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TEK SETE KARŞIN ÇOK SETLE YAPILAN SEKİZ HAFTALIK DİRENÇ ANTRENMANLARININ GENÇ ERKEKLERİN KEMİK TURN-OVER MARKERLERİ ÜZERİNE ETKİSİ

EFFECTS OF EIGHT-WEEK SINGLE VERSUS MULTIPLE SETS OF RESISTANCE TRAINING ON BONE TURN-OVER MARKERS IN YOUNG MALES

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Anahtar sözcükler: kemik turn-over markerleri, direnç antrenmanı, genç erkekler
Key words: bone turn-over markers, resistance training, young-adults

ÖZET

Çalışmamızın amacı farklı volümde (tek set-çok set) ancak aynı şiddette [8-12Tekrar Maksimum(RM) ve 12-15 RM] yapılan sekiz haftalık direnç antrenmanlarının kemik turn-over markerları üzerine etkisinin incelenmesi. Metod: 42 sağlıklı erkek üniversite öğrencisi rasgele tek set (TSG; n=14), çok set (ÇSG; n=13) ve kontrol grubu (KG; n=15) olarak ayrıldı. ÇSG tüm egzersizleri ilk üç hafta 3 set, 12-15RM ile; geri kalan 5 hafta ise süper set ve 3 set halinde 8-12RM ile, tüm çalışma boyunca haftada 3 gün olarak yaptı. TSG ise tüm egzersizleri tek set olarak yaptı. Çalışma döneminin başında ve sonunda serumda total alkalen fosfataz (ALP), kalsiyum (Ca^{2+}), fosfat, paratiroid hormonu (PTH), osteokalsin (OC), idrarda deoksipiridinolin (DPD) düzeyleri ölçüldü.

PTH TSG, ÇSG ve KG'de sırasıyla %15.32 (p<0.05), %34.88 (p<0.01) ve %20.76 (p<0.05) olarak arttı. ÇSG'de Ca^{2+} (p=0.054) ve OC (p=0.075)'de anlamlı olmayan artış görüldü. ÇSG'de Ca2+ ve OC'de gözlenen yüzde değişim CG'den yüksekti (sırasıyla p<0.05 ve p<0.01). Fosfat ve DPD'de grup içi ve gruplar arası farklılık saptanmadı. Sonuç olarak, çok set grubundaki genç sporcular daha iyi bir kemik metabolizması göstergesi olan yüksek PTH ve OC değerlerine sahiptiler. Bu nedenle, kemik mineral metabolizmasını arttırmak için çok setler halinde yapılan direnç antrenmanlarının önerilebileceği kanısına varıldı.

SUMMARY

The aim of the study was to investigate whether different training volume (single set vs. multiple sets) but equal intensity [8-12 Repeat Maximum (RM) and 12-15 RM] of 8-week weight training would lead to different changes in bone mineral metabolism.
Method: 42 healthy male college students were randomly placed into single set group (SSG; n= 14), multiple-set group (MSG; n= 13) or control group (CG; n= 15). MSG performed all exercises in 3 sets of 12-15 RM for the first three weeks; super sets and 3 sets of 8-12 RM were performed for the remaining 5 weeks; 3days/week. SSG performed all exercises in one set. Serum total alkaline phosphatase (ALP), calcium (Ca^{2+}) phosphate, parathyroid hormone (PTH), osteocalcin (OC), and urinary deoxypyridinoline (DPD) levels were measured before and at the end of the training period.

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PTH was increased by 15.32% (p<0.05), 34.88% (p<0.01) and 20.76% (p<0.05) in SSG, MSG, and CG, respectively. The increase observed in Ca2+ (p=0.054) and OC (p=0.075) in MSG was nearly significant. The percent change observed in MSG was greater than the change in CG in Ca2+ and OC (p<0.05 and p<0.01, respectively). There were no significant differences in phosphate and DPD levels within and between groups.

In conclusion the young-adult athletes of the MSG had elevated PTH and OC values, which indicate a better bone mineral metabolism. Thus, resistance training performed in multiple sets can be recommended to increase bone mineral content.

GİRİŞ

There is a link between physical exercise and strengthening of the skeleton. Many studies confirm that exercise enhances bone density and improves or prevents the decline of bone mass in older people of both sexes. Since it is difficult to increase bone mineral density (BMD) later in life, increasing peak BMD in the early stages of life is important (1). Weight-bearing physical exercise has been shown to maintain or increase bone mass in younger and elderly adults (2-6).

It is thought that the remodeling process of the bone is stimulated and activated during exercise. However, the understanding of how the hormonal and mechanical stimuli affect bone and interact throughout life is still incomplete (7). The remodeling cycle may start when the bone lining cells on the surface of the bone are activated by signals from osteocytes or by systemic or local factors. Parathyroid hormone (PTH), as well as mechanical load, is thought to play a role in this event. Activated bone lining cells may release still unknown factors that stimulate the osteoclast activity (8). Alkaline phosphatase (ALP) is essential for mineralization (9). ALP might be a causative agent for the calcification process. It splits inorganic phosphate from organic phosphate, and thereby increases the calcium-phosphate product, enabling mineralization. Thus, biochemical markers of bone metabolism have been used for some time now, particularly in clinical studies to evaluate bone metabolism in skeletal diseases (10,11). However, only a few studies have examined the effect of exercise on the skeleton using these markers, and the existing ones have revealed conflicting results (3,12-18). In some studies, serum PTH concentrations were found to increase during endurance exercise (14,15). PTH is important for the regulation of bone metabolism with catabolic as well as anabolic properties (19). Its major physiological function is the maintenance of plasma-ionized calcium (Ca^{2+}) and there is some belief that exercise influences calcium metabolism (20). However, data in the literature concerning the effect of exercise on Ca^{2+} are very controversial (21-23). The beneficial effects from resistance training depend on many factors such as intensity and frequency of training, and the volume of exercise needed to meet the goals of the individual (24). The debate over the amount of volume needed to elicit maximal strength gains has continued in recent years with the studies comparing single and multiple sets of training. However, to our knowledge, no studies comparing the effect of different-volume strength training (single set versus multiple sets) on biochemical markers of bone metabolism exist. Therefore, in order to investigate the effect of resistance training of different volumes on bone metabolism, we have examined several bone-associated parameters in weight-trained athletes and compared the results with their age-matched controls.

INTRODUCTION

There is a link between physical exercise and strengthening of the skeleton. Many studies confirm that exercise enhances bone density and improves or prevents the decline of bone mass in older people of both sexes. Since it is difficult to increase bone mineral density (BMD) later in life, increasing peak BMD in the early stages of life is important (1). Weight-bearing physical exercise has been shown to maintain or increase bone mass in younger and elderly adults (2-6).

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physiological function is the maintenance of plasma-ionized calcium (Ca^{2+}) and there is some belief that exercise influences calcium metabolism (20). However, data in the literature concerning the effect of exercise on Ca^{2+} are very controversial (21-23). The beneficial effects from resistance training depend on many factors such as intensity and frequency of training, and the volume of exercise needed to meet the goals of the individual (24). The debate over the amount of volume needed to elicit maximal strength gains has continued in recent years with the studies comparing single and multiple sets of training. However, to our knowledge, no studies comparing the effect of different-volume strength training (single set versus multiple sets) on biochemical markers of bone metabolism exist. Therefore, in order to investigate the effect of resistance training of different volumes on bone metabolism, we have examined several bone-associated parameters in weight-trained athletes and compared the results with their age-matched controls.

MATERIAL AND METHOD

Subject selection: Forty-two male students between the ages of 18 and 25 years who met the experimental criteria participated in this study. These students from the School of Physical Education and Sports were randomly divided into three groups as exercise and control groups. Exercise groups were assigned as the single set group (n= 14; mean age ± SD= 20.0±1.3) and multiple-set group (n= 13; mean age ± SD= 22.3±2.1). Control subjects consisted of 15 students (mean age ± SD= 22.4±2.1). Physical characteristics of the athletes and controls are given in table I. Baseline variables (means + SD) were normally distributed and did not differ statistically among the three groups.

Table 1. Baseline physical characteristics of the subjects (mean ± SD)

Variable	SSG (n= 14)	MSG (n= 13)	CG (n= 15)
Age (year)	20.01±1.3	22.33±2.1	22.4±2.1
Height (cm)	181.6±9.3	178.1±6.9	175.4±4.7
Body mass (kg)	73.6±9.3	72.8±5.3	67.8±4.9
BMI (kg/m^2)	22.2±1.3	22.9±1.4	21.7±1.4
Body fat (%)	15.59±4.2	18.6±5.1	17.88±4.2

SSG= Single-set group; MSG= Multiple-set group; CG= Control group; BMI= Body Mass Index

Participants completed a comprehensive screening process that included a health history questionnaire. Subjects were excluded if they regularly took any medication (including nutritional supplements and drugs known to affect bone metabolism); their calcium intake was below the recommended daily allowance, or if they had a history of bone disease. Participants were expected not to have been engaged in any resistance training program during the past 12 months. Because of these strict conditions, only a limited number of students were enrolled in the study. After being informed of the purpose and the risks associated with the study, consent was given by all subjects. This study was approved by the Ethical Council of the Faculty of Medicine of Celal Bayar University.

Calcium intake: At the beginning of the study, total calcium intake was assessed by recent meal composition. A sample one-week diet for calcium intake was analyzed by trained technicians and no differences were found among the groups (data not shown). Therefore, the participants were advised not to change their food habits throughout the study.

Experimental design: Before the experiment, all subjects were familiarized with the laboratory environment and the experimental procedures. Two exercise groups were tested on two occasions: at baseline to determine the heaviest loads that could be lifted maximum 12-15 times (12-15RM loads), and at the end of the third week of training to determine the heaviest loads that could be lifted maximum 8-12 times (8-12RM loads). Exercise groups performed resistance training for 8 weeks, 3 days per week (non-consecutive days). Control group members maintained their daily routine throughout the study period but they did not perform any type of exercise. Subjects had no alcohol or caffeine for 24 hours before the tests, which were performed at least three hours after a meal. In addition, the subjects were not tested within 48 hours of the previous training session. All testing and training took place at the same time of the day to control the circadian variation in performance. Subjects showed 100% compliance with exercise training. Bone mineral metabolism and body composition were assessed at baseline and week 8 of the study. Resistance training protocol: Appropriate periodization is essential In order to supply appropriate muscular development and to prevent injuries, thus, there should be resistance (amount of weight utilized) variations and the loads should gradually be increased. Therefore, a resistance-training program should start with increasing muscular endurance and continue with muscular hypertrophy. The recommended loads for muscular endurance and muscular hypertrophy are 12-15RM and 8-12RM, respectively (25). In our study, before the training period, the loads to be used for the first three weeks were determined as 12-15RM because the first period of the training program aimed at muscular endurance. At the end of the third

week, the loads to be used for the remaining five weeks were determined. The aim of the second period was muscular hypertrophy, thus the loads necessary for this purpose were determined as 8-12RM.

Table 2: Exercises performed by SSG and MSG for the first three weeks

Monday	Wednesday	Friday
bench press (FW)	half squat	leg press (M)
leg extension-right/left (M)	shoulder press (M)	leg curl-right/left (M)
leg curl (M)	leg curl (M)	chest press (M)
upright row (FW)	lat-pull-down (M)	upright row (FW)
seated row (M)	seated row (M)	happelman (FW)
biceps curl (FW)	biceps curl (FW)	lat-pull-down (M)
triceps press down (M)	triceps press down (M)	biceps curl (FW)
incline chest press (M),	incline chest press (M),	triceps press down (M)
		hip extension-right/left (M)

FW= Free Weights M= Machine

*Rest between sets was 90-120 seconds. Each exercise was performed as one set, with 12-15RM loads by SSG and as three sets with 12-15RM loads by MSG.

Table 3: Exercises performed by SSG and MSG for the last five weeks.

Monday		Wednesday		Friday	
First series	MSG #series	First series	MSG #series	First series	MSG #series
Seated press (FW) Upright row (FW) Happelman (FW)	2	Seated press (FW) Lat pull down (M)	3	Seated press (FW) Upright row (FW) Happelman (FW)	2
Second series	MSG #series	Second series	MSG #series	Second series	MSG #series
Half squat (M) Leg curl-right (M) Leg curl- left (M)	2	Leg curl-right (M) Leg curl-left (M)	3	Leg press (M) Leg curl-right (M) Leg curl-left (M)	2
Third series	MSG #series	Third series	MSG #series	Third series	MSG #series
Bench press (FW) Seated row (M)	3	Bench press (FW) Incline chest press (M) Decline chest press (M)	2	Bench press (FW) Seated row (M) Incline chest press (M) Lat-pull-down (M)	2
Fourth series	MSG #series	Fourth series	MSG #series	Fourth series	MSG #series
Triceps press down (M) Triceps curl (FW)	3	Biceps curl (FW) French curl (FW)	3	Triceps press down (M) Biceps curl (FW)	3
Fifth series	MSG #series	Fifth series	MSG #series	Fifth series	MSG #series
Biceps barbell curl (FW) Triceps press down (M)	3	Hip flexion-right (M) Hip extension-right (M) Hip flexion-left (M) Hip extension-left (M)	2	Hip flexion-right (M) Hip extension-right (M) Hip flexion-left (M) Hip extension-left (M)	2

FW= Free Weights M= Machine

*Rest between sets was 30 seconds, and rest between series (total of sets) was 3 minutes. Both exercise groups performed each exercise for 8-12 times with 8-12RM loads, SSG performed each exercise as only one series however MSG performed as two-three series.

The 12-15RM and 8-12RM were achieved by increasing the load by 5 kg after each successful set of lifts (12-15 and 8-12 lifts per set) until the maximum load sustainable for 12-15 and 8-12 lifts were obtained.One training session lasted ~25 min in SSG and ~60 min in MSG. During one training session 8-14 exercises that activated major and minor muscle groups from a total of 18 exercises were used. When differences occurred between the determined and the used loads, the load for the next session was increased by 5-10%. MSG performed three-set system in the first three weeks and in the remaining five weeks su-per-set system (that is, loading the same muscle from different angles and agonist-antagonist work) and three-set systems. SSG performed the same exercises in the same repetition, but in single set. For both single-set and multiple-set groups participants followed a training program with free weights and machine consisted of the following 18 different exercises performed on different days (Table II and III). Throughout the intervention period, each subject received appropriate instruction concerning warm-up and cool-down techniques. Training logs were kept for each session to monitor the progress of each participant and to adjust resistance loads as necessary.

Bone mineral turn-over markers and body composition measurements: Blood samples were collected from each subject at baseline and at the end of week eight, between 8.00-9.00a.m. Calcium, phosphorus, alkaline phosphatase and creatinine were assessed by enzymatic methods on autoanalyzer (Integra Roche Diagnostics Corporation, Indianapolis, USA) by commercial reagents. Bone turn-over markers including PTH, Osteocalcin, and urinary DPD were assessed by an enzyme-amplified chemiluminescence assay (IMMULITE®, Diagnostic Products Corp., Los Angeles, CA, USA). Inter and intra assay coefficients of variation for Ca^{2+}at 5.8 mg/dl level were 3.8%, and 2.2% respectively. The inter and intra assay coefficients of variation for phosphate at 3.4 mg/dl level were 0.95% and 1.4%, for ALP at level 45 U/L were 2.3% and 2.7%, for PTH at level 57 pg/ml were 6.3% and 8.6%, for OC at level 2.8 ng/ml were 2.8% and 3.5%, and DPD at 50nM/mmol creatinin level were 10% and 14%, respectively.

Height, weight and body mass index (BMI) were measured by traditional methods; percent body fat was measured by Dual-energy X-ray absorptiometry (DXA) at the beginning of the study.

Kruskall-Wallis test was used to compare percentage changes among the study groups. Mann-Whitney U test was used to determine the difference between the two groups. The differences between pre and post training values were determined by using Wilcoxon test. All comparisons were considered statistically significant at $p< 0.05$.

RESULTS

Table IV shows the bone turn-over marker measurements of each group. Baseline measurements of the three groups showed no significant differences in terms of their bone turn over marker values except for OC. As a result of 8-week weight training program, PTH increased by 15.32±50.60% ($p<0.05$), 34.88±16.70% ($p<0.01$) and 20.76±28.29% ($p<0.05$) in SSG, MSG, and CG, respectively (Fig. 1). The increase observed in Ca^{2+} (5.02±7.59%; p=0.054) and OC (16.67±22.68; p=0.075) in MSG was nearly significant. The percent change observed in MSG was greater than CG in Ca^{2+} ($p<0.05$). A similar change was obtained in MSG in OC ($p<0.01$ Fig. 2). There were no significant differences in phosphate and DPD levels within and between groups.

Table 4. Changes in bone turn-over markers for the SSG, MSG and CG following 8 weeks of resistance training period

	SSG (n= 14)		MSG (n= 13)		CG (n= 15)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
ALP,U/L	84.85±26.30	83.50±23.51	74.46±26.85	78.84±23.97	80.86±28.16	74.60±20.20
%Δ		-2.05±13.55		5.49±13.85		-7.18±14.82
Ca^{2+} mg/dl	9.59±0.90	9.92±0.41	9.22±0.85	9.70±0.38	9.84±0.42	9.77±0.35
%Δ		3.39±8.01		5.02±7.59 [c]		-0.84±5.11
Pmg/dl	3.52±0.52	3.80±0.48	3.56±0.58	3.89±0.61	3.46±0.40	3.61±0.47
%Δ		6.56±14.46		7.09±16.50		3.26±13.16
PTH pg/ml	46.72±21.08	63.66±28.34[a]	43.31±12.54	71.01±28.52[b]	40.91±17.79	53.56±18.76[a]
%Δ		15.32±50.60		34.88±16.70		20.76±28.29
OC ng/ml	36.00±11.57[e]	35.87±12.93	23.63±13.18[f]	28.42±11.76	35.06±16.83	30.01±15.21
%Δ		-2.54±16.59		16.67±22.68 [d]		-25.06±56.39
DPD nM/mmol kreatinin	6.07±1.97	6.06±2.09	5.32±1.84	5.09±1.61	6.12±2.48	5.49±2.01
%Δ		-3.56±26.51		-5.60±29.50		-12.05±23.35

SSG= Single-set group; MSG = Three-set group; CG= Control group
[a]p<0.05 change from baseline; [b]p<0.01 change from baseline; [c]p<0.05 vs. CG;
[d]p<0.01 vs. CG; [e]p< 0.01 vs. preMSG; [f] p< 0.05 vs. preCG;

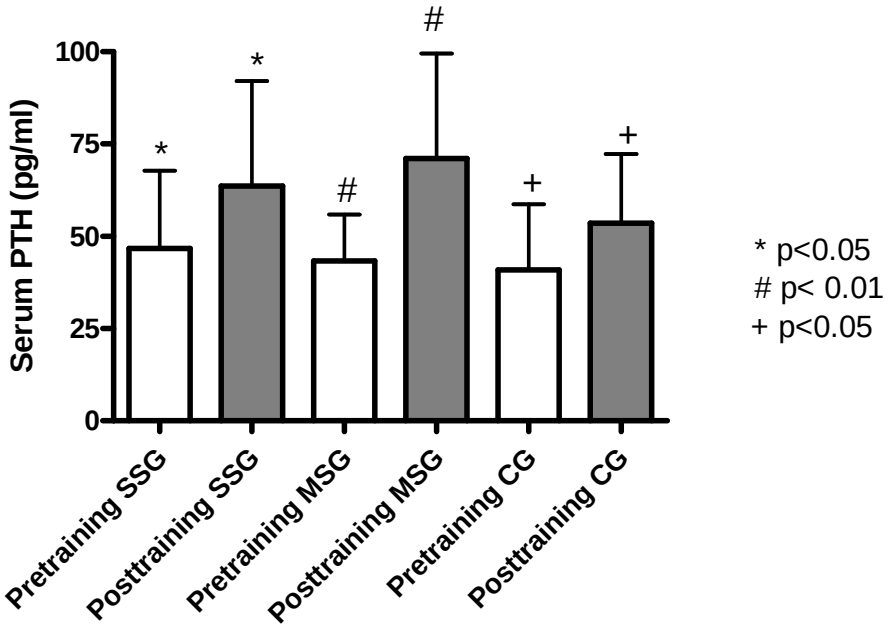

Figure 1. Changes in serum parathyroid hormone levels within groups from baseline to 8th weeks.

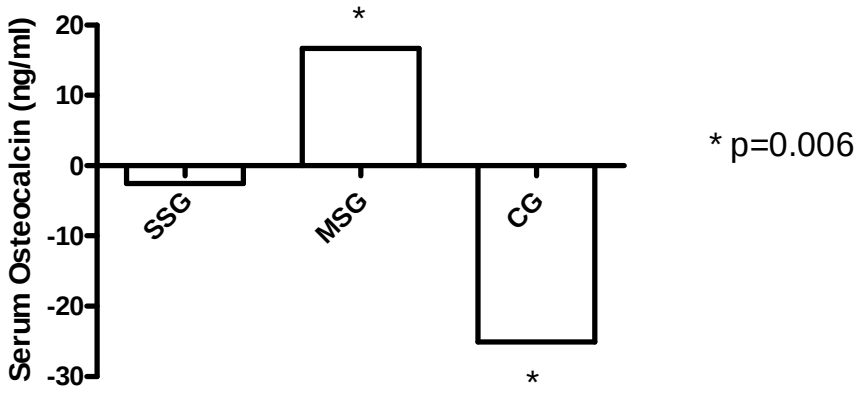

Figure 2. Comparison of percent changes observed in all groups in serum Osteocalcin hormone levels from baseline to 8th weeks

DISCUSSION

In the present study, we investigated the effects of 8-week single versus multiple sets of weight training with equal intensity on bone turn-over marker levels in young-adult male college students. The exercise regimen of MSG appeared to be more effective in increasing some bone formation markers than the program followed by SSG. Biochemical markers of bone metabolism have recently been used, particularly in clinical studies, to evaluate bone metabolism in skeletal diseases (10,11). However, attempts made to evaluate the effect of exercise on skeleton using these markers have revealed conflicting results. In recent studies, serum PTH, an important marker of bone formation, was found to increase during endurance training (15,20). Similarly, Rudberg et al (7) found significant increases in PTH levels after 30-40min jogging and the increase was significantly correlated with lactate levels.

However, Brahm et al (18) observed lower PTH levels in runners than in controls. The finding that there is an acute rise in PTH following exercise but reduced levels in the resting state might indicate that PTH could be involved in the responses of bone to exercise. On the other hand, Thorsen et al (8) found no alterations in PTH levels due to the moderate endurance exercise of a 90min brisk walking. They explained the lack of increase in PTH by the inadequate intensity of physical exercise. In longitudinal and cross-sectional studies in humans, resistance exer-

cise has been shown to increase bone mineral density with increased bone formation (26,27). This may be explained with the physiological adaptation process to increase bone mass with repeated cycles of exercise. Some researchers tried to explain this with the fact that strength-training increases lactate threshold and reduces blood lactate concentration at the same relative exercise intensities during submaximal exercise (28). It is also reported that the capacity to transport lactate is higher in athletes than in untrained and less-trained subjects (29). Negative effects of lactic asidosis on calcium and bone metabolism might be minimized or cleared in trained athletes, allowing an ostegenic response with loading to emerge. The alterations in hormonal factors may also be effective in this physiological adaptation because male muscle builders have been reported to have higher circulating levels of PTH than normal control subjects (30). In our study, we observed significant increases between the baseline and post-training period levels of all groups in regard to PTH. However, although it is not statistically significant among the groups, the greatest percent change within group determination was observed in MSG (Δ%=34.88±16.70; p=0.001 vs. 15.32±50.60; p=0.026 in SSG), indicating the beneficial effect of strength training on bone formation.

Osteocalcin (OC) and alkaline phosphatase (ALP) are other markers for bone formation and osteoblastic activity (31). Etherington et al (32) found significant decreases in OC and ALP with 10-week weight-bearing exercise. Brooke-Wavell et al (33) found no significant changes in OC as a result of 12-month brisk walking in post-menopausal women. Similarly, Rudberg et al (7) found no obvious exercise-induced changes of OC as a result of neither cycle ergometer until exhaustion nor 30-40min jogging. On the other hand, Milliken et al (2) found that exercisers, who performed both weight-bearing aerobic training and resistance training, showed a trend toward larger positive changes in both bone formation and resorption over 12 months vs. controls. In our study, the percent changes occurred in OC in MSG and CG were significantly different favoring MSG (16.67±22.68% vs. -25.06±56.39%, respectively; p=0.006). Within group comparisons also revealed a nearly significant increase in OC of MSG (p=0.075). This finding of ours is parallel with others who also found increases in OC levels as a result of strength training. (2,3,16,17). These findings may suggest that strength training is more effective than endurance training in increasing OC. In addition, Vincent et al (3), Fujimura et al (16) and Menkes et al (17) found increases in ALP activities of the athletes. However, we could not observe any changes in ALP activities in any of our groups. This might have resulted from the difference between the duration of the intervention period of the aforementioned studies all of which lasted 16 weeks and our study that lasted only 8 weeks.

DPD is a sensible marker of bone resorption. Brooke-Wavel et al (33) determined a significant increase in urinary DPD in control subjects. Fujimura et al (16) found that DPD was transiently suppressed and turned to the initial value but never was stimulated during the 4 months of resistance training. Some studies indicated that exhaustive running exercise (i.e. marathon or long-term cross-country running) (12,13) caused a tendency toward rising levels of bone resorption markers because of a direct result of disruption of cells due to the load applied to the skeleton during exhaustive exercise or because of a release from tendons that also could contain type I collagen. Our study was not as exhaustive as a marathon run; therefore, we did not observe an increase in DPD.

The major physiological function of PTH is the maintenance of plasma ionized calcium (Ca^{2+}) and there is evidence that exercise influences calcium metabolism (20). However, data in the literature concerning the effect of exercise on Ca^{2+} are very controversial. While some studies have found reductions after anaerobic exercise (21), others have observed increases after incremental exercise and short-term maximal work (22,23). However, to our knowledge, there are no studies investigating the Ca^{2+} changes in young-adult males, comparing two different weight- training programs of different volume and equal intensity. Our study revealed no changes in the mineral metabolism related to Ca^{2+} and phosphate differences in the athletes as a result of weight training. In conclusion, the greater increases observed in MSG than SSG in PTH and OC may suggest more beneficial effects of strength training programs performed with multiple sets rather than the ones conducted with single set on bone formation. If the findings of the present intervention study are supported by detailed longitudinal investigations, the presently reported observations might be important for increasing bone formation at early ages of life, thus in turn in the prevention of future osteoporotic fractures.

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