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Institute of Sport Sciences and Physiotherapy, Faculty of Medicine, University of Tartu, Tartu, Estonia

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications referred to in the text by the respective Roman numerals (I–III).

I Kaldur, T., Unt, E., Ööpik, V., Zilmer, M., Eha, J., Paapstel, K., Kals, J. (2016). The acute effects of passive heat exposure on arterial stiffness, oxidative stress, and inflammation. *Medicina* (Kaunas), 52(4):211–216. https://doi.org/10.1016/j.medici.2016.06.001.

II Kaldur, T., Kals, J., Ööpik, V., Burk, A., Kampus, P., Zagura, M., Zilmer, M., Unt, E. (2013). Heat acclimation increases arterial elasticity in young men. *Applied Physiology, Nutrition and Metabolism*, 38(9):922–927. https://doi.org/10.1139/apnm-2012-0389.

III Kaldur, T., Kals, J., Ööpik, V., Zilmer, M., Zilmer, K., Eha, J., Unt, E. (2014). Effects of heat acclimation on changes in oxidative stress and inflammation caused by endurance capacity test in the heat. *Oxidative Medicine and Cellular Longevity*, 2014:107137. https://doi.org/10.1155/2014/107137.

The author's contribution:

Papers I–III: Triin Kaldur had primary responsibility for the enrolment of the participants, data analysis, and the writing of the manuscripts. Triin Kaldur also had responsibility for developing the protocol and performing the measurements.

LIST OF ABBREVIATIONS

AIx	augmentation index
AIx@75	augmentation index corrected for heart rate of 75 beats *per* minute
AE	arterial elasticity
AS	arterial stiffness
BP	blood pressure
β2M	β_2-microglobulin
CDBP	central diastolic blood pressure
CPP	central pulse pressure
CRP	C-reactive protein
CSBP	central systolic blood pressure
CV	cardiovascular
EC	endurance capacity
EGF	epidermal growth factor
HA	heat acclimation
HAS	heat-acclimated status
HR	heart rate
hsCRP	high-sensitive C-reactive protein
IL-6	interleukin-6
IL-8	interleukin-8
LAE	large artery elasticity index
MAP	mean arterial pressure
MCP-1	monocyte chemoattractant protein-1
NHAS	non-heat-acclimated status
NO	nitric oxide
NT-proBNP	N-terminal pro-brain natriuretic peptide
OSI	oxidative stress index
oxLDL	oxidized low-density lipoproteins
PDBP	peripheral diastolic blood pressure
PHE	passive heat exposure
PPP	peripheral pulse pressure
PSBP	peripheral systolic blood pressure
PWA	pulse wave analysis
PWV	pulse wave velocity
PWVc-f	pulse wave velocity at carotid-femoral segment
PWVc-r	pulse wave velocity at carotid-radial segment
RH	relative humidity
ROS	reactive oxygen species
SAE	small artery elasticity index
sICAM-1	soluble intercellular adhesion molecule-1
TAC	total antioxidant capacity
Tr	travel time of the reflected wave
VEGF	vascular endothelial growth factor

VO_2peak	peak oxygen uptake
WBC	white blood cells
ΔLAE	the difference between large artery elasticity index value measured after the heat acclimation program and measured at baseline
ΔSAE	the difference between small artery elasticity index value measured after the heat acclimation program and measured at baseline
ΔTc	the difference between the values in core body temperature observed before and after the passive heat exposure

1. INTRODUCTION

The capacity of an athlete to exercise for a prolonged period of time is determined by several factors, one of which is ambient temperature. Besides, detrimental effect of heat exposure on physical performance has been observed (Galloway and Maughan, 1997). Because of the enhanced thermoregulatory demand for the skin blood flow coupled with dehydration and hyperthermia, exercise in the heat can pose a severe challenge to the human cardiovascular (CV) control (Gonzalez-Alonso *et al*., 2008). High ambient temperature during exercise increases the CV strain (Gonzalez-Alonso *et al*., 1997), which has been proposed as a possible mediator of severe fatigue and exhaustion (Cheung and Sleivert, 2004). Heat acclimation (HA) is a key CV strain mitigation strategy, and it has been widely employed to prepare athletes and military personnel to perform activities in hot environment. The improvement in heat tolerance and work performance can be produced by the physiological adaptations that arise from repeated exposure to exercise and heat stress (Houmard *et al*., 1990).

Despite the evidence-based research demonstrating that athletes have a more favourable overall CV disease risk profile, it has been shown that excessive training for and competing in marathons may be associated with an increased risk of accelerated atherosclerosis and CV diseases (Schwartz *et al*., 2014). Furthermore, it has been revealed that marathon runners may have higher arterial stiffness (AS) compared to recreationally active subjects (Vlachopoulos *et al*., 2010). In addition, environmental heat stress during exercise may increase the risk of the exertional heat stroke – a serious, life-threatening medical complication with dangerous elevations in core body temperature (Armstrong *et al*., 2007; DeMartini *et al*., 2014).

The pathogenesis of CV disease is multifactorial and therefore the importance of an integrated approach, including the measurement of different vascular functional properties and systemic metabolic markers, is relevant. Increased AS has emerged as an early indicator of CV disease and a contributor to the progression of the disease (Cheng and MacDonald, 2019). Increased arterial elasticity (AE), the inverse of AS, is a good prognostic indicator of arterial health, and non-invasive assessment of arterial functional properties is widely used in clinical settings (Laurent *et al*., 2006). The identification of changes in the vasculature is of great importance in estimating the CV strain and has significant clinical implications in monitoring the CV response to heat stress and HA. It has been established that CV adaptations are important contributors to improved exercise capacity and the reduced risk of a serious heat illness caused by exercise and HA (Sawka *et al*., 2011).

The worsening of AE has been shown to be directly linked to increased oxidative stress (Kals *et al*., 2006) and low-grade inflammation (Kals *et al*., 2011) as well as to prognosis in patients with atherosclerosis (Kals *et al*., 2014). There are only few reports regarding the impact of passive heat stress or HA on markers of oxidative stress and inflammation in humans, whereas the data are limited and

inconsistent. Exercise-induced oxidative stress response may be increased by high ambient temperature and hyperthermia (McAnulty *et al*., 2005; Quindry *et al*., 2013). It has been shown that HA is associated with decreased level of oxidative stress (Huang *et al*., 2012), but the impact of acclimation on the oxidative stress response to a single bout of exercise in a hot environment has not been fully explained (Souza-Silva *et al*., 2016; Williamson-Reisdorph *et al*., 2023). It has been concluded that a single bout of exercise induces an acute low-grade inflammatory and subsequent anti-inflammatory response, which is thought to be partly responsible for the protective effects of regular exercise (Gleeson *et al*., 2011; Petersen and Pedersen, 2005). However, the data about the effect of heat stress on inflammation markers are still scarce.

Therefore, the purpose of the present thesis is to evaluate the effect of acute heat stress exposure and heat acclimation on several parameters of arterial stiffness, oxidative stress and inflammation markers in healthy young men. This study aims to offer valuable guidance to athletes for the training process and competitions as well as to military personnel for missions in a hot climate.

2. REVIEW OF THE LITERATURE

2.1. Exercising in the heat and the importance of acclimation

Environmental heat stress and physical exercise interact synergistically to increase the strain on physiological systems (Sawka *et al*., 2011). A high ambient temperature may impair physical performance and increase the risk of developing health problems during exercise (Armstrong *et al*., 2007). Ely *et al.* (2007) demonstrated the unfavourable effect of hot weather on the performance in marathon-running. Early recognition and effective treatment of milder forms of heat illness as well as risk factor awareness are all crucial to the prevention of heat stroke and potential fatalities associated therewith (Coris *et al*., 2004; Periard *et al*., 2022). Heat stroke is the most serious of heat illness syndromes, where the body temperature is elevated to a level that causes damage to body tissues and affects multiple organs requiring urgent medical intervention (Coris *et al*., 2004).

Significant risk factors for heat illness include dehydration, a hot and humid climate, obesity, a low physical fitness level, lack of acclimatisation, underlying health problems and certain medications (Sawka *et al*., 2011). Fatigue and exhaustion, the most common causes of withdrawal from activity in hot conditions, occur more rapidly during exercise when an ambient temperature increases beyond 20 ºC and heat stress rises (Armstrong *et al*., 2007).

HA is the systemic process of repeated exposures to a thermally extreme environment in a natural or artificial setting that elicits beneficial physiological and perceptual adaptations (Casadio *et al*., 2017). Furthermore, HA is a heat mitigation strategy to attenuate heat-induced performance impairments and prevent heat illness (Benjamin *et al*., 2021). The study by Houmard *et al*. (1990) revealed that athletic and occupational activities performed in the heat consisting of continuous, moderate-intensity exercise for a relatively short duration can produce heat tolerance in trained subjects. Acclimation efficiency is usually evaluated by the beneficial changes in heart rate (HR) variability, core body temperature and physical performance.

2.2. Background and assessment of arterial stiffness

2.2.1. Background of arterial stiffness

AS describes the rigidity of arterial walls (Mackenzie *et al*., 2002) and the reduced capability of an artery to expand and contract in response to pressure changes (Cecelja and Chowienczyk, 2012). AS, measured also in terms of vascular compliance or AE, can be used as a surrogate marker for CV risk and target-organ damage in individuals with hypertension (Ben-Shlomo *et al*., 2014; Cohn *et al*., 2004; Spronck *et al*., 2024). AE is defined as the ability of an artery to expand and recoil with cardiac pulsation and relaxation. Decreased arterial function also

serves as a risk factor for CV disease (Miura, 2012). The assessment of AE provides valuable information for the identification of subclinical CV pathologies (Cohn, 2001) enabling prevention and targeted early treatment (Glasser *et al*., 1997). In addition, AS is correlated with several traditional and novel CV risk factors for atherosclerotic lesions (Laurent *et al*., 2006). In healthy sedentary male and female subjects, AE is progressively reduced by 40 to 50% between the ages of ~25 and ~75 years (Tanaka *et al*., 2000) and is also reduced in certain disease states that are themselves associated with an increased CV risk, including hypertension, diabetes mellitus, hypercholesterolaemia and the end-stage renal failure (Glasser *et al*., 1997). Arterial stiffening is considered a consequence of hypertension and have shown an independent predictor of CV and all-cause mortality (Mackenzie *et al*., 2002; Szalo *et al*., 2024). Arterial stiffening reduces the buffering capacity of the main elastic arteries, which leads to an increased systolic and pulse pressure, promotes left ventricular hypertrophy and dysfunction, and impairs the capacity for myocardial perfusion (Safar, 2007).

2.2.2. Assessment of arterial stiffness

There are several different methods for assessing AS, some of which are more widely applicable in clinical setting than others. The direct measurement of AS is based on the measurement of changes in the arterial diameter and pressure at the same site and can be performed invasively (O'Rourke *et al*., 2002). AS can be measured locally, regionally, or systemically. Local stiffness is measured primarily with ultrasonographic techniques that are suitable for the determination of this parameter in the superficial arteries, such as the carotid and femoral artery. Local stiffness of the deep arteries (e.g. aorta) can be measured by magnetic resonance imaging. Regional stiffness can be measured non-invasively by using specific anatomical sites of the arterial tree, and the determination of the regional pulse wave velocity (PWV) is the verified method to assess AS (Laurent *et al*., 2006). Systemic AS assessment by pulse wave analysis (PWA) is based on the model for pulse wave propagation and determines a proximal capacitive compliance, a distal oscillatory compliance and wave reflections (Laurent *et al*., 2006; Loboz-Rudnicka *et al*., 2022).

The PWA is a method that allows the non-invasive measurement of AS to identify patients at risk for CV events before the disease becomes clinically apparent (Mackenzie *et al*., 2002). The pulse wave should be analysed through three major parameters: central pulse pressure (CPP), central systolic blood pressure (CSBP) and augmentation index (AIx) (Laurent *et al*., 2006).

Applanation tonometry is used to record pressure at the radial artery, and a validated generalized transfer factor is then applied to derive the corresponding central waveform. Based on this, the AIx, which is the difference between the first and second systolic peaks expressed as a percentage of the pulse pressure and a measure of systemic stiffness, can be derived. It is also possible to estimate the central arterial pressure from the peripheral waveform.

The measurement of the PWV by applanation tonometry is generally accepted as the simplest and highly reproducible non-invasive method to determine AS (Laurent *et al*., 2006; Spronck *et al*., 2024). This technique has been validated by both invasive and non-invasive testing (Cohn *et al*., 1995; Prisant *et al*., 2002; Resnick *et al*., 2000; Wittrock *et al*., 2009). The velocity of the pulse wave as it traverses the arterial tree is dependent on the characteristics of the conduit artery wall. The central PWV is measured from the carotid artery to the femoral artery, whereas the peripheral velocity is assessed from the carotid to the radial or brachial arteries (Cohn *et al*., 2004).

The shape of the arterial pressure wave is influenced by the structure and function of the large and small arteries. The analysis of the diastolic pressure decay has involved the use of a modified Windkessel model of circulation (Cohn *et al*., 2004). This model depicts the arterial vasculature as a hydraulic filter converting the pulsatile flow from the left ventricle into the steady flow in the capillary bed. The principle of the model is that the arterial tree is loaded in systole by the stroke volume and the diastolic pressure contour is a function of resistance and compliance of an isolated arterial system. The modified Windkessel model separately identifies the total systemic large artery (capacitive) compliance and the small peripheral (oscillatory) artery compliance or elasticity. The capacitive component assesses the arterial storage capacity, which is predominantly a function of the larger conduit arteries. The oscillatory or reflective component is related to the cushioning effect of compliance at the arterial reflective sites that are thought to reside primarily in the small arteries and arterioles as well as at the branching sites of the small arteries.

2.3. Impact of exercise and heat stress on arterial stiffness

2.3.1. Exercise and arterial stiffness

There is evidence that AS is decreased for a short term through a single bout of endurance exercise in both younger and older adults (Kingwell *et al*., 1997; Nickel *et al*., 2011) and that regular exercise is associated with an improvement in AS (Maeda *et al*., 2008). The findings by Kobayashi and colleagues (2020) indicate that regular aerobic exercise may be important in reducing AS in middle-aged and older people regardless of the intensity or duration of aerobic exercise. Furthermore, regular endurance exercise attenuates age-related reductions in AE (Tanaka *et al*., 2000). Endurance exercise has been shown to reduce AS in healthy individuals and in patients with an established CV disease (Li *et al*., 2015). In older adults with multiple CV risk factors, short-term improvements in AS induced by aerobic training became attenuated over the long term (Madden *et al*., 2013).

There is evidence that the response of the AE parameters to endurance exercise is related to the subjects' maximal oxygen uptake (Kampus *et al*., 2008). Nevertheless, increased aortic stiffness has been observed in marathon runners as

compared to age-matched controls (Vlachopoulos *et al*., 2010). It has been demonstrated by Jürgenson and others (2021) that a half-marathon competition did not cause statistically significant changes in the AS parameters, whereas a mild increase in AS was more remarkably shown in high-level athletes with a better performance level.

The data about the impact of resistance exercise on AS is controversial. It has been found that acute resistance exercise and chronic resistance training may increase AS (DeVan *et al*., 2005; Miyachi *et al.*, 2004). Jürgenson and colleagues (2019) revealed that a 12-week strength training program had no significant effect on AS in well-trained powerlifting athletes. Nevertheless, it has been concluded that chronic resistance exercise has been shown not to impair CV health in terms of altered AS (Garcia-Mateo *et al*., 2020).

2.3.2. Heat stress and arterial stiffness

The data on the effect of the heat stress on AS are scarce and inconsistent. Many studies are performed in animal models but very little data are available in humans. *In vitro* evidence is suggesting that the direct heating of isolated iliac arteries to a very high temperature (60 ºC) increases vessel elasticity (Mitchel *et al*., 1994). Furthermore, it has been shown that acute local thermal therapy may result in a decrease in AS among healthy young and older women (Hu *et al*., 2012) and that warm water immersion may improve central and peripheral AS in healthy men (Sugawara and Tomoto, 2021).

It has also been demonstrated that passive heat using lower-limb hot water immersion reduced central and peripheral AS in patients with peripheral arterial disease as well as in healthy elderly controls (Thomas *et al*., 2017). In addition, it has been shown that passive heat stress leads to a transient reduction in AS (Caldwell *et al*., 2017). Furthermore, Lee with colleagues (2018) demonstrated decreased AS after a single sauna session.

In contrast, a core body temperature increase of up to 1.5 ºC above the baseline achieved with passive heating did not affect the average peripheral or central AE in a group of men and women varying in age (Ganio *et al*., 2011). The findings of the study by Schlader and others (2019) indicated that progressive whole-body passive heat stress did not affect AS in groups of older and younger adults. Although Moyen with colleagues (2016) reported that an acute bout of whole-body passive heat stress did not change peripheral AS, increased central AS was detected.

In addition to the scarce data about the acute effect of heat stress on AS, there is also limited data on the chronic effect of heat stress on AS. To our best knowledge, there is only one study showing that eight weeks of passive heat therapy improves AS in young, healthy and sedentary subjects (Brunt *el al*., 2016).

2.3.3. Combined impact of exercise and heat stress on arterial stiffness

Although there is inconsistent data available about the independent acute effects of exercise and heat stress on AS, the interaction of heat stress and exercise on AS is largely unexplored. There are very few studies providing compelling evidence about the combined effect of heat stress and exercise on AS.

Caldwell and others (2017) examined the acute effects of passive heating and exercise in the heat on AS in healthy men and women. This was accomplished by four trials: a control trial, a passive heating trial and two other trials, where participants cycled in a hot or cool environment. In this study, it was concluded that an acute exercise bout did not result in any acute changes in AS regardless of the heat stress. However, they demonstrated that passive heat stress independently leads to a transient reduction in peripheral stiffness.

To our best knowledge, the data about the effect of repeated exposure to combined exercise and heat stress on AS are limited. Lee with colleagues (2022) examined the impact of regular exercise in conjunction with sauna bathing on participants with a low physical activity level. They showed that neither an eight-week exercise intervention alone nor an eight-week exercise intervention together with sauna bathing had an impact on AS.

2.4. Effect of exercise and heat stress on oxidative stress and inflammation

2.4.1. Effect of exercise and heat stress on oxidative stress

2.4.1.1. Exercise and oxidative stress markers

All forms of exercise, both aerobic and anaerobic, possess the potential to result in increased reactive oxygen species (ROS) production and the subsequent oxidative stress in both human and animal models**.** There are many different biomarkers used in the oxidative stress measurement in humans, both biomolecules oxidized (lipids, proteins, and DNA) and antioxidants (enzymes and nonenzymatic antioxidants) in addition to ROS. The measurement of different oxidants and antioxidants molecules separately may not be practical because it is costly and time-consuming. Moreover, their oxidant and antioxidant effects are additive. Therefore, the measurement of total oxidant and antioxidant capacities and their ratio, called the oxidative stress index (OSI) is considered a more valid method (Sanches-Rodriguez and Mendoza-Nunez, 2019).

It has been shown that exercise of various intensities and durations is a sufficient stimulus to invoke increased ROS production in humans. While the body does possess a complex antioxidant defence system that provides protection against ROS, antioxidative capacity systems are often not capable of eliminating oxidative damage during and after exercise. Exercise-related oxidative stress appears to be

affected by several factors, including the intensity and duration of exercise as well as the age, health and training status of the subjects (Fisher-Wellman and Bloomer, 2009). There is evidence that oxidative stress is lower in physically fit and active adults compared with less fit or sedentary individuals (Shanely *et al*., 2011).

Furthermore, systematic endurance training may alleviate oxidative stress and elevate the antioxidant status following endurance training in sedentary older men, whereas the cessation of training may reverse these training-induced adaptations with the oxidative stress and antioxidant status markers returning to the pre-training values (Fatouros *et al*., 2004). In addition, intense endurance training for 12 weeks reduces oxidative stress after exhausting exercise in humans and up-regulates the antioxidant defence system (Miyazaki *et al*., 2001). Goto and colleagues (2003) showed increased oxidative stress after high-intensity exercise training for 12 weeks in healthy young men, whereas moderate exercise tended to decrease the oxidative stress markers.

2.4.1.2. Heat stress and oxidative stress markers

The results of animal studies suggest that the exposure to a high temperature may result in increased oxidative stress (Yang *et al*., 2010), and this should be considered as part of the stress response to heat exposure (Lin *et al*., 2006). Mills *et al*. (1996) showed an increase in oxidative stress in hyperthermic horses, who were used as a model of an elite athlete exercising to fatigue. Furthermore, in the latter study, the oxidative stress was exacerbated during exercise in a high temperature environment.

The investigations in humans also reveal that a high ambient temperature and hyperthermia may increase the exercise-induced oxidative stress response (McAnulty *et al*., 2005). Moderate-intensity exercise in a warm environment elicits a blood oxidative stress response not observed at comparable exercise performed at lower temperatures (Quindry *et al*., 2013). In addition, an elevated core body temperature is independently associated with a rise in oxidative stress (Laitano *et al*., 2010). Huang and others (2012) demonstrated that HA decreased the oxidative damage resulting from exposure to high heat in an occupational setting. In another study, four weeks of high-intensity interval training performed in the heat enhanced exercise-induced lipid peroxidation but reduced the oxidation of proteins following a maximal incremental exercise test in a temperate environment in healthy active men (Souza-Silva *et al*., 2016). In contrast, the investigation by Williamson-Reisdorph and colleagues (2023) failed to demonstrate the effects of three-week exercise-heat acclimation on blood oxidative stress response to an acute bout of moderate-intensity aerobic exercise.

2.4.2. Effect of exercise and heat stress on inflammation

2.4.2.1. Exercise and inflammation markers

It is known that chronic low-grade inflammation may play an important role in the development of atherosclerosis (Ross, 1999) and understanding the role of specific inflammatory markers can provide more effective treatment and guidelines for lowering the risk of CV events.

The potential targets for the measurement to identify and monitor the ongoing inflammatory process include proinflammatory risk markers, such as oxidized low-density lipoproteins (oxLDL), proinflammatory cytokines (e.g. interleukin-1, tumor necrosis factor alpha), adhesion molecules (e.g. intercellular adheshion molecule-1), inflammatory stimuli with hepatic effects (e.g. interleukin-6, IL-6) or the products of the hepatic stimulation, such as the C-reactive protein (CRP), and a host of other acute-phase reactants. In addition, other indicators of cellular responses to inflammation, such as elevated leukocyte count, might be evaluated (Pearson *et al*., 2003).

Physical activity has been shown to influence inflammatory markers. When a bout of exercise is of sufficient intensity and duration, it induces an acute low-grade inflammatory and a subsequent anti-inflammatory response, which is thought to be partly responsible for the protective effects of regular exercise (Gleeson *et al*., 2011; Petersen and Pedersen, 2005). The protective effect of a physically active lifestyle against chronic inflammation-associated diseases may be mediated not only *via* a reduction in the visceral fat mass (with a subsequent decreased production and release of proinflammatory adipokines) but also by the induction of an anti-inflammatory environment with each bout of exercise (Gleeson *et al*., 2011; Mathur and Pedersen, 2008).

The IL-6 responds most dramatically to acute exercise and has been suggested to be the main driver of the anti-inflammatory effects of exercise. The acute post-exercise peak of the IL-6 triggers the production of anti-inflammatory cytokines, thus providing an inhibitory effect on pro-inflammatory cytokines (Petersen and Pedersen, 2005).

Furthermore, regular physical activity is inversely associated with the CRP concentrations suggesting that physical activity may mitigate low-grade inflammation (Fernandez *et al*., 2018; Ford, 2002). The CRP is an acute-phase reactant and a marker of inflammation, and its level is a significant independent risk factor for coronary heart disease (Pearson *et al*., 2003; Tracy, 1998).

Cell adhesion molecules are shown to be strong biomarkers for vascular inflammation, and they are closely related to a variety of proinflammatory cytokines, such as interleukin-1, interleukin-8 (IL-8), the tumor necrosis factor alpha (Koh and Park, 2018) and thus, elevated cell adhesion molecules promote vascular inflammation. It has been concluded by Koh and Park (2018) that a favourable outcome, a decrease in cell adhesion molecules, is observed following low-to-moderate intensity aerobic exercise, while high intensity aerobic exercise elevates these molecules immediately following exercise.

Intensive physical exercise is associated with an acute inflammatory response (Liesen *et al*., 1977). Weight and colleagues (1991) reported an acute rise in inflammatory markers after a strenuous long-lasting exercise. There was a short-term, transient increase in serum CRP after strenuous exercise produced by an exercise-induced acute phase response (Kasapis and Thompson, 2005). Prolonged physical stress induced a remarkable increase of CRP in the study by Kampus and others (2008). Physical activity and exercise favourably affect the concentrations of acute phase reactants (Ford, 2002). Shanely and colleagues (2011) concluded that inflammation is lower in physically fit and active adults in comparison with sedentary ones. Akhtari-Shojaei *et al*. (2011) compared the response of inflammatory markers after acute moderate aerobic cycling in healthy young active and sedentary men and concluded that moderate exercise increased the inflammatory markers in both groups, but the increase was greater in sedentary men. Research has shown that the CRP is significantly reduced following nine months of endurance training in moderately trained runners and that regular exercise has a systemic anti-inflammatory effect (Mattusch *et al*., 2000).

2.4.2.2. Heat stress and inflammation markers

The data about the effect of acute passive heat stress on inflammation markers are scarce. It has been demonstrated that one hour of passive heating induces a two-fold increase in the IL-6 (Faulkner *et al*., 2017). Laing *et al*. (2008) reported a significant increase in the IL-6 concentration in healthy men immediately after two-hour passive heat stress by hot water immersion.

The epidemiological study by Laukkanen and Laukkanen (2018) revealed a significant inverse association between the frequency of sauna bathing and the level of the CRP indicating that sauna bathing may reduce systemic low-grade inflammation. Moreover, a two-week regular hot water immersion decreased concentrations of inflammatory markers in patients with chronic heart failure (Oyama *et al*., 2013). In contrast, Kanikowska and colleagues (2012) did not observe changes in inflammatory markers after a nine-day HA.

Hoekstra *et al*. (2018) investigated the acute and chronic effects of hot water immersion on the IL-6 and concluded that the concentration of the IL-6 was higher immediately after acute heat stress, but the resting level on the IL-6 was not altered after the two-week intervention period.

In summary, exhaustive physical effort in a hot environment is associated with the detrimental physiological responses, including the cardiovascular strain. To our best knowledge, there is practically no data available about the responses of arterial stiffness to heat acclimation. Furthermore, data from existing studies provide different results regarding the effects of heat stress on oxidative stress or inflammation markers, which may also play a role in the changes in arterial stiffness.

3. AIMS OF THE STUDY

The purpose of the study was to elucidate the effect of acute heat exposure and heat acclimation on arterial stiffness, oxidative stress and inflammation in healthy young men.

The specific aims of the study were:

1. To assess the effect of acute heat exposure on arterial stiffness, oxidative stress, and inflammation.
2. To assess the effect of 10-day heat acclimation on arterial stiffness and to establish whether acclimation-related changes in arterial stiffness are associated with endurance capacity.
3. To assess the effect of 10-day heat acclimation on oxidative stress and inflammation.
4. To assess the effect of 10-day heat acclimation on the responses of oxidative stress and inflammation markers to exhausting endurance exercise in the heat.

4. METHODS

4.1. Ethical approval

The study was approved by the Research Ethics Committee of the University of Tartu (protocol No. 186M-19, 26.10.2009; protocol No.198-M, 22.11.2010). Prior to the beginning of the study, all subjects gave a written informed consent.

4.2. Participants

The participants were recruited from the university population and the military community (military college cadets). The main characteristics of the participants are presented in Table 1. All the subjects were characterized as healthy, young, physically active men. The participants of the passive heat exposure (PHE) study and HA study exercised habitually from three to five times per week and from two to ten times per week, respectively. They had not been exposed to warm weather during the preceding two months. All the subjects were non-smokers, none of them took any medication nor had a history of heat illness or a CV disease.

To ensure the standardization of the nutritional status prior to the study, the subjects were instructed to follow a healthy diet, keep the diet stabilized and to avoid any use of additional food supplements for two months prior to participation in the study and during the study.

The sample of the PHE study consisted of nine participants from the HA study, in which a total of 21 subjects were included.

Table 1. Characteristics of the participants.

Parameter	**PHE study** (n = 9)	**HA study** (n = 21)
Age (years)	28.8 ± 3.4	24.9 ± 3.7
Height (m)	1.82 ± 0.05	1.83 ± 0.06
Weight (kg)	78.9 ± 11.2	80.3 ± 9.4
VO_2peak (mL · kg^{-1} · min^{-1})	53.6 ± 7.9	53.8 ± 7.1

Data are presented as mean ± SD; PHE – passive heat exposure; HA – heat acclimation; VO_2peak – peak oxygen uptake.

4.3. Study design

4.3.1. Passive heat exposure study

The study was conducted during the winter period in Estonia. The parameters of AS and the markers of oxidative stress and inflammation were measured in thermoneutral condition: before and immediately after passive heat stress exposed in a climatic chamber (Figure 1).

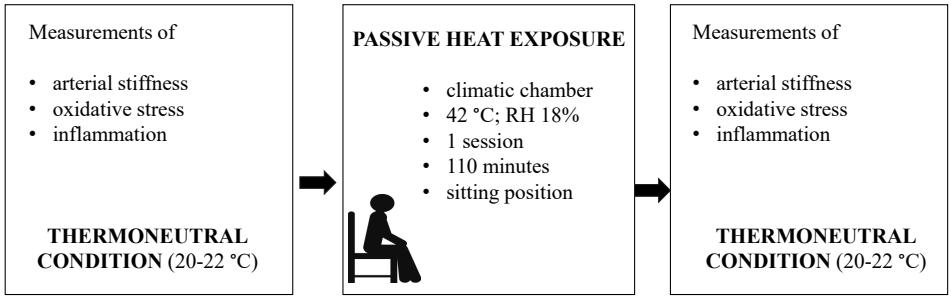

Figure 1. Design of the passive heat exposure study (Paper I). RH – relative humidity.

During the PHE, the climatic chamber (Design Environmental Ltd., Gwent, South Wales, UK) was maintained at a high temperature (42 ºC); relative humidity (RH) 18%, and the subjects stayed in a sitting position for 110 minutes. The subjects' core body temperature was monitored in real time using a rectal probe (TX-2, Columbus Instruments, Columbus, OH, USA) and the values observed before and immediately after the PHE were recorded by means of an electronic data logger (Iso-Thermex 256, Columbus Instruments, Columbus, OH, USA). The difference between the values in core body temperature observed before and after the PHE (ΔTc) was calculated.

4.3.2. Heat acclimation study

The study, which consisted of preliminary procedures and experimental trials was conducted in Estonia during the winter-spring period (from December to March) to avoid natural HA. Therefore, their natural HA state should have been at the annual nadir.

4.3.2.1. Heat acclimation and arterial stiffness

The parameters of AS and endurance capacity (EC) in the heat were measured at baseline and after the HA program (Figure 2).

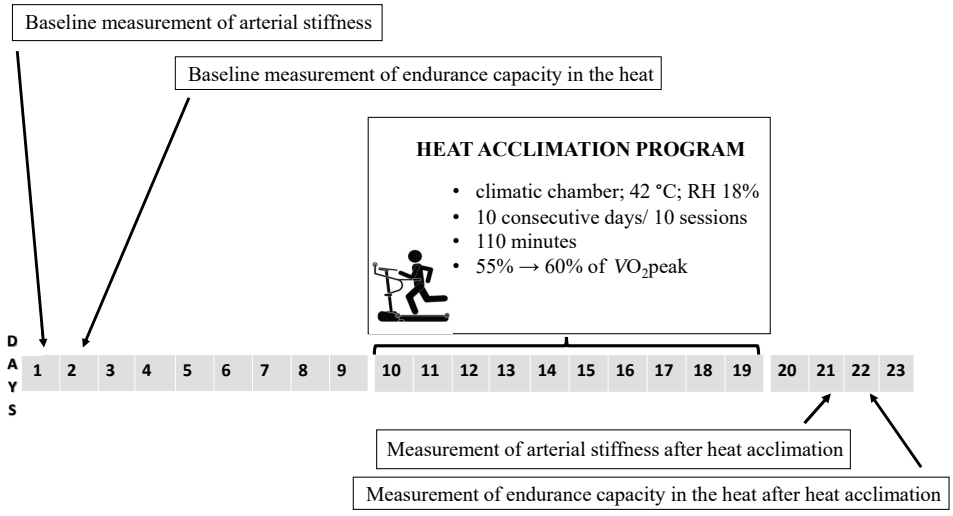

Figure 2. Design of the heat acclimation study (Paper II). RH – relative humidity; VO_2peak – peak oxygen uptake.

4.3.2.2. Heat acclimation, oxidative stress and inflammation

During the HA study, blood samples for oxidative stress and inflammation markers were taken four times: two times in the non-heat-acclimated status (NHAS) [at baseline and after the EC test in the heat] and two times in the heat-acclimated status (HAS) [at baseline and after the EC test in the heat] (Figure 3).

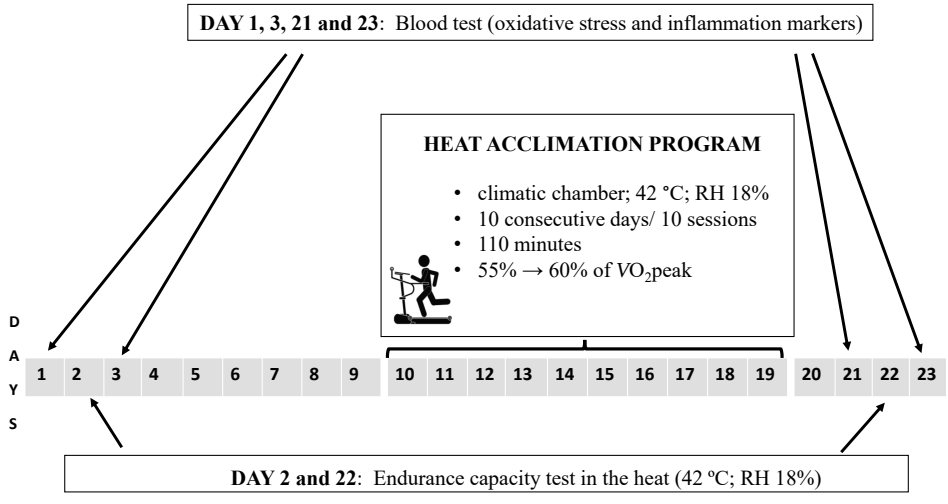

Figure 3. Design of the heat acclimation study (Paper III). RH – relative humidity; VO_2peak – peak oxygen uptake.

4.4. Preliminary proceedings and measurements

One week before the start of our study, the subjects visited the laboratory and were familiarized with the equipment to be affixed to their bodies as well with all the experimental procedures. In HA study the subjects completed a 30-min treadmill walk in hot-dry environmental conditions (42 ºC; RH 18%). These measures were undertaken in order to minimize any potential learning and anxiety effects during the main phase of the study.

4.4.1. Anthropometry

The subjects' body height, using Martin's metal anthropometer, and the nude body mass, using an electronic scale (CH3G-1501 Combics, Sartorius AG, Goettingen, Germany) were measured (± 0.001m and ± 0.001kg, respectively).

4.4.2. Measurement of peak oxygen uptake

Before the actual experiment, the subjects' peak oxygen uptake (VO_2peak) was measured. The walking exercise test was conducted on a motorized treadmill (Viasys/Jaeger LE300 C, Viasys Healthcare GmbH, Hoechberg, Germany) in thermoneutral conditions (20–22 ºC). The VO_2peak was determined by using an online breath-by-breath metabolic system (MasterScreen CPX, Viasys Healthcare GmbH, Hoechberg, Germany). After a 10-min warm-up at 5 km · h^{-1} on a level surface, the speed of the treadmill was increased to 6 km · h^{-1} and the grade to 5%. Thereafter, the grade was increased by 2.5% every two minutes, but the speed was kept constant until self-determined exhaustion. The mean of the three highest consecutive 15-sec recordings at the end of the test was considered as the VO_2peak. The secondary criteria for achieving the VO_2peak included the respiratory exchange ratio > 1.15 and HR > 95% of the subjects' age-predicted maximum.

In the HA study, the subjects' VO_2peak results were used to establish the workload for the protocol of the EC test and for the HA program.

4.5. Heat acclimation program

The subjects completed a HA program, where they exercised for ten consecutive days in a climatic chamber maintained at a hot temperature (42 ºC; RH 18%). According to the HA protocol, the daily exposure lasted for 110 minutes (two 50-min bouts of exercise with 10 min of rest between the bouts) and involved exercising on a treadmill at a workload predicted to elicit an oxygen uptake of 55% of the VO_2peak during the first five days and the workload was raised to the level of 60% of the VO_2peak for the last five days of the HA protocol. The intensity of exercise was controlled by changing the grade of the treadmill belt in the

range of 5–15%, whereas the speed was kept constant at 6 km · h^{-1}. The participants were wearing shorts, socks and athletic shoes. The sessions took place under the supervision of experienced personnel.

The subjects' HR and core body temperature were monitored continuously throughout the whole heat exposure to detect clinical symptoms of heat illness *via* a sport tester (Suunto Dual Belt, Suunto OY, Finland) and a rectal probe (TX-2, Columbus Instruments, Columbus, OH, USA), respectively.

The subjects were allowed to drink water *ad libitum*. The termination criteria included the following: 1) the completion of the protocol; 2) the rise of the core body temperature to 39.5 ºC for 5 min; 3) the rise of the HR to 95% of the individual maximal HR for 5 min; 4) the symptoms of the exertional heat illness; 5) a subject's request to stop.

4.6. Measurement of endurance capacity in the heat

The EC test in the heat was performed at baseline and after the HA program (Figure 2, Figure 3). The subjects were instructed to refrain from alcohol for 24 hours and from ingesting caffeine for 12 hours prior to each experimental trial. For the purposes of being adequately hydrated, the subjects were instructed to consume an additional 500 mL of water at the previous evening and in the morning of each experimental trial day. For controlling the subjects' hydration status before the EC test, the participants' urine samples were collected for a specific gravity measurement, which was performed by a digital clinical refractometer (PDX-CL, VeeGee Scientific Inc., Kirkland, WA, USA). The subjects with urine-specific gravity above 1.030 were considered dehydrated and were instructed to consume an additional 250 mL of water.

The EC test was performed in the heat (42 ºC; RH 18%) in a climatic chamber (Design Environmental Ltd., Gwent, South Wales, UK) on a treadmill. The intensity was individually adjusted to 60% of the subject's personal VO_2peak by employing a constant speed of 6 km · h^{-1} and regulating the grade of the belt of the treadmill in the range of 7–15%. The participants performed the test until exhaustion or until the indications for the termination of an EC test. The termination criteria for the EC test were the following: 1) the core body temperature above 40 ºC for more than 5 minutes; 2) the HR above 95% of the individual maximum HR for at least 5 minutes; 3) the occurrence of the symptoms of the exertional heat illness, such as nausea, headache, dizziness.

4.7. Assessment of arterial stiffness

The measurement procedures of AS were conducted in thermoneutral conditions:

a) twice in the PHE study (Figure 1): in the morning after an overnight fast and immediately after the PHE in the climatic chamber (Paper I);

b) twice in the HA study (Figure 2): in the morning after an overnight fast in thermoneutral conditions at baseline and in the morning of the second day after the termination of the HA program (Paper II).

The subjects' brachial blood pressure (BP) was measured supine in the left arm using an automated digital oscillometric BP monitor (OMRON M4-I; Omron Healthcare Europe, Hoofdorp, the Netherlands).

4.7.1. Systolic pulse wave analysis

The Sphygmocor apparatus (SCOR Px, 7.0; AtCor Medical®, Sydney, Australia) was used to assess AS by PWA. A high fidelity micromanometer (SPT-301B; Millar Instruments®, Texas, USA) was employed to record the peripheral pressure waveforms from the radial artery of the dominant arm at the wrist. The corresponding ascending aortic waveforms were then generated by using the transfer function. The central haemodynamics, AIx, and the travel time of the reflected wave (Tr) were calculated. The AIx was corrected for the HR of 75 beats per minute (AIx@75). The PWV was measured by the foot-to-foot method, using the Sphygmocor device. The PWV at the carotid-femoral (PWVc-f) and carotid-radial segments (PWVc-r) was determined.

4.7.2. Diastolic pulse wave analysis

The arterial waveform was measured in the dominant arm by the Cardiovascular Profiling Instrument (HDI/Pulse Wave CR-2000, Hypertension Diagnostics Inc®, Eagan, USA) (Gardner and Parker, 2011). The tonometer was applied to the subjects' radial artery at the wrist overlying the radial bony prominence. The cuff for the BP measurement was placed on the contralateral arm and inflated concurrently with the pulse waveform recording for calibration. The elasticity indices of the arteries were quantified during the diastolic portion of the cardiac cycle (mean of 30 sec recording). This method of vascular assessment allows for the calculation of elasticity of large central (capacitive) arteries and small peripheral (oscillatory) arteries, calculated as the large artery elasticity index (LAE) and the small artery elasticity index (SAE), respectively. To determine HA-related changes in the AE parameters (the HA study, Figure 2), we calculated the difference between the LAE and SAE value measured after the HA program and measured at baseline (ΔLAE and ΔSAE), respectively. All the measurements were made in duplicate and the mean values were used in the subsequent analysis.

4.8. Measurement of oxidative stress and inflammation

The blood measurements were performed at the Institute of Biomedicine and Translational Medicine (University of Tartu) and the United Laboratories of Tartu University Hospital. All manufacturers' recommendations regarding the determination procedures were carefully followed.

The venous blood samples were obtained from the *antecubital fossa*:

a) twice in the PHE study (Figure 1): the baseline samples were taken between 8:00 and 10:00 following an overnight fast and the second set of samples was obtained after the heating session in the climate chamber after the AS measurements (Paper I).

b) four times in the HA study (Figure 3): the samples were taken between 8:00 and 10.00 following an overnight fast two times in the non-heat-acclimated status (at baseline and after the EC test in the heat) and two times after the 10-day HA program (i.e. in the heat-acclimated status) both at baseline and after the EC test in the heat (Paper III).

The QBC Autoread Plus autoanalyzer (QBC Diagnostics, Inc., USA) was used to assess white blood cell (WBC) counts, haemoglobin and haematocrit in the whole blood. The other blood samples were centrifuged within 15 minutes after their collection at 3000 rpm for 15 minutes to obtain plasma or serum. All the plasma or serum samples were stored at –70 ºC until the analysis.

The plasma high-sensitive CRP (hsCRP) was measured by a validated latex particle-enhanced high-sensitivity immunoturbidimetric assay (CRP Latex HS, Roche Diagnostics Gmbh®, Mannheim, Germany), and analyzed by the Hitaci 912 analyzer (Roche Diagnostics®, Basel, Switzerland).

The total peroxide concentrations of the samples were determined by using OXYSTAT Assay Kit Cat. No BI-5007 (Biomedica Gruppe, Biomedica Medizinprodukte GmbH & Co Kg, Wien). The total antioxidant capacity (TAC) was measured by using the automated measurement method by Erel (2004). The percent ratio of the total peroxide concentration of plasma to the TAC of plasma was accepted as the OSI, which is an indicator of the degree of oxidative stress.

Commercially available enzyme-linked immunosorbent assay kits were used to determine serum oxLDL (Mercodia AB, Uppsala Sweden; Cat No 10-1143-01) and serum soluble intercellular adhesion molekule-1 (sICAM-1) (Human soluble ICAM-1 Immunoassay, catalogue number BBE 1B, R&D Systems Inc.®, Minneapolis, USA). The plasma β_2-microglobulin (β2M) concentration was measured by a chemiluminescent immunoassay using a commercially available kit (L2KBM2, Siemens Medical Solutions Diagnostics®, California, USA) in the IMMULITE 2000 automated analyzer (Siemens Medical Solutions Diagnostics®, California, USA).

The Evidence Investigator Cytokine and Growth Factors High-Sensitivity Array based on the sandwich chemiluminescent immunoassay (Randox Laboratories Ltd CTK HS Cat. No. EV 3623) was used for the simultaneous quantitative detection of multiple related cytokines: the IL-6, the IL-8, the epidermal growth factor (EGF), the vascular endothelial growth factor (VEGF), the monocyte chemoattractant protein-1 (MCP-1) from a single patient's sample. The core technology was the Randox Biochip containing an array of discrete test regions of immobilized antibodies specific to different cytokines and growth factors. The Evidence Investigator™ Metabolic Syndrome Array1 based on the sandwich chemoluminescent immunoassay (Randox Laboratories Ltd METS1 catalogue number EV 3755) was used in the PHE study for the simultaneous quantitative detection of the IL-6 from a single patient's sample.

The N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured using the electrochemiluminescence immunoassay ECLIA and an Elecsys® 2010 analyzer (Roche Diagnostics).

In the HA study (Figure 3, Paper III), the relative changes in the plasma volume after the EC test in the NHAS and the HAS were calculated on the basis of the hemoglobin and hematocrit values (Dill and Costill, 1974).

4.9. Statistical analysis

The data analysis was performed using The Statistical Package for the Social Sciences (SPSS, version 18.0 and 20.0) software. All the data were checked for normal distribution using the Kolmogorov-Smirnov test and are presented as means ± standard deviation (SD).

A dependent samples (paired) t-test was used to compare the values of the parameters measured before and after the PHE (Paper I), the values of the baseline parameters and the values of the parameters after the HA (Paper II) and the values of the parameters measured before and after the EC test in the NHAS and the values of the parameters measured before and after the EC test in the HAS (Paper III).

A Pearson and Spearman product moment coefficient of correlation was used to determine the relationships between variables. Independent Samples t-tests were used to determine the differences between the groups (Paper II).

Because of the skewed distribution of the hsCRP, logarithmic transformation was performed before the statistical analysis (Paper I, Paper III). The effect of acclimation was analyzed with repeated measures analysis of variance. The Bonferroni *post-hoc* analysis was used to evaluate the differences in the values of the parameters in the heat-acclimated and non-heat-acclimated status before and after the EC test (Paper III). For all the statistical analyses, the 0.05 level of significance was used.

5. RESULTS

5.1. Effect of acute heat exposure on arterial stiffness, oxidative stress and inflammation

After the PHE, the subjects' core body temperature was significantly higher compared to the core body temperature before the PHE: 37.01 ± 0.19 ºC and 36.4 ± 0.31 ºC ($p < 0.001$), respectively. The values of the haemodynamic and AS parameters before and after acute heat exposure are given in Table 2. There were no statistically significant changes in the HR, the central and peripheral BP. A significant increase was found in the peripheral pulse pressure (PPP) after the PHE as compared to the baseline value.

Table 2. The values of the haemodynamic and arterial stiffness parameters before and after the passive heat exposure (PHE).

Parameter	Before PHE	After PHE
HR (beats/min)	49 ± 10	51 ± 7
PSBP (mmHg)	117.22 ± 8.69	119.56 ± 7.04
PDBP (mmHg)	66.00 ± 7.35	64.78 ± 3.80
CSBP (mmHg)	99.17 ± 8.86	100.33 ± 7.83
CDBP (mmHg)	66.44 ± 7.49	65.22 ± 4.12
PPP (mmHg)	51.22 ± 4.15	54.67 ± 5.32 **
CPP (mmHg)	32.83 ± 4.09	35.11 ± 4.97
PPP/CPP	1.57 ± 0.13	1.57 ± 0.10
MAP (mmHg)	80.72 ± 8.06	78.33 ± 9.29
AIx (%)	0.06 ± 12.05	2.50 ± 8.29
AIx@75 (%)	–12.43 ± 13.01	– 8.71 ± 8.00
Tr (ms)	175.11 ± 12.97	173.33 ± 10.48
PWVc-f (ms)	5.91 ± 0.49	5.93 ± 0.71
PWVc-r (ms)	8.71 ± 0.86	8.41 ± 0.87
SAE (ml/mmHgx100)	11.05 ± 1.68	10.01 ± 1.90

Data are presented as mean ± SD; **HR –** heart rate; **PSBP –** peripheral systolic blood pressure; **PDBP** – peripheral diastolic blood pressure; **CSBP –** central systolic blood pressure; **CDBP** – central diastolic blood pressure; **PPP –** peripheral pulse pressure; **CPP –** central pulse pressure; **MAP –** mean arterial pressure; **AIx** – augmentation index; **AIx@75** – augmentation index corrected for heart rate of 75 beats *per* minute; **Tr** – travel time of the reflected wave; **PWVc-f** – pulse wave velocity at carotid-femoral segment; **PWVc-r** – pulse wave velocity at carotid-radial segment; **SAE –** small artery elasticity index; ** $p < 0.01$ as compared to the value measured before PHE.

There was a significant decrease in the LAE (17%) after the PHE (Figure 4), whereas other parameters of AS did not reveal significant changes (Table 2).

The magnitude of the decrease in LAE was negatively related to the normothermic value of this parameter measured before the PHE ($r = –0.878$; $p < 0.01$) (Figure 5). The correlation analysis revealed that the decrease in LAE was not associated with the increase in core body temperature (ΔTc) ($r = –0.432$; $p > 0.05$).

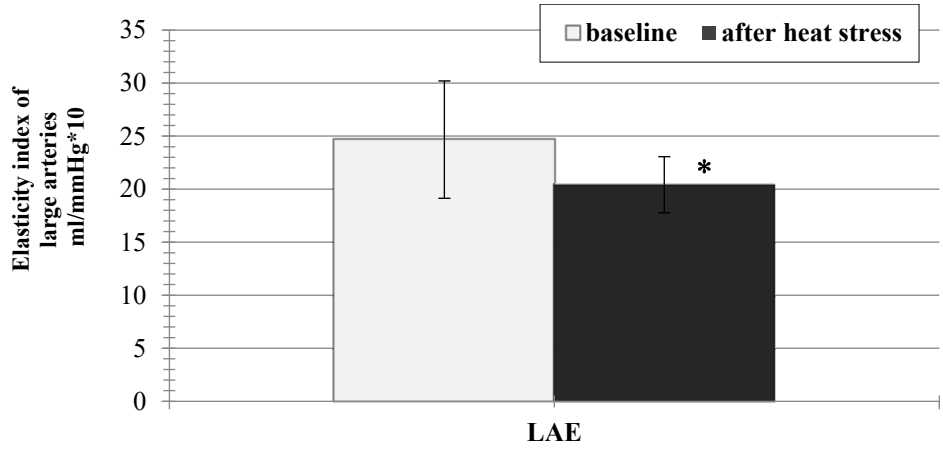

Figure 4. Large artery elasticity index (LAE) at baseline and after the passive heat exposure (x ± SD). A significant decrease compared with the baseline measurement: ∗ $p < 0.05$.

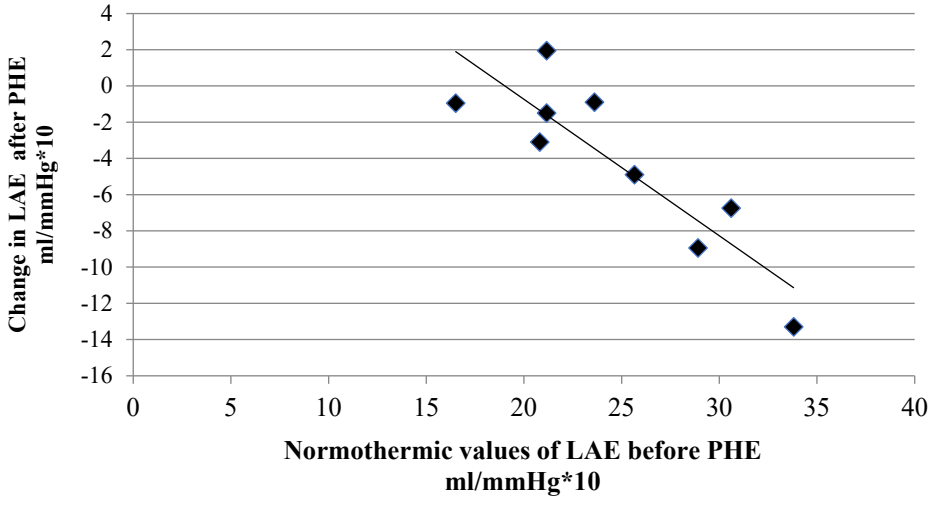

Figure 5. The relationship between the baseline large artery elasticity index (LAE) and the change in the LAE after the passive heat exposure (PHE) ($r = –0.878$, $p < 0.01$).

The values of oxidative stress and inflammation markers are given in Table 3. Oxidative stress markers remained unchanged ($p > 0.05$) after heat exposure and there were no significant associations between oxidative stress, AS and core body temperature ($p > 0.05$). The PHE induced a significant increase in the serum IL-6 concentration (30%, $p < 0.05$), but there was no significant association between the values of IL-6 measured before and after the PHE. Likewise, the pre- and post-values and the changes in blood IL-6 level did not correlate with the pre- and post-values or changes in core body temperature and LAE ($p > 0.05$).

Table 3. The values of inflammation and oxidative stress markers before and after the passive heat exposure.

Parameter	Before PHE	After PHE
WBC (x 10^9/L)	4.64 ± 1.14	4.93 ± 1.20
hsCRP (mg/L)	0.74 ± 0.35	0.72 ± 0.34
IL-6 (pg/mL)	0.44 ± 0.16	0.59 ± 0.27 *
Total peroxide concentration (μmol/L)	282.67 ± 114.37	305.90 ± 156.49
TAC (mmol Trolox equivalent/L)	1.54 ± 0.22	1.44 ± 0.20
OSI (%)	19.02 ± 7.94	22.13 ± 11.07

Data are presented as mean ± SD; **PHE** – passive heat exposure; **WBC** – white blood cells; **hsCRP** – high-sensitive C-reactive protein; **IL-6** – interleukin-6; **TAC** – total antioxidant capacity; **OSI** – oxidative stress index; * $p < 0.05$ as compared to the value measured before heat exposure.

5.2. Heat acclimation study

Subjects' acclimated state was successfully achieved confirmed by a significant decrease in resting HR from 56 ± 9 to 49 ± 8 beats per minute ($p < 0.001$), and in core body temperature from 37.2 ± 0.2 to 37.0 ± 0.2 ºC ($p < 0.01$), respectively. After the HA program, all subjects showed an improvement in the EC (individual variation from 10 to 128 minutes); the subjects' mean EC increased statistically significantly by 86%: from 88.62 ± 27.51 to 161.95 ± 47.80 minutes ($p < 0.001$). Over the 10-day HA program, the subjects' mean systolic BP (pre-HA 122.14 ± 9.65 and post-HA 120.38 ± 7.95 mmHg; $p > 0.05$) and diastolic BP (pre-HA 65.88 ± 7.52 and post-HA 63.86 ± 7.81 mmHg; $p > 0.05$) did not change significantly.

5.2.1. Effect of heat acclimation on arterial stiffness

Our study results showed that the subjects' AS improved after HA: the LAE and SAE increased statistically significantly after the acclimation as compared to the baseline values (Figure 6).

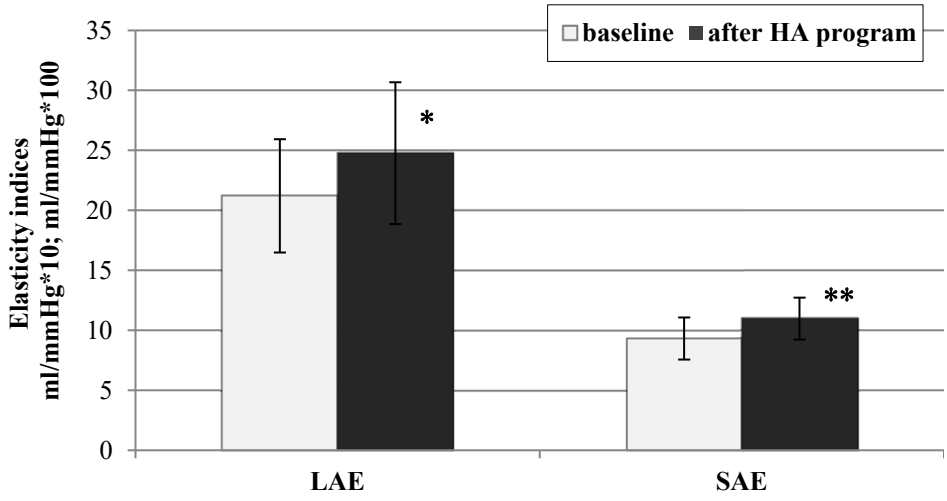

Figure 6. Large artery elasticity index (LAE) and small artery elasticity index (SAE) at baseline and after the heat acclimation (HA) program (x ± SD). A significant increase occurred in the LAE and SAE after the HA program compared with the baseline measurement: ∗ $p < 0.05$; ∗∗ $p < 0.01$.

For further analysis, we divided our subjects into four groups (median values were considered as respective cut-off values) to comprehensively elucidate the potential relationships between the EC and AE:

1) Based on the EC values after the HA program: 99–150 minutes (Group 1; n = 11) *vs* 170–300 minutes (Group 2; n = 10)

2) Based on the improvement in the EC (EC value after the HA program – EC value at baseline): 10–68 minutes (Group 3; n = 10) *vs* 73–128 minutes (Group 4; n = 11).

There were no statistically significant differences in the AE measured at baseline and after the HA or in the HA-related changes in the AE between the respective subgroups (Table 4).

However, a significant improvement was demonstrated in the LAE in group 2 and in group 4, which were characterized by a superior EC after the HA and a greater improvement in the EC due to the HA, respectively. A significant improvement in the SAE values only occurred in group 1, where the EC was modest after the HA.

Table 4. Between-group differences in arterial elasticity.

	Group 1 (n = 11)	**Group 2** (n = 10)	**p-value** between groups 1 and 2	**Group 3** (n = 10)	**Group 4** (n = 11)	**p-value** between groups 3 and 4
LAE at baseline ml/mmHgx10	20.7 ± 4.8	21.7 ± 4.8	NS	21.5 ± 4.7	21.0 ± 5.0	NS
LAE after HA ml/mmHgx10	22.5 ± 4.6	27.0 ± 6.4 ∗	NS	23.4 ± 4.2	25.9 ± 7.0 ∗	NS
SAE at baseline ml/mmHgx100	9.1 ± 1.9	9.5 ± 1.6	NS	9.1 ± 2.2	9.5 ± 1.3	NS
SAE after HA ml/mmHgx100	11.5 ± 1.7 ∗	10.4 ± 1.7	NS	11.3 ± 1.8	10.7 ± 1.7	NS
ΔLAE ml/mmHgx10	1.8 ± 5.1	5.3 ± 5.9	NS	1.9 ± 5.4	5.0 ± 5.7	NS
ΔSAE ml/mmHgx100	2.4 ± 3.0	0.9 ± 2.0	NS	2.2 ± 3.3	1.2 ± 1.9	NS

Data are presented as mean ± SD; **Group 1** – subjects who had the endurance capacity value 99–150 minutes after the HA; **Group 2** – subjects who had the endurance capacity value 170–300 minutes after the HA; **Group 3** – subjects who had an improvement in the endurance capacity of 10–68 minutes; **Group 4** – subjects who had an improvement in the endurance capacity of 73–128 minutes; **LAE** – large artery elasticity index; **SAE** – small artery elasticity index; **HA** – heat acclimation; **ΔLAE** – difference between elasticity index value measured after the HA program and value measured at baseline; **ΔSAE** – difference between elasticity index value measured after the HA program and value measured at baseline; **NS** – not significant; ∗ $p < 0.05$ as compared to the baseline value.

The correlation analysis did not reveal significant associations between the ΔLAE and ΔSAE and the subjects' VO_2peak or improvement in the EC. A statistically significant inverse association was found between the ΔSAE and the EC measured after the HA program ($r = –0.465$, $p < 0.05$) and between the ΔSAE and the value of the SAE measured at baseline ($r = –0.756$, $p < 0.001$).

5.2.2. Effect of heat acclimation on oxidative stress and inflammation

The values of the oxidative stress markers measured at baseline (before the EC test) in both the NHAS and the HAS are shown in Figure 7, Figure 8, Figure 9, Figure 10. HA significantly increased baseline total peroxide concentration and OSI in the HAS as compared to the baseline value measured in the NHAS (Figure 7, Figure 9). Baseline oxLDL decreased significantly in the HAS as compared to the baseline value measured in the NHAS before the EC test (Figure 10).

Analysing the effect of the EC test on oxidative stress markers in the NHAS status, total peroxide concentration and OSI increased significantly after the EC test, whereas the TAC and the oxLDL remained significantly unchanged. In the

HAS, the EC test significantly decreased OSI, but had no significant impact on the total peroxide concentration, the TAC and the oxLDL (Figure 7, Figure 8, Figure 9, Figure 10). The Bonferroni *post-hoc* analysis revealed a significant effect of the acclimation and the EC test interaction on total peroxide concentration ($F = 3.79$, $p < 0.05$), OSI ($F = 3.79$, $p < 0.05$) and oxLDL ($F = 2.83$, $p < 0.05$), but there was no significant effect on TAC ($F = 1.39$, $p > 0.05$).

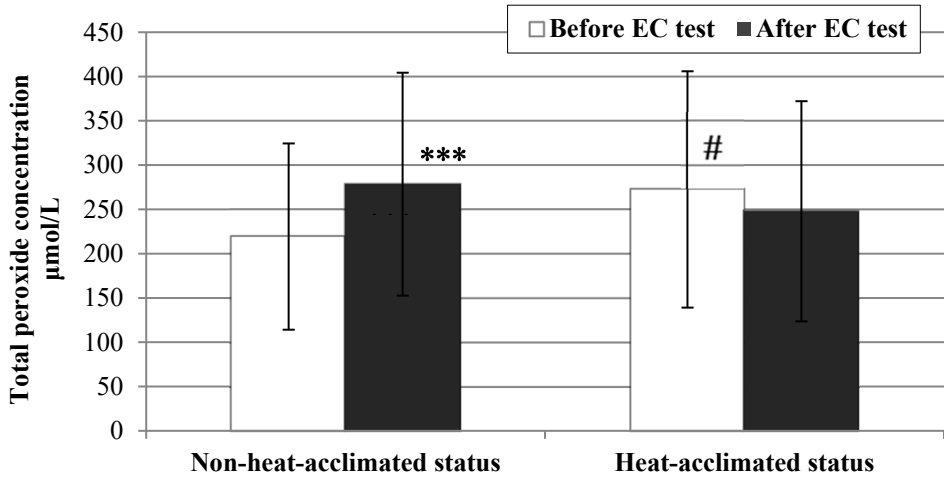

Figure 7. The values of the total peroxide concentration before and after the endurance capacity test in the heat measured before and after the heat acclimation (x ± SD). **EC** – endurance capacity; *** $p < 0.001$ as compared to the value measured before the EC test; # $p < 0.05$ as compared to the non-heat-acclimated status before the EC test.

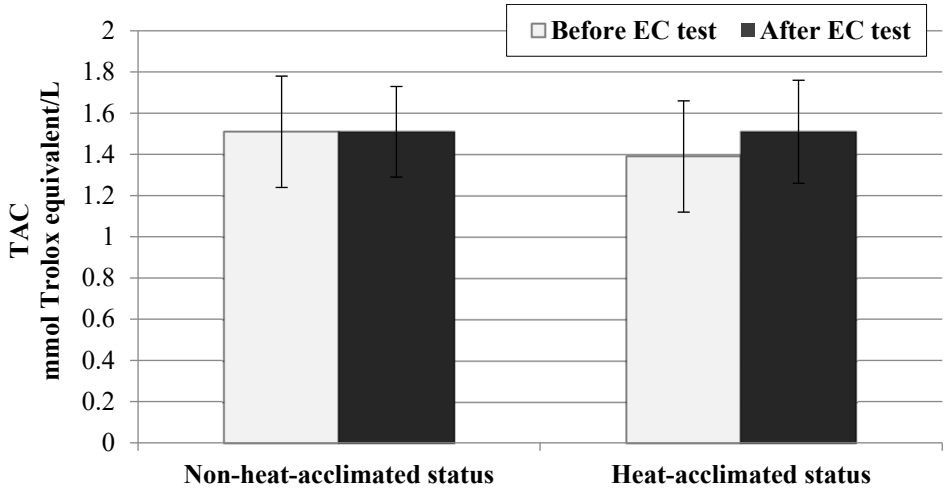

Figure 8. The values of the total antioxidant capacity before and after the endurance capacity test in the heat measured before and after the heat acclimation (x ± SD). **EC** – endurance capacity; **TAC** – total antioxidant capacity.

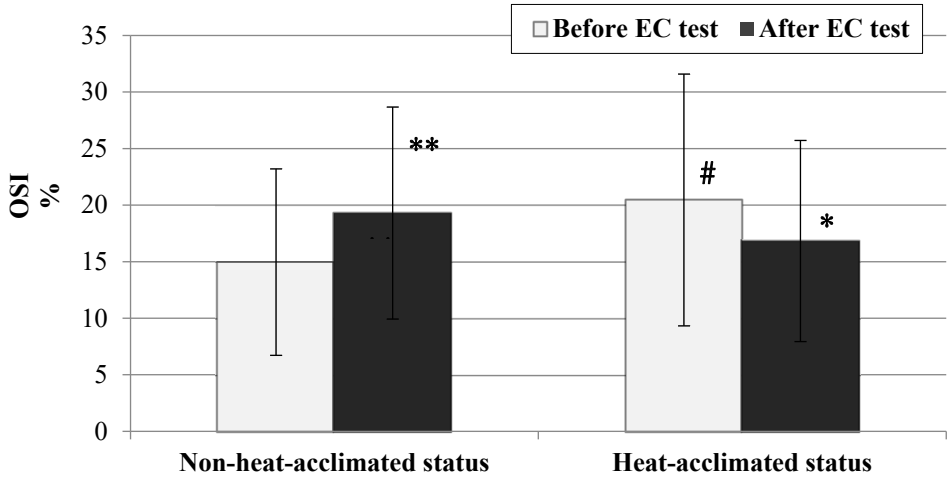

Figure 9. The values of the oxidative stress index before and after endurance capacity test in the heat measured before and after the heat acclimation (x ± SD). **EC** – endurance capacity; **OSI** – oxidative stress index; * $p < 0.05$; ** $p < 0.01$ as compared to the value measured before the EC test in the heat-acclimated status/ non-heat-acclimated status; # $p < 0.05$ as compared to the non-heat-acclimated status before the EC test.

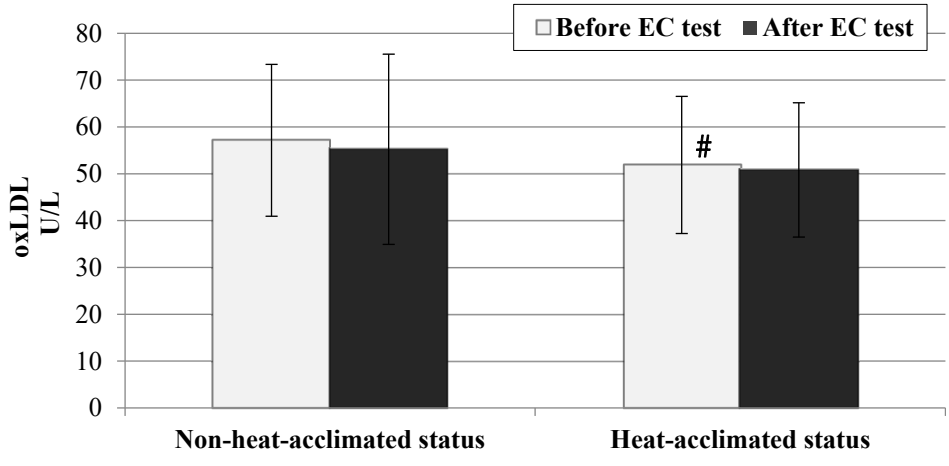

Figure 10. The values of the oxidized low-density lipoproteins before and after the endurance capacity test in the heat measured before and after the heat acclimation (x ± SD). **EC** – endurance capacity; **oxLDL** – oxidized low-density lipoproteins; # $p < 0.05$ as compared to the non-heat-acclimated status before the EC test.

The values of inflammation markers at baseline (before the EC test) in both the NHAS and the HAS are presented in Table 5. The 10-day HA program had no impact on the inflammation markers measured at baseline in the NHAS and the HAS before the EC test.

The EC test significantly increased the hsCRP in both the NHAS and the HAS. There was no impact ($p > 0.05$) of the EC test on any other inflammation marker in the NHAS. In the HAS, the EC test significantly decreased the concentrations of the MCP-1 ($p < 0.001$).

The Bonferroni *post-hoc* analysis revealed a significant effect of the acclimation and the EC test interaction on the inflammation markers: on the hsCRP ($F = 5.97$, $p < 0.01$) and on the MCP-1 ($F = 6.1$, $p < 0.001$).

In addition, the EC test increased the NT-proBNP level from 19.57 ± 10.43 to 28.79 ± 15.83 pg/mL in the NHAS ($p < 0.05$) but had no significant impact on this marker in the HAS (27.90 ± 18.15 and 30.90 ± 15.55 pg/mL, before and after EC test, respectively; $p > 0.05$). The Bonferroni *post-hoc* analysis revealed a significant effect of the acclimation and the EC test interaction on the NT-proBNP ($F = 2.98$, $p < 0.05$).

Table 5. Subjects' inflammation markers before and after the endurance capacity test in the non-heat-acclimated status and the heat-acclimated status.

Markers of inflammation	Non-heat-acclimated status		Heat-acclimated status		Main effect (F)	p-value
	Before EC test	After EC test	Before EC test	After EC test		
WBC x 10^9/L	5.33 ± 1.13	5.68 ± 1.42	5.30 ± 1.08	5.72 ± 1.22	1.72	NS
hsCRP mg/L	0.63 ± 0.52	1.04 ± 0.89 *	1.17 ± 2.59	3.53 ± 4.64 *	5.97	$p < 0.01$
log hsCRP mg/L	–0.32 ± 0.32	–0.12 ± 0.34 *	–0.31 ± 0.47	0.28 ± 0.46 ***	14.76	$p < 0.001$
sICAM-1 ng/mL	163.62 ± 29.62	169.20 ± 30.79	164.48 ± 28.17	169.22 ± 27.31	0.95	NS
EGF pg/mL	2.81 ± 1.99	3.19 ± 2.26	3.31 ± 2.33	2.46 ± 1.77	2.16	NS
VEGF pg/mL	53.14 ± 45.04	54.99 ± 36.04	54.87 ± 35.89	45.87 ± 29.38	1.16	NS
MCP-1 pg/mL	157.52 ± 76.74	148.64 ± 60.20	187.79 ± 76.37	138.36 ± 58.05 ***	6.10	$p < 0.001$
IL-6 pg/mL	0.78 ± 0.67	0.92 ± 0.67	1.11 ± 1.51	0.90 ± 0.93	0.93	NS
IL-8 pg/mL	8.55 ± 12.25	9.93 ± 17.48	9.92 ± 13.05	8.30 ± 12.63	2.11	NS
β2M µg/L	1478.29 ± 217.79	1465.42 ± 204.64	1453.24 ± 213.02	1528.18 ± 280.28	1.73	NS

The data are presented as mean ± SD; **EC** – endurance capacity; **hsCRP** – high-sensitive C-reactive protein**; sICAM-1** – soluble intercellular adhesion molecule-1; **WBC** – white blood cells; **EGF** – epidermal growth factor; **VEGF** – vascular endothelial growth factor; **MCP-1** – monocyte chemoattractant protein-1; **IL-6** – interleukin-6; **IL-8** – interleukin-8; **β2M** – β_2-microglobulin; * $p < 0.05$; *** $p < 0.001$ as compared to the value measured before the EC test.

6. DISCUSSION

6.1. Effect of acute heat exposure on arterial stiffness, oxidative stress and inflammation

This study examined the acute effect of passive heat exposure on the arterial stiffness, oxidative stress, and inflammatory markers in young healthy men. The participants were exposed to the heat stress in a climatic chamber (42 ºC; RH 18%) for 110 minutes in non-exercise settings.

The main finding of the study is that the acute PHE induced a significant decrease in the LAE (an increase in AS). Furthermore, the observed decrease in the LAE inversely correlated with the baseline values of this parameter, i.e., the greatest PHE-induced decrements in the LAE occurred in the individuals exhibiting the highest values of the LAE before the heat exposure. We also showed a remarkable increase ($p < 0.05$) of the IL-6 after the acute PHE in a climatic chamber. However, our data indicated that the PHE did not have a significant effect on oxidative stress markers.

The data on the effect of the heat stress on the AS are limited and contradictory results have been shown. *In vitro* evidence has demonstrated that heating vessels to a non-physiological temperature (60 ºC) decreased the stiffness of these vessels (Mitchel *et al*., 1994). Furthermore, it has been shown in humans that an acute local thermal therapy (footbath) resulted in a decrease in the AS in both healthy young and older women (Hu *et al*., 2012). The studies also indicate that a single sauna session decreased the AS (Lee *et al*., 2018) and that a short-term warm water immersion improved the central and peripheral AS in healthy men (Sugawara and Tomoto, 2021). In addition, a passive hot-water immersion of the lower limb reduced the central and peripheral AS in patients with peripheral artery disease and in healthy, elderly controls (Thomas *et al*., 2017). Caldwell and others (2017) demonstrated that passive heating *via* a water-perfusion suit reduced peripheral AS. However, in contrast, Moyen *et al*. (2016) showed a slightly increased central AS with an acute bout of a whole-body passive heat stress *via* a water-perfusion suit, while peripheral AS remained unchanged. The findings of the study by Schlader and others (2019) also indicate that a progressive whole-body passive heat stress does not affect the AS in older and younger adults. In addition, Ganio *et al*. (2011) studied the effect of a passive heat stress on the peripheral and central AS in a group of men and women and concluded that the core body temperature increase of up to 1.5 ºC above the baseline did not affect the AS.

In our study, the subjects' mean core body temperature increased significantly after the PHE ($p < 0.001$), but this rise in the core body temperature (0.61 ºC) was not sufficient to induce significant changes in most AS parameters. However, a significant decrease in the value of the LAE ($p < 0.05$) was registered showing a worsening of the central AS. Furthermore, we found that the magnitude by which the heat stress increased the AS was negatively related to the value measured at baseline – the decrease in the LAE was higher in the individuals with a higher

baseline value of the LAE. Our study results demonstrate that heat-stress-induced changes in AS are related to the baseline AS level and these findings are supported by other studies (Ganio *et al*., 2011; Moyen *et al*., 2016).

The response of AS to heat stress may be affected by several factors and mechanisms. The heat stress increases sympathetic activity (Low *et al*., 2011), which is associated with an increase in the AS (Swierblewska *et al*., 2010). Although this has not been evaluated in our study, we can speculate that the PHE increased sympathetic activity in our participants and therefore caused the increase in the AS. As it is not fully understood how heat stress affects the AS differently depending on the baseline tone of the arteries, we can only postulate that the individuals with lower baseline AS in our study had higher sympathetic activity in response to the heat stress, and this is part of the reason they were more responsive to the heat stress induced changes in the AS. Although Ganio and others (2011) did not evaluate the sympathetic activity in their study, they speculated that the magnitude of the elevation in the sympathetic activity to heat stress was greater in the individuals with the highest baseline arterial compliance.

Hyperthermia increases blood flow, which would increase shear stress and promote the release of nitric oxide (NO) (Thomas *et al*., 2017; Tinken *et al*., 2010). Vasodilatation, secondary to the NO release, can reduce AS (Kinlay *et al*., 2001). Therefore, our finding that the increase in the AS in response to the heat stress was higher in the individuals with a lower baseline AS may partially be explained by the possibility that in the vessel with low AS during normothermia, the amount of arterial tone changing factors released in response to heat stress were not adequate or these arteries were not sufficiently sensitive to these factors to cause changes in the arterial tone in response to heat stress.

The response of the AS on heat stress may also be affected by an increased low-grade inflammation. It has been found that heat exposure tends to stimulate the release of the inflammatory cytokine IL-6 (Cosio-Lima *et al*., 2011) and that a higher value of the IL-6 is associated with higher AS (Schnabel *et al*., 2008). It has been demonstrated that 1 hour of passive heating induces a 2-fold increase in the IL-6 (Faulkner *et al*., 2017), and a significant increase in the IL-6 concentration immediately following a 2-hour passive heat stress by hot water immersion in healthy men has been demonstrated by Laing and others (2008). In our study, we found a significant ($p < 0.05$) increase of the IL-6 after the passive heat stress exposure in a climatic chamber. Nevertheless, our study did not reveal significant associations between the values or changes in core body temperature, IL-6, and LAE. In another study (Patel *et al*., 2011), none of the markers of inflammation correlated with the AE indices in subjects, who were free of traditional CV risk factors.

The results of animal studies suggest that exposure to high temperature may result in increased oxidative stress (Yang *et al*., 2010), and oxidative stress should be considered as part of the stress response to heat exposure (Lin *et al*., 2006). In addition, the environmental temperature can influence exercise-induced oxidative stress (Quindry *et al*., 2013), and it has been suggested that the core body temperature is a participating factor in the induction of oxidative stress (Mestre-

Alfaro *et al*., 2012). It has been concluded that oxidative stress is directly linked to increased AS (Kals *et al*., 2006; Patel *et al.*, 2011). In the present study, we did not find any significant changes in oxidative stress markers after the heat stress exposure nor were there any associations between oxidative stress, AS and core body temperature values.

6.2. Effect of heat acclimation on arterial stiffness

The primary novel finding of this study is the beneficial impact of the combined heat and exercise stress on AS. A significant improvement in AS compared to baseline was demonstrated: the LAE was increased by 17% and the SAE by 18%. The study did not reveal significant correlations between the changes in the AS and the improvement in the EC or the subjects' $\dot{V}O_2peak$.

Significant acute improvements in AE after exercise have been seen in young and older adults (Kingwell *et al*., 1997; Nickel *et al*., 2011). It has been shown that the AE improved by 66% for a short term through a single 30-min bout of endurance exercise without heat stress (Kingwell *et al*., 1997). It has also been demonstrated that regular exercise is associated with decreased AS (Maeda *et al*., 2008), but the AE improving effect cannot be maintained without continuing regular physical exercise (Kakiyama *et al*., 2005).

There are studies showing that acute passive heat stress may result in a decrease in AS (Caldwell *et al*., 2017; Hu *et al*., 2012; Sugawara and Tomoto, 2021; Thomas *et al*., 2017) and chronic passive heat stress also decreases AS (Brunt *et al*., 2016). In contrast, there are studies revealing that passive heating does not affect AS (Ganio *et al*., 2011; Schlader *et al*., 2019).

Despite evidence regarding the independent acute effects of exercise and heat stress on AS, the interaction of heat stress and exercise on AS is largely unexplored. Caldwell and others (2017) examined the acute effects of passive heating and exercise in the heat on AS and concluded that an acute exercise bout did not result in any acute changes in AS regardless of heat stress. A recent study by Lee with colleagues (2022) examined the impact of regular exercise in conjunction with sauna bathing and showed that neither an eight-week exercise intervention alone nor the eight-week exercise intervention together with sauna bathing had an impact on AS.

The observed changes in the AE parameters in our study are likely a result of the combined effect of exercise and heat stress. While the specific factors and mechanisms driving these changes are not fully understood, they are physiologically significant for effective acclimation, particularly in terms of cardiac loading. The elasticity of the large arteries plays a crucial role in determining cardiac loading. More elastic aorta and large arteries can lead to an increased coronary blood flow during diastole, decreased cardiac afterload, and augmented left ventricular function. This, in turn, contributes to enhanced aerobic exercise capacity (Kingwell, 2002). The improvement in AE contributes to reducing the CV strain and delaying the onset of fatigue during exercise. Moreover, improved AE

contributes to minimizing the risk of CV morbidity and mortality (Ben-Shlomo *et al*., 2014; Mattace-Raso *et al*., 2006; Weber *et al*., 2004).

AS has two major elements: a passive component that reflects the structural composition of the artery wall (the composition of elastin and collagen) and an active component related to the arterial tone exerted by the arterial wall smooth muscle cells (Bank *et al*., 1996; Kinlay *et al*., 2001). It is unlikely that our acclimation intervention decreased AS *via* structural vascular adaptations because biochemical changes in the elastin-collagen composition of the arterial wall are believed to occur over the years (Kohn *et al*., 2015).

Functional dynamic changes, e.g. vasodilation, are most probably the mechanism underlying such short-term changes in AS. The NO bioavailability is thought to be one of the most potent modulators of a smooth muscle tone (Sugawara *et al*., 2007). The increased pulsatile flow in the arteries associated with exercise training might evoke an acute release of the NO, as well as lead to an upregulation of the NO production and an increase in the production of other vasodilating factors (Delp and Laughlin, 1997; Rubanvi *et al*., 1986; Spier *et al*., 1999), which may regulate the vascular tone. The relative loading of collagen and elastin fibers is altered by changes in the arterial smooth muscle tone (Belz, 1995), thus making the arterial wall more compliant – the wall stress from the stiffer collagen fibers is transferred to the more extensible elastin fibers.

The improvement in AE may also be partly explained by the elevations in core body temperature. An elevated core body temperature increases the blood flow through the conduit arteries and therefore causes increased shear stress, which in turn releases the NO and other endothelium-derived factors, which may regulate the vascular tone and thereby decrease AS (Bellien *et al*., 2010; Kellogg *et al*., 2003; Kinlay *et al*., 2001; Sugawara *et al*., 2007).

A substantial increase in the EC in the heat that occurred in our subjects over a rather short HA is in agreement with the findings of several previous studies (Nielsen *et al*., 1993; Yamada *et al*., 2007; Amorim *et al*., 2008). Research has established a link between a higher exercise capacity and reduced AS (Vaitkevicius *et al*., 1993). However, our study did not reveal significant associations between the improvement in AS or the improvement in the EC. There was only a significant inverse correlation between the improvement in the SAE and the EC measured after the HA – the individuals who had the biggest improvement in the elasticity of small arteries had the lowest EC after the HA. Our study data also showed that the individuals who had the biggest HA-induced improvement in the SAE had the lowest baseline SAE. Ganio and others (2011) similarly showed that changes in AS during passive heating are predicated on baseline AS. In our study, the AE parameters did not differ in the subgroups of the subjects formed either based on the EC values registered after the HA or based on the extent of the improvement in the EC over the HA. These findings suggest that the HA-induced changes in AS and the EC occur simultaneously but are not directly related to each other. Nevertheless, we demonstrated a significant improvement in the LAE in the groups, where the values of the EC after the HA were higher (Group 2) and where the improvement in the EC after HA was bigger (Group 4).

The finding of our study that there is no relationship between the baseline VO_2peak and the HA-related changes in AS differs from the results of a previous study (Kampus *et al*., 2008), which suggested that the response of the AE parameters to physical exercise may depend on the subjects' maximal oxygen uptake. However, in that study (Kampus *et al*., 2008), a very small group (n = 7) of well-trained cadets (mean maximal oxygen uptake 66.0 mL · kg^{-1} · min^{-1}, range 57.6 to 77.3 mL · kg^{-1} · min^{-1}) was monitored during a three days of extreme physical load in the absence of the environmental heat stress. Thus, substantial differences in the subjects' characteristics as well as in the physical load employed and the environmental factors could explain the discrepancy in the data between these two studies. Furthermore, all the participants in the current study were healthy young males with AE values in the normal range. This may partially explain the absence of associations between the improvement in the AE indices and the baseline VO_2peak.

6.3. Effect of heat acclimation on oxidative stress and inflammation

The primary finding of this study is that undergoing a 10-day HA period leads to an increase in the subjects' oxidative stress level. However, it also induces positive adaptive effects on the responses of oxidative stress and inflammation markers during exhausting endurance exercise in hot conditions among young healthy men.

We observed a higher OSI level (36.7%, $p < 0.05$) in baseline before the EC test in our subjects in the HAS compared to the NHAS, and the increase in the OSI was mainly caused by the elevated total peroxide concentration (24.2%, $p < 0.05$). Our finding is in agreement with Goto and colleagues (2003), who showed increased indices of oxidative stress after high-intensity exercise training for 12 weeks in healthy young men. In our study, the changes in oxidative stress were caused by the combined effect of exercise and the heat stress occurring simultaneously. The exercise-induced physiological strain and the oxidative stress response may be increased by a high ambient temperature and hyperthermia (Cheung and Sleivert, 2004; McAnulty *et al*., 2005), where the rise in core body temperature plays an important role (Laitano *et al*., 2010; Mestre-Alfaro *et al*., 2012).

The study by Quindry and others (2013) found that moderate intensity exercise in a warm environment elicits a blood oxidative stress response not observed at comparable exercise performed at lower temperatures and indicated that exercise-induced oxidative stress can be influenced by the environmental temperature.

Our study revealed that HA induces beneficial adaptive effects on the responses of oxidative stress and inflammation markers to an acute exhausting EC test in the heat. When the EC test was performed in the NHAS, the oxidative stress increased significantly after the EC test (*e.g.* an increase in the total peroxide

concentration and the OSI). However, when the EC test was performed in the HAS, the OSI decreased (17.7%, $p < 0.05$) as compared to the value measured before the EC test. The beneficial adaptive effect of the HA and the EC test interaction on the response of oxidative stress to an acute exhausting exercise is also supported by the finding of the Bonferroni *post-hoc* analysis – a significant effect was revealed on the total peroxide concentration ($F = 3.79$, $p < 0.05$) and on the OSI ($F = 3.79$, $p < 0.05$). Our results are similar to those of Miyazaki and colleagues (2001), who found that intense endurance training for 12 weeks reduces oxidative stress after exhausting exercise and up-regulates the antioxidant defence system in humans.

One explanation for this is that the observed beneficial adaptive effect of HA on the oxidative stress level caused by the EC test can at least partly be due to the heat shock proteins. The heat shock proteins may be important modifying factors in cellular responses to a variety of physiologically relevant conditions such as hyperthermia, exercise, oxidative stress, and modifying factors in acquired thermotolerance (Kregel, 2002). It is known that heat and exercise greatly accelerate the synthesis of the inducible heat shock proteins (especially heat shock protein 70) (Walsh *et al*., 2001; Lovell *et al*., 2007), which is thought to have both a cellular and systemic protective role (Kregel, 2002). Sandström and others (2008) found that the levels of the serum heat shock protein 70 increased significantly over 15 days of HA.

In addition to the reduction of oxidative stress after the EC test in the HAS, our results revealed a significantly decreased value of the inflammatory marker MCP-1 (26.3%, $p < 0.001$). The beneficial adaptive effect of the HA and the EC test interaction was revealed by the Bonferroni *post-hoc* analysis on the MCP-1 ($F = 6.1$, $p < 0.001$). In our subjects, the average log hsCRP increased significantly after the EC test at the NHAS as well at the HAS as compared to the values before the EC test. The highest value of the log hsCRP was detected after the EC test at the HAS. However, the absolute values of the hsCRP were relatively low (the mean value less than 4 mg/L) in our study. During prolonged exercise and/or heat stress, the level of inflammatory cytokines increases and heat exposure tends to stimulate the release of the IL-6 (Cosio-Lima *et al*., 2011). It is known that the short-term transient increase in the hsCRP after exercise is mediated by the cytokine system and mainly by the IL-6 (Kasapis and Thompson, 2005). Regular exercise and a good fitness level (Kampus *et al*., 2008; Kasapis and Thompson, 2005) may alleviate this response. In our study, the value of the IL-6 increased (17.9%, $p > 0.05$) in the NHAS and decreased (18.9%, $p > 0.05$) in the HAS after the EC test in the heat.

In the present study, evoked changes, although nonsignificant, were detected in the growth factors: after the EC test at the HAS, the mean decrease in the EGF was 25.7% ($p > 0.05$) and in the case of the VEGF 16.4% ($p > 0.05$). We suggest that the decreases in these angiogenesis promoters may be at least in partly the effect of our finding about the improved vascular elasticity after the HA and the improved AS may reduce the total CV burden. Our study results reveal that the CV strain was smaller after the EC test at the HAS as the EC test increased the

NT-proBNP value at the NHAS but not at the HAS. The Bonferroni *post-hoc* analysis revealed a significant effect of the HA and the EC test interaction on the NT-proBNP value ($F = 2.98$; $p < 0.05$). These results support the finding that our subjects were acclimated through a 10-day exhaustive exercise in the heat. In line with the findings of several studies (Amorim *et al.*, 2008; Nielsen *et al*., 1993; Yamada *et al*., 2007), after the HA, all our subjects showed an improvement in the EC, where the subjects' mean EC increased by 86%.

6.4. Limitations of the studies

To our best knowledge, our study was the first, in which the HA effect on AS was evaluated. Furthermore, our study was planned much more comprehensively, where the changes in AS were observed in relation to the oxidative stress and low-grade inflammation, which may affect the response of the AS to HA. It can be emphasized that due to a relatively exhausting study design (10-day HA and additional EC tests in hot conditions), the drop-out rate was minimal.

However, the present studies have some limitations. Firstly, the number of participants in the studies was relatively small. This is mainly explained by the extreme and intensive intervention schedule in the HA study, which presumed a good health and overall fitness status of the subjects for minimizing the drop-out problem from the study. Furthermore, specific inclusion criteria were needed (not being acclimated 2 months prior to the study, taking no medication, using no supplements, etc.). In addition, all the measurements were organized during a restricted period (winter-time period). At the same time, all the measurements as well the HA program in a climatic chamber were time-consuming. Climatic chamber and other devices (treadmill) for the present study design were maximally used during 2–2.5 months (winter-time period). In addition, the measurements of the AS and blood markers were time-dependent (in the mornings in the fasting state) and time-consuming due to standardization procedures (Laurent *et al.*, 2006),

Secondly, there was no intention to involve a control group separately in both studies. Due to the methodological point of view, the PHE study was designed for the evaluation for the independent effect of acute heat exposure without physical load.

6.5. Implications

The findings of our study have significant scientific and practical value. The results of our HA study support the previous findings that acclimation has a powerful effect on the EC during the physical activities in a hot environment, which could be effectively implemented by athletes or military personnel. From the practical point of view, improved HA could be very important for minimizing the heat

exertional stroke risk, which is still a serious problem in marathon and ultra-marathon events in hot environments.

The novel findings that the HA decreases AS and has favourable responses in the oxidative stress and inflammatory markers to acute physical load in the heat-acclimated status may have a significant long-term effect of lowering the CV risk in athletes. This is based on the understanding that impaired AS is considered a good prognostic indicator for further CV events (Szalo *et al*., 2024).

7. CONCLUSIONS

1) Acute passive heat exposure induces unfavourable increase in arterial stiffness and inflammatory IL-6 level but has no effect on the oxidative stress markers in young men.
2) The 10-day heat acclimation reduces stiffness of small and large arteries in young men. These favourable changes in arterial stiffness related to acclimation are not associated with the subjects' baseline aerobic fitness level or the extent of improvement in endurance capacity.
3) The 10-day heat acclimation increases the oxidative stress level but has no impact on inflammation markers in young men.
4) The 10-day heat acclimation induces beneficial adaptive responses of oxidative stress and inflammation markers to the exhausting endurance exercise in the heat in young men.

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SUMMARY IN ESTONIAN

Akuutse kuumastressi ja kuumas keskkonnas aklimatiseerumise mõju arterite jäikusele, oksüdatiivsele stressile ning põletikule tervetel noortel meestel

Sissejuhatus

Kuum keskkond on oluline tegur, mis tõstab südame- ja veresoonkonna haigustesse haigestumise riski ja suremust. Tugev kehaline koormus kõrge temperatuuriga keskkonnas koormab inimese füsioloogilisi süsteeme, vähendab vastupidavuslikku töövõimet ning suurendab kuumarabanduse riski. Aklimatiseerumisel kuumas keskkonnas käivituvad organismis mitmed füsioloogilised kohanemisprotsessid, mille tulemusena kuumastressi taluvus paraneb, terviseriski tase väheneb ja vastupidavuslik töövõime suureneb. Seetõttu on aklimatiseerumisel oluline roll nii spordis kui ka militaarses sfääris.

Arterite jäikus peegeldab kardiovaskulaarse süsteemi seisundit ja selle kohanemist erinevate keskkondlike teguritega. Arterite jäikust võivad mõjutada põletikulised protsessid ja oksüdatiivse stressi taseme tõus, mis tugeva kehalise koormusega kaasnevad. Seejuures on väga vähe uuringuid, mis käsitleksid akuutse kuumastressi ja kuuma keskkonnaga aklimatiseerumise mõju arterite jäikusele, oksüdatiivsele stressile ja põletikule.

Uurimistöö eesmärgid

Antud doktoritöö eesmärk oli välja selgitada akuutse kuumastressi ja kuumaga aklimatiseerumise mõju arterite jäikusele, oksüdatiivsele stressile ning põletikule tervetel noortel meestel.

Vastavalt töö eesmärgile püstitati järgmised ülesanded:

1. Hinnata akuutse kuumastressi mõju arterite jäikusele, oksüdatiivsele stressile ning põletikule.
2. Hinnata 10-päevase aklimatiseerumise mõju arterite jäikusele ja tuvastada, kas antud muutused on seotud uuritavate üldise treenituse tasemega või aklimatiseerumise tulemusena paranenud vastupidavusliku töövõimega.
3. Hinnata 10-päevase aklimatiseerumise mõju oksüdatiivsele stressile ja põletikule.
4. Hinnata 10-päevase aklimatiseerumise mõju oksüdatiivse stressi ja põletiku markerite muutustele suutlikkuseni sooritatud vastupidavuslikul tööl kuumas keskkonnas.

Uuritavad ja metoodika

Käesolev töö põhineb kahel sekkumisuuringul, mis viidi läbi Eestis kevad-talvisel perioodil kehaliselt aktiivsete tervete noorte meestega, kes eelnevalt kahe kuu jooksul ei olnud viibinud kuumas keskkonnas.

Akuutse kuumastressi uuringus osales 9 meest vanuses 21–31 eluaastat ja sekkumisena kasutati ühekordset 110-minuti pikkust passiivset kuumastressi spetsiaalses kuumakambris (42 ºC, suhteline õhuniiskus 18%). Enne ja pärast kuumastressi hinnati arterite jäikust pulsilaine analüüsi abil ja määrati oksüdatiivse stressi ning põletiku markerid vereseerumis.

Aklimatiseerumise uuringus osales 21 meest vanuses 19–32 eluaastat. Sekkumisena kasutati 10-päevast aklimatiseerumisprogrammi, mis viidi läbi kuumakambris (42 ºC, suhteline õhuniiskus 18%) ja mille ühe seansi raames (kestvusega 110 minutit) treenisid uuritavad liikuval jooksurajal spetsiaalse programmi alusel. Enne ja pärast aklimatiseerumisprogrammi läbimist sooritasid uuritavad samades tingimustes (42 ºC, suhteline õhuniiskus 18%) vastupidavusliku töövõime kõnnitesti intensiivsusel 60% VO_2peak'ist kuni suutlikkuseni. Antud uuringus hinnati enne ja pärast aklimatiseerumisprogrammi arterite jäikust ja analüüsiti oksüdatiivse stressi ja põletiku markereid vereseerumis. Lisaks hinnati 10-päevase aklimatiseerumise mõju oksüdatiivse stressi ja põletiku markerite muutustele suutlikkuseni sooritatud vastupidavuslikul tööl kuumas keskkonnas.

Tulemused ja arutelu

Akuutne kuumastress tõstis uuritavate arterite jäikust – suurte arterite elastsusindeks langes 17% ($p < 0.05$). Arterite jäikuse suurenemise ulatus oli suurem nendel uuritavatel, kelle kuumastressi eelne arterite jäikus oli madalam ($p < 0.01$). Arterite jäikuse suurenemise ulatust uuritavate keha süvatemperatuuri tõus ei mõjutanud ($p > 0.05$). Oksüdatiivse stressi markerid passiivse kuumastressi tagajärjel ei muutunud ($p > 0.05$) ning need ei seostunud oluliselt arterite jäikuse ega keha süvatemperatuuriga. Passiivse kuumastressi tagajärjel suurenes IL-6 kontsentratsioon, seda 30% ulatuses ($p < 0.05$).

Aklimatiseerumise uuringus vähenes 10-päevase kehalise koormuse ja kuumastressi koosmõju tulemusena noorte meeste arterite jäikus olulisel määral: suurte arterite elastsusindeks suurenes 17% ulatuses ($p < 0.05$) ja väikeste arterite elastsusindeks suurenes 18% ulatuses ($p < 0.01$). Statistiliselt olulisi seoseid arterite jäikuse muutuste, uuritavate treenituse ja aklimatiseerumise tulemusel paranenud vastupidavusliku töövõime vahel ei leitud ($p > 0.05$). Uuringus rakendatud aklimatiseerumisprogrammil uuritavate põletikumarkeritele olulist mõju ei olnud ($p > 0.05$). Samas tõusis uuritavate oksüdatiivse stressi tase – võrreldes algväärtustega suurenes peroksiidide üldkontsentratsioon 24% ja oksüdatiivse stressi indeks 36% võrra ($p < 0.05$). Enne aklimatiseerumisprogrammi läbimist suurendas suutlikkuseni sooritatud vastupidavuslik töö uuritavate oksüdatiivse stressi taset – tõusis nii peroksiidide üldkontsentratsioon ($p < 0.001$) kui ka oksüdatiivse stressi

indeks ($p < 0.001$). Peale aklimatiseerumisprogrammi läbimist langetas suutlikkuseni sooritatud vastupidavuslik töö oksüdatiivse stressi indeksit ($p < 0.05$). Aklimatiseerumine kutsus esile olulisi soodsaid muutusi ka põletikumarkerite osas vastupidavusliku töövõime testi ajal kuumas keskkonnas.

Järeldused

1. Akuutne passiivne kuumastress tõstab noortel meestel arterite jäikust ja põletikumarkeri IL-6 taset, kuid ei mõjuta oksüdatiivse stressi markereid.
2. 10-päevasel aklimatiseerumisel on noortel meestel arterite jäikusele soodne efekt. Olulisi seoseid arterite jäikuse muutuse ulatuse, treenituse taseme ja vastupidavusliku töövõime paranemise vahel ei esine.
3. 10-päevane aklimatiseerumine suurendab noortel meestel oksüdatiivse stressi taset, kuid ei mõjuta põletikumarkereid.
4. 10-päevane aklimatiseerumine kutsub noortel meestel esile soodsaid muutusi oksüdatiivse stressi ja põletiku markerite tasemes suutlikkuseni sooritatud vastupidavustööl kuumas keskkonnas.

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