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Evidence for Increased Bone Formation Following a Brief Endurance-Type Training Intervention in Adolescent Males

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ABSTRACT

The effect of exercise training, particularly relatively brief periods, on bone turnover markers in adolescents has been poorly studied. Thirty-eight healthy males (16 ± 0.7 years) participated in a 5-week summer school program in which 20 subjects were randomly assigned to a training group consisting of 2 h/day, 5 days/week of endurance exercise, and 18 subjects were assigned to a control group. Bone formation was assessed by measurements of circulating osteocalcin, bone-specific alkaline phosphatase (BSAP), and the C-terminal procollagen peptide (PICP). Bone resorption was assessed by urinary levels of free deoxypyridinoline cross-links (dPYR) and the C- (CTX) and N-terminal (NTX) telopeptide cross-links. Prior to training, there was a weak positive correlation between fitness and PICP ($r = 0.27, p < 0.05$), but no correlations were observed between fitness and either the other markers of bone formation or bone resorption. Training led to a significant increase in (1) osteocalcin (15 ± 4%, $p < 0.03$), (2) BSAP (21 ± 6%, $p < 0.02$), and (3) PICP (30 ± 11%, $p < 0.03$) and to a significant decrease in NTX (−21 ± 3%, $p < 0.05$). These bone turnover markers did not change in the control subjects (osteocalcin, 0 ± 4%; BSAP, 2 ± 4%; PICP, −4 ± 6%; NTX, −6 ± 4%). There was no change in urinary dPYR and CTX in either control or trained subjects. Fitness is only weakly, if at all, correlated with bone formation, but a relatively brief period of endurance training leads to a substantial increase in bone formation markers in adolescent males. School-based, short-term exercise training programs could play a role in enhancing bone formation in adolescents. (J Bone Miner Res 1997;12:1708–1713)

INTRODUCTION

Complete limb immobilization (e.g., bed rest[1] or space flight[2]) quickly leads to destructive bone loss, and bone formation dramatically increases when immobilized subjects resume exercise.[3] This has led to the popular conclusion that physical activity enhances bone formation and, consequently, bone mineral density (BMD). The potential of physical activity to increase bone mass is particularly important in children and adolescents when both BMD and markers of bone formation (e.g., osteocalcin) are rapidly increasing.[4] Moreover, BMD reaches about 90% of its peak by the end of the second decade,[5] supporting the idea that patterns of physical activity during childhood and adolescence can act to prevent bone disorders (like osteoporosis) later in life.

Despite strong indirect evidence in highly trained athletes or immobilized subjects linking physical activity with increased bone formation, direct evidence for this relationship in an otherwise healthy, mobile population is lacking. A variety of investigators have been unable to find a consistent relationship between habitual physical activity levels and bone mass in moderately active adults.[3,6–8] Moreover, in women engaged in intense training, bone loss may actually predominate if energy balance is impaired and estrogen production is decreased.[9]

In contrast, most cross-sectional studies in normally active children and adolescents suggest that higher levels of

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physical activity are indeed associated with increased bone mass.[10–12] The relatively new development of assays for circulating biochemical markers of bone turnover[13] now allows us to gain greater mechanistic insight into the effects of factors like exercise and maturation on bone development. These observations led us to form two hypotheses focused on adolescent males: (1) levels of fitness would be correlated with circulating markers of increased bone formation; and (2) a brief (5-week) training intervention would lead to increases in biochemical markers of bone formation.

Similar to adults, there have been few, if any, controlled prospective studies designed to examine the effect of a quantified training intervention on the serum biochemical markers of bone formation and resorption. Nor have there been studies designed to examine the relationship between an objective index of fitness (e.g., peak $\dot{V}O_2$) and circulating markers of bone turnover.

MATERIALS AND METHODS

Overall study design

The study consisted of two components: (1) a cross-sectional investigation of the correlation between fitness and bone turnover markers; and (2) a prospective, controlled, endurance exercise training intervention.

Sample population

Forty-four males volunteered to participate in the study. The subjects were all students at Torrance High School (Torrance, CA, U.S.A.) and enrolled in an anatomy and physiology class during the summer of 1995 (July–August) with class hours from 8 a.m. to 12:30 p.m. The ethnic configuration of the group was 71% Asian, 20% Caucasian, and 9% Hispanic. No attempt was made to recruit subjects who participated in competitive extramural athletic programs. The study was designed to examine late pubertal subjects with an age range of 15–17 years (mean 16 ± 0.7 years). Measurements of height and weight were made using standard techniques. Assessment of pubertal status was performed by physical examination in all of the subjects using Tanner criteria of penile length, testicular size, and pubic hair. Thirty-one (70%) of the subjects were found to be at Tanner level V, 11 (25%) at Tanner level IV, and 2 (5%) at Tanner level III.

The participants were randomized to a control ($n = 22$) or training group ($n = 22$). All subjects participated in the 2-h daily teaching program. During the remaining time, the training group members underwent endurance-type training consisting of running, aerobic dance, competitive sports (e.g., basketball), and occasional weight lifting. These activities were varied in duration and intensity throughout the week, primarily to encourage maximal participation of the subjects. On average, "aerobic" or endurance type activities accounted for about 90% of the time spent in training. Of these, about 50% involved running, 40% team sports, and 10% aerobic dance. Resistant training (i.e., weight lifting) accounted for the remaining 10% of the time spent in training. Training was directed by a member of the Torrance High School faculty.

The control group subjects participated in a computer workshop designed to improve their computer skills. No attempt was made to influence extracurricular levels of physical activity in either the control or trained groups. The study was approved by the Institutional Human Subject Review Board, and informed consent was obtained from the subjects and their parents or guardians.

One subject did not participate in the blood and urinary sampling protocols. Thus, the cross-sectional component of the study consisted of 43 subjects. During the course of the intervention period, five subjects (three from the control group and two from the training group) withdrew due to academic and/or disciplinary reasons. The two subjects who withdrew from the training group did so 2 days prior to the final examination and reported that the difficulty of the training protocol was not the reason for their decision. Thus, at the end of the study, 38 subjects (18 from the control group and 20 from the trained group) completed all pre- and postinterventional aspects of the protocol.

Measurement of peak $\dot{V}O_2$ and HR

Studies of peak $\dot{V}O_2$ and heart rate (HR) were made before and immediately after the 5-week protocol in all subjects. Each subject performed a ramp-type progressive exercise test on a cycle ergometer in which the subject exercised to the limit of his tolerance. Gas exchange was measured breath-by-breath,[14] and the peak $\dot{V}O_2$ and the peak O_2-pulse (i.e., peak $\dot{V}O_2$/peak HR) were determined as previously described in children and adolescents.[15,16]

Blood and urine markers of bone turnover

Assays are now available to detect serum and urinary markers of bone formation and resorption.[17,18] Bone osteoblastic activity was assessed by measurements of circulating osteocalcin, bone-specific alkaline phosphatase (BSAP), and the C-terminal procollagen peptide (PICP) levels which are released to the circulation during collagen synthesis.[13,19] Bone resorption was assessed by urinary levels of collagen degradation products including free deoxypyridinoline cross-links (dPYR), and the C- (CTX) and N-terminal (NTX) telopeptide cross-links which reflect osteoclastic activity.[20]

Blood and urine (early morning specimen) samples were collected the week before and during the week after the completion of the training intervention. Measurements of urinary bone resorption markers were normalized to urinary creatinine levels. No subjects trained during the day preceding the overnight blood sampling. All pre- and postintervention specimens were analyzed in the same batch by laboratory workers who were blinded to the group and order of the sample.

Osteocalcin

Circulating osteocalcin levels were measured by coated-tube immunoradiometric assays (IRMA), using the CISUS

Inc. ELSA-OSTEO kit (Bedford, MA, U.S.A.). Interassay coefficient of variation (CV) was 4.5–5.2%, and intra-assay CV was 3.8–3.9%. Assay sensitivity was 0.4 ng/ml.

Bone-specific alkaline phosphatase

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

C-terminal PICP

PICP levels were measured by a sandwich immunoassay using the Metra Biosystems, Inc. Procolagen-C kit. Inter-assay CV was 5.0–7.2%, and intra-assay CV was 5.5–6.8%. Assay sensitivity was 0.2 U/l.

Deoxypyridinoline cross-links

Urinary dPYR was measured by a competitive enzyme immunoassay in a microtiter plate format utilizing a monoclonal anti-dPYR antibody coated on the plate to capture dPYR. dPYR in the sample competes with conjugated dPYR-alkaline phosphatase for the antibody and the reaction is detected with a p-nitrophenyl phosphate substrate. We used the commercially available Pyriliks-D kit from Metra Biosystems, Inc. Inter-assay CV was 6.3–10.3%, and intra-assay CV was 3.6–9.5%. Assay sensitivity was 3 nM.

C-terminal telopeptide cross-links

Urinary CTX levels were measured by enzyme-linked immunosorbent assay (ELISA), using the Diagnostic System Laboratories, Inc. crosslaps DSL-10-1700 kit (Webster, TX, U.S.A.). Inter-assay CV was 4.7–9.4%, and intra-assay CV was 2.9–5.7%. Assay sensitivity was 50 μg/l.

N-terminal telopeptide cross-links

Urinary NTX levels were measured by ELISA, using the Ostex International Inc. Osteomark kit (Seattle, WA, U.S.A.). Inter-assay CV was 3–5%, and intra-assay CV was 5–8%. Assay sensitivity was 20 nM bone collagen equivalents.

Statistical analysis

Linear regression analysis was used to determine the correlation between fitness (as peak $\dot{V}O_2$ in absolute terms and as peak $\dot{V}O_2$ normalized to body weight—peak $\dot{V}O_2$/kg; the latter is a common, albeit imperfect, way to reduce the confounding factor of body size on the assessment of fitness[21,22]) and bone turnover markers. Linear regression techniques were used to determine the correlation between training-associated, percent changes in the markers of bone formation and markers of bone resorption. Unpaired *t*-tests were used to determine differences in markers of bone formation and resorption between control and training group subjects prior to the training intervention. Two-way repeated measures analysis of variance (ANOVA) was used to compare the effect of the intervention on bone markers and on the peak HR and peak O_2-pulse. In addition, for peak $\dot{V}O_2$ values, nonparametric chi-square analysis was also used. Statistical significance was taken at the $p < 0.05$ level. Data are presented as mean ± standard error (SE).

RESULTS

Cross-sectional study: Correlation between fitness and bone turnover markers

There were no correlations between the peak $\dot{V}O_2$ (either in absolute terms or normalized to body weight) and markers of bone resorption. There was a weak correlation between the absolute and normalized peak $\dot{V}O_2$ and PICP ($r = 0.27$, $p < 0.05$). No other significant correlations between fitness and bone formation markers were observed.

Prospective endurance type training intervention

Peak $\dot{V}O_2$ was not changed in the control subjects (pre, 2440 ± 110 ml/minute; post, 2484 ± 110 ml/minute). In the trained subjects, peak $\dot{V}O_2$ tended to increase (from 2419 ± 77, to 2501 ± 82 ml/minute), but this increase was significant only when we used nonparametric chi-square analysis. However, HR at peak $\dot{V}O_2$ was significantly lower (by ANOVA) following the intervention in the trained (pre, 191 ± 2 bpm; post, 184 ± 2 bpm) but not control subjects (pre, 193 ± 2 bpm; post, 193 ± 2 bpm). Consequently, the peak O_2-pulse was significantly greater following the intervention in the trained (pre, 12.7 ± 0.4 ml/beat; post, 13.6 ± 0.4 ml/beat, $p < 0.05$) but not control subjects (pre, 12.6 ± 0.5 ml/beat; post, 12.9 ± 0.6 ml/beat). This represented an increase in O_2-pulse of 7.6 ± 1.6%.

The effects of the training intervention on bone formation markers are summarized in Table 1 and Fig. 1. There were no significant differences in bone formation and resorption markers between the control and training group subjects prior to the intervention. There was a significant increase in circulating osteocalcin, BSAP, and PICP levels in the trained subjects but not in the control group subjects. There were no significant changes in urinary deoxypyridinoline and CTX in either group following the intervention. Urinary NTX levels decreased significantly in the trained but not in the control group subjects (Fig. 2).

In the trained subjects, the percent change in osteocalcin was inversely correlated with percent changes in NTX ($r = -0.49$, $p < 0.03$; Fig. 3) and CTX ($r = -0.43$, $p < 0.05$). However, significant correlations were not found comparing the changes in BSAP and PICP with changes in bone resorption markers.

DISCUSSION

In this study of healthy, adolescent males, we found no relationship between fitness and markers of bone resorp-

TABLE 1. EFFECTS OF 5 WEEKS OF ENDURANCE-TYPE TRAINING INTERVENTION ON BONE FORMATION AND BONE RESORPTION MARKERS IN ADOLESCENT MALES

	Control group (n = 18)		*Training group* (n = 20)	
	Pre	*Post*	*Pre*	*Post*
Osteocalcin (ng/ml)	84.3 ± 10.5	83.2 ± 9.9	92.7 ± 12.0	103.1 ± 12.7*
BSAP (U/l)	42.3 ± 4.2	43.1 ± 4.4	43.9 ± 4.9	51.5 ± 5.2*
PICP (ng/ml)	154.7 ± 13.2	144.6 ± 13.5	148.8 ± 12.9	179.2 ± 14.0*
dPYR (nM/mM creatinine)	8.1 ± 1.1	8.1 ± 0.8	10.8 ± 1.4	11.0 ± 1.5
CTX (μg/l/mM creatinine)	723.5 ± 101.3	783.6 ± 108.7	950.4 ± 112.1	960.3 ± 127.3
NTX (nM/mM creatinine)	203.9 ± 45.0	182.5 ± 36.7	323.3 ± 58.5	267.8 ± 53.9*

Results are shown as mean ± SEM. *Statistical significance by ANOVA, $p < 0.05$.

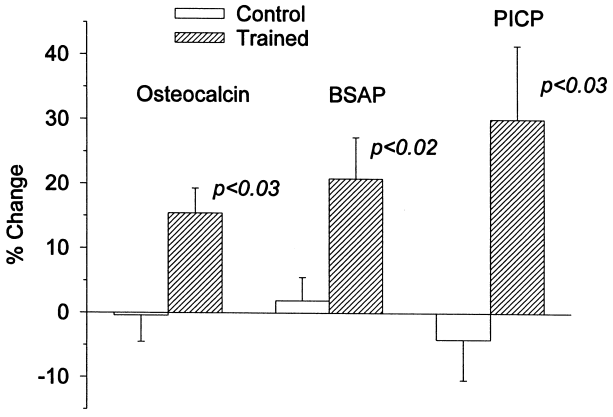

FIG. 1. Effect of exercise training on bone formation markers in adolescent males. Data are presented as mean percent change ± SE.

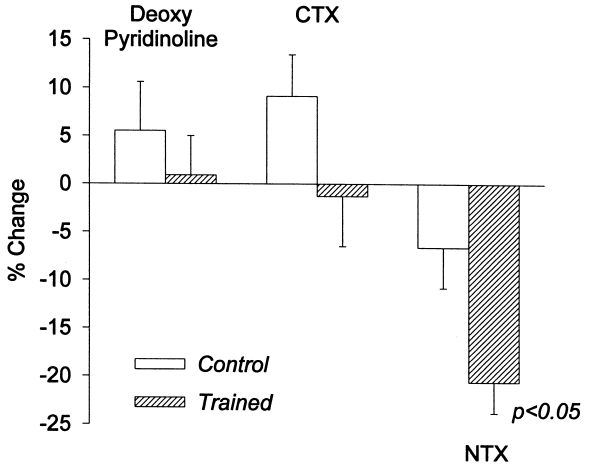

FIG. 2. Effect of exercise training on bone resorption markers in adolescent males. Data are presented as mean percent change ± SE.

tion. A weak correlation was observed between fitness and one marker of collagen formation (PICP), but not with osteocalcin or BSAP. Thus, our hypothesis that there would be a correlation between fitness and markers of bone formation was only partially supported by the data. In contrast, the 5-week training intervention increased fitness and led to substantial increases in all three markers of bone formation and a significant decrease in urinary NTX, a marker of bone resorption. In addition, training-induced increases in bone formation were correlated with reductions in bone resorption. These data from randomized, controlled prospective studies of endurance type training indicate that a relatively brief training intervention leads to a remarkably robust response of the circulating markers of bone formation in adolescent males.

The correlation between fitness and PICP, although weak, is intriguing because the PICP indicates new formation of type I collagen, which, while abundant in bone, is not solely limited to bone.[23] PICP is released from skin, cartilage, tendons, and other connective tissues, suggesting perhaps that there is a generalized increase in the synthesis of these tissues in fitter adolescents, those who are, presumably, more physically active. We found no positive correlation between fitness and either osteocalcin and BSAP (the more specific markers of bone formation) or a negative correlation between fitness and the markers of bone resorption.

The training input led to characteristic changes in the cardiorespiratory response to exercise (i.e., the increase in peak O_2-pulse). In addition, as we reported elsewhere in these subjects,[24] the training "input" was successful in that total energy expenditure in the training subjects was 15.5% greater than in the controls, and thigh muscle volume increased significantly only in the trained subjects.

In contrast to the cross-sectional results, the effect of the prospective training intervention on the markers of bone formation was clear (Fig. 1). It is important to note that puberty itself is characterized by increases in these markers, but we observed no significant increase in osteocalcin, BSAP, or PICP over the 5-week period in the control subjects. The finding of large increases in bone formation markers in the trained subjects strongly supports the hypothesis that relatively brief endurance type training in adolescent males specifically stimulates new bone formation independent of ongoing puberty-associated increases in these markers.

In some studies focused on young to middle-aged adults,

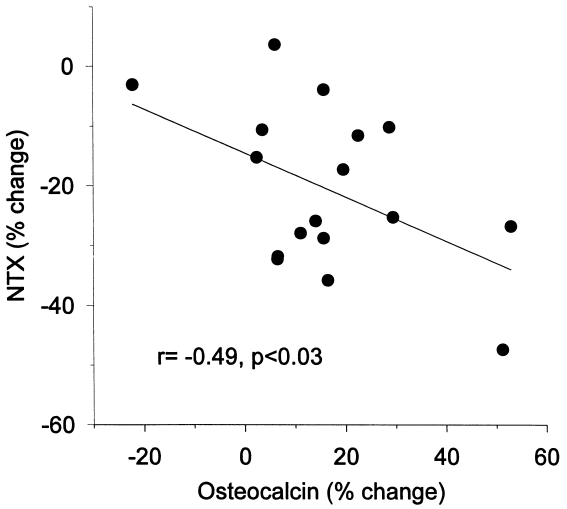

FIG. 3. Correlation between training-associated changes in a bone formation marker (serum osteocalcin) and changes in a bone resorption marker (urinary NTX) in the trained subjects.

exercise training led to increases in osteocalcin[25–27] after periods ranging from 12 weeks to 5 months. In contrast, Franck et al.[28] examined the effect of 8 weeks of endurance-type training in a group of sedentary young adult males and females (mean age 24 years) and found that circulating osteocalcin levels were significantly reduced after 4 weeks of training and only returned to basal levels by 8 weeks. Finally, in postmenopausal women, 11 months of endurance-type exercise training did not lead to changes in osteocalcin levels, but an increase in BMD was found.[29] These varying responses probably reflect the important roles played by gender, maturational status, and the type and duration of the training input.

An important result from our study was that in adolescent males a relatively moderate intensity training protocol lasting only 5 weeks led to changes in bone formation markers in the range of 15–30%. This is consistent with our recent data in adolescent females in whom 5 weeks of endurance training led to large increases (mean 39%) in circulating osteocalcin.[30] The apparent discrepancy that we found between relatively large increases in bone formation markers following a short, exercise training intervention and the lack of a strong correlation between the fit "state" and circulating levels of these markers is intriguing. Perhaps the maximal effect of exercise on bone formation occurs early in the course of the adaptation to training. In this paradigm, continued training does not result in further increases in bone formation markers, which may eventually fall. The course of bone formation in response to longer periods of training has yet to be determined in children and adolescents.

The effect of the training intervention on bone resorption, assessed by urinary collagen degradation products, was less consistent than the effects of training on bone formation. We did find a significant decrease in NTX in the trained but not the control subjects, as well as inverse correlations between training-induced changes in osteocalcin and changes in both NTX (Fig. 3) and CTX. Collectively, these observations would tend to support the concept that training led to a decrease in bone resorption. However, reductions in dPYR and CTX were not observed in the trained subjects. This is unexpected since the three markers of bone resorption are all derived from degradation of collagen and each reflects a different component of the collagen cross-links and their associated peptides.[23] There is no ready explanation for this discrepancy in our data. It may be that NTX is the most sensitive of bone resorption markers in the response to training. It is also possible that, had the training intervention continued for a longer period, all three markers of bone resorption would parallel the changes in NTX that were observed at 5 weeks.

We believe that the results of the present study in males, along with our previous demonstration of an even greater training-induced increase in osteocalcin in adolescent females,[30] are particularly important because of the widespread effects of physical activity on health during adolescence. Moreover, it is becoming apparent that there is currently a lack of school-based participation in noncompetitive types of exercise experiences for adolescents.[31] By working with local schools, we took advantage of existing facilities and an environment that was familiar to and relatively comfortable for high school students. The integration of a summer course in anatomy and physiology with a prospective study of physiological responses to exercise generated interest and enthusiasm among all the participants. As a result, we could successfully implement a training program with good compliance leading to substantial increases in bone formation markers. Whether or not this degree of intervention is optimal or can be accomplished under routine conditions in schools is not known; however, the approach might serve as a model for further investigations or programs designed to increase levels of physical activity and peak bone mass among children and adolescents and to prevent osteoporosis later on in life.

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