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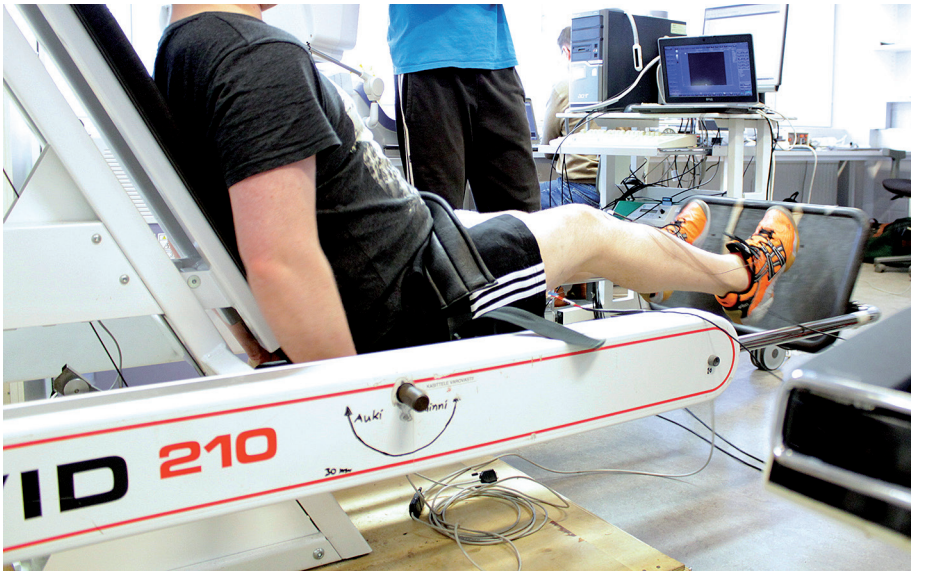
Johanna Ihalainen

Exercise and Inflammation with Special Reference to Resistance Training



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UNIVERSITY OF JYVÄSKYLÄ

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Editors
Taija Juutinen
Faculty of Sport and Health Sciences, University of Jyväskylä
Pekka Olsbo, Päivi Vuorio
Open Science Centre, University of Jyväskylä

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”I have not failed. I’ve just found 10.000 ways that won’t work.”

Thomas A. Edison

ABSTRACT

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The aim of the present dissertation was to examine the acute and chronic effects of resistance exercise on inflammation markers in young men. In addition, the effect of combined resistance and endurance training on inflammation was assessed. The present dissertation consisted of four studies. Acute inflammation response was evaluated using cross-sectional design after hypertrophic and maximal (n=12) resistance exercise bouts (I). In addition, acute inflammation was examined before and after resistance training (RT) consisting either hypertrophic or maximal explosive (n=8) resistance exercise bouts (RE) (II). The effect of RT on basal levels of markers of systemic inflammation was evaluated after an initial phase RT after which the participants were randomly assigned to hypertrophy-strength training (n=37) or to hypertrophy-strength-power training (n=31) (III). Additionally, the effect of combined training (resistance and endurance) was evaluated after 24 weeks of training (n=48) (IV). Hypertrophic RE led to greater acute responses in inflammation markers compared to maximal strength RE (I). An enhanced MCP-1 response was observed during the recovery phase after RT in hypertrophic resistance exercise along with acute enhanced IL-1ra and reduced IL-1β response. Thus, high-intensity RT modifies the RE-induced cytokine responses. Both training studies (III, IV) led to significantly improved lean mass and maximal strength as well as reduced abdominal fat mass. In the RT study (III), the initial four week phase of RT lead to increased circulating resistin, MCP-1, and IL-1ra concentrations, and decreased circulating leptin concentration. After the specialized RT periods, hypertrophy-strength RT elicited normalizing effects on inflammation markers, such as circulating resistin and leptin, whereas hypertrophy-strength-power did not have an effect on these markers. Combined training (IV) reduces concentrations of C-reactive protein, leptin, and resistin, and when performed on alternating days elicited the largest reductions in abdominal fat mass as well as circulating levels of TNF-α and MCP-1. The findings of the present dissertation suggest that the beneficial effects of resistance training could be due to the repeated effect of one bout of resistance exercise on inflammation markers as well as the favorable changes in body composition following RT.

Keywords: inflammation, cytokine, visceral fat, resistance training

Author's address

Johanna K. Ihalainen
Biology of Physical Activity
Faculty of Sport and Health Sciences
University of Jyväskylä
P.O. Box 35
FI-40014 University of Jyväskylä, Finland
johanna.k.ihalainen@jyu.fi

Supervisors

Professor Antti A. Mero, PhD
Biology of Physical Activity
Faculty of Sport and Health Sciences
University of Jyväskylä
Jyväskylä, Finland

Professor Eeva Moilanen, PhD
The Immunopharmacology Research Group
Faculty of Medicine and Life Sciences
Tampere University Hospital
University of Tampere
Tampere, Finland

Professor Heikki Kainulainen, PhD
Biology of Physical Activity
Faculty of Sport and Health Sciences
University of Jyväskylä
Jyväskylä, Finland

Reviewers

Professor Anthony C. Hackney, PhD, DSc
Department of Exercise & Sport Science
University of North Carolina
Chapel Hill, USA

Professor Ivan Bautmans, PhD
Department of Gerontology
Faculty of Medicine & Pharmacy
Free University of Brussels (VUB)
Brussels, Belgium

Opponent

Professor Neil Walsh, PhD
School of Sport, Health and Exercise Sciences
Bangor University
Bangor, UK

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LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original articles, which are referred to in the text by their Roman numerals

I Ihalainen, J., Walker, S., Paulsen, G., Häkkinen, K., Kraemer, W.J., Hämäläinen, M.,Vuolteenaho K., Moilanen E. & Mero, A.A. 2014. Acute leukocyte, cytokine and adipocytokine responses to maximal and hypertrophic resistance exercise bouts. European Journal of Applied Physiology, 114(12), 2607-2616.

II Ihalainen, J. K., Ahtiainen, J. P., Walker, S., Paulsen, G., Selänne, H., Hämäläinen, M., Moilanen E., Peltonen H. & Mero, A.A. 2017. Resistance training status modifies inflammatory response to explosive and hypertrophic resistance exercise bouts. Journal of Physiology and Biochemistry, 73(4), 595-604.

III Ihalainen, J. K., Peltonen H., Paulsen, G., Ahtiainen, J. P., Taipale R. S., Hämäläinen, M., Moilanen E. & Mero, A. A. 2017. Inflammation status of healthy young men: initial and specific responses to resistance training. Applied Physiology, Nutrition, and Metabolism. DOI: 10.1139/apnm-2017-0315.

IV Ihalainen, J. K., Schumann, M., Eklund, D., Hämäläinen, M., Moilanen, E., Paulsen, G., Häkkinen, K. & Mero, A. A. 2017. Combined aerobic and resistance training decreases inflammation markers in healthy men. Scandinavian Journal of Medicine & Science in Sports. DOI: 10.1111/sms.12906.

Additionally, some previously unpublished results are included in this dissertation.

ABBREVIATIONS

AD	combined training on alternating days
ANCOVA	analysis of covariance
ANOVA	analyse of variance
BIA	bioimpedance
BMI	body mass index
CT	combined endurance and resistance training
DXA	dual-energy x-ray absorptiometry
ET	endurance training
HS	hypertrophy-strength
HYP	hypertrophic resistance exercise bout
IL-1ra	interleukin-1 receptor antagonist
IL-1β	interleukin-1 beta
IL-6	interleukin-6
MAX	maximal resistance exercise bout
MAX	maximal resistance exercise bout
MCP-1	monocyte chemoattractant protein-1
POW	power (maximal explosive) resistance exercise bout
Rep	repetition
RM	repetition maximum
RE	resistance exercise
RT	resistance training
SD	standard deviation
SE	standard error
SHP	strength-hypertrophy-power
SS	combined training in same sessions
VO_{2peak}	peak oxygen consumption

CONTENTS

1 INTRODUCTION

More than 2000 years ago Hippocrates stated "*Eating alone will not keep a man well…he must also take exercise''*. Since then it has been clearly shown that individuals who maintain an active and fit way of life live longer and healthier lives than those who do not (Sallis 2009). At the same time the World Health Organization has estimated that 1.9 billion adults worldwide are overweight, with 600 million defined as clinically obese. Overweight and obesity are the fifth leading risk for global deaths and at least 2.8 million adults die each year as a result of being overweight or obese. The epidemic of inactivity, overweight and obesity presents a major challenge to chronic disease prevention and health across the life course around the world (Ng et al. 2014). Inactivity, overweight and obesity have been shown to be related to chronic diseases and premature death through chronic low-grade inflammation (Van Gaal, Mertens & De Block 2006; Mathur & Pedersen 2008). Even though chronic low-grade inflammation is associated with premature death, the acute inflammatory response is a beneficial response to various events threatening the body equilibrium (Medzhitov 2008).

The acute inflammation response is a transient immune response to harmful conditions such as traumatic tissue injury or an invading pathogen and it also facilitates the repair, turnover, and adaptation of many tissues (Franceschi & Campisi 2014). The acute inflammation response is characterized by a complex process in which a number of cells and molecules play different roles in a coordinated and well-controlled manner (Baizabal-Aguirre et al. 2014). A failure of the mechanism that self-regulates and resolves the process may lead to chronic inflammation, which is indicated by elevated levels of circulating inflammation markers, such as interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) and C-reactive protein (CRP). Chronic low-grade inflammation has been shown to be involved in the pathogenesis of several diseases (Goldfine & Shoelson 2017). Low-grade inflammation differs from classical inflammation in that there are no typical signs of inflammation, whereas similar but more modest increases can be observed in typical inflammation mediators and signaling pathways (Woods et al. 2012). In addition, it is notable, that chronic

low-grade inflammation links obesity to the development of several severe diseases, including cardiovascular diseases and type 2 diabetes (Ridker et al. 2000; Pradhan et al. 2001; Ridker et al. 2002; Petersen & Pedersen 2005; Walsh et al. 2011). A chronic modest increase in inflammation markers is observed with age, obesity, and inactivity, however, chronic modest increases in inflammation markers may be evident among apparently healthy men, which has predictive value for future adverse events (Koenig et al. 2006). Thus, there have been numerous efforts to use non-pharmacological as well as pharmacological approaches to reduce chronic low-grade inflammation (Simpson et al. 2012).

Exercise has a potential role in prevention of premature death. Growing evidence has demonstrated the beneficial effects of physical activity on chronic systemic inflammation status. Previous studies have indicated an inverse association between physical activity and chronic inflammation (Fischer et al. 2007; Lavie et al. 2011; Pinto et al. 2012). As such, lower inflammatory markers have been observed in individuals who report performing frequent moderate intensity physical activity (Beavers, Brinkley & Nicklas 2010). This supports emphasizing the increase of physical activity to improve fitness and body composition for improving inflammation status and health risk profiles of individuals (Lee, Blair & Jackson 1999). The benefits and adaptations achieved by exercising have been shown to be specific to the type of activity performed. Endurance training (ET) and resistance training (RT) represent the two extremes of the exercise continuum. Typically, endurance exercise contains a high volume of submaximal repetitive muscle actions with a relatively low load, whereas in resistance exercise the volume of muscle action is low and the load is rather high or even sometimes near maximal. Endurance training has been shown to beneficially reduce basal levels of pro-inflammatory markers and increase basal levels of anti-inflammatory markers in healthy participants (Kondo, Kobayashi & Murakami 2006; Bouassida et al. 2010). This net effect of exercise training on inflammation markers is thought to be related to changes in body composition, an increase in cardiorespiratory fitness, and the repeated anti-inflammatory effect of one exercise bout.

Less is known about the effects of RT on inflammation markers, and the data regarding the effects of RT on inflammation markers is sparse. RT requires a well-coordinated and controlled inflammatory response, which includes an increase in pro-inflammatory, as well as anti-inflammatory cytokines. There is recent evidence that RT could also have beneficial effects on inflammation in untrained young men (Forti et al. 2017). However, the beneficial anti-inflammatory effect of RT has not been consistent between all studies (Rall et al. 1996; Ara et al. 2006; Libardi et al. 2012). The mixed findings of the effects of RT could be due to large variety of training protocols utilized. Nevertheless, reducing inflammation markers at an early stage in life via regular exercise could serve as an efficient approach to prevent or delay the onset of low-grade inflammation and related diseases (Forti et al. 2017). The mechanisms behind the beneficial effects have been suggested to be similar to those in ET. In addition, recently it has been suggested that improvements in muscle mass

induced by RT could be directly related to the inflammation reducing effects of RT, through reduced pro-inflammatory cytokines in the skeletal muscle (Phillips et al. 2012). Both ET and RT appear to be important non-pharmacological strategies for improving inflammatory profiles. However, it is notable that both ET and RT can be performed in numerous ways. The mode, intensity, and duration of exercise can be modified and all these factors have been shown to affect the response in inflammation markers (Walsh et al. 2011).

The present dissertation aimed to investigate the acute and chronic effects of resistance and combined resistance and endurance exercise training on selected inflammation markers.

2 REVIEW OF THE LITERATURE

2.1 Inflammation

Inflammation is a complex defense mechanism in which leucocytes migrate from the vasculature into damaged tissues to destroy agents that can potentially cause tissue injury. Inflammation ultimately leads to the restoration of functional and morphological integrity of affected tissues (B. Lee & Lee 2014). The inflammatory response is a complex, highly coordinated process and is regulated by the interactions between the nervous, the endocrine, and the immune systems (Del Rey & Besedovsky 2017, 19-20). The causes as well as physiological and pathological outcomes of inflammation are presented in Figure 1.

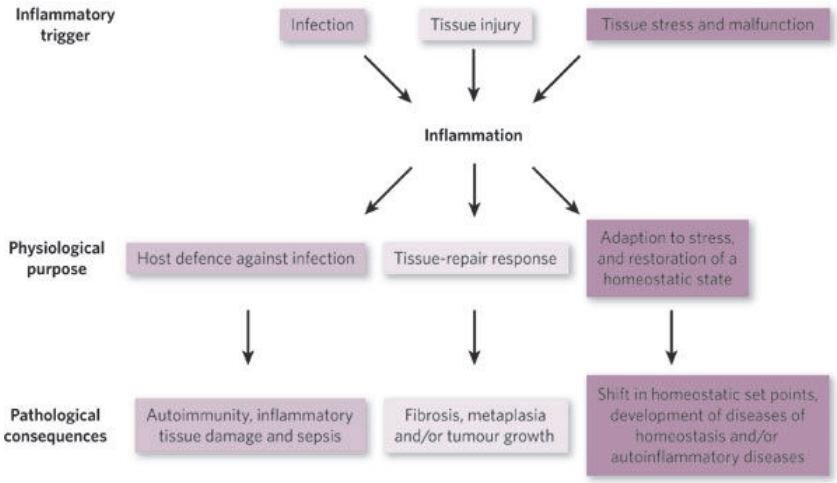


FIGURE 1 The causes and physiological and pathological outcomes of inflammation (Medzhitov 2008). Copyright Nature Publishing Group 2008, reproduced with permission.

2.1.1 Acute inflammation response

Inflammation is an essential mechanism that protects an organism from foreign pathogens by inducing a transient coordinated accumulation of blood cells and fluid. It can be triggered by various factors, such as invading microbes as well as damaged tissue. The aims of the inflammatory response are to remove foreign invaders and repair damaged tissue, which leads to restoration of host homeostasis (Rang et al. 2015, 88-89). The acute inflammatory response's complex cascade of events is initiated immediately and the acute response may last up to several days. The response starts with vasodilation and the activation of leukocytes. The activated cells release several inflammation mediators, including cytokines, chemokines, vasoactive amines, lipid mediators, and products of the proteolytic cascade. These mediators trigger an influx of lymphocytes, neutrophils, monocytes and other cells, which participate in the clearing of antigens (Rang et al. 2015). When the stimulus that triggered the inflammatory response has been eliminated, macrophages orchestrate the resolution and repair phase of inflammation. The resident macrophages have several functions: 1) phagocytosis of foreign particles (microbes, antigens, injured or dying cells) 2) antigen-presentation, 3) secretion of enzymes and oxidative derivatives to fight pathogens, 4) production of cytokines and growth factors that affect the parenchymal tissue and 5) recruit additional immune cells. The role of a monocyte/macrophage is consequently fundamental in destroying the pathogens in infection, and in assisting in tissue repair following injury (Pillon et al. 2013). Normally inflammation is self-limiting, however, if the acute inflammatory response fails to eliminate the pathogen, the inflammatory process persists and acquires new characteristics such as the characteristics of chronic inflammation (Rang et al. 2015, 88-89).

2.1.2 Chronic low-grade inflammation

Low-grade inflammation is characterized as a condition with sustained 2-4 fold elevations in circulating inflammation markers like CRP, TNF-α, and IL-6 (Petersen & Pedersen 2005; Woods et al. 2012). This chronic inflammation state is not caused by the classic instigators of inflammation: infectious agents and necrotic tissue. Instead, it is associated with the malfunction of tissue: that is, with the homeostatic imbalance of one of several physiological systems that are not directly functionally related to host defense or tissue repair (Medzhitov 2008). Chronic low-grade inflammation is strongly associated with increasing age (Beyer, Mets & Bautmans 2012), lifestyle factors, such as smoking (Yasue et al. 2006), and obesity (Cancello & Clement 2006). In addition, inflammation is considered to be a pathologic mediator of commonly co-occurring diseases including type 2 diabetes and cardiovascular diseases (Goldfine & Shoelson 2017)

2.1.3 Inflammation markers

Inflammation is characterized by the sequential release of cytokines. Cytokines are key modulators of inflammation, participating in acute and chronic inflammation via a complex and sometimes seemingly contradictory network of interactions. Cytokines include interleukins, interferons, growth- and colony-stimulating factors, chemokines and tumor necrosis factors (Turner et al. 2014). The main function of cytokines is to regulate immune function. Generally, the immune cells are the major origin of cytokines (Robinson, Harmon & O'Farrelly 2016). However, several other tissues, including adipose and skeletal muscle have been shown to be involved in the production of these markers (Raschke et al. 2013).

Cytokines are a group of low molecular weight (8 to 40 kDa) regulatory proteins secreted by white blood cells and a variety of other body cells in response to a number of inducing stimuli. They are involved in the regulation of cell trafficking during inflammation and the cellular arrangement within the immune organs. (Nathan & Sporn 1991; Klarlund Pedersen et al. 1998). Cytokines are secreted molecules that may exert specific effects both on the cell from which they are secreted (autocrine affects) and other cells (paracrine affects). Thus, three networks of intercellular communication are associated with cytokine secretion; one limited to cells of the immune system, one limited to parenchymal cells, and one involving interactions between immune and parenchymal cells. The cytokine connections between the cells determine the inflammatory response to injury and subsequent healing as well as the biologic consequences of the adaptive immune response to antigens. In many cases, the synergistic action of several cytokines is required to achieve the desired outcomes. (Frankenstein, Alon & Cohen 2006).

Traditionally, cytokines have been divided by their inflammatory activity into pro-inflammatory and anti-inflammatory subgroups. Pro-inflammatory cytokines promote inflammation whereas anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines or other inflammatory responses. Production of pro-inflammatory cytokines leads to stimulation of the hepatic acute phase response releasing acute phase reactants such as fibrinogen, serum amyloid A, and C-reactive protein (CRP). Even if the cytokines work on networks, each cytokine may have a different function on different targets and during different stages of the immune response. Taken together, the interplay between cytokines appears to be very complex but strictly controlled (Steinke & Borish 2006; Kapoor et al. 2011).

Cytokines are primarily considered as immune-regulatory molecules (Gleeson, Bishop & Walsh 2013). However, it has been shown that adipose as well as muscle tissue also expresses many pro-inflammatory and anti-inflammatory factors (Cancello & Clement 2006; Pedersen & Febbraio 2008). Thus, their origins and influence is far broader than the immune system alone. Metabolic inflammation induces a production of inflammatory cytokines that are often referred as adipocytokines. Adipocytokines include the adipose tissue

secreted adipokines and cytokines that are secreted by immune cells that are infiltrated to adipose tissue (Cao 2014). Similarly, cytokines that are secreted from muscle tissue are referred as myokines (Pedersen 2011). In addition, Görgens et al. (2015) have suggested that cytokines which are secreted from numerous places for example, IL-6 which is secreted from myocytes and adipocytes, should be called adipo-myokines (Görgens et al. 2015).

Chemokines are chemotactic cytokines that, together with adhesion molecules, control the migration and positioning of immune cells in tissues and are critical for the function of the innate immune system. Chemokines control the release of innate immune cells from the bone marrow during homeostasis as well as in response to infection and inflammation. In addition, chemokines recruit innate immune effectors out of the circulation and into the tissue where, in collaboration with other chemoattractants, they guide these cells to the very sites of tissue injury. Chemokine function also regulates the movement and positioning of innate immune cells in homeostasis and in response to acute inflammation, and plays an essential role in linking the innate and adaptive immune responses (Sokol & Luster 2015).

2.1.4 Relevant inflammation markers in the present dissertation

Interleukin-6 is the most studied and discussed cytokine in the context of exercise. IL-6 is a soluble factor that represents a keystone cytokine in infection, cancer, and inflammation in which it drives disease progression or supports the maintenance of immunological reactions (Hunter & Jones 2015). IL-6 is mainly known as a pro-inflammatory cytokine involved in T-cell differentiation and activation, and is involved in many diseases (Catoire & Kersten 2015). Almost all cells of the immune system produce IL-6, and while interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are major activators of IL-6 expression, other pathways such as Toll-like receptors, prostaglandins, adipokines, stress responses and other cytokines can promote the synthesis of IL-6. (Hunter & Jones 2015). In addition to pro-inflammatory properties, IL-6 has been suggested to lower inflammation status as it stimulates the appearance of anti-inflammatory cytokines, such as interleukin-1 receptor antagonist (IL-1ra) and IL-10 (Steensberg et al. 2003a; Petersen & Pedersen 2005). IL-6 is produced by a variety of white blood cells as well as in many non-immune cells, like endothelial and epithelial cells, adipocytes, fibroblasts, osteoblasts, synoviocytes, and myocytes (Pedersen & Febbraio 2008). It has been recently recognized that IL-6 has hormone-like attributes that affect vascular disease, lipid metabolism, insulin resistance, mitochondrial activities, the neuroendocrine system, and neuropsychological behavior (Hunter & Jones 2015).

The IL-1 family of cytokines has important roles in endocrinology and in the regulation of responses associated with inflammatory stress. The IL-1 cytokine family consists of inteleukin-1α (IL-1α), IL-1β, and IL-1ra. IL-1α and IL-1β are potent agonists and recognize the same type 1 IL-1 receptor, whereas IL-1ra acts as an antagonist to this receptor by binding to IL-1 receptors but

does not induce any intracellular response, thereby blocking the actions of IL-1α and IL-1β. IL-1 agonists are inducers of molecules essential for adhesion of leukocytes to the endothelial surface and it has been reported to have synergistic effects with TNF-α. In addition, IL-1 activates the release of histamine from mast cells, which trigger early vasodilation and increases vascular permeability, which is an important part of inflammation response (Banerjee & Saxena 2012).

Activated monocyte-macrophage lineage cells are the main origin for local and circulating TNF-α. TNF-α is a pro-inflammatory cytokine known to a have a central role in the initial host response to infections and in the pathogenesis of various chronic immune-mediated diseases. High levels of circulating TNF-α in an acute inflammation response can lead severe consequences, for example, to shock and tissue damage, catabolic hormone release, vascular leakage syndrome, and fever that have the potential to harm the body (Papadakis & Targan 2000). A chronic moderate increase in TNF-α concentrations leads to worsening insulin resistance and inflammation status (Maury & Brichard 2010). TNF-α has been shown to induce insulin resistance by downregulating the tyrosine kinase activity of the insulin receptor and decreasing the expression of GLUT-4 glucose transporters. (Halle et al. 1998). However, increases in circulating TNF-α in obesity may be viewed also as an attempt to fight excess adiposity. Increased TNF-α may resists adiposity gains by limiting further weight gain through lipolysis and insulin resistance, impaired pre-adipocyte differentiation, and increased adipocyte apoptosis (Prins et al. 1997). In addition, TNF-α has shown to have the potency to promote tissue remodeling and repair, inflammation, cytotoxic reactions, and anti-tumoral activity.

MCP-1 (also known as CCL2) is the most powerful chemoattractant cytokine and its action is mediated via its receptor CCR2. It is a small chemokine that directs circulating leukocytes, especially monocytes, to areas of inflammation or injury (Catoire & Kersten 2015) This MCP-1 activated migration of leukocytes have been shown to further contribute to the inflammatory cascade by releasing pro-inflammatory cytokines. Apart from monocytes and macrophages, MCP-1 also synchronizes recruitment and infiltration of memory T cells and natural killer cells to sites of inflammation (Yap, Frankel & Tam 2017). MCP-1 is the most studied member of chemokine family and, there have been a multitude of studies detailing its over expression and up-regulation in various diseases (Deshmane et al. 2009). MCP-1 has been suggested to be linked to tissue regeneration after traumatic injury (Harmon et al. 2010), whereas circulating and adipose tissue levels of MCP-1 are elevated in obesity and are considered as a marker of low-grade inflammation. Nevertheless, circulating MCP-1 that increases with visceral fat increase has been speculated to be one of the potential candidates linking obesity with obesity-related metabolic complications such as atherosclerosis and diabetes (Kim et al. 2006).

Leptin is an adipocytokine that centrally regulates body mass and links appetite, energy state, and neuroendocrine function. Obese individuals have

increased levels of circulating leptin as compared to their non-obese counterparts (Trayhurn & Bing 2006). An increase in leptin concentrations increases energy expenditure and reduces food intake by suppressing appetite, which should lead to a reduction in body weight. However in obese people, the hypothalamus does not react appropriately to increased levels of leptin (Vuolteenaho, Koskinen & Moilanen 2014). The term leptin resistance is used to describe this failure of leptin to elicit a response (Sáinz et al. 2015). Thus, the primary function of leptin may not be as a satiety factor. It is evident that the role of leptin is pleiotropic and in addition to the energy balance regulator it has a role as a pro-inflammatory regulator in the immune system. Leptin stimulates the production of pro-inflammatory cytokines and enhances T-helper cell 1 type of immune response (Faggioni, Feingold & Grunfeld 2001).

Resistin is a signaling protein that was initially characterized in mice. In mice resistin is secreted primarily by adipocytes and is known to be linked with obesity-related diseases like type 2 diabetes (Shuldiner, Yang & Gong 2001). However, in humans, resistin is expressed in multiple tissues, mainly in mononuclear leukocytes, macrophages, the spleen, and bone marrow cells. In addition, it is expressed at low levels in preadipocytes, endothelial cells, and vascular smooth muscle cells (Patel et al. 2003). Serum resistin concentrations have been associated with cardiovascular disease and all-cause mortality in patients with type 2 diabetes (Fontana et al. 2017). Fontala et al. (2017) suggested that the mechanism behind the association could be the deleterious effect exerted by resistin on intermediate metabolism, low-grade inflammation, and atherosclerotic processes. A more specific mechanism has been proposed and suggests that resistin is an endogenous agonist of toll-like receptor 4, which triggers major inflammatory pathways that are also activated by lipopolysaccharide (Benomar et al. 2013). Lastly, elevated serum resistin is associated with poor exercise capacity and exercise-induced cardiac ischemia in patients with stable coronary disease (Zhang et al. 2010).

Adiponectin, also known as AdipoQ and ACRP30 is an adipocyte delivered anti-inflammatory plasma protein (Bouassida et al. 2010). Adiponectin is a relatively abundant plasma protein that circulates in different oligomeric forms of trimeric, hexameric, and high-molecular weight. Adiponectin acts through two known cellular receptors, one (AdipoR1) found predominantly in skeletal muscle and the other (AdipoR2) mainly in the liver (Liu & Sweeney 2014). In contrast to most other adipocytokines, circulating adiponectin is negatively correlated with BMI (Brichard, Delporte & Lambert 2003; Arita 2012) and body fat mass (Bouassida et al. 2010), and is decreased in patients with type 2 diabetes or cardiovascular disease (Ouchi et al. 2011). The mechanism of action has been suggested to be due to adiponectins insulin sensitizing, anti-inflammatory, and anti-atherogenic properties (Simpson & Singh 2008). However, in patients with classical chronic inflammatory/autoimmune diseases, adiponectin levels positively correlate with inflammation markers. Furthermore, it has been reported that adiponectin has pro-inflammatory effects on tissues such as synovial joints and epithelium

of colon. This indicates that adiponectin may exert differential functions in different conditions (Kusunoki, Kitahara & Kawai 2016)

C-reactive protin (CRP) is produced in response to inflammatory stimulus and tissue damage (Pepys & Hirschfield 2003). CRP binds to several different species of bacteria, fungi and parasites and recognizes molecular ligands found on bacterial membranes and apoptotic cells. The plasma concentrations of CRP are very low in healthy individuals and therefore in the clinical settings CRP is mainly used as a marker of acute infection and during such, CRP levels can increase up to 1000-fold (Pepys & Hirschfield 2003; Ridker 2016). However, when CRP is used to predict the risk for cardiovascular diseases, it has been suggested that relative risk categories (low, average, high) corresponding to approximate tertiles of values (1.0, 1.0-3.0, and >3.0 mg·L^{-1}, respectively) could be used (Pearson et al. 2003; Ridker 2016). Even if the CRP has been shown to predict cardiovascular risk, recent clinical investigations have moved upstream to study IL-6 and il-1 as targets for anti-inflammatory atheroprotection (Ridker 2016).

2.1.5 Adipose tissue, muscle and inflammation

2.1.5.1 Adipose tissue

Two types of adipose tissue are present in mammals: white adipose tissue and brown adipose tissue (Gesta, Tseng & Kahn 2007). From 5 to 20% of A lean adult's body weight is white adipose tissue, whereas in an obese person up to 70% of body weight can be white adipose tissue (Clement et al. 2004). Interestingly, especially visceral fat mass increases by over 300% between the ages of 25 and 65 years, which creates an increased risk for the development of metabolic diseases in adults with normal body mass index but abdominal obesity (Allison et al. 2005). The worldwide epidemic of obesity has contributed to a better understanding of the biology of adipose tissue. Adipose tissue, which consists of adipocytes and pre-adipocytes, connective tissue matrix, nerve tissue, stromal vascular cells, and immune cells, was traditionally considered as inactive energy storage. Nowadays, the source of chronic low-grade inflammation is suggested to be specifically related to the expansion of truncal adipose tissue (Patel & Abate 2013).

There are several mechanisms that have been implicated to be involved in the generation of systemic low-grade inflammation. These mechanisms range from adipocyte hypertrophy and necrosis, tissue hypoxia, lipid spillover, metabolic endotoxemia, and endoplasmatic reticulum (ER) stress to the effects of other subtypes of adipose tissue immune cells (Maury & Brichard 2010). Figure 2 summarizes the mechanisms proposed to lead to increased inflammation with increased adipose tissue. The rapid expansion of adipose tissue leads to surpass of the perfusion capacity of the existing vasculature in adipose and this leads to hypoxia. Furthermore, the hypoxia leads to tissue necrosis followed by inflammatory response. The inflammation response elicits dysregulated secretion of adipocytokines and increased release of free fatty

acids (Trayhurn, Wang & Wood 2008). In addition, obesity induces a phenotypic switch in adipose tissue from anti-inflammatory (M2) to pro-inflammatory (M1) macrophages (Jung & Choi 2014). Macrophages are tissue-resident phagocytes that serve as sentinels of innate immunity reactions and fulfill a number of housekeeping tasks (Mraz & Haluzik 2014). Adipose tissue macrophages represent 4% of the visceral fat with an increase to 12% in excess enlargement of adipose tissue (Harman-Boehm et al. 2007). Infiltration of immune cells, i.e., macrophages and T cells in the adipose tissue has been shown to be at least partly responsible for the adipose secretion of inflammatory cytokines (Harford et al. 2011). The exact mechanisms of macrophage recruitment into the adipose tissue remains only partially elucidated. However, especially chemokines have an important role in the attraction of monocytes to migrate to the adipose tissue (Dahlman et al. 2005)

In recent decades, adipose tissue has been shown to secrete an array of signaling molecules called adipocytokines (Blüher & Mantzoros 2015). Adipocytokines may have pro-inflammatory and anti-inflammatory functions. Adipocytokines, like leptin, not only regulate feeding behavior and energy expenditure but are also involved in the regulation of inflammatory responses (Jaworek et al. 2003; Blüher & Mantzoros 2015; Aguilar-Valles et al. 2015). The first study reporting a link between obesity and inflammation reported that a high caloric diet led to an increase in the expression of the inflammation marker TNF-α in the adipose tissue of rats (Hotamisligil, Shargill & Spiegelman 1993). The expansion of the adipose tissue initiates a cascade of events causing a local response to spread systemically. The mechanisms responsible for this association are still unknown, but several hypotheses, which are not mutually exclusive, have been formulated (Maury & Brichard 2010).

The free fatty acids and pro-inflammatory adipocytokines move to metabolic tissues, including skeletal muscle and liver, and modify inflammatory responses as well as glucose and lipid metabolism, thereby contributing to metabolic syndrome.

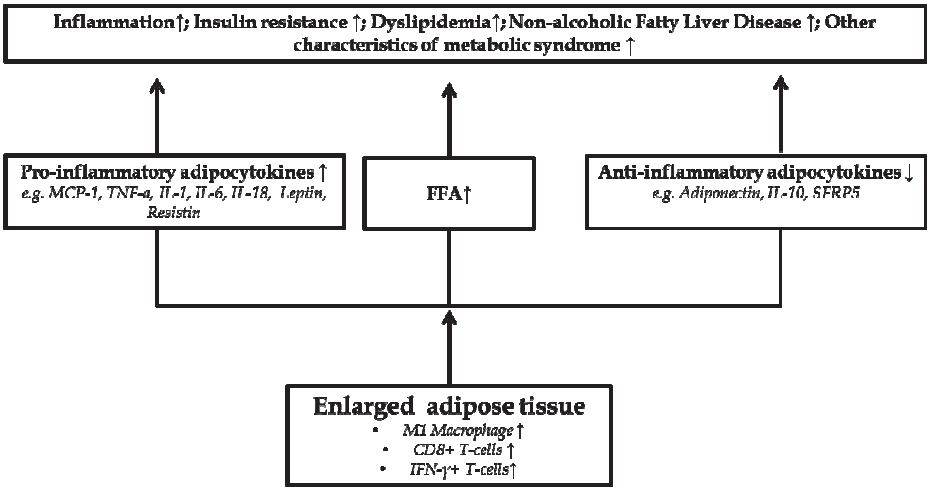


FIGURE 2 The enlargement of adipose-tissue contributes to metabolic syndrome. IL = interleukin; MCP-1 = monocyte chemotactic protein, SFRP5 =secreted frizzled-related protein 5; TNF-α = tumor necrosis factor-α. Modified from Jung & Choi 2014.

2.1.5.2 Muscle tissue

Skeletal muscle plays a key role in postural control, locomotion, and other physiological tasks requiring mechanical activity based on muscle fiber contraction (Baltzopoulos & Gleeson 2001). As the largest organ in the body, the energy production and consumption by skeletal muscles are fundamental for metabolic control and homeostasis. More recently, skeletal muscle has gained considerable interest as an endocrine organ that releases myokines while contracting (Raschke & Eckel 2013). Myokines are proteins secreted from skeletal muscle cells, excluding proteins that are secreted by other cell types in skeletal muscle tissue and excluding proteins that are only described on the mRNA level. Contractile activity has been suggested to be the key regulatory element for expression and secretion of myokines from muscle (Pedersen 2011). Interestingly, many of the contraction-regulated myokines described in the literature are also known to be secreted by adipocytes (Görgens et al. 2015).

Skeletal muscles contain resident immune cell populations and their abundance and type is altered in inflammatory myopathies, endotoxemia or different types of muscle injury/insult. Injuries in muscle tissue have been shown to lead to accumulation of inflammation cells, including macrophages, into the muscle tissue. The time-course and cell types activated in the muscle tissue following muscle damage are shown in Figure 3. The activated macrophages, depending on their type, secrete pro-inflammatory and anti-inflammatory cytokines that are needed in the regulation of muscle repair (Hyldahl & Hubal 2014; Peake et al. 2017). Given the extent of inflammation following injury it has been hypothesized that cross-talk between inflammatory cells and satellite cells, a specialized population of muscle stem cells, might be

an important factor in promoting muscle repair. Notably, especially cytokines released from the macrophages has been shown to be involved in satellite cell proliferation (Cantini et al. 1994). These events require a highly regulated cross-talk between myofibers and immune cells, involving paracrine/autocrine and contact interactions (Pillon et al. 2013). Recent reports show that inflammatory macrophage numbers within the muscle are elevated during obesity and that muscle cells in vitro can mount autonomous inflammatory responses under metabolic challenge (Pillon et al. 2013).

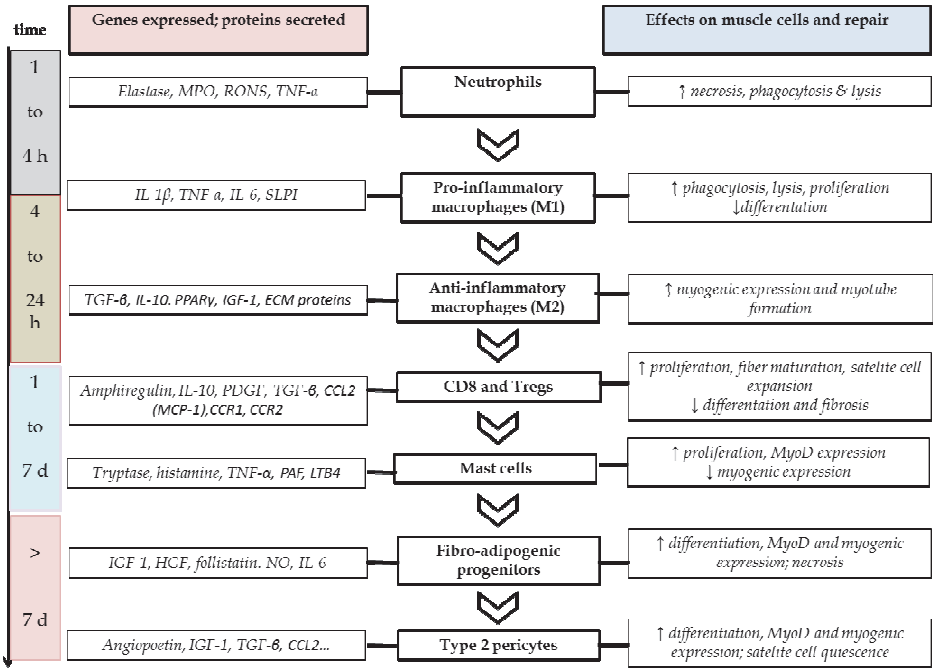


FIGURE 3 Muscle inflammatory response to injury. time = time after injury; MPO = myeloperoxidase; RONS = reactive oxygen and nitrogen species; TNF-α = tumor necrosis factor; IL = interleukin; TGF = transforming growth factor; SLPI = secretory leukocyte protease inhibitor; PPAR = peroxisome proliferator-activated receptor; IGF = insulin-like growth factor; PDGF = platelet-derived growth factor; CCR = C-C motif chemokine receptor; PAF = platelet activating factor; LTB = leukotriene; NO = nitric oxide; MCP-1 = monocyte chemotactic protein; VEGF = vascular endothelial growth factor. Modified from (Peake et al. 2017).

2.2 Exercise and inflammation markers

The first to investigate the effects of exercise on circulating levels of cytokine was Cannon et al. 1986. They were the first to report that plasma IL-1 activity was detected after moderate intensity endurance exercise (Cannon et al. 1986).

This was further confirmed in the 1990s as Northoff and Berg (1991) reported an elevation in several cytokines after the completion of a marathon. Since then it has been shown that an acute bout of physical activity is accompanied by responses that in many respects are similar to those induced by infection and sepsis, however the increase in classical pro-inflammatory cytokines, TNF-α and IL-1β after exercise is lesser in magnitude or absent (Mathur & Pedersen 2008). The acute effects of resistance and endurance exercise bouts are discussed below. To our knowledge there are no studies regarding the acute responses to combined resistance and endurance exercise bouts, thus the response is expected to be similar to separate resistance and endurance exercise bouts.

Exercise training presents a paradoxical situation with respect to inflammation as an acute exercise bout can produce a transient increase in inflammation markers whereas exercise training can lead to reduced inflammation marker concentrations (Petersen & Pedersen 2005). The benefits and adaptations brought by exercise are specific to the type of activity performed. ET and RT represent the two extremes of the exercise continuum. Typically, endurance exercise contains high volume submaximal repetitive muscle actions with a relatively low load, whereas in resistance exercise the volume of muscle action is low and the load is rather high or sometimes even near maximal.

Endurance training. Good cardiorespiratory fitness reduces all-cause mortality. Engaging in endurance training helps to maintain and to improve aerobic capacity. In addition, endurance training has been shown to be beneficial in preventing weight gain, and to some extent, in promoting clinically significant weight loss (Donnelly et al. 2009). An individual's aerobic capacity refers to the maximal amount of physiologic work that an individual can perform as measured by oxygen consumption (VO_{2peak}). Aerobic capacity depends on body size and composition, and is affected by age, sex, weight, genetic background, and physical activity level. The mechanisms that lead to an improvement in individuals' aerobic capacity and endurance performance are adaptations in the cardiorespiratory and neuromuscular systems that enhance the delivery of oxygen from blood to muscles cells (Jones & Carter 2000). The physiological demands and outcomes of endurance training are dependent upon the exercise volume and its determinants duration, intensity and frequency of training. In endurance training the training volume has been defined for example as hours per week or kilometers per week (Jones & Carter 2000).

TABLE 1 Primary demands and outcomes of the resistance training. (Kramer & Häkkinen 2002, 50-51; W. J. Kraemer & Ratamess 2004).

Training outcome	Intensity (% of 1RM)	Reps per set	Inter-set rest (s)	Physiological demands
Power	30-60	8-10	180	Neural
Maximal strength	80-100	1-6	180	↓
Hypertrophy	70-80	6-12	60-90	
Strength endurance	50-70	>15	60	Metabolic

Resistance training. Muscle strength and the ability of the muscles to develop force rapidly are important performance characteristics that have also been shown to contribute to health and several tasks of daily life (Bassey et al. 1992). However, generally RT has not been associated with clinically significant weight loss (Donnelly et al. 2009). Engaging in RT leads to neurological and morphological changes, such as increase in voluntary activation and fiber size that ultimately lead to increases in muscle size and strength (Folland & Williams 2007). The physiological demands and outcomes of RT (Table 1) depend on the training variables including mode (eccentric and/or concentric muscular actions), volume (total work of the session), load (weight lifted), rest periods, and intensity of RT. In RT the training volume can be defined as number of repetitions × load (Kraemer & Ratamess 2004).

Combined endurance and resistance training. As discussed, ET improves cardiorespiratory fitness while RT leads to adaptations in muscle size and strength. Both training modes are important and appear to be important strategies for improving overall health (Thompson, Gordon & Pescatello 2010). Thus both ET and RT are part of the American College of Sports Medicines (ACSM) position stand recommendations regarding quantity and quality of exercise (Pollock et al. 1998). Combined training can be performed in multiple ways, for example by performing endurance and resistance training in the same session with different orders or separated on alternating days (Eklund et al. 2016).

2.2.1 Endurance exercise bout and inflammation markers

The earliest research on the effects of an exercise bout on cytokines has been done in endurance running. Northoff et al. (1991) showed an increase in several circulating cytokines, like IL-6 and TNF-α, after a marathon (Northoff & Berg 1991). The earliest and most consistent finding has been the elevation in circulating IL-6 concentration (Petersen & Pedersen 2005). Later, increases in pro-inflammatory (IL-1β and TNF-α), anti-inflammatory (IL-10), cytokine inhibitors (IL-1ra) (Suzuki et al. 2000), chemokines (MCP-1) (Suzuki et al. 2002), and colony-stimulating factors (Suzuki et al. 2000) have been reported after endurance exercise. The relative changes in plasma concentrations of selected cytokines after strenuous exercise bout are presented in Figure 4.

Only a few studies have found a significant effect of an acute exercise bout on adipocytokine concentrations. Kraemer et al. (2003) reported a significant increase in adiponectin concentrations after a graded treadmill walk/run protocol in trained runners (Kraemer et al. 2003). In contrast, Jürimae et al. (2005) found a significant reduction in adiponectin levels after-high intensity rowing exercise (Jürimäe, Purge & Jürimäe 2005). Other studies have not observed significant effects of moderate intensity endurance exercise on adiponectin (Ferguson et al. 2004; Jamurtas et al. 2006) or resistin (Jamurtas et al. 2006). However, recently Vuolteenaho et al. (2014) reported a significant increase in adiponectin and resistin after marathon running, whereas leptin

remained unchanged. Bouassida et al. (2010) suggested that leptin levels decrease after long-term exercise (>60 min) stimulating free fatty acid release, or after exercise that generates energy expenditure greater than 800 kcal (Bouassida et al. 2010). The magnitude of the inflammation marker response to endurance exercise has been suggested to be proportional to exercise intensity, duration, mode, and the mass of skeletal muscle recruited (Pedersen & Hoffman-Goetz 2000; Febbraio & Pedersen 2002; K. A. Simpson & Singh 2008). Later, Peake et al. (2005) have showed that in endurance type exercise the cytokine response is more dependent on the intensity of the exercise than muscle damage (Peake et al. 2005).

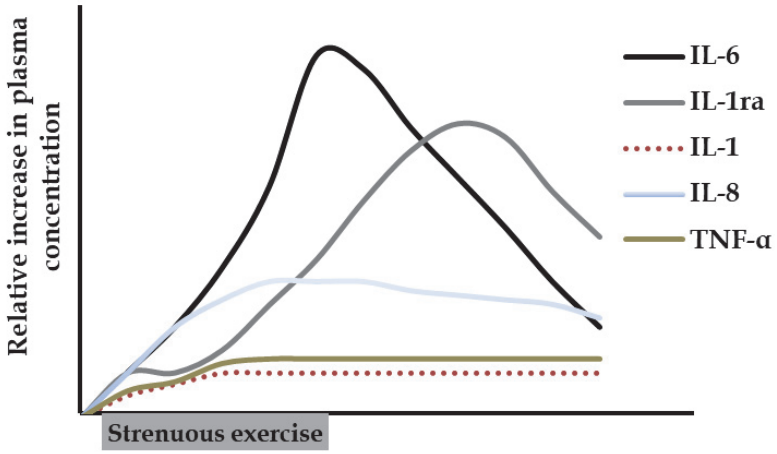


FIGURE 4 Schematic presentation of the changes in a number of cytokines in relation to strenuous endurance exercise. Increased CRP levels do not appear until 8 –12 h later. Modified from (Pedersen 2000). IL-6 = interleukin-6; IL-1ra = interleukin-1 receptor antagonist; IL-1 = interleukin-1; IL-8 = interleukin-8; TNF-α = Tumor Necrosis Factor-α.

2.2.2 Resistance exercise bout

The acute response to a resistance exercise bout seems to be similar to that of an endurance exercise bout but with a lower magnitude. A single exercise bout has been demonstrated to exert specific acute effects on cytokine levels (Bouassida et al. 2010). Recent studies on the effects of RE bout on selected inflammation markers (IL-6, IL-1ra, IL-1β, MCP-1, resistin, adiponectin and leptin) are presented in Table 2.

One bout of heavy resistance exercise triggers a transient inflammatory response comprising of an augmented white blood cell count and stimulation of pro- and anti-inflammatory cytokine production (Freidenreich & Volek 2012). The cytokine response induced by a bout of heavy resistance exercise involves enhanced production of pro-inflammatory cytokines, such as IL-1β and IL-6.

Similar to ET, the pro-inflammatory response is followed by increase in anti-inflammatory markers. Together these mediators play a crucial role in the containment and resolution of inflammation processes, and have been suggested to have a role in mediating the beneficial effects of resistance exercise (Steensberg et al. 2003a; Petersen & Pedersen 2005). Especially the increase in anti-inflammatory markers within the circulation has been suggested to provide positive metabolic changes through increased fat oxidation and glucose uptake (Petersen & Pedersen 2005; Pedersen & Febbraio 2008; Walsh et al. 2011).

TABLE 2 The effects of RE bout on selected inflammation markers in circulating (IL-6, IL-1ra, IL-1β, MCP-1, resistin, adiponectin and leptin).

Study	RE bout	Participants	Samples	IL-6	IL-1ra	IL-1β	MCP-1	Resistin	Adiponectin	Leptin
Ashtary-Larky et al. 2017	MH	Healthy young men (n=28)	pre, post	↑$	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Bautmans et al. 2005	MH	Healthy elderly men and women (n=31)	pre, post	↑	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Buford, Cooke & Willoughby 2009	MH	Post-menopausal women (n=24)	pre, post180, post24, Post48	↔	n.a.	↔	n.a.	n.a.	n.a.	n.a.
Izquierdo et al. 2009	MH	Healthy young men (n=12)	pre, post, post15, post45	↑ post45	↑	↑	n.a.	n.a.	n.a.	n.a.
Peake et al. 2006	ME MS	Healthy young men (n=10)	pre, post, post60, post180, post1-4d	↑ post180	↔	n.a	n.a	n.a	n.a	n.a
Phillips et al. 2010	MH	Obese postmenopausal women (n=23)	pre, post, post360	↑	n.a	n.a	n.a	n.a	n.a	n.a
Prestes et al. 2009	MH	Post-menopausal women (n=35)	pre, post, post1-2d	↓ post2d	n.a	n.a	n.a	↑¤ post1d	n.a.	↑¤
Uchida et al. 2009	ME	Healthy young men (n=35)	pre, post1-3d	↔	n.a	↔	n.a	n.a	n.a	n.a
Varady et al. 2010	MH	Healthy young men (n=43)	pre, post	n.a.	n.a.	n.a.	n.a.	↓*	↑*	↔
Wells et al. 2016	MH MS	Healthy young men (n=10)	pre, post, post30, post60, post300	n.a.	n.a.	n.a.	↑	n.a.	n.a.	n.a.
Zafeiridis et al. 2003	MH, MS; ME	Healthy young men (n=10)	pre, post, post45	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	↓ post30

*= Significant change only in participants with resistance training background; $ = significant change in untrained and trained. ¤ = Significant increase only before resistance training intervention; IL-6 = interleukin-6; IL-1ra = interleukin 1 receptor antagonist; IL-1β = interleukin 1β; MCP-1 = monocyte chemoattractant protein-1; ME = muscle endurance; MH = muscle hypertrophy; MS = maximal strength; post30 = sampled given minutes after exercise; post1d = sampled one day after the exercise; post2d = sampled two days after the exercise.

The extent of inflammation response to resistance exercise is affected by the physiological demands of RE, depending on the mode (eccentric and/or concentric muscular contractions), volume (total work of the session), load (weight lifted), and intensity (extent of neuromuscular and metabolic fatigue) (Peake et al. 2017). Previous studies have suggested that exercise training would significantly alter the acute inflammatory responses of high intensity resistance exercise and recovery process after a resistance exercise bout. Cross-sectional studies have suggested that training background affects the acute cytokine responses to an RE bout (Varady et al. 2010). Furthermore, Murton et al. (2014) highlighted that the response to the first RE bout in participants with no background in RT is significantly different and involves more inter-participant variability compared to the second RE bout (Murton et al. 2014). In addition, Izquierdo and colleagues (2009) reported a significantly lower inflammation-responsive cytokine IL-6 response to the same RE bout followed by a significantly enhanced response in the anti-inflammatory IL-1ra response after RT intervention compared to the response before training (Izquierdo et al. 2009). Thus, the overall effects of long-term RT appears to attenuate acute inflammation response but there are mixed findings on the effect of RT on specific markers (Vanhees et al. 2012).

2.3 Adaptations of basal inflammation markers to exercise training

Previous studies have indicated an inverse association between physical activity and low-grade inflammation (Pitsavos et al. 2003; Pischon et al. 2003; Fischer et al. 2007; Lavie et al. 2011; Pinto et al. 2012). As such, lower concentration of inflammation markers have been especially observed in individuals who report performing frequent moderate intensity physical activity (Beavers et al. 2010). Exercise training has been shown to influence the production of cytokines and adipocytokines towards an anti-inflammatory direction in various tissues including adipose tissue and skeletal muscle (Görgens et al. 2015). Thus, there seems to be a general consensus that exercise training is effective in suppressing inflammation (Peake et al. 2005; Nassis et al. 2005; Stewart et al. 2007; Calle & Fernandez 2010; Walsh et al. 2011; Forti et al. 2017).

2.3.1 Endurance training

The first studies on the effects of endurance exercise training on inflammation markers focused on the immunosuppressive effects of strenuous endurance training on athletes (Nieman 2000). Later, endurance training has been shown to be effective in reducing inflammation (Steensberg et al. 2003b). Beavers et al. (2010) summarized randomized controlled studies that have evaluated the effect of endurance training on systemic inflammation markers. They concluded

that small scale studies support the assertion that exercise training reduces inflammation, however, future studies are needed to refine our understanding of the effects of exercise training on systemic low-grade inflammation, the magnitude of such an effect, and the amount of exercise necessary to elicit clinically meaningful changes in the deleterious association between inflammation and disease (Beavers et al. 2010). For example, Kohut et al. (2006) showed that endurance exercise 3 $d \cdot wk^{-1}$, 45 $min \cdot d^{-1}$ for 10 months reduces CRP, IL-6, IL-18, and TNFα concentrations in aged participants (Kohut et al. 2006). In addition, there is strong evidence on the significant effects of endurance training on leptin and adiponectin concentrations (Bouassida et al. 2010). The mechanisms related to the improvements in inflammation status when endurance type of exercise training is done have been suggested to be related to reduction of visceral obesity and the improvements in insulin sensitivity (Zoppini et al. 2006).

2.3.2 Resistance training

Resistance training is associated with reduced risk of low grade inflammation related diseases, such as cardiovascular disease and type 2 diabetes. The effects of RT on inflammation markers have been reported to be more limited than in ET (Calle & Fernandez 2010). The results of the RT studies that have measured inflammation markers in blood are summarized in Table 3. To summarize the current data, RT has been associated with improvements in inflammation state in overweight adults (Olson et al. 2007), elderly individuals (Phillips et al. 2012), as well as in specific patient groups (Conraads et al. 2002; Moraes et al. 2014), whereas the results regarding healthy young men are mixed (Ara et al. 2006; Azizbeigi et al. 2015; Forti et al. 2017). The heterogeneity of the results may be explained by the range of different RT intervention used across the studies. Training variable differences included frequency duration, intensity, and dose of exercise. In addition, there may have been diversity in the initial strength and metabolic status of the participants. Nevertheless, the findings on the effects of the intensity and mode of training on inflammation markers are limited (Forti et al. 2017).

2.3.3 Combined resistance and endurance training

As both ET and RT have been suggested to have anti-inflammatory effects, adhering to both exercise modes (combined training, CT) could be considered to be important for reducing low-grade inflammation (Table 4). Data regarding the effects of combined ET and RT on inflammation markers is sparse. Steward et al. (2007) showed that healthy young participants can benefit from CT, as CRP decreased significantly after 12 weeks of training 3 $d \cdot wk^{-1}$ (Stewart et al. 2007). Later, Libardi et al. (2012) failed to observe significant reductions in inflammation markers after CT in sedentary middle-age men (Libardi et al. 2012), while other studies have found significant improvements in inflammation markers in healthy untrained men and women (Donges et al.

2013; Stefanov et al. 2014) as well as in obese men (Brunelli et al. 2015), participants with metabolic syndrome (Balducci et al. 2010) in overweight women, and in children (13 to 17 year-old). However, Changas et al. (2017) showed a significant reduction in TNF-α and increase in IL10/TNF-α in post-menopausal women that trained with moderate intensity three times a week (Chagas et al. 2017). Since both endurance training and resistance training have shown positive results in improving inflammation status, it has been proposed that combined training would be superior to only one mode of training when aiming for health improvements (Nimmo et al. 2013; Brunelli et al. 2015).

TABLE 3 Effects of resistance training on selected inflammation markers. Table continues on next page.

Study	Study design / Participants	Dur. / Freq.	Resistance Training	IL-6	IL-1ra	IL-1β	TNF-α	CRP	MCP-1	Resistin	Adipo	Leptin
Azizbeigi et al. 2015	MI-RT vs. HI-RT vs. C (young men, n = 30)	8 wk /3d·wk^{-1}	MI-RT 3 x 10-12, 65-70% of 1RM; HI-RT 3 x 3-6, 85-90% of 1RM.	↔	n.a.	n.a.	↔	n.a.	n.a.	n.a.	n.a.	n.a.
Ara et al. 2006	RT vs C (young men, n=18)	6 wk /3d·wk^{-1}	1-3 x 10(3) Intensity from 50→ 70% (90%) of 1RM.	n.a.	n.a.	n.a.	n.a	n.a.	n.a.	n.a.	n.a.	↔
Bautmans et al. 2005	HI-RT (older persons, n=31)	6 wk / 3d·wk^{-1}	3 x 10RM. Whole-body. Intensity from 50% → 70–80% of 1RM	↔	n.a.	n.a.	n.a	n.a.	n.a.	n.a.	n.a.	n.a.
Botero et al. 2013	RT vs. C (postmeno-pausal women, n=35)	1 yr / 2d·wk^{-1}	3 x 6-14RM.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	↓	n.a.	↓
Donges et al. 2013	ET vs. RT vs. CT (healthy men, n=47)	12 wk /3d·wk^{-1}	3(4) × 10(8). Whole body. 75-80% of 1RM	↓	↔	n.a.	↓	↔	n.a.	n.a.	n.a.	n.a.
Fatouros et al. 2005	HI-RT vs. LI-RT vs. MO-RT vs. C (inactive old men, n=50)	48 wk / 3d·wk^{-1}	3 x 8-10-14, 80-60-50% 1RM.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	↑ *	↓
Forti et al. 2014	Progressive RT vs C (old men and women, total n=40)	12 wk / 3d·wk^{-1}	wk 1-2: 3 × 10, 50% of 1RM; from wk 3→ 3 × 10, 70–80 % of 1RM	↓	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Forti et al. 2017	HI -RT vs. LI-RT vs. LI+MAX RT (young men, total n=36)	9 wk / 3d·wk^{-1}	HI: 1×10–12, 80% 1RM; LO: 1×10–12, 40% 1RM; LOmax: 1×10–12, 40% 1RM + 1×60, 20–25% 1RM.	↓	↑	↔	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

TABLE 3 Effects of resistance training on selected inflammation markers. Continues from the previous page.

Study	Study Design / Participants	Dur. / Freq.	Resistance Training	IL-6	IL-1ra	IL-1β	TNF-α	CRP	MCP-1	Resistin	Adipo	Leptin
Jorge et al. 2011	ET vs. RT vs. CT (men and women; diabetic patients; total n=48	12 wk / 3d·wk-1/	Whole-body, circuit training.	↔	n.a.	n.a.	↔	↓	n.a.	n.a.	↔	n.a.
Karabulut et al. 2013	HI-RT vs. LI-RT vs. C (older healthy men, total n=36)	6 wk / 3d·wk^{-1}	HI: 80% 1-RM LI-BFR: 20% 1-RM with vascular restriction	↔	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Levinger et al. 2009	Progressive RT (men and women with and without metabolic risk factors, n=27)	10 wk / 3d·wk^{-1}	3 x 10-12, 40– 50% to 75–85% of 1RM	↔	n.a.	↔	n.a.	↔	n.a.	n.a.	n.a.	n.a.
Libardi et al. 2012	ET vs. RT vs. CT vs. C (sedentary men, n=47)	16 wk / 3d·wk^{-1}	3 x 8-10RM. Whole body.	↔	n.a.	n.a.	↔	↔	n.a.	n.a.	n.a.	n.a.
Olson et al. 2007	RT vs C (overweight women, total n=28)	1 yr / 2d·wk^{-1}	3 x 8-10RM. Whole body.	↔	n.a.	n.a.	n.a.	↓	n.a.	n.a.	↑	n.a.
Phillips et al. 2012	RT vs. C (postmenopausal women, n=23)	12 wk / 3d·wk^{-1}	3 x 8RM. Whole body.	n.a.	n.a.	n.a.	↓	↓	n.a.	n.a.	↔	↓
Prestes et al. 2009	RT vs. C (postmenopausal women, n=35)	16 wk / 2d·wk^{-1}	3 x 6-14RM. Whole body.	↓	n.a.	n.a.	↔	n.a.	n.a.	↓	n.a.	↓
Rall et al. 1996	HI-RT (rheumatoid arthritis patients vs. young vs. old vs. C men, total n=30)	12 wk / 2d·wk^{-1}	80% of 1RM. Whole body.	↔	n.a.	↔	↔	n.a.	n.a.	n.a.	n.a.	n.a.

RT = resistance training, ET = endurance training, CT = combined endurance and resistance training, wk = week, HI-RT = high-intensity resistance training, LI-BFR = low-intensity resistance training with vascular restriction, C = no exercise control group. *= only in HI-RT group. IL-6 = interleukin-6; IL-1ra = interleukin-1 receptor antagonist; IL-1β = interleukin-1β; TNF-α = tumor-necrosis factor-α; CRP = C-reactive protein; MCP-1 = monocyte chemoattractant protein-1, adipo = adiponectin.

TABLE 4 Effects of CT on selected inflammation markers.

Study	Study design / Participants	Dur. / Freq.	Combined training	IL-6	IL-1ra	IL-1β	TNF-α	CRP	MCP-1	Resistin	Adipo	Leptin
Balducci et al. 2010	ET vs. CT vs. LI AT vs. C (patients with type 2 diabetes, n=82)	1 yr / 2d·wk^{-1}	Same-session: 40 min aerobic exercise at 70–80% VO2max + 20 min a whole body RT program 80% 1RM	↓	n.a.	↓	↓	↓	n.a.	↓	↑	↓
Brunelli et al. 2015	CT vs. C (obese men, n=30)	24 wk / 3d·wk^{-1}	Same session: a whole-body RT program 3x 10 + 30 minutes of AT	↔	n.a.	n.a.	↓	↓	n.a.	↓	↑	↓
Donges et al. 2013	ET vs. RT vs. CT (healthy men, n=47)	12 wk /3d·wk^{-1}	Different day: a whole-body training program 3 × 10 of each exercise at 75% of 1RM + 40 to 60 minutes of AT.	↓	↔	n.a.	↓	↔	n.a.	n.a.	n.a.	n.a.
Libardi et al. 2012	ET vs. RT vs. CT vs. C (sedentary men, n=47)	16 wk /3d·wk^{-1}	Same-session: 3 x 8-10RM. Whole body work-out + 30 minutes AT	↔	n.a.	n.a.	↔	↔	n.a.	n.a.	n.a.	n.a.
Lopes et al. 2016	CT vs. (overweight girls 13-17 yr, n=33)	12 wk / 3d·wk^{-1}	Same-session: 3 x 6-10 RM Whole body work-out + 27-29 minutes AT	↔	n.a	n.a	↔	↓	n.a	↔	↔	↓
Stewart et al. 2007	Combined training vs. C (older and younger men and women total, n=59)	12 wk / 3d·wk^{-1}	Same-session; aerobic 20 minutes + 2 x 8 70-80% of 1-RM	↔	n.a.	↔	↔	↓	n.a.	n.a.	n.a.	n.a.

RT = resistance training; ET = endurance training; CT = combined resistance and endurance training, wk = week; HI-RT = high-intensity resistance training; LI-BFR = low-intensity resistance training with vascular restriction; C = control group; IL-6 = interleukin-6; IL-1ra = interleukin-1 receptor antagonist; IL-1β = interleukin-1β; TNF-α = tumor-necrosis factor-α; CRP = C-reactive protein; MCP-1= monocyte chemoattractant protein-1, Adipo = adiponectin.

2.4 Mechanism of the anti-inflammatory effect of exercise

Exercise has been shown to influence the production of cytokines and adipocytokines towards an anti-inflammatory direction in various tissues including adipose tissue and skeletal muscle (Görgens et al. 2015). Therefore, it is likely that changes in adipocytokines, in addition to the classical inflammatory cytokines, contribute to established health benefits of exercise.

2.4.1 Reduction of visceral fat

Excess fat mass in the abdomen, liver, and muscles is associated with all-cause mortality (Pischon et al. 2008). Especially intra-abdominal fat mass has been shown to be an important risk factor for systemic low-grade inflammation. The distribution of excess fat in the abdominal region is known to modify the health risk profile, whereas excess adiposity in the periphery does not appear to increase the risk of developing cardiovascular disease (Strasser, Arvandi & Siebert 2012). Regular exercise can reduce total as well as visceral/abdominal fat mass, even in the absence of any loss of body weight (Ross & Bradshaw 2009). Therefore, exercise training can have a positive decreasing effect on the inflammation status via a reduction of pro-inflammatory adipocytokine secretion as a direct result of lowering the amount of active, pro-inflammatory mediator producing adipose tissue (Gleeson et al. 2011; Görgens et al. 2015).

Migration of monocytes towards the infection site, including muscle, adipose tissue and damaged vascular cells, is central to the development of sustained inflammation (Zeyda et al. 2011). Exercise has been suggested to limit the movement of monocytes into inflamed adipose tissue (Bishop et al. 2009). The mechanism is suggested to be related to the increased chemokine concentration after acute exercise stress. For example, as concentrations of MCP-1 increase it results in chemokine receptor internalization. This is thought to serve as a negative feedback mechanism that reduces migration and further accumulation of monocytes in inflamed tissue by downregulation of expression of receptors in monocytes and restricting the migration of these cells towards adipose tissue. However, this finding in humans does not report any independent effect of exercise training on adipose-tissue expression of MCP-1, despite the decrease in circulating MCP-1 concentrations. Another mechanism behind the anti-inflammatory effect of exercise has been suggested to be related to the switch of tissue-resident macrophages in adipose tissue from M1 to M2 phenotype (Gleeson et al. 2013, 303). Exercise has been shown to induce the phenotypic switch in mice but human studies are lacking (Kawanishi et al. 2012; Gleeson et al. 2013, 303-305).

2.4.2 Release of IL-6 from contracting muscle

In addition to adipose tissue, skeletal muscle has gained considerable interest as an endocrinal organ and the release of cytokines, known as myokines, from

contracting muscle is assumed to be at least partly responsible for the health-promoting effects of exercise that protect against low-grade inflammatory diseases (Mathur & Pedersen 2008). Even if IL-6 is pro-inflammatory nature, the anti-inflammatory effect of exercise has been suggested to be mediated by the increased production and release of IL-6 from the contracting skeletal muscle (Mathur & Pedersen 2008).

Pedersen et al. (2008) have shown that a contracting active skeletal muscle increases cellular and circulating concentrations of IL-6 (Pedersen & Febbraio 2008). Later, post-exercise muscle biopsy samples have been shown to contain muscle cells that can produce and release IL-6 and MCP-1 as well as resident macrophages that can produce IL-6, IL-10, IL-8, MCP-1, and Granulocyte-Colony Stimulating Factor (G-CSF). In addition, the muscle biopsies have contained endothelial/epithelial cells that can produce IL-8, MCP-1, IL-1ra, and G-CSF. Increased secretion of IL-6 from the contacting muscle has been suggested to be related to the depletion of muscle glycogen. Thus the IL-6 would work as an energy sensor in the muscle (Nieman et al. 2015).

The increases in IL-6 are transient but this acute increase appears to be responsible for an increase in the level of anti-inflammatory mediators, IL-1ra and IL-10, as well as the release of cortisol from the adrenal glands (Steensberg et al. 2003a). Increased IL-1ra secretion from macrophages and monocytes leads to inhibition of pro-inflammatory IL-1β (Kaplanski et al. 2003), whereas the increase in IL-10 leads to downregulation of the adaptive immune systems effector response, lesser tissue damage in infectious challenges (Couper, Blount & Riley 2008), and to the lower expression of several pro-inflammatory cytokines (Cassatella et al. 1993). There are limitations to the hypothesis that the release of IL-6 from contracting muscle is the main mechanism behind the anti-inflammatory effect of exercise training. The main critique for the hypothesis has been that there is no substantial increase observed in circulating IL-6 after low/moderate intensity exercise (Fischer et al. 2007) despite the known health benefits associated with moderate increases in physical activity. The biological effects of muscle-delivered IL-6 are presented in Figure 5.

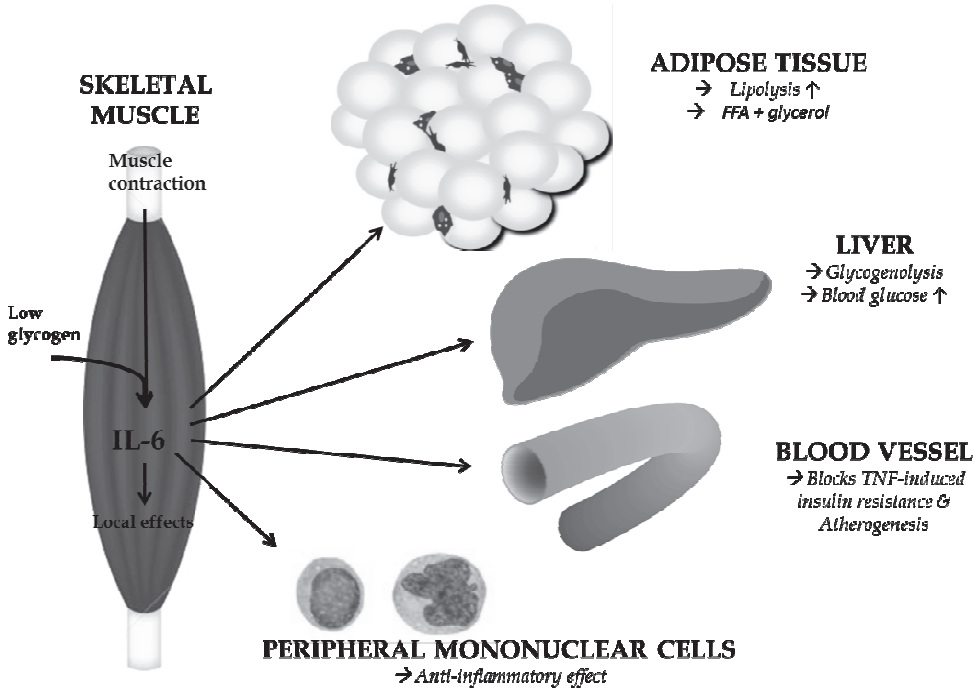


FIGURE 5 The biological aspects of the muscle delivered IL-6. Modified from (Pedersen et al. 2001). FFA = Free fatty acids; IL-6 = interleukin-6; TNF = tumor necrosis factor α.

2.4.3 Other mechanisms

Increased levels of circulating hormones. Short and prolonged high -intensity exercise bouts lead to acute elevation of cortisol (Kraemer & Ratamess 2005; Hackney et al. 2012) and adrenalin (Boutcher 2011). Secretion of adrenal hormones in response to activation of the sympathetic nervous system is usually observed before the increase in circulating cytokines (Kjaer & Dela 1996). Cortisol is known to have an anti-inflammatory effect (Pedersen & Hoffman-Goetz 2000) and catecholamines downregulate lipopolysaccaride-induced production of IL-6 as well as TNF-α (Bergmann et al. 1999). Thus, the exercise induced hormonal responses have been suggested to be among the mechanisms that are involved in the anti-inflammatory effect of exercise (McMurray & Hackney 2005; Gleeson et al. 2013, 307).

Downregulation of toll-like receptor expression. Activation of toll-like receptor (TLR) signaling results in increased expression and secretion of pro-inflammatory cytokines and thus has an important role in mediating systemic inflammation. It has been shown that especially TLR4 expression is lower in physically more active individuals (Flynn & McFarlin 2006) and this has been shown to be associated with decreased production of pro-inflammatory cytokines (Lancaster et al. 2005).

Increased number of regulatory T-cells. Regulatory T-cells suppress immune responses via cell contact-dependent mechanisms (Sakaguchi et al. 2009). Low intensity exercise has been shown to increase the number of regulatory T-cells (Yeh et al. 2006). Wang et al. (2012) have shown that only high-intensity training increases the regulatory T-cells in mice (Wang et al. 2012). Later it has been shown that aged athletes have greater regulatory T-cells response to exercise than the sedentary controls which might indicate that the training background changes the acute response to more anti-inflammatory direction (Minuzzi et al. 2017).

3 PURPOSE OF THE DISSERTATION

The purpose of this dissertation was to examine the acute and chronic effects of resistance training on inflammation markers in healthy men. In addition, the effect of combined resistance and endurance training on the inflammation markers was assessed. Lastly, the relationship between these markers, muscle damage, and body composition was evaluated.

Cross-sectional RE bout study (I) and longitudinal training study with acute RE bouts (II)

These two papers examined the acute effects of different types of resistance exercise bouts on inflammation markers in healthy young men. The main objectives were to:

1) Examine the acute effects of hypertrophic, maximal, and explosive resistance exercise bouts on selected inflammation markers in healthy young men.
2) Assess the effects of resistance training on the acute inflammation responses after hypertrophic and maximal explosive resistance exercise bouts.

Longitudinal resistance (III) and combined resistance and endurance training studies (IV)

These two papers examined the chronic effect of resistance training and combined resistance and endurance training in young men. The main objectives were to:

1) Evaluate the effects of the RT and combined ET and RT on selected inflammation markers
2) To evaluate the association between the changes in abdominal fat mass, total body lean mass, strength, and selected inflammation markers.

The primary hypothesis was that acute resistance exercise bouts would elicit a significant pro-inflammatory response, which would be followed by an anti-inflammatory response. The magnitude of responses could be affected by the volume and intensity of the RE as well as the muscle damage followed by the RE bout. As each RE bout leads to a shift towards an anti-inflammatory direction, it was expected that RT would lead to a decrease in circulating inflammatory markers. In addition, it was hypothesized that CT would lead to significant reduction in fat mass that would be followed by decrease in resting concentrations of inflammation markers.

4 RESEARCH METHODS

4.1 Participants and ethical considerations

A total of 146 healthy men, 18-40 years of age with no background in systematic resistance training (RT) were recruited to participate in these four studies comprising papers I - IV. The participants were moderately physically active as characterized by walking, cycling, or occasionally participating in team sports at light to moderate intensity with a frequency of 3 $d \cdot wk^{-1}$. The physical characteristics of the participants are presented in Table 5. The participants were carefully informed about the possible risks and benefits of all study procedures before providing a written informed consent. A completed health questionnaire and resting ECG were reviewed by a cardiologist prior to the first exercise testing and training. All participants were non-smokers, free of acute and chronic illness, as well as disease or injury and did not report use of any medications. All the studies were conducted according to the Declaration of Helsinki, and ethical approval was granted by the University of Jyväskylä Ethical Committee.

TABLE 5 Baseline data for included participants.

Original paper	Group	Age (years)	Height (m)	Weight (kg)	Bodyfat (%)
I	C-O (n=12)	28.2 ± 3.5	1.8 ± 0.1	78.6 ± 10.4	16.5 ± 3.5*
II	C-O, RT(n=7)	31.0 ± 0.9	1.8 ± 0.1	84.6 ± 1.9	25.3 ± 7.1#
III	HS (n=35)	33.2 ± 7.4	1.8 ± 0.1	84.9 ± 11.2	20.9 ± 8.5#
III	SHP(n=31)	34.8 ± 6.7	1.8 ± 0.1	81.1 ± 10.9	20.1 ± 9.5#
III	C (n=14)	34.5 ± 5.3	1.8 ± 0.1	83.3 ± 11.6	19.2 ± 9.0#
IV	SS (n=16)	30.9 ± 5.3	1.8 ± 0.1	80.1 ± 13.2	25.4 ± 7.1#
IV	AD (n=15)	29.2 ± 6.4	1.8 ± 0.1	81.8 ± 10.3	22.9 ± 6.1#
IV	C (n=16)	33.0 ± 4.7	1.8 ± 0.1	80.7 ± 11.8	23.1 ± 8.3#

C-O = cross-over experiment; C-O RT = cross-over experiment with 12 weeks of resistance training; C = Control; HS = hypertrophy-strength; SHP = strength-hypertrophy-power; SS = Same session; AD = training in alternating days. * measured by bioimpedance; # measured by dual-energy x-ray absorptiometry.

4.2 Research design

In order to study the effects of different types of resistance exercise bouts as well as resistance and combined resistance and endurance training, two separated acute studies with maximal, hypertrophic and explosive resistance exercise bouts and two different prolonged training interventions with resistance and combined resistance and endurance training were planned.

4.2.1 Cross-sectional resistance exercise bout study (I)

In order to determine the acute effects of two different resistance exercise protocols, participants performed a maximal (MAX) or a hypertrophic (HYP) resistance exercise bout in a randomized and counter-balanced manner. Acute effects of RE bouts on inflammation markers were assessed using blood samples before, immediately, 15 minutes, and 30 minutes after the RE bout. The durations of the RE bouts were 20 and 50 minutes for HYP and MAX, respectively.

4.2.2 Longitudinal training study with acute RE bouts (II)

A longitudinal study design was used to examine the effect of RT on the acute responses to a resistance exercise bout. The study design is presented in Figure 6. After pretesting, the participants went through a cross-over study design during which they were randomly assigned to acute HYP1 or POW1 RE bout and after an average of ten days of recovery to POW1 or HYP1, respectively. After the acute RE bouts, participants trained for 12 weeks after which they did the same acute RE bouts in the same order (HYP2 and POW2, respectively). The

first resistance exercise bout was performed after a four-week long preparatory RT period, during which participants were familiarized to RT. This RT period was conducted to reduce possible heterogeneity in performance and to minimize the effects of stressors related to unaccustomed exercise, which may occur in the initial phase of RT. The order of the protocols was randomized and counter-balanced. The exercise bouts were separated by one to two weeks. The same acute RE bout protocols were performed after 12 weeks of training. To assess the acute effects and recovery after the RE bouts, blood samples were drawn before (PRE), immediately after (POST0) as well as 24 (POST24) and 48 (POST48) hours after the RE bout before and after the RT period. The durations of the RE bouts were 20 and 30 minutes for HYP and POW, respectively.

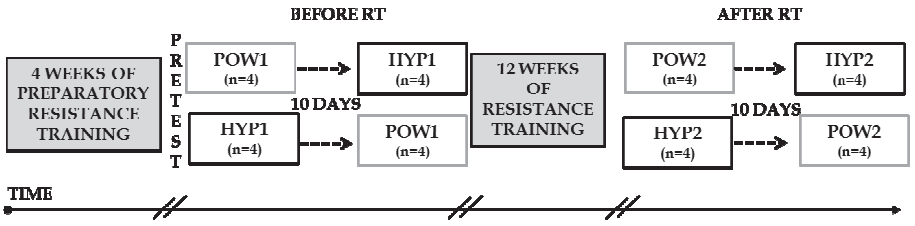


FIGURE 6 Study design in longitudinal training study (II) with acute RE bouts. POW1 = maximal explosive RE before RT; HYP1 = hypertrophic RE before RT; POW2 = maximal explosive RE after RT, HYP2 = hypertrophic RE after RT.

4.2.3 Longitudinal resistance training study (III)

This study is a part of a larger research project (TEKES Decision No. 70007/13). A total of 150 men contacted us to express their interest in the study. Of these, 92 men met the participation criteria and participated in pre-measurements. The participants were randomly assigned to hypertrophy-strength training (HS, n=44) or to strength-hypertrophy-power training (SHP, n=44). In addition, 14 men served as a control group. The participants were randomly assigned to hypertrophy-strength training (HS, n=44) or to hypertrophy-strength-power training (HSP, n=44). The number of the participants that completed pre-, 4wk, and 16wk measurements and were included in this study were HS = 37 and SHP = 31. The duration of the whole training intervention was 16 weeks. Measurements were performed before (wk0), after 4 weeks of familiarization (wk4) as well as after 16 weeks (wk16) of training. The C group was measured only before and after the intervention.

4.2.4 Combined endurance and resistance training study (IV)

This study is a part of a larger research project (Schumann et al. 2014; Eklund et al. 2016). Participants were recruited through general advertisements in local newspapers as well as posters and emails that were delivered to local companies and institutions. A total of 150 people contacted us to express their

interest toward the study. Of these, 93 people met the participation criteria. The participants were assigned to either of the two training interventions or the control group: combined resistance and endurance training performed in the same session (SS, n=16) or on alternating days (AD, n=16) or control group (C, n=16). Ultimately, a total of 48 healthy men completed pre- and post-measurements and were included in this study. The exercise order of SS training was randomized with half of the group performing endurance exercise bout immediately followed by resistance exercise bout and the other half performing the opposite exercise order. The overall training volume was equal in the SS and AD. Measurements were performed before (PRE), during (i.e. after 12 weeks, MID) and after (i.e. after 24 weeks, POST) the training intervention.

4.3 Measurement procedures

4.3.1 Blood samples

Venous blood samples were drawn from an antecubital vein. Fasting samples were taken in the morning (7:00-9:00 a.m.) after a 12 h overnight fast. Participants were instructed to abstain from strenuous physical activity for 48 h before the blood samples were taken. Venous blood was collected into EDTA and serum separator tubes for analysis of inflammation profiles. The serum samples were held for 15 min at room temperature before being centrifuged for 10 min at 2000 x g Megafuge 1.0 R, Heraeus, Germany). The plasma samples were centrifuged for 10 min at +4°C with 2000 x g (Megafuge 1.0 R, Heraeus, Germany). Both plasma and serum were kept at -80°C until analyzed.

4.3.1.1 Blood lactate

Blood lactate concentrations were determined during the incremental cycling test (IV) and after the acute resistance exercise bouts (I, II). Capillary blood samples were taken from the fingertip before, during, and after the experimental RE bouts into a reaction tube containing an anti-coagulant and hemolyzing agent. The samples were analyzed using a Biosen lactate analyzer (S-line Lab+ EKF, Magdeburg, Germany). The sensitivity for the blood lactate was 0.5 $mmol \cdot L^{-1}$. The inter-assay coefficient of variation was 6.2%.

4.3.1.2 C-reactive protein and cytokines

High-sensitivity C - reactive protein (hsCRP) from serum samples was analyzed using the Immulite 1000 and immunoassay kits (Immulite, Siemens, IL, USA). Concentrations of interleukin 6 (IL-6), interleukin 1 receptor antagonist (IL-1ra), interleukin 1β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), adiponectin, leptin, and resistin from plasma samples were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (R&D Systems, Europe Ltd, Abingdon, UK). The detection limit and inter-assay

coefficient of variations are reported in each paper. There were no samples that showed a concentration under the detection limit in any variable.

4.3.1.3 Muscle damage markers and serum hormones

Myoglobin concentration (I) was determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (USCN Life Science Inc., Wuhan, China) and creatine kinase (II) was analyzed from serum samples using the Immulite 1000 and immunoassay kits (Immulite, Siemens, IL, USA). The detection limit and inter-assay coefficient of variations are reported in each paper.

4.3.2 Cardiorespiratory performance

In the CT study (IV), endurance performance was assessed by a maximal graded protocol on a cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany). The protocol began at 50 W and increased by 25 W every 2 minutes. Participants were asked to maintain a pedaling frequency of 70 rpm throughout the test. The test was stopped when the participants failed to maintain the required cadence for more than 15 seconds. Oxygen uptake was determined continuously breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). Peak oxygen consumption (VO_{2peak}) was averaged over 60 second periods during the test.

4.3.3 Neuromuscular performance

Maximal bilateral concentric strength was assessed as one-repetition maximum (1 RM) using a David 210 weight stack horizontal leg press device (David Health Solutions Ltd., Helsinki, Finland). The participants were seated in the device with a starting knee angle of 60° (58° ± 2°). In preparation for the 1 RM trials, the participants performed 3 warm-up sets (5 × 70–75% estimated 1 RM, 3 × 80–85% estimated 1 RM, 2 × 90–95% estimated 1 RM) with 1 min rest between sets. When assessing 1 RM, a dynamic action to full leg extension (knee angle 180°) was performed when verbally instructed. The load was increased following each successfully completed repetition. After a maximum of five maximal trials, the trial with the highest successfully completed load was accepted as the 1 RM.

4.3.4 Anthropometrics and body composition

In order to control the conditions, each measurement was done after an overnight fast. Body height was measured to the nearest 0.5 cm using a wall-mounted scale. The participants were barefoot and wore shorts.

Bioimpedance (I). After an overnight fast, body composition (body mass (BM), total body muscle mass (MM), fat mass (FM) and percentage of body fat measurements were performed using an eight-point bioelectrical impedance device (Inbody 720 body composition analyzer, Biospace Co. Ltd, South Korea).

*Dual X-ray Absorptiometry (DXA) (*II, III, IV*).* Body composition was assessed by Dual X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems, Madison, USA). Total fat mass and total lean mass were automatically analyzed (Encore-software, version 14.10.022). Abdominal fat (II and IV) was calculated by manually defining a range of interest (ROI) confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest (Tallroth, Kettunen & Kujala 2013). This customized range was then copied to the DXA scans at wk4/MID and wk16/POST, respectively in RT and CT studies. The same technician analyzed all the scans.

4.3.5 Nutrition

The researchers gave participants both verbal and written nutritional recommendations based on the Finnish Nutrition Recommendations 2014 in one two hour lecture. As a rule, these follow the recommendations for the Nordic countries in Europe published in Autumn 2013 (NNR2012) and are very close to USDA and HHS dietary guidelines (2010) for normal healthy adults. Participants (II, III, IV) were asked to keep their dietary intake constant and the dietary intake was examined by nutritional diaries. In addition, the participants were instructed on how to report nutritional intake in the diaries. Dietary intake was recorded over three weekdays and one weekend day. The food diaries were analysed by nutrient analysis software (Nutri-Flow; Flow-team Oy, Oulu, Finland).

As resistance training study (III) was part of the larger project the participants were randomly given a post-workout supplement. One group received protein, one group carbohydrate, and one group protein plus carbohydrate. Protein and carbohydrates were provided by Northforce (Kuusamon Juusto Oy, Kuusamo, Finland). The protein group received 37.5 grams of whey concentrate (30 g of whey proteins, 5 g of lactose < 1 g of fat) and the carbohydrate group received 34.5 grams of maltodextrin. In contrast, the protein plus carbohydrate group received 37.5 grams of whey concentrate (30 g of whey proteins) and 34.5 grams of maltodextrin. Nutrients provided by the supplements were included in the analysis. There were no significant differences in the nutrient intake between the groups thus the nutritional subgroups were randomly assigned and evenly distributed within the training groups.

4.4 Acute resistance exercise bouts

The loads used during the first set of each acute resistance exercise were determined from the 1RM load of the familiarization session. The loads were adjusted during the sessions to enable completion of the required repetitions. If the participant was not able to complete the required repetitions, assistance was provided and the load reduced for the next set.

4.4.1 Pretesting and familiarization

All participants took part in a familiarization session, which included anthropometrics and body composition measurements, as well as the 1RM test performed as leg press (David D210 horizontal leg press device, David Health Solutions Ltd., Helsinki, Finland). The loads used during the first set in acute RE bouts were determined from the load of the 1RM measurement at pretesting. The loads were adjusted during the sessions to enable completion of the required reps.

4.4.2 MAX and HYP resistance exercise bouts (I)

The MAX protocol consisted of 15 sets of 1 repetition at 100% of 1RM and the HYP protocol was 5 sets of 10 reps at 80% of 1RM for the leg press exercise. The inter-set rest period was three minutes for MAX and two minutes for HYP. The exercise bouts were separated by one week. Blood samples were drawn before as well as immediately (POST0), 15 minutes (POST15) and 30 minutes (POST30) after the RE bout.

4.4.3 POW and HYP resistance exercise bouts (II)

The maximal explosive (POW) protocol included 10 sets of 5 repetitions, with the concentric phase performed as fast as possible at 60% of 1RM and the HYP protocol included 5 sets of 10 reps at 80% of 1RM for the leg press exercise. The inter-set rest period was three minutes for POW and two minutes for HYP.

4.5 Training programs

All participants (II, III and IV) were asked to maintain their habitual physical activity (light walking, cycling and occasional team sports) throughout the study period.

4.5.1 Resistance training (II and III)

RT programs were divided into four different training blocks. The intervention started with 4 weeks of progressive muscle endurance type RT twice a week for all group. A total of 8 training sessions were done during the initial phase of RT. After 4 weeks of training the participants were divided into two different RT regimens: 1) training aiming especially for muscle hypertrophy and strength (HS) and 2) training aiming for muscle strength, hypertrophy, and power (SHP) for 12 weeks. In the second block SHP group had 25% power and 75% maximal-strength training sessions, the third block 75% power and 25% maximal-strength training sessions and in the last block 87.5% power and 12.5% maximal-strength training sessions. By contrast, in the second block the HS

training had 100% hypertrophic training sessions, in the third block 75% HS and 25% maximal strength training sessions and in the last block 25% hypertrophic and 75% maximal strength of the total training sessions per block. A total of 28 training sessions were undertaken during the specialized training phase. Training consisted of exercises for the whole body 2-3 sessions per week, but lower limb exercises (bilateral leg press, knee extension, and knee flexion) were trained in every session. The training program also included exercises for the other main muscle groups of the body: chest and shoulders, upper back, trunk extensors and flexors, and upper arms conducted every second training session. The individual loads were determined by the strength tests to each exercise every fourth week. HS training contained mainly sets of 8–12 repetitions with 75–85% loads of 1 RM. Maximal strength training in both RT groups consisted of neural enhancing RT with lower repetitions per set (typically 4–6) and higher intensity (86–95% 1 RM), but also more traditional hypertrophy sets to increase muscle size. Power training consisted of sets with lower loads of 1 RM (50–80% 1 RM) performed with maximal concentric speed. In the longitudinal training study with acute RE bouts (II) the participants trained as in HS and the acute RE bouts were performed at wk4 and wk16.

4.5.2 Combined resistance and endurance training (IV)

The training was designed to reflect a program typically recommended for physically active populations (Thompson et al. 2010). The main objective was to improve both aerobic capacity and 1RM performance through a periodized program including moderate and vigorous intensity endurance exercise (Helgerud et al. 2007; Daussin et al. 2007) combined with hypertrophic and maximal strength protocols (Kraemer & Ratamess 2004). To assure the correct execution of the training prescribed, all training sessions were supervised by qualified instructors. AD group trained resistance and endurance training sessions separated on different days whereas SS group trained resistance and endurance on a same session in a random order resistance and endurance or endurance and resistance.

Endurance training sessions were carried out on a magnetic resistance cycle ergometer. Training intensities were controlled by heart rate zones corresponding to the aerobic and anaerobic threshold values. During weeks 1-7 and 13-16 the training consisted of 30-45 min of continuous cycling near the aerobic threshold and progressed to interval training at and above anaerobic threshold from weeks 8 and 17 onwards. Thresholds were re-evaluated after week 12 and applied to the training during weeks 13-24. The duration of each endurance training session was between 30 and 50 minutes.

Resistance training sessions were performed for all major muscle groups with special attention given to the lower extremities, focusing on knee extensors, hip extensors, and knee flexors. The training program included exercises for all major muscles and the loads used were determined by the number of repetitions and execution velocity. The training program was initiated with circuit training (as general, preparatory training, 2-4 x 15-20 repetitions at 40-

60%of 1RM) and progressing through hypertrophy-inducing training (2-5 x 8-10 repetitions at 80-85% of 1 RM) towards maximal strength training (2-5 x 3-5 repetitions at 85-95% of 1 RM) and during the last two weeks explosive strength (2 x 8-10 repetitions at 40% of 1RM). The periodization for the extensors and flexors of the arms followed a similar pattern. In addition, exercises for the trunk were included in all resistance training sessions. The lower body exercises were performed with weight-stack devices while dumbbells and cable pulley machines were used for upper body exercises. Trunk exercises were performed both with machines and body weight. The periodization was repeated during weeks 13-24 with increased training intensity and volume. The duration of one resistance exercise bout was between 50–60 minutes.

4.6 Statistical analyses

Conventional statistical methods were used to obtain means, standard deviations, standard error, and correlation coefficients. Normal distribution was determined through the Shapiro-Wilk test. Data that were not normally distributed was log transformed before applying parametric tests. Dependent variables were assessed by analysis of variance (ANOVA), or analysis of covariance (ANCOVA) where appropriate, with repeated measures and Bonferroni adjustments as post hoc tests. If a significant main effect or interaction was observed, the absolute change from pre-values for mid and post was compared between groups using paired t-tests with Bonferroni correction. Effect sizes (ES) are given as Cohen's d with an effect size of 0.20-0.50 being considered small, 0.50-0.80 medium, and >0.80 large (Cohen 1988, 25-27; Lakens 2013). The level of statistical significance was set at $p < 0.05$.

5 RESULTS

5.1 Acute inflammatory responses to RE bouts (I, II)

There were no significant differences between pre-exercise concentrations of cytokines or adipocytokines. In the cross-sectional study (I) total work in MAX was 2 500 ± 380 kg and 7 240 ± 1 190 kg in HYP. Whereas in the training study (II) the total work was 7 160 ± 272 kg in POW and 7 550 ± 427 kg in HYP and POW, respectively.

5.1.1 Interleukins

IL-6 responses were similar in all exercise protocols, as in HYP, MAX and POW a significant increase at POST0 was observed (I, II, Table 6). Also, in the cross-sectional study (I), IL-6 showed a progressive increase after the exercise bout with the highest concentrations being observed at POST30 (+90 and +94% for MAX and HYP, respectively). In the training study (II), IL-6 response was significantly affected by RT in HYP (time × training interaction, $p < 0.05$, ES = 0.534) and in POW (time × training interaction, $p < 0.05$, ES = 0.808). A significant increase in IL-6 was observed at POST0 in HYP1 ($p < 0.01$, ES = 0.719) and in POW1 ($p < 0.01$, ES = 0.878) before training, whereas no significant effects were observed after training in HYP2 and POW2.

In the cross-sectional study (I), the IL-1ra response was different between MAX and HYP. In HYP, IL-1ra concentrations were significantly increased at POST0 ($p < 0.05$) whereas in MAX no significant differences were observed in untrained participants. Conversely, in the training study (II) circulating IL-1ra remained unaltered before RT in HYP1 and in POW1 but after RT the circulating IL-1ra concentration increased significantly from PRE to POST0 after RT in HYP2 ($p < 0.05$) and in POW2 ($p < 0.05$). IL-1ra concentration returned to baseline levels at POST24 after both RE bouts.

TABLE 6 The acute effect of RE on interleukins (mean + SD).

PAPER I			PRE	POST0	POST15	POST30
HYP	IL-6 (pg·mL^{-1})		0.62 ± 0.26	0.84 ± 0.36*	0.85 ± 0.34*	1.21 ± 0.49**
	IL-1ra (pg·mL^{-1})		357 ± 94	457 ± 182*¤	403 ± 9	319 ± 93
MAX	IL-6 (pg·mL^{-1})		0.77 ± 0.47	1.11 ± 0.35*	1.22 ± 0.42**	1.46 ± 0.45**
	IL-1ra (pg·mL^{-1})		357 ± 144	338± 99	374 ± 222	339 ± 137
PAPER II			**PRE**	**POST0**	**POST24**	**POST48**
HYP	IL-6 (pg·mL^{-1})	HYP1	1.03 ± 0.28	1.64 ± 0.48*	1.20 ± 0.31	1.03 ± 0.51
		HYP2	1.14 ± 0.23	1.33 ± 0.27	1.20 ± 0.31	0.98 ± 0.21
	IL-1ra (pg·mL^{-1})	HYP1	398 ± 95	386 ± 75	350 ± 61	381 ± 75
		HYP2	341 ± 55	403 ± 65*	344 ± 53	330 ± 53
	IL-1β (pg·mL^{-1})	HYP1	1.28 ± 0.20	2.90 ± 0.88**	1.84 ± 0.49	1.56 ± 0.30
		HYP2	2.17 ± 0.40	1.72 ± 0.46	2.03 ± 0.68	1.22 ± 0.21
POW	IL-6 (pg·mL^{-1})	POW1	1.03 ± 0.28	1.64 ± 0.48*	1.20 ± 0.31	1.03 ± 0.51
		POW2	1.14 ± 0.23	1.33 ± 0.27	1.20 ± 0.31	0.98 ± 0.21
	IL-1ra (pg·mL^{-1})	POW1	398 ± 95	386 ± 75	350 ± 61	381 ± 75
		POW2	341 ± 55	403 ± 65*	344 ± 53	330 ± 53
	IL-1β (pg·mL^{-1})	POW1	1.28 ± 0.20	2.90 ± 0.88**	1.84 ± 0.49	1.56 ± 0.30
		POW2	2.17 ± 0.40	1.72 ± 0.46	2.03 ± 0.68	1.22 ± 0.21

HYP = hypertrophic RE bout, MAX = maximal RE bout; POW = maximal explosive RE; HYP1 = HYP before resistance training; HYP2 = HYP after resistance training; POW1 = POW before resistance training; POW2 = POW after resistance training. IL-6 = interleukin-6; IL-1ra = Interleukin-1 receptor antagonist; IL-1β = Interleukin-1 beta; ¤ = significant between RE bouts difference, * = significant difference from pre-value. *p<0.05, **p<0.01.

IL-1β was analyzed only in the training study (II). A significant main effect of time in circulating IL-1β was observed in HYP ($p < 0.05$, ES = 0.775) and in POW ($p < 0.05$, ES = 0.496). Significant increases from PRE to POST0 were observed in IL-1β in HYP1 before RT ($p < 0.05$, ES = 0.795) whereas after RT statistically significant changes were not observed. Similarly, in POW1 before RT a significant increase was observed in circulating IL-1β concentration from PRE to POST0 ($p < 0.05$), whereas after RT significant changes were not observed.

5.1.2 MCP-1

MCP-1 response to HYP (I, II), MAX (I) and POW (II) RE bouts are presented in Figure 7. In the cross-sectional study (I), a significant main effect of time was observed in MCP-1. There was a significant decrease ($p < 0.01$) in MCP-1 in HYP at POST30 when compared to PRE, whereas in MAX a significant decrease was not observed ($p = 0.102$). In the training study (II) similar results were observed in the untrained state as no significant differences were observed at POST0. However, a significant time × training effect was observed in circulating MCP-1 concentrations in HYP ($p < 0.01$, ES = 0.924). Thus, after RT in HYP2 a significant increase in MCP-1 was observed during recovery at POST24 ($p < 0.01$) and at POST48 ($p < 0.05$). In addition, an increasing trend in circulating MCP-1 concentrations was observed also at POST24 and POST48 in POW2 but that effect did not reach statistical significance ($p = 0.102$).

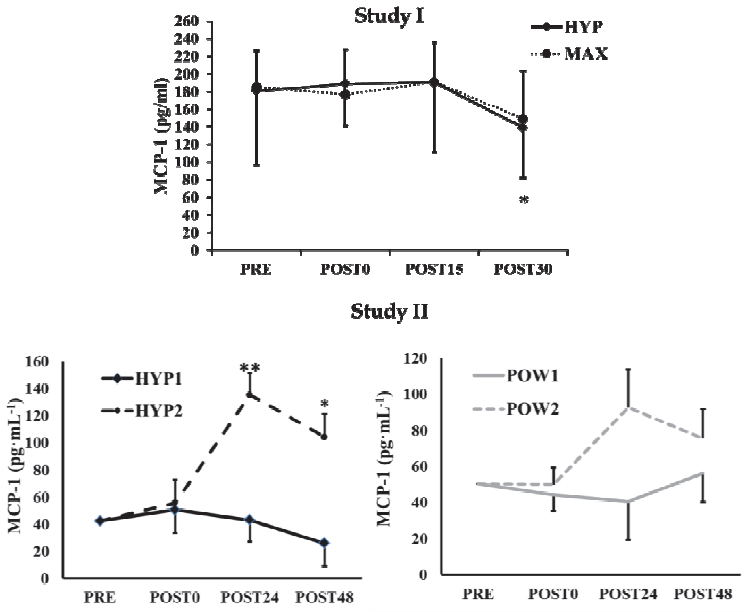


FIGURE 7 The effect of acute RE bouts on MCP-1 concentration. HYP = hypertrophic RE bout, MAX = maximal RE bout; POW = maximal explosive RE; HYP1 = HYP before resistance training; HYP2 = HYP after resistance training; POW1 = POW before resistance training; POW2 = POW after resistance training. MCP-1 = monocyte chemoattractant protein-1. *= significant difference from pre-value. *p<0.05, **p<0.01.

5.1.3 Adipocytokines

Adiponectin and leptin response to HYP (I, II), MAX (I) and POW (II) RE bouts are presented in Table 7. Resistin responses are illustrated in Figure 8.

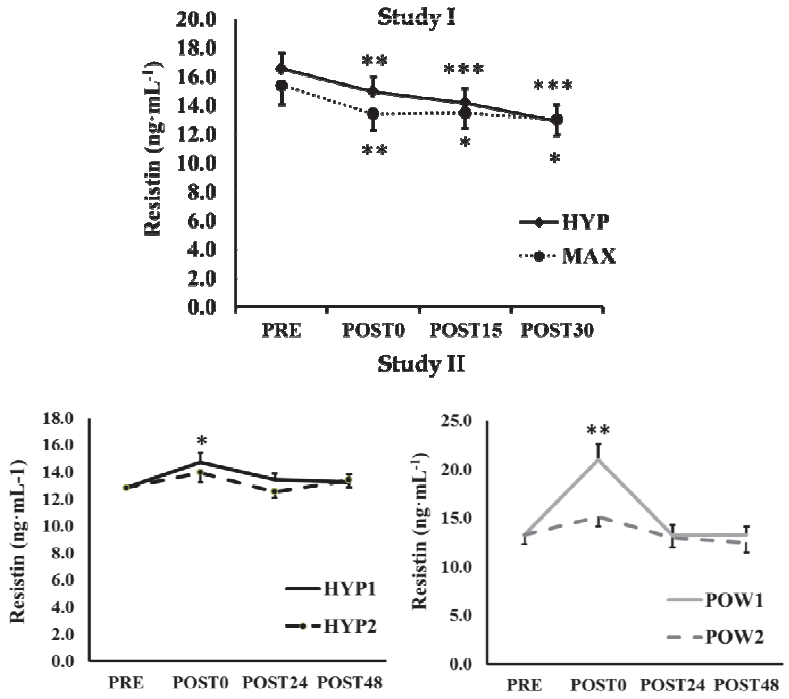


FIGURE 8 The effect of acute RE bouts on circulating resistin concentrations. HYP = hypertrophic RE bout, MAX = maximal RE bout; POW = maximal explosive RE; HYP1= HYP before resistance training; HYP2 = HYP after resistance training; POW1 = POW before resistance training; POW2 = POW after resistance training. * = significant difference from pre-value. *p<0.05, **p<0.01, ***p<0.001.

Adiponectin and leptin. In the cross-sectional study (I) adiponectin and leptin levels were unaffected by HYP and MAX exercise bouts. Following RT in the training study (II), the circulating leptin concentrations were significantly higher at PRE before both RE bouts. In HYP1, a significant reduction in circulating leptin concentrations was observed at POST24 ($p < 0.05$) and in HYP2 at POST0 ($p < 0.05$). Similarly to leptin, also pre-exercise circulating adiponectin concentrations were significantly higher after RT in HYP2 ($p < 0.05$) and in POW2 ($p < 0.05$) and stayed unaltered after RE bouts.

Resistin. In the cross-sectional study (I), a significant main effect of time ($p < 0.05$) was observed in resistin. Resistin decreased significantly ($p < 0.01$) in MAX and HYP at POST0 and was progressively decreased during recovery at POST15 and at POST30. Conversely, in the cross-sectional study (II), there was an increase in HYP1 from PRE to POST0 before RT ($p < 0.05$, ES = 0.789) whereas resistin stayed statistically unaltered by HYP2 after RT. In addition, there was a significant main effect of time ($p < 0.01$, ES = 0.732) and time × training interaction ($p < 0.05$, ES = 0.732) in circulating resistin levels in POW. A significant increase in resistin concentrations from PRE to POST0 was observed

before RT in POW1 ($p < 0.01$, ES = 0.997), whereas after RT in POW2 such a response was not observed.

TABLE 7 The acute effect of RE on leptin and adiponectin (mean ± SD).

PAPER I			PRE	POST0	POST15	POST30
HYP	Adiponectin (pg·mL^{-1})		4.4 ± 1.3	4.8 ± 1.4	4.7 ± 1.2	4.6 ± 1.3
	Leptin (pg·mL^{-1})		3.9 ± 2.5	3.8 ± 2.2	3.6 ± 2.3	3.3 ± 2.2
MAX	Adiponectin (pg·mL^{-1})		4.2 ± 1.2	4.3 ± 1.4	4.2 ± 1.2	4.3 ± 1.3
	Leptin (pg·mL^{-1})		3.3 ± 1.9	3.3 ± 1.9	3.3 ± 1.8	3.3 ± 1.9
PAPER II			**PRE**	**POST0**	**POST24**	**POST48**
HYP	Adiponectin (pg·mL^{-1})	HYP1	4.0 ± 0.9	4.5 ± 1.1	4.0 ± 0.9	4.8 ± 1.0
		HYP2	4.5 ± 0.7#	4.4 ± 0.6	4.6 ± 0.9	4.3 ± 0.7
	Leptin (pg·mL^{-1})	HYP1	7.2 ± 1.6	7.8 ± 2.0	5.8 ± 1.2*	7.6 ± 1.5
		HYP2	8.7 ± 2.0#	8.0 ± 1.9*	8.6 ± 1.8#	8.4 ± 1.6#
POW	Adiponectin (pg·mL^{-1})	POW1	3.9 ± 0.9	4.7 ± 0.7	4.2 ± 1.0	4.2 ± 0.9
		POW2	4.7 ± 0.7#	4.5 ± 0.7	4.9 ± 0.9	4.5 ± 0.7
	Leptin (pg·mL^{-1})	POW1	6.5 ± 1.7	6.6 ± 1.5	6.8 ± 1.6	7.5 ± 1.7
		POW2	8.9 ± 2.2#	7.9 ± 2.1#	9.3 ± 2.5#	8.5 ± 1.9#

HYP = hypertrophic RE bout, MAX = maximal RE bout; POW=maximal explosive RE; HYP1 = HYP before resistance training; HYP2 = HYP after resistance training; POW1 = POW before resistance training; POW2 = POW after resistance training. *= significant difference from pre-value. #significant difference from corresponding value before training. *p<0.05, **p<0.01, #p<0.05.

5.1.4 Muscle damage markers and blood lactate response

Myoglobin (I). There were no differences in serum myoglobin levels between resistance exercise bouts. Myoglobin concentrations increased significantly, and at a similar magnitude in MAX (+182%, $p < 0.01$) and in HYP (+217%, $p < 0.001$) at POST0, and did not increase further after POST0 but remained elevated during the follow-up at POST30. Muscle damage and blood lactate results are presented in Table 8.

TABLE 8 The immediate and prolonged effects of RE bouts on lactate and muscle damage markers.

I			PRE	POST0	POST15	POST30
HYP	Lactate (mmol·L^{-1})		2.1 ± 0.1	12.0±0.5***†††	11.2± 0.6***†††	n.a.
	Myoglobin (ng ·ml^{-1})		25.1 ± 8.0	41.7 ± 6.5**	50.4 ± 7.1***	48.1 ± 4.4***
MAX	Lactate (mmol·L^{-1})		1.8 ± 0.2	4.6 ± 0.6**	2. 1± 0.2*	n.a.
	Myoglobin (ng ·ml^{-1})		23.1 ± 4.2	44.0 ± 8.0**	49.1 ± 8.0***	51.0 ± 4.4***
II			**PRE**	**POST0**	**POST24**	**POST48**
HYP	Lactate (mmol·L^{-1})	HYP1	1.5 ± 0.4	13.0 ± 4.1***	n.a	n.a
		HYP2	2.2 ± 0.5	10.1 ± 3.4**#	n.a	n.a
	CK (pg·mL^{-1})	HYP1	250 ± 230	280 ± 390	360 ± 240***	240 ± 120
		HYP2	140 ± 40	150 ± 40#	280 ± 150**#	230 ± 90*
POW	Lactate (mmol·L^{-1})	POW1	1.9 ± 0.9	3.0 ± 1.1*	n.a.	n.a.
		POW2	2.2 ± 0.9	3.1 ± 1.8	n.a.	n.a.
	CK (pg·mL^{-1})	POW1	150 ± 80	140 ± 30	310 ± 90**	230 ± 70*
		POW2	130 ± 80	110 ± 60	330 ± 90**	250 ± 60**

HYP1 = hypertrophic resistance exercise bout before RT; HYP2 = hypertrophic resistance exercise bout after RT; POW1 = maximal explosive resistance exercise bout before RT; POW2 = maximal explosive resistance exercise bout after RT; CK = creatine kinase; n.a. = not analyzed. * = significant difference from pre-value. † = significant difference from corresponding value on MAX, #significant difference from corresponding value before training. *p<0.05, **p<0.01,***p<0.001, #p<0.05, †††p<0.001.

Creatine kinase (II). A significant increase in creatine kinase was observed at POST24 before RT in HYP1 (130 ± 50%, p<0.001) and in POW1 (140 ± 40%, p<0.01) and after RT in HYP2 (120 ± 50%, p<0.001) and in POW2 (200 ± 50%, p<0.01). Thus, RT significantly (p<0.05) suppressed the immediate increases in creatine kinase induced by HYP.

Blood lactate. In the cross-sectional study (I), there was a significant type × time ($p < 0.01$) and main effect ($p < 0.01$) of time observed in blood lactate concentrations. The peak lactate concentrations were observed at POST0 (4.6 ± 0.6 and 12.0 ± 0.5 mmol·L^{-1} in MAX and HYP, respectively). The increase was significant in both HYP (+670 ± 210%, $p < 0.001$) and in MAX (+160 ± 110%, $p < 0.01$) at POST0 when compared to PRE values, although the increase was significantly higher ($p < 0.001$) in HYP compared to the MAX protocol.

In the training study (II), peak lactate concentrations were observed at POST0 (13.0 ± 1.5 and 3.0 ± 0.4 mmol·L^{-1} in HYP and POW, respectively). A significantly increased lactate concentrations before RT was observed at POST0 in HYP (800 ± 340%, $p<0.001$) and in POW (80 ± 90%, $p<0.01$), whereas a significant increase in lactate after RT was observed only in HYP (360 ± 310%, $p<0.01$). Further, RT significantly ($p<0.05$) suppressed the immediate increases in lactate induced by both HYP and POW.

5.1.5 Effect of RT on body composition and 1RM (II)

In the training study with acute loadings (II), whole-body fat-free mass increased significantly ($p < 0.05$), whereas body weight, whole-body fat mass and abdominal fat mass stayed unaltered after RT. Additionally, maximal strength increased significantly (+13%, $p < 0.05$, Table 9).

TABLE 9 Effects of RT on body composition and 1RM (II).

Variable	Week 0	Week 12	Δ-%
Body weight (kg)	84.6 ± 5.1	83.8 ± 4.85	-0.8 ± 2.9
Height (m)	1.78 ± 0.1	1.78 ± 0.1	-
BMI (kg·m^{-2})	26.8 ± 1.4	26.6 ± 1.2	-0.8 ± 2.9
Body fat mass (kg)	21.6 ± 6.8	19.8 ± 5.8	-6.6 ± 13.8
Abdominal fat mass (kg)	2.9 ± 1.5	2.8 ± 1.0	-3.2 ± 6.4
Fat-free mass (kg)	59.6 ± 0.5	61.6 ± 0.6*	3.3 ± 0.3*
1 RM (kg)	225 ± 35	255 ± 30*	14 ± 8

1RM = 1 repetition maximum; *= significant difference from pre-value.*$p<0.05$.

5.2 Chronic effect of RT (III) and CT (IV) on inflammation markers

The training adherence for the included participants in the RT study (III) was 99 ± 2% and 95 ± 1% in HS and SHP groups, respectively. All participants included for the study completed at least 90% of the overall training volume. There were no significant differences between the groups in self-reported total energy intake. Total energy intakes in the RT study (III) (expressed in MJ) were 10.1 ± 1.8 and 11.2 ± 2.1 in HS and SHP groups, respectively.

In the CT study (IV), the training adherence for the included participants was 99 ± 2% and 100 ± 1% in same session (SS) and alternating days (AD) respectively. All participants completed at least 90% of the overall training volume. There were no significant differences between the groups in self-reported total energy intake. Total energy intakes in CT study (expressed in MJ) were in 10.2 ± 2.0 SS and 9.2 ± 2.6 in AD, respectively.

5.2.1 Effect of training on body composition, maximal strength and aerobic capacity

No significant changes in body mass were observed in either of the longitudinal studies. The body composition and physical fitness data from the longitudinal studies are presented in Tables 10 and 11.

Total fat mass. In the resistance training study (III), a significant reduction in total fat mass already from wk0 to wk4 (-3.2 ± 6.8%, $p < 0.01$, ES = 0.969) was observed. During the specialized training period a significant group × time interaction was observed ($p < 0.01$, ES=0.795) in total fat mass. Thus, from wk4 to wk16 a significant reduction in total fat mass was observed only in the HS group (-6.2 ± 10%, $p < 0.001$, ES = 0.993). Significant change in total fat mass in the CT study (IV) was not observed

Abdominal fat mass. In the RT study (III), abdominal fat mass decreased in line with the decreases in total fat mass. Independent of the change in total fat mass, in the CT study (IV), a significant main effect of time ($p < 0.001$, ES = 0.974) and interaction ($p < 0.05$, ES = 0.789) was observed in abdominal fat mass. A significant decrease in abdominal fat mass from PRE to POST was observed in SS (-7.4 ± 15.4%, $p < 0.05$, ES = 0.445) and AD (-21.1 ± 17.6%, $p < 0.001$, ES = 0.997). No significant change in abdominal fat mass was observed in C. Abdominal fat mass in AD at POST was significantly lower compared to SS and C ($p < 0.05$; $p < 0.05$ respectively).

Lean mass. In the RT study (III), lean mass increased significantly from wk0 to wk4 (1.4 ± 1.9%, $p < 0.001$, ES = 1.00), and a significant group × time interaction was observed during the specialized RT ($p < 0.05$, ES = 0.536). A significant increase in lean mass was observed from wk4 to wk16 both in HS and SHP groups (1.3 ± 1.9%, $p < 0.001$, ES = 1.00; 1.5 ± 1.7%, $p < 0.05$, ES = 0.669, respectively) but the increase was greater in HS. In the CT study (IV), a significant increase in lean mass was observed at POST in both training groups (2.8 ± 2.7% and 3.8 ± 3.5%, in SS and AD, respectively).

TABLE 10 Changes in body composition variables in resistance training (III) and combined training study (IV) studies.

		Resistance training (III)			Combined training (IV)		
		HS (n=37)	**SHP** (n=31)	**C** (n=14)	**SS** (n=16)	**AD** (n=15)	**C** (n=18)
Body weight (kg)	PRE	85 ± 11	81 ± 11	81 ± 13	80 ± 13	82 ± 10	81 ± 12
	wk4 (III)/ wk12 (IV)	85 ± 12	82 ± 11	n.a.	80 ± 12	82 ± 10	n.a.
	POST	85 ± 11	82 ± 11	82 ± 14	80 ± 11	81 ± 10	82 ± 12
BMI ($kg \cdot m^{-2}$)	PRE	25.8 ± 3.1	25.7 ± 3.3	25.2 ± 4.5	25.2 ± 3.0	25.3 ± 2.6	25.2 ± 3.9
	wk4 (III)/ wk12 (IV)	25.9 ± 3.20	25.9 ± 3.23	n.a.	25.2 ± 2.5	25.3 ± 2.9	n.a.
	POST	25.2 ± 4.5	26.0 ± 3.2	25.5 ± 4.5	25.4 ± 2.3	24.9 ± 2.9	25.5 ± 3.9
Body fat mass (kg)	PRE	20.9 ± 8.5	20.1 ± 9.5	19.2 ± 8.9	20.8 ± 8.1	22.9 ± 6.1	19.2 ± 7.4
	wk4 (III)/ wk12 (IV)	20.2 ± 8.5***	19.7 ± 9.3***	n.a.	20.0 ± 7.3	21.6 ± 6.7	n.a.
	POST	18.9 ± 8.4###	19.4 ± 9.1	20.3 ± 9.1#	19.0 ± 7.0	19.5 ± 7.3	20.4 ± 7.7
Abdominal fat mass (kg)	PRE	3.2 ± 1.5	3.2 ± 1.8	2.4 ± 1.2	2.6 ± 1.2	3.1 ± 1.0	2.3 ± 1.2
	wk12 (III) / wk4 (IV)	3.0 ± 1.5***	2.9 ± 1.8***	n.a.	2.3 ± 1.1	2.8 ± 1.0**	n.a.
	POST	2.8 ± 1.5###	3.0 ± 1.7	2.6 ± 1.2#	2.3 ± 1.1	2.5 ± 1.1###	2.5 ± 1.4
Lean mass (kg)	PRE	60.5 ± 6.0	57.8 ± 4.9	59.6 ± 5.9	53.3 ± 6.1	55.9 ± 5.1	59.5 ± 5.9
	wk12 (III) / wk4 (IV)	61.3 ± 6.1***	58.6 ± 5.1***	n.a.	54.1 ± 5.7	57.2 ± 5.7	n.a.
	POST	62.5 ± 6.1###	59.1 ± 5.0 ###	59.6 ± 5.9	54.8 ± 5.9#	58.0 ± 5.2#	58.7 ± 5.9

SS = same session; AD = alternating days; HS=hypertrophy-strength; SHP = strength-hypertrophy-power; n.a. = not analyzed; * = significant difference from pre-value; # = significant difference from wk12/wk4. *p<0.05; **p<0.01; ***p<0.001, #p<0.05; ##p<0.01; ###p<0.001.

Aerobic capacity. In the CT study (IV), a significant main effect of time ($p < 0.05$, ES = 0.748) and interaction ($p < 0.01$, ES = 0.877) was observed for VO_{2peak}. Both the SS and AD groups increased VO_{2peak} significantly from PRE to MID (6.80 ± 8.28%, $p < 0.01$ and 13.2 ± 11.9%, $p < 0.001$, respectively) and from PRE to POST (9.3 ± 8.85%, $p < 0.001$ and 18 ± 10.3%, $p < 0.001$, respectively), while no significant change was observed in C ($p = 0.637$, ES = 0.081).

Strength. In the RT study (III), a significant main effect of time was observed in 1RM from PRE to wk4 ($p < 0.001$, ES = 0.916) as an increased 1RM was observed from PRE to wk4 (17.6 ± 18.3%, $p < 0.001$, ES = 1.00). Both HS and SHP training increased 1RM from wk4 to wk16 (13 ± 8.1%, $p < 0.001$, ES = 1.00; 11 ± 6.5%, p <

0.001, ES = 1.00, respectively). 1RM did not change significantly in the control group (p = 0.399). In the CT study (IV), a significant main effect of time (p < 0.001, ES = 0.989) and interaction (p < 0.01, ES = 0.918) in 1RM was observed and 1RM increased in both training groups (p < 0.001). Both training groups as well as C increased 1RM from PRE to MID (p < 0.001) and from PRE to POST (p < 0.001). The increase in 1RM was significantly larger in SS and AD groups (+14.1 ± 11.4%, p<0.01 and +12.7 ± 7.24%, p<0.01; respectively) than in C group (+4.7 ± 4.7%).

TABLE 11 Changes in performance variables in resistance training (III) and in combined training (IV) studies.

		Resistance training (III)			Combined training (IV)		
		HS (n=37)	**SHP** (n=31)	**C** (n=14)	**SS** (n=16)	**AD** (n=15)	**C** (n=18)
Physical fitness							
1RM (kg)	PRE	207 ± 35	213 ± 32	173 ± 29	151 ± 32	145 ± 18	159 ± 30
	wk12 (III) / wk4 (IV)	222 ± 34 ***	226 ± 33 ***	n.a.	164 ± 27	159 ± 17	n.a.
	POST	244 ± 32 ###	251 ± 36 ###	180 ± 28	170 ± 26 ##	163 ± 16 ##	167 ± 29
VO2peak (L·min^{-1})	PRE	n.a.	n.a.	n.a.	3.1 ± 0.4	2.8 ± 0.3	3.1 ± 0.5
	wk12 (III) / wk4 (IV)	n.a.	n.a.	n.a.	3.3 ± 0.4	3.2 ± 0.3	n.a.
	POST	n.a.	n.a.	n.a.	3.4 ± 0.5 ##	3.3 ± 0.4 ##	3.1 ± 0.5

SS = same session; AD = alternating days; HS=hypertrophy-strength; SHP = strength-hypertrophy-power; n.a. = not analyzed; *= significant difference from pre-value; #significant difference from wk12/wk4. *p<0.05; **p<0.01; ***p<0.001, #p<0.05; ##p<0.01; ###p<0.001.

5.2.2 Basal interleukins and TNF-α

No significant main effects of time or interaction in IL-6 were observed in either of the training studies (III, IV). The adaptation in basal IL-1ra was analyzed only in the RT study (III), where a significant increase in IL-1ra concentration was observed after the 4wks of RT (p < 0.001, ES = 0.989, Figure 9). The specialized RT phase did not have any effect on IL-1ra.

In the combined training study (IV), a significant main effect of time was observed in circulating concentrations TNF-α (p < 0.01, ES = 0.926). A slight but statistically significant reduction in TNF-α concentration was observed in AD at POST (p < 0.05, ES = 0.418), while no significant changes in SS or C were observed (p = 0.056 and p = 0.218, respectively).

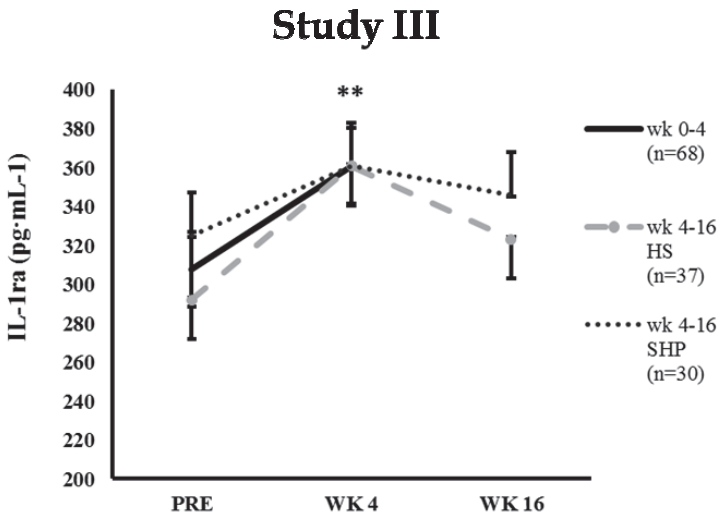


FIGURE 9 The effect of RT on IL-1ra. IL-1ra = interleukin-1 receptor antagonist; HS = hypertrophy-strength; SHP = strength-hypertrophy-power. Significant difference from pre to wk4, **p < 0.01.

5.2.3 Basal MCP-1

In the RT study (III), MCP-1 concentration increased after the initial phase of RT ($p < 0.05$, ES = 0.548). Thereafter, both HS and SHP had a significant lowering effect on MCP-1 levels ($p < 0.05$, ES = 0.522; $p < 0.05$, ES = 0.604, respectively). Whereas, in the CT study (IV), a significant main effect of time ($p < 0.05$, ES = 0.869) and interaction ($p < 0.05$, ES = 0.760) was observed in the levels of MCP-1 (Figure 10). At POST, a significant reduction in MCP-1 was observed in AD ($p < 0.05$, ES = 0.840) but not in SS and the control groups. In addition, the concentration of MCP-1 at POST in AD was significantly lower than in SS and C ($p < 0.05$ and $p < 0.01$ respectively).

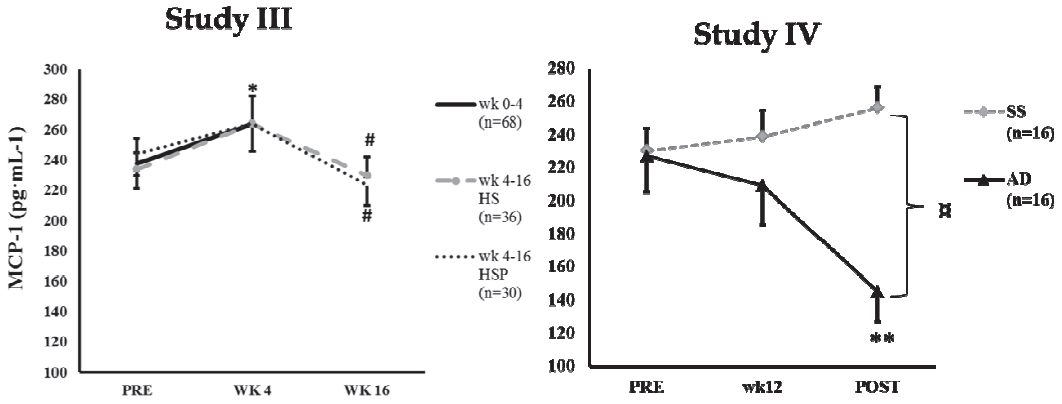


FIGURE 10 The effects of RT (left) and CT (right) on circulating monocyte chemoattractant protein 1 (MCP-1). There were no significant changes in MCP-1 concentration from pre to post in RT (240 ± 20 → 230 ± 20 pg·mL^{-1}) or in CT study (230 ± 30 → 220 ± 40 pg·mL^{-1}). SS = same session training, AD = alternating days training; HS = hypertrophy-strength; SHP = strength-hypertrophy power. *= significant difference from pre-value; # significant difference from wk4 value. *p < 0.05; **p < 0.01; #p < 0.05).

5.2.4 Basal adipocytokines

The chronic adaptations in adipocytokines (resistin, leptin and adiponectin) in RT (III) and CT (IV) studies are presented in Figures 11 and 12, respectively.

Resistin. In the RT study (III), after the initial phase of RT, circulating resistin concentrations increased significantly (1.25 ± 0.40%; $p < 0.05$, ES = 0.546) but were significantly reduced during the following 12 weeks only in HS training ($p < 0.05$, ES = 0.519). Additionally, there was a significant between-group difference in resistin at wk16 ($p < 0.05$). Whereas, in the CT study (III), a significant main effect of time ($p < 0.01$, ES = 0.942) was observed in concentrations of circulating resistin. Significant reductions in concentrations of circulating resistin were observed in SS ($p < 0.05$, ES = 0.582) and AD ($p < 0.05$, ES = 0.661) but in C resistin concentration remained unaltered.

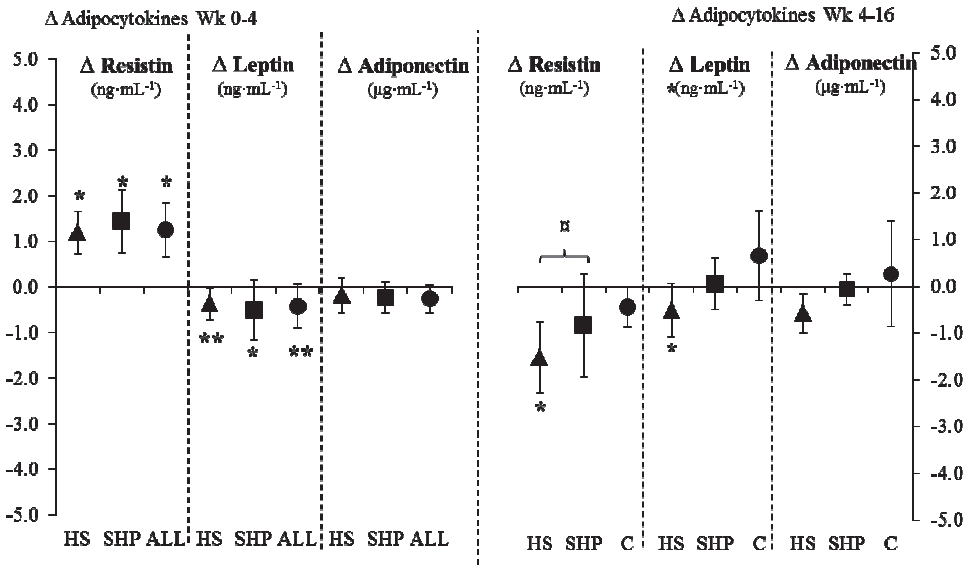


FIGURE 11 Mean changes in adipocytokines in the RT study (III) from wk0 to wk4 (left) and wk4 to wk16 (right). * Significant within-group change, ¤Significant between groups difference. HS = hypertrophy-strength, SHP = strength-hypertrophy power, ALL = HS+SHP, C = Controls .*p < 0.05; **p < 0.01; ¤p < 0.05.

Leptin. In the RT study (IV), significant changes in circulating leptin concentrations were observed already after the initial 4 weeks of RT ($p < 0.01$, ES = 0.799). Then, leptin concentrations were further reduced during the specialized HS training ($p < 0.05$, ES = 0.538). SHP had no effect on leptin concentration ($p = 0.821$). In the CT study (III), significant changes in concentrations of circulating leptin were observed in SS ($p < 0.05$) and AD ($p < 0.05$) after 24 weeks of training. There were no between groups differences.

Adiponectin. No significant changes in circulating adiponectin concentrations were observed in either of the training studies.

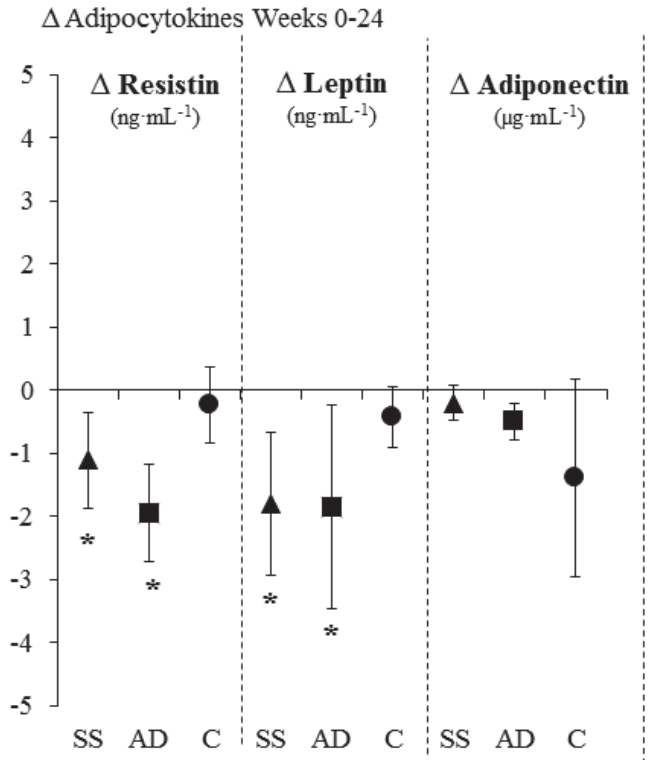
5.2.5 Basal c-reactive protein

Resistance training (III) did not have effect on circulating hs-CRP concentrations. In the CT study (IV), a significant main effect of time was observed ($p < 0.01$, ES = 0.785) for hs-CRP. Circulating concentrations of hs-CRP decreased significantly in the SS ($p < 0.05$) and in the AD ($p < 0.01$) from PRE to POST.

5.2.6 Selected correlations

Similar associations were observed in both training studies. Circulating leptin concentration showed a strong positive correlation with abdominal fat mass ($R = 0.522\text{-}0.775$, $p < 0.001$) in both CT and RT studies. Also, the change in abdominal fat mass from PRE to POST correlated with the change in leptin concentration in both training studies. In the CT study (IV), the change in MCP-1 and resistin was also associated with the change in abdominal fat mass. In addition, in the RT study (III), the pooled data of circulating adiponectin correlated negatively with 1RM ($R = 0.355$, $p < 0.01$). Whereas in the CT study (III), an inverse relationship between the change in concentrations of circulating adiponectin and change in maximal strength from PRE to POST was observed ($R = -0.459$, $p < 0.05$).

FIGURE 12 The effect of 24 weeks of combined endurance and resistance training (IV) on resistin, leptin, and adiponectin. * significant within-group change. SS = same session training, AD = alternating days training, C = Controls. *p < 0.05.



6 DISCUSSION

The main objectives of the present dissertation were to examine the acute and chronic effects of resistance training (RT) on selected inflammation markers. In addition, the effects of combined resistance and endurance training (CT) on selected markers were assessed. Lastly, the relationships between these markers, blood lactate, muscle damage, fitness, and body composition were evaluated when appropriate. While a vast majority of the research during the last decade has focused on endurance exercise, fewer studies have aimed to clarify the effects of resistance and combined resistance and endurance training on the inflammation. Thus, this dissertation provides new information about the specific cytokine and adipocytokine response after traditional resistance exercise (RE) bouts as well as after progressive RT and CT. The main findings of the present dissertation showed that generally higher number of muscle contractions as well as higher metabolic demands in RE and RT led to greater acute response and chronic adaptations in the inflammation markers measured, however, the magnitudes of differences, especially between acute responses, were modest. It must be acknowledged, that the magnitude of the acute inflammatory response after RE is affected by RT and thus the training status modifies the response. The present studies demonstrated that both RT and CT have beneficial effects on muscle mass, abdominal fat mass, and selected inflammation markers. Lastly, one RE bout can elicit a significant response and RT as well as CT can be effective in the reducing abdominal fat mass. Thus the findings of the present studies indicate that the beneficial effects of exercise training could be a combination of the repeated effect of one exercise bout as well as the favorable changes in body composition following more prolonged training.

6.1 Acute inflammatory responses to resistance exercise bouts (I, II)

Resistance training can be carried out in many ways. Muscle mass, strength, and the ability of the muscle to produce force rapidly are important factors when aiming for health benefits. Skeletal muscle is the largest organ in the human body and ground-breaking work during the last decade has demonstrated that skeletal muscle is an active endocrine organ releasing a host of cytokines, which work as messengers that mediate inflammatory response (Pedersen 2011). In recent years, studies reporting the acute effects of a resistance exercise bout on cytokines have been published quite extensively, whereas the specific time-course of the effect of different resistance exercise protocols on cytokines and adipocytokines remains unclear (Brown et al. 2015). Previous studies have shown that a hypertrophic type of resistance exercise bout induces a significant response in selected cytokines, like in IL-6 and IL-1ra concentrations (Izquierdo et al. 2009). Whereas, research on the effects of maximal strength and explosive power-type of resistance exercise bout on inflammation markers, is sparse. Thus, it is of interest to dissertation, in addition to HYP, the effects of MAX and POW on selected inflammation markers, as these RE protocols load the muscles in completely different ways (time under tension, metabolic cost, and the magnitude of acute force reduction). Interestingly, the hormonal responses to MAX and POW have been reported to be similar to HYP, just of lower magnitude (Linnamo et al. 2005; McCaulley et al. 2009; Walker, Ahtiainen & Häkkinen 2010). The results from the cross-sectional (I) and training study with acute RE bouts (II) are discussed here.

6.1.1 Interleukins

A significant progressive increase in circulating IL-6 concentrations were observed after HYP (I, II), MAX (I) and POW (II). It is important to note, however, that the increase in IL-6 levels was relatively modest, and only 1.5 to 2-fold increase in circulating IL-6 was observed. The magnitude of the response is in line with a previous study by Izquierdo et al. (2009). Nevertheless, it must be acknowledged that only leg press was used in the resistance exercise bout and the involvement of a higher number of muscles, i.e. in whole body resistance training, or training the arms, could have elicited a more pronounced systemic inflammatory response (Helge et al. 2011). The magnitude of the changes, however, has been relatively similar in studies using whole body resistance exercise bouts (Ashtary-Larky et al. 2017). It is also relevant that IL-6 response was progressive and the highest IL-6 concentration in the present study (I) was observed 60 minutes after the present RE bout. Thus, it is possible that peak concentrations could have been observed later and were beyond the measurement window in the present study. The progressive increase in IL-6

concentrations up to one hour after high-intensity resistance exercise has also been observed in previous study by Ashtary-Larky et al. (2017) in untrained but not in trained participants.

Resistance training (II) reduced the acute increase in IL-6, as there was no significant immediate increase in IL-6 after HYP or POW observed after 12 weeks of training. IL-6 has pro-inflammatory effects in inflammatory diseases and in connection to obesity and metabolic syndrome. Contracting skeletal muscle has been proposed to be the main source of increased IL-6 in circulation during and following exercise. Several mechanisms, including changes in calcium homeostasis, impaired glucose availability, and formation of reactive oxygen species, have been suggested to affect the secretion of IL-6 (Fischer 2006). It is known that IL-6 production is increased when glycogen is compromised suggesting that IL-6 has a role as an energy sensor (Nieman et al. 2015). Thus, IL-6 has been shown to enhance basal and insulin-stimulated glucose uptake in muscle cells and thus the adapted IL-6 response could have favorable effects on energy metabolism. Thus, the adaptation of the IL-6 response to heavy exercise induced by RT as observed in the RT study (II) can be regarded as a beneficial form of acclimatization considering that exercise training has also been found to increase IL-6 receptor expression and IL-6 sensitivity in skeletal muscle (Keller et al. 2005). Another mechanisms that could explain the suppressed IL-6 response are increased lean mass, maximal strength, and smaller relative mechanical stress, fatigue and muscle damage that all could contribute to the observed suppressed IL-6 response (Ahtiainen et al. 2003; Izquierdo et al. 2009).

The IL-1ra results in the acute studies (I, II) were mixed. The cross-sectional study (I) demonstrated that in an untrained state only the HYP RE with higher number of repetitions and with higher metabolic demands elicited a significant response in anti-inflammatory IL-1ra, while MAX did not elicit a significant response. Even if the IL-6 responses to HYP and MAX were similar, a significant increase in IL-1ra was observed only after HYP. In addition to the pro-inflammatory properties, IL-6 has anti-inflammatory effects as it stimulates the appearance of anti-inflammatory cytokines, such as IL-1ra and IL-10 (Steensberg et al. 2003a; Petersen & Pedersen 2005). The results of the cross-sectional study (I) could, however, indicate that higher stimulation of the cardiovascular system or higher number of muscle contractions might be needed to elicit a response in IL-1ra.

In the training study (II), a significant increase in IL-1ra was observed only after RT in HYP and POW, again regardless of the absence of acute increase in circulatory IL-6. In addition, the training study with acute RE bouts (II) demonstrated a significant increase in IL-1β concentrations before RT in HYP and POW, whereas after RT the response was blunted. The results in the training study are in line with the results in Izquierdo et al. (2009) who observed a significant increase in IL-1ra only after seven weeks of RT. However, it must also be noted that the increase in IL-1β was modest and for example, Forti et al. (2017) were unable to detect any IL-1β in resting state in most their

young healthy participants. Thus, the present dissertation demonstrated that RT modifies RE-induced cytokine responses towards an anti-inflammatory direction by suppressing increase in pro-inflammatory and enhancing the increase in anti-inflammatory markers.

6.1.2 MCP-1

The present cross-sectional (I) and RT training (II) studies are among the first studies that have measured MCP-1 concentration after traditional RE bouts. The results showed that MCP-1 response is exercise and time-point specific, as the acute RE studies demonstrated MCP-1 kinetics after an RE bout. A significant reduction in MCP-1 concentrations were observed 30 minutes after the RE bout (I), whereas in the trained state (II) a significant increase was observed 24 hours after HYP. In the cross-sectional study, there was a trend for MCP-1 concentrations to decrease below pre-exercise levels during recovery from both loadings, but this reached statistical significance only in HYP. The decrease in MCP-1 during recovery could be one sign of the anti-inflammatory milieu after the RE bout, which when repeated continually, might reduce chronic inflammation (Gleeson et al. 2011). Interestingly, in the training study, a significant increase in MCP-1 concentration was observed in HYP at POST24. While speculative, enhanced MCP-1 response after RT could be a marker of a more efficient inflammatory response and possibly a marker of muscle regeneration.

MCP-1 and its receptors are required for successful muscle regeneration, but at the same time MCP-1 has a negative role in chronic diseases that include low-grade inflammation (Kim et al. 2006; Paulsen et al. 2012; Peake et al. 2017). MCP-1 has been suggested to have an important role in the anti-inflammatory phase of the inflammation process that leads to muscle regeneration (Pillon et al. 2013; Peake et al. 2017). Thus, the significant prolonged increase in MCP-1 after RT could be considered as a marker of an enhanced inflammatory response and possibly as a marker of enhanced muscle regeneration. Efficient recovery, especially from resistance exercise, requires a well-coordinated and controlled inflammatory response, which includes an increase in pro-inflammatory, as well as anti-inflammatory cytokines. Hubal et al. (2008) showed that MCP-1 mRNA levels were significantly elevated after muscle-lengthening lower body exercise and the response was enhanced in repeated bouts. Furthermore, the immunohistochemistry analysis in their study showed that MCP-1 was localized with resident macrophages and satellite cell populations, which link MCP-1 to muscle regeneration.

6.1.3 Adipocytokines

The results of the present dissertation showed that immediate and prolonged cytokine and adipocytokine responses are modest, specific to the resistance exercise bout, and dependent on the training status of the participants. No significant immediate changes in leptin or adiponectin levels were observed in

participants in an untrained state in either of the acute studies. Interestingly, in the training study (II), regardless of the lack of total or abdominal fat loss, there was a beneficial significant increase in pre-exercise leptin and adiponectin concentrations. Furthermore, in a trained state, a significant immediate decrease in leptin concentration was observed in HYP after resistance training (HYP2). This is in line with Bouassida et al. (2010) who suggested that to induce significant decreases in leptin, some background in training might be needed. This could indicate that a significant adipocytokine response would be observed only in trained participants, however, it has to be acknowledged that e.g. Zafeiridis et al. (2003) reported a significant reduction after maximal, hypertrophic and muscle endurance RE bout but this was not different from the control group who did not exercise. Zafeiridis et al. suggested that leptin kinetics might have been affected by the overnight fast, which cannot be fully ruled out in the present study design either.

In the cross-sectional study (I), resistin decreased significantly immediately after both MAX and HYP. Previous studies have reported no effect (Jamurtas et al. 2006) or acute decreases (Varady et al. 2010) in circulating resistin concentrations following RE. Varady et al. (2010) observed beneficial resistin modulation after resistance exercise only in habitual resistance trainers, but the present study (I) indicates that HYP, as well as MAX, may have a beneficial decreasing effect on resistin concentrations also in people who do not have RT background when the exercise is performed with maximum effort. Thus, it should be noted that the response might not be observed immediately after an RE bout but during recovery. As in the study of Varady et al. (2010), the results from this study might only be applicable to heavy resistance exercise bouts and for young lean men and more research is needed in other populations.

Conversely, in the training study (II), a significant immediate increase in circulating resistin concentration occurred immediately after both HYP and POW bouts before but not after RT. It must be acknowledged that the resistin response was lesser after HYP than POW. Nevertheless, the mechanism of the resistin response has been hypothesized to be related to metabolic demand of exercise, which could explain the blunted response after training in HYP (Prestes et al. 2009). Our data does not fully support the hypothesis of metabolic demand as a main factor regulating the resistin response as the lactate after RT immediately after HYP was an average of 10.1 ± 3.4 mmol $\cdot L^{-1}$ and no significant response in resistin levels was observed. Nevertheless, in POW before RT with the average lactate of 3.0 mmol $\cdot L^{-1}$ a significant increase in resistin levels was observed. Hence, in addition to metabolic stress, other mechanisms seem to be involved in inducing resistin response. Skeletal muscle cells have been shown to release resistin (Dietze et al. 2002), while mechanical stress has been shown to enhance the expression of resistin in cardiac muscle (Wang et al. 2007). Thus, one origin of increased resistin concentrations could be the skeletal muscle as it has been shown with IL-6 (Pedersen 2011). Another mechanism could be related to the loading of joints. It has been shown that several adipocytokines

are produced also in joints and have a role in joint diseases such as osteoarthritis (Scotece et al. 2014). Vuolteenaho et al. (2014) reported a significant effect of marathon running on circulating resistin concentrations and one may hypothesize that it could be related to cartilage degradation or strenuous muscle exercise. Interestingly, in the RT study (II) a significant immediate increase in resistin concentration was observed only before training in both RE bouts. Especially the POW resistance exercise bout that is characterized by explosive muscle contraction produces stress on tendons and joints (Stengel et al. 2005). While speculative, the blunted resistin response after RT may suggest that RT could elicit protective mechanisms in cartilage and muscle, which could be observed as a reduced immediate resistin response to resistance exercise bout.

6.1.4 Acute RE bouts

Many types of RE sessions can be effectively used to improve muscular fitness. The ACSM position stand on Progression Models in Resistance Training for Healthy Adults (2009) recommends loads corresponding to a repetition range between 8-12 RM to be used for novice training and 1-12 RM in a periodized fashion for intermediate (individuals with approximately 6 months of consistent RT experience) to advanced training. The number of sets per exercise recommended is initially one to three for novice individuals and for progression into intermediate to advanced status it is recommended to use multiple sets with systematic variation of volume and intensity over time (ACSM 2009). To ensure optimal health and fitness gains, RT should be undertaken with proper preparation, guidance and surveillance (Williams et al. 2007).

The exercise protocols in the present dissertation were heavy and maximal, which must be taken into account when evaluating the present results. Studies with a lower number of sets (Buford et al. 2009) and smaller muscle groups (Uchida et al. 2009) did not observe significant immediate response in circulating cytokines after a resistance exercise bout. The reported cytokine responses to resistance exercise vary considerably; however, the results of the present dissertation suggest that heavy exercise protocols may be needed to elicit a significant change in inflammation markers in healthy participants. The physiological demands and outcomes of RT are dependent on the acute exercise bout variables including mode (eccentric and/or concentric muscular actions), volume (total work of the session), load (weight lifted), rest periods, and intensity of RT (Kraemer & Ratamess 2004). The total work (defined as number of sets ×number of repetitions × external load), number of muscle contractions nor the duration of resistance exercise bouts were not matched between the RE bouts. This must be considered comparing the acute effects of RE bouts and interpreting the findings of this studies.

It should be acknowledged that only leg press was used in RE bout and the involvement of higher number of muscles, i.e. in whole body resistance training, could have elicited more pronounced systemic inflammatory

responses. However, as stated earlier the magnitude of the changes observed for example in circulating IL-6 in the present dissertation were relatively similar or greater to those in studies using whole body resistance exercise bouts (Ashtary-Larky et al. 2017). The use of a single exercise, in this case leg extension, was a matter of standardization. A simple exercise protocol on a leg extension was introduced during familiarization and testing of muscle performance outcomes.

Unaccustomed exercise with eccentric muscle contractions and exhaustive exercise cause muscle damage, inflammation, leakage of muscle proteins into the circulation, and soreness several days after an RE bout (Kanda et al. 2013). In the training study (II), creatine kinase (CK) was elevated 24 hours after both after HYP and POW before the RT, but after 12 weeks of training increased concentrations in CK were observed only after POW. CK is considered as an indirect indicator of muscle damage (Ebbeling & Clarkson 1989), thus the lack of CK response suggests an improved tolerance to an exercise bout. This finding of no significantly elevated CK concentrations after RT in HYP emphasizes the effect of training specificity on muscle damage (Vincent & Vincent 1997). There was no POW type of RT included in the present training study (II). Interestingly, despite the fact that participants had RT training background at the end of the RT period, POW as an unaccustomed mode of training, elicited muscle damage, assessed as significant elevation in CK. While speculative, one of the mechanisms behind the suppressed pro-inflammatory response could be lesser muscle damage after RT. Previously Peake et al. (2005) suggested that untrained individuals are likely to experience greater muscle damage and a potentially greater anti-inflammatory cytokine response to downhill running. In contrast, the observations in the present dissertation, regarding RT, lead to a hypothesis that RT enhances anti-inflammatory effects which could suppress the pro-inflammatory response at a cellular level. It is noteworthy that such an anti-inflammatory response within the circulation may provide positive metabolic changes through increased fat oxidation and glucose uptake (Petersen & Pedersen 2005).

All the RE bouts, with an exception of POW after RT, induced a significant increase in lactate concentrations, which was significantly greater after HYP than MAX and POW. The mechanism of the inflammatory response has been hypothesized to be related to metabolic demand of exercise. Since lactate response in the training study (II) was suppressed after RT, it could explain the blunted response in HYP. Thus, even if it has been shown that the intensity, metabolic demand, and the following stress hormone release are more important regulators of inflammatory response in endurance exercise than the exercise-induced muscle damage, this might not be the case in resistance training (Peake et al. 2005).

6.2 Chronic effects of resistance and combined resistance and endurance training on inflammation markers (III, IV)

RT has been suggested to increase muscle mass and elicit RE bout induced anti-inflammatory effects (Phillips et al., 2012). In order to stimulate RT induced adaptations toward specific training goals, progressive RT protocols are necessary (Kraemer & Ratamess 2004). The unique aspect of the RT study (III) was the design with an initial preparatory phase of RT, comprising of muscle endurance type RT, followed by progressive hypertrophy-strength (HS) or strength-hypertrophy-power (SHP) RT.

Both ET and RT are part of the ACSM's guidelines for exercise prescription (American College of Sports Medicine 2013) and both ET and RT appear to be important strategies for improving inflammation profiles and general fitness. Indeed, Nimmo et al. (2013) concluded that the most marked improvements in the inflammation profile are probably achieved with a combination of high intensity ET and RT. Combined training can be performed in multiple ways, by performing ET and RT in the same session with different orders or separated on alternating days (Eklund et al., 2016). The special focus of the CT study was to investigate whether performing ET and RT in the same session (SS) or on alternating days (AD) affected the inflammation markers differently. The results from the resistance training (III) and combined resistance and endurance (IV) studies are discussed here.

6.2.1 Effects of CT and RT on body composition and fitness

Neither of the training studies (III, IV) led to a weight reduction. However, it seems that both RT and CT have potential to reduce abdominal fat mass (Strasser et al. 2012). In the RT study (III), abdominal fat mass was significantly reduced already after 4wks of RT and a significant group × time interaction was observed in abdominal fat mass after specialized training. Abdominal fat mass decreased significantly in HS only. Significant changes in abdominal fat mass were not observed in the SHP group after specialized training. Interestingly, in the control group a significant increase in total fat mass was observed. The frequency of training sessions was the same between the HS and SHP groups. The main difference between the training programs was that there were more metabolically demanding hypertrophic training in HS, whereas in SHP there were more maximal strength and power type RT where the physiological demands are more on the neural end of the spectrum (Kraemer & Ratamess 2004). The results of the RT study emphasize the fact that the intensity and especially the metabolic demands of the RT performed are factors that affect the health outcomes after a period of RT.

In the CT study, despite the same amount of training, abdominal fat mass decreased only in the AD group. Interestingly, the significant decrease in abdominal fat mass in AD group was observed already after 12 weeks of

training. The present findings indicate that AD training may be a more efficient strategy in decreasing abdominal fat mass and consequently contribute to improvement of inflammation status and further cardiovascular and metabolic health. It should be noted that in the CT study, the training volume in AD and SS groups were matched. The main difference in the training programs was that the AD group performed training sessions on alternating days, whereas the SS group trained longer in one session but more rarely, as they performed resistance and endurance training in the same session. Based on this difference between the groups in the reduction of abdominal fat mass, it would be reasonable to assume that several shorter and more frequent training sessions are more efficient in the reduction of abdominal fat mass and further inflammation status. One of the mechanisms behind this group difference in abdominal fat mass reduction observed in the CT study could be a difference in the overall energy expenditure. The greater frequency of exercise and higher post-exercise oxygen consumption could have increased the overall energy expenditure in the AD group, as shown previously by (Almuzaini, Potteiger & Green 1998). Another mechanism that could lead to more beneficial differences in AD group could be the higher frequency of anti-inflammatory milieu after acute exercise bouts, which would consequently accumulate and result in larger anti-inflammatory effect (Gleeson et al. 2011).

The beneficial effects of CT and RT on abdominal adipose tissue have been shown to be highly important. Abdominal adipose tissue compared with total body fat correlates significantly better with several health markers, including triglycerides, blood pressure, and insulin resistance as well as brain volume (Strasser et al. 2012). A prospective cohort study from Japan has shown that irrespective of BMI, changes in abdominal fat mass within 1 year follow-up correlated significantly with changes in the number of metabolic risk factors (Okauchi et al. 2007).

Regarding the effects of training on strength and lean mass, in the present training studies, both CT and RT increased muscle strength and lean mass. However, no between groups differences in RT study were observed in gains in maximal strength or in lean mass. Similarly, in CT study, both SS and AD led to similar changes in maximal strength and no between group differences were observed. Aerobic capacity was measured only in CT study and improved similarly in both training groups. While in the CT study it appeared that placing ET and RT on separate days could provide additional benefits in body composition and inflammation markers, this is not a consistent finding and especially performance outcomes might adapt differently. For example, Robineau et al. (2016) showed that training adaptations in aerobic capacity and strength following combined ET and RT might be compromised if the recovery time between the exercise bouts is shorter.

It is important to consider that the changes observed in fat mass could be partly mediated by the changes in nutrition. In the RT study (IV), one RT session lasted around 60 minutes and was done two to three times a week. Thus the total amount of exercise was around three hours. If we estimate that the

intensity of the RT sessions was moderate on average, then the energy expenditure would range from 300 to 600 kcal. As estimation, due to the moderate intensity, around 40 to 60% of the energy was delivered from the fats, which would indicate that in a typical exercise fat will be utilized between 12 and 40 grams, which is relative small amount for weight loss (Swain 2000; McMurray & Hackney 2005). It is notable, however, that the moderate but significant changes in fat mass were observed especially in the abdominal area. In addition, RT and CT induced changes in muscle mass and following greater resting metabolic rate can affect the training induced changes in body composition (Darveau et al. 2002).

6.2.2 Basal levels of interleukins and TNF-α

A significant reduction in IL-6 concentrations after training in healthy young men was not observed in either of the training studies (III, IV). This is in line with the previous studies (Rall et al. 1996; Olson et al. 2007; Levinger et al. 2009; Libardi et al. 2012; Karabulut et al. 2013; Azizbeigi et al. 2015). The studies that have observed a significant reduction in IL-6 concentrations have had elderly men and women (Prestes et al. 2009; Forti et al. 2014) as participants. Conversely, Forti et al. (2017) reported a significant reduction in circulating IL-6 concentrations after nine weeks of RT in healthy young men. However, efficient RT was executed with low-intensity (1x10–12, 40% 1 RM) or with low-intensity and fatiguing (60 x 20-25% 1RM followed by 1x10–12, 40% 1 RM) resistance exercise sessions, whereas after hypertrophic RT (1 x 10-12, 80% 1RM) significant reductions in IL-6 were not observed. Thus, the findings of the previous studies are similar to the results of the present training studies and it seems that high-intensity RT does not affect basal IL-6 concentration. Forti et al. (2017) suggested that different resistance exercise regimens might elicit different immunological adaptations and hypothesized that alternating between high load and low load resistance exercise might induce a more complete anti-inflammatory response.

In the RT study (IV), a significant increase in IL-1ra, which is known to inhibit the pro-inflammatory response, was observed already after the first four weeks of training. Lancaster & Febbraio (2014) have suggested that the anti-inflammatory effect of exercise would be elicited by the release of IL-6 from skeletal muscle and subsequent production of IL-1ra by monocytes and macrophages. Previous studies on circulating IL-1ra and resistance exercise, including the acute responses reported in the present dissertation, report a significant acute effect of exercise, rather than on longitudinal effect on basal levels. Recently, Forti et al. (2017) showed that training only with high external loads increases the basal levels of IL-1ra in untrained young men. The RT study suggests that in the initial phase of training a significant increase in basal IL-1ra concentration can be achieved with relatively low-intensity training, whereas further anti-inflammatory effects might require higher training frequencies and /or intensities. Even though the RT programs (IV) during the HS and SHP were

progressive, it might be that they were not sufficient enough to elicit a significant response in IL-1ra.

TNF-α is an important regulator of glucose and lipid metabolism (Halle et al. 1998). In the CT study, a slight but statistically significant reduction in TNF-α concentration was observed only in AD at POST, while no changes in SS or C were found. This is in line with other observations that training in alternating days may be superior to training in same session when inflammation status is considered.

6.2.3 Basal level of MCP-1

In the RT study (IV), interestingly, an increase in MCP-1 concentration was observed after four weeks of training in a pooled group (HS + SHP). Later, both HS and SHP led to reduction in MCP-1 and normalized MCP-1 concentrations. Thus, MCP-1 was significantly reduced after the specialized RT period in HS and SHP regardless of the absence of changes in fat mass in SHP. In the CT study (III), a significant reduction in circulating MCP-1 concentrations after 24 weeks was observed only when the training was separated into alternating days. Whereas, when ET and RT were performed in the same session the opposite result, a significant increase in MCP-1 concentrations, was observed.

In the acute part of the present dissertation (I, II), a significant reduction in MCP-1 was observed 60 minutes after HYP, whereas after training a significant increase was observed 24 hours after the RE bout. Trøseid et al. (2004) suggested that especially visceral fat mass would have an effect on plasma levels of MCP-1. However, MCP-1 is also a potent chemotactic and activating factor for macrophages, inflammation, and skeletal muscle regeneration (Shireman et al. 2007). While speculative, the mechanism that could lead to higher MCP-1 concentrations when starting RT after an initial phase of RT could be related to muscle damage and the following muscle regeneration experienced during the initial phase of RT (Peake et al. 2017). This is in line with the observation from the acute study (II) that in a trained state MCP-1 concentrations can be elevated still at 48 hours after an RE bout. Another mechanism that might lead to increased MCP-1 concentrations after the initial phase of RT could be related to shear stress in vascular smooth muscle cells experienced in early phase of RT (Shyy et al. 1994). Nevertheless, more research is needed on the mechanisms that lead to higher circulating MCP-1 concentrations in the initial phase of RT.

Long-term RT and CT seem to elicit a beneficial reduction in circulating MCP-1 concentrations, as a reduction in MCP-1 was observed in both training groups after RT despite the lack of abdominal fat mass reduction in SHP (III). The reason behind the increased MCP-1 concentration in SS remains unknown. Nevertheless, the results of the present dissertation suggest that changes in circulating MCP-1 concentrations are not entirely dependent on changes in adipose tissue mass.

6.2.4 Basal levels of adipocytokines

Leptin is a pro-inflammatory hormone mainly secreted by adipocytes and acts as a peripheral signal informing the central nervous system of changes in the amount of adipose tissue in the body (Bouassida et al. 2010). In the RT study (IV), already 4 weeks of RT reduced circulating leptin concentrations. There was an association between the change in abdominal and total fat mass and reduction in leptin concentrations, which emphasizes the fact that changes in leptin levels seem to depend on a reduction in fat mass. It is possible that the RT study (III) did not observe significant reductions in leptin concentrations in the SHP group because fat mass was not reduced during training. The present study does, however, demonstrate that a significant reduction in body mass is not needed for a significant reduction in circulating leptin concentrations; however the reduction in leptin seems to be dependent on a reduction in fat mass. These observations are in line with the previous studies on leptin and body composition (Baile, Della-Fera & Martin 2000). RT has been shown to favorably affect body composition (Kraemer, Ratamess & French 2002). The effect of RT on body composition and inflammation markers seems to be strongly dependent on the intensity and external load of RT. In the beginning of training, the beneficial effects in fat mass and leptin were observed with moderate intensity muscle endurance type RT. However, for further improvements, more metabolically demanding HS training, with higher volume (repetitions × load), slow velocity (longer time under tension), and shorter rest intervals than in SHP seems to be advantageous. Similar results were observed in the CT study, where significant reductions in leptin concentrations were observed after 24 weeks of training both in AD and SS groups. Surprisingly, no significant reductions in leptin concentrations were observed in the CT study after 12 weeks of training even though the training included both resistance and endurance training and was conducted twice a week for SS and four time a week for AD group. While speculative, it could be that the amount and intensity of the training during the first 12 weeks of combined training was not high enough to elicit a beneficial effect on leptin (Fatouros et al. 2005).

Resistin stimulates the production of pro-inflammatory cytokines, and has been associated with obesity and atherosclerosis (Zhang et al. 2010), as well as several other diseases (Cao 2014). In line with the previous studies, resistance HS training led to significant reductions in circulating resistin concentrations (Prestes et al. 2009; Botero et al. 2013). Similar results were observed after 24 weeks of CT. Consequently, a reduction in resistin concentration may be interpreted as a beneficial biological adaptation. The data in the present dissertation indicates that long-term exercise training can alter the concentrations of circulating resistin regardless of changes in abdominal fat mass. One of the possible mechanisms behind the anti-inflammatory effect of exercise has been suggested to be the acute release of IL-6 following an exercise session, possibly stimulating the accumulation of anti-inflammatory cytokines,

such as interleukin-10 and interleukin-1 receptor antagonist (Gleeson et al. 2011). IL-6 has been shown to be related to circulating resistin levels, but whether or not IL-6 release after exercise is mechanistically linked to reductions in circulating resistin levels awaits further investigation.

In RT study (III), an unexpected increase in circulating resistin concentrations was observed after the initial phase of RT. Moreover, a reduction in resistin was not observed after SHP. Our observation of increased circulating resistin concentrations after the initial phase of RT could be due to the increased secretion of resistin by the activated anti-inflammatory macrophages in muscle tissue (Schwartz & Lazar 2011; Filková, Šenolt & Vencovský 2013). It has been shown that unaccustomed exercise leads to muscle damage characterized by transient ultrastructural myofibrillar distribution, especially in untrained populations (Clarkson & Hubal 2002; Pillon et al. 2013). Exercise-induced muscle damage initiates tissue repair and remodeling, which leads to the accumulation of inflammatory cells, including macrophages, into the muscle tissue. The activated macrophages, depending on their type, secrete pro-inflammatory and anti-inflammatory cytokines, which are needed for the regulation of muscle adaptations after exercise (Hyldahl & Hubal 2014; Peake et al. 2017). The increased resistin concentrations in the RT study could be due to macrophage activation and could be part of the efficient inflammation resolving process that has been shown to lead to regeneration of muscle fibers (Peake et al. 2017). In our training study with acute RE bouts (II), a significant increase in resistin after HYP and POW was observed and the response was greater after POW. The RT study (IV) found that during the specialized training period, a significant reduction in resistin concentrations was observed only in the HS group. While speculative, it can be suggested that the new and unaccustomed training stimulus, the explosive training in the last block of 4 weeks in the SHP program, could have unbalanced the homeostasis of the body, which in turn induced muscle damage or stress to other tissues eliciting another initial response to a different stimulus.

Adiponectin is suggested to have numerous beneficial effects including anti-diabetic, anti-inflammatory and cardio protective outcomes on health. Skeletal muscle has been shown to be an important peripheral target for adiponectin's actions (Liu & Sweeney 2014). Interestingly, CT or RT did not have an effect on adiponectin. This is in line with previous RT studies (Pereira et al. 2011; Phillips et al. 2012) whereas the previous CT studies have observed a significant increase in adiponectin (Balducci et al. 2010; Brunelli et al. 2015). However, previous CT studies had obese (Brunelli et al. 2015), and type 2 diabetes patients (Brunelli et al. 2015), which could indicate that adiponectin is not affected by CT in healthy men. Furthermore, in the CT study, a negative association was observed between strength gains and the changes in adiponectin concentrations.

As a conclusion, the results of the present dissertation showed that adipocytokines are affected by exercise. Cytokines that are secreted by adipocytes are referred as adipocytokines, however, resistin has been shown to

be secreted from the other tissues as well (Schwartz & Lazar 2011; Filková et al. 2013). Thus, especially resistin and its response to exercise might provide unique information about the independent effects of exercise on inflammation status.

6.2.5 Basal level of C-reactive protein

Prior to the commencement of longitudinal training studies, the baseline levels of CRP allowed us to classify the participants in all groups as having "moderate cardiovascular risk" (1.0 to 3.0 $mg \cdot L^{-1}$) (Pearson et al. 2003). Despite the similar training induced changes in body composition in the CT and RT studies, RT did not have any effect on circulating CRP-levels, whereas, in the CT study, after 24 weeks of training the mean CRP was reduced to the level of "low cardiovascular risk" (< 1.0 $mg \cdot L^{-1}$) in both experimental groups. It is also notable that the observed reduction of 40% in CRP concentration in the CT study compares with reported reductions achieved through medications, such as statins (Ridker et al. 2008). These findings are in line with a study by Stewart et al. (2007), who suggested that a combination of ET and RT reduced the risk of cardiovascular disease development, as defined by a decrease in CRP concentrations in healthy populations.

The RT study did not find a significant decrease in CRP when only RT was done. Strasser et al. (2012) pooled a data from 8 RT studies and found a small but significant reduction in CRP concentrations; however it has to be acknowledged that some of the studies included to the analysis included also ET.

Interestingly, the present training studies (III, IV) did not show any significant changes in circulating inflammation markers after 12 weeks of CT or after 16 weeks of RT. In contrast to the studies by Stewart et al. (2007) and Libardi et al. (2012), the participants in the present training studies were young and healthy and reported to be moderately active. Thus, the findings of the present dissertation indicate that even moderately active young healthy participants benefit from prolonged combined ET and RT, but adaptations may be delayed in comparison to inactive and/or elderly people.

While hs-CRP concentrations are generally determined by genetic factors, centrally located adiposity is also considered to be a major determinant of CRP levels (Perry et al. 2008). Cross-sectional studies have found an inverse relationship between physical activity and CRP (Ford 2002) while training studies have reported reductions in CRP (Stewart et al. 2007). Interestingly, Libardi et al. (2012) did not find any significant differences in CRP, IL-6, or TNF-α in sedentary middle age men after 16 weeks of concurrent training in which ET and RT were performed in the same session, three times a week. These findings were opposed to those of Stewart et al. (2007), who found a significant improvement in CRP concentrations after 12 weeks of concurrent training in young and old sedentary participants. Thus, it is suggested that 24 weeks of progressive combined resistance (maximal strength and hypertrophic) and endurance training (aerobic and anaerobic) seems to be beneficial for the

reduction of CRP in healthy young men with a moderate risk for cardiovascular diseases.

6.2.6 Associations

Changes in body composition seem to be an important factor when an exercise intervention for reducing inflammation markers is planned (Strasser et al. 2012). The distribution of excess fat in the abdominal region is known to negatively modify the health risk profile, whereas excess adiposity in the periphery does not appear to increase the risk of developing cardiovascular disease (Strasser et al. 2012). The results of the present training studies support the previous findings that decreases in abdominal fat mass are associated with decreases in inflammation markers (Baile et al. 2000). It is also highlighted that a significant reduction in body mass might not be needed for a significant reduction in circulating MCP-1 and resistin concentrations. Nevertheless, in the CT study, a significant association between the change in abdominal fat mass and all measured circulating adipocytokine concentrations was observed. However, as expected, significant reductions in leptin levels seem to be associated with a significant reduction in fat mass (Baile et al. 2000). It is possible that the RT study did not observe significant reductions in leptin concentrations in the SHP group because fat mass was not reduced during training.

Previous studies suggest that physically active individuals and participants with a higher level of fitness have more favorable adipocytokine profiles in comparison to sedentary populations (Lavie et al. 2011). In addition, the increased adipocytokines are reported to enhance loss of muscle mass. In the CT study, participants with the highest leptin and TNF-α concentration had the lowest VO_{2peak} before training. This confirms the assumption that inflammation status is associated with aerobic capacity. However, associations between training induced increase in $VO2_{peak}$ and inflammation markers were not observed. Changes in muscle strength were not associated with the beneficial changes in inflammation markers.

6.3 Methodological strengths and limitations

This dissertation had several strengths in its design as well as in practical procedures. The cross-sectional studies used balanced, cross-over designs. Physical activity was not done 48 hours before the acute exercise bouts or basal measurements to avoid the interference of the acute effects of previous exercise on the basal levels. Additionally, participants in the training studies were randomized and controlled and the durations of the training studies (24 and 16 weeks in III and IV, respectively) were rather long when compared to earlier studies. The prolonged length of the supervised training intervention and a relatively high number of participants are important. Another strength of the present dissertation is that the same investigator performed all measurements

and all analyses to eliminate differences in measurement and analysis technique between investigators. All training sessions were supervised by researchers from the Faculty of Sport and Health Sciences. Supervised training ensured that the quality of the training sessions remained high throughout training studies. Furthermore, the present dissertation included a strict training adherence of more than 90% and participants were given written and verbal instructions how to prepare for each set of measurements with the goal of minimizing the interference of the differences in the basal values. A limitation to the prolonged training period is the possible seasonal variation in physiological and psychological variables as well as in physical activity modes and volume. Both training studies were initiated in the winter and completed in the summer. Nevertheless, the training studies did not observe significant improvements in the control group in measured variables.

The participants were selected by convenience sampling from the local area, which may have resulted in volunteers that are not representatives of the entire population. All the studies included to this dissertation aimed to recruit the participants that did not have systematic training background but were healthy and capable of high intensity strength training. Consequently, the participants in this dissertation were young healthy adults and therefore the results should be generalized to other populations with caution. Furthermore, both the smokers and participants with acute and chronic illness or disease, or other injuries preventing high-intensity training were excluded from the studies to ensure the safety of the subjects and retain a homogeneous sample from which accurate data could be gathered.

When evaluating the acute response to a resistance exercise bout, it is important to have several measurement points to get an overall picture on the cytokine kinetics. For example, the acute RE study (I) showed that IL-6 increased progressively up to the last measurement point, 60 minutes after RE bout. Thus, it cannot be ruled out that the peak values could have been observed later. Due to our recovery window and the different durations of the exercise protocols, the present acute RE studies (I, II) could have been unable to detect it. More accurate indications of the cytokine kinetics could have been obtained with extra time-points. Although in the present study several different factors are suggested to be important markers or regulators of inflammation, there are many other pro- or anti-inflammatory factors that could have been measured.

Nutrition is extremely important in terms of body composition and the body's inflammation status. Nutrition was not strictly controlled in any of the included studies. In longitudinal studies, dietary intake was followed only once during the whole 16 to 24 weeks of training. Thus, it cannot be ruled out that there could have been an effect of nutritional alterations undertaken by the participants that were not reported to the investigators. In addition, in the longitudinal RT study (IV), as it was part of a larger research projects, participants received a post-exercise supplement that included either carbohydrates or whey or whey and carbohydrates. It has been suggested that

both whey (Krissansen 2007) and carbohydrates (Gleeson, Pedersen & Nieman 2004) could effect the inflammatory response after exercise and this could have affected the results. The nutritional subgroups were randomly assigned and evenly distributed within the training groups. Thus, due to a careful randomization and such a large sample size it is improbable that there would be a consistent difference due to the supplement between the training groups.

7 MAIN FINDINGS AND CONCLUSIONS

The main objectives of the present dissertation were to examine the acute and chronic effects of resistance on inflammation markers. In addition, the effects of combined resistance and endurance training on the selected inflammation markers were assessed. Last, the relationships between these markers, blood lactate, muscle damage, and body composition were evaluated. While the vast majority of the research during the last decade has focused on endurance exercise, fewer studies have aimed to clarify the effects of traditional resistance training on inflammation markers. Thus, the present studies provides new information about the inflammatory response after traditional RE bouts and progressive RT. The results of this dissertation indicate:

1) Inflammation markers' responses to hypertrophic and maximal explosive resistance exercise bouts were generally similar to each other. Hypertrophic compared to maximal resistance exercise led to greater responses in IL-1ra. Thus, an anti-inflammatory effect, in terms of increased IL-1ra concentrations, was observed only when the resistance exercise bout had higher number of muscle contractions and was metabolically more demanding.
2) Concentrations of resistin increased the most in the maximal explosive resistance exercise bout, which could be due to the higher mechanical load required in maximal explosive compared to hypertrophic resistance exercise bout.
3) An enhanced anti-inflammatory, increased IL-1ra, and reduced pro-inflammatory, suppressed IL-1β response, effect was observed after RT when the participants were in a trained state. Thus, resistance training modifies resistance exercise induced cytokine responses towards an anti-inflammatory direction.
4) A significantly increased MCP-1 concentration was observed after resistance training in hypertrophic resistance exercise bout at POST24 and POST48. This could be considered as a marker of enhances muscle regeneration after resistance training.

5) Combined training reduced inflammation as demonstrated by lowered circulating concentrations of hs-CRP, leptin, and resistin. It was also efficient in reducing abdominal fat mass. It is suggested that 24 weeks of progressive combined resistance (maximal strength and hypertrophic) and endurance training (aerobic and anaerobic) elicits beneficial changes in inflammation markers in healthy young men.
6) In the combined training study (IV), training performed on alternating days elicited the largest reductions in abdominal fat mass and circulating levels of TNF-α and MCP-1. Thus, training on alternating days could be more suitable than training in same-sessions when inflammation status is considered.
7) The short-term initial phase (four weeks) of resistance training led to significant effects on cytokines and adipocytokines: circulating pro-inflammatory resistin and MCP-1 concentrations increased, anti-inflammatory IL-1ra concentrations increased, and circulating leptin concentrations decreased along with the increasing muscle mass and decreasing abdominal fat mass. This emphasizes the fact that even short term of resistance training can have beneficial effects on body composition and inflammation status. However, it must be acknowledged that the changes in inflammation markers might not only represent systemic inflammation status but might be also related to training adaptations. While speculative, the increase in resistin and MCP-1 could be markers of the muscle recreation process.
8) After the specialized resistance training periods, hypertrophic-strength resistance training reduced inflammation markers, such as circulating resistin and leptin. Strength-hypertrophy-power resistance training did not have any effect on inflammation markers. Also notable was that the anti-inflammatory effect of resistance training was achieved without a concomitant loss in body mass. However, both hypertrophy-strength and strength-hypertrophy-power resistance training programs led to further increases in muscle mass, whereas significant reduction in fat mass was observed only after hypertrophy-strength resistance training.

These results outline the importance of resistance exercise as a non-pharmacological tool to reduce systemic inflammation in young untrained men. In summary, more metabolically demanding RE and RT led to greater responses in the inflammation markers measured in the present dissertation. However, the magnitude of differences between loadings was modest. In addition, it is notable that the acute response in inflammation markers is affected by RT. Lastly, the present studies demonstrated that RT and CT have beneficial effects on muscle mass, abdominal fat mass, and inflammation markers. Thus, the findings of the present studies indicate that the beneficial effects of RT could be due to the repeated anti-inflammatory effect of one bout of RE as well as the favorable changes in body composition following more prolonged RT. However, there are several mechanisms involved in the

beneficial effects of exercise on inflammation and it remains unclear how much of this effect is mediated via cellular events causing changes in the within tissue via auto-, para and/or endocrine actions; and, how much of this effect is mediated via changes in the adiposity. Thus, further studies are needed to identify the molecular mechanisms underlying the anti-inflammatory effect of exercise and what the role of skeletal muscle is in this action.

YHTEENVETO (FINNISH SUMMARY)

Voimaharjoittelun akuutit ja pitkäaikaiset vaikutukset tulehduksen merkkiaineisiin

Tulehdus (inflammaatio) on kehon luontainen tapa puolustautua ulkopuolisia uhkatekijöitä vastaan. Elimistöön tunkeutuneen taudinaiheuttajan aikaansaama infektio aktivoi tulehdusvasteen, jonka tarkoituksena on hankkiutua eroon taudinaiheuttajasta. Akuutti tulehdusvaste voi olla seurasta myös kudosvauriosta. Tulehdustilaa kuvaavat tulehduksen merkkiaineiden, kuten sytokiinien ja c-reaktiivisen proteiinin, kohonneet pitoisuudet. Pitkäaikaisesti kohonneet merkkiaineiden pitoisuudet voivat olla seurausta rasva- ja lihaskudoksen epänormaalista aineenvaihdunnasta, jolloin puhutaan matala-asteisesta tulehduksesta. Matala-asteinen tulehdus on oireeton, ja tulehduksen merkkiaineet ovat vain lievästi kohollaan.

Väestön fyysinen inaktiivisuus ja lihavuus ovat yleistyneet maailmanlaajuisesti, ja niiden on osoitettu olevan yhteydessä matala-asteiseen tulehdukseen. Samaan aikaan matala-asteiseen tulehdukseen liittyvien kroonisten sairauksien, kuten tyypin 2 diabeteksen, määrä on noussut räjähdysmäisesti. Liikunnan on todettu lieventävän matala-asteista tulehdusta useiden mekanismien kautta. Vielä ei ole kuitenkaan selvää, miten erityyppinen liikunta vaikuttaa matala-asteiseen tulehdukseen. Tästä syystä on tärkeää selvittää, miten voimaharjoittelu vaikuttaa tulehdustilaa kuvaaviin merkkiaineisiin.

Tämän väitöskirjan tarkoituksena oli: 1) tarkastella akuutteja vasteita tulehduksen merkkiaineissa kolmeen erilaiseen voimakuormitukseen (hypertrofinen eli lihaskasvuun tähtäävä kuormitus, maksimivoimakuormitus ja maksimaalinen räjähtävän voiman kuormitus), 2) tutkia tulehduksen merkkiaineiden akuuttien vasteiden muutoksia 12 viikon voimaharjoittelun seurauksena, 3) seurata progressiivisen voimaharjoittelun ja yhdistetyn voima- ja kestävyysharjoittelun aikaansaamia muutoksia tulehdusmerkkiaineissa, sekä 4) tarkastella kehonkoostumuksessa ja suorituskyvyssä tapahtuneiden muutosten yhteyttä tulehduksen merkkiaineiden muutoksiin.

Tutkimukseen suoritti loppuun akuuttien kuormitusten osalta 20 tutkittavaa (osaraportti 1: n = 12; osaraportti 2: n=8), voimaharjoittelun osalta 68 tutkittavaa ja yhdistetyn voima- ja kestävyysharjoittelun osalta 32 tutkittavaa. Lisäksi harjoittelututkimuksissa 16 (osaraportti 3) ja 14 (osaraportti 4) miestä muodostivat kontrolliryhmän. Kaikki tutkittavat olivat terveitä nuorehkoja miehiä (ikä 18–40 vuotta), joilla ei ollut voimaharjoittelutaustaa. Akuutteja vasteita selvitettiin kolmen erilaisen voimakuormituksen jälkeen. Kaikki voimaharjoitukset toteutettiin jalkaprässilaitteella: maksimivoimakuormituksessa suoritettiin viisitoista sarjaa yhden toiston maksimipainolla, hypertrofisessa kuormituksessa suoritettiin viisi sarjaa 10 toistolla 80 %:lla maksimipainosta ja maksimitehokuormituksessa suoritettiin 10 sarjaa viidellä toistolla 60 %:lla maksimipainosta. Voimaharjoittelututkimus (osaraportti 3) sisälsi kokonaisuudessaan 16 viikkoa

voimaharjoittelua, joista ensimmäiset neljä viikkoa sisälsivät kaikilla tutkittavilla kestovoimaharjoittelua. Tämän perehdyttämisjakson jälkeen tutkittavat jaettiin hypertrofia-maksimivoima-ryhmään ja maksimivoima-hypertrofia-tehoryhmään. Yhdistetyssä voima- ja kestävyysharjoittelututkimuksessa (osaraportti 4) harjoittelu koostui aerobisesta kuntopyöräilystä sekä hypertrofisesta ja maksimivoimaharjoittelusta. Yhdistetyssä voima- ja kestävyysharjoittelututkimuksessa puolet tutkittavista suoritti harjoitukset eri päivinä (EP, 4–6 kertaa viikossa) ja puolet samassa harjoituksessa (SP, 2–3 kertaa viikossa). Harjoittelun kokonaismäärä ryhmien välillä oli sama.

Tutkimuksen tulokset osoittivat, että voimaharjoitus saa aikaan akuutin vasteen tulehdusmarkkereissa. Akuutti vaste on yleisesti ottaen suurempi aineenvaihdunnallisesti kuormittavamman ja suuremman määrän lihassupistuksia sisältävän hypertrofiakuormituksen seurauksena. Poikkeuksena oli vaste resistiinissä, joka näytti olevan suurin maksimaalisen räjähtävän voimakuormituksen jälkeen. Voimaharjoittelu pienensi voimaharjoituksen aikaansaamaa vastetta tulehduksen merkkiaineissa. Lisäksi voimaharjoittelu vahvisti hypertrofiakuormituksen aikaansaamaa MCP-1-vastetta kuormitusta seuraavina päivinä. Tämän voidaan ajatella kuvaavan vahvistunutta lihaksen regeneraatioprosessia, jonka avulla lihassyyt paranevat lihasvaurioista.

Molemmissa harjoittelututkimuksissa havaittiin merkitsevä lihasmassan kasvu sekä vatsanalueen rasvamassan ja tulehdusmarkkerien väheneminen. Voimaharjoittelututkimuksessa havaittiin vatsan alueen rasvamassan lasku jo neljän viikon harjoittelun jälkeen. Samaan aikaan resistiini ja MCP-1 nousivat merkitsevästi ensimmäisen neljän viikon harjoittelun seurauksena. Huomionarvoista on, että vatsan alueen rasvamassa laski voimaharjoittelun seurauksena vain hypertrofia-maksimivoima-ryhmässä. Samoin vain hypertrofia-maksimivoima-ryhmällä resistiini ja leptiini laskivat merkittävästi. Yhdistetyssä voima- ja kestävyysharjoittelussa harjoitusten jakautuminen useammalle päivälle vähensi enemmän vatsan alueella olevaa rasvakudosta ja tulehdusmerkkiaineidenpitoisuuksia (TNF-α ja MCP-1). Tämän väitöskirjatutkimuksen tulokset osoittavat, että hyvin erilaiset voimakuormitukset saavat aikaan merkitsevän akuutin vasteen tulehduksen merkkiaineissa. Yleisesti vaste on suhteellisen lievä. Vasteen vahvuuden ja kuormituksen väliset yhteydet riippuvat mitattavasta muuttujasta, harjoittelutaustasta sekä kuormituksen aineenvaihdunnallisesta kuormittavuudesta ja lihassupistusten määrästä.

Progressiivisen voimaharjoittelun aloittaminen johtaa lisääntyneeseen lihasmassaan ja lihasvoimaan. Voimaharjoittelu voi johtaa positiivisiin muutoksiin kehonkoostumuksessa, etenkin vatsanalueen rasvamassassa sekä pitkäaikaista tulehdustilaa kuvaavissa merkkiaineissa jo neljän viikon harjoittelun jälkeen. On kuitenkin huomioitava, että uusi ärsyke, tässä tapauksessa voimaharjoittelu, saattaa harjoittelun alussa nostaa tulehduksen merkkiainepitoisuuksia, etenkin resistiini- ja MCP-1-tasoja. Tämä ei kuitenkaan välttämättä ole seurausta tulehduksen lisääntymisestä, vaan saattaa ennemmin kuvata harjoittelun aikaansaamaa lihaksen uudistumisprosessia. Harjoittelun jatkuessa aineenvaihdunnallisesti kuormittavampi lihaskasvuun tähtäävä hypertrofinen harjoittelu

näyttää olevan tehokkaampaa, jos tavoitellaan merkitseviä muutoksia vatsan alueen rasvamassassa ja tulehdusta kuvaavien merkkiaineiden pitoisuuksissa. Myös yhdistetty voima- ja kestävyysharjoittelu saa aikaan positiivisia vasteita tulehdusmarkkereissa. Yhdistetyssä voima- ja kestävyysharjoittelussa merkittävä vatsan alueen rasvan väheneminen havaittiin vain harjoiteltaessa eri päivinä. Myös useammassa tulehdusta kuvaavassa merkkiaineessa havaittiin positiivisia vasteita, kun harjoittelu toteutettiin eri päivänä. Tämän seurauksena eri päivinä toteutettu yhdistetty voima- ja kestävyysharjoittelu vaikuttaa olevan tehokkaampaa kuin harjoitusten yhdistäminen samalle päivälle vatsanalueen rasvamassan ja tulehdusmerkkiaineiden pitoisuuksien vähentämisessä.

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ORIGINAL PAPERS

I

ACUTE LEUKOCYTE, CYTOKINE AND ADIPOCYTOKINE RESPONSES TO MAXIMAL AND HYPERTROPHIC RESISTANCE EXERCISE BOUTS.

by

Johanna K. Ihalainen, Simon Walker, Göran Paulsen, Keijo Häkkinen, William J. Kraemer, Mari Hämäläinen, Katriina Vuolteenaho, Eeva Moilanen, Antti A. Mero.
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Johanna Ihalainen · Simon Walker · Gøran Paulsen · Keijo Häkkinen · William J. Kraemer · Mari Hämäläinen · Katriina Vuolteenaho · Eeva Moilanen · Antti A Mero

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Abstract The purpose of this study was to examine the acute immune response (circulating levels of leukocytes, cytokines and adipocytokines) to maximal resistance (MAX, 15 × 1RM) and hypertrophic resistance (HYP, 5 × 10RM) exercise bouts. Twelve healthy men (age = 28.2 ± 3.5 years, weight = 78.6 ± 10.4 kg, height 178.8 ± 5.0 cm, fat percentage = 16.5 ± 3.5 %) participated in the study. Blood was sampled before, immediately after and 15 and 30 min after exercise. Leukocytes (WBC) significantly increased immediately after HYP ($p < 0.01$), whereas in MAX, increases in WBC became significant after 30 min ($p < 0.05$). Lymphocytes increased only after HYP ($p < 0.001$), while MAX induced lymphopenia during recovery ($p < 0.01$). Monocyte chemoattractant protein-1 (MCP-1) decreased ($p < 0.05$) and interleukin-1 receptor antagonist (IL-1ra) increased after HYP, which were not observed after MAX. Adipsin and resistin decreased after both exercise bouts ($p < 0.05$), which suggest that heavy resistance exercise is at least transiently beneficial for adipocytokine profile. Immediate mechanical stress seemed similar as no differences in myoglobin response were observed. The higher magnitude of metabolic demand reflected in higher lactate response in HYP could be the reason for the significantly high responses in WBC, IL-1ra and decrease in MCP-1.

Keywords Resistance exercise · White blood cells · Cytokines · Adipocytokines

A list of abbreviations

HYP	Hypertrophic resistance exercise bout
IL-1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
MAX	Maximal resistance exercise bout
MCP-1	Monocyte chemoattractant protein-1
WBC	White blood cell count

Introduction

Resistance training is associated with a reduced risk of low-grade inflammation and improvement in metabolic diseases such as cardiovascular disease and type 2 diabetes (Kraemer et al. 2002; Gordon et al. 2009; Calle and Fernandez 2010). Resistance training has been associated with improvements in inflammation state in overweight adults (Olson et al. 2007), elderly (Phillips et al. 2012) as well as in specific patient groups (Conraads et al. 2002; Moraes et al. 2014). A bout of heavy resistance exercise triggers a transient inflammatory response comprising augmented white blood cell count and stimulation of pro- and anti-inflammatory cytokines production (Freidenreich and Volek 2012). The physiological stress caused by heavy resistance exercise acts as a major stimulus for muscle fiber

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J. Ihalainen (✉) · S. Walker · K. Häkkinen · A. A. Mero
Department of Biology of Physical Activity, University of Jyväskylä, P.O Box 35, 40014 Jyväskylä, Finland
e-mail: johanna.stenholm@jyu.fi

G. Paulsen
Norwegian School of Sport Sciences, Oslo, Norway

W. J. Kraemer
Human Performance Laboratory, Department of Kinesiology, University of Connecticut, Storrs, USA

M. Hämäläinen · K. Vuolteenaho · E. Moilanen
The Immunopharmacology Research Group, University of Tampere School of Medicine and Tampere University Hospital, Tampere, Finland

hypertrophy, and efficient repair of muscles requires a well-coordinated and controlled inflammatory response (Peake et al. 2010).

Well-known immunological responses after intensive and prolonged endurance exercise include neutrophilia (high neutrophil counts), lymphopenia (low lymphocyte counts) and decreased cytotoxic activity in natural killer cells (Nieman 1997). The inflammatory response to heavy resistance exercise and especially after a traditional maximal resistance exercise, which is primarily designed to maximize neural adaptation and places low metabolic demands (few repetitions and long inter-set rest periods) (Simonson and Jackson 2004; Hakkinen and Pakarinen 1993; Hulmi et al. 2012) is much less investigated (Paulsen and Peake 2013). Resistance exercise may impact the magnitude of circulating WBC in similar manner to endurance exercise, but the exact mechanisms that induce responses in the immune system during resistance exercise are not known (Freidenreich and Volek 2012).

The cytokine response induced by a bout of heavy resistance training involves enhanced production of pro-inflammatory cytokines, such as IL-1β and IL-1α and IL-6. In addition to the pro-inflammatory properties, IL-6 has anti-inflammatory effect as it stimulates the appearance of anti-inflammatory cytokines, such as IL-1ra and IL-10. These mediators play a crucial role in the containment and resolution of inflammatory processes, and have been suggested to link the acute effect of exercise to the decrease of cardiovascular diseases associated with low-grade inflammation (Steensberg et al. 2003; Petersen and Pedersen 2005); (Izquierdo et al. 2009) suggested that the magnitude of metabolic demand and fatigue, which is experienced during hypertrophic resistance exercise, affects both the cytokine and hormone response patterns. Complementarily, Miles et al. (2003) found evidence to support the hypothesis that the increase in blood lactate levels may be part of the mechanism to increase lymphocyte counts in the circulation. Moreover, heavy resistance exercise also induces an acute endocrine response, e.g., increased circulating levels of cortisol and epinephrine (Kraemer et al. 1996; Nieman et al. 1995b) which are known to be potential effectors for the immune system (Pedersen and Hoffman-Goetz 2000).

One of the physiological changes responsible for the positive effects of exercise on cardiovascular and metabolic health has been suggested to be changes in adipocytokines (Robinson and Graham 2004). Adipocytokines (leptin, adiponectin, resistin and adipsin) are hormones that were first discovered to be secreted by adipose tissue and to regulate energy metabolism and appetite. More recent findings on the ubiquitous expression of their receptors and on their cellular effects have revealed that they are also involved in the regulation of a variety of biological functions related to immune responses and inflammatory diseases (Ouchi et al. 2011). A single exercise bout has been demonstrated to exert specific acute effects on adipocytokine levels and the response appears dependent on the duration of the exercise and on energy expenditure (Bouassida et al. 2008).

Heavy resistance exercises for improving maximal strength and muscle growth are important parts of a progressive resistance training program of athletes and could be beneficial for the general population as well as for elderly people with consistent resistance training experience. Therefore, it is important to characterize the immediate effects of different types of resistance exercise on blood WBC, cytokines and adipocytokines, and the possible mechanisms that link the type of resistance exercise to the magnitude of the response. The purpose of the present study was to examine the acute immune response (changes in WBC, cytokines and adipocytokines) during two heavy but specifically different resistance exercise bouts: MAX (15 × 1RM) and HYP (5 × 10RM). Secondly, we investigated the relationship of muscle injury, lactate accumulation and hormonal changes after the exercise sessions. We hypothesized that, as the metabolic demand is lower in maximal resistance type resistance exercise, the acute inflammatory response (changes in blood WBC counts and cytokine levels) would also be lower after MAX than after HYP but beneficial changes in adipocytokine profiles would be observed during recovery after both loadings.

Methods

Participants

Twelve healthy, young males volunteered for this study (age = 28.2 ± 3.5 years, weight = 78.6 ± 10.4 kg, height 178.8 ± 5.0 cm, fat percentage = 16.5 ± 3.5 %; mean ± SD). All participants reported taking part in sport activities on a weekly basis, but none were competitive athletes or had a background in systematic strength training. Participants filled in a health questionnaire prior to participation in the study. All subjects reported that they were non-smokers, free from injury, and were not using any medications. Each subject was informed of the potential risks and discomforts associated with the measurements, and all the subjects gave their written informed consent to participate. The study was conducted according to the declaration of Helsinki, and the ethics committee of the University of Jyväskylä, approved the study.

Pretesting

All subjects in this investigation participated in a familiarization session, which included anthropometrics and body

composition measurement, as well as the one repetition maximum (1RM) test performed in the leg press exercise (David D210 leg press device, David Health Solutions Ltd., Helsinki, Finland). This bilateral 1RM test was used to determine the loads used in each acute exercise protocol. The starting position (flexed) was approximately 60° for knee angle and 70° for hip angle, whereas the finishing position, at full extension was 180° for the knee angle. As part of their warm-up, the subjects performed 4 progressive submaximal sets (1 set of 10 repetitions with 70 % of estimated 1RM, 1 × 7 × 75 % estimated 1RM, 1 × 5 × 80 % estimated 1RM, and 1 × 1 × 90 % estimated 1RM). After the estimated submaximal sets, single repetitions were performed with increasing loads (2.5–5 kg increments) until the subject could not lift the load to the finishing position of 180° knee angle. The rest period between attempts was 3 min. The last successful repetition (rep) was considered to be the subject's 1RM.

Experimental protocol

The first exercise session was performed 1 week after the familiarization session. The order of the protocols was randomized and counter-balanced. The MAX protocol was 15 sets of 1 repetition (reps) at 100 % of 1RM and the HYP protocol was 5 sets of 10 reps at 80 % of 1RM for the leg press exercise. The loads used during the first set were determined from the 1RM load of the familiarization session. The loads were adjusted during the sessions to enable completion of the required reps. If the subject was not able to complete the required reps, assistance was provided and the load is reduced for the next set. The inter-set rest period was 3 min for MAX and 2 min for HYP. The exercise bouts were separated by 1 week.

Body composition

After an overnight fast, body composition body mass (BM), total body muscle mass (MM), fat mass (FM) and percentage of body fat measurements were performed using an eight-point bioelectrical impedance device (Inbody 720 body composition analyser, Biospace Co. Ltd, South Korea.) The subjects were barefoot and wore shorts. Body height was measured to the nearest 0.5 cm using a wall-mounted scale.

Blood samples and analyses

Blood lactate was measured to determine the metabolic effect of work performed in exercises. Blood samples were obtained from the fingertip and collected into capillary tubes (20 μL), which were placed in a 1-mL hemolyzing solution and analyzed automatically after the completion of testing according to the manufacturer's instructions (EKF diagnostic, C-line system, Biosen, Germany). The lactate data has already been published previously (Walker et al. 2012).

Venous blood samples were drawn by repeated venepunctures from an antecubital vein using standard procedures. To assess the acute impact and short-term recovery from exercise protocols, blood samples were collected pre-exercise, immediately after (POST0), 15 min after (POST15), and 30 min after (POST30) the exercises (4 blood samples in total during each exercise bout ×10 mL/sample = 40-mL blood). Total (WBC) and differential white blood cells, platelets, as well as hemoglobin and hematocrit were determined from EDTA-treated blood (Venosafe, Terumo, Belgium) with Sysmex KX-21 N (TOA Medical Electronics Co., Ltd., Kobe, Japan). Of WBC, neutrophils, lymphocytes and mixed cells (monocytes, eosinophils, basophils and immature precursor cells) were analyzed. In addition, venous blood was collected into serum separator tubes (Venosafe, Terumo, Belgium). The samples were centrifuged for 10 min at +4 °C with 2,000×*g* (Megafuge 1.0 R, Heraeus, Germany). Serum was kept at −80 °C until analyzed for serum cortisol using the Immulite 1,000 and hormone-specific immunoassay kits (Immulite, Siemens, IL). Detection limit for cortisol was 5.5 nmoL/l and inter-assay coefficient of variation (CV %) 7.9 %. Concentrations of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), L-selectin (L-sel), interleukin-1 receptor antagonist (IL-1ra), adipsin, adiponectin, leptin, resistin and myoglobin in serum samples were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (IL-6: eBioscience, San Diego, CA, USA; MCP-1, L-selectin, IL-1ra, adipsin, adiponectin, leptin, and resistin: R&D Systems, Europe Ltd, Abindgon, UK; myoglobin: USCN Life Science Inc., Wuhan, China). The detection limits and inter-assay coefficients of variation, respectively, were 0.2 pg/mL and 3.4 % for IL-6, 3.9 pg/mL and 5.9 % for MCP-1, 19.5 pg/mL and 2.9 % for L-selectin, 31.3 pg/mL and 7.6 % for IL-1ra, and 0.78 ng/mL and 9.2 % for myoglobin, 15.6 pg/mL and 4.5 % for adipsin, 31.3 pg/mL and 4.2 % for adiponectin, 15.6 pg/mL and 5.8 % for resistin and 15.6 pg/mL and 3.8 % for leptin. Prior to the statistical analyses, because of significant fluctuations in plasma in HYP, all data were corrected for changes in plasma volume calculated using hematocrit and hemoglobin described by Dill and Costill (1974).

Statistical analysis

Data is presented as mean ± SE. Before applying further statistical methods, the data were checked for sphericity and normality. If a specific variable violated the assumptions of

parametric tests then rank-transformation was used. Rank-transformation was used for cortisol, IL-6, myoglobin, adiponectin and leptin. Absolute and relative changes (e.g., WBC, acute cytokine and hormonal adaptations) were analyzed via two-way repeated analysis of variance for main (type, time) and interaction (type × time) effects. This was followed by one-way repeated measures ANOVA on each MAX and HYP trials to examine a main effect of time. If main or interaction was observed $p \leq 0.05$ the change from pre-values for POST0, POST15 and POST30 was compared between type or time using paired t tests with Bonferroni correction. Spearman's rank correlation coefficient was used to examine the relations. Data were analyzed using PASW statistic 18.0 (SPSS, Chicago, IL, USA). The level of statistical significance was set at $p \leq 0.05$.

Results

Duration, total work, lactate, hormones and myoglobin

The duration of MAX was 50 min, whereas HYP took 20 min to complete. Total work in MAX was 2,500 ± 380 kg and 7,240 ± 1,190 kg in HYP. There were no significant differences between pre-exercise lactate, hormone or myoglobin concentrations. Circulating levels of lactate, cortisol and myoglobin are presented in Table 1. There was a significant type × time ($p < 0.01$) and main effect ($p < 0.01$) of time and type effect in lactate. The peak lactate concentrations were observed at POST0 in MAX (4.6 ± 0.6) and in HYP (12 ± 0.5) and the increase was significant in both HYP (+670 %, $p < 0.001$) and in MAX (+160 %, $p < 0.01$) at POST0 when compared to the pre-exercise values, although the increase was significantly higher ($p < 0.001$) in the HYP compared to the MAX protocol. Results for cortisol indicated significant type × time interaction ($p < 0.05$) and a significant main effect for type ($p < 0.05$) and time ($p < 0.01$). Post hoc comparison revealed that statistical differences between HYP and MAX was significant at POST15 ($p < 0.000$) and POST30 ($p < 0.01$). Serum cortisol concentrations increased significantly only after HYP ($p < 0.01$). There were no differences in serum myoglobin levels between resistance exercise bouts. Myoglobin concentrations increased significantly, and at a similar magnitude in MAX (+182 %, $p < 0.01$) and in HYP (+217 %, $p < 0.001$) protocols, and did not significantly increase after POST0 but remained elevated during the follow-up of 30 min.

White blood cells

There were no significant differences between pre-exercise white blood cells or, platelet counts between exercise bouts. White blood cell and subgroup counts and platelets are presented in Table 2. For the WBC a significant group × time interaction ($p < 0.05$) was observed, and a significant main effect for the type ($p < 0.05$) and time ($p < 0.05$) was found. WBC ($p < 0.001$) significantly increased immediately after HYP, whereas in MAX, increases in WBC counts became significant only at POST30 ($p < 0.05$). A significant group × time interaction ($p < 0.05$) was observed in neutrophils and post hoc comparison revealed a significant difference in acute increase on neutrophils ($p < 0.05$). During recovery, neutrophil counts tended to decrease from POST0 in HYP and were at basal levels at POST30. Results for lymphocytes indicated a significant type × time interaction ($p < 0.05$) and main effect of time ($p < 0.01$) and type ($p < 0.05$). Lymphocyte responses were noticeably different, as lymphocyte numbers increased immediately after HYP and returned to pre-exercise levels during recovery, whereas in MAX slight, but significant, decreases from pre-exercise values were observed at POST15 ($p < 0.05$) and POST30 ($p < 0.05$).

Cytokines, adipocytokines and L-selectin

There were no significant differences between pre-exercise concentrations either in cytokines or adipocytokines

Table 1 Myoglobin, l-selectin, lactate and cortisol

	PRE		POST 0		POST 15		POST30	
	MAX	HYP	MAX	HYP	MAX	HYP	MAX	HYP
Myoglobin (ng/mL)	23 (4.2)	25 (8.0)	44 (8.0)**	42 (6.5)***	49 (8.0)**	50 (7.1)***	51 (6.8)**	48 (4.4)***
L-selectin (ng/mL)	1,100 (16)	1,100 (14)	1,100 (16)	1,100 (18)	1,100 (17)	1,100 (20)	1,100 (15)	1,100 (19)
Lactate (mmol/L)	1.8 (0.2)	2.1 (0.1)	4.6 (0.6)**	12 (0.5)*** †††	2.1 (0.2)*	11 (0.6)*** †††	–	–
Cortisol (nmol/L)	290 (27)	300 (38)	300 (36)	330 (33)** ††	270 (33)	520 (24)** †††	240 (30)*	600 (40)*** †††

Lactate was only measured at PRE, POST and POST15

MAX maximal, *HYP* hypertrophic

* significant difference to pre-exercise value

† significant difference between the exercise bouts. (mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$)

Table 2 White blood cells, subgroups, and platelets

	PRE		POST 0		POST 15		POST30	
	MAX	HYP	MAX	HYP	MAX	HYP	MAX	HYP
Total WBC ($\times 10^9$/l)	6.4 (0.4)	7.0 (0.6)	7.1 (0.6)	9.9 (0.6)*** †††	7.2(0.7)	9.2 (0.6)*** ††	7.6 (0.6)*	8.0 (0.5)*
Lymphocytes ($\times 10^9$/l)	2.2 (0.2)	2.3 (0.5)	2.2 (0.6)	3.8 (0.7)*** †††	2.0 (0.5)*	3.4 (0.2)*** †††	1.9 (0.5)*	2.4 (0.6)††
Neutrophils ($\times 10^9$/l)	3.6 (0.3)	4.0 (0.4)	4.1 (0.4)	5.1 (0.5)* †	4.6 (0.5)*	5.0 (0.4)*	4.9 (0.5)*	4.7 (0.4)
Mixed cells ($\times 10^9$/l)	0.6 (0.1)	0.7 (0.1)	0.7 (0.1)	0.9 (0.1)* †	0.7 (0.1)	0.8 (0.1)*	0.7 (0.1)*	0.7 (0.1)
Platelets ($\times 10^9$/l)	220 (11)	220 (12)	230 (12)	240 (12)**	230 (11)*	240 (11)*	230 (11)*	230 (11)

MAX maximal, *HYP* hypertrophic, *Mixed cells* monocytes, eosinophils, basophils and immature precursor cells

* Significant difference to pre-exercise value

† Significant difference between the exercise bouts. (mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$)

between exercise bouts. For the IL-6 only significant main effect of time ($p < 0.05$) was observed. IL-6 responses were similar in both exercise protocols, as both showed a progressive increase after the exercise with the highest concentrations being observed at POST30 (+90 and +94 % for MAX and HYP, respectively, Fig. 1a). A significant main effect of time was observed in MCP-1. Interestingly, there was a significant decrease ($p < 0.01$) in MCP-1 in HYP at POST30 when compared to the PRE (Fig. 1b). A significant type × time interaction ($p < 0.05$) was observed in IL-1ra. In HYP, IL-1ra concentrations were significantly increased at POST0 ($p < 0.05$, Fig. 1c) whereas in MAX we did not observe significant differences. Significant changes in serum L-selectin were not observed.

The effects of resistance exercises on adipocytokines are presented in Fig. 2. For the adipsin response a significant main effect for time ($p < 0.01$) was observed. Adipsin concentrations decreased immediately after MAX ($p < 0.05$) and HYP ($p < 0.01$) and kept progressively decreasing after both exercise protocols up to POST30. Adiponectin and leptin levels were unaffected by exercises. A significant main effect of time ($p < 0.05$) was observed in resistin. Resistin decreased significantly ($p < 0.01$) in MAX and HYP and progressively decreased during recovery.

Correlations

A negative correlation ($r = -0.643$, $p < 0.05$) was observed between fat mass and adiponectin pre-exercise (Fig. 3a). In HYP, a correlation was observed between myoglobin and IL-1ra at POST0 ($r = 0.701$, $p < 0.05$, Fig. 3b) as well as between myoglobin and neutrophils ($r = 0.601$, $p < 0.05$, Fig. 3c). In MAX, there was a significant negative correlation between the acute increase in WBC and the acute increase in cortisol at POST0 ($r = -0.622$, $p < 0.05$, Fig. 3d). Interestingly, the peak lactate (POST15) correlated negatively with lymphocytes in HYP ($r = -0.874$, $p < 0.05$, Fig. 3e) and in MAX ($r = -0.697$, $p < 0.05$, Fig. 3f).

Discussion

This study demonstrated that both hypertrophic and maximal resistance exercise bouts alter immediate responses in WBC and cytokine concentrations. Interestingly, the acute increase in white blood cells was significantly higher after HYP. The increase in total WBC was dominated by neutrophils in MAX, whereas in the HYP both neutrophils and lymphocytes increased significantly. The higher metabolic demand in HYP demonstrated by significantly higher lactate response might explain the difference in lymphocyte response. Both exercise bouts induced similar signs of muscle injury, demonstrated by increased myoglobin concentrations. There was a significant decrease in lymphocytes during recovery at POST15 in MAX. IL-6 increased significantly after both exercise sessions. Interestingly, both exercise protocols decreased adipsin and resistin levels immediately after and continuously during the 30 min follow-up after the exercise. In addition, there was a significant increase immediately after exercise in IL-1ra and decrease at 30 min in MCP-1 in HYP. These observations support the hypothesis that resistance exercise has beneficial anti-inflammatory effects, which might be more pronounced in HYP because the metabolic demand is higher than during MAX. The findings of this study show the immediate immune responses to MAX and HYP, however, the evaluation is limited by the difference in duration of the exercise bouts.

White blood cells

An increased mobilization of white blood cells into the circulation was observed after both bouts. An acute increase in blood WBC counts has been shown to occur after resistance exercise (RE) in several studies (Nieman et al. 1995a; Kraemer et al. 1996; Simonson 2001; Natale et al. 2003; Ramel et al. 2003, 2004; Simonson and Jackson 2004; Mayhew et al. 2005; Risoy et al. 2003). The type of resistance exercise seems to affect the amplitude and time course

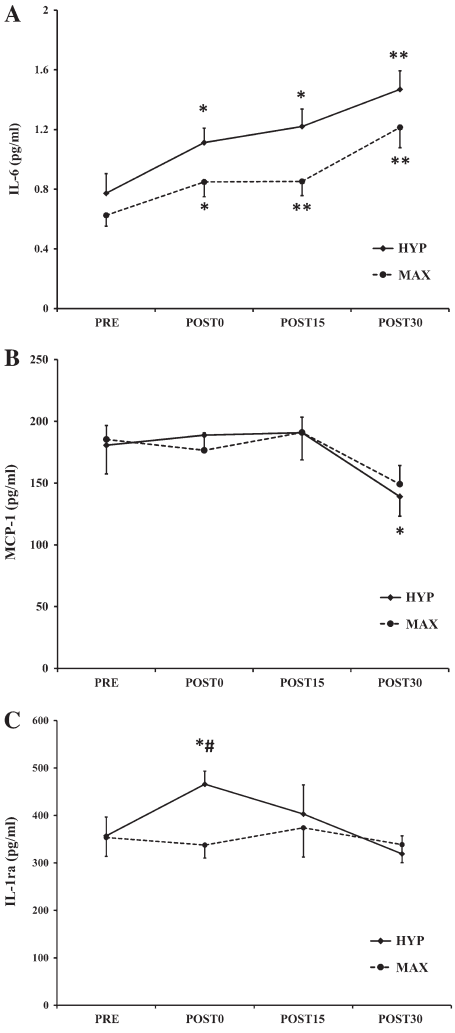

Fig. 1 The effect of exercise bouts on serum IL-6, IL-1ra and MCP-1. *MAX* maximal, *HYP* hypertrophic. *significant difference to pre value, #significant difference between the exercises. (mean ± SEM *$p < 0.05$, **$p < 0.01$, #$p < 0.05$)

of the leucocytosis. In this study, the increase in total WBC was significantly higher after HYP than MAX. Risoy et al. (2003) demonstrated that white blood cells infiltrate into exercised muscles and that might be related to impaired recovery. In the present study, neutrophil concentrations peaked immediately after HYP, whereas in MAX the acute response was slower and became significant 15 min after the end of the exercise session. In accordance with previous study (Mayhew et al. 2005), the peak values might have been observed later than our 30 min time point.

Previous studies (Suzuki et al. 1999; Peake et al. 2005b; Paulsen and Peake 2013) have reported that post-exercise myoglobin concentrations correlate with neutrophil counts especially in the delayed phase of leukocytosis. In the present study, during the recovery window of 30 min, a significant correlation was observed only in HYP, even though both MAX and HYP induced similar acute increase in serum myoglobin concentrations. To avoid repeated bout effect on inflammatory response that has been shown in muscle damaging, especially eccentric exercise bouts, the participants took part into familiarization session in which 1RM was measured and counter-balanced design was used (McHugh 2003). The main limitation in the present study was the narrow recovery window which might have hidden the peak myoglobin levels. On the other hand significant increase in myoglobin was not observed between POST15 and POST30. The present study observed significant increases in lymphocytes only after HYP. Interestingly, a slight, but significant decrease in lymphocyte counts was observed in MAX at 15 and 30 min. This might indicate that a high-intensity maximal resistance exercise bout might induce lymphopenia, which has been associated with so called "open window theory" of higher risk for infections after exercise (Nieman and Pedersen 1999). Again, the last recovery sample was collected 30 min after the exercise bouts and lymphopenia might be observed later than that in HYP. We cannot ignore the fact that a decrease in lymphocytes below pre-exercise values might have been also observed in HYP, but due to our recovery window and the different durations of the exercise protocols, we were unable to observe it. Shear stress and hormonal signals have been suggested to induce the release of WBC from marginated pool (Freidenreich and Volek 2012). Elevations in cortisol are thought to lead to reductions in circulating lymphocyte counts during post-exercise recovery (Shinkai et al. 1996). We did not observe a significant correlation between cortisol response and increase in WBC in HYP but interestingly, cortisol levels measured immediately after exercise correlated negatively with WBC in MAX. The acute WBC response after exercise is most likely a result of several mechanisms, especially in HYP, which might blunt the effects of cortisol. Whereas in MAX, even though the cortisol concentrations decreased, they correlated negatively with WBC and lymphocyte counts. Miles et al. (2003) observed an association between lactate accumulation and increased number of lymphocytes after squat exercise. In the present study, as expected, peak lactate levels

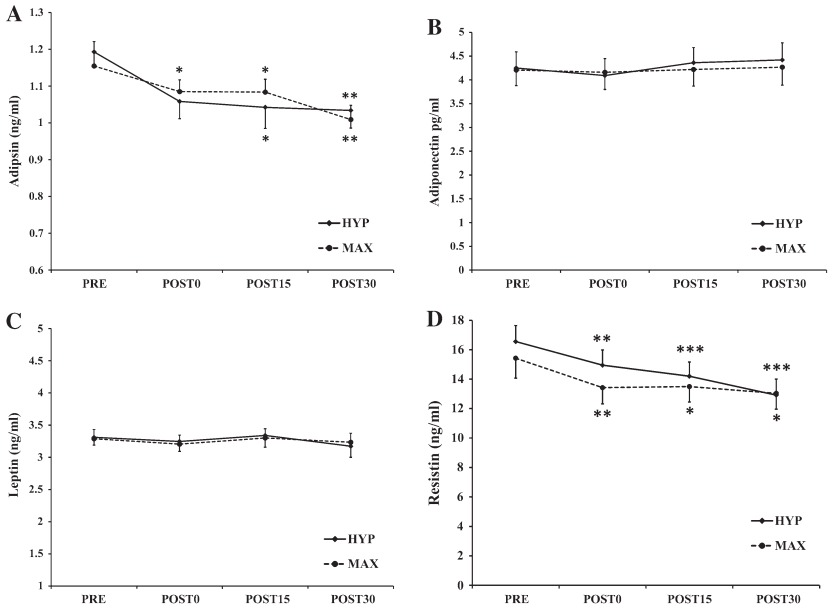

Fig. 2 The effect of exercise bouts on serum adipsin, adiponectin, leptin and resistin. *MAX* maximal, *HYP* hypertrophic. *significant difference to pre value. There were no significant differences between loadings. (mean ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$)

correlated positively with lymphocyte counts after exercise bout in HYP whereas a negative correlation between lymphocyte counts and lactate concentrations was observed at POST0 and POST15.

Cytokines

The results of this study demonstrated that IL-6 increased progressively after both HYP and MAX. Contracting skeletal muscle synthesizes and releases IL-6 into the interstitium as well as into systemic circulation during exercise. Local factors seem to be necessary in IL-6 release and synthesis from contracting muscle but systemic factors may modulate the response. Muscle contraction has been linked to IL-6 release in several mechanisms, including change in calcium homeostasis, impaired glucose availability and formation of reactive oxygen species (Fischer 2006). HYP lead to a significantly higher blood lactate concentration, which demonstrates the higher metabolic demand. Whereas, mechanical stress, due to the higher loads, was expected to be higher in MAX. It is important to note, however, that the increase in IL-6 levels was relatively modest, which is in-line with previous studies (Izquierdo et al. 2009). Izquierdo et al. (2009) reported significant increases in IL-6 45 min after 5 × 10RM leg press exercise in untrained men while IL-1ra increase in a statistically significant manner only immediately after a training period of 7 weeks. Secreted IL-6 has shown to have an anti-inflammatory effect as it stimulates IL-10 and IL-1ra (Steensberg et al. 2003). Interestingly, even if the exercise bouts were very different significant differences in IL-6 response were not observed, we observed a significant increase in IL-1ra after HYP, but not after MAX, which might indicate that higher stimulation of the cardiovascular system might be needed to elicit a response in IL-1ra. In addition, there was a trend for MCP-1 concentration to decrease below pre-exercise levels in both loadings during recovery, but this reached statistical significance only in HYP. MCP-1 has been reported to increase immediately after muscle damaging exercise (Peake 2005a; Crystal 2013); however, the immediate increase in MCP-1 was not observed. MCP-1 and its receptors are required for successful muscle regeneration, but at the same time MCP-1 has a negative role in chronic diseases that include low-grade inflammation (Paulsen et al. 2012; Kim et al. 2006). In addition to acute increase in IL-1ra in the present study, the significant decrease in MCP-1 after the HYP could be considered as an acute anti-inflammatory response to HYP.

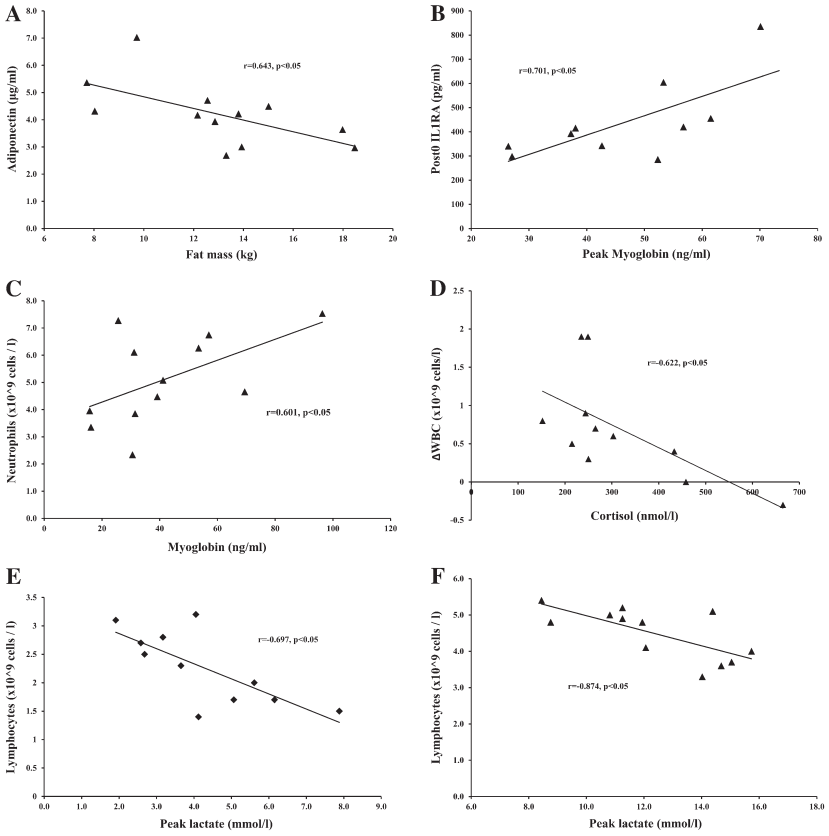

Fig. 3 Significant correlations were observed between fat mass and adiponectin ($r = -0.643$, $p < 0.05$, (**a**), in HYP, between myoglobin and IL-1ra at POST0 ($r = 0.701$, $p < 0.05$, (**b**), in MAX, between the acute increase in WBC and the acute increase in cortisol at POST0 ($r = -0.622$, $p < 0.05$, (**c**). In addition, the peak lactate (POST15) correlated negatively with lymphocytes in HYP ($r = -0.874$, $p < 0.05$, (**d**) and in MAX ($r = -0.697$, $p < 0.05$, (**e**)

Adipocytokines

Altered adipocytokine levels (especially low adiponectin) have been proposed to be a link between obesity and diabetes (Kanaya et al. 2004). In the present study, in-line with previous reports (Ronti et al. 2006), resting adiponectin levels negatively correlated with body fat. Bouassida et al. (2008) suggested that to induce significant acute decreases in leptin, a background in training might be needed and thus significant adipocytokine response would be observed only when performed by trained athletes. In the present study, no significant changes in leptin or adiponectin levels were observed. In contrast, resistin and adipsin decreased significantly immediately after both resistance exercise bouts. (Varady et al. 2010) observed beneficial adipocytokine modulation after resistance exercise only in habitual weight-trainers, but the present study indicates that HYP, as well as MAX, may have a beneficial effect on adipocytokine profile also in people who do not have weight training background when the exercise is performed with maximum effort. As in the study of (Varady et al. 2010), the results from this study might only be limited to heavy resistance exercise bout and for young lean men, and more research is needed in other populations.

Exercise protocols

Many types of resistance exercise sessions can be effectively used to improve muscular fitness. The ACSM position stand on Progression Models in Resistance Training for Healthy Adults (2009) recommends loads corresponding to a repetition range 8–12RM to be used for novice training and 1–12RM in a periodized fashion for intermediate (individuals with approximately 6 months of consistent resistance training experience) to advanced training. The number of sets per exercise recommended is initially one to three for novice individuals and for progression into intermediate to advanced status it is recommended to use multiple sets with systematic variation of volume and intensity over time (American College of Sports Medicine 2009). To ensure optimal health and fitness gains, resistance training should be undertaken with proper preparation, guidance and surveillance (Williams et al. 2007). The exercise protocols in this study were heavy, which must be taken into account when evaluating the present results. Studies with a lower number of sets (Buford et al. 2009) and smaller muscle groups (Uchida et al. 2009) did not observe significant immediate response in circulating cytokines after a resistance exercise bout. The reported cytokine responses to resistance exercise vary considerably; however, this study suggests that heavy exercise protocols may be needed to elicit a significant change in inflammation markers in healthy subjects.

Conclusion

The findings of this study suggest that a resistance exercise bout, in general, produces changes in WBC, cytokines and adipocytokines. Due to the greater metabolic demand and stress with shorter rest periods, the WBC and concentrations of cortisol and cytokines increased to a greater extent after HYP than after MAX. There was notable individual variability in the cytokine responses, especially in MCP-1, whereas WBC and adipocytokine responses were more coherent. Efficient recovery, especially from resistance exercise, requires a well-coordinated and controlled inflammatory response, which includes a rise in pro-inflammatory, as well as anti-inflammatory cytokines. One heavy resistance exercise bout could lead to an anti-inflammatory environment, which might reduce inflammation markers that are related to low-grade inflammation while repeated in regular training (Gleeson et al. 2011). Future well-controlled studies are needed to determine the mechanisms that regulate the observed responses and the long-term effects of repeated heavy resistance exercise bouts on cytokine and, especially, adipocytokine profiles.

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II

RESISTANCE TRAINING STATUS MODIFIES INFLAMMATORY RESPONSE TO EXPLOSIVE AND HYPERTROPHIC RESISTANCE EXERCISE BOUTS

by

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ORIGINAL ARTICLE

Resistance training status modifies inflammatory response to explosive and hypertrophic resistance exercise bouts

Johanna K. Ihalainen[1] · Juha P. Ahtiainen[1] · Simon Walker[1] · Gøran Paulsen[2,3] · Harri Selänne[4] · Mari Hämäläinen[5] · Eeva Moilanen[5] · Heikki Peltonen[1] · Antti A. Mero[1]

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Abstract The purpose of the present study was to examine the immediate and prolonged immune response in circulating cytokine and adipocytokine concentrations after two different resistance exercise bouts: hypertrophic (HYP1, 5 × 10, 80% of 1RM) and maximal explosive (POW1, 10 × 5, 60% of 1RM) resistance exercise bouts and how 12 weeks of resistance training (RT) modifies these responses (HYP2, POW2). Eight men completed the study. RE-induced interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1ra), monocyte-chemoattractant protein-1 (MCP-1), leptin, resistin, and adiponectin were measured before (PRE) and immediately (POST0), 24 (POST24) and 48 (POST48) hours after RE bouts before and after RT. In the untrained state, IL-6 increased immediately after RE in HYP1 ($p = 0.002$) and in POW1 ($p = 0.003$) whereas no changes were observed after RT. Similar results were observed in IL-1β, whereas conversely, IL-1ra increased only after RT in HYP2 and POW2 ($p < 0.05$). Resistin increased before RT in HYP1 and in POW1 ($p = 0.011$ and $p = 0.003$, respectively), but after RT, significant responses were not observed. Interestingly, in HYP2, MCP-1 increased significantly at POST24 ($p = 0.009$) and at POST48 ($p = 0.032$) only following RT. The present study shows that RT modifies RE-induced cytokine responses towards an anti-inflammatory direction.

Keywords Initial response · Hypertrophic resistance exercise · Power · Cytokines · Inflammation

✉ Johanna K. Ihalainen
johanna.k.ihalainen@jyu.fi

[1] Biology of Physical Activity, Faculty of Sport and Health Sciences, University of Jyväskylä, P.O Box 35, 40014 Jyväskylä, Finland

[2] The Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway

[3] Norwegian School of Sport Sciences, Oslo, Norway

[4] Department of Psychology, University of Jyväskylä, Jyväskylä, Finland

[5] The Immunopharmacology Research Group, Faculty of Medicine and Life Sciences, University of Tampere and Tampere University Hospital, Tampere, Finland

Introduction

Skeletal muscle is the largest organ in the human body, and ground-breaking work during the last decade has demonstrated that skeletal muscle is an active endocrine organ releasing a host of cytokines [1]. In recent years, studies reporting the acute effects of exercise on cytokines have been published quite extensively, whereas the specific time course of the effect of different resistance exercise protocols on cytokines remains unclear [2]. Cytokines are glycoproteins involved in the regulation and modulation of the immune response, and they are produced by a broad range of cells, including immune cells, skeletal muscle, connective tissue, and adipose tissue cells [3]. Resistance exercise (RE) is a potent activator of the immune system demonstrated by the changes in circulating pro-inflammatory and anti-inflammatory cytokine concentrations after exercise bouts [4]. For instance, a single bout of resistance exercise of moderate to high intensity has been shown to promote a transient increase in pro-inflammatory interleukins IL-6 and IL-1β as well as in the circulating levels of C-reactive protein (CRP), which is associated with a later increase in the levels of the anti-inflammatory IL-1 receptor antagonist (IL-1ra) [4, 5].

Far less is known about the effects of resistance exercise bout on so-called adipocytokines, e.g., leptin, adiponectin, and resistin. Adipocytokines are hormones that were first

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discovered to be secreted by adipose tissue and to regulate both energy metabolism and appetite. More recent findings on the ubiquitous expression of their receptors and on their cellular effects have revealed that adipocytokines also are involved in the regulation of a variety of biological functions related to immune responses and inflammatory diseases [6]. Previous studies have demonstrated that a single heavy resistance exercise bout exerts a specific acute effect on circulating adipocytokine concentrations, and the immediate response appears to be dependent on the duration and intensity of the exercise, as well as on training status and background [7–9].

Previous studies have suggested that exercise training can significantly alter the acute inflammatory responses to high intensity resistance exercise and recovery processes after the resistance exercise bout. Murton et al. [10] highlighted that the response to the first RE bout in participants with no background in resistance training (RT) is significantly different and has more inter-subject variability compared to the second resistance exercise. Izquierdo and colleagues [4] reported a significantly greater inflammation-responsive cytokine IL-6 response followed by a significantly enhanced response in the anti-inflammatory IL-1ra after a heavy resistance training intervention compared to the response before training. Cross-sectional studies have also suggested that training background affects the acute cytokine responses to a RE bout [9]. Thus, the overall effects of long-term RT appears to attenuate the acute inflammation response, but there are mixed findings on the effect of RT on specific markers [11].

It has been proposed that the acute anti-inflammatory (immunosuppressive) effect following a bout of resistance exercise could be beneficial for patients with autoimmunity disease, for senior citizens, and for obese individuals [3]. It is important to identify the specific resistance exercise-induced changes in circulating cytokines as well as the longitudinal effect of resistance training on immediate and prolonged responses after specific resistance exercise bouts in order to better understand possible health benefits, but also the possible health hazards, related to resistance training [12]. Pedersen and Febbraio [13] have linked skeletal muscle contraction to cytokine production. The extent of inflammation response to resistance exercise is affected by the physiological demands of RE, depending on the mode (eccentric and/or concentric muscular contractions), volume (total work of the session), load (weight lifted), and intensity (extent of neuromuscular and metabolic fatigue) of resistance exercise [14]. Many types of resistance exercises can be effectively used to improve muscular fitness and overall health [15]. Muscle strength and the ability of the muscles to develop force rapidly are important performance characteristics, which have also been shown to contribute to health and several tasks of daily life [16]. Since the cytokine responses appear to be related to the intensity of the exercise protocol, it is of interest to examine the cytokine responses of exercise protocols that aim for gains in muscle mass and strength [17], as well as in rapid force development [18, 19].

If the cytokine responses are related to the amount of muscle mass activated and respective metabolic changes, it is expected that the greatest responses will be observed after hypertrophic resistance exercise. However, in explosive RE, muscles are also activated maximally but with a shorter duration of each repetition, accompanied by a lower metabolic response. Thus, it is not clear whether this type of stimulus is large enough to cause considerable hormonal changes. We expected that the hypertrophic resistance exercise (HYP, 5 × 10, 80% of 1RM) would induce a significant response to the variables measured [4, 7]. The hormonal responses to a maximal explosive (POW, 10 × 5, 60% of 1RM) RE bout have been shown to be similar as in HYP but with a lower magnitude [18, 19]. Thus, we expected to observe a significant but lower response in the measured variables after POW RE bout. However, several studies have showed that hormonal responses to same hypertrophic [20] as well as explosive [21] RE are modified by 7–21 weeks of resistance training. Hence, we found it justified to assess whether the inflammatory response is modified after progressive resistance training. Therefore, the purpose of the present study was to examine the immediate and prolonged immune response, by measuring circulating cytokine and adipocytokine concentrations, induced by two different resistance exercise bouts: hypertrophic (HYP, 5 × 10, 80% of 1RM) and maximal explosive (POW, 10 × 5, 60% of 1RM) resistance exercise bouts. In addition, we measured how typically preiodized 12 weeks of RT possibly modified these responses.

Materials and methods

Subjects This study was a part of a larger research project (TEKES Finland, Decision No. 70007/13). Eight healthy, slightly overweight, young men were selected for this study (age 31.0 ± 0.9 years, body weight 84.6 ± 1.9 kg, height 1.78 ± 0.04 m, fat percentage 25.3 ± 7.1%). All participants were physically active on a weekly basis, but none were competitive athletes or had a background in systematic strength training. The subjects' physical activity was characterized by walking, cycling, or occasionally participating in team sports at light to moderate intensity and a frequency of 3 d week^{-1}. Participants filled in a health questionnaire prior to participation in the study. All subjects reported that they were non-smokers, free from injury, and were not using any medications. Each subject was informed of the potential risks and discomforts associated with the measurements, and all the subjects gave their written informed consent to participate. The study was conducted according to the Declaration of Helsinki, and ethical approval for the study procedures were granted by the Ethical Committee at the University of

Jyväskylä and by the Ethical Committee of the Central Hospital, Jyväskylä.

Study design and experimental resistance exercise bouts The study design (A) and experimental RE protocols (B) are presented in Fig. 1. The first phase of the study was a 4-week long preparatory RT period, during which the subjects were familiarized to RT and underwent pretesting. Subsequently, the cross-over study was started, and the subjects were randomly assigned to perform HYP1 or POW1 RE bout first and then after 10 days of recovery, POW1 or HYP1, respectively. Thereafter, they trained for 12 weeks according to supervised progressive RT protocol and did the same RE bouts with the same order as before training (HYP2/POW2). The POW RE bout included 10 sets of 5 repetitions of the concentric phase as fast as possible at 60% of 1 repetition maximum (RM) and the HYP RE bout was 5 sets of 10 repetitions at 80% of 1RM for the leg press (David D210 horizontal leg press device, David Health Solutions Ltd., Helsinki, Finland) exercise. The loads used during the first set were determined from the 1RM load measured during pretesting. The loads were adjusted during the sessions to enable completion of the required repetitions. The inter-set rest period was 3 min in POW and 2 min in HYP. The duration of POW was 32 min, whereas HYP was completed in 20 min. Total volume (load × repetitions) was 7160 ± 272 and 7550 ± 427 kg in POW and HYP, respectively. All experiments were conducted at the same time of day (± 1 h) for each subject.

Pretesting All subjects participated in a pre-test session, which included anthropometrics and body composition measurement, as well as the 1RM test performed in the leg press device. This bilateral 1RM test was used to determine the loads used in each acute RE bout. Three warm-up sets (5 × 70–75, 3 × 80–85, and 2 × 90–95% of estimated 1RM) with 1 min of rest between sets were performed before the 1RM trials. Upon verbal instruction, subjects performed a full leg extension (knee angle 180°) from a starting knee angle of below 60°. After each successful completion, the load was increased. Subjects were allowed a maximum of 5 trials. The trial with the highest completed load was accepted as the 1RM.

Training Subjects were asked to maintain individual habitual physical activity (e.g., light walking, cycling, and occasional team sports) throughout the study period. All prescribed training in the study was consistently supervised by qualified instructors. The training was designed to reflect a program designed for physically active populations according to recommendations outlined by the American College of Sports Medicine [15]. The detailed training program (hypertrophic-strength-group) has been previously reported by Hulmi et al. [22]. Briefly, in the preparatory 4-week RT period before POW1/HYP1, see above, the subjects exercised using whole-body workouts two times per week to standardize training status, to minimize the effects of stressors related to unaccustomed exercise, and to overcome strong neural and learning adaptations known to occur within the first few weeks of RT. Training loads were 50–80% of one repetition maximum (1 RM) increasing throughout the preparatory RT period.

The veritable 12-week progressive RT program was started after POW1/HYP1 and divided into three different blocks. Each block consisted of 4 weeks of RT. The first block consisted only of hypertrophic RE sessions. In the second block, 75% of the sessions were hypertrophic and 25% were maximal-strength sessions, and in the last block, 25% were hypertrophic and 75% maximal-strength RE sessions. The subjects did on average nine exercises in each session, 2–3 sets per exercise. Bilateral leg press, bilateral knee extension, and bilateral knee flexion exercises were performed during each RE session.

Body composition Whole body composition was estimated before and after RT by dual X-ray absorptiometry (DXA, LUNAR Prodigy, GE Medical Systems) after an overnight fast and 48 h without training. Abdominal fat was calculated manually defining a range of interest confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest [23] at PRE. This customized range was then copied to the DXA scans at week 4 and week 16, respectively.

Nutrition Dietary intake was recorded over three weekdays and one weekend day during the training period. The subjects were instructed to follow the same diet before all the acute exercise bouts. The breakfast before RE bouts was standardized and served at the laboratory.

Subjective muscle soreness Muscle soreness was rated on a visual analogic scale (VAS) of 0 (= no pain) to 100 (= maximum pain) in millimeters for the overall muscle soreness of the quadriceps muscles at PRE, POST24, and POST48.

Blood samples and analyses Blood lactate was measured to determine the metabolic effect of work performed in RE bouts. Blood samples were obtained from the fingertip and collected into capillary tubes (20 μL), which were placed in a 1-mL hemolyzing solution and analyzed automatically after the completion of testing according to the manufacturer's instructions (EKF diagnostic, C-line system, Biosen, Germany). To assess the immediate and prolonged (up to 48 h) impact from exercise protocols, blood samples were collected pre-exercise (PRE) and during recovery as follows: immediately (POST0), 24 h (POST24), and 48 h (POST48) after the exercises.

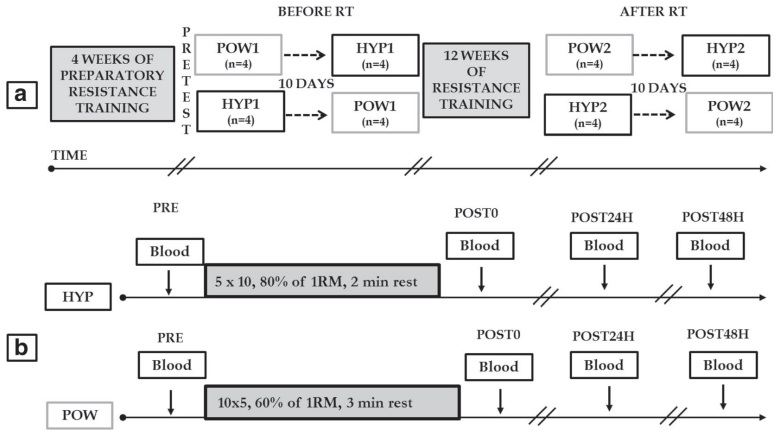

Fig. 1 Study design (**a**) and experimental protocols (**b**). *1RM* repetition maximum, *POW* power, *HYP* hypertrophic resistance exercise bout, *RT* resistance training, *PRE* before loading, *POST0* immediately after exercise, *POST 24H* 24 h after the loading, *POST48H* 48 h after the loading

Venous blood samples were drawn from the antecubital vein into EDTA tubes (Venosafe, Terumo, Belgium). Hemoglobin and hematocrit were determined with Sysmex KX-21N (TOA Medical Electronics Co., Ltd., Kobe, Japan), and plasma volume change was determined after exercise at POST0 from hemoglobin and hematocrit concentrations using the equation by Dill and Costill [24]. Plasma glucose (GLU) was measured using the KONELAB 20XTi analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

The serum samples were held for 15 min at room temperature before being centrifuged for 10 min at 2000×*g* (Megafuge 1.0 R, Heraeus, Germany). The serum was kept at − 80 °C until analyzed. High-sensitivity C-reactive protein (hsCRP), creatine kinase (CK), cortisol (COR), and interleukin-1 beta (IL-β) in serum samples were analyzed from using the Immulite 1000 and immunoassay kits (Immulite, Siemens, IL, USA). The detection limits and inter-assay coefficients of variation, respectively, were 0.1 mg L^{-1} and 10% for hsCRP, 3.9 pg mL^{-1} and 5.9% for CK, 5.5 nmol L^{-1} and 7.9% for COR, and 1.5 pg mL^{-1} and 2.8% for IL-1β.

The EDTA-treated samples were centrifuged for 10 min at + 4 °C with 2000×*g* (Megafuge 1.0 R, Heraeus, Germany). The plasma was kept at − 80 °C until analyzed. Concentrations of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), interleukin-1 receptor antagonist (IL-1ra), adiponectin, leptin, and resistin were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (R&D Systems, Europe Ltd., Abingdon, UK). The detection limits and inter-assay coefficients of variation, respectively, were 0.2 pg mL^{-1} and 1.8% for IL-6, 3.9 pg mL^{-1} and 5.0% for MCP-1, 31.3 pg mL^{-1} and 2.0% for IL-1ra, and 0.78 ng mL^{-1} and 2.2% for adiponectin, 15.6 pg mL^{-1} and 4.0% for resistin, and 15.6 pg mL^{-1} and 5.1% for leptin.

Statistical analyses Conventional statistical methods were used for the calculation of means and standard deviations and standard errors. Before applying further statistical methods, the data was checked for sphericity and normality. If a specific variable violated the assumptions of parametric tests, then log-transformation was used. For IL-6, adiponectin, and leptin, the log-transformation provided sufficient remedy for normality or homogeneity of variance. Absolute changes were analyzed via two-way repeated analysis of variance for time (PRE, POST0, POST24, POST48), training (before, after), and interaction (time × training) effects. This was followed by one-way repeated measures ANOVA on each RE bout to examine a main effect of time. If a main or interaction effect was observed at $p \leq 0.05$, the change from pre-values to POST0, POST24, and POST48 was compared between type or time using paired *t* tests. Effect sizes (ES) are given as Cohen's *d* with an effect size of 0.20–0.50 being considered small, 0.50–0.80 medium, and > 0.80 large. Data was analyzed using PASW statistic 18.0 (SPSS, Chicago, IL, USA). The level of statistical significance was set at $p \leq 0.05$.

Results

Descriptive statistics of the anthropometric characteristics and 1RM are presented in Table 1. After RT, whole body fat-free

Table 1 Anthropometric characteristics of the participants

Variable	Week 0	Week 12	Δ-%
Body weight (kg)	84.6 ± 5.09	83.8 ± 4.85	*− 0.8 ± 2.9*
Height (m)	1.78 ± 0.04	1.78 ± 0.04	–
BMI (kg m^{-2})	26.8 ± 1.37	26.6 ± 1.24	*− 0.8 ± 2.9*
Body fat mass (kg)	21.6 ± 6.8	19.8 ± 5.8	− 6.6 ± 13.8
Abdominal fat mass (kg)	*2.9 ± 1.5*	*2.8 ± 1,0*	*− 3,2 ± 6.4*
Fat-free mass (kg)	59.6 ± 0.53	61.6 ± 0.63*	3.3 ± 0.3*
1 RM (kg)	*225 ± 34.5*	*255 ± 30.1**	*13.8 ± 7.6*

*Significant difference to pre-training value $p < 0.05$ (mean ± SD)

mass was increased significantly ($p < 0.05$), whereas body weight, whole body fat mass, and abdominal fat mass stayed unaltered after RT. 1RM increased significantly (+ 13%, $p = 0.032$). Table 2 shows that RT significantly ($p < 0.05$) suppressed the immediate increases in lactate and glucose and prolonged those of creatine kinase and muscle soreness induced by HYP. A significant increase in cortisol was observed before RT in HYP ($p < 0.001$), whereas after RT, this was not observed. POW reduced average circulating concentrations before and after RT, but the decrease was statistically significant only after RT ($p < 0.05$).

Effects of HYP and POW RE on the circulating levels of inflammatory markers before and after RT intervention are shown in Table 3. The most notable changes were seen in MCP-1 and resistin (Fig. 1). A significant time × training effect was observed in circulating MCP-1 concentration in HYP ($p = 0.002$, ES = 0.924) (Fig. 1a): after RT in HYP2, a significant increase in MCP-1 was observed during recovery at POST24 ($p = 0.009$) and at POST48 ($p = 0.032$). An increasing trend in circulating MCP-1 concentration was seen also at POST24 and POST48 in POW2 but that effect did not reach statistical significance.

There was a significant main effect of time ($p = 0.005$, ES = 0.560) and time × training ($p = 0.028$, ES = 0.732) in circulating resistin levels in POW (Fig. 1b). A significant increase in resistin concentration from PRE to POST0 was observed before RT in POW1 ($p = 0.003$, ES = 0.997); however, after RT in POW2, such significant response was not observed ($p = 0.102$). In HYP, a similar effect, although smaller in quantity, in circulating resistin concentration was found: there was an increase in HYP1 from PRE to POST0 before RT ($p = 0.011$, ES = 0.789) whereas resistin stayed statistically unaltered by HYP2 after RT ($p = 0.248$) (Fig. 1b).

Neither of the RE bouts, before or after RT, elicited significant changes in circulating CRP concentrations (Table 3). IL-6 response was significantly affected by RT in HYP (time × training interaction, $p = 0.048$, ES = 0.534) and in POW (time × training interaction, $p = 0.013$, ES = 0.808). A significant increase in IL-6 was observed at POST0 in HYP1 ($p = 0.002$, ES = 0.719) and in POW1 ($p = 0.003$, ES = 0.878) before training whereas no effect was observed after training (Table 3).

A significant main effect of time in circulating IL-1β was observed in HYP ($p = 0.022$, ES = 0.775) and in POW ($p = 0.043$, ES = 0.496) (Table 3). Significant increases from PRE to POST0 were observed in IL-1β in HYP1 before RT ($p = 0.019$, ES = 0.795) whereas after RT, statistically significant changes were not observed. Similarly in POW1 before RT, a significant increase was observed in circulating IL-1β concentration from PRE to POST0 ($p = 0.048$), whereas after RT, significant changes were not observed (Fig. 2).

Circulating IL-1ra remained unaltered before RT in HYP1 and in POW1, but after RT, the circulating IL-1ra concentration increased significantly from PRE to POST0 in HYP2 ($p = 0.048$) and in POW2 ($p = 0.024$), see Table 3.

Following RT, the circulating leptin concentrations were significantly higher at PRE before both loadings. In HYP1, a significant reduction in circulating leptin concentration was observed at POST24 ($p < 0.05$) and in HYP2 at POST0 ($p < 0.05$). Pre-exercise circulating adiponectin concentration was significantly higher after RT in HYP2 ($p = 0.048$) and in POW2 ($p = 0.026$) and stayed unaltered after RE bouts.

Discussion

The purpose of the present study was to examine the immediate and prolonged immune response, by measuring circulating cytokine and adipocytokine concentrations, induced by two different resistance exercise bouts: hypertrophic (HYP, 5 × 10, 80% of 1RM) and maximal explosive (POW, 10 × 5, 60% of 1RM) resistance exercise bouts, and how 12 weeks of RT may modify these responses. We expected HYP RE to elicit greater responses in the selected cytokines. However, the differences between the loadings were cytokine-specific. Nevertheless, the pro- and anti-inflammatory responses were modified differently: the pro-inflammatory IL-6, IL-1β, and resistin response was blunted, whereas anti-inflammatory IL-1ra response was enhanced in both HYP and POW as a consequence of training. Interestingly, the prolonged MCP-1 response was enhanced after RT in HYP, possibly as a marker of muscle regeneration. The present data also demonstrated that progressive RT increases muscle strength and alters immediate and prolonged response of cytokine and adipocytokine concentrations to HYP and POW similarly. Interestingly, RT affected responses to POW similarly even though the training did not include explosive type of training. This could indicate that the effect of training on immediate prolonged cytokine response is more dependent on overall training status than on specific RE training background.

Regular exercise reduces the risk of chronic metabolic and cardiorespiratory diseases, and this reduction has been linked

Table 2 Lactate (Lac), creatine kinase (CK), muscle soreness (DOMS), glucose and cortisol responses to hypertrophic (HYP), and explosive (POW) RE before and after the 12-week resistance training (RT) intervention

		PRE	POST0	POST24	POST48
HYP RE					
Lac (mmol L^{-1})	Pre RT	1.5 ± 0.4	13.0 ± 4.1***	na	na
	Post RT	2.2 ± 0.5	10.1 ± 3.4**#	na	na
CK (pg mL^{-1})	Pre RT	250 ± 230	280 ± 730	362 ± 240***	239 ± 120
	Post RT	140 ± 36	150 ± 360#	279 ± 150**#	229 ± 91*
DOMS (mm)	Pre RT	2.4 ± 6.4	na	42 ± 19**	44 ± 22**
	Post RT	0.9 ± 1.5	na	33 ± 25*#	22 ± 25#
Glucose (mmol L^{-1})	Pre RT	5.1 ± 0.4	6.2 ± 0.4**	na	na
	Post RT	5.3 ± 0.6	5.6 ± 0.5#	na	na
Cortisol (nmol mL^{-1})	Pre RT	400 ± 170	562 ± 130***	380 ± 140	330 ± 110
	Post RT	400 ± 120	450 ± 120#	470 ± 110	450 ± 140
POW RE					
Lac (mmol L^{-1})	Pre RT	1.9 ± 0.9	3.0 ± 1.1*	na	na
	Post RT	2.2 ± 0.9	3.1 ± 1.8	na	na
CK (pg mL^{-1})	Pre RT	150 ± 75	140 ± 27	310 ± 100**	240 ± 75*
	Post RT	130 ± 80	110 ± 59	330 ± 94**	250 ± 65**
DOMS (mm)	Pre RT	1.3 ± 2.4	na	20 ± 23	10 ± 12
	Post RT	3.9 ± 5.7	na	22 ± 21	14 ± 25
Glucose (mmol L^{-1})	Pre RT	5.7 ± 1.0	5.6 ± 1.3	na	na
	Post RT	5.3 ± 0.7	5.7 ± 0.3	na	na
Cortisol (nmol mL^{-1})	Pre RT	370 ± 49	310 ± 70	420 ± 120	428 ± 93
	Post RT	410 ± 58	340 ± 55*	490 ± 120	371 ± 130

*Significant difference to pre-exercise value in the corresponding RE bout

Significant difference to corresponding value before resistance training in the corresponding RE bout (mean ± SD *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, #$p < 0.05$)

to the anti-inflammatory effect of exercise [25]. It has been suggested that this anti-inflammatory effect of exercise is mediated via the introduction of an anti-inflammatory environment following each bout of endurance or resistance exercise [25]. The present study showed increased IL-6 and IL-1β levels immediately post-exercise before RT but not after RT, whereas circulating IL-1ra increased only after RT in both resistance exercise bouts. This modified IL-6 response is in line with the study by Izquierdo et al. [4], which showed exercise-induced IL-6 response only in the initial phase of resistance training. Contracting skeletal muscle has been proposed to be the main source of increased IL-6 in circulation during and following exercise but also connective tissue, brain, and adipose tissue contribute to the exercise-induced increased IL-6 levels [1]. IL-6 production in the muscle cells is increased when glycogen is compromised suggesting that IL-6 has a role as an energy sensor in the exercising muscle, and IL-6 has been shown to enhance basal and insulin-stimulated glucose uptake in muscle cells and to its favorable effects on energy metabolism, and its anti-inflammatory effects, IL-6 has also pro-inflammatory effects in inflammatory diseases and in connection to obesity and metabolic syndrome. Therefore, the observed adaptation of the IL-6 response to heavy exercise induced by resistance training as observed in the present study can be regarded as a beneficial form of acclimatization considering that exercise training has also been found to increase IL-6 receptor expression and IL-6 sensitivity in skeletal muscle [26]. The present study also demonstrated a significant increase in IL-1β concentrations before RT in HYP and POW, whereas after RT, the response was blunted. IL-1 has been reported to enhance the secretion of hypothalamic corticotropin-releasing factor, which further stimulates glucocorticoid release [20]. In the present study, the increase in IL-1β was accompanied by an increase in cortisol before RT in HYP, and neither were increased following RT supporting a possible link between those two. Nevertheless, in POW, we did not observe a significant cortisol response before RT; however, a significant increase in IL-1 β was observed. To summarize, in the present study after RT, a blunted IL-6 response, increased IL-1ra concentration, and suppressed IL-1β response were observed regardless of the type of RE bout. These observations lead to a hypothesis that RT enhances anti-inflammatory effects and that might suppress the immediate pro-inflammatory response at the cellular level. Such an anti-inflammatory response within the circulation may provide positive metabolic changes through increased fat oxidation and glucose uptake [13, 27]. In line with the observed responses in HYP, the present study observed a

Table 3 High-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin 1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1ra), adiponectin, leptin, and resistin responses to hypertrophic (HYP) and maximal explosive (POW) resistance exercises (RE)

		PRE	POST0	POST24	POST48
HYP RE					
hs-CRP (mg L^{-1})	Pre RT	1.17 ± 0.47	1.27 ± 0.53	1.55 ± 0.63	1.35 ± 0.64
	Post RT	1.21 ± 0.50	1.43 ± 0.56	1.46 ± 0.43	1.66 ± 0.33
IL-6 (pg mL^{-1})	Pre RT	0.99 ± 0.24	1.67 ± 0.45*	1.10 ± 0.19	1.27 ± 0.38
	Post RT	1.11 ± 0.32	1.27 ± 0.29	1.30 ± 0.36	1.57 ± 0.40
IL-1ra (pg mL^{-1})	Pre RT	373 ± 60.1	395 ± 78.8	350 ± 79.8	380 ± 107.1
	Post RT	370 ± 74.7	425 ± 92.2*	315 ± 49.5	364 ± 43.0
IL-1β (pg mL^{-1})	Pre RT	1.79 ± 0.38	2.63 ± 0.58*	2.15 ± 0.48	2.47 ± 0.54
	Post RT	1.51 ± 0.51	2.13 ± 0.60	2.52 ± 0.92	2.50 ± 0.99
Adiponectin (pg mL^{-1})	Pre RT	4.00 ± 0.86	4.47 ± 1.13	3.98 ± 0.92	4.78 ± 0.99
	Post RT	4.50 ± 0.71#	4.35 ± 0.64	4.56 ± 0.89	4.33 ± 0.70
Leptin (pg mL^{-1})	Pre RT	7.21 ± 1.57	7.78 ± 1.96	5.81 ± 1.23*	7.57 ± 1.45
	Post RT	8.73 ± 2.04#	7.98 ± 1.92*	8.62 ± 1.87#	8.39 ± 1.60#
POW RE					
hs-CRP (mg L^{-1})	Pre RT	0.89 ± 0.32	0.89 ± 0.37	1.42 ± 0.60	1.72 ± 0.73
	Post RT	1.52 ± 0.60	1.42 ± 0.52	1.18 ± 0.33	1.22 ± 0.37
IL-6 (pg mL^{-1})	Pre RT	1.03 ± 0.28	1.64 ± 0.48*	1.20 ± 0.31	1.03 ± 0.51
	Post RT	1.14 ± 0.23	1.33 ± 0.27	1.20 ± 0.31	0.98 ± 0.21
IL-1ra (pg mL^{-1})	Pre RT	398 ± 94.9	386 ± 74.8	350 ± 60.9	381 ± 74.9
	Post RT	341 ± 55.2	403 ± 64.9*	344 ± 52.8	330 ± 53.3
IL-1β (pg mL^{-1})	Pre RT	1.28 ± 0.20	2.90 ± 0.88**	1.84 ± 0.49	1.56 ± 0.30
	Post RT	2.17 ± 0.40	1.72 ± 0.46	2.03 ± 0.68	1.22 ± 0.21
Adiponectin (pg mL^{-1})	Pre RT	3.85 ± 0.88	4.69 ± 0.73	4.22 ± 0.99	4.17 ± 0.93
	Post RT	4.72 ± 0.67#	4.49 ± 0.72	4.87 ± 0.86	4.48 ± 0.69
Leptin (pg mL^{-1})	Pre RT	6.5 ± 1.70	6.63 ± 1.49	6.81 ± 1.56	7.47 ± 1.72
	Post RT	8.93 ± 2.17#	7.88 ± 2.09#	9.33 ± 2.48#	8.47 ± 1.86#

A significant time × training interaction was observed in IL-6 in HYP ($p = 0.048$) and in POW ($p = 0.013$)

*Significant difference to pre-exercise value in the corresponding RE bout

Significant difference to corresponding value before resistance training in the corresponding RE bout (mean ± SD *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, #$p < 0.05$)

significant increase in blood glucose before RT, whereas after RT, blood glucose response was suppressed and a similar blunted response was observed in cortisol.

MCP-1 is a potent chemotactic and activating factor for macrophages, inflammation, and skeletal muscle regeneration [28]. MCP-1 response has been studied mostly after eccentric exercise [29, 30]. To our knowledge, only two other studies have examined changes in plasma MCP-1 after acute traditional RE. Our previous study found a significant decrease 30 min after hypertrophic RE [7] whereas Wells and colleagues [31] reported a significant increase in circulating MCP-1 immediately following damaging RE. The present study added longer tracking into recovery with two measurement points to the existing data; namely 24 and 48 h after the present RE. Interestingly, MCP-1 significantly increased after resistance training in HYP 24 h after the RE. Hubal et al. [30] showed that MCP-1 mRNA levels were significantly elevated after muscle-lengthening lower body exercise and the response was enhanced in repeated bouts. Furthermore, the immunohistochemistry analysis in their study showed that MCP-1 was localized with resident macrophages and satellite cell populations, which link MCP-1 to muscle regeneration. Skeletal muscle has an intrinsic protective mechanism to adapt after muscle damaging exercise to resist future muscle damage [32]. Deyhle et al. [32] measured intracellular MCP-1 after initial lengthening contraction and 27 days after the first bout and suggested that the muscle or the immune system becomes sensitized to the initial bout of damaging exercise such that inflammatory cell infiltration into the muscle is enhanced upon a repeated bout of damaging exercise. In the present study, a significant level of muscle damage, as assessed by CK increase, was observed after all RE bouts, which could be one of the mechanisms that lead to increased MCP-1, however, the mechanisms behind the enhanced MCP-1 response after RT remains unclear. The present study demonstrates that not only the initial response to unfamiliar resistance exercise differs

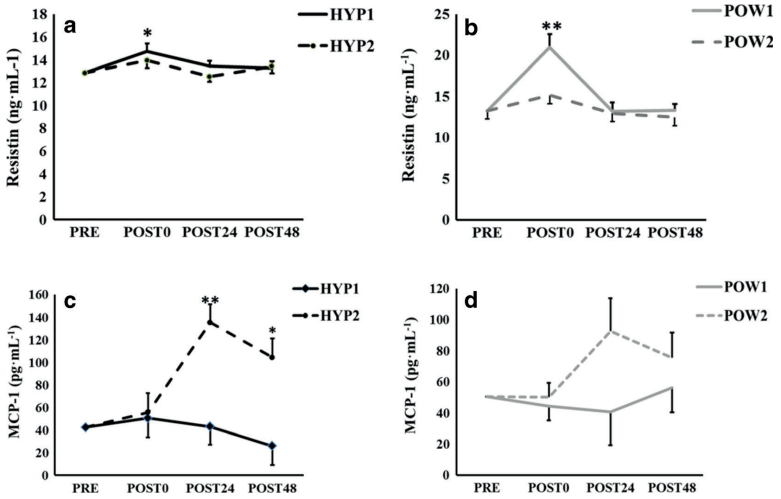

Fig. 2 MCP-1 (**a**, **b**) and resistin (**c**, **d**) responses to hypertrophic (HYP, left) and explosive (POW, right) resistance exercises before (HYP1/POW1) and after (HYP2/POW2) RT (mean ± SD). A significant time × training interaction was observed in MCP-1 in HYP ($p = 0.002$) and in resistin in POW ($p = 0.028$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared to pre value in the corresponding RE bout

from the later responses but also that RT significantly affects the MCP-1 response.

Adipocytokines are released from the adipose tissue and have been associated strongly not only with metabolism but also with inflammation [33]. Previous studies have reported no effect [34] or acute decreases [7, 9] on circulating resistin concentration following RE. Interestingly, regardless of the lack of total or abdominal fat loss, there was a beneficial significant increase in pre-exercise leptin and adiponectin concentrations. The present study observed a significant immediate increase in circulating resistin concentration immediately after both HYP and POW bouts before but not after RT. This is in line with the observation by Varady and colleagues [9] that resistance training background modifies the resistin response. However, they reported a significant reduction after RE bout in resistin in the subjects with RT background whereas no significant response was observed in participants with sedentary and running background. The mechanism of the resistin response has been hypothesized to be related to metabolic demand of exercise, which could explain the blunted response in HYP. However, our data does not fully support this hypothesis as the lactate after RT immediately after HYP was an average of 10.1 ± 3.4 mmol L^{-1} and no significant response in resistin levels was observed whereas in POW before RT with the average lactate of 3.0 mmol L^{-1}, a significant increase in resistin levels was observed. Hence, in addition to the metabolic stress, other mechanisms have to be involved. Skeletal muscle cells have been shown to release resistin [35]. In addition, mechanical stress has been shown to enhance the expression of resistin in cardiomyocytes [36]. Thus, one origin of increased resistin concentration could be the skeletal muscle as it has been shown with IL-6 [1]. Another mechanism could be related to the loading of joints. It has been shown that adipokines are produced also in joints and have a role in joint diseases such as osteoarthritis [37]. Vuolteenaho et al. [38] reported a significant effect of marathon running on circulating resistin concentrations, and one may hypothesize that it could be related to the cartilage degradation or strenuous muscle exercise. Interestingly, in the present study, a significant immediate increase in resistin concentration was observed only before training in both resistance exercise bouts. Especially, maximal explosive resistance exercise bout characterized by explosive muscle contraction produces stress on tendons and joints [39]. While speculative, the blunted resistin response after RT may suggest that RT could elicit protective mechanisms in cartilage, which could be observed as a reduced immediate resistin response to resistance exercise bout.

Limitations of this study should be noted. The most notable limitation of the present study was the small sample size. In addition, the mechanisms by which resistance training alters the inflammatory response to an acute resistance exercise bouts remain to be explored. Serial blood samples and additional muscle biopsies are needed to investigate the series of events initiated by resistance exercise bouts on the cytokine kinetics after traditional RE bouts. Hence, the present study cannot go further than to state that the acute pro- and anti-inflammatory response is altered by resistance training towards an anti-inflammatory direction. It is also possible that

the nutritional status of the participants had an effect on the acute cytokine responses as the nutrition on the day before the RE bout was not strictly controlled [13]. It is notable that the exercise protocols in this study were intense, which must be taken into account when evaluating the present results. The present study showed that the pro-inflammatory response in novice trainers is blunted by the resistance training. Generally, a repetition range between 8 and 12 RM is used for novice training and 1–12 RM in a periodized fashion for individuals with resistance training experience are recommended [15]. Our findings support the recommendation. However, to ensure optimal health and fitness gains, resistance training should be undertaken with proper preparation, guidance, and surveillance.

Conclusion

Twelve weeks of resistance training blunted IL-6, IL-1β, and resistin responses in the circulation in both HYP and POW, whereas the response of the IL-1ra was enhanced. In addition, enhanced MCP-1 response in HYP was observed only after the RT intervention. This study emphasizes the importance of reporting training background when investigating immediate cytokine and adipocytokine responses after resistance exercise. The improvement in the anti-inflammatory and the blunted pro-inflammatory response achieved in the present study by resistance training may be an effective means for reducing systemic low-grade inflammation and thus improve the future health trajectory of young men.

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Compliance with ethical standards

The study was conducted according to the Declaration of Helsinki, and ethical approval for the study procedures was granted by the Ethical Committee at the University of Jyväskylä and by the Ethical Committee of the Central Hospital, Jyväskylä.

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

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III

INFLAMMATION STATUS OF HEALTHY YOUNG MEN: INITIAL AND SPECIFIC RESPONSES TO RESISTANCE TRAINING

by

Johanna K. Ihalainen, Heikki Peltonen, Göran Paulsen, Juha P. Ahtiainen, Ritva S. Taipale, Mari Hämäläinen, Eeva Moilanen & Antti A Mero.

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INFLAMMATION STATUS OF HEALTHY YOUNG MEN: INITIAL AND SPECIFIC RESPONSES TO RESISTANCE TRAINING

Ihalainen Johanna K.[1], Peltonen Heikki[1], Paulsen Göran[23], Ahtiainen Juha P[1], Taipale Ritva S.[14], Hämäläinen Mari[5], Moilanen Eeva[5], Mero Antti A.[1]

[1]*Biology of Physical Activity, Faculty of Sport and Health Sciences, University of Jyväskylä, Finland*

[2]*The Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway*

[3]*Norwegian School of Sport Sciences, Oslo, Norway*

[4]*Kajaani University of Applied Sciences, Kajaani, Finland*

[5]*The Immunopharmacology Research Group, Faculty of Medicine and Life Sciences, University of Tampere and Tampere University Hospital, Tampere, Finland*

Correspondence to: Johanna K. Ihalainen, MSc
Biology of Physical Activity
University of Jyväskylä
P.O Box 35
40014 Jyväskylä, Finland
Tel. 358-40-8347106
Fax 358-14-2602071
Email: johanna.k.ihalainen@jyu.fi

Email adresses of the other authors : Peltonen Heikki (heikki.peltonen@jyu.fi); Paulsen, Gøran (Goran.Paulsen@olympiatoppen.no); Ahtiainen Juha (juha.ahtiainen@jyu.fi); Taipale Ritva (Ritva.Taipale@kamk.fi); Hämäläinen Mari (Mari.Hamalainen@uta.fi); Moilanen Eeva (eeva.moilanen@uta.fi); Mero Antti A. (antti.a.mero@jyu.fi)

Abstract

Purpose: Our primary aim was to study the effects of a 4 wk preparatory resistance training (RT) period followed by 12 wks of two specific RT protocols (either hypertrophic-strength, HS, or strength-hypertrophy-power, SHP, training) on inflammation markers and the possible relationship of the changes in abdominal fat and lean mass to the changes in inflammation status.

Methods: A total of 82 healthy men were included in the study. Maximal concentric leg press strength (1RM), total body lean mass, total body and abdominal fat mass, circulating high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), monocyte chemoattractant protein 1 (MCP-1), and selected adipocytokines (resistin, adiponectin and leptin) concentrations were measured before (PRE) and after 4 (wk4) and 16 weeks (wk16) of RT.

Results: After the initial phase of RT, on wk4, abdominal and total fat mass as well as plasma leptin concentrations were significantly reduced ($p<0.05$), whereas muscle mass, IL-1ra, resistin and MCP-1 concentrations were significantly increased ($p<0.05$). During specialized training phase, at wk16, only HS led to further reduction in abdominal and total fat mass, resistin, and leptin ($p<0.05$), whereas both training modes led to lower MCP-1 concentrations ($p<0.05$).

Conclusion: Abdominal fat mass and circulating leptin were reduced already after 4 wks of RT. Simultaneously, circulating MCP-1 and resistin concentrations increased, possibly as markers of muscle adaptation and regeneration. The present findings also suggest that RT with hypertrophic focus is beneficial for further reductions in abdominal fat mass and to decrease circulating inflammatory markers.

Keywords: lean mass, muscle strengthening, adipokines, cytokines, body mass maintenance

Introduction

Systemic inflammation is an independent risk factor for several diseases like type 2 diabetes (Pradhan et al. 2001) and atherosclerosis (Hansson 2005). Resistance training (RT) has been associated with improvements in inflammation state in overweight adults (Olson et al. 2007), elderly people (Phillips et al. 2012) as well as in specific patient groups (Conraads et al. 2002, Moraes et al. 2014). Suppressing chronic inflammation at an early stage in life via regular exercise could serve as an efficient approach to prevent or delay the onset of inflammation and related diseases (Forti et al. 2017). There is recent evidence that RT could also have beneficial effects on inflammation in untrained young men (Forti al. 2017), however, this has not been consistent between all studies (Ara et al. 2006, Libardi et al. 2012, Rall et al. 1996).

A bout of heavy resistance exercise (RE) triggers a transient inflammatory response comprising of an augmented white blood cell count and stimulation of pro- and anti-inflammatory cytokine production (Freidenreich and Volek 2012). The cytokine response induced by a bout of heavy resistance exercise involves enhanced production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-1β (IL-1β). In addition to its pro-inflammatory and metabolic activities, IL-6 produced by the exercising muscle has been proposed to have anti-inflammatory properties through its stimulatory effects on the production of anti-inflammatory cytokines interleukin-1 receptor antagonist (IL-1ra) and interleukin-10 (IL-10). These mediators play a crucial role in the containment and resolution of inflammatory processes, and have been suggested to have a role in mediating the beneficial immune-modulating effects of resistance exercise (Petersen and Pedersen 2005). It has been suggested that not only the initial response to unfamiliar RE differs from the later responses but also that RT significantly affects the acute inflammation response to RE and modifies it into an anti-inflammatory direction (Ihalainen et al. 2017, Murton et al. 2014). Thus, it could be possible that the initial phase of RT in untrained participants might also induce different effects on inflammation status that have been observed in long-term RT studies.

Another mechanism explaining the anti-inflammatory effects of exercise, observed as lower circulating inflammation markers, is suggested to be the reduction in visceral fat mass with a subsequent decrease in

release of proinflammatory adipocytokines and increase in the release of anti-inflammatory adipocytokines (Gleeson et al. 2011, Robinson and Graham 2004). Adipocytokines (e.g. leptin, adiponectin, and resistin) are hormones that were first discovered to be secreted by adipose tissue and to regulate energy metabolism and appetite. More recent findings on the ubiquitous expression of their receptors and cellular effects have revealed that they also are involved in the regulation of a variety of biological functions related to immune responses and inflammatory diseases (Ouchi et al. 2011, Cao 2014, Scotece et al. 2014). A single resistance exercise bout has been demonstrated to exert specific acute effects on adipocytokine levels and the response appears to be dependent on the duration of the exercise as well as on energy expenditure (Bouassida et al. 2010, Ihalainen et al. 2014).

RT has been shown to have a positive effect on several inflammation markers, including adiponectin (Fatouros et al. 2009). However, the time course, the effects of starting RT and the effects of different RT regimens on inflammation markers are not fully understood. Consequently, our primary aim was to study the effects of 4 wk preparatory RT period followed by 12 wks of either hypertrophic-strength (HS) or strength-hypertrophy-power (SHP) RT on inflammatory markers in previously untrained men. As a secondary purpose, we evaluated the association between the changes in abdominal fat mass, total body lean mass, and strength performance variables and the selected inflammation markers. We hypothesized that both the preparatory and the more specific RT would induce significant improvements in inflammation markers and that the magnitude of these adaptations would be related to the protocol used in longer-term RT and to the changes in body composition.

Materials and methods

Participants. A total of 150 men contacted us to express their interest in the study. Of these, 92 men met the participation criteria and participated in pre-measurements. The participants were randomly assigned to hypertrophy-strength training (HS, n=44) or to strength-hypertrophy-power training (SHP, n=44). In addition, 14 men served as a control group. The number of the participants that completed pre-, 4wk, and 16wk measurements and were included in this study were HS = 37 and SHP = 31. All participants reported taking part in sport activities on a weekly basis, but none were competitive athletes or had a background in systematic strength training. Participants filled in a health questionnaire prior to participation in the study. A completed health questionnaire and resting ECG were reviewed by a physician prior to exercise testing and training. All subjects reported that they were free from injury, and were not using any medications or smoking. The subjects were informed about the importance of maintaining their previous dietary habits throughout the study. Each subject was informed of the potential risks and discomforts associated with the measurements, and all of the subjects gave their written informed consent to participate. The study was conducted according to the Declaration of Helsinki, and the Ethics Committee of the University of Jyväskylä, approved the study.

Study design. The duration of the whole training intervention was 16 weeks. Measurements were performed prior to (wk0), after 4 weeks of preparatory RT (wk4), and after 12 weeks of specialized RT at week 16 (wk16) of training. The control group was measured only before and after the RT as a follow-up.

Training. The intervention started with 4 weeks of progressive muscle endurance training twice a week as one group (HS+SHP). A total of 8 training sessions were done during this initial phase RT. The participants used eight to ten exercises in one workout, 2–3 sets of every exercise, and 10–15 repetition in every set. Recovery time between the sets lasted two minutes. Training loads were 50–80 % of one repetition maximum (1 RM) increasing throughout the preparatory phase. Bilateral leg press, bilateral knee extension, and bilateral knee flexion exercises were performed with weight-stack devices during each RT session. The preparatory RT period also included exercises for the other main muscle groups of the body and were conducted once a week using machines. Chest and shoulders, upper back, trunk extensors and flexors, and

upper arms rotated during 2 weekly exercises. After 4 weeks of training, the subjects were divided into two different RT regimens: 1) training aiming especially for muscle hypertrophy and strength (HS) and 2) training aiming for muscle strength, hypertrophy and power (SHP) for 12 weeks. A total of 28 training sessions were undertaken during this specialized training phase. Participant had 2 or 3 training sessions a week, depending on the phase of the training period. The exercises used were the same as those used in the 4 wk preparatory phase. The specific RT programs performed over the next 12 wks was periodized and thus divided further into three different blocks. Every block consisted of 4 wks of RT. In the first block, the SHP group had 25 % power-strength (PS) and 75 % maximal-strength (MS) training sessions, in the second block 75 % PS and 25 % MS training sessions, and in the last block 87.5 % PS and 12.5 % MS training sessions. In contrast, the HS training group's first block consisted of 100 % HS sessions, the second block 75 % HS and 25 % MS training sessions, and the last block 25 % HS and 75 % MS training sessions. HS training contained mainly of sets including 8–12 repetitions with 75–85 % loads of 1 RM. MS training in both RT regimens consisted of neural enhancing RT with lower repetitions per set (typically 4–6) and higher intensity (86–95 % 1 RM), but also more traditional hypertrophy sets to increase muscle size. PS training consisted of sets with lower loads of 1 RM (50–80 % 1 RM) performed with maximal concentric speed. The individual loads were determined by strength testing for each exercise every fourth wk. The training techniques were carefully supervised and the training was controlled throughout the whole RT period. The sets were conducted such that the last repetition could still be performed with good technique or until concentric failure. The exception to this was the power-strength (PS) sets in SHP that were conducted with maximal concentric speed and, thus, not close to concentric failure. The training program has been described in detail by Hulmi et al. (Hulmi, Laakso, Mero et al. 2015). Subjects were advised to continue their normal recreational physical activities such as low-intensity walking, skiing, cycling, and swimming throughout the study.

Abdominal fat. Whole body composition was estimated by Dual X-ray Absorptiometry (LUNAR Prodigy, GE Medical Systems, Madison, USA). The DXA-scans were performed in the morning with the participant in a fasted (12 h) state. Automatic analyses (Encore-software, version 14.10.022) provided total body fat mass and total body lean mass. Abdominal fat was calculated manually defining a range of interest confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by

the iliac crest (Tallroth et al. 2013) at wk0. This customized range was then copied to the DXA scans at wk4 and wk16, respectively.

Nutrition. Subjects kept 4-day food diaries during the second block of the 12-week RT period. The researchers gave subjects both verbal and written nutritional recommendations based on the Finnish Nutrition Recommendations 2014 in a one two-hour lecture. As a rule, these follow the recommendations for the Nordic countries in Europe published in Autumn 2013 (NNR2012) and are very close to USDA and HHS dietary guidelines (2010) for normal healthy adults. The subjects were instructed on how to report nutritional intake in the diaries. During the intervention subjects were randomly given a post-workout supplement. One group received protein, one group carbohydrate, and one group protein plus carbohydrate. Protein and carbohydrates were provided by Northforce (Kuusamon Juusto Oy, Kuusamo, Finland). The protein group received 37.5 grams of whey concentrate (30 g of whey proteins, 5 g of lactose < 1 g of fat) and the carbohydrate group received 34.5 grams of maltodextrin. In contrast, the protein plus carbohydrate group received 37.5 grams of whey concentrate (30 g of whey proteins) and 34.5 grams of maltodextrin. The nutritional subgroups were randomly assigned and evenly distributed within the training groups. Nutrients provided by the supplements were included in the analysis. The food diaries were analyzed by nutrient analysis software (Nutri-Flow; Flow-team Oy, Oulu, Finland).

Maximal-strength performance. Maximal strength was measured by a one-repetition maximum (1RM) test of dynamic leg press exercise performed using David D210 leg press device (David Health Solutions Ltd., Helsinki, Finland). The starting position (flexed) was at a knee angle of 60 degrees, and 1RM was accepted as the highest load that the subjects could lift to a full knee extension (180 degrees). Subjects performed 3 warm-up sets and 3 to 5 trials, after which the highest load was accepted as the 1RM.

Venous blood samples. Fasting samples were taken in the morning (7:00-9:00 a.m.) after a 12 h overnight fast. Participants were instructed to abstain from strenuous physical activity, including RT sessions in the training programme, for 48 h before the blood samples were taken at wk0, wk4 and wk16. Venous blood samples were drawn from an antecubital vein using standard procedures and the blood was transferred into serum and EDTA tubes (Venosafe, Terumo, Belgium). The serum samples were held for 15 min at room temperature before being centrifuged for 10 min at 2000 x g (Megafuge 1.0 R, Heraeus, Germany). The EDTA-treated samples were centrifuged for 10 min at +4°C with 2000 x g (Megafuge 1.0 R, Heraeus,

Germany). Both plasma and serum were kept at -80°C until analysed. High-sensitivity C - reactive protein (hsCRP) in serum samples was analysed by using the Immulite 1000 and immunoassay kits (Immulite, Siemens, IL, USA). The detection limit and inter-assay coefficient of variation was 0.1 $g \cdot mL^{-1}$ and 10 %, respectively. Concentrations of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), interleukin-1 receptor antagonist (IL-1ra), adiponectin, leptin and, resistin in plasma samples were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (R&D Systems, Europe Ltd, Abingdon, UK). The detection limits and inter-assay coefficients of variation, respectively, were 0.2 $pg \cdot mL^{-1}$ and 7.8 % for IL-6, 3.9 $pg \cdot mL^{-1}$ and 6.5 % for MCP-1, 15.6 $pg \cdot mL^{-1}$ and 4.1 % for IL-1ra, 19.5 $pg \cdot mL^{-1}$ and 4.4 % for adiponectin, 15.6 $pg \cdot mL^{-1}$ and 6.0 % for resistin and 15.6 $pg \cdot mL^{-1}$ and 3.2 % for leptin.

Statistical analyses. All data are expressed as means ± SD, except where indicated. Data was analysed using PASW statistic 22.0 (SPSS, Chicago, IL, USA). The final analysis for the present study was performed only on adherent participants (90 % adherent to exercise intervention) with outliers **(n=3)** removed. Outliers were defined as a variable greater than 3 SD above the mean for wk0, wk4 or wk16. Results were similar when the same analysis was performed with outliers included. Before applying further statistical methods, the data was checked for sphericity and normality. If a specific variable violated the assumptions of parametric tests, log-transformation was used. This concerned values of adiponectin, leptin, IL-6, MCP-1 and hs-CRP. Absolute changes were analysed via two-way repeated analysis of variance for main (time and group) and interaction (group × time) effects. For each analysis, the corresponding baseline values and the nutritional supplement were used as a covariate when appropriate. If a significant main effect or interaction was observed, the change from wk0-values for mid and from wk4 to post were compared between groups using paired t-tests with Bonferroni correction. Effect sizes (ES) are given as Cohens d with an effect size of 0.20-0.50 being considered small, 0.50-0.80 medium, and >0.80 large. Spearmans correlation coefficients were used to examine the associations between depending variables. The level of statistical significance was set at $p < 0.05$.

Results

Training adherence. The training adherence was 99±2% and 95±1% in HS and SHP groups, respectively. All subjects completed at least 90% of the overall training volume.

Nutrition. Total energy intake (MJ) was 10.1 ± 1.8 and 11.2 ± 2.1 in HS and SHP groups, respectively. There was no significant difference in dietary intake between the groups.

Inflammation markers. Effects of RT on the circulating levels of inflammatory markers are shown in Table 1. There were no significant differences in the initial 4wk RT phase responses between the HS and SHP groups in any of the inflammation markers. All the variables stayed statistically unaltered in the control group (C) after the follow-up.

Table 1 somewhere here

IL-6, CRP, and adiponectin concentrations remained unaltered by RT. Interesting statistically significant alterations were observed in IL-1ra, MCP-1, resistin, and leptin (Figure 1). A significant increase in IL-1ra concentration was observed after the initial phase of RT ($p < 0.001$, ES = 0.989). The specialized RT phase did not have any additional effect on IL-1ra. MCP-1 concentration increased after the initial phase of RT ($p = 0.039$, ES = 0.548), however, both HS and SHP had a significant lowering effect on MCP-1 levels ($p = 0.045$, ES = 0.522; $p = 0.027$, ES = 0.604, respectively). In the initial phase of RT, circulating resistin concentrations increased significantly (1.25 ± 0.40 %; $p = 0.039$, ES = 0.546) but were reduced during the following 12 wks of HS training ($p = 0.046$, ES = 0.519). Significant changes in circulating leptin concentrations were observed after the initial 4 wk phase of RT ($p = 0.006$, ES = 0.799) and it was further reduced during the specialized HS training period ($p = 0.037$, ES = 0.538). SHP had no effect on leptin ($p = 0.821$).

Figure 1 somewhere here

Total and abdominal fat mass. Changes in body composition and 1RM are summarized in Table 2. No significant changes were observed in body mass. We observed a significant reduction in total fat mass from wk0 to wk4 (-3.2 ± 6.8 %, p = 0.001, ES = 0.969). During the specialized training period a significant group × time interaction was observed (p = 0.006, ES=0.795) in total fat mass. Thus, from wk4 to wk16 we observed a significant reduction in total fat mass only in the HS group (-6.2 ± 10 %, p < 0.001, ES = 0.993). In line with the reduction in total fat mass, abdominal fat mass was significantly reduced after 4wk of RT (p=0.001) and a significant group × time interaction was observed (p = 0.004, ES = 0.848) in abdominal fat mass after specialized training. Abdominal fat mass decreased significantly (-7.9 ± 11.4 %, p < 0.001, ES = 1.00), in HS only. Significant changes in total and abdominal fat mass were not observed in the SHP group after specialized training (0.1 ± 6.6 % and 3.3 ± 9.4 %, respectively). In the C group a significant increase in total fat mass was observed (+8 ± 22 %, p = 0.018).

Lean mass. Lean mass increased significantly from wk0 to wk4 (1.4 ± 1.9 %, p = 0.001, ES = 1.00), and a significant group × time interaction was observed during the specialized RT (p = 0.041, ES = 0.536). A significant increase in lean mass was observed from wk4 to wk16 both in both HS and SHP groups (1.3 ± 1.9 %, p < 0.001, ES = 1.00; 1.5 ± 1.7 % p = 0.018, ES = 0.669, respectively) but the increase was greater in HS.

1RM. A significant main effect of time was observed in 1RM from PRE to wk4 (p=0.001, ES = 0.916) as an increased 1RM was observed from PRE to wk4 (17.6 ± 18.3 %, p < 0.001, ES = 1.00). Both HS and SHP training increased 1RM from wk4 to **wk16** (13 ± 8.1 %, p < 0.001, ES = 1.00; 11 ± 6.5 %, p < 0.001, ES = 1.00, respectively). 1RM did not change significantly in the control group (p = 0.399).

Table 2 here somewhere

Associations between changes in 1RM, body composition and inflammatory markers. At wk0 measurements circulating leptin concentration showed a strong positive correlation with total fat mass (R = 0.843, p<0.001) and abdominal fat mass (R = 0.775, p<0.001) when all the subjects were pooled. In

addition, in the pooled data circulating adiponectin correlated negatively with 1RM ($R = 0.355$, $p=0.001$) and IL-6 had a weak positive correlation with abdominal fat mass ($R = 0.263$, $p = 0.024$). The greater reduction in fat mass was associated with greater reduction in circulating leptin concentration. As in the initial phase of RT when the groups are pooled, the change in total fat mass and abdominal fat mass correlated significantly with the change in circulating leptin concentrations ($R=0.406$, $p<0.001$; $R=0.391$, $p<0.001$, respectively). The same was observed during specialized training as the change in circulating leptin concentrations correlated with the change in abdominal fat mass as well as with the change in total fat mass ($R = 0.363$, $p=0.004$; $R = 0.539$, $p<0.001$, respectively). The greater increase in muscle mass was associated with a reduction in circulating adiponectin concentrations. As in the pooled data, an inverse relationship between the change in concentration of circulating adiponectin and the change in total lean mass from wk4 to wk16 was observed ($R = -0.255$, $p = 0.039$).

Discussion

The present study assessed the effects of RT on inflammatory markers, i.e., cytokines and adipocytokines, in previously untrained men. The unique aspect of the present study was the design with an initial preparatory phase of RT, comprising of muscle endurance type of RT, followed by either hypertrophy-strength (HS) or strength-hypertrophy-power (SHP) RT. In the present study, RT modified systemic inflammation measured as significant modifications in concentrations of selected cytokines. A primary finding was that the short-term initial phase of RT (4 wk) had significant effects on cytokines and adipocytokines: circulating pro-inflammatory resistin and MCP-1 concentrations increased, anti-inflammatory IL-1ra concentrations increased, and circulating leptin concentrations decreased along with the increasing muscle mass and decreasing fat mass. Furthermore, during the specialized RT periods, HS RT elicited normalizing effects on inflammation markers, such as circulating resistin and leptin. Also notable was the enhanced anti-inflammatory effect of RT that was achieved without a concomitant loss in body mass. It is notable that RT elicited a beneficial effect on body composition as muscle mass increased and fat mass decreased significantly. Interestingly, these adjustments in body composition were observed already after 4 wks of training at the end of the initial phase of training. Later, both HS and SHP RT further increased muscle mass, but reduction in fat mass was observed only after HS RT whereas, in the control group, a significant increase in fat mass was observed. Finally, we report that decreases in abdominal fat mass seem to be associated with the magnitude of the reductions in leptin concentrations.

Adipocytokines (also refered as adipokines) adiponectin, leptin, and resistin, are hormones that were initially found to be secreted by adipocytes and to regulate energy metabolism linking nutritional status to neuroendocrine function (Ouchi et al. 2011). Resistin stimulates the production of pro-inflammatory cytokines, and has been associated with obesity and atherosclerosis (Zhang et al. 2010) and several other diseases (Cao 2014). In the present study, we observed an increase in circulating resistin concentrations during the initial phase of RT, despite reductions in total and abdominal fat mass. However, muscle mass and muscle strength were increased. Our observation of increased circulating resistin concentrations could be due to the increased secretion of resistin by the activated anti-inflammatory macrophages in muscle

tissue (Schwartz and Lazar 2011, Filková et al. 2013). It has been shown that unaccustomed exercise leads to muscle damage characterized by transient ultrastructural myofibrillar distribution (Clarkson and Hubal. 2002, Pillon et al. 2013). Exercise-induced muscle damage initiates tissue repair and remodeling, which leads to the accumulation of inflammatory cells, including macrophages, into the muscle tissue. The activated macrophages, depending on their type, secrete pro-inflammatory and anti-inflammatory cytokines, which are needed for the regulation of muscle adaptations after exercise. (Hyldahl and Hubal 2014, Peake et al. 2017). The increased resistin concentrations in the present study could be due to macrophage activation and could be part of the efficient inflammation resolving process that has been shown to lead to regeneration of muscle fibers (Peake et al. 2017). Previous studies have demonstrated reduced resistin levels immediately after hypertrophic RT (Ihalainen et al. 2014, Varady et al. 2010). The present study found that during the specialized training period a significant reduction in resistin concentrations was observed only in the HS group. While speculative, we suggest that the new and unaccustomed training stimulus, explosive training, in the SHP program could have unbalanced the homeostasis of the body, which in turn induced muscle damage and elicit another initial response to a different stimulus.

Leptin is a pro-inflammatory hormone mainly secreted by adipocytes and acts as a peripheral signal informing the central nervous system of changes in the amount of adipose tissue in the body (Bouassida et al. 2010). In the present study, already 4 weeks of RT reduced circulating leptin concentrations in healthy young men. There was a correlation between the change in abdominal and total fat mass and reduction in leptin concentrations, which emphasizes the fact that changes in leptin levels seem to depend on a reduction in fat mass. It is possible that we did not observe significant reductions in leptin concentrations in the SHP group because fat mass was not reduced during training. The present study demonstrates that a significant reduction in body weight is not needed for a significant reduction in circulating leptin concentrations, although this study is in line with the previous studies, which have shown that a reduction in leptin is dependent on a reduction in fat mass (Baile et al. 2000). RT has been shown to favorably affect body composition (Kraemer et al. 2002). The effect of RT on body composition and inflammation markers seems to be strongly dependent on the intensity and external load of RT. In the beginning of the training, the beneficial effects in fat mass and leptin were observed with moderate intensity muscle endurance type

resistance training. However, for further improvements, more metabolically demanding HS training, with higher volume (repetitions × load), slow velocity (longer muscle contraction), and shorter rest intervals than in SHP seems to be advantageous (Kraemer and Ratamess 2004).

An increase in MCP-1 concentrations was observed after four weeks of training. Later, both HS and SHP led to normalized MCP-1 concentrations. Thus, MCP-1 was significantly reduced after the specialized RT period in HS and SHP regardless of an absence of changes in fat mass in SHP. MCP-1 has been shown to increase after acute strenuous endurance type exercise (Suzuki et al. 2003), whereas a significant increase (Wells et al. 2016) or reduction (Ihalainen et al. 2014) has been observed after resistance exercise. Troseid et al. (2004) suggested that especially visceral fat mass would have an effect on plasma levels of MCP-1. However, MCP-1 is also a potent chemotactic and activating factor for macrophages, inflammation, and skeletal muscle regeneration (Shireman et al. 2007). While speculative, the mechanism that could lead to higher MCP-1 concentration could be related to the muscle damage and the following muscle regeneration experienced during the initial phase of RT (Peake et al. 2005, Peake et al. 2017). Other mechanism that might lead increased MCP-1 concentrations after the initial phase of RT could be related to shear stress in vascular smooth muscle cells experienced in early phase of resistance training (Shyy et al. 1994). Nevertheless, more research is needed on the mechanisms that lead to higher circulating MCP-1 concentrations in the initial phase of RT. Furthermore, long-term resistance training seems to elicit a beneficial reduction in circulating MCP-1 concentrations as at the end of the study, after 12 weeks of specialized RT, a reduction in MCP-1 was observed in both training groups. The results of the present study suggest that changes in circulating MCP-1 concentrations are not entirely dependent on changes in adipose tissue mass.

In the present study a significant increase in IL-1ra, which is known to inhibit the pro-inflammatory response, was observed already after the first four weeks of training. Previously Lancaster & Febbraio (2014) suggested that the anti-inflammatory effect of exercise would be elicited by the release of IL-6 from skeletal muscle and subsequent production of IL-1ra by monocytes and macrophages. The previous studies on circulating IL-1ra and resistance exercise studies report on the acute effect of exercise, rather than on

basal levels (Peake et al. 2005, Izquierdo et al. 2009). Recently, Forti et al. (2017) showed that training only with high external load increases the basal levels of IL-1ra. Even if the RT program during the HS and SHP was progressive, it might be that it was not sufficient to elicit a significant response in IL-1ra.

Limitations of the study. The strength of this study is its prolonged randomized design training intervention. In addition, this study showed how starting RT affects the inflammation status and further compared the effects of two progressive and specific RT program on inflammation markers. Despite these strengths, our study has some limitations. First, the participants in this study were young healthy adults and therefore the results may not be applicable to other populations. Second, nutritional status has been shown to affect inflammation status. We followed the dietary intake of participants only once during the 16 weeks of training. Thus, we cannot rule out the effect of nutritional alterations on the present results, however, subjects were instructed to maintain their normal diet throughout the study. In addition, subjects received a post-exercise supplement that included carbohydrates, whey, or whey and carbohydrates. It has been suggested that whey could produce an anti-inflammatory effect (Krissansen 2007). However, due to a careful randomization and such a large n-size we found it improbable that there would be a consistent difference due to the supplements between the training groups. Lastly, although in the present study several different factors are suggested to be important markers and/or regulators of inflammation, there are many other pro- or anti-inflammatory factors that could be measured.

Summary and Conclusion

In the present study, we show an overall anti-inflammatory effect of RT in healthy, young previously untrained men. Circulating leptin concentrations as well as total and abdominal fat mass decreased and IL-1ra increased already after four weeks of RT. Interestingly, we also observed increased circulating resistin and MCP-1 concentrations after 4 wk of RT, and those reduced towards their initial levels during prolonged RT probably as a consequence of muscle adaptation and regeneration. When aiming for reductions in abdominal fat mass and a more favorable inflammation status, metabolically more demanding RT, e.g. hypertrophic type of training should be preferred over strength and power type of RT.

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Conflict of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

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Table 1. Effects of RT on the circulating levels of inflammatory markers *: Significant within-group change from pre to wk4. #: Significant within-group change from wk4 to wk16, ¤: significant between group difference. na= not analysed.

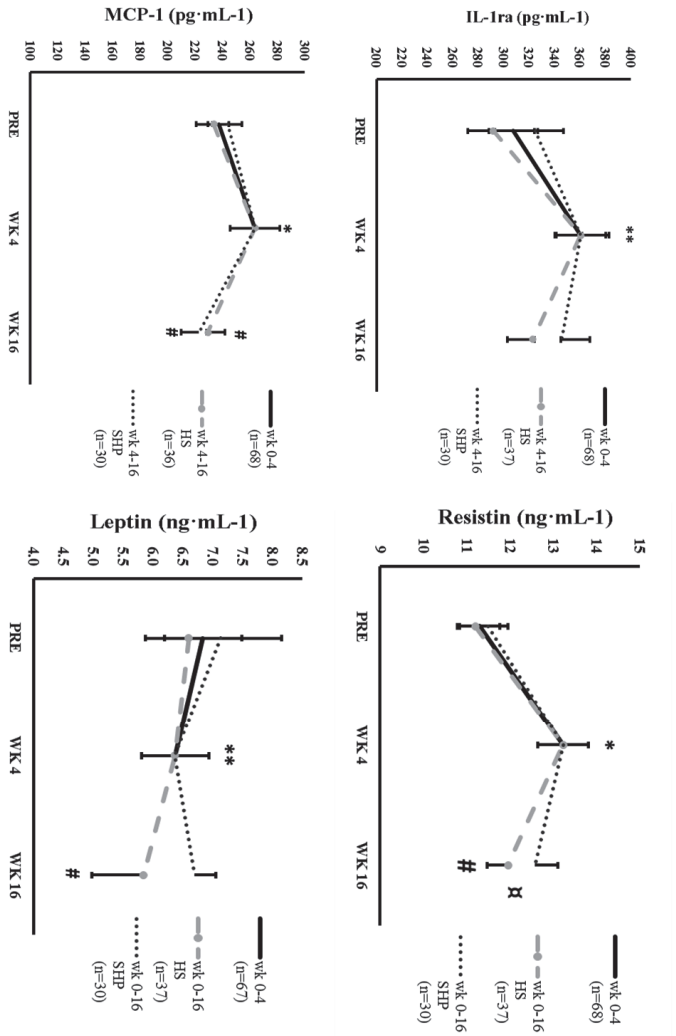
		HS + SHP (n=68)	SH (n=37)	SHP (n=31)	C (n=14)
hs-CRP (mg·L^{-1})	wk0	1.5 ± 0.1	1.4 ± 0.1	1.6 ± 0.2	1.9 ± 0.4
	wk4	1.7 ± 0.2	1.5 ± 0.2	1.9 ± 0.3	na
	wk16	1.5 ± 0.2	1.4 ± 0.2	1.7 ± 0.3	1.6 ± 0.5
MCP-1 (pg·mL^{-1})	wk0	248.1 ± 16.7	234.1 ± 21.1	250.0 ± 26.1	236.2 ± 23.6
	wk4	264.2 ± 16.8*	258.8 ± 22.5*	278.3 ± 21.2*	na
	wk16	233.8 ± 14.7#	233.7 ± 20.0#	239.1 ± 21.0#	224.1 ± 18.8
IL-6 (pg·mL^{-1})	wk0	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
	wk4	1.2 ± 0.2	1.4 ± 0.3	1.0 ± 0.1	na
	wk16	1.2 ± 0.2	1.3 ± 0.2	1.1 ± 0.2	1.0 ± 0.1
IL-1ra (pg·mL^{-1})	wk0	307.2 ± 19.0	292.2 ± 12.9	330.0 ± 39.0	323.2 ± 26.7
	wk4	361.1 ± 19.5***	352.1 ± 27.3*	377.2 ± 27.7*	na
	wk16	334.0 ± 17.4	312.1 ± 17.2	367.4 ± 31.6	298.1 ± 13.9
Adiponectin (µg·mL^{-1})	wk0	7.0 ± 0.3	7.5 ± 1.2	6.9 ± 0.4	9.6 ± 0.9
	wk4	6.8 ± 0.4	7.4 ± 0.5	6.2 ± 0.5	na
	wk16	6.5 ± 0.3	7.0 ± 0.5	5.8 ± 0.4	9.3 ± 1.0
Leptin (ng·mL^{-1})	wk0	6.8 ± 0.7	6.6 ± 0.7	7.3 ± 1.1	6.0 ± 0.8
	wk4	6.4 ± 0.5*	6.2 ± 0.7*	6.7 ± 0.9*	na
	wk16	6.2 ± 0.6	5.7 ± 0.7##¤¤	7.0 ± 1.0	6.3 ± 1.2
Resistin (ng·mL^{-1})	wk0	12.1 ± 0.5	11.8 ± 0.6*	12.5 ± 0.9	11.6 ± 1.1
	wk4	13.4 ± 0.7*	12.9 ± 0.7*	13.9 ± 1.2*	na
	wk16	12.2 ± 0.5	11.6 ± 0.6#	12.9 ± 1.0#	11.8 ± 1.2

Table 2. The effect of RT on 1RM and body composition. *: Significant within-group change from pre to wk4. #: Significant within-group change from wk4 to wk16.

	wk0				wk4			wk16		
	HS + SHP	**HS**	**SHP**	**C**	**HS + SHP**	**HS**	**SHP**	**HS**	**SHP**	**C**
	(n=68)	**(n=37)**	**(n=31)**	**(n=14)**	**(n=68)**	**(n=37)**	**(n=31)**	**(n=37)**	**(n=31)**	**(n=14)**
Physical fitness										
1RM (kg)	210 ± 33.8	207 ± 35.1	213 ± 32.3	173 ± 29.3	224 ± 33.3***	222 ± 33.7***	226 ± 33.3***	244 ± 32.0###	251 ± 36.1###	180 ± 27.9
Body Composition										
Body Mass (kg)	83.2 ± 11.0	84.9 ± 11.1	81.1 ± 10.9	80.7 ± 13.4	83.5 ± 10.8	84.9 ± 11.9	81.6 ± 10.5	84.9 ± 10.7	81.9 ± 10.6	81.7 ± 13.6
BMI ($kg \cdot m^{-2}$)	25.8 ± 3.2	25.8 ± 3.1	25.7 ± 3.3	25.2 ± 4.5	25.9 ± 3.2	25.9 ± 3.2	25.9 ± 3.2	25.2 ± 4.5	26.0 ± 3.2	25.5 ± 4.5
Body Fat Mass (kg)	20.4 ± 9.0	20.9 ± 8.5	20.1 ± 9.5	19.2 ± 8.9	19.8 ± 9.0***	20.2 ± 8.5***	19.7 ± 9.1***	18.9 ± 8.4###	19.4 ± 9.1	20.3 ± 9.1#
Body Fat Percentage (%)	23.8 ± 8.1	24.0 ± 7.3	23.5 ± 7.4	24.7 ± 8.5	22.7 ± 8.1***	23.2 ± 7.4***	22.1 ± 9.8***	21.7 ± 7.4###	21.8 ± 9.4	25.8 ± 9.3#
Abdominal Fat Mass (kg)	3.1 ± 1.6	3.2 ± 1.5	3.2 ± 1.8	2.4 ± 1.2	2.9 ± 1.6***	3.0 ± 1.5***	2.9 ± 1.8***	2.8 ± 1.5###	3.0 ± 1.7	2.6 ± 1.2#
Lean Mass (kg)	59.5 ± 5.9	60.5 ± 6.0	57.8 ± 4.9	59.6 ± 5.9	60.3 ± 6.1***	61.3 ± 6.1***	58.6 ± 5.1***	62.5 ± 6.1###	59.1 ± 5.0###	59.6 ± 5.9

Figure caption

Figure 1. Effects of of RT on IL-1ra, MCP-1, resistin and leptin. *: Significant within-group change from pre to wk4. #: Significant within-group change from wk4 to wk16, ¤: significant between group difference.



IV

COMBINED AEROBIC AND RESISTANCE TRAINING DECREASES INFLAMMATION MARKERS IN HEALTHY MEN

by

Johanna K. Ihalainen, Moritz Schumann, Daniela Eklund, Mari Hämäläinen, Eeva Moilanen, Göran Paulsen, Keijo Häkkinen & Antti A Mero.

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ORIGINAL ARTICLE

WILEY

Combined aerobic and resistance training decreases inflammation markers in healthy men

J. K. Ihalainen[1] | M. Schumann[1,2] | D. Eklund[1] | M. Hämäläinen[3] | E. Moilanen[3] | G. Paulsen[4,5] | K. Häkkinen[1] | A. A. Mero[1]

[1]Neuromuscular Research Center, Biology of Physical Activity, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

[2]Department of Molecular and Cellular Sport Medicine, German Sport University Cologne, Cologne, Germany

[3]Faculty of Medicine and Life Sciences, The Immunopharmacology Research Group, University of Tampere, Tampere University Hospital, Tampere, Finland

[4]The Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway

[5]Norwegian School of Sport Sciences, Oslo, Norway

Correspondence
Johanna K. Ihalainen, Neuromuscular Research Center, Biology of Physical Activity, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland.
Email: johanna.k.ihalainen@jyu.fi

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Our primary aim was to study the effects of 24 weeks of combined aerobic and resistance training performed on the same day or on different days on inflammation markers. Physically active, healthy young men were randomly divided into three groups that performed: aerobic and resistance training consecutively in the same training session (SS) 2-3 days wk^{-1} or on alternating days (AD) 4-6 days wk^{-1} as well as control (C). The total training volume was matched in the training groups. The control group was asked to maintain their habitual physical activity and exercise level. Maximal leg press strength (1RM) and peak oxygen uptake ($VO2_{peak}$) were measured. Abdominal fat mass was estimated with dual-energy absorptiometry (DXA). High-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor alpha (TNF-α), and adipocytokines resistin, adiponectin, and leptin were analyzed from plasma samples. Training significantly reduced circulating hs-CRP, leptin, and resistin in both training groups ($P<.05$), whereas MCP-1 and TNF-α decreased only in AD ($P<.05$). Significant correlations were observed between changes in abdominal fat mass and corresponding changes in MCP-1, leptin, adiponectin, and resistin. Long-term combined aerobic and resistance training reduced markers of subclinical inflammation in healthy young men. The results indicate that a higher frequency of individual exercise sessions might be more beneficial with respect to the anti-inflammatory effects of physical activity. The decreases in inflammation markers seem to be related to decreases in abdominal fat mass.

KEYWORDS
abdominal fat, adipokines, low-grade inflammation, physical exercise

1 | INTRODUCTION

It is well recognized that the pathogenesis of chronic metabolic diseases such as type 2 diabetes[1] and atherosclerosis[2] involve prolonged low-grade inflammation indicated by increased circulating levels of inflammatory mediators.[3] Thus, previous studies have indicated an inverse association between physical activity and low-grade inflammation.[4–6] As such, lower inflammatory markers have been observed especially in individuals who report performing frequent moderate intensity physical activity.[7]

Both aerobic (AT) and resistance training (RT) have been shown to be important strategies for improving inflammatory profiles.[8] Interestingly, Nimmo et al.[9] concluded that the most marked improvements in the inflammatory profile are probably achieved with a combination of high-intensity AT and RT. While the effects of either AT or RT on inflammation are relatively well studied, data regarding the effects of

combined AT and RT on inflammatory markers are sparse. Libardi et al.[10] failed to observe significant reductions in inflammatory markers after combined training in sedentary middle-age men, while other studies have found significant improvements in inflammation markers in healthy untrained men and women[11,12] as well as in obese men[13] and in subjects with metabolic syndrome.[14] However, combined training can be performed in multiple ways, for example, by performing AT and RT in the same session with different orders or separated on alternating days.[15]

Training intensity and frequency have been shown to affect inflammation markers in a dose-dependent manner.[16] As changes in fat mass have previously been associated with alterations in low-grade inflammation,[17] it can be assumed that the mode of combined training could have a significant effect on the inflammatory profiles as well. A previous study from our group reported a significant reduction in fat mass after a training intervention, but only in a group that separated aerobic and resistance exercises on alternating days, thus increasing the frequency of training while keeping the total training volume constant.[15] Thus, we hypothesized that the combined training mode with sufficient frequency may have a beneficial effect on inflammatory profiles. A secondary purpose was to assess whether training-induced changes in body composition and physical performance influence inflammation markers.

2 | METHODS

2.1 | Participants

This study is a part of a larger research project.[15,18] Participants were recruited through general advertisements in local newspapers as well as posters and emails that were delivered to local companies and institutions. A total of 150 people contacted us to express their interest toward the study (Figure 1). Of these, 93 people met the participation criteria: healthy non-obese (BMI<30 kg m^{-2}) men who were non-smokers, free of acute and chronic illness, disease or injury, and did not report use of any medications (diabetes, cardiovascular diseases, cancer, hypertension, rheumatism, osteoporosis). Ultimately, a total of 48 healthy men completed pre- and post-measurements and were included in this study (age=31±6 years, height=1.79±0.06 m, body mass=80.9±12.3 kg, BMI=25.2±3.5 kg m^{-2}). The subjects were moderately physically active as characterized by walking, cycling, or occasionally participating in team sports at light to moderate intensity and a frequency of 3 days wk^{-1}. The subjects were informed about the possible risks of all study procedures before providing a written informed consent. A completed health questionnaire and resting ECG were reviewed by a cardiologist prior to participation. The study was conducted according to the Declaration of Helsinki, and ethical approval was granted by the University of Jyväskylä Ethical Committee.

2.2 | Study design

The subjects were assigned to either of the two training interventions or the control group: combined aerobic and resistance training performed in the same session (SS, n=16) or on alternating days (AD, n=16) or control group (C, n=16). In another data set from our research group, which was analyzed from the same group of previously untrained subjects, we did not observe significant changes in fat mass

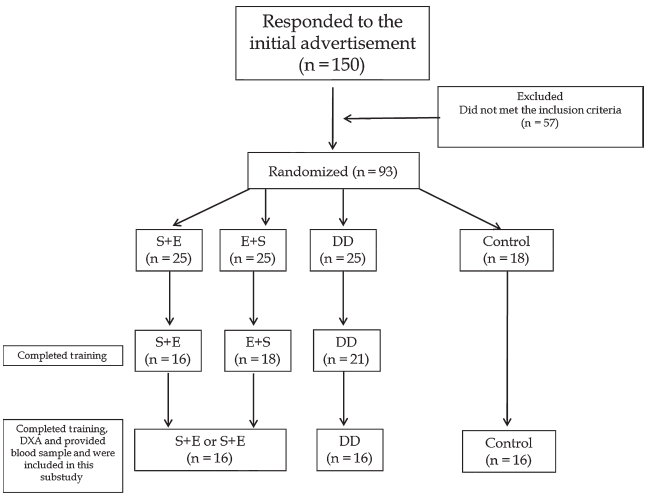

FIGURE 1 Flowchart of study participants

or performance variables between the participant who trained endurance and strength in a same session but with a different order, and thus, we pooled the data of SS for the purpose of this study. The exercise order of SS training was randomized with half of the group performing aerobic immediately followed by resistance training and the other half performing the opposite exercise order. The overall training volume was equal in the two groups but SS consisted of only 2-3 combined training sessions per week, whereas AD performed 4-6 sessions per week (2-3×aerobic and 2-3×resistance, respectively) for 24 weeks. Measurements were taken before (PRE), during (ie, after 12 weeks, MID), and after (ie, after 24 weeks, POST) the training intervention. The control group was measured at PRE and POST. Participants were asked to keep their dietary intake constant and the dietary intake was examined by nutritional diaries.

2.3 | Training

All training sessions were supervised, and the detailed content has been described elsewhere.[15] Briefly, the endurance training was conducted on a cycle ergometer. During weeks 1-7, steady-state cycling of low to moderate intensity (below and above the aerobic threshold) was performed, and during the remaining weeks, additional high-intensity interval sessions (below and above the anaerobic threshold) were incorporated into the training program. The duration of endurance cycling progressively increased from 30 to 50 minutes. During the second half of the study, training volume and intensity were further increased. The resistance training program included exercises for all major muscle groups with a focus on lower extremities. During the first 2 weeks, training was performed as a circuit using low loads. Thereafter, protocols aiming for muscle hypertrophy and maximal strength were performed. During the last 2 weeks also, protocols targeting explosive strength development were performed. During the subsequent 12-week period, both training volume and frequency were slightly increased in an attempt to avoid a training plateau. The overall duration of each resistance training session was 30-50 minutes.

2.4 | Abdominal fat

Whole-body composition was estimated by Dual X-ray Absorptiometry (LUNAR Prodigy, GE Medical Systems, Madison, USA). The DXA scans were performed in the morning with the participant in a fasted (12 hours) state. Automatic analyses (Encore Software, version 14.10.022) provided total body fat mass and total body lean mass. Abdominal fat was calculated manually defining a range of interest confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest.[19] This customized range was then copied to the DXA scans at MID and POST, respectively.

2.5 | Cardiorespiratory performance

A graded protocol on a cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) was used to determine $VO2_{peak}$ and metabolic thresholds for the aerobic training. The initial load for all subjects was 50 Watts and increased by 25 Watts every two minutes until volitional exhaustion. Oxygen uptake was determined continuously breath by breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). Peak oxygen consumption ($VO2_{peak}$) was averaged over 60-s periods during the test.

2.6 | Maximal strength performance

Maximal strength was measured by a one-repetition maximum (1RM) test of dynamic leg press exercise performed by a David 210 leg press device (David D210, David Health Solutions Ltd., Helsinki, Finland). The starting position (flexed) was at a knee angle of approximately 60 degrees, and 1RM was accepted as the highest loads the participants could lift to a full knee extension (180 degrees). Subjects performed three warm-up sets and 3-5 maximal trials, after which the highest load was accepted as the 1RM.

2.7 | Venous blood samples

Fasting venous blood samples were drawn from an antecubital vein in the morning (7:00-9:00 AM) after a 12-h overnight fast. Participants were instructed to abstain from strenuous physical activity for 48 hours before the blood samples were taken. Venous blood was collected into EDTA tubes for analysis of inflammatory profiles. The samples were centrifuged for 10 minutes at +4°C with 2000×*g* (Megafuge 1.0 R, Heraeus, Germany). Plasma was kept at −80°C until analyzed for high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) using the Immulite 1000 and immunoassay kits (Immulite, Siemens, IL). Concentrations of monocyte chemoattractant protein-1 (MCP-1), adiponectin, leptin, and resistin in plasma samples were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (R&D Systems, Europe Ltd, Abingdon, UK). The detection limits and interassay coefficients of variation, respectively, were 0.1 pg mL^{-1} and 10% for hs-CRP, 0.2 pg mL^{-1} and 3.4% for IL-6, 3.9 pg mL^{-1} and 5.0% for MCP-1, 19.5 pg mL^{-1} and 2.2% for adiponectin, 15.6 pg mL^{-1} and 4.0% for resistin, and 15.6 pg mL^{-1} and 5.1% for leptin.

2.8 | Statistical analysis

Data were analyzed using PASW statistic 22.0 (SPSS, Chicago, IL, USA). Data are presented as mean±SD Before applying further statistical methods, the data were checked

for sphericity and normality. If a specific variable violated the assumptions of parametric tests, log-transformation was used. This concerned values of adiponectin, leptin, IL-6, MCP-1, and hs-CRP. Absolute changes were analyzed via two-way repeated-measures analysis of variance for main (time) and interaction (group×time) effects. For each analysis, the baseline values were used as a covariate to control between-subject and between-group differences at baseline. This was followed by one-way repeated-measures ANCOVA on each group to examine a main effect of time. If a significant main effect or interaction was observed, the change from pre-values for MID and POST was compared between groups using paired t tests with Bonferroni correction. Effect sizes (ES) are given as Cohen's d with an effect size of ≥0.20 being considered small, ≥0.50 medium, and ≥0.80 large. Spearman's correlation coefficients were used to examine the associations between depending variables. The level of statistical significance was set at $P \leq .05$.

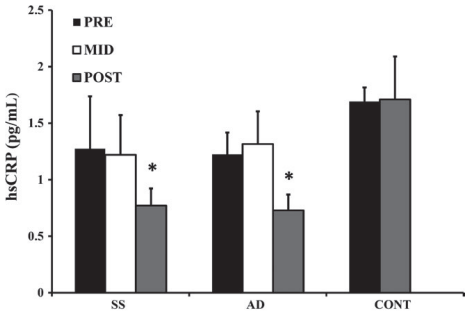

FIGURE 2 Mean (SD) in hs-CRP at weeks 0, 12, and 24. *significant within-group change. AD, alternating days training; SS, same-session training; C, controls

3 | RESULTS

3.1 | Training adherence

The training adherence was 99±2% and 100±1% in SS and AD, respectively. All subjects completed at least 90% of the overall training volume.

3.2 | Circulating inflammatory markers

Circulating hs-CRP is presented in Figure 2. For hs-CRP, a significant main effect of time was observed (P=.010, ES=0.785). Circulating concentrations of hs-CRP decreased significantly in the SS (P=.021) and in the AD (P=.004) from PRE to POST.

Figure 3 illustrates the changes in circulating adipocytokine and cytokine concentrations. A significant main effect of time (P=.010, ES=0.942) was observed in concentrations of circulating resistin. Significant reductions in concentrations of circulating resistin were observed in SS (P=.031, ES=0.582) and AD (P=.022, ES=0.661) but remained unaltered in C. At POST, significant changes in concentrations of circulating leptin were observed in SS (P=.031) and AD (P=.019) at POST. Significant changes in adiponectin concentrations were not observed.

In the inflammatory cytokines, a significant main effect of time (P=.02, ES=0.869) and interaction (P=.027, ES=0.760) was observed in the levels of MCP-1. At POST, a significant reduction was observed in AD (P=.02, ES=0.840) but not in SS and the control groups. In addition, the reduced concentration of MCP-1 in AD was significantly lower than in SS and C (P=.019 and P=.007, respectively). A significant main effect of time was observed in circulating concentrations of TNF-α (P=.001, ES=0.926). Slight but statistically significant reduction in TNF-α concentration was observed in AD at POST (P=.048, ES=0.418), while no changes in SS or C

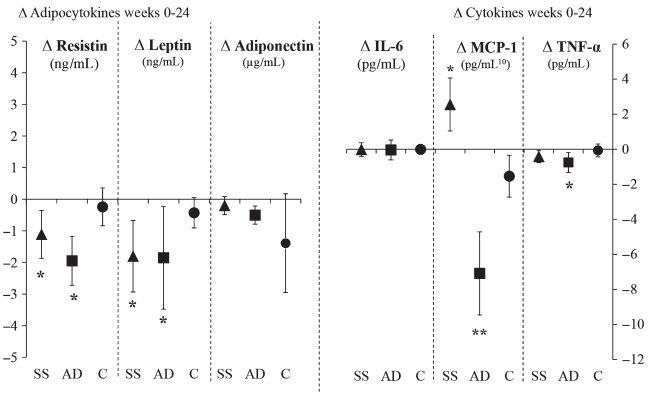

FIGURE 3 Mean (SD) changes in adipocytokines (left) and cytokines (right). *significant within-group change. SS, same-session training; AD, alternating days training; C, Controls

were found (P=.056 and P=.218, respectively). Significant main effects of time or interaction in IL-6 were not observed.

3.3 | Body composition, aerobic performance, and strength

Changes in body composition, 1RM, and $VO2_{peak}$ are summarized in Table 1 and have been partly published elsewhere[15,18] (Schumann et al. 2014). No significant changes were observed in body weight. A significant main effect of time (P<.001, ES=0.974) and interaction (P=.014, ES=0.789) was observed in abdominal fat mass. After 12 weeks of training, fat mass did not decrease in either of the two experimental groups. However, a significant decrease in abdominal fat mass from PRE to POST was observed in SS (−7.4±15.4%, P=.041, ES=0.445) and AD (−21.1±17.6%, P<.001, ES=0.997). No significant changes in abdominal fat mass was observed in C. Abdominal fat mass in AD at POST was significantly lower compared with SS and C groups (P=.050, P=.019, respectively).

A significant main effect of time (P=.015, ES=0.748) and interaction (P=.007, ES=0.877) was observed in $VO2_{peak}$. Both the SS and AD groups increased $VO2_{peak}$ significantly from PRE to MID (6.80±8.28% P=.001 and 13.2±11.9% P<.001, respectively) and from PRE to POST (9.3±8.85% P<.001 and 18±10.3% P<.001, respectively), while no significant change was observed in C (P=.637, ES=0.081). A significant main effect of time (P<.001, ES=0.989) and interaction (P=.003, ES=0.918) in 1RM was observed. 1RM increased in all groups (P<.001). Both training groups and C group increased 1RM from PRE to MID (P<.001) and from PRE to POST (P<.001). The increase in 1RM was significantly larger in SS and AD groups (+14.1±11.4%, P<.01 and +12.7±7.24%, P<.01; respectively) than in C group (+4.7±4.65%).

3.4 | Associations between changes in performance, body composition, and inflammatory markers

Leptin correlated significantly with abdominal fat mass at all measurement points (PRE R=.732, P<.001, MID R=.650, P<.001 and POST R=.522 P<.001) when all the subjects were pooled. In addition, in the pooled data, the changes from PRE to POST in abdominal fat mass correlated positively with the change in leptin (R=.433, P=.002), MCP-1 (R=.581, P=.023), and resistin (R=.343, P=.016) and negatively with adiponectin (R=−.290, P=.043). Changes in inflammation markers and performance variables were not associated, but a significant negative correlation was observed between TNF-α and $VO2_{peak}$ as well as between leptin and $VO2_{peak}$ at PRE (R=−.389, R=.018 and P=−.654, all P<.05). In the experimental groups, an inverse relationship

TABLE 1 Physical fitness and body composition at before (PRE) after 12 wk (MID) and after 24 wk (POST) of training

	PRE			MID		POST		
	SS (n=16)	AD (n=15)	CONT (n=18)	SS (n=16)	AD (n=15)	SS (n=16)	AD (n=15)	CONT (n=18)
Physical fitness								
1RM (kg)	151±32.2	145±18.3	159±29.9	164±26.5	159±16.7	170±26.2	163±16.0	167±28.5
$VO2_{peak}$ (L min^{-1})	3.13±0.40	2.82±0.32	3.07±0.53	3.33±0.42	3.17±0.26	3.41±0.49	3.34±0.36	3.11±0.53
Body composition								
Height (m)	1.78±0.06	1.80±0.08	1.78±0.06	1.78±0.06	1.80±0.08	1.78±0.06	1.80±0.08	1.78±0.06
Body weight (kg)	80.1±13.2	81.8±10.3	80.7±11.7	80.1±11.9	81.9±10.3	80.4±11.1	80.6±10.4	81.7±11.5
BMI ($kg \cdot m^{-2}$)	25.2±3.00	25.3±2.60	25.2±3.9	25.2±2.50	25.3±2.93	25.4±2.34	24.9±2.85	25.5±3.89
Body fat mass (kg)	20.8±8.12	22.9±6.11	19.2±7.42	20.0±7.27	21.6±6.67	19.0±7.00	19.5±7.28	20.4±7.66
Body Fat-% (%)	25.4±7.1	27.0±4.3	23.1±8.3	24.5±6.6*	27.6±4.4	23.2±6.2 **	25.9±5.5 **	24.4±8.9
Abdominal fat mass (g)	2571±1190	3060±993	2310±1210	2340±1060	2810±1040**	2330±1080#	2490±1120***	2450±1361
Lean mass (kg)	53.3±6.13	55.9±5.12	59.5±5.85	54.1±5.74	57.2±5.73	54.8±5.93*	58.0±5.22*	58.7±5.87

AD, Different-day training; SS, Same-session training; C, Controls.
difference from PRE value (P<.05, **P<.01, ***P<.001) #difference between the groups (#P<.05). Mean±SD.

between change in concentration of circulating adiponectin and change in maximal strength from PRE to POST was observed (R=−.459, P=.014).

4 | DISCUSSION

The present study assessed the effects of 24 weeks of combined aerobic and resistance training on inflammation markers in young, healthy men. Herein, we provide evidence that combined AT and RT reduces inflammation as demonstrated by lowered circulating concentrations of hs-CRP, leptin, and resistin. The special focus of the present study, however, was to investigate whether the performing AT and RT in the same session (SS) or on alternating days (AD) affected the inflammation markers differently. The main finding of the study was that combined training performed on alternating days elicited the largest reductions in circulating levels of TNF-α and MCP-1. Furthermore, the beneficial effects of exercise on inflammation markers were achieved without concomitant weight loss; however, a decrease in abdominal fat mass was associated with reductions in the inflammation markers, which emphasizes meaningfulness of this change in body composition.

In the present study, we showed that the baseline levels of hs-CRP allowed us to classify the participants as having "moderate cardiovascular risk" (1.0-3.0 mg L^{-1}) prior to commencement of the study in all groups. At POST the mean concentration of hs-CRP was reduced to the level of "low cardiovascular risk" (<1.0 mg L^{-1}) in both experimental groups.[20] These findings are in line with a study by Stewart et al.,[21] who suggested that a combination of AT and RT reduced the risk of cardiovascular disease development, as defined by a decrease in hs-CRP concentrations in healthy populations. While C-reactive protein (CRP) concentrations are generally determined by genetic factors, centrally located adiposity is also considered to be a major determinant of CRP levels.[22] Cross-sectional studies have found an inverse relationship between physical activity and CRP,[23] and training studies have reported reductions in CRP.[20] Interestingly, Libardi et al.[10] did not find any significant differences in CRP, IL-6, or TNF-α in sedentary middle-age men after 16 weeks of concurrent training in which AT and RT were performed in the same session, three times a week. These findings were opposed to those of Stewart et al.,[20] who found a significant improvement in CRP concentrations after a 12-wk concurrent training period in young and old sedentary subjects. Interestingly, in the present study we did not observe any significant changes in circulating inflammation markers after 12 weeks, but only after 24 weeks of training. In contrast to the studies by Stewart et al.[20] and Libardi et al.,[10] the subjects in the present study were young and healthy and reported to be moderately active. Thus, our findings indicate that even moderately active young healthy subjects benefit from prolonged combined AT and RT, but adaptations may be delayed in comparison with inactive and/or elderly subjects. However, it is notable that the training in the present study was progressive as both training volume and frequency were increased during the training intervention. Therefore, it is also possible that the training was not intensive enough to elicit anti-inflammatory effect during the first 12 weeks of training.

Beavers et al.[7] concluded that AT interventions for healthy individuals are beneficial for reducing inflammatory biomarkers, although reductions in body weight are small. In the present study, we did not observe significant reductions in body weight. Interestingly, the abdominal fat mass decreased significantly only when combined training was performed on alternating days as opposed to AT and RT in the same session. This group difference in abdominal fat mass could be due to the greater frequency of exercise that probably resulted in increased overall energy expenditure.[24] Intra-abdominal obesity has been shown to be an important risk factor for low-grade inflammation. The distribution of excess fat in the abdominal region is known to modify the health risk profile, whereas excess adiposity in the periphery does not appear to increase the risk of developing cardiovascular disease.[25] In the present study, we observed a significant association between the change in abdominal fat mass and all measured circulating adipocytokine concentrations. Previous studies suggest that physically active individuals or subjects with higher fitness level have more favorable adipocytokine profiles compared with sedentary populations.[5] This was supported by our findings as the initial $VO2_{peak}$ was significantly associated with circulating leptin concentration at baseline. However, we did not observe a significant correlation between changes in $VO2_{peak}$ and changes in adipocytokine concentrations. Interestingly, we observed a significant reduction in circulating MCP-1 concentrations after 24 weeks when the training was separated into alternating days as opposed to AT and RT in the same session. Moreover, reductions in MCP-1 are associated with the changes in abdominal fat mass, irrespective of intervention group, which indicates that fat mass in the abdominal area has a significant effect on MCP-1 concentration.

We observed that the circulating resistin levels were reduced in both experimental groups after 24 weeks of training, even if we did not observe a significant reduction in visceral fat mass in the SS group. Resistin is a signaling protein that has been linked to inflammation and coronary heart disease,[26] and consequently, a reduction in resistin concentrations may be interpreted as a beneficial biological adaptation. Our data indicate that long-term combined AT and RT alter the concentrations of circulating resistin regardless of changes in abdominal fat mass. Gleeson et al.[17] suggested that both the reduction in visceral fat mass and the anti-inflammatory

environment induced by each exercise session might elicit long-term anti-inflammatory effects. One of the possible mechanisms behind the anti-inflammatory effect of exercise has been suggested to be the acute IL-6 release following an exercise session, possibly stimulating the accumulation of anti-inflammatory cytokines, such as interleukin-10 and interleukin-1 receptor antagonist.[17] IL-6 has been shown to be related to circulating resistin levels, but whether IL-6 releases are mechanistically linked to reductions in circulating resistin levels awaits further investigation. Nevertheless, we observed no significant changes in circulating IL-6 concentration in the experimental groups.

Changes in body composition or, more precisely, changes in abdominal fat mass seem to be an important factor when an exercise intervention for reducing inflammation markers is planned. In the present study, we showed that a significant reduction in adipokines is possible also in the absence of change in abdominal fat mass, as seen in the decrease in resistin levels. However, significant reductions in leptin levels seem to be dependent on a significant reduction in fat mass.[27] There are several mechanisms involved in the beneficial effects of exercise on immunological function, and recent research has focused on its role in the improvement of the inflammatory profile. However, further studies are needed to identify the molecular mechanisms underlying the anti-inflammatory effect of exercise and what the role of skeletal muscle is in this action.

The strengths of this study include its careful measurement of a wide range of potential confounding variables and a prolonged supervised training intervention. However, several limitations should be considered when interpreting our results. First, the participants in this study were young healthy men, and therefore, a generalization of our results to other populations might be problematic. Secondly, although in the present study several different factors are suggested to be important markers and/or regulators of inflammation, there are many other pro- or anti-inflammatory factors that could have been measured. Nevertheless, CRP, in particular, has proven to be a relatively useful marker of systemic inflammation and predictor of clinically relevant outcomes and is the most commonly measured inflammatory marker (Pearson et al. 2003). Lastly, we cannot determine the directions of the associations nor causality observed in this study with absolute certainty.

4.1 | Perspectives

Combined AT and RT without concomitant body weight loss may induce anti-inflammatory effects, leading to improvements in levels of circulating inflammation markers in men. These effects could be enhanced with a reduction in visceral fat mass that was observed only when AT and RT were performed on alternating days. The findings of this study indicate that a higher frequency of exercise sessions should be recommended in the prevention of inflammation-related diseases. The improvement in the inflammatory profile achieved in the present study may be an effective strategy for reduction in low-grade systemic inflammation and improving the health trajectory of young men.

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CONFLICT OF INTEREST

The authors do not have conflict of interests and state that the results of the present study do not constitute endorsement by ACSM. The authors alone are responsible for the content and writing of the manuscript.

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