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ORIGINAL ARTICLE

Effects of sauna bathing on stress-related genes expression in athletes and non-athletes

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Abstract

Introduction and objective. Heat stress induces the expression of genes encoding heat-shock proteins and immune response mediators. The aim of this study was to determine the differences in the expression of genes encoding heat-shock proteins 70 kDa and27 kDa**,** interleukin 6**,** interleukin 10and C-reactive protein, between athletes and non-athletes after sauna bathing.

Materials and method. Athletes (n=9) and non-athletes (n=9) were exposed to a Finnish sauna twice during one session at a temperature of 98.2 °C and humidity of 10% ± 2%, with a 5 min break for cooling down under a shower. The groups did not differ in terms of age, height or body mass. Blood samples were taken before and after sauna exposure in order to assess gene expression, using reverse transcription polymerase chain reaction.

Results. Differences were observed in leukocyte mRNA levels of tested genes between athletes and non-athletes. In the non-athlete group, all the tested genes were expressed at higher levels as a response to the same heat challenge.

Conclusion. It appears that expression of stress-related genes induced by heat stress is dependent on the level of physical activity.

Key words

steam bath, gene expression, *HSPA1A*, interleukins, athletes

INTRODUCTION

The analgesic, anti-inflammatory and relaxant effects of high temperatures on the human body are used for medical reasons as well as for relaxation and biological regeneration. However, high temperature is a stressor causing a number of defensive reactions, and it does not have only positive effects; e.g. it can consequently reduce the capacity for physical work [1]. Adaptation to stress conditions, including overheating, is very important for exercise capacity. Many reports indicate comparable physiological changes in a sauna and during exercise [2]. Expression analysis of stress-related genes and their signalling pathways has shown that cellular response to stress, irrespective of the stressor, results in changes in the expression of several hundred stress-related genes, associated with the production of heat-shock proteins (HSPs) and interleukins [3, 4]. Although multiple genes are described as stress genes, only three signalling pathways regulating their expression are currently known [5]. The most important pathway involves the protein nuclear factor kappa B (NF-kB), which induces genes encoding, early and late inflammatory response interleukins (IL-1, IL-6, IL-8), and interferes with the two another pathways, dependent on heat-shock factor protein 1 (HSF1) and p53 protein [5].

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HSP70 encoded by *HSPA1A* is rapidly induced by stress factors such as heat or physical activity [6, 7, 8, 9]. Its over-expression under stress conditions is associated with the function of HSP proteins, i.e. protection of cells from apoptosis or degradation of damaged and denatured proteins [10, 11, 12]. Moreover, in recent years, genes encoding interleukins and HSPs have been increasingly tested under physical stress conditions. For example, Radom-Azik et al. [13] studied the expression of these genes in leukocytes after 30 min of physical effort. The authors suggested that high intensity exercises results in over-expression of genes associated with apoptosis, e.g. *HSPA1A*. Similar results were obtained by Neubauer et al.[14]) who examined gene expression 2h after exercise consisting of 1h running and cycling. Furthermore, some authors reported lower levels of *HSPA1A* expression in trained subjects in comparison to untrained group, as measured after physical activity [14, 15]. Induction of genes encoding interleukins as a result of exercise was also shown by Buttner et al. [15]. The cited authors suggest that the over-expression of genes encoding HSPs, and in particular *HSPA1A,* may highlight the major role of stress factors in body response to heat. Therefore, there is a need to study their expression in order to improve training programmes [15].

One of the most common sources for imitating heat-shock is the Finnish sauna used by athletes and non-athletes alike. However, there have been relatively few reports using the sauna for heat-stress induction, and they have mainly focused on the effects of sauna on the immune system [16] or prevention of colds [17]. Furthermore, no research has been undertaken on the expression of HSPs and interleukins

as a function of physical activity. Occasional reports on the effects of sauna on the bodies of athletes refer to changes in the number of leukocytes and the profile of the white blood cells [18]. In the opinion of the cited authors and the authors of the current study, adaptive changes to exercise should be reflected in differential induction of stress-related genes by sauna bathing. Therefore, the aim of this study was to determine whether there are differences in the expression of genes encoding HSP70, HSP27, IL-6, IL-10 and CRP between trained and untrained subjects in peripheral blood leukocytes in response to a single sauna bath.

MATERIALS AND METHOD

18 healthy men participated in the study: 9 athletes (soccer players): aged 19.8±0.65 years; 188.9±8.16 cm; 82.68± 8.24 body mass, 6 yr average training period, and 9 non-athletes: aged 19.7±0.87 years; 183.78±6.83 cm; 77.58±7.74 body mass. No significant differences were found between the groups for age, height, and body mass (Tab. 1). All volunteers avoided using a sauna for a month before the study, which was performed in December 2014, during the break in football training. Subjects in the control group as well as the soccer players exhibited spontaneous physical activity during the study period (2–3 sessions per week, on average). These data were collected via a questionnaire.

The subjects in both groups led a healthy lifestyle and did not take any supplementation, either before or during the study. They gave written informed consent to participate in the study and were aware of the resulting risks..

The study was approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk (KB14/14), and the authors are obliged to respect the principles of the Helsinki Declaration.

The subjected were divided into two groups (athletes and non-athletes) and remained in the Finnish sauna room at the same time of day (athletes at 16:00 and non-athletes at 17:00) The subjects remained in the Finnish sauna room at the temperature of 98.2 °C and humidity 10 +/- 2% twice for 15 minutes each during the same session, with a 5 min break for cooling under a shower (water temperature – 18–20ºC). Rectal temperature was monitored during sauna bathing at 5 min intervals using a MRV-55044-A electric thermometer with an accuracy of 0.05 °C (Denmark).

Before and after the session in the sauna, the weight loss and percentage of body fat were measured (scales Tanita BC-418 MA (II) with the accuracy of body mass and fat measurement 0.1kg and 0.1%, respectively).

RNA extraction and quantitative real-time polymerase chain reaction (qRT–PCR). Two ml of peripheral blood were collected from the ulnar vein of each participant before and after sauna session. Prior to RNA extraction, erythrocytes were lysed and discarded using RBCL buffer (A&A Biotechnology, Poland). The leukocytes were lysed using Fenozol (A&A Biotechnology, Poland) and the RNA subsequently precipitated by the method described by Chomczynski and Sacchi[19]. The extracted RNA was treated with DNaseI (Invitrogen) to digest any remaining DNA. cDNA was synthesized using the Transcript Me system (Blirt, Gdańsk, Poland), according to the manufacturer's instructions. A mix of 10 µL master mix, 2 µL enzyme mix with M-MuLV reverse transcriptase, 2 µg RNA and water, up to a final volume of 20 µL, was added to each tube. Samples were incubated at 25 °C for 10 min, 55 °C for 30 min and 85 °C for 5 min. qRT-PCR analyses for *HSPA1A* and *HSPB1*were performed using the Step One real-time PCR system (Applied Biosystems). Each qRT-PCR reaction mix consisted of 10 µl Semi Fast Sybr Green qPCR master mix (Biolone, UK), 4 µl of cDNA, 0.8 µL of each primer at the concentration of 10 pM, in a final volume of 20 µL. Thermal cycling conditions included an initial hold at 95ºC for 2 min, and then 40 cycles of 95ºC for 15 sec, 60ºC for 10 sec and 72ºC for 20 sec. All samples were assayed in triplicates. Primer sequences are listed in Table 2. Target gene expression was normalized to the expression of the reference gene *Tata Box Protein* (*TBP*). To amplify the genes, the following primer sequences were applied:

TBP	F: ACTCCCGTTGTCCCAAGGCTTC
	R: TCTGTCGGCTCCGCTCTGAGAT
HSPA1A	F: TGGACTGTTCTTCACTCTTGGC
	R: TTCGGAGAGTTCTGGGATTGTA
HSPB1	F: AAGGATGGCGTGGTGGAGATCA
	R: GAGGAAACTTGGGTGGGGTCCA
IL-6	F: TCCACGGCCTTGCTCTTGTTT
	R: GACATCAAGGCGCATGTGAAC
IL-10	F: GAATCCAGATTGGAAGCATCC
	R: AATTCGGTACATCCTCGACGG
CRP	F: TCGTTAACGGTGCTTTGAGG
	R: TCTTGGTCTTGACCAGCCTCT

Statistical analysis. Data were collected and relative gene expressions analysed in Excel 2005. In order to calculate the level of gene expression, the method of Schmigtten and Livak[20] was used. To assess statistical significance, the following tests were used: the normality of distribution was checked with the Shapiro-Wilk's test, and the non-parametric Wilcoxon test (comparing results before and after the test). To determine the differences between the two groups, a one-way ANOVA was used. All calculations and graphs were prepared using GraphPad Prism 6.0 (ftx.pl/program/graphpad-prism). Statistically significant differences were considered at the level of $p \leq 0.5$.

RESULTS

Mean anthropometric parameters for athletes and untrained young men are presented in Table 1. There were no significant differences in age, height, body mass and BMI, nor in fat % between the tested groups.

Weight loss after the sauna session was similar in both groups, and the average values were as follows: -0.79±0.31 kg in the group of athletes and -0.77±0.25 kg in the group of

Table 1. General profile of subjects involved in the study

Groups	Age (years)	Height (cm)	Body mass (kg)	BMI (kg/m²)	Fat (%)
Athletes (mean ± SD)	19.8±0.65	188.9±8.16	81.68±8.24	19.8±0.53	8.75±3.00
Non-athletes (mean ± SD)	19.678±0.87	183.78±6.83	77.58±7.74	22.89±2.33	9.08±3.81
Differences	0.13 (ns)	5.12 (ns)	4.1 (ns)	3.09 (ns)	0.33 (ns)

Table 2. Body mass loss during sauna session

Groups	Body mass (kg)		Difference
	PRE-	POST-	
Athletes (n=9) (mean ±SD)	81.68 ±8.24	80.89 ±8.07	-0.79±0.31
Non-athletes (n=9) (mean ±SD)	77.58 ±7.74	76.82 ±7.66	-0.77±0.25

untrained people (Tab. 2). The time spent in the sauna room was 30 min, during which rectal temperature increased by 1.2 °C in the group of athletes, and in the untrained group 37.06±0.28 – 38.26±0.31 in athletes, and from 37.09±0.19 -38.29±0.18 in the control group). There were no significant differences between the groups in body mass, total duration of sauna bathing, or increase in rectal temperature.

Gene expression. Changes in gene expression after sauna bathing in the athlete and non-athlete groups are presented in Figure 1. The data are presented as fold changes compared to the value at rest (i.e. before sauna bathing).

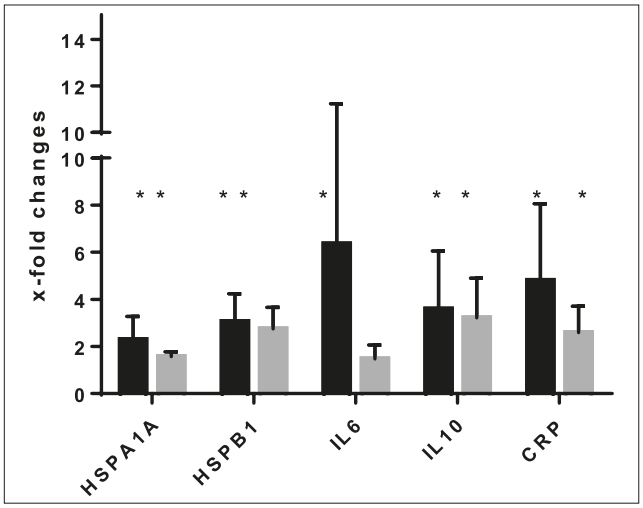

Figure 1. x-fold changes in expression of tested genes in the non-athletes (black bars) and athletes (gray bars). x-fold changes was calculated as: relative expression after sauna/relative expression before sauna

The basal level of gene expression was similar in both examined groups. There was a significant increase in expression of all tested genes, except IL6, both in the control and athlete groups following sauna bathing, but the extent of the increase was different. In the group of non-athletes, a significant increase was observed in the expression of: *HSPA1A* (~2.3-fold, p≤.01), *HSPB1* (~3-fold, p≤.01), *IL-6* (~6.4-fold, p≤.01), *IL-10* (~3.6-fold, p≤.01) and *CRP* (~4.8-fold, p≤.01). In the group of athletes, the changes in gene expression were smaller: ~ 1.5-fold change for *HSPA1A*(p≤.01), 2.8-fold for *HSPB1* (p≤.04), 1.5-fold for *IL-6* (p≥.05), 3.2-fold for *IL-10* (p≤.01) and 2.6-fold for *CRP* (p≤.01). The smallest difference in the expression level between the groups was noted for *IL10* while the greatest for *IL6*. With regard to *HSPB1* and *CRP* genes, the differences between the groups were substantial and statistically significant (p≤.05). The results of two-way ANOVA are shown in Table 3.

Table 3. Results of two-way Anova

Gene		Relative expression (mean+/-SD)		Inter-action	Row factor	Time	Subject
		Athletes (n=9)	Non-athletes (n=9)				
HSPA1A	PRE	0.761515 0.138583	0.550913 0.213214	ns	ns	ns	ns
	POST	1.164539 0.201702	1.109347 0.430418				
HSPB1	PRE	0.46371 0.14555	0.272446 0.064998	.04*	ns	ns	.03*
	POST	1.183324 0.348363	0.662795 0.137974				
IL-6	PRE	0.541102 0.294002	6.355109 4.879734	ns	ns	ns	ns
	POST	2.824537 1.95512	0.502084 0.229948				
IL-10	PRE	0.43069 0.161744	0.393241 0.259623	ns	ns	ns	ns
	POST	1.389949 0.727234	0.948875 0.388738				
CRP	PRE	0.612097 0.267151	0.258632 0.132443	.005*	.0044*	.05	ns
	POST	1.985216 0.686276	0.591512 0.225282				

* significant differences p<0.5

DISCUSSION

It is well-known that overheating and physical exercise result in the induction of genes encoding HSPs and interleukins. Hyperthermia is a limiting factor in physical performance [21]; therefore, upregulation of genes encoding HSPs and interleukins could be essential for gaining thermotolerance [22]. There are not many reports in the literature on changes in gene expression upon one-off Finnish sauna bath that would include people who train regularly and those who do not exhibit regular physical activity. Despite the supposedly similar thermoregulation processes in trained and untrained subjects, in the presented study t was found that these two groups displayed differences in the expression levels of all tested genes. Significant increase in the expression of all genes was observed in the untrained group. In the group of athletes, the increase in the expression of ***HSPA1A***, ***IL-10***, ***HSPB1*** and ***CRP*** mRNA was also significant. Increase in ***IL6*** mRNA was small and not significant in this group. Stimulation of synthesis of heat-shock proteins and interleukins in leukocytes under stress factors, such as high temperature, was studied by Pizurki and Polla [23], Jacquier-Sarlin et al. [24], and Polla and Cassarizza[25]. These authors have shown in ***in vitro*** studies of leukocytes that the increase in temperature to 41˚C causes a 10-fold increase in HSP concentration, which is significantly higher than that observed in the current study [23, 24]. However, these experiments were performed ***in vitro,*** which makes a direct comparison with these results is impossible. The increase was much smaller in the presented study, which confirmed the substantial differences obtained in the tests ***in vivo*** vs. ***in vitro***[26]. Furthermore, the current results demonstrate a relationship between gene expression and physical activity. Trained people have a higher thermotolerance, which is manifested in a lower expression of genes encoding HSPs and interleukins such as

IL-6 or IL-10. The greater thermotolerance has been acquired during many years of sports training and may have been also induced in response to other stressors. On the other hand, comparable expression of ***HSPA1A*** may be required for leukocytes to survive under heat-stress conditions [27]. It could be suggested that relative heat-stress was smaller for athletes. The changes in expression of ***IL6*** and ***CRP*** mRNA are very interesting: there was no significant increase in ***IL6*** mRNA, and the increase in CRP mRNA was half as large as in the group of athletes. Heat stress in sauna therefore caused a less severe pro-inflammatory response in the group of athletes and similar anti-inflammatory response. Thus, in athletes, the pro-inflammatory response to heat-stress was smaller despite the same heat load.

Stress-related genes are also upregulated during exercise, as reported by Buttner et al. [15]. Radom-Azik et al.[13] and Neubauer et al. [14] stated that the upregulation of genes related to apoptosis and immune response in neutrophils, represents an integrated response to various physiological dysfunctions. The presented results, obtained from the experiment which was carried out under heat-stress conditions, are consistent with those reported by Neubauer [14] and Buttner [15], who noted lower expression of genes encoding HSPs and interleukins in trained vs. untrained people after exercise using the same load.

The results obtained in this study show a greater thermotolerance in athletes' blood leukocytes compared to untrained people, which is manifested in lower expression of ***HSPA1A***, ***HSPB1***, ***IL-6***, ***IL-10*** and ***CRP***. This effect may have been caused by lower expression of transcription factors, such as ***HSF-1*** or ***NF-kB***. Changes in gene expression during sauna bathing were similar to those reported during exercise, but they are also dependent on the stress load [28]. On this basis, the authors suggest that the sauna can be used to acquire or maintain thermotolerance of cells during the transition period, training time, and in the case of injury, whenever the influence of heat is recommended. Moreover, it may constitute a means to increase tolerance to exercise for people with lower physical activity.

CONCLUSIONS

There were differences in the expression of ***HSPA1A***, ***HSPB1***, ***IL-6***, and ***CRP*** depending on the level of physical activity. The immunization potential of sauna bathing may be used for medical purpose, but upregulation of ***HSPA1A*** in all subjects indicates that the sauna can improve thermotolerance, which is an important aspect of the sports practice. It is therefore important to use its influence during the breaks in training or in case of injury.

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