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ORIGINAL ARTICLE

Does the Rhythmic Gymnastics Training Affect Serum Bone Resorption and Oxidative Stress Markers?

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ABSTRACT

Introduction: Rhythmic gymnastics training (RGT) includes intensive anaerobic loads, therefore it may cause oxidative stress (OS) and then consequently might negatively affect bone metabolism and nitric oxide (NO) levels which is related to vasodilation and bone metabolism. This study investigated the effects of chronic RGT on the markers of serum bone metabolism, OS, and NO levels together, which is yet unclear.

Materials and methods: 16 girls rhythmic gymnasts (athletic group, AG; 10.33 ± 1.79, years) and 13 controls (CG; 9.23 ± 1.00 years) participated in this study. Type 1 collagen carboxy-terminal cross-linked telopeptide (ICTP) as bone resorption marker (using ELISA method), alkaline phosphatase (ALP), parathyroid hormone (PTH), calcium (Ca), and growth hormone (GH) as bone metabolism markers; Total oxidant /antioxidant status ratio (TOS/TAS) as OS index (OSI) and NO levels; Troponin-I (Tn-I), and CK-MB levels, alanine aminotransferase (ALT), aspartate amino transferase (AST), creatine kinase (CK) as muscle injury markers; Some hematological parameters including hemogram were measured by standard methods.

Results: Serum ICTP1 (p= 0.01) and GH (47.4 %) levels of AG were significantly higher than CG, whereas Ca level (p= 0.04) and body fat rate values was lower (p= 0.00). There is no significant difference between the groups for other parameters.

Conclusions: The results suggest that RGT training did not significantly affect OS and NO levels that can cause anemia. But besides the increased GH levels, bone development can be affected negatively in child gymnasts due to the increased ICTP and decreased Ca levels. Thus, a calcium-rich diet and regular observation of bone metabolism markers are recommended.

Keywords: Rhythmic gymnastics, Oxidative stress, Bone destruction, Nitric oxide.

INTRODUCTION

Rhythmic gymnastics is an artistic and aesthetic sportive performance in which both the ability to use various hand apparatuses (rope, hoop, ball, clubs, ribbon) and many motoric skills (especially flexibility, strength, coordination) are used at a high level[1]. However, the rhythmic gymnastics branch basically requires anaerobic power and endurance, therefore for the desired performance, long-term training and heavy loads are applied. Although it is not recommended to perform long term anaerobic loads in child athletes in general, the trainings are started and continued in childhood due to the nature of this sport.

In reality, it is also known that physical exercise has many beneficial effects on health[2]. For example, moderate regular physical exercises (40-60% maxVO2) are recommended for primary and secondary prevention of many chronic diseases like cardiovascular diseases, diabetes, osteoporosis and obesity[3,4]. But, the long-term anaerobic loads as in rhythmic gymnastics may lead to some health risks due to oxidative stress (OS) in children. There are pieces of evidence indicating the reactive oxygen species (ROS) genesis and OS occurrence in muscle, liver and blood during severe exercises[4]. The increased oxygen consumption may increase 10-15 times compared to rest during severe exercise, therefore, free radical production in mitochondria increases[5,6]. These formations of oxidant and antioxidant events are in equilibrium[7]. As long as this balance is achieved, the organism is not damaged by the negative effects of free radicals. But, if the oxidative balance shifts towards free radicals due to an insufficient antioxidant defense mechanism, then OS occurs[8]. In a study, Gougoura et al.[9] found that the chronic swimming training significantly reduced levels of the endogenous antioxidant glutathione (GSH) compared to the control group, whereas it significantly increased thiobarbituric acid reactive products (TBARS) levels used as an index of lipid peroxidationin in 8-12-year old swimmers. In the other study, the low-density lipoprotein (LDL)'s sensitivity to peroxidation was higher in gymnasts[10]. In another study, the hydroperoxide level was found to be significantly higher in post-training in amateur rhythmic gymnasts[11].

As known, oxidized LDL (OxLDL) plays an important role in the initiation of atherosclerosis (AS) process, and AS begins in childhood, thus lipid peroxidation is an important risk factor for AS as well as the other diseases also in children[12]. In addition, nitric oxide (NO) is an antioxidant and metabolic regulator gas and has an important effect on the preservation of vascular homeostasis and protects endothelial cells against OS damage. Furthermore, NO has also an important role in exercise adaptations[13]. Besides these, NO can play role as a mediator of estrogen effects in osteocytes[14]. Therefore, the increase of NO caused by exercise may contribute to improvement of both exercise capacity[13] and bone health as well as prevention of CVD (decreasing OxLDL formation)[14]. If OS occurs, it decreases NO bioavailability[13] and may cause NO dysfunction, which limits exercise capacity and increases CVD risk[13,14,15,16,17,18,19,20,21]. It is reported that lipid peroxidation increases bone resorption by directly activating osteoclasts[15,16]. In addition, the decreased NO

bioavailability can increase OxLDL formation[17]. If OS levels increase much, NO reaction with increasing superoxide (SO) anions may cause the formation of peroxynitrite radical, which may lead to endothelial cells injury and reduce the bioavailability of NO[13,14,15,16,17]. There is a relationship between decreased bone density and increased OS[18,19]. Increased OS can also cause toxic effects in many organs including the heart[20,21,22]. Furthermore, it was reported that structural and functional disorders in erythrocytes occurred in iron deficiency anemia due to oxidative damage[23]. But, there are limited studies the literature that examine the effects of chronic rhythmic gymnastics training (RGT) on especially markers as to the formation and resorption of bone tissue. In addition, a study examining bone turnover, OS and NO parameters together with hematological has not yet been found in the literature in rhythmic gymnast in children. Therefore, the present study investigated the effects of chronic rhythmic gymnastics training on the mentioned parameters in rhythmic gymnasts with the ages of 8-14 year-old girls.

MATERİAL AND METHODS

The Study Design: The gymnasts were instructed not to take any vitamin and antioxidant dietary supplements for a week before the blood taking and physical measurements. They were invited to the laboratory for the tests. The physical measurements were performed and blood samples were taken. The athletes and sedentary were informed about the purpose of the study, the benefits, the tests to be done as well as possible risks, and an informed consent form was completed. It was taken written and signed their acceptance of the families of the participants. Ege University Faculty of Medicine, Scientific Research Ethics Committee approved the project, which is in compliance with the “Helsinki Declaration of Ethical Principles in Medical Research on Humans”.

Inclusion Criteria: Healthy athletes (athletes group; AG) and sedentary (control group; CG) girls between the ages of 8-14 who did not regularly use any medications or vitamins, were not menarche, participated in the study. AG is made up of 15 licensed rhythmic gymnasts athletes who have been regular rhythmic gymnastics for at least 5 years. CG is made up of 13 sedentary students who do not do any exercise. AG's general training content includes a branch-specific warm-up, strength and conditioning training, ballet exercises, body difficulties (rotations, balances, jumps and flexibility exercises), apparatus technique and routines. AG trains 24 hours a week, 6 days a week, an average of 4 hours a day.

Physical Measurements: Height, body weight, and body mass index, measurements were made with an electronic medical weighing device (Seca 769, Hamburg, Germany) without clothes and shoes. Skinfold measurements were measured from the right side, triceps, subscapular, abdomen, and supra-iliac regions using the skinfold caliper tool (Holtain, London, UK) (0.02 mm apart) and calculated using the Yuhasz Method.

Collection, Storage and Analysis of Blood Samples: Venous blood samples were taken from all participants after 9 hours of fasting for biochemical analysis between 09.00-10.00 in the morning, into tubes with a vacuum with EDTA and one serum tube of 9 mL. After the blood samples were kept at room temperature for 20 minutes, serums were obtained by centrifuging at 2500 g for 15 minutes. Serum samples were stored in the freezer (at -20° C) until analysis. Serum total antioxidant status (TAS), total oxidant status (TOS), total oxidant/antioxidant status (OSI = TOS / TAS ratio), NO (colorimetrically), uric acid, albumin (ALB); As bone metabolism indicators; Serum type 1 collagen carboxyterminal cross-linked telopeptide (ICTP) and alkaline phosphatase (ALP), parathyroid hormone (PTH), calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), growth hormone (GH) levels are accepted as a marker of bone turnover. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK),gamma-glutamyl transferase (GGT) and myoglobin (MYB) are used as indicators of muscle damage; furthermore, indicators of heart muscle injury-specific serum Troponin I (Tn I) and CK-MB levels. As anemia markers, hemogram, iron-binding capacity, total iron binding capacity (TIBC) and ferritin (FER) levels frequently are used. Analysis of all the biochemical parameters was performed within a month.

Hemogram Analysis: Hemogram analysis from blood in EDTA tube was performed on the Automatic Hematology Analyzer (BC 3200 Mindray, Shenzhen, PRC) within 3-4 hours the same day. Hemogram measurement; It included erythrocyte, leukocyte, hematocrit, hemoglobin, platelet and mean erythrocyte volumes.

ICTP Analysis: Serum ICTP levels were performed from the serum samples with the microplate reader (Dialab EL X800G, Neudorf, Austria) using the ELISA (Enzyme-Linked Immunosorbent Assay Method Cusabio Biotech Co. Ltd., Chine).

Nitric Oxide (NO) Analysis: Serum NO analysis was done with a spectrometer device (Shimadzu UV 1700, Kyoto, Japan). Analysis of serum NO levels was performed with a minor modification of the method using the NO kit (Oxford Biomedical Research Inc, Michigan, USA). The method is based on the measurement of the absorbance of the pink-colored nitrogen dye formed by the reduction of nitrate (NO3) to nitrite (NO2) with cadmium (CD + 2) and using the "Griess Color Reagent". With this method, the nitrite levels formed by reducing the NO2 and NO3 present in the sample are measured in total.

TAS and TOS Analysis: Serum levels TAS and TOS levels were determined with an autoanalyzer (Abbott Architect C8000, Illinois, USA) using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) by a spectrophotometric method in the chromogenic methods performed within a month. Serum TOS to the TAS ratio is accepted as OS index (OSI).

Analysis of Biochemical Parameters: AST, ALT, CK, LDH, ALP, GGT, Ca, Pi, Mg, iron and TIBC parameters are determined using standard enzymatic methods by the autoanalyzer (Abbott Architect C16000, Illinois, USA). CK-MB, Tn I, MYB and FER parameters were analyzed by the chemiluminescent immunoassay method (Abbott Architect i 2000, Illinois, USA). The transferrin saturation (TS %) value was calculated by the calculation method according to (the iron / TIBC x100) formula. Parathormon (Abbott Architect i4000, Illinois, USA) and GH (Cobas 6000, Basel,

Switzerland) were analyzed by the chemiluminescent immunoassay method.

Statistical Analysis: Windows SPSS 20 program was used for statistical analysis. Whether there is a significant difference between the groups was analyzed with the nonparametric test "Mann Whitney U Test". The relationships between the biochemical parameters and physical measurement values of both groups were revealed by "Spearman Correlation Analysis". In statistical analysis, the $p< 0.05$ level was accepted as the statistical significance level.

RESULTS

Physical data and comparisons between groups of AG and CG are given in Table 1. The body fat rate value of AG was found significantly lower than CG (p= 0.00; Table 1). There was no significant difference between the groups for other parameters (p> 0.05; Table 1).

Table 1: Descriptive data of groups (mean±SD) and comparison between groups.

	Age (year)	Height (cm)	BW (kg)	BFR (%)	BMI (Kg/m^2)	SE (year)
AG (n=15)	10.3±1.80	144±11.10	31.0±6.45	11.9±1.49	14.8±1.14	4.50±2.34
CG (n=13)	9.2±1.00	137±6.10	29.6±4.88	17.9±4.50	15.8±1.90	0.0±0.0
Difference	p= 0.09	p= 0.11	p= 0.71	p= 0.00	p= 0.174	p= 0.00

AG: Athlete group, **CG:** Control group, **BW:** Body weight, **BFR:** Body fat rate, **BMI:** Body mass index, **SE:** Sports experience.

There was no significant difference for the OS markers and NO between the groups (p> 0.05) (Table 2).

Table 2: Oxidative stress parameters (mean±SD) of the athlete group and the control group and comparison.

	TAS (mmol Trolox Equiv/L)	TOS (µmol H2O2 Equiv/L)	OSI (Ratio)	NO (µM)
AG (n=15)	1.30±0.07	5.16±3.54	3.93±2.68	107.94±6.41
CG (n=13)	1.32±0.12	5.50±3.49	4.06±2.30	107.01±17.73
Difference	p= 0.60	p= 0.91	p= 0.84	p= 0.10

AG: Athlete group, **CG:** Control group, **TAS:** Total antioxidant status, **TOS:** Total oxidant status; **OSI:** Oxidative stress index, **TOS/TAS:** Total oxidant / antioxidant status, **NO:** Nitric oxide.

Seum ICTP value of AG was significantly higher than CG (p= 0.01) and the Ca value was significantly lower than CG (p= 0.04) (Table 3). No significant differences were found between the two groups for other parameters (p> 0.05; Table 3). But although there is no significant difference, GH values were higher (47.4%) than the control.

Table 3: Bone metabolism parameters (mean±SD) and comparison of the athletes group and the control group.

	ICTP (ng/mL)	PTH (pg/mL)	ALP (U/L)	Ca (mg/dL)	Mg (mM)	Pi (mg/dL)	GH (ng/mL)
Referance ranges	-	9-52	<500	8.8-10.8	0.70-0.86	4.5-5.5	1-16.4
SG (n=15)	65.23±16.99	36.05±13.1	214.73±52.57	9.59±0.44	0.98±0.05	4.63±0.43	4.18±4.82
CG(n=13)	51.70±22.88	30.26±15.28	206.85±40.88	9.97±0.47	0.95±0.06	4.50±0.33	2.20±1.82
Difference	p= 0.01	p= 0.11	p= 0.52	p= 0.04	p= 0.38	p= 0.39	p= 0.26

AG: Athlete group, **CG:** Control group, **ICTP:** Type 1 collagen carboxy terminal cross linked telopeptide, **PTH:** Parathyroid hormone, **ALP:** Alkaline phosphatase, **Ca:** Calcium, **Mg:** Magnesium, **Pi:** İnorganic phosphorus, **GH:** Growth hormone.

HCT levels of athletes were higher than the control (p= 0.04). It was not found a significant difference in the other hematological parameters and all the parameters were within normal ranges (Table 4). Biochemical parameters were found within the normal value ranges (Table 4). No significant difference was found between the groups for CK, CK-MB, LDH, Tn I, MYB, ALT, AST, GGT values, and WBC count (Table 5).

Table 4: Hematological parameters (mean±SD) and comparison of AG and CG.

	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	Platelets (PLT) (10^3/µL)	Iron (µg/dL)	Iron Binding (µg/dL)	TDBK (µg/dL)	Transferrin (%)	Ferritin (ng/mL)
Referance ranges	3.7-5.8	10.1-15	34-45	71-90	140-450	25-156	-	250-450	12–45	4.63-204.00
AG (n=15)	4.73±0.26	12.81±0.57	39.45±1.58	83.58±4.19	286.87±52.68	55.60±27.36	290.73±59.83	346.33±41.06	16.72±9.10	26.48±13.24
CG (n=13)	4.55±0.30	12.53±0.72	37.66±2.15	82.96±3.32	283.92±63.43	70.38±27.19	276.54±41.63	346.92±25.73	20.52±8.21	28.46±15.60
Difference	p =0.13	p =0.28	p =0.04	p =0.58	p =0.52	p =0.20	p =0.68	p =0.82	p =0.31	p =0.84

AG: Athlete group, **CG:** Control group, **RBC:** Erythrocyte, **HGB:** Hemoglobin, **HCT:** Hematocrit, **MCV:** Mean erythrocyte volüme, **TIBC:** Total Iron Binding Capacity.

Table 5: Muscle damage parameters (mean±SD) and comparison of AG and CG.

	CK (U/L)	CKMB (ng/mL)	LDH (U/L)	Tn-I (ng/mL)	MYB (ng/mL)	ALT (U/L)	AST (U/L)	GGT (U/L)	WBC (10^3/µL)
Referanc e ranges	29-168	0-3.4	125-243	-	0-106	0-55	5-34	9-36	3.0-10.0
AG (n=15)	80.27±23.4 1	0.89±0.3 7	173.27±30.9 1	0.00047±0.00 1	18.8±5.48	7.27±1.5 8	20.33±2.5 5	10.13±2.0 3	6.89±2.9 3
CG (n=13)	77.92±26.5 8	0.93±0.4 3	178.38±17.8	0.0000±0.000 0	19.36±5.3 0	7.85±2.3 7	20.23±5.3 1	10.69±1.3 7	6.86±1.9 8
Differenc e	p= 0.61	p= 0.93	p= 0.84	p= 0.35	p= 0.98	p= 0.71	p= 0.82	p= 0.70	p= 0.53

AG: Athlete group, **CG:** Control group, **CK:** Creatine kinase, **CK-MB:** Creatine kinase-MB, **LDH:** Lactate dehydrogenase, **Tn-I:** Troponin-I, **MYB:** Myoglobin, **ALT:** Alanine amino transferase, **AST:** Aspartate amino transferase, **GGT:** Gamma glutamly transferase, **WBC:** Leukocyte.

DISCUSSION

The main findings of the present study: Serum ICTP 1 (p= 0.009) and GH (47.4 %) levels of the athletes were significantly higher than the control group, whereas the calcium (p= 0.04) and body fat rate (BFR) (p= 0.01) values of the AG group was significantly lower (p= 0.04). But no significant differences were found between groups for OS markers and NO. The lower BFR value of AG compared to the control is consistent with the literature[24,25], which emphasizes that rhythmic gymnastics body fat rate is significantly lower than the CG. In addition to low body fat rate, it is necessary to have an aesthetic body structure in the rhythmic gymnastics to perform body movements such as branch-specific rotation, jump, balance and flexibility with the right technique and the required competence. Moreover, although not statistically significant, the body weight of AG was higher than CG. This difference may be due to the increase in muscle mass in AG with the training effect.

The rhythmic gymnastics is an anaerobic sport, therefore, the required energy during the competition is dominantly supplied by anaerobic glycolysis, that causes metabolic acidosis leading to muscle damage and OS. Because, in this condition, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NADP+) way is mainly activated as well as the other ways, which causes more SO radicals formation[26]. There is some piece of evidence that severe physical exercise dramatically increases oxygen use and thus free oxygen radical formation[27]. In a study examining the systemic responses of the oxidant / antioxidant status in rhythmic gymnasts, who train at different intensities, the hydroperoxide level was found to be significantly higher in post-training and 48-hour rest in the rhythmic gymnasts. No significant difference was observed between total antioxidant capacities. While the hydroperoxide level decreased significantly in the low-intensity training group, it was found at the base level after 48-hours of rest. In the high-intensity group, hydroperoxide level increased significantly after 48 hours and reached a high oxidative level, while total antioxidant capacity was found to be lower than pre-training value. It has been emphasized that hydroperoxide and total antioxidant capacity levels may be affected by different regulation mechanisms in terms of low and high-intensity training effects, while it is emphasized that OS induced by high-intensity training in rhythmic gymnastics branch may need a 48-hours rest time to restore redox balance capacity to achieve redox balance[11]. Similarly, Çakmak et al.[28] found that TOS, TAS, OSI and lipid hydroperoxide values of fifteen age group amateur basketball players were significantly higher than those of the control group. In the mentioned study, it was found that long-term regular exercise causes oxidant formation on the one hand and increases antioxidant capacity, in other words, it shows a double-sided effect. Unlike these studies, Ortenblad et al.[29] found no significant difference in muscle or blood antioxidant enzyme activity and malondialdehyde levels between elite volleyball players and the sedentary group after severe jump exercise. Miyazaki et al.[30] emphasized that the antioxidant defense mechanism increased defense against increasing lipid peroxidation during exercise. Similarly, Bloomer and Bloomer et al.[31] suggested that chronic anaerobic exercise training has an antioxidant production stimulant and exercise-related OS suppressive aspect.

In contrast, in the present study, it was no significant differences between the groups for WBC count as inflammation marker and the parameters of skeletal muscle damage (AST, ALT, LDH and CK). besides, Tn I and CK-MB and MYB as specific indicators of heart injury markers; which were within normal ranges and were not associated with OS parameters. In addition, no significant difference was found between groups for OS markers (TAS, TOS, and OSI) (Table 3). Therefore, these findings can indicate that chronic rhythmic gymnastic training does not cause OS. The absence of a difference between the two groups in terms of OS indicators may demonstrate that OS levels caused by rhythmic gymnastics training may have been balanced by the antioxidant system improved by the training. Thus, it can be expressed that the rhythmic gymnastics trainings similar to those of the present study didn't increase OS to create a risk for heart injury.

NO is a vasodilator, antioxidant, and important metabolic regulator gas. NO protects vessel endothelium and prevents AS formation. However, in high OS conditions, its bioavailability decreases and peroxynitrite may occur, which can cause occurrence of OxLDL (as AS

marker). Maeda et al.[32] reported that there was an increase in plasma NO level after 8 weeks of moderate exercise (3 times a week, 70% VO2max.) and this persisted for 4 weeks after stopping workouts. In the study by Cuzzolin et al.[33], it was reported that it can lead to NO formation after the exercise with 75% maximal oxygen consumption in the participants (6 active and 6 sedentary) in cycling ergometer. In another study, Özkol et al.[34] reported that regular aerobic exercise training can improve basal serum NO levels, while NO may play a role in aerobic and anaerobic exercise adaptations. The absence of a significant difference between the athletes and the control group for NO in the present study indicates that it was not negatively affected by this training. Therefore, it may indicate that NO's antioxidant, anti-inflammatory and antiatherosclerotic and bone health-related effects continue during exercise training in children[13]. In differences between the results of the above studies may be the role of the factor as the type, severity, and duration of the training as well as the age and gender.

Anemia is a condition defined by inadequate red blood cells (RBC) or HGB. When the body lacks sufficient amounts of iron, the production of the protein HGB is reduced. The main reasons are that athletes have lower (iron levels iron deficiency) due to mechanical hemolysis (destruction of red blood cells from physical impact), loss of iron through sweat and urine, gastrointestinal blood loss which occur generally in endurance athletes[35]. The hematocrit value of AG was significantly higher than CG (p= 0.01; Figure 3), one reason for this may be explained by increased hemoconcentration due to intense exercises, which may mainly be from splanchnic circulation to circulation[36]. These findings may put the need to replace water loss and mineral loss during this type of training. As a result, in the present study, it can be said that chronic rhythmic gymnastics training does not create anemia risk in children, based on the results of hematological parameters that are in normal value ranges and are not related to OS parameters.

In bone remodeling (or bone metabolism), there are two main types of cells; the first is osteoblasts that create new bone, and the other is osteoclasts that break a bone. The structure of bones besides a sufficient supply of calcium requires close intractions between these two cell types and other cell populations present at the bone remodeling locations. Bone metabolism is based on complex signaling pathways and control mechanisms to perform proper rates of growth and differentiation. The controls mechanism includes the action of several hormones, including PTH, vitamin D, GH and calcitonin and the other factors[37]. In the present study, serum ICTP and GH values of AG (p= 0.01; Table 4) were higher than that of the CG, whereas Ca value was lower (p= 0.04; Table 4). ICPT is a marker of bone destruction, which is liberated during the degradation of mature type I collagen and the extension fragment of procollagen III, thus this is accepted as an indexe of bone and collagen turnover[38].

Robinson et al.[39] found that rhythmic gymnasts have above-average values for their ages in their studies comparing bone mineral density (BMD) of rhythmic gymnasts and runners. Tournis et al.[40] found that calcium value, cortical bone mineral content, and density were significantly (38%) higher in rhythmic gymnasts between the ages of 9-13, without menarche unlike the present study (for Ca). There was no significant difference between the groups in C terminal telopeptide (ICTP) as the rate of bone turnover, unlike the present study. De La Piedra et al.[38] reported that the collagen 1 carboxy-terminal telopeptide α isomer (α CTX) has a high level of sensitivity in bone remodeling in adolescents similar to the present study. Munoz et al.[41] found the trochanter and femur BMD values significantly higher in rhythmic gymnastics and ballet dancers in the 14-18 age group, who performed intensive training for 20 hours a week than in ballet dancers and control groups. While bone ALP and type 1 procollagen amino-terminal propeptide values were normal in all participants, the C-terminal telopeptide (a CTX) / creatinine (Cr) ratio was found to be higher in rhythmic gymnasts like the present study. In a study examining specific bone mass gain in elite female rhythmic gymnasts and swimmers, whose ages ranged from 10.8 to 18.0 years, the osteogenic effect of gymnastics sports was mainly achieved by mechanically loaded bone mass (ie. proximal femur) around the menarche period. Moreover, it is emphasized that bone mass gain in gymnasts lasts longer, which can be explained by the delay in sexual maturation[42].

In another study, it was reported that the baseline concentrations of biomarkers such as ICPT of specifically, synchronized swimming and rhythmic gymnastics female athletes were different compared to athletes doing different sports. Age can be the main reason for the difference, as especially in the case of collagen turnover biomarkers. It was reported that physical fitness and specific training workload in different sports can affect ICTP serum levels, but these variations, although statistically significant in many cases, are within normal athlete population ranges like the present study[43]. Similarly, in another study with 1103 elite athletes, it was reported that a wide range of ethnic backgrounds, age, gender, BMI, and ethnicity accounted for up to 54% of the total variability of the GH-responsive markers including insulin-like growth factor 1 (IGF-I) and ICTP. And it was shown that age was negatively correlated with all the markers including ICTP. The contribution of gender was smaller than that of age[44]. In a study, it was shown that all molecular isoforms of GH measured increased and peaked at the end of acute exercise consisting of continuous cycle ergometry at 80% of maximal oxygen uptake for 20 min.[45]. Although it is not clear if the various biological actions of different GH isoforms impact postexercise homeostasis in this study, the other reason of the increased ICTP may be the increase for GH caused by training as well as the above-mentioned reasons similar to the previous literature. Because, although there is no significant, GH values of the athletes were higher (47.4 %) than the control. Considering the fact that menstruation has not started in the participants and the significant differences in ICTP and Ca values in the present study, it may be thought that bone destruction may be a risk factor for the athletic group. Because the increase in training loads with age as well as low calcium levels may cause the impairing of bone health. Although Ca values of the AG were significantly lower than the control, it is within the normal value range, suggesting that rhythmic

gymnasts, whose daily diets are not controlled and trying to have low body fat rate values, which may also be caused by insufficient Ca intake due to low-calorie intake. Furthermore, as known, it was that the young female rhythmic gymnasts have been a potential risk group for malnutrition due to weight reduction and leanness. Therefore it may be the role of these factors in the decreasing of calcium levels in the athletes.

As a result, it can be said that rhythmic gymnastics training may negatively affect bone metabolism and increase bone destruction, while decreasing calcium levels, but it did not OS and NO levels. These results indicate that ICP and Ca levels are sensitive markers for child rhythmic gymnastic sportswomen to observe chronic exercise training effects.

CONCLUSIONS

The results suggest that RGT did not significantly affect OS and NO levels and create anemia risk. But besides the increased GH levels, bone development can be affected negatively in child gymnasts due to the increased ICTP and decreased Ca levels. Thus, a calcium-rich diet and regularly observing of bone metabolism markers are recommended.

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