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Investigating the biochemical parameters and bone mineral density in active and sedentary adults

Karakuzulu M[1], Mendeş B[2*], Çiçek H[3], Elboğa U[4], Mendeş M[5]

[1]Gaziantep University Institute of Health Sciences Department of Physical Education and Sports, Gaziantep, Turkey.

[2] Gaziantep University Faculty of Physical Education and Sports, Gaziantep, Turkey.

[3] Gaziantep University School of Medicine, Department of Medical Biochemistry, Gaziantep, Turkey.

[4] Gaziantep University School of Medicine, Department of Nuclear Medicine, Gaziantep, Turkey.

[5]Canakkale Onsekiz Mart University Faculty of Agriculture, Animal Science Department, Biometry and Genetics Unit, Canakkale, Turkey.

*__Corresponding author:__ Bekir Mendeş, Gaziantep University Faculty of Physical Education and Sports, Elazığ, Turkey, Email: zeynimendes@hotmail.com

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ABSTRACT

Propose: This study aims to identify the change in the relationship between biochemical parameters and bone mineral density (BMD) in adults with different sex and lifestyles.

Methods: The sample group of the study consists of football players and sedentary men and women in the 18-25 age group, 80 participants in total. The participants were tested in terms of certain biochemical parameters such as glucose, insulin, creatinine, calcium, phosphorus, vitamin D, homocysteine, vitamin B12, folate and bone mineral density (BMD). Multidimensional Scaling Technique was used in investigating the relations between BMD and biochemical parameters.

Results: Results of Multidimensional Scaling Analysis showed that there was a strong relationship in all groups between T and Z scores and other parameters except vitamin B12 and glucose levels.

Conclusions: The biochemical parameters related to bone, muscle and heart health in football players were found more normal than sedentary group. While no significant gender-related difference was identified among the groups in terms of the relationship between BMD, T and Z scores of lumbar and femur areas and biochemical parameters.

Keywords: *biochemical parameters, football player, sedentary, bone mineral density*

INTRODUCTION

In adults and children, the bodyweight is the static mechanical load on bones. Therefore, the bone mineral density (BMD) is rather dependent on the body weight, while age, sex, height, weight, smoking, hormones, genetic, physical exercises, recreational sporting events and alcohol use can affect the bone mineral density (1). Physical inactivity can result in a serious loss of bone mineral density, and people who do not exercise experience skeletal deformation earlier than others.

It was stated that the individuals engaged in physical exercises have higher bone mass density (2), so physical inactivity can result in a serious loss of BMD and people who do not exercise experience skeletal deformation earlier than others (3). It has been reported that recreational football training has contributed significantly to BMD, in addition, regular football training for older sedentary men has increased by 1.8% on BMD at the end of 4 months and 5.4% at the end of 12 months on femoral bone region (4,48,49).

Regular exercise seems to be able to reduce the risk of fractures in football players as well as in other people. Therefore, in order to minimize the risk of fracture, bone health improvement strategies should be adopted in the early stages of life (5).

The Dual-Energy X-ray Absorptiometry (DEXA) is considered as the most efficient method in BMD evaluations and thus it is one of the most widespread methods used in today (6). However, some biochemical parameters are reported to be used in diagnosis and treatment follow-up as indicators either development or destruction of bone and muscle (7,50). Thus, it has been reported that adequate vitamin D levels should be maintained for healthy bones in the literatüre (8).

Also, vitamin B 12 is assumed to affect BMD in addition to calcium and vitamin D (9).

On the other hand, there are studies stating that there is no direct relationship between homocysteine and BMD, as well as high homocysteine level and folate and vitamin B-12 deficiency are associated with osteoporosis and bone fracture risk (10).

In addition, high homocysteine and low folate levels were associated with decreased BMD in women, but not in men (11).

Since the results of these studies in the literature are contradictory, it was thought that it would be appropriate to investigate the parameters such as vitamin D, creatinine, calcium, homocysteine, vitamin B12, folate, insulin, glucose and phosphorusus in groups that exercise and non-exercise.

This study aimed at identifying the gender-related change in biochemical parameters and BMD values of adults in 18-25 age groups consisting of individuals who perform regular physical exercises and sedentary lifestyles.

MATERIAL AND METHODS

Research Design

Participants

This study was applied to 80 individuals consisting of 20 males and 20 females who educating at the School of Physical Education and Sports (Group I) and 20 male and 20 female students educating at different schools (Group II) in Gaziantep University. Participants of the first group are football players who do regular exercises, while the other group consisted of people living sedentary. Participants were informed about the work plan and purpose, and a written confirmation was obtained from them indicating that they voluntarily participated in the study. This study was conducted upon the approval of the ethics committee of Gaziantep University Faculty of Medicine (2016/317-28.11.2016) and in accordance with the Helsinki Declaration Rules. Bone mineral density of each individual participating in the study was measured and vitamin D, creatinine, calcium, homocysteine, vitamin B12, folate, insulin, glucose and phosphorus levels were detected in serum samples of subjects.

Data Collection

Identification of Age, Height and Bodyweight

The age of each individual is determined by the relevant personal identification information. Their height was measured using a height scale with a sensitivity of 0.01 cm which mounted on a bascule, and each subject was barefoot or wearing socks during the measurement. On the other hand, body weight was measured using a scale weighting with a sensitivity of 0.01 kg when each subject wearing suitable cloths.

Identification of Body-Mass Index

Body Mass Index is computed by using following formula.

$$\mathrm{BMI} = \frac{\mathrm{kg}}{\mathrm{height}^2}$$

Measurement of the bone mineral density

Measurement of BMD values was conducted using the Hologic QDR 4500 Elite densitometer. DEXA method was used to identify bone mineral densities. Subjects laid down on the device and the process was performed in the supine position. The lower back bone area and hips were scanned during the process, in order to flatten the lower back area (lordosis), subjects' legs were pulled up to the abdominal region and placed on a support surface. When the hips were scanned, legs (femur) were turned inwards (internal rotation). Lumbar backbone areas of the participant subjects (L1, L2, L3, L4) were tested in terms of BMD values and T and Z scores.

The T-score defines the difference between the BMD of the patient under examination and the average BMD of the standard young adult (20-30 years old) population as standard deviation (SD)and expresses peak bone mass. Normal BMD value T-score is defined as 0 for healthy young adult.

Z-score expresses the average BMD difference of the patient's BMD results and the controls in the same gender and age group as SD. According to the recommendation of WHO, a uniform, standard reference database should be used in all ethnic groups, women and men in the T-score calculation.

Measurement of biochemical parameters

The blood samples were taken after at least 8 hours of fasting, between 8:00-11:00 in the morning. Within the first one hour, all samples were centrifuged at 2500 rpm for 15-20 minutes and then kept at −80 ° C until the measurements were completed. Among the biochemical parameters, the serum glucose, calcium, phosphorus and creatinine levels were measured with the enzymatic colorimetric method using Architect C 4000. The process was automatized using commercial kits (Architect c4000, Abbott, ABD). Reference ranges of serum glucose, calcium and phosphorus were 70-110, 8,5-10,5 and 2,5-4,5 mg/dL for each parameter respectively. Creatinine reference ranges were 0,64-1,20 mg/dL for male and 0,42-1,06 mg/dL for female participants. Serum homocysteine concentrations were identified with a high performance liquid chromatography method (HPLC) with Agilent 1200 Analyzer (Agilent Technologies, Waldbronn, Germany) supported with commercial kits (Chromsystems Instruments & Chemicals GmbH, München, Germany). The reference range was determined as 5,0-12 µmol/l.

Insulin, vitamin B12, folate and vitamin D values were tested using an automatized electrochemiluminescence method on Roche Cobas e 411analyzer supported with commercial kits. (Roche Diagnostics GmbH, Germany). The reference values were2.6-27 µIU/ml for insulin, 193-982 pg/ml for vitamin B12, and 3-17 ng/ml for folate. Vitamin D levels below 12 ng/mL were identified as a deficiency, while those between 12-20 ng/mL, and those above 20 ng/mL were considered as dysfunction and normal, respectively.

Statistical Analysis

Multidimensional Scaling Technique (MDS) was used in investigating relations between biochemical parameters and bone mineral density values. MDS Technique was performed for each gender in sedentary and football players groups separately. SPSS for Windows (Ver.20) statistical package programs was used in performing analyses (IBM, Armonk, NY, USA). p value less than 0.05 was considered statistically significant.

RESULTS

Descriptive statistics for the measured characteristics of males and females in the sedentary and football players groups were presented in Table 1, 2, 3, and 4, respectively.MDS analysis performed to investigate the relations between biochemical parameters and bone mineral density of adults in sedentary and football player groups (Figure 1, 2, 3, 4). Two different goodness-of-fit criteria namely R2 and stress coefficient have been used to determine the suitability of MDS technique to assess the relations between biochemical parameters and bone mineral density. R2 and stress coefficient values (≥0.991 and 0.023) indicated that MDS technique was one of a good choice to evaluate the relations between biochemical parameters and bone mineral density of males and females in the sedentary and football player groups.

TABLE 1: Descriptive statistics for sedentary males

Variables	N	Minimum	Maximum	Mean	Standard Error of Mean
Age (year)	20	18.00	23.00	19.40	0.34
Weight (kg)	20	55.00	95.00	70.90	2.44
Height (cm)	20	1.70	1.89	1.77	0.01
BMI	20	0.00	28.68	21.40	1.30
Glucose (mg/dl)	20	59.00	100.00	84.55	2.68
Insulin(mg / dl)	20	5.60	17.10	10.35	0.81
Creatinine (mg/dl)	20	0.84	1.06	0.88	0.01
Calcium (mg/dl)	20	8.90	9.90	9.41	0.05
Phosphorus (mg/dl)	20	1.90	4.50	3.27	0.16
Vitamin D (ng/ml)	20	4.20	14.70	8.35	0.66
Homocysteine(µmol/l)	20	7.73	52.70	20.80	2.61
Vitamin B12 (pg/ml)	20	132.00	386.00	230.80	14.63
Folate(ng/ml)	20	4.31	13.03	8.56	0.45
T score 1 (g/cm2)	20	-2.80	-0.30	-1.53	0.16
Z score 1 (g/cm2)	20	-2.70	0.00	-1.19	0.18
T score 2 (g/cm2)	20	-1.60	0.60	-0.43	0.12
Z score 2 (g/cm2)	20	-1.60	0.60	-0.45	0.13

TABLE 2: Descriptive statistics for sedentary females

Variables	N	Minimum	Maximum	Mean	Standard Error of Mean
Age (year)	20	18.00	21.00	18.95	0.25
Weight (kg)	20	44.00	76.00	56.35	1.89
Height (cm)	20	1.52	1.75	1.64	0.013
BMI	20	16.77	27.34	20.83	0.63
Glucose (mg/dl)	20	61.00	102.00	85.90	2.54
Insulin(mg / dl)	20	4.60	15.30	8.47	0.62
Creatinine (mg/dl)	20	0.60	0.80	0.66	0.01
Calcium (mg/dl)	20	8.90	9.70	9.42	0.04
Phosphorus (mg/dl)	20	1.90	4.30	3.01	0.13
Vitamin D (ng/ml)	20	4.20	9.00	5.26	0.27
Homocysteine(µmol/l)	20	5.79	38.70	13.21	1.77
Vitamin B12 (pg/ml)	20	116.00	364.00	225.50	17.57
Folate(ng/ml)	20	4.46	11.89	8.08	0.43
T score 1 (g/cm2)	20	-2.40	-0.10	-1.12	0.14
Z score 1 (g/cm2)	20	-2.60	0.10	-1.00	0.16
T score 2 (g/cm2)	20	-2.40	0.90	-0.81	0.19
Z score 2 (g/cm2)	20	-2.50	0.90	-0.82	0.19

TABLE 3: Descriptive statistics for male football players

Variables	N	Minimum	Maximum	Mean	Standard Error of Mean
Age (year)	20	20.00	25.00	22.60	0.30
Weight (kg)	20	60.00	95.00	74.15	1.80
Height (cm)	20	1.65	1.89	1.77	0.01
BMI	20	0.00	29.00	22.06	1.24
Hand grip strength (kg)	20	30.00	64.01	40.18	2.09
Back strength (kg)	20	70.00	151.05	110.52	4.85
Leg strength (kg)	20	63.05	147.00	101.82	4.55
Glucose (mg/dl)	20	58.00	102.00	81.55	2.70
Insulin (mg / dl)	20	4.30	12.60	7.68	0.67
Creatinine (mg/dl)	20	0.84	1.02	0.90	0.01
Calcium (mg/dl)	20	9.00	9.90	9.50	0.05
Phosphorus (mg/dl)	20	2.80	4.70	4.04	0.10
Vitamin D (ng/ml)	20	9.00	26.40	14.77	0.98
Homocysteine (μmol/l)	20	7.18	22.50	12.65	0.91
Vitamin B12 (pg/ml)	20	226.00	356.00	283.45	10.60
Folate (ng/ml)	20	6.41	13.06	8.99	0.44
T score 1 (g/cm2)	20	-1.90	2.50	0.51	0.25
Z score 1 (g/cm2)	20	-1.50	2.50	0.61	0.24
T score 2 (g/cm2)	20	-0.20	3.40	1.31	0.22
Z score 2 (g/cm2)	20	-0.10	3.40	1.31	0.21

TABLE 4: Descriptive statistics for female football players

Variables	N	Minimum	Maximum	Mean	Standard Error of Mean
Age (year)	20	18.00	26.00	20.95	0.48
Weight (kg)	20	38.00	79.00	56.40	2.28
Height (cm)	20	1.50	1.75	1.65	0.01
BMI	20	16.89	27.73	20.67	0.70
Glucose (mg/dl)	20	58.00	97.00	79.20	2.92
Insulin(mg / dl)	20	3.60	11.50	7.1400	0.51
Creatinine (mg/dl)	20	0.60	0.90	0.73	0.02
Calcium (mg/dl)	20	8.50	9.80	9.21	0.07
Phosphorus (mg/dl)	20	2.80	4.50	3.78	0.10
Vitamin D (ng/ml)	20	4.30	33.20	12.68	1.43
Homocysteine (μmol/l)	20	7.77	20.20	11.22	0.80
Vitamin B12 (pg/ml)	20	162.00	362.00	269.90	13.71
Folate(ng/ml)	20	4.89	11.76	8.48	0.42
T score 1 (g/cm2)	20	-2.50	2.30	-0.62	0.26
Z score 1 (g/cm2)	20	-2.60	2.60	-0.52	0.28
T score 2 (g/cm2)	20	-2.20	3.10	-0.20	0.26
Z score 2 (g/cm2)	20	-2.20	3.00	-0.18	0.25

MDS results for the sedentary males and females are given figure 1 and 2, respectively. When the results given in Figure 1 regarding biochemical parameters of sedentary males are examined, it is seen that the Z and T scores are compatible to all parameters except for particularly vitamin B12 and relatively the glucose levels. These findings can be interpreted as indicators showing that the Z and T scores have highly strong relationship with all chemical parameters except for particularly vitamin B12 and relatively the glucose levels.

When figure 2 is examined, it is seen that the Z and T scores are compatible to all parameters except for particularly vitamin B12 and relatively the glucose level. As it is noticed, the results in the figure 1 and figure 2 are almost the same, mean that the relationship between biochemical parameters and bone mineral density is not affected by the gender difference.

Figure 1. MDS Results for Sedentary Males

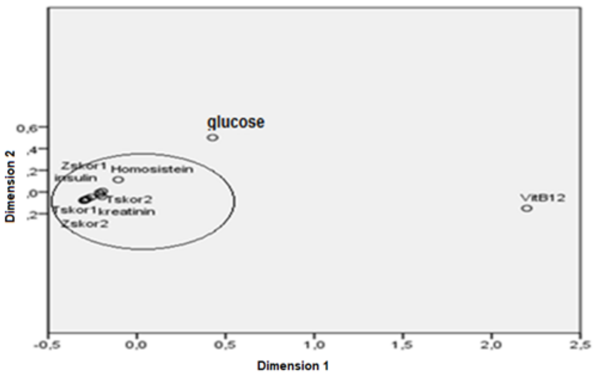
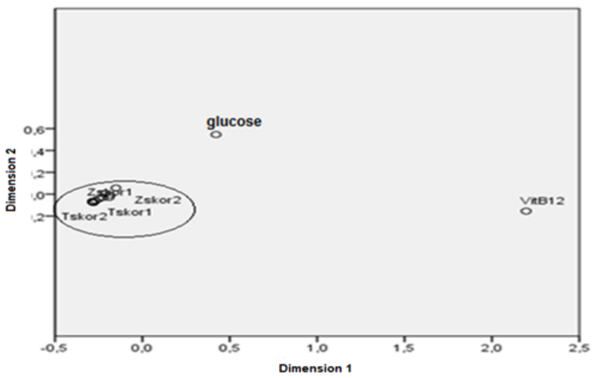


Figure 2. MDS Results for Sedentary Females



MDS results for the males and females' participants in the football player group are presented in Figure 3 and 4, respectively. As the Figure 3 and 4 show that all biochemical parameters except for vitamin B12 and glucose level are placed in the same group that means these parameters are highly related or there is a strong relation between / among those parameters. On the other hand, the glucose level and vitamin B12 are located in different places. Consequently, it is not possible to say that the relations between these parameters (glucose level and vitamin B12) and other parameters are high or strong. It is also possible to conclude that the relation glucose level and vitamin B12 with the other parameters is obviously different.

One of the other important points to be considered based on the MDS results is that the results outcomes for sedentary and football player groups are similar. Therefore, it is possible to conclude that the relationships between biochemical parameters and bone mineral density are changed based on group and gender differences. On the other hand, it should be keep in our min that the relations between / among the variables may be changed based on sample size, number of variables to be considered, and type of measurement level of the variables.

Figure 3. MDS Results for Football Player Males

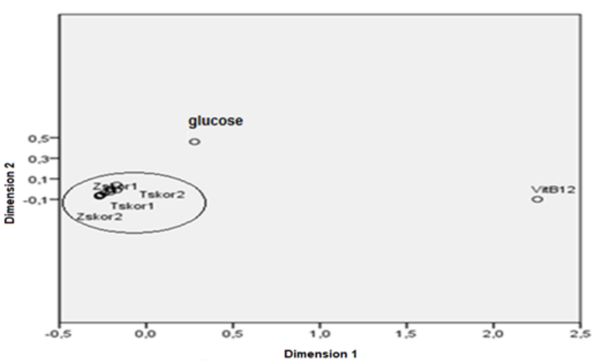
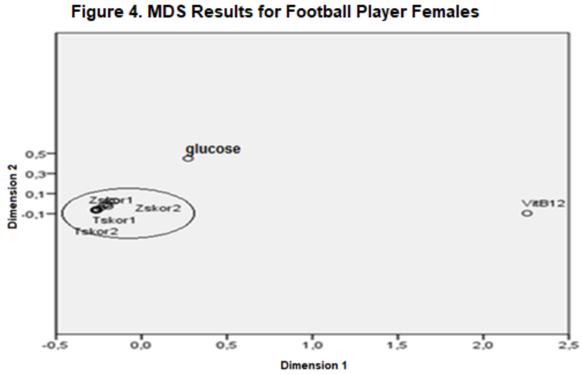


Figure 4. MDS Results for Football Player Females



DISCUSSION

This study was conducted to investigate the relations between/among selected biochemical parameters and BMD values of two different groups. The first consisting of adult males and females in 18-25 age group who are engaged in regular football exercises and the second consisting of sedentary individuals of the same age group. The study also reveals the impact of regular exercises on these parameters.

Bone is a mineralized connective tissue formed by organic and inorganic components, 30% of a bone is formed by the organic matrix while 70% is composed of the inorganic matrix (12).

In the relevant literature, most of the studies prefer vertebra and femoral regions for measurement of BMD (13,14). It is often stated that these two areas are preferred in measurement of bone mineral density as in these areas. Changes inside the bone mineral content and the trabecular structure are dense and the fracture incidence is relatively high (15).

In this study, the measurements are made on the vertebra and femur, which are emphasized as significant areas using the Dual-Energy X-ray Absorptiometry (DEXA) method.

According to the multidimensional scaling analysis, which was conducted to examine the relationship between biochemical parameters as glucose, insulin, creatinine, calcium, phosphorus, vitamin D, homocysteine, vitamin B12, folate and BMD values; the T and Z scores of sedentary males (stress coefficient: 0.027, R2=99.1%), sedentary females (stress coefficient: 0.027, R2=99.5%), male football players (stress coefficient: 0.026,R2=99.3%) and female football players (stress coefficient: 0.027,R2=99.3%) are in fairly close positions to all biochemical parameters, except for particularly vitamin B12 and relatively the glucose levels. According to these findings, it was identified that the Z and T scores have highly strong relationships with all chemical parameters except for particularly vitamin B12 and relatively the glucose levels.

In the literature, it is emphasized that both the problems in protection of the general health of bones (16) and the low BMD values in lumbar and femur areas can be explained with inadequate calcium intake (17). As it is a living tissue with vital functions such as being a structural support mechanism and a mineral storage, a bone is affected by genetics, physical activity and particularly adequacy levels of calcium and vitamin D (18). A study analyzing the relationship between physical activity and muscular force reported that BMD and muscular force are interrelated (19) It was also stated that aging causes a decrease in BMD values and the results of this situation are seen as falls in the muscular force (20).

Kohrt et al. reveal that any decrease in the density of physical exercises causes an increase in osteoporotic fractures (21). The same study suggests that those who regularly exercise have higher BMD values (22).

Alfredson et al. analyze the impact of aerobic exercises on women's BMD values. This study's sample group consists of 23 female participants who do regular 3-hour exercises each week and 23 other women who have sedentary lifestyles. The measurements are conducted with the DEXA instrument. In conclusion of the research, it is revealed that the aerobic exercise group have higher values of BMD considering the whole body, particularly in lumbar backbone, femur and tibia areas, than BMD values of sedentary women. This research shows that regular exercises have a positive impact on BMD and this suggestion supports the findings of our study (23).

It has been noted that recreational handball practice applied to inactive postmenopausal women has positive effects on BMD in the lumbar and femoral regions. (24).

Despite its general existence in the whole body, the vitamin D receptors are more intensely located in the cells of the skin, heart, brain, pancreas and the immune system (25).

It is emphasized that vitamin D deficiency can be seen in all age groups, however, aging can increase its incidence (26).

The reasons for the high frequency of vitamin D deficiency cases include covering the whole body (27, 28), genetic factors, staying in closed places, lack of physical activity, smoking and use of certain medications that affect the vitamin D metabolism (29).

Vitamin D deficiency; poor muscular tone and force (30), as well as decreases in lower extremity muscular force (31) which increased incidence of fallings and cause balance disorders at later ages (32).

10-15% of the calcium and 60% of the phosphorus content are absorbed in case of vitamin D deficiency, while the absorption rates reach to 30-40% for the calcium content and 80% for the phosphorus content if vitamin D exists in adequate amounts (28).

A study on athletes in Spain reports that the participants' athletes who do outdoor exercises have higher vitamin D values than those who exercise in gymnasiums (33).

Considering the fact that the participants of our study is in an aging process, it is better understood that vitamin D is particularly significant for the members of the sedentary group.

Moreover, vitamin D values of the sedentary male and female groups, which were identified as lower than the group of football players in our study, can indicate that sedentary individuals spend more time indoor, while the athletes intake adequate amounts of vitamin D as a result of outdoor exercises and matches.

Relevant studies in the literature refer to a relationship between low vitamin D levels and muscle weakness. Children with genetic vitamin D deficiency (who cannot produce 1.25 (OH)D) face significant muscle weakness, however, 1.25 OHD treatments can ensure a quick recovery. In the meta-analysis of three studies where 800-1000 international unit vitamin D additives were given to the participants each day, the lower extremity muscle force and balancing ability were slightly but significantly improved (34).

Lloyd et al. analyzed the impact of daily calcium intake trends of young women in the 12-22 age groups on bone development, where no relationship was identified between calcium intake and body bone gain (35).

It is generally accepted that the ATP experiences a fast renewal process during high density exercises and this leads to an increase in creatine amounts in storages (36).

Pearson et al. identified that the athletes who intake 5 g creatine every day for a period of 10 weeks show more distinct increases in power index and bodyweight compared to placebo groups who follow the same training program (37).

Three other studies in the literature identified an increase in homocysteine levels of swimming athletes after 3 weeks of high tempo interval training (38).39 triathletes in the 19-49 age group after 4 weeks of high density training (39), and 32 males, with an average age of 22, after 4 weeks of training (40) examined under normoxic conditions, at throb rates between 70-85%. We understand from these three studies that high density physical exercises increase homocysteine levels. As it is seen in this finding, intensive trainings scheduled in high density intervals increase homocysteine levels together with the risk of cardiovascular diseases. As for the differences between these studies and our research, the reasons among others are considered to be the duration, type, density and scope of the chosen exercises and age ranges of the participants.

Hermann et al. examine the acute impacts of the durability exercises performed by three different groups, including 100 athletes in total (marathon runners, mountain bikers and 100-km runners), on the athletes' homocysteine levels. Homocysteine levels of the marathon runners increased by 64% after the contest, while no significant difference was seen in homocysteine levels of mountain bikers and 100-km runners. In durability athletes, on the other hand, the results were seen as moderate hyperhomocysteinemia, vitamin B12 deficiency and low folate levels (38).

In a study on 24 middle-aged men, Gaume et al. compare 12 individuals who have been doing 8 hours of durability exercises every week for more than 15 years and a group of sedentary males who only walk for two hours in a week. In this study, the Homocysteine values of the training group was found lower than those of the sedentary group, while the folate and vitamin B12 values of the training group were measured as far higher than those of the sedentary group (41). As in our study, the aforementioned research makes a comparison between athletes and sedentary groups and findings of this study support the data we have obtained as a result of our measurements.

Randeva et al. conduct an analysis on 21 women with polycystic ovarian syndrome. In the study, they include 12 of these women in the walking exercises for 6 months while not allowing the other 9 women to participate in any of the exercises. As a result of this study, a significant decrease was identified in homocysteine levels of females who participated in the 6-month walking program (42). Also, Yilmaz et al. found that higher homocysteine levels in women who have lower BMD their study (43). The literature supports the findings that we obtained as a result of our measurements. Our study also shows lower homocysteine values for those who do sportive exercises than the values of those who do not.

Zoladz et al. identified in a study about insulin density that there was a decrease in insulin values of all 15 participants who performed a longtime cycling exercise (44). Likewise, insulin values of female and male football players who do regular exercises were found lower than those of the sedentary group in our study.

Sütken et al. conducted a research on 7 males and 7 females (14 in total) licensed professional athletes and stated that insulin levels of both male and female athletes were significantly lower after aerobic exercises than the levels measured before the trainings (45).

Aydın et al. asked 9 male football players to do aerobic exercises and identified lower insulin values at the end of the trainings (46). These findings we see in the relevant studies within the literature support our idea which suggests that physical exercises result in lower insulin levels.

Moreover, Boyd et al. found out in a study on college students that aerobic exercises decreased their glucose levels (47). Similarly, our findings throughout the study show that glucose levels of male and female athletes who do exercises are lower than those who do not.

CONCLUSION

Based on the results of statistical analyses, it can be suggested that the relationships between biochemical parameters and bone mineral density (Z and T scores) are highly strong except for particularly vitamin B12 and relatively the glucose levels. In conclusion; the relationship between T and Z scores and the identified biochemical parameters show fair similarities in males and females, as well as the groups of football players and sedentary participants. Based on this finding, it was concluded that there is no significant sex-related change in the relationships between T and Z scores and the biochemical parameters among the groups of participants. The biochemical parameters related to bone, muscle, heart and however general health in football players were found more normal than sedentary group. Individuals should participate in sportive exercises especially at ages when the skeletal system matures, in order to ensure stronger BMD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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