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Assessment of fatigue and the overtraining syndrome in female spinning instructors based on a survey and on the expression of selected genes

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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
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abstract

Background: The study aimed to assess the occurrence of fatigue and overtraining in spinning instructors based on subjective feeling (a questionnaire) and the expression of selected genes (*IL6*, *IL10* and *NF-kB* mRNA).

Material/Methods: Two research methods were used in the study: a subjective assessment in the form of a survey and an objective evaluation of the expression of genes related to the cellular stress response. The survey involved a group of 12 female spinning instructors 24–32 years old. Five subjects (mean age 26.4 years) agreed to have a blood sample taken for genetic analyses. The control group comprised four physically active women (mean age 25.5 years). For genetic analyses, two ml of venous blood were taken in the morning hours after a night's rest. The relative evaluation of gene expression was performed using real-time quantitative PCR.

Results: According to the questionnaire declarations, the instructors feel overtraining. It was observed that the concentration of the transcription factor *NF-kB* in peripheral blood at rest was significantly lower ($p < 0.05$) in the study group compared to the control one. The measurement of the *IL6* encoding gene expression significantly differed ($p < 0.05$) between the two groups. In the case of the pro-inflammatory cytokine *IL10*, there were no significant differences between the two groups.

Conclusions: According to the questionnaire declarations, spinning instructors feel overtraining, but simultaneously they are very well adapted to physical effort, which was confirmed by the analysis of the expression of genes related to the cellular stress response.

Key words: spininng, gene expression, overtraining syndrome.

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INTRODUCTION

Lack of proper biological regeneration after intense physical training leads to the development and overlapping of fatigue changes, which, in turn, may lead to overtraining of the body. Overtraining and its effects has been a subject of many studies, the majority of which concerned active athletes [1, 2]. The subject of overtraining has been relatively rarely studied in coaches, instructors or judges who perform heavy physical efforts for a long time, which may lead to the development of overtraining. One of such forms of physical activity is spinning (indoor cycling), where physical exercise is performed with an intensity of approx. 60–90% HRmax [3], and instructors often conduct several classes per day. Therefore, it seems that the level of fatigue can disturb homeostasis and lead to impaired functioning of the body not only in athletes. Sometimes such a condition may last several months being diagnosed in sport as the overtraining syndrome [4]. According to Meur et al. [5], this syndrome is a serious health hazard. The best way to prevent overtraining is a properly tailored training plan taking rest into account [6]. However, it is more often possible in the training of an athlete rather than in the professional work of an instructor who performs physical exercises together with a group of exercisers.

Training loads contribute to multifaceted metabolic changes in the entire human body. However, so far, diagnosing fatigue and overtraining has not been simple and is based on a variety of methods, including psychological [7, 8, 9] or biochemical ones, e.g. determining cortisol concentration, which varies in the circadian rhythm [10]. Research on the overtraining syndrome indicates an increased concentration of cortisol with a simultaneous decrease in testosterone concentration [11]. In recent years, adaptive changes and those indicating fatigue of an organism have been determined in the expression of genes responsible for encoding proteins „sensitive" to physical effort [12, 13]. This group of genes includes, among others, genes encoding heat shock proteins (HSP) associated with anti-apoptotic action and interleukins [14]. Despite few studies on the expression of genes under the influence of physical effort [12, 13, 15], a growing level of *IL10* and a falling one of *IL6* seem to be crucial for adaptation to physical effort [16]. In turn, chronic inflammation, which is identified with the state of fatigue or overtraining, is characterized by a much higher increase in the concentration of some cytokines as well as the expression of genes encoding them, especially *IL6* mRNA [17]. Intense activity increases plasma *IL6* concentration up to 100-fold and modulates anti-inflammatory processes, increasing the production of anti-inflammatory cytokines, such as *IL10* and *IL1*ra [18]. Locally produced *IL10* acts as a natural feedback mechanism that controls inflammation and maintains immunity at the correct level [19]. *IL10* is important in the anti-inflammatory response and promotes the maintenance of IkB, thus inhibiting the nuclear transcription factor kappa B (*NF-kB*), the main *TNF-α* transcription factor [18]. Fatigue changes may be sought in the absence of this balance between the expression of *IL6* and *IL10*.

In view of the above, the aim of this research was to assess the occurrence of fatigue and overtraining in female spinning instructors based on their subjective feelings (questionnaire) and the expression of selected genes (*IL6*, *IL10* and *NF-kB* mRNA). Based on the available literature, it was assumed that, due to the high intensity of training, the profession of a spinning instructor might contribute to the development of negative fatigue changes.

MATERIAL AND METHODS

Two research methods were used in the presented study: a subjective one in the form of an original questionnaire and an objective assessment of the expression of genes related to the cellular stress response.

PARTICIPANTS

The survey involved a group of 12 female spinning instructors aged 24–32 years old. Five of the surveyed instructors (mean age 26.4 years) agreed to have a blood sample taken for genetic analyses. The control group consisted of four physically active women (mean age 25.5 years). The participants were instructed to keep their normal diet and exercise intensity until the day of taking the blood sample. The instructors were physically active as part of their profession, while the control group maintained a recreational level of activity (3 times a week for about 1 hour). Blood collection and testing was conducted in May 2017 in the genetics laboratory at the University of Physical Education and Sport in Gdańsk. Approval of the Bioethical Commission No. KB-10/16 was obtained for the study.

QUESTIONNAIRE "PROTOCOL OF SPINNING INSTRUCTORS' OVERTRAINING"

The questionnaire asked 21 questions to obtain information on professional work and the related health aspects. A significant number of the questions required self-assessment of one's health and well-being. The respondent's overtraining protocol was filled in by means of a created online questionnaire. Spinning instructors had at least 2 years of professional experience, and each of them conducted at least 3 training sessions a week (6 hours). Each training session involved endurance work with the heart rate over 75% HRmax, which was measured with a heart rate monitor.

GENETIC METHODS

For genetic analyses, 2 ml of venous blood were taken in the morning hours after a night's rest. In order to eliminate erythrocytes, erythrocyte lysing buffer (RBCL, A&A Biotechnology, Gdynia, Poland) was used. 800 μL of Fenozol (A&A Biotechnology, Gdynia, Poland) was added to the obtained white blood cells for their lysis. Further RNA isolation was conducted with reference to Chomczyński and Sacchi's method (1987). The quality and the quantity of pure RNA were determined spectrophotometrically. cDNA synthesis from 2 μg of RNA was conducted with a use of the Transcriba kit (Roche, Department Poland). To evaluate the expression of genes, quantitative real-time PCR was applied in duplicate for each sample using LightCycler polymerase (Roche, Department Poland). The temperature profile and the reaction time were consistent with the manufacturer's instructions. As the housekeeping gene, TUBB (the gene encoding tubulin B) was used.

RESULTS

Ten spinning instructors declared that they were using a heart rate monitor during exercises. However, the whole group of respondents declared a rapidly growing feeling of fatigue, and eight of them felt muscle stiffness and pain. Ten subjects felt a lack of full post-workout restoration, reflected in an inability to withstand a full training unit, although four of them indicated that they usually had enough hours to rest. In the case of four instructors, the amount of rest was almost never adequate to their physical effort, and two respondents often rested the appropriate amount of time.

Five of the respondents controlled the amount of food intake, but four instructors declared that they often ate too little. Two were not influenced by energy balance, although they tried to eat healthy. The instructors were aware of the need for biological regeneration, and mostly they chose: stretching and "rolling" (8), sauna (6), massage (4), water baths (1). One of the respondents did not use biological regeneration treatments. As many as nine of the examined women complained about the occurrence of night sweats, and six had gastric disorders and menstrual disorders. A significant number (9) of the instructors indicated that they were sensitive to stress, and they had their blood tested relatively rarely (6 participants declared blood tests once a year and 6 only when they had to).

The results of the expression of genes collected in blood samples during the resting period are presented below. Figure 1 shows a comparison of the examined indicators between the group of spinning instructors (SG) and the control group (CG) made of physically active women.

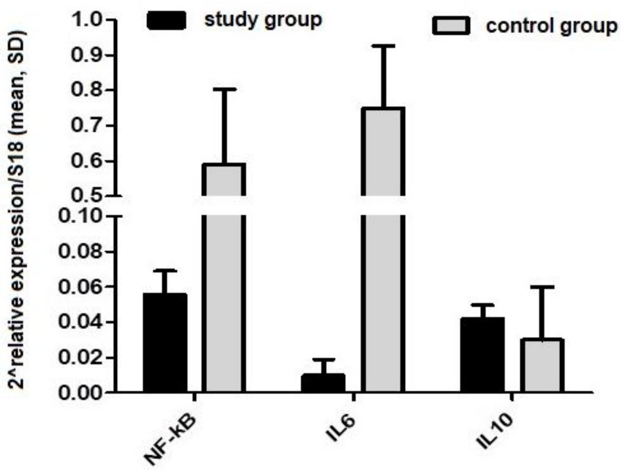

Fig. 1. Mean values, standard deviation (SD), a comparison of the expression genes *Nf-kB*, *IL6, IL10* in concentration at rest between spinning instructors and a control group

It was observed that the resting concentration of the transcription factor *NF-kB*, also called the "stress factor", in peripheral blood was significantly lower ($p < 0.05$) in SG in comparison to CG. The measurement of the *IL6* gene expression significantly differed ($p < 0.05$) between the two groups. In the case of the pro-inflammatory cytokine *IL10*, there were no significant differences between the two groups SG and CG. Only slightly higher *IL10* values were observed in the case of SG.

DISCUSSION

Analysing the survey results, it can be concluded that most of the instructors feel overtraining manifested in the lack of post-workout regeneration (10) and a feeling of skeletal muscles stiffness and pain (8), despite the appropriate amount of sleep and the use of biological regeneration treatments.

An analysis of the overtraining protocol proposed for the needs of the presented study indicates too frequent incidence of symptoms related to fatigue and overtraining. This confirms the assumption that the spinning instructor's profession may contribute to the development of fatigue changes.

Changes in the gene expression in peripheral blood leukocytes show a systemic response to effort or training and prove the stress load to the body [20, 21, 22]. The expression of numerous genes may increase locally in skeletal muscles, but there are differences between local and systemic changes in the mRNA levels. High training intensity affects many metabolic changes in the body, changing the production of interleukins, the availability of substrates, the activation of metabolic enzymes and others. Each change starts in the process of gene transcription. Changes in the expression of genes which are dependent on physical effort are mainly related to the genes associated with apoptosis and inflammation [23].

The literature data show that *IL6* and *IL10* encoding genes are highly sensitive to effort. Cytokine responses depend on the type, intensity and duration of physical activity [12]. Factors such as oxidative stress [24], damaged muscle proteins [25], hyperthermia or energy imbalance probably induce cytokine production during exercise [26].

The nuclear factor kappa B (*NF-kB*) is a transcription factor important for the development of inflammation. In SG, the level of expression of the *NF-kB* nuclear factor was lower compared to CG. In the present study, no significant changes in *NF-kB* and *IL6* mRNA were observed in SG, which could indicate the occurrence of inflammation associated with overstraining the organism. Furthermore, after a night's rest this level was lower in the group of instructors.

In addition to health benefits, intense exercise increases interleukin 6 (*IL6*) concentration by increasing its production in skeletal muscles, active in the regulation of energy supply in the muscles [18, 27]. Moreover, higher expression of *IL6* results in increased expression of the anti-inflammatory cytokine *IL10*. Scientific research confirms that *IL10* can inhibit a high level of *IL6* secretion, and it has an inhibitory function for the *NF-kB* pathway [28]. The mutual proportions of *IL6*/*IL10* seem to be a very good indicator of training adaptation [14, 23]. In view of the above, the instructors clearly show adaptation to heavy physical exercise, manifested by a decrease in the expression of *IL6* with a higher level of *IL10* mRNA than in the control group.

The results of the study suggest that the elevated level of *IL10* mRNA in the SG plasma triggers favourable changes in the resting inflammatory profile. A significantly lower level of the *NF-kB* transcription factor as well as *IL6* m RNA in SG may be associated with good training adaptation. It is likely that with regular spinning training, the anti-inflammatory effects of intense activity protect the body against chronic systemic inflammation.

Working professionally as a spinning instructor can contribute to the development of negative fatigue changes. In order to avoid overtraining, preventive measures should be taken, including a proper diet, hydration and rest combined with properly selected biological regeneration. Such behaviour will allow performing this profession for a longer time.

CONCLUSIONS

According to questionnaire declarations, spinning instructors feel overtraining, but at the same time they are very well adapted to physical effort, which is apparent in the analysis of the expression of genes related to the cellular stress response.

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