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Original Article

A Comparison of Glutathione and Malondialdehyde Concentrations in Athletes Engaged in Certain Sports

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Abstract

Sports stimulate the body to adapt to physical activity, causing biochemical and functional metabolic changes in various systems and organs of the body due to exercise. Antioxidants and free radicals affect the biological activity of living organisms, leading to physiological changes in all body systems due to exercise. This study, conducted from December 2023 to February 2024, included 120 blood samples from male basketball (40), football (40), and volleyball (40) players ages 18-27 years, from the College of Physical Education and Sports Sciences at Tikrit University. Blood samples were collected ten minutes before and ten minutes after 70 minutes of exercise on the same day. The study found no significant differences in glutathione (GSH) and malondialdehyde (MDA) concentrations before and after exercise among the groups. The findings suggest that the type of sport (football, basketball, or volleyball) with an anaerobic system does not significantly affect antioxidant and fat peroxidation levels, though slight variations due to increased lactic acid in muscles were noted, leading to muscle fatigue and minor changes in GSH and MDA concentrations.

Keywords: Exercise Physiology; Exercise; Antioxidants; Glutathione; Free Radicals; Malondialdehyde

1. Introduction

Sports are necessary to activate the biological processes carried out by the various body systems. Exercise affects a person's metabolic energy by increasing the consumption of energy generated after exercise. Exercise also raises the level of performance of the body's organs and systems, such as the lungs, stomach, and muscles, regulates the work of the heart, and stimulates blood circulation and the respiratory system [1]. Sports stimulate the body to adapt to physical activity, which causes biochemical and functional metabolic changes in various systems and organs of the body as a result of exercise, all these changes determine the training effectiveness they are manifested in the multiple functions improvement of the body and fitness level improvement of the trainee [2]. Physical conditioning is one of the essential foundations for success and progress in exercise [3]. Physical activity is vital for the prevention of many diseases [4]. It has broad-ranging positive health implications, including those that improve the metabolic health of the liver [5].

Energy in the human body is the source of movement, various types of sports performances, and the source of muscle contraction, which cannot occur without energy production. The body's energy needs vary according to the duration of physical exertion, its strength, and the level of performance [6]. When performing physical effort, energy production systems are divided into two primary sections: 1- anaerobic system and 2- aerobic system. Skill performance is one of the fundamental pillars through which a reasonable level can be achieved. The effectiveness of basketball, football, and volleyball depends on the anaerobic system at a ratio of 80-90% with short, continuous, intermittent, and moderate high-stress exercises, respectively. Antioxidants and free radicals affect the biological activity of living organisms, causing physiological changes in all body systems as a result of exercise [7]. Antioxidants are compounds with high reducing power, characterized by their ability to inhibit free radical activities [8]. Antioxidants are produced naturally in the body and can also be obtained from food or as synthetic products added to food [9]. Antioxidants protect cells from injury caused by oxidative stress and prevent cell destruction. Thus, they form a line of defence against the destructive activity of free radicals. Antioxidants also work to strengthen the body's immunity [10]. GSH is a unique molecule essential for life that participates in critical aspects of cellular homeostasis, having a paramount role in defending against the oxidative damage that occurs during various diseases. GSH plays a central participation in trans-hydrogenation reactions needed to maintain a reduced state of sulfhydryl groups of other molecules, proteins, and enzymes, as well as the formation of deoxyribonucleotides and vitamin reduction [11,12].

Free radicals are a natural product of cellular metabolism. The increase in their number in cells occurs due to the inability of antioxidants to neutralize them, as well as the imbalance between free radicals and antioxidants, causing oxidative stress. This imbalance causes tissue damage in the body, leading to diseases [13]. Malondialdehyde is the final product of the fat peroxidation process that occurs automatically in the body. It is one of the oxidation processes that occurs at low rates in all types of cells and tissues. While free radicals are a natural physiological process, increasing their production enables them to bind to fats, causing fat peroxidation and forming malondialdehyde [14]. This compound has a high inhibitory activity on antioxidants, so it is an indicator of many diseases related to fats, such as obesity and atherosclerosis, and is also a contributing factor to cancer through the interaction of malondialdehyde with the genetic material DNA, causing cancerous mutations [15]. The study compares concentrations of the antioxidant glutathione and lipid peroxidation malondialdehyde in athletes playing football, basketball, and volleyball before and after physical exertion.

2. Materials and Methods

2.1 Collecting Samples

The current study included 60 male students, volunteers from the College of Physical Education and Sports Sciences at Tikrit University, aged between 18-27 years (who played football, basketball, and volleyball). A total of 120 blood samples were collected per day. The exact times were once ten minutes before the start of the exercise and a second time ten minutes after the end, which lasted for 70 minutes. Information was recorded through a questionnaire that included age, gender, and type of game from December 2023 to February 2024; blood samples were collected from each student within the study twice on the same day before performing the physical exertion and after completing the physical exertion. Five ml of venous blood was collected before physical exertion using a medical syringe; the blood samples were collected using clean and tightly covered gel tubes. The blood samples were left for 30 minutes at room temperature until the blood was clotted; then, the blood samples were centrifuged for 15 minutes at 3000 rpm to obtain serum. The serum was pipette using a micropipette, placed in eppendorf tubes, and frozen at -200C until biochemical tests. Five ml of venous blood was also collected immediately after performing the physical exertion, which lasted for 70 minutes, in the same manner as before, and the serum was preserved in the same way.

2.2 Measurement of Glutathione Concentration

The glutathione concentration in blood is measured using the modified Allman reagent. This method is based on the use of Ellman reagent that contains DTNB (5,5'-dithiobis-2-nitrobenzoic acid), where Ellman's reagent reacts quickly with glutathione and is reduced by the sulfhydryl group (SH group) of glutathione, which gives a colored product whose absorption is measured at a wavelength of 450nm. The concentration of the product formed depends on the level of glutathione in the blood [16].

2.3 Measurement of Malondialdehyde Concentration

The concentration of malondialdehyde in blood is measured by determining the level of MDA, an end product of lipid peroxidation. This method relies on a reaction between lipid peroxides, mainly MDA, and thiobarbituric acid, which occurs in an acidic medium. The absorbance of the colored product is measured at a wavelength of 532 nm [17].

2.4 Statistical Analysis

The results were analyzed statistically to find the arithmetic mean (M) ± standard error (SE), and the t-test was used to show the difference between the study groups (football group, basketball group, volleyball group) at the probability level ($P \leq 0.05$) using the SPSS program.

3. Results and Discussion

3.1 Results

The results in Tables 1, 2, and 3 indicated a low, non-significant decrease in the concentration of glutathione in the athletes' blood ($P < 0.05$) after performing the physical exertion compared to before performing the physical exertion in the football, basketball, and volleyball groups.

Table 1: Comparison between the level of glutathione and malondialdehyde in serum before and after training for the football group

Biochemical variables M ± SE	Before performing physical exertion M ± SE	After performing physical exertion M ± SE
Glutathione GSH (mmol/l)	0.10 ± 0.01 A	0.08 ± 0.01 A
Malondialdehyde MDA (mmol/l)	0.07 ± 0.01 A	0.09 ± 0.01 A

Table 2: Comparison between the level of glutathione and malondialdehyde in serum before and after training for the basketball group.

Biochemical variables M ± SE	Before performing physical exertion M ± SE	After performing physical exertion M ± SE
Glutathione GSH (mmol/l)	0.10 ± 0.01 A	0.09 ± 0.01 A
Malondialdehyde MDA (mmol/l)	0.02 ± 0.01 A	0.10 ± 0.01 A

Table 3: Comparison between the level of glutathione and malondialdehyde in serum before and after training for the volleyball group.

Biochemical variables M ± SE	Before performing physical exertion M ± SE	After performing physical exertion M ± SE
Glutathione GSH (mmol/l)	0.09 ± 0.01A	0.07 ± 0.01A
Malondialdehyde MDA (mmol/l)	0.06 ± 0.01 A	0.08 ± 0.01 A

The results in Table 4 showed that there were no significant differences ($P < 0.05$) in the concentration of glutathione when comparing the three exercises. We noticed a slight non-significant decrease in the level of glutathione concentration in volleyball players.

Table 4: Comparison between the level of glutathione and malondialdehyde in serum basketball, football and volleyball group.

Biochemical variables M ± SE	Basketball After performing the Physical exertion M ± SE	Football After performing the Physical exertion M ± SE	Volleyball After performing the Physical exertion M ± SE
Glutathione GSH (mmol/l)	0.09 ± 0.01 A	0.07 ± 0.01 A	0.07 ± 0.01 A
Malondialdehyde MDA (mmol/l)	0.10 ± 0.01 A	0.07 ± 0.01 A	0.08 ± 0.01 A

3.2 Discussion

The reason for this may be attributed to the increased consumption rate of glutathione by liver cells and skeletal muscles, as it is one of the essential non-enzymatic antioxidants that work to remove free radicals and their products directly due to the presence of the ethanol group (SH) in the synthesis of glutathione, which plays a vital role in protection. It is present in cases of severe oxidation, as it reacts with the hydroxyl radical (OH) and the peroxynitrite radical (ONOO), converting from the active form (GSH) to the inactive form (GSSG), which is glutathione disulfide, or indirectly by being the raw substance for some antioxidant enzymes such as the enzyme glutathione peroxidase. The sulfur group in the composition of glutathione is a reducing agent, as it quickly gives away a hydrogen atom due to the weakening bond between sulfur and hydrogen (S-H) and the strengthening of the bond between carbon and hydrogen (C-H) in the free radicals. Therefore, it protects cellular membranes from damage caused by free radicals [18].

The concentration of glutathione also decreases due to its oxidation during oxidative stress, and this oxidation transforms it into the oxidized form of disulfide (GSSG). This form is still toxic and stimulates the production of more free radicals [19]. The reason for the decrease in the concentration of glutathione may also be attributed to a decrease in the primary materials for its construction, especially the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), resulting from the pentose sugar pathway, which is the companion enzyme to the enzyme glutathione reductase, which works to restore the active form of glutathione (GSH) ineffective form (GSSG), and during physical exertion with high exertion, the activity of the enzyme glutathione peroxidase decreases. The decrease in GSH concentration may be due to the use of glutathione in the body to defend cells against free radical attacks resulting from increased oxidative stress and accumulation of free radicals [20]. The high concentration of GSH in the blood of athletes in the group before performing physical exertion is attributed to good nutrition containing flavonoids, catechins, vitamins, phenols, and other substances in food. These substances are essential in inhibiting the activity and work of free radicals, thus increasing Glutathione concentration. These substances are used as a cofactor in synthesizing enzymes that control all physiological processes in the body, especially when energy is released from fatty and sugary substances by raising the glucose level in the blood. Hence, the concentration of glutathione increases [21].

Vitamins such as vitamins E and C raise the glutathione concentration you get from food. Vitamin C helps prevent oxidative damage caused by active free radicals, including the powerful oxidizing agents, the hydroxyl radical (OH) and the superoxide radical, in the blood and body fluids and thus reduces the consumption of glutathione, which is a non-enzymatic antioxidant present within the body, which causes an increase in the level of glutathione concentration in the blood [22]. The results in tables 1, 2, and 3 showed a slight, non-significant increase ($P < 0.05$) in the concentration of malondialdehyde in the athletes' blood after performing the physical exertion compared to before performing the physical exertion in the football, basketball, and volleyball groups. The reason for the high concentration of MDA in blood after high-exertion exercise may be attributed to the generation of free radicals (ROS) reactive oxygen species due to oxidative stress to which skeletal muscles are exposed as a result of high physical exertion, which works to oxidize unsaturated fatty acids in cellular membranes, leading to increase lipid peroxidation and then oxidative stress in cellular membranes, which leads to an increase in the level of MDA concentration, which is one of the essential products of the lipid peroxidation process resulting from the interactions of free radicals with biomembrane molecules [23].

The level of MDA concentration increases after exercise for two hours. Free radicals oxidize fats in cellular membranes, as fatty acids in cellular membranes are considered the most exposed to free radical reactions. MDA results from the oxidation of these fatty acids resulting from active oxygen species (ROS) in lipid peroxidation [24]. In many pathological conditions resulting from oxidative stress that causes damage to tissues, The body increases the activity of free radicals beyond the ability of antioxidants to remove or neutralize them, leading to the occurrence of lipid peroxidation and then increasing the level of MDA concentration and losing the balance between the activity of free radicals and the activity of antioxidants, causing oxidative stress, and increasing the level of MDA concentration causes cellular membranes to lose their elasticity [25]. The low concentration of MDA in the blood of athletes in a group before performing physical exertion may be attributed to the presence of compounds that enter the body through food that act as antioxidants, which contribute to a decrease in the level of MDA concentration in the blood. These compounds include phenols, flavonoids, tannins, and vitamins that inhibit the activity and work of free radicals. These compounds have properties that reduce fats in the blood. These compounds also contribute to protecting the body from diseases.

Vitamin E reduces the level of MDA concentration by increasing the effectiveness of several antioxidant enzymes, such as the superoxide enzyme, the glutathione peroxidase enzyme, the superoxide dismutase (SOD) enzyme, and catalase dismutase, which breaks down several active types of oxygen (ROS) and converts them into a water molecule. Vitamin E is also highly concentrated in cell membranes, especially in the liver, muscles, and fatty tissue, so it reduces the products of fat peroxidation. These compounds minimize fat peroxidation and oxidative stress, thus decreasing MDA concentration [26].

The reason for this is that practicing physical exercises leads to a reduction in the activity of the glutathione reductase enzyme as a result of a decrease in the primary materials for building GSH, which is the enzyme NADPH, the enzyme glutathione reductase, as a result of its increased consumption by liver cells and skeletal muscles. Hence, the level of glutathione concentration in volleyball players decreases to lower than that of football and basketball players.

As for football players, the slightly higher concentration of glutathione is due to the player's body being exposed to intermittent high exertion. During periodic high-exertion sports training, the player's body is exposed to a decrease in the production of free radicals and an increase in enzyme activity. Catalase and the enzyme glutathione peroxidase break down free radicals and transform them into a harmless water molecule in the body. Thus, energy consumption by skeletal muscles increases, and glucose concentration increases, leading to a slightly higher glutathione concentration in football players than in volleyball players and a lower concentration in basketball players. As for basketball players, the high level of GSH concentration is due to the continuous high exertion that the player's body is exposed to due to the constant movement of the player's body along the stadium. The skeletal muscles need energy, as they resort to using glucose and fats as their energy source, leading to the production of power within the skeletal muscles; where This energy is used to raise the level of glucose in the blood and muscles, which leads to an increase in the level of glutathione concentration in basketball players, which is higher than that of football and volleyball players [27].

The results in Table 4 showed no significant differences ($P < 0.05$) in concentration. When comparing the three exercises. We noticed a slight, non-significant increase in the level of MDA concentration in basketball players. This is due to the player's body being exposed to continuous stress. During ongoing high-exertion training, the skeletal muscles' need for oxygen and energy increases, so the muscles' need for oxygen is more significant. Through the flow of large amounts of oxygen-laden blood from the liver, kidneys, and stomach, the muscles need energy by oxidizing fats in the cellular membranes. This increase is accompanied by an increase in the metabolism process and an increase in energy production with an increase in oxygen consumption. When training is suddenly stopped, fat oxidation will lead to an increase in oxidative stress in cellular membranes and blood quickly flows to the organs from which it came. This process causes an increase in free radicals, so the level of MDA concentration in basketball players rises higher than that of football and volleyball players. Football players are exposed to intermittent high exertion, which leads to a decrease in the level of MDA concentration due to the athlete's body being exposed to high exertion, then low exertion, then high exertion—intermittent exertion. During high-exertion intermittent exercise, the energy level decreases, and the body's metabolism decreases, which reduces the production of free radicals, as it works to balance the radicals. Free radicals and antioxidants lead to a slightly lower level of MDA concentration in football players than in basketball players and are higher than in volleyball players. As for playing volleyball, we notice a slight decrease in MDA concentration. The reason for this among volleyball players is that their bodies are exposed to moderate, high-exertion exercise due to the movement of part of the player's body, which reduces the consumption of oxygen and energy by the skeletal muscles. Hence, the oxidation process of unsaturated fats in cellular membranes decreases slightly. The production of free radicals decreases somewhat, causing the decrease in the level of MDA concentration in volleyball players to be less than in basketball and football players [28].

4. Conclusions

The lack of significant differences is likely due to the type of game (football, basketball, volleyball) in the anaerobic system not significantly affecting the concentration of antioxidants and fat peroxidation but only slightly. In the short anaerobic system with high-intensity exertion, lactic acid accumulates in muscles as a result of the high exertion to which the body is exposed, which causes muscle fatigue for the player, leading to a decrease in glucose, followed by a decrease in the metabolic process, causing a reduction in the body's energy, thus reducing the efficiency of the player's body, leading to a slight change in the concentration of GSH and MDA before and after exercise, to eliminate the lactic acid in the muscles, which causes muscle fatigue in the player's body, it is necessary to practice prolonged, moderate-intensity or low-intensity aerobic exercise, which leads to an increase in glucose, then an increase in the metabolic process in the body and an increase in the body's energy. In the aerobic system, the power generated is almost 50 times greater than the energy generated in the anaerobic system, the efficiency of the player's body increases, and there is a significant change in the concentration of GSH and MDA before and after the exercise. Therefore, I suggest studying aerobic exercises such as jumping, walking, and cycling, as well as anaerobic exercises such as football or volleyball, or studying aerobic exercises only to get the required variables.

List of abbreviations

Glutathione (GSH)

Malondialdehyde (MDA)

Deoxyribonucleic Acid (DNA)

Dithiobis-2-Nitrobenzoic Acid (DTNB or Elman's Reagent)

Mean (M)

Standard Error (SE)

Sulfhydryl Groups (SH)

Hydroxyl Radical (OH)

Peroxynitrite (ONOO)

Oxidized Glutathione (GSSG)

Carbon-hydrogen (C-H)

Nicotinamide Adenine Dinucleotide Phosphate (NADPH)

Reactive Oxygen Species (ROS)

Superoxide Dismutase (SOD)

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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