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Comparison of Some Plasma Inflammation Markers in Elite Master Athletes, Recreational Athletes and Sedentary Males

Elit Master Atletler, Rekreasyonal Atletler ve Sedanter Erkeklerde Bazı Plazma İnflamasyon Markerlerinin Karşılaştırılması

Fatma GİRGİNER[a],
Gürbüz BÜYÜKYAZI (1954-2018)[b],
Cevval ULMAN[c],
Yeliz DOĞRU[d],
Fatma TANELİ[c],
Raziye YILDIZ[e],
Murat TAŞ[f],
Pembe KESKİNOĞLU[g]

[a]Hacı Serpil Kabaklıoğlu Secondary School, Denizli, TURKEY
[b]Department of Trainer Training, Dokuz Eylül University Faculty of Sports Sciences, İzmir, TURKEY
[c]Department of Biochemistry, Manisa Celal Bayar University Faculty of Medicine, Manisa, TURKEY
[d]Deparment of Sports, İzmir Kâtip Çelebi University Health, Culture and Sports Office, İzmir, TURKEY
[e]Biochemistry Laboratory, Akçaabat Haçkalı Baba State Hospital, Trabzon, TURKEY
[f]Manisa Celal Bayar University Faculty of Sports Sciences, Manisa, TURKEY
[g]Department of Biostatistics, Dokuz Eylül University Faculty of Medicine, İzmir, TURKEY

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Correspondence:
Cevval ULMAN
Manisa Celal Bayar University Faculty of Medicine, Department of Biochemistry, Manisa, TURKEY/TÜRKİYE
cevval.ulman@bayar.edu.tr

ABSTRACT Objective: It is known that high-intensity exercises lead to muscle damage and fibrosis. This study aims to compare some plasma inflammation markers in elite master athletes, recreational athletes and sedentary males to determine muscle injury. **Material and Methods:** 60 healthy male participants (min-max 45-65 years) were divided into 3 groups: Elite master athletes performing high intensity training (EMAG; n=22), recreational athletes performing moderate intensity training (RG; n=21) and sedentary controls (CG; n=17). Resting serum levels of the monocyte chemoattractant protein-1 (MCP-1), High-Sensitivity C-Reactive Protein (hsCRP), Tumor Necrosis Factor Alfa (TNF-α), Transforming Growth Factor Beta-1 (TGF-β-1), and Klotho hormone were compared among the groups. **Results:** Body weight, fat percentages, body mass index were lower and $MaxVO_2$ was higher in EMAG than those of both RG and CG. TGF-β-1 and Klotho Hormone levels of EMAG and RG were higher than CG. Vitamin D levels of EMAG were found higher than those of CG. **Conclusion:** Regular long-term endurance training has a long-term positive impact on the regeneration of muscle damage and inflammation. The high levels of Klotho hormone determined in EMAG provides a longer and healthier life by extending the initiation process of many illnesses including metabolic diseases.

Keywords: Master athletes; recreational athletes; hsCRP; MCP-1; TGF-β-1; klotho hormone

ÖZET Amaç: Yüksek şiddette egzersizin kas hasarı ve fibrozise yol açtığı bilinmektedir. Bu çalışma kas yaralanmasını belirlemek için elit master atletler, rekreasyonal atletler ve sedanter erkeklerde bazı plazma inflamasyon markerlerini karşılaştırmayı amaçlamaktadır. **Gereç ve Yöntemler:** 60 sağlıklı erkek (min-maks: 45-65 yıl) yüksek şiddette antrenman yapan elit master atletler (EMAG; n=22), orta şiddette antrenman yapan rekreasyonal atletler (RG; n=21) ve sedanter kontroller (KG; n=17) olmak üzere üç gruba ayrıldı. Monocyte Chemoattractant Protein-1 (MCP-1), Yüksek hassasiyetli C-Reaktif Protein (hsCRP), Tumor Necrosis Factor Alpha (TNF-α), Transforming Growth Factor Beta-1 (TGF-β-1) ve Klotho hormon istirahat düzeyleri üç grupta karşılaştırıldı. **Bulgular:** EMAG'ın vücut ağırlığı, yağ yüzdesi ve vücut kütle indeksi değerleri RG ve CG'den daha düşük; $MaxVO_2$ değerleri ise daha yüksekti. EMAG'ın TGF-β-1 ve Klotho hormon düzeyleri hem RG'den hem de CG'den istatistiksel olarak daha yüksekti. EMAG'ın Vitamin D değerleri ise CG'den istatistiksel olarak daha yüksek bulundu. **Sonuç:** Uzun süre yapılan dayanıklılık antrenmanının kas hasarı ve inflamasyonun rejenerasyonu üzerine olumlu etkisi vardır. EMAG'da belirlenen yüksek düzeylerdeki Klotho hormonu metabolik hastalıklar da dahil pek çok hastalığın başlangıç sürecini uzatarak daha uzun ve daha sağlıklı bir yaşam sağlar.

Anahtar Kelimeler: Master atletler; rekreasyonal atletler; hsCRP; MCP-1; TGF-β-1; klotho hormon

Regular physical exercise especially walking, running, or swimming has positive effects on human health. When performed regularly, it prevents the development of coronary artery disease (CAD), helps patients to heal from cardiovascular disease (CVD), reduces the risk of developing diseases such as type 2 diabetes, osteoporosis, obesity, depression, and cancer.[1-3] In addition, there is literature suggesting physical exercise induces muscle damage and non-specific inflammatory response manifested

by high concentrations of circulating pro- inflammatory growth factors and cytokines.[4,5]

hsCRP is a major acute phase reactant that acutely and rapidly rises in response to infection and tissue damage in humans. hsCRP has emerged as a risk factor for predicting cardiovascular disease.[6-10] It has been reported that exercise in men aged 65-95 years has decreased plasma hsCRP levels after moderate intensity training lasting 24 weeks.[11] Similarly, plasma CK and hsCRP levels were found to be higher in ultra-marathon runners (308km) than marathon runners (100km).[12]

Monocyte Chemoattractant Protein-1 (MCP-1) is required for reperfusion in normal skeletal muscle regeneration after ischemic tissue disease. Macrophages and inflammation are important components in the formation of collateral arteries and regeneration of skeletal muscle.[13] MCP-1 has been found to be positively correlated not only with obesity, but also with other metabolic diseases such as type 2 diabetes, arteriosclerosis and cardiovascular diseases.[14,15] MCP-1 response to exercise has been examined in some studies. Glintborg et al. investigated the effect of strength training in elderly male subjects on some inflammatory and hormonal parameters.[16] They found a decrease in MCP-1 levels during the last three months of the exercise period. In another study, 100 people (65 to 95 yrs) performing endurance training and high intensity interval training were observed and MCP-1 levels were found to be decreased after 30 minutes of exercise in the endurance training group.[17]

Many cell types secrete the cytokine, Tumor Necrosis Factor (TNF) during acute inflammation. TNF causes a diverse range of signaling events within cells, leading to necrosis or apoptosis (cell death). The protein also provides resistance against infection and cancers. Increased local concentration of TNF results in bacterial infections. TNF activates the clotting system by altering the balance between procoagulant and anticoagulant activities of vascular endothelin.[18] The effects of exercise on TNF-a have been a concern for researchers; however, the results of the studies have revealed contradictory findings.[19-21]

Transforming Growth Factor-Beta-1 (TGF-β-1) is an effective cytokine in the formation of many tissue and cell activities, supporting early development, differentiation, angiogenesis (new vascular formation), immunological functions, and apoptosis.[22] TGF-β-1 levels have been found to be increased right after exercise.[23]

The Klotho Hormone (Kl) gene was first described in mice in 1997 by Kuro-o et al. These mice, defective in this gene and identified as Klotho, develop normally until 3 weeks, then slow down in their development and die within 8-9 weeks with the increase in signs of aging. Klotho mice exhibit typical aging phenotypes such as atherosclerosis, ectopic calcification, pulmonary emphysema, deep atrophy and osteoporosis.[24] Analyses at the molecular level have shown that the genetic locus (KL gene) localized on the 5th chromosome does not function normally in the appearance of these phenotypic features in mice.[25,26] Plasma klotho levels have been found to be associated with muscle strength in humans. Hand grip strength is a strong determinant of total muscle strength and a sign of weak strength in elderly adults. Therefore, Richard et al. compared the plasma klotho levels by measuring hand grip strength in the elderly.[27] They found that the lower level of klotho was significantly associated with weak skeletal muscle strength in the elderly. Likewise, there was an association between age, gender, smoking, physical activity status, nutrition, chronic diseases, cognitive level and klotho level and handgrip strength.

Intervention studies examining the effect of exercise on inflammation markers such as hsCRP, CK, MCP-1, TGF-β-1, and the levels of klotho hormone are quite limited. In addition, the underlying mechanism between exercise and these parameters is not completely clear yet. Moreover, we found no studies which examine the effect of high-intensity exercises and recreative exercises performed for more than a decade on plasma inflammation markers and the klotho hormone levels. Thus, this study aims to compare some plasma inflammation markers in elite master athletes, recreational athletes and sedentary males to determine the muscle injury.

MATERIAL AND METHODS

Subjects: Forty-three athletes and 17 control males participated in our study. Table 1 gives the characteristics and training background of the athletes and controls.

Elit Master Athletes (High intensity and long duration exercise training group: EMAG): We evaluated twenty-two male endurance athletes (mean age±SD: 57.28±6.38 years) from İzmir Athletics Masters' Club. Inclusion criteria were as follows: 1) age between 45-65 yr, 2) under training for more than 10 years, 3) under training regularly at least 5 days/10 hours per week, 4) participating in national or international competitions regularly, 5) living in a city at the sea level for at least 10 years.

Recreational Athletes (Moderate intensity and duration exercise training group: RG): Twenty-one (walking, jogging, gymnastics) male athletes (mean age±SD:62.38±2.12 years) from a fitness-center and exercising for endurance were evaluated. We invited the participants meeting the following criteria: 1) age between 45-65 yr, 2) exercising aerobically for recreational purposes, 3) under training for at least 10 years, 5) under training for at least 3 days/3 hours per week, 6) enrollment for training in a fitness center regularly, 7) living in a city at the sea level for at least 10 years.

Sedentary individuals (Control group: CG): By means of local advertisements seventeen sedentary men (mean age±SD: 52.23±6.05 years) were invited for the study. Not performing any type of physical exercise even for 15 minutes twice per week for the previous 6 months was accepted as the criterion for being sedentary. The other criteria for participation in the study were being between 45 and 65 years old and living at the sea level for at least 10 years.

The ones having chronic diseases, being under regular medical treatment, having Vitamin D and CK supplements, having a BMI beyond the acceptable range (20-32 kg/m^2), having musculoskeletal diseases, and smokers were excluded. Subjects were given questionnaires to get information about their training history. Necessary information about the study design was given, and they signed an informed consent form. Nutritional habits of the participants were determined using "the nutritional habits section" of the "health and lifestyle profile" survey developed by Walker et al. No statistically significant differences were found in nutritional habits among groups.[28] The ethical council of Celal Bayar University, Faculty of Medicine approved the study.

TABLE 1: Physical and physiological parameters of participants.

	EMAG (n= 22)		RG (n= 21)		CG (n= 17)	
Parameters	**Mean ± SD**	**Min-max**	**Mean ± SD**	**Min-max**	**Mean ± SD**	**Min-max**
Age (years)	57.28±6.38	47-65	62.38±2.12	52-65	52.23±6.0 5	45-64
Body weight (kg)	70.58±7.45	61-87.30	78.21±9.6	64.1-93.2	80.04±5.8 3	68.8-91.5
BMI (kg/m^2)	24.30±1.14	21.90- 26.20	27.2±2.78	22.1-32.2	26.33±1.1 8	24.4-27.7
Body fat (%)	9.27±3.82	4.60-18	19.55±6.01	10.8-31.3	19.64±2.6 5	12.3-22.3
MaxVO$_2$ (ml/min/kg)	46.60±3.07	42.30-54	32.63±1.72	29.79-35.73	23.31±2.6 9	18.41-29.5
MCP-I (ng/ml)	568.84± 490.33	2.2- 2393.65	700.75± 588.08	203.93- 3086.39	454.57± 218.61	203.93- 1027.08
hsCRP (mg/L)	.98±.79	.20-2.96	.98±.89	.08-3.20	1.70±.99	.18-2.96
TNF-α (ng/ml)	2.22±0.11	2.2-2.72	3.4±5.38	2.2-26.88	2.2±.0	2.2-2.2
TGF-β-1(pg/ml)	87774.56± 80859.11	2.2- 296297.66	38882.16± 40798.08	2.2- 111718.64	37059.65 ± 46034.16	2.2-155654.96
Klotho (pg/ml)	820.08± 335.65	426.10- 1677.54	796.70± 357.13	249.22- 1584.67	569.94± 229.52	333.24- 1279.55
Vitamin D (ng/ml)	21.85±8.63	9-39	18.28±7.81	5-32	14.52±8.2 1	.86-36
CK (ng/ml)	124.09±73.0 9	53-335	71.52±27.42	28-134	102.70±4 8	50-223

Group comparisons were made using Kruskal-Wallis test.

EMAG: High intensity and long duration exercise training group; RG: Moderate intensity and duration exercise training group; CG: Control group; BMI: Body mass index; MCP-1: Monocyte Chemoattractant Protein-1; hsCRP: High-Sensitivity C-Reactive Protein; TNF-α: Tumor Necrosis Factor-Alfa; TGF-β-1: Transforming Growth Factor-Beta-1.

Study design: The evaluations of the participants were conducted within the same month to avoid the seasonal variation. On the first day, their body compositions were measured using bioelectric impedance method, using a Tanita body composition analyzer (Tanita TBF 300 M, Tokyo-Japan). On the second day, their aerobic capacity ($MaxVO_2$) was measured indirectly using 2-km walking test. Using the following formula for men, $MaxVO_2$ of the participants was estimated:[29]

184.9- 4.65 x duration (min-sec)-0.22 x HR-0.26 x age (years) -1.05 x BMI (kg/m2)

Participants were warned not to perform any physical activity within 48 hours preceding the assessment day.

Blood analyses: Blood samples were taken from the antecubital vein (20 ml) in the sitting position after a 20-minute rest. MCP-1, hsCRP, TNF alpha, TGF-β-1, and Klotho hormone, CK and vitamin D were studied in the blood samples taken between 8.00-10.00 a.m. Serum and plasma were separated by centrifugation and stored at -80°C; they were analyzed all at once. hsCRP levels were studied from plasma samples using DRG, an international kit (New Jersey, USA) and enzyme-linked immunosorbent assay (ELISA) method. The assay range of hsCRP kit is 0.1mg/L-10mg/L and its sensitivity is 0.1 mg/L. Intra-Assay: CV <7.5% Inter Assay: CV <4.1%. The results were calculated by comparing with the standard curve. In accordance with the manufacturer's recommendation, samples were diluted 20 times and then multiplied by the dilution factor of 20.

MCP-1 levels were studied from plasma samples with eBioscience, BenderMed (Vienna, Austria) kit and ELISA method. The assay range of MCP-1 kit was 2 ng-/ mL →- 1000 ng/mL and sensitivity was reported as 2.3 pg/mL. Intra-Assay: CV 4.7%; Inter-Assay: CV 8.5%. The results were calculated by comparing with the standard curve. In accordance with the manufacturer's recommendation, the samples were diluted 5 times and then multiplied by the dilution factor of 5 to obtain the results. TNF-α levels were measured using eBioscience, BenderMed (Vienna, Austria) brand kit and plasma samples were analyzed using ELISA method. According to the information given by the manufacturer, the lowest level of TNF-α kit has been reported to be 2.3 pg/ml. Intra-Assay CV: 6.0%; Inter-Assay CV: 7.4%. The results were calculated by comparing with the standard curve. In accordance with the manufacturer's recommendation, the samples were diluted 2 times and then multiplied by the dilution factor 2 to obtain the results.

TGF-β-1 levels were studied from plasma samples using eBioscience, BenderMed (Vienna, Austria) brand kit and ELISA method. The minimum sensitivity of TGF-β-1 is 8.6 pg/ml has been reported. Intra-Assay CV was 3.2%; Inter-Assay CV was reported as 4.9%. The results were calculated by comparing with the standard curve. In order to separate the disulfide bonds, samples were first diluted with 1 Normal HCl for 1 hour, then neutralized with 1 Normal NaOH, 30 times diluted, and then multiplied by dilution factor of 30 to obtain the results.

Human soluble alpha klotho levels were studied from plasma samples with IBL (Gunma, Japan) brand kit and ELISA method. The range of measurement of human soluble alpha klotho kit is 93.75-6.000 pg/ml, and sensitivity of 6.15 pg/ml has been reported. Intra- Assay CV is 3.5%; Inter-Assay CV is 6.5%. The results were calculated by comparing with the standard curve. In accordance with the manufacturer's recommendation, the samples were diluted 2 times and then multiplied by the dilution factor 2 to obtain the results.

Measurements of creatine kinase levels in plasma samples are based on the recommendations of the International Federation of Clinical Chemistry (IFCC). They were analyzed with The Beckman Coulter original commercial kits (Beckman Coulter Chemistry Analyzer AU 5800, Tokyo, Japan) in an autoanalyzer.

Vitamin D levels in the plasma samples were analyzed by the electrochemiluminescence binding method using the ROCHE original commercial kit (Roche Cobas e411, Sandhofer Strasse, Mannheim).

STATISTICAL ANALYSIS

For data analysis SPSS 15.00 statistical package program was used. Results were presented as mean+standard deviation (SD). We compared the groups using a Kruskal-Wallis test. When a significant difference was obtained among the groups, Mann-Whitney U test was used to determine the difference between the two groups. $p<0.05$ was accepted as the statistical significance level.

RESULTS

Body weight, percent body fat (%) and BMI results of the groups revealed statistically significant differences, favoring EMAG. The highest $MaxVO_2$ values among the three groups were observed in EMAG ($p<0.01$; Table 2). Table 3 presents the comparisons of biochemical parameters among groups. There was not any significant difference in serum levels of MCP-1, hsCRP, TNF-α and CK among groups. EMAG and RG were significantly higher than CG in their TGF-β-1 and Klotho hormone levels ($p<0.01$ $p<0.05$, respectively). Vitamin D levels of EMAG (21.85±8.63 ng/ml) were statistically higher than those of CG (14.52±8.21 ng/ml) ($p<0.05$).

DISCUSSION

In our study, we tried to evaluate the associations between long-term training with different intensity and duration being performed for more than 10 years and some inflammation markers. Higher levels of TGF-β-1 and Klotho hormone were obtained in EMAG than the other two groups.

Exercise not only enhances muscle function, but also reduces pro-inflammatory cytokines through circulation. MCP-1 is required for reperfusion in normal skeletal muscle regeneration after

TABLE 2: Physical and physiological parameters of participants.

Parameters	EMAG (n=22)	RG (n=21)	CG (n=17)	p	EMAG / RG	EMAG / CG	RG / CG
Age (years)	57.28±6.38	62.38±2.12	52.23±6.05	.548	.269	.016	.587
Body weight (kg)	70.58±7.45	78.21±9.6	80.04±5.83	.001**	.010*	.000**	.419
Body fat (%)	9.27±3.82	19.55±6.01	19.64±2.65	.000**	.000**	.000**	.713
BMI (kg/m^2)	24.30±1.14	27.2±2.78	26.33±1.18	.000**	.000**	.000**	.258
$MaxVO_2$ (ml/min/kg)	46.60±3.07	32.63±1.72	23.31±2.69	.000**	.000**	.000**	.000**

*p<0.05; **p<0.01

Group comparisons were made using Kruskal-Wallis test.

Mann-Whitney U test was used to determine the differences between the two groups (two- sided).

EMAG: High intensity and long duration exercise training group; RG: Moderate intensity and duration exercise training group; CG: Control group; BMI: Body mass index.

TABLE 3: Comparison of biochemical parameters among groups.

Parameters	EMAG (n=22)	RG (n=21)	CG (n=17)	p	EMAG / RG	EMAG / CG	RG / CG
MCP-I (ng/ml)	568.84±490.3 3	700.75±588.08	454.57± 218.61	.180	.285	.502	.060
hsCRP (mg/L)	.98±.79	.98±.89	1.70±.99	.412	.535	.368	.197
TNF-α (ng/ml)	2.22±0.11	3.4±5.38	2.2±.0	.100	.056	.083	.780
TGF-β-1 (pg/ml)	87774.56±80859.11	38882.16±40798.08	37059.65± 46034.16	.009**	.850	.002**	.023*
Klotho (pg/ml)	820.08±335.6 5	796.70± 357.13	569.94± 229.52	.032*	.166	.002**	.023*
Vitamin D (ng/ml)	21.85±8.63	18.28±7.81	14.52± 8.21	.009**	.126	.010*	.060
CK (ng/ml)	124.09±73.09	71.52±27.42	102.70± 48	.166	.195	.692	.060

*p<0.05; **p<0.01

Group comparisons were made using Kruskal-Wallis test.

Mann-Whitney U test was used to determine the differences between the two groups (two- sided).

EMAG: High intensity and long duration exercise training group; RG: Moderate intensity and duration exercise training group; CG: Control group;

MCP-1: Monocyte Chemoattractant Protein-1; hsCRP: High-Sensitivity C-Reactive Protein; TNF-α: Tumor Necrosis Factor-Alfa; TGF-β-1: Transforming Growth Factor-Beta-1.

ischemic tissue disease.[13] Glintborg et al. found a reduction in MCP-1 levels in the last three months of a strength training program conducted with elderly male subjects.[16] They stated that longer physical activity has more positive effects on inflammatory parameters. Plasma MCP-1 levels were also investigated in endurance training, high-intensity interval training and sedentary elderly subjects (65-95 yr). Researchers determined a decrease in MCP-1 levels after 30 minutes of exercise in the endurance training group.[17] Some studies have evaluated changes in the release of cytokines in skeletal muscle after long-term exercise intervention. In a study conducted with 8 young (28 yr) and 8 elderly male participants (70 yr) performing isokinetic exercises for 12 weeks, MCP-1 was found to be significantly increased after exercise period. However, post-exercise MCP-1 levels of the elderly were higher than the young participants. The researchers suggested that regular exercise can be an important factor in normalizing this inflammatory response in the elderly through muscle regeneration and adaptation.[30] In another study, a group of regularly exercising elderly (65-72 yr.) were compared to younger adults (23-27 yr) as controls; plasma MCP-1 and hsCRP levels were found to be lower in younger individuals. This is explained by the fact that the exercise intensity performed by the elderly is lower and the physiological functions of the elderly are weaker than the young although they exercise regularly.[31]

We did not find a statistically significant difference among our groups in MCP-1 levels. Even so, the lower MCP-1 levels in the master athletes than those of recreative athletes, (568.84± 490.33ng/ml vs. 700.75±588.08ng/ml), which shows similarities with some experiments , may be clinically important for further research in terms of exercise intensity and volume (Table 3).[16,17]

However, our results are contradictory to the results of Della Gatta et al. since they applied isokinetic exercises in their study.[30] Due to the reduced levels of MCP-1 observed in our EMAG, we can suggest that aerobic endurance exercise may have more positive effects on the inflammatory parameters as with Glintborg et al.[16] Since MCP-1 parameter is found to be lower in the Elite master athletes, this could have led to normalizing the inflammatory response in the elderly through muscle regeneration and adaptation.

Plasma CK and hsCRP levels were investigated in long-distance endurance and ultra long-distance endurance male runners. The results revealed that plasma CK and hsCRP levels were higher in ultra-marathon runners than in marathon runners.[12] Muscle and cartilage biomarkers and cytokine release were evaluated during a 200-km race and the biomarkers associated with muscle and cartilage damage and inflammation were found to be increased during the 200-km race, particularly in the second half of the race, proving that ultra-marathon exercises are an important influence on muscle and cartilage structures.[21] However, in a 24-week moderate-intensity aerobic exercise program led to a decrease in post-training plasma hsCRP levels in men aged 65-95.[11] Our exercising groups had insignificantly lower hsCRP levels than our CG. These lower levels may be clinically important because high levels of hsCRP are generally related with high-intensity exercises like ultra-marathon run. Relatively higher levels of hsCRP of the CG led us to think some other underlying mechanisms for inflammation. Recommendations of the Joint committee of American Heart Association on CRP testing for coronary heart disease, state the decision interval <1 mg/L as low risk; 1-3 mg/L intermediate risk and >3 mg/L as high risk. They also do not recommend universal hsCRP screening of the adult population. Our results in all groups are in the normal reference ranges and much lower than even the low risk threshold. We are of the opinion that leading a sedentary life style may be equally harmful for the human body in terms of inflammation. However, this view needs support with additional experimental and longitudinal data. In brief, the exercise programs of our exercising groups seem appropriate for preventing muscle and cartilage injuries.

TNF-α is a cytokine released from various acute and chronic inflammatory adipocytes and has

been found to be increased in the presence of metabolic syndrome, diabetes, and cancer. Therefore, its response to exercise has been investigated in some intervention studies. Research investigating the effect of exercise on TNF-α has revealed contradictory results. In a study performed by Ho et al. three different exercise models -12 weeks of regular intensive aerobic exercise, resistance exercise and combined exercise-were applied.[19] TNF-α levels significantly decreased in the highest proportion in the combined exercise group compared to the baseline values. As the aforementioned study results suggest, both intensive aerobic and resistance exercises are required for reductions in TNF-α levels. In our belief, since our exercising groups did not include resistance exercises in their training programs, there were no differences between them in their TNF-α levels. Studies examining the acute effects of exercise on TNF-α revealed contradictory results. Though insignificantly, TNF-α levels increased up to day 7 after the ultra-marathon run of 52-64-year-old men.[20] In another study, Kim et al. evaluated the muscle and cartilage biomarkers and cytokine release during a 200 km race and they found no significant changes in TNF-α levels.[21]

TGF-β-1 is an effective cytokine in many tissue and cell activities, promoting early differentiation, angiogenesis, immunological functions and apoptosis. TGF-β-1 may be associated with tumor suppression due to the suppressive effect of apoptosis. The induction capacity of apoptosis has begun to be investigated in many cell types. Studies at the cell level show that the role of TGF beta apoptosis is important in stabilizing B and T cells. Studies have also shown that TGF-β-1 is effective in the death of prostate epithelial cells and in the control of liver cell death. It is also an important cytochrome in the ontogenetic nerve death stages, tissue regeneration and shape alteration.[22] The role of exercise on TGF-β-1 was examined by Czarkowska-Paczek et al. They compared exercising and sedentary rats. Exercising rats participated in a 6-week study in which they exercised 5 days/week, starting from 10 minutes with ten-minute increments reaching 60 min/week. They reported that exercising rats showed a significant increase in their TGF-β-1 levels right after the exercise and 2 hours following exercise. However, the most significant increase was seen 3 hours after the exercise.[23]

In another study, blood samples were taken on the 4th, 8th and 16th weeks from the healthy mice performing intensive exercise. Although TGF-β-1 levels significantly increased in all three analyses, the highest increase was observed on the 16th week.[32]

We observed parallel results in our exercising groups, which enabled us to suggest that regular exercise either in high or moderate intensity can lead to positive outcomes in many tissue and cell activities, in the support of early development, differentiation, and angiogenesis. Even so, the higher TGF-β-1 levels observed in our EMAG compared to RG may suggest that aerobic exercise should be performed at a slightly higher intensity than moderate intensity.

The importance of Vitamin D increases as people get older since it has an important role on bone health and muscle strength. The role of exercise on Vitamin D is the concern of recent studies. Some researchers are on the belief that Vitamin D supplementation is required for healthier bones. After a 9-month intervention with and without Vitamin D supplementation, the group of the elderly performing resistance training and taking supplements showed higher muscle strength and higher levels of Vitamin D than the other group.[33] We observed higher Vitamin D levels in our EMAG than the other exercising group although they did not use any Vitamin D supplements. The open-field exercise program followed by our EMAG might have enabled them to utilize the sun rays for Vitamin D synthesis. In addition, long distance run hypothetically puts more strain on the bones, which in turn may help bones to become stronger. However, we did not perform any tests for bone strength such as DEXA for our groups. Therefore, we do not have any data to make any further comment.

Klotho hormone has been a concern in research in recent years, but the number of studies examining the effects of exercise on this hormone is very limited. Increases in plasma Klotho concen-

trations by means of aerobic exercise could result in exercise-induced decreases in arterial stiffness.[34] It is suggested that this leads to enhancing vascular protection and ameliorating endothelin-induced arterial stiffness. Secreted Klotho protects endothelial cells and smooth muscle cells through NO production.[35]

Men and women between 24-102 years old were investigated to find out a relationship between cardiovascular diseases and plasma klotho levels.[36] Researchers determined correlations between Plasma klotho levels, hsCRP levels, and age. Richard et al. compared plasma klotho levels with hand grip strength in 804 elderly individuals at the age of 65 and over.[27] They found that the lower level of klotho was significantly associated with weak skeletal muscle strength in the elderly. Klotho hormone levels were higher in younger footballers than their sedentary counterparts.[37] We found a statistically significant difference among our groups, but could find no significant observed in EMAG difference between EMAG and RG. Even so, the higher Klotho hormone levels (820.08± 335.65 pg/ml vs. 796.70±357.13 pg/ml) may be clinically important in terms of the exercise intensity and volume to gain better Klotho hormone levels for further research (Table 3).

In a recent study by Lucas Jurado-Fasolia et al., alcohol consumption and sKlotho plasma levels in middle aged sedentary adults were found to have negative association. Furthermore they found higher consumption of total alcoholic drinks is associated with lower S-Klotho plasma levels controlling for BMI (P=0.011), Lean Mass Index (P=0.010) and for Fat Mass Index β=-P=0.049) using multiple linear regression models.[38] This is in accordance with our findings in our groups. We found statistical differences among EMAG and RG, EMAG and CG for body fat (%), BMI, $MaxVO_2$ (P= .000) and a statistically difference in EMAG and CG for body weight (P= .000). Klotho hormone is associated with the regulation process of the body composition assessed with measurements such as BMI, body fat, fat mass index etc. In a study examining the associations of plasma klotho levels with BMI in patients with restricting-type anorexia nervosa (r-AN) and obesity was examined. Plasma klotho levels were markedly lower in the obesity group than in the control group. Moreover, plasma klotho levels increased significantly after the recovery of BMI in r-AN patients.[39] In another study it was found that concentrations of serum Klotho were associated with body weight.[40] The final report from the clinical trial of Amaro-Gahete et al. will be very interesting and could uncover the relationship mechanism of klotho with the metabolism.[41]

CONCLUSION

The comparison of two different groups of 45-65 year-old males in terms of inflammation markers revealed that regular long-term endurance training has long-term positive effects on muscle regeneration and regeneration of inflammation. However, performing an exercise program similar to the one followed by EMAG in our study seems more favorable since such a program appeared to be more effective in delaying the initiation period of cardiovascular, immune system, joint and skin diseases, high cholesterol, and Type II diabetes due to aging. Our present study provides the field with clear evidence on the positive effects of regular long-term endurance training for a longer and healthier life.

Informed Consent

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical Council of Manisa Celal Bayar University approved the study (date: 03.08.2016; number: 20.478.486.283).

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Fatma Girginer, Gürbüz Büyükyaz;* ***Design:*** *Fatma Girginer, Gürbüz Büyükyazı;* ***Control/Supervision:*** *Fatma Girginer, Gürbüz Büyükyazı;* ***Data Collection and/or Processing:*** *Fatma Girginer, Yeliz Doğru, Murat Taş, Cevval Ulman, Fatma Taneli, Raziye Yıldız;* ***Analysis and/or Interpretation:*** *Fatma Girginer, Gürbüz Büyükyazı, Cevval Ulman, Fatma Taneli, Raziye Yıldız;* ***Literature Review:*** *Fatma Girginer, Yeliz Doğru, Murat Taş;* ***Writing the Article:*** *Fatma Girginer, Gürbüz Büyükyazı, Cevval Ulman;* ***Critical Review:*** *Cevval Ulman, Fatma Taneli;* ***References and Fundings:*** *Fatma Girginer, Gürbüz Büyükyazı, Cevval Ulman, Fatma Taneli;* ***Materials:*** *Cevval Ulman, Fatma Taneli, Raziye Yıldız, Gürbüz Büyükyazı;* ***Diğer:*** *Pembe Keskinoğlu.*

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