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Case Study

COMPARISON OF ADAPTIVE CHANGES IN THE EXPRESSION OF SELECTED GENES BETWEEN AN ACTIVE AND FORMER SPEED SKATER – A CASE STUDY

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Abstract- Adaptive changes to exercise include, inter alia, the level of *IL6* and *IL10* expression. The aim of the study was to compare adaptation to exercise in the expression of the *IL6* and *IL10* genes in peripheral blood leukocytes between an active and former speed skater. The two-year period after ending the professional career resulted in differences in anaerobic capacity, and consequently, significantly lower results in exercise tests by the former athlete (difference in maximum power of 1.46 W/kg, time to attain MAP 0.14; VO_2max at the average population level). The resting-state and post-exercise expression levels of the tested genes in the former athlete was lower than that of the active athlete, and there was no increased *IL10* expression immediately after the test (2^0.2 of baseline after WAnT and 2^0.5 after exercise until refusal in the former athlete). Based on analysis of gene expression results in response to laboratory tests, it was found that 2 years of training interruption resulted in the reduction of adaptive changes to intense physical exercise.

Keywords-adaptive changes, expression of *IL6* and *IL10* genes, physical fitness.

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Introduction

Acquisition of adaptive changes to physical activity is crucial from the point of view of training and sports success. The properly conducted training process at the highest level of sports can result in faster adaptation to increasing training loads, where the individual characteristics of the athlete's body are also of great importance. Adaptive changes to physical activity have been studied for many years, resulting in knowledge about, for example, heart changes, especially after endurance exercise, the so-called "sports heart", "sports anaemia" or other changes in biochemical parameters [1,2], cytokine concentrations and stress response proteins occur [3,4]. In recent years, the process of adaptation to physical exercise has also been investigated at the level of the expression of genes exhibiting sensitivity to physical exercise, including those connected with the stress-response of a cell, such as those coding HSP or interleukins [5,6]. According to Ziemann, *et al*., [3] and Fisher, *et al*., [4], adaptation to exercise is the decrease in the level of proinflammatory interleukins and the increase of those which are anti-inflammatory. The level of cytokine expression is also related to the type of effort [7], training load or type of training [8,9]. Due to the specifics of speed skating, where distances vary in length, intensive training is often used in high intensity interval training. The popularity of this type of training has increased in both sport and recreation in recent years. The positive effects of training in the aspect of anaerobic and aerobic capacity can be achieved with less work and the effects are similar to those achieved in classic endurance training [10,11].

Despite many reports on the changes at the level of transcripts, induced in response to various forms of physical activity, there is little research on the sustainability of these changes, *i.e*., the duration of their retention at the end of sports careers. Therefore, the purpose of the study was to compare changes in adaptation to exercise in the expression of the *IL6* and *IL10* genes in peripheral leukocytes between an active and former high speed skater.

Material and Methods

Methods 1. Description of the studied athletes

Two speed skaters participated in this study. One of the athletes (A.W.) is active and at a high level of sports. He is a national representative at international competitions, a specialist in long-distances. His main distance is 5,000 m. The other participant (P.S.) went through the same training process from the beginning of his career to the end of the junior category and was at the same level of sport as the first study participant. However, he ended his career in January 2015, but remained physically active. Both participants had no injuries or injuries in the previous three months. At the time of the test, the active athlete was in the final stage of the start phase, while the other participant was physically active 3-4 times a week for about 1.5 hours. [Table-1] shows the basic characteristics of the studied subjects.

Table-1 *Basic parameters of the study participants*

Dimension	sym./un.	Subject A.W.	Subject P.S.
Sex	[ ]	Male	Male
Date of birth	[ ]	10 Feb. 1993	16 Apr. 1993
Age	[years]	24	24
Body mass	[kg]	80.0	83.0
Body height	[cm]	179.0	179.0
Date of study	[ ]	27 Feb. 2017	27 Feb. 2017

Methods 2. Procedure of performing the Wingate test and the progressive "refusal" test on a cycle ergometer

The Wingate Anaerobic Test (WAnT) [12] was performed before noon on the Monark 894E, Peak Bike ergometer. Before each test, the athlete warmed-up on a cycle ergometer for 5 minutes at 60 rpm and with a load of 1 W/kg. Then, each participant performed the 30-second Wingate test with a maximum load of 75 g/kg of total body mass. Before the test, a 3 second countdown was used, and each participant received verbal encouragement to achieve the best possible result.

After a 3 h break, the progressive test was performed on a cycle ergometer (Monark 894E, Peak Bike). Prior to the proper test, the subjects performed a two-minute warm-up (without a load). Then, they pedalled for 90 minutes with a load of 90 W. After 7 minutes, the main part of the test took place. The test started at 150 W and Qa increased by 50W at 2 min intervals. The test ended the moment the athlete was unable to continue the exercise. During the test, HR values were measured with a Polar heart rate monitor. The participants performed both tests in the same order so that both had an identical break between the efforts.

Methods 3. Genetic testing

2 ml of venous blood were collected four times: immediately before and after each of the performed tests [5, 6]. To eliminate erythrocytes, 2 ml of venous blood were treated with a red blood cell lysis buffer (RBCL). The isolated white blood cells were lysed with Fenozol AA Biotechnology, Gdynia, Poland. Further isolation of RNA was carried out using the Chomczyński and Sacchi chemical methods [13]. The purity and concentration of the isolated RNA was determined by spectrophotometry (Spectrophotometer Eppernorf, Germany). Two µg of RNA (TranscriptMe KIT, Blirt, Poland) were used for reverse transcription. For gene expression analysis, real-time PCR was performed in 2/3 repetitions for each sample (Light Cycler polymerase, Roche, Poland). The temperature and reaction time profile corresponded with the manufacturer's recommendations., *TUBB* was used as a housekeeping gene. For amplification of each tested genes the following primers were used:

TUBB F:CTA GAA CCT GGG ACC ATG GA
TUBB R:TGC AGG CAG TCA CAG CTC T
IL6 F: AAT TCG GTA CAT CCT CGA CGG
IL6 R: GAA TCC AGA TTG GAA GCA TCC
IL10 F:GAC ATC AAG GCG CAT GTG AAC
IL10 R:TCC ACG GCC TTG CTC TTG TTT. All primers have been programmed by the authors.

Statistics 1

Relative expression was calculated using the delta C_t method in Exel 2017 [14]. The change in expression induced by the test effort was expressed as 2^x-fold, and was calculated: 2^ post-exercise expression / 2^ resting-state expression.

Results

Results 1. Results of anaerobic performance tests

Both athletes, in periods when they both trained and competed with each other, were at a similar athletic level regarding the Wingate test [12], as well as results obtained during sprints. In the currently performed WAnT test, the athlete (A.W.) gained a maximal power of 12.65 W/kg, and the time needed to reach it was 5.48 s. In contrast, the second participant (P.S.) obtained a maximal power of 11.19 W/kg and needed 5.62 s. The mean power during the test was 9.22 W/kg in A.W. and 8.10 W/kg for P.S. A significant value during this test was also the decrease index from maximal power, which (A.W.) was 0.23W/kg/s [Fig-1].

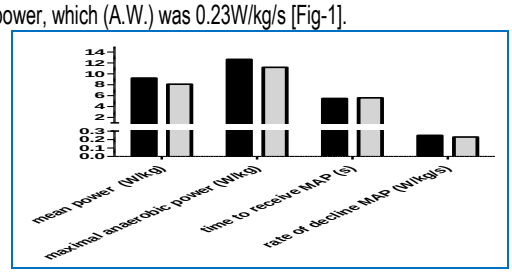

Fig-1 The results of Wingate Anaerobic Test for A.W. (black bars) and P.S. (gray bars).

Results 2. Results obtained in the "refusal" test

The second test performed by the subjects was the so-called "refusal" test, carried out on a cycle ergometer. The athletes performed the second test after a 3 hour resting period and in the same order.

The subject A.W. - active athlete, achieved VO_{2max} at 56.0 ml/min/kg. This result was obtained with a load of 450 W. The maximal heart rate reached by the athlete was 200 bpm. The second participant - P.S. obtained VO_{2max} at 43.6 ml/min/kg at 300 W. The maximal heart rate measured during the test was 203 bpm [Fig-2].

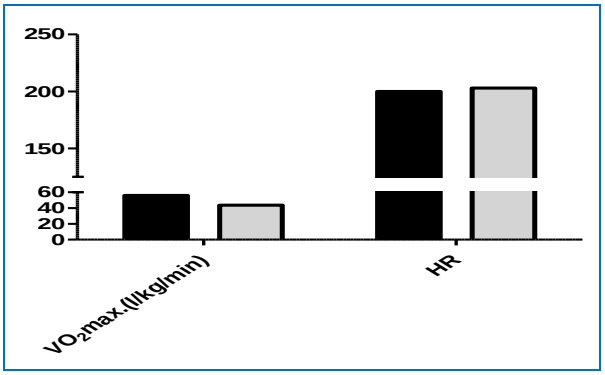

Fig-2 VO_2 max and maximal heart rate in the "refusal" test, black bars – A.W., gray bars – P.S.

Results 3. Changes in the expression of genes coding selected interleukins

[Fig-3] shows the resting-state values of the relative transcript copies (2^) before the athletes performed the stress tests.

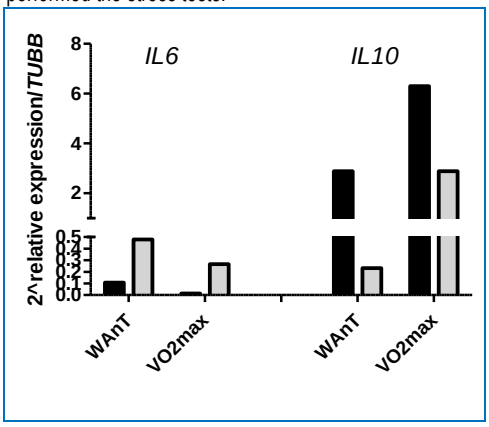

Fig-3 Rest value of *IL6* and *IL10* mRNA before WAnT and test to "refuse" of work (A.W. – dark bars, P.S. – gray bars).

The resting-state number of *IL6* copies for A.W. was lower for both resting-state collections (2^0.1 and 2^0.02) while *IL10* was higher (2^2.9 and 2^6.3). [Fig-4 and 5] show the multiplicity of *IL6* and *IL10* expression changes induced by the Wingate test and the "refusal" effort.

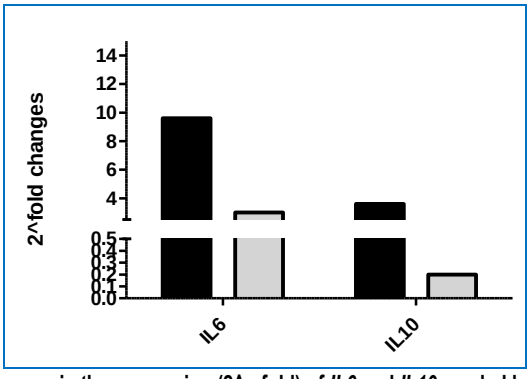

Fig-4 Changes in the expression (2^x-fold) of *IL6* and *IL10* cauded by WAnT (black bars – (A.W.), gray bars – (P.S.)).

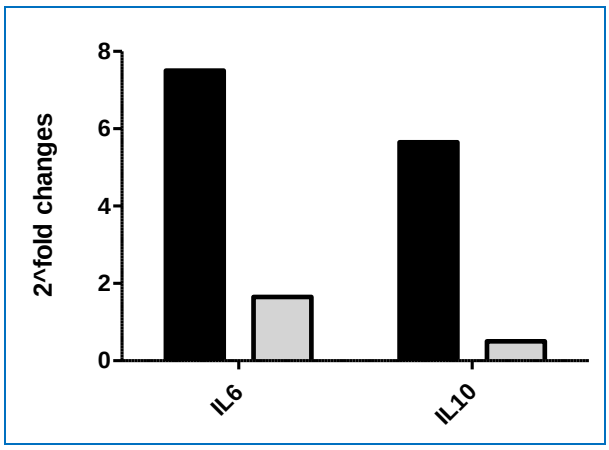

Fig-5 Changes in the expression (2^x-fold) of *IL6* and *IL10* caused by the "refusal" test, black bars – (A.W.), gray bars – (P.S.).

[Fig-4 and 5] show changes in *IL6* in both participants after the conducted stress tests. In both athletes, *IL6* expression levels increased significantly after exercise. In blood samples taken immediately after the test, for P.S., there was a 2^3-fold increase in cytokine content, while for A.W., this level increased 2^9.5-fold. After performing the "refusal " test, *IL6* increased by 2^1.5, while for athlete continuing his career, this value was 2^7.5. For both athletes, the Wingate test caused greater variation in the expression of this gene. *IL6* changes were accompanied by a change in expression of *IL1*0. During the Wingate test as well as the "refusal" test, for P.S., the transcriptional *IL10* was decreased (2^0.2 of baseline after WAnT and 2^0.5 after the "refusal" test). For A.W., expression of *IL10* increased more than 2^3.5-fold (after WAnT) and 2^5-fold after the "refusal" test.

Discussion

The active athlete (A.W.), who is still in the process of completing the training process at the highest level, achieved better results in the Wingate test. The difference between the subjects was not very large, however, this may be due to the fact that speed parameters are mainly genetically determined [16]. In addition, it should be noted that the athlete (A.W.) is not a sprinter but a specialist in long-distances. In comparison, athletes representing Poland at international competitions in this sport, who specialize in shuttles, achieved a maximal power of around 13.5-14.0 W/kg in this test [17,18]. In addition, the Wingate test is not specific to this discipline and the results are largely influenced by technical-tactical components. During his career – A.W. – obtained better results in long-distances than P.S., in the years in which they both competed. The difference reached in the VO_{2max} level between competitors was high. The VO_{2max} level drops very quickly during cessation of the training process. According to Wilmore and Costill [19], and the values presented in [Fig-1], the study participant (A.W.) was within the values typical for speed skaters. In contrast, P.S. was within the range of VO_{2max} values achieved by non-training individuals in the age range corresponding to participants of the study. However, the athlete continuing to pursue his career, still has a reserve in terms of the result obtained in this test. The best results of VO_{2max} which are achieved by the representatives of the Polish national team in speed skating are at the level of 60-66 ml/min/kg [18].

Changes in gene expression in both athletes show that 2 years of discontinuation of specialized training was enough to reduce adaptation to exercise at the systemic level in blood cells. High expression of tested genes in professional athlete may be due to the fact that it was tested during the immediate post-heavy period. According to Buttner, *et al*., [20], changes occurring at transcriptional levels in white blood cells show the general load of the physical exercise. Furthermore, Ziemann, *et al*., [3] believe that the key to adaptation is the decrease in *IL6* levels and the increase in *IL10*. Such adaptive changes were apparent in the athlete (A.W.), whose resting transcript level of *IL6* was significantly lower than before both stress test, and *IL10* was higher. Beginning with *IL6*, the increase in this cytokine was observed in both subjects, however, for P.S., this increase was 2^3 and for (A.W.), it was as great as 2^9.5. The much higher change in the active athlete is due to, among others, the much lower level of resting-state *IL6* as well as the easy induction of this gene acquired in the course of sports training [10]. A similar dependency was also found in the case of the "refusal" test, although the change under the influence of the Wingate test was much greater. Such an increase in *IL6* levels after intense exercise may be evidence of inflammation, as well as decreased glycogen levels in the muscles. Elevated *IL6* is accompanied by an adequate increase in *IL10* [5,6]. However, this dependence was reported only for the active athlete, which again confirms the reduction of adaptability (P.S.) 2 years after the end of his career. The increase in *IL6* was not accompanied by an increase in *IL10* immediately after exercise. This allows us to state that the athlete (A.W.), as a result of a long-term training process, is adapted to high intensity efforts and the regeneration process occurs more quickly, and hence the ability to repeat the intense effort. Evidently, there is no simultaneous increase in *IL10* with the decrease in adaptation to exercise. Changes in gene expression in both athletes show that 2 years of discontinuation of specialized training was enough to reduce adaptation to exercise at the systemic level in blood cells.

Conclusion

The two-year period from the end of the athlete's career resulted in differences in the level of anaerobic and aerobic capacity. The former athlete obtained weaker Wingate test results and his oxygen threshold was typical of the average population. In response to the test efforts, lower expression of *IL6* and *IL10* genes in the former athlete was observed, with no increase in the expression of *IL10* after the tests, which is essential for exercise adaptation. Hence, it should be noted that two years after the end of one's career, there is a reduction in exercise capacity with a decrease in adaptation to intense physical effort at the molecular level.

Application of research:

Recreational activity after stopping a professional sport career is not enough to maintain adaptation to hard exercises at molecular level. To maintain decrease in IL6 mRNA and increase in IL10 mRNA heavier workouts are needed.

Author Contributions:

All author equally contributed (DD – study design, writtining the article, performance of the experiment; PS - performance of the experiment, statistical analysis, PF – genetics research, data collection; JW. – literature search, supervision, WP. - literature search, supervision.

Abbreviations:

IL6 – gene encoding interleukin 6
IL10 – gene encoding interleukin 10
MAP – maximal anaerobic power
VO_2max – maximal oxygen uptake
WAnT – Wingate Anaerobic Test
HSP – heat shock protein
HR – heart rate
PCR – polymerase chain reaction
TUBB – gene encoding tubulin B

Conflict of Interest:

The authors have declared that no competing interests exist.

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Conflict of Interest: None declared

Ethical approval:

The study was approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk (KB14/14). The authors were obliged to respect the principles of the Helsinki Declaration. Participants gave written, informed consent to participate in the study, and could withdraw consent at any time for any reason

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