ORIGINAL ARTICLE

Changes in expression of selected cellular stress response genes after passive body overheating in sauna and moderate exercise in judo athletes and untrained people

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
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Abstract

Background and Study Aim: Passive overheating and exercises influence the genes expression, especially encoding heat shock protein and interleukin. The aim was the effect of one-time sauna and moderate exercise on selected genes expression in judo athletes and untrained people.

Material and Methods: Ten athletes trained judo (JA group aged 21.5 ±0.43 years) and 10 untrained (CON group aged 20 ±0.7 years) were subjected to 30 min cycling with load 80% maximal oxygen consumption (VO_2max). and 30 min one-time sauna (temperature of 98.2°C and humidity 10 ±2%). Venous blood samples were collected before and immediately after exercise or sauna to measured express of genes related to cellular stress response using quantitative real-time PCR (qPCR).

Results: Sauna induced higher changes in genes expression than exercise in both groups. After passive overheating *HSPA1A* and *HSPB1* mRNA increased significantly in both groups ($p<0.05$), while *IL6* mRNA only in CON and *IL10* mRNA only in JA. Exercise significantly affected *HSPA1A* in both groups. *HSPB*1 and *IL6* mRNA significantly increased only in CON ($p = 0.03$ for *HSPB1* and $p = 0.02$ for *IL6*) but in JA these changes were not significant. Generally in JA the expression of tested genes was lower both after exercise and sauna compared to CON.

Conclusion: Applied exercise caused lower (but insignificant) changes in the expression of tested genes than passive overheating in the sauna. Sauna induced similar changes to exercise and can be used in maintain adaptive changes to physical effort in judo training or can be useful during forced breaks in training, e.g. due to injury.

Keywords: genes expression • heat shock protein • interleukin

Conflict of interest: Author has declared that no competing interest exists

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INTRODUCTION

JA – judo athletes

CON – control group

VO_2max – maximal oxygen consumption

qPCR – quantitative real time PCR (polymerase chain reaction)

HSP 70 – heat shock protein 70

HSPA1A – gene encoding heat shock protein 70 kDa

HSP 27 – heat shock protein 27 kDa

HSPB1 – gene encoding heat shock protein 27 kDa

IL6 – interleukin 6

Il6 – gene encoding IL6

IL10 – interleukin 10

IL10 – gene encoding IL10

TBP – gene encoding TATA box protein

TUBB – gene encoding tubulin B

BMI – body mass index

mRNA – messenger ribonucleic acid

cDNA – cyclic deoxyribonucleic acid

The popularity of sauna bathing as a wellness treatment in sport and relaxation has led to an interest among researches regarding changes within the body appear after the application of external heat. It is well known that sauna exposure has significant influences many aspects of the human body, including relaxation of skeletal muscle [1], reduction of body fat [2], reduction of oxidative stress [3], stimulation the immune system [4], and activation of the hormonal system, especially in secretion of adrenocorticotropic hormone (ACTH) [5].

Although the sauna is most commonly used as a relaxation treatment, some of the changes caused by such external heat stress are similar to those obtained after exercise-associated internal stress. For instance, increased body temperature resulting from environmental heat stress or exercise can result in oxidative stress [6], mobilization of the immune and hormonal systems [7], sweat activity [2], and heat shock protein [8], interleukin, and others.

Overheating and dehydration are stressors that activate homeostatic mechanisms, especially on the cellular level, such as the expression of stress-related genes. After sauna exposure or exercise, genes associated with apoptosis, such *HSPA1A* or *HSPB1*, are overexpressed [9-11]. These genes are easily induced by physiological stress, changes in temperature, or oxidative stress [12-14]. Furthermore, many reports show that the cellular response to stress is independent of the stressor and involves the production of pro- and anti-inflammatory proteins [15]. According to Ziemann et al. [16], changes in heat shock protein and interleukin levels are crucial for training adaptation. These adaptive changes in transcription levels result in a decrease in basal *HSPA1A* mRNA, lower expression of *IL6* mRNA, and higher *IL10* mRNA compared to sedentary people [17, 18].

Numerous studies of judo athletes in the literature examine a variety of factors, such as reduction of body mass in individuals that participate in combat sports [19], testing fights in a vertical posture as a criterion of talent for combat sports [20], as well as physiological possibilities, kind of training, etc. However, there are no many data associated with genes expression so after exercise as the overheating during biological regeneration.

Physiological requirements in judo include both anaerobic and anaerobic possibilities. Judo athletes must demonstrate skills in accordance with technical, tactical, or conditioning improvements [21]. Development of these skills is associated with lower and upper body exercise [22, 23]. Training programs that feature high-intensity intermittent training (HIIT) are often used in judo training sessions [21]. Given the potential for the intensity of training and participation of direct combat to result in injury, judo is one of the more traumatic sports. Therefore, whether sauna baths can be useful to maintain adaptive changes during forced breaks in training for judo athletes is an important question. Unfortunately, there is currently only one study in the literature that examines changes caused by sauna exposure compared to those obtained by exercise [6]. The authors found that both sauna and exercise influence the pro-oxidant-antioxidant balance and that oxidative stress was lower after exercise compared to sauna. Oxidative stress and changes in temperature are important factors that affect the cellular stress response, including changes in the expression of heat shock proteins and interleukins.

The aim was the effect of one-time sauna and moderate exercise on selected genes expression in judo athletes and untrained people.

For practical purposes, the main question addressed was whether probable changes in the expression of some genes in the sauna are similar to changes observed after exercise, and whether such changes may be useful for maintaining adaptive changes to exercise.

MATERIAL AND METHODS

Ethics Statement

This study was approved by the Bioethics Committee for Clinical Research (KB14/14). The authors are obliged to respect the principles of the Helsinki Declaration. All participants gave written informed consent to participate in the study. All participants were informed that they could withdraw consent at any time for any reason.

Table 1. Age, anthropometric and physiological characteristics of the study groups (mean and standard deviation).

Group	Age (years)	Height (cm)	BMI (kg/m^2)	% body fat	VO_2max (ml/min/kg)
JA	21.5 ± 0.43	181.5 ±7.45	20.5 ±1.64	9.21 ±2.24	44.25 ±1.86
CON	20.0 ±0.77	182.0 ±6.53	21.5 ±2.27	11.24 ± 4.47	44.44 ±1.78

Participants

A total of 20 healthy males took part in the experiment. The volunteers were divided into two groups, including 10 athletes trained in judo with an average of 8 years' experience (JA group) and 10 physically active men without judo training as a control group (CON group). There were no significant differences between the groups in body mass, body mass index (BMI), and maximal oxygen consumption (VO_2 max) (Table 1).

The experiment took place in the end of May and volunteers were asked to avoid using a sauna for at least one month before the experimental sauna bath. Physically active men making up the control group (CON) reported regular recreational participation in sports such as running, swimming, soccer, and other team sports on average 2-3 times a week for a duration of 45 minutes/. All participants had a normal life style and did not have any injuries to the bone or muscle tissue; reported no intake of drugs, nicotine, or alcohol during the study, and had no other diseases that might directly affect the results. The participants were informed of the nature and possible inconveniences associated with the experiment.

Experimental Overview

During the experiment, participants were exposed to 30 min in the sauna and 30 min of cycling on a cycle ergometer with an individually selected load at 80% VO_2 max (Monark 894E, Peak Bike from Sweden) to assess genes expression. Each activity was performed on Saturday with one week break in between. The judo athletes (JA) and the control group (CON) were subjected to a one time of exposure to the dry sauna. The subjects remained in the Finnish sauna room at a temperature of 98.2°C and humidity 10 ±2% twice for 15 minutes during the same session with a 5 minute break for cooling under the shower (water temperature was 18-20°C). The total time spent in the sauna was 30 minutes (2 x 15 minutes). Before and after the sauna exposure, blood samples were collected to determine genes expression. One week later, participants in both groups returned at the same time of day (morning) to perform 30 min of cycling on a cycle ergometer with individually selected load at 80% VO_2 max (Monark 894E, Peak Bike from Sweden). The saddle height was adjusted for each participant and toe clips were used to ensure stability of the feet. Before and up to 5 min after the test, blood samples were collected to determine gene expression.

RNA extraction, qRT–PCR

Erythrocytes were eliminated from 2 ml of venous blood using red blood cell buffer (RBCL, A&A Biotechnology, Gdynia, Poland). The remaining white blood cells were lysed using Fenozol (A & A Biotechnology, Gdynia, Poland). Further isolation of RNA was carried out by a chemical method as described by Chomczyński and Sacchi [24]. Purity and concentration of the isolated RNA was determined by spectrophotometry (Eppendorf BioPhotometer Plus, Germany). cDNA synthesis from 2 µg RNA was performed using TranscriptMe Kit, containing oligo dT and random hexamers (Blirt, Gdańsk, Poland). Obtained cDNA was diluted 10 times and 2µl was used to qPCR.

Quantitative polymerase chain reaction (q-PCR) assay to determine gene expression

For the analysis of gene expression, Real-Time PCR (LightCycler 480II, Roche, Poland) was applied twice in triplicate for the same sample using a LightCycler polymerase (Roche, Poland). The temperature-time profile of the reaction was in accordance with the manufacturer's instructions. For each reaction, melt curve analysis was performed. The TATA box protein (*TBP*) and B-tubulin (*TUBB*) were used as housekeeping genes. To amplify the genes, the following primer sequences were applied (Table 2).

Statistical Analysis

Data were collected and relative gene expression was analyzed in Microsoft Excel 2017.

Table 2. Primers used for real-time PCR (designed by the author).

HSPA1A	Forward primer: TGGACTGTTCTTCACTCTTGGC Reverse primer: TTCGGAGAGTTCTGGGATTGTA
HSPB1	Forward primer: AAGGATGGCGTGGTGGAGATCA Reverse primer: GAGGAAACTTGGGTGGGGTCCA
IL6	Forward primer: TCCACGGCCTTGCTCTTGTTT Reverse primer: GACATCAAGGCGCATGTGAAC
IL10	Forward primer: GAATCCAGATTGGAAGCATCC Reverse primer: AATTCGGTACATCCTCGACGG
TBP	Forward primer: TGGACTGTTCTTCACTCTTGGC Reverse primer: TTCGGAGAGTTCTGGGATTGTA
TUBB	Forward primer: CTAGAACCTGGGACCATGGA Reverse primer: TGCAGGCAGTCACAGCTCT

In order to calculate the level of gene expression, the Schmigtten and Livak method [25] was applied. The results of relative expression were analyzed in GraphPad Prism 5.0 (www.graphpad.com). Data were transformed to linear and then the normality of distribution was checked with Shapiro-Wilk's test and a paired t-test was applied to determine p-value. To calculate the differences between groups after sauna and exercise as well as within the groups (sauna vs. exercise) two-way and one-way ANOVAs were performed, respectively. Additionally, a paired t-test was performed to check for differences within each group and an unpaired t-test was performed to check for differences between groups. A p-value of less than 0.05 was considered significant.

RESULTS

Exposure to 30 min of sauna bathing caused a significant increase in *HSPA1A* and *HSPB1* mRNA in both groups. *HSPA1A* increased 2^1.6-fold in JA (p = 0.003) and 2^2.8-fold in CON (p = 0.001). *HSPB1* increased 2^1.3-fold in JA (p = 0.02) and 2^1.5-fold in CON (p = 0.001) (Fig. 1A). The increase in *IL6* mRNA was not significant in JA, but was significant in CON (2^1.7-fold, p = 0.02). A significant increase in *IL10* mRNA was observed only in athletes (2^2.1-fold, p = 0.003).

After 30 min of exercise, a significant increase in both groups was observed only for *HSPA1A* mRNA (2^1.4-fold, p = 0.001 in JA; 2^2.2-fold, p = 0.002 in CON, Fig. 1B). Significant increases in *HSPB*1 and *IL10* mRNA were noted only in CON (2^1.2-fold, p = 0.03 for *HSPB1*; 2^1.6-fold, p = 0.02 for *IL6*). Overexpression at a level greater than 2-fold (clinically significant) was observed for *HSPA1A* in CON after both sauna and exercise and for *IL10* after sauna in JA.

To better explain the differences observed in the experiment between groups and between stressors, Figure 2 displays differences in 2^-fold change in the response of the JA vs. the CON to sauna exposure (black bars) and 30 min exercise (gray bars).

Lower and significant changes in expression were observed in JA for *HSPA1A* (2^-1.2-fold,

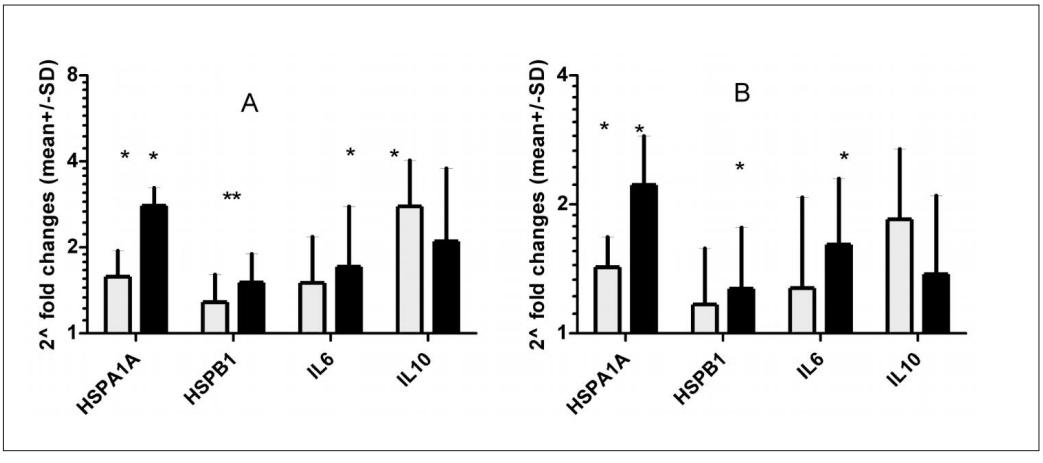

Figure 1. 2^-fold changes in relative expression after (A) sauna and (B) exercise in JA (gray bars) and CON (black bars).

*significant increase in expression p<0.05.

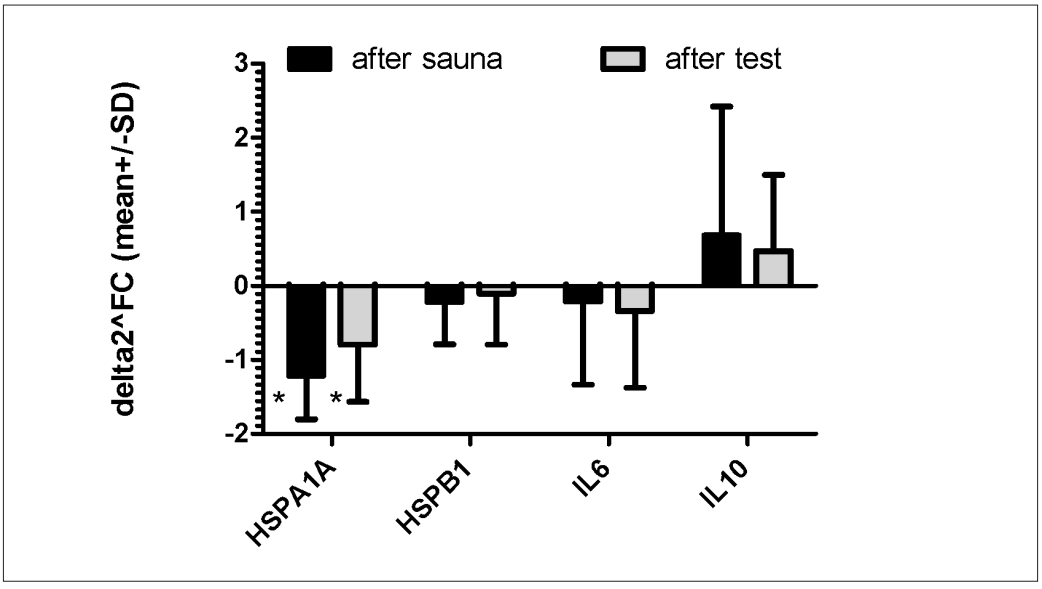

Figure 2. Delta 2^fold changes in genes expression between judo vs control group after sauna (dark bars) and exercise (gray bars). Delta 2^-fold changes were calculated as: 2^-fold changes after sauna in the judo/ 2^-fold changes in the control group.

*significant decrease in expression p<0.05.

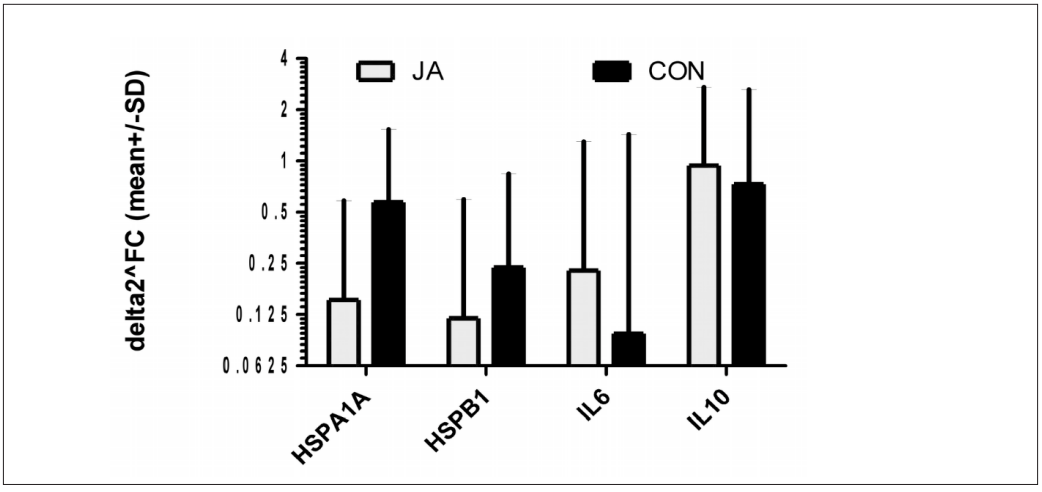

Figure 3. Delta 2^-fold changes in genes expression between sauna vs exercise in judo athlete group (dark bars) and control group (gray bars). Delta 2^fold changes were calculated as: 2^-fold changes after sauna / 2^-fold changes after exercise in the judo and control group, respectively.

p = 0.0001 after sauna; 2^-0.8-fold, p = 0.01 after exercise compare to CON). For *HSPB1* m-RNA the lower expression in JA was observed, but this difference was not significant (2^-0.2-fold after sauna; 2^-0.1-fold after exercise)as well as for *IL6* (2^-0.2-fold after sauna; 2^-0.3-fold after exercise). Higher expression in JA for *IL10* mRNA was observed after sauna and after exercise (2^0.68-fold; p = 0.01 after sauna, 2^0.67-fold, not significant after exercise).

The expression of all genes was higher after sauna exposure compared to exercise in both groups. For *HSPA1A* mRNA in JA and CON, 2^-fold changes were 0.15-fold and 0.57-fold, respectively. For *HSPB1* mRNA, the 2^-fold differences were 0.2-fold and 0.1-fold for the JA and CON groups, respectively. For *IL10* mRNA, the 2^-fold differences were 0.7-fold and 0.9-fold. None of these differences were significant.

DISCUSSION

To my knowledge, this is the first study in which the effects of one time sauna bath exposure and physical exercise on transcript levels was assessed. Judo athletes and control group participating in the experiment had a similar mean VO_2 max and internal load applied on the test and external in sauna was the same.

According to Pałka et al. [26], the effect of many years of specific training in judo is characterized by marked changes in anaerobic indicators, but report no significant difference in VO_2 max between judo athletes and a control group (40.8 ml/kg/min in judo athletes; 42.6 ml/kg/min in control group). Results obtained in this experiment are in accordance to cited data. Judo athletes had VO_2 max. on mean level 44.25 ±1.86 ml/kg/min and control group VO_2 max. 44.44 ±1.78 ml/kg/min. Despite minor differences, all participants performed a 30 min ergometer test in normal humidity (40%) and room temperature with individually selected load (80% max). The training load during exercise and time spent in the sauna room were the same for all subjects.

The changes in *HSPA1A* mRNA were significantly lower in JA after both sauna bath and exercise exposure. Initially, there was a difference in the basal level of *HSPA1A* mRNA, which was lower, but not significantly so, in judo athletes. Lower basal expression in athletes was reported by Buttner [18] and Neubauer [27] and could be an adaptive effect caused by long-term training. Changes in three other genes: *HSPB1*, *IL6*, and *IL10* mRNA for exercise and sauna were observed in judo athletes and controls for decreases in *HSPB1* and *IL6* mRNA and increases in *IL10* mRNA. According to Rutkowski et al. [28] and Szołtysek et al. [15], regardless of the stressor, the cellular stress response is the result of activity in three pathways, two of which are dependent on the influence of *HSF1* and *NF-kB* on the expression of genes examined in this study. In judo athletes, the stress load after both exercise and sauna bath was lower than in controls and there difference in *HSPA1A* and *HSPB1* expression after exercise and sauna within the judo group were very low. On the other hand the changes in expression after exercise were clearly lower than after sauna in this group. These differences suggest specific adaptive mechanisms not only to exercise, but also to external stress.

Evidence suggests that the level of expression of *HSPA1A* and *HSPB1* is associated with temperature [8, 12, 13] and oxidative stress [29, 30] and these indicators are changing under the conditions of sauna and exercise. Pilch et al. [6] examined the association of the tested genes with oxidative stress and antioxidant status, reporting that athletes had higher antioxidant levels than sedentary people, which may be a reason for lower expression of stress-related genes in this group. Furthermore, in a study performed in sauna conditions and during exercise, the authors observed that oxidative stress was different depending on thermal stressor [6]. Smaller changes in expression in the judo athletes may be associated with high heat tolerance and a better functioning thermoregulatory system [31-33].

Stimulation of the synthesis of heat shock protein and interleukin in leukocytes after heat stress was studied by Pizurki et al. [34], Polla et al. [35] and Jacquier-Sarlin et al. [36]. The authors reported a 10-fold increase in the expression heat shock protein after *in vitro* studies of leukocytes in 41°C [34-36].

Changes in *IL6* and *IL10* mRNA confirm better adaptation to exercise and heat stress in judo athletes. The differences between groups were associated with lower expression of *IL6* mRNA and higher expression of *IL10* mRNA. Insignificant increases in *IL6* mRNA in judo athletes confirm the small stress load caused by exercise in this group. There are many reports in which moderate intensity of exercise fails to affect *IL6* expression [37]. However, these interleukins are regulated by transcription factor *NF-kB* which is known as redox-sensitive transcription factor. Adaptive changes to training caused by higher adaptation to oxidative stress rely on activation of the NF-kB pathway as well as HSF-1 [38]. There are some reports in the literature that associate down regulation of *NF-kB* expression with *IL10* mRNA and it is possible that higher expression of *IL0* in athletes simultaneously lowers expression of *IL6* mRNA [39-41]. This association was also observed in this experiment (in exercise and sauna). Observed differences may also be associated with hormonal system (i.e. activation of endocrine system, especially stress hormones). Some data in the literature indicate that changes in cortisol level occur only in people regularly trained, but not in control groups [42, 43]. Since it is known that stress hormones may influence IL1

and IL6 levels, these differences between groups may also affect genes expression [44].

CONCLUSIONS

In conclusion, the judo athletes were characterized by lower expression of *HSPA1A* at rest and lower expression of tested genes after exercise or sauna exposure compared to the control group. In both groups, physical exercise caused lower changes in the expression of tested genes compared to passive overheating in the sauna. Therefore, because of similar changes after sauna and exercise it seems, that sauna can be used not only for biological regeneration but also to maintain adaptive changes to physical effort in judo training or during forced breaks in training, e.g. due to injury.

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