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ARTICLE

Effects of different exercise training on antioxidant potential and mood profile in elite female athletes

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ABSTRACT: Several metabolic changes caused by regular exercise, can lead to adaptive mechanisms in athletes. These changes create a new dynamic balance that ensures better performance. On the other hand, because most studies on changes in the metabolic profile of the blood have been conducted in male athletes, this study investigated the effects of different exercise conditions on reactive oxygen species (ROS), adiponectin, and subjective mood in female athletes.

A cohort of 30 females, 20 professional players (10 target shooters and 10 basketball players), and 10 sedentary females as control group were enrolled in the study. Capillary blood samples and the Brunel Mood Scale questionnaire (BRUMS) were collected at noon before the training session. ROS and adiponectin were determined by the ELISA method. To compare among groups, one-way analysis of variance and Tukey's post hoc test were used at the significance level of $P<0.05$.

Physiological evaluations showed a significant high level of adiponectin in basketball players ($p=0.005$). The anger subscale in the basketball group had a significant increase compared to other groups ($p=0.04$). The tension subscale exhibited a higher level in the shooting group than in the control group. ($p=0.017$). Regarding the vigor subscale, there was no significant difference in experimental groups, and there was a significant difference in the non-athlete group (control) ($p=0.001$).

Basketball, being a team sport, exhibits a greater degree of aggression in contrast to shooting, which is classified as an individual sport. More focus of coaches on adjusting in another sport with non-competitive and non-confrontational features to reduce anger is suggested.

KEYWORDS: Mood, Reactive oxygen species (ROS), Adiponectin, Basketball, shooting.

1 Introduction

It has been shown that exercise can alter reactive oxygen species (ROS) levels and further affect a wide range of signaling processes and, thus brain function (Radak et al., 2016). One of the main sources of ROS production is nerve cells, the highest amount of ROS production is in complexes I and III in brain mitochondria. Complex III plays a stronger role in ROS production (Radak et al., 2016). We aimed to investigate the relationship between physiological assessments and subjective mood assessments by measuring ROS and using mood questionnaires in athletes who regularly practice basketball and shooting to

confirm that differences in these sports it is noteworthy. During physical exercises, the level of ROS production increases, but regular exercise improves the physiological function of skeletal and cardiac muscles and prevents the occurrence of a wide range of diseases, including cardiovascular diseases, certain types of diseases such as cancer, diabetes II, etc.

in the last decade, it has been proven that regular exercise affects in the prevention and treatment of stroke, Alzheimer's and Parkinson's diseases (Radak et al., 2010). If the athletes use the correct training program, the identification of changes in redox homeostasis has led to the clarification of the mechanism of adaptation to acute and chronic exercise in well-trained athletes, which can be considered as a possible indicator of exercise stress. In addition, the effect of redox signals can be used to understand the mechanisms of improving athletes' performance and preventing overtraining. In overtraining, we see an increase in pro-inflammatory markers, followed by a decrease in performance (Cardoos, 2015) Therefore, the beneficial or harmfulness of exercise can be seen as the result of the balance between oxidative stress and inflammation ((Varamenti et al., 2020), (Luti et al., 2020)). Chronic exercise-induced stress causes changes in several metabolic hormones that affect inflammation, among them, adiponectin, which is an anti-inflammatory hormone and it secreted in greater amounts from adipose tissue (Fang & Judd, 2018). As well as skeletal muscles (Liu & Sweeney, 2014). In well-trained athletes, an increase in adiponectin levels was observed, which reveals the effect of training on adiponectin response ((Guy Hornsby et al., 2020), (Horowitz & Klein, 2000)).

Therefore, it seems interesting to investigate the level of adiponectin in athletes who do regular exercise. Bump's training pyramid (2009) states that mental health is a key factor that preparation athletes in sports (Edition, 2019) Periodization: theory and methodology of training. It is a fact that mood is a leading indicator for assessing a person's performance which broadly influences performance during exercise, training, or competition (Berger & Motl, 2000).

Mood can affect people's attitude, performance, and sense of self-efficacy (Kavanagh, D. J., & Hausfeld, 1986). Engaging in physical exercise stimulates the release of endorphins, thereby contributing to the increase of individuals' overall mood (Kim & Kim, 2007). The feeling of excitement, alertness, and physical energy shows the positive mood of an athlete (Anshel et al., 1990).Anxiety, depression, anger, fatigue, and confusion have also been introduced as negative moods (Terry et al., 1999).

Anger is an individual reaction that people show to a real or perceived threat to a person or group of people (Lazarus, 1991).

Fatigue is an emotional feeling that refers to both mental and physical fatigue but tension includes stress, apprehension, worry, and anxiety (Anshel et al., 1990).

About depression which is one of the negative feelings, Mousavi and Salamander (2003) described depression as a negative self-schema characterized by ideas such as helplessness, personal inadequacy, worthlessness, and self-blame(Mousavi, M.K., Samandar, 2003).

Confusion is described as the uncertainty of doing work, the feeling of being confused, and the general inability to control individual attention and emotions (Mousavi, M.K., Samandar, 2003) .

2 Methods

2.1 Participants

A group of 30 female participants, 10 professional shooters (SH), and 10 professional basketball players (B), who had national experience in the summer season period (May–June 2023), and 10 women who did not perform regular heavy sports as healthy controls (C) were included in this study. All participants were adults who were volunteers and selected based on their age between 20 and 30 years and they were all Iranians, also they completed a questionnaire that included medical history, professional sports and using food, and history of recent illness or injury to determine eligibility. None of them used antioxidant or nutritional supplements and were non-smokers. The athletes did technical 5 times a week according to specific training programs, related to their sport with sessions lasting 2 h per day. Training program for basketball athletes: aerobic and interval training, which includes running from 3 km to 12 km daily, as well as bodybuilding exercises including working with weights, barbell chest press and one-arm chest press with dumbbells, and rehabilitation for basketball next Speed and simulation of different game situations. Training program for shooting athletes: general bodybuilding exercises including running, Swedish swimming, jumping rope, strengthening shoulder muscles, and strengthening back and lower back muscles, as well as simulating shooting posture. The female control group was selected from the students of the Faculty of Humanities and the graduates of the Mathematics Department of Imam Reza International University. All biological samples were collected in noon from 1 pm until 2 pm, and in the case of athletes, samples were collected away from sports competitions and before starting training. Also a mood assessment using the Profile of Mood States (brums) was conducted before starting sampling.

After understanding all the experimental procedures, completed the informed consent enrollment in the study. This study was approved by the ethics committee of the University of International University Imam Reza, Mashhad (IR.IMAMREZA.REC.1403.002).

Weight and height were measured with high accuracy, and then body mass index (BMI) was calculated from the ratio of body weight (kilograms) to body height (square meters).

Venous blood samples of 7 cc were collected from the subjects' anterior brachial veins before the start of the training session in laboratory conditions. The prepared samples are left at room temperature for about 10 to 15 minutes to coagulate. Then it is centrifuged at 3000 rpm for 10 minutes. After separating the serum, it is kept at minus 80 degrees. The diagnostic kit was ordered from Zell Bio, Germany.

2.1 Measurement and Tools

Plasma reactive oxygen species (ROS) measurements: High-intensity exercise has been showing to induce excessive amounts of ROS, which may react with macromolecules such as proteins, lipids, and DNA (Halliwell & Gutteridge, 2015). That is important the balance between oxidative stress, caused by the extensive production of reactive species, and reductive stress, caused by the excessive presence of antioxidants, must be maintained (Egea et al., 2017).

Detection principle: DCFH-DA (2, 7-dichlorofluorescein diacetate) is a fluorescent probe without fluorescence that can freely cross the membrane. After entering the cell, it can be hydrolyzed by intracellular esterase to form DCFH (dichlorofluorescin). In the presence of reactive oxygen species (ROS), DCFH is oxidized to DCF (dichlorofluorescein) which is a strong green fluorescent substance that cannot penetrate the cell membrane. DCF has a maximum wave peak near the excitation wavelength of 502 nm and the emission wavelength of 525 nm, and the intensity is proportional to the level of intracellular reactive oxygen species.

Adiponectin analysis: To measure the adiponectin level of the participants by ELISA method an antibody specific for Adiponectin has been pre-coated onto a 96-well microliter plate. The standards and test

samples are added into the wells and the Adiponectin present in each sample is bound to the wells by the immobilized antibody. Following incubation, the wells are washed and then incubated with a Biotinylated Anti-Adiponectin Antibody, which binds the captured Adiponectin present in each well. Following incubation, the unbound biotinylated detection antibody is removed by washing, an HRP-Streptavidin conjugate is added to the wells and the microliter plate is incubated. Following incubation and washing, the TMB substrate solution is then used to visualize the HRP enzymatic reaction by catalysis to produce a blue-colored product that changes to yellow after the addition of an acidic stop solution. The density of yellow is proportional to the amount of Adiponectin captured in each well. The concentration of Adiponectin can then be calculated by reading the O.D. absorbance at 450nm in a microplate reader and referring to the standard curve.

Mental Index: Subjective Mood Assessment: Brunel's mood scale consists of 24 simple mood descriptors, including anger, confusion, depression, fatigue, and tension a sign of negative mood and so positive mood including vigor. This questionnaire was completed by the participants one hour before the start of daily practice at 1-2 pm. In Iran, this questionnaire was validated by Mousavi and Vaez Mousavi (2017).

3 Results

Participants' characteristics: are reported in Table 1. In detail for basketball players mean weight was 62.3 kg and height was170cm; also, for shooting players mean weight was 59.6 kg and height was162cm while for controls mean weight was 63.3 and height was 166. Just in height were found between basketball and shooting significant differences. (P-value = 0.005). Although no significant differences were shown between the body mass index (BMI) of participants of the three groups.

Table 1. Participants' characteristics. Females of group controls (C), basketball players (B) and shooting players (Sh); BMI = body mass index. Tukey's test was performed (*p-value < 0.05)

Tukey's testb			Meana (SD)			**Characteristics**
B vs. SH	**C vs. SH**	**C vs. B**	**shooting player**	**Basketball players**	**Control**	
0/76	0/73	0/99	24/6	23/4	23/3	Age (year)
0/81	0/67	0/96	59/8	62/3	63/3	Weight (kg)
*0/005	0/32	0/13	1/62	1/7	1/66	Height (cm)
0/69	0/94	0/5	22/51	21/43	22/93	BMI (kg/m2)

Plasma adiponectin determination: Plasma adiponectin levels were detected by ELIZA in three groups covering both athletes and healthy subjects, and The results of the one-way analysis of variance related to adiponectin in the research groups showed that there is a significant difference between the groups (p=0.005 F(6.418=.

Post hoc tests showed that the level of adiponectin in the basketball group was significantly higher than in the control group (p=0.014) and shooting (p=0.010). There was no significant difference between the shooting and control group.

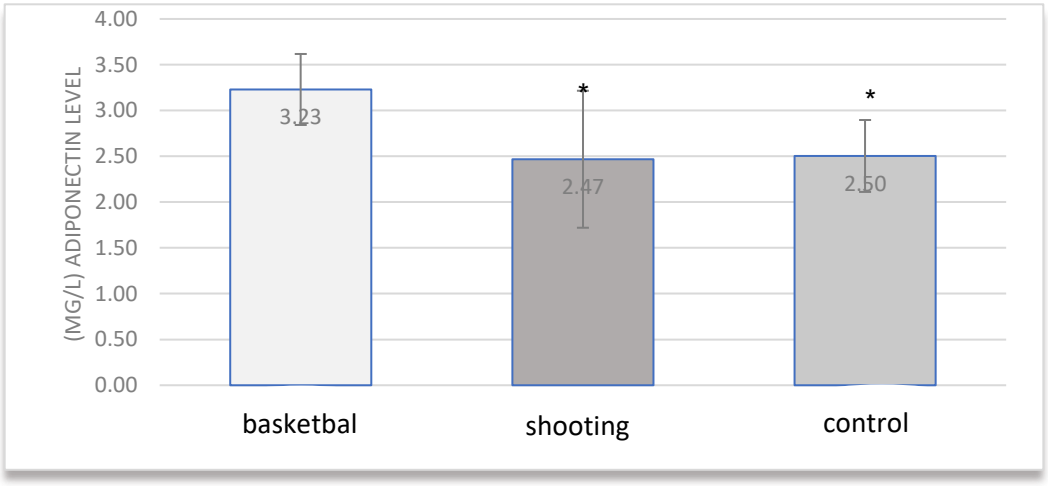

Figure1. Assessed Adiponectin level was by ELIZA methods ($p<0.05$)

Plasma Reactive Oxygen Species: The average value of ROS for the shooting was 338.30 ± 82.83 unit/liter and for the control group was 29.40 ± 311.70 u /liter, for basketball players it was 24.87 ± 353.70 unit/liter. The basketball group was more than other groups while No significant difference was observed.

Mental Assessment: The mental assessment indicated that both of athlete groups had more vigor than participants who were not engaged in sports. The results of Turkey's post hoc test showed that there is a significant difference between the two groups of shooting and control ($p=0.001$) and basketball and control ($p=0.001$) in the Vigor subclass. However, no significant difference was observed between the two basketball and shooting groups ($p=0.974$).

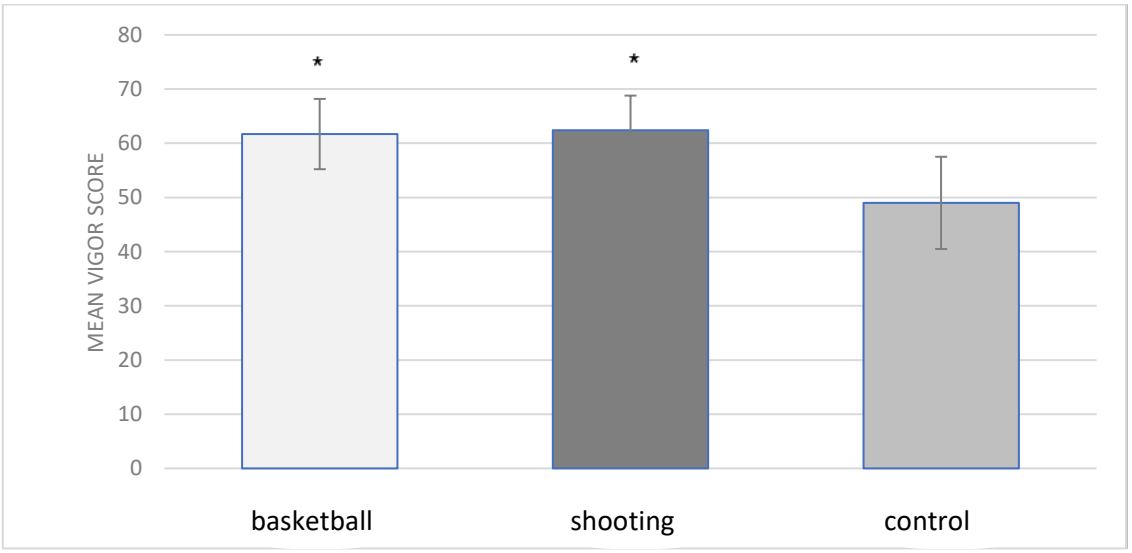

Figure 2. Comparison of vigor scores in research groups ($p<0.05$)

Also, the subjective assessment showed that the anger subscale was higher in basketball players than in other groups, which was a significant difference ($p=0.033$).

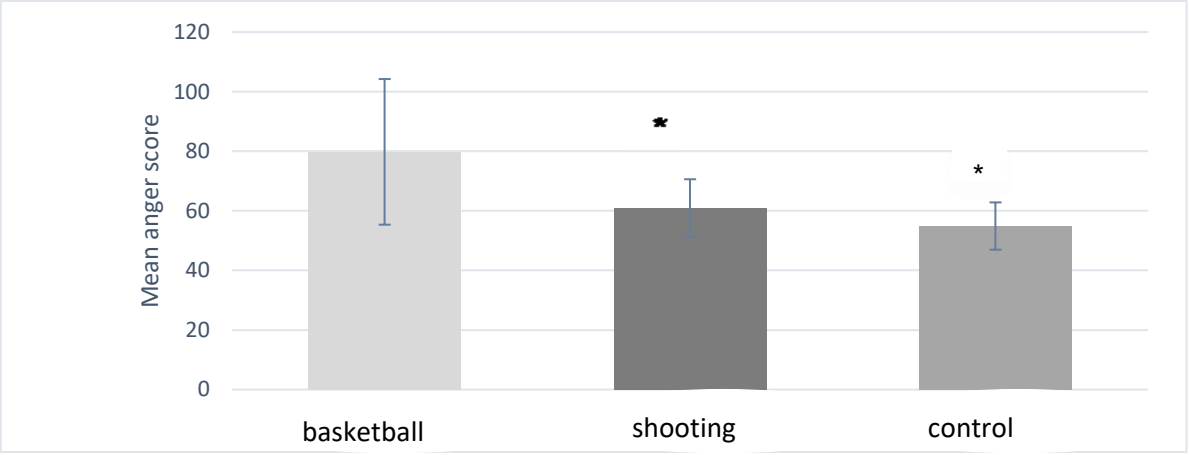

Figure 3. Comparison of anger scores in research groups ($p<0.05$)

One-way analysis of variance related to the standard tension score in the research groups showed that there was a significant difference in the mood subscale in the shooting and control groups p=0.017).

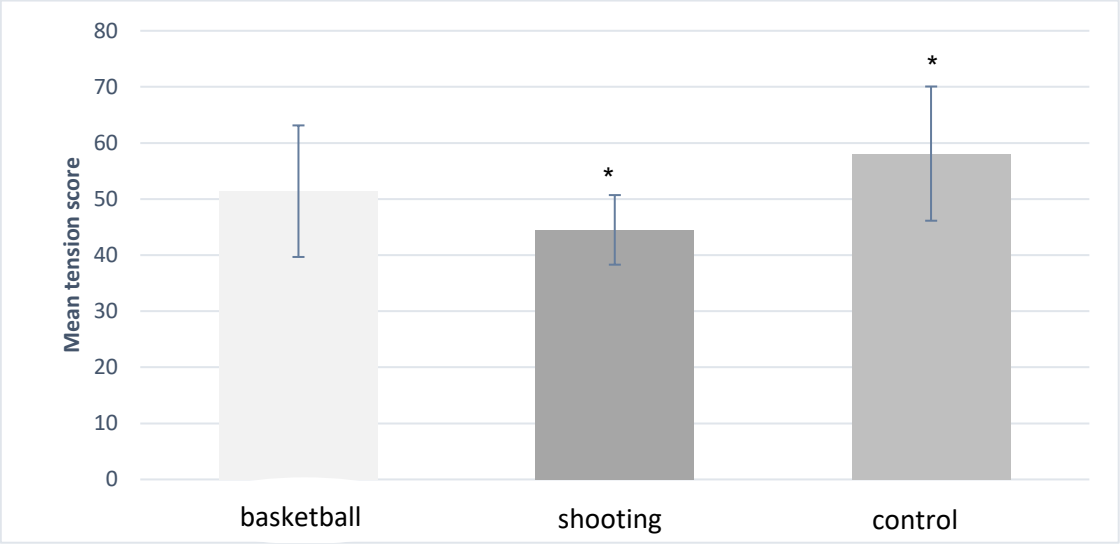

Figure 4. Comparison of tension scores in research groups ($p<0.05$)

In other mood subcategories (confusion, depression, and fatigue) was no significant difference in research groups.

5. Discussion and Conclusion

In this study, the metabolomic effects of regular exercise were evaluated by examining the amount of ROS and adiponectin in professional female basketball and shooting athletes. In addition, the relationship between these blood factors and mood states was investigated.

Study on oxidative stress in female athletes is limited. Female athletes are more susceptible to oxidative stress than male athletes (Dopsaj et al., 2010). So we studied female athletes and chose two different

sports, basketball as a team sport and shooting as an individual sport. We observed in our study obtained in elite athletes with similar BMI that reactive Oxygen Species in the basketball group were little more than shooting and control group but these differences were not significant. These results have shown that the defense mechanism of antioxidative can adapt to the type of sport (Luti et al., 2022).

Adiponectin: From skeletal muscle, adiponectin is secreted and because of its anti-inflammatory effect considered as a good adipokine. However, there is little information about the relationship between adiponectin levels and muscular fitness. In addition, few studies have been done to measure the associations between adiponectin and training in female athletes (Militello et al., 2021) . In this study, we observed that basketball athletes have a significant difference in adiponectin in comparison to shooting players and the control group this confirms what was reported by several authors that well-trained athletes present higher baseline adiponectin than non-athletes (Duzova et al., 2018).

Mood state: Observing the results of anger between both groups of athletes shows that anger in the basketball group was higher than in the shooting and control groups. These results are the same as Baghel's (2023) research (Ladiun et al., 2021). It can be concluded that in shooting, athletes may need more focus and accuracy with a lower level of anger for better performance. On the other hand, in basketball as a field with high physical contact, it is an advantage to have a higher anger score(Ladiun et al., 2021) Gilbert's (2006) research studies have found in sports with more physical contact athletes are willing to be more aggressive (Guilbert & Grand-Jardin, 2006).

Tension is associated with feelings of panic, anxiety, worry and nervousness (Anshel et al., 1990)In our study, there is no significant difference in tension between basketball as a team sport and shooting as an individual sport. However, in the control group, the tension was significantly different from the shooting group. This shows that elite athletes, regardless of the type of sport, experience the same amount of stress on themselves and have also learned how to moderate this stress.

Vigor can be seen as a vital positive factor in predicting players' athletic skills. (LIM et al., 2011),(Yatabe et al., 2021)). The results showed that there was no significant difference in strength in both groups of professional athletes had higher vigor than the control group. This may be because professional athletes feel more energetic during the season and training than non-athletes. These results were consistent with the Abu Talip results (LIM et al., 2011).

Our results show that there are differences in mental and physical factors in basketball as a team sport and shooting as an individual sport.

We suggest a significant increase in plasma adiponectin levels in basketball players can counteract the local inflammatory effects of exercise during off-season training.

On the other hand, according to the results that the researchers found, athletes do need different mood subscales, including anger and vitality scores, to achieve success in different sports, and also different sports increase the mood subscales in athletes. That adjusting it is one of the duties of the specialized staff of the sports team. In the current study, the researcher emphasizes that measuring and examining the emotional states of athletes are important both to achieve success and to maintain physical and mental health.

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