**UNIVERSITY OF THESSALY**

**DEPARTMENT OF PHYSICAL EDUCATION AND SPORT SCIENCES**

TITLE

# The effect of High Intensity Interval Training (HIIT) on the immune system in cold, thermo-neutral and hot environment

BY

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# ABSTRACT

**Introduction:** HIIT is a type of exercise which has many benefits for someone either he is recreationally active or a professional athlete. Literature indicates that immune system can be affected beneficially by exercise although the proved benefits refer mainly to moderate intensity training and in thermo-neutral conditions. The aim of this study is to investigate changes on immunological and biochemical variables during HIIT training in three different environments (cold, thermo-neutral and hot) in systematically trained healthy men.

**Methods:** Fourteen male volunteers (age: 22,53±0,74 yr., BMI: 23,42±0,89 kg/$m^2$) have participated in the study. The participants were randomly allocated to three groups - one for each environment - where they were age and BMI matched. The main HIIT workout is consisting of 4 rounds of exercises with ratio 35":25" (exercise: rest) and 2' rest between the rounds. The measurements were implemented in an environmental chamber in three different environmental conditions: cold (14°C, 60% Relative Humidity), thermo-neutral (24°C, 60% RH) and hot (31°C and 45% RH). HR and $T_{skin}$ were measured continuously. There were 4 blood samplings: a) before the experimental protocol, b) after it, c) 24h after, d) 72h after. Repeated measures analysis of variance (Repeated measures ANOVA) was conducted to immunological (WBC, LYM, RBC, HCT, HGB, PLT, MPV, PDW, PCT) and biochemical (CPK, CRP) markers to assess differences among the 4 different time measurements independently from each environment.

**Results:** WBC and LYM decreased significantly between $2^{nd}$ (post exercise) and $3^{rd}$ (24h after) blood sampling in all environments (all $p<0.05$) and neutral respectively whereas they increased significant between the $1^{st}$ and the $3^{rd}$ blood sampling (C, N) ($p<0.05$). RBC and HCT showed a significant decrease between post and 24h after ($p<0.05$ in all environments). HCT also, increased after HIIT (C) but decreased between $1^{st}$-$3^{rd}$ and $2^{nd}$-$4^{th}$ blood sampling (C, N). PLT, PDW and PCT instantly increased after HIIT (C, N) but decreased significantly 24h and 72h after (C, N). Only MPV decreased 24h and 72h after HIIT (H). CPK increased significantly after HIIT (C, N) and continued to increase up to 72h after (N). No significant changes were found for CRP and HGB throughout the time in any of the different environments.

**Conclusion:** This specific HIIT protocol did not generate significant changes on the analysed immunological and biochemical markers throughout time among different environmental conditions.



**KEY WORDS:** HIIT, immune system, thermoregulation, heat, cold, biochemical markers

# ΠΕΡΙΛΗΨΗ

**Τίτλος:** Η επίδραση της διαλειμματικής προπόνησης υψηλής έντασης (HIIT) στο ανοσοποιητικό σύστημα σε ψυχρό, θερμοουδέτερο και θερμό περιβάλλον

**Εισαγωγή:** Το HIIT είναι ένα είδος άσκησης που έχει πολλά οφέλη είτε κάποιος ανήκει στο μαζικό αθλητισμό είτε είναι επαγγελματίας αθλητής. Η βιβλιογραφία δείχνει ότι το ανοσοποιητικό σύστημα μπορεί να επηρεαστεί ευεργετικά από την άσκηση, ωστόσο τα μέχρι τώρα οφέλη αναφέρονται κυρίως σε μέτριας έντασης άσκηση και σε θερμοουδέτερες περιβαλλοντικές συνθήκες. Ο στόχος αυτής της μελέτης είναι να διερευνήσει τις αλλαγές σε ανοσολογικές και βιοχημικές μεταβλητές κατά τη διάρκεια της προπόνησης HIIT σε τρία διαφορετικά περιβάλλοντα (ψυχρό, θερμοουδέτερο και θερμό) σε συστηματικά ασκούμενους υγιείς άνδρες.

**Μεθοδολογία:** Στη μελέτη συμμετείχαν 14 άνδρες εθελοντές (ηλικίας: 22,53±0,74 ετών, ΔΜΣ: 23,42±0,89 kg/m2). Οι συμμετέχοντες κατανεμήθηκαν τυχαία σε τρεις ομάδες - μία για κάθε περιβάλλον - με αντιστοιχία στην ηλικία και στο ΔΜΣ. Το κύριο πρωτόκολλο HIIT αποτελούνταν από 4 γύρους ασκήσεων με αναλογία 35":25" (άσκηση: ανάπαυση) και 2' ανάπαυση μεταξύ των γύρων. Οι μετρήσεις πραγματοποιήθηκαν σε ειδικό περιβαλλοντικό θάλαμο σε τρεις διαφορετικές περιβαλλοντικές συνθήκες: ψυχρό (Ψ) (14°C, 60% RH), θερμοουδέτερο (Ο) (24°C, 60% RH) και θερμό (Θ) (31°C και 45% RH) περιβάλλον. Η καρδιακή συχνότητα και θερμοκρασία δέρματος καταγράφονταν συνεχώς. Έγιναν 4 αιμοληψίες: α) πριν από το πειραματικό πρωτόκολλο, β) αμέσως μετά, γ) 24 ώρες μετά, δ) 72 ώρες μετά. Η ανάλυση διακύμανσης επαναλαμβανόμενων μετρήσεων (Repeated measures ANOVA) διεξήχθη σε ανοσολογικούς (WBC, LYM, RBC, HCT, HGB, PLT, MPV, PDW, PCT) και βιοχημικούς (CPK, CRP) δείκτες για να αξιολογηθούν οι διαφορές μεταξύ των 4 διαφορετικών χρονικών μετρήσεων στις διαφορετικές περιβαλλοντικές συνθήκες.

**Αποτελέσματα:** Τα λευκοκύτταρα μειώθηκαν μεταξύ της 2ης και της 3ης αιμοληψίας σε όλα τα περιβάλλοντα (όλα $p<0,05$)ενώ τα λεμφοκύτταρα μόνο στο Θ αντίστοιχα ενώ αυξήθηκαν και τα 2 μεταξύ της 1ης και της 3ης αιμοληψίας (Ψ, Ο) ($p <0,05$). Τα ερυθροκύτταρα και ο αιματοκρίτης μειώθηκαν μεταξύ $2^{ης}$ και $3^{ης}$ αιμοληψίας ($p<0,05$ σε όλα τα περιβάλλοντα). Ο αιματοκρίτης αυξήθηκε επίσης μετά από HIIT (Ψ) αλλά μειώθηκε μεταξύ 1ης-3ης και 2ης-4ης αιμοληψίας (Ψ, Ο). Οι δείκτες των αιμοπεταλίων αυξήθηκαν επίσης στιγμιαία μετά το HIIT (Ψ, Ο) αλλά μειώθηκαν σημαντικά 24 ώρες και 72 ώρες μετά (Ψ, Ο). Μόνο ο μέσος όγκος τους επηρεάστηκε από το θερμό περιβάλλον και μειώθηκε 24 ώρες αλλά και 72 ώρες μετά. Η CPK αυξήθηκε σημαντικά μετά την προπόνηση (Ψ, Ο) και συνέχισε να αυξάνεται έως και 72 ώρες μετά (Ο). Η C-RP και η αιμοσφαιρίνη παρέμειναν αμετάβλητες καθ' όλη τη διάρκεια του χρόνου σε όλα τα περιβάλλοντα.

**Συμπεράσματα:** Το συγκεκριμένο πρωτόκολλο HIIT δεν προκάλεσε σημαντικές αλλαγές στους ανοσολογικούς και βιοχημικούς δείκτες μεταξύ των διαφορετικών περιβαλλοντικών συνθηκών αλλά και κατά τη διάρκεια του χρόνου πέρα από τις αναμενόμενες αντιδράσεις.

**ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ:** HIIT, ανοσοποιητικό σύστημα, θερμορύθμιση, θερμό περιβάλλον, ψυχρό περιβάλλον, βιοχημικοί δείκτες

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# LIST OF ABBREVIATIONS

WHO (World Health Organization)

HIIT (High Intensity Interval Training)

HR (Heart Rate)

T2D (Type 2 Diabetes)

METs (Metabolic Equivalents)

ICU (intensive care units)

$VO_{2max}$ (Maximal aerobic capacity)

RPE (Rate of Perceived Exertion)

COPD (chronic obstructive pulmonary disease)

MCT (moderate-intensity continuous exercise training)

$T_{core}$ (core body temperature)

BAT (brown adipose tissue)

$T_{skin}$ (skin temperature)

WBC (White blood cells)

LYM (lymphocytes)

RBC (red blood cells)

PLT (platelets)

MPV (mean platelet volume)

IL6 (interleykin-6)

IL3 (interleykin-3)

PDW (platelet distribution width)

PCT (Platelecrit)

HGB (hemoglobin)

HCT (hematocrit)

ESR (erythrocyte sedimentation rate)

CRP (C-reactive protein)

CPK (creatine phosphokinase)

ATP (adenosine triphosphate)

RA (rheumatoid arthritis)

RH (relative humidity)

IPAQ (international physical activity questionnaire)

BMI (Body Mass Index)

DXA (Dual energy X-ray absorptiometry)

MHR (maximum HR)

RCF (relative centrifugal force)

NK cells (natural killer cells)

hs-CRP (high sensitivity C-reactive protein)

USG (Urine Specific Gravity)

ES (Effect sizes)

C (cold)

N (thermo-neutral)

H (hot)

# CHAPTER 1: INTRODUCTION

It is widely accepted that since the pandemic of COVID-19 broke out, our daily lives changed variously. People started to exercise in the outdoors such as parks, stadiums or even in the centre of their city the so-called "urban running". As a result, the outdoor activities for 2021 ranked in the 3rd place according to the fitness trends for 2021[1]. As we go through a post covid era, people are adjusting to their pro covid pace of life which is quite fast and challenging as they are always trying to save time. That is a reason why High Intensity Interval Training (HIIT) is among the top 10 worldwide fitness trends during the last decade with some years being on the top. According to the updated guidelines of WHO physical activity has to be an integral part of peoples' lives in order to gain the significant health benefits and mitigate their health risks[2]. WHO strongly suggests at least 150-300min of moderate intensity or 75-120min of vigorous intensity aerobic physical activity per week for substantial health benefits. As training sessions are time consuming, HIIT's structure help people save time having simultaneously the health benefits they need.

HIIT became popular during the early 1950s after its utilisation of a long-distance runner named Emil Zatopek who also won the gold metal in the Olympics in Helsinki 1952 in 10.000m [3, 4]. HIIT is a form of exercise which is characterized by repeated intervals of high intensity activity bouts separated by active rest at low intensity or even passive rest [5]. Its main advantage is that a greater volume of higher intensity exercise is accumulated during a single session compared to the steady state of other forms of exercise such as the moderate-intensity continuous exercise training which was used regularly before the emergence of the former.

As almost every training protocol, HIIT performance is affected by environment. HIIT workouts can be performed in different environmental conditions including heat or cold but most research studies have been conducted in controlled temperate environments[6].

Furthermore, there is limited literature regarding studies connecting HIIT with variations of immune system factors in populations without underlying chronic diseases. The majority of literature in this field focuses on clinical populations and a reason for that could be there is a high percentage of them globally who can improve their health and their disease through physical activity. HIIT has been shown from literature that can affect clinical populations -such as cardiovascular patients, diabetic people and people with cancer- except healthy people. Important cardiovascular responses -such as the heart rate (HR)- are affected at the same time by heat stress. In a study of Wingo and his team who participated ten male volunteers the exercise intensity of the HIIT protocol is modified and adjusted accordingly due to heat[7]. Only to think that an increase in the cardiovascular drift provokes higher HR and reduced stroke volume over time although the work rate seems steady[8]. Previous research has shown that heat acclimation can induce thermoregulatory, metabolic,

cardiovascular and many more adjustments which help the human body reduce the negative effects of heat stress during exercise [9].

According to Li and his team HIIT may have many benefits in the cardiac rehabilitation therapy for patients with COVID-19 infection[10]. Other researchers are in the same line with Li saying that HIIT is so beneficial in many chronic diseases such as type 2 diabetes[11], hypertension[12, 13], stroke[14, 15] and coronary artery disease[16]. According to Ochi et al., a home based unsupervised HIIT programme during the COVID-19 era led to improved both cardiorespiratory fitness and muscle strength in early-stage breast cancer survivors (50 women with I-IIa breast cancer) [17].

Nevertheless, there is a great variety in the markers of the immune system that are investigated each time. Durrer and his team investigated that after one single bout of HIIT in type 2 diabetes (T2D) patients (10 participants with T2D + 9 healthy age-matched controls) training increased immediately after exercise the total number of leukocytes in the blood and then declined after 1 hour. These results indicate that HIIT is an efficient type of training with anti-inflammatory effects[18].

In conclusion, immune system is a critical factor in HIIT trainings as it can provide us with information that affect the performance of a training session in different ways.

## AIM OF THE STUDY

The aim of this study is to investigate changes on immunological and biochemical variables during HIIT training in three different environments (hot, thermo-neutral and cold) in systematically trained healthy men.

## NULL HYPOTHESIS

The temperature of the environment does not affect the immunological and biochemical variables during HIIT training.

## ALTERNATIVE HYPOTHESIS

The immunological and biochemical variables are affected by the temperature of the environment during HIIT training.

# CHAPTER 2: LITERATURE REVIEW

The literature review provides general information about exercise, HIIT and thermoregulation. Furthermore, results from previous studies are discussed about the effect of HIIT and temperature of the environment on the immune system. To achieve the purposes of the present literature review, two databases were included in the searching procedure: Pub Med and Scopus. Keywords for the searching procedure were: HIIT, immune system, thermoregulation, heat, cold, biochemical markers.

## Physical activity & exercise

Physical activity is vital for humans' life. It is known that WHO provides evidence-based recommendations for people about the amount of physical activity which is required I order to stay healthy. In the updated version of these guidelines WHO emphasizes the effects of sedentary life compared with the health out comes and additionally announced special guidelines for pregnant and postpartum women and also people with disabilities[2]. As physical activity is defined any movement of the human body which produced by muscles and requires energy expenditure such as cleaning, gardening, dance, yoga, tai chi, spots or other household tasks[19]. On the other hand, exercise has planned, structured, repetitive and purposeful character[2]. Either one or the other opposes to the sedentary lifestyle is desired. Recent study shows that high levels of prolonged sedentary lifestyle are associated with abnormal glucose metabolism and cardiometabolic morbidity and as a result overall morbidity[20]. According to Kokkinos et al. men (65 up to 92 years old) who were able to exercise in intensities over 5METs (Metabolic Equivalents) decreased their mortality risk by ~50% whereas men who transitioned in lower intensities increased their mortality risk by ~50%[21]. Other studies support the opinion that physical activity is a kind of non-invasive therapy for mental health improvements in cognition[22], depression[23], anxiety[24], neurodegenerative diseases such as Parkinson disease[25].

## Cost of inactivity

Inactivity is a global public health problem. Number of studies through the years have been referred to the malfunction of organs because of physical inactivity. Brain can lose specific types of cognition and heart has a maximal pumping volume/minute. About peripheral circulation there is maximal capacity to supply blood to the working muscles. Skeletal muscle may have less mass and strength and also lower insulin sensitivity. Pancreas in lack of activity has loss of its β cells while at the same time bones have less mass and strength and liver has an excessive fat storage[26].

The increased percentages of sedentary lifestyle globally have led to increased hospitalisation. If for one-year physical inactivity in Greece was eliminated, the Greek economy would have notably additional financial resources. In a scenario where all of

us stay active for a year, we could build a new hospital, hire about five thousand physicians or buy 180 new ICU (intensive care unit) beds. In many countries inactivity levels are increased due to transportation, technology as well as urbanisation. According to European Commission in Greece the percentages of population showing hypoactivity has increased from 54% in 2022 to 68% in 2018. Increased percentages of hypoactivity also have financial implications. For the period between 2000-2019 the total cost of underactivity for Greece was over 2 billion. If everyone would be physically active this budget alternatively could be used for new schools, children's homes, meals for homeless people, school computers, new fire trucks and rescue boats[27]. Globally, if prevalence of physical inactivity does not change 499,2 million of new cases of preventable major non-communicable diseases will occur globally by 2030[28].

## High Intensity Interval Training (HIIT)

HIIT is an interval training model. It is characterised by short bursts of vigorous activity performed at either submaximal efforts that require ≥90% of $VO_{2max}$ (Maximal aerobic capacity), 75% of maximal power, ≥90% minimal running speed of $VO_{2max}$ or at the range of "hard" to "very hard" RPE (Rate of Perceived Exertion) and at a range of "hard" to "very hard" rate of perceived exertion (≥6 on a 10 Borg scale and ≥15 on a 6–20 scale)[29, 30]. Each bout lasts from a few seconds to several minutes depending to the exercise intensity. The equipment of HIIT can include dumbbells, barbells, sprints, bodyweight exercises even climbing of the stairs[1]. Furthermore, HIIT session can be adjusted according to the level of physical activity by changing the work/recovery ratio or adding or removing number of cycles[31]. This kind of training can also be specialised for specific sports such as soccer, basketball and swimming taking into consideration the characteristics and the aim of each sport especially for professional athletes[31]. For sedentary people HIIT provides a high stimulus for central cardiovascular system and metabolic stress[32]. A meta-analysis by Reljic et al. stated that HIIT- based interventions have lower dropout rates than traditional exercise programs making HIIT a tolerable and acceptable type of training[33]. Many studies investigated the advantages of HIIT in healthy kai clinical populations and the results were quite encouraging. Original research and meta-analysis papers showed that HIIT based programs with duration from 5 to 12 months are effective in improving $VO_{2max}$ [34], insulin sensitivity[35] and can affect positively the cognition[36]. Literature has shown that HIIT can decrease the risk of metabolic syndrome[37], breast cancer[38] and cardiovascular diseases[39]. It is also evaluated in chronic obstructive pulmonary disease (COPD) patients. Results showed that participants produced beneficial physiological effects and also reported less discomfort and dyspnea[40]. Other than that, another study showed that HIIT had significant decreases in minute ventilation and oxygen consumption in COPD patients compared to with moderate-intensity continuous exercise training (MCT) (*Figure 1*).

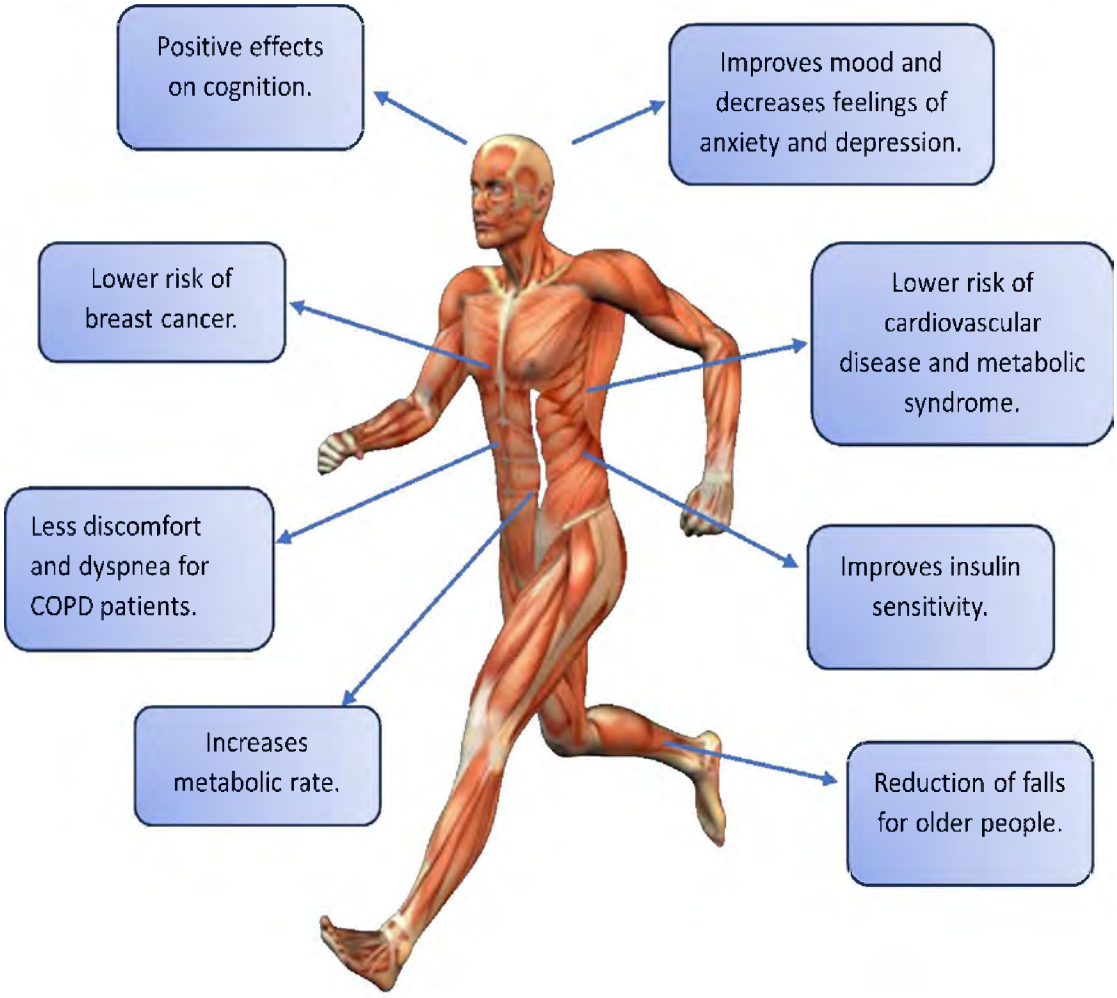


*Figure 1: Health benefits of High Intensity Interval Training*

## Thermoregulation system

Humans are classified as homeothermic animals as they are organisms who can maintain their core body temperature ($T_{core}$) in quite constant levels independently from their environment. Normal $T_{core}$ is around 37°C with a narrow range throughout the day because of the circadian rhythms. This is achieved because the rate of heat production and the rate of heat loss are in equilibrium [41]. Hypothalamus is the central coordination system for thermoregulation and thermoreceptors are the input for the hypothalamus. Thermoreceptors are responsible for transmitting information regarding thermal homeostasis. There are two types of them: central and peripheral, each of them with specific role and functions. They have the ability to change the level of heat transfer as well as its production in either some parts of the body or as a whole. This is succeeded through nerve fibers, hormones and many other biological auxiliary functions. This thermoregulatory mechanism allows in the temperature fluctuations of the environment to be felt in both the outer levels of the body and its core resulting in the adaptation and the thermoregulation of the organism [41].

### Thermoregulation in cold environment

When temperature decreases the corresponding effectors of human body are skin blood vessels, arrector pili muscles, skeletal muscles, endocrine tissue, changes in the behavior and brown adipose tissue (BAT) [41]. Exposure to cold environment leads to a reduction in skin's temperature, normal respiratory flow and pulmonary ventilation due to vasoconstriction and pulmonary alveoli. Furthermore, "shivering" or "non-shivering" is caused activating an involuntary or voluntary muscle contraction in order to maintain the $T_{core}$. In the initial stages of cold exposure skin temperature ($T_{skin}$) is decreased to succeed vasoconstriction aiming to reduce heat loss. As cold stress becomes more severe and $T_{skin}$ reduces further, the metabolic cold effectors of shivering thermogenesis (in BAT) and non-shivering thermogenesis (in muscles) are activated to stimulate heat production[42]. If cold stress continues, peripheral blood flow continues to be reduced leading to further decreases of hand and foot temperatures[43]. Regarding voluntary muscle contraction blood pressure increases along with the blood flow to the muscles. It has been shown that physical activity in winter months is reduced [44] and as a result this leads to lower daily energy expenditure [45, 46] and decreased maximal oxygen uptake ($VO_{2max}$) [47]. Simultaneously, blood pressure, blood glucose, blood lipids, C- reactive protein, hemoglobin, body mass index and arterial stiffness are increased both in men and women in all ages [48-51].

### Thermoregulation in hot environment

When a human is exposed to a warm environment there is an elevation in $T_{core}$, $T_{skin}$ and HR. The body tries to remove the additional heat from the body and in this point the metabolic skin effectors are activated. These are skin blood vessels, sweat glands, endocrine tissue and a change in the behaviour. Skin blood flow has the ability to get adapted accordingly to the temperature of the environment through sympathetic vasodilation or vasoconstriction. Heat is evaporated from the body when blood is closer to the skin's surface. Sweat production plays a principal role in heat loss. As ambient temperature elevates $T_{core}$, $T_{skin}$ and sweat rate is increased. Evaporation of sweat allows the heat to dissipate to the environment as water vapor through respiratory passages and skin surface[41]. Another metabolic effector is behavior. Our body can be adapted through behavioral adaptations such as the seek to a shelter, stay in the shade than the sun, grab a sweater or consume ice-drinks[41]. Previous research has shown that the ingestion of cold drinks can change thermoregulation and lead to improved perpetual response highlighting the role of gut thermoreception in homeostasis[52].

## The immune system & immunological markers

The main role of the immune system is to defend the body against any kind of infection. It also affects other physiological systems such as tissue repair, metabolism, thermoregulation and mental health. During the last decades, exercise immunology has evolved enough and gained the recognition that the immune system mediates

many exercise effects and that stress responses of nervous and endocrine systems play a key role in determining exercise-induced immune changes[53].

## Immunological markers

### White blood cells

White blood cells (WBC) or leukocytes are the effector cells of the immune system. They circulate throughout the bloodstream and the lymphatic system such as the lymphatic vessels, lymph nodes, lymphoid organs and tissues and the lymph[54]. One of their main functions are to defend the human body against invading pathogens. All WBC are produced and derived from multipotent cells in the bone marrow named as hematopoietic stem cells[55]. The existence of the nucleus is what differentiates them from the other blood cells (red blood cells (RBC) and platelets (PLT)). Based on differences in their physical and functional characteristics, they are classified into five main subcategories named as: neutrophils, basophils and eosinophils (differently called as granulocytes), lymphocytes and monocytes (differently called as agranulocytes)[56].

### Lymphocytes

Lymphocytes (LYM) are round cells with a large nucleus and they constitute 20-45% of the total number of WBCs. They are divided into B LYM and T LYM [57]. Both types of LYM participate in the function of the immune system, but the mechanism of each one has a different biological pathway. B- LYM produce antibodies for each antigen, while T- LYM produce specific chemicals that activate other cells of the immune system (either B or other T LYM or phagocytes) so that they can effectively confront each particular infection[58].

### Red blood cells

RBCs are responsible for transporting oxygen to tissues through the blood[59]. Every second, 2-3 million of RBCs are produced in the bone marrow and released into circulation. They can remain and circulate up to 120 days after this period the old and damaged ones are removed by other specialised cells called macrophages. Given the fact that they do now own a nucleus there is more space to store hemoglobin allowing to the RBC to transport more oxygen. In people who have low levels of hemoglobin appears a condition called anemia. These people may feel tired more easily than others or they feel short of breath as there is less oxygen transferred from RBCs to the lungs [60].

## Platelet indices

### *Platelets*

PLTs are fragments of other cells with irregular shape that circulate in the blood until they are either activated to form a blood clot or are removed by the spleen. They have no nucleus, originate from the bone marrow and their circulated duration is about 9 days. One of their major functions is to contribute to the hemostasis which is the procedure to prevent, stop bleeding and keep the blood within the damaged blood vessel. They have a major effect on the formation of atherosclerotic plaques and therefore their role is decisive for the pathogenesis of atherothrombosis. The stress caused by exercise is considered as a positive factor for hemostasis system but the effect of training is dependent on many factors such as the type, exercise intensity, duration and training condition[60].

### *Mean platelet volume*

Mean platelet volume (MPV) is the average apparent volume of all the particles in a blood sample that are counted as individual platelets[61]. MPV is determined in the progenitor cell, the bone marrow megakaryocyte. The platelet volume is found to be associated with cytokines and result in the production of larger platelets. When platelet production is decreased, young platelets become bigger and more active, and MPV levels increase [62].

### *Platelet distribution width*

Platelet distribution width (PDW) is a measurement of platelet anisocytosis (RBC which are of different sizes) calculated from the distribution of individual platelet volumes. Under physiological conditions, there is a direct relationship between MPV and PDW; both usually change in the same direction [62].

### *Platelecrit*

Platelecrit (PCT) is the volume occupied by platelets in the blood as a percentage. The normal range for PCT is 0.22–0.24% [62].

## Hemoglobin & Hematocrit

Hemoglobin (HGB) is the protein contained in RBC that is responsible for delivery of oxygen to the tissues. To ensure adequate tissue oxygenation, a sufficient hemoglobin level must be maintained. When the hemoglobin levels are low, the patient has anemia. On the other side, HGB levels above normal show erythrocytosis which is the consequence of too many RBCs[63].

Hematocrit (HCT) measures the volume of packed RBC relative to whole blood[59]. It is the ratio between RBC per unit volume in the blood and it is a key health indicator.

Hematocrit levels can reflect a person's hydration levels, the oxygen-carrying capacity, and even possible blood loss [64].

## Markers of inflammation

Inflammation is defined the set of mechanisms that are activated after contact with an allergic reaction or other antigens, a pathogenic microorganism or some injury to body tissues[65]. The first stage is the dilation of the blood vessels, the flow increases and fluid accumulates in the area consisting mainly by LYM of the immune system. During inflammation there are specific clinical symptoms which are used for diagnosis develop such as swelling, pain, increased local temperature and redness[66]. Inflammations are mainly divided into three main categories: acute, subacute and chronic. Acute inflammation includes cases related to allergic reactions or injuries while in the contrary a chronic inflammation concerns the ones caused by an autoimmune disease [65]. A diagnosis for any kind of inflammation can be accomplished either with hematological or biochemical tests:

- Hematological tests: the erythrocyte sedimentation rate (ESR) increases, whereas the number of leukocytes (leukocytosis) and their type changes form (leukocyte inversion).
- Biochemical tests: In acute inflammations the amount of C-reactive protein (CRP) (acute phase) and alpha and beta-globulins are increased though in chronic inflammations gamma-globulins are increased. In cases where the inflammation lies on the skeletal muscles or the heart, there may also be an increase in the phosphorylated form of creatine (CPK).

From a molecular point of view, inflammation is connected strongly with cytokines which are divided into inflammatory and anti-inflammatory. The first ones enhance the inflammation while the second ones soothe it [67].

Low-grade inflammations are characterised by both increased levels of CRP and cytokines. Their role is predicted for cardiovascular diseases but they also are increased along with the normal aging process [67].

### C-Reactive Protein

CRP is a ring shaped pentameric protein found in blood plasma *(Figure 2)*. Its circulating concentrations rise in response to inflammation. It is an acute-phase protein that is increased greatly more than 25% [68]. Under normal conditions, it binds to phosphocholine -a protein found in the plasma membrane of dead cells- and activates important components of the immune system to remove dead cells and pathogens. CRP is composed in the liver after the activation of the macrophages [69]. Detection is done in the blood by various methods, whereas the most accurate is measuring the sedimentation rate of RBC [70].

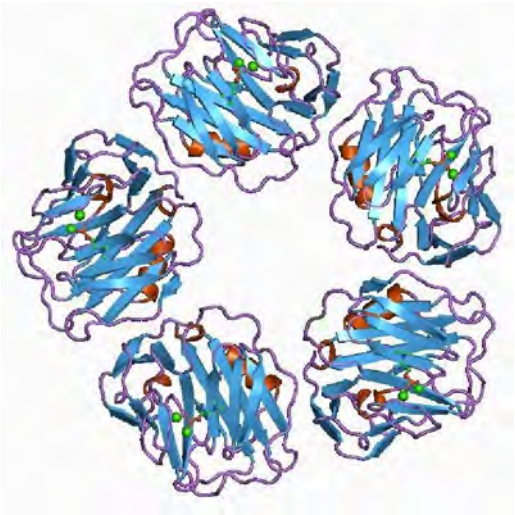

*Figure 2: The molecular structure of C- reactive protein*

## Creatine phosphokinase

Creatine phosphokinase (CPK) or creatine phosphokinase is an enzyme in heart and skeletal muscles but it is also found in smaller amount in the brain. Its role is determinant as it provides energy when there is no residual in the muscles. Through CPK the reaction is conducted and the required energy of the muscles is released. Its function is to catalyze the conversion reaction of creatine into creatinine *(Figure 3)*. Increased levels in blood serum can be observed after prolonged duration of physical exercise and high volume of training. Under these circumstances muscles have suffered muscle strain [71]. Clinically, it is an indicator of catabolism, in response to tissue injury, however it seems to participate also in the immune system. It enhances the function of macrophages and also is involved in the production of interferons and cytokines those associated with inflammation[72].

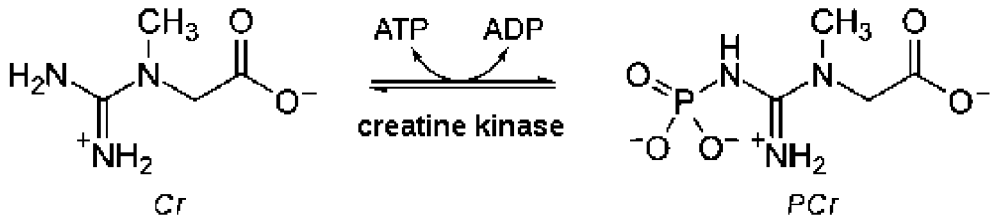


*Figure 3: The catalyzed reaction of CPK*

## Immune system & exercise

Exercise is a factor able to modify the total number of blood cells and affecting their functions. While training, there is an increasing demand for oxygen which is something that simultaneously increases blood circulation. RBCs produce ATP which effectively helps the muscles function [73]. Whilst exercise can be extremely beneficial, is also an inflammation marker. Exercise inevitably causes micro-injuries to tissues resulting in the production of substances such as hormones. Furthermore, structured physical

activity changes both the number and the function of immune cells [74]. Exercise-induced fatigue exists on a continuum. Repeated bouts of intense exercise on the same day or over several days may cause acute fatigue, as indicated by an inability to maintain exercise workloads [59]. An athlete who trains intensely for more than a week may experience a state of "functional overreaching," which is associated with a temporary performance decrement, followed by improved performance. A study of Nehlsen-Kannarella and her colleagues on WBCs showed that moderate exercise for 15 weeks provoked no differences on LYM function although there was observed a reduction in the circulation T-cells [75]. Aerobic exercise on the contrary seems to have no effect on the total blood cells counts [76].

Latest literature has recognised that skeletal muscles are organs that can produce and release cytokines -and more specifically myokines. Taking this into consideration, the relationship between the musculoskeletal system and the immune system was established. Although the main cytokine produced by muscles is IL6 [77] this increase does not seem to be accompanied by an increase in other pro-inflammatory cytokines [78]. In the same line with this, more recent studies have shown that frequent exercise can improve immune system function and reduce the pro-inflammatory cytokine response, thereby reducing systemic inflammation [79]. Exercise has inevitably the ability to confront the long-term systemic inflammation which is caused by sedentary lifestyle. Regular exercise works as a protection against atherosclerosis (deposition and accumulation of lipids and fibrous elements in large arteries) [71].

### Immune system & HIIT

Bogdanis and his team showed that a 3-week intervention with HIIT training managed to increase the WBC count 30min after exercise in recreationally active men but with no previous structured training program or cycling experience. Regarding lymphocyte percentage there was a decrease 24h after exercise. Furthermore, WBC and LYM responses to HIIT showed a decrease by 42% and 9,6% respectively giving a hint that short-term HIIT may enhance exercise-induced immune responses [80]. A study demonstrated in professional canoe polo athletes showed that a 3-week HIIT program with variable intensity increased significantly MPV compared with pretraining whereas LYM were significantly decreased during continuous endurance training but not in HIIT groups. These findings suggest that this form of HIIT is a time-efficient strategy to induce hematological adaptations compared to other forms of exercise such as continuous endurance training [81]. In people with a sedentary lifestyle, immune indicators remained unchanged during a 9-week HIIT training program [82].

Regarding HIIT exercise and immune system the majority of scientific papers concerns clinical populations. Besides, each study focuses on different indicators.

In pre-diabetic patients a study showed that HIIT can have similar or even greater positive effects on insulin sensitivity than moderate-intensity interventions. Specifically, HIIT has been shown to improve endothelial function and insulin

sensitivity. Similarly, football-based games have been shown to reduce these markers and improve glycemic indices [82]. In people with rheumatoid arthritis (RA), the creation of a HIIT protocol, even though it was based on walking, led to the reduction of rheumatoid inflammation and improvement of cardiovascular system. These effects, although not accompanied by changes in cytokine production, managed to change pathways related to the non-specific defense of the immune system [83]. According to a study of Joisten and his colleagues concerns that quite interesting results about Multiple Sclerosis patients. In this disease the ratio of neutrophils to lymphocytes plays an important role in the health of the patient, and the symptomatology. Exercising with HIIT protocols for three weeks reduced this ratio index. This positive effect is attributed to the alternation secretion between the pro-inflammatory and anti-inflammatory cytokines [84].

# CHAPTER 3: MATERIALS AND METHODS

## Participants

Fourteen male volunteers (age: 22,53±0,74 yr., weight: 73,25±2,26 kg, height: 1,76±0,01 m, body mass index: 23,42±0,89 kg/m$^2$) have participated in the study. The participants were selected based on the following criteria: a) men aged 18-30 years, b) healthy, without having been sick with COVID-19 for at least 3 months or with a common cold for at least one week before the protocol was implemented, c) abstinence from smoking or alcohol during participation in the protocol, d) systematically exercising men. All participants were informed verbally about the procedures of the study including possible risks and discomforts. Following their agreement to participate, they signed a written informed consent and completed a medical history questionnaire. The study was approved by the University of Thessaly Ethics Review Board with protocol number 1889 (see Appendix).

## Environmental conditions

The measurements were implemented in an environmental chamber in three different environmental conditions, a cold, a thermo-neutral and a hot environment. The environmental conditions which used were decided based on data collected from the weather station of Spata in Athens. Athens was determined according to the city of Athens as the most accurate representation due to its population.

To determine the environmental conditions of the hot environment the following procedure was held: average temperature and humidity were calculated concerning the three summer months (June, July and August) during the last four years (2018-'19-'20-'21) resulting in 31°C and 45% relative humidity (RH) for the hot environment.

Regarding thermo-neutral and cold environment, the protocol was performed at 24°C & 60% RH and 14°C & 60% RH respectively. The characteristics of these environments were selected based on previous studies. All the environmental conditions are shown in the figure below *(figure 4).*

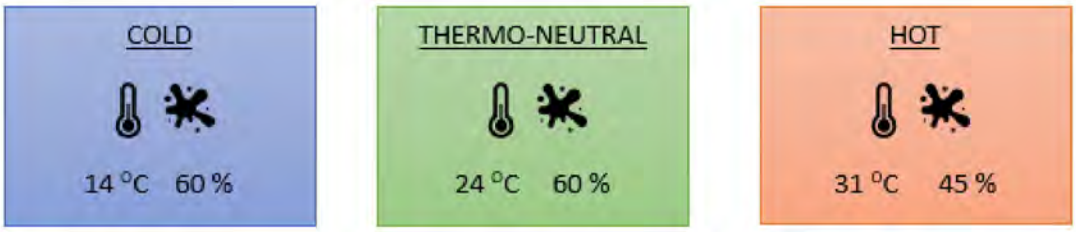


*Figure 4: Environmental conditions of the experimental protocol*

## Experimental Protocol

### Preliminary Session

Prior to the experimental session, was determined a preliminary familiarization session with the main exercises of the protocol. During the same day participants completed a medical history and a short international physical activity questionnaire (IPAQ) (see Appendix). Furthermore, some basic anthropometric characteristics were collected, such as weight using a precision scale (Version 5.3 KERN & Sohn GmbH) and height using a statiometer (Seca Appl. Sci. 2019, 9, 3170 9 of 20 213; Seca GmbH & Co. KG; Hamburg, Germany), to find the Body Mass Index (BMI), while a dual energy X-ray absorptiometry (DXA -Dual energy X-ray absorptiometry) measurement was performed in order to see the percentages of body components (fat mass and lean mass). Also, the volunteers underwent a $VO_{2max}$ assessment with simultaneous continuous recording of maximum HR (MHR) using polar. MHR was used to calculate their 90% MHR, which was used while performing the HIIT program.

#### *$VO_{2max}$ protocol:*

To measure maximal oxygen uptake, a $VO_{2max}$ protocol was followed on a treadmill ergometer *(Figure 6)* with an increase in incline and speed every three minutes, starting with walking and gradually reaching high speed *(Figure 5)*.

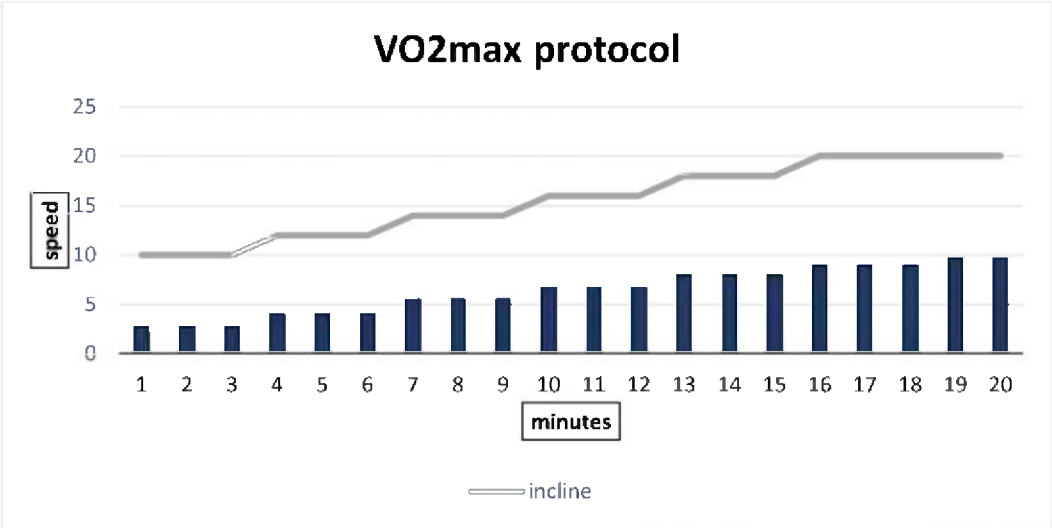


*Figure 5:* $VO_{2max}$ *protocol*

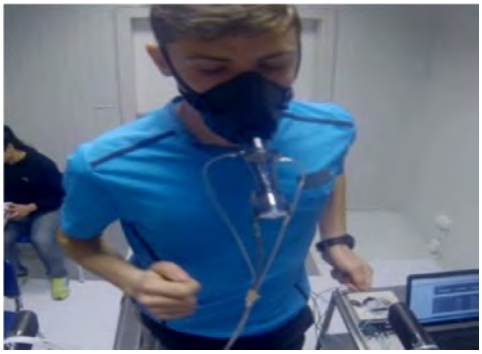

*Figure 6: $VO_{2max}$ measurement on a treadmill ergometer*

### Experimental session

The participants were randomly allocated to three groups - one for each environment - where they were age and BMI matched. Each participant abstained from exercise 72 hours before and 72 hours after carrying out the protocol, in order not to affect the hematological indices measured during the blood draws. There were four blood samplings in four different times: a) before the experimental protocol, b) immediately after it, c) 24 hours after, d) 72 hours after the main protocol.

On the day of the experimental session, volunteers arrived at the laboratory thirty (30) minutes earlier. Specific clothes were given, which were the same for all volunteers. Polar was recording HR continuously. iButtons (for measuring $T_{skin}$) were placed at four points (pectoralis major, biceps brachii, quadriceps and gastrocnemius) on the right side. After this procedure, the level of hydration was assessed using a refractometer and the body weight using a scale. At this point he first blood sample was taken. The exercise protocol was attained in a special environmental chamber with strictly defined conditions throughout the protocol and included:

#### *WARM-UP:*

Prior to the main session the participants had a warm-up period consisting of 2 parts. First of all, 5 min of moderate intensity on the treadmill ergometer and after dynamic stretches for the whole body -each one for about 15'' *(Table 1)*.

| **FULL BODY DYNAMIC STRETCHES** | |
|---|---|
| **Upper body & Core** | **Lower body** |
| Rolling shoulder circles & Shoulder rotations | Knee flexions & knee extensions |
| Elbows flexions & extensions in frontal & sagittal plane | Hip flexions & hip extensions |
| Arm abductions and adductions in sagittal plane | Front & Lateral lunges |
| Flexions & extensions of lateral line | Stretch of gastrocnemius and soleus muscles |

*Table 1: Warm up dynamic stretching*

### *MAIN WORKOUT SESSION*

The main workout is consisting of 4 rounds of exercises. Each round includes 6 different exercises for different muscle group and all of them in total are a total body workout. Each exercise durates 35" and there is a rest period 25". Among the rounds the rest period in 2min. Below there is a visual representation of the protocol exercises *(Figure 7)*.

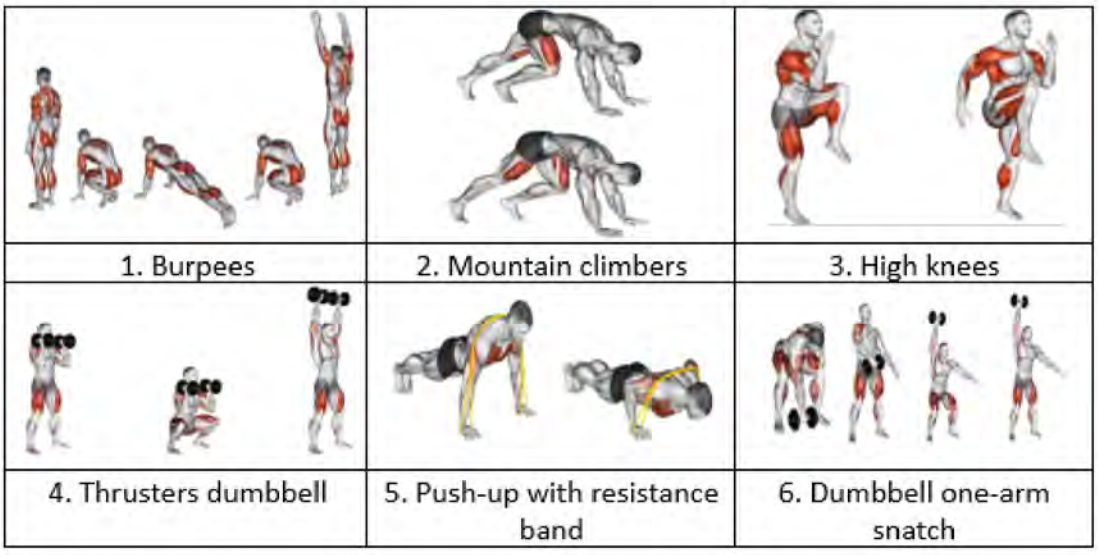


*Figure 7: Main protocol exercises*

### *COOL DOWN*

The cool down had duration of about 10min and was performed at the same stretched but in static form. Each static stretch duration was about 20sec.

Immediately after the protocol, the second blood sample was taken, followed by the assessment of the hydration level (with a refractometer) and the weighing of the volunteer.

## Blood Sampling Protocol

Blood samples were obtained from a brachial vein by a certified phlebotomist. There were four blood samplings throughout the protocol. The first was taken before the main experimental protocol and while the volunteer was in rest. The second immediately after the main experimental protocol. The third and the fourth 25h and 72h after the protocol respectively *(Figure 8).*

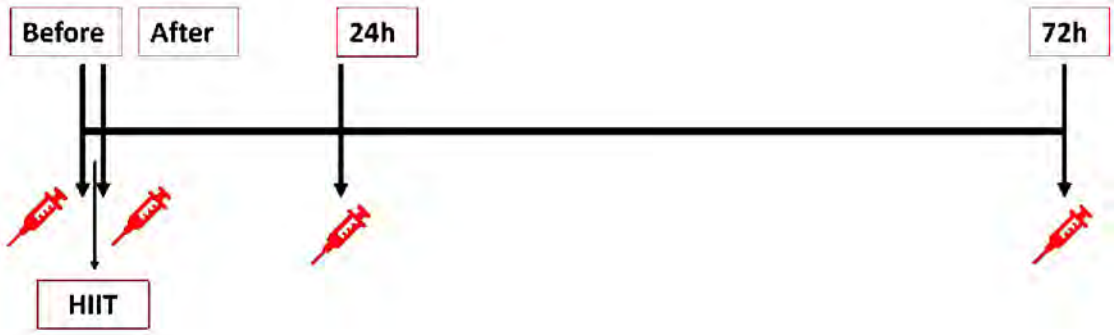


*Figure 8: Time protocol of blood samplings*

Blood count:

The complete blood count was resumed twice (2) for each blood draw on the Mythic 18 automatic analyzer (Orphée S.A., Geneva, Switzerland). The blood samples were centrifuged for at 4°C in 1370 relative centrifugal force (RCF) for 10min.

The indicators analyzed were both immune (WBC, LYM, RBC, PLT, MPV, PDW, HCT, HGB, PCT) and biochemical markers (CRP and CPK). There are also other markers who are highly affected by exercise such as IL6, cytokines, NK cells (natural killer cells), cortisol and hs-CRP (high sensitivity C-reactive protein) but we chose these ones above for our measurements.

### Mean $T_{skin}$

The skin surface temperature was measured using iBUTTON sensors (DS1921H, Maxim/Dallas Semiconductor Corp., USA). The iBUTTON sensor is a small disk-shaped system (16x 6mm$^2$) which is applied temporarily to specific points in the volunteers' body so as to measure and record the temperature in its memory. Each sensor placed in four different points: pectoralis major, biceps brachii, quadriceps femoris and gastrocnemius. All of them on the right side of the body *(Figure 9)*. The time and temperature data are transferred to a computer to analyze the data and calculate the mean $T_{skin}$ [Tsk (0.47* biceps brachii) + (0.38*quadriceps) + 4.56] [85].

*Figure 9: iBUTTON points*

## Ambient temperature area

To control the stability of the conditions we used the Kestrel 5400FW (Fire Weather Pro WBGT, Nielsen-Kellerman Corp., USA). Prior to the start of the protocol, we placed the Kestrel on a point where could record unaffected and RH of the environmental chamber *(Figure 10).*


*Figure 10: Kestrel 5400FW*

## HR recording

HR data were collected every second during the protocol using an oscilloscope (H7, Polar Electro Oy, Kempele, Finland). The data were converted into per minute for the analysis *(Figure 11).*


*Figure 11: Polar zone*

## Urine Specific Gravity Measurement (USG measurement)

In order to assess hydration levels before and after the experimental protocol, each participant gave a urine sample in a special collection container. Then, with the use of a refractometer (PAL-10 S, Atago, Tokyo, Japan), the specific gravity of urine was measured and the hydration level of the volunteer was assessed *(Figure 12).*


*Figure 12:* Refractometer

## Body Mass Index Measurement

Each volunteer's Body Mass Index was calculated using equations for height and body mass data. Specifically, body weight was measured with a precision scale (Version 5.3 KERN & Sohn GmbH), while height was measured using a stadiometer (Seca Appl. Sci. 2019, 9, 3170 9 of 20 213; Seca GmbH & Co. KG; Hamburg, Germany) *(Figure 13).*

*Figure 13: Precision scale and stadiometer*

## Body composition measurement

The body composition was analyzed using the DXA method, which is ideal for body analysis. More specifically, it measures three elements of the body - muscles, fat and bones - with great accuracy.

## Questionnaires

Two questionnaires were given to the participants during the preliminary session. Specifically, the laboratory's medical history form and the IPAQ (see appendix).

## Clothing

For the regular implementation of the protocol, the same specific clothes had been chosen in all three environmental conditions. The participants were wearing a pair of shorts and a Dri-fit short-sleeved top. Both the shorts and the shirt were weighted before and after the HIIT protocol.

## Statistical Analysis

The minimum required sample size to perform the study was calculated using the G*power program (version 3.1.9.2). The Shapiro-Wilk, Levene's and Mauchly´s tests were used in order to verify the normality, homogeneity of variance and sphericity, respectively, for all hematological kai biochemical variables. For the variables that met those criteria there was conducted a repeated measures analysis of variance (Repeated measures ANOVA) to assess differences among the 4 different time measurements independently from each environment. Therefore, post hoc t tests incorporating Bonferroni were used to assess statistical differences among the three environments and the 4-time measurements. Effect sizes (ES) were calculated interpreted as 0.2- 0.49 for small, 0.5-0.79 for medium-sized and ≥0.8 for large. For those that did not meet the normality criteria was conducted a Friedman test and for the post hoc tests where needed were conducted Wilcoxon signed rank tests.

The Shapiro-Wilk and Levene's test were used in order to verify the normality and sphericity, respectively, for HR and Tskin. Kruskal-Wallis ANOVA was conducted to find differences in the HR and Tskin of the participants among the three different environments. For the post-hoc tests needed were used Mann-Whitney tests.

Dependent samples T-tests and Wilcoxon signed rank tests were conducted respectively for USG and W_vol (weight of clothing) and for W_shorts and W_shirt.

# CHAPTER 4: RESULTS

The participants were examined in pairs who had similar BMI and belonged to the same category (normal) and similar age. From the questionnaires given (medical history and IPAQ), it was observed that all participants were healthy and had a high level of physical activity. Based on HR data we confirmed that all participants performed the protocol at high intensity, from 80%-90%, as literature defines for HIIT programs. All statistical procedures were performed using the SPSS Statistics v.29 software (IBM Corporation, USA). Their anthropometric characteristics are shown in the table below *(Table 2).*

| | **Age** | **Weight** | **Height** | **Body Mass Index (BMI)** |
|---|---|---|---|---|
| **Mean ± SD** | 22.53±0.74 | 73.25±2.26 | 1.76±0.01 | 23.42±0.89 |

*Table 2: Participants characteristics*

From the statistical analysis we observed that there were already differences in the three groups before the beginning of the HIIT protocol. As a result, our analyzes were focused on differences within groups which means changes that occurred over time.

## Immunological responses

A significant increase was found for WBC in all three environments between the 2$^{nd}$ (cold (C): 6.17±1.07, thermo-neutral (N): 7.61±2.60, hot (H): 8.07±2.36) and the 3$^{rd}$ blood sampling (C= 4.52±0.72, N= 5.45±1.94, H= 6.32±1.32) which means 24h after the protocol (C: p=0.029, N: p=0.008, H: p=0.023). Furthermore, there was a statistically decrease between the 1st and the 3$^{rd}$ blood sampling for the thermo-neutral environment (p=0.029). There was also a large ES (≥0.8) between 2$^{nd}$ and 3$^{rd}$ blood sampling only for cold and thermo-neutral environment *(Figure 14).*

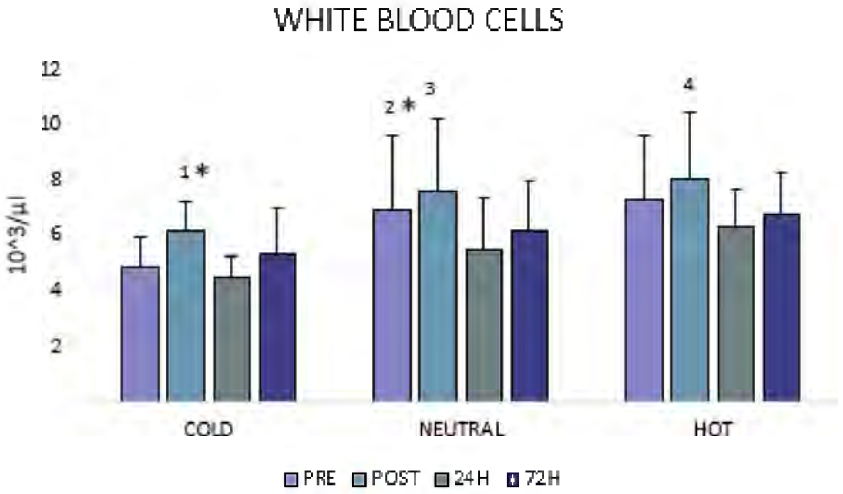


*Figure 14: WBC over time during the three different environments. 1= p=0.029 between post exercise and 24h after, 2= p= 0.029 between pre-exercise and 24h after, 3= p=0.008 between post exercise and 24h after, 4= p=0.023 between post exercise and 24h after, * ES=1.62 & 0.85*

A significant decrease with a large ES (≥0.8) was found for LYM only in the thermo-neutral environment between the 2nd (2.65±0.46) and the 3rd blood sampling (1.82±0.34), p=0.035. Regarding hot environment between the 1st (2.37±0.78) and the 3rd (1.95±0.43) blood sampling there was a significant decrease, p=0.027 *(Figure 15).*

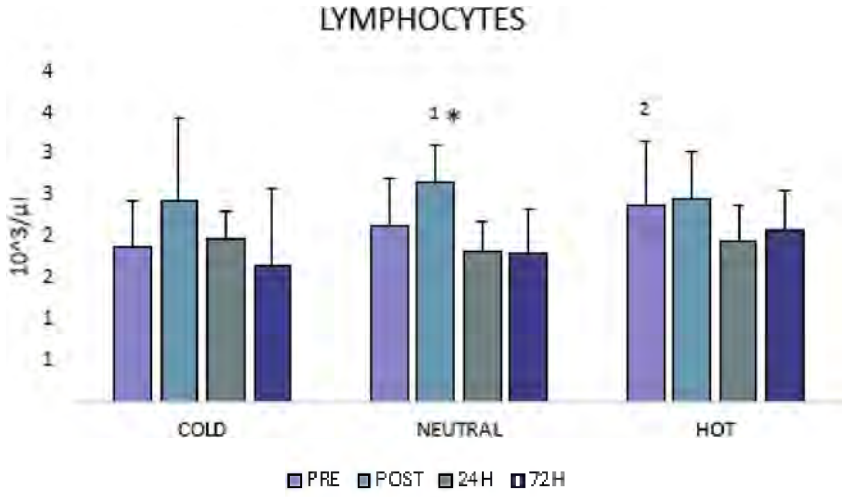


*Figure 15: LYM over time during the three different environments. 1= p=0.035 between post exercise and 24h after, 2= p=0.027 between pre-exercise and 24h after, *ES= 1.81*

RBC decreased significantly for the three environments during the period between the 2nd (C= 5.01±0.29, N= 5.05=±0.25, H= 5.69±0.64) and the 3rd blood sampling (C= 4.72±0.30, N= mean=4.67±0.37, H= 5.38±0.62) with p-values: C: p=0.002, N: p<0.001,

H: p=0.001. For cold and neutral environment there was a large ES (≥0.8) between these two blood samplings *(Figure 16).*

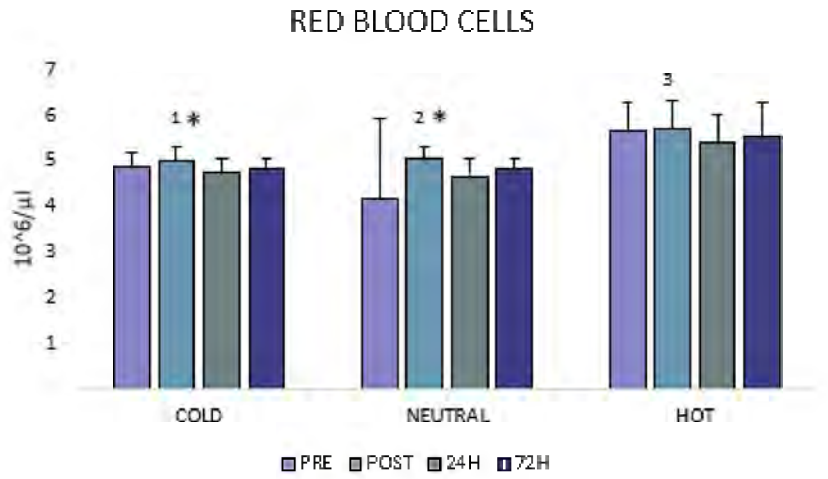


*Figure 16: RBC over time during the three different environments. 1= p=0.002 between post exercise and 24h after, 2= p<0.001 between post exercise and 24h after, 3= p=0.001 between post exercise and 24h after, *ES= 0.88 & 1.06*

In HCT we found many statistically significant changes over time among all environments. HCT significantly increased in all three environments between the 2nd (C= 43.92±3.66, N= 45.33±1.50, H= 45.25±2.73) and the 3rd blood sampling (C= 41.62±3.73, N= 41.46±2.39, H= 43.60±3.01), C: p=0.002, N: p<0.001, H: p=0.015. Furthermore, there was also a significant decrease with a large ES (≥0.8) between the 2nd (C= 43.92±3.66, N= 45.33±1.50) and the 4th (C= 41.12±1.21, N= 42.21±2.30) blood sampling for cold and thermo-neutral environment with p=0.049 and p=0.032 respectively. A significant change was observed also between the 1st (N= 44.02±3.09, H= 45.61±3.51,) and the 3rd (N= 41.46±2.39, H= 43.60±3.01) blood sampling for thermoneutral and hot environment with the followed p values: N: p<0.001, H: p=0.004. For the cold environment a significant increase in HCT was found before and after the protocol with p=0.049 (before= 42.60±3.77, after: 43.92±3.66). A large ES (≥0.8) was observed for thermo-neutral environment between 1st and 3rd & 2nd and 3rd blood samplings *(Figure 17).*

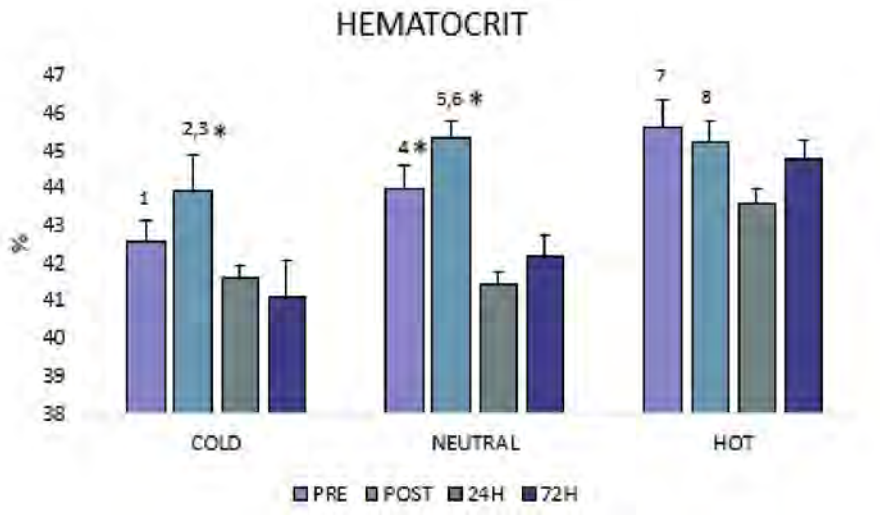


*Figure 17: HCT over time during the three different environments. 1= p=0.049 between pre and post exercise, 2,3= p=0.002 & p=0.049 between post exercise and 24h and 72h after, 4= p<0.001 between pre-exercise and 24h after, 5,6= p<0.001 & 0.032 between post exercise and 24h and 72h after, 7= p=0.004 between pre-exercise and 24h after, 8= p=0.015 between post exercise and 24h after, *ES= 0.93 (post-72h), 0.84, 1.75 (post-24h) & 1.45 (post-72h)*

We observed a significant increase (p=0.043) in PLT concentration for the cold environment before (246.2±67.71) and after (296.2±70.75) the HIIT protocol. Nevertheless, PLT decreased after exercise and for the following 24h. In line with this, a significant decrease was observed 72h (256±73.42) after exercise with p-value =0.043. As for the thermoneutral conditions there was a significant increase with a large ES (≥0.8) before (231.5±36.2) and after (271.5±26.09) the HIIT protocol (p=0.043) which followed by a significant decrease 72h (214.7±33.92) after it (p=0.043) *(Figure 18).*

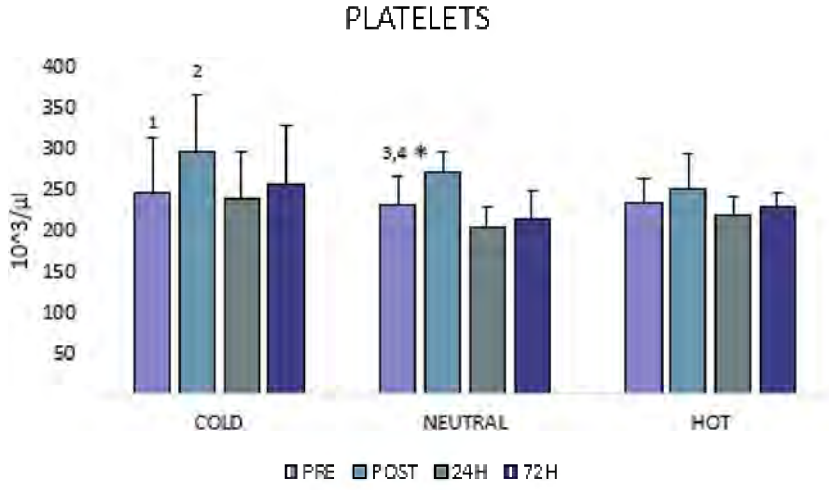


*Figure 18: PLT over time during the three different environments. 1= p=0.043 between pre and post exercise, 2= p=0.043 between post exercise and 72h after, 3,4= p=0.043 & p=0.043 between pre and post exercise & 72h after, *ES= 1.14 (pre-post) & 1.69 (post-72h).*

For MPV a significant decrease was detected only in the hot environment between the 1st (8.77±0.76) and the 3rd (8.35±0.67) with p=0.008 but also between the 1st and the 4th (8.33±0.57) blood sampling with p=0.034. No other significant changes were observed for the other environments *(Figure 19).*

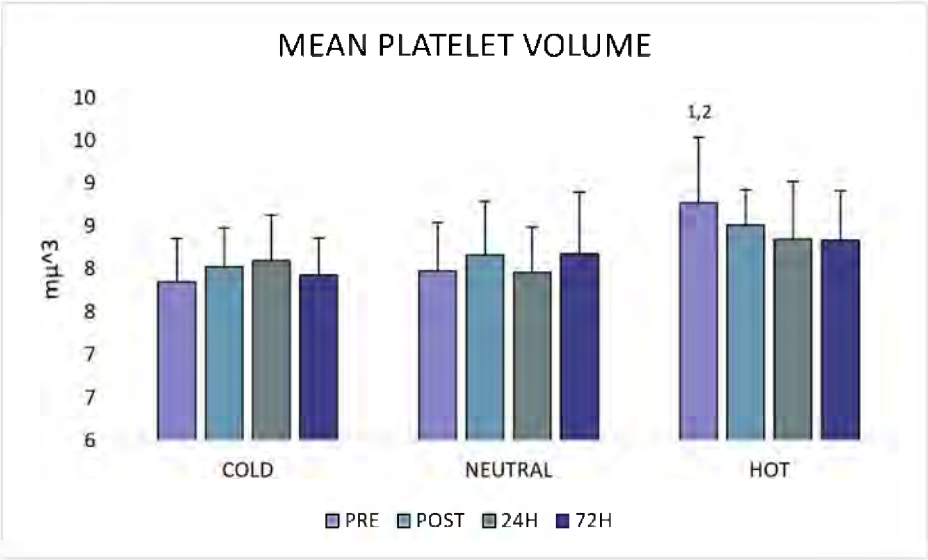


*Figure 19: MPV over time during the three different environments. 1,2= p=0.008 & p=0.034 between pre-exercise and 24h and 72h after.*

No significant changes were found for PDW regarding hot environment. Although in neutral conditions there was a significant increase (p=0.032) between the 1st (14.78±1.14) and the 2nd (16.93±1.31) blood sampling. Regarding cold conditions PDW decreased significantly between 2nd (17.5±2.30) and 3rd (14.57±1), p=0.045 & 2nd and 4th (14.92±1.75) blood sampling with p=0.048. All these changes were observed to have a large ES (≥0.8) *(Figure 20).*

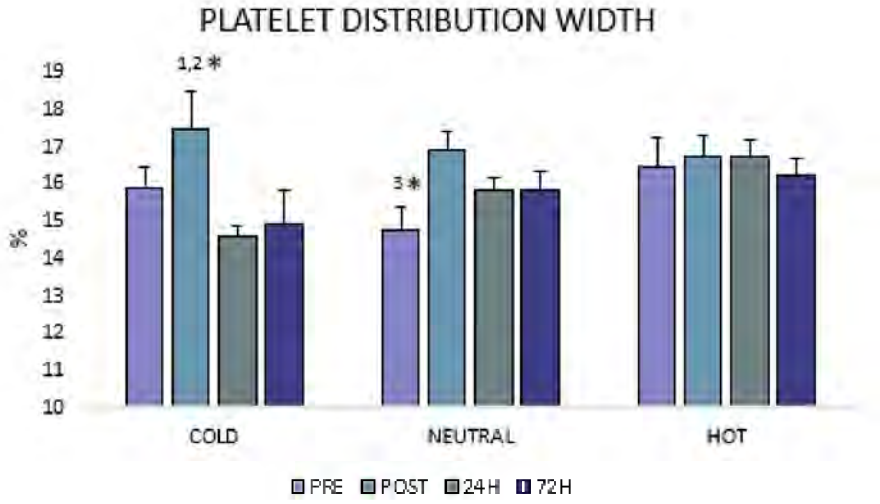

*Figure 20: PDW over time during the three different environments. 1,2= p= p=0.032 & p=0.045 between post exercise and 24h & 72h after for cold, 3= p=0.048 between pre and post exercise for thermo-neutral environment, *ES= 0.98 (post-24h), 1.13 (post-72h) & 1.58*

No significant changes were observed for PCT in hot conditions in contrast with the two other environments. A significant increase in PCT was found after exercise for both cold (before= 0.194±0.04, after= 0.334±0.21) and thermoneutral (before= 0.184±0.02, after= 0.224±0.01) environment (C: p=0.043, N: p=0.041) which was followed by a significant decrease 72h after exercise for the same two environments with p-values 0.043 and 0.039 respectively [C- 72h= 0.198±0.04, N- 72h= 0.174±0.01). All changes had a large ES (≥0.8) *(Figure 21).*

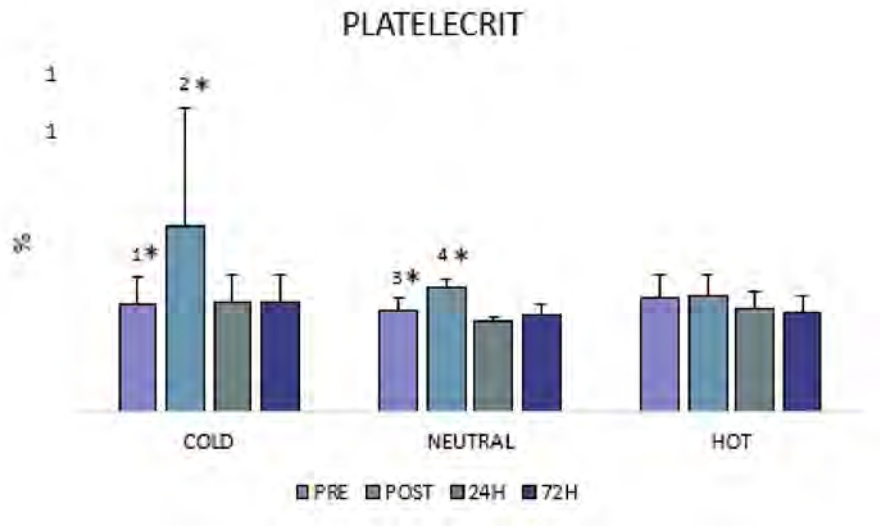


*Figure 21: PCT over time during the three different environments. 1= p=0.043 between pre and post exercise, 2= p=0.043 between post exercise and 72h after, 3= p=0.041 between pre and post exercise, 4= p=0.039 between post exercise and 72h after, *ES= 0.83, 0.81, 2.07, 2.83*

## Biochemical responses

No significant changes were observed about hot conditions related to CPK. However, in the other two conditions things were different. We found that in cold environment there was a significant increase before (201.9±77.64) and after (289±148.97) the protocol (p=0.043). Regarding thermoneutral conditions was observed a significant increase between pre (177.8 ±109.29) and post (226±126.06) exercise (p=0.043) which continued by a significant increase between pre and 72h (1223.58±825.04) after it (p=0.043). Also, CPK increased significantly (p=0.043) after (226±126.06) exercise and 72h after it (1223.58±825.04). Only the last two changes had a large ES (≥0.8) *(Figure 22).*

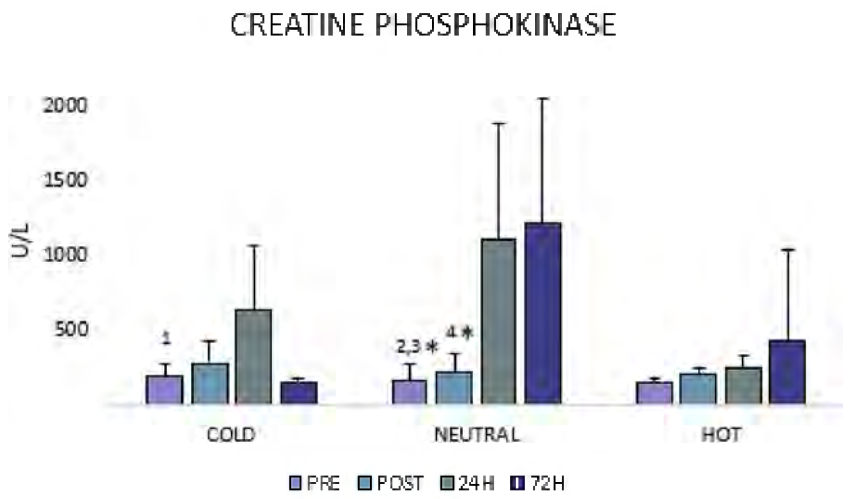


*Figure 22: CPK over time during the three different environments. 1= p=0.043 between pre and post exercise, 2,3= p=0.043 & p=0.043 between pre and post exercise & 72h after, 4= p=0.043 between post exercise and 72h after, *ES= 1.6 (pre-72h) & 1.53*

No statistical differences were found for CRP *(Figure 23)* and HGB *(Figure 24)* between the different blood samplings (p>0.05). Effect sizes were conducted for HGB and CRP for each different period but only two of them were in medium size. Regarding CRP the ES was 0.64 between 24h and 72h after HIIT. As for HGB, ES was 0.57 between post and 24h after.

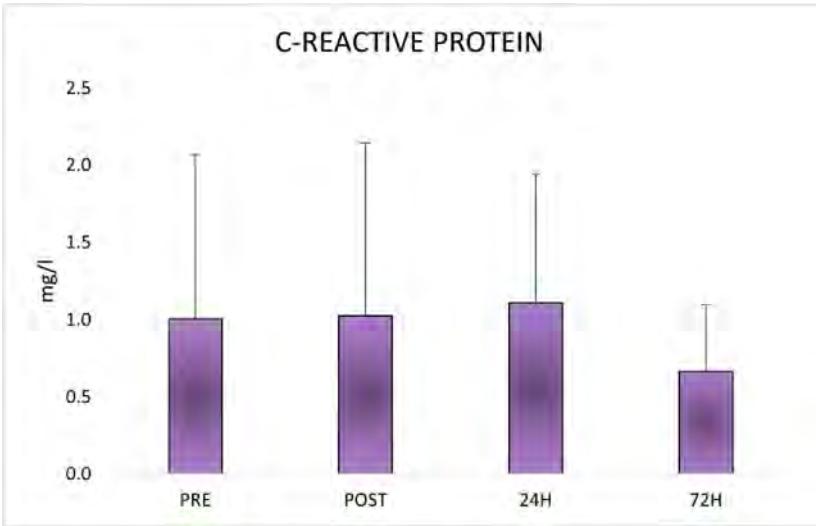


*Figure 23: CRP over time in all environments*

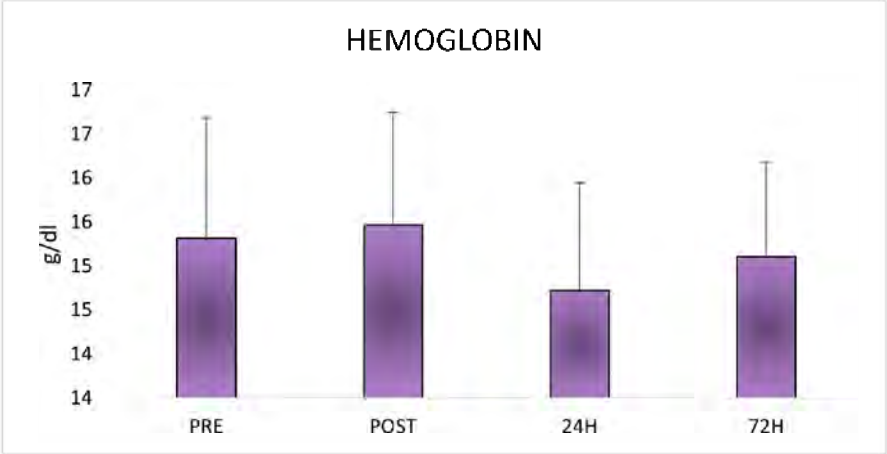


*Figure 24: HGB over time in all environments*

## Mean $T_{skin}$ responses

Regarding mean $T_{skin}$ as we can observe from the following graph (figure 25) from the time of placing the iButtons until the warm-up, the average $T_{skin}$ is at similar levels. From the 10' minute onwards when the participants entered the special chamber there is a big difference of the cold in contrary to the others. In the first minutes after the end of the HIIT protocol there is a tend for temperature decrease especially for hot and thermoneutral environments. From the analysis ran we found that there were statistically significant changes between the mean $T_{skin}$ of every group with the other two ($p<0.001$) which is also observed from the graph. A large ES (≥0.8) was found for the thermo-neutral group of participants in contrary with the two others (thermo-neutral & cold, thermo-neutral & hot).

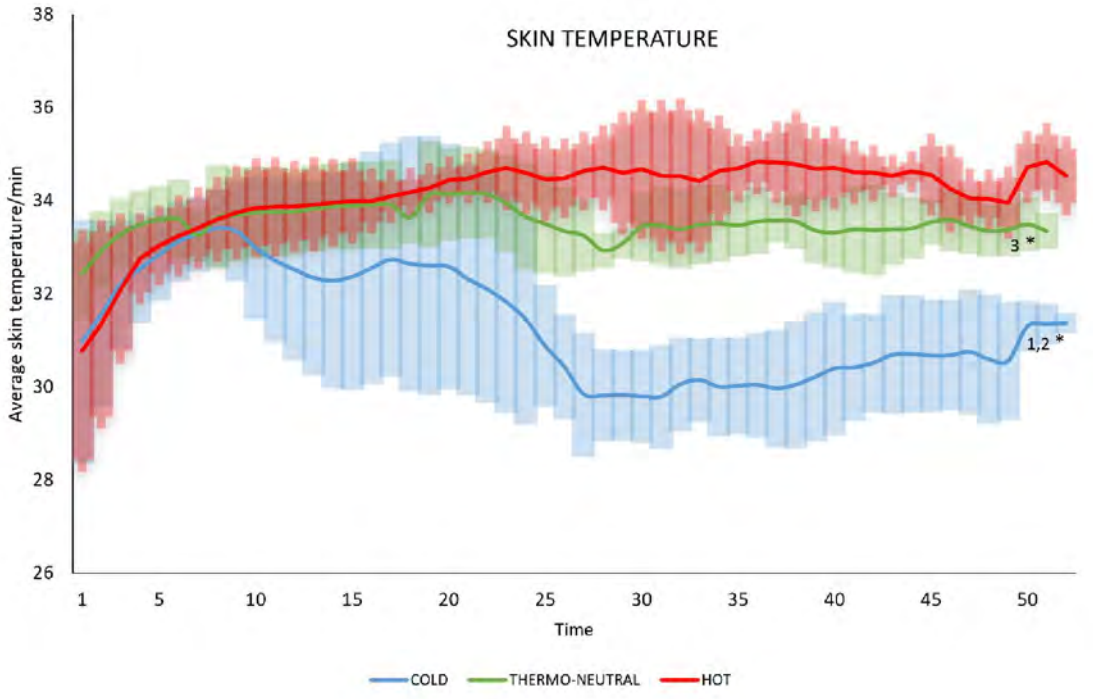

*Figure 25: Mean $T_{skin}$ over time during the three different environments. 1,2= p<0.001 & p<0.001 between cold and thermoneutral environment & cold and hot environment, 3= p<0.001 between thermoneutral and hot environment. * ES= 1.77 (cold-neutral environment) & 2.28 (neutral-hot environment)*

## Mean HR responses

Mean HR in cold environment was clearly lower during the whole protocol whereas in the other two the mean HRs were similar despite the difference in temperature. For this reason, we conducted a statistical analysis to observe further the two means of thermoneutral and hot environment. The results, as it seems also in the graph (figure 26), showed that there was no statistically significant difference between these two environmental conditions (p>0.05) and in conclusion the means of HR of the participants of thermoneutral and hot environment were similar. However, the mean HRs of participants for cold - thermoneutral and cold -hot (effect size=0.91) were significant different (p<0.001) which can be seen clearly in the followed figure.

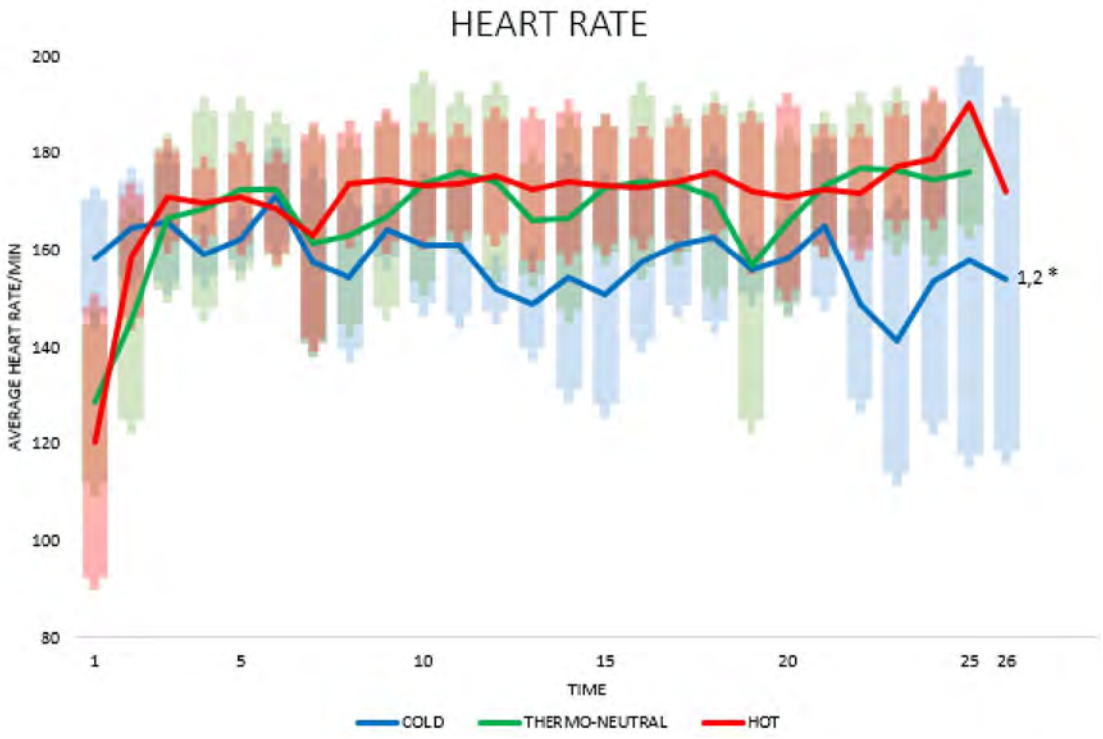


*Figure 26: Mean HR over time during the three different environments. 1,2= p<0.001 & p<0.001 between cold and thermoneutral environment & cold and hot environment. *ES= 0.91 (cold-hot environment)*

# CHAPTER 5: DISCUSSION

The aim of this study was to investigate changes on immunological and biochemical variables in three different environments (cold, thermo-neutral and hot) in systematically trained healthy men during HIIT training. We also examined how HR and Tskin changed over time during HIIT protocol.

WBC and LYM

To our knowledge, this is the first study that investigates the effect of HIIT exercise on immunological and biochemical variables in three different environmental conditions in healthy recreationally young men. In our study WBC were decreased significantly 24 hours after HIIT protocol in all environments (cold, thermo-neutral, hot). Similar with these results was a study of Belviranli and his colleagues in which their participants had decreased levels of leukocytes 6h after exercise[86]. The participants in this study were at similar age with ours but they had a sedentary lifestyle in contrary with our study where they were systematically exercised. In the same line are the results of another study in which the participants were about 18 years old and elite junior soccer players. Their HIIT protocol duration was 8 weeks and WBC were also decreased significantly [87]. Another study observed that leukocyte count was remained at the same levels after cycling HIIT sessions with either active or passive rest between the bouts[88]. However, the same study showed that after sprint training protocols the leucocyte count was increased immediately after exercise and for the next 3h. Our study showed that WBC increased significantly immediately after exercise and there are also other studies that showed the same result [89][80, 90]. In our study WBC in cold environment although increased after exercise in the next blood sampling 24h after the levels had been statistically decreased. Another study observed that there was exactly the same reaction of WBC also in their results: an increase after the exercise and a decrease in the next 3h [91]. On the other hand, there were studies with HIIT protocols that the WBC reaction was quite different. Mora-Rodriguez and his team showed that after 6 months of HIIT the leukocyte count remained unchanged [92] and in the same line was the study of Khammassi et al in which WBC showed an improvement in a MCT (Moderate Continuous Training) but not in a HIIT one[93]. A reason for that could be the fact that moderate intensity exercise tends to increase instantly the immune system levels. This has not been proved for higher intensity exercise protocols (such as HIIT). These levels return to their baseline during the 3 following hours[94].

3 weeks of HIIT protocol were not enough to affect WBC according to a study of Sheykhlouvand and his team whereas in the same line was also a study of Baria and his team in which after a HIIT-based Tabata session WBC remained the same[95].

LYM in our study followed a statistically significant decrease 24h after our protocol in the thermo-neutral and hot environment after an increase immediately after the exercise. Similar with these results were also two previous studies those of Bogdanis

and his team and Belviranli and his colleagues[80, 86]. The interesting point is that in these two studies participants were also young and non—smokers men like in ours. Furthermore, a study of Jamurtas and his team in healthy young men showed exactly the same reaction of LYM[90]. In contrary with ours, a study of Rhibi and his team observed a direct decrease of LYM after exercise whereas we observed exactly the opposite[96]. In our case LYM increased after the exercise but in the next blood sampling 24h after exercise had been decreased significantly. A reason for that could be a LYM apoptosis which is a normal process of cell death[97]. Exercise induced fatigue seems to be a factor affects cell apoptosis. Furthermore, LYM apoptosis can be affected by cortisol response to exercise. According to Wahl et and his team, LYM were decreased 30min and 60min after the training protocol regardless of the type of rest between the HIIT bouts[88].

RBC

Regarding RBC our results have shown that there was a significant decrease 24h after HIIT protocol regardless of the environmental conditions which means that this result was the same for cold, thermo-neutral and hot environment. A large ES was also observed for both cold and thermo-neutral environments which is interpreted as an applicable result consistent in reality. Our results are concordant with a previous study of Belviranli et al [86] in which they observe that after a 30sec Wingate test, RBC although they immediately increased after exercise , they followed a significant decrease 3h and 6h later. Our results are in the opposite line with the results of Heidari et al [98] who found that erythrocytes decreased immediately after exercise but increased 30min later again. Jastrzebska and the team observed that RBC decreased significant after 8 weeks of HIIT training sessions on elite junior soccer players[87]. The last result consists of a chronic effect of HIIT training whereas all the previous ones referring to acute effects. We are surprising found that other previous studies [80, 90, 92, 93, 95] in different HIIT protocols and population groups RBC were remained unchanged. Some of the possible reasons of this could be the duration, time, intensity or volume of the HIIT protocol.

HCT

Our results about HCT showed that it increased significantly instantly after exercise only for cold environment. This result is in accordance with previous studies where HCT increased immediately after exercise [86], [98]. The levels started decreasing 24h after the protocol in all environments (ES ≥0.8) and remained statistically decreased until 72h after for thermo-neutral and hot environment (ES ≥0.8). In agreement with this , Belviranli et al and Heidari et al [86], [98] found also that their HCT levels had decreased 3h & 6h and 30min after respectively. When Bogdanis and his team ran a 3-week intervention consisting of 9 HIIT sessions in recreationally active male participants they found out that HCT levels increased significantly up to 30min after their sessions but they returned to their baseline values 24h after[80]. An explanation

for that could be a decrease in plasma volume which could be detected because of the increase rate of sweat rate. Regarding chronic HIIT effects on hematocrit previous authors observed that HCT levels decreased statistically after 8 weeks of HIIT sessions. Not every different protocol affects in the same way the hematological variables. Hematocrit levels remained unchanged in other studies regarding acute (immediately post, 24h, 48h and 72h post-exercise) [90] or chronic (2 days after the successful completion of a 9-week training program) [93] effects of HIIT.

PLATELET INDICES

Our study found that platelets values increased notably exactly after exercise but followed by a decrease for the following 72h. This instant increase of platelets after exercise could be explained by a release of new platelets from the spleen or the bone marrow[98, 99]. In agreement with these results are also the ones of a previous study who observed that PLT increased by 13% after their HIIT protocol [100]. In the same line with this instant increase are also other studies [60, 80, 86, 95] whereas the later decrease was in accordance with the study of Belviranli and his colleagues [86]. On the other hand, other studies with HIIT protocols found in their results that there were not significant differences in HCT levels before and after the main sessions [81, 87, 90, 92]. Important to say the abovementioned studies had included either healthy participants or participants with chronic medical problems (such as metabolic syndrome patients) or participants with active or sedentary lifestyle.

Another indicator analysed was the MPV. In our case MPV decreased significantly after 24 up to 72h after in hot environment. However, Sheykhlouvand and his team found that MPV increased statistically after HIIT and that changes in HIIT protocol with variable intensity were larger than the ones with the variable volume [81]. Furthermore, MPV has increased in well-trained athlete after a 30km race with ascents and descents[101]. Another previous study found that MPV decreased after HIIT protocol in patients with coronary artery disease[100].

Another platelet indicator was the PDW. In cold environment decreased significantly 24h after and 72 after our HIIT protocol with a large ES also. Regarding thermo-neutral conditions there was a significant increase immediately after exercise (ES≥0.8). In this line are also the results of Ahmadizad et al, who found that after their HIIT protocol accomplished in clinical populations, PDW decreased significantly [100]. Scientists of another study observed that PDW decreased after a protocol in under HIIT intensities (60-75% $VO^2_{peak}$) in T2D sedentary female women[102].

Regarding PCT our results showed that in cold and thermo-neutral conditions PCT increased immediately after exercise and decreased significant statistically 72h after (ES≥0.8). According to a previous study, PCT followed a significant increase in contrary with the other interventional group (moderate continuous training)[100]. Whereas Akbarinia and the colleagues underline in their results that PCT remained unchanged while an under-HIIT intensity protocol[102].

CPK

CPK was one of our analysed biochemical markers. CPK was increased instantly after exercise. The levels stayed increased up to 72h after our session in thermo-neutral conditions with a large ES also. This result agrees with previous literature which says that CPK levels reach a peak in the next 1-4 days after exercise and started to decrease again in the next few days. In the same line were the results of other studies where CPK increased after exercise and up to 24h after [103, 104]. According to Rhibi et al, CPK levels decreased 30min after their protocol in physical education students[96]. This result is also in the same environment with ours (thermo-neutral). Another study observed that their CPK levels increased immediately after exercise but they decreased 72h after [105]. Last but not least, a study by Cirpyan et al, showed that CPK levels do not affected after their HIIT protocols in highly trained males [91].

In order to gain a more fulfilled understanding of our study we chose to also measure two other markers except the immunological and biochemical ones. $T_{skin}$ and HR gave us a clearer comprehension in general.

Physiological point of view of our immunological and biochemical markers

In our study we found that WBC and LYM showed an instant increase immediately after HIIT whereas in the next hours returned to their baseline levels. This can be easily explained as physical activity causes many changes in the circulation of WBC. One of them is leukocytosis which is called as the increase in the number of WBC circulating the blood[106] and It is accompanied by cytokines production after prolonged exercise. Another reason for the increased WBC and LYM is that exercise intensity and duration affect them directly[107]. Furthermore, this increase may attribute to the increased catecholamine levels[108]. Regarding RBC, it is known from literature that strenuous exercise can cause structural damage in the erythrocytes, resulting in hemolysis. Increasing the number of erythrocytes deformed by exercise can be reflected as an increase of mean cell volume in the circulation [86].

As for platelet indices which in our study increased after training protocol and returned to baseline levels in the next hours. This result is in accordance with Ahmadizad and his team who found also a significant increase in the PLT after a single session of anaerobic exercise. PLT secretion increases by increasing the secretion of epinephrine during HIIT[86]. Another explanation for the increased levels of PLT could be the release of new PLT from the spleen or the bone marrow [98]. MPV increased after HIIT for cold and thermoneutral environments may be caused by platelet hyperreactivity. HCT in this study increased after HIIT in the cold and thermoneutral conditions but decreased slightly in hot ones. This result is quite common in high intensity trainings because of the increase in thermoregulatory capacity, the cardiac filling pressure and the stroke volume. On the other hand, a decrease in HCT could mean a possible decrease in the number of RBC which means that there is no dehydrated condition.

Regarding CPK, literature underlines that when skeletal muscle fibers sustain injury, such as membrane damage or myofibrillar disruptions marked by myofilament disorganization and loss of Z-disk integrity, CK leaks into the plasma, reflecting the mechanical-muscular strain of training [105]. Another possible cause for the high values of CPK after exercise may caused to the characteristics of our HIIT with many different exercises in the whole body which ultimately results in the musculoskeletal system's microinjuries and muscle soreness[105]. In our case we observed a different reaction of CPK in different environments. A possible reason for this could be that some people are not respond in the same way like others due to a lower permeability of muscle cell membranes [109].

Skin temperature

$T_{skin}$ in our study followed lower values in cold environment whereas the highest values of it were showed in hot conditions. Significant differences were observed between the group of cold vs thermo-neutral and thermo-neutral vs hot environment (ES≥0.8). In a previous study of O'Connor and his team where participants were divided in two groups according to their sport (cycling and American football ) and attained a sport-based HIIT protocol indoor and outdoor with solar radiation, Tskin seemed to be higher in cyclists in compared to the American football players [110].

Heart rate

In our study we observed a group x time interaction for cold vs hot group (ES≥0.8). HR was significantly increased in hot group compared with the cold one but there was no significant difference between the group of thermo-neutral vs hot which means that HR in these two environments was ranged in similar levels. Acute heat increases peripheral blood flow to the skin and encourages perspiration to help with cooling [111]. Except that, increased heart rate and decreased stroke volume are main results of heat stress. Cardiac output is increased via this mechanism[8]. It is known from the literature that humans' initial reaction to heat is to increase blood flow to the periphery in order to cool the skin [41]. Under these environmental condition HR is accelerated by about 40% [112]. Furthermore, heat stress-induced catecholamine circulation is likely another contributor accelerating HR. All these mechanisms above increased HR in thermoneutral and hot environments compared to the cold [113]. Previous authors in their results suggested that mean HR was also higher outdoors with solar radiation compared with indoors in their HIIT protocol[110]. In another study about CrossFit with three different protocols and one HIIT among them, one of the greater mean HRs were found in the HIIT protocol (as many double skip rope jumps in 8 sets of 20sec with 10sec of rest between sets)[114].

## PRACTICAL RECOMMENDATIONS

Analysing and thinking of our results on a second level we reach at the following conclusions: WBC and LYM in hot conditions were seemed to be more increased than the other environments without this result to be statistically significant. A reason of this could be a higher stress of the immune system. At the same time HCT values were also higher in hot environment which possibly shows a more intense decrease of plasma volume and simultaneously a higher dehydration. Furthermore, LYM after exercise in hot environment followed a lower increase and then started to decrease to their prior levels. This shows a further fatigue of immune system (we do not have an instant increase after exercise to boost it) and as a result the jeopardy of URTI is higher.

HCT values are shown higher in hot conditions (although not significant) which means a higher decrease in plasma volume and as a conclusion higher dehydration. All these information indicate that there is a higher risk for training in the heat especially when we are talking about maximal intensity exercise such as HIIT. In the category of professional athletes, it is recommended to trainers and coaches to spread their insight about immunological variables and how they adapt to the heat. HIIT and heat together can be a tricky and hazardous combination for professional athletes. As we already know the benefits of HIIT a practical recommendation could be to avoid the hours of the day with intense heat and train earlier or later than that. The majority of our results followed the same line with other previous studies but our advantage is that we collect information for hot environmental conditions along with the cold and thermo-neutral ones.

## LIMITATIONS

Although in our study many immunological markers seemed to be affected by HIIT, our results could be affected variously by the limitations. The small sample size may be a factor that affected our results. Because of it, we had many significant results in many hematological indicators. In order to weak this limitation we chose to make effect sizes so as to understand which of them respond indeed in reality. Other limitations could be the time duration -only 30 min- or the time sessions (only one session for each participant). The ideal scenario was each participant to be exposed in all three environments. Another factor that could affect our final results could be the weather station in Spata. This station is near Athens but not every place in Greece has the same prevailing temperatures such as the city where our study accomplished (Trikala, Thessaly). The months of measurements may also have played a role, especially for the hot environment. Our experiments were attained during April and May, months that temperature in our region has already increased and as a result the participants could be already acclimatized in heat. Last but not least, a wider difference among each temperature environment maybe could lead us to a clearer result.

CONCLUSION

This specific HIIT protocol did not generate significant changes on the analysed immunological and biochemical markers through among different environmental conditions.

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## APPENDIX: INTERNAL ETHICS COMMITTEE

**ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ**
**ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΦΥΣΙΚΗΣ ΑΓΩΓΗΣ ΚΑΙ ΑΘΛΗΤΙΣΜΟΥ**

Εσωτερική Επιτροπή Δεοντολογίας

Τρίκαλα:

9/2/2022

Αριθμ. Πρωτ:1889

**Βεβαίωση έγκρισης της πρότασης για διεξαγωγή Έρευνας με τίτλο:** Επίδραση της διαλειμματικής άσκησης, υψηλής έντασης στο ανοσοποιητικό σύστημα σε θερμο-ουδέτερο και θερμό περιβάλλον.

**Επιστημονικώς υπεύθυνος-η / επιβλέπων-ουσα:** Ανδρέας Δ. Φλουρής
**Ιδιότητα:** Αναπληρωτής καθηγητής του ΣΕΦΑΑΔ
**Ίδρυμα:** Πανεπιστήμιο Θεσσαλίας
**Τμήμα:** Φυσικής Αγωγής και Αθλητισμού
**Κύριος ερευνητής-τρια / φοιτητής-τρια:** Τετρίγγα Μαρία

**Πρόγραμμα Σπουδών:** Βασικό πτυχίο στην Επιστήμη Φυσικής Αγωγής και Αθλητισμού.
**Ίδρυμα:** Πανεπιστήμιο Θεσσαλίας
**Τμήμα:** Φυσικής Αγωγής και Αθλητισμού

**Η προτεινόμενη έρευνα θα είναι:** Διπλωματική εργασία

Αφαίρεση προσωπικών δεδομένων (Υπηρεσία Βιβλιοθήκης & Πληροφόρησης Πανεπιστημίου Θεσσαλίας)

**Τηλ. επικοινωνίας:** **********
**Email επικοινωνίας:** tetringamaria@gmail.com

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Η Εσωτερική Επιτροπή Δεοντολογίας του Τ.Ε.Φ.Α.Α., Πανεπιστημίου Θεσσαλίας μετά την υπ. Αριθμ. 1-4/9-2-2022 συνεδρίασή της εγκρίνει τη διεξαγωγή της προτεινόμενης έρευνας.

Ο Πρόεδρος της
Εσωτερικής Επιτροπής
Δεοντολογίας – ΤΕΦΑΑ

Τσιόκανος
Αθανάσιος
Καθηγητής

**ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ**
**ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΦΥΣΙΚΗΣ ΑΓΩΓΗΣ ΚΑΙ ΑΘΛΗΤΙΣΜΟΥ**

Εσωτερική Επιτροπή Δεοντολογίας

Τρίκαλα:

9/2/2022

Αριθμ. Πρωτ:1895

**Βεβαίωση έγκρισης της πρότασης για διεξαγωγή Έρευνας με τίτλο:**
Επίδραση της διαλειμματικής άσκησης, υψηλής έντασης στο ανοσοποιητικό σύστημα σε θερμο-ουδέτερο και κρύο περιβάλλον.

**Επιστημονικώς υπεύθυνος-η / επιβλέπων-ουσα:** Ανδρέας Δ. Φλουρής
**Ιδιότητα:** Αναπληρωτής καθηγητής του ΣΕΦΑΑΔ
**Ίδρυμα:** Πανεπιστήμιο Θεσσαλίας
**Τμήμα:** Επιστήμης Φυσικής Αγωγής και Αθλητισμού

**Κύριος ερευνητής-τρια / φοιτητής-τρια:** Ντικούλη Βασιλική
**Πρόγραμμα Σπουδών:** Βασικό πτυχίο στην Επιστήμη Φυσικής Αγωγής και Αθλητισμού.
**Ίδρυμα:** Πανεπιστήμιο Θεσσαλίας
**Τμήμα:** Επιστήμης Φυσικής Αγωγής και Αθλητισμού

**Η προτεινόμενη έρευνα θα είναι:** Διπλωματική εργασία

**Τηλ. επικοινωνίας:** **********
**Email επικοινωνίας:** vasilikhntikoulh@gmail.com



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Η Εσωτερική Επιτροπή Δεοντολογίας του Τ.Ε.Φ.Α.Α., Πανεπιστημίου Θεσσαλίας μετά την υπ. Αριθμ. 1-6/9-2-2022 συνεδρίασή της εγκρίνει τη διεξαγωγή της προτεινόμενης έρευνας.

Ο Πρόεδρος της
Εσωτερικής Επιτροπής
Δεοντολογίας – ΤΕΦΑΑ

Τσιόκανος
Αθανάσιος
Καθηγητής

## (IPAQ) Σύντομο Διεθνές Ερωτηματολόγιο Φυσικής Δραστηριότητας

### Μία συνηθισμένη εβδομάδα

Οι παρακάτω ερωτήσεις αφορούν το χρόνο που έχετε αφιερώσει για κάποια σωματική δραστηριότητα **κατά τη διάρκεια μιας συνηθισμένης εβδομάδας**. Περιλαμβάνουν ερωτήσεις σχετικά με δραστηριότητες που κάνατε κατά την εργασία σας, στις μετακινήσεις σας, στις δουλειές του σπιτιού, του κήπου και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή άθληση. Σας παρακαλώ να απαντήσετε όλες τις ερωτήσεις, ακόμα και εάν πιστεύετε ότι δεν είστε ένα σωματικά δραστήριο άτομο.

Πριν απαντήσετε τις ερωτήσεις 1 και 2, σκεφτείτε όλες τις **έντονες** σωματικές δραστηριότητες που κάνατε **κατά τη διάρκεια μιας συνηθισμένης εβδομάδας.** Μια έντονη σωματική δραστηριότητα αναφέρεται σε δραστηριότητες που απαιτούν έντονη σωματική προσπάθεια και σας κάνουν να αναπνέετε σημαντικά δυσκολότερα από ότι συνήθως. Σκεφθείτε μόνο τις **έντονες** σωματικές δραστηριότητες που κάνατε και είχαν διάρκεια **μεγαλύτερη από 10 λεπτά** κάθε φορά.

1. **Κατά τη διάρκεια μιας συνηθισμένης εβδομάδας, πόσες ημέρες κάνατε κάποια σωματική δραστηριότητα, όπως σκάψιμο, έντονη άσκηση με βάρη, τρέξιμο σε διάδρομο με κλίση, γρήγορο τρέξιμο, aerobics, γρήγορη ποδηλασία, γρήγορη κολύμβηση, τένις μονό, αγώνας σε γήπεδο (ποδόσφαιρο, basketball, volleyball, handball);**

   ..... ημέρες ανά εβδομάδα

   αν δεν κάνατε έντονες σωματικές δραστηριότητες, σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 3.

2. **Τις ημέρες που κάνατε κάποια έντονη σωματική δραστηριότητα πόση ώρα αφιερώνατε συνήθως;**

   ..... λεπτά ανά ημέρα  δεν γνωρίζω/δεν είμαι βέβαιος ☐

Πριν απαντήσετε τις ερωτήσεις 3 και 4, σκεφτείτε όλες τις **μέτριας έντασης** σωματικές δραστηριότητες που κάνατε κατά **τη διάρκεια μιας συνηθισμένης εβδομάδας.** Μια μέτριας έντασης σωματική δραστηριότητα αναφέρεται σε δραστηριότητες που απαιτούν μέτρια σωματική προσπάθεια και σας κάνουν να αναπνέετε κάπως δυσκολότερα από ότι συνήθως. Σκεφθείτε μόνο τις **μέτριας έντασης** σωματικές δραστηριότητες που κάνατε και είχαν διάρκεια **μεγαλύτερη από 10 λεπτά** τη φορά.

3. **Κατά τη διάρκεια μιας συνηθισμένης εβδομάδας, πόσες ημέρες κάνατε κάποια μέτρια σωματική δραστηριότητα, όπως το να σηκώσετε και να μεταφέρετε ελαφρά βάρη (μικρότερα από 10 κιλά), συνολική καθαριότητα του σπιτιού, ήπιες ρυθμικές ασκήσεις σώματος, ποδηλασία αναψυχής με χαμηλή ταχύτητα, χαλαρή κολύμβηση; <u>Σας παρακαλώ να μη συμπεριλάβετε το περπάτημα</u>.**

   ..... ημέρες ανά εβδομάδα
   αν δεν κάνατε μέτριας έντασης σωματικές δραστηριότητες, σημειώστε Χ

   εδώ ☐ και προχωρήστε στην ερώτηση 5.

4. **Τις ημέρες που κάνατε κάποια μέτρια σωματική δραστηριότητα πόση ώρα αφιερώνατε συνήθως;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

Πριν απαντήσετε στις ερωτήσεις 5 και 6, σκεφτείτε το χρόνο που περπατήσατε **<u>κατά τη διάρκεια μιας συνηθισμένης εβδομάδας.</u>** Να συμπεριλάβετε το περπάτημα στο χώρο της εργασίας σας, στις μετακινήσεις σας και στον ελεύθερο χρόνο σας για ψυχαγωγία, άσκηση ή άθληση.

5. **Κατά τη διάρκεια μιας συνηθισμένης εβδομάδας, πόσες ημέρες περπατήσατε για περισσότερο από 10 συνεχόμενα λεπτά;**
   ..... ημέρες ανά εβδομάδα

   αν δεν περπατήσατε καμία ημέρα περισσότερο από 10 συνεχόμενα

   λεπτά, σημειώστε Χ εδώ ☐ και προχωρήστε στην ερώτηση 7.

6. **Τις ημέρες που περπατήσατε, για περισσότερο από 10 συνεχόμενα λεπτά, πόση ώρα περάσατε περπατώντας;**

   ..... λεπτά ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

7. **Πόσο χρόνο περάσατε καθισμένοι σε μία συνηθισμένη μέρα κατά τη διάρκεια μιας συνηθισμένης εβδομάδας; Ο χρόνος αυτός μπορεί να περιλαμβάνει το χρόνο που περνάτε καθισμένοι στο σπίτι, στο γραφείο, όταν επισκέπτεστε φίλους, όταν διαβάζετε, μελετάτε ή βλέπετε τηλεόραση, <u>αλλά δεν περιλαμβάνει τον ύπνο</u>.**

   ..... ώρες ανά ημέρα δεν γνωρίζω/δεν είμαι βέβαιος ☐

**Τέλος ερωτηματολογίου. Σας ευχαριστούμε για τη συμμετοχή σας**

# MEDICAL HISTORY QUESTIONNAIRE

**Εργαστήριο Περιβαλλοντικής Φυσιολογίας FAME Lab**
**Φόρμα Ιατρικού Ιστορικού**

Όνομα: _____ Ημερ.: __/ __/ ____ Ημερ. __/ __/ ____
Γέννησης:
HH MM XXXX HH MM XXXX

ΠΑΡΟΥΣΑ ΚΑΤΑΣΤΑΣΗ

1. Από όσο γνωρίζετε, είστε σε καλή υγεία την παρούσα περίοδο; Ναι ☐ Όχι ☐
   Αν απαντήσατε "Όχι", παρακαλώ εξηγήστε τους λόγους:

_____
_____
_____
_____
_____
_____

2. Είστε υπό τη φροντίδα γιατρού την παρούσα περίοδο; Ναι ☐ Όχι ☐
   Αν απαντήσατε "Ναι", παρακαλώ εξηγήστε τους λόγους:

_____
_____
_____
_____
_____
_____

3. Λαμβάνετε κάποια συνταγογραφούμενη φαρμακευτική αγωγή την παρούσα περίοδο;
Ναι ☐ Όχι ☐
   Αν απαντήσατε "Ναι", παρακαλώ αναφέρετε λεπτομέρειες:

| Συνταγογραφούμενη αγωγή: | Δοσολογία: |
|---|---|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

4. Λαμβάνετε κάποια μη συνταγογραφούμενη φαρμακευτική αγωγή την παρούσα περίοδο;
Ναι ☐ Όχι ☐
   Αν απαντήσατε "Ναι", παρακαλώ αναφέρετε λεπτομέρειες:

| Μη συνταγογραφούμενη αγωγή: | Δοσολογία: |
|---|---|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

_____ _____
_____ _____
_____ _____
_____ _____

5.Έχετε ιστορικό υψηλής αρτηριακής πίεσης; Ναι ☐ Όχι ☐
6. Έχετε ιστορικό διαβήτη; Ναι ☐ Όχι ☐
7. Έχετε ιστορικό καρδιακού εμφράγματος, θωρακικού πόνου,
ή άλλων καρδιακών παθήσεων; Ναι ☐ Όχι ☐
8. Έχετε ιστορικό πηξίματος των ποδιών; Ναι ☐ Όχι ☐
9. Έχετε ιστορικό αγγειακής νόσου; Ναι ☐ Όχι ☐
10.Έχετε ιστορικό συχνών πονοκεφάλων; Ναι ☐ Όχι ☐
11.Έχετε ιστορικό συχνών ημικρανιών; Ναι ☐ Όχι ☐
12.Έχετε ιστορικό δυσκοιλιότητας; Ναι ☐ Όχι ☐
13.Έχετε ιστορικό γλαυκώματος; Ναι ☐ Όχι ☐
14.Έχετε ιστορικό υπνικής άπνοιας; Ναι ☐ Όχι ☐
15 Έχετε ιστορικό δυσλιπιδαιμίας; Ναι ☐ Όχι ☐
16 Έχετε ιστορικό υψηλών τριγλυκεριδίων; Ναι ☐ Όχι ☐
17.Έχετε ιστορικό υψηλής γλυκόζης; Ναι ☐ Όχι ☐
18.Έχετε ιστορικό μεταβολικού συνδρόμου; Ναι ☐ Όχι ☐
19 Έχετε ιστορικό νεφρικής ανεπάρκειας; Ναι ☐ Όχι ☐
20. Έχετε ιστορικό πόνου/αδυναμίας των αρθρώσεων; Ναι ☐ Όχι ☐
21. Έχετε ιστορικό πόνου/αδυναμίας των μυών; Ναι ☐ Όχι ☐
22. Έχετε ιστορικό ασθένειας του θυρεοειδούς; Ναι ☐ Όχι ☐
23. Έχετε ιστορικό ανοσολογικών ασθενειών; Ναι ☐ Όχι ☐
24. Έχετε ιστορικό καρκίνου; Ναι ☐ Όχι ☐
25. Έχετε ιστορικό κατάχρησης αλκοόλ; Ναι ☐ Όχι ☐
26. Έχετε ιστορικό χρήσης ναρκωτικών ουσιών; Ναι ☐ Όχι ☐
27. Έχετε ιστορικό αιμορροφιλίας; Ναι ☐ Όχι ☐
28. Έχετε ιστορικό ηπατίτιδας; Ναι ☐ Όχι ☐
29. Έχετε ιστορικό υπερθερμίας; Ναι ☐ Όχι ☐
30. Έχετε ιστορικό καπνίσματος; Ναι ☐ Όχι ☐

Αν απαντήσατε ''Ναι'' αναφέρετε πόσα πακέτα καπνίζετε ή καπνίζατε ημερησίως:
_____

Αν απαντήσατε "Ναι" αναφέρετε πόσα χρόνια καπνίζετε ή καπνίζατε: _____

31. Καπνίζετε την παρούσα περίοδο; Ναι ☐ Όχι ☐

32. Χρησιμοποιείτε οποιαδήποτε άλλη μορφή καπνού; Ναι ☐ Όχι ☐
Αν απαντήσατε ''Ναι'', παρακαλώ εξηγήστε:

_____
_____
_____
_____
_____

33. Έχετε ιστορικό σοβαρών τραυματισμών; Ναι ☐ Όχι ☐

Αν απαντήσατε "Ναι", παρακαλώ αναφέρετε λεπτομέρειες και ημερομηνίες:

_____

_____

_____

_____

_____

_____

34. Έχετε ιστορικό χειρουργικών επεμβάσεων; Ναι ☐ Όχι ☐

Αν απαντήσατε "Ναι", παρακαλώ αναφέρετε λεπτομέρειες και ημερομηνίες:

_____

_____

_____

_____

_____

_____

ΠΛΗΡΟΦΟΡΙΕΣ ΣΩΜΑΤΙΚΟΥ ΒΑΡΟΥΣ:

35. Ποιο είναι το επιθυμητό σας βάρος; _____ (kg)

36. Ποια είναι το μέγιστο βάρος που έχετε φτάσει (όχι σε κατάσταση εγκυμοσύνης); _____ (kg)

37. Ποια ήταν το βάρος σας πριν 1 χρόνο; _____ (kg)

38. Ποια ήταν το βάρος σας στην ηλικία των 20 ετών; _____ (kg)

39. Ποια ήταν το βάρος σας κατά τη γέννηση σας; _____ (kg)

ΠΛΗΡΟΦΟΡΙΕΣ ΒΡΕΦΙΚΗΣ ΗΛΙΚΙΑΣ:

40. Που έχετε γεννηθεί; _____ _____ _____

Χωριό/Πόλη Νομός Χώρα

41. Πόσο χρονών ήταν η μητέρα σας όταν γεννηθήκατε; _____

42. Πόσο χρονών ήταν ο πατέρας σας όταν γεννηθήκατε? _____

43. Για πόσους μήνες θηλάζετε; _____

ΙΣΤΟΡΙΚΟ ΟΙΚΟΓΕΝΕΙΑΣ

| | Ηλικία | Ασθένειες | Αν έχουν αποβιώσει, δηλώστε αιτία θανάτου | |
|---|---|---|---|---|
| Πατέρας | | | | Υπέρβαροι; Ναι ☐ Όχι ☐ |
| Μητέρα | | | | Υπέρβαροι; Ναι ☐ Όχι ☐ |
| Αδελφοί | | | | Υπέρβαροι; Ναι ☐ Όχι ☐ |
| Αδελφές | | | | Υπέρβαροι; Ναι ☐ Όχι ☐ |
| | | | | Υπέρβαροι; Ναι ☐ Όχι ☐ |
| | | | | Υπέρβαροι; Ναι ☐ Όχι ☐ |

| | | | |
|---|---|---|---|
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |
| _____ | Υπέρβαροι; | Ναι ☐ | Όχι ☐ |

Έχετε συγγενείς εξ αίματος που έχουν τα ακόλουθα;

| | | | | Συγγένεια |
|---|---|---|---|---|
| Γλαύκωμα | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Άσθμα | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Υψηλή αρτηριακή πίεση | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Νεφρική ανεπάρκεια | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Διαβήτη | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Καρδιακά νοσήματα | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Δυσλιπιδαιμία | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Υψηλά τριγλυκερίδια | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Υψηλή γλυκόζη | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |
| Μεταβολικά σύνδρομο | Ναι ☐ | Όχι ☐ | Δεν γνωρίζω ☐ | _____ |

# AFFIRMATION

Η κάτωθι υπογεγραμμένη Στυλιανή Ζιάκα, ΑΕΜ: 0716235 (μεταπτυχιακή φοιτήτρια του τμήματος Επιστήμης Φυσικής Αγωγής και Αθλητισμού του Πανεπιστημίου Θεσσαλίας του προγράμματος «Άσκηση και Υγεία»

δηλώνω υπεύθυνα ότι αποδέχομαι τους παρακάτω όρους που αφορούν

τα πνευματικά δικαιώματα και τη διαχείριση του επιστημονικού υλικού ή των ερευνητικών δεδομένων που θα έχω στη διάθεσή μου κατά την πορεία της συμμετοχής μου στο ερευνητικό έργο με τίτλο: " Η επίδραση διαφορετικών προγραμμάτων άσκησης σε διαφορετικούς δείκτες υγείας"

και του οποίου επιστημονικώς υπεύθυνος είναι ο καθηγητής κ. Ανδρέας Φλουρής

1. Τα πνευματικά δικαιώματα του έργου αυτού μετά την αποπεράτωσή του ανήκουν στον/στην επιστημονικώς υπεύθυνο-η ή/και στον φορέα που υποστηρίζει ή χρηματοδοτεί το έργο.
2. Οποιαδήποτε επιστημονική δημοσίευση ή ανακοίνωση (αναρτημένη ή προφορική), ή αναφορά που προέρχεται από το υλικό/ δεδομένα της εργασίας αυτής και η πιθανή συμμετοχή του ονόματός μου σε αυτήν αποφασίζεται εκ των προτέρων από εμένα και τον/την επιστημονικώς υπεύθυνο-η του έργου και αυτό θα πιστοποιηθεί εγγράφως μεταξύ εμού και του/της υπεύθυνου-ης.
3. Η σχετική σύμβαση εργασίας και οι υποχρεώσεις μου από τη συμμετοχή μου στο έργο αυτό, ρυθμίζονται με βάση τους κανόνες και τις αρχές της Επιτροπής Ερευνών του ΠΘ.

**Τέλος, δηλώνω ότι γνωρίζω τους κανόνες περί δεοντολογίας στην έρευνα της σχετικής με το συγκεκριμένο ερευνητικό έργο περιοχής, τους κανόνες περί λογοκλοπής και πνευματικής ιδιοκτησίας και ότι θα τους τηρώ απαρέγκλιτα καθ' όλη τη διάρκεια της συμμετοχής μου στο έργο αυτό και κατά την πιθανή μου συμμετοχή σε διαδικασίες δημοσίευσης που θα προκύψουν μετά το τέλος του έργου.**

11/1/2024

Η δηλούσα

Ζιάκα Στυλιανή