No effect of acute exercise on the dynamic change in the expression of genes involved in epigenetic modification in professional athletes

Witold Józef Światowy
Poznan University of Medical Sciences: Uniwersytet Medyczny imienia Karola Marcinkowskiego w Poznaniu

Jacek Zieliński
Akademia Wychowania Fizycznego im Eugeniusza Piaseckiego w Poznaniu

Maria Aleksandra Osielska
Poznan University of Medical Sciences: Uniwersytet Medyczny imienia Karola Marcinkowskiego w Poznaniu

Krzysztof Kusy
Akademia Wychowania Fizycznego im Eugeniusza Piaseckiego w Poznaniu

Dariusz Wielinski
Akademia Wychowania Fizycznego im Eugeniusza Piaseckiego w Poznaniu

Andrzej Pławski
Polish Academy of Sciences: Polska Akademia Nauk

Paweł Jagodzinski ( pjagodzi@ump.edu.pl )
Poznan University of Medical Sciences: Uniwersytet Medyczny imienia Karola Marcinkowskiego w Poznaniu https://orcid.org/0000-0002-9046-6802

Research Article

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Abstract

Background:

The adaptation of the organism to exercise in the context of gene expression profile is an interesting phenomenon. Exercise can change the expression of individual genes due to changes in the degree of DNA methylation, changes in miRNA expression, or through methylation or acetylation of histones.

Hypothesis:

Acute exercise increases the expression of genes such as HDAC1, DNMT1, and JHDM1D that can affect epigenetic modifications in PBMCs.

Methods:

The aim of this study was to determine whether there was a change in gene expression in the blood cells during acute exercise and after a 1-hour recovery. The transcriptions of genes involved in epigenetic modifications (HDAC1, HDAC1 and JHDM1D) were examined in 9 professional athletes at rest, during consecutive stages of a treadmill exercise until exhaustion, and following recovery.

Results:

No significant differences in the level of transcript were observed in the course of the experiment in the tested PBMC cells. On the other hand, a significant (p = 0.007) correlation was observed in the level of the JHDM1D gene transcript and the number of monocytes in the samples obtained after reaching peak exercise intensity, but in the initial samples this correlation was not significant (p = 0.053).

Conclusion:

Acute physical exercise does not rapidly alter the transcript levels of the JHDM1D, DNMT1 and HDAC1 genes in PBMCs. The observed correlation between the level of JHDM1D mRNA and the level of monocytes and HDAC1 with lymphocytes requires further investigation.

Introduction

Gene expression is influenced by epigenetic modifications as well as DNA sequences. Both biological and environmental factors induce these modifications. One of these factors is exercise (acute and chronic) that can alter the expression of individual genes due to changes in the degree of DNA methylation, changes in miRNA expression, or through methylation or acetylation of histones (Ling and Rönn 2014; Simmonds and Seebacher 2017; Grazioli et al. 2017; Basso and Suzuki).

Higher levels of global methylation were found in people exercising 26-30 minutes per day compared to people exercising only 10 minutes per day. However, the increase in methylation was statistically insignificant following multivariate adjustment for age, gender, BMI, and other data (Hughes et al. 2011).
A 6-month exercise training increased the level of methylation in adipose tissue (Rönn et al. 2013) and decreased the level of global methylation in peripheral blood mononuclear cells (PBMC) (Dimauro et al. 2016) and muscle tissue (Barrès et al. 2012).

DNA methylation and histone acetylation and their effect on gene expression during single exercise and training were intensively explored. However, relatively little is known about histone methylation and its impact on gene expression during exercise. Some data is available on the effect of acute exercise on histone methyltransferase (HMT), such as protein arginine methyltransferase (PRMT), or the effect of exercise on methylation of specific regions, such as the H3K36 region. The methyl group can be removed from this region by the demethylases having a JmjC domain, such as the HMT of the JmjC domain-containing histone demethylation protein 1 group (JHDM1) (Dimauro et al. 2020).

The potential changes in DNA (cytosine-5)-methyltransferase 1 (DNMT1) expression during exercise appear to be interesting. The transcription of DNMT1 in PBMCs is relatively constant and its expression linearly declines with age (Cicccarone et al. 2016). It is of interest to explore the effect of acute exercise on DNMT1 transcriptions. Considering studies that show that interleukin-6 (IL-6) stimulated cells upregulated DNMT1 expression, and the release of IL-6 is observed during exercise and inflammation (Horsburgh et al. 2015), we can expect increased DNMT1 expression during acute exercise.

The same is true for Sirtuin 1 (SIRT1) deacetylase which reduces SIRT1’s activity and expression under the influence of oxidative stress. Then, SIRT1 affects the acetylation of the promoter region of the DNMT1 gene, i.e. the expression of this gene (Dimauro et al. 2020).

Histone deacetylases (HDAC) are an interesting class of enzymes which indirectly influence the development and function of muscles, e.g. by affecting myocyte enhancer factor-2 (MEF2) (McGee and Hargreaves 2004) and myoblast determination protein 1 (MyoD) (Mal et al. 2001). The research on the effects of exercise on histone deacetylases primarily focused on class Ila HDAC. Class Ila nuclear export of HDAC under the influence of exercise, and HDAC3 impact on skeletal muscle adaptation after exercise, by acetylation of histones in the nucleus, were observed (McGee et al. 2009). Moreover HDAC5 can regulate MEF-2 and Glucose transporter type 4 (GLUT4) in response to exercise in muscle cells by exporting HDAC5 from the testicle, not changing its quantity (McGee and Hargreaves 2004). Stable expression of HDAC1 in PBMC prompts the investigation of whether acute exercise affects transcription of this gene.

It may also be interesting to check the expression of some enzymes responsible for epigenetic control in the blood, as most research focused on muscle or fat tissue. In our study, we selected genes with documented expression in blood mononuclear cells (Nawrocki et al. 2015, 2017) i.e. DNMT1, HDAC1, and JHDM1D genes.

Most studies on athletes focused on muscle and fat cells. The aim of this study was to determine whether there is a change in gene expression in the blood cells during acute exercise and after a 1-hour
recovery. We hypothesized that acute exercise increases the expression of genes HDAC1, DNMT1, and JHDM1D that can affect epigenetic modifications in PBMCs.

**Methods**

Nine professional triathletes were invited to participate in the research conducted at the Poznan University of Physical Education in Poznań. The group consisted of adult men aged 23.8 ± 3.4 years with training experience of 9.7±1.6 years. The weight, height and BMI of the athletes were 74.1±6.4 kg, 180.2±5.4 cm, and 22.56±2.33 kg/m², respectively. All participants were informed about the purpose and protocol of the study and signed a written consent. The project was approved by the Ethics Committee at the Poznan University of Medical Sciences (Poznań, Poland) and implemented in accordance with the Helsinki Declaration.

**Exercise test**

Two days before the visit to the laboratory, the athletes reduced the amount and intensity of their training. All measurements were performed in the morning, about 2 hours after a light breakfast. First, weight and height were measured using the SECA 285 measuring station (SECA GmbH, Hamburg, Germany). Then, subjects performed an incremental treadmill test until exhaustion on the h/p Cosmos Pulsar treadmill (Sports & Medical GmbH, Nussdorf-Traunstein, Germany). After application of the necessary equipment, once on the treadmill, the athlete stood still on the treadmill for 3 minutes to record resting parameters and check the functioning of the measuring system. The initial speed was set at 4 km·h⁻¹ and after 3 minutes increased to 8 km·h⁻¹. After that point, the speed of the moving strip was progressively increasing by 2 km·h⁻¹ every 3 minutes until voluntary exhaustion. The main part of the test was followed by a 30-minutes recovery phase consisting in walking at a speed of 4 km·h⁻¹ for 3 minutes and then sitting for another 27 minutes. During the test, a number of cardiorespiratory variables were measured constantly (breath by breath) using the MetMax 3b-R2 ergospirometer and analyzed using the MetaSoft Studio 5.1.0 software package (Cortex Biophysik GmbH, Leipzig, Germany). Heart rate was measured continuously with the Polar Bluetooth Smart H6 monitor (Polar Electro Oy, Kempele, Finland). The whole measuring system was calibrated according to the manufacturers' instructions. During the measurements, constant ambient temperature was kept at 20-21°C. At the final stage of the test, maximal oxygen uptake (\(\dot{V}O_2\)max) and the values of accompanying cardiorespiratory variables were determined. \(\dot{V}O_2\)max was considered achieved if at least three of the following criteria were met: (i) a plateau in \(\dot{V}O_2\) despite an increase in speed and minute ventilation; (ii) blood lactate concentration \(\geq 9\) mmoll⁻¹; (iii) respiratory exchange ratio \(\geq 1.10\); and (iv) heart rate \(\geq 95\%\) of the age-predicted maximum heart rate (Edvardsen et al. 2014).

**Blood sampling**

Blood samples were obtained via peripheral catheter 1.3 x 32 mm (BD Venflon Pro, Becton Dickinson, Helsingborg, Sweden) inserted retrogradely into the antecubital vein which was kept patent with isotonic
saline (0.9% NaCl) during the entire procedure. The blood for molecular analysis was collected into tubes with EDTA (S-monovette K3 EDTA, 7.5 mL, Sarstedt, Nümbrecht, Germany). For lactate concentration measurement, lithium heparin was used as an anticoagulant (S-monovette, 2.7 mL KE, Sarstedt, Nümbrecht, Germany). Lactate in whole blood (20 µL) was immediately assayed using the spectrophotometric enzymatic method (Biosen C-line, EKF Diagnostics, Barleben, Germany).

Blood samples were drawn 12 times: at rest (before the test), at the end of each 3-minute stage beginning from the 10 km h⁻¹, and then during the recovery phase. Detailed timing of blood sampling is presented in Table 1.

| Collection point | Test stage                        | Total time (min) |
|------------------|-----------------------------------|------------------|
| 1                | At rest (before the test)         | 0                |
| 2                | Running – at 10 km/h              | 12               |
| 3                | – at 12 km/h                      | 15               |
| 4                | – at 14 km/h                      | 18               |
| 5                | – at 16 km/h                      | 21               |
| 6                | – at 18 km/h                      | 24               |
| 7                | Test termination (exhaustion)     | 24-27*           |
| 8                | Recovery – 5 minutes              | 32               |
| 9                | – 10 minutes                      | 37               |
| 10               | – 15 minutes                      | 42               |
| 11               | – 20 minutes                      | 52               |
| 12               | – 30 minutes                      | 62               |

* The range is due to different levels of aerobic capacity and exercise tolerance of individual athletes.

**Isolation of PBMC and RNA**

On the day of blood sampling, PBMCs were isolated using a Ficoll density gradient (Lu et al. 2015). 3 ml of blood was applied gently to 1.5 ml of Ficoll solution (from Sigma) in a 15 ml tube so that the layers did not mix. The samples were centrifuged for 35 minutes at 400 g at room temperature. The resulting PBMC-containing interphase was transferred to a new tube and washed 3 times in PBS. Washing was performed by adding 2 ml PBS, vortexing, centrifuging for 10 minutes at 340 g and discarding the supernatant. Finally, the pellet in PBS was transferred to a centrifuge tube and subjected to final
centrifugation for 10 minutes at 250g. The obtained pellet was suspended in Trizol (RiboEx® GeneAll) and frozen at -80°C.

Further RNA was isolated from the obtained PBMCs according to the protocol of the manufacturer of the RiboEX® reagent from point three. The manufacturer allows the storage of the isolated cells in the RiboEX reagent at -80°C, which takes place in the second step.

The quality and concentration of the obtained RNA were checked on an agarose gel under denaturing conditions and by measuring the absorption spectrum on the NanoDrop apparatus.

**RT-PCR and qPCR**

The obtained RNA samples were reverse transcribed into cDNA using M-MLV Reverse Transcriptase® (Invitrogen). The reactions were performed according to the manufacturer's protocol. An equal concentration of templates in each reaction was prepared for RT-PCR reactions in the amount of 100 ng RNA. From each performed reaction, 1 µl of cDNA was collected for the pooled standard sample to derive the standard curves.

*HDAC1, DNMT1, and JHDM1D were selected as test genes, and Esterase D (ESD) and Porphobilinogen deaminase (PBGD) genes were selected for internal control and as a reference to assess the relative amount. The matrices for these genes are presented in the table.*

### Table 2

**Primer sequences**

| Gene   | Sequence (5’–3’) | Product size (bp) |
|--------|------------------|-------------------|
| **DNMT1** | F: GATGAGAAGAAGCACAAGAAGT | 149 |
|        | R: TCTTTGGGGGTCTGTTCG | |
| **HDAC1** | F: GAGACGGGATTGATGACGA | 104 |
|        | R: TGAGCCACACTGTAAGACC | |
| **JHDM1D** | F: TCCCTTCACCTACATTTTCTG | 89 |
|        | R: TGCTGCTCGCCACATC | |
| **ESD**  | F: ACCACCAAAGGCAGAAACAG | 135 |
|        | R: GGAGCAATGACACAAAGACC | |
| **PBGD** | F: GCCAAGGACCAGGACGAC | 160 |
|        | R: TCAGGTACAGTTGCCCAC | |
Real-time PCR reactions were performed using "5X Hot Fire Eva Green qPCR Mix"® and the manufacturer's reaction protocol. Light Cycler 480 Real-time PCR by ROCHE was used for the reaction (check carefully). Agarose gel electrophoresis was performed to check the specificity of the amplification products obtained.

**Statistical analysis**

The normality of the distribution was checked with the Shapiro-Wilk test, and then the correlation matrix was used to assess the significance of the related data. Statistical analyses were performed using the STATISTICA 13 software.

**Results**

Maximal oxygen uptake ($V_{O_2}^{\text{max}}$) in the tested athletes was 4,96±0,49 l/min/kg, and the maximal heart rate (HRmax) was 187,9±6,47 bpm. The level of lactate was also measured in the examined subjects: it was 1,02±0,19 mmol/l at rest, 10,29±1,59 mmol at exhaustion and 4,16±1,73 mmol/l after 30 minutes of recovery.

When analyzing changes in gene expression during incremental exercise, one can see an initial decrease in the amount of transcript of the studied genes, followed by an increase and subsequent fluctuations in the level of the transcript. However, taking into account the standard deviations and the coefficients of variation, the changes in expression seem to be more of an individual matter. A potential tendency would have to be confirmed on a larger sample of participants.

**Table3**

| mRNA concentration of individual genes during progressive exercise until exhaustion and 30-min post-exercise recovery | phase | 0 | 12 | 15 | 18 | 21 | 24 | 27 | 5 | 10 | 15 | 20 | 30 | mean values |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mean HDAC1** | 1,4361 | 1,3601 | 1,2207 | 1,2716 | 1,3846 | 1,4284 | 1,2816 | 1,2549 | 1,4997 | 1,3408 | 1,3895 | 1,2944 | 1,347 | 1,347 |
| **sd HDAC1 (%)** | 0,4882 | 0,5944 | 0,4605 | 0,3610 | 0,5176 | 0,4926 | 0,4762 | 0,3570 | 0,4942 | 0,4501 | 0,3984 | 0,4330 | 0,460 | 0,460 |
| **Mean DNMT1** | 1,4496 | 1,2004 | 1,4531 | 1,4289 | 1,6279 | 1,8138 | 1,5813 | 1,3091 | 1,5139 | 1,3696 | 1,5154 | 1,5495 | 1,484 | 1,484 |
| **sd DNMT1 (%)** | 0,8225 | 0,6233 | 0,6721 | 0,5762 | 0,8035 | 0,9224 | 0,7136 | 0,5057 | 0,5817 | 0,8134 | 0,7383 | 0,7886 | 0,714 | 0,714 |
| **Mean JHDM1D** | 1,9942 | 1,7237 | 1,4935 | 1,4137 | 1,6655 | 1,8187 | 1,8286 | 1,9465 | 1,5314 | 1,6136 | 1,9141 | 1,7869 | 1,721 | 1,721 |
| **sd JHDM1D (%)** | 1,7629 | 1,9024 | 0,7634 | 0,9711 | 1,1478 | 1,4513 | 1,5970 | 1,6141 | 1,0649 | 1,3600 | 1,1897 | 0,9716 | 1,315 | 1,315 |
| **Mean JHDM1D (%)** | 88,4 | 110,4 | 54,2 | 68,4 | 68,9 | 79,8 | 87,3 | 82,9 | 69,5 | 84,3 | 61,1 | 54,4 | 75,8 | 75,8 |

In further stages of the statistical analysis, the results of expression obtained from individual athletes were correlated with the biochemical data. Among the average levels of transcripts of given genes in individual athletes, the values of $JHDM1D$ mRNA in athletes 3 and 4 are outliers, being about three times
higher than in the other subjects. Several parameters were found, where Athletes 3 and 4 stood out from all athletes on the basis of biochemical data.

Athletes 3 and 4 had elevated monocytes compared to all subjects both in the rest phase (MonRest) and after reaching the maximum speed (MonMax). Some parameters, such as the level of monocytes, were assessed only at rest (collection point 1) and at maximum speed (collection point 7).

In athletes 3 and 4, the number of lymphocytes (LymRest, LymMax), the number of thrombocytes (PltRest, PltMax), the average volume of platelets (MPVRest, MPVMax) and procalcitonin (PCTRest, PCTMax) were different from the mean resting value. However, these values came from two different poles, where one athlete had the highest value in his pool, while the other had the lowest value in another pool.

The correlation of the transcript level with all available parameters of athletes was also checked. It was checked whether the level of transcripts at rest (before exercise), after running at maximum speed, and the difference between these conditions correlated with the available parameters.

The parameters of monocytes at maximum velocity, i.e. the value of MonMax with the statistical significance (p = 0.007), correlated with the value of \textit{JHDM1D}_{Max} at the level of 0.82. The values of MonRest and \textit{JHDM1D}_{Rest} and the differences between the values at rest and at exhaustion did not give significant results (p > 0.05) with high correlation. The correlation MonRest and \textit{JHDM1D}_{Rest} was 0.6598 (p = 0.053), and for the Rest-Max difference, the correlation was -0.2122 (p = 0.584).

A significant correlation was observed between the change in the amount of \textit{HDAC1} transcript from resting conditions to maximum speed and the change in the number of lymphocytes at the same time. The number of increased lymphocytes during exercise was inversely proportional to the \textit{HDAC1} transcript level. Correlation was -0.7645 (p = 0.016)

**Discussion**

Many studies involving various exercise protocols have been conducted on the effect of acute exercise and training periods on the changes in genome methylation and gene expression. The changes in expression at two moments were usually reported, i.e. before and after a single exercise, e.g. a marathon run. Research conducted so far has primarily focused on tissues that undergo significant changes, i.e. muscle and fat (McGee and Hargreaves 2011; Ling and Rönn 2014; Dimauro et al. 2020). However, changes in blood cells were also studied (Horsburgh et al. 2015; Hunter et al. 2019).

Available research demonstrated a global decrease or increase in DNA methylation depending on the tissue, suggesting that the proteins responsible for methylation and demethylation change their activity, whether through activating factors or increasing the expression of genes encoding methylases and demethylases (Dimauro et al. 2020).
It was observed in many studies that physical exercise causes hypermethylation of the genome in adipose tissue. In muscle tissue, acute exercise induces hypomethylation of numerous genes responsible for mitochondrial function. Chronic exercise induces genetic adaptation of a muscle through demethylation of genes involved in mitochondrial, lipid and glucose metabolism, muscle growth and angiogenesis (Dimauro et al. 2020). This may demonstrate that exercise stimulates muscle tissue cells to intensify their activity, but the activity of adipose tissue cells is reduced. In contrast, studies on blood cells showed a reduction in global methylation in PBMCs with exercise (Hunter et al. 2019). Despite the epigenetic changes observed in the literature, the results obtained in this study indicate that acute exercise has no effect on the production of $DNMT1$, $HDAC1$ and $JHDM1D$ mRNA in PBMC cells.

Increased levels of $DNMT1$ mRNA can be expected based on an increase in $DNMT1$ mRNA production by $IL-6$ (Hodge et al. 2007, p. 1; Horsburgh et al. 2015) and the fact that $IL-6$ is released from the general circulation by acute exercise (Fischer 2006). However, the lack of effect of exercise on $DNMT1$ transcription in PBMCs was observed in the work of D.J. Hunter et al. (Hunter et al. 2019). The observed global DNA hypomethylation in PBMCs after acute exercise probably did not involve the $DNMT1$ gene and was independent of this demethylase (Hunter et al. 2019).

Histone methylation, depending on the type and position of methylated amino acids, can affect gene expression, for example by activating transcription (Ntanasis-Stathopoulos et al. 2013). One of the first observed differences between acetylation and methylation of histones was that methylation increased the basicity and hydrophobicity of the histones, and hence tightened DNA packing and reduced the expression (Rice and Allis 2001). Later, it was observed that acute exercise contributed to a change in the degree of methylation of some histones, such as increased lysine methylation in histone H3-k36 in skeletal muscle in nonathletic men (Naghavi Moghadam et al. 2019). Histone methyltransferase PRMT (protein arginine methyltransferase) is responsible for arginine methylation, which may contribute to activation or repression of gene expression. The expression and activity of this enzyme do not change under the influence of severe exercise, but its subcellular location changes, namely it moves to the nucleus where the degree of histone methylation increases (Barrès et al. 2012). On the other hand, one of the proteins responsible for lysine demethylation is $JHDM1D$ (JmjC domain-containing histone demethylase 1D) that acts, among others, on histone H3k36 (Klose et al. 2006). Due to the lack of observed differences in the transcription of $JHDM1D$ in our athletes we examined, it can be assumed that during acute exercise the gene is not subject to increased expression and possible subcellular translocation, as it is the case in a large number of enzymes involved in epigenetic modification (McGee and Hargreaves 2004; McGee et al. 2009; Horsburgh et al. 2015).

Myoblast determination protein 1 ($MyoD$) is one of the proteins that affect the development and repair of skeletal muscle. It was shown that it has greater expression in humans after acute exercise than without exercise (Caldow et al. 2015). Also, acute exercise increased MyoD expression before and after a 12-week resistance training (Mal et al. 2001; Kadi et al. 2004).
HDAC1 has the ability to repress MyoD transcription by maintaining deacetylation (Mal et al. 2001). One can also expect that in people who exercise regularly HDAC1 will less interfere with MyoD deacetylation and its transcription in muscle tissue should not increase. In some researches, an increase in HDAC activity in blood after strenuous exercise was observed in obese subjects. The effect of exercise may be linked to epigenetic control of inflammation (Dorneles et al. 2016). Moreover, the effect of acute exercise on the reduction of HDAC2 activity in PBMC (Dorneles et al. 2017) was observed. Our study showed that HDAC1 transcription is not altered by acute exercise.

In the leukocytes of our athletes, an increase can be seen between the value at rest and after intense exercise. The increase in these leukocytes in the same conditions was also observed by other researchers (Dorneles et al. 2016). We could not find any research on the relationship between the effects of exercise on leukocytes and genes. Also, the correlation between the JHDM1D transcript and the level of monocytes and HDAC1 transcript with level of lymphocytes should be confirmed. Further research should focus on the level of proteins encoded by HDAC1, DNMT1 and JHDM1D genes and their subcellular locations.

Summary

Acute physical exercise does not alter the transcript levels of the JHDM1D, DNMT1 and HDAC1 genes in PBMCs. The observed correlation between the level of JHDM1D mRNA and the level of monocytes and HDAC1 with lymphocytes requires further investigation.

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Figures
Figure 1

JHDM1D, DNMT1, and HDAC1 transcript levels in peripheral blood mononuclear cells (PBMCs) in athletes during a progressive exercise until exhaustion and a 30-min post-exercise recovery. The JHDM1D, DNMT1, and HDAC1 mRNA levels were corrected for PBGD and ESD levels. The amounts of JJHDM1D, DNMT1, and HDAC1 mRNA were expressed as the decimal logarithm of multiples of these cDNA copies in the calibrator.