SUMMARY

It is well known that physical activity promotes mental health. Physically active people relatively rarely suffer from psychosomatic and depressive disorders. It is possible that the differences in the gene activity in peripheral blood leukocytes may be associated with depression, especially those genes participating in an inflammatory response. Thus, the aim of our study was to investigate the levels of $IL6$ and $IL10$ mRNA and $IL10/IL6$ ratio in peripheral blood leukocytes in healthy, physically active individuals.

One hundred healthy young men (20-23 years old) participated in this study. All of them declared their regular participation in physical activity. Participants were non-smokers, and consumed alcohol occasionally. To access genes expression, 2 ml of venous blood was collected. RNA isolation was performed and then the relative expression of $IL6$ and $IL10$ was calculated using the quantitative real-time polymerase chain reaction (Q-RT-PCR). The pro and anti-inflammatory balance was calculated as $2^{\text{relative of } IL10} / 2^{\text{relative expression of } IL6}$.

A low expression of the tested genes was found in healthy young men. Mean expression for $IL6$ was $2^{0.051}$ (n=90) and for $IL10$ $2^{0.08}$ (n=98). Mean ratio $IL10/IL6$ was $2^{1.58}$.

A higher expression of $IL10$ compared to $IL6$ may be essential not only for physical performance but also for any mental health evaluation. Diverse reports in the subject literature may be associated with choosing control groups that were too diverse, i.e., sedentary or older individuals. It is possible that measuring the expression of $IL6$ and $IL10$ (especially the ratio $IL10/IL6$) in peripheral blood leukocytes may be useful in the assessment of depressive disorders. Thus, a molecular study of active young men can confirm the need for physical activity among people suffering from depression, but further studies are needed, particularly among people with psychosomatic disorders to establish the potential therapeutic effect.

Key words: depressive disorder, gene expression, inflammation, physical activity, peripheral blood mononuclear cells

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BACKGROUND

According to WHO, depression is the most common mental disease, one which affects approximately 300 million people of all ages worldwide, and results in as much as 800,000 deaths caused by suicides each year (WHO, 2018). Currently the most common type of treatment is pharmacological management along with psychotherapy (Gautam et al., 2017) but due to the various side effects of medication, safer treatments are being researched. A few dietary interventions have shown promising results, such as introducing various seafood and leafy greens into the diet (LaChance et al., 2018). The promising results are mainly based on the anti-inflammatory and anti-oxidative properties of those foods (Fruutos et al., 2018). Physical activity also seems to be an effective treatment strategy for patients with depression, but the underlying mechanisms have yet to be discovered (Agudelo, 2014). This kind of treatment might be less burdensome and more affordable than pharmacotherapy (Moore, 2018). The objective of the present study was to show the changes in gene expression of the genes \(IL6\) and \(IL10\) with the emphasis on the \(IL10/IL6\) ratio in healthy, physically active subjects. Regarding the well known fact that physically active people are less likely to develop depressive disorders, it is important to determine the differences between both groups. In our opinion it is important to determine the marker genes and the dysregulation of their expression that will enable professionals to diagnose their patients easier.

MATERIAL AND METHODS

Subjects and study design

One hundred healthy young men (20-23 years old) participated in this study. All of them declared participation in regular physical activity (minimum 3 times a week of 45 minutes of activity). Participants were on a standard, healthy diet, all were non-smokers and consumed alcohol occasionally. No individual declared depression episodes prior to the experiment. All subjects were informed of the purpose of this study and the possible risks involved before giving their written consent.

The study was approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk. The authors of this study were obliged to respect the principles of the Helsinki Declaration.

Experimental Procedure

The methodology used in this experiment was consistent with one described earlier (Kochanowicz et al. 2017; Żychowska et al. 2017). To access gene expression, 2 ml of venous blood samples were taken from the antecubital vein with vacutainers with EDTA as a anticoagulant. The collected blood was then mixed with the RBCL buffer (A&A Biotechnology, Gdynia) and incubated on ice for at least 15 minutes to lyse red blood cells. The samples were then spun at
3000 rcf for 10 minutes at 4°C. The resulting pellet was washed again with the hemolysis buffer and the remaining white blood cells were lysed using Fenozol (A&A Biotechnology, Gdynia).

RNA extraction and reverse transcription

The isolation of the total RNA was carried out using the Chomczynski and Sacchi method (Chomczynski & Sacchi, 1987). The purity and integrity of the RNA were determined by a spectrophotometer (Eppendorf, BioPhotometer Plus, Germany) by absorbance at UV 260/280 and the ratio >1.9 was accepted as a pure RNA suitable for further analysis. RNA was then reverse transcribed to cDNA using oligo(dT) and Transcriptor First Strand cDNA Synthesis Kit as per the manufacturer’s instructions (Roche, Poland).

Quantitative Polymerase Chain Reaction assay

Quantitative real-time PCR (qPCR) was carried out on the AriaMx Real-Time PCR System (Agilent Technologies, Poland) using FastStart Universal SYBR® Green Master (ROX) according to the manufacturer’s protocol (Roche, Poland) on 96-well PCR plates in triplicate for each sample. The thermal cycling conditions were as follows – activation step: 10 min at 95°C followed by 40 cycles of annealing and extension step: 15 sek at 95°C, 1 min at 60°C. Additionally melt curve analysis was performed for each reaction. The β-tubulin (TUBB) was used as a reference gene. The relative expression of IL10 and IL6 was calculated using a quantitative real-time polymerase chain reaction (Q-RT-PCR). The primer sequences used in this study are written down below:

**TUBB**
Forward primer: TCCACGGCCTTGCTCTTGT
Reverse primer: GACATCAAGGCGCATGTGAAC

**IL6**
Forward primer: TCCACGGCCTTGCTCTTGT
Reverse primer: GACATCAAGGCGCATGTGAAC

**IL10**
Forward primer: GAATCCAGATTGGAAGCATCC
Reverse primer: AATTCGGTACATCCTCGACGG

Statistical analysis

To determine relative expression Schmitten and Livak delta delta C_t method (2008) was used (Microsoft Excel, 2017). The mean value and standard deviation was calculated in GraphPad Prism 6.0. The pro and anti-inflammatory balance was calculated as $2^{\Delta \Delta C_t}$ relative of IL10/ $2^{\Delta \Delta C_t}$ relative expression of IL6.

RESULTS

A lower expression of IL6 mRNA compared to IL10 mRNA in rest samples was observed in the samples of all participants as well as in the mean value.
Relative expression was $2^{0.05}$ for IL6 and $2^{0.075}$ for IL10 mRNA. The IL10/IL6 ratio was $2^{1.58}$. These results indicate that the anti-inflammatory response was higher than the inflammatory in physically active people.

DISCUSSION

Physically active people showed a lower expression of IL6 compared to IL10 mRNA, which suggests that the anti-inflammatory response prevails over the inflammatory response. This observation is also visible in the IL10/IL6 ratio which indicated 3.16 fold more copies of IL10 ($2^{1.58}$ fold responds 3.16 fold in linear value). In our study active people constituted a model group, as no individual had previously suffered from depressive disorders.

Devorak et al. (2015) postulated that the main biological mechanism triggering depression is inflammation. Therefore, in our opinion, physical activity for people affected by depression is needed and could support their therapy. One of the main adaptive changes to exercises on the molecular level depends on the decrease of the IL6 expression and the increase of the IL10 expression (Żychowska 2017; Ziemann et al. 2013). That is the reason why active people had a higher expression of IL10 in the rest state.

There are some reports in the subject literature in which changes in genes encoding interleukins were analyzed. Horowitz et al. (2014) postulated that the high expression of IL6 could be associated with depression. Furthermore, up-regulation of the expression of genes associated with inflammation was postulated by Pace et al. (2006). Trystuła et al. (2017) in their research evaluated genes expression in a 54-old patient with depressive disorder following a transient ischemic attack and found a higher expression of IL6, IL10 and CRP mRNA compared to the control group. Despite the relatively high IL10 mRNA of this patient, IL6 mRNA was definitely higher ($2^{1000}$-fold compared to $2^{400}$-fold for IL10).
Therefore, in our opinion the calculation of the $IL10/IL6$ mRNA ratio in the rest samples is crucial.

In recent years, the development of medical diagnostics is closely related to molecular diagnostics, especially with the analysis of genes expression. In our opinion the determination of genetic markers and/or dysregulation in genes expression will be a major advance to facilitate the diagnosis of mental illnesses. Unfortunately, there are still only a few studies in this area. Only some studies in the literature are associated with the expression of genes encoding interleukines. Usually these are studies conducted on stuttering patients (Kahl et al., 2004, Pace et al., 2006, Półrola et al., 2016), sometimes in patients with depression or schizophrenia (Freudenreich et al., 2010, Chase et al., 2015). In our opinion, it is important, for further diagnostics, to carry out more well-planned studies on a larger group of patients. It is also crucial, to perform such studies before introducing pharmacotherapy to the patients, as the medication modulates the immune response.

In conclusion, we suggest that in patients with depressive disorders, the determination of genes expression, especially associated with interleukin, could be useful in difficult diagnostics. Moreover, because of the positive changes caused by physical activity, including appropriate exercising programs in the treatment process, it might be beneficial for the patients.

REFERENCES


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