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FULL PAPER

Biomarkers in predicting myocardial damage in athletes

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The dynamics of autoantibodies (AAB) to cardiomyocyte proteins in the blood of basketball athletes in the dynamics of a one-year training cycle was shown. Men aged 19-23 years old with different amounts of total motor activity were examined. Anthropo- and physiometric examinations, electrocardiogram measurements, general blood analysis, quantitative determination of AAB to cardiomyocyte proteins, and the concentration of sex hormones were carried out. In individuals with high total motor activity, the amount of AAB to myocardial proteins (alpha-actin 1, beta-myosin 7B, and troponin I) was higher compared to peers who did not engage in sports. At the end of the competition period, two players had a more than 5-fold excess of AAB to troponin I compared to the average values determined in the group of other team members. At the same time, there were minor changes in electrocardiogram and the absence of complaints about the condition of the cardiovascular system. A multiple increase in the content of AAB to myocardial proteins during the period of intensification of training loads is recorded in the absence of obvious signs of pathological changes in the heart and complaints from the subject. This indicates the prospects of determining AAB to cardiomyocyte proteins and, first of all, AAB to troponin I as an indicator of early signs of incipient pathological changes in the myocardium.

KEYWORDS

Auto-antibodies to cardiomyocyte proteins; exercise; sex hormones; cardiospecific immunoglobulins.

Introduction

Sports activities of modern athletes are often carried out at the limit of their functional capabilities, which is often accompanied by a breakdown of adaptive mechanisms and the development of various pathologies. Violations can sometimes be so serious that they cause sudden death of athletes. In 90% of cases, the cause of death of athletes is cardiovascular disorders leading to sudden cardiac death (SCD) [1]. In most cases, SCD is provoked by myocardial hypertrophy [2-5]. Currently, the term SCD implies death that occurred directly during exercise or within 1-24 hours from the moment of the first signs

that caused the athlete to change or stop his activity [6]. It is known that intense and prolonged physical activity increases the risk of developing SCD by 7-10 times [7]. An unpleasant fact is the constant increase in the number of cases of SCD among athletes is 2-3 times higher than that of people of the same age and gender who have the usual level of total motor activity [8,9]. For example, according to Maron *et al.*, in 1994 to 2006, 1,290 cases of SCD were noted and in 1989 to 1993 – 576 [10]. In the presence of pronounced gender differences, the frequency of SCD in men is more than 9 times higher than that in women [11,12]. According to the frequency of SCD cases among athletes, football is the second most common, followed by basketball [13,14].

A high percentage of cases of SCD among basketball players is associated with the peculiarities of their anthropometric data. High height and long limbs create certain difficulties in hemodynamics and heart function [15].

At the moment, the Russian Federation does not keep official statistics on the frequency of SCD occurrence among athletes. Accordingly, there are no programs for the prevention of SCD and recommendations for the early recovery of athletes of the Rix group. Thus, the urgent task is to find new approaches and means of early diagnosis of the development of pathological changes in the myocardium of athletes. An informative method for the prenosological diagnosis of emerging disorders in the myocardium may be a technique for determining the serum content of autoantibodies (AAB) to cardiomyocyte proteins [16,17].

The essence of this method lies in the fact that in the athlete's body, any violations of the normal functioning of the cardiovascular system in the early stages may not affect general and special performance. Nevertheless, the range of products released by damaged cells into the bloodstream is changing as well as the content of autoantibodies that utilize this product. Such biological markers are the first indicators of a possible problem, and timely preventive measures taken in many cases can prevent the threat of developing disorders.

At the same time, there are few studies highlighting the dynamics of AAB to cardiomyocyte proteins during training sessions and competitions. Previously conducted experimental studies [18-21], according to the definition of AAB to troponin I, to alpha-actin 1, to the heavy chain of beta-myosin 7B, allowed us to establish a clear dependence of the immune system response on the processes occurring in cardiac myocytes under conditions of adaptation to muscle loads of varying duration and intensity. The data of experimental studies suggest that the determination of AAB to cardiomyocyte proteins in the body of athletes is highly informative in determining the nature of adaptive processes at different stages of sports activity.

The purpose of this study is to study the dynamics of AAB to cardiomyocyte proteins in the blood of basketball athletes in the dynamics of a one–year training cycle. To solve these issues, a comparative study of the concentrations of autoimmune antibodies to troponin I, alpha-actin 1, and beta-myosin 7B in the blood serum of sportsmen was carried out.

Experimental

As part of the current scientific study, 27 men with different amounts of total motor activity were examined. Group 1 (control) included 12 people with the usual amount of total motor activity. Group 2 (athletes) included 15 people - members of the North Caucasus Federal University basketball team with at least 8 years of training experience. Written consent was received from all the subjects to participate in the study.

In the course of the research, anthropometric and physiometric parameters

(height, weight, lung capacity, blood pressure, and heart rate) were evaluated in accordance with Hurezeanu *et al.* [22]. All subjects underwent electrocardiography on the ATES MEDICA Easy ECG device (ECM, Russia) using 12-channel parameters. The assessment of the overall physical performance of the body was carried out by the step test method, determining the maximum oxygen consumption.

The examination of the control group and blood collection were carried out once at the initial stages of the research work. Blood collection in the group of athletes was carried out at the beginning of the experiment, as well as at the end of the preparatory, competitive and transitional stages of training (within 24 hours from the last day of the cycle). It is worth noting that preparatory, competitive, and transitional stages of training were initially planned and set as graphic by the major coach of the team.

Blood for analysis was taken from the ulnar vein in from 8:00 a.m.-10:00 a.m., strictly on an empty stomach, in the absence of intense muscle exertion for 24 hours. Test tubes with a separating gel forming a barrier between serum and coagulated blood after centrifugation were used.

The general blood analysis was performed using the hematology analyzer of the XN SYSMEX hematology analyzer (Sysmex Corporation, Japan) using the regents of the company - Beckman culter (Japan). Determination of the concentration of testosterone, follicle-stimulating and luteinizing hormones was determined by immunochemistry analysis on an automatic immunochemical analyzer Beckman Coulter Unicel Dxi 800 (Beckman Coulter, USA).

The quantitative indicator of estradiol was measured by enzyme immunoassay using reagents from Bio – Rod Laboratories.ins (USA) in accordance with the attached instructions.

The criterion for early detection of possible pre-pathological and pathological changes in the myocardium was the determination of autoantibodies (AAB) to cardiomyocyte proteins in blood serum. In particular, AAB was determined for troponin I (Anti-cTnI), alpha-actin 1 (Anti-ACTC 1), and beta-myosin 7B heavy chain (Anti-MYH7B) using a highly sensitive and highly specific kit from Cloud-Clone Corp. (China). The work used a microplate photometer for scientific research hermo Scientific Multiskan FC (Thermo Fisher Scientific, Finland), a medical thermostatically controlled ST-3L shaker (Elmi, Latvia), an automatic microplate washer Thermo Scientific (Thermo Fisher Scientific, USA). The complex of medical research was conducted on the basis of the City Clinical Polyclinic No. 1 (Stavropol, Russia).

The results of the study were statistically processed using the Biostat statistical package (version 4.03). The Student's t-test was used to assess the statistical significance of the differences. The reliability of the differences in the values of the studied indicators was judged at $p < 0.05$.

Results and discussion

Basketball is a dynamic game that places high demands on the general and special endurance of athletes. In this regard, great attention is paid to the development of these physical qualities in the dynamics of the training process. Conducting anthropometric and physiometric studies in groups of people with different amounts of total motor activity revealed a significant superiority ($P < 0.05$) in height and body weight of athletes compared with people who do not engage in sports. Such an advantage is primarily associated with the selection of children with tall stature and strong physique to the sports sections [23]. Table 1 shows comparative analysis of the physical development of young men with different levels of total motor activity.

TABLE 1 Comparative analysis of the physical development of young men with different levels of total motor activity

Investigated indicators, units of measurement	Control group	Athletes
Height, cm	170.5 ± 8.6	195.0 ± 4.4 *
Weight, kg	76.1 ± 2.6	84.4 ± 2.3*
Respiratory rate, qty/min	16.0 ± 1.7	15.3 ± 2.4
Vital capacity of the lungs, mL	3501 ± 209.1	5120 ± 319.2*
Maximum oxygen consumption, mL/kg of body weight	4600 ± 101.5	6597 ± 252.4*
Heart rate, beats/min	76.3 ± 2.3	54.6 ± 3.8*
Systolic blood pressure, ml.hg.	116.5 ± 19.08	117.0 ± 11.08
Diastolic blood pressure, ml.hg.	70.3 ± 4.15	71.5 ± 3.0
Brush strength, kg	34.6 ± 2.5	50.9 ± 3.0*

* – the reliability of differences between the groups of subjects ($p<0.05$).

The indicator of an athlete's overall physical performance is the amount of the maximum oxygen consumption, in the group of athletes it corresponded to 6597±252.4 ml/kg with a value of 4600±101.5 mL/kg in persons with a normal level of total motor activity. Significant superiority was recorded in lung vital capacity indicators – 5120±319.2 and 3501±209.1 mL, respectively. The hand strength in the group of athletes corresponded to 50.9±4.3 kg at 34.6±2.5 in individuals with the usual volume of total motor activity. When calculating the heart rate, bradycardia (54.6±3.8 beats/min) was noted in athletes. In the control group, it was 71.3±2.3 beats/min, which corresponded to the norm for people with a low level of total motor activity.

The values of blood pressure in the examined groups corresponded to the norm. Blood pressure is not an indicator of fitness, and significant deviations from the norm indicate developing disorders in the cardiovascular system.

Repeated anthropometric and physiometric examinations during one-year training cycles did not reveal significant differences in the determined indicators in the group of athletes. Fluctuations in body weight did not exceed 2 kilograms – a decrease from 85.3±3.2 kg to 84.7±5.2 kg in the competitive period and a repeated increase to 86.9±3.9 in the transitional training cycle were unreliable. Fluctuations in the values of vital capacity of the lungs, the maximum oxygen consumption and hand strength during the year also did not have significant differences. All determined blood parameters in the examined groups were within the physiological norm. But at the same time, athletes have a high hematocrit, a significantly significant superiority in the number of red blood cells, hemoglobin content in the blood and in the cell, which indicates a high oxygen capacity of the blood, as a result of long-term sports training associated with the development of general and special endurance [24-27]. Table 2 presents data from general clinical blood tests of men of both groups. Table 3 indicates the content of sex hormones in the blood of men of both groups.

TABLE 2 Data from general clinical blood tests

Groups of subjects and the training stage	WBC $*10^9$/L 4-9	RBC $*10^{12}$/L 4-5	HGB g/L 130-160	HCT % 39 - 50	MCH pg 27 - 31	PLT $*10^9$/L 180-320	PCT % 0.15 – 0.40	ESR Mm/h
Data at the beginning of the research								
Control group	4.5 ±0.75	4.1 ±0.25	140.1 ±11.3	40.2 ±1.36	24.0 ±1.25	295 ±15.9	0.29 ±0.08	2.0 ±0.1
Athletes	4.67 ±0.39	5.79 ±0.61*	178.0 ±12.4*	46.9 ±2.5*	30.2 ±2.4*	297 ±20.5	0.30 ±0.05	2.4 ±0.08*
Data of athletes determined during the end of annual training cycles								
Preparatory period	5.51 ±0.38	5.76 ±0.45*	172.1 ±10.2*	48.7 ±2.9*	29.5 ±1.7*	305 ±19.1	0.32 ±0.06	2.6 ±0.09*
Competitive period	6.49 0.34*	5.79 ±0.55*	169.0 ±11.2*	49.0 ±3.4*	31.4 ±1.9*	315 ±20.3	0.36 ±0.07	4.3 ±0.9*
Transition period	5.15 ±0.64	5.62 ±0.49*	163.1 ±12.3*	48.0 ±2.2*	30.4 ±1.6*	309 ±26.0	0.35 ±0.08	4.0 ±1.2

Note: * – the reliability of the differences compared to the data of the control group ($p<0.05$); α – the reliability of the differences in the group by the athlete compared to the data at the beginning of the experiment (or with the preparatory period ($p<0.05$). WBC – white blood cells, RBC – red blood cells, HGB – hemoglobin, HCT – hematocrit, MCH - mean corpuscular hemoglobin, PLT – platelets, PCT – platelet crit, and ESR – erythrocyte sedimentation rate.

TABLE 3 The content of sex hormones in the blood of young men with different levels of total motor activity

Detectable hormones, units of measurement	Control group (n-12)	Athletes (n-15), training cycles		
		Preparatory period	Competitive period	Transition period
Testosterone, ng/dl	610.2±38.0	586.3±23.6	433.1±40.2*	712.2± 25.2*α
Estradiol nmol/l	0.19±0.08	0.28±0.04	0.31±0.03*	0.29±0.06
FSH mMEd/ml	3.4± 0.19	3.1± 0.25	2.5± 0.34	2.8± 0.39
LG mMEd/ml	3.4± 0.27	2.7± 0.25	3.2± 0.18	3.7± 0.13

Note: * – the reliability of differences compared to the data of the control group ($p<0.05$); α – the reliability of differences compared to the data determined at the preparatory stage of training cycles ($p<0.05$). FSH – follicle stimulating hormone and LG – luteinizing hormone

The testosterone concentration in the blood of young men with the usual level of total motor activity was within the physiological norm (610.2±37.3 ng/dl) for this age group. In the blood of athletes, the concentration of testosterone from 586.3±23.6 ng/dl, determined in the preparatory period, decreased to 433.1±40.2 ng/dl in competition and increased to 712.2± 25.2 ng/dl in transition [28].

The level of estradiol in the blood of athletes was higher compared to those in the boys of the control group. During the end of the competitive stage of training, this superiority was reliable. Changes in estradiol concentration at different stages of training cycles did not exceed 11% and were unreliable ($p>0.1$). There were no significant differences in the dynamics of luteinizing hormone and follicle stimulating hormone during training cycles. There were no significant differences compared with the data determined in the control group [29].

Notably, the recent studies provide evidence that troponin I, alpha-actin 1, and beta-myosin 7B are the main biomarkers for myocardial damage [30-33]. In individuals with high total motor activity, the amount of AAB to myocardial proteins (troponin I and beta-myosin 7B heavy chain) was higher

compared to peers who did not engage in sports, achieving a significantly significant superiority at the end of the competitive and transitional periods [28]. Thus, the content of AAB to troponin I proteins increased from 0.44±0.05 ng/mL to 0.62±0.09 ng/mL and alpha-actin from 2.89±0.30 ng/mL to 3.41±0.41 ng/mL (Table 4).

TABLE 4 The content of AAB in the blood of young men with different levels of total motor activity

Groups	Autoantibodies to cardiospecific myocardial proteins, units of measurement		
	AAB to Troponin, ng/mL	AAB to Actin, ng/mL	AAB to Myosin, ng/mL
Control group, n=12	0.34±0.03	2.54±0.60	1.45±0.31
Athletes (preparatory period), n=15	0.44±0.05	2.89±0.30	2.37±0.29
Athletes (competition period), n=13	0.62±0.09* α	3.41±0.41	2.80±0.45*
Athletes (transition period), n=15	0.51±0.03*	3.36±0.28	2.65±0.39*

Note: * – the differences in relation to the control group are significant ($p<0.05$); α – the reliability of the differences compared to the data determined at the preparatory stage of training cycles ($p<0.05$).

In the blood of two athletes, a five- and more than seven-fold excess of AAB to troponin I was recorded compared with the average values determined in the group of other team members (athlete 1-3.1 ng/ml; athlete 2-4.8 ng/mL, in the group 0.62±0.09 ng/ml). It is worth noting, that difference in the groups were statistically significant ($p<0.05$).

The analysis of ECG data revealed no significant changes in the groups of subjects. Sinus rhythm (bradycardia) with a heart rate of 50-55 beats/min. The position of the electrical potential axis of basketball athletes is vertical, which anatomically also corresponds to the vertical position of the heart in the chest due to high height and asthenic physique [34]. No significant deviations in the size and morphology of the electrocardiogram teeth were recorded. At the same time, in the group of athletes, in 25% of cases, there is a downward elevation of the ST segment in V2-V3 to 2.5 mm (normal to 1.5 mm), which indicates functional myocardial hypertrophy of the septum of the left ventricle.

At the end of the competition period, an increase in the width of the QRS complex was noted in the group of athletes by 0.026 ± 0.009 msec, compared with the results of the 1st measurement. Otherwise, no diagnostic significant ECG changes were detected [35-39].

In two sportsmen with a high level of AAB, changes in the ECG were noted: the interval PQ = 0.10 sec was shortened, with a norm of 0.12 sec (Figure 1). Incomplete blockade of the right Gis beam. The ST segment is higher than the isoline up to 4 mm in the isoline V2-V3, which may indicate a violation of the processes of repolarization of the myocardium of the septum of the left ventricle, probably due to its functional hypertrophy. At the same time, sinus bradycardia with a heart rate of 51 per minute and the absence of complaints of the subjects about the state of the cardiovascular system were recorded.

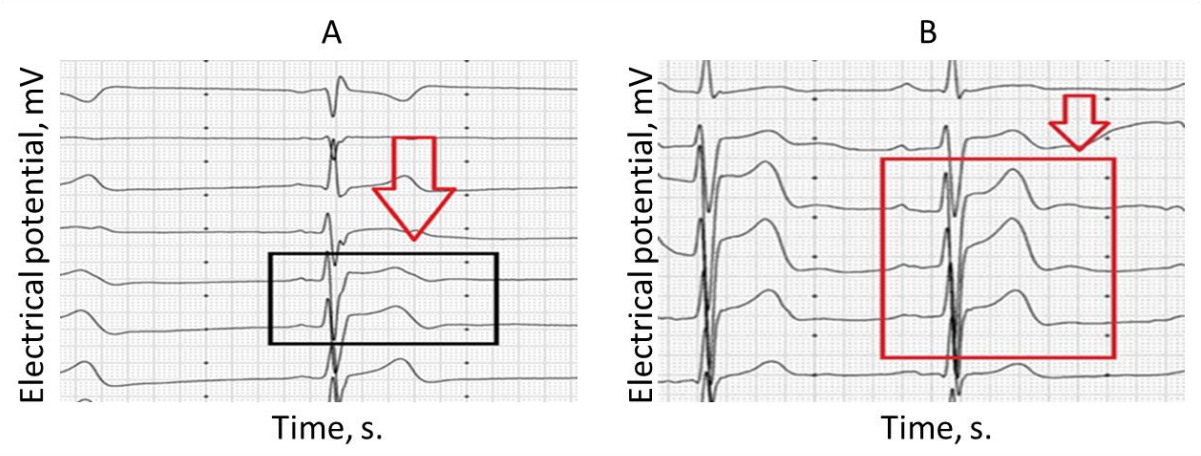

FIGURE 1 Electrocardiogram data. A: Athlete 1, signs of ST segment change up to 4 mm above the isoline in V2-V3 and B: Athlete 2, signs of an increase in the degree of ST elevation, initially 2.5 mm, then 6 mm and tightens the leads V2-V4.

Conclusion

A multiple increase in the content of AAB to myocardial proteins during the intensification period of training loads is recorded in the absence of obvious signs of pathological changes in the heart and complaints from the subject. This indicates the prospects of determining AAB to cardiomyocyte proteins and, first of all, AAB to troponin I as an indicator of early signs of incipient pathological changes in the myocardium. Based on the data obtained during the research, it can be mentioned that cardiospecific immunoglobulins to myocardial proteins are a highly informative diagnostic method in the early diagnosis of functional and structural changes of the heart and can provide significant assistance in situations with an atypical clinical picture and the absence of diagnostic changes on the ECG.

Thus, the results obtained demonstrate the dependence of the quantitative dynamics of AAB on morphofunctional processes occurring in cardiomyocytes in conditions of adaptation to muscle loads of varying duration and intensity. At the same time, it is possible to speak with full confidence about the AAB dynamics to cardiomyocyte proteins as a predictor only with available information about the state of the immune system under conditions of physical overstrain and the possibility of using AAB to cardiomyocyte proteins for the prenosological detection of incipient pathological processes in the myocardium in cases of chronic physical overstrain requires additional research. This is the main limitation of this study and, at the same time, the aim for conducting further research.

Therefore, further work should be aimed at establishing acceptable norms in the content of AAB to specific cardiomyocyte proteins at the stage of intensification of training loads of representatives of various sports, which will allow practitioners to make recommendations in correcting the training process in order to prevent morphofunctional disorders in the athlete's cardiovascular system.

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Authors' Contribution

All the authors took an equal part in the organization of the experiment and the preparation of the manuscript.

Conflict of Interest

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

Ethical Approval

The examinations were conducted in accordance with the standards of good clinical practice and the principles of the Helsinki Declaration of the World Medical Association (WMA Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects, 2013). The work was examined and approved by the Ethics Committee of the North Caucasus Federal University in Stavropol. The preliminary survey made it possible to exclude persons with somatic and acute infectious diseases from participating in the survey during the last month.

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