THE EFFECT OF FINNISH SAUNA ON THE ACTIVITY OF SELECTED LYSOSOMAL ENZYMES ON HEALTHY SUBJECTS

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Abstract

Purpose. The aim of this study was to determine the effect of sauna on the activity of arylsulfatase (ASA), cathepsin D (CTS D), acid phosphatase (AcP) and α-1-antitrypsin (AAT) in serum of healthy subjects.

Methods and procedures. Subjects (n=16) performed 30 min Finnish sauna in temperature of 85°C and humidity of 40%. The blood samples were taken from cubital vein before the entry to sauna, 5 and 30 min after the exit. The obtained results were statistically analyzed by using ANOVA test. The changes of the level $p<0.05$ were accepted as statistically significant.

Results. Statistically significant increase of ASA and AAT as well as decrease of CTS D activity 5 and 30 min after the exit from sauna was revealed as compared to the value before entry to sauna. There was not changed in AcP activity after sauna. Statistically significant correlations between studied parameters were also revealed.

Discussion and conclusions. Sauna is a very popular regeneration method among sportsmen. This procedure is also very stressful for human organism. Statistically significant increase of activity of lysosomal enzymes revealed in the paper proves that single entry to Finnish sauna significantly decreases stability of lysosomal membranes in healthy volunteers.

Key words: Finnish sauna, arylsulfatase, cathepsin D, acid phosphatase, α-1-antitrypsin.

Introduction

The sauna bathings origin from very primitive steam baths (R. Livingston, 2010). The place where sauna bathings were started was probably Central Asia and this practice arrived to Europe just from there (J. Bruchac, 1993, Sauna, 2004 a). Very primitive steam baths were used by Vikings, Aztecs, North American Indians, ancient African people (R. Livingston 2010, Sauna, 2004 a) and Maya people (Harvard Men's Health Watch, 2005). The practice of bathing in hot air fumes reached to Syria, Greece and also to Egypt (Sauna, 2004 a). The sauna bathings supersaturated by water steam were especially widely practiced among the ancient Slavs and in the eastern part of the Scandinavian Peninsula. The Finland is a place where about 2000 years ago techniques of hot steam bath with hot dry bath were joined together and Finnish sauna was created. It gradually got popularity in whole Europe (similarly like the "banya" in Russia which is steam sauna) (Sauna, 2004 a, b). In the Middle Ages using of sauna was almost completely stopped. The revival of this procedure occurred not early as in the middle of 20th century, mainly due to Finnish sportsmen (T. Prystupa, 2009). Nowadays, Finnish sauna is the most popular type of such procedures.

In the first part of the whole sauna procedure subjects stay in a wood room (sauna) by 5-20 min at temperature of 80-120°C. Inside of sauna of relative air humidity is 5-10%. After exit fast and precise cool down of whole body is done by immersion in cold water or a shower (Harvard Men's Health Watch, 2005, R. Jeffrey et al., 2009, T. Prystupa, 2009). Extremely high temperatures inside of the sauna are obtained due to an oven which heats the stones located on it. Red-hot stones can be sprinkled with water what causes an increase of air humidity and decrease of temperature. In this way other types of dry sauna can be also obtained: wet sauna (70-90°C, 25-39% of air humidity) and steam sauna (45-65°C, 40-65% of air humidity). The steam sauna belongs to the mildest kind of sauna bathings and it is often combined with aromatherapy (using of essential oils, herbs) (Sauna, 2004 a). A relatively new type of sauna is far-infrared sauna. Its course is similarly like in Finnish sauna, source only difference is the heat. In infrared sauna it is far-infrared radiation. Its energy is absorbed by the skin (4cm in depth) and deeper located tissues, and then it is converted into heat energy. The temperature inside of far-infrared sauna is lower than in a dry sauna (40-60°C, air humidity to 5%). Nevertheless, the body sweating is more intensive and due to lower air humidity it is easier to breathe (R. Beever, 2009). For this reason far-infrared sauna does not load an organism and therefore it can be used in patients with diseases of circulatory system (S. Il-Suk et al., 2010).

During sauna bathing the sympathetic nervous system and hypothalamus-pituitary-adrenal hormonal axis is intensively activated to compensate the excessive increase of body temperature (K. Kauppinen and I. Vuori, 1986, K. Kukkonen-Harjula and K. Kauppinen, 1988). The consequences of this activation are changes in hormonal profile resulting in water retention and "fight-or-flight" organism response. It is followed by reduced perception of pain, stimulation and increase of vigilance. Thus, the thermal impulse is connected i.a. with increased concentration of noradrenaline (D. Jezovà et al., 1994, K. Kauppinen et al., 1989). The level of adrenaline does not change or it can be increased. The alterations revealed by different authors probably result from different conditions of doing bath into sauna, especially from various techniques of organism cooling down (K. Kauppinen et

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al., 1989, K. Kukkonen-Harjula and K. Kauppinen, 1988, K. Vähä-Eskeli et al., 1992). The overall heart work determined by the heart rate and systolic blood decrease and the systolic pressure do not change significantly (A. Eisalo and O. Luurila J., 1988). Sauna improves blood circulation in the mucosa of the upper respiratory tract (W. Pilch et al., 2006, O. Hanninen, 1986) and positively effects on the locomotor system. It increases mobility in the joints by increasing the flexibility of the fibrous tissue of joint capsules and ligaments, by improving of blood flow in the tissues surrounding joint and by reducing the viscosity of synovial fluid. Sauna relaxes the skeletal muscles and their intergrowths, too (T. Brzostek et al., 2007). Sauna session increases endurance performance and psychological efficiency of organism (D. Groves, 1987, M. Chorąży and K. Kwaśny, 2005, W. Pilch et al., 2006, G.S.M. Scoon et al., 2007).

Sauna bathings are often used as a supplement of treatment in patients with circulatory system diseases, depression, respiratory system diseases (especially in chronic obstructive pulmonary disease – COPD) as well as in diseases of locomotor system (usually in fibromyalgia but also in rheumatoid arthritis) (K. Kukkonen-Harjula and K. Kauppinen, 2006). However, sauna is a procedure the most often applied for biological restoration in sportsmen both professional and amateur (T. Prystupa et al., 2009, Sauna, 2004 a, b, G.S.M. Scoon, 2007). Special indication for supporting treatment in patients with circulatory system diseases can be far-infrared sauna, because this type of sauna does not load the patient's heart (S. Il-Suk et al., 2010, M. Imamura, 2001, M. Miyata et al., 2008). The contraindication for sauna

The cathepsin D activity was estimated by the Anson method (S. P. Colowick and N. C. Kaplan, 1955). Substrate was 2% denatured bovine hemoglobin dissolved in 100 ml in 0.1 M solution of citrate-phosphate buffer about pH = 3.8. The cathepsin D activity was measured by the following scheme:

Reagent (ml)	**Assay**		
	Blank	Subject	Control
2% bovine hemoglobin	-	0.4	0.4
0.1 M citrate-phosphate buffer pH=3.8	2.5	2	2
	15 min incubation at temp. of 37°C		
Serum	-	0.1	0.1
5% TCA	60 min incubation at temp. of 37°C		no incubation
	1.0	1.0	1.0
	centrifugation 5 min by 960xg		
Supernatant	2.0	2.0	2.0
5 N NaOH	1.0	1.0	1.0
Reagent of Folin-Ciocalteu	3.0	3.0	3.0
	centrifugation 5 min by 960xg		

Extinction of the samples was detected after 15 min at a wavelength λ = 600 nm versus blank. The enzyme activity was calculated by the formula:

$$C\ (nmol/min) = E(B) – E(K) \times K$$

pressure, which indicate myocardial oxygen demand, does not increase greatly (K. Kauppinen and I. Vuori, 1986) because the diastolic and mean arterial pressures bathing is its using to fast reduction of body mass by sportsmen of disciplines depending on weight classes. It often results in considerable decrease of endurance performance due to too large and rapid dehydration (T. Prystupa et al., 2009). The sauna procedures are also contraindicated for patients with abnormal heart rhythms, unstable angina, advanced heart failure or heart valve disease (Harvard Men's Health Watch, 2005).

The purpose of this paper was to study the effect of single Finnish sauna procedure on the activity of selected lysosomal enzymes and proteases inhibitor, α-1-antitrypsin in peripheral blood of healthy volunteers.

Methods and procedures

The studied group consisted of 16 healthy subjects (3 women and 13 men) which did not use sauna at least 3 months before the study. Mean age was 36.7 ± 12.3 years. The study in Finnish sauna was performed at "Olsztyńska Szkoła Wyższa" in Olsztyn. Subjects stayed inside of the sauna in swimsuit by 30 min at temperature of 85°C and 40% relative air humidity.

Blood samples were taken from cubital vein three times: before the entrance to the sauna and 5 and 30 min after the exit from the sauna. The venous blood was taken into dry tubes in order to obtain the blood serum. In the serum the activity of cathepsin D, arylsulfatase and acid phosphatase and also α-1-antitrypsin was determined.

where: E(B) – extinction of subject
E(K) – extinction of control
K – gradient coefficient of calibration curve

The activity of the enzyme was expressed as nM of tyrosine/mg of protein/min.

The activity of acid phosphatase was measured according to Bessy method which was modified by Krawczyński (1972). P-nitrophenylphosphate disodium (substrate) in 0.5 M citrate-tartaric-formaldehyde buffer about pH = 4.9 was used for study. The level of enzyme activity was the amount of p-nitrophenol released during enzymatic hydrolysis of the substrate. Acid phosphatase activity was determined by the following scheme:

Reagent (ml)	**Assay**			
	Standard	Subject	Control	Blank
Working solution of substrate	-	1.0	-	1.0
H_2O	1.0	-	1.0	-
30 min incubation at temp. of 37°C				
Standard solution of p-nitrophenol	2.0	-	-	-

Serum	-	2.0	2.0	-
H_2O	-	-	-	2.0
30 min incubation at temp. of 37°C				
0.1 N NaOH	0.5	0.5	0.5	0.5

Working solution was prepared on the same day as an experiment. 0.4% solution of p-nitrophenylphosphate disodium was mixed with 0.5 M citrate-tartaric-formaldehyde buffer (pH = 4.9) in 1:1 ratio. Extinction was detected versus distilled water at the wavelength λ = 405 nm. Acid phosphatase activity was calculated according to the formula:

C (nmol/ml) = (E(B) – E(K) – E(S))/E(W) x n

where: E(B) – extinction of subject
E(K) – extinction of control
E(W) – extinction of standard
E(S) – extinction of blank
n – the concentration of p-nitrophenol in standard

The activity of the acid phosphatase was expressed as nM of p-nitrophenol/mg of protein/min.

The arylsulfatase activity was estimated according to Roy method which was modified by Błeszyński (W. Błeszyński and L. M. Działoszyński, 1965). For the determination 0.01 M sulphate of 4-p-nitrocatechol (4-NCS) in 0.5 M acetate buffer (pH = 5.6) was used. The measure of enzyme activity was the amount of released 4-nitrocatechol (4-NC) during enzymatic hydrolysis of the substrate. Arylsulfatase activity was determined according to the following schedule:

Reagent (ml)	**Assay**	
	Subject	Control
0.01 M 4-NCS	0.5	0.5
0.5 M acetate buffer pH 5.6	0.4	0.5
Serum	0.1	-
10 min incubation at temp. of 37°C		
1 N NaOH	2.0	2.0

Extinction was detected versus the control at a wavelength λ = 510 nm. Amount of released 4-NC was calculated due to the formula:

C (nmol/ml) = K x E(B)

where: K – gradient coefficient of calibration curve
E(B) – extinction of subject

The arylsulfatase activity was expressed as nM of 4-NC/mg of protein/min.

The activity of α-1-antitrypsin in blood serum was determined by Eriksson method (E. Szczeklik, 1974, M. Szmidt et al., 1991). The basis of the assay in measuring a decrease of enzymatic activity of trypsin due to short incubation with defibrinated blood serum. As a substrate a synthetic amide derivative of arginine – benzoyl-DL-arginine-p-nitroanilide was used. The samples consisted of 0.1 M Tris-HCl buffer (pH = 8.2) which contained 0.02 M $CaCl_2$, trypsin solution (10 mg of trypsin in 50 ml of 0.0025 N HCl) and the serum of the subjects. After 15 min incubation at temp. of 25°C into the tubes a solution of substrate was added and it was still incubated at temp. of 25°C by 10 min. After this time the reaction was stopped by acetic acid and then absorbance was detected at a wavelength λ = 410 nm versus the blank which did not contain a solution of trypsin. Control samples contained the same ingredients as the subject samples but without the serum. The inhibitor activity was calculated in accordance with to the formula:

TIC = (Ec – Es)/Ec x T x 1/ V x F

where: Ec – extinction of control
Es – extinction of subject
T – number of μg of trypsin
V – the volume of the serum expressed at μl
F – coefficient that expresses the ratio of the trypsin combined with soy inhibitor which is used for standardization of trypsin solution

The TIC means mg of trypsin which activity was inhibited by 1 ml of serum (mg of trypsin/ ml).

The laboratory studies were done in the biochemical laboratory of the Chair of Medical Biology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń. Subjects were informed about the purpose of study and gave their written consent. The study received the approval of the Bioethics Committee at the Collegium Medicum in Bydgoszcz. The results were statistically analyzed by using ANOVA test. The changes of the level $p<0.05$ were accepted as statistically significant.

Results

In the paper statistically significant decrease of cathepsin D activity both 5 min (about 84%; $p<0.001$) and 30 min (about 75%; $p<0.01$) after sauna was revealed as compared to activity before sauna bathing. On the other hand, the arylsulfatase activity increased about 94% 5 min ($p<0.001$) and about 83% 30 min after sauna ($p<0.001$) as compared to ASA activity before entrance into sauna. An increase of α-1-antitrypsin activity after bath into sauna was also reported. Comparing AAT activity before and after the sauna procedure (tab. 1), it was about 34% higher 5 min ($p<0.05$) and 54% higher 30 min ($p<0.001$) after exit from sauna.

The tendency to increase of AcP activity 5 min after sauna was also found as compared to the activity of this enzyme before the sauna ($p>0.05$). Although activity of acid phosphatase decreased statistically insignificant 30 min after exit from sauna compared with the value measured 5 min after sauna session, it was still higher than before the entrance to the sauna ($p>0.05$).

Moreover, statistically significant correlations were reported between studied parameters. 5 min after exit from sauna strong, positive correlation between AcP and ASA activity ($r=0.94$; $p<0.001$) and also

between CTS D and AAT activity (r=0.5; p<0.05) was found. Positive correlation was also determined in the third term of study (30 min after exit from sauna) between activity of AcP and ASA (r=0.7; p<0.01).

Table 1. The activity of lysosomal enzymes and α-1-antitrypsin in the blood of volunteers after Finnish sauna

	Term of study		
Parameter	**Before entrance to sauna (control)**	**5 min after exit from sauna**	**30 min after exit from sauna**
ASA (10^{-2} nM of 4-NC/mg of protein/min)	0.48±0.12	0.93^{***}±0.38	0.88^{***}±0.23
CTS D (10^{-2} nM of tyrosine/mg of protein/min)	19.89±18.82	3.12^{***}±1.22	4.9^{**}±0.71
AcP (10^{-3} nM of p-nitrophenol/ mg of protein/min)	110.58±26.89	125.59±12.28	118.55±12.17
AAT (mg of trypsine/ml of serum)	0.61±0.33	0.82^{*}±0.13	0.94^{***}±0.14

The results were shown as mean ± SD

* statistically significant difference as compared to study before entrance to the sauna ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$)

Discussion and conclusions

Among sportsmen sauna is often used as a training of the respiratory and cardiovascular system without necessity of the locomotor system loading. Sauna activates many physiological reactions in the organism which are very similar to the reactions after physical exercise. Therefore, it is regarded as a type of physical training (W. Pilch, 2010). It has been proved that during the sauna session, the organism burns about 300 kcal what is equivalent to the energy needed for 3-4 km run (A. Tanny, 1995). During sauna bathing the heart rate increases about two times as compared to the value at rest (K. Kauppinen, 1989, K. Kukkonen-Harjula et al., 1989). The frequency of lung ventilation significantly rises what is caused i. a. by an increase of lung tissue extensibility (W. Pilch et al., 2006, O. Hanninen, 1986). Regular sauna using increases vital capacity (VC), peak expiratory flow rate (PEF) and also the forced expiratory volume in the first second (FEV_1) (L.A. Laitinen et al., 1988). Similarly like after exercise due to sauna effect, the increase of organism temperature induces synthesis of heat shock proteins. In the blood serum of subjects which performed single sauna bathing significantly increase of Hsp70 concentration 2 hours after procedure ending was reported (J-É. Blatteau et al., 2008). As an effect of exercise-induced hyperthermia the synthesis of heat shock protein increases in skeletal muscle, heart and liver cells of rats (D. C. Salo et al., 1991). In addition to increase of body temperature sauna also affects the endocrine glands and especially the adrenal glands on the hypothalamic-pituitary-adrenal axis as well as the renin-angiotensin-aldosterone system. Both as a result of the sauna and exercise the increase the human growth hormone (hGH), ACTH, prolactin, cortisol and catecholamines concentration in blood is observed. After sauna like after physical effort the concentration of LDL cholesterol reduces and HDL cholesterol and free fatty acids (FFA) concentration increases (W. Pilch, 2010).

During aerobic exercise in the organism the generation of reactive oxygen species (ROS) is increased which occurs mainly during the reduction of oxygen in the respiratory chain in mitochondria (R. R. Jenkins, 1988, A. Woźniak et al., 2005). In a natural way some part of the oxygen is incompletely reduced leading to production oxygen free radicals (OFR) (R. R. Jenkins, 1988, L. L. Ji, 1995) what may also take place as a result of the sauna.

During sauna in the overheating phase cardiovascular system combats thermal stress by cutaneous vasodilatation and increase of cutaneous blood flow. The overheating phase is followed by a rapid phase of cool down and contraction of blood vessels (vasoconstriction) (K. Kukkonen-Harjula et al., 1989). These phases form a sauna cycle which is performed at least twice (T. Prystupa, 2009, Sauna, 2004 a, b). The phenomenon ischaemia (cool down phase) and then hyperaemia (overheating phase), in the endothelium of blood vessels leads to convertion of xanthine dehydrogenase to xanthine oxidase which catalyzes the reaction of superoxide anion radicals generation (B. Halliwell and J. M. C. Gutteridge, 1993). In this way OFR can be formed also during the sauna.

Sauna procedure statistically significant increases noradrenaline and adrenaline concentration (D. Jezovà et al., 1994, K. Kauppinen et al., 1989, K. Kukkonen-Harjula and K. Kauppinen, 1988). Autooxidation of catecholamines due to higher supply of oxygen (hyperventilation during overheating phase) is another possible source of higher than physiological concentration of OFR (B. Halliwell and J. M. C. Gutteridge, 1993) as the sauna effect.

The generation of reactive oxygen species (ROS) intensifies the processes of lipid peroxidation. These are free radical reactions which are one of the most specific consequences of free radicals reactive action. The process is based on self-stimulation chain reactions according to the principle of transfer free electrons to each other. Free radical molecules which are still produced accelerate chain reaction (J. Kedziora, 1998). Peroxidation initiated by ROS leads to the split of polyunsaturated fatty acids that build protein-lipid membranes (P. L. Marino, 2001).

The statistically significant increase of ASA activity and tendency to increase of AcP activity after single sauna procedure revealed in this study, can be

caused by oxidative damage of lysosomal membranes in organism and the leak of these enzymes first into the intercellular space and then into the peripheral blood. The existence of such the phenomenon as an effect of oxidative stress caused by exercise was proved by Wozniak et al. (2001). The confirmation of the increased lability of lysosomal membranes after the sauna is also correlations between the activity of AcP and ASA both 5 and 30 min after exit from the sauna found in this paper.

The increase of the lysosomal enzymes after the sauna can be also explained by another theory. It is supposed that the source of the increased permeability of lysosomal membranes during and/or after exercise is an increase of pH in lysosomes and decrease of the aggregation of enzymes molecules in their interior. This is a consequence accumulation in the lysosomes of ammonia which concentration in skeletal muscle and in blood increases significantly during exercise (A. Wozniak, 2005). The physiological effects of sauna on the organism are believed to be are similar to the effects of physical exercise thus, it is possible that after sauna procedures ammonia also accumulates in lysosomes.

This study also revealed statistically significant decrease of cathepsin D activity both 5 min (about 84%; $p<0.001$) as well as 30 min after sauna (about 75%; $p<0.01$) as compared to the activity before sauna bathing. However, simultaneous the increase AAT activity was found in subjects' blood serum in the same terms of study. Alfa-1-antitrypsin as a glycoprotein of blood is the main protease inhibitor in the organism (I. Graziadei et al., 2000). Hence, it could inhibit the activity of CTS D after the sauna. AAT is included to acute-phase proteins. The increase of its concentration occurs during infection, inflammation and cancer (D. Kolarich et al., 2006). Reported in this study statistically significant increase of arylsulfatase activity and the tendency to increase of acid phosphatase activity may indicate on the organism response similar to inflammation. Increase of the AAT activity in the blood serum due to sauna effect therefore, can be explained by the stress caused in the organism as a result of this procedure (W. Pilch, 2010, T. Prystupa et al., 2009).

The statistically significant increase in activity of lysosomal enzymes and correlations between them observed in this study may testify to the fact that single staying in Finnish sauna by 30 min at temperature of 85°C and with 40% relative air humidity significantly decrease of stability of lysosomal membranes in healthy volunteers both sexes.

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