

Type of training has a significant influence on the GH/IGF-I axis but not on regulating miRNAs

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ABSTRACT: The growth hormone (GH)/insulin-like growth factor-1 axis is responsible for glucose homeostasis. In the present study we assessed the expression levels of miRNA-124, miRNA-210 and miRNA-375 and immunoexpression of IGFBP-3 in relation to the concentrations of IGF-1 and glucose in athletes performing different types of effort. Sixty-six young male athletes (age 25.4±4.1 years) were divided into: group EN (33 male athletes; age 25.6±4.4 years) with endurance-type efforts (disciplines: triathlon, long distance running, cycling) and group ST (33 male athletes; age 25.2±3.9 years) with strength-type efforts (disciplines: weightlifting, body building, CrossFit). The control group consisted of 28 non-training men (age 29.1±4.7 years). Statistically significantly higher IGF-1 concentration and lower glucose concentration ($P<0.05$) in serum were observed in the group of athletes (vs. controls). Immunoexpression of IGFBP-3 was higher in athletes (vs. controls), and a higher value of immunoexpression was obtained in athlete group ST vs. group EN ($P>0.05$). Levels of expression of miRNA-210 and miRNA-375 were higher in athletes vs. controls ($P>0.05$). The obtained data confirmed the importance of the somatotrophic axis in the regulation of metabolic adaptation to physical exercise. The detected variation in the concentrations and expression levels of the studied molecules involved in the somatotrophic axis in athletes confirmed the role of the somatotrophic axis in adaptation to physical effort. Statistically significant reduction of glucose concentration and the highest expression of IGF-1 in serum in athletes suggest the anabolic effect of IGF-1 through insulin receptors on many tissues under the influence of moderate physical exercises (mainly during resistance training).

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INTRODUCTION

The GH/IGF-1 axis is a system of growth mediators, receptors and binding proteins playing an important role in glucose homeostasis, growth of tissue and muscle hypertrophy [1,2]. It was documented that during the training cycle the GH/IGF-1 axis exhibits biphasic behaviour, beginning with a catabolic phase characterized by a reduction in hormone concentrations lasting 3 to 5 weeks, followed by an anabolic phase characterized by an increase in hormone concentrations, after 5 to 6 weeks of training [3,4]. All types of exercise potentially stimulate the secretion of growth hormone (GH) and within approximately 10 to 20 min after onset of exercise, the plasma GH level starts to increase [5]. Insulin-like growth factor-1 (IGF-1), a polypeptide hormone with a homologous structure similar to proinsulin, mediates many of the somatic effects of growth hormone (GH). It is the most important downstream mediator of GH's effect in most

tissue via IGF-1R and the insulin-like growth factor-binding proteins (IGFBPs) [6,7]. IGF-1 is synthesized in the liver and secreted into the blood, where it circulates as complexes associated with specific binding proteins (IGFBPs) [8]. This complex is regulated in both endocrine and tissue-specific auto- and paracrine manners. It was documented that IGFBPs may act as IGF-1 transporting proteins to target cells, but they are also able to modify the interaction between IGF-1 and its cellular receptors. Furthermore, IGFBPs may act independently from IGF-1 [9].

IGF-1 was identified as one of the key mediators of carbohydrate, lipid and protein metabolism, thus maintaining the body's energy balance, which is important for athletes during systematic physical effort [10-12].

So far, six IGF-1 binding proteins (IGFBP-1 to IGFBP-6), which have different affinities for IGF-1, have been well studied. It was confirmed that IGFBP-3 is the main important binding protein for IGF-1 in serum, which may enhance or inhibit IGF activity. [10,13,14]. Interestingly, IGFBP-3 can also bind to cellular targets and directly regulate gene transcription in the nucleus [15].

A high endogenous concentration of IGF-1 may increase insulin sensitivity in healthy persons but also has insulin-like effects on peripheral uptake of glucose and fatty acids [16].

Both insulin and IGF-1 seem to work together to maintain normal glucose levels. It was recognized that IGF-I might exert a coordinating effect on glucose homeostasis, especially in the absence of insulin, e.g. in the fasting state [17]. The mechanism of regulation of insulin and IGF-I-mediated glucose metabolism may be multifactorial. However, it has been documented that short-term (2 weeks) training was unable to reduce the fasting insulin level while a 10–12-month endurance training programme reduced fasting insulin and improved insulin action [18,19].

Physical activity can stimulate growth hormone and IGF-1 secretion and therefore IGF-1 may correlate with intensity of physical exercise [20,21]. According to literature data, duration and intensity of physical exercise and the type of training have a significant impact on IGF-1 concentration. Moreover, they have an impact on the concentration of IGFBP-1 and IGFBP-3 proteins [22]. It has been documented that mainly IGF binding protein 3 and 1 increase (IGFBP1 and 3), while IGF-1 may not change during exercise.

It should be noted that it is difficult to clearly recognize the physiological role of the GH/IGF-1 axis during exercise. So far, widespread individual variation in levels of total IGF-1, “free” IGF-1 (unbound to IGFBPs) and the IGFBPs in healthy persons has been confirmed [8,23]. There are insufficient published scientific data focused on modification of physiological GH/IGF-1/IGFBP molecule concentrations in blood in relation to type of training and/or enhance of exercise performance. It has been confirmed that the maximal oxygen consumption (VO_2 max; aerobic capacity or the maximum ability to

use oxygen) is increased, especially during systematic endurance training. It was confirmed that GH secretion correlates positively with duration of exercise when intensity is constant. A linear dose-response relationship between exercise intensity and the GH secretory response was confirmed, with rising GH release across the range (25 to 175% VO_2 max of lactate threshold) [24].

Recent studies have documented that concentration of growth factors in body fluids may be regulated by miRNAs, which are recognized as gene expression regulators. Especially the IGF1/PI3K/AKT/mTOR signalling pathway, which influences cardiac and skeletal muscle adaptation during exercises, may be regulated by various miRNAs [25]. The level of expression of particular miRNAs varies and may correlate with physiological indicators of adaptation to exercise, depending on the type of training and its duration [26,27]. Some of them – miRNA-124, miRNA-210 and miRNA-375 – may regulate molecules of the GH/IGF-1 axis.

MiRNA-375 is associated with the regulation of insulin secretion and glucose homeostasis. The overexpression of miRNA-375 in pancreatic cells impairs glucose-stimulated insulin secretion [28,29].

MiRNA-210 is involved in mitochondrial metabolism, angiogenesis, response to DNA damage, cell proliferation and apoptosis. Its expression is also induced during hypoxia, which plays an important role in the adaptive response to low oxygen levels [30].

MiRNA-124 stimulates the differentiation of embryonic nerve cells and astrocytes, and is involved in the process of neurodegeneration (Alzheimer’s and Parkinson’s disease). It has been shown to regulate IGFBP-1 and IGFBP-3 activity [28].

The aim of our study was to evaluate the IGFBP-3 immunoexpression level and expression level of miRNAs (miRNA-124, miRNA-210 and miRNA-375) under the influence of physical training.

Assessment of the concentration of IGF-1 binding proteins, in addition to the level of the growth factor (IGF-1) itself, seems to be important in estimating the condition of the athlete’s training preparation as well as in assessing the possible effects of overtraining.

TABLE 1. Selected biological features of study athletes and selected training features.

PARAMETER \bar{x}	Control	Group EN	Group ST
Age [years]	29.07±4.26	25.57±4.38	25.15±3.86
Body height [cm]	181.85±5.94	179.70±4.57	179.00±5.61
Body weight [kg]	89.70±16.50	74.23±9.17	83.21±7.50
BMI [kg/m ²]	26.98±3.86	22.98±2.64	25.84±1.94
Body fat percentage BFP [%]	22.04±6.11	14.57±5.58	14.74±3.93
Number of training units per week [n]	-	5.36±4.21	4.93±4.19
Training experience [months]	-	68.33±1.78	76.36±4.38
Training unit duration [min]	-	90±3.52	90±5.21
Total number of times participating in competitions during last year [n]	-	43.7±3.78	13.3±5.27

MATERIALS AND METHODS*Subjects*

Ninety-four athletes – clinically healthy males aged 18-35 (26.5 ± 4.5 years) – were included in the study and divided into three groups: group EN – 33 athletes with predominant endurance training character of dynamic effort with minimal 2 years experience in one of the following sport disciplines: triathlon, long runs, cycling; group ST – 33 athletes with predominant resistance training character of dynamic effort with minimal 2 years experience in one of the following sport disciplines: powerlifting, Olympic weightlifting, bodybuilding, CrossFit; control – a group of 28 healthy males with average or low physical activity in the same range age.

All the athletes enrolled in the study were in the training cycle lasting for at least 8 weeks. The training cycle consisted of standard training for every individual. The test group subjects had been training regularly for at least two years in one of the sports disciplines (endurance or strength sports). The experiment was performed after the eight-week training cycle.

Selected biological features of study athletes and selected training features are presented in Table 1.

The study was approved by the Medical University of Lodz Ethics Committee (RNN/93/15/KB). All participants gave written informed consent prior to study commencement.

METHODS

Detailed medical examinations were carried out on study participants. An interview was included the history on their sports career and physical activity.

The biochemical and molecular examination was performed after the end of the 8-week training cycle. Participants visited the laboratory in a fasted state and at least 12 h after training and before anthropometric measurements and the cardiopulmonary exercise test (CPET) (Figure 1).

Collection of biological material

Samples for biochemical tests (glucose, insulin, IGF-1 concentrations) were taken on an empty stomach in the morning after qualification by a doctor.

Selected blood samples from all athletes were collected in morning hours after 12 hours overnight fasting at one point. Samples were collected in Eppendorf tubes and left for about 30-45 minutes at 37°C (until clot formation). Then the tube was placed in a refrigerator at a temperature of 4°C for several hours (0.5-4 h) until the total organization of the clot. Next, the tube was centrifuged ($1200 \times g$ 10 min, 4°C), and serum was separated from the clot carefully into a new sterile tube, frozen and stored at -80°C .

Biochemical study

Prepared material was then analysed for selected blood biochemical parameters – concentration of glucose, insulin and insulin growth factor-1 (IGF-1) – using the standard haematology method (Sysmex K-4500) and the Roche COBAS Integra 400 plus analyser. All samples were analysed in duplicate within the same assay. The intra-assay variations were 4.1-5.5 mmol/L for glucose, 2.56-24.9 $\mu\text{U/ml}$ for insulin and 91-355 ng/ml for IGF-1.

Physical fitness tests

For all well-trained males the cardio-pulmonary exercise test (CPET) was conducted and maximal oxygen uptake ($\text{VO}_2\text{max/kg}$) and VO_2/kg at anaerobic threshold (VO_2/kg at AT). The test was conducted on Schiller Cardiovit CS-200 mechanical treadmill kit according to the modified Costill-Fox protocol [31,32]. To establish maximal effort achieved during the treadmill test, we modified the protocol to be more versatile for tested subjects representing different sport disciplines [33]. The modified test began with a 1-min warm-up stage at 0% grade and minimal speed and was continued for seven 3-minute stages during which the speed and incline were increased respectively

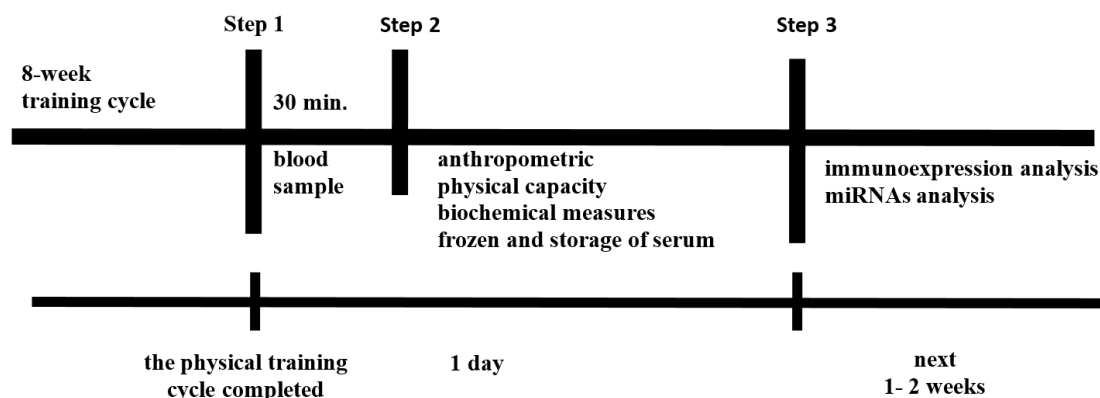


FIG. 1. Study design diagram.

to maximal 24% of incline and 11.6 km/h speed. The protocol ended with an additional 3-min stage for those most trained athletes who did not achieve maximal effort. For them the incline was established at 16% and the speed increased from 16.1 km/h to the moment they could no longer continue the test and grasped the front handrail support. The intensity of the effort yielded was evaluated by measuring the heart rate (HR) and metabolic equivalents (METs).

Anthropometric measurements

Anthropometric body weight, body height, BMI and body fat percentage (BFP) measurement from four skin folds, according to Durnin-Womersley, were collected by standard methods on certified scales [34].

Molecular study

RNA extraction and reverse transcription

All molecular and statistical analyses were conducted at the Medical University of Lodz. RNA was extracted from serum exosomes using the Total Exosome Isolation Reagent (from serum) and Total Exosome RNA & Protein Isolation Kit (Applied Biosystems, USA), according to the manufacturer's instructions, with a starting volume of 200 μ l.

From the resulting RNA eluate, complementary DNA (cDNA) was transcribed from 1000 ng of total RNA, using a high-capacity TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) using 5 μ l of template RNA. cDNA products were diluted to 250 μ l with double-distilled water and loaded on plates for storage at -20°C until further analysis.

miRNA analysis

cDNA was transcribed from 10 ng of total RNA using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) in a total volume of 15 μ l according to the manufacturer's protocol. The relative expression of miRNAs was assessed by qPCR reactions using TaqMan probes for the studied miRNA (Applied Biosystems, Carlsbad, CA) according to the manufacturer's protocol. The following microRNA probes were used for the study:

miR-124 (CGUGUUCACAGCGGACCUUGAU),
hsa-miR-210 (CUGUGCGUGUGACAGCGCUGA),
hsa-miR-375 (UUUGUUCGUUCGGCUCGCGUGA).

The PCR mixture contained: 1.0 μ l of TaqMan MicroRNA Assay (20X) 1.33 μ l of product from RT reaction, 10.00 μ l of TaqMan 2X Universal PCR Master Mix, No AmpErase UNG, and 7.76 μ l of nuclease-free water (Applied Biosystems, Carlsbad, CA). The plates were placed in the Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA) according to the manufacturer's protocol. The expression levels (RQ values) of the studied miRNA were calculated using the delta delta CT method.

Immunoexpression analysis

Immunoexpression analysis was performed using commercial ELISA kits for IGF1 (Cloud-clone Corp, USA). The intensity of the final colorimetric reaction, in proportion to the amount of protein bound,

was measured in a plate reader (ELx800, BioTek) at 450 nm. The obtained results were compared to the standard solution of concentrations (0.156–10 ng/ml).

Statistical analysis

The Mann–Whitney *U* test was used to evaluate differences between relative expression level (RQ values) of the miRNAs, immunoexpression level of the protein IGF1, HR, VO_2 max/kg values, VO_2 /kg at AT, and concentrations of glucose, insulin and IGF1. Spearman's rank correlation coefficient was used to evaluate the relationships between resting HR, VO_2 max/kg values, VO_2 /kg at AT, BFP, concentration of glucose, insulin and IGF1, expression levels of the miRNAs and immunoexpression levels of IGF1. Multivariate analysis of MANOVA the parametric one-dimensional analysis of ANOVA, Fisher's test *F*, The Tukey test were used to identify differences between resting HR, VO_2 max/kg values, VO_2 /kg at AT, BFP, concentrations of glucose, insulin and IGF1, expression level of the miRNAs and immunoexpression level of IGF1. The value of $P < 0.05$ was considered statistically significant. In this study all statistical analyses were performed using the Statistica 12.0 program and in the statistical environment R v.3.5.3.

RESULTS

The average values of selected performance parameters – heart rate (HR), oxygen uptake (VO_2 max/kg), VO_2 /kg at AT (maximal oxygen uptake measured at anaerobic threshold) – and biochemical parameters – glucose, insulin and IGF-1 concentration – at the studied time point were calculated (Figures 2 and 3).

Statistically significant greater VO_2 max/kg, VO_2 /kg at AT and IGF-1 concentration and lower HR and glucose were observed in the group of athletes compared to the control ($P < 0.05$, Mann–Whitney *U* test) (see Figure 2).

We also observed statistically significantly higher VO_2 max/kg in group EN vs. group ST and lower HR in group EN vs. group ST ($P < 0.05$, Mann–Whitney *U* test) (see Figure 3).

Relative expression levels of miRNAs in athletes vs. control group

In both studied groups (athletes vs. control), the expression of miR-210 and miR-375 was overexpressed in relation to the calibrator (RQ > 1). In the case of miR-124, the RQ value was under the level of detection. The reporter's fluorescence phase was below the detection of cDNA amplification detection threshold compared to the control (calibrator).

The expression levels (mean RQ) of both studied miRNAs were higher in athletes vs. control, but the differences were not statistically significant ($P > 0.05$, Mann–Whitney *U* test) (see Table 2).

Relative expression levels of the miRNAs (miR-210, miR-375) in athlete group according to type of training

In both groups EN and ST miR-210 and miR-375 were overexpressed according to the calibrator (RQ > 1). We observed higher expression levels (RQ) of miRNAs in group ST than in group EN, but the differences were not statistically significant ($P > 0.05$, Mann–Whitney *U* test).

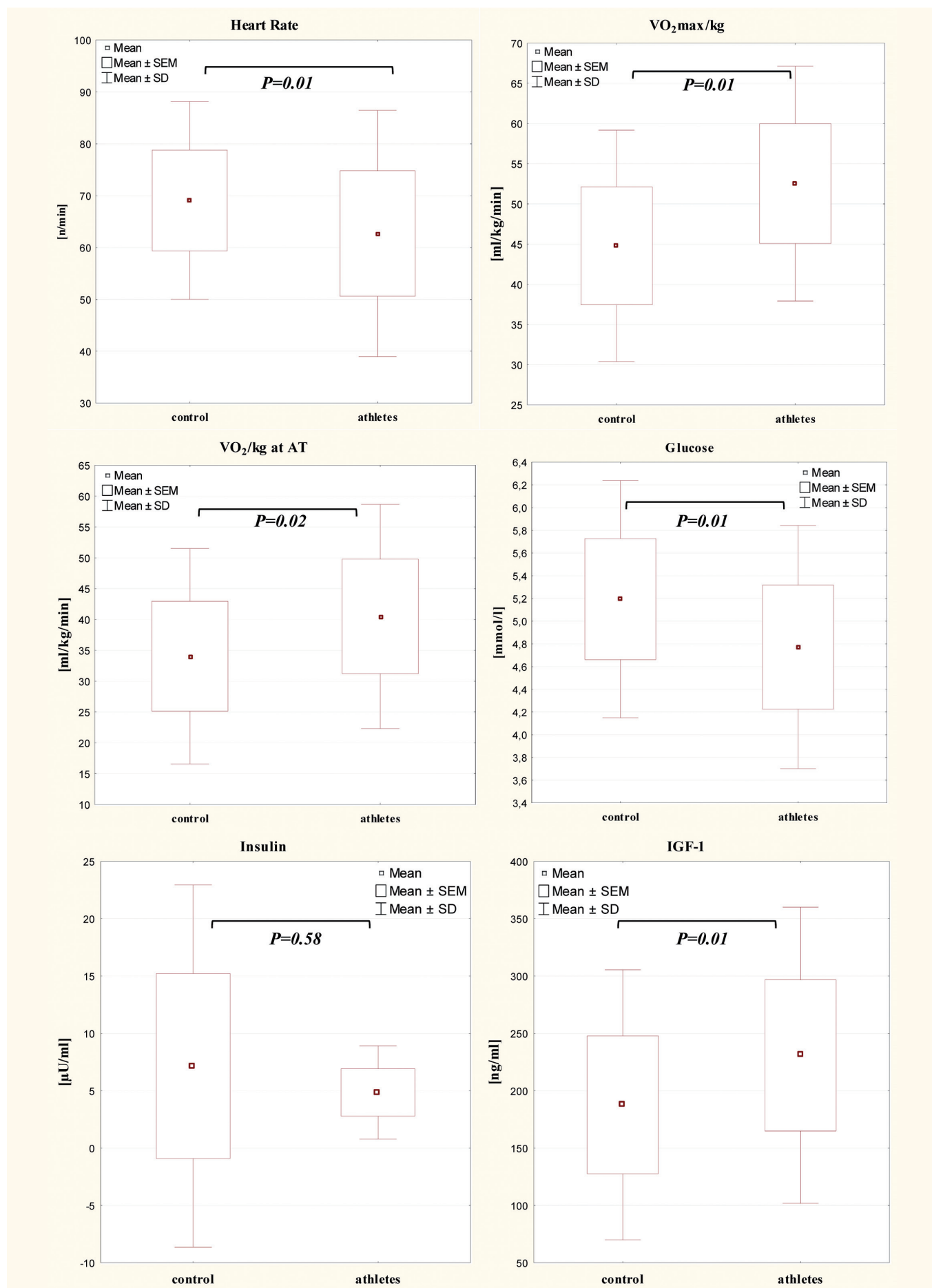


FIG. 2. HR, VO₂max/kg, VO₂/kg at AT, glucose, insulin, IGF-1 in athletes compared to controls (Mann-Whitney U test).

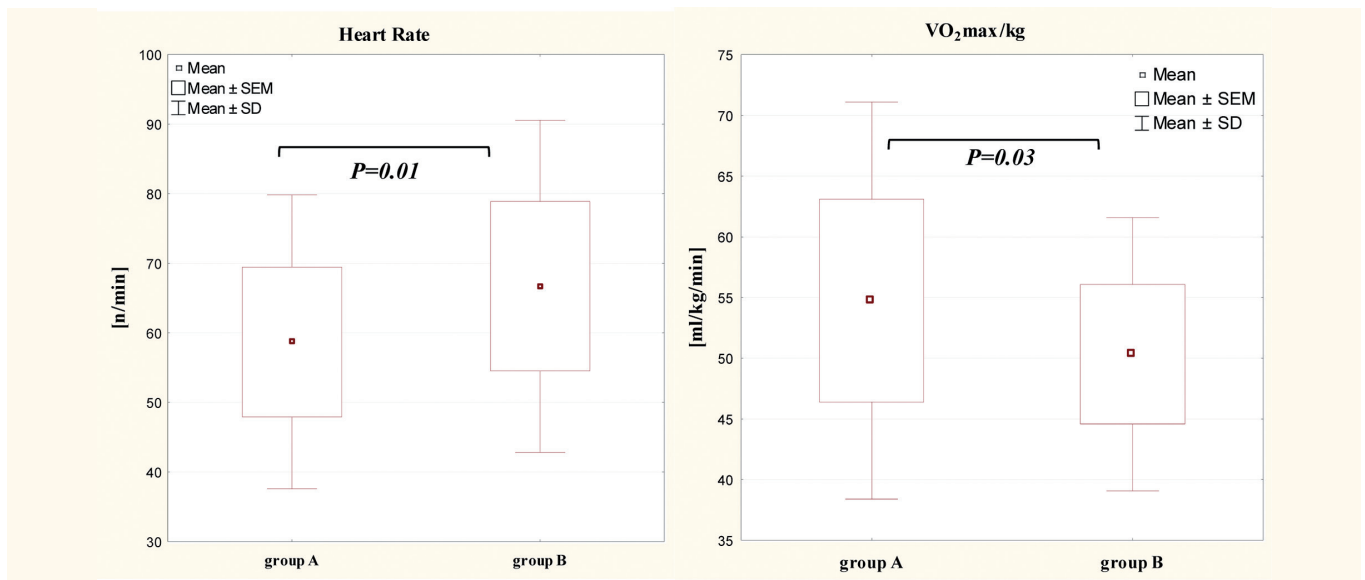


FIG. 3. HR, VO₂max/kg in group EN (group A) vs. group ST (group B) (Mann–Whitney U test).

TABLE 2. Expression levels (mean RQ value) of studied miRNAs in athlete and control groups.

	Mean RQ value in athletes	Mean RQ value in controls	P
miR-210	1.84	1.01	0.38
miR-375	1.83	1.51	0.33

TABLE 3. Expression levels (mean RQ value) of studied miRNAs in athletes: group EN vs. group ST.

	Mean RQ value		P
	Group EN	Group ST	
miR-210	1.65	2.03	0.50
miR-375	1.37	2.30	0.28

TABLE 4. Immunoexpression levels of IGFBP-3 (ng/ml) in athletes: athletes vs. control.

	Mean concentration (ng/ml)		P
	Athletes	Control	
IGFBP-3	3.67 ± 1.97	3.22 ± 2.39	0.81

TABLE 5. Immunoexpression levels of IGFBP-3 (ng/ml) in athletes: group EN vs. group ST.

	Mean concentration (ng/ml)		P
	Group EN	Group ST	
IGFBP-3	3.31 ± 1.51	3.63 ± 2.03	0.50

Additionally, higher expression of miR-375 was noticed in ST group, compared to EN, and this differences were higher than in the case of miR-210, although without statistical significance ($P > 0.05$, Mann–Whitney U test); see Table 3.

Immunoexpression levels of the studied protein IGFBP-3 in serum of athletes vs. controls

In the studied groups (athletes vs. controls), the immunoexpression of IGFBP-3 revealed values of 3.67 ng/ml vs. 3.22 ng/ml. The difference was not statistically significant ($P > 0.05$, Mann–Whitney U test) (see Table 4).

Immunoexpression levels of the studied protein IGFBP-3 in serum according to type of training

We observed higher immunoexpression levels of IGFBP-3 in athletes of group ST compared to group EN, but without statistical significance $P > 0.05$, Mann–Whitney U test) (see Table 5).

In addition, a correlation analysis between the parameters resting HR, VO₂ max/kg, VO₂/kg at AT, BFP, concentrations of glucose, insulin and IGF1, expression levels of the miRNAs (miR-210, miR-375) and immunoexpression levels of IGFBP-3 was performed (Spearman's rank correlation).

We found several positive and negative correlations between the several biochemical, physical and molecular parameters (miRNA, mean RQ value) of studied miRNAs in all study groups: athletes (group EN and group ST) and the control group. Table 6 shows the correlation values for those parameters for which statistically significant results were obtained ($P < 0.05$).

In the control group, statistically significant increase of IGF-1 concentration in correlation with reduced IGFBP-3 immunoexpression

TABLE 6. Correlations between biochemical, physical and molecular parameters in all study groups; Spearman's rank correlation.

Type of training	Parameter	Parameter	Rho value	P value
control	IGF-1	IGFBP-3	-0.44	0.01
	IGF-1	BFP	-0.33	0.01
	HR	BFP	0.41	0.01
	miRNA-210	miRNA-375	-0.64	0.0001
group EN	IGFBP-3	BFP	0.33	0.01
	IGFBP-3	HR	0.487	0.004
	IGFBP-3	VO ₂ max/kg	-0.41	0.018
	VO ₂ max/kg	BFP	-0.80	0.0001
group ST	IGFBP-3	VO ₂ max/kg	0.49	0.0037
	IGFBP-3	BFP	-0.31	0.01
	VO ₂ max/kg	BFP	-0.46	0.001
	miRNA-210	miRNA-375	-0.50	0.001

and BFP was confirmed. However, with the increase of the BFP we observed a statistically significant increase of the HR value. Additionally, the strongest negative correlation in the control group between the expression level of miRNA-210 and miRNA-375 was shown, similar results in the ST group were observed.

In the EN group a significant increase of IGFBP-3 immunoexpression with an increase of BFP and HR values was observed. In contrast, the value of VO₂max/kg significantly decreased in correlation with IGFBP-3, while in the ST group VO₂max/kg positively correlated with IGFBP-3. Among EN group athletes, the increase in the value of VO₂max/kg was strongly associated with a decrease of BFP, as in the ST group, but the strength of the relationship in the ST group was moderate (see Table 6).

Multivariate analysis of variance between values of parameters: resting HR, VO₂max/kg, VO₂/kg at AT, BFP, concentrations of glucose, insulin and IGF-1, expression levels of miRNAs and immunoexpression levels of IGFBP-3

Multivariate analysis of variance (MANOVA) revealed significant differences in the values of the following parameters: resting HR, VO₂max/kg, VO₂/kg at AT, BFP, concentrations of glucose, insulin, IGF-1, expression levels of miRNAs (miR-210, miR-375) and immunoexpression levels of IGFBP-3 between the three groups: control group, group EN, group ST. The Wilks test statistic was 0.6041 at $P < 0.001$. Box plots are presented in Figure 4.

Analysis of variance (ANOVA) and Fisher's F test were carried out and revealed significant differences in group variables for resting HR ($P = 0.0009832$), VO₂max/kg ($P < 0.001$), VO₂/kg at AT ($P = 0.002285$), BFP ($P < 0.001$), and immunoexpression level (mean value) of IGF-1 ($P = 0.006774$). For the variables miR-210

(RQ) ($P = 0.2174$), miR-375 (RQ) ($P = 0.4474$), IGFBP-3 ($P = 0.7082$), concentration of glucose ($P = 0.3569$) and insulin ($P = 0.5342$), there were no statistically significant differences.

Tukey's test confirmed significantly higher levels of resting HR in group ST compared to the control group ($P = 0.0011782$). A significantly higher level of resting HR in group ST compared to group EN was observed ($P = 0.0110396$).

Additionally, Tukey's test confirmed significantly higher levels of VO₂max/kg in group EN ($P = 0.0000018$) and for group ST ($P = 0.0101376$) compared to the control group. A significantly higher VO₂max level in group EN vs. group ST was found ($P = 0.0387144$).

Tukey's test confirmed a significantly higher level of BFP for the control group in comparison to group ST ($P = 0.0000018$) and to group EN ($P = 0.0000009$) and a lower IGF-1 level in the control group compared to group ST ($P = 0.0077$).

DISCUSSION

It has been suggested that serum IGF-1 concentration and IGFBP-3 immunoexpression are sensitive markers of training status [35]. Therefore it was proposed that they may be used as guides for training monitoring and modulating its intensity [36]. The results from this study demonstrate that IGF-1 concentration may be used as a sensitive marker of acute and chronic effects of training. It was documented that the catabolic phase, followed by anabolic, was characterized by a reduction of IGF-1 concentrations in serum during the intensive stage of activity. The anabolic phase was marked by a significant increase in serum IGF-1 concentrations in the post-training stage, but short intensive aerobic activity (above the anaerobic threshold) may significantly increase the IGF-1 level. Our

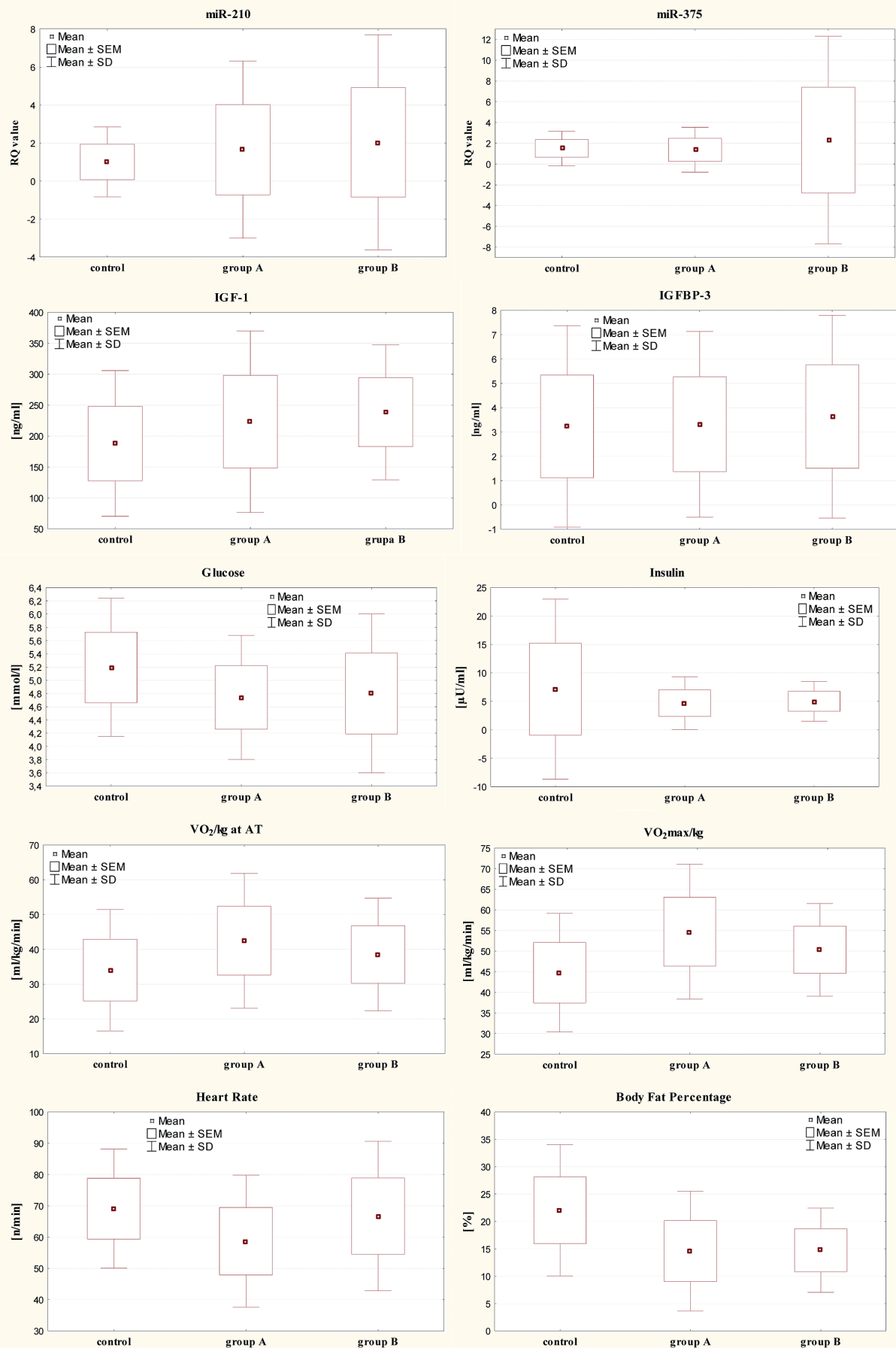


FIG. 4. Box plots presenting differences in mean values of tested parameters (miR-210, miR-375, IGF-1, IGFBP-3, glucose, insulin, VO₂max/kg, VO₂/kg at AT, HR, BFP) in individual groups: control vs. EN (group A) vs. group ST (group B).

results showing an increase in IGF-1 and especially in athletes after resistance exercise suggest that isometric/eccentric/concentric exercises cause an increase in IGF-1 not only locally in muscle but also in serum. Moreover, our data supported the hypothesis that the ability to increase circulating IGF-1 concentration correlates with athletes' accurate physical condition [37]. Interestingly, IGFBP-3 was the only sensitive marker of training effects, with a reduction in serum concentrations during the post-training intensive stage and an increase during the tapering stage of training. Moreover, increases of IGFBP-3 concentration in serum may correlate with increases in peak and average force as a chronic effect of exercising [36]. Our data confirmed that the increased immunoexpression of IGFBP-3 may suggest the chronic effect of exercises. What is more, the increased immunoexpression of IGFBP-3 suggests the best athletic performances and deficiency of high fatigue of athletes in our study group. In a different study on rugby players a strong negative correlation between IGF-1 and IGFBP-3 was observed. In particular, significant reduction in IGFBP-3 concentration in serum after the match was observed in more fatigued, overtrained sportsmen [35].

In our study we evaluated the heart rate (HR) as one of the main parameters of physical fitness and we observed statistically significantly lower HR in the group of athletes compared to the control group and statistically significantly higher HR in the group of resistance training athletes. These data are consistent with the statement that endurance-trained athletes have a decreased submaximal exercise HR due to reduced sympathetic activity for a given submaximal work rate [38]. Also in our study we evaluated aerobic endurance (VO_{2max} ; maximal oxygen uptake), which is important in aerobic cardiovascular endurance monitoring. We observed a statistically significantly higher VO_{2max}/kg level in athletes compared to the control group but we also observed significantly higher aerobic endurance in the group of endurance training athletes. Interestingly, the published results in this area seem to be controversial [39]. Most of the previous studies confirmed the positive effects of training on the VO_{2max} kinetics during interval or endurance exercises [40]. Indeed, mainly the endurance training of running athletes was associated with a significant increase of aerobic endurance or increase in running performance as a result of the simultaneous strength and endurance improvement [41]. In our study we observed an increase of VO_{2max} in training athletes and a higher level of VO_{2max} in the endurance training group. Moreover, our resistance training athletes improved their aerobic endurance. However, in contrast, Kraemer et al. (1995) reported that aerobic athletes who followed an intense resistance programme (for 3 months) did not improve VO_{2max} [42]. All considered, it is difficult to conclusively determine the effectiveness of different types of training on VO_{2max} evaluation. Moreover, in our opinion, resistance training is effective in increasing VO_{2max} independently of the protocol, but the training programme should last many weeks (about 20) at high intensively (60-90%). Our athletes trained for 8 weeks, which was too short to significantly improve their aerobic fitness.

So far it is well known that regular physical activity influences tissue adaptation to exercises, mainly skeletal, muscle, circulatory and respiratory systems, affecting health. However, the mechanisms of molecular adaptation to exercise are insufficiently understood. Recently, the role of miRNAs as potential regulators of training response in the human body has been widely discussed [43-46]. MicroRNAs (miRNAs) regulate post-transcriptionally gene expression and are involved in the regulation of a wide range of molecular targets (i.e. genes, other miRNAs, transcription factors or proteins), and biological processes: mitochondrial metabolism, cell lipid and glucose metabolism and energy homeostasis [47,48]. Some types of circulating miRNAs, and myomiRs, identified in cardiac and skeletal muscle, have been under consideration as molecular biomarkers of physical adaptation to activity related to the type of training [43,45,49]. Moreover, c-mRNAs are well documented as regulators of physiological processes under aerobic and resistance exercises [50-52].

In our study we chose miRNAs (miR-124-3p, miRNA-210 and miRNA-375) which are related to numerous biological and cellular processes involved in adaptation to physical activation. Firstly we evaluated the miR-124-3p expression level, but we did not detect its expression at any time point during our experiment. Our observations are consistent with the study of another author who also found that miR-124-3p remained undetectable [53]. Interestingly, this undetectable miRNA is associated with bone formation and turnover and may regulate bone turnover markers (e.g. osteocalcin) and DKK1 (Dickkopf-related protein) [54]. Remarkably, bone metabolic markers may be minimally disturbed during brief exercises, which can be associated with low/undetected levels of their regulators. Another miRNA researched by us –MicroR-210 –is recognized as hypoxia-induced [55]. It influenced mitochondrial metabolism, cell apoptosis and erythropoiesis regulation [56,57]. In an animal model it was demonstrated that expression of miR-210 in rat cardiac tissue (in myocytes) increased in a group of animals subjected to 8 weeks training of voluntary exercise. Profiles of circulating miRNAs vary under different exercise conditions and types of training [58]. In our research we observed non-significantly increased expression of miRNA-210 in the group of athletes and higher miR-210 expression level in resistance training athletes. In contrast, Li et al. documented decreased circulating miR-210 in response to acute exercise in basketball athletes [55]. Our data were partially confirmed by others who observed a non-significant exercise-dependent increase of miR-210 expression level immediately following exhaustive exercises and no change in the post-training resting period [50]. On the other hand, strong miR-210 upregulation was observed in rats undergoing physical exercises [58]. The proposed mechanism of this observation is induction of miR-210 via HIF-1 during exercise, mainly in cardiac muscle but also in circulation. Moreover, circulating miR-210 levels were most strongly increased in healthy women with low VO_{2max} [30,59]. Supposedly growing release of miR-210 from the endothelium in people with low VO_{2max} may be associated with subclinical or persistent chronic cardiovascular diseases or pathological processes

(atherosclerosis, hypoxia, myocardial infection, diabetic ischaemic heart failure or inflammation) [60-62]. In our study, we observed that resistance exercises may increase more effectively the circulating miRNA-210 expression level. Resistance exercise finally may initiate hypoxia-induced changes (HIF-1/VEGF pathway) in skeletal and cardiac muscle metabolism and in circulation (vessels' endothelium), influencing maximal oxygen uptake (VO₂max). Surprisingly, in our study we did not confirm the relationship between miR-210 expression level and VO₂max in either study group.

Additionally in our studies, we focussed on miRNA-375. This miRNA takes part in regulation of insulin secretion, and is proposed as a significant determinant of glucose homeostasis. It was demonstrated that miR-375 is implicated in pancreatic β -cell development and its deletion influences loss of pancreatic β -cell mass [63,64]. In human pancreatic islets insulin secretion positively correlated with miR-375 expression level [65]. Based on mentioned data and the conducted research, it is confirmed that all types of physical exercises, including resistance exercises, decrease blood insulin levels. Our data confirmed a published study on insulin concentrations in active persons [66]. We observed a decrease in insulin concentration in blood of our athletes compared to controls, but higher insulin concentration was observed in resistance training athletes. Moreover, our observation of a higher expression level of miR-375 with a simultaneous decrease in insulin levels in athletes in both groups may be interesting. We also observed a higher expression level of miR-375 in serum of athletes from the group training with resistance-type exercises. Unfortunately, we cannot discuss this result because, to our

knowledge, there are no similar published data on this subject. However, we confirmed a possible association of miRNA-375 with the regulation of insulin secretion under physical exercises; therefore we also confirmed the role of miRNA-375 in glucose homeostasis. Our data may partially correlate with research of Keller *et al.*, who demonstrated that antagonized action of miR-375 could potentially enhance in diabetes patients the effects of exendin-4, (homolog of glucagon-like peptide-1), which revealed anti-diabetic actions [67,68].

CONCLUSIONS

Evaluation of the protein concentration of IGFBP-3 and the concentration of IGF-1 as well as the expression levels of miR210 and miR-375 seems to reflect exercise adaptation at the cellular level. Increased levels of IGF-1 and IGFBP-3 in athletes who did not present the effect of overtraining indicate the protective effect of growth factors and proteins binding these factors on the athlete's body. The obtained results confirm the importance of the somatotropic axis in the regulation of the metabolic adaptation process to physical effort.

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Conflict of interest declaration

The authors declare that there is no conflict of interest for this manuscript.

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