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"Influence of a 6-month progressive resistance training on the TGF-beta pathway of institutionalized elderly"

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Anmerkung: Im Zuge der „Active Ageing“ Studie wurden auch weitere Magisterarbeiten verfasst, wobei parametrische statistische Testverfahren für die Auswertung der körperlichen Leistungsfähigkeit älteren Frauen zum Einsatz kamen (siehe Wohlmuth, 2015).

ABSTRACT - ENGLISH

Background: The process of ageing is related to various changes in the human body including alterations in cytokine expressions often concomitant with a low-grade chronic inflammation.

Objectives: The aim of the present study was to investigate possible effects of progressive resistance training alone or in combination with a dietary supplement on the cytokine levels of transforming growth factor β (TGF-β), the expression of TGF-β, its receptors TGF-β receptors 1 (TGF-βRI) and 2 (TGF-βRII) as well as microRNA-21 (miRNA-21) expression in peripheral blood mononuclear cells (PBMCs) in older persons.

Methods: For this randomized controlled study, 117 older adults (age 65+; 14 men and 103 women) living in retirement homes were assigned to a resistance exercise group (RT), a RT group with additional nutritional supplementation (RTS) or a cognitive training group (CT) for a 6-month intervention period. During this time the RT and RTS groups performed a resistance exercise routine twice a week using elastic bands and the own body weight for the major muscle groups. Enzyme-linked immunosorbent assays (ELISA) were used for the measurement of circulating TGF-β, whereas quantitative real-time RT-PCRs were applied for the determination of the intracellular markers at baseline (0m), 3 months (3m) and 6 months (6m). Furthermore, the 6-minute walking test, the chair stand test, isometric handgrip strength and isokinetic peak torque measurements for the quadriceps (PTQ) and the hamstrings (PTH) were applied for testing the physical performance of the subjects. Statistical analyses were performed using Friedman's test to detect changes over time in the intervention groups (RT, RTS, CT).

Results: The statistical analyses revealed a significant time effect for the 6-minute walking test (RT: +11.5%, p = 0.011 [0m – 6m]), the chair stand test (RT: +27.3%, p = 0.002 [0m – 3m], +27.3%, p = 0.010 [0m – 6m]; RTS: +15.4%, p = 0.028 [0m – 6m]) as well as PTH at 60°/s (RT: +23.1%, p = 0.004 [0m – 6m], +10.3%, p = 0.006 [3m – 6m]). Additionally, time effects were found for PTQ at 120°/s (RT: +10.7%, p = 0.003 [0m – 3m]; RTS: +9.6%, p = 0.009 [0m – 3m], -0.2%, p = 0.049 [3m – 6m]) and PTH at 120°/s (CT: +36.4%, p = 0.010 [0m – 3m], +18.2%, p = 0.029 [0m – 6m]; RT: +10%, p = 0.006 [0m – 3m], +8%, p = 0.047 [0m – 6m]). Neither the serum markers of inflammation (high-

sensitive C-reactive protein, TGF-β) nor the intracellular markers (TGF-β, TGF-βRI, TGF-βRII, miRNA-21) were significantly influenced by the study interventions.

Conclusion: The present study could show that progressive resistance training with elastic bands and the own bodyweight improved physical performance of community-dwelling older persons. The additional protein supplementation could not further increase the positive training effects of the training intervention. Interestingly, the inflammatory serum markers and the intracellular biomarker were not affected by the applied training regime and nutritional supplementation, indicating that maybe a different type of exercise program, like endurance training or a combination of resistance training with endurance training, might be more effective in altering low-grade chronic inflammation.

ABSTRACT - DEUTSCH

Hintergrund: Der Alterungsprozess steht im menschlichen Körper mit verschiedensten Veränderungen, wie zum Beispiel Änderungen der Zytokin-Expression und des häufig damit einhergehenden chronischen, geringgradigen Entzündungszustandes, in Verbindung.

Zielsetzung: Das Ziel der vorliegenden Studie war, die möglichen Effekte eines progressiven Krafttrainings alleine oder in Kombination mit einem Nahrungsergänzungsmittel auf die Zytokin-Level des transformierenden Wachstumsfaktors β (TGF-β), die Expression seiner Rezeptoren 1 (TGF-βRI) und 2 (TGF-βRII) als auch auf die Expression der microRNA-21 (miRNA-21) in mononukleäre Zellen des peripheren Blutes (PBMC).

Methodik: Für diese randomisierte kontrollierte Studie wurden 117 in Pensionistenheimen lebende Senioren und Seniorinnen (Alter 65+, 14 Männer und 103 Frauen) einer Widerstandstrainingsgruppe (RT), einer RT Gruppe mit ergänzender Einnahme eines Nahrungssupplements (RTS) oder einer Kognitivtrainingsgruppe (CT) für einen 6-monatigen Interventionszeitraum zugeteilt. Während dieser Zeit führten die RT und RTS Gruppen zweimal wöchentlich ein Widerstandstrainingsprogramm für die Hauptmuskelgruppen mit elastischen Gymnastikbändern und dem eigenen Körpergewicht durch. Enzyme-linked immunosorbent assays (ELISA) wurden für die Messung des zirkulierenden TGF-βs eingesetzt, wohingegen das Verfahren der quantitativen Echtzeit RT-PCR für die Bestimmung der intrazellulären Marker zu Studienbeginn (0m), nach 3 Monaten (3m) und nach 6 Monaten (6m) angewandt wurden. Des Weiteren wurden der 6 Minuten Gehtest, der 30 Sekunden Aufstehtest, ein Test für die isometrische Handgriffkraft sowie eine isokinetische Drehmomentmessung der Knieextensoren (PTQ) und Knieflexoren (PTH) durchgeführt um die körperliche Leistungsfähigkeit der Teilnehmer und Teilnehmerinnen zu erheben. Die statistische Analyse wurde mittels Friedman's Test durchgeführt um Zeiteffekte in den einzelnen Gruppen (RT, RTS, CT) aufzudecken.

Ergebnisse: Mithilfe der statistischen Analysen konnten signifikante Zeiteffekte für den 6 Minuten Gehtest (RT: +11.5%, p = 0.011 [0m – 6m]), den 30 Sekunden Aufstehtest (RT: +27.3%, p = 0.002 [0m – 3m], +27.3%, p = 0.010 [0m – 6m]; RTS: +15.4%, p = 0.028 [0m – 6m]) sowie das PTH bei 60°/s (RT: +23.1%, p = 0.004 [0m – 6m], +10.3%, p = 0.006 [3m – 6m]) festgestellt werden. Ergänzend wurden Zeiteffekte für die PTQ bei 120°/s (RT:

+10.7%, p = 0.003 [0m – 3m]; RTS: +9.6%, p = 0.009 [0m – 3m], -0.2%, p = 0.049 [3m – 6m]) und die PTH bei 120°/s (CT: +36.4%, p = 0.010 [0m – 3m], +18.2%, p = 0.029 [0m – 6m]; RT: +10%, p = 0.006 [0m – 3m], +8%, p = 0.047 [0m – 6m]) gefunden. Weder die im Serum gemessenen Entzündungsmarker (high-sensitive C-reactive protein, TGF-β) noch die intrazellulären Marker (TGF-β, TGF-βRI, TGF-βRII, miRNA-21) wurden durch die angewandten Interventionen signifikant beeinflusst.

Conclusio: Die aktuelle Studie konnte zeigen, dass progressives Widerstandstraining mit elastischen Gymnastikbändern und dem eigenen Körpergewicht die körperliche Leistungsfähigkeit von in Pensionistenheimen lebende Senioren und Seniorinnen verbessert. Die ergänzende Einnahme eines Nahrungssupplements führte zu keinem weiteren positiven Anstieg des Trainingseffektes. Interessanter Weise wurden die zirkulierenden Entzündungsmarker als auch die intrazellulär gemessenen Marker durch die Trainingsintervention und die Einnahme des Nahrungsergänzungsmittels nicht beeinflusst. Dies könnte darauf hindeuten, dass die Anwendung eines alternativen Trainingsprogramms, wie zum Beispiel der Einsatz eines Ausdauertrainings oder eines Kombinationstrainings von Ausdauer und Kraft, effektivere Veränderungen eines chronischen, geringgradigen Entzündungszustandes bewirken könnte.

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1 INTRODUCTION

Research has shown that the ageing process is accompanied by severe alterations in the human immune system, like changes in cytokine secretion patterns, antibody production, T lymphocytes (T cells) subpopulations size or cell replicative capacity, culminating in a proinflammatory state (de Araujo, Silva, Fernandes, & Benard, 2013). In addition, they are related to the increased morbidity and mortality rates observed in older people (de Araujo et al., 2013). Slowing down these ongoing changes could improve the health status of elderly and the process of ageing itself (Cevenini, Monti, & Franceschi, 2013). Therefore it is important to identify the pathways that control age-related inflammation across multiple systems in order to find out whether interventions may be beneficial or not (Franceschi & Campisi, 2014). Due to the fact that the cytokine transforming growth factor beta (TGF-β) and its signaling pathway are important for medically relevant processes of inflammation, immunity, cancer, fibrosis as well as the homeostasis of different tissues, they provide an interesting field of research (Massague, 2012). The analysis of micro RNAs (miRNA) could also be an effective tool for the development of targeted exercise and dietary interventions for muscle preservation (Pasiakos & McClung, 2013). Concerning inflammation, circulating micro RNA-21 (miRNA-21) might be a possible inflammatory biomarker, which is linking age-related diseases and the ageing process (Olivieri et al., 2012).

1.1 IMMUNE SYSTEM

The physiological function of the human immune system is the defense against infectious microbes or, more explicitly, components of microbes, also to macromolecules, like proteins and polysaccharides, and small chemicals, when they are recognized as 'foreign' (Abbas, Lichtman, & Pillai, 2012). It evolved to maintain the homeostatic balance between the host tissues and the external and internal microbiological environment in order to maintain the integrity of the host as well as the microbiome, like the gut microbiota (Müller, Fülöp, & Pawelec, 2013). Under some circumstances, even self molecules can evoke the so-called autoimmune responses (Abbas et al., 2012). The defense against microbes is mediated by two different mechanisms, the early reactions of the *innate immunity* and the later responses of the *adaptive immunity* (Abbas et al., 2012; Sirisinha, 2014; Vivier et al., 2011).

1.1.1 INNATE IMMUNE SYSTEM

The early line of defense against microbes is provided by the innate immunity (Le Blanc & Mougiakakos, 2012; Solana et al., 2012), which is also called native or natural immunity (Abbas et al., 2012). The innate immunity itself comprises biochemical and cellular defense mechanisms, which are in place even before infection and are prepared to respond rapidly to them. These defense mechanisms respond to products of injured cells and microbes, and they react fundamentally the same way to repeated infections (Abbas et al., 2012). There are four main components of innate immunity: firstly, the chemical and physical barriers (Le Blanc & Mougiakakos, 2012) (like antimicrobial chemicals produced at epithelial surfaces and the epithelia itself), secondly, dendritic cells, natural killer (NK) cells and phagocytic cells (macrophages, neutrophils) (Shaw, Joshi, Greenwood, Panda, & Lord, 2010) thirdly, blood proteins (including members of the complement system and other mediators of the inflammation process), and fourthly, proteins named cytokines (which coordinate and regulate a lot of activities of the innate immunity cells) (Abbas et al., 2012).

1.1.2 ADAPTIVE IMMUNE SYSTEM

The immunity responses oft the adaptive immunity are stimulated by the exposure to infectious agents (Abbas et al., 2012). With each successive exposure to a special microbe the immune responses increase in defensive capabilities as well as in magnitude (Abbas et al., 2012). The exquisite specificity for distinct molecules and the ability to ‘remember’ and to respond more strenuously to repeated exposures to the same microbe, are the defining characteristics of adaptive immunity (Abbas et al., 2012; Flajnik & Kasahara, 2010).

Furthermore it can recognize and react to a great number of nonmicrobial and microbial substances (Abbas et al., 2012). Moreover, it has the remarkable capability to distinguish between different molecules and microbes, even if they are closely related. Therefore, the adaptive immunity is also called specific immunity. Cells named lymphocytes and their secreted products, such as antibodies, form the main components of adaptive immunity. Antigens are foreign substances that evoke specific immune response or are recognized by antibodies or lymphocytes. Individuals that have not been confronted with a particular antigen, are referred to as naïve, signifying their immunological inexperience. On the contrary, individuals are said to be immune, if they have responded to a microbial antigen and

are protected from subsequent exposure to that microbe. The adaptive immunity can be subdivided into the *humoral immunity* and the *cell-mediated immunity* (Abbas et al., 2012).

Molecules in the blood and mucosal secretions, termed antibodies, mediate the *humoral Immunity* (Abbas et al., 2012). Antibodies are produced by cells named B lymphocytes (B cells), which detect microbial antigens, neutralize the infectivity of microbes and can target them for elimination by several different effector mechanisms. Antibodies bind to microbes and their toxins and support their elimination, thus humoral immunity is the main defense mechanism against extracellular pathogens. Antibodies are specialized and initiate different effector mechanisms, such as the ingestion of microbes by host cells (phagocytosis), or they are bind to and trigger the release of inflammatory mediators from cells (Abbas et al., 2012).

So-called T cells mediate the c*ell-mediated immunity,* or also named cellular immunity (Abbas et al., 2012). Viruses and some bacteria can survive and proliferate inside phagocytes and other host cells so that they are inaccessible to circulating antibodies. One function of cell-mediated immunity is the defense against such infections by promoting the destruction of these intracellular microbes residing in phagocytes, or the elimination of infected cells, consequently the reservoirs of infection (Abbas et al., 2012).

1.1.3 CELLS OF THE IMMUNE SYSTEM

Phagocytic cells can be found in the tissues and the circulation and belong to two major lineages, the *mononuclear phagocytes* (monocytes and macrophages) and the *polymorphonuclear granulocytes* (neutrophils, basophils and eosinophils) (Male, Brostoff, Roth, & Roitt, 2007). The primary function of phagocytes is to identify, ingest and eliminate microbes (Abbas et al., 2012).

The cells of the *mononuclear phagocyte* systems can be found in virtually all organs of the body and their primary function is phagocytosis, which plays a central role in innate and adaptive immunity (Abbas et al., 2012). They arise from a common precursor in the bone marrow and enter the peripheral blood in an incompletely differentiated cell type named monocytes (Abbas et al., 2012). They can be rapidly recruit from the blood stream into sites of tissue inflammation or may be the origin of tissue resident macrophages and some dendritic cells (Abbas et al., 2012; Geissmann et al., 2010; Male et al., 2007). Monocytes migrate through the blood vessel walls into organs where they mature and become

different types of macrophages (Le Blanc & Mougiakakos, 2012), determined by their local microenvironment (Abbas et al., 2012; Gomez, Nomellini, Faunce, & Kovacs, 2008; Male et al., 2007). Macrophages actively phagocytose and eliminate microorganisms, cells that died from apoptosis and even tumor cells by enzymatic generation of reactive oxygen and nitrogen species that are toxic to the microbes or by proteolytic digestion (Abbas et al., 2012; Male et al., 2007). Activated macrophages can secrete cytokines that can instruct other cells to respond in ways that contribute to host defense, by binding to their signaling receptors (Abbas et al., 2012).

Neutrophils, also named polymorphonuclear leukocytes, are the most common population of circulating white blood cells (Abbas et al., 2012; Jiao et al., 2014; Le Blanc & Mougiakakos, 2012). They originate from a common lineage with mononuclear phagocytes and are produced in the bone marrow (Abbas et al., 2012). Neutrophils circulate in the blood for only about 6 hours and may migrate to sites of infection (Le Blanc & Mougiakakos, 2012) after the entry of pathogens (Abbas et al., 2012). Neutrophils that are not recruited into a side of inflammation within this short period of time, undergo apoptosis and are normally phagocytosed by resident macrophages in the spleen or liver (Abbas et al., 2012). Neutrophils constitute the primary immune defense against yeast, fungal infections and rapidly dividing bacteria by deploying microbicidal mechanisms, like the generation of reactive oxygen and nitrogen species, the release of proteolytic enzymes or microbicidal peptides from cytoplasmic granules (Panda et al., 2009; Shaw et al., 2010).

Like neutrophils, basophils and eosinophils are bone marrow derived and circulate in the blood (Abbas et al., 2012). Normally, *basophils* are not present in tissue, but may be recruited to inflammatory sites. Less than 1 % of blood leucocytes are constituted by basophils. Their importance in host defense and allergic reactions is uncertain, due to their low number in tissues (Abbas et al., 2012).

Eosinophils comprise 2-5 % of blood leukocytes in healthy humans and they appear to be able to phagocytose and kill ingested microorganisms (Male et al., 2007). They express enzyme containing cytoplasmic granules that are harmful to the cell walls of parasites but can also cause damage to host tissues (Abbas et al., 2012). Also in peripheral tissues some eosinophils are normally present, especially in mucosal linings of the genitourinary, respiratory and gastrointestinal tracts (Abbas et al., 2012). In the setting of inflammation their numbers can increase by recruitment from the blood (Abbas et al., 2012; Kariyawasam & Robinson, 2006).

Lymphocytes are unique cells of the adaptive immunity and they are the only cells in the human body that express clonally distributed antigen receptors (Abbas et al., 2012). Each of those receptors has a fine specificity for a different antigenic determinant (Abbas et al., 2012). Lymphocytes initiate the adaptive immune responses because they are wholly responsible for the specific immune recognition of pathogens (Male et al., 2007). The two major subpopulations, named B cells and T cells, arise from bone marrow stem cells, but T cells develop in the thymus, while B cells develop in the bone marrow (in adult human beings) (Male et al., 2007). Naïve B cells and T cells encounter antigens in specialized lymphoid organs and go through a process of maturation and cell division before exerting their effector function (Vivier et al., 2011). T and B cells differ in their functions and in the way they recognize antigens. B cells are the only cells that are able to produce antibodies (Abbas et al., 2012). They discern extracellular antigens, including those on the cell surface and differentiate into antibody-secreting plasma cells. These plasma cells produce antibodies that have the same antigen-binding site as the B cell receptors that first recognized the antigen. T cells recognize the antigens of intracellular microbes and either directly eliminate the infected cell or help phagocytes to destroy these microbes. Their antigen receptors are membrane molecules that are distinct from antibodies but are structurally related to them. T cells detect peptides derived from foreign proteins that are bound to host proteins (major histocompatibility complex molecules), which are expressed on the cell surface of other cells. Therefore, T sells recognize and respond to cell surface-assosiated antigens but not to soluble ones (Abbas et al., 2012).

T cells subsist of functionally distinct populations, such as *helper T cells* ($CD4^+$ T cells) and *cytotoxic T lymphocytes* (CTLs or $CD8^+$ T cells) (Abbas et al., 2012; Male et al., 2007). When helper T cells encounter antigens they secrete cytokines, which activate other cells like B cells, macrophages and other leucocytes and stimulate differentiation and proliferation of the T cells themselves (Abbas et al., 2012). CTLs kill foreign antigen producing cells, such as cells infected by intracellular microbes and viruses (Abbas et al., 2012).

As mentioned before, exposure of the immune system to foreign antigens improves its ability to respond to the same antigens in the future, so that the so-called secondary immune response is larger and more rapid then the first one (Abbas et al., 2012; Rolle, Pollmann, & Cerwenka, 2013). Therefore the immune system generates long-lived memory T cells that react much more vigorously and rapidly to antigen challenge (Weng, 2006) and memory B cells that produce antibodies that bind antigens with higher affinities (Abbas et al., 2012).

The *NK cells* are a third type of lymphocytes and they are involved in the defense against a variety of viral infections and malignancies (Abbas et al., 2012; Rolle et al., 2013). They are a part of innate immunity (Gayoso et al., 2011), characterized by invariant, germ-line encoded receptors for the detection of infected cells and pathogens (Rolle et al., 2013; Vivier et al., 2011). Moreover, they play a significant role in the inhibition of tumor growth and metastases and in the defense against a broad variety of infections (Gomez et al., 2008). Recent findings indicate that NK cells also share some of the features of adaptive immunity (Rolle et al., 2013; Vivier et al., 2011).

Dendritic cells (DC) have phagocytic capabilities (Satpathy, Wu, Albring, & Murphy, 2012), long membranous projections and are distributed in mucosal epithelium, lymphoid tissues and organ parenchyma (Abbas et al., 2012). They express receptors similar to macrophages that detect microbial and not mammalian molecules and respond to them by secreting cytokines (Abbas et al., 2012). When the so-called *conventional dendritic cells* or *classical dendritic cells* (cDC) (Jiao et al., 2014), which constitute a major part of the DCs, are activated by microbes they become mobile and migrate from the mucosa, the skin and organ parenchyma to lymph nodes (Abbas et al., 2012). There they present (Satpathy et al., 2012) the microbial antigens to T cells (Abbas et al., 2012; Male et al., 2007).

Follicular dendritic cells (FDCs) can be found intermingled in in specialized collections of activated B cells, named germinal centers, in the spleen, musical lymphoid tissues and lymphoid follicles of the lymph nodes (Abbas et al., 2012). FDCs are unrelated to the dendritic cells that display antigens to T cells (Abbas et al., 2012). However, they trap antigens complexed to antibodies or complement products and present them on their surfaces for recognition by B cells (Abbas et al., 2012; Male et al., 2007).

DCs and FDCs form the two main types of the antigen-presenting cells (APCs) (Abbas et al., 2012). Further B cells and macrophages also function as APCs. Macrophages display antigens to helper T cells at the sites of infection. This antigen presentation leads to the activation of helper T cell and to the production of molecules, which in turn activate the macrophages. B cells display antigens to T helper cells in the spleen and lymph nodes, which is a crucial step in the cooperation of B cells with helper T cells in humoral immune responses to protein antigens (Abbas et al., 2012). APCs link the innate and the adaptive immune systems by producing cytokines (Male et al., 2007).

1.2 CYTOKINES AND THE ACUTE PHASE RESPONSE

Cytokines are intercellular signaling polypeptides that are produced by activated cells and have multiple functions, multiple sources and multiple targets (Gabay & Kushner, 1999). They act as key modulators of the immune system as well as of inflammation (Bak & Mikkelsen, 2010). Generally, cytokines bind to their cognate receptors on the cell surface, trigger transcriptional changes and also balance cellular activities like growth, cell survival and differentiation (Bak & Mikkelsen, 2010). For example, they have an important role during T cell differentiation (Shachar & Karin, 2013). Cytokines that are produced during inflammatory processes and which also participate in those processes are the main stimulators of the production of acute phase proteins (APPs) (Gabay & Kushner, 1999).

APPs can be characterized as proteins whose plasma concentration increases with inflammatory reactions (positive APPs) or decrease with inflammatory reactions (negative APPs) (Ahmed, Jadhav, Hassan, & Meng, 2012) and include anti-proteasis, transport proteins, complement factors and clotting proteins (Black, Kushner, & Samols, 2004). Inflammatory cytokines including tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) (Ahmed et al., 2012; Venteclef, Jakobsson, Steffensen, & Treuter, 2011) and interleukin 1β (IL-1β) are the main inducers of the acute phase response (APR) and are predominantly produced by monocytes and macrophages at centers of inflammation (see Fig. 1) (Venteclef et al., 2011). Mediators like IL-6 affect subsets of acute phase genes and can either synergistically inhibit or increase their respective effects by affecting transcriptional and to some extent also posttranscriptional levels (Bauer, Press, & Trauner, 2013). These signaling events can lead to an up regulation, e.g. of the APP C-reactive protein (CRP), or down regulation of proteins like albumin (Bauer et al., 2013; Tanaka & Kishimoto, 2012).

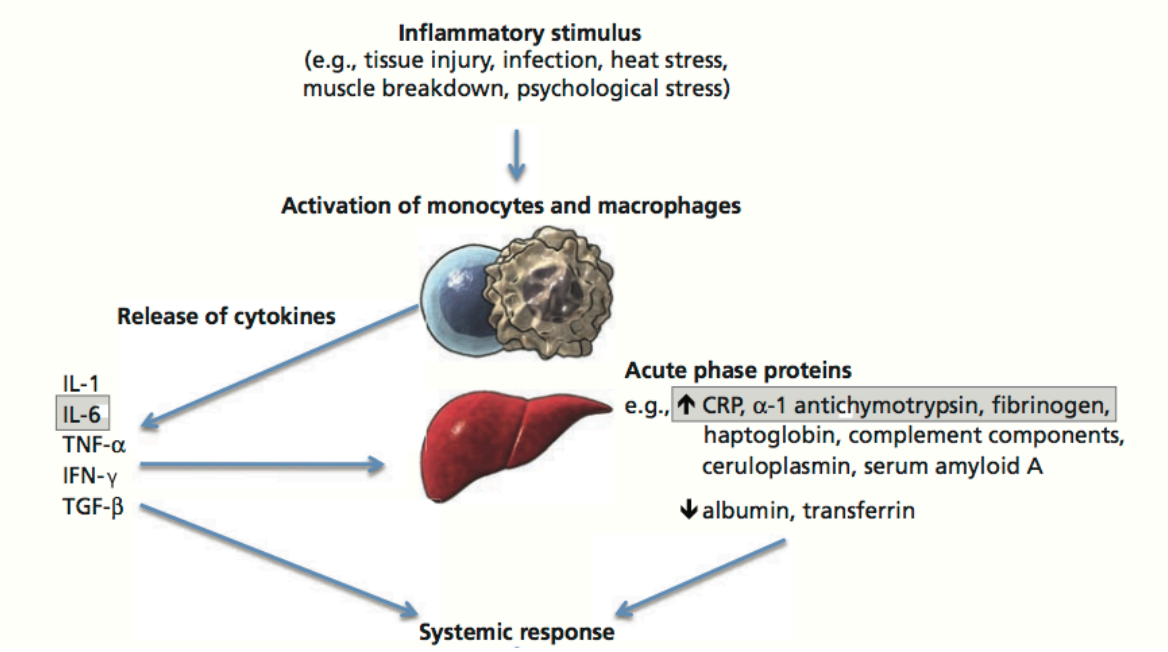

Figure 1. *Schematic representation of the acute phase response (IFN = interferon) (Anglin, Rosebush, & Mazurek, 2010)*

The APR is part of the early defense mechanisms of the human body and its principal physiological role is to trigger the innate immune response (Venteclef et al., 2011). The 'classic' APR is a primary and temporary response, which is linked to acute inflammation triggered by injury and infections with the goal of restoring tissue homeostasis in the human body. In contrast, low-grade 'chronic' APR can be seen as a prolonged response linked to an inflammation that is triggered metabolically. It can be caused by metabolic disturbances, tissue stress and malfunction, nutrients or is implicated in metabolic syndrome disorders, thus obesity, atherosclerosis, type 2 diabetes, insulin resistance or cardiovascular complications (Venteclef et al., 2011). In the liver, more than 200 APPs are primarily synthesized in and secreted from hepatocytes (Bauer et al., 2013; Venteclef et al., 2011) and then transported to peripheral tissues (Venteclef et al., 2011).

1.2.1 C-REACTIVE PROTEIN (CRP)

CRP is an effector and recognition molecule from the innate immune system that plays a role in innate responses and also as an adaptor to the adaptive immune system (Du Clos & Mold, 2004). It is binding to specific molecular configurations that are normally found on the surface of pathogens or are exposed during cell death (Black et al., 2004). At the peak of inflammatory responses CRP is very rapidly synthesized by hepatocytes (Black et al., 2004), so that the plasma level of CRP in humans can rise markedly and rapidly as much

as 1,000-fold or more (Black et al., 2004; Nicklas & Brinkley, 2009). Plasma levels of CRP mirror the level of circulating IL-6 (Du Clos & Mold, 2004; Fischer, Berntsen, Perstrup, Eskildsen, & Pedersen, 2007), because CRP is mainly controlled by IL-6 (Yao et al., 2014) and also correlates with markers of the APR (Du Clos & Mold, 2004). CRP increases and decreases more dramatically and rapidly than many other APPs (Du Clos & Mold, 2004). This characteristic makes it a useful marker for the monitoring of the clinical course of diseases or response to treatment (Du Clos & Mold, 2004; Pepys & Hirschfield, 2003) and is one of the most commonly measured biomarker of systemic inflammation (Lapice et al., 2009; Yao et al., 2014). Beyond that it has been confirmed that it is an independent risk factor for all-cause mortality and cardiovascular disease (Kengne, Batty, Hamer, Stamatakis, & Czernichow, 2012). However, it is unlikely that CRP concentration itself is even a moderate causal factor in coronary heart disease (Collaboration et al., 2011). Besides it was demonstrated, that stable chronic obstructive pulmonary disease (COPD) patients have higher circulating CRP levels than healthy patients so that those values can be used for long-term prediction of prospective COPD outcomes in persons with airway obstruction (Deng et al., 2014). There has also been described a graded association of CRP with progression and extent of atherosclerosis, whose strength is dependent on the used measure of atherosclerosis (Elias-Smale, Kardys, Oudkerk, Hofman, & Witteman, 2007). Further association between high-sensitivity CRP (hs-CRP) and abdominal adiposity (Lapice et al., 2009) as well as with non-vertebral fractures in men and women (Dahl et al., 2015) have been confirmed.

1.2.2 TUMOR NECROSIS FACTOR α (TNF-α)

Proteins of the TNF family include secreted cytokines and membrane proteins that bind to receptors on the cell surface (Caminero, Comabella, & Montalban, 2011), which can be found on virtually all cells in the body (Hajeer & Hutchinson, 2000). TNF-α is a proinflammatory cytokine that is mainly produced by infiltrating macrophages (Finck & Johnson, 2000; Hajeer & Hutchinson, 2000) and also from adipose tissue (Coppack, 2001). It has the ability to induce the production of additional pro-inflammatory mediators like IL-6 and IL-8 as a main feature of its pro-inflammatory activity (Williams et al., 2008). TNF-α is involved in inflammatory and cellular immune reactions and its circulating levels underlie individual variations (Hajeer & Hutchinson, 2000). Moreover, TNF-α has been implicated in the severity of various immune-regulated diseases like autoimmune diseases and transplantation (Hajeer & Hutchinson, 2000). TNF-α is described as one of those inflammatory mediators that increase across various age-related diseases (Franceschi &

Campisi, 2014). Therefore it is one of the inflammatory markers that are most commonly associated with disability and chronic diseases related to age (Singh & Newman, 2011). In 100-year-old persons TNF-α was confirmed as an independent prognostic marker for mortality (Bruunsgaard, Andersen-Ranberg, Hjelmborg, Pedersen, & Jeune, 2003), but in individuals aged 80 and older elevated TNF-α levels could not be associated with mortality (Giovannini et al., 2011). Also the soluble TNF-α receptor 1 (sTNF-RI), which is a member of the TNF-α superfamily, was shown to be a powerful predictor of 10-year mortality in community-dwelling older adults, measured in 1,155 participants of the large InCHIANTI study population (Varadhan et al., 2014). Schaap et al. (2009) observed strong associations between TNF-α and its soluble receptors and a decline in grip strength, so that they might also be important markers of loss of muscle strength and mass.

1.2.3 INTERLEUKIN-6 (IL-6)

The pleiotropic cytokine IL-6 (O'Reilly, Cant, Ciechomska, & van Laar, 2013) is produced by a broad spectrum of activated cell types involved in autoimmunity, like B cells, T cells, plasmacytoid dendritic cells (pDC) and cDCs (Yao et al., 2014). The molecular mass of IL-6 lies between 21-28 kDa and depends on glycosylation of the protein (O'Reilly et al., 2013).

The cytokine IL-6 uses two different mechanisms to trigger its biological effects (Calabrese & Rose-John, 2014; Hunter & Jones, 2015). Leukocytes including B cells and T cells as well as hepatocytes express IL-6 receptors (IL-6R) (O'Reilly et al., 2013). IL-6R is used for the so-called classic signaling (see Fig. 2A), whereas the cytokine IL-6 binds the IL-6R and a common shared subunit, the signaling molecule glycoprotein130 (gp130) (Hunter & Jones, 2015; O'Reilly et al., 2013). The presence of gp130 is required for signal transduction, although IL-6R is sufficient for low-affinity binding (O'Reilly et al., 2013). Finally, after some further steps of the signaling pathway it leads to the transcription of target genes (O'Reilly et al., 2013). The second mechanism, termed trans-signaling enables cells that do not express IL-6R on their cell surface to respond to IL-6 cytokines (Hunter & Jones, 2015). Therefore, a soluble IL-6R (sIL-6R) is binding the IL-6 cytokine and gp130 so that IL-6 can act on these cells (see Fig. 2B) (Hunter & Jones, 2015; O'Reilly et al., 2013).

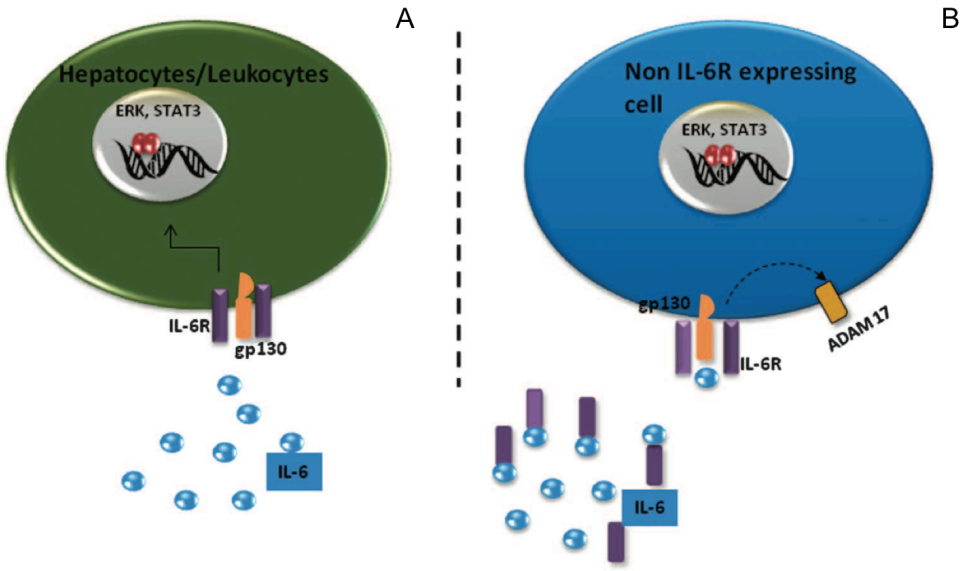

Figure 2. *IL-6 signaling pathways (classic pathway; trans-signaling pathway) (O'Reilly et al., 2013)*

IL-6 takes part in a wide range of biological activities in immune regulation, acute-phase reaction, hematopoiesis, inflammation (Kimura & Kishimoto, 2010; Kishimoto, 2010) and oncogenesis (Kishimoto, 2010; Singh & Newman, 2011). Yao et al. (2014) also stated, that biological functions of IL-6 contribute to pathogenesis of cancer and inflammatory autoimmune diseases. Additionally a deviant expression of IL-6 is associated with a variety of diseases like Castleman's disease, osteoporosis and rheumatoid arthritis (O'Reilly et al., 2013) or systemic juvenile arthritis and Crohn's disease (Kishimoto, 2006). Further, it is an important cytokine for plasma B cell survival and B cell differentiation (Yao et al., 2014). IL-6 induces activation of hematopoietic stem cells and the maturation of megakaryocytes into platelets (Tanaka & Kishimoto, 2012). In addition, it promotes angiogenesis and the differentiation of osteoclasts, the proliferation of mesangial cells and keratinocytes, and the growth of myeloma and plasmacytoma cells (Tanaka & Kishimoto, 2012). IL-6 is concomitantly involved in the regulation of proinflammatory and anti-inflammatory activities and contributes to the development as well as the resolution of the acute inflammatory response (Hunter & Jones, 2015; Maggio, Guralnik, Longo, & Ferrucci, 2006). Varadhan et al. (2014) concluded, that IL-6 is a powerful predictor of mortality for 10 years in older adults living in retirement homes, based on the results from their research with the InCHIANTI study cohort. Another finding from the InCHIANTI study suggests, that IL-6 is associated with low plasma levels of high density lipoprotein cholesterol (HDL-C), but due to the applied cross –sectional study design it was not possible to distinguish whether high IL-6 levels causes low HDL-C levels or vice versa (Zuliani et al., 2007). Aside from that, IL-6 is a commonly used marker of inflammatory status (Franceschi & Campisi, 2014). In

the literature IL-6 is also described as a myokine, which are cytokines or other peptides that are produced, expressed and released by muscle fibers (Pedersen & Febbraio, 2012; Pedersen & Fischer, 2007; Pedersen et al., 2003; Petersen & Pedersen, 2005).

1.3 AGEING AND THE IMMUNE SYSTEM

1.3.1 IMMUNOSENESCENCE

The process of ageing is a complex phenomenon that involves gradually and homogeneously the integrity of all organs of the human organism (Malaguarnera et al., 2001). The progressive decay of tissue functions results in the long term in organ dysfunction and death (Montoya-Ortiz, 2013). Senescence is not represented by a pre-established moment, rather it consists of a slow and continuous preparation of the organism for a morpho-functional involution as part of the normal biological cycle (Malaguarnera et al., 2001). The complex alterations in connection with the senescence may interfere with functions of the immune system (Malaguarnera et al., 2001).

The progressive deterioration of adaptive and innate immune responses is the result of age-dependent decreases in immunological competence, often referred to as 'immunosenescence' (Goronzy & Weyand, 2013; Montoya-Ortiz, 2013). The ageing process is associated with changes in the numbers of innate immune cells, which can be explained in some cases by the redistribution of cell subsets (Solana et al., 2012). In general, a reduction of the NK cell mediated cytotoxicity and disturbances in macrophage-derived cytokine release lead to increased prevalence of infections (Ongradi & Kovesdi, 2010). Moreover, ageing is associated to decreased main functions of innate immune cells, caused by an altered signal transduction pathways and changes in the expression of various innate immune cell receptors (Solana et al., 2012). As a consequence of these changes, the ability to collaborate in the initiation of the adaptive immune response can be impaired (Solana et al., 2012). Panda et al. (2009) also note that normal human ageing affects several aspects of the innate immune response, which leads to a reduced ability to provide the prompt response to viral and bacterial pathogens and also to influence and integrate with the adaptive immune response.

Sansoni et al. (2008) mention, that the innate immune system, in comparison to the more sophisticated adaptive compartment, is relatively preserved during ageing, while the latter

manifests more profound modifications. Müller et al. (2013) also concludes that in general the adaptive arm of immunity has a grater susceptibility to immunosenescence, relative to the innate arm. A possible explanation for this is the necessity of maintaining clonal expansion of memory cells, which are unable to self-renew in the way that cells of the innate immune system can (Müller et al., 2013).

Pawelec (2007) points out that immunosenescence contributes to the heightened susceptibility of the elderly to infectious diseases. The defense against pathogens is impaired mainly because of alterations in adaptive immunity mediated by B and T cells (Pawelec, 2007). The ability of B cells to produce antibodies against novel antigens is dampened by the ageing process, which leads to a replacement of naïve cells with exhausted memory B cells (Ongradi & Kovesdi, 2010). Multiple changes (see Fig. 3A) like reduced output of new T cells, thymic involution, deficiencies in cytokines production, and accumulation of anergic memory cells results in a decline of cell-mediated immunity (Ongradi & Kovesdi, 2010). The accumulation of senescent T cells, which are phenotypically characterized by the absence of cluster of differentiation 28 (CD28) expression and the acquisition of killer cell lectinlike receptor G1 (KLRG1) and CD57 (Simpson, 2011), is another notable feature of immunosenescence (see Fig. 3C) (de Araujo et al., 2013). Moreover, Spielmann et al. (2014) reported about relations between obesity and T cell differentiation that are associated with immunosenescence in adolescents.

The presence of linear chromosomes is a defining feature of the eukaryotic genome, which poses several challenges regarding chromosomal replication and maintenance (Andrews, Fujii, Goronzy, & Weyand, 2010). Linear chromosomes are capped by repetitive nucleoprotein structures named telomeres (Goronzy, Fujii, & Weyand, 2006; Simpson, 2011) to suppress gross chromosomal rearrangements and the loss of coding sequences (Andrews et al., 2010). Human telomeres are 10- to 15-kb-long, guanine-rich, tandem repeats of hexarmer sequences (TTAGGG), which are associated with diverse specific proteins (Goronzy et al., 2006; Montoya-Ortiz, 2013; Weng, 2006). Each cell division leads to a progressive shortening of telomeres (see Fig. 3D), which promotes genome senescence, instability and apoptosis if it comes below a certain threshold (Andrews et al., 2010; Tarazona, Solana, Ouyang, & Pawelec, 2002; Weng, 2006). Telomer loss can be considered a mitotic clock, which is approximately reflecting the life history of divisions of individual cells by telomere length (Goronzy et al., 2006). Montoya-Ortiz (Montoya-Ortiz, 2013) states that immunosenescence is caused by molecular regulatory machinery alterations and is closely related to telomere erosion in the chromosomes.

In recent years, there has been an increasing amount of literature on the so-called cytomegalovirus (CMV) and its role in immunosenescence (Derhovanessian, Larbi, & Pawelec, 2009; Khan et al., 2002; Olsson et al., 2000; Pawelec, 2012; Sansoni et al., 2008; Vescovini et al., 2014). This virus is a persistent activating virus of the β-herpesvirus family (Pawelec, Koch, Franceschi, & Wikby, 2006; Pawelec, McElhaney, Aiello, & Derhovanessian, 2012). It resides in the myeloid cell compartment but can also infect other cell types (Pawelec et al., 2006). Infection can occur in neonates of CMV-infected mothers via mother´s milk, or at any time thereafter via intimate contact of different kinds (Derhovanessian et al., 2009; Pawelec et al., 2006). In most Western populations, the main part of people are infected by middle age and it can be hard to find persons >65 who are not infected (Pawelec et al., 2006). The infection is usually asymptomatic and seems to pass unnoticed in immunocompetent hosts (Derhovanessian et al., 2009; Pawelec et al., 2006) clinical problems emerge only in immune-deficient persons (Pawelec et al., 2012). The CMV leads to an expansion of very large numbers of CMV-specific T cells, which results in a reduction of the CD4/CD8 ratio and a suppression of the numbers of naïve T cells (Savva et al., 2013). The CMV-specific T-cell populations in $CD4^+$ as well as $CD8^+$ T-cell subsets are differentiated to a high degree and have shorter telomeres (Pawelec et al., 2005). Derhovanessian et al. (2009) argued that CMV contributes substantially to immunosenescence. According to Pawelec et al. (2012) the possibility that indirect consequences of CMV infection could include numerous age-associated disease syndromes should be considered.

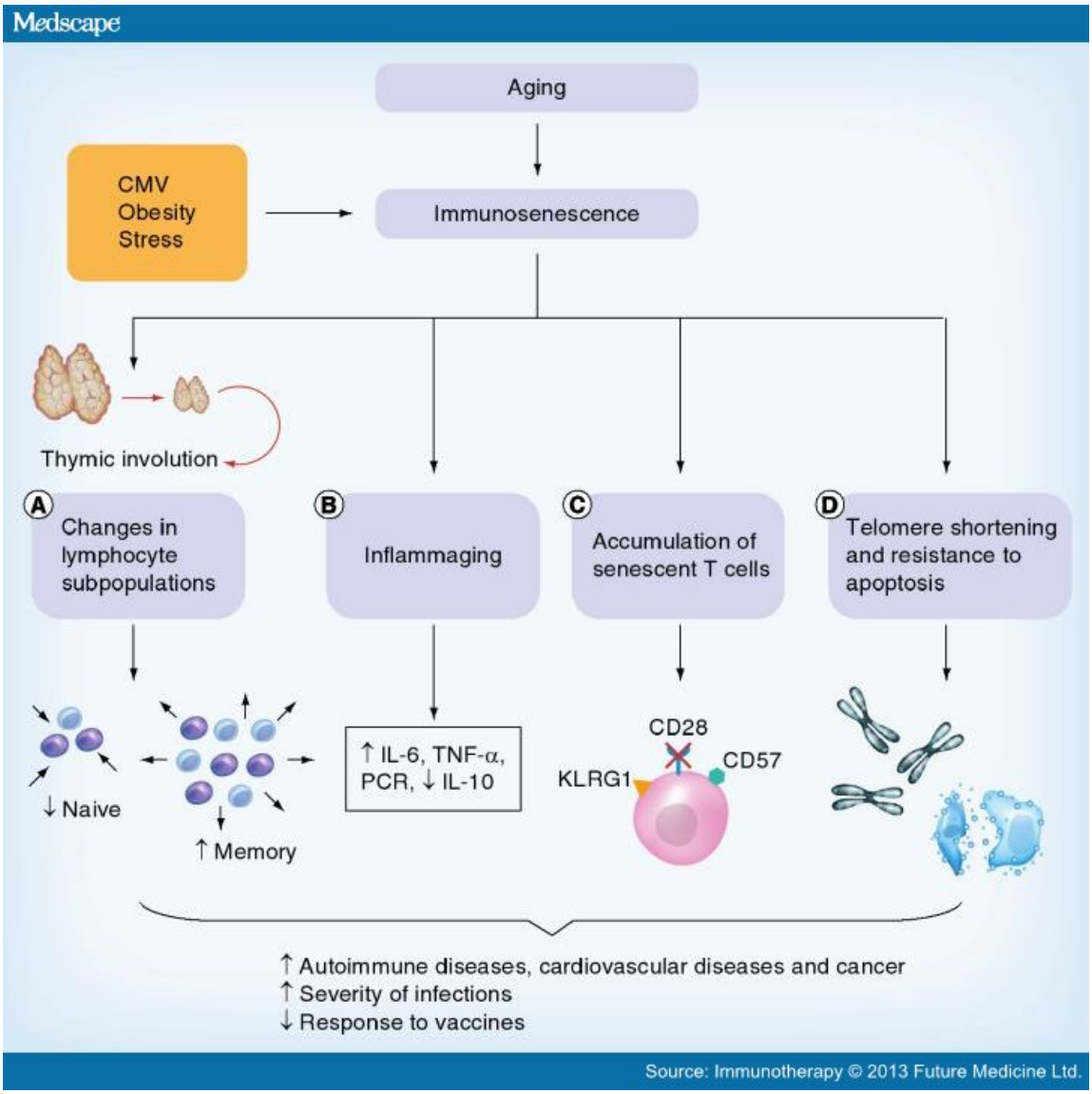

Figure 3. *Age-related immunological changes (de Araujo et al., 2013)*

1.3.2 LOW-GRADE CHRONIC INFLAMMATION (INFLAMMAGEING)

Generally, the inflammatory response is thought to operate during serious disturbances of homeostasis, like traumatic tissue injury, infection and the invasion of pathogens (Medzhitov, 2008) and can be beneficial as a transient, acute immune response to these harmful conditions (Franceschi & Campisi, 2014). In addition, it facilitates the turnover, repair and adaption of many tissues (Franceschi & Campisi, 2014). The cytokines TNF-α, IL-1β, IL-6, interleukine 1 receptor antagonist (IL-1ra), sTNF-RI and interleukin 10 (IL-10) appear in the circulation in relation to an acute infection in the mentioned order (Pedersen & Febbraio, 2008; Petersen & Pedersen, 2006). Acute as well as chronic inflammation are different types of adaptive response that are commonly activated when other homeostatic mechanisms are either not competent or insufficient (Medzhitov, 2008). Injuries and infec-

tions are at the extreme end of a range of conditions that can cause inflammation and the triggered responses are of the highest magnitude. Very low stress levels like mild tissue-specific malfunctions might be handled by tissue-resident cells (primarily mast cells and macrophages) whereas more extensive damage or malfunctions may require additional leukocytes to be recruited and plasma proteins to be delivered to the affected areas (Medzhitov, 2008).

Chronic inflammation has many characteristics of acute inflammation but it is normally persistent and of low grade and in further consequence it results in responses that lead to tissue degeneration (Franceschi & Campisi, 2014). The term 'chronic low-grade systemic inflammation' was introduced for conditions in which the systemic concentrations of TNF-α, IL-1, IL-6, IL-1ra, sTNF-R and CRP are increases two- to three times (Pedersen & Febbraio, 2008). Further, it is a pervasive feature of a majority of age-related diseases as well as of ageing tissue (Cevenini et al., 2013; Franceschi & Campisi, 2014; Zhuang & Lyga, 2014). Obesity, atherosclerosis, diabetes type II, asthma and neurodegenerative diseases are examples of these human diseases that are characterized by low-grade inflammation (Medzhitov, 2008). A great number of these chronic inflammatory diseases that are not caused by injury or infection seem to be connected to conditions that were not present in the early stages of evolution of human beings such as physical inactivity, continuous supply of high-caloric nutrients, exposure to toxic compounds, low levels of physical activity and age (Medzhitov, 2008).

There are different mechanisms that could potentially trigger chronic inflammation. For example, constant production of reactive molecules by infiltrating leukocytes designed to eliminate pathogens, eventually damages the cellular and structural components of tissues (Franceschi & Campisi, 2014). Besides, activated immune cells and damaged nonimmune cells induce the production of cytokines that alter the phenotypes of nearby cells and modulate or intensify the inflammatory response, often to the disadvantage of the normal tissue function (Rodier & Campisi, 2011). Another mechanism could be the interference with 'anabolic signaling', like TNF-α and IL-6 downregulate insulin, insulin-like growth factor-1 (IGF-1), erythropoietin and signaling protein synthesis after a bout of exercise or the intake of a meal (Franceschi & Campisi, 2014).

The term 'inflammageing' was established by Franceschi et al. (2000) to describe the low-grade chronic systemic inflammation in the ageing process, in the absence of obvious infection. It is described as a highly significant risk factor for mortality as well as morbidity in the elderly people. Moreover a progressive increase of proinflammatory status is char-

acteristic for the process of ageing (Franceschi et al., 2000) and is revealed by constantly high serum levels of TNF-α, IL-6, CRP and reduced IL-10 levels (see Fig. 3B) (de Araujo et al., 2013).

In the current literature, several different sources of inflammageing are described (Franceschi & Campisi, 2014): (1) Damaged cells (self-debris) and macromolecules that accumulate with age because of an increased production and/or inadequate elimination could be a source of inflammageing. Cell and organelle injury lead to the emission of self-debris, which can function as endogenous 'damage'-associated molecular patterns and mimic bacterial products, so that the innate immunity is activated. (2) The human gut or oral microbiota change during the ageing process and may thereby produce harmful products and metabolites, which have local and systemic inflammatory effects. The gut appears to diminish its ability to sequester these microbes and their harmful products with age (Franceschi & Campisi, 2014). In elderly, defects of the mucosal barrier can lead to an overcoming stimulation of immune cells by nonpathogenic products of bacteria (Guigoz, Dore, & Schiffrin, 2008). Furthermore, the complex bacterial community from the gut seems to limit the accumulation of potentially pathogenic bacteria and infections, being able to influence the efficiency of the immune system (Cevenini et al., 2013). (3) Additionally, the increasing activation of the coagulation system with age could expedite the inflammageing process (Franceschi & Campisi, 2014). Coagulation and the inflammation system share many components and strong interactions, so that it might be considered as a part of the latter. (4) Cellular senescence as a response to stress and damage can be defined as being a further source for inflammageing. Usually, the senescence response contributes to optimal wound healing in healthy tissue and prevents cancer by suppressing the proliferation of cells with a compromised genome, but persistent senescent cells likely promote age-associated pathologies and ageing itself by their secretory phenotype. (5) Likewise, immunosenescence is described as a driver of inflammageing (Franceschi & Campisi, 2014).

1.4 TRANSFORMING GROWTH FACTOR β (TGF-β)

TGF-β superfamily members are secreted polypeptides that are expressed in most cell types and they play essential roles in differentiation and tissue morphogenesis (Feng & Derynck, 2005). In humans the TGF-β family consists of over 30 members (see Fig. 4) (Massague, 2012; Wakefield & Hill, 2013). It comprises TGF-βs, NODAL, activins, growth

differentiation factors (GDFs), anti-Müllerian hormone (AMH) and bone morphogenetic proteins (BMPs) (Wakefield & Hill, 2013). Most of them function as paracrine factors on cells nearby the source (Massague, 2012).

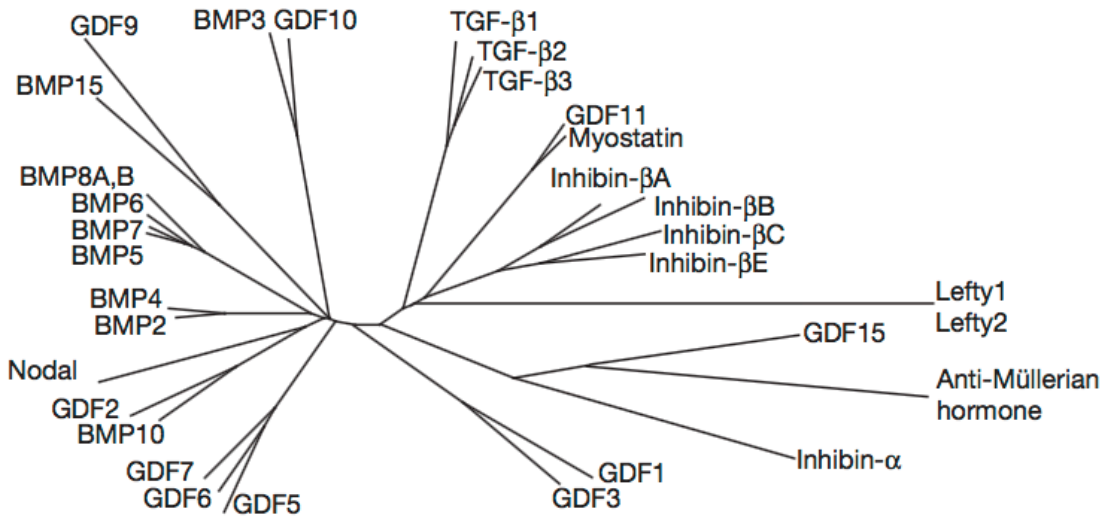

Figure 4. *TGF-β superfamily members depicted in form of a phylogenetic tree (Shi et al., 2011)*

There are three homologous isoforms of TGF-β (TGF-β1, TGF-β2, TGF-β3), which are encoded by specific genes (Blobe, Schiemann, & Lodish, 2000; Govinden & Bhoola, 2003; Munger & Sheppard, 2011) and are expressed in a developmentally regulated and a tissue-specific fashion (Blobe et al., 2000). The messenger RNA (mRNA) of the isoform TGF-β1 is primarily expressed in connective-tissue, hematopoietic and endothelial cells, TGF-β2 mRNA in neuronal and epithelial cells, and TGF-β3 mRNA in mesenchymal cells (Blobe et al., 2000). TGF-β1 is also produced by different lineages of leukocytes and stromal cells (Li & Flavell, 2008). The immune system predominantly expresses the isoform TGF-β1, but all of the three isoforms have similar properties (Li, Wan, Sanjabi, Robertson, & Flavell, 2006). TGF-β1 was the first factor that was characterized at the molecular level and can be considered as the prototype factor of the TGF-β superfamily (Derynck & Feng, 1997).

TGF-β is acting as a sustainer of tissue homeostasis by promoting tolerance in the immune system and by regulating pluripotency and differentiation in stem and progenitor cells (Macias, Martin-Malpartida, & Massague, 2015). Furthermore, signaling of TGF-β itself inhibits proliferation of many cell types like hematopoietic and epithelial cells and controls tumorigenesis (Feng & Derynck, 2005) by repressing the oncogenic progression

of premalignant cells (Macias et al., 2015). TGF-β plasma concentrations have also been described as stable prognostic maker of coronary artery disease (Tashiro, Shimokawa, Sadamatu, & Yamamoto, 2002). Additionally, TGF-β modulates a regulatory network on which crucial processes of T cell development, homeostasis, tolerance and differentiation highly depend on (Li & Flavell, 2008). In general, naïve $CD4^+$ T cells can differentiate into several subsets of T helper cells (e.g. T_H1, T_H2 and T_H17) (Bettelli, Korn, Oukka, & Kuchroo, 2008). Two subsets of $CD4^+$ T lymphocytes, the $CD4^+$ T_H17 cells and the regulatory T (T_{reg}) cells are linked to the mediation and regulation of autoimmune responses (Eisenstein & Williams, 2009). The pathogenic effector T_H17 cells (Oukka, 2007) show pro-inflammatory functions and play a critical role in diverse autoimmune disorders (Kimura & Kishimoto, 2010). In contrast, T_{reg} cells are pivotal in the maintaining of immune homeostasis. Hence, reduced numbers of T_{reg} cells or defects in T_{reg} functions as well as increased numbers of T_H17 cells or an excess in T_H17 functions, may trigger inflammatory disorders (Kimura & Kishimoto, 2010).

Under the influence of the TGF-β1 cytokine T_H17 as well as T_{reg} cells can develop from naïve $CD4^+$ T lymphocyte precursors (Eisenstein & Williams, 2009; Walsh et al., 2011). The presence of TGF-β leads to a differentiation of naïve T cells into T_{reg} cells that express the transcription factor forkhead box p3 (Foxp3) (see Fig. 5) (Bettelli et al., 2008). In contrast, TGF-β together with IL-6, which is produced by the activated innate immune system in case of infection or inflammation (Kimura & Kishimoto, 2010), induces the differentiation of naïve T cells into T_H17 cells that express the transcription factors Retinoic acid receptor γ (RORγ) and RORα (Kishimoto, 2010). Simultaneously IL-6 inhibits the T_{reg} cell differentiation that is induced by TGF-β (Kimura & Kishimoto, 2010; Kishimoto, 2010).

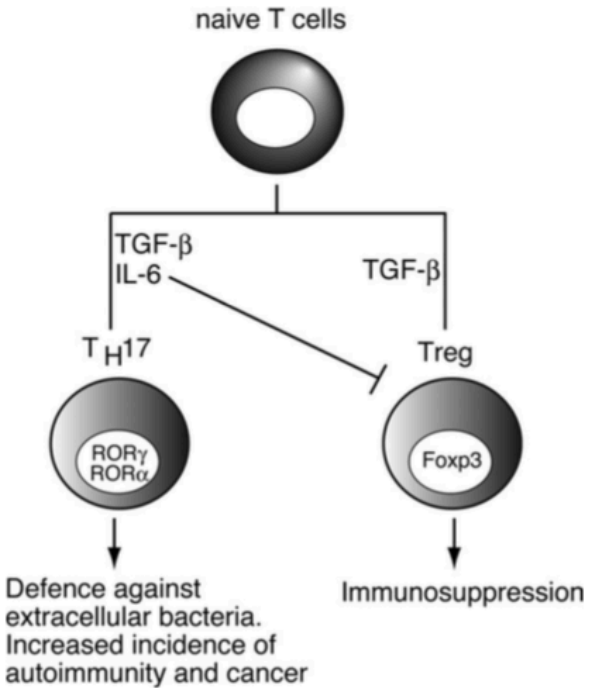

***Figure 5**. T cell differentiation by TGF-β (Kishimoto, 2010)*

In summary, TGF-β has essential functions in controlling both, pro-inflammatory and anti-inflammatory T cell responses in the presence of other cytokines, which are dictating the functional outcome of TGF-β signaling in T cells (Travis & Sheppard, 2014). TGF-β is associated with immunosuppressive functions (Li et al., 2006; Shachar & Karin, 2013) like inducing tolerance, containing and resolving inflammation (Li et al., 2006) on the one hand and also seems to facilitate pro-inflammatory responses by promoting TH17 cell development on the other hand (Mangan et al., 2006; Shachar & Karin, 2013).

1.4.1 TGF-β RECEPTORS AND TGF-β SIGNALING PATHWAY

Effects of the TGF-β ligands are mediated by signaling through transmembrane serine/threonine kinase type I (TGF-βRI) and type II (TGF-βRII) receptors (Kamato et al., 2013; Santibanez, Quintanilla, & Bernabeu, 2011). These receptors exist as homodimers at the cell surface (Feng & Derynck, 2005). The following structural features are characteristic for TGF-βRI and TGF-βRII (see Fig. 6): They have small (101 residues TGF-βRI and 136 residues TGF-βRII) extracellular domains (ECDs) and heavy disulfide bonds (Hinck, 2012). Further, they possess a three-finger toxin fold in their ligand binding ECDs, a single trans-membrane domain (TMD) as well as an intracellular serine-threonine kinase domain (S/TKD) (de Caestecker, 2004). Unlike TGF-βRII, TGF-βRI includes a ~ 20 amino

acid juxta-membrane glycine-serine rich regulatory domain, called the GS box (Hinck, 2012).

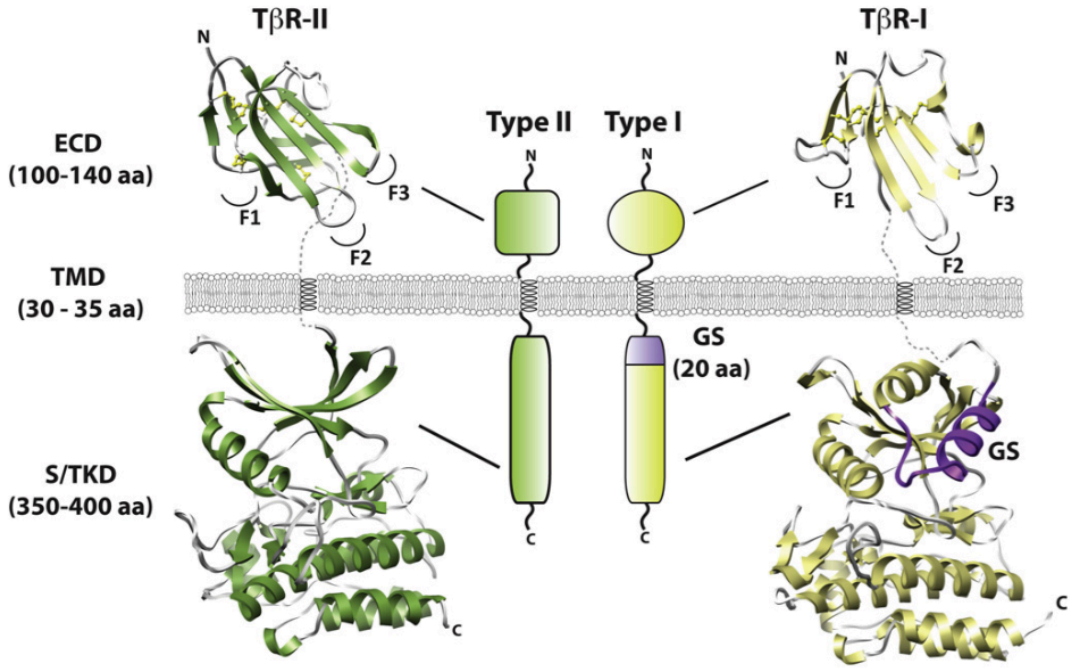

Figure 6. *Structural featrures of TGF-βRI and TGF-βRII; ECD = extracellular domain; TMD = transmembrane domain; S/TKD = serine-threonine kinase domain; aa = amino acid; GS = glycine-serine rich regulatory domain; c = c-terminal; n = n-terminal; F1-3 = the three fingers of receptor three-finger toxin fold (Hinck, 2012)*

The TGF-β superfamily signaling pathway is regulated at all levels, starting at the ligand level (Gordon & Blobe, 2008). An inactive precursor protein, which consists of a signal peptide named latency associated peptide (LAP) domain and a mature TGF-β1 (Gordon & Blobe, 2008), forming the small latent complex (SLC) (Annes, 2003; Hyytiainen, Penttinen, & Keski-Oja, 2004), is synthesized. In the SLC it is not possible for TGF-β to bind to its surface receptors (Rifkin, 2005). The SLC usually associates with a latent TGF-β binding protein (LTBP) and is secreted as a large latent complex (LLC) to the extracellular matrix (ECM) (Hyytiainen et al., 2004). The LLC is then covalently bound to the ECM through an isopeptide bond (Annes, 2003). Latent TGF-β can be activated by several different mechanisms like the activation by thrombospondin 1, enzymatic activation, regulation by glycoprotein A repetitions predominant protein (GARP), or the activation by integrins (Travis & Sheppard, 2014). Concentration of extracellular TGF-β activity is mainly regulated by the conversion of latent TGF-β to active TGF-β (Annes, 2003).

The signaling pathway initiates by a TGF-β ligand binding to and bringing together TGF-βRI and TGF-βRII serine/threonine kinases on the cell surface (see Fig. 7) (Shi &

Massagué, 2003), forming a tetrameric complex (Travis & Sheppard, 2014). As a result, TGF-βRII is able to phosphorylate the TGF-βRI kinase domain, which then transfers the signal through phosphorylation of so-called SMAD proteins (Kang, Liu, & Derynck, 2009; Shi & Massagué, 2003).

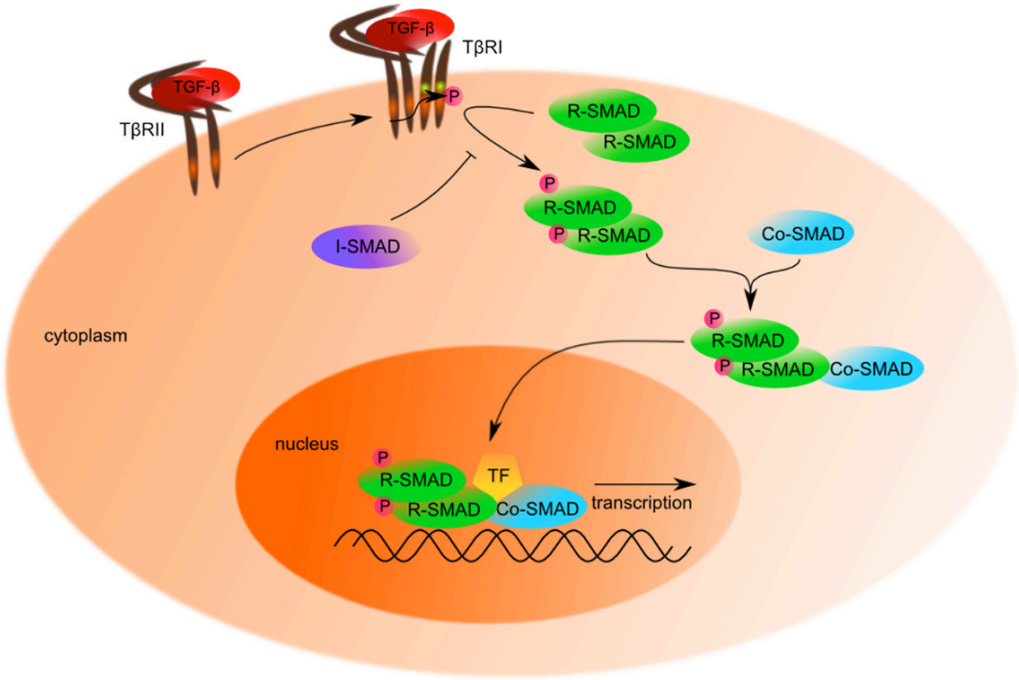

Figure 7. *TGF-β signaling pathway (Kubiczkova, Sedlarikova, Hajek, & Sevcikova, 2012)*

These SMAD transcription factors are the primarily used factors for the mediation of TGF-β responses (Shachar & Karin, 2013). There are eight different SMAD proteins that can be subdivided in three functional classes, namely receptor-regulated SMAD (R-SMAD), the inhibitory SMAD (I-SMAD) and the common-mediator SMAD (Co-SMAD) (Shi & Massagué, 2003). The R-SMAD group, consisting of SMAD1, SMAD2, SMAD3, SMAD5 and SMAD8, are directly phosphorylated and activated by TGF-βRI kinases (Shi & Massagué, 2003). Then they undergo homotrimerization and formation of heteromeric complexes with SMAD4 from the Co-SMAD class (Pardali & Ten Dijke, 2012; Shi & Massagué, 2003). These SMAD complexes are subsequently transported to the nucleus where they bind with specific transcription factors (TF) and induce the transcription of TGF-β dependent target genes (Kubiczkova et al., 2012; Pardali & Ten Dijke, 2012). By competing with R-SMADs for receptor or Co-SMAD interaction and by targeting the receptors for degradation, the two I-SMADs, SMAD6 and SMAD7 negatively regulate TGF-β signaling (Shi & Massagué, 2003).

Moreover, there are non-SMAD pathways that result in the activation of JNK, Akt/PKB, MAP kinases, small GTPases and other factors (Horbelt, Denkis, & Knaus, 2012; Zhang, 2009). These pathways activate transcriptional responses and also direct cellular responses without transcriptional regulation (Horbelt et al., 2012).

1.4.2 MICRORNAS (MIRNAS)

The 'classic' RNA can be subdivided in three main types, the mRNA, the transfer RNA (tRNA) and the ribosomal RNA (rRNA). mRNAs are translated into proteins and tRNA and rRNA have housekeeping roles during this translation process (Grosshans & Filipowicz, 2008). In comparison, small RNAs, like miRNAs, are not translated into proteins. Lin-4 and let-7 were the first two miRNAs that were discovered and identified in the worm Caenorhabditis elegans in the 1990s (Grosshans & Filipowicz, 2008).

miRNAs are about 22 nucleotides (nt) long, endogenous RNAs (Bartel, 2004) that are important regulators of post-transcriptional gene expression (Wang, Keys, Au-Young, & Chen, 2009). These non-coding RNAs (Carissimi, Fulci, & Macino, 2009) seem to be key regulators of immune cell function and development as well as disease pathogenesis (O'Connell, Rao, Chaudhuri, & Baltimore, 2010).

At the beginning of miRNA-biogenesis (see Fig. 8) miRNA genes are transcribed by RNA polymerase II (Ambros & Chen, 2007; Garzon, Calin, & Croce, 2009; Kim & Nam, 2006; Shenouda & Alahari, 2009) or III to generate primary miRNA transcripts (pri-miRNA) in the nucleus (Winter, Jung, Keller, Gregory, & Diederichs, 2009). In the following step the pri-miRNA transcripts are processed by the microprocessor complexes Drosha-DGCR8 (Choudhuri, 2010; Kim & Nam, 2006; Winter et al., 2009), resulting in about 70 nt long pre-miRNAs (Choudhuri, 2010; Kim & Nam, 2006). The hairpin formed pre-miRNAs are exported from the nucleus by Exportin 5 (Garzon et al., 2009; Jung & Suh, 2012; Winter et al., 2009). Then pre-miRNAs are processed into about 22 nt long miRNA duplexes (miRNA / miRNA*) by RNAse III Dicer (Choudhuri, 2010; Kim & Nam, 2006; Shenouda & Alahari, 2009). In the further course of miRNA biogenesis the functional strands of the miRNA duplexes are bound by Argonaute proteins to form RNA-induced silencing complexes (RISC), whereas the other strands of the duplexes are degraded (Kim & Nam, 2006). The single strands of mature miRNA guide RISC to its target mRNAs (Winter et al., 2009) so that it can downregulate gene expression by suppressing mRNA translation or mRNA degradation (Davis & Ross, 2008; Jung & Suh, 2012).

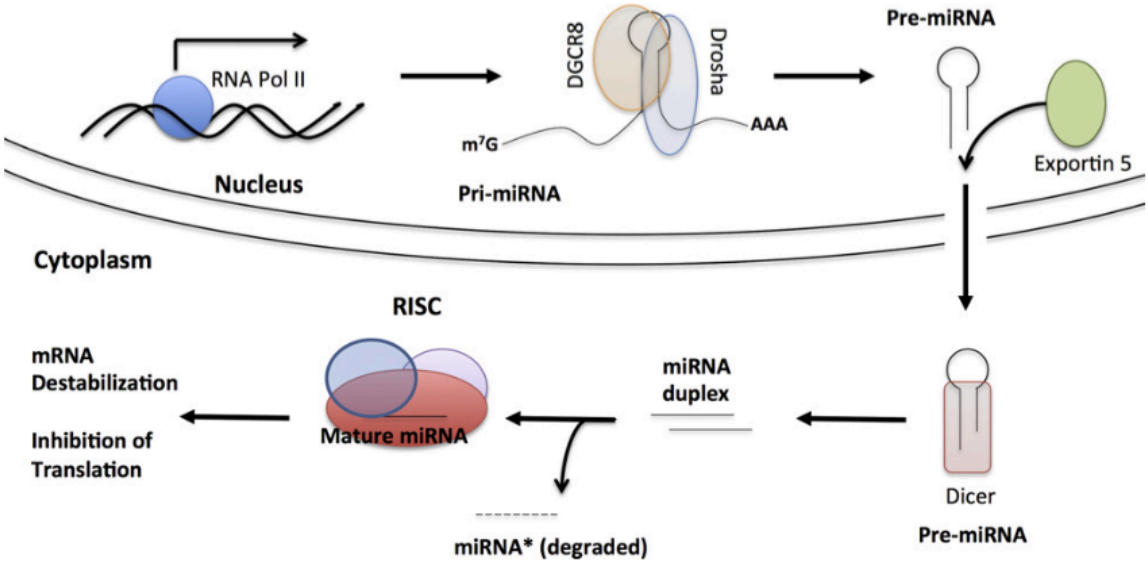

Figure 8. *Biogenesis of miRNAs (Blahna & Hata, 2012)*

miRNA regulation itself can be influenced by SMADs (see Fig.9). This regulation can be divided into two different mechanisms (Blahna & Hata, 2012). On the one hand there is the transcriptional regulation of miRNAs by SMADs, which is similar to the canonical TGF-β signaling pathway. The binding of a TGF-β ligand results in phosphorylation of an R-SMAD and the formation of an R-SMAD / Co-SMAD heterodimer. Then the complex is translocated to the nucleus and binds to the SMAD-binding element (SBE) to positively or negatively regulate the transcription of miRNA genes, so that the pri-miRNAs then undergo regular miRNA processing (Blahna & Hata, 2012). On the other hand there is the post-transcriptional regulation of miRNA biogenesis that acts on pri-miRNAs in the cell nucleus (Blahna & Hata, 2012; Davis, Hilyard, Nguyen, Lagna, & Hata, 2010). The phosphorylation of an R-SMAD leads to its import into the nucleus where it recognizes and binds an SBE-like sequence that is located in the stem section of the pri-miRNA. Next the R-SMAD recruits the microprocessor complex Drosha-DGCR8 to the pri-miRNA where it stimulates processing of the pri-miRNA into pre-miRNA (Blahna & Hata, 2012; Davis et al., 2010). Davis et al. (2008) showed for example that TGF-β and BMP modulate the fast post-transcriptional induction of miRNA-199a and miRNA-21 in human pulmonary smooth muscle cells (PASMCs). Further research revealed even more miRNAs, like miRNA-105, miRNA-215, miRNA-421 and miRNA-509 are regulated post-transcriptionally by BMP and TGF-β (Davis et al., 2010).

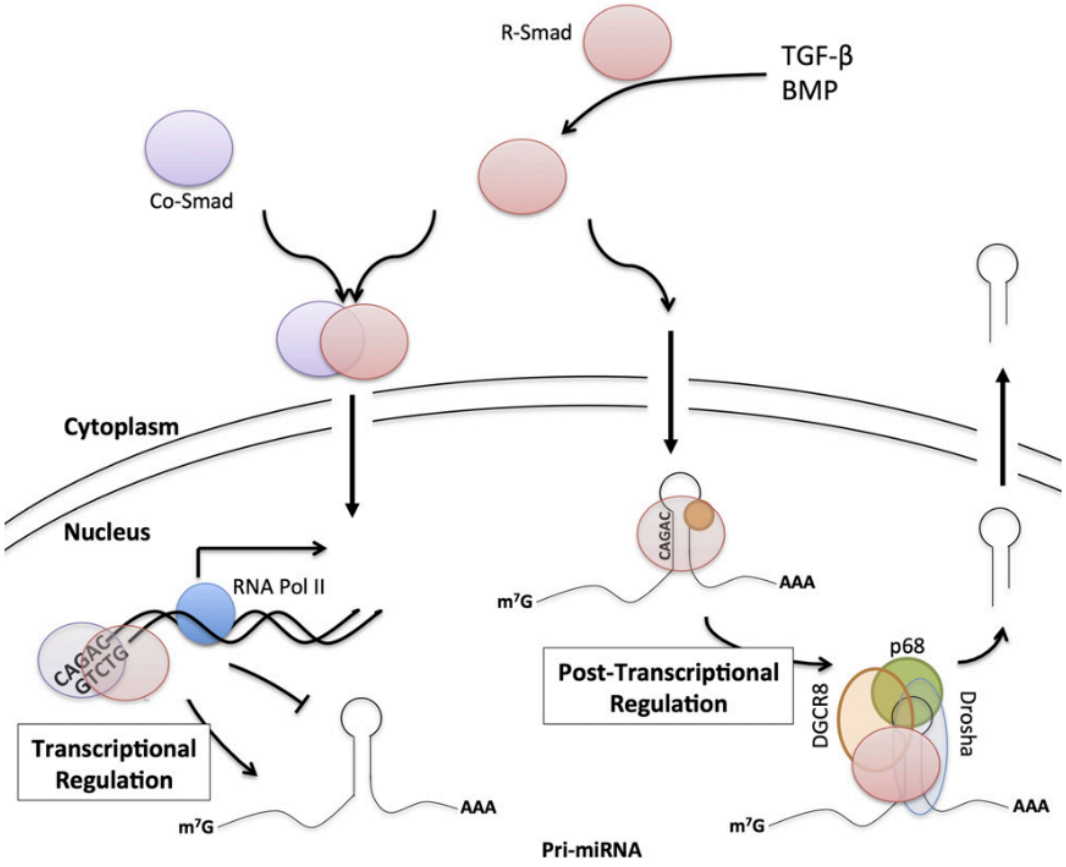

Figure 9. *miRNA expression regulated by SMADs (Blahna & Hata, 2012)*

Likewise, miRNAs can target SMAD proteins (Blahna & Hata, 2012), as depicted by Olivieri et al. (2012) (see Fig. 10). A big part of the SMAD proteins is targeted from even one or more miRNAs (Blahna & Hata, 2012). For instance, Marquez et al. (2010) were able to show that miRNA-21 represses SMAD7, which is a negative regulator of TGF-β signaling. The fact that miRNA-21 is able to down-regulate SMAD7 was confirmed by Wang et al. (2014).

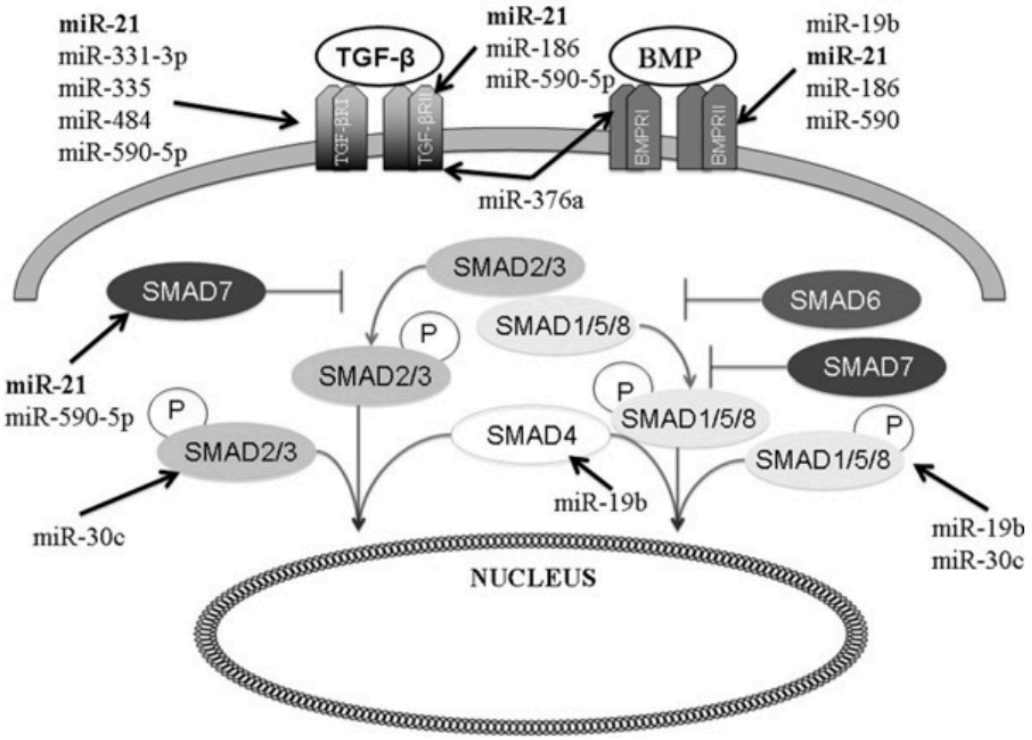

Figure 10. *Influence of miRNAs on SMADs (Olivieri et al., 2012)*

Investigations about roles of miRNAs in the regulation of mammalian hematopoiesis showed that various hematopoietic cell types and organs have special miRNA profiles and the expressions of distinct sets of miRNAs are dynamically regulated during B cell and T cell development (Sonkoly, Stahle, & Pivarcsi, 2008). Moreover, miRNAs seem to play a crucial role in embryotic stem cell maintenance, lineage determination and differentiation (Wang et al., 2009). Their expression is altered abnormally in most cancer types (Fabbri et al., 2012), but the function of miRNAs as tumor suppressors or as oncogenes depends on the expression and the tissue of their targets (Garzon et al., 2009). Furthermore, miRNAs are also detectable in a stable form outside the cell (Mooren, Viereck, Kruger, & Thum, 2014). These circulating miRNAs (c-miRNA) are protected from degradation by RNase by incorporation into microvesicles (Collino et al., 2010; Hunter et al., 2008) exosomes (Kosaka et al., 2010; Valadi et al., 2007) or protein complexes like Ago2 (Arroyo et al., 2011; Turchinovich, Weiz, Langheinz, & Burwinkel, 2011). As shown by an increasing number of studies, analyses of miRNA expression could be valuable in diagnosis, treatment and prognosis of tumors (Wei et al., 2011). Noren Hooten et al. (2010) identified several miRNAs in humans, which are down regulated with age. In contrast, Jung and Suh (2012) point out that altered miRNA function is linked to processes of ageing and a range of age-related diseases. It is likely that miRNAs are involved in T cell ageing and

play causal roles in immunosenescence (Kroesen et al., 2014). According to Franceschi and Campisi (2014) circulating miRNAs can maintain and propagate inflammageing and, to some degree the ageing phenotype itself. Olivieri et al. (2012) also conclude that circulating miRNAs, including miRNA-21 might be important for inflammageing, hence affecting the risk of major diseases that are related to the ageing process.

1.5 CYTOKINES AND EXERCISE

In general, exercise can protect against chronic low-grade inflammation associated diseases (Mathur & Pedersen, 2008; Petersen & Pedersen, 2005). According to Pedersen and Febbraio (2008) myokines might be involved in the mediation of health-beneficial effects of exercise and have a pivotal role in the protection against diseases associated with low-grade inflammation, hyperlipidemia, cardiovascular diseases, type 2 diabetes, insulin resistance and cancer. Myokines are secreted by contracting skeletal muscles into the circulation (Brandt & Pedersen, 2010; Pedersen, 2011; Pedersen & Fischer, 2007). However, some myokines directly act on the muscle itself (Pedersen & Febbraio, 2012). Important identified myokines are inter alia IL-4, IL-6, IL-7, IL-8, IL-15, myostatin, leukaemia inhibitory factor (LIF), IGF-1, fibroblast growth factor 2 (FGF-2), follistatin-like 1 (FSTL-1), irisin and brain-derived neurotrophic factor (BDNF) (Pedersen & Febbraio, 2012). Myokines are able to create a systemic anti-inflammatory environment and also bring about specific endocrine effects on visceral fat (Brandt & Pedersen, 2010).

The myokine IL-6 is expressed from type I and also type II muscle fibers (see Fig. 11) and acts locally within the muscle, for example through the activation of AMP-activated protein kinase (AMPK), or peripherally in different organs in a hormone like way (Pedersen & Fischer, 2007). In resting muscle, the cytokine encoding gene is silent, but it is rapidly activated by muscle contractions (Pedersen & Fischer, 2007). Commonly, IL-6 is the first myokine that is released into the circulation during physical exercise (Pedersen, 2009, 2011). Its level can increase up to 100-fold in an exponential fashion and declines after the exercise (Pedersen, 2009, 2011). The carbohydrate availability in muscles influences the production of IL-6 and, in addition, IL-6 works as an energy sensor (Pedersen & Fischer, 2007; Petersen & Pedersen, 2005). It has been shown that skeletal muscle maintains its function as an endocrine organ in age and that even a normal capability of IL-6 cytokine production and release is preserved in healthy elderly people in comparison to younger individuals (Pedersen et al., 2004).

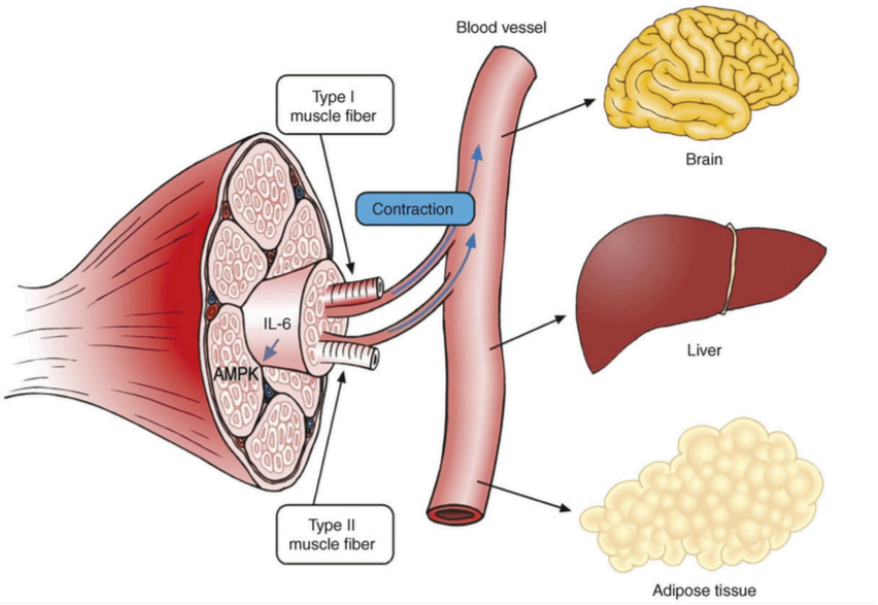

Figure 11. *Contractions from skeletal muscles lead to the release of the myokine IL-6 (Pedersen & Fischer, 2007).*

The increase in IL-6 induced by exercise is related to exercise duration, intensity, recruited muscle mass and the persons endurance capacity (Febbraio & Pedersen, 2002). IL-6 is inducing a pro-inflammatory response when it is signaling in macrophages or monocytes, whereas the activation and signaling of IL-6 in the muscle is completely independent of a previous TNF-response (Brandt & Pedersen, 2010). Hence, IL-6 derived from muscles seems to inhibit the low-grade production of TNF-α, thus TNF-α induced insulin resistance (Pedersen & Fischer, 2007). These differences can be seen in comparison between cytokine cascade in sepsis and in exercise. The cytokine response in sepsis (see Fig. 12A) includes the secretion of TNF-α, IL-1β, IL-6, IL-1ra, TNF-R and IL-10 in the first few hours (Petersen & Pedersen, 2005). In contrast, in the reaction to exercise (see Fig. 12B) TNF-α and IL-1 are not included (Petersen & Pedersen, 2005), but it is characterized by a distinct increase in IL-6, followed by IL-1ra, TNF-R and IL-10 (Pedersen, 2011; Petersen & Pedersen, 2005). This appearance of IL-1ra and IL-10 also contributes to the mediation of the anti-inflammatory effects of exercise (Petersen & Pedersen, 2005).

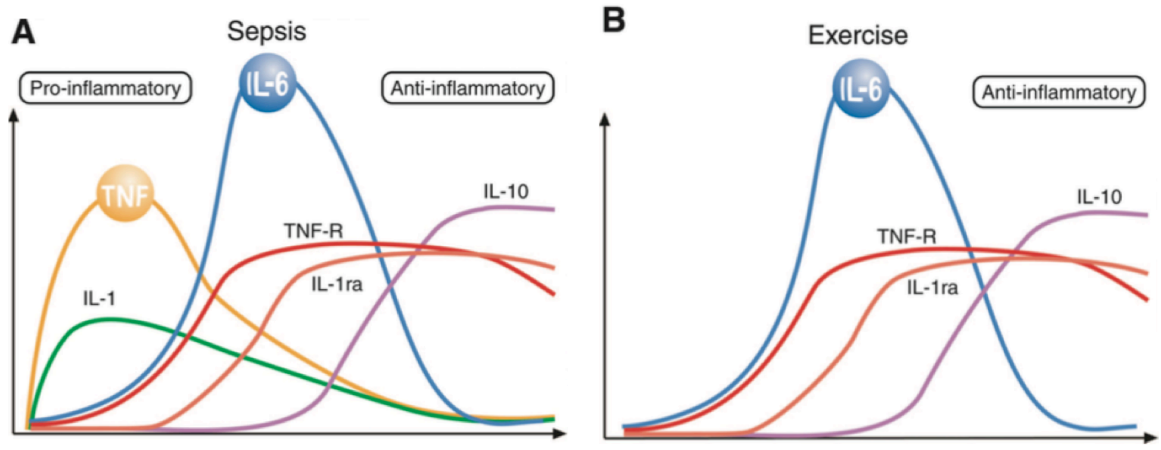

Figure 12. *Differences in cytokine levels between sepsis and exercise (Petersen & Pedersen, 2005)*

1.6 MIRNAS AND EXERCISE

Recent studies investigated the influence of different forms of exercise on miRNAs (Davidsen et al., 2011; Drummond, McCarthy, Fry, Esser, & Rasmussen, 2008; Mueller et al., 2011; Nielsen et al., 2010; Pasiakos & McClung, 2013; Radom-Aizik, Zaldivar, Oliver, Galassetti, & Cooper, 2010; Radom-Aizik, Zaldivar, Haddad, & Cooper, 2014; Russell et al., 2013; Wessner, Gryadunov-Masutti, Tschan, Bachl, & Roth, 2010). Davidsen et al. (2011) examined the differences in miRNA expression levels of 21 miRNAs of 56 young, healthy men after performing a 12-week resistance training program 5 days per week. The subjects were divided into 'high responders' and 'low responders' depending on the gains in muscle mass induced by the resistance training. The results showed an up-regulation of miRNA-451 only in low responders and a down-regulation of miRNA-29a, miRNA-26a and miRNA-378 in low responders with no changes in high responders (Davidsen et al., 2011). Drummond et al. (2008) demonstrated that pri-miRNAs (pri-miRNA-1-1, pri-miRNA-1-2, pri-miRNA-133a-1, and pri-miRNA-133a-2) were higher expressed in older men than in younger men at rest. Furthermore, a maximal protein anabolic stimulus consisting of resistance exercise and the intake of an essential amino acid solution leads to changes of the expression of pri-miRNAs and miRNAs. The pri-miRNAs levels of pri-miRNA-1-2, -133a-1 and -133a-2 as well as the level of mature miRNA-1 decreased after the exercise in comparison to baseline only in the young men (Drummond et al., 2008).

In addition, chronic and acute endurance exercise can provoke rapid miRNA adjustments (Nielsen et al., 2010). Russel et al. (2013) investigated the effects of an acute bout of endurance cycling at a moderate intensity and 10 days of endurance training on different

miRNAs in 9 healthy, untrained, male subjects. The results from this study demonstrated that miRNA-9, miRNA-23a, miRNA-23b and miRNA-31 were decreased and miRNA-1, miRNA-133a, miRNA-133-b and miRNA-181a were increased 3 hours post exercise. The 10 days short-term training resulted in an elevation of miRNA-1 and miRNA-29b, while miRNA-31 remained on a decreased level (Russell et al., 2013). Brief exercise consisting of ten 2-min bouts of cycle ergometry with a 1-minute rest in between each bout also significantly altered a number of miRNAs in circulating monocytes in 12 healthy, male participants (Radom-Aizik et al., 2014). These alterations might be able to attenuate pathological activation of the monocytes (Radom-Aizik et al., 2014). Even plasma-based, circulating miRNAs seem to be altered by exercise as tested by Baggish et al. (2011). Therefore, young, competitive, healthy endurance athletes performed an acute, exhaustive cycling exercise testing before and after a 90 day training period of sustained rowing exercise training (Baggish et al., 2011). The measurements of circulating miRNAs showed that miRNA-146a and miRNA-21, which regulate various functions relevant to exercise (Baggish et al., 2011) and were described in connection with inflammation (Taganov, Boldin, Chang, & Baltimore, 2006; Urbich, Kuehbacher, & Dimmeler, 2008) were significantly up-regulated directly after acute exercise and decreased after one hour of rest. This may indicate that these alterations are a true response to exercise (Baggish et al., 2011). Moreover, the plasma levels of miRNA-146a and miRNA-21 were significantly elevated at rest after the 90 day training period in comparison with each individual´s resting baseline data. Only miRNA-146a displayed a further significantly increased plasma concentration immediately following acute exercise after the training period (Baggish et al., 2011). Similarly, Nielsen et al. (2014) studied the response of circulating mi-RNAs to acute aerobic exercise and endurance training in plasma samples from trained healthy men. Interestingly, they observed a significant decrease in the circulating miRNA-146a, but no effects on miRNA-21 expression directly after the exercise bout. In addition, no changes in the expression of miRNA-146a after the endurance training as well as a decreased expression of miRNA-21 after the training period were found (Nielsen et al., 2014). These findings differ from the results reported by the research team of Baggish et al. (2011). These varieties were ascribed to differences in the applied acute exercise interventions and also the distinct post-processing methods of the samples by the authors (Nielsen et al., 2014). In conclusion it can be said that miRNAs expression can be influenced by various forms of physical exercise.

1.7 RESEARCH QUESTIONS

Morrisette-Thomas et al. (2014) recommended using multiple markers for researching inflammation and its biological processes instead of simply using IL-6 or CRP as single inflammation markers. In the course of the 'Vienna Active Ageing Study' (VAAS) many different blood parameters and biomarkers have been measured, but the main focus of attention in the present master thesis is lying on the cytokine TGF-β, its receptors TGF-β receptors 1 (TGF-βRI) and 2 (TGF-βRII) and the miRNA-21. More precisely, the lack of research results concerning the alteration of circulating TGF-β and the expression of TGF-βRI and 2 TGF-βRII as well as miRNA-21 in leukocytes in response to chronic progressive resistance training in older adults can be considered as the main incitement for present investigations. For a better understanding of the ongoing mechanisms on the cellular level, the following research questions will be examined:

- Is the TGF-beta blood serum concentration altered by a 6-month progressive resistance training with or without nutritional supplementation in elderly persons?
- Does a 6-month lasting progressive resistance training with or without nutritional supplementation influence TGF-βRI and TGF-βRII in leukocytes of older people?
- Are miRNA-21 levels affected by a 6-month progressive resistance training in elderly?

Additionally, the effects of progressive resistance training in combination or without a nutritional supplement on physical performance and body composition of the subjects were examined.

2 METHODS

The 'Vienna Active Ageing Study' (VAAS; Clinical Trial Registration Number: NCT01775111) was initiated and conducted by the Department of Sports and Exercise Physiology and the Department of Nutritional Science of the University of Vienna in cooperation with the Curatorship of Viennese retirement homes (KWP) in order to gain further information concerning the influence of resistance training with or without nutritional supplementation on blood biomarkers and functional parameters on institutionalized elderly.

2.1 PARTICIPANTS

All participants of the VAAS were residents of the Curatorship of Viennese retirement homes (KWP). The following 5 of a total of 31 houses took part in the study:

- Retirement home *Am Mühlengrund*, Breitenfurter Straße 269-279, 1230 Vienna
- Retirement home *Atzgersdorf*, Gatteredererstraße 12, 1230 Vienna
- Retirement home *Tratzerberg*, Schrutkagasse 63, 1130 Vienna
- Retirement home *Hohe Warte*, Hohe Warte 8, 1190 Vienna
- Retirement home *Leopoldau*, Kürschnergasse 10, 1210 Vienna

In the course of the weekly conducted, obligatory morning briefing of the retirement homes general information about aims, procedures and requirements of the study was given/passed on to the residents. About one week later an information meeting was organized to provide more detailed information to the potential participants and to answer further questions. In addition individual appointments were arranged, where arising questions were resolved and the screening of the inclusion and exclusion criteria were performed.

Inclusion criteria for the study were, that the study participants had to be (1) older than 65 years of age, (2) they should reach a *Minimal-Mental-State* (Folstein, Folstein, & McHugh, 1975) of ≥23, also comprising a Clock-Drawing-Test, and (3) reach ≥6 points at the *Short Physical Performance Battery* (SPPB). The *SPPB* includes balance tests, a timed 4 m usual-pace walk and chair stands (Vasunilashorn et al., 2009). All of the mentioned inclusion criteria had to be fulfilled to participate in the study.

Individuals were excluded if they met one of the following exclusion criteria, which referred to the scientific statement of the American Heart Association (AHA) (Williams et al., 2007): (1) chronic diseases, which contraindicate a medical training therapy, (2) serious cardiovascular diseases (decompensated heart failure, severe or symptomatic aortic stenosis, instable angina pectoris, uncontrolled arterial hypertension, uncontrolled cardiac arrhythmias), diabetic retinopathy, regular use of cortisone-containing drugs, regular strength training (> 1x / week) in the last 6 months before inclusion. In total 117 individuals participated in the VAAS (see chapter 3.1 for participant flow (page 47)) (Oesen et al., 2015).

2.2 STUDY DESIGN

The VAAS study was conducted as a randomized, observer-blind, controlled intervention study in accordance to the Austrian laws, (doctors Act, CISA, Data Protection Act), the ICH-GCP Guidelines and the Declaration of Helsinki (as revised in Edinburgh 2000) (Franzke et al., 2015). Further, it was approved by the ethics committee of the City of Vienna (EK-11-151-0811). The organization of the study was carried out with special ethical and scientific care. Written informed consent was obtained from all participating subjects. The total span of the study was 2 years and it comprised 5 points of measurement (baseline, 3 months, 6 months, 12 months and 24 months) (Franzke et al., 2015). The 117 participants (men n=14, women n=103) aged 83 ± 6.0 were allocated randomly but stratified by gender and retirement home to one of the 3 groups [Resistance Training (RT), RT + nutritional supplement (RTS), Cognitive Training (CT)] after the first medical assessment (Oesen et al., 2015). A commercial randomization tool was used for the allocation of the subjects. Due to the stratification procedures, gender and allocation to a specific retirement home was equal in all intervention groups (Oesen et al., 2015).

2.3 INTERVENTION

All intervention sessions were supervised by qualified instructors in groups of 10 participants at the maximum, to assure correct exercise techniques. An attendance check of the participants was performed at the beginning of each session.

2.3.1 RESISTANCE TRAINING

Twice a week the participants of the RT and RTS group performed progressive resistance training (Oesen et al., 2015). To increase sustainability elastic bands (Thera-Band®, The Hygenic Corporation, Akron, OH, USA) sticks and chairs were the only additional equipment used during the training sessions. Every exercise session lasted 55-60 minutes, and was divided into 10 minutes of warming-up, 35-40 minutes of strength training and a cool down of 10 minutes at the end (Oesen et al., 2015).

The strength training included 10 exercises for the six major muscle groups (arms, shoulder, chest, abdomen, back and legs), which were performed from the lager to the smaller muscle groups (Oesen et al., 2015). The exercise program was based on the American College of Sports Medicine (ACSM) guidelines for resistance training for older adults (Nelson et al., 2007). Although the exercises were performed with elastic bands and own body weight, progressive resistance was ensured by adapting the execution of the own-body weight exercises or the adaption of the resistance of the Thera-Bands® (Oesen et al., 2015). At the beginning, each participant started with the lightest level of resistance of the Thera-Bands® (yellow) and progressed to higher levels of resistance (red followed by black Thera-Bands®) if it was necessary (Page & Ellenbecker, 2011).

During the settling-in period (4 weeks) one set of 15 repetitions was conducted with low resistance to learn the correct execution of the exercises (Franzke et al., 2015). Only if the individuals were strongly underutilized, advanced exercises were performed. From the fifth week onwards the exercise volume and intensity were progressively increased, so that the participants completed two sets of light exercises. If those sets were ‘easily done’, one light exercise set was changed to one set of heavy exercise and eventually both light exercise sets were replaced by two heavier sets of exercise. An exercise was considered to be ‘easily done’ when 15 repetitions of the second set were attainable without any problems, which meant, that 2 more repetitions in the 2nd set would have been possible (Franzke et al., 2015). During the following 6 months one weekly training session was performed supervised and the second one self-organized. For the next half-year the participants completed the exercises independently two times a week.

2.3.2 RESISTANCE TRAINING AND NUTRITIONAL SUPPLEMENT

Individuals assigned to the RTS group were asked to consume the supplement (FortiFit, Nutricia GmbH, Vienna, Austria), which was distributed every morning after breakfast and also directly after each training session by the staff of the KWP and the sport scientists, respectively (Franzke et al., 2015). The intake of the nutritional supplement drinks was also controlled by the same persons. Each portion of the supplement contained 20.7g protein (56 En%, 19.7 g whey protein, 3 g leucine, > 10 g essential amino acids), 9.3 g carbohydrates (25 En%, 0.8 BE); 3.0 g fat (18 En%), 1.2 g dietary fibers (2 En%), 800 IU (20µg) of vitamin D, 250 mg calcium, vitamins B6 and B12, folic acid and magnesium and had a total caloric value of 150 kcal. The subjects of the RTS group completed the same progressive resistance training as the RT group as mentioned above (Franzke et al., 2015).

2.3.3 COGNITIVE TRAINING

Twice a week activities, based on cognitive and coordinative tasks, were provided for the participants of the CT group. The program included exercises for memory training and also finger dexterity (Gatterer & Croy, 2004). Further, the subjects were asked to maintain their usual physical activity and dietary habits.

2.4 PHYSICAL PERFORMANCE

2.4.1 6-MINUTE WALKING TEST

For the evaluation of the aerobic endurance of the subjects a 6-minute walking test was performed (Oesen et al., 2015). The subjects walked as fast as possible on a 30-meter shuttle track for the duration of 6 minutes. The participants walked on their own and were allowed to rest or reduce the chosen speed during the test to ensure their comfort and safety. The walked distance of each person was recorded to the nearest meter (Steffen, Hacker, & Mollinger, 2002).

2.4.2 CHAIR STAND TEST

To assess the function of the lower extremities a chair stand test was carried out according to Rikli and Jones (2013). The participants had to rise from a chair as often as possible within 30 seconds with folded arms. For the testing a chair with a seat height of 46 cm, which was placed against a wall, was used. When a person reached the standing position with fully stretched hip and knees and sat down on the chair again, the repetition was considered as valid. When a participant attempted to stand up during the last of the 30 seconds and thereby reached more than 50 % of the standing position it was counted as a full stand (Rikli & Jones, 2013).

2.4.3 ISOMETRIC HANDGRIP STRENGTH

To determine the isometric handgrip strength of the participants, a handgrip dynamometer (JAMAR®, Sammons Preston Inc., Bolingbrook IL, USA) with an adjustable handle was used, which could be adapted to various sized hands (Oesen et al., 2015). The test was performed with the dominant hand in a sitting position, with an elbow angle of 45° and the lower arm resting on an armrest. In total, two trials with a maximal isometric contraction of 4-5 seconds duration were performed with a rest of 1 minute in between (Mijnarends et al., 2013).

2.4.4 ISOKINETIC PEAK TORQUE

The isokinetic peak torque measurements were administered by performing concentric isokinetic torque measurements (Lido, Loredan Biomedical Inc., Davis, USA) of the hamstrings and quadriceps with a range of motion (ROM) of 30° - 80° and a speed of 60°/s or 120°/s (Halper et al., 2015). Using the left leg for the testing guaranteed an easier positioning of the subjects, whereby the feasibility of the measurements was increased. Subjects with acute impairments of the left leg were tested on the right leg. Two trials with a two-minute rest in between were conducted for each speed setting and muscle. The better result of the two attempts was used for further statistical calculations (Halper et al., 2015).

2.5 ANTHROPOMETRIC MEASUREMENTS

The body mass of the subjects was measured by using a digital scale (SECA Model 877, Seca GmbH & Co. KG, Hamburg, Germany) to the nearest 0.1 kg (Hofmann et al., 2015). Therefore the participants were barefoot and lightly dressed. The standing height was measured to the nearest 0.5 cm with a commercial stadiometer (Seca Model 217, Seca GmbH & Co. KG, Hamburg, Germany). The individuals were instructed to keep their shoulders in a relaxed position and they were allowed to keep their arms hanging freely. The body mass index (BMI) was determined by dividing the body mass in kilograms by the square of the height in meters (Hofmann et al., 2015). Body composition data, like muscle mass and fat mass, were collected by utilizing bioelectric impedance analyses (BIA), which has been shown to afford reliable data (Roubenoff et al., 1997). Beyond that it was validated against dual-energy X-ray absorptiometry (Hofmann et al., 2015). For the measurements a BIA Analyzer 2000-S (Data-Input GmbH, Darmstadt, Germany) was employed. The BIA was conducted in the morning after an overnight fast (Hofmann et al., 2015). Additionally, waist and hip circumferences were measured with a circumference tape (Seca Model 201, Seca GmbH & Co. KG, Hamburg, Germany) and were further used to calculate the waist to hip ratio (waist circumference [cm] / hip circumference [cm]).

2.6 BLOOD COLLECTION AND ROUTINE ANALYSES

Whole Blood samples were obtained from all participants at baseline, after 3 months and after 6 months (Halper et al., 2015). The blood samples were drawn from the antecubital vein, in the morning (06:30-08:00 AM) after an overnight fast, in a sitting position using Z Serum Clot Activator collection tubes (Vacuette®, Greiner Bio-One GmbH, Kremsmünster, Austria) and EDTA tubes, for cytokine analyses and to determine the subpopulation numbers of leukocytes, respectively. BD Vacutainer® CPT™ cell preparation tubes containing ~130 IU Na-Heparin and 2 ml Ficoll™ (Becton, Dickinson and Company, Franklin Lakes, NJ) were used for the isolation of peripheral blood mononuclear cells (PBMCs) from whole blood (Halper et al., 2015).

Thirty to 60 minutes after the blood taking the serum tubes were centrifuged at 3,000 x g for 10 minutes (Halper et al., 2015). For the immediate hs-CRP, insulin and glucose analysis an aliquot of 1 ml was used. The residual serum was stored in aliquots at a temperature of -80 °C for following procedures. Glucose was determined by the hexokinase method and insulin was analyzed by using a solid-phase, enzyme-labeled chemiluminescen-

timmunometric assay (IMMULITE 2000, Siemens Healthcare Diagnostics Inc., Llanberis, UK). Further, hs-CRP, cholesterol, HDL cholesterol, LDL cholesterol and triglyceride were quantified on a Cobas 8000 (Roche Diagnostics, Vienna, Austria). Leukocytes, granulocytes, monocytes and lymphocytes were quantified by using flow cytometry on a Sysmex XE-2100™ Automated Hematology System (Sysmex Austria GmbH, Vienna, Austria) (Halper et al., 2015). The analyses stated above were performed in a routine laboratory (*study lab GmbH*, Davidgasse 85-89, 1100 Vienna, Austria).

2.7 TOTAL RNA ISOLATION FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

The BD Vacutainer® CPT™ cell preparation tubes were centrifuged at 1,650 x g for 20 minutes (Rotina 420R, Hettich AG, Bäch, Switzerland) to separate the PBMCs from neutrophils and red blood cells (Halper et al., 2015). Afterwards, 2 ml of the plasma supernatant was transferred into separate tubes. For recovering the separated PBMCs, they were resuspended in the remaining plasma and transferred into 15 ml size conical centrifuge tubes with caps. (Halper et al., 2015). The following cell washing steps were performed as specified by the manufacturer (Becton, Dickinson and Company, Franklin Lakes, NJ; retrieved February 24, 2014, from https://www.bd.com/vacutainer/pdfs/bd_cpt_VDP40105.pdf):

- Phosphate buffered saline (PBS) was added to bring the volume to 15 ml in the cap tube and the cells were mixed by inverting the tube 5 times.
- After 15 minutes of centrifugation at 300 x g as much supernatant as possible was aspirated without disturbing the cell pellet.
- The cell pellet was resuspended by tapping on the tube using the index finger or by gently vortexing the tube.
- PBS was added to bring the volume to 10 ml in the cap tube and the cells were mixed by inverting the tube 5 times.
- After 10 minutes of centrifugation at 300 x g as much supernatant as possible was aspirated without disturbing the cell pellet.
- The cell pellet was resuspended in 700µl of QIAzol Lysis Reagent (QIAGEN, Hilden, Germany) and stored at a temperature of -80 °C for following procedures/until the day of analysis.

Subsequent to these steps the samples were thawed and incubated for 5 minutes at room temperature prior to RNA isolation (Halper et al., 2015). For purification and isolation of total RNA and small RNAs the automated QIAcube® (Qiagen, Hilden, Germany) system was used in combination with the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany) was used to obtain a miRNA-enriched fraction separated from lager RNAs (>200nt). Moreover, the reverse transcription of the lager RNAs was carried out by using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). The miRNA-enriched fraction was transcribed using the miScript II RT Kit (Qiagen, Hilden, Germany) (Halper et al., 2015).

2.8 QUANTITATIVE REAL-TIME REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

The following primer assays were used respectively for the determination of mRNA of TGF-β, TGF-βRI and TGF-βRI (Hs_TGFB1_1_SG (QT00000728), Hs_TGFBR1_1_SG (QT00083412), Hs_TGFBR2_1_SG (QT00014350), Qiagen, Hilden, Germany) in combination with the QuantiTect SYBR® Green PCR kit (Qiagen, Hilden, Germany) (Halper et al., 2015). The standard curve was obtained by pooling equal parts of cDNA of PBMCs from 7 young and 23 old participants who were randomly selected from the study population. Additionally, GAPDH (Hs_GAPDH_2_SG (QT01192646), Qiagen, Hilden, Germany) was used as endogenous control and for data normalization. An Applied Biosystems® 7500 Real-Time PCR System was used for detection and quantitation (Halper et al., 2015).

For the measurement of miRNA-21 expression levels a miScript Primer Assay (hs_miR-21_2 (MS00009079), Qiagen, Hilden, Germany) was used (Halper et al., 2015). A commercially available totalRNA (Total RNA (R1234148-10), BioChain, Newark, USA) from peripheral blood leukocyte cells of a 24 years old female donor was used for the preparation of the standard curve. An Applied Biosystems® 7500 Real-Time PCR System was used for detection and quantitation (Halper et al., 2015).

2.9 ENZYME-LINKED IMMUNOSORBENT ASSAY

For the determination of the TGF-β1 serum levels, a DuoSet® ELISA (enzyme-linked immunosorbent assay) development kit (DY240, R&D Systems, Abingdon, UK) was used (Halper et al., 2015). The standard curve (31-2,000 pg/ml) was prepared by using recombinant human TGF-β1 (Standard, Part 840118, R&D Systems, Abingdon, UK). Ten µl of 1N HCl was added to 20 µl of each serum sample for activation of latent TGF-β1 to immunoreactive TGF-β1. The samples were incubated for 10 minutes and then neutralized with 10 µl of 1.2N NaOH/0.5 M Hepes. The activated samples were diluted 20-fold with Reagent Diluent (0.05% Tween® 20 in PBS) (Halper et al., 2015). The subsequent steps were performed for plate preparation, followed by a description of the assay procedure according to the protocol provided by the manufacturer (DY240, R&D Systems, Abingdon, UK; retrieved August 04, 2014, from https://resources.rndsystems.com/pdfs/datasheets/dy240.pdf) :

Plate preparation:

- The Capture Antibody (2 µg/ml of mouse anti-TGF-β1, Part 840116, R&D Systems, Abingdon, UK) was diluted to the needed concentration in PBS without the carrier protein. A 96-well microplate was immediately coated with 100 µl of the diluted Capture Antibody per well. The plates were sealed and incubated overnight (12 hours) at room temperature.
- Capture Antibody was removed and all wells were washed with Wash Buffer, which was filled in by using a pipette controller. The washing process was repeated three times and after the last wash all the remaining Wash Buffer was removed by inverting the microplate and blotting it against clean paper towels.
- The plates were blocked by adding 300 µl of Block Buffer to each well and then incubated for a minimum of 1 hour at room temperature.
- After incubation the described washing process was repeated again for three times.

Assay procedure:

- 100 µl of sample or standards were added per well. Then the wells were covered with an adhesive strip and incubated for 2 hours at room temperature.
- The washing process from plate preparation was repeated.

- 100 µl Detection Antibody (300 µg/ml of biotinylated chicken anti-human TGF-β1, Part 840117, R&D Systems, Abingdon, UK) diluted in Reagent Diluent was added to each well. Then the wells were covered with a new adhesive strip and incubated for 2 hours at room temperature.
- The washing process from plate preparation was repeated.
- 100 µl of Streptavidin-HRP (Part 890803, R&D Systems, Abingdon, UK) diluted to the working concentration was added to each well. Then the plate was covered to protect it from direct light and incubated for 2 hours at room temperature.
- The washing process from plate preparation was repeated.
- 100 µl of Substrate Solution was added to each well. Then the plate was covered to protect it from direct light and incubated for 20 minutes at room temperature.
- 50 µl of Stop Solution was added to each well and the microplate was gently tapped to ensure thorough mixing.
- The optical density of the wells was immediately determined by using a Victor ³ 1420 Multilabel Counter (Perkin Elmer, MA, US) at 450 nm. The wavelength correction was set to 570 nm.

2.10 STATISTICAL ANALYSES

Only data from the first 3 points of measurement (baseline, 3 months, 6 months) were consulted for the statistical analyses of the present thesis. The statistical data analyses were conducted by using commercial software (IBM SPSS 20, SPSS Inc. Chicago, IL, USA). For the general data from the participants a descriptive statistical analysis was performed. All data were assessed for normality using Kolmogorov–Smirnov test. Since most of the variables were not normally distributed, baseline gender differences and group differences were tested using Mann-Whiney U-Test and Kruskal-Wallis test, respectively. Friedman´s test was performed in order to detect changes over time in the different intervention groups. If the results from Friedman´s test were significant ($p < 0.05$), a Wilcoxon test with Bonferroni adjustment was performed to assess the differences in between each time point. All values are reported as median (minimum-maximum) and the significance level was set at $p < 0.05$. Further, Spearman rank correlation tests were used to evaluate correlations between physical performance and inflammatory markers and in between inflammatory markers among themselves.

3 RESULTS

3.1 STUDY PARTICIPATION

The following diagram (see Fig. 13) represents the participant flow of the present study and therefore numbers of tested subjects for each parameter in each group are shown.

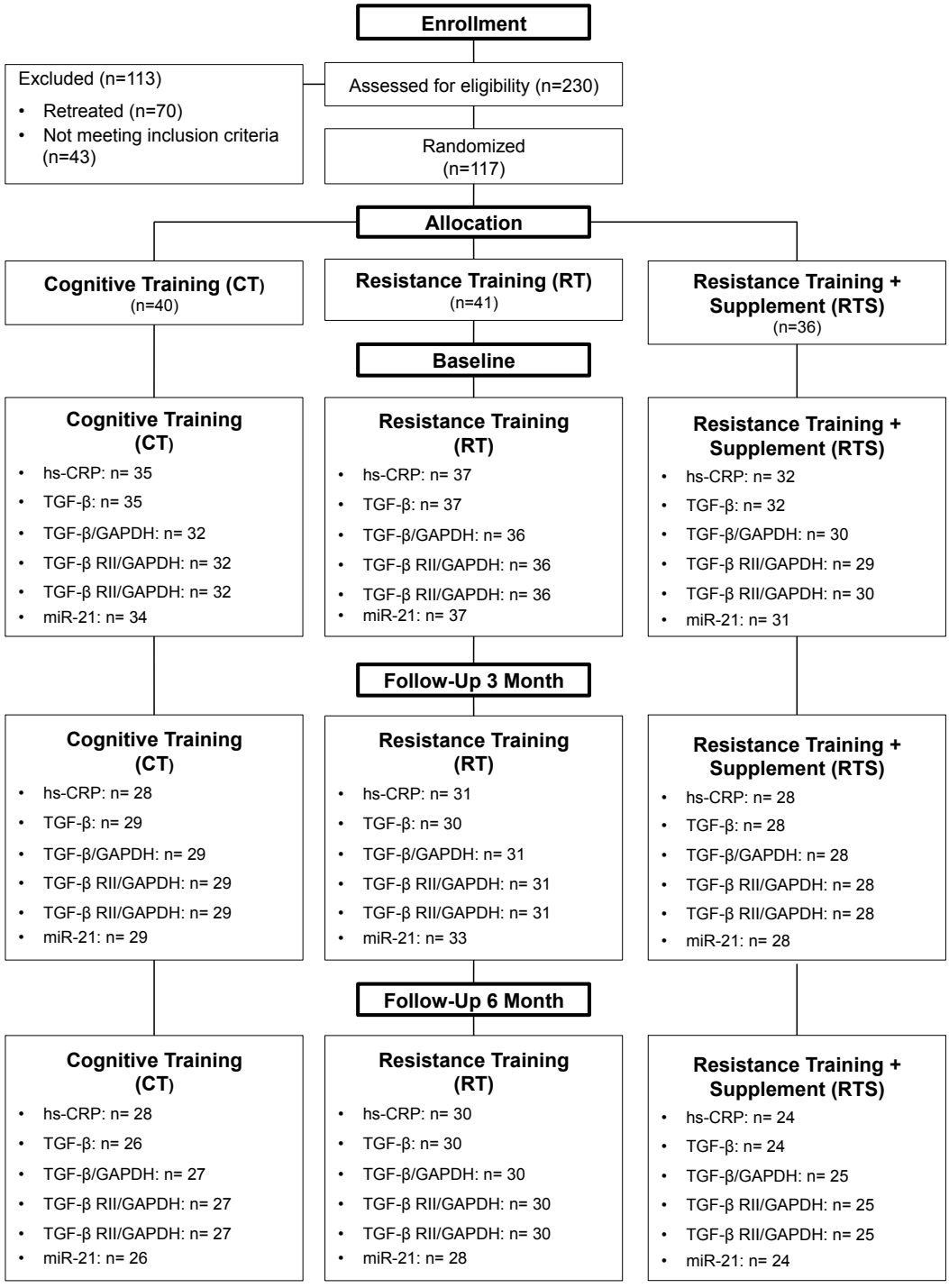

Figure 13. *Participation of individuals at the different stages of the study protocol (study profile)*

At the beginning a total number of 230 persons was assessed for eligibility, among whom 117 individuals were randomly allocated to the three groups of the study.

3.2 BASELINE CHARACTERISTICS

3.2.1 GENDER DIFFERENCES

As shown in Table 1, significantly more females (88%) participated in the study than males (12%) ($p < 0.001$). The age of the participants was 83.9 (65.0 – 97.4) years. At baseline, significant gender differences were shown in the handgrip test ($p < 0.001$), the relative peak torque, 60°/s ($p = 0.002$) and 120°/s ($p = 0.001$), of the hamstrings (PTH) and the relative peak torque 120°/s ($p = 0.009$) of the quadriceps (PTQ). Medians of strength related tests were significantly higher in male individuals. No gender differences were found for the parameters age ($p = 0.373$), 6-minute walking test ($p = 0.229$), the chair stand test ($p = 0.339$) as well as for the relative peak torque 60°/s of the quadriceps ($p = 0.080$).

Table 1. *Baseline subject characteristics (physical performance)*

	Total	**Females**	**Males**	***p*-value**
Subjects [number (%)]	117 (100%)	103 (88%)	14 (12%)	**< 0.001**
Age n=117	83.9 (65.0 – 97.4)	83.6 (65.0 – 92.2)	84.7 (72.8 – 97.4)	0.373
6-minute walking test [m] n=101	365 (114 – 620)	362 (114 – 558)	393 (193 – 620)	0.229
Chair stand test [repetitions] n=101	12 (0 – 25)	12 (0 – 22)	12 (0 – 25)	0.339
Handgrip test [kg] n=93	19 (10 – 43)	18 (10 – 30)	32 (21 – 43)	**< 0.001**
relative PTQ 60°/s [Nm/kg] n=93	1.01 (0.19 – 2.10)	1.00 (0.19 – 1.92)	1.16 (0.55 – 2.10)	0.080
relative PTH 60°/s [Nm/kg] n=93	0.52 (0.17 – 1.20)	0.51 (0.17 – 0.97)	0.75 (0.36 – 1.20)	**0.002**
relative PTQ 120°/s [Nm/kg] n=93	0.81 (0.24 – 1.67)	0.80 (0.24 – 1.54)	1.15 (0.40 – 1.67)	**0.009**
relative PTH 120°/s [Nm/kg] n=93	0.48 (0.15 – 1.18)	0.46 (0.15 – 0.80)	0.63 (0.41 – 1.18)	**0.001**

Values are presented as median (minimum - maximum). P-values refer to gender differences (chi-square and Mann-Whitney U-Test). PTQ = peak torque quadriceps; PTH = peak torque hamstrings

Gender differences were also found for parameters of the body composition (see Table 2.). Male participants were significantly heavier ($p = 0.016$), taller ($p < 0.001$) and had more body fat mass ($p = 0.004$) as well as significant higher body cell mass ($p < 0.001$) and waist to hip ratio ($p < 0.001$) than the female participants. Only for the BMI no significant differences were found ($p = 0.417$).

Table 2. *Baseline subject characteristics (body composition)*

	Total	**Females**	**Males**	***p*-value**
Weight [kg] n=104	72.2 (46.2 – 114.7)	71.2 (46.2 – 112.4)	81.9 (59.8 – 114.7)	**0.016**
Height [m] n=104	1.58 (1.40 – 1.82)	1.57 (1.40 – 1.72)	1.68 (1.63 – 1.82)	**< 0.001**
BMI [kg/m²] n=104	29.03 (18.14 – 49.96)	29.24 (18.14 – 49.96)	27.05 (21.19 – 37.32)	0.417
Body fat mass [kg] n=100	24.8 (5.4 – 54.3)	25.7 (6.3 – 54.3)	18.8 (5.4 – 31.8)	**0.004**
Body cell mass [kg] n=100	20.3 (12.9 – 36.9)	19.5 (12.9 – 26.4)	28.5 (17.6 – 36.9)	**< 0.001**
Waist / Hip Ratio [-] n=94	0.85 (0.70 – 1.06)	0.85 (0.70 – 1.01)	0.92 (0.86 – 1.06)	**< 0.001**

Values are presented as median (minimum - maximum). P-values refer to gender differences (Mann-Whitney U-Test). BMI = body mass index

At baseline, no genders differences between the medians of the inflammatory serum markers and intracellular markers were observed (see Table 3.).

Table 3. *Baseline subject characteristics (inflammatory markers)*

	Total	**Females**	**Males**	***p*-value**
Serum makers				
hs-CRP [mg/l] n=104	2.25 (0.3 – 56.7)	2.3 (0.3 – 56.7)	1.9 (0.6 – 22.5)	0.840
TGF-β [ng/ml] n=104	35.3 (16.7 – 73.7)	34.5 (16.7 – 73.7)	37.2 (17.2 – 49.7)	0.691
Intracellular markers				
TGF-β /GAPDH [-] n=98	0.52 (0.06 – 3.36)	0.53 (0.06 – 3.36)	0.39 (0.26 – 2.34)	0.255
TGF-βRI /GAPDH [-] n=97	2.05 (0.14 – 28.81)	2.11 (0.14 – 28.81)	1.76 (0.86 – 13.5)	0.734
TGF-βRII /GAPDH [-] n=98	1.60 (0.51 – 14.86)	1.74 (0.51 – 14.86)	1.28 (0.66 – 6.54)	0.126
miR-21 [copies/pg] n=102	2,529 (57 – 5,480)	2,456 (57 – 5,480)	3,045 (504 – 3,400)	0.622

Values are presented as median (minimum - maximum). P-values refer to gender differences (Mann-Whitney U-Test). hs-CRP = high-sensitive C-reactive protein; TGF-β = transforming growth factor beta; TGF-βRI = transforming growth factor beta receptor I; TGF-βRII = transforming growth factor beta receptor II; miR-21 = mircoRNA 21

3.2.2 GROUP DIFFERENCES

At the beginning of the study there were no between group differences for any of physical performance parameters (see Table 4.). Furthermore, neither the body composition markers nor the inflammatory maker levels differed at baseline between the groups (see Tables 5 and 6).

Table 4. *Baseline between group differences (physical performance)*

	CT	**RT**	**RTS**	***p*-value**
Age n=117	84.5 (69.4 – 97.4)	83.2 (71.7 – 93.2)	83.7 (65.0 – 92.2)	0.767
6-minute walking test [m] n=101	362 (114 – 620)	366 (134 – 558)	360 (180 – 552)	0.904
Chair stand test [repetitions] n=101	12 (0 – 22)	11 (0 – 20)	13 (0 – 25)	0.705
Handgrip test [kg] n=93	18 (10 – 36)	20 (11 – 43)	19 (11 – 41)	0.065
relative PTE 60°/s [Nm/kg] n=93	1.01 (0.50 – 1.77)	1.03 (0.19 – 1.54)	1.02 (0.42 – 2.10)	0.924
relative PTF 60°/s [Nm/kg] n=93	0.51 (0.26 – 0.93)	0.52 (0.17 – 0.81)	0.53 (0.19 – 1.20)	0.498
relative PTE 120°/s [Nm/kg] n=93	0.79 (0.40 – 1.54)	0.84 (0.24 – 1.25)	0.83 (0.27 – 1.67)	0.911
relative PTF 120°/s [Nm/kg] n=93	0.44 (0.26 – 0.94)	0.50 (0.15 – 0.77)	0.47 (0.24 – 1.18)	0.500

Values are presented as median (minimum - maximum). P-values refer to group differences (Kruskal-Wallis Test). PTQ = peak torque quadriceps; PTH = peak torque hamstrings

Table 5. *Baseline between group differences (body composition)*

	CT	RT	RTS	*p*-value
Weight [kg] n=104	74.4 (46.2 – 114.7)	72.5 (54.0 – 89.6)	69.7 (56.3 – 112.4)	0.988
Height [m] n=104	1.58 (1.42 – 1.80)	1.58 (1.40 – 1.82)	1.58 (1.47 – 1.72)	0.993
BMI [kg/m²] n=104	29.84 (18.14 – 36.86)	28.46 (22.68 – 40.15)	27.87 (22.43 – 49.96)	0.895
Body fat mass [kg] n=100	26.3 (6.3 – 48.2)	23.8 (12.0 – 39.8)	24.4 (5.4 – 54.3)	0.932
Body cell mass [kg] n=100	19.3 (14.4 – 32.8)	19.8 (14.3 – 30.4)	21.4 (12.9 – 36.9)	0.235
Waist / Hip Ratio [-] n=94	0.88 (0.71 – 0.99)	0.85 (0.70 – 0.98)	0.85 (0.72 – 1.06)	0.434

Values are presented as median (minimum - maximum). P-values refer to group differences (Kruskal-Wallis Test). BMI = body mass index

Table 6. *Baseline between group differences (inflammatory markers)*

	CT	RT	RTS	*p*-value
Serum makers				
hs-CRP [mg/l] n=104	2.3 (0.5 – 14.3)	2.2 (0.3 – 56.7)	2.1 (0.6 – 40.5)	0.983
TGF-β [ng/ml] n=104	39.0 (18.7 – 73.7)	33.3 (16.7 – 55.4)	33.1 (21.0 – 51.2)	0.061
Intracellular markers				
TGF-β/GAPDH [-] n=98	0.46 (0.23 – 2.66)	0.54 (0.21 – 2.52)	0.58 (0.06 – 3.36)	0.521
TGF-βRI/GAPDH [-] n=97	1.92 (0.14 – 22.39)	2.05 (0.69 – 19.25)	2.38 (0.29 – 28.81)	0.944
TGF-βRII/GAPDH [-] n=98	1.44 (0.66 – 7.79)	1.75 (0.66 – 14.86)	1,70 (0.51 – 11.75)	0.477
miR-21 [copies/pg] n=102	2,609 (343 – 4,500)	2,520 (57 – 4,720)	2,452 (550 – 5,480)	0.900

Values are presented as median (minimum - maximum). P-values refer to group differences (Kruskal-Wallis Test). hs-CRP = high-sensitive C-reactive protein; TGF-β = transforming growth factor beta; TGF-βRI = transforming growth factor beta receptor I; TGF-βRII = transforming growth factor beta receptor II; miR-21 = mircoRNA 21

3.3 EFFECTS OF INTERVENTION

3.3.1 INFLUENCE OF INTERVENTION ON PHYSICAL PERFORMANCE

Results of the statistical analysis of the influence of the intervention on physical performance are summarized in Table 7. The values of the 6-minute walking test did not change in the CT group, whereas the walked distance of the RT and RTS group significantly changed over time ($p = 0.021$ and $p = 0.015$ respectively). However, the significant changes in the RTS group did not persist after post-hoc analysis, only the results of the RT group showed a significant increase (+11.5%, $p = 0.011$) between baseline and 6 months (see Fig. 9A). Similarly, chair stand test showed no changes in the CT group but the number of repetitions was significantly influenced by time in the RT ($p = 0.001$) and the RTS group ($p = 0.003$). Post-hoc test revealed, that the improvements in chair stand test were significant between the beginning of the study and the 3 months follow up (+27.3%, $p = 0.002$) as well as for the time period between baseline and the 6-month followup (+27.3%, $p = 0.010$) for the RT group. Also in the RTS group a significant improvement (+15.4%, $p = 0.028$) was detected between 0 months and 6 months (see Fig. 9B) for the chair stand test. No statistical significant alterations of handgrip strength were identified between any of the time points. For the relative PTQ 60°/s a significant time effect was observed ($p = 0.050$) but did not persist after post-hoc testing and Bonferroni correction. On the contrary, the significant results from Friedman test for the PTH 60°/s for the RT group ($p = 0.003$) also was confirmed by the post-hoc testing (see Fig. 9E), which revealed an improvement between 0 months and 6 months (+23.1%, $p = 0.004$) as well as between 3 months and 6 months (+10.3%, $p = 0.006$). Moreover, relative PTQ 120°/s did not significantly change in the CT group, but in the RT ($p = 0.003$) and RTS group ($p = 0.001$). Wilcoxon test showed a significant improvement (+10.7%, $p = 0.003$) in the RT group and the RTS group (+9.6%, $p = 0.009$) between baseline and 3 months and also a reduction (-0.2%, $p = 0.049$) between 3 and 6 months of the intervention in the RTS group (see Fig. 9F). Additionally a significant time effect was found for relative PTH 120°/s for CT ($p = 0.002$) and RT group ($p = 0.004$). In more detail, post hoc analysis showed, that these changes were significant between 0 months and 3 months (36.4%, $p = 0.010$) as well as between 0 months and 6 months (18.2%, $p = 0.029$) for the CT group. Likewise, significant changes were noted in the RT group for the same periods of time (10%, $p = 0.006$ and 8%, $p = 0.047$ respectively). No significant change was observed in the RTS group.

Table 7. *Influence of intervention on physical performance*

Parameter	Group	Baseline	3 months	6 months	*p* value
6-minute walking test [m]	CT	362 (114 – 620)	378 (180 – 648)	369 (150 – 633)	0.650
	RT	366 (134 – 558)	375 (165 – 559)	408 (231 – 600)	**0.021**
	RTS	360 (180 – 552)	400 (175 – 912)	373 (207 – 900)	**0.015**
Chair stand test [repetitions]	CT	12 (0 – 22)	12 (0 – 18)	11 (0 – 17)	0.943
	RT	11 (0 – 20)	14 (3 – 23)	14 (8 – 25)	**0.001**
	RTS	13 (0 – 25)	14 (0 – 24)	15 (0 – 35)	**0.003**
Handgrip test [kg]	CT	18 (10 – 36)	17 (9 – 39)	17 (4 – 38)	0.525
	RT	20 (11 – 43)	22 (11 – 40)	22 (9 – 38)	0.309
	RTS	19 (11 – 41)	19 (9 – 41)	20 (13 – 40)	0.701
relative PTQ 60°/s [Nm/kg]	CT	1.01 (0.50 – 1.77)	1.10 (0.45 – 2.11)	1.02 (0.29 – 1.79)	**0.050**
	RT	1.03 (0.19 – 1.54)	1.12 (0.35 – 1.45)	1.10 (0.21 – 1.51)	0.060
	RTS	1.02 (0.42 – 2.10)	1.05 (0.61 – 2.37)	1.09 (0.57 – 2.29)	0.104
relative PTH 60°/s [Nm/kg]	CT	0.51 (0.26 – 0.93)	0.57 (0.37 – 0.86)	0.51 (0.29 – 0.99)	0.311
	RT	0.52 (0.17 – 0.81)	0.58 (0.13 – 0.89)	0.64 (0.13 – 0.83)	**0.003**
	RTS	0.53 (0.19 – 1.20)	0.58 (0.36 – 1.23)	0.63 (0.40 – 1.32)	0.104
relative PTQ 120°/s [Nm/kg]	CT	0.79 (0.40 – 1.54)	1.06 (0.41 – 1.45)	0.81 (0.35 – 1.49)	0.084
	RT	0.84 (0.24 – 1.25)	0.93 (0.41 – 1.51)	0.91 (0.26 – 1.24)	**0.003**
	RTS	0.83 (0.27 – 1.67)	0.91 (0.46 – 2.02)	0.91 (0.46 – 1.95)	**0.001**
relative PTH 120°/s [Nm/kg]	CT	0.44 (0.26 – 0.94)	0.60 (0.42 – 1.01)	0.52 (0.25 – 1.06)	**0.002**
	RT	0.50 (0.15 – 0.77)	0.55 (0.15 – 0.83)	0.54 (0.17 – 0.82)	**0.004**
	RTS	0.47 (0.24 – 1.18)	0.57 (0.32 – 1.03)	0.55 (0.36 – 1.25)	0.260

P-values are calculated by using Friedman test. PTQ = peak torque quadriceps; PTH = peak torque hamstrings; CT = cognitive training; RT = resistance training; RTS = resistance training + nutritional supplement

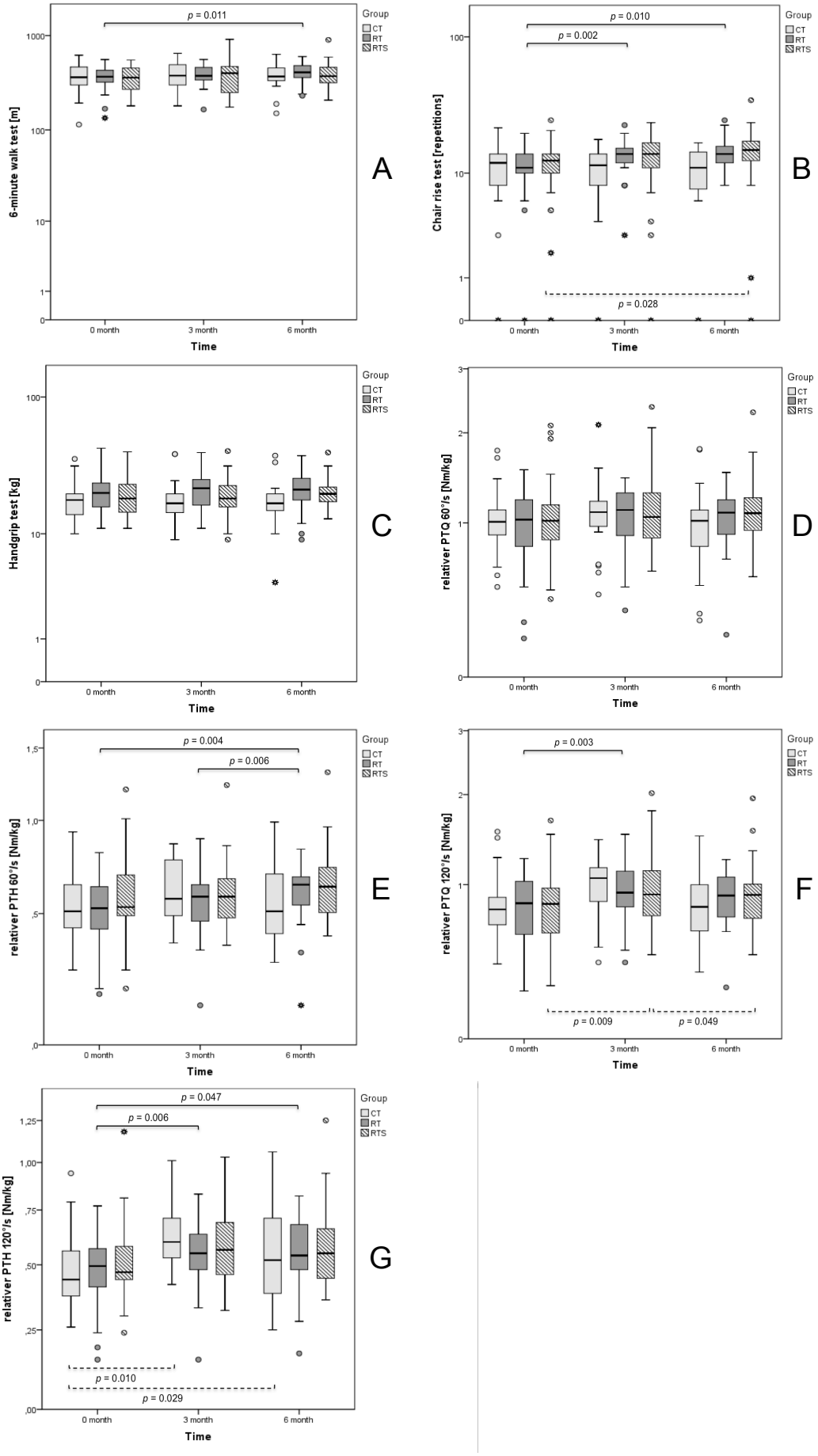

Figure 14. *Influence of intervention on physical performance (p-values are Bonferroni corrected)*

3.3.2 INFLUENCE OF INTERVENTION ON INFLAMMATORY MARKERS

The influence of the intervention on inflammatory markers is presented in Table 8. Moreover, these results are visualized as boxplots (see Fig. 10A - F). Friedman test showed no significant time effects for any of the inflammatory serum markers or intracellular markers with the only exception that TGF-βRI values decreased significantly in the RT group (p = 0.039). Though, the results of the post-hoc analysis revealed that there were no significant changes detectable between baseline, 3-month and 6-month followup.

Table 8. *Influence of intervention on inflammatory markers*

Parameter	Group	Baseline	3 months	6 months	*p* value
Serum markers					
hs-CRP [mg/l]	CT	2.3 (0.5 – 14.3)	2.5 (0.2 – 52.2)	2.5 (0.3 – 22.1)	0.350
	RT	2.2 (0.3 – 56.7)	2.5 (0.5 – 22.9)	2.3 (0.6 – 12.7)	0.938
	RTS	2.1 (0.6 – 40.5)	2.3 (0.6 – 11.8)	2.2 (0.4 – 13.4)	0.575
TGF-β [ng/ml]	CT	39.0 (18.7 – 73.7)	39.8 (18.4 – 67.8)	41.8 (21.9 – 68.0)	0.446
	RT	33.3 (16.7 – 55.4)	34.8 (18.3 – 59.7)	35.7 (12.7 – 57.3)	0.146
	RTS	33.1 (21.0 – 51.2)	37.7 (22.9 – 50.8)	36.3 (22.1 – 64.5)	0.417
Intracellular markers					
TGF-β /GAPDH [-]	CT	0.46 (0.23 – 2.66)	0.47 (0.27 – 4.10)	0.44 (0.25 – 1.93)	0.834
	RT	0.54 (0.21 – 2.52)	0.50 (0.16 – 2.63)	0.52 (0.19 – 2.30)	0.629
	RTS	0.58 (0.06 – 3.36)	0.48 (0.15 – 2.65)	0.54 (0.09 – 2.92)	0.469
TGF-βRI /GAPDH [-]	CT	1.92 (0.14 – 22.39)	1.82 (0.46 – 29.89)	2.06 (0.11 – 9.24)	0.553
	RT	2.05 (0.69 – 19.25)	1.83 (0.34 – 9.21)	1.83 (0.54 – 15.91)	**0.039**
	RTS	2.38 (0.29 – 28.81)	1.69 (0.28 – 15.06)	2.38 (0.52 – 15.04)	0.959
TGF-βRII /GAPDH [-]	CT	1.44 (0.66 – 7.79)	1.39 (0.68 – 9.17)	1.48 (0.67 – 6.32)	0.772
	RT	1.75 (0.66 – 14.86)	1.68 (0.57 – 13.50)	1.66 (0.63 – 8.79)	0.651
	RTS	1,70 (0.51 – 11.75)	1.73 (0.44 – 5.25)	1.87 (0.63 – 6.67)	0.326
miR-21 [copies/pg]	CT	2,609 (343 – 4,500)	2,830 (276 – 5,120)	2,430 (1,050 – 4,700)	0.468
	RT	2,520 (57 – 4,720)	2,300 (555 – 5,600)	2,515 (330 – 5,020)	0.182
	RTS	2,452 (550 – 5,480)	2,535 (353 – 4,780)	2,750 (1,600 – 4,700)	0.846

P-values are calculated by using Friedman test. hs-CRP = high-sensitive C-reactive protein; TGF-β = transforming growth factor beta; TGF-βRI = transforming growth factor beta receptor I; TGF-βRII = transforming growth factor beta receptor II; miR-21 = mircoRNA 21; CT = cognitive training; RT = resistance training; RTS = resistance training + nutritional supplement

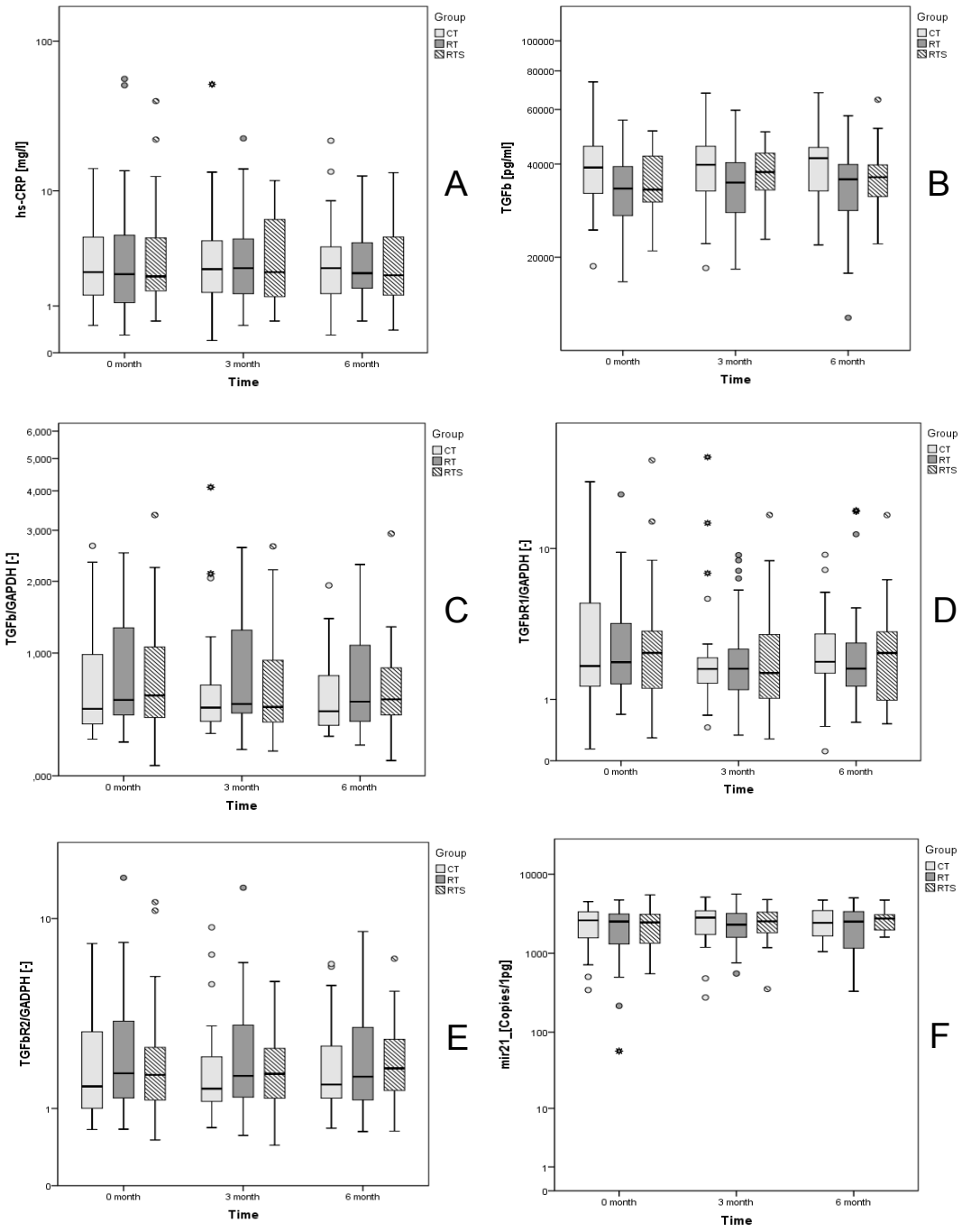

Figure 15. *Influence of intervention on inflammatory markers*

3.4 CORRELATIONS

3.4.1 CORRELATIONS BETWEEN PHYSICAL PERFORMANCE AND INFLAMMATORY MARKERS AT BASELINE

Spearman rank correlation tests were applied to determine the relations between physical performance parameters and the inflammatory markers of the subjects. The results from these Spearman correlations are summarized in Table 9. There was a significant negative association between the age of the subjects and the hs-CRP level ($r = -0.222$, $p = 0.024$). Moreover, there was also a negative association between age and the 6-minute walking test ($r = -0.304$, $p = 0.002$) as well as between the 6-minute walking test and the TGF-βRII ($r = -0.053$, $p = 0.002$). A negative correlation has also been found between the chair stand test and TGF-βRI ($r = -0.213$, $p = 0.038$). Further, there was a significant negative association between handgrip strength and age ($r = -0.306$, $p = 0.003$) and a significant positive association between handgrip strength and miR-21 ($r = 0.218$, $p = 0.038$). Also for the relative PTQ 120°/s, a positive correlation to hs-CRP was observed ($r = -0.260$, $p = 0.012$). On the contrary, no interactions were found between the physical performance parameters relative PTQ 60°/s, PTH 60°/s and PTH 120°/s and any of the inflammatory markers. No significant correlation between TGF-β (serum marker and intracellular marker) and any of the physical performance markers was detected.

Table 9. *Correlations between physical performance and inflammatory markers*

			Serum markers			**Intracellular markers**		
		Age [years]	hs-CRP [mg/l]	TGF-β [ng/ml]	TGF-β /GAPDH [-]	TGF-βRI /GAPDH [-]	TGF-βRII /GAPDH [-]	miR-21 [copies/pg]
Age [years]	Spearman rho	1.000	**-0.222**	-0.075	0.136	0.099	0.183	-0.030
	p-value	-	**0.024**	0.452	0.182	0.335	0.071	0.768
6-minute walking test [m]	Spearman rho	**-0.304**	0.074	-0.016	-0.118	-0.097	**-0.199**	0.000
	p-value	**0.002**	0.464	0.872	0.251	0.349	**0.053**	1.000
Chair stand test [repeti-tions]	Spearman rho	-0.060	-0.023	0.022	-0.140	**-0.213**	-0.166	0.090
	p-value	0.549	0.819	0.826	0.175	**0.038**	0.105	0.376
Handgrip test [kg]	Spearman rho	**-0.306**	0.163	-0.062	-0.104	-0.107	-0.127	**0.218**
	p-value	**0.003**	0.118	0.556	0.330	0.319	0.232	**0.038**
relative PTQ 60°/s [Nm/kg]	Spearman rho	-0.166	-0.198	-0.009	0.025	-0.116	-0.031	0.085
	p-value)	0.111	0.057	0.930	0.818	0.279	0.771	0.426
relative PTH 60°/s [Nm/kg]	Spearman rho	-0.104	-0.133	-0.004	-0.079	-0.145	-0.128	0.046
	p-value	0.323	0.202	0.971	0.458	0.175	0.229	0.666
relative PTQ 120°/s [Nm/kg]	Spearman rho	-0.059	**-0.260**	0.002	0.050	-0.063	-0.007	0.052
	p-value	0.573	**0.012**	0.987	0.642	0.559	0.949	0.625
relative PTH 120°/s [Nm/kg]	Spearman rho	-0.035	-0.173	-0.093	0.015	-0.067	-0.006	0.061
	p-value	0.740	0.096	0.374	0.885	0.533	0.953	0.563

Data are calculated by using Spearman rank correlation; PTQ = peak torque quadriceps; PTH = peak torque hamstrings; hs-CRP = high-sensitive C-reactive protein; TGF-β = transforming growth factor beta; TGF-βRI = transforming growth factor beta receptor I; TGF-βRII = transforming growth factor beta receptor II; miR-21 = mircoRNA 21

3.4.2 CORRELATIONS BETWEEN BODY COMPOSITION AND INFLAMMATORY MARKERS AT BASELINE

The parameters of body composition were also tested for correlations with the inflammatory markers (see Table 10.). The results showed, that there is a significant positive correlation between hs-CRP and body weight (r = 0.288, p = 0.003), the BMI (r = 0.292, p = 0.003), body fat (r = 0.335, p = 0.001) and the body cell mass (r = 0.216, p = 0.031). TGF-β (intracellular marker) was negatively correlated with the waist to hip ratio (r = -0.273, p = 0.009). In addition, the waist to hip ratio was significantly associated with TGF-βRII (r = -0.268, p = 0.010). No significant correlations were found between the inflammatory markers TGF-β (serum marker), TGF-βRI and miR-21 and the parameters of body composition.

Table 10. *Correlations between body composition and inflammatory markers*

		Weight [kg]	Height [m]	BMI [kg/m²]	Body fat mass [kg]	Body cell mass [kg]	Waist / Hip Ratio [-]
Serum markers							
hs-CRP [mg/l]	Spearman rho	**0.288**	0.110	**0.292**	**0.335**	**0.216**	0.115
	p-value	**0.003**	0.268	**0.003**	**0.001**	**0.031**	0.268
TGF-β [ng/ml]	Spearman rho	0.140	-0.049	0.175	0.131	-0.006	0.201
	p-value	0.157	0.621	0.078	0.197	0.956	0.052
Intracellular markers							
TGF-β /GAPDH [-]	Spearman rho	-0.073	-0.015	-0.075	-0.123	-0.030	**-0.273**
	p-value	0.477	0.888	0.468	0.240	0.779	**0.009**
TGF-βRI /GAPDH [-]	Spearman rho	0.028	0.094	-0.009	0.052	-0.023	-0.206
	p-value)	0.788	0.361	0.932	0.619	0.830	0.051
TGF-βRII /GAPDH [-]	Spearman rho	-0.111	-0.073	-0.094	-0.131	-0.059	-**0.268**
	p-value	0.278	0.475	0.362	0.210	0.577	**0.010**
miR-21 [copies/pg]	Spearman rho	-0.062	0.003	-0.107	-0.186	0.153	-0.008
	p-value	0.537	0.978	0.289	0.068	0.135	0.942

Data are calculated by using Spearman rank correlation. hs-CRP = high-sensitive C-reactive protein; TGF-β = transforming growth factor beta; TGF-βRI = transforming growth factor beta receptor I; TGF-βRII = transforming growth factor beta receptor II; miR-21 = mircoRNA 21; BMI = body mass index

3.4.3 CORRELATIONS BETWEEN INFLAMMATORY MARKERS

Finally, inflammatory markers were tested for correlations in between themselves (see Table 11.). Hs-CRP showed a significant positive correlation (r = 0.294, p = 0.002) to TGF-β (serum marker) and a negative correlation to TGF-βRII (r = -0.205, p = 0.038). TGF-β (intracellular marker) was positively associated with TGF-βRI (r = 0.612, p < 0.001) and TGF-βRII (r = 0.902, p < 0.001). Besides, TGF-βRI was positively correlated to TGF-βRII (r = 0.562, p < 0.001), but negatively correlated to miR-21 (r = -0.256, p = 0.012).

Table 11. *Correlations between inflammatory markers*

		Serum markers		**Intracellular markers**			
		hs-CRP [mg/l]	TGF-β [ng/ml]	TGF-β /GAPDH [-]	TGF-βRI /GAPDH [-]	TGF-βRII /GAPDH [-]	miR-21 [copies/pg]
Serum markers							
hs-CRP [mg/l]	Spearman rho	1.000	**0.294**	-0.184	-0.111	**-0.205**	0.046
	p-value	-	**0.002**	0.070	0.280	**0.043**	0.645
TGF-β [ng/ml]	Spearman rho	**0.294**	1.000	-0.172	-0.172	-0.175	-0.047
	p-value	**0.002**	-	0.091	0.092	0.084	0.639
Intracellular markers							
TGF-β /GAPDH [-]	Spearman rho	-0.184	-0.172	1.000	**0.612**	**0.902**	0.113
	p-value	0.070	0.091	-	**< 0.001**	**< 0.001**	0.275
TGF-βRI /GAPDH [-]	Spearman rho	-0.111	-0.172	**0.612**	1.000	**0.562**	**-0.256**
	p-value)	0.280	0.092	**< 0.001**	-	**< 0.001**	**0.012**
TGF-βRII /GAPDH [-]	Spearman rho	**-0.205**	-0.175	**0.902**	**0.562**	1.000	0.122
	p-value	**0.043**	0.084	**< 0.001**	**< 0.001**	-	0.238
miR-21 [copies/pg]	Spearman rho	0.046	-0.047	0.113	**-0.256**	0.122	1.000
	p-value	0.645	0.639	0.275	**0.012**	0.238	-

Data are calculated by using Spearman rank correlation. hs-CRP = high-sensitive C-reactive protein; TGF-β = transforming growth factor beta; TGF-βRI = transforming growth factor beta receptor I; TGF-βRII = transforming growth factor beta receptor II; miR-21 = mircoRNA 21;

4 DISCUSSION

The aim of the current study was to obtain an insight into alterations of circulating TGF-β as well as TGF-βRI, TGF-βRII and miRNA-21 levels in PBMCs caused by a progressive exercise intervention with or without nutritional supplementation in institutionalized elderly women and men. Therefore 117 institutionalized individuals were allocated to one of the three study groups (RT, RTS or CT) and performed supervised progressive resistance training with Thera-Bands® with or without dietary supplementation or participated in cognitive training sessions over a period of 6 months, respectively. Anthropometric measurements, physical performance tests and blood sample analyses were conducted at the beginning of the study, after 3 months and after 6 months.

The present study cohort consisted of 88% female and only 12% male participants. According to the statistic data from the Curatorship of Viennese retirement homes, the gender distribution of the residents of all retirement homes in the age group 80-89 years is 80% female to 20 % male individuals (KWP, 2014). Hence, the gender distribution of the study population is approximately the same as in the old people's homes in Vienna and is therefore representative for this particular age group.

Taking a closer look at the baseline values, it can be seen that there were no significant gender differences in age, the chair stand test, the relative PTQ 60°/s and the BMI. Also, the 6-minute walking test did not show any gender differences at the beginning of the study. Camarri et al. (2006) investigated the maximum walk distance in 70 healthy male and female subjects between 55 an 75 using the 6-minute walking test and found significant differences in the 6-minute walking distance between male and female participants, yet it should be noted that these persons were generally younger than the study population of the VAAS (64.5 years and 83.9 years respectively). Sugimoto et al. (2014) came to similar conclusions, when they performed a 6-minute walking test and other physical performance tests with a group of elderly men and women with cardiac and other serious diseases during maintenance period. These people had attended a one-year exercise therapy program, which also included 6-minute walking twice a week (Sugimoto et al., 2014). These individuals at the average age of 80.2 (men) and 79.1 (women) (Sugimoto et al., 2014) were also younger than those of the present study, which could be a reason for the differences in the results. Normative values from 7,183 community-residing older

adults between 60 to 94 years of age (Rikli & Jones, 1999) also indicate that men perform significantly better in the 6-minute walking test as well as in the chair stand test.

In strength related tests, men reached significantly better values than women, which is in concordance with the available literature (Cooper et al., 2011; Goodpaster et al., 2006; Hughes et al., 2001; Sugimoto et al., 2014). In grip strength, relative PTH 60°/s and 120°/s as well as relative PTQ 120°/s men achieved significantly higher baseline results. The meta-analysis from Cooper et al. (2011), which included 5 studies with 14,213 subjects between 50 and 90+ years of age, described the same trend in handgrip strength. The mean difference in grip strength of men and women was 12.62 kg after adjustments for age and body size, though evidence was found indicating that these gender differences diminish with increasing age (Cooper et al., 2011). Likewise, Werle et al. (2009) reported higher grip strength in male than in female persons in their study testing a population of 1,023 persons in an age range from 18 to 96 years. The women aged 75 and older reached 64% of the mean values of the men in the same age group (Werle et al., 2009). The situation of relative PTQ and PTH is quite similar to the situation of grip strength. The study conducted by Lindle et al. (1997) assessed age and gender differences in concentric peak torque of the knee extensors and flexors among other muscle parameters at various velocities in 654 individuals (aged 20 to 93 years) and observed significantly greater peak torque values in men than those of women across all velocities and ages. However, more recent investigation from Cramer et al. (2015) and Borda et al. (2014) supported these findings. Though the results from Borda et al. (2014) were only significant in terms of absolute values, but normalized to body mass significance was no longer met.

As expected, men of the VAAS population were significantly taller and heavier than women, which was in line with diverse studies (Fragala et al., 2012; Perissinotto, Pisent, Sergi, Grigoletto, & Enzi, 2002; Rea, Gillen, & Clarke, 1997). A large cross sectional study by Gavriilidou et al. (2015) with 3,360 elderly persons from the ‘Good Aging in Scania’ population study showed similar anthropometric data. The non-significant difference in BMI between the sexes of the present study resembles the described results by Fragala et al. (2012) for their age groups 71-77 and 78+ years. The present findings for waist to hip ratio also correspond with results from other research teams (Perissinotto et al., 2002; Sanchez-Garcia et al., 2007), as the significantly higher body fat mass in women from the current study does (Fragala et al., 2012). Due to stratification by gender, the gender differences were compensated during group allocation. The assessment of group differences at baseline did not show any significant differences, hinting at the effectiveness of

the randomized distribution and therefore minimizing the variability of its evaluation (Suresh, 2011).

The loss of muscle strength and the related weakness in elderly causes problems related to daily activities and is also associated with a higher risk of falling (Liu & Latham, 2009). This age-related state called sarcopenia is a common phenomenon and has enormous personal and financial costs (Cruz-Jentoft et al., 2010). In general, it has been shown that resistance training leads to improvements in strength, even in older persons (Kosek, Kim, Petrella, Cross, & Bamman, 2006; Mayer et al., 2011; Stout et al., 2013). An increase in strength (Liu & Latham, 2009; Peterson, Rhea, Sen, & Gordon, 2010) and an improvement of lean body mass (Peterson, Sen, & Gordon, 2011) in elderly people was also confirmed by a systematic review as well as two meta-analyses, respectively.

Martins et al. (2013) conducted a meta-analysis in order to investigate the efficiency of exercise trainings with elastic bands in elderly. After analyzing 11 studies including 834 older individuals the research team came to the conclusion that elastic resistance training is effective in increasing their muscle strength (Martins et al., 2013). The results from the VAAS showed that the performed 6-month progressive resistance training with Thera-Bands® led to significant alterations of the results of aerobic endurance as well as strength and physical function in in older individuals. In more detail, the applied strength training routine was able to positively influence the walked distance of the 6-minute walking test in the RT group. Previous investigations showed that the walk distance of the 6-minute walking test is strongly related to power and strength of the lower limbs in elderly (Bean et al., 2002). Since the values of PTQ and PTH also increased significantly in the RT group and partly in the RTS group, the positive alterations of the 6-minute walking test distance could thereby be explained. Interestingly, in some of the tested physical performance parameters like the relative PTQ 120°/s and relative PTH 120°/s the highest values were already reached after the third month of progressive resistance training. As recommended by the ACSM (Nelson et al., 2007), in the present study different variables were altered to systematically increase the demands placed upon the bodies of the individuals. Nevertheless, it could be conjectured that the progression in form of different Thera-Bands® with higher resistance, the modification of the execution of the own-body weight exercises as well as the increase of sets did not provide a stimulus high enough to cause further improve strength after the third month of the training intervention. Maybe selecting different exercises for the same muscle groups after 3 months would have helped to counteract this some kind of habituation effect and would have led to additional gain of strength.

The results of the chair stand test obtained in the present study demonstrate that the study participants significantly improved over time in the RT and the RTS groups. Similar outcomes were described by the research team of Fahlman et al. (2011). In their study functionally limited elderly men and women (65-93 years) performed progressive resistance training with Thera-Bands® three times per week, whereof one time in a group setting and two times at home with an overall duration of 16 weeks. The exercise intervention led to significantly increased chair stand test results in the exercise group but not in the control group at both measuring points, at week 9 and 17 (Fahlman et al., 2011). According to data published by Egaña et al. (2010), postmenopausal, elderly women were able to significantly improve their performance in the 30-second chair stand test after taking part in a 12 week progressive resistance training program. This program comprising lower body and upper body exercises with elastic bands was attended twice a week by the participants (Egaña et al., 2010). These results are in line with the study conducted by Rogers et al. (2002) who also reported significant improvements of chair stand test performance. They tested the efficiency of a combined elastic band and dumbbell exercise program for the major muscle groups on physical function of older women (62-94 years) (Rogers et al., 2002). Something that might have influenced the results of the chair stand test of the VAAS is the fact that during the training intervention sessions, chairs were used regularly for exercises in the sitting position. By changing the exercise position several times the people practiced the movement of the chair stand test to a certain extend. This fact may also have led to improvements in the respective test results.

Interestingly, Roger et al. (2002) found significant improvements in grip strength, though no exercises specifically targeting grip strength were included in the study program. The alterations might be attributable to gripping the Thera-Bands® and dumbbells during the training (Rogers et al., 2002). In comparison, Zion et al. (2003) were not able to show significant differences in handgrip strength in 4 male and 4 female persons, who participated in an 8-week home-based progressive resistance training program using Thera-Bands®. Though, the authors pointed out that the small sample size reduced the power of their study (Zion et al., 2003). In the VAAS handgrip strength also was not significantly affected by the training intervention. A possible explanation could be that no special training exercises were performed for handgrip strength and that the stimulus from just holding the Thera-Bands® during the exercises was insufficient.

Recent papers reported positive effects of protein supplementation alone (Shahar et al., 2013) but also of combinations of exercise and nutrition interventions (Kim et al., 2012;

Shahar et al., 2013) on muscle strength in older subjects. According to Malafarina et al. (2013) the positive effects of nutritional supplementation increase when accompanied by physical exercise. The meta-analysis of 22 randomized controlled studies with a total of 1,287 young and older subjects by Cermak et al. (2012) revealed that supplementation of proteins is effective in increasing skeletal muscle in response to prolonged exercise-type resistance training in young and older persons. The authors noted that the individuals in the category 'older' were just 50+ years old (mean age: 62 ± 6 years) and speculated that in older and specially frail elderly the protein supplementation could be even more effective (Cermak et al., 2012). These findings are inconsistent with results from other studies. Verdijk et al. (2009) reported that a 12-week resistance training with 3 sessions per week and additional 10 g protein supplementation before and after the sessions did not further enhance muscle strength or mass in elderly men in comparison to the gains from resistance exercise alone. Investigations by Tieland et al. (2012) and also by Leenders et al. (2013) with even higher amounts of protein supplementation (additional ingestion of 30 g protein per day) in combination with 24 weeks of different resistance training routines did not reveal further augmentations in strength in elderly compared to the values of the groups that just performed resistance training without supplementary protein intake. In the present study, supplementation with proteins also did not induce an additional increase in muscle strength. Protein supplementation in elderly persons who have a habitually adequate consume of dietary protein (Verdijk et al., 2009) and who are well-nourished could result in limitations of the expected margin of improvement in strength (Malafarina et al., 2013).

Generally, chronic low-grade inflammation is an essential contributory factor to the pathophysiology of diverse chronic health states (Beavers, Brinkley, & Nicklas, 2010). This type of inflammation was also recognized as one of the major risk factors for age-related diseases (Cevenini et al., 2013). According to a review by Beavers et al. (2010), numerous publications of observational studies reported an inverse association between effects of self-reported exercise or aerobic exercise interventions and inflammatory markers. (Beavers et al., 2010). In their systematic review de Salles et al. (2010) reported that resistance training was not able to significantly reduce TNF-α levels. Contrary to these results, TNF-α was reduced by a whole-body resistance training of moderate to high intensity (Phillips et al., 2012). In addition, Ogawa et al. (2010) found that a 12-week strength training is effective in decreasing chronic inflammation in elderly women. As demonstrated by Mavros et al. (2014), a progressive resistance training with a duration of 12 months could reduce systemic inflammation in elderly with type 2 diabetes. A recent study by Forti at al. (2014) showed that progressive strength training significantly reduced IL-6 levels in

community-dwelling elderly persons between 62 and 72 years. Walsh et al. (2011) pointed out that the positive effects of endurance exercise on chronic inflammation are well known, while the anti-inflammatory role of strength training has not been inadequately defined yet. In case of the commonly used inflammatory marker CRP, this description also seems to be partly valid when taking a look at the available literature. The meta-analysis by Kelley and Kelley, (2006) of randomized controlled studies, which included different types populations (persons with diabetes, cardiovascular diseases, overweight/obesity or breast cancer), showed that aerobic exercise could not lower the CRP levels in adults. On the other side, a more recent meta-analysis by Hayashino et al. (2014) with type two diabetes patients reported a significant reduction of CRP levels by aerobic exercise programs but only a non-significant change in trials that focused on resistance training. Further studies by Kohut et al. (2006), Libardi et al. (2012) and Swift et al. (2012) support this lack of effectiveness of resistance training on CRP, whereas Donges et al. (2010) and Martins et al. (2010) found contradictory results. Beyond that, resistance training interventions were reported to be effective in decreasing CRP levels in older individuals and obese people, whereby interventions of 16 weeks or longer might be necessary to attain significant results (de Salles et al., 2010). The levels of hs-CRP were not altered by the intervention employed in the VAAS. It seems that the progressive resistance training regime was not ideal for this purpose. Although there is no conclusive evidence for a most potent intervention so far, it may be hypothesized that in case of hs-CRP, it is more effective to use endurance and aerobic training programs (Campbell et al., 2009) or a combination of resistance and endurance training (Brunelli et al., 2015; Daray et al., 2011; Kim, Jung, & Kim, 2008; Stewart et al., 2007) to lower hs-CRP levels in future studies.

According to Touvra et al. (2011), the effects of exercise on TGF-β1 have been examined only by a small number of studies. Furthermore, Czarkowska-Paczek et al. (2006) emphasized that data concerning regulatory effects of physical exercise on TGF-β is conflicting and very limited. This research team investigated the effects of strenuous physical exercise in form of graded cycling on a treadmill on serum levels of TGF-β in 14 young cyclists. Their results showed that the TGF-β serum levels significantly increased directly after the exercise and decreased after two hours to a level that was still significantly higher than the initial values directly before the exercise (Czarkowska-Paczek et al., 2006). Treadmill running until exhaustion was reported to lead to a non-significant increase in plasma TGF-β1 levels in 6 healthy, young male individuals that might be in connection to mechanical loading of tissues during the exercise testing (Heinemeier, Langberg, Olesen, & Kjaer, 2003). A study done by Toft et al. (2002) compared TGF-β1 levels between young and elderly persons at rest and revealed that the levels of the younger participants

were significantly elevated compared to the levels of the elderly. Moreover, a one-hour eccentric exercise on a cycle ergometer was performed in order to evaluate the alterations in TGF-β1 plasma cytokine levels of the individuals. In both, the young and the older subjects, eccentric exercise had a small positive effect on TGF-β1 (Toft et al., 2002).

Other authors evaluated how different types of exercise training influence circulating TGF-β1 levels in groups of different age. Ten patients (median age: 55,5) with diabetes mellitus II took part in a systemic training regimen that combined strength training for the upper and lower body (60-70% of the one repetition maximum [1RM]; 3 sets of 15 repetitions) and aerobic training (70-80% of the maximal heart rate) and was performed 4 times per week over a period of 8 weeks (Touvra et al., 2011). The researchers tested serum concentrations of pro- and anti-inflammatory cytokines at baseline and also 3 days after the end of the training intervention. TGF-β1 was found to be significantly elevated, whereas hs-CRP levels were significantly reduced after the combined training, underscoring its anti-inflammatory effects. Throughout the study no significant changes were found for levels of IL-6, IL-10, TNF-α and INF-γ (Touvra et al., 2011). Another study reported that a 4-week intensive strength training (2 hours a day; 5 days a week) for the lower body in 6 healthy, young students significantly elevated plasma levels of latent TGF-β1 after the second week of training, slowly declining to lower levels after the third week, followed by a stronger decline in week 4 (Hering et al., 2002). This decrease of latent TGF-β1 concentration after the third week was interpreted as the completion of adaptation to the applied mechanical stimuli by the resistance training (Hering et al., 2002). In elderly men and women a 6-week intensive strength training with training machines resulted in a non-significant increase in circulating IL-10 and TGF-β levels and a slight decrease in circulating IL-6 (Bautmans et al., 2005). As demonstrated by most of the presented studies, exercise interventions led to an increase in circulation TGF-β1 levels. In contrast to these results, the progressive resistance training of the current study could not influence the circulating levels of TGF-β. Nevertheless, it is difficult to directly compare the data from the mentioned studies due to variations in the different research designs, such as study populations, age groups, applied training interventions and measuring methods.

With regard to intracellular markers Heinemeier et al. (2013) tested the effects of a one-hour, one-leg kicking exercise (at 67% of maximum work load) in 31 young men on levels of mRNA of TGF-β and TGF-βRII among various other parameters. Biopsies from muscle (vastus lateralis) and tendon (patellar tendon) were taken from both legs of the participants (control and exercise leg) 2 hours, 6 hours or 26 hours after the exercise depending on a previous group allocation. The results demonstrated no changes of gene expression

in the tendon but a significant overall effect on TGF-β1 mRNA in muscle tissue by exercise. Further, a significant increase in TGF-β2 mRNA at 2 hours and in TGF-βRII at 6 hours in the muscle due to the performed physical exercise was reported, indicating an anabolic response (Heinemeier et al., 2013). Despite this, the effects of a graded cardiopulmonary exercise test on TGF-β signaling gene expression patterns in PBMCs of young, healthy, trained cyclists were analyzed in a study by Kimsa et al. (2012). For the extraction of RNA from PBMCs blood samples were drawn before, directly after and 15 minutes after the exercise testing. Finally, the results from the microarray analysis indicated that the levels of TGF-β1 were increased 1.5-fold at the end of the exercise but its expression decreased to the basic level 15 minutes after the exercise task. According to the authors, these alterations might support the anti-inflammatory role of TGF-β1 (Kimsa et al., 2012).

The oncogenic miRNA-21 (Merline et al., 2011; Pan, Wang, & Wang, 2010) was also described as one of the so-called 'inflamma-miRNAs' that are involved in the regulation of inflammatory and immune response (Olivieri, Rippo, Procopio, & Fazioli, 2013). Abnormal expressions of these miRNAs might contribute to low chronic inflammation in major age-related diseases and also in the normal ageing process (Olivieri et al., 2013). Measurements of a subset of c-miRNAs in trained, young, male endurance and strength athletes showed that plasma levels of c-miRNA-21 were significantly higher in the endurance group than in the strength athletes (Wardle et al., 2015). Besides, it was reported that a resistance exercise comprising bench press and bilateral leg press (5 sets of 10 repetitions; 70% of the 1RM) in 12 healthy, male subjects did not affect the levels of c-miRNA-21 (Sawada et al., 2013). These results differ from the reported effects of endurance exercise on c-miRNA by Baggish et al. (2011), which could mean that the influence of exercise on c-miRNAs might be depending on the types of performed exercise (Sawada et al., 2013). Moreover, Sawada et al. (2013) underline that no standardized detection method has been established yet, so that the differences in the applied methods can lead to inconsistencies between the study outcomes. On contrary to the discussed alterations in the expressions of miRNA-21 and TGF-β by exercise, in the present study none of the evaluated intracellular markers significantly changed over time in any of the three study groups.

Based on data from available literature, age was, as expected, significantly inversely correlated with the physical performance of the 6-minute walking test and handgrip test (Martin, Ramsay, Hughes, Peters, & Edwards, 2015; Werle et al., 2009). Surprisingly, hs-CRP was also negatively associated with age, which stands in contrast to the previous findings showing that CRP tends to be elevated with age (Ballou et al., 1996; Hutchinson

et al., 2000; Woloshin & Schwartz, 2005). Although hs-CRP only negatively correlated with one of the physical performance parameters, namely PTQ 120°/s, this result points towards the general tendency that higher physical performance is related to a lower inflammatory status (Abramson & Vaccarino, 2002; Cesari et al., 2004; Geffken et al., 2001; Reuben, Judd-Hamilton, Harris, & Seeman, 2003). Olivieri et al. (2012) suggested using miRNA-21 as a marker of inflammation and showed that older persons have higher levels of miRNA-21 expression than younger subjects, indicating that high levels of miRNA-21 would rather be associated with lower physical performance. This argumentation may be supported by the data of Wardle et al. (2015) who demonstrated a negative association between total handgrip strength and circulating levels of miRNA-21, but with the addition that their subjects were young athletes and the results were only significant in an uncorrected model. Nevertheless, in the present study handgrip strength and the expression of miRNA-21 were positively associated. The correlations between inflammatory markers and the body composition revealed a significant positive association between BMI, bodyweight, body fat mass and hs-CRP, as already reported earlier (Festa et al., 2001; Mavros et al., 2014; Mediano et al., 2013; Pannacciulli et al., 2001; Timpson et al., 2011).

Unsurprisingly, intracellular TGF-β showed a high positive correlation with the expression of its two receptors TGF-βRI and TGF-βRII due to their naturally given, functional interaction. Hence, the positive association between TGF-βRI and TGF-βRII may also be explained by their mutual necessity for the SMAD-dependent signaling pathway (Kang et al., 2009; Shi & Massagué, 2003). On the basis of the positive correlation between serum hs-CRP and TGF-β, it might be speculated that higher values of TGF-β could be representative for an immunosuppressive answer (Li et al., 2006; Shachar & Karin, 2013) of the human body to elevated hs-CRP serum levels. Though, for this hypothesis more detailed information about other circulating inflammatory biomarkers, especially IL-6, would be required.

Another essential point is the interplay between miRNA-21 and TGF-β. On the one hand, Davis and Ross (2008) stated that elevated basal miRNA-21 expression could be due to the contribution of autocrine TGF-β signaling. Additionally, the TGF-β pathway seems to promote the invasive and metastatic potential of cancer cells by modulating the biosynthesis of the oncogenic miRNA-21 (Davis & Ross, 2008). On the other hand, Yu et al. (2012) speculated that miRNA-21 directly targets the TGF-βRII, based on the observation that the receptors three prime untranslated region (3' UTR) contains a complimentary site to miRNA-21 and that a downregulation of TGF-βRII mRNA occurred in miRNA-21 overexpressing colon cancer cells. TGF-βRII and miRNA-21 are also involved in the adipogen-

ic differentiation of human adipose tissue-derived mesenchymal stem cells (hASCs) (Kim, Hwang, Bae, & Jung, 2009). TGF-β seems to block adipogenic differentiation in its early phase and an increase of miRNA-21 counteracts this inhibitory action by down regulating the TGF-βRII expression (Kim et al., 2009). Moreover, Olivieri et al. (2012) stated that TGF-βRI and also TGF-βRII are targeted by miRNA-21. Interestingly, in the present study miRNA-21 was only significantly negative associated with TGF-βRI but not with TGF-βRII.

As mentioned before, TGF-β in combination with IL-6 leads to T_H17 differentiation whereas TGF-β alone causes T_{reg} differentiation of naïve T-cells (Kimura & Kishimoto, 2010). T_{reg} cells play an important role in maintenance of immune homeostasis (Kimura & Kishimoto, 2010) and Foxp3 functions as their cell lineage specification factor (Fontenot & Rudensky, 2005). Rouas at al. (2009) examined the miRNA expression profile of human thymus-derived natural T_{reg} cells and discovered that miRNA-21 positively regulates the expression of Foxp3. The presented facts regarding the relationship between the TGF-β pathway and the miRNA-21 highlight the complexity of their interaction. Due to the fact that IL-6 plays an important role in the way how TGF-β exerts its pro- or anti-inflammatory effects it would be interesting, as mentioned above, to additionally analyze the serum concentrations of this cytokine to perform further statistically analyses of correlations and changes.

Overall, the findings from this randomized controlled intervention study were able to show that progressive resistance training with Thera-Bands® is generally able to improve physical performance in community-dwelling elderly with no additional positive effect of protein supplementation. Complementary to these improvements, the training intervention neither affected the serum markers hs-CRP and TGF-β nor the intracellular expression of TGF-β, TGF-βRI, TGF-βRII and miRNA-21.

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APPENDIX

ABBREVIATIONS

1RM	One repetition maximum
ACSM	American College of Sports Medicine
AHA	American Heart Association
AMH	Anti-Müllerian hormone
AMPK	AMP-activated protein kinase
APC	Antigen-presenting cells
APP	Acute phase proteins
APR	Acute phase response
B cells	B lymphocytes
BDNF	Brain-derived neurotrophic factor
BIA	Bioelectric impedance analyses
BMI	Body mass index
BMP	Bone morphogenetic protein
c-miRNA	Circulating microRNA
cDC	Conventional dendritic cells or classical dendritic cells
$CD4^+$ T cells	Helper T cells
CD28	Cluster of differentiation 28
CD57	Cluster of differentiation 57
CMV	Cytomegalovirus
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
Co-SMAD	Common-mediator SMAD
CTLs or $CD8^+$ T cells	Cytotoxic T lymphocytes
CT	Cognitive training
DC	Dendritic cells
ECD	Extra cellular domain
FDC	Follicular dendritic cells
FGF-2	Fibroblast growth factor 2
FSTL-1	Follistatin-like 1
GARP	Glycoprotein A repetitions predominant protein

gp130	Glycoprotein130
GDF	Growth and differentiation factor
HDL-C	High density lipoprotein cholesterol
hs-CRP	High-sensitivity CRP
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IL-1β	Interleukin 1β
IL-1ra	Interleukine 1 receptor antagonist
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
IL-10	Interleukin 10
I-SMAD	Inhibitory SMAD
KLRG1	Killer cell lectinlike receptor G1
KWP	Curatorship of Viennese retirement homes
LAP	Latency associated peptide
LIF	Leukaemia inhibitory factor
LLC	Large latent complex
LTBP	Latent TGF-β binding protein
miRNA	Micro RNA
mRNA	Messenger RNA
MMST	Mini Mental State Test
NK	Natural killer
PASMC	Pulmonary smooth muscle cells
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
pDC	Plasmacytoid dendritic cells
pri-miRNA	Primary miRNA
PTQ	Peak torque quadriceps
PTH	Peak torque hamstrings
RISC	RNA-induced silencing complex
ROM	Range of motion
RORα	Retinoic acid receptor α
RORγ	Retinoic acid receptor γ
rRNA	Ribosomal RNA
R-SMAD	Receptor-regulated SMAD
RT	Resistance training

RTS	Resistance training + nutritional supplement
SBE	SMAD-binding element
sIL-6R	Soluble interleukin 6 receptor
SLC	Small latent complex
SPPB	Short Physical Performance Battery
S/TKD	Serine-threonine kinase domain
sTNF-RI	Soluble TNF-α receptor 1
T cells	T lymphocytes
TGF-β	Transforming growth factor β
TGF-βRI	Transforming growth factor β receptor 1
TGF-βRII	Transforming growth factor β receptor 2
TMD	Trans-membrane domain
TNF-α	Tumor necrosis factor α
TF	Transcription factor
T_{reg}	Regulatory T cells
tRNA	Transfer RNA
VAAS	Vienna Active Ageing Study

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EIDESSTATTLICHE ERKLÄRUNG

„Ich erkläre, dass ich die vorliegende Arbeit selbstständig verfasst habe und nur die ausgewiesenen Hilfsmittel verwendet habe. Diese Arbeit wurde weder an einer anderen Stelle eingereicht noch von anderen Personen vorgelegt."

Wien, 2015 Thomas Wolf