Chapter 8

General discussion

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The studies described in this thesis investigated higher inflammatory marker concentrations and lower sex hormone concentrations as determinants of changes in muscle mass and muscle strength and decline in physical functioning in older persons. In addition, the association between low testosterone concentrations and increased mortality risk in older men was examined. In this chapter the main conclusions are discussed and some methodological issues related to the studies are explored. Further, implications of the findings for public health are discussed and recommendations for future research are made.

Main findings and comparison with the literature

Relationship between inflammatory markers and sarcopenia and muscle strength

Chapter 2 describes the relationship between concentrations of IL-6, CRP and ACT and changes in muscle mass and muscle strength during three years of follow-up in the LASA study. The findings of this study suggest that higher serum concentrations of IL-6 and CRP increase the risk of substantial muscle strength loss, whereas higher concentrations of ACT decrease the risk of muscle strength loss in older men and women. Persons with higher concentrations of ACT tended to have a smaller decline in muscle mass compared with persons with low concentrations of ACT. To our knowledge, this is the first study that examined whether concentrations of ACT are associated with changes in muscle mass and strength. Other studies have shown associations between increased levels of ACT and cognitive decline,[1] Alzheimer's disease[2] and depression.[3] In our study however, increased concentrations of ACT were protective of decline in muscle strength. Future studies may help to define possible mechanisms by which ACT is associated with smaller decline in muscle mass and/or strength.

We also examined the relationship between inflammatory marker concentrations and five year changes in muscle mass and muscle strength in the Health ABC study (chapter 3). Although higher serum concentrations of IL-6, CRP and TNF-α were associated with greater decline in thigh muscle area, most associations were attenuated after adjustment for weight change. TNF-α and TNF soluble receptors 1 and 2 showed the strongest associations with decline in muscle mass and muscle strength in both men and women. The results of these studies support the hypothesis that the relationship between inflammation and

physical performance can be explained by decline in muscle mass strength.[4] The attenuation of associations by weight change suggests a mediating role for weight change in the association between inflammatory marker concentrations and change in muscle mass and muscle strength. However, analyses in a subgroup of weight stable persons still showed associations between TNF-α and its soluble receptors with change in muscle mass, suggesting that weight change does not completely account for the observed associations. More research on the possibly mediating role of weight change is warranted.

Several other studies have shown independent associations of inflammation with muscle mass, muscle strength and physical performance.[5-9] Moreover, associations between inflammation and functional limitations[10] and incidence of mobility limitations[11] have been reported. In combination with evidence from animal studies that have shown that administration of IL-6 or TNF-α in rats induces muscle protein breakdown and decreases the rate of protein synthesis,[12-14] this strongly suggests that there is an inflammatory mechanism involved in the development of sarcopenia, loss of muscle strength and disability, independent of the presence of diseases.

Relationship between sex hormone concentrations and muscle strength, physical functioning and mortality

In chapter 4 we examined the cross-sectional associations between serum sex hormone concentrations (testosterone and estradiol) with grip strength, mobility and the incidence of falls. The results of this study, using data from the LASA study, showed that low concentrations of estradiol and testosterone are associated with low grip strength and impaired mobility (measured as physical performance as well as self-reported functional limitations) in men, but not in women. No association was observed between lower concentrations of sex hormones and falls during three years of follow-up.

In a subsequent longitudinal study, which is described in chapter 5, we examined the relationship between testosterone concentrations and three-year change in grip strength and three-year change in physical performance in older men from LASA and Health ABC. Lower concentrations of total and free testosterone were neither associated with decline in physical performance, nor with decline in muscle strength with consistent results in both study samples.

Several cross-sectional observational studies suggest that low serum testosterone concentrations are associated with low muscle mass, strength and mobility,[15-20] which is in agreement with our findings in chapter 4. There are several methodological explanations that

may (partly) account for the absence of associations in our longitudinal study or that can account for the cross-sectional associations. First, a possible explanation might be the selective loss to follow-up, resulting in healthier samples. Respondents not included in the statistical analyses were older, had a poorer physical performance, were less physically active, were more often smokers, were more likely to be cognitively impaired, to have depressive symptoms and had more chronic diseases, all important determinants of physical decline, compared to respondents with complete follow-up data. Second, it is possible that persons with extremely low testosterone concentrations still show a significantly greater decline in physical performance or muscle strength. However, additional analyses using an extra category of persons with very low total testosterone concentrations < 8 nmol/l (n=21 in LASA, n=159 in Health ABC), suggested no associations with either decline in physical performance or loss of muscle strength. Third, it is possible that testosterone only has an acute and short-term effect on muscle or physical performance,[21] which can account for the cross-sectional associations found in previous studies and lack of association with long-term changes in physical performance or muscle strength. Another explanation for the absence of associations might be a possible ceiling effect of the physical performance scores. This might account for the Health ABC respondents, as they had no self-reported physical limitations at baseline. The LASA sample, however, includes physically impaired persons and showed similar results. Fourth, perhaps the measurement of muscle strength and physical performance itself is not sensitive enough to measure any changes in these outcomes, or the time between the measurements is too short. Fifth, cross-sectional data do not allow causal inference. In the cross-sectional analyses in chapter 4, it is not clear whether a decline in testosterone levels preceded a decline in muscle strength and performance, whether both happened at the same time or whether a decline in muscle strength and performance preceded a decline in testosterone concentrations. Due to these limitations, the observed associations between low testosterone levels and low muscle strength and impaired mobility may be biased due to the cross-sectional nature of the study, while the longitudinal data may present the true finding of no associations between testosterone concentrations and change in muscle strength and physical performance.

Trials examining the effects of testosterone treatment on muscle mass and strength are inconsistent.[22-27] A recent large study among Dutch older men with low testosterone concentrations (<13.7 nmol/l), investigating the effects of testosterone supplementation, found an increase in muscle mass, but this was not accompanied by an increase in muscle strength or physical performance after six months of treatment.[27]

Contrary to men, there is a lack of knowledge on the role of testosterone in healthy aging in women. Chapter 6 describes the relationship between testosterone concentrations and muscle strength and physical performance, using cross-sectional as well as prospective (three year change) outcomes in older women from the Health ABC study. Lower concentrations of total and free testosterone were associated with low muscle strength and greater three-year decline in muscle strength. Although we did not find a cross-sectional association with physical performance, lower concentrations of total and free testosterone were significantly associated with a greater decline in physical performance during three years in older women. These findings in women are in contrast to our findings in men. More research is needed to investigate the role of testosterone in women and to explore these sex differences.

In older women, testosterone has mainly been administered to relieve menopausal symptoms or to enhance sexual function.[28-30] Only few studies have examined the effects of testosterone treatment on body composition in younger and post-menopausal women.[31-33] Except for one small study that showed an increase in muscle strength in women who were treated with testosterone,[32] the effects of testosterone therapy on muscle strength and physical functioning have not been studied. Based on our results, it might be worth examining changes in muscle strength and physical functioning in women participating in testosterone treatment trials.

The association between sex hormones and falls has been examined in one other longitudinal study among participants of the Osteoporotic Fractures in Men Study.[34] Fall risk in men in the lowest quartile of baseline bioavailable testosterone concentration was more than 40% greater than that in men in the highest quartile, before and after adjustment for physical performance.[34] In this study, 5-year fall follow-up data were used, while in our study, we used data of 3-year fall follow-up. This may possibly (partly) account for the difference in findings between the two studies. More observational research is needed to confirm or reject these findings and to explore the testosterone-related mechanisms that might contribute to falling.

The association between low concentrations of testosterone and mortality was examined in older men from LASA (chapter 7). Although there was a strong inverse association between testosterone concentrations and age, there were no associations with all-cause or cause-specific mortality. Total testosterone concentrations and, to a greater extent, free testosterone concentrations decline with aging.[35] Recently, several studies reported associations between low testosterone concentrations and mortality in older men.[36,37] The Rancho Bernardo study[37] was the first prospective, population based study to demonstrate an increased mortality for men in the lowest quartile of total testosterone concentrations. These

results were unaffected when adjusted for co-morbidities and when deaths in the first 5 years were excluded. Only a few smaller studies had shown this association.[38,39] A recent Swedish study reported a positive association between serum testosterone and estradiol concentrations and mortality.[36] Two other prospective studies – the Caerphilly study[40] and the Massachusetts Male Aging Study[41] did not confirm the association between testosterone and mortality. Possible explanations for the conflicting results may be differences in age of the participants, differences in testosterone concentrations, length of the follow-up, and number of deaths. Further longitudinal observational studies are required to clarify if a link between low testosterone concentrations in old age and mortality does exist.

Methodological considerations

Selection bias

In cohort studies of older persons such as LASA and Health ABC, selective non-response and loss to follow-up of the most frail and unhealthy persons is an inevitable problem. This might have led to an under-representation of older persons with lower muscle mass and muscle strength, lower physical performance, lower testosterone concentrations or higher inflammation in our study, which could have resulted in an under-estimation of the associations of low testosterone concentrations or inflammation with sarcopenia loss of strength and decline in physical performance. However, in studies examining the effect of attrition on different outcomes, attrition only had effect on the description of the sample but not on the outcomes,[42,45] so the effect of attrition on the risk of adverse outcomes is not clear.

Muscle mass measurement

There is currently a lack of standardized methodologies to assess sarcopenia. MRI and CT are considered the reference methods to assess body composition, as they are very precise, making it possible to measure small changes in body composition. However, MRI and CT are expensive, CT produces high radiation exposure and both methods are time-consuming. An attractive alternative is DXA, which is less expensive, produces less radiation exposure and is becoming increasingly common in clinical and research applications and is more widely available.[46,47] DXA measures the complete arms and legs, contrary to CT, which only examines a few slices of the body. DXA divides soft tissue into fat-free mass and fat mass, whereas CT provides muscle tissue mass and adipose tissue mass. Because the fat-free soft tissue of the

extremities is almost entirely skeletal muscle, except for a small amount of skin and connective tissues, DXA provides the opportunity to measure appendicular skeletal muscle.[48,49]

In LASA, muscle mass was assessed with DXA in a subgroup (people living in the Western part of the Netherlands). We used these data in chapter 2, in which we examined the association between inflammatory marker concentrations and three-year changes in muscle mass and muscle strength. In this study, we did not find any significant associations between high inflammatory markers and decline in muscle mass. This may be due to the small number of people with data on change in muscle mass (n=328), resulting in limited power of the analyses. However, we did not see a trend towards an association between high CRP or IL-6 with decline in muscle mass either. Second, the persons who agreed to the DXA scans were significantly younger, healthier (they had less chronic diseases) and were more physically capable than those who did not, because they had to be able to visit the VU University Medical Center. This selection of healthier persons might have resulted in an underestimation of the association between concentrations of inflammatory markers and change in muscle mass.

In chapter 3, the relationship between inflammatory markers and changes in muscle mass and strength in persons from the Health ABC study has been examined. In this study, muscle mass was assessed as mid-thigh cross-sectional muscle area by CT. We found associations between several inflammatory markers with five-year change in muscle mass, contrary to our findings in LASA. Differences in age (persons in Health ABC are older and may experience more loss in muscle mass), the higher statistical power due to a larger number of people in the analyses, longer time to follow-up and more accurate assessment of muscle mass (CT vs DXA) in the Health ABC study compared to LASA might (partly) explain our different findings.

Muscle strength measurement

Grip strength has been suggested to be a less valid instrument to determine overall muscle strength, as it might not represent the muscle strength in the lower extremity and mainly measures isometric aspects of muscle strength. However, previous studies have shown that grip strength is positively correlated with both total body strength and lower extremity strength in older persons, with reported correlation coefficients between 0.47 and 0.63.[50,51] Moreover, grip strength is an important marker for future health and function outcomes and easy to perform in clinical practice. In Health ABC, isokinetic strength of the knee extensors (a measure of lower extremity strength) was determined at 60° with a dynamometer (model 125 AP, Kin-Com, Chattanooga, TN). Electrical stimulation superimposed on maximal

voluntary contraction has been demonstrated to produce no additional torque in elderly men and women,[52,53] suggesting that the maximal voluntary effort is a valid measure of lower body strength.

Definition of sarcopenia

In the literature, different definitions of sarcopenia have been used, based on low muscle mass, loss of muscle mass, low muscle strength and loss of muscle strength.[54-58] In one study (chapter 2) in this thesis, we used a cut-point of >3% change in muscle mass and >40% change in muscle strength after three years of follow-up as cut-points for sarcopenia. These cut-points represent the lowest 15% of the LASA study sample. However, because there is no consensus regarding the definition of sarcopenia in old age, we did not dichotomize changes in muscle mass or strength, but rather used absolute or relative change in muscle mass and muscle strength as study outcomes in our other longitudinal studies in this thesis. However, for identification of persons at risk of sarcopenia, and to start prevention/treatment trials, it is important to develop cut-points of sarcopenia.

Measurement of physical performance

In this thesis, physical performance was measured using objective tests: a walk test, the ability to rise from a chair and a balance test. The use of these tests in the Health ABC study resulted in a ceiling effect, which might give problems when examining changes in physical performance over time. This ceiling effect is due to the fact that the Health ABC study comprises healthy persons without functional limitations at baseline. In chapter 5, describing the associations between testosterone and three-year changes in grip strength and physical performance, it was shown that most older men in the Health ABC sample scored high on physical performance, with a mean score of 10.4 (SD 1.3) on a range from 0 to 12. In an attempt to overcome these limitations, the Health ABC performance battery has been developed.[59] This battery of tests expanded on the established performance battery with increased test duration, a single foot stand, and a narrow walk test of balance. This battery was used in chapter 6. When we compared the results of the analysis with the Health ABC performance battery with the results of the analysis with the regular performance tests, we did not find considerable differences. This suggests that the possible ceiling effect of the performance tests seem to have a minor effect on the results of our studies.

Inflammatory markers

Currently, CRP is the most often measured inflammatory marker in clinical practice as it has shown consistent positive associations with the risk of future cardiovascular events and angina pectoris.[60-62] Increased concentrations of inflammatory markers are also associated with other medical conditions such as diabetes mellitus,[63] atherosclerosis[64] and chronic obstructive pulmonary disease (COPD). [65]

These findings have led to the hypothesis that increased concentrations of inflammatory markers could be the common root in the pathophysiological mechanisms leading to an age-related decline in physical function. Several studies have shown cross-sectional and longitudinal associations between higher inflammatory marker concentrations and (onset of) disability[66,67] and it was suggested that these associations are mediated by loss of muscle mass and strength.[4] Cross-sectional studies have demonstrated that higher concentrations of inflammatory markers are associated with lower muscle mass and strength.[5, 8] One prospective study examining the relationship between inflammation and sarcopenia showed that IL-6 was a predictor of sarcopenia only in women. In chapter 2, reporting results from the LASA study, we found associations between IL-6 and CRP and loss of muscle strength in older men and women, with greatest odds ratios for persons with IL-6 concentrations in the highest tertile. In chapter 3, describing results from the Health ABC study, we found no associations of CRP with loss of muscle mass or strength, some associations for IL-6 and most consistent associations for TNF-α and its soluble receptors. TNF-α is known to be an important cytokine in skeletal muscle wasting and reduced muscle function.[68] TNF-α has long been associated with muscle pathology and was originally designated "cachectin" in recognition of its catabolic action. It has been proposed that TNF-α can act directly on muscle cells to stimulate protein loss, an action mediated by NF-κB.[69]

In LASA, the immunoassay that was used to measure IL-6 concentrations had a high detection limit, which resulted in few respondents with valid IL-6 concentrations. This has limited the power of the analyses for the study described in chapter 2. Another limitation is the single assessment of the inflammatory markers in LASA and Health ABC at baseline. Inflammatory markers are quickly released in response to various stimuli (e.g. pathogens and physical trauma). It is possible that assessment of inflammatory markers resulted in measurement of acute infections rather than the age-associated chronic inflammatory state that we aimed for. However, during infection, concentrations of inflammatory markers are very high, while with aging, inflammatory markers rise slightly and this phenomenon is therefore often referred to as "low-grade inflammation".[70]

Soluble receptor concentrations are more stable over time and thus more accurately detected with a single blood draw.[71, 72] Higher concentrations of soluble receptors may indicate the body's attempt to control inflammation and therefore reflect a more chronic, systemic state of inflammation.[73, 74] It seems therefore unlikely that the observed associations between soluble receptors of IL-6 and TNF-α and five-year decline in muscle mass that were observed in chapter 3 are due to an acute infection, but are rather due to chronic age-associated inflammation.

Testosterone

In LASA as well as in Health ABC, total testosterone concentrations were measured by immunoassays. Radio- (RIA) and chemiluminescent immunoassays are the most widely used and accepted methods for measuring total testosterone concentrations. Wang et al.[75] compared serum testosterone measurements of eugonadal and hypogonadal adult men using liquid chromatography-tandem mass spectrometry (gold standard method) vs. several automated immunoassay instruments. The authors concluded that most immunoassays tested were capable of distinguishing eugonadal from hypogonadal males if the adult male reference range was established in each individual laboratory.

In the Health ABC study, a direct measure of free testosterone concentrations was available, but it has been suggested that assays to measure free testosterone are not accurate and may give an underestimation of the actual free fraction of testosterone.[17, 76] Therefore, we decided not to report any results for directly measured free testosterone concentrations. Instead, free testosterone concentrations were calculated using the method described by Vermeulen which takes concentrations of total testosterone, SHBG and albumin into account.[76] Calculated free testosterone concentrations obtained by the Vermeulen method are used widely in the endocrinology literature. However, the results of free testosterone calculation depend on a proper determination of the concentrations of total testosterone and SHBG and it has been shown that results differ considerably between commercially available assays for testosterone.[75, 77, 78] Despite these limitations, calculation is currently the best accepted approach to obtain free testosterone concentrations until better laboratory methods are being developed.

The currently available assays for determining serum testosterone concentrations were developed for application in men, who have much higher concentrations than women, or in women with abnormally high concentrations of testosterone. Consequently, the current assays are less sensitive for measuring low testosterone concentrations, as are present in

women. We experienced this problem in Health ABC, as half of the women had total testosterone concentrations below the detection limit. Therefore, we used sample-specific categories of total and free testosterone in this study. Another issue is the lack of normal reference ranges for (older) women, which complicates the definition of a reference group. We were able to compare the testosterone concentrations obtained in women from Health ABC with a few other studies and found fairly similar concentrations,[79,80] suggesting that the concentrations of testosterone found in the women in our study might be representative of testosterone concentrations in older women.

A limitation of our studies is that testosterone measurements were performed at a single time-point in LASA and Health ABC, which may not be representative of a person's testosterone profile over time. In the future, it would be interesting to measure testosterone concentrations at several time points to examine changes over time in relation to changes in muscle mass, strength and performance. This is especially interesting since testosterone might only have a short-term effect on muscle and performance.[21]

Interrelationship between inflammatory markers and sex hormones

In this thesis we did not investigate the possible interrelationship between inflammatory markers and sex hormones. With aging, changes occur in the endocrine system that affect the immune system and vice versa. The few existing studies that have evaluated the interrelationship between sex hormone concentrations and inflammatory marker concentrations have shown inconsistent results.[81,83] A recent cross-sectional study found a significant inverse relationship between testosterone concentrations and the soluble receptor of IL-6, but not with IL-6 itself, TNF-α, CRP or IL-1β.[82] Also, testosterone administration is followed by a marked reduction in inflammatory marker concentrations in hypogonadal men.[84,85] In vitro studies showed that testosterone downregulates the IL-6 gene through androgen receptors,[86] although conflicting data are available regarding the effects of IL-6 on testosterone as well.[87] On the other hand, higher concentrations of estradiol have been associated with increased concentrations of CRP and IL-6 in older men.[81,83] Moreover, estrogen therapy increases CRP concentrations in postmenopausal women.[88-90] Recent studies have shown that adipose tissue is not only a major source of estradiol but also produces adipokines, inducing chronic low-grade inflammation. Therefore, the positive association between estradiol and inflammatory marker concentrations is possibly explained by the amount of adipose tissue.[81]

Physical activity, inflammation and decline in muscle mass

Physical inactivity is a significant contributing factor to sarcopenia. It is well established that older men and women who are less physically active have less skeletal muscle mass and increased prevalence of disability.[91] A recent study showed that higher activity energy expenditure levels were associated with more fat-free mass, but higher levels were not associated with less decline in fat-free mass after five years of follow-up.[92] Many studies have now documented that exercise training can built muscles well into old age,[93] and that people who retain a high level of physical activity throughout their lives maintain a higher level of physical functioning and live longer.[93-95] Resistance exercise is feasible and can improve muscle mass, strength and balance in frail older people.[94, 96]

IL-6, but not TNF-α, increases during acute exercise in proportion to intensity, duration and level of fitness. It has been suggested that this acute increase in IL-6 concentrations is important for the process of muscle repair and cell turnover, as well as for some of the beneficial health effects of exercise, especially on lipids.[97] In contrast to these acute effects, persons who are long-term physically active tend to have lower concentrations of inflammatory markers.[98-100]

In unpublished data from the Health ABC study, we found a significant interaction of physical activity in the association between IL-6 and decline in muscle mass ($p<0.10$). After stratification of physical activity in three groups: persons who were inactive (less than 1000 kcal/wk of exercise and 2719 kcal/week or less total physical activity), persons who were lifestyle active (less than 1000 kcal/wk or exercise activity and more than 2719 kcal/wk or total physical activity), and persons who exercised (1000 kcal/wk or more of exercise activity), there was a significant inverse association between IL-6 concentrations and decline in muscle mass in the inactive group (B -3.13 (SE 1.23), $p=0.01$) and in the lifestyle active group (B -1.53 (SE 0.65), $p=0.02$). In the exercise group however, higher concentrations of IL-6 were no longer significantly associated with decline in muscle mass (B -0.53 (SE 0.86), $p=0.54$). These results show that physical activity, and in particular exercise, modifies the association between IL-6 concentrations and decline in muscle mass in older persons. Another study reported that leisure-time physical activity is not enough to prevent loss of muscle mass and that resistance training is the best approach to maintain and improve muscle mass and strength in older persons.[101]

Sarcopenia and obesity

Weight changes are frequently present in older persons. Weight loss is associated with loss of fat and lean mass, with the greatest proportion being fat. With weight gain however, fat replacement is greater than lean mass.[102] Loss of muscle mass also occurs in persons who are weight stable, due to a corresponding increase in fat mass.[103] It has recently been suggested that persons with a combination of both high fat mass and low muscle mass or muscle strength are at increased risk of functional decline.[58, 104, 105]

It is now recognized that adipose tissue is an active metabolic tissue that secretes hormones and proteins. Adipose tissue produces inflammatory markers and adipokines such as leptin and adiponectin, which upregulate the inflammatory response.[106, 107] It has been reported that inflammatory markers are positively associated with fat mass, and negatively associated with muscle mass.[108] Moreover, the association between inflammation and fat mass appeared to account for all of the association between inflammation and sarcopenia, suggesting that obesity-associated inflammation may precede sarcopenia. In this thesis however, we found associations between inflammatory markers and decline in muscle mass in weight stable persons as well, suggesting a direct pathway from inflammation to loss of muscle mass (chapter 3).

Other determinants of sarcopenia and decline in physical performance

In this thesis, we have focused on inflammatory markers and sex hormones as important determinants of sarcopenia and decline in physical functioning. However, there are of course other factors that contribute to the process of sarcopenia and disability, which have not been investigated, such as life style factors, genetic factors or other metabolic markers. Vitamin D for example, plays an important role in the skeletal muscle metabolism, and low serum 25-hydroxyvitamin D concentrations are associated with low muscle mass and muscle strength in older persons.[109] We did however adjust for most of these factors. Furthermore, although we carefully selected the most relevant confounders on theoretical grounds, it might be possible that some confounders (e.g. nutritional status) have been missed. Diet may affect inflammatory marker secretion. For instance, a high-fat diet increases IL-6 and TNF-α concentrations,[101] while a Mediterranean diet is associated with significantly lower IL-6 concentrations.[111] Older persons tend to obtain too little proteins in their diet, which impairs muscle protein turnover, especially during periods of weight loss.[112-114]

Also, although we were able to adjust for several chronic diseases, we did not have information on the severity of the diseases. Some chronic diseases have a greater impact

on physical functioning than other chronic diseases. The severity of a chronic disease will, together with sarcopenia, determine the consequences for physical functioning.

Relevance, implications and recommendations for future research

This thesis provides evidence that higher concentrations of inflammatory markers are associated with loss of muscle mass, loss of muscle strength and decline in physical performance and shows that weight change is an important factor in these associations. With regard to low testosterone concentrations in men, we found cross-sectional associations with some measures of muscle strength and physical performance, but there were no longitudinal associations between low testosterone concentrations and changes in muscle strength or performance. These findings, the lack of an association with mortality and the adverse effects of testosterone add to the increasing concern regarding testosterone therapy for the prevention of negative outcomes associated with aging.

Contrary to our non-significant findings in older men, low testosterone levels were associated with decline in muscle strength and physical performance in older women. These results should be confirmed in other observational studies. The associations between low testosterone concentrations with muscle strength and physical performance in women suggests that intervention studies in older women should include physical outcomes such as change in muscle mass, muscle strength and physical performance to confirm our findings. Also more understanding of the physiological mechanisms regulating levels of testosterone and other androgens in women is needed. Better insight into these mechanisms might also reveal possible sex differences that have contributed to our contradicting findings in men and women.

Testosterone supplementation

Several intervention studies, which have been reviewed by Matsumoto,[115] have shown positive associations between testosterone treatment and increases in muscle mass in older men. However, the association between testosterone administration and muscle strength is less clear.[23, 25, 116, 117] A recent meta-analysis revealed a small to moderate increase in muscle strength, with larger effects on lower extremity and total body strength than on upper extremity strength measures.[118] Based on the results described in this thesis, suggesting no associations between low testosterone concentrations and decline in muscle strength and physical performance, testosterone treatment in older men does not seem to be the most

obvious therapy for improvement of physical functioning. The lack of consistent effects of T therapy on muscle strength may be due to differences in the methods used to assess strength and the dosages and duration of T that were administered.

There is significant controversy with respect to the safety of testosterone administration in older men. Possible risks of testosterone therapy may include increased risk of cardiovascular disease, increase in haematocrit concentrations and prostate cancer.[119, 120] Results from a large Dutch study on testosterone treatment in older men showed no effects of testosterone on the prostate, and no effects on liver and kidney functions. Haematocrit concentrations were elevated, but this was without any clinical consequences.[117] In 2008, a large randomized clinical trial (TOM Trial)[121] has been set up to determine the effects of testosterone treatment on muscle strength and physical function in older men with mobility limitations and low endogenous testosterone concentrations. Results are not available yet, but will be very important for future directions of testosterone therapy research in older men. Results from the TOM Trial will further provide insight into the risks and safety aspects of testosterone treatment.

As described in this thesis, we found significant associations between low testosterone concentrations with greater decline in grip strength and physical performance in older women. Clinical trials with testosterone treatment in older women should aim to study outcomes such as body composition, muscle strength and physical performance.

The concerns about the long-term risks of prostate and cardiovascular disorders in older men treated with testosterone have encouraged efforts to develop selective androgen receptor modulators (SARMs) that have the desired anabolic effects on the muscle, but that do not have adverse effects on prostate and cardiovascular outcomes.[122, 123] One of the biggest potential advantages of SARMs is that these drugs might be safe to use in women. With improved tissue selectivity, SARMs maintain the anabolic actions of androgens without the undesirable virilising effects that occur with traditional androgen therapies. A number of first-generation SARMs are currently in phase I or phase II trials.

Anti-inflammatory drugs

It can be hypothesized that drug treatment of inflammation or treatment of diseases that lead to inflammation could reduce the risk of sarcopenia. The great majority of persons with diabetes and cardiovascular disease are currently being treated with cholesterol lowering drugs named statins. Statin medications have anti-inflammatory effects and therefore may be candidates for preventing sarcopenia.[124-126] Data from the Women's Health Initiative Observational study (WHI-OS) showed that current statin use was not associated with incident

frailty (including self-reported low physical function, exhaustion, low physical activity, and unintentional weight loss) after three years of follow-up. Longer duration of statin use was associated with reduced risk of frailty.[127] However, a side effect of statin use is myopathy, including muscle pain and weakness with or without a concomitant increase in creatine kinase concentrations (indicating muscle damage).[128] A recent study revealed that statin use predicted an increased risk of falls over 2.6 years and decline in muscle strength.[129]

Recently, a clinical trial among 355 men with type 2 diabetes showed that treatment with statins was associated with reduced total testosterone, but not with free or bioavailable testosterone.[130] Another small study in diabetic men and women did show a small decline in total testosterone but this was non-significant.[131] These results implicate that statin use might lead to loss of muscle mass through the decline in testosterone concentrations. The clinical trials described here were conducted in diabetic patients with a mean age of 60 years. Randomized controlled trials in older patients are needed to confirm the association between statin use and low testosterone concentrations. Further, intervention studies are needed to assess the effects of statins on inflammatory marker concentrations and androgen status in various patient groups. In intervention studies more attention should be given to physical outcomes such as muscle strength.

Somewhat promising are angiotensin-converting enzyme (ACE) inhibitors to prevent decline in physical function. Clinical and genetic studies in humans and experimental evidence in animals suggest that modulation of the renin-angiotensin system is associated with metabolic and biochemical changes in skeletal muscle and fat, changes that are associated with declining physical function. ACE inhibitors may modulate this process through a variety of molecular mechanisms including their influence on oxidative stress and on metabolic and inflammation pathways.[132] ACE is an important component of the renin-angiotensin system, the central hormonal regulator of blood pressure. Currently, the indications for use of ACE inhibitors include CHF, hypertension and possibly diabetes. Results from studies investigating long-term use in both aged persons with these conditions and animal models suggest an attenuation of the various pathologies and increases in mean life expectancy.[133] Recent evidence suggest that ACE inhibitors may improve physical function by means of direct effects on body composition in older persons,[134-136] rather than through its blood-pressure-lowering effects.

One definition of sarcopenia

To answer the questions as to "who is sarcopenic?" and "who is at risk for becoming sarcopenic?", one definition of sarcopenia should be developed. Several definitions have been

developed, but there is still no consensus which definition should be used. The first US population-based study made a correction of appendicular skeletal muscle mass (ASM) for height, with sarcopenia defined as ASM in kg divided by height in m^2 (ASM/height2) less than 2 standard deviations below the mean for a young healthy reference group.[54] The developed cut-points were 7.26 kg/m^2 for men and 5.45 kg/m^2 for women. The young reference group was a volunteer sample and might not have been representative for young men and women in the US.

More recently, the concept that functionally relevant loss of muscle may be relative rather than absolute was introduced.[55-57] Skeletal muscle mass was estimated from bioimpedance analysis measurements and expressed as skeletal muscle mass index (SMI = skeletal muscle mass/body mass x 100). Subjects were considered to have a normal SMI if their SMI was greater than one standard deviation above the sex-specific mean for young adults (aged 18-39). Class I sarcopenia was considered present in subjects whose SMI was within one to two standard deviations of young adult values, and class II sarcopenia was present in subjects whose SMI was below-two standard deviations of young adult values. A potential disadvantage of this approach is that the ratio of skeletal muscle mass divided by total body mass is highly dependent on the amount of fat since the between-person variation in total body fat is much larger compared to the between-person variation in skeletal muscle.

Another approach that incorporates both height and total body fat has been suggested recently. Linear regression was used to model the relationship between muscle mass with height (meters) and fat mass (kg). The residuals of the regression were used to identify those whose muscle mass was much lower or higher than the predicted value. The 20th percentile of the distribution of residuals was used as the cut-point for sarcopenia in men and women separately (men: 7.23 kg/m^2) and women: 5.67 kg/m^2). Recently, an international working group was established to work on the definition of sarcopenia.[137] Based on our finding that weight change plays an important role in the association between inflammation and loss of muscle mass and strength, this definition should include some measure of fat to account for this factor. Once a cut-point of sarcopenia is established, it will be possible to start with the development of interventions to prevent or treat sarcopenia. Different factors have to be considered when selecting a target population for clinical trials, including the goal of the intervention (treatment or prevention), feasibility, safety issues, expected prevalence and generalizability of the study findings to the population.[138]

It was first believed that the age-associated decline in muscle strength was largely due to a parallel decline in muscle mass, but this idea has not proven to be true. Both muscle mass and muscle strength decline with aging, but the decline in strength exceeds what is

expected based on the decline in mass.[139-141] It has also been reported that muscle strength, rather than muscle mass, is a more important determinant of functional limitation and poor health outcomes in older persons.[139-142] Therefore, it is also questionable if sarcopenia (irrespective of the definition used) should be used as a marker of age-related muscle impairment or whether loss of muscle strength is a better choice.[143] In data from a large Italian cohort study, low muscle strength and power were strongly associated with poor mobility, while small calf muscle cross-sectional area, an indicator of muscle mass was only weakly associated with poor mobility. These findings suggest that measures of muscle mass should be complemented with measures of strength. This is consistent with data demonstrating that muscle strength, but not muscle mass, is independently associated with lower extremity performance.[144-147]

Measurement of testosterone concentrations

Only recently, interest in testosterone administration in women started growing and so far not much is known about normal reference ranges for testosterone concentrations in women. Few studies,[79, 80] including the study described in chapter 6 of this thesis, have provided values of total testosterone in various age ranges. These studies however, used different types of assays, which makes it difficult to compare the observed testosterone concentrations. Reference values for total and free testosterone in women across all age ranges are needed before a definition of "female androgen deficiency" can be developed. Because testosterone assays are currently developed for testosterone measurement in men, who in general exhibit higher testosterone concentrations than women, measurement of testosterone concentrations in women may be difficult due to a lack of sensitivity of the assays. Improvement of the sensitivity of testosterone assays is needed to be able to reliably measure testosterone concentrations in older women.

Prevention of obesity

In this thesis, we showed an important role of weight change in the associations between inflammation and loss of muscle mass and strength. In an observational study, the mediating effects of inflammation on the association between weight change and changes in muscle mass, muscle strength and physical functioning should be further explored. Intervention studies focussing on exercise training and/or prevention of obesity should investigate changes in muscle mass, strength and performance and examine whether inflammation plays a mediat-

ing role. Much public policy is aimed at preventing weight gain and the attendant complications of obesity. Excessive weight gain can clearly cause more disability, and the combination of low muscle mass and high fat mass is the worst of both worlds. However, it has been shown that weight loss in older persons is accompanied not only with loss of fat mass, but also with loss of muscle mass. A combination of weight loss and exercise training might be the best approach in older obese or overweight persons to prevent sarcopenia.[148]

Biological pathway leading from inflammation to sarcopenia

Currently, the pathway leading from inflammation to sarcopenia is still unclear. The findings in this thesis suggest an association between increased concentrations of inflammatory markers, in particular concentrations of TNF-α, and decline in muscle mass and muscle strength, which is not independent of weight change.

As sarcopenia is believed to have multifactorial origins, other determinants than those investigated in this thesis may play an important role in the loss of muscle mass, strength and decline in physical functioning. To date, no study has attempted to develop a model with adequate data on all variables that (possibly) contribute to sarcopenia. However, Baumgartner et al. performed cross-sectional analyses evaluating the relative contributions of physical activity, dietary energy and protein, health status, body composition measures, serum testosterone, estrone, SHBG and IGF-1 in men and women aged 65 to 87 years.[149] Muscle mass in men was significantly associated with free testosterone, physical activity, heart disease and IGF-1. In women, however, muscle mass was only associated with total fat mass and physical activity. Grip strength was associated with muscle mass in both sexes. These data are limited by the cross-sectional nature of the analyses, the absence of data on inflammatory markers and the relatively small sample size (121 men and 180 women). Future observational studies with a large number of older men and women should investigate the relative contributions of these variables and interactions between these variables on loss of muscle mass and strength.

As inflammatory markers are all part of a complex immune mechanism, it is important to identify a set of inflammatory markers (and perhaps other biomarkers) that are most strongly related to physical function. This might provide more clinically useful measures of underlying chronic inflammation. Recently, in Health ABC, inflammatory indexes were created using principal component analysis in order to improve classification of underlying inflammatory status. Two different components were found: a TNF-α related component, including TNF-α, TNF soluble receptors 1 and 2, the IL-2 soluble receptor and IL-6 soluble

receptor, and a CRP-related component, including CRP, IL-6 and plasminogen activator inhibitor-1 (PAI-1). Only the TNF-α related component was related to knee extensor strength and physical performance, but both components were associated with grip strength. Further, the contribution of the components compared with the individual inflammatory markers to physical function was examined, and showed that none of the components had the highest correlation with any of the physical function measures. More research using techniques to cluster various biomarkers is needed to better identify older persons with chronic inflammation who are at risk physical decline.

Conclusion

The findings described in this thesis show that higher concentrations of inflammatory markers are associated with increased decline in muscle mass and muscle strength in older persons, supporting the evidence that inflammation is associated with decline in physical functioning through the effects of higher inflammatory marker concentrations on muscle. Also, change in weight should be considered as an important factor and future studies should further explore the effects of weight change on these associations. Future studies should also focus on clustering of inflammatory markers and/or other biomarkers to better identify older persons at risk of sarcopenia and physical impairments.

Low testosterone concentrations were not associated with decline in muscle strength, physical performance and mortality in men, but showed significant associations with decline in muscle strength and physical performance in women. More research is needed to elucidate the exact role of testosterone in women and to explore these sex differences. Also, large trials of testosterone treatment in older men and women are needed to answer the question whether testosterone replacement can help to prevent or treat sarcopenia, decline in muscle strength and physical impairment. These trials should also focus on the safety aspects of testosterone treatment so that the benefits and adverse affects of testosterone treatment can be weighted.

As the population grows older, more people will experience sarcopenia and a decline in physical functioning. Insight into the mechanisms underlying sarcopenia and loss of muscle strength is important to prevent disability and represents a high public health priority. Anti-inflammatory drugs and/or testosterone supplementation may be considered to reduce the risk of sarcopenia, but more research is needed to assess the effects and the risks of such treatments.

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