The role of inflammation in muscle aging

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Chapter 1

General Introduction

Muscle aging

Skeletal muscle tissue accounts for 40% by mass for men and 30% by mass for women (Janssen *et al.*, 2000). Age-related decline in muscle mass and strength starts between the age of 30 and 50 years. After the age of 70 years the loss of muscle mass is around 1% per year and the loss of muscle strength around 3% per year (Goodpaster *et al.*, 2006). Low muscle mass and strength is a major contributor to disability and mortality (Filippin *et al.*, 2015).

Sarcopenia

To provide more recognition from the scientific community Rosenberg proposed in 1989 to give age-related low muscle mass and function a name. He suggested to call it *sarcopenia*, derived from the Greek words $\sigma\acute{\alpha}\rho\xi$ = sarx = flesh and $\pi\epsilon\nu\acute{\iota}\alpha$ = penia = poverty, so "poverty of flesh" (Rosenberg, 1989, 1997). Although the number of scientific articles using the term sarcopenia has increased to 750 per year in 2015, its definition is still topic of debate (Reijnierse *et al.*, 2015). There is no consensus on

1. whether the diagnosis also involves low muscle strength (next to low muscle mass) — Clark and Manini (2008) postulated that low muscle strength is a different phenomenon and named it *dynapenia*, Greek for "poverty of strength";

2. whether the diagnosis also involves low physical function (next to low muscle mass and strength) and how this should be measured;

3. which threshold values should be used for the diagnosis;

4. whether the focus should be on muscle mass in the whole body (equivalent to lean body mass) or only on muscle mass in the arms and legs (equivalent to appendicular lean mass);

5. how to adjust muscle mass and strength and physical function for fat mass, and height;

6. which method should be used to measure muscle mass and strength and physical function.

Notwithstanding these uncertainties, in the past decades it has been become more and more clear that the etiology and pathogenesis of age-related low

muscle mass and strength is a complex interplay of a myriad of factors, including physical inactivity, hormonal, metabolic, and nutritional factors (Ali & Garcia, 2014). Research has also implicated inflammation in its pathogenesis (Budui, Rossi & Zamboni, 2015).

Acute and chronic inflammation

Inflammation involves a wide variety of physiological and pathological processes in response to infection, tissue injury, tissue stress (such as hypoxia, which occurs in muscle tissue during exercise), and tissue malfunctioning. One should make a distinction between the *acute inflammatory response* and *chronic inflammation* (Medzhitov, 2008). On the one hand, during the acute inflammatory response, triggered cytokine producing leukocytes such as neutrophils and monocytes from the blood migrate to the site of infection or injury, eliminate the pathogen or injured tissue and stimulate repair (Chazaud, 2014). On the other hand, chronic inflammation leads to tissue damage and the formation of fibrosis (Mann *et al.*, 2011).

Cytokines are involved in the acute inflammatory response as well as in chronic inflammation, but their source differs. Cytokines produced during the acute inflammatory response have as their main source monocytes. Cytokines involved in chronic inflammation have as their main source a wide variety of cell types, including lymphoid cells as well as non-lymphoid cells such as endothelial cells, fibroblasts, and adipocytes (Naka, Nishimoto & Kishimoto, 2002). Moreover, it is known of cytokines like interleukin-6 (IL-6) that they can have pleiotropic effects (Munoz-Canoves *et al.*, 2013). Therefore, it remains an open question whether cytokines like IL-6 have the same effect during the acute inflammatory response as during chronic inflammation, and whether these effects are dependent or independent of each other. We notice in this connection that blood levels of circulating IL-6 are known to be two-fold higher in 90-years old subjects compared to 65-years old subjects, indicating an age-related increase in chronic inflammation (Puzianowska-Kuznicka *et al.*, 2016). However, there are also indications that the acute IL-6 production response decreases over age (Nyugen *et al.*, 2010).

The acute inflammatory response can be estimated using a whole blood stimulation assay. With this assay it is possible to measure the amount of cytokines that are produced within 24 hours in whole blood samples upon stimulation with lipopolysaccharide (LPS), a bacterial component (Damsgaard *et al.*, 2009a). The acute inflammatory response estimated using this method is called immune responsiveness, cytokine production capacity or cytokine production response. Chronic inflammation can be estimated using the data

on circulating levels of cytokines and C-reactive protein (CRP). These levels are referred to as circulating markers of inflammation.

Inflammation and muscle aging

Most research on the relation between inflammation and muscle aging has been focused on the detrimental role of chronic inflammation on muscle mass and strength. For instance, higher levels of circulating IL-6, tumor necrosis factor-alpha (TNF-α) and CRP have been associated with poorer physical performance and a decrease in muscle mass and strength (Ferrucci *et al.*, 2002; Schaap *et al.*, 2009). In mice it has been shown that after acute injury the cytokine producing monocytes infiltrate muscle tissue and are crucial for the stimulation of muscle regeneration (Arnold *et al.*, 2007). This beneficial acute inflammatory response is often overlooked or confused with damaging chronic inflammation. The author of this thesis has encountered this especially in the literature on the effect of physical exercise on chronic inflammation and muscle strength. While several studies show evidence that physical exercise temporarily decreases the acute inflammatory response, for example, Woods *et al.* (2012) instead speak of a decrease in chronic inflammation. This has made the literature on inflammation often confusing and its relation with muscle aging unclear.

Outline of the thesis

The objective of this thesis is to investigate the role of chronic as well as acute inflammation on muscle aging.

In *chapters two and three* we examine the role of chronic inflammation on muscle aging. We studied patients with rheumatoid arthritis (RA), a chronic disease characterized by high levels of circulating inflammatory mediators. RA is used as a disease model for excessive chronic inflammation. If chronic inflammation is one of the main driving factors in muscle aging, we expect to find signs of accelerated muscle aging in RA patients. In *chapter two* we describe the association between muscle strength and age in RA patients and compare it with subjects from the general population. This was investigated using pooled data from 185 studies involving 10149 subjects. In *chapter three* we investigate the age-related histological muscle characteristics in RA patients in comparison to control patients with osteoarthritis (OA). This was investigated using muscle biopsy data from 10 RA and 27 OA patients undergoing elective knee replacement.

In *chapter four* we investigate whether chronic inflammation and the acute inflammatory response are two mutually dependent or independent endo-

types. This was investigated in 403 subjects from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trail. Firstly, we study the correlation between circulating serum markers of inflammation (as an estimate of chronic inflammation) and cytokine production response measured by a whole blood stimulation assay (as an estimate of acute inflammatory response). Secondly, we study whether the acute inflammatory response has an association with cardiovascular mortality independent of chronic inflammation. Unlike the potential beneficial effects of the acute inflammatory response in skeletal muscle tissue, in the cardiovascular system the acute inflammatory response may have potential damaging effects. This has been suggested by authors reporting a transient increase in risk for a vascular event after infection (Smeeth *et al.*, 2004). If the acute inflammatory response indeed increases the risk for a vascular event, then this should be taken into account in future therapies that increase the acute inflammatory response for the potential benefit of muscle mass and strength.

When the relation between inflammation and diseases is analyzed, the potentially confounding effect of sex differences needs to be addressed. It is known that sex differences in muscle mass and strength are substantial. In order to better interpret the association between the acute inflammatory response and muscle mass and strength, we investigate in *chapter five* the sex differences in the cytokine production response. For this study we used cytokine data from 4020 subjects originating from 15 study populations, either from the general population or from patient populations with specific diseases.

Finally, in *chapter six* we then study the association between cytokine production capacity (as an estimate of acute inflammatory response) and muscle mass and strength in middle-aged elderly. The data from this study originates from 191 men and 195 women from the Leiden Longevity Study, a study consisting of offspring from long-lived Caucasian siblings and the partners thereof.

In *chapter seven* we further explore this association by investigating the relation between interleukin 10 (IL-10) gene variants, known to be associated with cytokine production response (acute inflammatory response) and muscle strength. The research presented in this chapter was performed in 554 elderly from rural Ghana, where muscle strength is of vital importance and pro-inflammatory IL-10 gene variants are enriched. In *chapter eight* the key findings of this thesis are discussed in relation to current literature and recommendations for future research are given.