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The acute mobilization of leukocytes and their subpopulations after different intensities of endurance exercise.

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Zusammenfassung

Diese Arbeit untersuchte die akuten Effekte unterschiedlich intensiver Ausdauertrainingseinheiten auf die Immunmobilisierung bei gesunden jungen (n = 12; Alter: 28,2 ± 2,4 Jahre; Body Mass Index: 25,4 ± 2,9 kg/m^2; peak oxygen uptake ($VO_{2\ peak}$): 44 ± 8,9 ml/kg/min) und älteren (n = 6; Alter: 65,5 ± 1 Jahre; Body Mass Index: 24,2 ± 2,1 kg/m^2; $VO_{2\ peak}$: 39 ± 2,8 ml/kg/min) Probanden. Von den Teilnehmern wurde in zufälliger Reihenfolge eine Fahrradergometer-Einheit mit gleichem Arbeitsvolumen unter (moderate) und über der ersten ventilatorischen Schwelle (schwere Intensität) durchgeführt. Blutproben wurden vor der Einheit und bis zu vier Stunden nach Belastungsende entnommen. Es wurde eine Leukozytose nach beiden Intensitäten bei jungen Probanden beobachtet, welche ihren Höhepunkt zwischen zwei und vier Stunden nach der Belastung hatte und nach schwerer Intensität akzentuierter auftrat. Diese Leukozytose war hauptsächlich durch Neutrophilen Anstieg bedingt. Der Lymphozyten-Anteil verringerte sich parallel um die zwei Stunden-Marke unter Baseline Niveau. Bei älteren Probanden konnten ähnliche Immunantworten beobachtet werden, welche in ihrer Ausprägung abgeschwächt waren. Die Ergebnisse bestätigen einen intensitätsabhängigen immunmodulierenden Effekt von akuten Ausdauersporteinheiten.

Abstract

This study investigated the acute effects of endurance exercise of different intensities on immune cell mobilization in healthy young (n = 12; age: 28.2 ± 2.4 years; body mass index: 25.4 ± 2.9 kg/m^2; $VO_{2\ peak}$: 44 ± 8.9 ml/kg/min) and older (n = 6; age: 65.5 ± 1 years; body mass index: 24.2 ± 2.1 kg/m^2; $VO_{2\ peak}$: 39 ± 2.8 ml/kg/min) individuals. The participants performed a bicycle ergometer session with the same workload below (moderate intensity) and above (heavy intensity) the first ventilatory threshold in a randomized order. Blood samples were taken before the session and up to four hours after the end of the exercise. Leukocytosis was observed after both intensities in young subjects, peaking between two and four hours after exercise and showing greater increase after heavy intensity. This leukocytosis was mainly due to an increase in neutrophils. In parallel the lymphocyte percentage decreased around the two-hour mark to below baseline levels. Similar immune responses were observed in older subjects, but these were less pronounced. The results confirm an intensity-dependent immunomodulatory effect of acute endurance exercise sessions.

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1. Introduction

With advancing age structural and functional changes occur in the organism which include reduction of cardiovascular fitness, hormonal changes as well as the development of age associated diseases including cardiovascular diseases, diabetes, neurodegenerative diseases and cancer (Graff et al., 2022; Großkopf & Simm, 2022). The immune system is also affected by aging – a phenomenon called “immune senescence”, leading to functional changes in both parts of the immune system, the non-specific and specific immune system. Cells of the non-specific immune system, which protect the body through a short-term initial defense reaction lose functionality and their subpopulations are altered towards pro-inflammatory cell types (Weyh et al., 2020).

The specific or “adaptive” immune system is characterized by more differentiated immune cells in old age, i.e. immune cells that are already specialized to a particular pathogen. The number of naïve, unspecialized cells decreases, which impairs the immune system's flexibility of response. Proliferation is restricted to senescent cells which contributes to a pro-inflammatory milieu (Graff et al., 2022; Großkopf & Simm, 2022; Weyh et al., 2020). In consequence, risk of infection is increased, and the development of age-specific diseases becomes more likely (Weyh et al., 2020).

Regular exercise seems to have an opposite effect on these age-related changes, e.g. physically active older people show an improved immune response and a lower number of senescent cells (Graff et al., 2022; Nieman & Wentz, 2019; Weyh et al., 2020).

Even acute exercise sessions can have beneficial effects on the immune system. Through the elevated mobilization of immune cells into peripheral parts of the body, the immune surveillance is increased. There are indications that senescent cells of the specific immune system, such as T-cells, can be removed through apoptosis after mobilization. Apoptosis is the natural death of the cell, which may counteract the accumulation of senescent cells and has rejuvenating effects (Graff et al., 2022; Weyh et al., 2020). These acute effects of exercise are thought to accumulate with regular training, contributing to a sustainably healthy immune system (Nieman & Wentz, 2019).

However, it’s not fully understood which acute exercise intensity and time has the optimal effects on immune modulation (Sellami et al., 2018). Nieman and Wentz (2019) state that, in general, moderate to vigorous exercise performed for up to 60 minutes (min) has beneficial effects on the immune system; it may mobilize anti-inflammatory cytokines, immunoglobulins, neutrophils, immature B- and cytotoxic T- cells, NK cells as well as improve the antipathogenic function of macrophages. In a study by Neves et al. (2015), there were some immune-modulating effects found after acute vigorous intensity exercise,

such as a general increase in leukocytes, lymphocytes and monocytes immediately after exercise or increase in neutrophils two hours (h) after the exercise. However, no changes were found after moderate intensity exercise. Other works, by contrast, have reported immune-modulating effects of moderate intensity exercise (Graff et al., 2022; Sellami et al., 2018), which emphasizes the need for further research in this area. In addition, knowledge of immune modulation through exercise in older individuals is limited (Sellami et al., 2018).

This thesis focuses on the acute influence of exercise on immune components. The response of the immune system to different intensities of exercise as well as the potential difference between age groups were addressed in the present study.

To date, there are few studies that focus on the age aspect of the immune response to different intensities of exercise (Baskerville et al., 2024; Sellami et al., 2018).

Therefore, the present thesis will first provide an overview on what is meant by the aging process, which mechanisms have an influence specifically on the aging of the immune system and what effects this aging process has on immune function. This will be followed by a clarification of the chronic and acute influence of exercise on the immune system and an overview of how different exercise intensities affect the immune parameters acutely according to the current state of research.

1.1. The aging process and its mechanisms

Aging is understood to be a physiological process that leads to a reduction in the function of organismic processes that are essential for survival (Behr et al., 2023; Dodig et al., 2019). A distinction can be made between chronological and biological age, where chronological age refers to the period of life since birth. Biological age on the other side is defined by the maintenance of physiological and cognitive body functions, whereby people with the same chronological age can have different biological ages, as shown in *Figure 1*. Biological aging is a non-uniform phenomenon that can occur at an individual rate in each person (Behr et al., 2023).

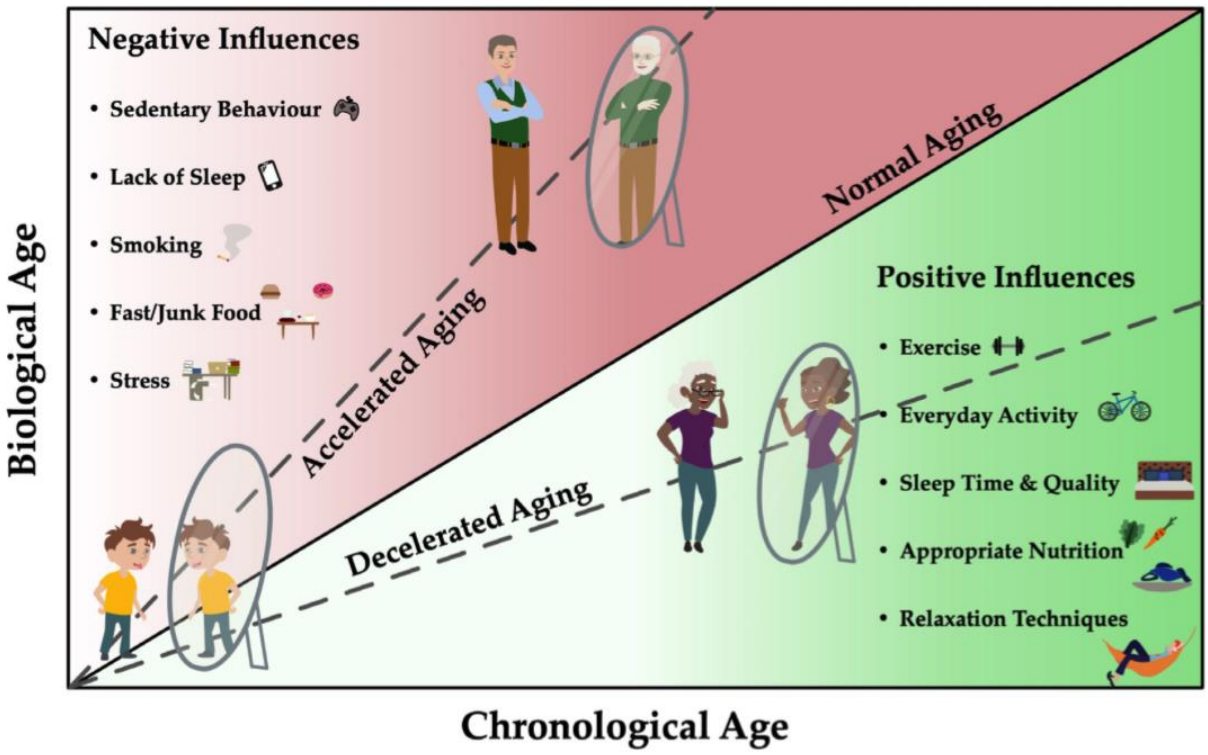

Figure 1: The correlation between chronological and biological age and its influencing factors (Haupt et al., 2022).

Deterioration of tissue and organ function, which becomes more frequent with advancing biological age, has a major impact on the aging process. These detrimental changes can be caused by intrinsic factors, e.g., because of metabolic pathways. This includes for example increased levels of oxidative stress caused by reactive oxygen species (ROS) with time. Also, extrinsic factors, such as UV-radiation or environmental substances, contribute to the development of senescent cells and age-related functional decline (Behr et al., 2023; Dodig et al., 2019).

The result of aging is a variety of altered phenotypes over time, which have a limiting effect on the organism's physiology. These alterations of phenotypes are characterized by accumulation of damage and insufficient repair mechanisms of damaged cells leading to an increased risk of age specific diseases and mortality (Keshavarz et al., 2023).

A key question in the study of aging is why and how people age in the first place. There are several theories that define the cause of aging (da Costa et al., 2016; Lipsky & King, 2015).

For example, a distinction can be made between program theories and damage theories. The program theory states that aging has an evolutionary purpose and is predetermined by biological programs and "aging genes" (da Costa et al., 2016; Lipsky & King, 2015). The damage theory, on the other hand, sees the outcome of aging as an accumulation of intrinsic

and extrinsic damage that causes cell and tissue dysfunction over time (da Costa et al., 2016; Lipsky & King, 2015). Combined theories define the outcome of aging as an interaction of different theories, i.e. a complex interplay of feedback mechanisms of different biological processes (da Costa et al., 2016). It should be noted here that no theory fully explains the cause of aging and that the aging process is multifactorial (Lipsky & King, 2015).

Dodig et al. (2019) describe aging as the changes in phenotype that occur to varying degrees in different cells and tissues, which are triggered both genetically and by environmental factors and time. Changes that are more likely with aging include somatic complaints such as reduced mobility and functional limitations as well as an increase in the prevalence of chronic diseases. Additionally, cognitive, psychological and social changes can occur. Diseases such as diabetes, cardiovascular diseases, osteoporosis, osteoarthritis, cancer or neurological disorders become more likely with age (Dodig et al., 2019).

According to Dodig et al. (2019), there are three key factors that influence aging: cellular senescence, immune aging and inflammation (Dodig et al., 2019).

The concept of cell senescence is of particular importance in the literature, as it represents one of the fundamental mechanisms of aging (Behr et al., 2023; Dodig et al., 2019). Senescence is an irreversible developmental arrest of the cell, which is triggered by critically short telomeres; other important trigger mechanisms are oncogenetic markers, oxidative stress, mechanical stress, genotoxic damage, incompletely functioning mitochondria, viral and bacterial infections or imbalance in nutrients (López-Otín et al., 2023). Damage accumulates in the cell, which cannot fully be eliminated by compensatory repair mechanisms and is consequently accompanied by changes in the cell and the organism (Behr et al., 2023; Dodig et al., 2019). The removal of damaged cells can take place in two steps, with the senescent cell triggering an immune response that eliminates the aged cell in the next step. However, if this important second step fails or if this repair mechanism is exhausted, this causes an accumulation of senescent cells (López-Otín et al., 2023).

Finally, the senescent cell enters a resting phase in which it is metabolically active, but malignant cell transformations and the transfer of damage to the next generation are avoided. This still phase is characterized by altered cell functions, a senescence-associated secretory phenotype (SASP) and associated inflammation (Behr et al., 2023; Dodig et al., 2019). Cell aging is a mechanism that protects against the onset of disease and is also characterized by its tumor-suppressive effect (López-Otín et al., 2023).

Senescence can occur to varying degrees in different tissues, mainly affecting immune cells, endothelial cells and fibroblasts (López-Otín et al., 2023).

A distinction is also made here between acute senescence, which is part of biological processes such as tissue repair and in which affected cells are eliminated by the activated immune system, and chronic senescence, which shows heterogeneity in their SASP exhibition, characterized by persistent cellular stress and resistance to immune cells, which can lead to an accumulation of senescent cells and subsequently to age-specific diseases and cancer (Dodig et al., 2019).

This brings up the immune system as the next central factor of aging. With age, the sensitivity of the immune system becomes impaired. The work of the innate components of the immune system and thus phagocytosis may be impaired. The thymus plays a central role in the maturation of immune cells, but when it undergoes degenerative changes with age, B- and T- cells cannot work efficiently and anti-inflammatory and pro-inflammatory mechanisms become disrupted. This decline in immune system function leads, for example, to impaired elimination of senescent cells. Sensitivity to antigens that trigger infections or malignant cells decreases. This also leads to inflammation and an increased risk of autoimmune diseases. The next chapter will discuss the development of the immune system in old age in more detail (Dodig et al., 2019).

Chronic inflammation also occurs in old age, with macrophages and lymphocytes always present in the tissue. Mediators of inflammation such as cytokines trigger such chronic inflammation. Normally, pro- and anti-inflammatory mediators are in balance; aging is associated with an imbalance and frequent pro-inflammatory cytokines (Dodig et al., 2019).

Models attempt to describe molecular processes and characteristics of aging and connect them to each other. As research in this area progresses, the view of aging has changed. Aging was initially believed to be unavoidable and unchangeable due to the predetermination of genetic programs. However, research in recent years has shown that specific molecular characteristics determine the aging process, making it possible to apply targeted interventions aimed at influencing the rate of aging and the occurrence of age-related diseases. This work will focus primarily on the sensitivity of the immune system and age-related changes in this sensitivity (Behr et al., 2023).

Before discussing the immune system in detail, it is important to mention another important work by López-Otín et al. (2023), which is a collection of molecular hallmarks that change with progressive aging. These “hallmarks of aging” are manifestations associated with age, which can be emphasized experimentally, thereby accelerating aging, or can be slowed down or even reversed by interventional therapy (López-Otín et al., 2023).

Twelve central characteristics of aging are discussed. The primary hallmarks include genome instability, epigenetic changes, telomere attrition, impaired macroautophagy and loss of proteostasis. Primary hallmarks represent the fundamental forms of cellular and molecular damage that accumulate with age and give rise to subsequent hallmarks of aging. Further, there are the antagonistic characteristics that are a consequence of this damage, including cell senescence, deregulated nutrient sensing and mitochondrial dysfunction (López-Otín et al., 2023). These characteristics can also initiate protective processes against specific diseases, whereby they play a differentiated role. Finally, integrative characteristics are discussed, where the cause lies in the accumulation of primary and antagonistic characteristics. These include stem cell depletion, chronic inflammation, altered intercellular communication and dysbiosis. The characteristics interact with each other and an interplay of all hallmarks of aging determines how quickly the organism ages. Interventions aimed at slowing down aging therefore usually have an impact on several hallmarks of aging (López-Otín et al., 2023).

As already mentioned, all “hallmarks of aging” influence each other and can never be seen separately as a single factor for aging. Nevertheless, two of these hallmarks, namely cell senescence and chronic inflammation, are central to the purposes of the present thesis and, therefore, will be addressed in more detail in the next section with an emphasis on their relation to the immune system.

1.2. The immune system

The immune system is the body's defense system, which protects it from invading substances and germs and breaks down pathogens that are harmful to the body.

The immune system can be broadly divided into two components: the innate (non-specific) immune system, which provides immediate, general protection against pathogens, and the adaptive (specific) immune system, which recognizes specific pathogens and can mount a stronger, faster response upon future exposures (Murphy & Weaver, 2018, p. 9).

As mentioned above, the non-specific immune system is the body's initial protection, whereby a distinction can be made between two parts: The external non-specific immune system protects the body through anatomic barriers such as the skin; it also includes the respiratory tract, the digestive tract and the urinary tract. These structures either prevent contact with pathogens by making it physically difficult for them to infiltrate the mucous membranes or protect the body with additional cells on the mucous membranes, such as the body's own antimicrobial proteins (Murphy & Weaver, 2018, p. 8).

The internal parts of the non-specific immune system include cells capable of phagocytosis, i.e. the engulfment and elimination of harmful substances such as pathogens or cellular

debris. These phagocytic cells include neutrophil granulocytes, monocytes, which can differentiate into dendritic cells, and macrophages (Murphy & Weaver, 2018, p. 9).

In addition, natural killer cells, while not phagocytic, contribute to the innate immune defense by destroying virus-infected or cancerous cells through cytotoxic mechanisms. Eosinophilic and basophilic granulocytes are also present in the innate immune response, though in significantly smaller numbers compared to other immune cells. Eosinophils are especially important in the defense against large extracellular parasites that cannot be eliminated by phagocytosis. Basophils, on the other hand, contribute to immune responses by releasing inflammatory mediators such as histamine, particularly during allergic or parasitic reactions (Murphy & Weaver, 2018, p. 12).

The complement system is a part of the non-specific immune system, which supports phagocytic immune cells by covering pathogenic cells with complement proteins. These are marked by antibodies and activated by antigens, making them easily recognizable to phagocytic cells. Antigens are components of pathogens that are recognized by the immune system (Murphy & Weaver, 2018, p. 5).

The non-cellular components of the non-specific immune system include cytokines, chemokines and interferons. Cytokines and chemokines are inflammation initiators. Through their activation they can increase the activity of other cells or attract other cells, e.g. neutrophils or monocytes, to the site of infection (Murphy & Weaver, 2018, p. 14). Finally, “interferons” also play an important role as part of the non-specific immune system, preventing viruses from proliferating (Murphy & Weaver, 2018, p. 149).

The task of the specific or “adaptive” immune system is to identify invading pathogens and mark them so that the body can prepare itself for future infections and take measures to defend itself against known pathogens more quickly – a process also referred to as “immunological memory”. Compared to the short-term response of the non-specific immune system, which can react just within a few minutes, the response of the specific immune system takes longer and can take up to a few hours (Murphy & Weaver, 2018, p. 10).

The role of antibodies (immunoglobulins) is very central in the specific immune system; they are responsible for binding to antigens and initiating an immune response. The antibodies do not destroy pathogens, but initiate the activity of other immune cells, such as the complement system (Murphy & Weaver, 2018, pp. 17-18).

B cells are important components of the specific immune system and mature in the bone marrow. These B cells react to antigens previously presented by macrophages by dividing. On the one hand, B cells can divide into plasma cells, which release antibodies. In addition, B memory cells are formed because of the division, which “store” the characteristics of these

antigens for the long term, making a targeted defense possible in the future (Murphy & Weaver, 2018, p. 29).

In contrast, T cells, which are the second most important component of the adaptive immune system, function without the formation of antibodies and mature in the thymus. When a T cell encounters an antigen presented by a macrophage, it is activated and forms four subunits: Cytotoxic T cells are responsible for the destruction of antigen-bearing substances. T helper cells initiate the activity of other immune cells, including B cells for antibody production and macrophages for the degradation of harmful substances. Regulatory T cells have a regulating effect on immune cell activity and can also reduce it. In addition, T memory cells as well as B memory cells contribute to the formation of an immunological memory and thus to sustainable protection against specific pathogens (Murphy & Weaver, 2018, p. 17).

1.3. Immune senescence

As discussed, immune cells also enter a senescent state, leading to a change in the composition of their subpopulations and their general function. With advancing age, limitations in the function of the immune system occur, making the body more susceptible to disease. All parts of the immune system, the innate non-specific immune system and the cells of the adaptive specific immune system are affected by age-specific changes. On the one hand, the properties of the skin such as the pH and lipid composition, as well as the hydration level of the outermost epidermal layer change, leading to a worsened barrier function (Großkopf & Simm, 2022). On the other hand, the composition of the immune cells also changes, although many of the alterations occurring in the non-specific and specific immune systems are still complex to interpret and disentangle when it comes to aging (Großkopf & Simm, 2022). Irrespective of this, many immune cell populations have been shown to function less effectively. For example, both the activity and number of complement proteins increase with age, and more cytotoxic NK cells are observed in older individuals, although without a parallel increase in their cytotoxic effect (Großkopf & Simm, 2022). Conversely, neutrophils exhibit reduced directional migration, intercellular signal transduction, and activity (Weyh et al., 2020). Monocytes, which act as an important interface between the non-specific and specific immune system through their presentation of antigens, have been shown to possess a decreased ability to migrate and to have a heightened proportion of pro-inflammatory subpopulations with short telomeres (Großkopf & Simm, 2022; Weyh et al., 2020). Likewise, an increased presence of a proinflammatory macrophage subpopulation is also observed, which in turn contributes to increased levels of inflammation (Weyh et al., 2020). Phagocytosis and antigen presentation are also less pronounced. An additional factor that contributes significantly to the senescence of the

immune system is a reduction in the number of naïve T and B cells - cells that have not yet been “imprinted” on a specific antigen - often accompanied by an increase in the number of effector and memory cells (Großkopf & Simm, 2022; Weyh et al., 2020). This results in less diversity, including in antibodies, and reduced flexibility of the immune system. With age, cellular proliferation becomes increasingly restricted (Großkopf & Simm, 2022). In addition, the production of pro-inflammatory cytokines gradually increases with age. This contributes to less active movement of the immune cells (Großkopf & Simm, 2022).

These factors - reduced ability for activation and efficiency of phagocytic cells, reduced diversity in naïve immune cells and an increase in inflammation levels contribute to the aging of the immune system (Großkopf & Simm, 2022).

With time, the general level of pro-inflammatory cells in the blood increases, which is sometimes referred to as “inflammaging”. Chronic inflammation is influenced by an interplay of different “hallmarks of aging” and typically increases with age. This leads to a higher amount of circulating inflammatory biomarkers and cytokines. Diseases and pathological changes such as osteoarthritis, intervertebral disc degeneration, neuroinflammation and arteriosclerosis are additionally promoted through a higher state of inflammation. Furthermore, the efficient functioning of the immune system decreases with increasing levels of inflammation (López-Otín et al., 2023). Additionally in a study by Santoro et al. (2021), it has been shown that people who were able to reach very old age show a rather low level of inflammation. There was even a trend towards anti-inflammatory effects, which in turn may help mitigate adverse consequences of chronic inflammation typically associated with advancing age (Santoro et al., 2021).

1.4. Effects of exercise on immune aging

The following section discusses the role of exercise in relation to these age-related changes in the immune system. Immune aging is accompanied by many changes in immune cell subgroups, which generally impair immune functioning and promote “inflammaging.” Current literature on the effects of regular and acute exercise will be presented.

Effects of regular exercise

In general, physical activity (PA) helps to counteract the dysregulating effects of immune senescence and promotes the regulation of immune cells (Nieman & Wentz, 2019). More specifically, regular exercise can reduce the risk of infection by 31% and mortality from infectious diseases by 37% (Chastin et al., 2021). Specifically, regular exercise in the elderly is thought to improve NK cell and neutrophil function in the innate immune system and reduce the accumulation of senescent, pro-inflammatory monocyte subpopulations. In

general, regular exercise is believed to result in a lower risk of infection and potential for inflammation (Weyh et al., 2020).

In terms of “inflammaging”, regular exercise results in a decreased release of pro-inflammatory cytokines (Nieman & Wentz, 2019; Sellami et al., 2018; Weyh et al., 2020) and shifts the number of macrophage subunits to anti-inflammatory M2 macrophages. Myokines and peptides, which play an important mediating role in immune function and have an anti-inflammatory effect, are also increased with regular exercise (Weyh et al., 2020). Visceral adipose tissue, which contributes to the rate of inflammation in the body, is more likely to be reduced with regular endurance sports, and the increased energy requirements resulting from regular exercise appear to have an additional positive effect on the rate of inflammation and thus on the development of inflammaging (Weyh et al., 2020). Furthermore, vaccinations are more effective in physically active older people (Sellami et al., 2018; Weyh et al., 2020).

Regarding the specific immune system, there are also clear improvements in function. Fewer signs of immune senescence or thymus dysfunction have been reported in physically active 55–79-year-olds (Weyh et al., 2020). In this regard, a physically active lifestyle is considered a factor that helps maintain a generally low level of inflammation in the body.

Regular practice of endurance sports increases athletic performance capacity. In this regard, a study by Spielmann et al. (2011) describes that people with an above-average value of maximum oxygen uptake ($VO_{2\,max}$) during physical exertion have a lower proportion of senescent T cells in their blood.

Caution is often advised with very intensive exercise over a long period of time. In a study, Moro-García et al. (2014) examined young and old athletes and compared their immune function with non-athletes at resting conditions. High volumes of exercise throughout life - in this case six days of rowing a week for several hours in young athletes - can severely impair the efficiency of the immune system and increase the risk of infection. In this study blood draws were taken 18 hours after the last training session. The results showed a generally reduced number of leukocytes, neutrophils and lymphocytes in young and old athletes, which leads to reduced immune function (Moro-García et al., 2014).

Summarizing several intervention studies, Chastin et al. (2021), concluded that aerobic training over twelve weeks on average, carried out 3-5 times a week for 30 minutes with moderate to severe intensity, has positive effects on immune function. According to the authors, the endurance training interventions lead to an increase in the number of CD4+ T-helper cells, which has a regulating function on the immune response as well as chronic inflammation and strengthens the memory and response of the immune system. In addition,

immunoglobulin A (IgA) levels in the saliva are increased, which leads to a more effective response against pathogens in the mucous membrane. IgA also has an anti-inflammatory effect. In addition, the proportion of neutrophils also seems to be reduced after aerobic training. These effects on neutrophils may appear as a depression of the immune system, but no functional impairments regarding the immune system were observed after the intervention, and total leukocyte counts were unaffected. Neutrophils serve, in addition to fulfilling important roles in muscle regeneration and the activation of other immune cells, as markers of chronic inflammation and tissue damage (Brown et al., 2015; Chastin et al., 2021). In this regard it is important to recognize the role of catecholamines, which are responsible for increased neutrophil activity observed during prolonged exercise. Additionally, cortisol and growth hormones released during exercise further regulate neutrophile function (Neves et al., 2015; Sellami et al., 2018).

Physical exercise, especially endurance sports at moderate intensity, is believed to hold potential to reverse age-associated changes to a certain extent, such as the age-related decline in the function of T-cells or changes in lymphocyte subpopulations. There is still a lack of data to be able to make clear statements about the effects of other types of sport, such as resistance training. It should also be noted that regular exercise may exert even stronger effects on immune aging in individuals with an initial health risk or immune risk profile (IRP), potentially targeting the multiple hallmarks of aging more effectively (Weyh et al., 2020).

Further studies are needed to find the optimal dose and type of sport and to better understand the immune-preserving effect of sport and the underlying mechanisms (Nieman & Wentz, 2019; Weyh et al., 2020). Although the anti-inflammatory and antioxidant effects of regular exercise are well established, they primarily result from the acute immune responses triggered by each individual exercise session. Over time, the transient effects accumulate, promoting a healthy metabolism and improved immune activity (Nieman & Wentz, 2019). The following section will provide more detailed insights into the short-term changes in immune cell activity that occur within minutes or hours after exercise. This thesis will ultimately focus on these acute effects.

Effects of acute exercise

Acute bouts of moderate- to vigorous-intensity endurance exercise lasting up to 60 minutes appear to present the dose most effective in eliciting protective effects on the immune system (Nieman & Wentz, 2019). Inactivity, by contrast, negatively impacts immune function and the acute immune defense, potentially leading to excessive inflammation and susceptibility to infection. A similar transient state of immune suppression, known as the

"open window" effect, can also occur following very intense or prolonged exercise (Gonçalves et al., 2020; Suzuki & Hayashida, 2021). If training sessions are very intensive and/or prolonged, the positive effects of exercise may diminish or even reverse, as seen in cases of overtraining or during periods of intense competition. The physiological, psychological and metabolic stress negatively affects immune function and increases the risk of illness (Nieman & Wentz, 2019; Suzuki & Hayashida, 2021).

In agreement with this rationale, Nieman (1994) assumed a J-curved model, which describes the risk of infection after an acute exercise bout. The author suggested that prolonged, very high intensity exercise leads to a higher risk of upper respiratory tract infection (URTI), whereas moderate intensity exercise is expected to lower the respective risk (Nieman, 1994).

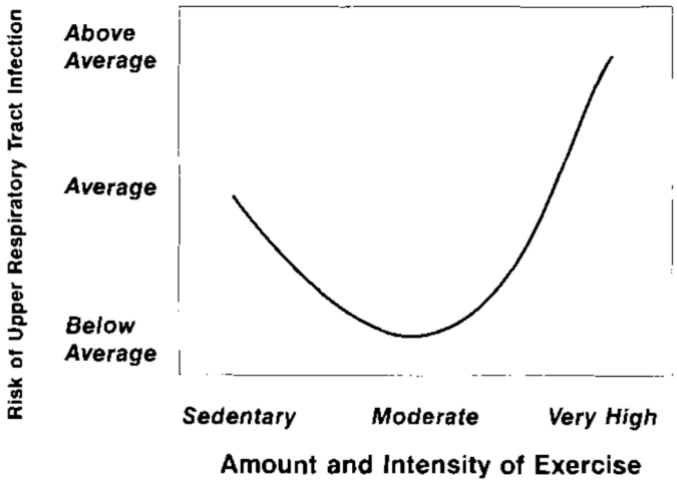

Figure 2: A J-shaped curve is assumed, where moderate exercise is suggested to reduce risk of upper respiratory tract infection (URTI) in contrast to very high intensity exercise or a sedentary lifestyle (Nieman, 1994).

The "open window" theory of immune suppression, which leads to higher risk of infection, is commonly believed to occur in response to extremely long, intense periods of exercise. While initially it has been assumed that acute immune suppression might also occur in response to shorter periods of moderate to vigorous intensity exercise, current data rather suggest that the function of the immune system may be temporarily increased after acute bouts of moderate to vigorous intensity exercise (Campbell & Turner, 2018; Graff et al., 2022). In general, when the exercise session is kept at a moderate to vigorous intensity, it is assumed that the circulation of immune cells between bloodstream and tissue would be

increased, leading to an increase in macrophage activity, anti-inflammatory cytokines, NK cells, neutrophils, immature B cells, cytotoxic T cells, and immunoglobulins after acute exercise (Nieman & Wentz, 2019). Gonçalves et al. (2020) showed an acute increase in the number of neutrophils, NK cells, monocytes, CD4+ helper and cytotoxic CD8+ T cells as well as in immunoglobulin G (Gonçalves et al., 2020). Campbell and Turner (2018), by contrast, reported a decline in lymphocytes in the circulating blood after exercise. This is believed to be caused by the migration of lymphocytes to the periphery, which increases immune surveillance immediately after a training session (Campbell & Turner, 2018).

In general, leukocytosis, an elevated state of white blood cell numbers, occurs after exercise, however this is less pronounced in older individuals. It has been reported that in response to acute exercise the proliferation rate decreases with age (Sellami et al., 2018). The level of lymphocyte proliferation specifically seems to be correlated to the acute effect of exercise on the adaptive immune system; however, it has to be added here that data is not consistent with some studies reporting acute increases and others reporting decreases in lymphocyte proliferation (Sellami et al., 2018). Mechanisms that regulate lymphocyte activity and proliferation are glutamine, epinephrine, norepinephrine, noradrenaline or catecholamines which are intensity-dependent with a higher release after heavy intensity (Neves et al., 2015; Sellami et al., 2018).

Exercise and sport also have acute effects on inflammation markers such as the cytokine IL-6, contributing to immune response and signal transmission (Brown et al., 2015; Gonçalves et al., 2020). On average, acute increases of 145% are recorded in IL-6 concentration, which can last up to two hours after acute cycling exercise at 55-60 % $VO_{2\,max}$ over one hour. This exercise-induced increase in IL-6 is followed by improved lipid and glucose metabolism or insulin sensitivity. Also, proinflammatory cytokines are inhibited subsequently. Mechanisms such as cellular stress of reactive oxygen species (ROS) activation are responsible for the increased IL-6 level (Brown et al., 2015).

Of course, also other factors such as nutrient deficiencies, psychological stress, fatigue or environmental influences can affect the modulation effects of exercise and sport on the immune system (Sellami et al., 2018).

Acute exercise influences the components of the immune system that are represented in the blood circulation. This also stimulates the mobilization of hematopoietic stem cells or senescent immune cells. In older people, these mobilization mechanisms are impaired, which is one of the factors why the immune response to exercise in these individuals often differs from those typically observed in younger individuals. However, as suggested by Sellami et al. (2018), more research is needed to make clear statements about the effects

of exercise on immune modulation in people over 65 years of age (Sellami et al., 2018). So far, only a limited number of studies have specifically examined the response of older people to acute exercise sessions (Baskerville et al., 2024; Cannon et al., 1994; Cannon et al., 1990; Graff et al., 2022; Sellami et al., 2018).

As regards the difference between acute moderate and vigorous intensity immune cell count changes, no clear conclusions can be drawn at this point (Sellami et al., 2018). However, some studies have focused specifically on the impact of exercise intensity. The following section provides an overview of these studies and their results. In line with the aims of the present thesis, the focus in on studies examining the effects of endurance exercise.

Exercise intensity as a potential moderator of immune response to acute exercise

The studies included in the review of Sellami et al. (2018), which focused specifically on older age groups, differed in exercise modalities. Despite this, it has been shown that acute exercise (45 minutes of running or cycling at 78% of maximum heart rate (HR_{max})) leads to an increase in the number of neutrophils in young (20-32 years) and older subjects (61-72 years) which remain elevated up until four- to six hours after exercise cessation. The modulating effect of exercise was more pronounced in young subjects compared to the older age group (Cannon et al., 1994). According to Brown et al. (2015), increases of neutrophile numbers of up to 51% can occur after acute exercise. However, it should be noted that exercise intensity and duration may strongly influence the reaction: Davison (2011) investigated the modulating effect of brief, vigorous exercise (Cycling Wingate Test, 4x30 sec), where blood collection was performed at baseline, immediately post-exercise and 30 minutes after exercise. After exercise, an increase in neutrophils was observed compared to baseline numbers (Davison, 2011). On the other hand, walking at moderate intensity (50% $VO_{2\,max}$) over 30 minutes did not have affect neutrophile counts for up to 168 hours after exercise (Markovitch et al., 2008). Nieman et al. (1991) reported elevated neutrophile counts both immediately after moderate-intensity exercise (+34%) and three hours (+33%) after exercise (walking at 60% $VO_{2\,max}$ for 45 min).

In general, leukocytosis is observed after bouts of vigorous aerobic exercise (Jamurtas et al., 2018; McCarthy et al., 1987). After 30 minutes of running at vigorous intensity, leukocyte levels reportedly peak between 2.75 (+75%) and 4.25 hours (+67%) after exercise (McCarthy et al., 1987), although this response is less pronounced in older people (Sellami et al., 2018). The observed leukocytosis was closely linked to the observed increase in neutrophils after exercise (McCarthy et al., 1987). The work of Nieman et al. (1991) support those findings, where a bout of moderate walking exercise at 60% $VO_{2\,max}$ for 45 minutes caused an increase in leukocyte numbers immediately after exercise (+27%) and three

hours (+21%) after exercise, after which the concentration decreased again until five-hour post-exercise follow-up (Nieman et al., 1991).

In an intervention study by Neves et al. (2015), the immune-modulating effects of high and moderate exercise were compared. Both exercise bouts were standardized to an energy expenditure of 350 kcal, with the moderate- and high-intensity bouts performed at 40% and 80% $VO_{2\ max}$, respectively. The study sample included healthy participants (21 ± 2 years), and blood samples were taken at baseline, immediately post-exercise and two hours after the exercise. After vigorous exercise, leucocyte numbers increased, with the increase persisting until two hours after exercise cessation. Additionally, lymphocyte and monocyte subpopulations were elevated post-exercise, however, they decreased by the two hour measurement. Neutrophile numbers remained unchanged post-exercise but were found to be increased after two hours. No similar changes were observed after the moderate intensity bout (Neves et al., 2015). By contrast, Cerqueira et al. (2020) reported that moderate exercise may equally have a modulating effect on immune cells, although its effectiveness may be lower compared to vigorous exercise. In a study by Nieman et al. (2012) cyclists with different fitness levels completed a 1.75-hour cycling session at 60% of peak power (P_{max}), immediately followed by a maximum effort over ten km (18.3 ± 0.3 min). White blood cells were measured at baseline, post-exercise and one hour after completion of the exercise session. It was observed that the leucocyte count increased immediately after cycling (+133%) and increased further until the one-hour measurement (+187%). The phagocytosis activity of granulocytes (+69%, +128%) and monocytes (+43%, +28%) was also increased. In this study, no correlations were found between these changes and $VO_{2\ max}$, age, body composition or training volume (Nieman et al., 2012).

Regarding lymphocyte subpopulations, it has been reported that numbers generally rise during exercise and stay elevated in the first regeneration phase, due to increased recruitment through marginalized, ‘resting’ leucocytes (Sellami et al., 2018). It appears that the degree of lymphocytosis is elevated with increasing intensity, where also B- and T lymphocyte levels are increased after exercise. It has been claimed that the elevation in B- and T cell numbers would be caused by redistributed cells that previously showed high replication activity (Sellami et al., 2018). More precisely, an acute moderate bout of cycling at 50% of P_{max} for 20 minutes has been shown to increase CD4+ and CD8+ T cells immediately after exercise, whereby their number decreased one hour after exercise (Mazzeo et al., 1998). Regarding NK-cells**,** a vigorous bout of exercise (an incremental cycling step test to exhaustion) has been found to contribute to a rise of NK cells, whereby the numbers decreased after two hours and returned to baseline 20 hours after exercise (Sellami et al., 2018). Graff et al. (2022) investigated the effects of a moderate intensity

walking session on a treadmill at 60-70% of heart rate reserve for 30 minutes on the acute change of lymphocyte counts. The authors reported an increase in total lymphocyte number and lymphocyte subpopulations immediately after exercise, with numbers dropping below baseline levels after one hour (Graff et al., 2022). This data is supported by the work of Cerqueira et al. (2020) that summarizes several studies investigating the effects of either moderate or vigorous exercise sessions on these responses. According to these authors, a decrease of lymphocytes and monocytes was reported one hour after vigorous intensity after an initial postexercise increase.

Apoptosis of lymphocytes, the programmed cell death of senescent, damaged cells, is a mechanism that occurs more frequently after vigorous training sessions (progressive exercise at 80% $VO_{2\ max}$ till exhaustion) compared to moderate (60% $VO_{2\ max}$) in young subjects between 20-30 years (Sellami et al., 2018).

In summary, previous studies have shown that both moderate and vigorous intensity exercise can have an immune-modulating effect. However, the findings are not consistent. For example, some studies found moderate-intensity exercise not to affect lymphocytes (Neves et al., 2015), whereas others reported significant effects (Graff et al., 2022; Sellami et al., 2018). Additionally, the timing of blood collection varied between studies, complicating the assessment of concentration changes over time. Most studies measured blood leucocytes only until one hour after exercise cessation, although evidence suggests that further changes may be observed at later points of recovery (Davison, 2011; Graff et al., 2022; Islam et al., 2024; Jackson et al., 2022; Koivula et al., 2023; Nieman et al., 1991; Nieman et al., 2012; Vider et al., 2001). Moreover, few studies (Cerqueira et al., 2020; Neves et al., 2015) compared intensities and the rather heterogenous intensity prescription in the literature makes it difficult to draw precise conclusions about immunomodulation associated with moderate and vigorous intensity-exercise. It should also be noted that previous studies defined exercise intensity based on several different measures such as HR_{max} (Cannon et al., 1994), heart rate (HR) reserve (Graff et al., 2022), $VO_{2\ max}$ (Nieman et al., 1991), P_{max} (Nieman et al., 2012) or subjective ratings of perceived exertion (McCarty et al., 1987), which further complicates the comparison between studies. Similarly, the exercise durations also varied greatly between studies, ranging between 30 seconds (Davison, 2011) and two hours (Nieman et al., 2012). The differences in duration and intensity within the moderate training intensity range described above (varying between 40-60% $VO_{2\ max}$ and 30-45 min in the studies examined) make it difficult to understand the individual effect of each immune-modulating factor. Hence, studies are urgently needed that tightly control the training duration and energy expenditure or total work, allowing for the effects of intensity to be properly investigated.

1.5. Summary and research question

There is substantial evidence to suggest that regular endurance exercise exerts beneficial effects on immune cell function and represents an effective strategy to counteract immune senescence and inflammaging (Chastin et al., 2021; Nieman & Wentz, 2019).

Illustrated in simplified form in the J-curve model (Nieman, 1994), moderate to vigorous exercise of up to 60 minutes has a protective effect on the immune system (Nieman & Wentz, 2019). Acute functional limitations of the immune system, excessive inflammation levels and susceptibility to infection due to the "open window" effect can be recorded at very high, prolonged intensity (Baskerville et al., 2024; Nieman, 1994).

However, it is not yet possible to say with certainty how exercise intensity affects the immune response to exercise. There are still knowledge gaps when it comes to the effects of exercise intensity as well as the impact of age on these responses (Baskerville et al., 2024; Sellami et al., 2018).

Accordingly, the present thesis aimed at addressing the following research questions:

- Does a single bout of endurance exercise alter total leukocyte counts and subpopulation distribution in peripheral blood?
- Are there time-dependent changes in leukocyte concentrations following an acute exercise bout?
- Does exercise intensity (moderate vs. heavy) differentially affect leukocyte responses?

2. Methods

2.1. Design of the study

As shown in *Figure 3*, subjects from two age groups, younger (18 – 35 years) and older (> 65 years) were included in the study. Each participant attended a total of three sessions. In the first session, subjects were medically screened to confirm their eligibility for participation, whereas in the second and third session participants completed a moderate and high intensity exercise bout in a randomized order.

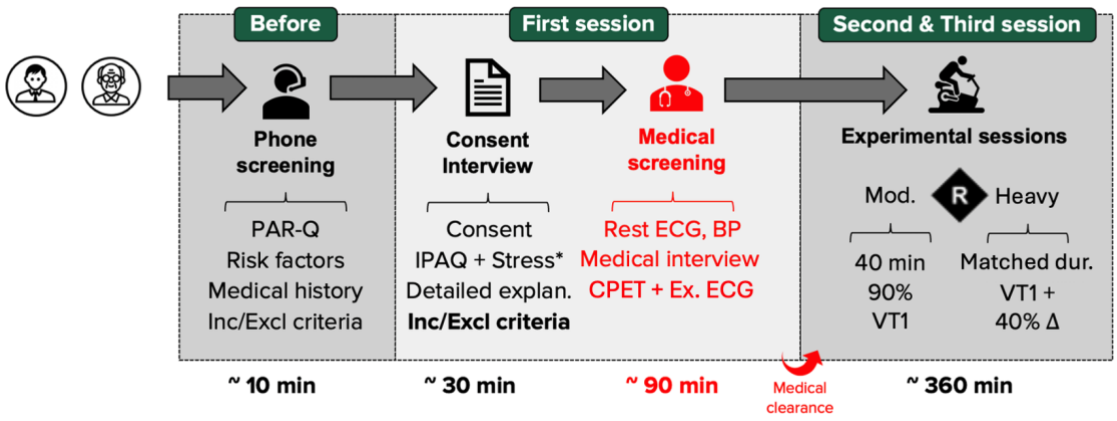

Figure 3: Design of the study. PAR-Q = Physical Activity Readiness – Questionnaire; IPAQ = International Physical Activity Questionnaire; ECG = Electrocardiogram; CPET = Cardio-pulmonary Exercise Test; Ex. ECG = Exercise Electrocardiogram; BP = Blood Pressure; Mod. = Moderate; VT1 = Ventilatory Threshold 1; 40% Δ = 40% of the Difference between VT1 and $VO_{2\ peak}$; Matched dur. = matched duration of the heavy bout with total work done at moderate bout.

The three sessions were preceded by a telephone screening, in which participants were checked for inclusion criteria. During the phone screening, volunteers were inquired about physical activity readiness and medical history. If initially cleared, they were subsequently invited for an in-person medical screening session, where their eligibility was confirmed and informed consent was acquired. In this first screening session, several clinical tests were performed (anthropometric measurements, resting electro cardiogram (ECG), blood pressure measurement, medical history, medication, physical activity readiness questionnaire (PAR-Q)). After completion of the assessments, the test results were reviewed and discussed with the participants in a medical consultation led by a qualified

specialist in sports medicine and cardiology. Following this consultation, a graded exercise test was performed. The specific intensities for the exercise interventions were determined individually based on the incremental test performed (explained in detail below). After the incremental test the study physician assessed the exercise ECG data for abnormalities and subsequently for inclusion of the participant. If participants were cleared and completed the incremental test during the screening, they were invited to attend two additional visits. During these visits, participants performed either a moderate or a heavy intensity exercise, the order of which was randomized. The study sessions were interspersed by a minimum of three days. Before the first experimental session, participants were asked to complete a 24-h food recall that spanned the day before and the morning of the first experimental session. They were then requested to replicate the same food intake on the day before the second experimental session to balance nutritional intake before both exercise sessions.

2.2. Subjects

In the study, young male subjects aged 18-35 years and older male subjects aged 65 years or older were recruited. Subjects met the inclusion criteria if they were considered healthy as per the medical screening described above and participated voluntarily in the study. In addition to the medical clearance, the physical readiness to participate was determined using the PAR-Q questionnaire (Warburton et al., 2011).

Exclusion criteria for the study included acute injuries or illnesses that could lead to disability in daily life or require treatment. Furthermore, acute infections from the week before or during the measurement period were considered exclusion criteria. Exclusion criteria also included known cardiovascular, immune, muscular or skeletal diseases that could prevent sports practice, severe chronic diseases (e.g., severe congenital heart defect (CHD), cancer, chronic obstructive pulmonary disease, uncontrolled hypertension), as well as the presence of abnormal bleeding or coagulopathy, auto-immune diseases, diabetes, smoking, obesity or shift work. In addition, regular intake of non-steroidal anti-inflammatory medication or steroid use for at least two weeks before the start of the study were also considered exclusion criteria. All participants gave their written informed consent to participate in the study that was approved by the University of Vienna Research Ethics Committee (processing number: 00959).

2.3. Examination procedure

The examination comprised a total of three test days, each separated by one week. The data collection was preceded by a screening session in which the subjects were tested for the above criteria and their endurance fitness was determined.

2.3.1. Screening

An abbreviated version of the Physical Activity Readiness Questionnaire (PAR-Q) (Canadian Society for Exercise Physiology, 2002) was used for telephone screening to ascertain, that subjects met the inclusion criteria. The call further served to explain the general study conditions and inquire anthropometric characteristics, physical activity levels and individual medical histories.

If candidates were deemed suitable for inclusion based on the initial telephone screening, an on-site medical screening was conducted. During this visit, clinical data were collected (including body mass and height, resting ECG, blood pressure, medical history, medication, PAR-Q health questionnaire).

In preparation for all three sessions, participants were asked to follow several recommendations prior to each appointment. These measures aimed to minimize factors that could affect the study outcomes investigated or influence exercise capacity on the test days. Specifically, subjects were instructed to abstain from caffeine and alcohol consumption 12 and 24 h, respectively, before testing, and to avoid moderate-to-vigorous exercise for at least 48 hours. They were also asked to consume only a light breakfast at least two hours before the start of each session.

All parameters were subsequently reviewed by a qualified physician to assess physical aptitude and verify that the inclusion criteria were met. Those medically cleared at this stage then completed an incremental Cardio-pulmonary Exercise Test (CPET) on a bicycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, The Netherlands) to determine their peak oxygen uptake ($VO_{2\ peak}$). The protocol started with a two-minute rest period for baseline assessment of gas exchange, lactate (Biosen C-Line, EKF Diagnostics, Penarth, United Kingdom), heart rate (HR) and blood pressure (BP), followed by a one-minute familiarization phase without resistance, during which participants maintained a cadence of approximately 60-80 revolutions per minute (rpm). This cadence was to be sustained throughout the test.

The incremental phase started at zero resistance, and workload was then increased in a ramp fashion according to age (20 W/min for younger and 15 W/min for older participants, respectively). Participants then continued the ergometer test until volitional fatigue set in or the participants could no longer maintain the minimum cadence. During the test, lactate, HR and rate of perceived exertion (RPE) were assessed every minute, whereas blood pressure was measured manually every 2 minutes using a cuff and stethoscope. All measurements were performed by trained medical personnel. After exhaustion, participants

completed a 6-minute cool-down phase, during which heart rate and blood pressure were recorded every minute until BP returned to baseline levels.

Breath-by-breath gas exchange and HR were continuously monitored using a metabolic system (Metamax 3B, Cortex Biophysik, Leipzig, Germany) and a HR monitor (Polar H10, Polar Electro, Kempele, Finland). Before each test, the gas analyzers were calibrated with standardized gases according to the manufacturer's instructions. The test was performed as described in the exercise testing guidelines of the American College of Sports Medicine (Liguori, 2021). Oxygen uptake data from the graded exercise test was 15-s time-averaged, and $VO_{2\ peak}$ was considered as the highest 15-s average. In addition, respiratory thresholds were determined visually by an experienced exercise physiologist and used to prescribe the intensity of the two experimental sessions.

2.3.2. Experimental sessions

Each participant completed two experimental sessions consisting of one moderate-intensity and one vigorous heavy-intensity exercise bout. The order of these sessions was randomized, and both were conducted on separate days to avoid carry-over effects. Exercise intensities for both conditions were determined from each participant's incremental test, based on their maximum performance and ventilatory thresholds.

Pre-exercise procedures

Upon arrival, participant's anthropometric data, including height, body mass (Seca GmbH & Co KG, Hamburg, Germany), and waist circumference (Seca 201, Seca GmbH & Co KG, Hamburg, Germany) were collected. Participants then rested in a seated position for 40 minutes, after which additional anthropometric measurements were obtained using bioelectrical impedance analysis (BIA) in the supine position (BIA, Nutriguard-MS + NutriPlus-Software, Version5.1, Data-Input, GmbH, Germany). Subsequently, a baseline venous blood sample was drawn from the antecubital vein while participants were seated.

Exercise protocol

Following a 6-min warm-up at 50 Watts (W) on the same cycle ergometer used during the incremental test (Lode Excalibur Sport, Groningen, The Netherlands), participants completed one of two exercise bouts: (i) a moderate-intensity bout, consisting of 40 minutes of continuous cycling at an intensity corresponding to 90% of the first ventilatory threshold (VT1), where VT1 was considered the boundary between the moderate and heavy intensity domains (Poole et al., 2016); and (ii) a heavy-intensity bout, performed at an intensity corresponding to VT1 + 40% Δ, where Δ (delta) represents the difference in power output between VT1 and the power associated with $VO_{2\ peak}$. This workload typically falls within the

heavy-intensity domain, below the critical power that demarcates the transition to the severe domain (approximately 50% Δ) (Jones et al., 2010).

Determination of VT1

VT1 was identified as the point where ventilation (VE) begins to rise disproportionately relative to oxygen uptake (VO_2). The determination followed standard multi-criteria assessment procedures, including: (i) identification of the first systematic increase in the VE/VO_2 ratio while the VE/VCO_2 ratio remained stable or decreased slightly; (ii) identification of the point where end-tidal oxygen pressure ($PETO_2$) began to increase systematically while end-tidal carbon dioxide pressure ($PETCO_2$) remained stable; (iii) inspection of the VE/VO_2-relationship to locate the inflection point where VE beings to rise disproportionately to VO_2; (iv) confirmation using respiratory exchange ratio (RER) value, as VT1 typically corresponds to an RER of approximately 0.85-0.90. All thresholds were determined independently by one experienced exercise physiologist and, when necessary, a second researcher was consulted to resolve discrepancies.

Mean response time correction

Because physiological responses (VO_2, ventilation, lactate, HR) typically lag changes in external workload during ramp tests, raw power values derived from these tests tend to overestimate the true steady-state intensities. To account for this mean response time and more accurately represent steady-state physiological conditions during constant-load exercise, power outputs corresponding to VT1 and $VO_{2\ peak}$ were corrected. Specifically, power output values obtained from the incremental test were adjusted by subtracting two-thirds of the ramp rate applied during testing (13.3 W for young participants and 10 W for older participants). A representative example of the mean response time correction and the determination of exercise intensities for the moderate and heavy bouts is shown in *Figure 4*.

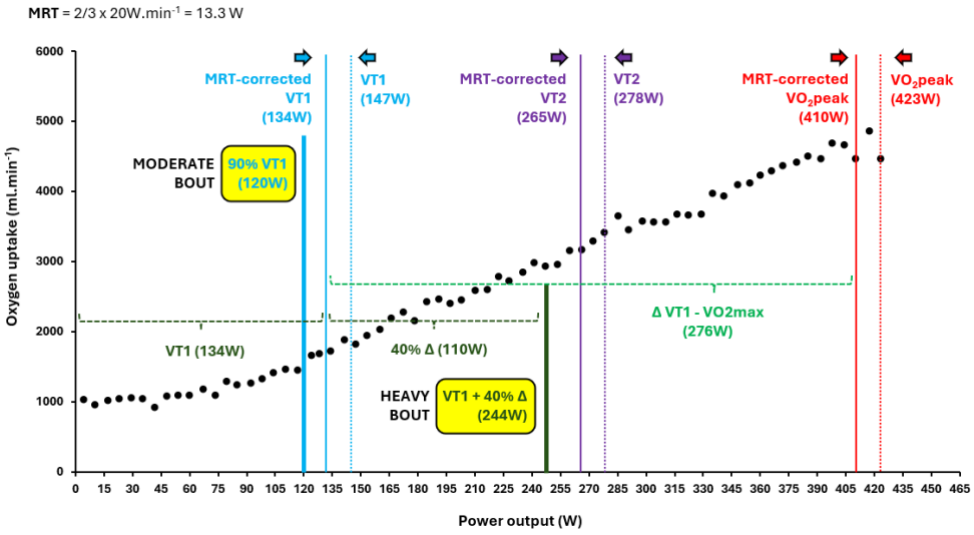

Figure 4: Representative example of the mean response time (MRT) correction applied to ventilatory threshold and VO2 peak data obtained from an incremental ramp test. The MRT correction (13.3W in this example) accounts for the delay between external workload and internal physiological responses, aligning ramp test thresholds with steady-state conditions. The moderate-intensity bout corresponds to 90% of the MRT-corrected VT1 (120 W; cyan), and the heavy-intensity bout corresponds to VT1 + 40% Δ (244 W; dark green). The second ventilatory threshold (VT2) is also displayed for reference.

Work-matching between intensities

To allow a direct comparison between the two intensity domains, the total work performed during the moderate-intensity session was used to determine the duration of the heavy-intensity bout. Total work (in Joule (J)) for the moderate bout was calculated as:

$$Total\ work\ (J) = Power\ (W) \times\ Duration\ (\mathrm{s})$$

Formula 1: Calculation of total work.

The required duration for the heavy-intensity session was then determined as:

$$Heavy\ duration\ (s) = \frac{Moderate\ total\ work\ (J)}{Heavy\ power\ output\ (W)}$$

Formula 2: Determination of heavy-intensity duration.

Thus, both sessions involved an equivalent total external workload, differing only in exercise intensity.

Post-exercise procedures

Following the completion of each bout, participants performed a 5-min cool-down at 50 W. Ratings of perceived exercise (RPE) were recorded midway through and immediately after each exercise session, and HR was continuously monitored using a Polar H10 chest strap. To assess the acute effects of exercise on immune cell mobilization, venous blood samples were collected at rest, and 30 minutes, one-, two-, and four-hours post-exercise. Participants remained in the laboratory resting quietly between collections. Further, a standardized light meal was provided after the 2-hour post-exercise blood draw. An overview of the experimental timeline is presented in *Figure 5* below.

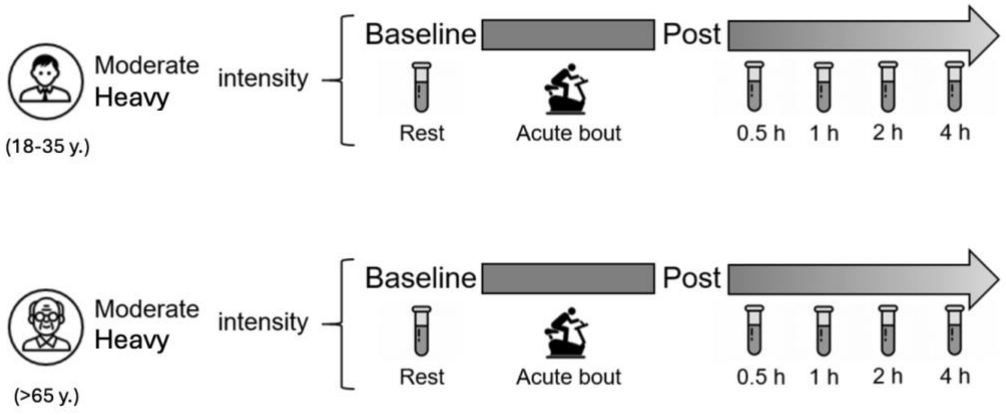

Figure 5: Overview of the experimental session protocol for young and older participants.

2.4. Evaluation of the data

Hematological Analysis

Whole blood was collected using 4 mL EDTA-coated Vacuette® tubes (Greiner Bio-One, Kremsmünster, Austria) according to the manufacturer's instructions. Immediately after sampling, tubes were gently inverted 8-10 times to ensure proper mixing with the anticoagulant. Samples were then analyzed using an MS4s® automated hematology analyzer (Melet Schloesing Laboratoires, Osny, France). A complete blood count was performed to determine leucocytes (10^3/µl), lymphocytes (%), neutrophil (%), monocyte (%),

eosinophil (%), basophil (%) levels, as well as erythrocyte count (10^6/µl), hematocrit (%), hemoglobin (g/dL), platelet count (10^3/µl) and mean platelet volume (fL).

Physical Activity and Perceived Stress Assessment

Physical activity levels were evaluated using the long version of the International Physical Activity Questionnaire (IPAQ), with results expressed in metabolic equivalent minutes per week (MET-minutes/week) in accordance with the official International Physical Activity Questionnaire Scoring Protocol (IPAQ Research Committee, 2005). Perceived stress, which may influence immune parameters, was assessed using the 10-item Perceived Stress Scale (PSS-10; Schneider et al., 2020). The PSS-10 scale provides a total score ranging from 0 to 40-points, with higher scores indicating greater perceived stress (Schneider et al., 2020).

2.5. Statistical analysis

Data normality and homogeneity of variances were tested using Shapiro-Wilk and Levene's tests, respectively. Differences in variables assessed during the experimental sessions (RPE, HR, and exercise duration) were analyzed using paired samples *t*-tests, effect sizes are reported via Cohen's d. Immune cell responses were examined using two-way repeated-measures ANOVAs (*intensity* (moderate vs. heavy) × *time* (five time points)) in the younger group. Additionally, to compare immune modulation between age groups, two-way repeated-measures ANOVAs (*group* (young vs. older participants) × *time* (five time points)) were conducted. Violations of sphericity were corrected using the Greenhouse-Geisser adjustment. Effect sizes are reported via partial eta squared. When significant main or interaction effects were detected, Sidak's post hoc tests were applied. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27 (IBM Corp., NY, USA). The significance level was set at $\alpha = 0.05$.

3. Results

3.1. Participants enrollment

A total of 23 participants provided their informed consent and were initially included in the present investigation, of which 13 belonged to the young and 10 to the older participants' group. During the course of the study, one young participant had an accident not related to the study and had to be excluded, whereas one participant in the older participants group was excluded by the study physician due to low overall fitness. An additional three participants in the older group presented abnormalities in the ECG results during the incremental exercise test and were, therefore, excluded from the study due to safety reasons, resulting in a final sample of six participants in this age group. The characteristics of the final sample are presented in *Table 1*. All resting values (HF, BP) were in the normal range, with four young subjects showing tendencies towards high blood pressure, which were not confirmed in follow-up assessments. Three young participants (Antihistamines: n = 1, Selective Serotonin Reuptake Inhibitors: n = 1, Antidepressants: n = 1) and two older participants (Alpha Receptor Blockers: n = 1, Antihypertensives: n = 1) were taking medication during the study process. Participants were all nonsmokers, showed similar (p = 0.178) levels of stress and slept between 7-9 hours per day.

Table 1: Characterization of the participants included in the study.

	Young	Old
n	12	6
Age (yrs.)	28.2 ± 2.4	65.5 ± 1
Body mass (kg)	85.9 ± 10.8	79.0 ± 13.8
Height (m)	1.88 ± 0.12	1.81 ± 0.07
BMI (kg/m^2)	25.4 ± 2.9	24.2 ± 2.1
Body fat %	20.9 ± 6.6	17.2 ± 4.9
Lean mass (kg)	68.4 ± 6.6	65.4 ± 5.5
Waist circ. (cm)	88 ± 8	87.4 ± 6.1
Resting HR (bpm)	65 ± 13	61.3 ± 1.5

Systolic / diastolic resting BP (mmHg)	130 ± 28.8 / 75 ± 5	122.5 ± 21.3 / 82.5 ± 13.8
PA level (MET-min/week)	1682.3 ± 760.4	1671 ± 1427.3
High	1	2
Moderate	10	4
Low	1	
Study years	14.5 ± 3.8	13 ± 2.5
Perceived stress score (PSS)	12.4 ± 4.1	9 ± 2

BMI: body mass index; HR: heart rate; BP: blood pressure; PA: physical activity; Values are mean ± SD (if normally distributed) or median ± IQRs.

3.2. Incremental test results (first session)

The participants' performance in the incremental test is shown in *Table 2*. On average, the test lasted between 16- 18 min in young and older participants, respectively, with no difference between groups ($p = 0.179$). Peak HR and peak power output, on the other hand, were higher in the younger participants ($p < 0.001$, $d = 3.035$ and $p = 0.002$, $d = 1.800$, respectively). This was accompanied by a higher aerobic capacity ($VO_{2\ peak}$) in younger compared with the older group (44.0 ± 8.9 ml/kg/min vs. 39.0 ± 2.8 ml/kg/min; $p = 0.023$, $d = 1.253$). Furthermore, all participants included in the study reached at least two of the secondary criteria, supporting the assumption that maximal effort was attained (Howley et al., 1995).

Table 2: Results of the incremental test.

	n	Young	Old
Test duration (s)	18	982.9 ± 107.7	1050 ± 60
HR_{max}	17	189.3 ± 5.3 *	157.5 ± 24.5
RER_{max}	17	1.2 ± 0.1	1.1 ± 0.1
LAC_{max} (mmol/L)	9	11.9 ± 4.3	8.1

RPE $_{final}$	18	20	20 ± 0.4
Peak power (W)	18	333.9 ± 43.5 *	267 ± 23.8
rel. VO_{2peak} (ml/kg/min)	18	44 ± 8.9 *	39 ± 2.8
VT_1 (W)	18	164.5 ± 40.5 *	129.5 ± 27.8
VT_2 (W)	18	251 ± 74.5 *	189.5 ± 48.8

*HR_{max}: maximal heart rate; RER_{max}: maximal respiratory exchange ratio; LAC_{max}: maximal lactate concentration; RPE: rate of perceived exertion; $VO_{2\ peak}$: relative aerobic capacity; VT_1: first ventilatory threshold; VT_2: second ventilatory threshold; LAC_{max} values are presented for 6 and 3 participants in the young and older adults' groups due to technical issues with the lactate measurement; * = significantly higher than older group; Values are mean ± SD (if normally distributed) or median ± IQRs.*

Due to a technical malfunction of the lactate analyzer, reliable maximal lactate values were obtained only in six young participants and three older participants. The median of the lactate measurements of the older group is shown in *Table 2*. For one young participant, HR_{max} couldn't be assessed because of a malfunction of the ECG-device during the test. Also, the RER_{max} value is missing for one young participant. In all these cases, the remaining measures were used to assess whether a maximal effort was made.

3.3. Exercise sessions (second and third sessions)

The prescribed intensity and duration of the two experimental sessions, which were determined based on the results from the incremental test, are summarized in *Table 3*. For the young group, the intensity of the sessions corresponded to 143 ± 30.6 W and 225.6 ± 38.8 W for the moderate and heavy bouts, respectively ($t = -22.223$, $p < 0.001$, $d = 6.415$). Based on the intensity and the duration of the moderate session, the total work of the session was calculated (342.4 ± 72.9 kJ), and used alongside the intensity established for the heavy bout to equate the duration of the latter (1512.4 ± 108.9 s) to match the total work of the moderate bout. In the older group, the intensities of the two bouts corresponded to 107.5 ± 24.8 W and 173 ± 24.5 W for the moderate and heavy bouts, respectively ($t = -62.051$, $p < 0.001$, $d = 25.332$). In this group, the total work of the moderate session was calculated to be 258.1 ± 59.9 kJ, resulting in a heavy intensity session that lasted, on average, 1479.9 ± 129.7 s.

In general, both young and older participants finished the moderate and heavy intensity bouts as intended, but some deviations occurred during the sessions that affected the data collection of a few of the outcomes. Specifically, the first two young participants completed shorter heavy intensity sessions due to exhaustion and, consequently, RPE was only recorded at the test termination. Another two young participants, in turn, paused for two and one minute, respectively, due to exhaustion towards the end of the heavy bout, but concluded the session which lasted longer to compensate for the pause duration. In the former, however, RPE was not acquired midway through the session. Heart rate data were missing for one participant in which the HR sensor malfunctioned during the heavy session.

Table 3: Characteristics of the two exercise sessions prescribed in the study for each of the groups investigated.

	Young		Old	
	Moderate	Heavy	Moderate	Heavy
Work (kJ)	342.4 ± 72.9		258.1 ± 59.9	
Time (s)	2400	1512.4 ± 108.9	2400	1479.9 ± 129.7
Intensity (W)	143 ± 30.6	225.6 ± 38.8 *	107.5 ± 24.8	173 ± 24.5 *

*Sessions were matched based on the total work of the 40-min moderate session; * = significantly higher than moderate intensity; Values are mean ± SD*.

Despite this, no significant differences were observed between the prescribed and the actual duration of the moderate and heavy bouts neither in the young ($V = 15$, $p = 0.057$; $Z = -0.866$, $p = 0.409$, respectively), nor in the older participants (moderate: $V = 15$, $p = 0.058$; heavy: $Z = -2.023$, $p = 0.058$, respectively). As expected, the peak and average HR responses were significantly lower ($t = -6.756$; $p < 0.001$, $d = 1.927$ and $t = -9.187$; $p < 0.001$, $d = 3.914$, respectively) in the moderate bout (147.1 ± 17.2 bpm and 127 ± 13.2 bpm) than in the heavy bout (176 ± 12 bpm and 157.9 ± 10.3 bpm), a result that was also mirrored by the participants' subjective perceptions of effort as assessed halfway through (11.8 ± 2.8 vs 14.8 ± 0.9; $t = -9.507$; $p < 0.001$, $d = 3.169$) and immediately after the two bouts (13.2 ± 1.7 vs. 17.8 ± 2.8; $t = -14.432$; $p < 0.001$, $d = 4.166$). In the older participants, the average HR corresponded to 108.2 ± 16 in the moderate and 129 ± 20.75 in the heavy bout ($t = -5.295$; $p = 0.003$, $d = 2.162$). Participants' rating of perceived exertion at the middle (11.8 ± 2.6 vs. 14.3 ± 1.9; $t = -4.564$; $p = 0.006$, $d = 1.863$) and at the end of the bouts (12 ± 2.4 vs. 16.5 ± 1.5; $t = -6.090$; $p = 0.002$, $d = 2.486$) were also lower in the moderate

session for this age group. A visual comparison between the HR values of the two exercise bouts is presented separately for each group in *Figure 6*, collected rate of perceived exertion (RPE) data is presented for each group in *Figure 7*.

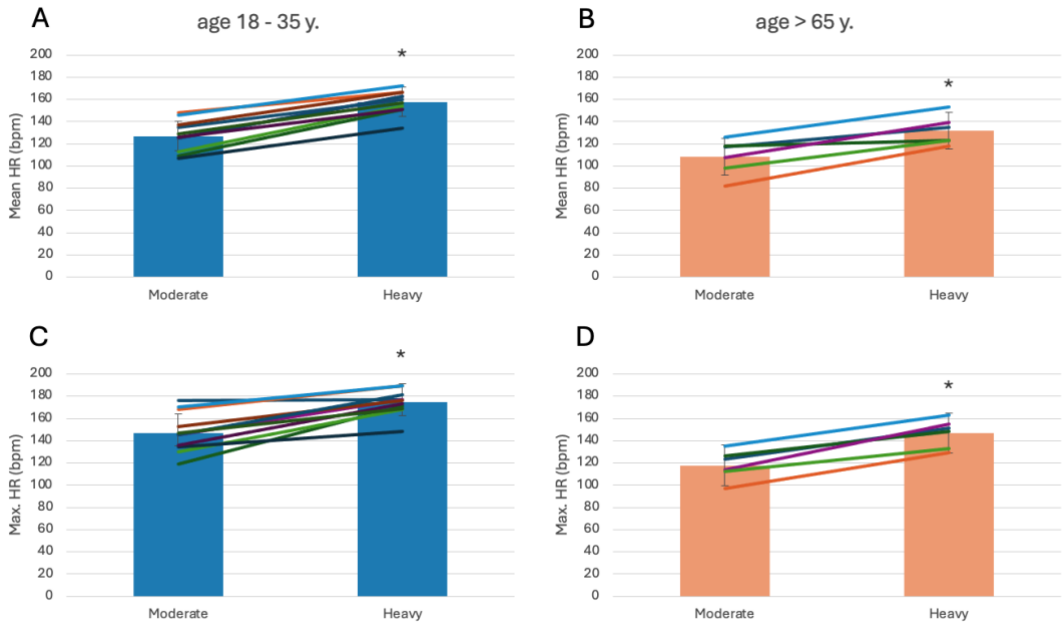

*Figure 6: Pairwise comparison of the mean and maximal heart rate (HR) responses between moderate- and heavy-intensity exercise bouts in young (A, C) and older (B, D) participants. Bars represent group means ± SD, while connecting lines indicate individual participant data. * = significantly different from the moderate-intensity bout ($p < 0.05$).*

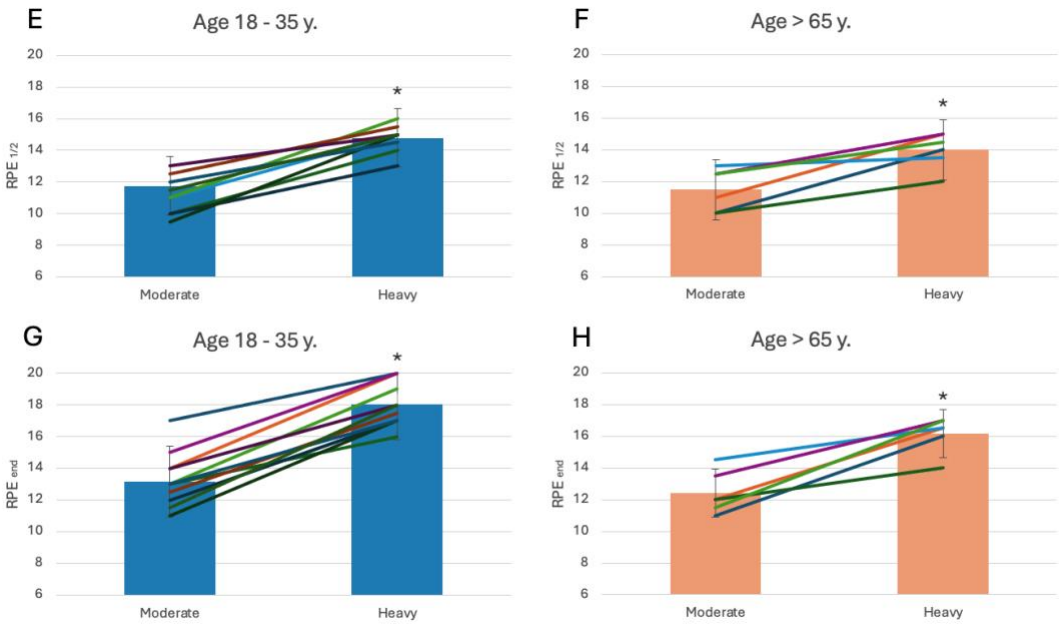

*Figure 7: Pairwise comparison of the rate of perceived exertion (RPE) responses between moderate- and heavy-intensity exercise bouts in young (E, G) and older (F, H) participants. Bars represent group means ± SD, while connecting lines indicate individual participant data. * = significantly different from the moderate-intensity bout (p < 0.05).*

3.4. Immune cell mobilization following acute exercise in young participants (second and third sessions)

Due to the small sample size in the older participants group, the main analysis in the present thesis focused on the effects of exercise intensity on immune cell mobilization in the young cohort. The influence of age on these responses is presented separately in a subsequent chapter; however, those findings should be viewed as exploratory. A summary of all immune cell measures across time points for the young participants is provided in *Table 4*.

3.4.1. Leukocytes

Total leukocyte counts in the young participants (N = 10) are shown in *Figure 8*. Since Mauchly's test indicated a violation of the assumption of sphericity for both the main effect of time (p = 0.004) and the time × intensity interaction (p = 0.019), the Greenhouse-Geisser correction was applied. A significant time × intensity interaction (F (6, 2.349) =5.062, p = 0.013, ηp^2 = 0.360), as well as a significant main effect of time (F (6, 1.751) = 24.099, p < 0.001, ηp^2 = 0.728) were found, whereas the main effect of intensity approached but did not reach significance (F (9, 1) = 4.788, p = 0.056). Simple effects analyses revealed that total leukocyte counts increased significantly over time after both exercise bouts, with a greater

magnitude of change following the heavy-intensity exercise. The leukocyte response peaked between two- and four-hours post-exercise. Compared with baseline, total leukocyte counts were significantly elevated at two hours ($p = 0.003$) and four hours ($p = 0.004$) post-exercise. When comparing between intensities, leukocyte counts were significantly higher following the heavy-intensity exercise at two hours ($p = 0.010$) and four hours ($p = 0.015$), whereas no differences were observed between the other time points (all $p > 0.05$)

Table 4: Mean values of immune cell types across all time points in the young participants group (N = 10).

		Baseline	**0,5h**	**1h**	**2h**	**4h**
Leukocytes (× 10^3/µl)	Moderate	4.94 ± 1.07	5.44 ± 1.30	5.95 ± 1.58	6.67 ± 1.55 *†‡	6.69 ± 1.73 *†‡
	Heavy	5.48 ± 1.10	5.70 ± 1.38	6.23 ± 1.40	8.50 ± 2.28 *†‡#	8.19 ± 2.22 *†‡#
Lymphocytes (%)	Moderate	35,27 ± 8,31	32,34 ± 9,97	29,65 ± 6,97 *†	28,94 ± 6,22 *†‡*f*	30,36 ± 5,38 *†
	Heavy	33,80 ± 11,12	33,13 ± 9,50	27,32 ± 8,59 *†	20,65 ± 7,38 *†‡*f*	24,66 ± 6,64 *†
Monocytes (%)	Moderate	5.20 ± 1.17	5.50 ± 1.51	5.33 ± 1.36	4.94 ± 1.13	5.36 ± 1.65
	Heavy	5.33 ± 1.63	5.05 ± 1.02	5.02 ± 0.98	4.98 ± 1.46	4.57 ± 1.51
Neutrophils (%)	Moderate	55.06 ± 8.94	59.01 ± 9.98	62.01 ± 7.65 *†	63.69 ± 6.26 *†‡*f*	61.55 ± 5.69 *†
	Heavy	56.39 ± 13.44	58.57 ± 11.31	65.17 ± 9.80 *†	72.29 ± 9.39 *†‡*f*	68.73 ± 8.05 *†
Eosinophils (%)	Moderate	3.98 ± 2.09	2.53 ± 1.65 *	2.46 ± 1.55 *	1.95 ± 0.99 *†	2.09 ± 1.16 *
	Heavy	4.06 ± 2.23	2.77 ± 1.62 *	1.97 ± 1.07 *	1.56 ± 1.32 *†	1.53 ± 0.60 *
Basophils (%)	Moderate	0.49 ± 0.29	0.62 ± 0.24	0.55 ± 0.32	0.48 ± 0.20	0.64 ± 0.28
	Heavy	0.42 ± 0.20	0.48 ± 0.32	0.52 ± 0.31	0.52 ± 0.27	0.51 ± 0.30
Erythrocytes (× 10^6/µl)	Moderate	4.92 ± 0.20	5.03 ± 0.22	5.02 ± 0.18	5.00 ± 0.19	4.98 ± 0.20
	Heavy	4.88 ± 0.21	5.027 ± 0.29	5.025 ± 0.28	4.96 ± 0.25	4.93 ± 0.23

Hematocrit (%)	Moderate	44.08 ± 1.60	45.29 ± 1.75 *	45.32 ± 1.50 *	45.17 ± 1.70	45.08 ± 1.91
	Heavy	43.66 ± 1.75	45.31 ± 2.10 *	45.11 ± 2.21 *	44.68 ± 2.37	44.59 ± 1.93
Hemoglobin (g/dl)	Moderate	14.65 ± 0.70	14.80 ± 0.68	14.81 ± 0.75	14.65 ± 0.70	14.62 ± 0.82
	Heavy	14.71 ± 0.67	14.90 ± 0.68	14.69 ± 0.76	14.64 ± 0.80	14.61 ± 0.67
Thrombocytes (× 10^3/µl)	Moderate	282.40 ± 104.96	296.50 ± 138.96	259.40 ± 90.78	264.00 ± 100.11	303.40 ± 149.50
	Heavy	345.60±192.720	315.10 ± 157.78	344.70 ± 222.83	322.90 ± 179.07	300.10 ± 171.73
Mean Platelet Volume (fL)	Moderate	7.61 ± 0.80	7.69 ± 0.90	7.88 ± 0.68	7.83 ± 0.75	7.58 ± 0.75
	Heavy	7.69 ± 1.02	7.92 ± 0.78	7.59 ± 0.95	7.74 ± 0.94	7.88 ± 0.93

× 10^3/µl= × 10^3 cells /micro liter; × 10^6/µl= × 10^6 cells /micro liter; g/dL = grams/deciliter; fL = femtoliter; % = proportion to total leucocytes (or total blood volume for Hematocrit). * = significantly different from baseline ($p < 0.05$); † = significantly different from the 0.5 h time point ($p < 0.05$); ‡ = significantly different from the 1 h time point ($p < 0 .05$); *ƒ* = significantly different from the 4 h time point ($p < 0.05$); # = significantly different from the moderate-intensity response ($p < 0.05$). *Values are mean ± SD.*

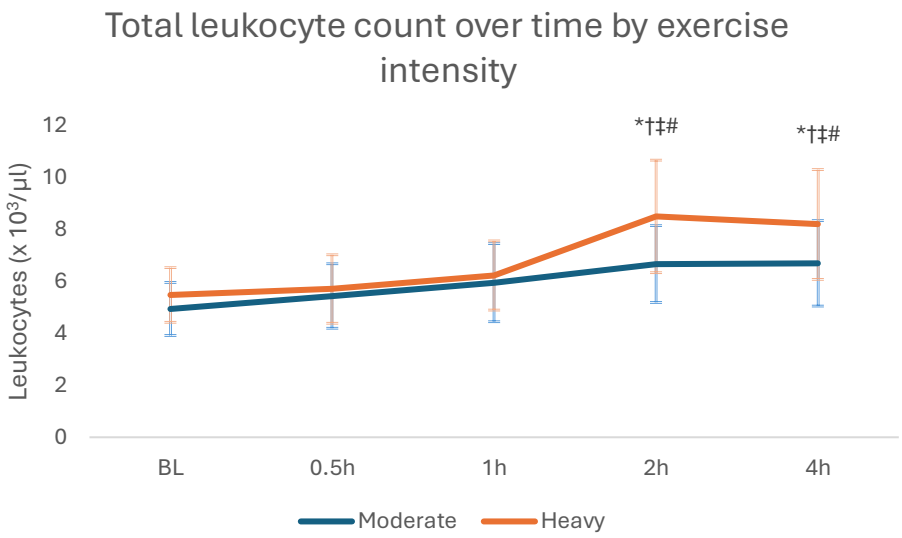

*Figure 8: Time course of total leukocyte counts following moderate- and heavy-intensity exercise in healthy young men (n = 10). × 10^3/µl = × 10^3 cells /micro liter; * = significantly different from baseline for both intensities (p < 0.05); † = significantly different from the 0.5 h time point for both intensities (p < 0.05); ‡ = significantly different from the 1 h time point for both intensities (p < 0 .05); # = significantly different from the moderate-intensity response (p < 0.05). Values are mean ± SD.*

3.4.2. Lymphocytes

The temporal changes in lymphocyte percentages are summarized in *Table 4* and depicted graphically in *Figure 9*. Both intensities exhibited an initial decrease in lymphocyte percentage, followed by a partial recovery at four hours post-exercise. A significant main effect of time was detected (F (6, 1.848) = 27.172; p < 0.001; ηp² = 0.751), whereas the intensity × time interaction was not significant (F (6, 1.270) = 3.616; p = 0.076). No main effect of intensity was observed (N = 10; F (9, 1) = 2.040; p = 0.187). Post hoc analyses revealed that the proportion of lymphocytes relative to total leukocyte decreased significantly at one hour (p = 0.006), two hours (p > 0.001), and four hours (p = 0.021) compared to baseline. The lowest lymphocyte percentage occurred around two hours post-exercise, which differed significantly from the values at one hour (p = 0.001) and four hours (p = 0.009).

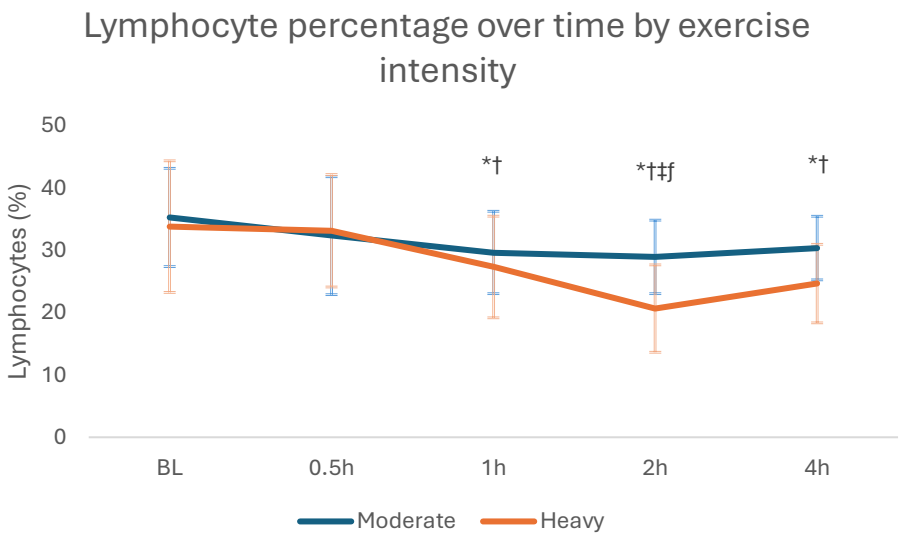

*Figure 9: Time course of lymphocyte percentages following moderate- and heavy-intensity exercise in healthy young men (n = 10). * = significantly different from baseline for both intensities (p < 0.05); † = significantly different from the 0.5 h time point for both intensities (p < 0.05); ‡ = significantly different from the 1 h time point for both intensities (p < 0 .05); ƒ = significantly different from the 4 h time point for both intensities (p < 0.05). Values are mean ± SD.*

3.4.3. Neutrophils

A significant main effect of time was observed for neutrophil proportion relative to total leucocytes (F (6, 1.654) = 25.264; p < 0.001; ηp^2 = 0.737), whereas no significant main effect of intensity was detected (F (9, 1) = 2.167; p = 0.175). The time × intensity interaction was also not significant (F (6, 1.349) = 3.318; p = 0.085). The temporal progression of neutrophil percentages is illustrated in *Figure 10* and summarized in *Table 4*.

Post hoc comparisons revealed that neutrophil percentages were significantly higher at one hour (p = 0.007), two hours (p = 0.002) and four hours (p = 0.02) post-exercise. The neutrophil percentage peaked around two hours, with values at this time point differing significantly from those at one hour (p = 0.008) and four hours (p = 0.025), respectively, for both intensities.

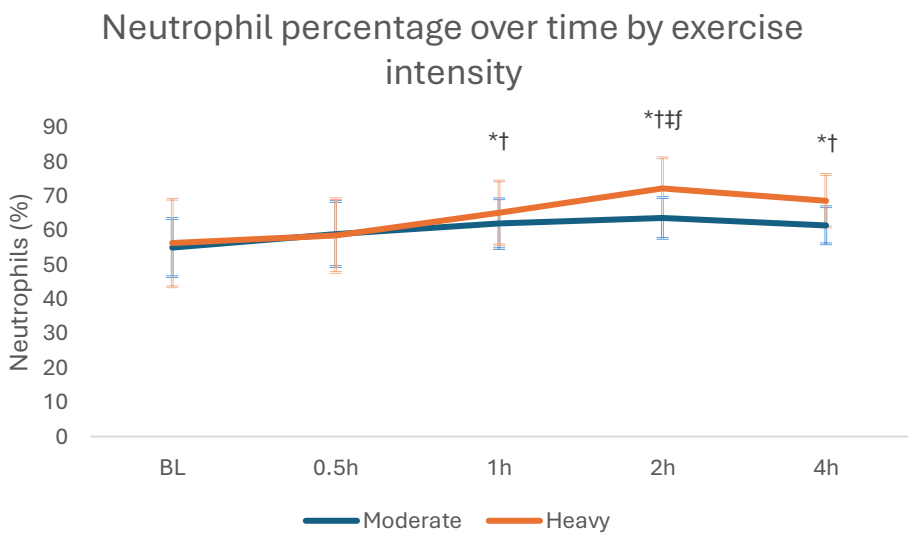

*Figure 10: Time course of Neutrophile percentages following moderate- and heavy-intensity exercise in healthy young men (n = 10). * = significantly different from baseline for both intensities (p < 0.05); † = significantly different from the 0.5 h time point for both intensities (p < 0.05); ‡ = significantly different from the 1 h time point for both intensities (p < 0 .05); ƒ = significantly different from the 4 h time point for both intensities (p < 0.05). Values are mean ± SD.*

3.4.4. Eosinophils

The temporal changes in eosinophil proportion relative to total leukocytes are shown in *Figure 11* and summarized in *Table 4*. No significant main effect of intensity was found (F (9, 1) = 0.454; p = 0.517). In contrast, a significant main effect of time was observed (F (6, 1.737) = 22.084; p < 0.001; ηp^2 = 0.710), while the time × intensity interaction was not significant (F (6, 4) = 1.714; p = 0.168). Post hoc analyses revealed that eosinophil percentages were significantly lower than baseline at 30 minutes (p = 0.025), one hour (p = 0.009), two hours (p < 0.001) and four hours (p = 0.008) post-exercise.

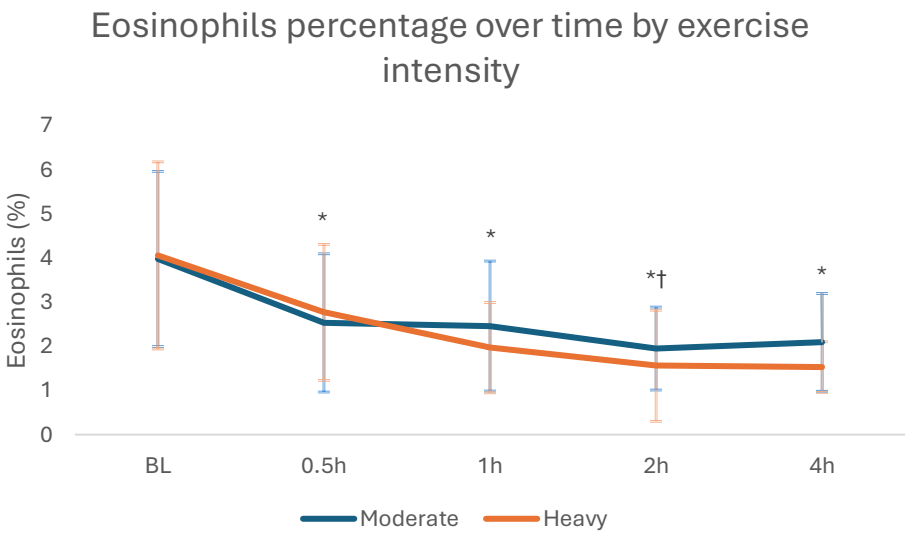

*Figure 11: Time course of Eosinophile percentage following moderate and heavy endurance exercise in healthy young men (n = 10). * = significantly different from baseline for both intensities (p < 0.05); † = significantly different from the 0.5 h time point for both intensities (p < 0.05). Values are mean ± SD.*

3.4.5. Monocytes and Basophils

No significant main effects of intensity or time, and no significant intensity × time interactions were observed for monocytes (p = 0.428, p = 0.626, p = 0.435, respectively) or basophils (p = 0.135, p = 0.658, p = 0.886, respectively). These data indicate stable proportions of these leukocyte subpopulations across both exercise intensities and all time points.

3.4.6. Red Blood Cells and Platelet-Related Measures

Temporal changes in erythrocyte counts are shown in *Figure 12*. Although a significant main effect of time was detected ($F(6, 4) = 4.094$; $p = 0.008$; $\eta p^2 = 0.313$), the post hoc test did not reveal any pairwise differences. Neither a main effect of intensity ($F(9, 1) = 0.312$; $p = 0.608$), nor a significant intensity × time interaction was observed ($F(6, 4) = 0.283$; $p = 0.868$).

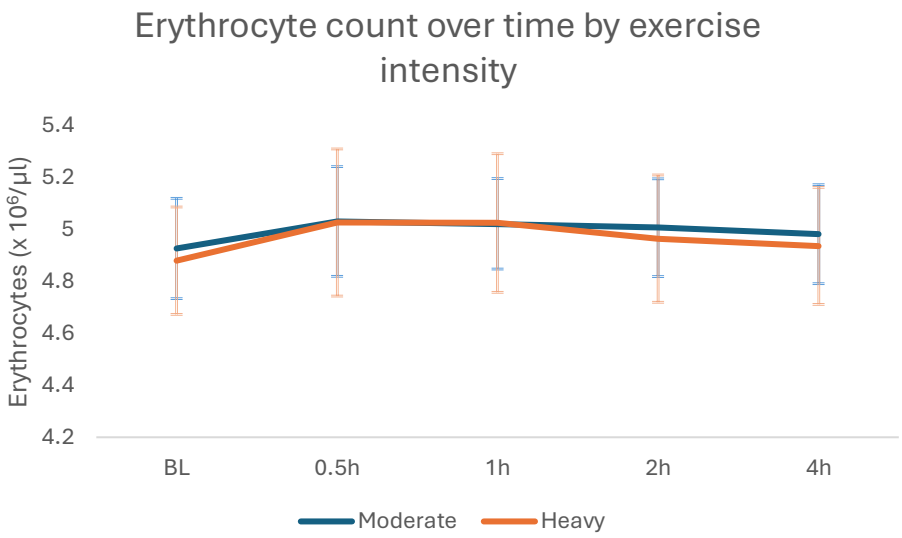

Figure 12: Time course of Erythrocyte counts following moderate- and heavy-intensity exercise in healthy young men (n = 10). × 10^6/µl = × 10^6 cells / micro liter. Values are mean ± SD.

The changes in hematocrit, i.e., the proportion of red blood cells relative to total blood volume, are shown in *Figure 13*. No significant main effect of intensity was found (F (9, 1) = 0.563; p = 0.472), while a significant main effect of time was detected (F (6, 4) = 5.355; p = 0.002; ηp^2 = 0.373). Post hoc analyses revealed significant increases from baseline at 30 minutes (p = 0.025) and one hour measurement (p = 0.022) post-exercise for both intensities. No significant intensity × time interaction was found (F (6, 4) = 0.249; p = 0.909).

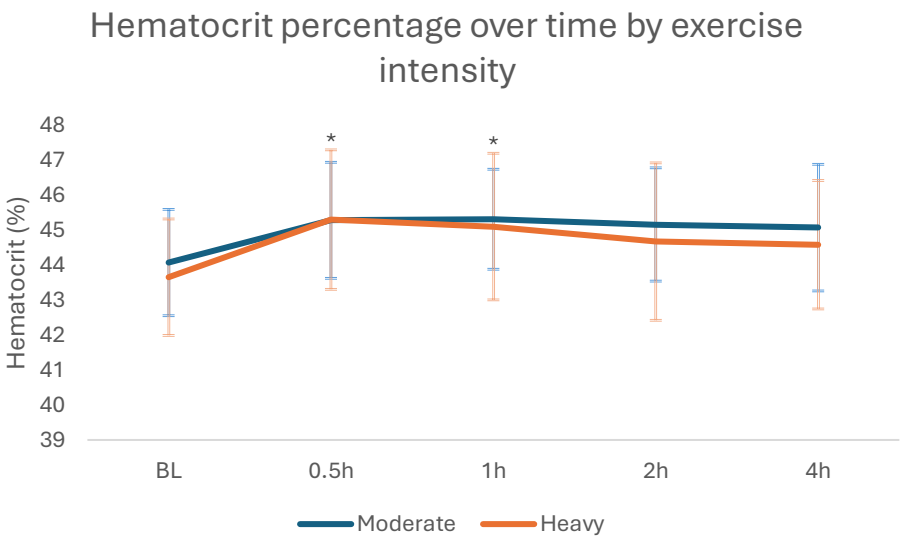

*Figure 13: Time course of Hematocrit percentage following moderate- and heavy-intensity exercise in healthy young men (n = 10). * = significantly different from baseline for both intensities (p < 0.05). Values are mean ± SD.*

Finally, neither a significant main effect of intensity or time, nor significant interactions were observed for hemoglobin (p = 0.972, p = 0.069, p = 0.704, respectively), platelet concentration (p = 0.332, p = 0.726, p = 0.233, respectively) and mean platelet volume (p = 0.871, p = 0.696, p = 0.073, respectively).

3.5. Age-related differences in the time course of immune cell responses (exploratory analysis)

Due to the small sample size in the older adult group (n = 6), comparisons between young and older participants were conducted separately for each exercise intensity (moderate and heavy). This approach was selected to minimize confounding effects and to provide a preliminary, exploratory assessment of age-related differences in blood cell responses.

3.5.1. Leukocytes

The temporal profiles of leukocyte concentrations following moderate- and heavy-intensity exercise for both age groups are shown in *Figure 14*. For the moderate-intensity bout, no significant main effect of age was observed ($F(15, 1) = 0.884$; $p = 0.362$), whereas a significant main effect of time ($F(12, 2.127) = 10.492$; $p < 0.001$; $\eta p^2 = 0.412$), as well as a significant time × age interaction ($F(12, 2.127) = 3.59$; $p = 0.037$; $\eta p^2 = 0.193$) were

observed. Simple main effects showed that in the moderate-intensity session younger participants had a lower leukocyte concentration at baseline (p = 0.033).

For the heavy-intensity bout, no significant main effect of age (F (14, 1) = 0.02; p = 0.888) or time × age interaction (F (11, 1.733) = 1.721; p = 0.202) was found. There was, however, a significant main effect of time (F (11, 1.733) = 26,970; p < 0.001; ηp² = 0.658). Post hoc analyses revealed that leukocyte concentrations at baseline were significantly lower than at 30 minutes (p = 0.004), two hours (p < 0.001) and four hours (p < 0.001).

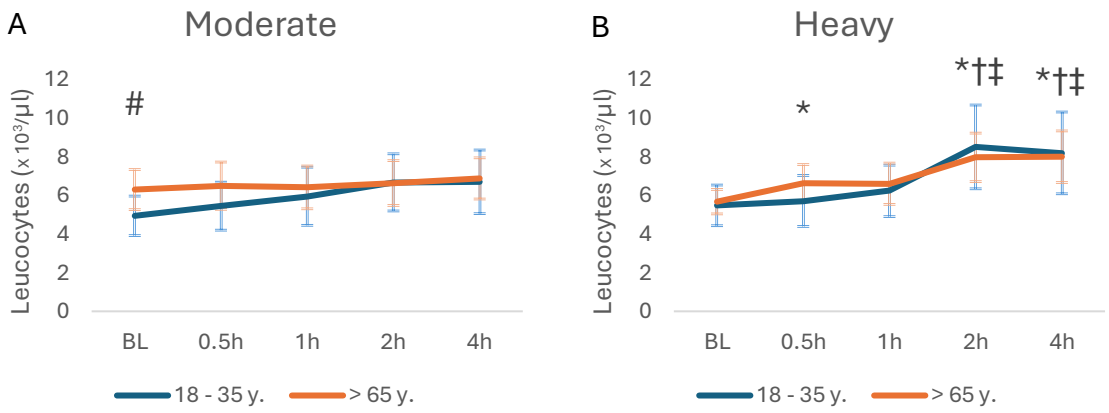

*Figure 14: Time course of Leucocyte counts following moderate- (A) and heavy-intensity (B) exercise in the young (n = 11) and older (n = 6 and 5, respectively) participants. × 10^3/µl = × 10^3 cells / micro liter; * = significantly different from baseline for both groups. (p < 0.05); † = significantly different from 0.5-h for both groups. (p < 0.05). ‡ = significantly different from 1-h for both groups (p < 0.05); # = different from younger group. (p < 0.05). Values are mean ± SD.*

3.5.2. Lymphocytes

The temporal profiles of lymphocyte percentages relative to total leucocytes following moderate- and heavy-intensity exercise for both age groups are shown in *Figure 15*. For the moderate-intensity bout, no significant main effect of age (F (15, 1) = 0.265; p = 0.614), nor significant time × age interaction (F (12, 2.082) = 0.383; p = 0.817) was observed. A main effect of time, however, was detected (F (12, 2.082) = 5.157; p = 0.011; ηp² = 0.256), with lymphocyte percentages differing between baseline and one-hour post-exercise (p = 0.026).

For the heavy-intensity bout, a significant main effect of time was observed (F (11, 1.340) = 19.35; p < 0.001; ηp² = 0.580), whereas no main effect of age (F (14, 1) = 0.022; p = 0.884) or significant time × age interaction (F (11, 1.340) = 1.196; p = 0.227) was detected. Post

hoc comparisons indicated that lymphocyte percentages at baseline differed significantly from one hour (p = 0.01) and two hours (p = 0.002) post-exercise. Additionally, values at two hours were significantly lower than at one hour (p = 0.004) and four hours (p < 0.001).

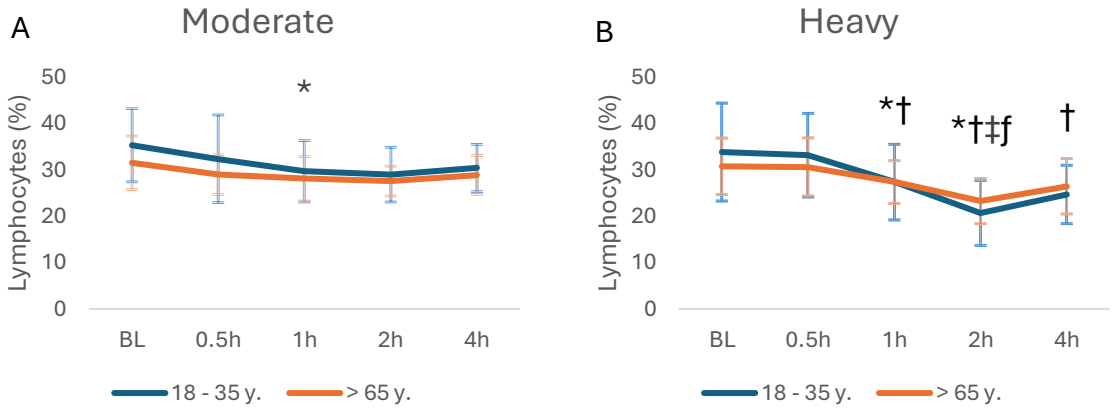

*Figure 15: Time course of Lymphocyte percentage following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) and older (n = 6 and 5, respectively) participants. * = significantly different from baseline for both groups. (p < 0.05); † = significantly different from 0.5-h for both groups. (p < 0.05). ‡ = significantly different from 1-h for both groups (p < 0.05). ƒ = significantly different from 4-h for both groups (p < 0.05). Values are mean ± SD.*

3.5.3. Neutrophils

The time courses of neutrophile percentages following moderate- and heavy-intensity exercise across age groups are shown in *Figure 16*. After the moderate-intensity bout, a significant main effect of time was observed (F (12, 2.400) = 5.985; p = 0.004; ηp^2 = 0.285), indicating that neutrophil proportions changed significantly through time. No main effect of age (F (15, 1) = 0.001; p = 0.970) and no time × age interaction (F (12, 2.400) = 0.817; p = 0.519) were detected. Compared to baseline, values were significantly higher at one hour (p = 0.044) and two hours (p = 0.044) post-exercise for both intensities, but not after four hours (p = 0.067).

Following the heavy-intensity bout, a main effect of time (F (11, 1.366) = 21.541; p < 0.001; ηp^2 = 0.606) was found, where neutrophile percentages increased from baseline, peaking around two hours post-exercise, before declining until the four hour measurement. Values at one hour (p = 0.002), two hours (p = 0.001) and four hours (p = 0.018) were significantly elevated compared with baseline post-exercise. No main effect of age (F (14, 1) = 0.214; p = 0.651) or time × age interaction (F (11, 1.366) = 0.894; p = 0.287) was found, suggesting

that the neutrophil response to both exercise intensities followed a similar temporal pattern in young and older adults.

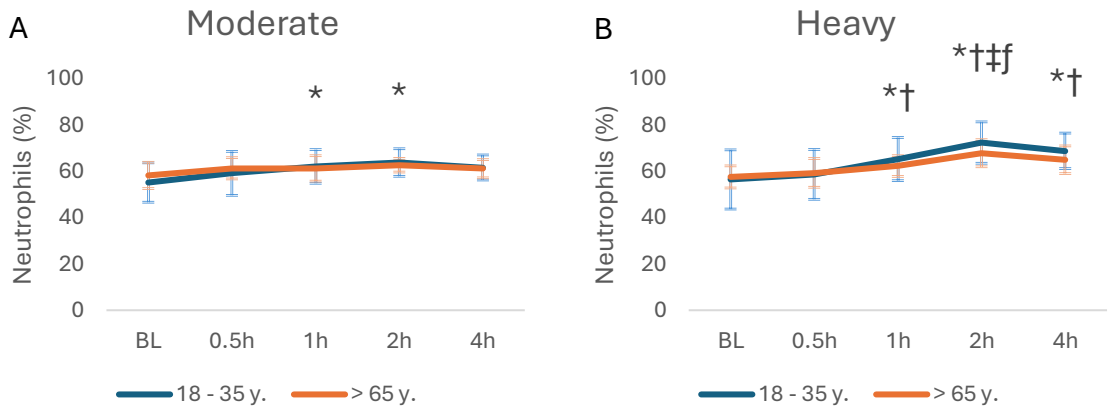

*Figure 16: Time course of Neutrophile percentage following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) and older (n = 6 and 5, respectively) participants. * = significantly different from baseline for both groups. (p < 0.05); † = significantly different from 0.5-h for both groups. (p < 0.05). ‡ = significantly different from 1-h for both groups (p < 0.05). ƒ = significantly different from 4-h for both groups (p < 0.05). Values are mean ± SD.*

3.5.4. Eosinophils

The time course of eosinophil percentages across both age groups are shown in *Figure 17*. After moderate-intensity exercise, a significant main effect of time was observed (F (12, 2.264) = 10.426; p < 0.001; ηp^2 = 0.410), whereas no main effect of age (F (15, 1) = 0.015; p = 0.905), and no time × age interaction (F (12, 2.264) = 0.6; p = 0.331) was found. Compared with baseline, eosinophil percentages were significantly lower at 30 minutes (p = 0.031), two hours (p = 0.012) and four hours (p = 0.005) post-exercise.

Following heavy-intensity exercise, no main effect of age (F (14, 1) = 0.287; p = 0.601) or time × age interaction (F (11, 2.166) = 0.199; p = 0.934) was observed. However, a significant main effect of time was evident (F (11, 2.166) = 20.948; p < 0.001; ηp^2 = 0.599), with eosinophil proportions being significantly reduced at all post-exercise time points compared to baseline (30 minutes: p = 0.001; one hour: p < 0.001; two hours: p < 0.001; four hours: p < 0.001).

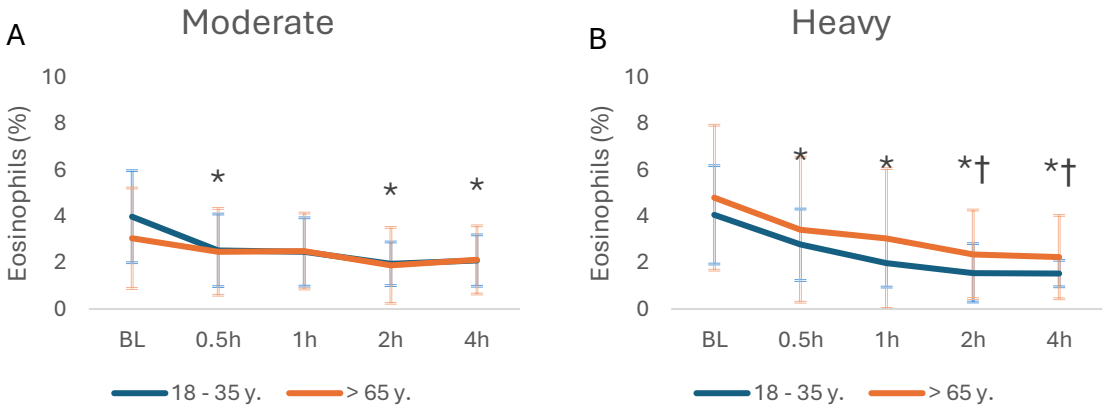

*Figure 17: Time course of Eosinophile percentage following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) and older (n = 6 and 5, respectively) participants. * = significantly different from baseline for both groups. ($p < 0.05$); † = significantly different from 0.5-h for both groups. ($p < 0.05$). Values are mean ± SD.*

3.5.5. Monocytes

The temporal profiles of monocytes percentages following moderate- and heavy-intensity exercise in young and older adults are presented in *Figure 18*. After the moderate-intensity bout, a significant main effect of age was observed ($F(15, 1) = 10.777$; $p = 0.005$; $\eta p^2 = 0.418$), indicating higher overall monocyte proportions in the older adults. However, no significant main effect of time ($F(12, 4) = 0.611$; $p = 0.656$) or time × age interaction ($F(12, 4) = 1.401$; $p = 0.244$) was detected. For the heavy-intensity exercise, no significant main effect of age ($p = 0.342$), time ($p = 0.447$), or time × age interaction ($p = 0.700$) was found.

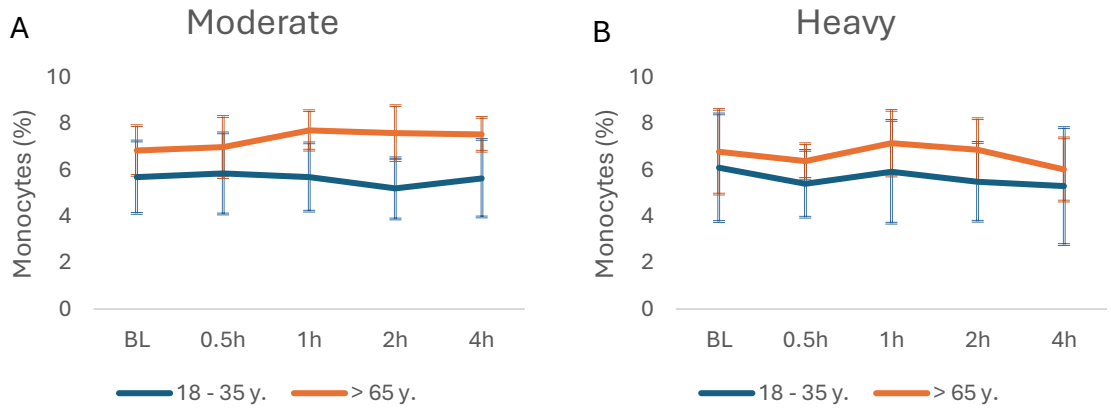

Figure 18: Time course of Monocyte percentage following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) and older (n = 6 and 5, respectively) participants. Values are mean ± SD.

3.5.6. Basophils

For both exercise intensities, basophils showed no significant changes over time or between age groups. After the moderate-intensity bout, there was no main effect of age ($p = 0.531$), no main effect of time ($p = 0.816$), and no time × age interaction ($p = 0.632$). Similarly, following the heavy-intensity exercise, no main effect of age ($p = 0.840$), time ($p = 0.326$), or interaction ($p = 0.105$) was detected.

3.5.7. Red Blood Cells and Platelet-Related Measures

The time courses of erythrocyte concentrations following moderate- and heavy-intensity exercise across age groups are depicted in *Figure 19*. After the moderate-intensity bout, no main effect of age was observed ($F (15, 1) = 3.415$; $p = 0.084$), whereas a significant main effect of time was detected ($F (12, 4) = 2.595$; $p = 0.045$; $\eta p^2 = 0.147$); the post hoc test did not reveal significant differences. In addition, a significant time × age interaction was present ($F (12, 4) = 4.759$; $p = 0.002$; $\eta p^2 = 0.241$). Simple effect analysis revealed that erythrocyte concentrations at two hours postexercise were significantly lower in older adults compared with young participants ($p = 0.014$).

Following heavy-intensity exercise, no main effect of age ($F (14, 1) = 1.475$; $p = 0.245$) or time × age interaction ($F (11, 4) = 0.437$; $p = 0.727$) was found. However, a significant main effect of time was found ($F (11, 4) = 7.096$; $p < 0.001$; $\eta p^2 = 0.336$). Erythrocyte concentrations were significantly higher at 30 minutes post exercise compared with baseline ($p = 0.005$), but subsequently declined, with values at four hours being significantly lower than at 30 minutes ($p = 0.02$).

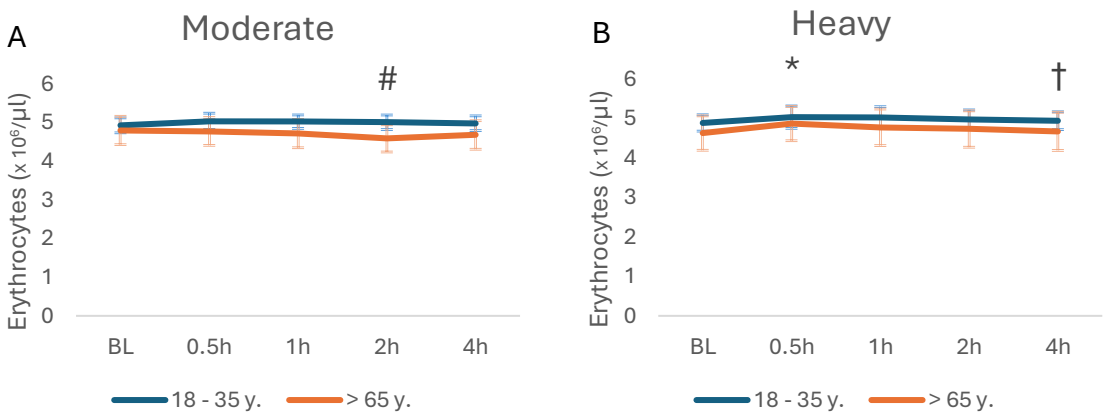

*Figure 19: Time course of Erythrocyte counts following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) and older (n = 6 and 5, respectively) participants. × 10^6/µl = × 10^6 cells / micro liter. * = significantly different from baseline for both groups. (p < 0.05); † = significantly different from 0.5-h for both groups. (p < 0.05).; # = different from younger group. (p < 0.05). Values are mean ± SD.*

Figure 20 shows the time course of hematocrit percentages following moderate- and heavy-intensity exercise in both age groups. After the moderate-intensity bout, no main effect of age (F (15, 1) = 1.553; p = 0.232) or time (F (12, 4) = 2.158; p = 0.085) was found. However, a significant time × age interaction was observed (F (12, 4) = 3.790; p = 0.002; ηp^2 = 0.249). Simple effect analyses indicated that hematocrit values at two hours postexercise differed significantly between young and older participants (p = 0.022). Following heavy-intensity exercise, a significant main effect of time was observed (F (11, 4) = 6.973; p < 0.001; ηp^2 = 0.332), with hematocrit values at 30 minutes postexercise being significantly higher than at baseline (p = 0.001). No main effect of age (F (14, 1) = 0.426; p = 0.525) and no time × age interaction (F (11, 4) = 0.452; p = 0.771) were found.

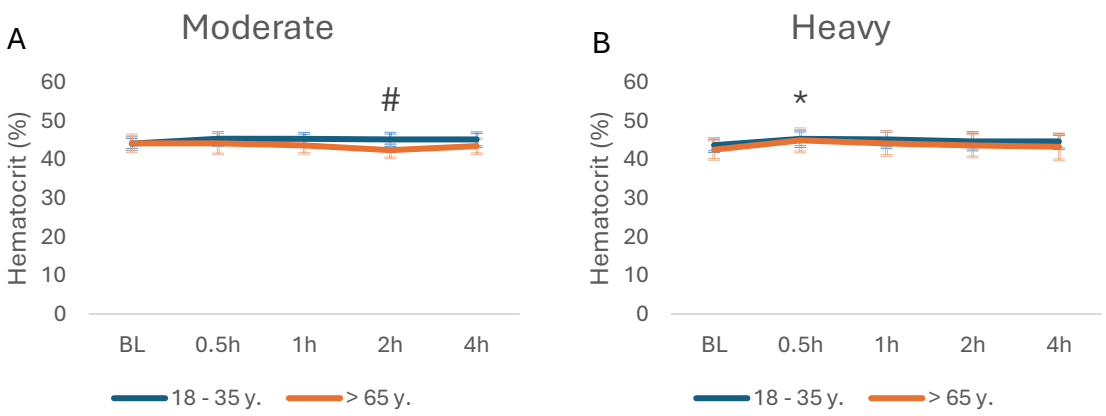

*Figure 20: Time course of Hematocrit percentage following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) older (n = 6 and 5, respectively) participants. * = significantly different from baseline for both groups. (p < 0.05); # = different from younger group. (p < 0.05). Values are mean ± SD.*

The time courses of hemoglobin concentration following moderate- and heavy-intensity exercise are shown in *Figure 21*. After the moderate-intensity bout, no main effect of age ($F(15, 1) = 3.064$; $p = 0.100$) or time × age interaction ($F(12, 4) = 1.275$; $p = 0.29$) was detected. However, a significant main effect of time ($F(12, 4) = 3.396$; $p = 0.014$; $\eta p^2 = 0.185$) was found. The post hoc test did not indicate any differences between time points.

Similarly, following heavy-intensity exercise, there was no main effect of age ($F(14, 1) = 2.920$; $p = 0.11$) or time × age interaction ($F(11, 4) = 0.669$; $p = 0.616$). However, there was a main effect of time ($F(11, 4) = 4.904$; $p = 0.002$; $\eta p^2 = 0.259$), with post hoc comparisons revealing that hemoglobin concentrations at 30 minutes postexercise differed significantly from baseline ($p = 0.017$), two hours ($p = 0.025$) and four hours ($p = 0.039$).

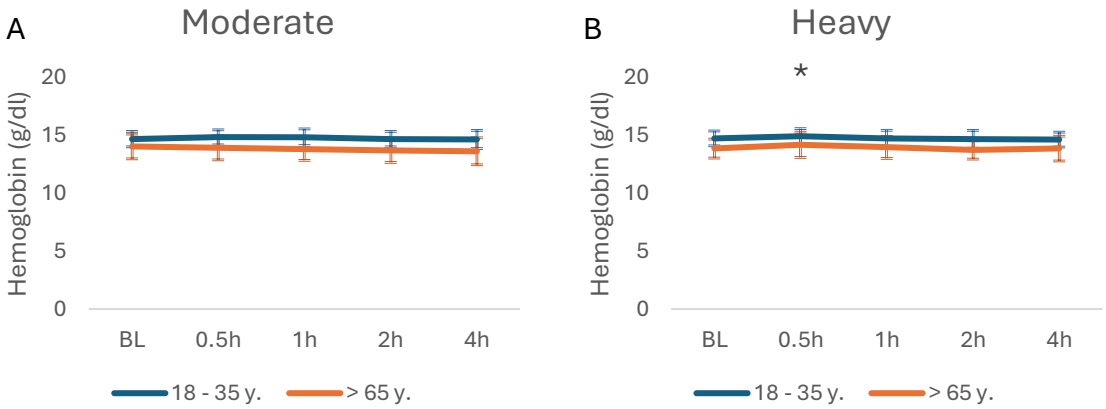

*Figure 21: Time course of Hemoglobin concentration following moderate- (A) and heavy-intensity (B) exercise in young (n = 11) and older (n = 6 and 5, respectively) participants. g/dL = grams/deciliter. * = significantly different from baseline for both groups. (p < 0.05). Values are mean ± SD.*

For platelet counts, there was neither a main effect of age (p = 0.09), time (p = 0.19), nor a significant time × age interaction (p = 0.419) after moderate-intensity exercise. Similarly, after heavy-intensity exercise, no main effect of age (p = 0.476), or time (p = 0.261) was detected, and no interaction between time and age was found (p = 0.816). Similarly, for *mean platelet volume* there was no main effects of age (p = 0.181), time (p = 0.833), and no time × age interaction (p = 0.339) following moderate-intensity exercise. The same pattern was observed after heavy-intensity exercise, with no significant effects of age (p = 0.181), time (p = 0.833), or their interaction (p = 0.339).

4. Discussion

The aim of the present thesis was to investigate whether exercise intensity modulates the mobilization of immune cells after a moderate and heavy endurance exercise bout. Ultimately, changes in total leukocyte count and leukocyte subpopulation proportions were observed in young subjects after both intensities investigated, with the extent of these changes depending on the intensity.

Regarding total leukocyte count, a significant increase in concentration was observed after both intensities. This increase in concentration peaked at around the two-hour mark, with cell counts then decreasing slightly until the four-hour-measurement. The values at two and four hours differed significantly between the two intensities, with more pronounced increases after the heavy intensity bout. The time course is consistent with previous studies reporting significant leukocytosis after high intensity endurance exercise (Gustafson et al., 2017; Islam et al., 2024). In the work by Gustafson et al. (2017), healthy participants (31 ± 4 years; $VO_{2\ peak}$ = 44 ± 10 ml/kg/min) completed two exercise protocols: a moderate-intensity bout at 60% of maximal workload for 45 min, and a graded step test increasing by 30 W every 2 minutes until exhaustion. Like in the present study, the moderate-intensity bout showed peak leukocytosis approximately three hours post-exercise, whereas leukocyte counts peaked immediately after exercise following their maximal test. Since the first blood sample in the present study was collected 30 minutes after exercise cessation, immediate post-exercise changes in leukocyte count could not be determined. Notwithstanding this, the exhaustive nature of their high-intensity effort may partly explain the different responses observed.

It should be also noted that our protocol was designed to match the total work in the moderate bout and thus it represents a more adequate approach for comparison of the effect of exercise intensity on these responses. In this sense, the definition of intensities used in this study seems appropriate for a comparison between moderate and heavy intensity, since the two intensities sessions differed physiologically, mechanically and perceptually in comparison to previous studies with a rather general prescription of exercise intensity based on several different measures such as HR_{max} (Cannon et al., 1994), HR reserve (Graff et al., 2022), $VO_{2\ max}$ (Nieman et al., 1991), P_{max} (Nieman et al., 2012) or subjective rate of exertion (McCarty et al., 1987).

Within this context, and in a comparable cohort of healthy participants (30 ± 3 years; $VO_{2\ peak}$ = 51 ± 6 ml/kg/min), Islam et al. (2024) examined the effects of two endurance exercise intensities matched for energy expenditure (350 kcal). In this study, moderate intensity was defined as 70% of the lactate threshold (45 ± 3% of peak power for 38 ± 6 min), while heavy

intensity was set at 10% of the difference between the lactate threshold and $VO_{2\ peak}$ (68 ± 4% peak power for 28 ± 4 min) added to the power output generated at first lactate threshold. These intensities are therefore highly comparable to those of the present study (40 min at 43% peak power and 25 min at 68% peak power for the moderate and heavy bouts, respectively). Blood samples were taken immediately after the sessions, at 30- and 90 minutes post-exercise, and leukocytosis was observed after both sessions, with a greater and more rapid increase following the high-intensity session, peaking immediately after cessation and declining by the 30 minutes mark (Islam et al., 2024). Unfortunately, as the final sample was obtained at 90 minutes post-exercise, it remains unclear whether a secondary rise in leukocyte concentration might have occurred at later time points, since in the present study leukocyte counts remained until four hours after exercise.

Overall, these studies demonstrate that leukocytosis occurs even after moderate exercise, although the magnitude of increase is smaller compared with higher-intensity exercise. However, there are also studies that reported no significant changes in leukocyte concentration following moderate-intensity exercise. For example, Neves et al. (2015) examined treadmill running sessions also standardized to an energy expenditure of 350 kcal, with moderate intensity set at 40% (~60 ± 5 min) and heavy intensity at 80% $VO_{2\ max}$ (29 ± 3 min). In contrast to the moderate condition, the heavy-intensity session elicited clear leukocytosis immediately after exercise, with leukocyte counts remaining significantly elevated up to two hours post-exercise, a pattern consistent with the present findings (Neves et al., 2015).

These intensity-dependent patterns were also reflected in the leukocyte subpopulations. Because neutrophils typically constitute the majority of circulating white blood cells (approximately 50-70%; Sellami et al., 2018), changes in total leukocyte count are often largely driven by alterations in this subgroup. In the present study, the proportion of neutrophils increased progressively following both exercise intensities, mirroring the time course of total leukocyte counts and reaching a peak around at two hours post-exercise. Several studies similarly reported an increased proportion of neutrophils in circulation immediately after endurance exercise (Brown et al., 2015; Jackson et al., 2022; Ottone et al., 2022; Sellami et al., 2018). Neves et al. (2015) observed no change in neutrophil percentage immediately post-exercise but noted a significant rise two hours after heavy-intensity exercise. In Gustafson et al. (2017), granulocyte counts (predominantly neutrophils) showed an intensity-dependent increase, peaking three hours post-exercise in both intensities. Notably, granulocytes were the only cell type to differ significantly from baseline after moderate-intensity endurance exercise in that study. Similarly, Islam et al. (2024) found a marked increase in neutrophils immediately after heavy exercise, followed

by a transient decline at 30 minutes and a secondary rise at 90 minutes after exercise, whereas no significant changes from baseline were observed after moderate exercise (Islam et al., 2024). The increase in circulating immune cells supports a rapid immune response following exercise. Especially the rise in neutrophils is believed to be driven by exercise-induced hormonal and inflammatory signaling, including catecholamines, cytokines, cortisol, and muscle-derived factors (Gustafson et al., 2017). Among these mediators, IL-6 appears to play a central regulatory role: it increases promptly during endurance exercise and subsequently promotes a release of IL-10 and IL-1Ra, contributing to a long-term anti-inflammatory response (Baskerville et al., 2024).

Beyond neutrophils, some studies also reported monocyte-related leukocytosis (Natale et al., 2003; Ottone et al., 2022). For example, Islam et al. (2024), observed a transient increase in monocyte proportion immediately after heavy endurance exercise, followed by a decline to baseline within 30 minutes. In the present study, however, no significant changes in monocyte percentages were detected across the monitored time points. In contrast, lymphocytes displayed distinct kinetics: their proportion decreased following both exercise intensities, reaching a minimum approximately two hours post-exercise, at which point values were lower than at baseline. This decrease is consistent with the findings of previous studies (Cerqueira et al., 2020; Gustafson et al., 2017; Islam et al., 2024). Importantly, the overall lymphocyte response in the present study aligns well with the established biphasic pattern described in the literature. Following an acute, intensity-dependent rise immediately post-exercise, a redistribution-driven decline to or below baseline values takes place within the subsequent hours (Gustafson et al., 2017; Koivula et al., 2023; Natale et al., 2003). A similar pattern can also be observed for absolute lymphocyte concentration, where lymphocyte numbers (baseline: 1.74 × 10^3/µl, 30 minutes: 1.77 × 10^3/µl) show below baseline levels at one hour (1.55 × 10^3/µl) and two hours post exercise (1.59 × 10^3/µl), and subsequently increase until four hours (1.86 × 10^3/µl) after cessation of heavy exercise. After moderate exercise, by contrast, the respective values initially remained constant (baseline: 1.62 × 10^3/µl, 30 minutes: 1.68 × 10^3/µl, one hour: 1.64 × 10^3/µl) until they increased by two hours (1.85 × 10^3/µl) and four hours (1.88 × 10^3/µl) after exercise.

In support of this, Islam et al. (2024) reported a significant increase in lymphocytes immediately after both moderate- and heavy-intensity endurance exercise, which a more pronounced effect in the latter, followed by a reduction below baseline levels as early as 30 minutes post-exercise (Islam et al., 2024). This decline in circulating lymphocytes was originally interpreted as exercise induced immune suppression (the “open window”). However, increasing evidence suggests that it more likely reflects enhanced lymphocyte

trafficking to peripheral tissues, where immune surveillance is temporarily strengthened, possibly reducing the risk of infection (Baskerville et al., 2024; Campbell & Turner, 2018).

The behavior of eosinophils closely resembles that of lymphocytes, showing a decrease in concentration after the end of exercise for both intensities, and reaching a minimum two hours post-exercise, whereas no significant changes were detected in basophil proportions over time. Evidence in the literature regarding exercise-induced alterations in eosinophils and basophils is scant. Ottone et al. (2022) reported no effect of a 90-minute heavy running session at 70% $VO_{2\ max}$ on either cell type, whereas Jukema et al. (2023) found a direct post-exercise decrease in the proportion of eosinophil leukocytes.

Hematocrit values were elevated at 30 minutes and one hour after exercise at both intensities, returning to baseline values thereafter. This transient increase probably reflects hemoconcentration due to plasma volume loss rather than a true rise in red blood cell mass. Plasma volume reductions can result from fluid shifts and sweating during exercise and are influenced by environmental factors such as temperature (Sawka et al., 2000; Schmidt & Prommer, 2008). Although acute spleen contraction may transiently release stored erythrocytes into circulation, (Holmström et al., 2021; Shephard, 2016), an actual increase in erythropoiesis would only be expected after weeks of training. In a comparable study, Jamurtas et al. (2018) found no significant post-exercise changes in hematocrit, erythrocyte count, or hemoglobin after 30 minutes of cycling at 70% $VO_{2\ max}$ in healthy young participants (22 ± 1 years; $VO_{2\ max}$: 45 ± 8 ml/kg/min). Similarly, no acute changes in hemoglobin concentration were detected after exercise in the present study. Regarding white blood cells, part of the post-exercise increase may be attributable to hemoconcentration. However, additional mechanisms such as neurohormonal activation and cytokine signaling, particularly via IL-6, are also known to contribute to the mobilization of immune cells. The distinct temporal profiles among leukocyte subtypes further suggest active, regulated mobilization processes, rather than purely passive changes in cell concentration (Baskerville et al., 2024; Gleeson et al., 1995; Szymczak et al., 2021).

No significant changes were observed in platelet count or mean platelet volume immediately after either exercise session. In contrast, Jamurtas et al. (2018) reported an acute increase in platelet counts immediately after exercise. Just as for leukocytes and erythrocytes, fluctuations in platelet concentration may be influenced by exercise-induced reductions in plasma volume or by spleen contraction, which can release stored platelets into circulation (Shephard, 2016). Additional mechanisms, such as increased mobilization from the bone marrow or pulmonary reservoirs, may also play a role, although these responses appear to be intensity-dependent, with significant increases occurring primarily after high-intensity

exercise (Skouras et al., 2023). In the present study, even the intense exercise bout may have been not have been strenous enough to induce such differences.

Similar dynamics as the ones found in the present study have also been observed in highly trained endurance athletes. For example, Nieman et al. (1994) reported an immediate post-exercise increase in lymphocyte proportion in young, well-trained participants (22 ± 1 years; $VO_{2\ max}$: 66 ± 2 ml/kg/min), followed by a decline below baseline levels 1–3.5 hours after exercise. This decline was accompanied by a transient decrease in lymphocyte proliferative capacity 1–2 hours post-exercise. The immune-modulatory effect was less pronounced following moderate-intensity exercise performed at 50% of $VO_{2\ max}$ compared with high-intensity exercise at 80% of $VO_{2\ max}$ for 45 minutes (Nieman et al., 1994). Consistent findings were reported by Vider et al. (2001), in a similarly young, highly trained cohort (22 ± 6 years; $VO_{2\ max}$: 69 ± 8 ml/kg/min) using a step test to exhaustion. They observed pronounced leukocytosis, primarily driven by neutrophils, immediately after exercise, which returned to baseline within 30 minutes. T lymphocytes and their subsets also increased immediately after exercise and fell below baseline values within 30 minutes, a response partly attributable to enhanced lymphocyte apoptosis. Hematocrit and hemoglobin concentrations were also elevated immediately after exercise and normalized within 30 minutes (Vider et al., 2001).

Taken together, the present study demonstrates that endurance exercise acutely modulates circulating immune cells, with high-intensity exercise eliciting greater mobilization than moderate intensity. However, it remains unclear whether the executed type of endurance sport influences the degree of immune cell mobilization (Baskerville et al., 2024; Sellami et al., 2018). Nevertheless, temporal patterns observed across individual leukocyte subtypes are consistent with previous literature (Gustafson et al., 2017; Islam et al., 2024; Neves et al., 2015).

4.1. Comparison of immune cell responses between age groups

When comparing immune cell responses between the young and old groups, certain, intensity-dependent differences were observed.

Regarding with total leukocytes, baseline values differed between the young and older participants before the moderate exercise session. Several factors can contribute to such differences at rest, including genetic disposition, nutrition, medication, stress, and lifestyle, all of which influence immune function and leukocyte counts. Age-related increases in systemic inflammation may also contribute to higher baseline leukocyte concentrations, although this chronic inflammation is not universally present in all older adults (Chmielewski et al., 2016). Furthermore, immune senescence, the gradual decline in immune efficiency

with age, can increase susceptibility to infections and alter baseline immune parameters (Großkopf & Simm, 2022). Other factors, such as prior exposure to cytomegalovirus – a very widespread virus infection with an estimated prevalence of 45 % in developed countries (Al Mana et al., 2019), which, however, was not assessed in this study - could also have contributed to systemic inflammation. Although we did not include participants who reported infections up to two weeks prior to the study, subclinical infections on study days could have contributed as well. Taken together, a combination of these factors likely explains the observed baseline differences in this session. On the other hand, no significant difference was observed before the heavy session, which increases confidence that post-exercise leukocyte responses are intensity-driven and not confounded by baseline disparities.

Following moderate-intensity exercise, there was an interaction that showed leukocyte numbers in the older participants remained unchanged, whereas that of the young participants were increased two and four hours after exercise. In contrast, heavy-intensity exercise induced a clear leukocytosis in both groups, with leukocyte concentrations at two- and four-hours post-exercise being significantly higher than those at 30 minutes and one hour. Older adults appeared to respond primarily to heavy exercise, whereas young participants showed increases after both intensities, although the increase was more pronounced after the high intensity bout. Possibly, the elevated levels of leukocytes prior to moderate-intensity exercise led to a minor response to exercise in older individuals. These findings align with previous research showing that the acute immune cell response to exercise is generally more pronounced in younger adults compared to older individuals (Cannon et al., 1994; Sellami et al., 2018).

For lymphocytes, a decrease was observed after moderate exercise in both age groups, with below baseline values after one hour. After heavy exercise, the proportion of lymphocytes also declined, reaching their lowest levels at two hours after the end of exercise, where values were significantly lower than at all other time points. No significant differences were observed between age groups. Similar results to show that lymphocyte mobilization increases with exercise intensity were also reported in the literature (Gustafson et al., 2017; Sellami et al., 2018). Leukocytosis, partly driven by the transient mobilization of lymphocytes immediately after exercise, has also been observed in older subjects (Sellami et al., 2018). Graff et al. (2022) observed that 30 minutes of treadmill walking at 60-70% of HR reserve led to an immediate post-exercise increase in lymphocytes and their subpopulations in adults aged 60-65 years; following this initial rise, values dropped to below baseline levels within one hour. All these studies assessed blood leukocytes only over relatively short post exercise, typically up to 90 minutes (Davison, 2011; Graff et al.,

2022; Islam et al., 2024; Jackson et al., 2022; Koivula et al., 2023; Nieman et al., 1991; Nieman et al., 2012; Vider et al., 2001). However, additional changes have been reported beyond this window (2-4 hours post-exercise; Davison, 2011; Graff et al., 2022; Islam et al., 2024; Jamurtas et al., 2018; Koivula et al., 2023; Nieman et al., 1991; Nieman et al., 2012; Ottone et al., 2022; Vider et al., 2001), which is consistent with the findings of the present study.

No significant temporal changes were observed in monocyte proportions after either intensity, however, older participants consistently showed higher monocyte proportion. This aligns with previous findings reporting an age-related increase in monocyte frequency, with lower monocyte levels correlating with greater longevity (Chmielewski et al., 2016). The results of neutrophil granulocytes followed a similar pattern in both age groups. After the moderate session, the proportions of neutrophils were significantly increased at one and two hours post-exercise compared to baseline. Also, after the intense exercise bout, neutrophils peaked at the two-hour mark. These results are consistent with previous studies reporting more pronounced exercise-induced increases in neutrophils in younger compared to older individuals (Cannon et al., 1990; Cannon et al., 1994). No significant temporal changes or age-related differences were observed in basophilic granulocytes. For eosinophilic granulocytes, a decrease in proportion was found in both age groups after both intensities.

For hematocrit and erythrocytes, values measured two hours after moderate-intensity exercise differed significantly between age groups, with younger participants showing higher hematocrit and erythrocyte concentrations. In older individuals, altered plasma volume regulation following exercise may contribute to these differences. Age-related factors such as reduced sweating, greater interstitial fluid accumulation, and diminished hemostatic capacity can all affect post-exercise plasma volume shifts (Bjerre-Bastos et al., 2022; Coull et al., 2021). The present study did not document water intake during the post-exercise period, which may also have influenced these outcomes. After intense exercise, no age-specific differences were observed for these measures.

Taken together, differences between age groups were observed at some time points for hematocrit and erythrocyte concentrations after the moderate-intensity bout, and there was also a higher level of monocytes present in the older age group. However, many immune responses were found to be similar across the two age groups, as seen in lymphocytes, neutrophils and eosinophils.

There is growing evidence that acute moderate to vigorous exercise of limited duration can transiently boost immune function. In contrast, prolonged or very intense endurance

sessions lasting more than 60-90 minutes, such as marathons, may induce temporary immune suppression, leading to the “open window” effect. The precise thresholds of exercise intensity and duration at which this transition occurs remain unclear (Baskerville et al., 2024). Moreover, the mechanisms underlying immune cell homeostasis in response to exercise are not yet fully understood (Gustafson et al., 2017). This study focused primarily on the acute effects of endurance sports on the mobilization of immune cells. Each exercise session has an immediate effect on the immune system, and the cumulative impact of regular training is well-known to improve immune function over time (Nieman & Wentz, 2019).

4.2. Limitations

One limitation of the study regarding immune cell measurements is the absence of blood samples taken immediately after the endurance sessions. Early post-exercise changes in leukocyte and its subtypes may occur within minutes and could provide additional insight into the acute mobilization of immune cells. Future studies in this field should also examine immune cell subpopulations, as their relative frequencies change across the lifespan and contribute to immune senescence and “inflammaging” (Großkopf & Simm, 2022).

Additionally, plasma volume may decrease after exercise, possibly due to sweating or fluid shifts into the interstitial space. As total blood volume is reduced while absolute leukocyte numbers in circulation may remain similar, measured immune cell number can appear elevated. This effect is nonspecific and affects all circulating blood cells, including leukocytes, erythrocytes, and platelets. The increases in leukocytes and subpopulations that were measured at between 30 minutes to two hours after exercise could be partially influenced by hemoconcentration rather than actual mobilization. Conversely, decreases (e.g., in lymphocytes at two hours) may be underestimated if plasma volume starts to recover at the same time. In the present study these potential changes in plasma volume were not corrected for.

Also, in older individuals, the higher baseline leukocyte counts before moderate intensity are likely multifactorial. However, it remains difficult to determine the exact causes, as relevant factors such as cytomegalovirus serostatus – known to affect leukocyte profiles on older adults – were not assessed (Graff et al., 2022; Weltevrede et al., 2016).

Regarding older participants, it should be noted that the inclusion criteria for the study were quite strict, ensuring a “healthy” group of participants without age-related diseases that are already common at this age, such as cardiovascular disease, diabetes, etc. This is a major strength of the study design.

However, a key limitation is the small number of participants included in the older age group. As a result, the findings in this cohort should be considered exploratory and as an overview of potential trends. Due to the limited sample size, it was not possible to compare the effect of intensity in responses between the two age groups. Nevertheless, the present results suggest that acute endurance exercise may elicit a reduced immune modulation response in older adults compared to younger individuals in some of the outcomes investigated. To obtain more robust and generalizable data, future studies should include larger cohorts of older participants. Lastly, it should be noted that the older group demonstrated above-average cardiorespiratory fitness ($VO_{2\ peak}$ = 39 ± 3 ml/kg/min), whereas the younger group was slightly below the age-specific average ($VO_{2\ peak}$ = 44 ± 9 ml/kg/min; Edvardsen et al., 2013). Consequently, the observed differences (or lack thereof) between age groups and across time points should be interpreted considering the aerobic fitness of our samples. The responses of our highly fit older participants may not be representative of typical older cohorts. Future studies that recruit participants with comparable relative fitness levels within each age group may help clarify the influence of fitness on the outcomes investigated.

4.3. Conclusion

In summary, a single session of endurance exercise induced measurable modulation of the immune system, with stronger effects observed following high-intensity exercise in a cohort of young individuals. Older participants also exhibited immune modulation, although the magnitude of the response was lower than in younger individuals for select outcomes. The temporal patterns of immune cell subtypes largely align with previous literature, suggesting that acute exercise enhances immune surveillance.

Literature

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List of abbreviations

BIA *Bioelectrical Impedance Analysis*

BP *Blood Pressure*

CHD *Congenital Heart Defect*

CPET *Cardio-Pulmonary Exercise Test*

dl Deciliter

e.g. *Exempli Gratia*

ECG *Electro Cardiogram*

fL Femtoliter

g Gram

h *Hours*

HR *Heart Rate*

HR_{max} *Maximum Heart Rate*

i.e. *Id Est*

IgA *Immunoglobulin A*

IPAQ *International Physical Activity Questionnaire*

IQR Interquartile Range

IRP *Immune Risk Profile*

J *Joule*

kcal *Kilo Calories*

km *Kilometer*

MET *Metabolic Equivalent*

min *Minute*

MRT Mean Response Time

PA *Physical Activity*

PAR-Q *Physical Activity Readiness Questionnaire*

$PETCO_2$ *End-Tidal Carbon Dioxide Pressure*

$PETO_2$ *End-Tidal Oxygen Pressure*

pH *potentia Hydrogenii*

P_{max} *Peak Power*

PSS-10 *Perceived Stress Scale*

RER *Respiratory Exchange Ratio*

ROS *Reactive Oxygen Species*

RPE *Rate of Perceived Exertion*

rpm *Revolutions per Minute*

s *Second*

SASP *Senescence-Associated Secretory Phenotype*

SD Standard Deviation

URTI *Upper Respiratory Tract Infection*

VE *Ventilation*

VO_2 *Oxygen Uptake*

$VO_{2\ max}$ *Maximum Oxygen Uptake*

$VO_{2\ peak}$ *Peak Oxygen Uptake*

VT1 *First Ventilatory Treshold*

VT2 Second Ventilatory Treshold

µl Microliter

W *Watts*

Erklärung

„Ich erkläre, dass ich die vorliegende Arbeit *selbstständig verfasst habe* und nur die ausgewiesenen Hilfsmittel verwendet habe. Diese Arbeit wurde weder an einer anderen Stelle eingereicht (z. B. für andere Lehrveranstaltungen) noch von anderen Personen (z. B. Arbeiten von anderen Personen aus dem Internet) vorgelegt.“