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Association between expression level of the miR-320, miR-182, miR-223 and miR-486 and body composition among young Polish female volleyball players

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Abstract: The expression of circulating microRNAs appears to be a promising indicator of physical strength. The objective of this study was to determine whether there is an association between the expression level of four selected microRNAs and body composition over time among young female volleyball players. Blood samples and body composition measurements were taken from 7 females who are Polish volleyball players before and after 5 matches played out between the years 2017 and 2018. The blood spots were used to assess the expression of four microRNAs: miR-320, miR-182, miR-223, and miR-486. Fat mass, PFB% and BMI were positively correlated with expression level (exp.l) of miR-182. The miR-320 the exp.l was positively correlated with muscle mass and TBW. There were inverse correlations between miR-486 exp.l and PBF%, as well as between miR-486 exp.l and body mass, muscle mass, TBW, FFM, and BMR. Conversely,

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there were positive correlations between miR-486 exp.l and body mass and fat mass. The miR-182 may be positively correlated with fat tissue, miR-320 was positively correlated with muscle mass, and miR-486 was negatively correlated with fat mass. Overall, our study shows that the expression of miR-182, miR-320, and miR-486 is associated with body composition. The results of our study also suggest that exercise may decrease the level of miR-486.

Key words: body composition, microRNA, epigenetic, volleyball.

Introduction

MicroRNAs are small noncoding molecules which may influence expression of various genes causing various phenotypic changes (Großhans and Filipowicz 2008). From a methodological perspective, microRNAs appear to be stable, reliable sources of biological material for epigenetic analysis (Jung et al. 2010).

To the best of our knowledge, there is a lack of research that addresses the relationship between microRNAs and physical activity over an extended period of time. The longitudinal effect of microRNAs on IGF1 and muscle tissue is not yet known. The miR-320, miR-182, miR-223 and miR-486 seem to be important molecules associated directly and indirectly with IGF1 and muscle tissue. The regulation of IGF is important for sport achievements due to maintaining processes of cells proliferation and differentiation, energy metabolism and glucose homeostasis (Jung and Suh, 2015). Consequently, alterations in selected miRNAs may prove pivotal in monitoring physical condition and athletic performance.

The microRNA (miRNA) miR-320 is associated with glucose levels. Increased levels of glucose concentration are associated with decreased level of miR-320 expression. The downregulation of miR-320 has been observed in individuals with diabetes (Zampetaki et al. 2010). Moreover, there is a link between the expression of miR-320 and muscle tissue. In diabetic rats, the expression level of miR-320 was increased in cardiac microvascular cells (Wang et al. 2009). Furthermore, Yerlikaya and Mehmet (2019) demonstrated that high-fat and high-sucrose diets resulted in the downregulation of miR-320. In a 2018 study, Munetsuna and colleagues (2018) observed a correlation between miR-320 expression and fat tissue and BMI. They also noted a significant decrease in miR-320 levels among Japanese adults with excess body fat.

Olivieri et al. (2014) reported significantly lower levels of miR-182 and miR-223 in muscle samples during periods of enhanced IGF-1 signaling among monozygotic twin pairs. As reported by Zhang et al. (2016), miR-182 is associated with glucose metabolism and muscle tissue. The authors showed that in murine models, a reduction in miR-182 expression results in alterations in muscle fiber composition and glucose metabolism.

Aoi et al. (2013) showed that a reduction in miR-486 may be associated with metabolic changes during physical activity, which are influenced by training. The miR-486 is encoded by ankyrin and myosin heavy chain, which are muscle-specific genes (McCarthy et al. 2009; Small et al. 2010). It has been observed that exercise may alter the concentration of miR-486, which may result in phenotypic changes (Safdar et al. 2009; Nielsen et al. 2010). Furthermore, Prats-Puig et al. (2013) demonstrated that miR-486

was upregulated in prepubertal obese children, which may be associated with a reduction in physical activity. It is important to note that the expression of certain microRNAs can be influenced by the presence or absence of physical activity. This underlying aspect forms the foundation of our investigation.

The objective of this pilot study was to assess the association between the expression levels of four microRNAs (miRNAs) that have been linked to insulin-like growth factor I (IGF-I) factor, strength, and muscle structure. In addition, we aimed to determine the association between the selected microRNAs and body composition among young Polish female volleyball players during the seasonal matches of the volleyball academic league.

Material and methods

Blood samples were taken from 7 Polish volleyball female players of volleyball academic league before and after 5 matches played between November 2017 and November 2018. The blood spots were taken from fingertips and collected using the Whatman™ FTA™ classic cards from each player, approximately 2 hours before and after each match, by a professional medical nurse. In addition, all players underwent body composition analysis before each of five matches (11.11.2017, 18.11.2017, 2.12.2017, 16.12.2017, 10.11.2018) using the InBody 230 BIA analyzer. Several parameters were recorded: body mass, muscle mass in kilograms, fat mass in kilograms, total body water (TBW), fat free mass in kilograms (FFM), percentage of body fat (%PBF), waist-to-hip ratio (WHR) and basic metabolic rate (BMR). The BMI was calculated based on the measure of height and weight [kg/m^2]. In total, 70 measurements were taken by a professional anthropologist.

The study was approved by Senate's Ethic Committee of Scientific Research at University School of Physical Education in Wroclaw 4/2020.

Laboratory work

Expression of selected microRNAs was associated with IGF1 and muscle tissue according to current literature (Tab. 1).

To isolate microRNAs RNA/miRNA the Purification kit was used (EURx) according to modified protocol by Skonieczka et al. (2016). To perform reverse transcription miRCURY LNA RT Kit was used. The analyses were done according to protocol supplied by the producer using in every sample 6 ng/ml of RNA. The reactions were performed in thermocycler Labcycler 48 (SensoQuest) according to reaction parameters supplied by

Table 1. Characteristic of the included microRNAs

MicroRNA	Description
miR-182	diminishing miR-182 expression enhances IGF-1 signaling in skeletal muscle (Olivieri et al. 2016)
miR-320	inhibition of miR-320 expression significantly increased IGF-1 and IGF-1R mRNA levels (Song et al. 2016)
miR-486	Correlation with VO2max R=0.58 (Aoi et al. 2013), downregulation during exercise (Xu et al. 2014)
miR-223	Up-regulation during exercise (Xu et al. 2014)

kit producer. Further, we diluted the obtained cDNA according to protocol 1:50 using RNAse DNAse free water. During the final step qPCR was performed for each sample in duplicates and each microRNAs (using proper starters: hsa-miR-223-3p miRCURY LNA miRNA PCR Assay; hsa-miR-182-3p miRCURY LNA miRNA PCR Assay; hsa-miR-320-3p miRCURY LNA miRNA PCR Assay, hsa-miR-486-3p miRCURY LNA miRNA PCR Assay) using Real-Time PCR Rotor-Gene Q 5-plex HRM (Qiagen) containing reaction mix micRCURY LNA SYBR Green PCR kit (Qiagen) according to the protocol. The final results were obtained using the Rotor-Gene Q Series Software 2.1.0 which calculated Ct for each sample. The sample over Ct=39 cycles were precluded from the further analyses. The spike in kit was used to normalize obtained microRNAs expression level. The dCt method was used to make final calculation using the following formula: 2-(mean CtmiR- mean Ct of reference) (Livak and Schmittgen, 2001). Due to insufficient quality of the analysis, 8 probes (3 of mirR-182 and 5 of miR-320) were unaccounted for in further calculations.

Statistical analysis

Differences in the level of expression of each microRNAs before and after each of five subsequent matches in 7 players were assessed by the Wilcoxon matched pair test, nonparametric equivalent to t-student test for dependent samples. The Spearman Rank correlation was applied to examine the relationship between each body composition parameter and level of expression of microRNAs before and after five subsequent matches. All the calculations were performed using the Statistica 13.1 software (Dell Inc. 2016).

Results

Descriptive statistics of microRNA before and after five successive matches are presented in Table 2. Changes of all body composition parameters in five successive matches are shown in Table 3. The Spearman Rank test showed (Tab. 4) that fat mass was positively correlated with expression level of miR-182 after the 3rd match (R=0.857; p=0.0137). Moreover, before the 5th match the level of miR-182 was positively correlated with BMI (R=0.786; p=0.0362) and after the match positively correlated with PBF% (R=0.829; p=0.0416). In the case of miR-320 the expression level after 5th match was positively correlated with muscle mass (R=0.786; p=0.0362) and TBW (R=0.786; p=0.0362).

There were statistically significant and negative correlations between miR-486 expression level before the 3rd match and PBF% (R=-0.857; p=0.0140); between miR-486 expression level after the 3rd match and body mass (R=-0.786; p=0.0362), muscle mass (R=-0.929; p=0.0025), TBW (R=-0.929; p=0.0025), FFM (R=-0.929; p=0.0025) and BMR (R=-0.929; p=0.0025). Moreover, after the 4th match there were statistically significant and positive correlations between miR-486 expression level and body mass (R=0.786; p=0.0362) and fat mass (R=0.786; p=0.0362).

The Wilcoxon test showed that the level of expression of miR-320 after 1st match was significantly (although marginally) lower (Z=2.028; p=0.0425). In the case of miR-486 the level of expression was statistically significantly lower after the 4th match (Z=2.028; p=0.0425).

The micro-RNA 486 had, although nonsignificant, lower level after each match (Z=1.769; p=0.0769) (Fig. 1).

None of the forward stepwise multiple regression models for muscle mass were statistically significant.

Table 2. Descriptive statistics of each microRNA expression level among players before and after successive matches

		DeltaCT before			DeltaCT after		
microRNA	Players	N	mean	SD	N	mean	SD
182	P1	4	-12.55	4.28	5	-12.45	7.19
	P2	5	-13.06	1.51	5	-13.00	7.20
	P3	5	-10.99	3.32	5	-13.89	4.15
	P4	5	-13.41	2.74	5	-13.29	6.09
	P5	5	-11.18	5.32	5	-11.03	2.63
	P6	4	-16.87	4.11	5	-11.44	7.73
	P7	5	-10.47	7.26	4	-11.02	5.86
223	P1	5	-9.54	2.12	5	-10.14	2.09
	P2	5	-8.98	2.80	5	-10.76	2.59
	P3	5	-10.62	0.79	5	-9.62	0.77
	P4	5	-9.39	0.85	5	-10.07	1.01
	P5	5	-9.01	1.26	5	-8.01	1.46
	P6	5	-12.53	7.00	5	-10.28	2.70
	P7	5	-10.90	2.57	5	-13.95	4.38
320	P1	5	-14.45	2.74	4	-15.94	3.55
	P2	5	-11.91	2.69	5	-14.48	3.47
	P3	5	-13.14	1.68	5	-14.11	1.81
	P4	5	-15.78	2.31	4	-13.17	1.93
	P5	5	-11.24	2.57	5	-12.21	1.80
	P6	4	-15.82	5.31	4	-16.51	5.91
	P7	5	-14.36	3.47	4	-14.01	1.08
486	P1	5	-11.18	1.02	5	-14.79	2.67
	P2	5	-12.44	3.98	5	-12.14	5.68
	P3	5	-11.82	2.14	5	-12.75	1.98
	P4	5	-11.33	1.45	5	-12.99	2.44
	P5	5	-12.54	1.86	5	-10.27	3.38
	P6	5	-10.16	5.60	5	-13.67	2.50
	P7	5	-13.54	2.15	5	-14.73	2.84

Table 3. Descriptive statistics of parameters of body composition by five successive matches in all players

matches		Body mass (kg)		Muscle mass (kg)		Fat mass (kg)		TBW		FFM		BMI		%PBF		WHR		BMR	
	N	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
I	7	70.67	15.62	29.60	5.83	17.43	5.77	38.89	7.19	53.24	9.94	23.23	2.55	24.26	2.54	0.86	0.04	1520.00	215.17
II	7	70.49	16.60	30.56	6.20	15.74	6.41	39.96	7.66	54.74	10.67	23.13	2.80	21.71	3.89	0.86	0.04	1552.71	230.20
III	7	69.84	16.74	30.66	6.18	15.01	7.37	40.03	7.60	54.83	10.59	22.91	2.87	20.70	6.20	0.85	0.05	1554.29	228.62
IV	7	69.90	16.72	30.73	6.51	14.94	6.47	40.13	7.87	54.94	10.91	22.94	2.88	20.71	4.21	0.85	0.04	1556.86	235.56
V	7	69.44	17.54	31.54	8.33	13.40	7.99	41.04	9.81	56.04	13.42	22.74	3.15	18.80	7.83	0.84	0.03	1524.00	242.87
		F=0.01; n.s.		F=0.08; n.s.		F=0.32; n.s.		F=0.06; n.s.		F=0.06; n.s.		F=0.03; n.s.		F=1.00; n.s.		F=0.39; n.s.		F=0.04; n.s.	

Table 4. The Spearman Rank correlation showing relationship between each body composition parameters and level of expression of microRNAs before and after five subsequent matches

	Match I		Match II		Match III		Match IV		Match V	
	Before	After	Before	After	Before	After	Before	After	Before	After
					microRNA - 182					
Body mass (kg)	0.257	-0.286	0.214	-0.250	-0.143	0.714	0.571	-0.071	0.571	
Muscle mass (kg)	0.257	-0.286	0.143	-0.179	-0.257	0.500	0.607	0.001	0.714	0.429
Fat mass (kg)	0.600	-0.286	0.001	0.036	-0.371	0.857*	0.500	-0.107	0.001	0.725
TBW	0.257	-0.378	0.143	-0.179	-0.257	0.500	0.607	0.000	0.714	0.429
FFM	0.257	-0.286	0.143	-0.179	-0.257	0.500	0.607	0.000	0.679	0.429
BMI	0.714	-0.464	-0.054	-0.036	-0.600	0.679	0.393	0.000	0.786*	0.600
%PBF	0.600	-0.250	0.143	-0.036	-0.714	0.750	0.536	-0.500	0.107	0.829*
WHR	0.231	-0.200	0.382	-0.509	0.200	0.679	0.234	-0.018	0.727	0.579
BMR	0.257	-0.286	0.143	-0.179	-0.257	0.500	0.607	0.000	0.679	0.429
					microRNA - 223					
Body mass (kg)	-0.143	-0.179	-0.429	-0.429	-0.286	-0.036	-0.250	0.214	-0.143	0.179
Muscle mass (kg)	-0.143	-0.179	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.143	0.464
Fat mass (kg)	-0.107	-0.107	-0.357	-0.643	-0.143	0.107	-0.107	0.179	-0.450	-0.234
TBW	-0.054	-0.288	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.143	0.464
FFM	-0.143	-0.179	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.036	0.286
BMI	-0.036	-0.286	-0.270	-0.577	-0.143	-0.071	-0.321	0.357	0.286	0.321
%PBF	-0.321	-0.071	-0.357	-0.714	0.250	0.000	0.143	-0.036	-0.214	-0.214
WHR	-0.273	0.327	-0.346	-0.309	0.214	0.428	0.162	0.234	0.036	0.091
BMR	-0.143	-0.179	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.036	0.286

	Match I		Match II		Match III		Match IV		Match V	
	Before	After	Before	After	Before	After	Before	After	Before	After
						microRNA - 320				
Body mass (kg)	0.143	0.000	-0.250	-0.100	-0.107	-0.371	0.086	0.086	0.321	0.643
Muscle mass (kg)	0.143	0.000	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.143	0.786*
Fat mass (kg)	0.321	0.214	-0.214	-0.300	-0.214	-0.314	0.143	0.143	0.432	0.054
TBW	0.054	-0.091	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.143	0.786*
FFM	0.143	0.000	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.214	0.678
BMI	0.071	0.036	-0.036	0.154	0.071	-0.371	-0.257	0.371	0.357	0.643
%PBF	0.286	0.250	-0.036	0.100	-0.250	-0.258	-0.257	-0.200	0.571	-0.143
WHR	0.436	0.164	0.000	0.410	-0.107	-0.143	0.290	0.087	0.618	0.509
BMR	0.143	0.001	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.214	0.679
						microRNA - 486				
Body mass (kg)	-0.036	-0.286	-0.429	0.071	-0.143	-0.786*	-0.429	0.786*	-0.286	0.143
Muscle mass (kg)	-0.036	-0.286	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.179	0.393
Fat mass (kg)	0.000	-0.250	-0.357	0.000	-0.393	-0.714	-0.571	0.786*	-0.162	-0.342
TBW	0.036	-0.306	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.179	0.393
FFM	-0.036	-0.286	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.214	0.250
BMI	0.107	-0.179	-0.270	0.000	-0.536	-0.607	-0.536	0.500	0.143	0.179
%PBF	-0.036	-0.393	-0.357	0.000	-0.857**	-0.179	-0.464	0.750	0.036	-0.536
WHR	-0.309	0.200	-0.346	0.236	-0.143	-0.393	-0.652	0.739	-0.109	-0.091
BMR	-0.036	-0.286	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.214	0.250

* $p<0.05$; ** $p < 0.01$

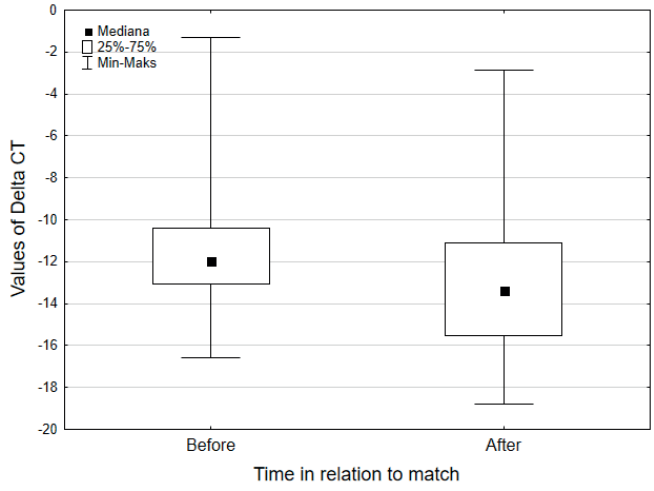

Fig. 1 The micro-RNA 486 expression before and after each match (Z=1.769; p=0.0769)

Discussion

The objective of our study was to evaluate the role of microRNA expression in body composition, with a particular focus on the muscle tissue in athletes. MicroRNAs, which are small, non-coding RNA molecules, perform essential regulatory functions in a variety of fundamental biological processes.

Despite the small sample size, we presented in this study a novel approach to longitudinal changes of the microRNAs connected with IGF1 and muscle mass and revealed some correlations and tendencies.

The association between microRNA expression as well as the composition of skeletal muscle and adipose tissue has been documented. Recent studies have demonstrated a considerable utility of microRNA analyses in a variety of contexts, including clinical, forensic studies (Barreiro et al. 2019; Svingos et al. 2019; Zampetaki et al. 2010) and the assessment of their role in competitive sport (Aoi et al. 2013). It is evident that body composition is responsive to physical exercise. Consequently, the question arises as to whether a modified level of selected microRNAs in the bloodstream translates into more favorable parameters of body composition in athletes. MicroRNAs are characterized by significant stability and may be introduced into sports practice as biomarkers (Jung et al. 2010). The utility of microRNA methodologies and analyses in the context of sport can be employed to elucidate their functions within the human body, particularly in relation to body composition (muscle tissue, adipose tissue, TBW). It is possible that further research will demonstrate the value of the advanced genetic biomarker methods in conjunction with the currently employed physiological methods utilized in preparing individuals for competitive sport. As such, our research represents a considerable advancement in the field of diagnostics, as well as contributes to the growing body of knowledge regarding the expression function of selected microRNAs.

The results of our study showed a positive correlation between miR-182 and fat mass following the third match. Furthermore, a positive correlation was observed between miR-182 and both before the 5th BMI and with percent body mass (PBF%) after the 5th match. Given that muscle and fat mass are inversely correlated, our results align with those reported by Olivieri et al. (2014), who observed significantly lower miR-182 levels in muscle samples. Furthermore, a study conducted on rats indicated that miR-182 levels were responsive to exercise in muscle tissue (Song et al. 2017). It was hypothesized that the level of miR-182 may serve as an indicator of the composition of adipose and skeletal muscle tissue in the body. Moreover, Zhang et al. (2016) demonstrated that miR-182 regulates glucose utilization in skeletal muscle, influencing blood glucose levels.

Our findings showed a positive correlation between miR-320 and muscle mass and TBW following the fifth match. In contrast, Munetsuna et al. (2018) identified a negative association between miR-320 expression and excess body fat. In addition, Yerlikaya and Mehmet (2019) demonstrated that high-fat conditions downregulated miR-320. Therefore, it can be postulated that the level of miR-320 is positively correlated with muscle mass and negatively correlated with fat mass. However, there is few studies that addresses the issue of miR-320 expression and total body water. Nevertheless,

the proper metabolism and body condition are hallmarks of organism hydration (Chumlea et al. 1999).

In contrary to clinical and forensic research, we are the first who tackled the problem of longitudinal effect of the circulation microRNAs expression level among athletes (Zampetaki et al. 2010; Barreiro et al. 2019; Svingos et al. 2019; Di Pietro et al. 2017; Goljanek-Whysall et al. 2020; McCrae et al. 2016; Woo et al. 2018). As stated by Nielsen et al. (2010), coordinated expression of selected microRNAs and non-single microRNA expression is crucial for skeletal muscle in endurance and speed training and adapts to the level of physical activity. The authors emphasized that the details on which the selected microRNA assembly can directly affect human physiology in response to physical exercise remain unknown. In addition, McCarthy et al. (2009) highlighted the role of several microRNAs (MyomiRs) as regulators of skeletal muscle cell function. Conversely, Small et al. (2010) observed that microRNA-486 exhibited particularly high levels in skeletal muscle cells and heart muscle, reaching ten to twenty times the concentration observed in other tissues. Aoi et al. (2013) suggested that microRNA-486 may regulate insulin-dependent glucose uptake in skeletal muscle tissue and may facilitate glucose uptake by activating insulin signaling during exercise. In the studies conducted by the authors among 10 healthy men, they observed that after a 4-week workout, the level of microRNA-486 decreased significantly. However, 24 hours after exercise, the microRNA returned to the baseline level and increased with insulin. Thus, microRNA can be considered to mediate adaptive muscle responses to physical exercise following training. In addition, we showed that PBF% is negatively correlated with miR-486; in contrast, Prats-Puig et al. (2013) showed that among obese prepubertal children the level of miR-486 was elevated.

The heterogeneous picture of body composition compounds and selected microRNAs suggests the need for further research on this problem. At the same time, we underline that the research conducted during the series of match games could also be associated with the level of sports advancement in subsequent matches. Specifically, in the case of the 3rd, 4th and 5th matches we observed the highest number of microRNA associations with body composition parameters.

It should be emphasized that the majority of studies on the role and functions of microRNA expression have been conducted on animals or through clinical observations and forensic analyses, thus applicative use in the sport industry may be limited. In this context, our research represents a novel approach to the application of microRNAs in the field of sport. Our findings show a correlation between body composition and microRNA expression levels. This suggests that the selection of microRNAs may be applied as a useful tool in identifying young athletes who are predisposed to different types of sports.

The study's limitations include a relatively small sample size thus further studies with larger sample sizes are needed to validate our results. In addition, further studies should include both sexes and other age groups. Nevertheless, we showed a longitudinal effect of each of the players. Moreover, a machine error in the case of one measurement of PBF% due to outliers' detection cannot be discarded.

We showed that expression levels of miR-182, miR-320 and miR-486 are associated with body composition. We also showed that the miR-182 is positively correlated with fat tissue, miR-320 is positively correlated with muscle mass while miR-486 is negatively correlated with fat mass. In addition, the results of our study indicated that exercise may result in a reduction in the level of miR-486.

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Ethical approval statement

The study was approved by Senate's Ethic Committee of Scientific Research at University School of Physical Education in Wroclaw 4/2020.

Conflict of interests

The authors declare no potential conflict of interest.

Authors' contributions

PPP – participating in designing the study, performed laboratory work, analyzed the data, prepared the draft and final version of the manuscript, SK – designed the study, conducted the analysis and prepared the draft and checked the final version; MK – collected the blood samples, prepared samples for analysis, build the database, EŻ, ZF, AR, IC, AD, MŚ, KK, AS – recruited and instructed participants, conducted measurements and prepare database, prepare first draft.

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