

The Effects of a Single and a Series of Finnish Sauna Sessions on the Immune Response and Heat Shock Protein Levels in Trained and Untrained Men

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Abstract

The aim of the study was to investigate the effect of a Finnish sauna on the immune status markers. Healthy males (20–25 years old) were divided into groups: the trained (T; n = 10), and the untrained group (N; n = 10). All participants were subjected to 10 baths (3X15-minute with cooled down for 2 minutes). Blood was collected before the 1st and 10th sauna bath, and 10 minutes after their completion. The levels of: cortisol, IL-6, HSP70, IgA, IgG, IgM and blood cells (WBC), leukocyte populations counts: neutrophils, lymphocytes, eosinophils, monocytes, and basophils were determined. No differences were found in the increase in rectal temperature, cortisol and Ig between groups. In response to the 1st sauna bath, a greater increase in HR was observed in the U group. After the last one, the HR value was lower in the T group. The impact on WBC, CD56+, CD3+, CD8+, IgA, IgG and IgM shows a differences in trained and untrained body responses. It seems that in trained people, the non-specific immune response increases, while in untrained, the specific one. Series of sauna baths can be a way of acclimation to high ambient temperatures for athletes and a solution to improve immune response.

Introduction

In athletes, as a result of intense physical training, the immune system is often suppressed [1]. Thermal treatments, e.g. sessions in the Finnish sauna, can be used to restore the disturbance in homeostasis [2]. Dry sauna baths have a beneficial effect on almost all vital organs. During a treatment, the skin temperature is the first to increase. Prolonged exposure to elevated ambient temperatures, despite thermoregulation mechanisms, leads to the accumulation of heat in the body [3]. In response to the thermal stressor, the body begins to sweat in an attempt to maintain a healthy internal temperature which can lead to dehydration [4]. Previous studies have evaluated the influence saunas can have on the immune system. The effects reported include changes in the number of leukocytes [5, 6], expression of pro and anti-inflammatory interleukins [7, 8], and reducing the risk of respiratory system diseases [9].

Overheating stresses the body and causes the increased production of interleukins and heat shock proteins (HSP) which are easily induced and are involved in the cellular response to heat stressors [10, 11]. However, the current research does not have a clear answer as to the reason for the increased amount of HSP in the plasma. The mechanisms may include increased intracellular expression and exocytosis of HSP, changes in the number of cells responsible for the production of HSP, or apoptosis or necrosis of these cells and release of their contents.

According to Zychowska et al., the response to heat stress involves the increased production of the anti-apoptotic HSP70 or the degradation of damaged HSP27 [12]. Their research demonstrated that heat stressors induce the expression of genes encoding HSP, and mRNA levels for the HSP70 and HSP27 genes differed between athletes and non-training participants. In the group of untrained men, the mRNA levels of all tested genes were higher in response to the same heat challenge. The authors concluded that the expression of genes related to heat-induced stress depends on the level of physical activity [12].

The currently available literature supports that there are differences in the cellular response to the same stressors in trained and untrained subjects. Despite numerous studies on the body's response to bathing in a Finnish sauna, it is impossible to know whether the changes in plasma or blood cell markers are the result of changes in the plasma volume or changes in the protein-blood profile as a result of adaptation to heat stress. Improving the immune system strength through exogenous stress is of great importance in a pandemic situation and may be an alternative for people with reduced immunity and an inability to exercise [13]. Therefore, this study aimed to investigate the effects of a single session and a series of 10 baths in a Finnish sauna on the profile of white blood cells (WBC), the body's immune response, and the concentrations of selected interleukins, cortisol, and HSP70 in the blood of trained and untrained men.

Material And Methods

Study participants

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Bioethics Committee of the Regional Medical Chamber in Krakow (66/KBL/OIL/2011).

All participants gave written informed consent to participate in the study. This study included two groups of healthy males between the ages of 20–25. The training group (T; n = 10) consisted of middle and long-distance runners with 5 ± 1.5 years of training and were members of the Academic Sports Association. The study took place between October and December. All runners were in their detraining period and had last competed and/or trained 2 months prior to the study. The untrained group (N; n = 10) consisted of volunteers, whose physical activity was estimated to be at the medium level (category 2) using the International Physical Activity Questionnaire (IPAQ) [14]. The inclusion criteria for participation in the study included no abnormalities in baseline complete blood count (CBC) and electrocardiogram (ECG), absence of an acute infection, and no history of chronic diseases such as arterial hypertension, diabetes, and epilepsy. No study participants attend sauna sessions before the initiation of this study. The anthropometric characteristics of the study participants in both groups are listed in Table 1. There were no differences in these parameters at baseline between the T and U groups.

Table 1

Anthropometric characteristics of the studied men from trained (T) and untrained (U) groups [x mean ± SD]

	<i>Age</i> <i>[yeas]</i>	<i>BH</i> <i>[m]</i>	<i>BM</i> <i>[kg]</i>	<i>BMI</i> <i>[kg•m⁻²]</i>	<i>PF</i> <i>[%]</i>	<i>FM</i> <i>[kg]</i>	<i>LBM</i> <i>[kg]</i>
T	20.8 ± 0.79	1.81 ± 0.04	73.43 ± 8.36	22.37 ± 1.75	9.36 ± 1.81	6.98 ± 1.96	66.45 ± 6.65
U	21.2 ± 1.93	1.82 ± 0.06	77.78 ± 11.35	23.51 ± 2.28	12.06 ± 3.96	9.70 ± 4.63	68.07 ± 7.38

Before participation in the study, in accordance with the requirements of the Declaration of Helsinki, all subjects were informed about the objective and methodology of the research project, the possible side effects, and the ability to resign from participation in the study at any time without stating a cause. The subjects were instructed to refrain from modifying their daily food intake, taking dietary supplements, and consuming alcohol.

Experimental procedure

The study protocol consisted of a series of 10 bath treatments in a traditional Finnish (dry) sauna. Each treatment consisted of three 15-minute sessions in the sauna chamber. Between each treatment, the body was cooled down for 2 minutes using running water at a temperature of approximately 20°C. The mean temperature in the sauna chamber at head level was $90 \pm 2^\circ\text{C}$ with a relative humidity of 5–16%. All sessions took place before noon. The subjects fasted prior to each treatment but were allowed to drink 0.5 L of water in the morning before treatment. The subjects went into the sauna chamber without clothes and did not drink anything during the session. The intervals between treatments were 1 or 2 days (weekends) and all treatments were completed within 3 weeks. All treatments were supervised by a physician.

Anthropometric measures

Before the 1st and 10th treatment, the following parameters were measured: body weight (BW), body height (BH), heart rate (HR), and skinfold thickness. BW was measured using the F1505-DZA scale manufactured by Sartorius (Germany) which had an accuracy of up to 1 g. BH was measured with medical scales with an accuracy of 1 cm. HR was determined using the Sporttester Polar R400 (Finland). Skinfold thickness measurements were performed with a Harpenden skinfold caliper with 20 g pressure strength on the contact surface with an accuracy of up to 0.1 mm. The percentage of body fat (PF) was calculated using the formula described by Slaughter et al. Before entering the sauna, rectal temperature sensors were placed 15 cm deep into the rectum. Rectal temperature (T_{re}) and HR were taken in 5-minute intervals. The T_{re} [$^\circ\text{C}$] was monitored with a CTD85M electrothermometer from Ellab, Radiometer (Denmark) with an accuracy of 0.1°C. HR was recorded using a Polar Elektro P-3000 heart rate monitor (Finland).

Blood analyses

Prior to and after completion of the 1st and 10th sauna baths, I – before the 1st sauna, II – after the 1st sauna, III – before the 10th sauna, and VI – after the 10th sauna, a venous blood draw was performed on each fasting subject. Twelve milliliters were collected from each participant while in a sitting position and placed in three test tubes (Vacutainers: two with EDTA and one with a clotting activator). In the samples with EDTA, the total number of WBC with differential counts of neutrophils (NEUT), lymphocytes (LYMPH), eosinophils (EO), monocytes (MONO), and basophils (BASO) were determined. These analyses were performed by flow cytometry using a Sysmex XE 2100D laser hematology analyzer (Roche Diagnostics, USA). On the surface of LYMPH, the expression of the following cellular markers were determined: CD3+ (T lymphocytes), CD4+ (Th helper lymphocytes), CD8+ (cytotoxic lymphocytes, Tc),

CD56+ (NK cells), and CD19+ (B lymphocytes). The analysis of LYMPH surface markers was performed by flow cytometry using fluorescently labeled monoclonal antibodies. The determination was performed with the Multitest CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 APC and Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC antibodies from Becton Dickinson using the FACSCalibur flow cytometer (Becton Dickinson, USA). The results were analyzed using the CellQuest Pro program (Becton Dickinson, USA).

Blood collected in the tubes containing a clot activator was centrifuged at 3500 rpm (1200 g) for 10 minutes at 4°C (MPW-351R, Med. Instruments, Poland). After centrifugation, serum, free from any traces of hemolysis, was evaluated for total protein using the biuret method on the Architekt ci8200 analyzer. Cortisol concentrations were determined using the enzyme-linked immunosorbent assay by DRG (DRG Instruments GmbH, Germany; sensitivity of 2.5 ng • ml⁻¹). The concentration of IL-6 and IL-10 cytokines were determined using the enzyme-linked immunosorbent assay by DRG (DRG Instruments GmbH, Germany; sensitivity for IL-6 0.03 pg • ml⁻¹, for IL-10 0.05 pg • ml⁻¹). IgA, IgG, IgM immunoglobulins concentrations were determined using the turbidimetric method with the Architekt ci8200 analyzer (Abbott Diagnostic, USA). The concentration of HSP from the HSP-70 family was determined using the enzyme-linked immunosorbent assay (ELISA) by DRG (DRG Instruments GmbH, Germany), sensitivity 0.039 ng • ml⁻¹).

The change in plasma volume (% ΔPV) was calculated using the changes in total protein concentration determined before and after the selected sauna treatment sessions using the following formula [15]:

$$\% \Delta PV = (B_p / B_k \cdot 100) - 100$$

B_p – protein concentration determined before the sauna bath, B_k – protein concentration determined after the sauna bath.

Due to the dynamic changes in plasma volume during sauna baths, the individual leukocyte fractions, lymphocyte subpopulations, and concentrations of cortisol, IL-6, IL-10, HSP-70, and immunoglobulins were corrected to reflect the changes in plasma volume. The corrected values were calculated using the formula described by Kraemer and Brown [16]:

$$W_c = (\% \Delta PV \cdot 0.01 \cdot W_b) + W_a$$

W_c – corrected value, W_b – value measured before sauna bath, W_a – value measured after sauna bath

Statistical analyses

Statistical analyses were carried out using Statistica 10.0 for Windows software developed by StatSoft (Poland). The normality of the distributions was assessed using the Shapiro-Wilk test. Friedman's rank test was performed to compare the significance of differences in the dependent groups. A non-parameter equivalent of one-reagent variance analysis for repeated measurements was used, and, additionally, the data was checked with the Wilcoxon signed-rank test. The significance of the mean value differences between groups of trained and untrained males was calculated using the Mann-Whitney U test. To assess

the relationship between physiological and biochemical indices, Spearman's rank correlation coefficients were used. A significance level of $p < 0.05$ was assumed as statistically significant. All data are presented as the arithmetic mean values: $\bar{x} \pm$ standard deviation (SD).

Results

Table 2 shows the changes in T_{re} after sauna baths. In both study groups, a statistically significant increase in T_{re} was observed after the 1st and 10th sauna sessions. Significantly lower initial temperature values were observed in the T group in response to a series of sauna baths. In response to a sauna bath, no significant differences were found in the increase in T_{re} between groups: T and U.

After the 1st and 10th heat baths, a statistically significant decrease in body mass (BM) was observed in both study groups. A greater difference in BM was noted after the 10th session compared to the 1st session in both groups. In response to sauna baths, no significant differences were found in BW loss between the two groups (Table 2).

In both study groups, a statistically significant increase in HR was found after the 1st and 10th sauna baths (Table 2). After the last sauna bath, the HR value was significantly lower compared to the 1st bath in the T group. In response to the 1st sauna bath, a significantly greater increase in HR was observed in the U group than in the T group.

Sauna baths resulted in a statistically significant increase in cortisol concentration after the 1st and 10th baths in both study groups. The increase in cortisol concentration observed after completing 10 thermal exposures was lower compared to the 1st treatment (Table 2). In response to sauna baths, no significant differences in the increases in cortisol concentrations were found between groups. A statistically significant positive correlation between the increase in cortisol concentrations and increase in internal temperatures after the 1st sauna was found in the T ($r = 0.72$) and U group ($r = 0.77$).

Table 2

Changes in rectal temperature (Tre), body mass (BM), heart rate (HR) and cortisol concentration after the 1st and 10th sauna baths in trained (T) and untrained (U) men

<i>Group</i>	<i>I</i>	<i>II</i>	<i>ΔI-II</i>	<i>III</i>	<i>IV</i>	<i>ΔIV-III</i>
Tre [°C]						
T	37.04 ± 0.18	38.51 ± 0.28*	1.47 ± 0.18	36.68 ± 0.27*	38.09 ± 0.22**&	1.41 ± 0.25
U	37.05 ± 0.23	38.66 ± 0.24*	1.61 ± 0.21	36.64 ± 0.32*	38.20 ± 0.22**&	1.56 ± 0.37
BM [kg]						
T	73.43 ± 8.36	72.16 ± 8.14*	-1.27 ± 0.37	73.58 ± 9.14	72.17 ± 8.96**	-1.41 ± 0.38
U	77.78 ± 11.35	76.89 ± 11.22*	-0.88 ± 0.33	77.48 ± 11.51	76.56 ± 11.34**	-0.92 ± 0.28
HR [beats·min⁻¹]						
T	67.6 ± 6.1	131.6 ± 10.1*	64.0 ± 11.9	66.4 ± 6.8	124.4 ± 11.1**&	58.0 ± 7.8
U	74.0 ± 6.9	136.4 ± 11.4*	62.4 ± 8.7	72.0 ± 5.7#	129.6 ± 9.1**	57.6 ± 12.4
Cortisol [ng·ml⁻¹]						
T	188.02 ± 38.91	225.16 ± 64.08*	37.13 ± 27.46	179.50 ± 22.11	211.71 ± 36.46**	32.21 ± 30.60
U	186.70 ± 43.90	244.41 ± 54.10*	57.63 ± 49.01	188.14 ± 26.85	241.21 ± 44.55**	53.07 ± 44.83
<p>I – before the 1st sauna bath, II – after the 1st sauna bath, III – before the 10th sauna bath, VI – after the 10th sauna bath.</p> <p>* – Significant differences at the level of $p < 0.05$ compared to the value before the 1st sauna.</p> <p>** – Significant differences at $p < 0.05$ compared to the value before the 10th sauna.</p> <p>& – Significant differences at $p < 0.05$ compared to the value after 1st sauna.</p> <p># – Significant differences between T and U at $p < 0.05$.</p>						

Table 3 shows the % ΔPV of study participants after sauna baths. After the 1st and 10th sauna baths, a decrease in plasma volume was observed in both groups. In both study groups, the mean loss of plasma volume was greater after the 10th bath compared to the 1st bath. The % ΔPV after the 10th bath was smaller in the U group compared to that observed in the T group, which was statistically significant.

Table 3

Changes in plasma volume (% ΔPV) after the 1st and 10th sauna baths in trained (T) and untrained (U) men

% ΔPV			
Group		% $\Delta PV1$ (II-I)	% $\Delta PV10$ (VI-III)
T	$\bar{x} \pm SD$	-8.91 ± 3.85	-11.17 ± 2.54
U	$\bar{x} \pm SD$	-7.31 ± 2.52	$-7.91 \pm 1.64^{\#}$
I – before the 1st sauna bath, II – after the 1st sauna bath, III – before the 10th sauna bath, VI – after the 10th sauna bath.			
# – Significant differences in % $\Delta PV10$ between T and U groups ($p < 0.05$).			

Table 4

Peripheral blood leukocytes counts and their individual populations expressed in absolute values ($\bar{x} \pm SD$) before and after the 1st and 10th sauna baths in men from trained (T) and untrained (U) men

<i>Parameter</i>	<i>Group</i>	<i>I</i>	<i>II</i>	$\Delta I-II$	<i>III</i>	<i>IV</i>	$\Delta IV-III$
WBC [$10^9 \cdot l^{-1}$] ¹	T	5.59 \pm 1.42	6.26 \pm 1.93*	0.67 \pm 1.16	5.54 \pm 1.62	5.89 \pm 1.81**	0.44 \pm 0.53
	U	5.31 \pm 0.95	5.88 \pm 1.37	0.57 \pm 1.16	5.21 \pm 0.85	5.53 \pm 0.82	0.32 \pm 0.60
NEUT [$10^9 \cdot l^{-1}$]	T	3.30 \pm 1.32	4.11 \pm 1.84*	0.81 \pm 0.89	3.12 \pm 1.64	3.61 \pm 1.84**	0.49 \pm 0.40
	U	2.87 \pm 0.93	3.71 \pm 1.37*	0.84 \pm 1.10	2.75 \pm 0.58	3.23 \pm 0.78**	0.48 \pm 0.59
LYMPH [$10^9 \cdot l^{-1}$]	T	1.76 \pm 0.46	1.69 \pm 0.25	-0.08 \pm 0.34	1.78 \pm 0.38	1.72 \pm 0.29	-0.06 \pm 0.18
	U	1.88 \pm 0.48	1.70 \pm 0.50	-0.18 \pm 0.35	1.92 \pm 0.52	1.76 \pm 0.46	-0.16 \pm 0.27
MONO [$10^9 \cdot l^{-1}$]	T	0.37 \pm 0.11	0.34 \pm 0.12	-0.03 \pm 0.10	0.36 \pm 0.12	0.36 \pm 0.11	0.01 \pm 0.06
	N	0.37 \pm 0.13	0.34 \pm 0.14	-0.03 \pm 0.10	0.39 \pm 0.15	0.39 \pm 0.13	0.00 \pm 0.07
EOS [$10^9 \cdot l^{-1}$]	T	0.14 \pm 0.07	0.11 \pm 0.07*	-0.03 \pm 0.02	0.17 \pm 0.08	0.15 \pm 0.07**	-0.02 \pm 0.02
	U	0.15 \pm 0.12	0.11 \pm 0.10*	-0.04 \pm 0.03	0.14 \pm 0.07	0.12 \pm 0.08**	-0.02 \pm 0.03
BASO [$10^9 \cdot l^{-1}$]	T	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	U	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
I – before the 1st sauna bath, II – after the 1st sauna bath, III – before the 10th sauna bath, VI – after the 10th sauna bath.							
* – Significant differences at the level of $p < 0.05$ compared to the value before the 1st sauna.							
** – Significant differences at $p < 0.05$ compared to the value before the 10th sauna.							

Table 5
Populations of peripheral blood leukocytes expressed as percentage values (

± SD) before and after the 1st and 10th sauna baths in men from trained (T) and untrained (U) men

<i>Parameter</i>	<i>Group</i>	<i>I</i>	<i>II</i>	<i>ΔI-II</i>	<i>III</i>	<i>IV</i>	<i>ΔIV-III</i>
NEUT [%]	T	57.12 ± 11.30	62.36 ± 10.33*	5.24 ± 2.97	54.61 ± 11.68	58.81 ± 10.97**	4.20 ± 1.79
	U	53.51 ± 11.31	61.66 ± 12.03*	8.15 ± 8.50	52.75 ± 7.91	58.16 ± 8.81**	5.41 ± 4.52
LYMPH [%]	T	32.73 ± 9.91	29.30 ± 8.11*	-3.43 ± 3.23	34.96 ± 10.56	31.55 ± 9.33**	-3.41 ± 1.79
	U	36.00 ± 8.71	29.96 ± 9.33*	-6.04 ± 8.31	36.63 ± 7.06	32.03 ± 7.09**	-4.60 ± 4.72
MONO [%]	T	6.73 ± 1.56	5.64 ± 1.53	-1.09 ± 1.86	6.56 ± 1.77	6.21 ± 1.26	-0.35 ± 1.09
	U	7.01 ± 2.17	5.82 ± 1.99*	-1.19 ± 1.42	7.48 ± 2.68	7.20 ± 2.53&&	-0.28 ± 1.24
EOS [%]	T	2.87 ± 2.08	2.19 ± 2.00*	-0.68 ± 0.35	3.38 ± 1.92	2.92 ± 1.69**	-0.46 ± 0.32
	U	2.97 ± 2.36	2.08 ± 2.16*	-0.89 ± 0.56	2.75 ± 1.40	2.16 ± 1.59**	-0.59 ± 0.63
BASO [%]	T	0.29 ± 0.15	0.32 ± 0.19	0.03 ± 0.15	0.27 ± 0.15	0.31 ± 0.23	0.04 ± 0.22
	U	0.31 ± 0.14	0.27 ± 0.19	-0.04 ± 0.16	0.24 ± 0.18	0.28 ± 0.13	0.04 ± 0.17
<p>I – before the 1st sauna bath, II – after the 1st sauna bath, III – before the 10th sauna bath, VI – after the 10th sauna bath.</p> <p>* – Significant differences at the level of $p < 0.05$ compared to the value before the 1st sauna.</p> <p>** – Significant differences at $p < 0.05$ compared to the value before the 10th sauna.</p> <p>&& – Significant differences at the level of $p < 0.05$ compared to the value after 1st sauna.</p>							

After the 1st and 10th sauna baths, an increase in leukocyte count was observed in both study groups. However, only after the last sauna session did this change reach statistical significance in the T group. In response to the sauna baths, no significant differences were found in the WBC between the U and T groups after both the 1st and 10th heat exposures (Tables 4 and 5). There was a directly proportional relationship ($r = 0.74$) between the increase in the total number of leukocytes and HR in the T group after the 1st sauna bath.

Changes in the number of cells from LYMPH subpopulations are shown in Table 6. Statistically significant changes in the T group were found only in NK cells (CD56+) between results obtained before the 1st and 10th sauna baths and before and after the 10th. For the U group, significant differences were shown for NK cells before and after the 1st exposure and T (CD3+) and Tc (CD8+) LYMPH before and after the 10th treatment. There was also a significant difference in the number of B LYMPH (CD19+) between samples drawn before the 1st and the 10th sessions. There was a significant difference between groups in B cell counts (CD19+) after the 1st heat exposure.

Table 6

Peripheral blood lymphocyte subpopulations expressed in absolute values before and after the 1st and 10th sauna baths in men from trained (T) and untrained (U) men

<i>Parameter</i>	<i>Group</i>	<i>I</i>	<i>II</i>	<i>ΔII-I</i>	<i>III</i>	<i>IV</i>	<i>ΔIV-III</i>
CD3+ [10 ⁹ .l ⁻¹]	T	1.22 ± 0.41	1.2 ± 0.28	-0.02 ± 0.22	1.16 ± 0.27	1.13 ± 0.28	-0.02 ± 0.18
	U	1.29 ± 0.38	1.23 ± 0.29	-0.06 ± 0.28	1.39 ± 0.38	1.26 ± 0.30**	-0.13 ± 0.18
CD4+ [10 ⁹ .l ⁻¹]	T	0.63 ± 0.15	0.66 ± 0.14	0.02 ± 0.12	0.64 ± 0.12	0.63 ± 0.15	-0.01 ± 0.10
	U	0.69 ± 0.23	0.72 ± 0.24	0.03 ± 0.19	0.79 ± 0.33	0.74 ± 0.28	-0.05 ± 0.12 [#]
CD8+ [10 ⁹ .l ⁻¹]	T	0.48 ± 0.26	0.45 ± 0.19	-0.03 ± 0.11	0.43 ± 0.17	0.42 ± 0.17	-0.01 ± 0.08
	U	0.48 ± 0.17	0.42 ± 0.11	-0.06 ± 0.08	0.5 ± 0.07	0.43 ± 0.06**	-0.07 ± 0.06 [#]
CD19+ [10 ⁹ .l ⁻¹]	T	0.18 ± 0.05	0.24 ± 0.08	0.06 ± 0.09	0.19 ± 0.05	0.19 ± 0.04	0 ± 0.04
	U	0.16 ± 0.06	0.17 ± 0.06 [#]	0.01 ± 0.04	0.19 ± 0.08	0.19 ± 0.07&&	0 ± 0.03
CD56+ [10 ⁹ .l ⁻¹]	T	0.31 ± 0.18	0.24 ± 0.12	-0.07 ± 0.17	0.25 ± 0.10*	0.20 ± 0.07**	-0.05 ± 0.05
	U	0.36 ± 0.18	0.28 ± 0.13*	-0.08 ± 0.11	0.32 ± 0.20	0.25 ± 0.08	-0.07 ± 0.15
<p>I – before the 1st sauna bath, II – after the 1st sauna bath, III – before the 10th sauna bath, VI – after the 10th sauna bath.</p> <p>* – Significant differences at the level of $p < 0.05$ compared to the value before the 1st sauna.</p> <p>** – Significant differences at $p < 0.05$ compared to the value before the 10th sauna.</p> <p>&& – Significant differences at the level of $p < 0.05$ compared to the value after 1st sauna.</p> <p># – Significant differences between T and U groups at $p < 0.05$.</p>							

Table 7 summarizes the measured immunological parameter mean values at each time point for both groups. A directly proportional positive relationship was demonstrated between the increase in IL-6 and cortisol concentrations in the T group after the 1st treatment ($r = 0.64$). In the same group of men, after the 1st bath, a positive correlation was found between the increase in IL-10 concentration and internal temperature ($r = 0.75$) and between the increase in IL-6 and IL-10 ($r = 0.69$) concentrations.

Table 7

Selected immunological markers expressed in absolute values before and after the 1st and 10th sauna baths in men from the trained (T) and untrained (U) men

<i>Parameter</i>	<i>Group</i>	<i>I</i>	<i>II</i>	<i>ΔII-I</i>	<i>III</i>	<i>IV</i>	<i>ΔIV-III</i>
IL-6 [pg·ml ⁻¹]	T	0.21 ± 0.09	0.37 ± 0.21*	0.16 ± 0.21	0.29 ± 0.27	0.38 ± 0.33**	0.09 ± 0.09
	U	0.31 ± 0.26	0.68 ± 0.53*	0.37 ± 0.34	0.5 ± 0.39	0.61 ± 0.4	0.11 ± 0.17
IL-10 [pg·ml ⁻¹]	T	0.94 ± 0.24	1.13 ± 0.25*	0.19 ± 0.12	1.13 ± 0.3*	1.35 ± 0.28**&&	0.22 ± 0.22
	U	0.99 ± 0.28	1.21 ± 0.44*	0.22 ± 0.31	1.08 ± 0.29	1.34 ± 0.34**	0.26 ± 0.25
IgG [g·l ⁻¹]	T	9.6 ± 1.49	9.45 ± 1.47	-0.15 ± 0.07	10.25 ± 1.48*	10.14 ± 1.45&&	-0.11 ± 0.29
	U	10.54 ± 2.93	10.55 ± 2.89	0.01 ± 0.24	10.93 ± 2.96	10.96 ± 2.88&&	0.03 ± 0.24
IgM [g·l ⁻¹]	T	0.87 ± 0.25	0.86 ± 0.23	-0.01 ± 0.04	0.97 ± 0.29*	0.94 ± 0.27&&	-0.03 ± 0.08
	U	0.87 ± 0.38	0.86 ± 0.36	-0.01 ± 0.04	0.93 ± 0.40	0.92 ± 0.41	-0.01 ± 0.04
IgA [g·l ⁻¹]	T	1.45 ± 0.35	1.43 ± 0.33	-0.02 ± 0.03	1.64 ± 0.3*	1.63 ± 0.29&&	-0.01 ± 0.05
	U	1.66 ± 0.74	1.66 ± 0.73	-0.01 ± 0.05	1.75 ± 0.69	1.69 ± 0.70	-0.05 ± 0.13
HSP-70 [ng·ml ⁻¹]	T	0.025 ± 0.015	0.061 ± 0.04*	0.036 ± 0.08	0.029 ± 0.02	0.041 ± 0.03	0.012 ± 0.02
	U	0.021 ± 0.01	0.078 ± 0.05*	0.057 ± 0.05	0.033 ± 0.01*	0.042 ± 0.03&&	0.009 ± 0.03
<p>I – before the 1st sauna bath, II – after the 1st sauna bath, III – before the 10th sauna bath, VI – after the 10th sauna bath.</p> <p>* – Significant differences at the level of $p < 0.05$ compared to the value before the 1st sauna.</p> <p>** – Significant differences at $p < 0.05$ compared to the value before the 10th sauna.</p> <p>&& – Significant differences at the level of $p < 0.05$ compared to the value after 1st sauna.</p>							

Discussion

Despite a similar increase in Tre during the first and last sauna baths, the athletes showed greater efficiency in thermoregulatory mechanisms (greater sweat secretion and greater weight loss). This study

demonstrates the beneficial effect of sports training, which enabled the athletes' bodies to respond more effectively to heat stress, as previously indicated by Pilch et al. [17]. The decrease in the observed resting temperature after a series of sauna treatments in the trained group indicates acclimation to the high temperatures. This finding was also observed by Bartolom et al. in young semi-professional football players who participated in 3 weeks of passive overheating in the sauna [18].

The reduction in plasma volume was greater during the last bath in both groups, which is an adaptive effect. These changes were significantly higher in the T group compared to the U group. The greater degree of dehydration observed in athletes reflects the positive effect endurance training has on the body's adaptation to high temperatures and the functioning of the circulatory system [19].

An increase in HR was observed during sauna treatments [20]. Activation of the autonomic nervous system in response to the sudden dilation of the skin's blood vessels results in an accelerated HR to maintain normal blood pressure [21]. In our study, a statistically significant increase in HR was observed during both monitored sauna treatments. During the last treatment, the trained men had a significantly lower increase in HR than the untrained men.

In the T group, a significant increase in the total number of leukocytes and NEUT was observed in response to both a single sauna bath as well as a series of 10 sessions. This finding was also observed in two additional studies [5, 22]. According to Shephard et al. [23], the mechanism responsible for leukocytosis after systemic hyperthermia is an increase in cardiac output and, consequently, an increase in leukocyte demargination. In our study, a positive correlation was observed between the increase in the total number of leukocytes and HR in the T group after the first sauna bath ($r = 0.74$, $p < 0.05$). This is in support of the mechanism described by Shephard et al. [23]. Others have proposed alternative mechanisms which include increased expression of granulocyte colony-stimulating factor (G-CSF) [24] and the increased secretion of cortisol causing NEUT to migrate from the bone marrow into the bloodstream [25]. In our study, there was an increase in cortisol levels after the 1st and 10th sauna baths. Similar results have been presented before [26, 27], suggesting hypercortisolemia after baths could explain the leukocytosis. Activation of the adrenergic system in response to heat stressors increases cortisol levels [20]. In both study groups, a lower increase in cortisol was observed in response to consecutive sauna sessions indicating thermal adaptation to similar thermal conditions [28].

In the present study, the number of EO decreased significantly after both the 1st and 10th sauna baths in both study groups. The sauna baths did not increase the number of LYMPH and MONO. The number of BASO after single and repeated sauna baths in both study groups did not significantly change. In both study groups, there was a significant increase in the number and percentage of NEUT, a decrease in the number and percentage of EO, and a reduction in the percentage of the LYMPH pool. This finding is difficult to interpret, on the one hand, it may indicate an improved phagocytic function of the blood (NEUT), while on the other hand indicate a reduction in specific and an increase in non-specific immunity.

The sauna baths caused a slight decrease in the number of T LYMPH (CD3+) after the 1st and 10th treatment. However, a significant difference in the number of CD3 + LYMPH was only noticed after the

10th bath in the untrained group. The absolute number of Tc (CD8+) decreased significantly only in the untrained men after the 10th treatment. There were no significant changes in the number of Th LYMPH (CD4+) and B LYMPH (CD19+) after both single and repeated sauna baths in both studied groups. The absolute number of NK cells (CD56+) decreased immediately after the 1st and 10th sauna baths in both study groups. A statistically significant change in the number of NK cells was noted in the U group after the 1st treatment and T group after the 10th sauna bath. The present study seems to confirm the direction of changes observed by Giannopoulos et al [29] where 13 healthy volunteers participated in a one-time bath in a Finnish sauna. Researchers observed a negligible increase in the total number of leukocytes and NEUT. As in the study by Giannopoulos et al [29], our study demonstrated a significant increase in the absolute number of NEUT after the 1st and 10th sauna in both groups. There were no significant changes in the total number of leukocytes and NEUT between the U and T groups. Similarly, no significant differences were found in the number of MONO after the 1st and 10th baths in both groups. In contrast, the absolute value and percentage of EO decreased significantly in both studied groups. There were no changes in the number of BASO. In our research, slightly different from previous observations [29], there were changes in the absolute number of T LYMPH (CD3+). After the 1st and 10th bath in the sauna, a slight decrease in the absolute value of CD3 + cells was observed, but only in the U group after 10 baths did this reach statistical significance. There were no significant changes in the absolute number of Th LYMPH (CD4+). The absolute number of Tc (CD8+) decreased slightly after the 1st and 10th baths but was only statistically significant after the 10th treatment in the U group. The immune system's response to sauna bathing in trained and untrained men is similar to its activation during physical activity in athletes. Leukocytosis occurs after exercise and its elevation is directly proportional to the intensity and duration of exercise, and inversely proportional to the level of training [30]. This is due to an increase in NEUT, LYMPH, and, to a lesser extent, MONO. Post-exercise EO counts decline while BASO levels do not change significantly [31]. The lymphocytosis that occurs during and after exercise is associated with an increase in the sub-population of T LYMPH (CD4 + and CD8+), B LYMPH (CD19+), and NK cells (CD56+). NK cells increase faster than any other lymphocyte subpopulation. At the same time, the CD4+/CD8 + cell ratio changes as the number of CD8 + LYMPH increases faster than CD4+ [32].

The changes in cortisol levels caused by stress stimulation are associated with changes in cytokine and leukocyte levels. Glucocorticoids inhibit the expression of pro-inflammatory cytokines: IL-1, IL-2, IL-6, IL-8, IL-11, IL-12, TNF α , and INF γ , while stimulating the production of the anti-inflammatory cytokines, IL-4 and IL-10. The reduction of pro-inflammatory cytokines is achieved by destabilizing and suppressing the transcription of mRNA [33]. As reported in previous studies, passive exposure to heat causes an increase in the concentration of circulating IL-6 [34–36]. In the present study, both the endocrine and immune systems activity increased after exposure to a thermal stimulus. After the 1st and 10th sauna baths, an increase in the concentrations of cortisol, IL-6, and IL-10 was shown. There were no significant differences in the overall increase and concentration of IL-6 and IL-10 after the 1st and 10th sauna baths between the T and U groups.

In the present study, an increase in IL-6 concentration was seen after the 1st and 10th sauna baths. In an experiment by Dugué and Leppänen [22], the reaction to the thermal load of a single sauna bath was examined. A significant increase in the concentration of cortisol and IL-6 was observed. Similar results were obtained by Brenner et al. [37] after subjecting 7 volunteers to an hour-long water bath at 35 or 38°C, and then cooling the body in a climate chamber with a temperature of 5°C. In a study by Behzadi et al. [38], middle-aged persons were subjected to two 10-minute baths in a sauna. This resulted in a strong increase in IL-6, a slight increase in IL1-RA, and no changes in CRP concentrations. The authors concluded that in passive heating, the increase in IL-6 is correlated with the intensity of the thermal stimulus.

In our study, increases in IL-10 were recorded after completion of the series of sauna baths and before the last treatment, but only in the T group was this change statistically significant. One can hypothesize that the adaptive increase in IL-10 was designed to limit the inflammatory reaction and production of pro-inflammatory cytokines after the applied hyperthermia. We analyzed three immunoglobulin classes, IgG, IgM, and IgA. Their levels in the T and U groups were within the normal range for healthy people. There were no significant differences in the level of immunoglobulins between the athletes and men who did not train after the 1st and 10th sauna baths.

Little information is available on the effects of hyperthermia on serum immunoglobulin levels. In the research of Hietal et al. [39], the 1st and 10th heat baths increased the internal temperature by 1.2°C but did not produce any significant changes in the serum concentrations of immunoglobulins. In our study, after a series of ten sauna baths, there was an increase in the baseline values of immunoglobulins in both study groups, but only in the T group were these changes significant. This indicates a better and faster adaptation to difficult environmental conditions by athletes (increased plasticity of the athletes' immune system) [23]. There was no correlation between immunoglobulin levels and the number of WBC, including B cells.

The traditional Finnish sauna aims to support skeletal muscle hypertrophy by stimulating HSP70, which acts as a molecular chaperone in the folding of functional skeletal muscles. In this study, the influence of an increase in internal temperatures during sauna bathing on the concentration of HSP from the HSP70 family was determined. After bathing in the sauna, a statistically significant increase in the level of HSP70 was obtained in both studied groups with an increase in Tre of $1.47 \pm 0.18^\circ\text{C}$ in the T group and $1.61 \pm 0.21^\circ\text{C}$ in the U group. However, no correlation was found between the increase in HSP70 concentrations and the increase in Tre. After the 1st sauna bath, the serum concentration of HSP70 increased by 144% in the T group and by 271% in the U group compared to the level obtained before the bath. This highlights the fact that one-time heat treatments are a heavy burden on the body. However, repeated treatments (series of treatments) reduce the body's stress response [40] and thus should only be recommended in this capacity. After a series of 10 baths, a smaller increase in the concentration of HSP70 was found compared to the increase observed after the 1st treatment. The lower increase in HSP70 after the 10th session may be a result of the lower Tre experienced by the subjects after the 10th sauna. It was not possible to identify differences between changes in HSP70 concentrations in T and U

groups due to large individual differences, but there was a tendency of a stronger reaction to heat stress in people from the U group.

In the study by Blatteau et al. [41], a thermal stimulus using an infrared sauna was applied and the concentrations of HSP70 in the serum were measured before and 30 minutes and 2, 8, and 24 hours after the thermal exposure. An increase in serum HSP70 was only demonstrated 2 hours after the end of the bath. The concentration of HSP70 in the present study, determined 10 minutes after the end of the sauna bath, increased significantly after the 1st bath in both groups. This may be explained by the higher increases in Tre than in the studies by Blatteau et al. [41].

In summary, a significant increase in WBC was only noted in the T group after a series of ten thermal baths in the sauna. This group also experienced an increase in IL-6 concentrations and a decrease in the number and percentage of CD56 + cells. In the U group, a decrease in the number of CD3 + and CD8 + cells was noted. This may indicate a difference in the adaptive responses to heat stresses between the groups. The results show a transient weakening of the non-specific response in the T group (NK cells), and the specific immune response in the U group. A significant increase in the concentrations of IgA, IgG, and IgM may indicate an improvement in humoral immunity in response to a series of treatments. A single sauna bath in both groups caused a significant increase in the concentrations of the cytokines IL-6 and IL-10 which may indicate the anti-inflammatory effect of hyperthermia on the human body.

Summary

The impact of sauna treatments on WBC, CD56+, CD3+, CD8+, IgA, IgG, and IgM in trained and untrained participants exemplifies the differences in body responses. In trained people, the non-specific immune response increases, while in the untrained, the specific response increases. Regardless of the changes in the WBC profile, the sauna caused a significant increase in the level of the anti-inflammatory cytokine IL-10, which indicates the indirect activation of the body's cells to produce it.

The use of sauna baths can be a good solution for physically inactive people to improve specific cellular responses and increase the production of anti-inflammatory cytokines. However, such treatments should be recommended as a series. Sauna baths can also be a way for athletes to acclimate to high ambient temperatures.

Declarations

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Data Availability Statement

All data obtained in this project will be made available to interested persons. Contact: Marta Szarek PhD

Conflict of Interest

The authors have no conflicts of interest to declare.

Authors Contribution Statement

Conceptualization, WP. and MS.; Methodology, WP, MS, ESK, MŽ; Software, OCzL; Validation, AP, OCzL. and TP; Formal Analysis, MS; Investigation, MS.; Resources, MS.; Data Curation, OCzL; Writing – Original Draft Preparation, WP, MS, MŽ and ESK; Writing – Review & Editing, AP, TP, SA; Visualization, AP; Supervision, WP, MS and MŽ; Project Administration, WP and MŽ.; Funding Acquisition: WP, MS.

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