

ORIGINAL ARTICLE

The effect of a single Finnish sauna bath after aerobic exercise on the oxidative status in healthy menPAWEŁ SUTKOWY¹, ALINA WOŹNIAK¹, TOMASZ BORACZYŃSKI²,
CELESTYNA MILA-KIERZENKOWSKA¹ & MICHAŁ BORACZYŃSKI²¹The Chair of Medical Biology, Nicolaus Copernicus University Ludwik Rydygier Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland, and ²Central Research Laboratory, Józef Rusiecki Olsztyn University College, Olsztyn, Poland**Abstract**

Background. The aim of this study was to determine the effect of Finnish sauna as a regeneration method post-exercise on the oxidant-antioxidant balance in healthy men. **Material.** 43 men aged 24.0 ± 4.3 years performed a 30-min aerobic exercise on a cycle ergometer and rested for 39 min at a room temperature (Day 1; 20°C) or in a sauna for post-workout recovery (Day 2; 90°C, air humidity 10%). Blood was taken 3 times during both study days: Before the exercise (baseline), 20 and 40 min after the recovery. **Methods.** The activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was determined in the subjects' erythrocytes. The concentration of thiobarbituric acid reactive substances (TBARS) was measured both in plasma (TBARSpl) and in the erythrocytes (TBARSer). **Results.** A 12.7% increase in the TBARSpl concentration versus the baseline was observed 40 min after the Finnish sauna ($p < 0.01$). The CAT activity observed 20 and 40 min after the sauna was also found higher by 8.1% and 8.9%, respectively, in comparison with the baseline ($p < 0.05$). In turn, the TBARSer concentration was lower by 17.5% 40 min after the recovery in the sauna, as compared with the TBARSer concentration 40 min after the recovery at the room temperature ($p < 0.05$). **Conclusions.** A single Finnish sauna bath as a source of free radicals *per se* is able to reduce oxidative stress induced by a 30-min aerobic exercise in healthy men.

Key Words: Adaptation, catalase, glutathione peroxidase, oxidative stress, physical exertion, steam bath, superoxide dismutase, thiobarbituric acid reactive substances

Introduction

Sauna baths, procedures known for thousands of years, are currently used as a successful method of treating numerous diseases, for post-workout recovery, in wellness and SPA centers, as well as in swimming pools for improving beauty and health. Nowadays, especially in Europe, the most popular type of sauna is the Finnish sauna [1–3]. Finnish sauna baths significantly increase the endurance of the locomotor and cardiorespiratory systems, as well as the psychological efficiency of the patient [4–6]; therefore, such baths are most often practiced for recovery after exercise [2,6–7].

The aim of the study was to determine the effect of the Finnish sauna used directly after aerobic exercise on the balance between the oxidation and reduction reactions in the human organism. Most of

the biochemical processes in human organism are based on free radical reactions which are dependent on the presence of aerobic environment. Although aerobic respiration, by means of glucose oxidation, provides energy necessary for the processes that we call life, free radicals called reactive oxygen species (ROS) are generated at the same time. There are many sources of ROS in human organism but the main source is the mitochondrial respiratory chain [8,9]. Thus, the respiratory chain is also the main source of ROS during physical effort. Oxygen uptake during aerobic physical exertion increases even 20-fold, while the use of oxygen in skeletal muscles increases 100–200 times, which causes oxidative stress. During physical exercise with a predominance of anaerobic processes, i.e. strength training, oxidative stress is observed during the recovery period as

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a result of the ischemia/reperfusion phenomenon [10].

In the study subjects, the concentration of thiobarbituric acid reactive substances (TBARS) in blood plasma (TBARSpl) and in the erythrocytes (TBARSer), as well as the activity of antioxidant enzymes (catalase, CAT; superoxide dismutase, SOD; glutathione peroxidase, GPx) were determined as indicators of oxidative stress. Thus, the assumption of the study was to check whether the Finnish sauna procedure has any influence on the generation of ROS, which are greatly important to maintain homeostasis. Homeostatic imbalance is associated with the pathogenesis of many diseases, such as cancers and neurodegenerative, autoimmune or infectious diseases [8,9]. Moreover, impaired elimination of exercise-induced ROS leads to the microdamage of the skeletal muscle and connective tissues (e.g. articular cartilages, ligaments) and therefore, prolongs recovery [11,12].

Methods

Participants

Forty-three men aged 24.0 ± 4.3 years volunteered for the study. The participants were randomly selected among the 110 physical education students at Józef Rusiecki University College in Olsztyn, Poland. The students who participated in the study performed similar levels of physical activity and had similar aerobic capacities. The subjects either had never visited a Finnish sauna or had visited it only occasionally before the study. Every subject was healthy and there were no contraindications for practicing the Finnish sauna or physical effort. No subject suffered from cardiorespiratory or pulmonary diseases, or claustrophobia. The volunteers were also requested not to change their eating habits and the level of their physical activity during the study period. The characteristics of the study subjects are presented in Table I. The study obtained the approval of the Bioethics Committee at Nicolaus Copernicus University Ludwik Rydygier Collegium Medicum in Bydgoszcz (no. KB 189/2012) and all subjects were informed about the purpose of the study and signed their informed consent.

Experiment overview

The study was divided into two days. On Day 1 (Monday), the subjects performed a 30-min aerobic exercise on a cycle ergometer, followed by a recovery at a room temperature (20°C in a gym) in a sitting position for 40 min (time of the sauna bath on Day 2 of the study). On Day 2 (Friday), the subjects performed the same 30-min exercise followed by a Finnish sauna bath (three sessions of 10 min at 90°C and 10% relative air humidity; after each session the

Table I. Age and anthropometric data of the studied men (mean \pm SD).

Number of men	43
Age (years)	24.0 ± 4.3
BM (body mass before the start of the study/kg)	79.2 ± 11.4
BH (body height/cm)	180.9 ± 7.0
BF (body fat/%)	14.0 ± 4.7
FM (body fat mass/kg)	11.4 ± 5.0
FFM (fat free mass/kg)	67.8 ± 8.2
BMI (body mass index/kg m^{-2})	24.2 ± 2.5

whole body was cooled down under a cold shower). During both days, blood for laboratory assays was taken from the basilic vein three times: Before the exercise, as well as 20 and 40 min after the recovery at the room temperature or in the sauna (i.e. 20 and 40 min after the end of the recovery, i.e. 90 and 110 min after the start of the exercise). During the recovery the subjects were rehydrated with 200 mL of still mineral water three times at regular intervals (between the sauna sessions, where applicable). The results obtained before the exercise preceding the recovery at the room temperature (Day 1) and in the sauna (Day 2) were similar; therefore, the values were averaged and described in this paper as the 'baseline'.

Procedures

The estimation of the parameters of physical endurance was conducted at the Central Research Laboratory of Józef Rusiecki University College in Olsztyn, Poland.

Before the exercises in the examined subjects, the values of basic somatic indices were determined. The assessment of body composition was performed with the Tanita BC 418 MA body composition analyzer (Tanita Corporation, Japan), according to the BIA (Bioelectric Impedance Analysis) method. The following somatic structure indices were measured: Body mass (BM) and its components – fat free mass (FFM), the percentage of body fat (BF) and body fat mass (FM) (Table I). The body mass of the subjects after the exercise followed by the recovery at the room temperature decreased by 0.3 kg, while the body mass of the subjects recovering in the Finnish sauna decreased by 0.9 kg.

For a subjective assessment of the workload during the exercise, the Borg Category-Ratio-10 Scale was used [13]. This is the second version of The Borg Rating of Perceived Exertion Scale (RPE Scale) developed by Borg, with ratings between 0 and 10. No exertion at all is marked on the scale as zero. Extremely hard (maximal) exertion is marked as 10. The level of exertion induced by the 30-min aerobic exercise on a cycle ergometer ranged between moderate and relatively hard for the subjects according to the RPE scale (3.44 ± 0.85).

To assess the level of physical activity, the International Physical Activity Questionnaire (IPAQ) was

used (Polish/short version – last 7 days) [14]. The physical activity of the subjects the week before the study was reported as high (3762.91 ± 2342.41 MET min wk^{-1} , MET = $3.5 \text{ mL O}_2 \text{ min}^{-1} \text{ kg}^{-1}$ as the baseline oxygen consumption).

To determine the subjects' physical fitness, their maximum oxygen consumption ($\text{VO}_{2\text{max}}$) was estimated. $\text{VO}_{2\text{max}}$ is the best single indicator of aerobic capacity which expresses the overall efficiency of the cardiovascular and respiratory systems as well as the skeletal muscles of the organism, to perform heavy and prolonged physical work [15]. $\text{VO}_{2\text{max}}$ was assessed via an indirect method using the PWC_{170} (Physical Working Capacity) test [16,17], according to the Astrand-Ryhming nomogram [18]. Physical fitness of the men was average with accordance to Shvartz and Reibold reference values [19] and it amounted to $43.9 \pm 5.6 \text{ mL kg}^{-1} \text{ min}^{-1}$ in the subjects.

The PWC_{170} test was based on the performance of two 5-min cycle ergometer tests on the Monark 828 E cycle ergometer (Monark Exercise AB, Sweden) with individual loads [16,17]. The load of both exercises was determined based on heart rate (HR) which was close to 130–150 bpm (heart beats per minute). Heart rate was measured with the Polar Sport Tester (Polar Electro Oy, Finland) recorder. The PWC_{170} index was calculated based on the mean of the HR values recorded at the end of each 5-minute exercise. PWC_{170} was calculated as the workload (power expressed in watts, W) at the heart rate of 170 bpm. The results of the test were presented graphically or calculated using the following formula: $\text{PWC}_{170} = P_1 + (P_2 - P_1) (170 - \text{HR}_1) (\text{HR}_2 - \text{HR}_1)^{-1}$, where: P_1 – power of the first exercise test, P_2 – power of the second exercise test, HR_1 – heart rate during the first exercise test, HR_2 – heart rate during the second exercise test [16,17]. In the examined men, the PWC_{170} index was 215.5 ± 47.0 W.

The 30-min aerobic exercise on the cycloergometer was carried out with the load determined individually for each subject according to a PWC_{140} test (two 5-min standard cycle ergometer tests at the heart rate of 130–150 bpm). The PWC_{140} index was calculated according to the formula analogous to that of the PWC_{170} index and amounted to 153.6 ± 34.4 W. The intensity of the exercise was 81.3% of HR_{max} on Day 1 and 78.8% of HR_{max} on Day 2 ($\text{HR}_{\text{max}} = 205 - (\text{age} \cdot 2^{-1})$).

Earlier pilot studies conducted at the Central Research Laboratory of Józef RUSIECKI University College revealed that some students could not finish the 30-min exercise definite by PWC_{170} index; therefore, the PWC_{140} index in the study was used.

Biochemical assays of the studied parameters were performed at the Chair of Medical Biology of Nicolaus Copernicus University Ludwik RYDYGIER Collegium Medicum in Bydgoszcz, Poland.

The TBARS concentrations were measured using the method described by Buege and Aust [20] and

modified by Esterbauer and Cheeseman [21]. Lipid peroxidation products were identified by assessing the concentration of thiobarbituric acid (TBA). Malondialdehyde (MDA) is the main product of lipid peroxidation which reacts with TBA; therefore, for the sake of simplicity, the concentrations of the substances reacting with TBA (TBARS) were expressed as the concentration of MDA. The identification of TBARS in blood samples is achieved via the measurement of extinction at a wavelength of 532 nm versus the baseline sample. The concentration of TBARS_{pl} was expressed in nmol of MDA per mL of plasma, while that of TBARS_{ser} was expressed in nmol MDA per g of hemoglobin.

The activity of antioxidant enzymes was determined in the subjects' erythrocytes. The CAT activity was determined using the Beers and Sizer method [22]. The principle of the method is based on a decrease in the absorbance of a hydrogen peroxide (H_2O_2) solution. H_2O_2 is decomposed by the enzyme, so the decrease in absorbance is directly proportional to the CAT activity, expressed in IU per g of hemoglobin, in the solution. The GPx activity was determined according to the Paglia and Valentine method [23]. The method is based on the decomposition of hydrogen peroxide by GPx with the simultaneous oxidation of reduced glutathione. Oxidized glutathione is then reduced in a reaction catalyzed by glutathione reductase. The role of a coenzyme in this reaction is played by reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is converted into an oxidized form and induces a change in the absorbance of light. The SOD activity was assayed using the method by Misra and Fridovich [24]. The method is based on the inhibition of oxidation of adrenaline to adrenochrome in alkaline environment, which induces a change in the extinction of the solution. The activities of GPx and SOD were expressed in U per g of hemoglobin.

Statistical analyses

The obtained results were subjected to statistical analysis. The hypothesis of the equality of the two mean values was tested using the one-way ANOVA test with post-hoc analysis. Before running ANOVA, the model assumptions were also tested using the Kolmogorov-Smirnov test for normality and the Levene's test to assess the homogeneity of variance. Differences at a significance level of $p < 0.05$ were assumed as statistically significant.

Results

The increase in the concentration of TBARS_{pl} versus the baseline was 12.7% 40 min after the recovery in the Finnish sauna and 10.1% 40 min after the recovery at the room temperature ($p < 0.01$; Table II).

Table II. Parameters of oxidative stress after the recovery at room temperature or in the Finnish sauna preceded by the 30-min aerobic exercise in healthy men (mean \pm SD).

	Before the exercise (baseline)*	Exercise and recovery at the room temperature		Exercise and recovery in the sauna	
		20 min after the recovery	40 min after the recovery	20 min after the recovery	40 min after the recovery
TBARSpl [nmol MDA mL ⁻¹]	0.347 \pm 0.064	0.359 \pm 0.072	0.382 \pm 0.084 ^a	0.373 \pm 0.063	0.391 \pm 0.085 ^{aa}
TBARSer [nmol MDA g Hb ⁻¹]	22.7 \pm 8.4	24.5 \pm 7.3	28.9 \pm 11.7 ^{aab}	24.3 \pm 8.9	24.6 \pm 9.2 ^c
CAT [10 ⁴ IU g Hb ⁻¹]	49.3 \pm 8.7	47.9 \pm 8.8	48.6 \pm 9.4	53.3 \pm 9.0 ^{abb}	53.7 \pm 10.1 ^{acc}
GPx [U g Hb ⁻¹]	8.5 \pm 6.95	6.5 \pm 5.6	9.3 \pm 8.1	5.9 \pm 4.0	7.5 \pm 5.6
SOD [U g Hb ⁻¹]	849.6 \pm 133.4	863.2 \pm 88.7	912.8 \pm 111.3 ^{aa}	891.0 \pm 118.9	874.0 \pm 120.1

Statistically significant differences: ^a $p < 0.05$, ^{aa} $p < 0.01$ – versus baseline; ^b $p < 0.05$, ^{bb} $p < 0.01$ – versus 20 min after the recovery at the room temperature; ^c $p < 0.05$, ^{cc} $p < 0.01$ – versus 40 min after the recovery at the room temperature. TBARSpl, thiobarbituric acid reactive substances in blood plasma; MDA, malondialdehyde; TBARSer, thiobarbituric acid reactive substances (TBARS) in erythrocytes; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase.

*Averaged results obtained before the exercise on Day 1 (no sauna) and Day 2 (sauna).

The CAT activity was higher by 8.1% and 8.9% 20 and 40 min after the sauna recovery, respectively, in comparison with the baseline ($p < 0.05$). Moreover, the CAT activity observed 20 and 40 min after the recovery in the sauna was 11.3% and 10.5% higher, respectively, than after the recovery at the room temperature (Table II).

The TBARSer concentration was lower by 17.5% 40 min after the recovery in the sauna, as compared with the TBARSer concentration 40 min after the recovery at the room temperature ($p < 0.05$). Furthermore, 40 min after the recovery at the room temperature the concentration of TBARSer increased by approx. 18% versus that of TBARSer measured 20 min after the recovery at the room temperature ($p < 0.05$) and by 27.3% versus the baseline ($p < 0.01$; Table II).

The activity of SOD measured 40 min after the recovery at the room temperature increased by 7.4% versus the baseline in a statistically significant way ($p < 0.01$; Table II).

Discussion

Oxidative stress induces the peroxidation of blood plasma lipids (lipoproteins of the LDL fraction), the lipids present in muscle sarcolemma and in the membranes of erythrocytes. Subsequently, the microdamage to cell membranes leads to cell necrosis and inflammation. The necrosis of red blood cells (hemolysis) leads to the release of heme with iron from denatured hemoglobin. This is another factor responsible for the formation of free radicals [10,25].

There are two theories describing the initiation of peroxidation of the erythrocyte membrane lipids. The first theory claims that the hydroxyl radical ($\cdot\text{OH}$) is responsible for this process. $\cdot\text{OH}$ is formed inside red blood cells in the Fenton's reaction and/or Haber-Weiss's reaction. The radicals necessary for these processes are formed in the self-oxidation of

oxyhemoglobin to methemoglobin, which occurs due to the SOD activity. The second theory is based on the hypothesis that the lipid peroxidation process is caused by ROS derived from outside of the cells. The ROS are hydroperoxyl radicals ($\text{HO}_2\cdot$) which can easily penetrate the hydrophobic membrane and enter the cell. The $\text{HO}_2\cdot$ radicals are formed by the protonation of the superoxide anion ($\text{O}_2\cdot^-$) and hydrogen peroxide (H_2O_2) at a high concentration of hydrogen cations (H^+) associated with physical exercise [10]. The results of the study confirm that an exercise bout is a source of ROS (Table II). A 10.1% increase in the TBARSpl concentration versus the baseline was observed 40 min after the recovery at the room temperature preceded by a 30-min aerobic exercise ($p < 0.01$). Furthermore, 40 min after the recovery at the room temperature the concentration of TBARSer increased by approx. 18% versus that of TBARSer measured 20 min after the recovery at the room temperature ($p < 0.05$) and by 27.3% versus the baseline ($p < 0.01$). TBARS are secondary products of the lipid peroxidation process, thus they are very reliable indicators of oxidative stress [25,26]. The significant increase in the TBARS concentration in blood after an exercise bout has been confirmed by other authors [25–27]. During the study phase involving the post-exercise recovery at the room temperature, the activity of SOD measured 40 min after the recovery also increased, as compared with that measured before the exercise ($p < 0.01$). Superoxide dismutase is the enzyme which catalyzes the dismutation of the superoxide anion ($\text{O}_2\cdot^-$) to another ROS, hydrogen peroxide (H_2O_2). During this reaction, pure oxygen (O_2) is also formed [8].

The Finnish sauna *per se* is also a source of ROS. A 12.7% increase in the TBARSpl concentration versus the baseline was observed 40 min after the recovery in the Finnish sauna ($p < 0.01$) (Table II). Moreover, the CAT activity 20 min and 40 min after the recovery in the sauna was higher by 8.1% and

8.9%, respectively, in comparison with the baseline ($p < 0.05$). The higher CAT activity is associated with an increase in the H_2O_2 concentration [8]. Some papers confirm that even a single exposure to an extremely high temperature is a source of free radicals. Sutkowy et al. [28] found that a sauna bath increases the SOD activity in a statistically significant way. The subjects in that study ($n = 7$) had a single dry sauna bath at a temperature of 90°C and 10% relative air humidity. The SOD activity was higher both 15 and 60 min after the sauna bath, as compared with the SOD activity determined directly before the beginning of the bath ($p < 0.05$). Zinchuk and Zhad'ko [29] confirmed that a single dry sauna bath ($85\text{--}90^\circ\text{C}$, 10–15% air humidity) causes oxidative stress. Mila-Kierzenkowska et al. [30] revealed a statistically significant decrease in the arylsulfatase (ASA) activity 30 min after a single 30-min sauna bath (85°C , 40% relative air humidity), as compared with the activity of this lysosomal enzyme in the blood of volunteers ($n = 40$, men) 5 min after the sauna bath. The authors suggested that the decrease could be due to ROS generation, as the activation of arylsulfatase is inhibited by ROS. Moreover, the authors reported that ASA, as a heat shock protein, plays the role of an anti-inflammatory molecule and probably possesses antioxidant properties [30]. Furthermore, higher activities of the lysosomal enzymes are a result of their release from lysosomes due to oxidative shock and the peroxidation of lipids of lysosomal membranes [25,26]. Mila-Kierzenkowska et al. [30] revealed the higher activity of ASA assessed 5 min after a sauna bath versus that assessed before the study ($p < 0.001$). The formation of radicals due to a sauna bath, as described above, was also confirmed by a decrease in the activity of cathepsin D (CTS D) with a simultaneous increase in the activity of alpha-1-antitrypsin (AAT), an inhibitor of CTS D [30]. The higher AAT activity had to be preceded by an increase in the CTS D activity. In another article, it was also reported that the CTS D activity observed 60 min after a single sauna bath (90°C and 10% relative air humidity) in healthy volunteers (seven men) was lower than at the start of the experiment ($p < 0.01$) [31].

The results of the study also demonstrated that a single Finnish sauna bath affects the oxidant-antioxidant balance after physical exertion. The CAT activity observed 20 and 40 min after the recovery in the sauna was higher than that after the recovery at the room temperature ($p < 0.01$), and the concurrent decrease in the TBARS concentration 40 min after the recovery in the sauna, as compared with that observed 40 min after the recovery at the room temperature ($p < 0.05$), may suggest that a single Finnish sauna bath, in accordance with the theory of hormesis, helps to retain the oxidant-antioxidant balance in the subjects after a 30-min aerobic exercise on a cycloergometer. The theory of hormesis is

based on the hypothesis that small amounts of apparently harmful agents can be beneficial for the health [30]. There are even papers, in which multiple sauna treatments repeated at regular intervals have been shown to reduce oxidative stress, to possess anti-inflammatory properties and to improve the impaired vascular endothelial function [29,32,33]. Nonetheless, to confirm the hypothesis that the Finnish sauna could be used to maintain oxidant-antioxidant balance after exercise bout, it is necessary to perform further studies.

Conclusions

- (1) A 30-min aerobic exercise on a cycloergometer is a source of reactive oxygen species in healthy men.
- (2) The exposure of healthy men to a single Finnish sauna bath directly after a 30-min aerobic exercise changes the antioxidant response of the organism.

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