

CATECHOLAMINES AND β 2-ADRENOCEPTOR GENE EXPRESSION BEFORE AND AFTER MAXIMAL INCREMENTAL CYCLE TEST IN YOUNG ICE HOCKEY PLAYERS: RELATION TO WORK PERFORMED

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ABSTRACT: The aim of this study was to assess the plasma adrenaline and noradrenaline concentrations as well as whole blood β ₂-adrenoceptor gene (*ADRB2*) expression in young ice hockey players before and immediately after exercise in relation to performed work. Nineteen Youth National Team ice hockey players were subjected to the maximal incremental cycle ergometer exercise. The test was done in the pre-competitive phase of training. Among many parameters the plasma adrenaline and noradrenaline concentrations and *ADRB2* gene expression in peripheral blood mononuclear cells (PBMC) were determined before and after exercise. The average performed work was $3261.3 \pm 558.3 \text{ J} \cdot \text{kg}^{-1}$ and maximal oxygen consumption (VO_2max) for all players was $53.85 \pm 3.91 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The geometric mean of the *ADRB2* gene expression was statistically significantly different before and after exercise ($P \leq 0.05$), while adrenaline and noradrenaline levels in plasma significantly increased after exercise. In the analysed group of athletes we found that initial level of plasma noradrenaline correlated with the performed work ($r = -0.55$, $P < 0.014$) and normalized *ADRB2* expression before the exercise correlated with the work done by them ($r = 0.48$, $P < 0.039$). However, no statistically significant correlations were found between the plasma adrenaline or noradrenaline concentrations and *ADRB2* gene expression in peripheral blood of the players. The performed work in the maximal incremental exercise test of regularly training young ice hockey players depends on the initial levels of noradrenaline in plasma and *ADRB2* mRNA in PBMC.

KEY WORDS: ice hockey players, catecholamines, *ADRB2*, maximal incremental cycle test

INTRODUCTION

Catecholamines (adrenaline, noradrenaline) react with several subtypes of α and β adrenoceptors. Adrenaline has a stronger effect on the β receptors while noradrenaline has a stronger effect on the α receptors. The final effect of both hormones on individual tissues or organs is the result of stimulation of either one or the other type of receptors [11,19]. Catecholamine secretion is caused by different kinds of physical activity and high psychological stress [26,33]. Plasma concentrations of adrenaline and noradrenaline show an exponential increase during progressive exercise and in incremental exercise is emphasized by the close relationships between these hormones [2,6]. Catecholamines modulate metabolic and cardiocirculatory reactions and adaptations to physical and psychological work [26]. Preliminary studies showed that the nature and extent of metabolic changes evaluated in the players during training and the results achieved by them may be responsible for gene expression [10]. The exercise has an influence on gene expression profile in various tissues including peripheral blood mononuclear

cells (PBMC). This mainly applies to genes involved in the process of inflammation, repair, stress, apoptosis, cell-to-cell signalling and interaction and functioning of the immune and oxidative system [4,7,32]. One of the genes that change expression level after exercise is *ADRB2* [32], a human gene encoding beta-2 adrenergic receptor (β ₂-adrenoceptor), which is a member of the G protein-coupled receptor superfamily, intronless with different polymorphic forms, point mutations and genetic markers determining the haemodynamic phenotype [23]. *ADRB2* is expressed in human peripheral lymphocytes as mRNA and protein [31]. Down-regulation of this gene is associated with nocturnal asthma, obesity and type 2 diabetes [21,22,24]. *ADRB2* is mainly related to the respiratory tract and blood vessels widening but also affects the heart [17,30].

Previous studies trying to explain the relation between catecholamines and *ADRB2* during exercise did not examine the correlation between these variables in terms of work done by participants. Moreover, these studies were not carried out in young, regularly training

■ Accepted
for publication
15.01.2013

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ice hockey players performing intense exercise. Therefore, the aim of this work was to assess the effect of the maximal incremental cycloergometer test on the changes of adrenaline and noradrenaline plasma concentrations and *ADRB2* gene expression in PBMC in a group of regularly training young ice hockey players who underwent this test in the pre-competitive phase of training.

MATERIALS AND METHODS

Participants

The test group consisted of 19 Youth National Team ice hockey players in the pre-competitive phase of training. Ten of the players were defensemen and 9 forwards. The mean values for the subjects' basal characteristics were as follows: age of the players 17.1 ± 0.5 years, weight 80.0 ± 6.7 kg, height 1.83 ± 0.05 m and BMI 24.4 ± 1.5 kg m⁻². Participants were on a carbohydrate diet. In 15 players, BMI was normal but in 6 indicated overweight.

Exercise protocol

The players performed physical exercise on a Monark cycloergometer, type 824 E (Sweden). After a five-minute warm-up with a load of 0.5 W·kg⁻¹, the test with 1.0 W·kg⁻¹ load was performed. The load was increased by 0.5 W·kg⁻¹ every 3 minutes until voluntary exhaustion. Respiratory gas exchange was determined using a K4 b² system (COSMED, Italy).

Permission from the Bioethical Committee of the Medical University of Silesia in Katowice no. KNW/0022/KB1/122/I/09 was obtained to conduct the test.

Blood collection

The blood for full blood count and determination of plasma adrenaline and noradrenaline concentrations as well as *ADRB2* and *ACTB* (β -actin) gene expression in PBMC was collected into coated EDTA tubes before the exercise test and immediately after its completion.

Blood count results (haematocrit, haemoglobin and mean corpuscular volume [MCV]) allowed evaluation of the proportional change of plasma volume that was caused by exertion. If MCV values did not change after the exercise, the change of plasma volume was evaluated based on the haematocrit value before and after the exercise. If MCV values were changed, the percentage change of plasma volume was evaluated based on the value of haematocrit and haemoglobin before and after the physical exercise [18]. These volumes decreased by about 6.7%. The changes in plasma volume were taken into account in the determination of hormone concentrations after the exercise.

Catecholamine levels and gene expression

Adrenaline and noradrenaline were extracted from plasma and then their concentrations were determined twice using the radioimmunoassay method. The KIPL 0100 and KIPL 0200 kit by BioSource Europe S.A. was used. The limit of detection in plasma was

7.5 pg·mL⁻¹ and 37.5 pg·mL⁻¹ for adrenaline and noradrenaline, respectively. Intra-assay precision had 4.5 and 4.6 coefficients of variation CV (%) for the adrenaline and noradrenaline concentrations and were 8.9 ± 0.4 ng·mL⁻¹ and 58.0 ± 2.7 ng·mL⁻¹ respectively. Radioactivity of the obtained complexes was measured using a Wallac Wizard 1470 automatic gamma counter.

Total RNA was extracted from fresh whole blood (collected from players) using the Chomczynski method [5], and extracts were purified using RNeasy Mini Spin Columns (Qiagen, GmbH, Hilden). Total RNA was estimated by the Gene Quant II RNA/DNA Calculator (Pharmacia Biotech, Cambridge, UK) and used as a matrix for RT-PCR.

The number of copies of mRNA in micrograms (μ g) of the total RNA from PBMC for *ADRB2* and *ACTB* (as internal control) was determined by the RT-PCR method using the ABI PRISM 7000 sequence detector (Applied Biosystems). The reaction mixture was prepared according to application of suitable products and contained the QuantiTect SYBR Green RT-PCR Kit (Qiagen) with RT enzyme, forward and reverse starters, matrix (total RNA) and bi-deionized water to the final capacity. Specific oligonucleotide primers for investigated genes were designed using Primer Express™ Version 2.0 software (PE Applied Biosystems, Inc., Foster, CA) and the following were used in the above-mentioned reaction mixture: *ADRB2*, forward CCAATAGAAGCCATGCGCCG, reverse GCAGACGCTCGAA CTTGGCA; and *ACTB*, forward TCACCCACACTGTGCCATCTACGA, reverse CAGCGGAACCGCTCATTGCCAATGG.

Thermal conditions of the reaction were as follows: reverse transcription at 50°C for 30 minutes, initial denaturation (95°C, 15 minutes), 50 cycles at 94°C for 15 s, 60°C for 30 s, 72°C for 30 s and then final extension at 72°C for 10 minutes. At the same time a calibration curve based on five concentrations (400–8000 copies per 1 μ l) of the cDNA fragment - β -actin commercially available (TaqMan® DNA Template Reagents Kit and β -actin Control Reagent Kit, PE Applied Biosystems) as recommended by Bustin [3] was prepared. The specificity of amplification was assessed by electrophoresis in 6% polyacrylamide gel and on the basis of the melting curve and T_m value. The expression of *ADRB2* and *ACTB* genes for each player was determined twice.

Statistical methods

Statistical evaluation of the results was carried out using the Microsoft Excel 2007 and Statistica 09 software. Normality of the data was checked by the Shapiro-Wilk test. For all normally distributed results the arithmetic mean values and standard deviations were calculated. To obtain better approximation of the normal distribution of the expression of genes under research and catecholamine concentrations results were transformed to logarithmic values (normalized data) and geometric means and geometric standard deviation errors (STD error) were calculated. The obtained mean values before and after the physical exercise were compared using Student's t-test for dependent variables. Differences at $P \leq 0.05$ were defined as

statistically significant. The ANOVA post-hoc Tukey's test was used to compare the differences between the groups of sportsmen. Pearson correlations were calculated for all obtained results and were regarded as statistically significant when $r > 0.45$ and $P < 0.05$.

RESULTS

The obtained results of weight, height, body mass index (BMI) and performed exercise of the trained young ice hockey players were normally distributed. The average duration of the maximal incremental cycle exercise test was 21.0 ± 2.4 min, and the mean value of performed work was 3261.3 ± 558.3 J·kg⁻¹. The maximal oxygen consumption (VO_{2max}) for all players was 53.85 ± 3.91 mL·kg⁻¹·min⁻¹. Using the ANOVA post-hoc Tukey test it was found that statistically significant differences ($P < 0.02$) existed only between work performed by 10 defensemen (3230.3 ± 467.9 J·kg⁻¹) and 9 forwards (2707.6 ± 418.0 J·kg⁻¹). For that reason the statistical assessment of hormone concentration and gene expression was done for all players (N = 19).

Plasma catecholamine levels and gene expression

Catecholamine concentrations and transcriptional activity of inves-

tigated genes were log-normally distributed. Before the exercise, the geometric mean of adrenaline concentration in the analysed group was 19.50 ± 0.88 pg·mL⁻¹ and in all players this parameter was within the physiological range and did not exceed the concentration of 100 pg·mL⁻¹. Directly after the physical exercise, the geometric mean of plasma adrenaline concentration increased statistically significantly to 75.65 ± 0.69 pg·mL⁻¹ ($P < 0.001$). Plasma adrenaline concentration increased about four times (Fig. 1 a).

Before the exercise, the geometric mean of plasma noradrenaline concentration in the analysed group was 142.00 ± 0.44 pg·mL⁻¹ and in all players it was within the limits of the physiological range and did not exceed 600 pg·mL⁻¹. Immediately after the exercise (physical exertion), the geometric mean of noradrenaline concentration increased statistically significantly to 3458.01 ± 0.42 pg·mL⁻¹ ($P < 0.001$), and thus increased up to 24.4 times (Fig. 1 b). The geometric mean values of the *ADRB2* gene expression before and after the exercise were $28\,539 \pm 9$ and $222\,865 \pm 3$ mRNA copies per 1 μg of total RNA, respectively, and there were statistically significant differences between them ($P < 0.004$) (Fig. 2a).

The respective geometric mean values of the *ACTB* gene expression before and after exercise were $9\,787 \pm 5$ and $51\,310 \pm 2$

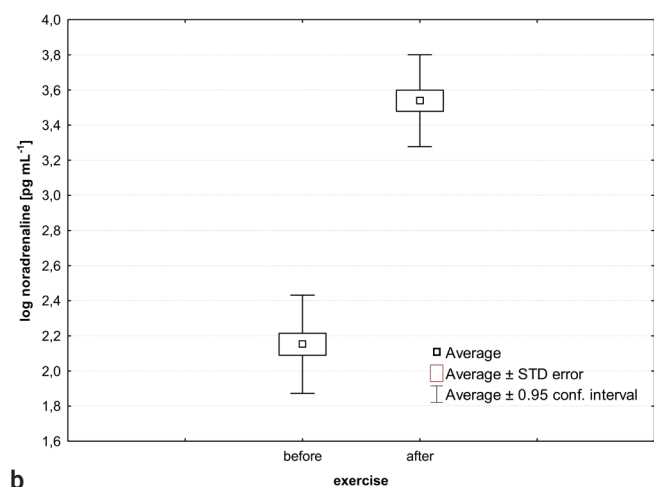
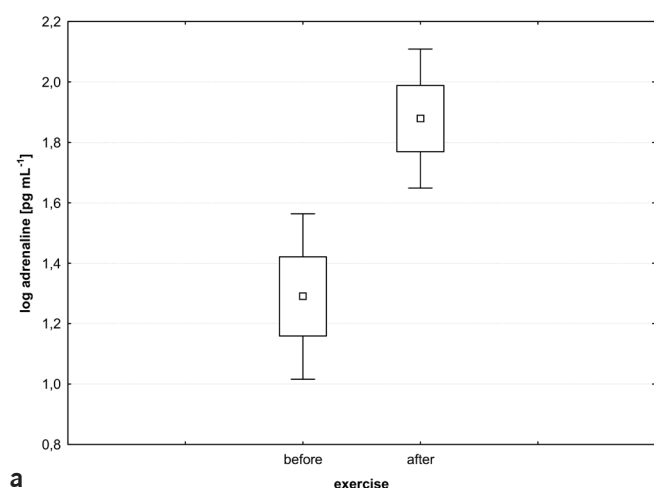


FIG. 1. CATECHOLAMINE LEVELS IN ICE HOCKEY PLAYERS BEFORE AND AFTER PHYSICAL EFFORT (A) ADRENALINE (B) NORADRENALINE

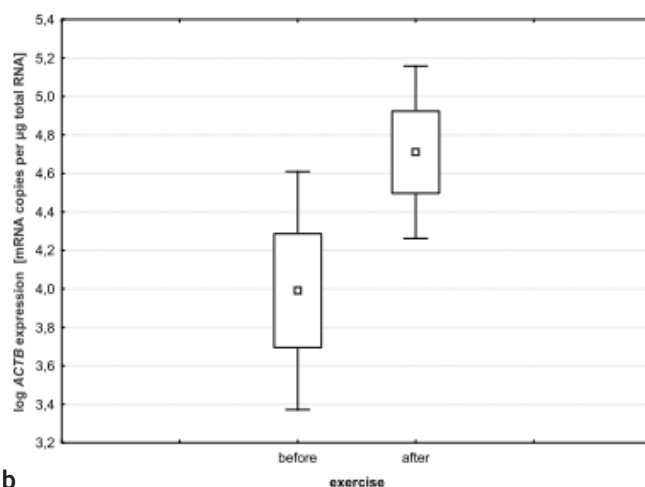
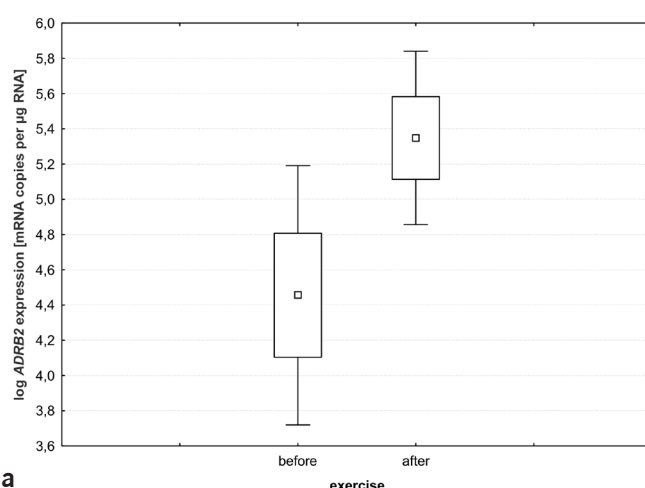


FIG. 2. EXPRESSION OF DETERMINED GENES IN ICE HOCKEY PLAYERS BEFORE AND AFTER PHYSICAL EFFORT; (A) *ADRB2*, (B) *ACTB*

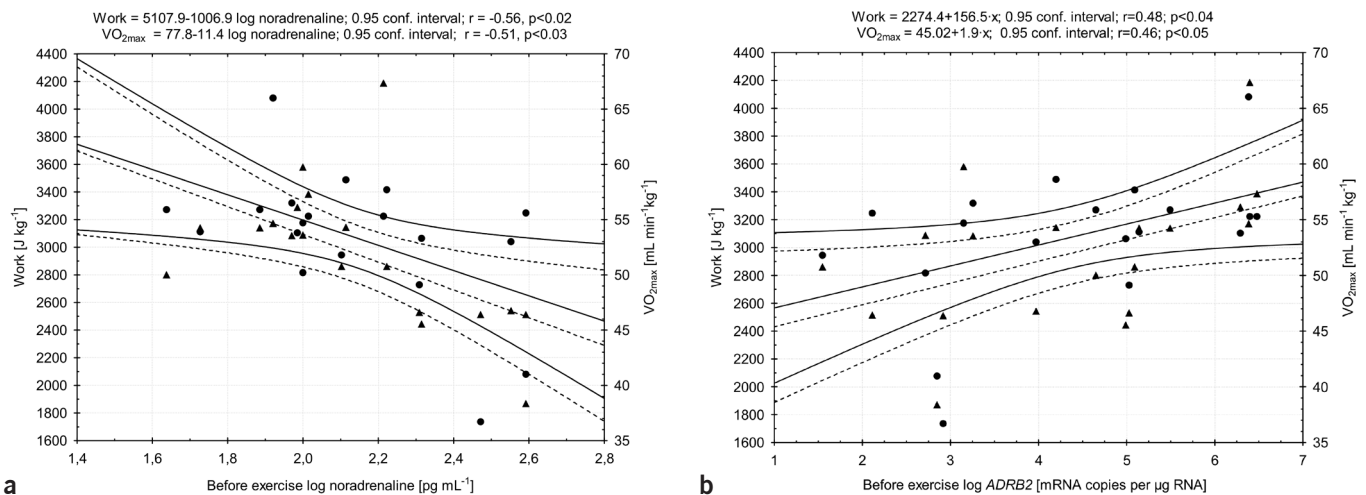


FIG. 3. CORRELATION BETWEEN NORMALIZED (A) PLASMA NORADRENALINE CONCENTRATION (B) *ADRB2* GENE EXPRESSION BEFORE PHYSICAL EFFORT AND PERFORMED WORK AND VO_{2max} FOR YOUNG ICE HOCKEY PLAYERS

mRNA copies per 1 μg of total RNA, respectively, and there were statistically significant differences between them ($P < 0.044$) (Fig. 2b). Correlations between initial logarithmic plasma concentration of noradrenaline and logarithmic *ADRB2* gene expression, performed work or VO_{2max} (Fig. 3) were found. The initial level of noradrenaline correlated with the performed work ($r = -0.55$, $P < 0.014$) and with VO_{2max} ($r = -0.51$, $P < 0.03$) of the players, which is shown in Figure 3a.

Correlations between initial logarithmic *ADRB2* gene expression of the players and performed work and VO_{2max} ($r = 0.48$, $P < 0.039$ and $r = 0.46$, $P < 0.05$, respectively) were found (Fig. 3b).

No correlations were found between the normalized values of *ADRB2* or *ACTB* expression and catecholamine concentrations. However, positive linear correlations between *ADRB2* before and after exercise ($r = 0.54$; $P < 0.018$) and adrenaline concentrations before and after exercise ($r = 0.69$, $P < 0.001$) were found.

DISCUSSION

The present study shows that maximal exercise performance and VO_{2max} inversely correlated with the initial plasma concentration while the correlations between work performed or VO_{2max} and the initial *ADRB2* expression in PBMC were positive. We found a statistically significant increase in plasma catecholamine concentrations and in levels of β₂-adrenoceptor expression.

Psychological pressure accompanying the test (for as long as possible to exercise as much as possible under load) activates the adrenergic system and hypothalamic-pituitary-adrenal axis, which is reflected in increased plasma concentrations of catecholamine. The situation can also cause stress waiting for the test result, as it affects the player's future (whether he will take part in a competition). Physical and mental stress associated with the test could lead to a significant increase in plasma noradrenaline levels. Circulating catecholamines have effects on haemodynamics and metabolism of athletes. They mobilize the energy substrates from sources outside of the muscle, such as adipose tissue and the liver. Adrenergic

activation in the direct start-up period are preferred because secreted catecholamines mobilize the body to be active and to exercise. Probably physical exercise can induce a decrease of *ADRB2* and the attenuation of cellular responsiveness to sympathoadrenomedullary activity [14]. The increased number of β₂-adrenoceptors under the influence of physical activity in our study may indicate the opposite effect, namely, increased sympathoadrenomedullary activity.

Our maximal incremental cycle exercise test caused an increase in adrenaline secretion (Fig. 1a), probably because of the mental stress and reactivity of the cardiovascular system in those extreme conditions. Noradrenaline is usually secreted together with adrenaline in stress situations and in exercise [9]. The changes of plasma noradrenaline concentrations were so high (Fig. 1b) probably because of the situation of anxiety associated with a performance test. Results of the exercise test in the group of young ice hockey players were simultaneously the basis for qualifying for the European Championship. Therefore, during the physical exercise test the athletes probably were under mental stress (serum cortisol level increased – data not shown). In response to exercise, catecholamine concentrations are influenced by several factors such as exercise characteristics, training status and gender [33]. The assessment of the changes in catecholamine concentrations resulting from maximum exercise in young ice hockey players confirmed that the increase in plasma noradrenaline concentration (24.4 times) was almost 6 times higher than the increase in adrenaline concentration (4 times), which may indicate a high activity of the sympathetic nervous system.

Bogacz et al. [1] examined hockey players during the period of pre-start preparation and demonstrated that the noradrenaline concentration in a similar group of players increased 2.5 times. The increases in noradrenaline concentration depend on many factors, not only on diet but also on the type and duration of the exercise, type of sport, and degree of being trained [16,33]. For example, in two groups of men practising sports and using various diets,

the mean values of noradrenaline concentration before the exercise were different [8]. Studies indicated that physical exercise significantly increased the noradrenaline level and were more visible in untrained people [26].

We found a statistically significant negative correlation between initial level of plasma noradrenaline and work done by the players (Fig. 3a). Lehmann et al. [20] studied the correlation between noradrenaline and maximum oxygen uptake ($r = -0.77$) and in their research circulating free plasma noradrenaline level decreased after training. Additionally, we found that magnitude of exercise performance depends on relative (concentration before exercise minus concentration after exercise)/(concentration before exercise) noradrenaline increase ($r=0.74$; $p<0.01$).

The changes in expression of the investigated genes (*ADRB2* and *ACTB*) in the analysed blood samples of the ice hockey team did not depend on whether the players were defensemen or forwards. The effect of physical exertion on the changes of the *ADRB2* and *ACTB* expression in the group of young sportsmen is shown in Figures 2a and 2b, respectively. In 74% of players the gene expression of *ADRB2* and *ACTB* increased after the exercise and in 26% decreased, which did not concern in the same competitors. Different studies demonstrated that a genetic variation of *ADRB2* influences vascular function in response to an endogenous and exogenous agonist and that particular variation of this gene sequence influences systemic vascular resistance [25]. In the investigated PBMC of the young ice hockey players statistically significant correlations between the noradrenaline or adrenaline concentration and *ADRB2* gene expression were not found. Possibly, changes in the *ADRB2* gene expression when compared to changes in catecholamine levels would be visible in another time frame (possibly in the recovery period), which would allow us to observe more interesting correlations between them. The obtained individual values of the *ACTB* expression indicate that it changes after exertion and it may not be regarded as a reference gene. Jemiolo and Trappe [15] determined the changes in housekeeping genes in skeletal muscles due to physical exercise and showed that exercise influenced expression of certain genes in human skeletal muscles and their results demonstrated that α -actin and *GAPDH* (glyceraldehydes-3-phosphate dehydrogenase) are appropriate internal controls because the mRNA was stable. In our case intensive exercise in the maximal incremental cycle test influenced expression of the *ACTB* in peripheral blood (Fig. 2b); thus the results had to be presented as normalized values of number of mRNA copies per microgram of total RNA [27,28].

We found that *ADRB2* mRNA in whole blood of investigated athletes increases after the maximal incremental test. Fragala and colleagues [12] showed that the *ADRB2* expression on monocytes was elevated in anticipation of the exercise protocol, but it decreased on monocytes and granulocytes during the exercise while on lymphocytes it was elevated during the recovery time points. Fuji and

colleagues also observed that the number of β -adrenergic receptors in lymphocytes after exercise was much higher than that at rest ($p<0.01$) [14], similar to our results of mRNA *ADRB2* from PBMC.

In spite of everything, we found that the performed work in the incremental exercise test had an effect on the changes in the *ADRB2* level in PBMC of young hockey players and all the above-mentioned parameters can influence it. However, we found the presence of a statistically significant correlation between initial level of *ADRB2* mRNA and performed work or VO_{2max} of the Polish Youth National Team ice hockey players (Fig. 3b). We did not obtain a correlation between magnitude of exercise performance and relative mRNA *ADRB2*. White et al. [29] also observed that maximal exercise oxygen consumption (VO_{2max}) showed the highest correlation with beta-adrenergic receptor density ($r^2 = 0.61$, $P < 0.001$) in patients with idiopathic cardiomyopathy, who underwent maximal exercise testing.

Richterova and colleagues [24] analysed, among other things, the effect of physical exercise on catecholamine levels and expression of adrenoceptors and the lack of changes in the *ADRB2* gene expression in adipose tissue in obese women observed due to training. We found the lack of a correlation between the *ADRB2* mRNA level in peripheral blood and plasma adrenaline concentration. Similar results were obtained in groups of trained and untrained men. It was connected with the regulation of *ADRB2* mRNA level and the reflection of the chronic effect of training and thus of the chronic change in the concentrations of catecholamines [13].

CONCLUSIONS

We demonstrated inverse correlations between the initial plasma concentrations of noradrenaline and the performed work or maximal oxygen uptake and positive correlations between *ADRB2* gene expression before exercise and performed work or maximal oxygen uptake. The performed work in this maximal incremental cycle exercise test of the regularly training young ice hockey players depends on the initial levels of noradrenaline and *ADRB2* mRNA. Future investigation in this subject will probably contribute to finding a biochemical marker of trained sportsmen.

Acknowledgments

This study was supported by the Medical University of Silesia, Katowice, Poland, grants KNW-1-026/10 and KNW-1-042/P/1/0. We thank all the ice hockey players who participated in this exercise test and their coach Tomasz Rutkowski. We thank Mrs Anna Palugniok for checking the English version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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