Medical and Biological Sciences, 2012, 26/3, 33-38

REVIEW / PRACA POGLĄDOWA

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THE ACTIVITY OF LYSOSOMAL ENZYMES IN THE HEALTHY MEN'S BLOOD AFTER SINGLE FINNISH SAUNA PROCEDURE – PRELIMINARY STUDY

AKTYWNOŚĆ ENZYMÓW LIZOSOMALNYCH WE KRWI ZDROWYCH MĘŻCZYZN PO JEDNORAZOWYM ZABIEGU SAUNY FIŃSKIEJ – DONIESIENIE WSTĘPNE

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Summary

Introduction. The aim of the paper was to investigate the effect of single Finnish sauna procedure on the activity of acid phosphatase (AcP), cathepsin D (CTS D), arylsulfatase (ASA) and inhibitor of proteases α-1-antitrypsin (AAT) in healthy men's blood.

Material and methods. Men (n=7) performed sauna procedure (3 entries, each 10 min) in temperature of 90^{o}C and relative air humidity of 10%. Men cooled their whole body (cold shower) after each exit from sauna. The blood was taken from basilic vein before the entry to sauna, 15 and 60 min after the procedure. Obtained results of activity of AcP, AAT and ASA were statistically analyzed by using ANOVA test. Statistically analysis of CTS D activity was performed by Mann-Whitney test. The changes of the level $p<0.05$ were accepted as statistically significant.

Results. Statistically significant ($p<0.01$) decrease of the CTS D activity of about 51.4% 60 min after sauna was revealed as compared to the activity of this enzyme before entry to sauna (control). Furthermore, the tendency to decrease of the activity of CTS D and AcP 60 min after sauna was found in comparison to the activity of these hydrolases measured 15 min after the procedure ($p>0.05$). The tendency was also observed when comparing the activity of AcP, ASA, CTS D and AAT 15 min after sauna to their activity before sauna ($p>0.05$). In turn, the activity of AAT and ASA insignificantly increased 60 min after Finnish sauna comparing to the activity measured 15 min after procedure ($p>0.05$).

Conclusion. Single Finnish sauna procedure increases stabilization of lysosomal membranes.

Streszczenie

Wstęp. Celem pracy było zbadanie wpływu jednorazowego zabiegu sauny fińskiej na aktywność kwaśnej fosfatazy (AcP), arylosulfatazy (ASA), katepsyny D (CTS D) i inhibitora proteaz α-1-antytrypsyny (AAT) we krwi zdrowych mężczyzn.

Materiał i metody. Mężczyzn (n=7) poddano działaniu sauny (3 wejścia, każde 10 min) w temperaturze 90^{o}C i 10% względnej wilgotności powietrza. Po każdym wyjściu z sauny mężczyźni schładzali całe ciało pod zimnym prysznicem. Krew pobrano z żyły odłokciowej przed wejściem do sauny oraz 15 i 60 min po zabiegu. Uzyskane wyniki aktywności AcP, AAT i ASA poddano analizie statystycznej za pomocą testu ANOVA. Analizę statystyczną aktywności CTS D wykonano testem Manna-Whitneya. Zmiany na poziome $p<0,05$ uznano za istotne statystycznie.

Wyniki. Badania wykazały istotne statystycznie ($p<0,01$) obniżenie aktywności CTS D o 51,4% 60 min po saunie w porównaniu do aktywności tego enzymu przed

wejściem do sauny (kontrola). Wykazano ponadto tendencję do obniżenia aktywności CTS D i AcP 60 min po saunie w porównaniu do aktywności tych hydrolaz zmierzonych 15 min po zabiegu (p>0,05). Tendencję do obniżenia obserwowano również porównując aktywność AcP, ASA, CTS D oraz AAT 15 min po saunie z ich aktywnością przed sauną (p>0,05). Aktywność AAT i ASA z kolei nieznacznie wzrosła 60 min po saunie fińskiej w porównaniu do aktywności zmierzonej 15 min po zabiegu (p>0,05).

W n i o s k i. Jednorazowy zabieg sauny fińskiej zwiększa stabilność błon lizosomalnych.

Key words: Finnish sauna, acid phosphatase, cathepsin D, arylsulfatase, α-1-antitrypsin
Słowa kluczowe: sauna fińska, kwaśna fosfataza, arylosulfataza, katepsyna D, α-1-antytrypsyna

INTRODUCTION

Finnish sauna (dry) among all types of saunas is the most popular and the most often used kind. The heating inside sauna progresses due to hot and dry air (80-120°C), whereas the cooling , outside its, is done by cold water and air [1, 2].

There are many papers which characterize the effect of Finnish sauna on circulatory system, locomotor system and skin both in healthy patients and patients that require permanent medical care [1, 3, 4 5]. Sauna improves vascular endothelial function, increases metabolism rate, decreases the level of total cholesterol (TC) and low density lipoproteins (LDL) in blood and augments the level of high density of lipoproteins (HDL) due to the fact that it improves health of patients with obesity, diabetes and atherosclerosis [3, 6]. It has been also proved that regular using of sauna procedures may be helpful for improvement physical endurance [7]. Scoon et al. revealed that dry sauna used for 3 weeks immediately after training increased the runners shape as a result of increase levels of blood serum and erythrocytes in comparison to 3 weeks training without using the sauna. According to the authors, the increase of physical endurance of 6 subjected runners was probably caused by the increase of erythropoietin synthesis as a result of dehydration after sauna [7]. There are also data presenting negative effect of sauna proved by the fact that cases of acute myocardial infractions after sauna procedures in patients with coronary atherosclerosis resulting from plaque rupture due to rapid temperature changes were noticed [8]. Statistical studies of rapid deaths in Finland show that a small percentage (1.7%) occurred 24h after sauna. However, most of these cases were related to the intake of alcohol [2].

The aim the paper was determination of the influence of single Finnish sauna procedure on the activity of selected lysosomal enzymes in blood of healthy men. Lysosomal enzymes are hydrolases which etch proteins, fats, carbohydrates and nucleic acids inside cells of organism that is why they can contribute to apoptosis or exist as an ingredient of blood plasma [9]. They determine efficient cell working and affect inflammation in a course of disease [9, 10] and after physical exercise [11, 12]. Some lysosomal hydrolases are markers of diseases, i.e.: atherosclerosis, degenerative diseases and neoplasms [9, 10].

MATERIAL AND METHODS

In the study 7 healthy men 23.57±2.15 years old participated. They performed sauna effect (3 entries, each lasting 10 min) in temperature of 90^{o}C and relative air humidity of 10%. After each entry men were cooling their whole body by cold shower. Peripheral blood that was taken 3 times from basilic vein: before the entry to sauna, 15 and 60 min after the procedure (15 and 60 min from last cooling of the body) constituted the material for study. The 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.

The cathepsin D activity was estimated by Anson method [13] - 2% denatured bovine hemoglobin with subject sample was incubated in 37°C. Next, extinction of subject sample at a wavelength λ = 600 nm versus blank (no serum and bovine hemoglobin) was measured and compared to extinction of control sample (2% bovine hemoglobin and serum – no incubation). The activity of the enzyme in nM of released tyrosine from hydrolyzed hemoglobin per time of unit (nM of tyrosine/mg of protein/min) was expressed.

The activity of acid phosphatase was measured according to Bessy method modified by Krawczyński [14]. The level of enzyme activity was the amount of released p-nitrophenol during enzymatic hydrolysis of the substrate (p-nitrophenylphosphate disodium). The activity of AcP in the subjected men's serum was expressed in nM of p-nitrophenol/mg of protein/min.

The arylsulfatase activity was estimated using the method described by Roy and modified by Błeszyński

[15]. The result of the enzyme activity was the amount of released 4-nitrocatechol (4-NC) during enzymatic hydrolysis of the substrate (4-nitrocatechol sulfate). The arylsulfatase activity in the blood serum was expressed in nM of 4-NC/mg of protein/min.

For assayed activity of inhibitor of proteases α-1-antitrypsin in the blood serum, Eriksson method was used [16, 17]. The basis of the assay is a result of decrease of enzymatic activity of trypsin due to short incubation with defibrinated blood serum. The absorbance of subject sample versus blank sample (without trypsin solution) was measured at wavelength λ = 410 nm. Control sample contained the same ingredients as the subject sample but without the serum. The AAT activity was 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 Chair of Medical Biology, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz. 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 of NCU in Toruń. Obtained results of activities of AcP, AAT and ASA were statistically analyzed by used ANOVA test. Statistical analysis of CTS D activity was performed by Mann-Whitney test. The changes of the level $p<0.05$ were accepted as statistically significant.

RESULTS

Statistically significant decrease of the CTS D activity in the healthy men's blood serum on average 51.4% 60 min after sauna in comparison to the activity of CTS D measured before entry to the sauna (control) was found (fig. 1).

Moreover, the tendency to decrease of CTS D and AcP activity within 60 min after sauna as compared to the activity of these enzymes determined 15 min after the procedure was revealed ($p>0.05$) (fig. 1, 2).

Comparing the activity of AcP, ASA, CTS D and inhibitor of proteases AAT 15 min after sauna to their activities before the sauna it was proved that their activity showed also a tendency to decrease ($p>0.05$) (fig. 1-4). On the other hand, the activity of AAT and ASA insignificantly increased 60 min after Finnish sauna in comparison to the activity determined 15 min after the procedure (fig. 3, 4).

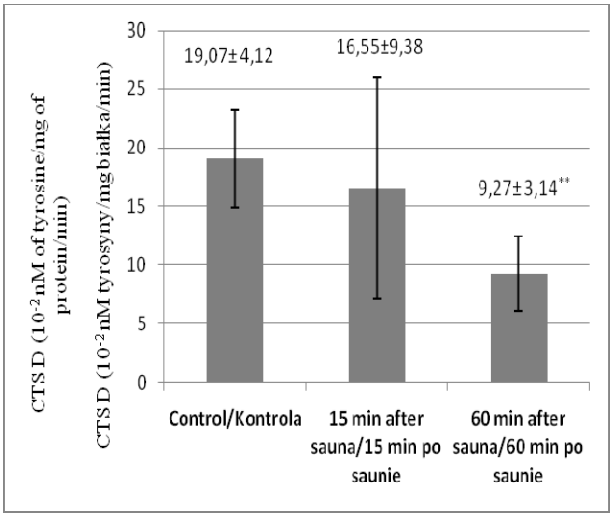


Fig. 1. *The activity of cathepsin D (CTS D) in the healthy men's blood serum after Finnish sauna. The results presented as X_{mean}±SD*

** *- statistically significant difference as compared to control (**$p<0.01$)*

Ryc. 1. *Aktywność katepsyny D (CTS D) w surowicy krwi zdrowych mężczyzn po saunie fińskiej. Wyniki przedstawiono jako $X_{śr}$±SD*

** *- różnica istotna statystycznie w porównaniu do kontroli (**$p<0,01$)*

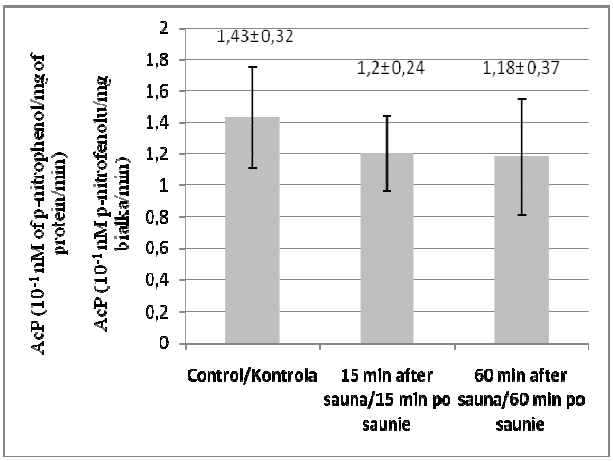
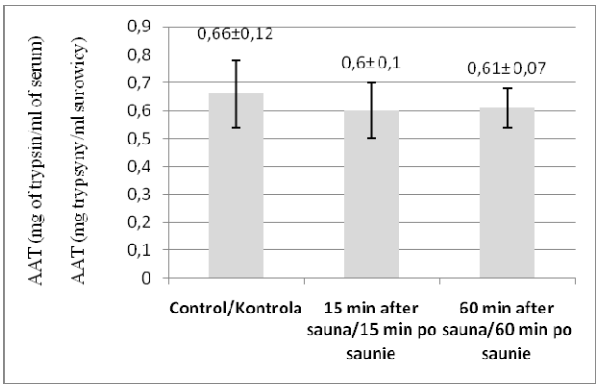


Fig. 2. *The activity of acid phosphatase (AcP) in the healthy men's blood serum after Finnish sauna. The results presented as X_{mean}±SD*

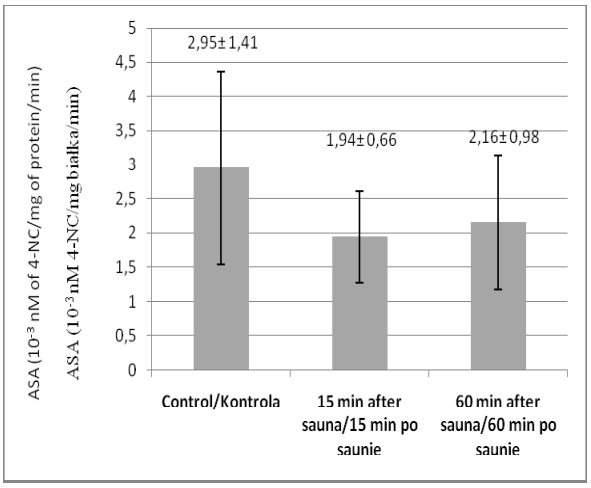
Ryc. 2. *Aktywność kwaśnej fosfatazy (AcP) w surowicy krwi zdrowych mężczyzn po saunie fińskiej. Wyniki przedstawiono jako $X_{śr}$±SD*



AAT (mg trypsyny/ml surowicy)
AAT (mg of trypsin/ml of serum)

Fig. 3. *The activity of α-1-antitrypsine (AAT) in the healthy men's blood serum after Finnish sauna. The results presented as* X_{mean}±*SD*

Ryc. 3. *Aktywność α-1-antytrypsyny (AAT) w surowicy krwi zdrowych mężczyzn po saunie fińskiej. Wyniki przedstawiono jako* $X_{śr}$±*SD*



ASA (10^{-3} nM 4-NC/mg białka/min)
ASA (10^{-3} nM of 4-NC/mg of protein/min)

Fig. 4. *The activity of arylsulphatase (ASA) in the healthy men's blood serum after Finnish sauna. The results presented as* X_{mean}±*SD*

Ryc. 4. *Aktywność arylosulfatazy (ASA) w surowicy krwi zdrowych mężczyzn po saunie fińskiej. Wyniki przedstawiono jako* $X_{śr}$±*SD*

DISCUSSION

The decrease of CTS D activity by 51.4% 60 min after sauna as compared to the activity of CTS D measured before entry to sauna was found ($p<0.01$). Similar changes were observed in own studies presented in other papers. Sutkowy et al. [18] revealed the decrease of cathepsin D activity in blood serum of women and men after Finnish sauna procedure. However, in that paper temperature inside sauna was slightly lower (85°C), air humidity higher (40%), and determinations were done after 5 and 30 min after sauna. Moreover, the subjects stayed in the sauna for 30 min only once, and then they continued with cooling stage [18]. Besides, in the study of Sutkowy et al. [18] the activity of CTS D in the second (30 min) period of determinations after sauna was insignificantly higher as compared to the first (5 min) period of determinations. However, in this paper inverse tendency was revealed. Lower CTS D activity 60 min after sauna in comparison to the activity determined 15 min after the procedure was observed. Change of the cathepsin D activity presents definite inverse tendency of change of activity of its inhibitor α-1-antitrypsin (AAT). That correlation for all three periods of study determinations was found both in present and in the previously mentioned own studies. Whole-body thermal therapies, including sauna, induce whole-body vasodilatation and vasoconstriction [3] since these are procedures based on a combination of warming and cooling of human organism with short impact of high air humidity [1]. Thus, first ischemia then hyperemia may cause an increase of production of reactive oxygen species (ROS) as a consequence of reaction catalysed by xanthine oxidase. On the other hand, increased production of ROS may lead to membranes cell damage [19]. However, there is no information which could confirm this hypothesis in reference to sauna effect. Furthermore, Pall ML [20] revealed that regularly repeated sauna procedure increases availability of tetrahydrobiopterin (BH4) as cofactor of nitric oxide synthase (NOS) [21] due to two possible mechanisms. Increased blood flow in subcutaneous blood vessels during heating sauna leads to an increase of pressure affecting wall of these vessels. It may cause an increase of GTP cyclohydrolase I (GTPCH-I) by the cells of blood vessels. This enzyme catalyses BH4 biosynthesis. Second possible mechanism is based on production of heat shock protein Hsp90 by mammalian tissues. Hsp90 saves GTPCH-1 complex from degradation and extends its functionality. Similar mechanisms increasing the concentration of BH4 in blood may act in intensive physical exercise [20]. The increase of BH4 availability may significantly decrease a disintegration of active protein complex of nitric oxide synthase (NOS) [20]. That phenomenon for endothelial nitric oxide synthase (eNOS) in hamsters performed regularly affected higher temperature [20]. The eNOS

activity in people after sauna is also thought as higher because sauna baths decrease systolic and diastolic blood pressure [1, 2, 5]. Moreover, an improve of hemodynamic parameters, functions of cardiac and endothelium blood vessels in patients with congestive heart failure, hypertension, diabetes, hypercholesterolemia, obesity and smokers after regular sauna procedures were evidenced [3]. At lower than physiological concentration of BH4, by-products of eNOS activity are reactive oxygen species (ROS) – superoxide anion radical (O_2^{-}) and nitric oxide ($NO^{\cdot}$) [20, 21,]. Rapid temperature change in cooling stage can also lead to increased production of ROS as a result of catecholamines oxidation [22]. Significant increase of adrenaline and noradrenaline concentration after sauna was observed if a body cooling was done by rapid dive into cold water (a few Celsius degrees) [5]. However, there is a lack of data in literature confirming the disturbance of oxidative-antioxidative equilibrium due to sauna effect.

Potential oxidative stress caused by sauna may lead to damage of biological membranes of lysosomes and release of lysosomal hydrolases to peripheral blood [11, 12, 18]. It could be confirmed by cited previously own studies where activity of ASA ($p<0.001$) and AcP ($p>0.05$) increased after sauna as compared to their activity before entry to sauna [18]. However, in this paper inverse tendency was revealed. Nonetheless, Sutkowy et al. in the same studies found also the tendency to decrease of ASA and AcP activities 30 min after sauna comparing to determinations carried out 5 min after the procedure [18]. Similar tendency was observed in the paper. Activity of AcP and ASA 60 min after sauna comparing to their activity 15 min after the procedure were lower. Variances between activities of lysosomal hydrolases after sauna may result from selective release of these enzymes from lysosomes. During physical exercise increase the permeability of membranes of lysosomes [12], increase pH inside their and decrease the aggregation level of some lysosomal hydrolases what enable their penetration to cytoplasm [23].

It should be emphasized that the statistically significant change of activity was observed in the paper only for CTS D where the decrease of its activity after sauna was revealed. It allows a conclusion that sauna has a stabilizing effect on lysosomal membranes.

CONCLUSIONS

Obtained results confirm that Finnish sauna increases stability of lysosomal membranes. However, this is a preliminary study and further studies are required.

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Received: 10.07.2012
Accepted for publication: 31.08.2012