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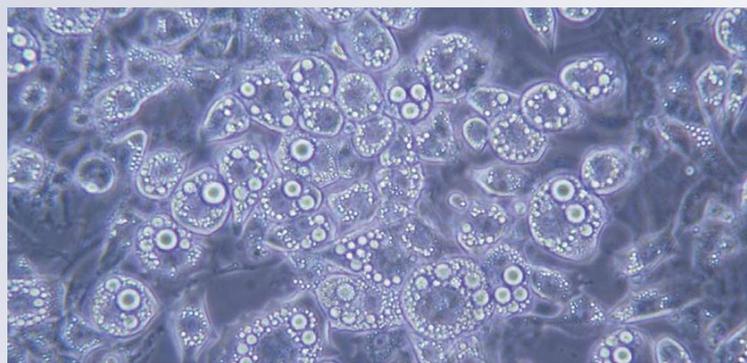
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Effects of resistance training on biomarkers of bone formation and association with red blood cell variables

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Abstract We previously showed that resistance training significantly increased the red blood cell count (RBC) and hematocrit (Hct) and decreased the mean cell hemoglobin concentration (MCHC) in physically inactive men. Since the enhanced hematopoiesis may result, at least partly, from bone metabolism, the purpose of this study was to further investigate the effect of resistance training on serum bone-specific alkaline phosphatase activity (B-ALP), a biomarker of bone formation, and focus on the relationship between the change in B-ALP from baseline to 20-week follow-up and the corresponding changes in RBC, Hct and MCHC. Seventy-four men aged 20–45 years were randomly assigned to training and control groups. The training group underwent a 20-week progressive resistance training. Fasting blood samples were analyzed for serum B-ALP at baseline, and at 10-week and 20-week follow-up. B-ALP in the control group exhibited no significant

change. In contrast, the training group increased its B-ALP from baseline at 10-week and 20-week follow-up (both $P < 0.01$ compared to control group). Within the training group, B-ALP was elevated at 10-week and 20-week follow-up when compared to baseline (both $P < 0.001$). A significant correlation was found between change in B-ALP from baseline to 20-week follow-up and the corresponding changes in RBC, Hct and MCHC in the training group ($r = 0.49$, $P < 0.01$; $r = 0.56$, $P < 0.01$, and $r = -0.38$, $P < 0.05$, respectively). We concluded that resistance training increased biomarkers of bone formation, which had association with RBC turnover. Adaptive changes of bone metabolism induced by resistance training might facilitate erythropoiesis.

Keywords Resistance training · Bone-specific alkaline phosphatase · Red blood cell · Hematocrit · Mean cell hemoglobin concentration

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Introduction

Ossification and hematopoiesis are two important processes taking place inside bone. Although the mechanisms of bone and blood formation have traditionally been viewed as distinct and unrelated, compelling evidence suggests that bone and blood are two tissues whose fates are intertwined [23]. Adult blood cells develop in the bone marrow, whose cavity serves as the main site of human hematopoiesis. There exist specific microenvironments or niches, in which hema-

topoietic stem cells (HSC) reside. These niches are essential for the survival, self-renewal and differentiation of HSC, as well as to sustain the lifelong process of constant replenishment of mature hematopoietic cells. A crucial component and regulator of the HSC niche are osteoblasts—the bone-forming cells derived from mesenchymal stem cells [3, 28].

It has been documented that disuse of bone (e.g., by immobility) can cause a severe loss of bone mass and strength, accompanied by anemia [17], whereas exercise training has been suggested to act as a safe, economical method of improving various symptoms of anemia [4, 5, 8, 12, 18]. Exercise training, such as climbing, increased bone formation and osteoblast differentiation in male mice [14]. Resistance training increased biomarkers of bone formation, indicated by enhanced serum osteocalcin concentration and serum bone-specific alkaline phosphatase activity (B-ALP) in young male subjects [6]. Furthermore, Kilgore et al. [10] reported that 6 weeks of moderate-to-intense resistance training of healthy untrained males could cause the serum total alkaline phosphatase (ALP) and the count of red blood cells (RBC) to rise. It was observed that exercise training might accelerate erythropoiesis or red cell mass expansion [9, 19, 22]. In a cross-sectional study, Schumacher et al. [20] found that athletes had higher RBC than sedentary subjects. Moreover, higher RBC and hemoglobin concentration were found in strength-trained athletes when compared with endurance- or mixed-trained subjects. Here, we hypothesized that there is a crosstalk between osteoblasts and HSCs involved with resistance training.

Generally, red blood cell variables can reflect bone erythropoiesis status. Previously, we showed that a 20-week resistance training could increase RBC and hematocrit (Hct) and decrease mean cell hemoglobin concentration (MCHC) in physically inactive men [7]. Hence, the purpose of this study was to further investigate the effect of resistance training on B-ALP, focusing on its association with the changes in RBC, Hct and MCHC induced by resistance training.

Materials and methods

Subjects

This study was a part of a larger investigation to explore the effects of resistance training on healthy,

physically inactive men. The subject recruitment and screening procedures as well as the resistance training program have been described previously [7]. Briefly, 74 healthy men, aged 20–45 years participating in regular physical activity no more than once a week during the past 3 years met the inclusion criteria. They were assigned randomly into two different resistance training groups ($n=26+26$) and one control group ($n=22$) based on their physical characteristics and level of physical activity. There were no significant differences in age, height, weight or isometric knee extension with right leg at 120° knee angle (180°=full extension) and height of counter-movement jumps between the groups. Since the volume and intensity of training was equal for both training groups, for the purposes of this report, the results of the two training groups were pooled.

Written informed consent was obtained from the subjects prior to the measurements, and the study was conducted according to the Declaration of Helsinki. Approval for the project was obtained before its initiation from the Ethics Committee of the Central Finland Health Care District.

Progressive resistance training

The progressive resistance training program has been presented in detail elsewhere [7]. Briefly, the 20-week progressive resistance training program included whole body exercise, with emphasis on increasing muscle strength and power of the lower limbs. The entire training period consisted of two 10-week training regimes. During the first 10 weeks, one training group trained with Natural Transmission™ machines (Ab Hur Oy, Kokkola, Finland), which use the pneumatic resistance principle. Another training group used conventional weight stack machines and free weights. In the following 10 weeks, groups were combined and they trained using pneumatic and weight stack training machines and also free weights. Each workout included a 10-min warm-up followed by 30–40 min of resistance training. The typical heavy resistance training was combined with explosive training. The typical heavy resistance training included comprehensive exercise using high load. Half squats, loaded squat jumps, leg presses and knee extension were the primary exercises used. Explosive training was high speed training with relatively lower load. Besides the high-load/high-speed training, part

of the leg exercise was performed with a special focus on the hypertrophy protocol [7]. Training loads were updated at weeks 10 and 15 on the basis of the present one-repetition maximum (1-RM) load that the training muscles could make. All training sessions were supervised by experienced personnel.

Subjects were asked not to alter their habitual physical activity during the study period. Training logs were kept to measure compliance and progress throughout the study.

Red blood cell variables measurement

Overnight fasting blood samples were drawn by a trained phlebotomist at 7:00–9:00 AM in supine position at baseline, and at 10-week and 20-week follow-up. Hematological variables were assayed with a fully automated haematology analyzer (Sysmex KX-21 N, Japan) immediately after sample collection. In this study, we calculated changes in RBC, Hct and MCHC from baseline to 20-week follow-up.

Assay of biomarkers of bone formation

Fasting blood samples for different time points were drawn and serum was separated from blood by centrifugation and stored immediately at -70°C until analyzed. Serum B-ALP at baseline, and at 10-week and 20-week follow-up was assessed using enzyme immunoassay (MicroVue, Quidel, San Diego, CA). The coefficient of variation in both inter- and intra-assay was less than 4%.

Maximal voluntary contraction

At baseline and after 20-week follow-up, peak maximal voluntary contraction (MVC) torque during isometric knee extension was measured bilaterally using a strength testing device (The Research Line, Ab Hur Oy, Finland). For isometric performances, the lever arm of the device was secured at the knee angle of 120° . Extraneous movement of the upper body was limited by two belts across the chest and abdomen respectively, with hip joint fixed at about 110° . The axis of the dynamometer was aligned with the knee flexion-extension axis, and the lever arm was attached to the right shank using a strap. After several warm-ups of submaximal knee extensions, the subjects were asked to exert maximum force as rapidly as possible

and to maintain that force for 3 s, during which verbal encouragement was given. Here, the results from the right leg are reported.

Body composition

At baseline and 20-week follow-up, body composition assessments were performed using a bioelectrical impedance analysis instrument (Tanita BC 418 MA Segmental Body Composition Analyzer, Japan). Lean body mass was given as the result. Anthropometric measurements (weight, height) were made using standard procedures.

Statistics

Analyses were performed using SPSS 15.0 for Windows. Data were checked for normality by Shapiro-Wilk's *W* test and for homogeneity by Levene's test before each analysis. All variables presented normal distribution except for B-ALP. Independent sample *t*-test was used to compare the differences between the groups at baseline. Comparisons of body weight, lean body mass and MVC before and after training in the two groups were tested using paired *t*-test. A natural logarithmic transformation was applied to normalize the B-ALP distribution. The effect of resistance training on B-ALP was assessed using analysis of variance (ANOVA) for repeated measures [time (baseline, 10- and 20-week follow-up) \times group (training, control)] with repeated contrast. An analysis of variance controlled for age was also performed. Correlations between change of serum B-ALP from baseline to 20-week follow-up (end-base) and corresponding changes of RBC, Hct and MCHC were examined in each group using Pearson's correlation coefficients. Significance was set at the level of $P < 0.05$.

Results

Of the enrolled subjects, 36 subjects in the training group and 15 in the control group completed all measurements. The main reasons for dropping out or exclusion were lack of time ($n=2$), no interest ($n=9$), diseases not related to the study ($n=3$), and inadequate participation rate in the training sessions ($n=3$). In addition, the serum of six subjects (one in the

control group and five in the training group) were missing for one time point.

There were no significant group differences with respect to physical characteristics and MVC torque at baseline (Table 1). After the 20-week intervention, the training group increased their lean body mass ($P<0.01$) and MVC of right leg extension ($P<0.01$, Table 1).

Serum B-ALP activity increased already at 10-week follow-up in the training group ($P<0.001$), and this significant elevation remained throughout the experimental period. A time \times group interaction was found ($P<0.01$). In contrast to the control group, the training group had higher serum B-ALP activity at both 10-week and 20-week follow-up than the initial value (Fig. 1). After adjusting for age, the results remained the same.

Significant correlations between the change in serum B-ALP activity from baseline to 20-week follow-up and the corresponding change in RBC and Hct were observed in the training group (Figs. 2, 3, $r=0.49$, $P<0.01$; $r=0.56$, $P<0.01$, respectively).

There was an inverse correlation between the change in serum B-ALP activity from baseline to 20-week follow-up and the corresponding change in MCHC in the training group ($R = -0.38$, $P<0.05$, Fig. 4).

However, no significant correlation between the change in serum B-ALP activity from baseline to 20-week follow-up and the corresponding change in RBC, Hct and MCHC was found in the control group (Figs. 2, 3, 4).

Discussion

A novel feature of this study was that the 20-week resistance training in physically inactive men had a

significant effect on serum B-ALP—a bone formation marker—whose change correlated with the corresponding changes in the hematopoietic indicators RBC, Hct and MCHC.

Resistance training increased serum B-ALP, concordant with a previous longitudinal study [6, 10]. In their study, Fujimura et al. [6] reported that the serum B-ALP increment of young adult men was detected already within the first month after beginning resistance training. B-ALP is a specific product of osteoblasts and may play a role in matrix mineralization [21]. Increased B-ALP is usually explained by awakening of bone remodeling and skeletal loading modulates remodeling rates. Studies revealed the function of osteocytes as mechanosensors in the early stage of bone remodeling. Loaded mechanical stress including fluid shear stress and tension or compression is converted to a series of biochemical signals, finally activating osteoblasts to induce bone formation [16, 24].

In the current study, the possible effect of age on bone formation capacity should be considered, since the subjects had an age range of between 20 and 45 years. However, after adjusting for age in the analysis of variance, the serum B-ALP still changed in the same way.

The effectiveness of the training program was demonstrated by a pronounced increment in MVC and lean body mass in the training group after 20-week follow-up. We previously found that a 20-week resistance training led to a pronounced RBC and Hct increment and MCHC decrement, which were probably due to accelerated erythropoiesis [7]. The conventional belief is that the mechanical stress is one of the main reasons for erythrocyte destruction. Also, activation of large muscle masses during

Table 1 Physical characteristics and maximal voluntary contraction (MVC) at the baseline and 20-week follow-up. Values are means \pm SD

Variable	Training ($n=36$)		Control ($n=15$)	
	Baseline	20-week follow-up	Baseline	20-week follow-up
Age (years)	32.1 \pm 7.3		32.7 \pm 6.8	
Height (cm)	180.2 \pm 4.8		178.4 \pm 7.7	
Body weight (kg)	81.7 \pm 10.9	82.1 \pm 10.8	79.9 \pm 10.2	79.5 \pm 10.9
Lean body mass (kg)	65.3 \pm 7.3	66.8 \pm 7.1**	64.1 \pm 7.7	64.7 \pm 8.4
MVC torque (Nm)	248 \pm 56	271 \pm 48**	218 \pm 47	219 \pm 47

** $P<0.01$, comparison between baseline and 20-week follow-up

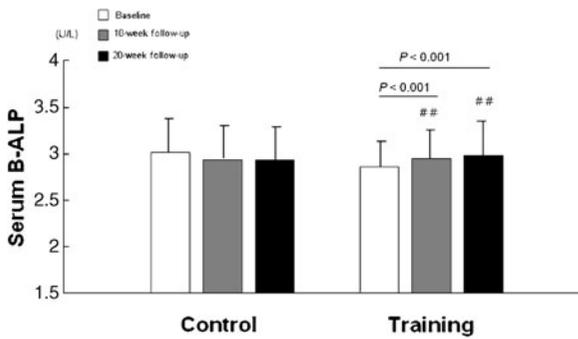


Fig. 1 Effects of resistance training on serum bone-specific alkaline phosphatase (B-ALP) activity (error bars SEM). ## Significant time × group interaction ($P < 0.01$). In contrast to the control group, serum B-ALP activity in the training group increased significantly from baseline to 10-week and 20-week follow-up

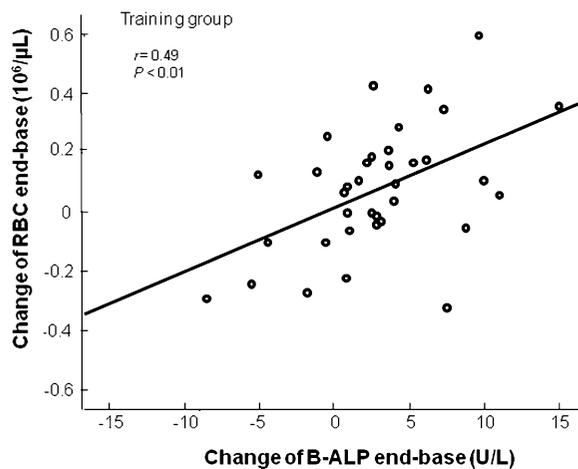
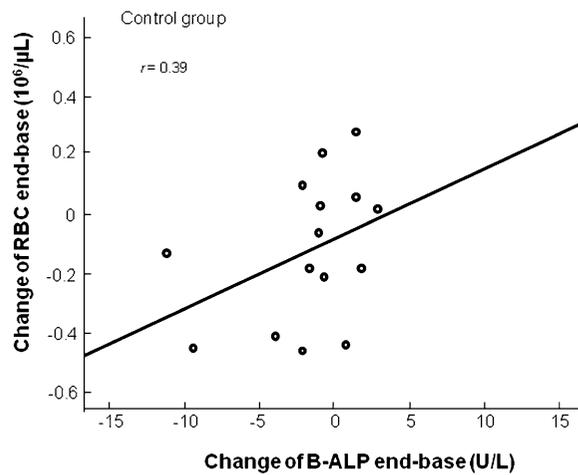


Fig. 2 Correlation of changes in serum B-ALP activity with changes in red blood cell count (RBC) from baseline to 20-week follow-up

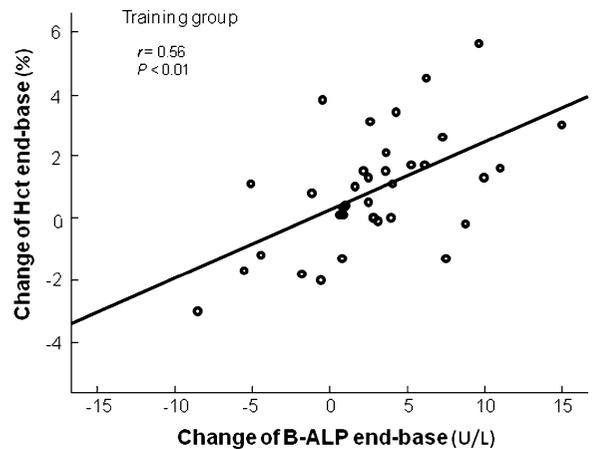
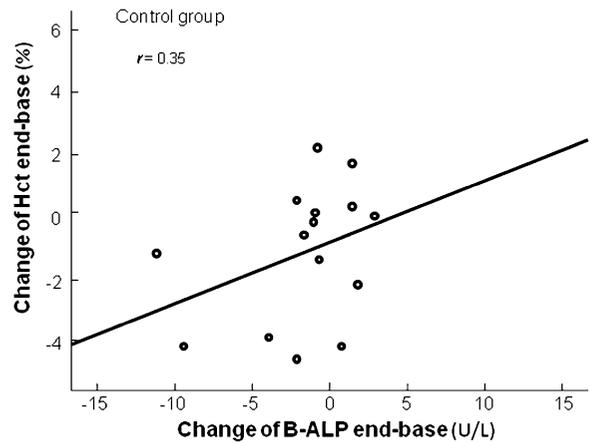


Fig. 3 Correlation of changes in serum B-ALP activity with changes in hematocrit (Hct) from baseline to 20-week follow-up

resistance training has been shown to increase intravascular haemolysis with slightly elevated erythropoiesis [19]. We like to describe this as the “haemolysis-pull” model.

Hematopoietic precursors reside close to endosteal surfaces. Osteoblasts might play a central role in hematopoiesis. It has been shown that osteoblasts produce many factors essential for the survival, renewal, and maturation of HSCs. Signals from osteoblasts can directly initiate and modulate HSC proliferation in the context of mobilization [13]. On the contrary, with an induced osteoblast deficiency, hematopoiesis was severely altered in mice [26]. HSCs receive regulatory information from bone, accounting for the localization of hematopoiesis in bone marrow [23]. Earlier observations in female pigeons of recurrent trabeculation of the medullary cavity in the reproductive cycle showed that the

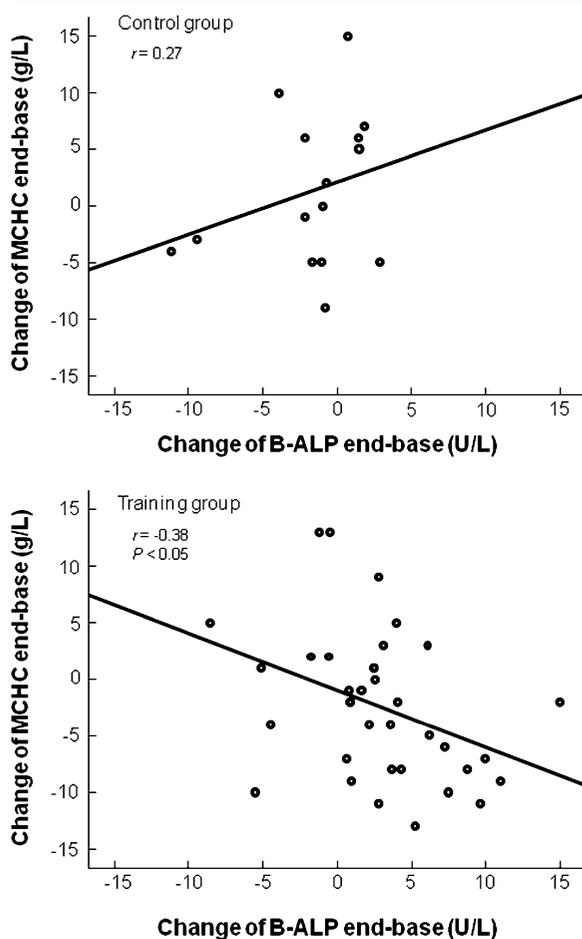


Fig. 4 Correlation of changes in serum B-ALP activity with changes in mean cell hemoglobin concentration (MCHC) from baseline to 20-week follow-up

hematopoietic tissue regressed as bone encroached the marrow space, strongly suggesting that osteogenesis and hematopoiesis are functionally linked [2]. Kilgore et al. [10] reported that 6 weeks of moderate-to-intense resistance training of healthy untrained males could cause ALP and RBC levels to rise. In healthy adults, a correlation between B-ALP and ALP is shown by most methods [27]. Once liver disease is ruled out, ALP provides a good impression of the extent of new bone formation and osteoblast activity [1, 25]. Therefore, the results reported in Kilgore et al. potentially indicate the association between osteoblast activity and RBC turnover, although the authors did not explore it. In the present study, B-ALP increased already at 10-week follow-up, which preceded RBC, Hct and MCHC changes [7]. This was in agreement

with Kilgore et al. [10], who observed ALP elevation earlier than RBC changes. This implies that changes in osteoblast activation possibly play a role in the observed changes of RBC, Hct and MCHC.

The present study showed that changes in B-ALP correlated positively with changes in RBC and Hct in the training group. This suggests that the higher activity of osteoblasts resulted in greater amounts of red blood cells in the blood. Meanwhile, as a lower MCHC reading is regarded as one of the characteristics of younger red blood cells, an inverse correlation between changes in serum B-ALP and the change of MCHC implies that the higher activity of osteoblasts resulted in younger red blood cells in the blood. Therefore, the present results support that view that changes in osteogenesis and hematopoiesis during resistance training are functionally linked, although the exact mechanism is not clear.

Another possible factor that needs to be confirmed is bone-marrow adipocytes. It was noted that voluntary resistance training, such as climbing, not only enhanced bone formation and osteoblast differentiation, but also inhibited adipogenic differentiation in bone marrow [14]. A recent study showed that bone-marrow adipocytes are negative regulators of the hematopoietic microenvironment [15]. Theoretically, this might also contribute to hematopoietic microenvironment improvement after resistance training. Taken together, we propose that osteoblast activation and inhibited adipogenic differentiation induced by moderate-to-intense resistance training might improve the hematopoietic microenvironment in the bone marrow. This “hematopoietic microenvironment-push” model could complement the “haemolysis-pull” model in explaining elevated erythropoiesis during exercise training.

Previous studies have shown that bone mass is associated with lean body mass because of the muscular forces leading to localized stresses on the bone, which then result in osteogenesis [11]. We examined the association between the change in lean body mass and changes in B-ALP from baseline to 20-week follow-up, but no correlation was found in either group.

The limitations of the present study should be recognized. Circulating RBC precursor cells (i.e., reticulocytes) were not measured in the study, which allows us to speculate but not directly verify the link between bone and erythropoiesis during resistance training. Further, the short period of the intervention did not allow us to measure bone mineral content and

density with current available densitometry to verify if increased B-ALP corresponded to increased bone mass. However, this does not affect the main outcome of the study, which is focused on the association between changes in B-ALP and the changes in RBC, Hct and MCHC induced by resistance training. On the other hand, the observed association could suggest that osteoblast activity possibly plays a role in the elevated erythropoiesis induced by exercise training, but it does not mean a cause–effect relationship, and other factors such as growth hormone and testosterone may also be involved in this process.

In conclusion, we found that resistance training could increase serum B-ALP—a biomarker of bone formation—in physically inactive men. The change in B-ALP level correlated with corresponding changes in RBC, Hct and MCHC. We propose that the adaptive changes of bone metabolism induced by resistance training might improve the hematopoietic microenvironment and facilitate erythropoiesis. Further studies are needed to clarify the crosstalk between osteoblast activation and hematopoietic stem cell mobilization during resistance training.

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