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Acute effects of different types of exercises on insulin-like growth factor-1, homocysteine and cortisol levels in veteran athletes

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Abstract

This study aimed to investigate acute effects of table tennis (physical+cognitive exercise), aerobic running (physical exercise), and chess (cognitive exercise) exercise sessions of veteran male athletes in their branches on the serum homocysteine (Hcy), insulin growth factor-1 (IGF-1), and cortisol (Cor) levels. Thirty veteran athletes [10 table tennis players (TT), 10 long-distance runners (LR), 10 chess players (CP)] and 10 sedentary controls (SC) between 50 and 65 years of age participated in the study. Blood samples were obtained before and immediately after exercise to determine serum Hcy, IGF-1, and Cor levels. According to their branch, each veteran athlete performed exercise sessions (70-75% of the participants' heart rate reserve) of 10-min of warm-up followed by 40-min of table tennis, aerobic running, or chess. TT and LR groups demonstrated significant increases in the serum IGF-1, Cor, and Hcy levels from pre to post-exercise ($p<0.05$). In contrast, the CP group showed significant increases only in the serum Hcy levels ($p<0.05$). Serum IGF-1 and Hcy, in response to exercise, were not significantly different between exercise groups ($p>0.05$). LR group had a greater serum Cor increase than all exercise groups ($p<0.05$). The TT group showed significantly greater changes in serum Cor levels than the CP group ($p<0.05$). In conclusion, although a single bout of aerobic running and table tennis exercise induces a remarkable increase in all measured biomarkers, chess exercise only elicits an increase in Hcy levels. Although aerobic running is more effective in increasing Cor levels than other types of exercise, the current study's findings suggest that serum Hcy and IGF-1 levels in veteran male athletes are not affected by the type of exercise.

Keywords: Aging, cortisol, exercise, homocysteine, IGF-1

Introduction

Cognitive aging involves various functional and structural changes in the whole brain or specific regions [1]. Invasive or pharmacologically effective treatment for cognitive aging remains elusive. In this regard, it is of great scientific interest to identify methods that can be used to slow down, control, or ameliorate structural and functional declines in the aging process [2].

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Veteran athletes', middle-aged and older individuals who do active, regular, and high-level exercise in a sports branch, whether they have a competition background or not; are generally accepted that have physical, social, and psychological well-being [3].

Many examples of successful aging, such as in veteran athletes, have led us to deal with cognitive decline as we age and to seek a lifestyle that prevents neurodegenerative diseases. These individuals can show us that regular exercise can make healthy cognitive aging possible.

Regular physical exercise (PE) is a promising method to increase brain health across the lifespan [4]. Research that examines the benefits of exercise on the human body has generally focused on the cardiovascular and musculoskeletal systems [5], but lately, there has been much focus on the underlying mechanisms of PE and healthy brain relation [6].

Exercise is the key regulator of multifaceted molecules with many specific neuroprotective mechanisms [7,8]. Leading studies have shown that different circulation factors with potential neuroprotective functions are released into the bloodstream via PE throughout the life span [9]. Despite the precise relationship between PE and brain health in humans, our knowledge of the cellular and molecular mechanisms that trigger such benefits is still limited.

Insulin-like growth factor 1 (IGF-1) has benefits synapse density and neurotransmission by modulating synaptic morphology and function [10]. IGF-1 levels can increase via exercise in the periphery and can cross the blood-brain barrier to mediate the trigger of neurogenesis in the brain [11]. Ploughman et al. (2019) indicated that after the aerobic (treadmill running) and cognitive exercises (working memory task or computer games), participants who provided better cognition status showed higher levels of serum IGF-1 [12]. Cho and Roh (2019) showed that taekwondo training (physical exercise which requires cognitive demand, 50-80% maximum heart rate for 60 min, 5d/w for 16 weeks) training enhances IGF-1 levels in women aged 65 or older [13]. Contrary to this, Mass et al. (2016) suggest that the relationship of IGF-1 with changes in the hippocampus is not exercise-induced [14]. A review study by Borba et al. (2020) indicated that IGF-1 serum levels might be affected by exercise type [15]. But researchers pointed out that there is no consensus on the conditions of the exercise, which is poorly defined.

Unlike IGF-1, elevated homocysteine (Hcy) levels are related to cardiovascular diseases, Alzheimer's disease, dementia, and cerebrovascular diseases. Thus, according to Sachdev (2005), Hcy called a "neurotoxin" can impair brain health [16]. Ford et al. (2012) pointed out that high Hcy levels are putatively one of the cognitive deterioration indicators, especially in older adults [17]. Both animal [18] and human [19] studies demonstrated that exercise could change Hcy levels in the blood. In another study, Joubert and Manore (2006) indicated a lack of clear evidence that whether physical fitness affects Hcy levels [21]. Lately, the review study of Silva et al. (2014) revealed that acute exercise could cause induced levels of Hcy [20].

Cortisol (Cor) is a corticosteroid and can be released in response to the stress induced by an acute bout of exercise despite the mechanisms still being poorly understood. Kraemer et al. (1992) stated that acute cortisol responses were not related to training status [22]. A study by Tremblay et al. (2004) revealed that endurance athletes had lower cortisol levels after the same resistance exercise protocol compared to well-resistance trained men [23]. According to Martínez-Díaz et al. (2020), high-intensity and long-duration exercise can modulate cortisol levels [24]. In addition, there is an inverted U-shaped relationship between cortisol and executive functions, and optimum cortisol levels may be beneficial for cognition. Moreover, although counter-evident exists [25], older adults generally have higher cortisol levels, and this accumulation can have deleterious effects on memory function [26].

The controversial results of these molecular mechanisms may be due to the wrong generalized concept of exercise. Generalizations made by ignoring exercise variables make it difficult to understand the results. Because exercise is directly or indirectly related to potential cognitive decline mechanisms depending on duration, frequency, type, and intensity [6-8]. Understanding how exercise types trigger different neurobiological mechanisms for brain adaptation can be crucial for creating an efficient exercise program for brain health in older adults.

To our knowledge, no study has examined the acute effect of PE (aerobic running), CE (chess), and a combination of them (table tennis) on homocysteine, cortisol, and IGF-1 levels in veteran athletes. This study aimed to investigate the acute effects of table tennis (PE+CE), aerobic running (PE), and chess (CE) exercise sessions of veteran male athletes in their branches on the serum Hcy, insulin growth factor-1 IGF-1, and Cor levels. We hypothesized that there would be a difference between the effects of different exercise types on serum IGF-1, Hcy, and Cor levels.

Materials and Methods

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Bursa Uludağ University, Medical Faculty Clinical Research (protocol code: 2020-5/13). Informed consent was obtained from all subjects involved in the study.

Participants

Forty healthy male participants between 50 and 65 years of age were recruited for this study (Table 1). According to their branches, veteran athletes were recruited into three exercise groups (TT=table tennis athletes, LR=long-distance runners, CP=chess players). Veteran athletes were recruited from national veteran sports clubs. Veteran athletes must train regularly (at least three days a week) for five years. The sedentary control (SC) group who had not been involved in regular PE or CE (i.e., playing chess and card games, doing puzzles, playing console or mobile games) was specified through community announcements from veteran athletes' social circle. Study information (including all potential risks) was given to the participants at the information meeting.

Participants were required to meet the following criteria;
- normal or corrected hearing and eyesight,
- no plans for major lifestyle changes during the study (i.e., changes in exercise routine, diet, or hobbies),
- Mini-Mental State Exam (MMSE) scores ≥26
- Beck Depression Inventory (BDI) scores ≤9

The exclusion criteria were given below;
- smoking, drug, and alcohol habits,
- history of depression, neurological illness, brain damage, neuromotor or musculoskeletal disorders,
- Positive Covid-19 or contact with a positive person

Design and Procedure

The study procedure consisted of a pretest-posttest design (Figure 1). Each participant was recruited to the laboratory in two sessions at least 5 days apart between 9-12 a.m. to control circadian influences. Participants were asked to maintain their rutin diet throughout the study and to forgo strenuous exercise for at least 72 h before the exercise bout. Participants also were prohibited from food, caffeine, and alcohol intake for 8 h before the exercise.

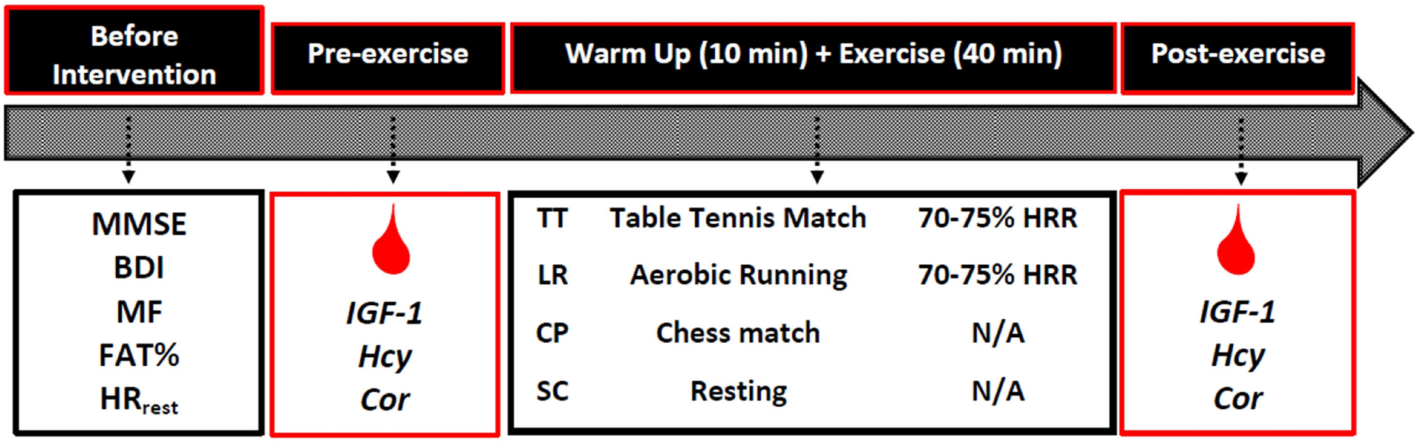

Figure 1. Experimental design. MMSE: Mini-mental state exam; BDI: Beck depression inventory; MF: Measurement of fitness; HRrest: Heart rate rest; HRR: Heart rate reserve; FAT%: percentage of fat mass; IGF-1: Insulin-like growth factor-1; Hcy: Homocysteine; N/A: Not available; TT: Table tennis athletes; LR: Long-distance runners; CP: Chess players; SC: Sedentary control

Table 1. Characteristics of participants. Data presented as means ± SD.

	TT (n=10)	LR (n=10)	CP (n=10)	SC (n=10)
Demographics				
Age (years)	56.0±4.4	56.7±5.7	57.3±5.7	57.5±8.4
Education (level)	3.8±1.1	3.8±0.6	3.9±0.9	3.3±0.9
Training history (years)	9.8±2.4	10.8±4.2	11.3±4.6	N/A
Weekly exercise (hours)	5.4±1.3	6.4±1.8	8.5±2.9	N/A
Physical Fitness				
Height (cm)	175.4±3.9	172,1±3.2	173.9±6.7	171.7±8.1
Weight (kg)*	84.4±7.6	72.8±5.3[a]	89.1±15.5[b]	82.5±14.2[b]
FAT (%)*	20.1±4.2	15.3±3.1	25.0±7.1b	24.5±5.1[b]
HR_{rest} (beat/min)*	63.8±7.1	52.8±8.1	78.3±7.2[a,b]	68.6±5.4[a,c]
VO_{2max}(mL/kg/min)*	43.6±6.0	57.7±3.2[a]	28.3±2.8[a,b]	28.3±4.9[a,b]
HR_{max} (beat/min)	165.9±7.2	167.1±9.4	156.7±5.0	159.4±6.1

(*p<0.05) indicates a significant difference a: Indicates a significant difference compared with TT b: Indicates a significant difference compared with LR c: Indicates a significant difference compared with CP VO2max: Maximum rate of oxygen consumption HRrest: Heart rate rest HRmax: Maximal heart rate Education level: 1= elementary school, 2= high school, 3= university, 4= postgraduate FAT %: Percentage of fat mass TT: Table tennis athletes LR: long-distance runners CP: Chess players SC: Sedentary control N/A: Not available

On the first visit, participants were familiarized with the experimental procedure and asked the participants to complete medical history and demographic questionnaire and perform an MMSE, DBI. Then, HRrest was measured in a sitting position with a Polar HR monitor (V800, Polar Electro OY, Kempele, Finland). Afterward, VO_{2max} was estimated by the graded maximal treadmill testing, during which their heart rate (HR) was continuously recorded using a Polar HR monitor, respectively.

On the second visit, the intervention started with 10 minutes of seated rest. After the rest, blood samples were obtained to determine serum IGF-1, Hcy, and Cor levels. Afterward, the participants performed a 10-minute warm-up and 40-minute exercise according to their branches (table tennis, aerobic running, and chess) under the supervision of a healthcare worker and accompanied by a specialist trainer. Immediately after the exercises, blood samples were obtained again.

Exercise protocol

In this study, table tennis was considered a PE+CE, aerobic running exercise was a PE, and chess was a CE. Participants performed approximately 50 minutes (10 min warm-up+40 min exercise) at a heart rate corresponding to 70–75% of participants' heart rate reserve (HRR) (Figure 1). The exercise intensity was based on HRR, which was calculated as 'HRmax' minus 'HRrest' [27]. Then, the target HR was calculated as follows:

"Target HR={HRR × 70%-75%}+ HRrest "

The exercise sessions were initiated with the subjects in the table tennis group. To not disrupt the structure of the table tennis game, which simultaneously requires a response to physical and cognitive demands, no restrictions were made to the participants in this group during the exercise. All subjects finished the table tennis exercise at between 70–75% of individual HRR. Then the participants in the running group were provided to finish their exercises at the same heart rate zone. HR variability was measured using a chest-strap HR polar monitor. Each subject in the CH group performed a standard chess match with the human opponent (equal national chess rating) and received 30 s for each movement. The subjects in the SC were only seated and rested at the laboratory without a phone, book, etc.

Measurement of fitness

Participants performed to exhaustion using Bruce protocol after a 5-min warm-up period to determine VO_{2max} . The submaximal Bruce protocol was used for all participants in sedentary control and chess groups. In the test, Tanaka et al. (2001)'s formula was used for estimating HRmax [28].

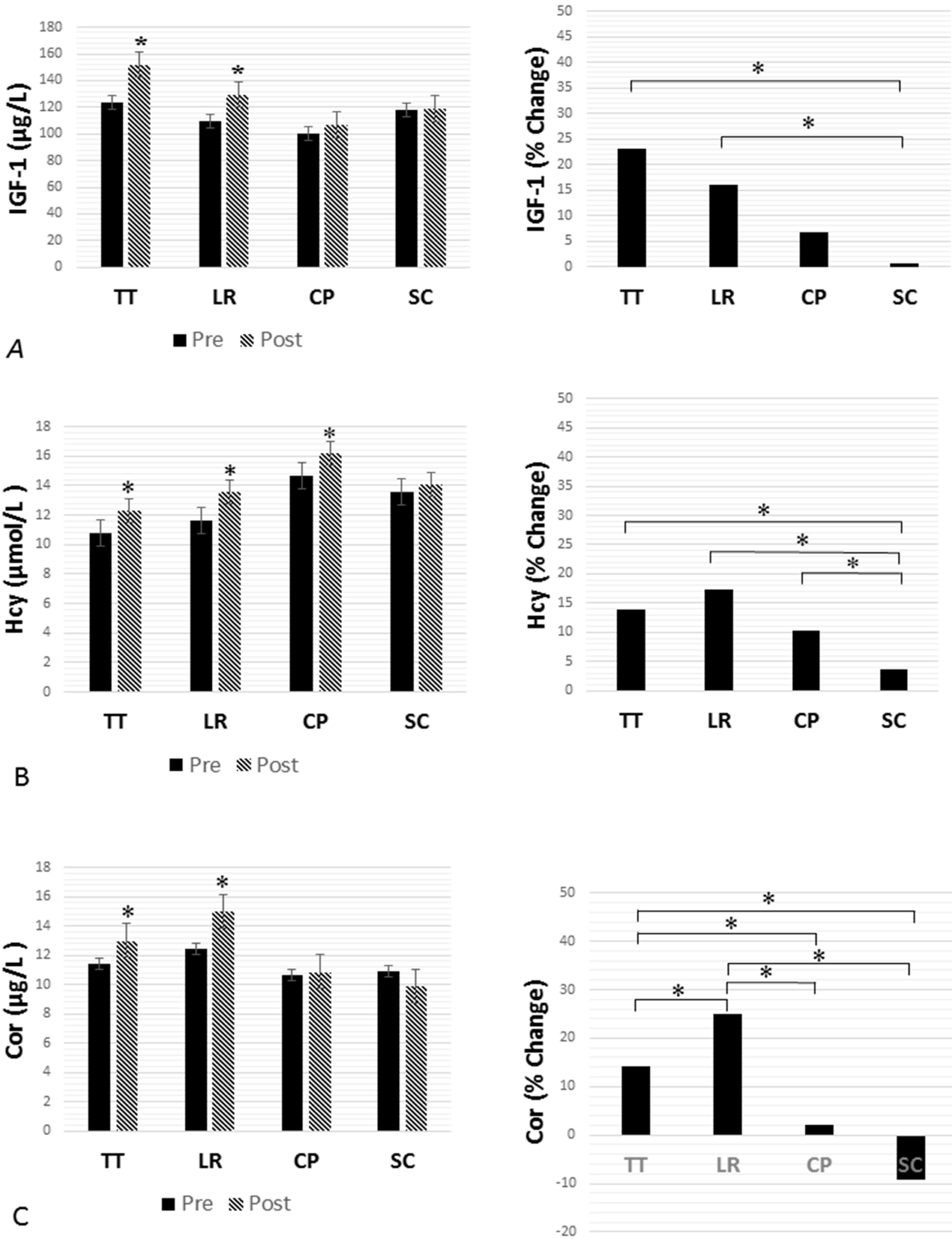

Figure 2. Changes in peripheral factors from before to after exercise. *Denotes significant difference from corresponding after exercise ($p<0.05$). IGF-1: Insulin-like growth factor-1 Hcy: Homocysteine Cor: Cortisol µmol/L: micromol/liter µg/L: microgram/liter TT: Table tennis athletes LR: Long-distance runners CP: Chess players SC: Sedentary control

"HRmax={208-(0.7×90%)}"

The time values obtained from the graded maximal treadmill test were written as a "T" value in fractional minutes. The formula for calculating VO_{2max} (ml-1. kg-1. min-1) was as follows:

"VO_{2max}=14.76-(1.379×T)+(0.451×T2)-(0.012×T3)"

Blood sampling and analysis

Blood samples (10 ml) were obtained from the antecubital vein before and immediately after exercise. The blood samples were assigned to clot at room temperature for 30-min and centrifuged at 3000×g for 15-min. The serum layer was removed and frozen at −80°C for further analysis.

Serum IGF-1 was analyzed by a Siemens immulite 2000 xpi immunoassay kit (manufacturing, GERMANY) using the enzyme-linked chemiluminescent immunometric method. Serum Hcy and Cor were measured by an Abbott Architect Plus i2000 immunoassay analyzer (manufacturing, USA) using the chemiluminescent microparticle immunoassay method.

Statistical analysis

Statistical analysis was performed using the SPSS software version 20.0. Shapiro-Wilk's test was used to determine whether or not the variables are normally distributed. Values are presented as the mean±standard deviation (SD) with normal distribution and the median, interquartile range (IQR) for the non-normally distributed. The % changes obtained by subtracting the pre-test values from the post-test values were used in the comparisons between the groups. One-way ANOVA was used to compare the variables normally distributed. Levene test was used to assess the homogeneity of the variances. The pairwise posthoc test was performed using Tukey's and Dunn-Bonferroni's test when an overall significance was observed. Kruskal Wallis was used to comparing the variables non-normally distributed among the groups. A dependent sample t-test was used for normally distributed variables, and Wilcoxon signed-rank test was used for non-normally distributed variables for intragroup comparisons. An overall 5% type-I error level was used to infer statistical significance.

Results

Demographic and physical characteristics of participants

Demographic variables, including age and education level, did not differ between the groups. Training history and the weekly exercise were significantly different between the groups. TT, LR and CP had longer training years (p=0.008, p=0.002, p=0.000, respectively) and weekly exercise hours (p=0.018, p=0.001, p=0.000, respectively) than SC. However, there was no significant difference between athlete groups. As to physical characteristics, height and HRmax did not differ between groups. LR had a lower weight than TT and CP (p=0.038), (p=0.030), respectively. TT (p=0.027, 0.025) and LR (p=0.000) had higher VO_{2max} levels than SC and CP. LR and TT had lower %FAT than SC (p=0.001). LR and TT had lower HRrest levels than CP (p=0.000). Furthermore, LR had lower HRrest levels than SC (p=0.001).

Insulin-like growth factor-1

As can be seen in figure 2 A, the TT (pre=123.7µg/dL, post=152.2µg/dL), and LR (pre=109.8 µg/dL, post=129.8 µg/dL) groups demonstrated significant increases in the serum IGF-1 levels from pre to post-exercise (p=0.001, p=0.005, respectively). But there were no significant difference in the serum IGF-1 levels from pre (100.1 µg/dL) to post (106.7 µg/dL) exercise in CP group (p=0.149). In the inter-group %change comparison, there were no significant differences in the serum IGF-1 levels from pre to post-exercise in between all exercise groups (p>0.05) while TT and LR groups had a greater increase than the SC group (p<0.05).

Homocysteine

As can be seen in figure 2 B, the TT (pre=10.8µmol/L, post=12.3 µmol/L), LR (pre=11.6µmol/L, post=13.6 µmol/L), and CP (pre=14.7µmol/L, post=16.2µmol/L), groups demonstrated significant increases in the serum Hcy levels from pre to post-exercise (p=0.011, p=0.001, p=0.022, respectively). However, after resting, SC (pre=13.6µmol/L, post=14.1µmol/L), group showed no significant differences in serum Hcy levels (p=0.074). In inter-group %change comparison, there were no significant difference in the serum Hcy levels from pre to post-exercise in between all exercise groups (p>0.05) while TT, LR, and CP groups had a greater increase than the SC group (p<0.05).

Cortisol

As can be seen in figure 2 C, the TT (pre=11.4 µg/dL, post=13.1µg/dL), and LR (pre=12.5 µg/dL, post=14.9 µg/dL) groups demonstrated significant increases in the serum Cor levels from pre to post-exercise (p=0.005, p=0.001, respectively). However, SC (pre=10.9 µg/dL, post=9.9 µg/dL), and CP (pre=10.6 µg/dL, post= 10.8µg/dL) groups showed no significant differences in serum Cor levels (p=0.061, p=0.069, respectively). In inter-group %change comparison, LR group had a greater increase than all the groups (p<0.05). In addition, TT group had a greater increase than CP and SC groups (p<0.05).

Discussion

This study investigated the acute effects of table tennis, aerobic running, and chess exercises on veteran male athletes' serum IGF-1, Hcy, and Cor levels. We showed that immediately after a 40-min of aerobic running or table tennis exercise performed by veteran athletes in their branches at an HRR of 70-75% significantly increased serum IGF-1, Hcy, and Cor levels, but chess exercise increased Hcy levels only. The blood concentration of serum IGF-1 and Hcy, in response to exercise, was not significantly different between exercise groups. Aerobic running had a greater serum Cor increase than other exercise types, and table tennis induced greater changes in serum Cor levels than the chess exercise. However, the current study's findings are insufficient to infer which type of exercise is more effective in the secretion of brain health-related molecules in veteran athletes.

Insulin-like growth factor-1

In the literature review examining the effects of exercise on IGF-1, the lack of study evidence evaluating the acute effects of different exercises on the veteran athlete population makes it difficult to conclude the results of the current study. No study has been found that evaluates the effects of cognitive exercises or sports with open skills that combine cognitive and physical exercise (table tennis, badminton, football, etc.) on serum IGF-1 levels in the veteran athlete population. The literature has generally focused on evaluating the effect of resistance and aerobic exercises on the relationship of IGF-1 with muscle hypertrophy.

We found a significant increase in serum IGF-1 levels after a 40-min of aerobic running or table tennis exercise sessions at an HRR of 70-75% performed by veteran athletes in their branches. In line with our findings, Wallace et al. (1999) showed that acute continuous aerobic-type exercise (4d/w/30-min cycling, 80% of VO2max) increases serum IGF-1 in trained men (n=17, mean age= 26.9 yr), [29]. Stein et al. 2021 showed that after the acute running exercise (until the 80% of HRmax), higher levels of IGF-1 levels in Alzheimer's disease patients (n=34, age=75 yr) compared to participants without dementia (n=40, age=74 yr) [30].

Contrary to these results, Kliszczewicz et al. (2021) showed no acute response to serum IGF-1 levels in Parkinson's patients who performed high-intensity functional exercise [31]. Hasani-Ranjbar et al. (2012) investigated the effect of resistance exercise [4exercises, 3series ×1 One Repetition Maximum (1RM):70%–80%] on IGF-1 levels in 19 healthy trained men (age=20-30 yr, a regular resistance training program for 3d/w over the last 10 months) and 15 healthy untrained men. They observed no significant alterations in serum IGF-1 levels after exercise in trained and untrained participants [32].

Previous systematic reviews and meta-analyses suggest that IGF-1 serum levels can increase by exercise type [15]. Nevertheless, exercise variables and subjects' fitness levels can also change serum IGF-1 blood concentrations [32]. The observations from previous studies are controversial. IGF-1 synthesis and release mechanisms depend on age, body composition, physical fitness level, diet, other hormones, and plasma volume. Moreover, the release of IGF-1 blood concentration is dynamic and complex. These factors may explain contradictory exercise results on the IGF- 1.

Homocysteine

PE can alter Hcy levels in the blood, but the duration, intensity, and type of acute exercise on Hcy blood concentration are still unclear. Our study results showed a significant increase in serum Hcy levels after 40-min aerobic running, chess, or table tennis exercise sessions at an HRR of 70-75% performed by veteran athletes in their branches. Some studies examining the young population are in line with our results. Wright et al. (1998) demonstrated an increase in the plasma Hcy after running at 70% of VO2max for 30-min in physically active healthy men aged 24-39 [33]. Similarly, Gelecek et al. (2007) showed a significant increase in plasma Hcy level post-exercise (walking for 30-min at 70–80% of HRmax) in sedentary healthy subjects (mean age=21yr, n=22), [34].

Contrary to these results, Gaume et al. (2005) compared endurance exercise (cycling until exhaustion on a cycle ergometer) effect on Hcy levels in trained (age=52 years, had endurance training experience of more than 15 years and training average was 8.2 h/week) and sedentary (age=56 yr) middle-aged men. The results showed that baseline Hcy levels were lower in trained subjects and a decrease was observed in Hcy levels at the submaximal and maximal levels of exercise [35]. In another study, De Crée et al. (2000) demonstrated that Hcy levels were not affected by acute submaximal exercise (cycle ergometer at 60% of VO2max) in moderately trained young males (n=7, age=21yr) [36].

After table tennis and aerobic running exercise sessions, the accumulation of various exercise-related metabolites (e.g., lactate, creatinine, uric acid, urea, and cortisol) might have increased Hcy levels. An interesting finding of our study is the increased serum Hcy levels after chess exercise. According to Troubat et al. (2009), chess games are considered a mental stressor [37]. Chess is a strategy game that requires to respond high cognitive demand tasks. Players have to decide through various movement patterns to find the more suitable win strategy. This situation may be elicited mental stress in chess players. According to studies, mental stress could increase Hcy levels significantly [38]. We think that the relationship between mental stress and Hcy may cause an increase in Hcy levels in chess players.

Cortisol

Cortisol is generally considered a hormonal marker of training stress. However, studies are still insufficient to determine how cortisol levels are affected by which type of exercise, especially in older adults or middle-aged veteran athletes [39].

We showed that immediately after a 40-min of aerobic running or table tennis exercise performed by veteran athletes in their branches at an HRR of 70-75% significantly increased serum Cor levels. Moreover, aerobic running had a greater serum Cor increase than other exercise types, and table tennis induced greater changes in serum Cor levels than the chess exercise. Our results are in line with Heuser et al. (1991) who showed that middle-aged men endurance athletes had significantly greater cortisol responses [40]. A possible reason why table tennis players and long-distance runners achieve higher serum cortisol levels than sedentary individuals and chess players may be the ability of trained individuals to exhibit greater hormonal responses than untrained individuals.

In contrast, Heaney et al. (2013) investigated the acute effect of exercise on serum cortisol levels in older adults with different training statuses. 49 community-dwelling older adults [age= 60-77 yr, sedentary (n=14), moderately active (n=14), endurance-trained (n=21)] performed an incremental submaximal treadmill test. The authors found that cortisol significantly decreased post-exercise. They also pointed out that the basal level of Cor and the response to exercise of Cor levels did not influence training status [26].

The type of stress can activate the hypothalamic-pituitary-adrenal (HPA) axis differently because there are various pathways of HPA axis activation. Thus, a physical stressor can be directly linked with a paraventricular nucleus, and metabolic variables regulate the response more. In contrast, as induced by chess exercise, a psychological stressor will go through the limbic system. These results may explain the differences between physical exercise and the effect of cognitive exercise on serum cortisol levels.

We only measured IGF-1, Hcy, and Cor among the various biomarkers related to brain health. Our sample size is small, and studies with larger samples are needed to determine the generalizability of our results. Next, we tested cross-sectionally healthy and motivated samples of middle-aged adults who are well-trained veteran athletes; hence, whether the results generalize to the sedentary population as a whole needs to be investigated in future studies.

Conclusion

In conclusion, although a single bout of aerobic running and table tennis exercise induces a remarkable increase in all measured biomarkers, chess exercise only elicits an increase in Hcy levels. Although aerobic running is more effective in increasing Cor levels than other types of exercise, the current study's findings suggest that serum Hcy and IGF-1 levels in veteran male athletes are not affected by the type of exercise.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

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Ethical approval

This research was carried out with the approval of the Bursa Uludağ University, Medical Faculty Clinical Research Ethics Committee numbered 2020-5/13 and conducted under the Declaration of Helsinki. This study was derived from a doctoral dissertation.

This study was previously presented at the 19th International Sports Sciences Congress, Turkey.

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