus preterm formula–fed infants.

We found that the increase in circulating IGF-I in the daily movements subjects was greater than the increase in the control subjects, but this difference was not statistically significant ($p<0.09$). We previously reported in adolescent males[27] and females[19] two phases in the IGF-I response to a more vigorous endurance exercise training. The sudden imposition of an endurance training program in sedentary adolescents first leads to reductions in IGF-I suggestive of a catabolic state. At some point, an anabolic rebound occurs, resulting in increased IGF-I levels. Exactly when and how this change occurs remains unknown. The daily movements protocol used in this study was anabolic at least to the skeleton,[13,14] and resulted in weight gain. However, because we measured IGF-I after 4 weeks of training, the possibility exists that a longer training program (or use of larger sample size) is needed to induce significant effects in circulating IGF-I during this period of rapid growth in premature infants.

Interestingly, the daily movements–associated increase in IGF-I was statistically significant in premature infants with BPD. It is not clear whether this suggests that daily movement exercise has a stronger effect in the more catabolic premature infants (i.e., BPD) and, therefore, needs to be investigated in a larger sample size.

In summary, a brief passive range of motion daily movement intervention was associated with increased weight gain and increased circulating leptin in premature infants, suggesting an increase in adipose tissue. A possible beneficial effect of applying a daily movement protocol may include earlier discharge from the NICU (due to increased weight gain). In addition, early exercise interventions for preterm infants may increase muscle mass and lead to an earlier maturation of the neuromuscular system. Moreover, if continued, exercise may perhaps improve their low level of physical fitness later in life.[28] The role and effects of exercise as part of the standard of care for premature infants are yet to be determined.

Acknowledgement

We thank MaryAnn Hill, PhD, from the University of California, Irvine, CA, USA, for her help in performing the statistical analysis.

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