



## **Chapter 2**

### **Inflammatory markers and loss of muscle mass (sarcopenia) and strength**

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## Abstract

**Purpose:** The objective of this study was to investigate whether high levels of serum interleukin (IL)-6, C-reactive protein (CRP), and  $\alpha$ 1-antichymotrypsin (ACT) were associated with the loss of muscle strength or muscle mass (sarcopenia) in older persons.

**Subjects:** The study included 986 men and women of the Longitudinal Aging Study Amsterdam, with a mean age of 74.6 years (standard deviation 6.2).

**Methods:** Grip strength (n = 986) and appendicular muscle mass (n = 328, using dual-energy x-ray absorptiometry) were obtained in 1995 and 1996 and repeated after a 3-year follow-up. Loss of muscle strength was defined as a loss of grip strength greater than 40%, and sarcopenia was defined as a loss of muscle mass greater than 3%, approximating the lowest 15% of the study sample.

**Results:** Multiple linear and logistic regression analyses revealed that higher levels of IL-6 were associated with greater decline in muscle strength, which decreased by -3.21 kg (standard error 0.81) per standard deviation increase in log-transformed IL-6. After adjustment for confounders, including sociodemographic, health, and lifestyle factors, high IL-6 (>5 pg/ mL) and high CRP (>6.1 g/ $\mu$ L) were associated with a 2 to 3-fold greater risk of losing greater than 40% of muscle strength. Persons with high levels of ACT (>181% of the normal human pooled plasma) were 40% less likely to experience loss of muscle strength and tended (p=.07) to have a smaller decline in muscle mass compared with those in the lowest quartile of ACT. No consistent associations of IL-6 and CRP with sarcopenia were found.

**Conclusion:** The findings of this prospective, population-based study suggest that higher levels of IL-6 and CRP increase the risk of muscle strength loss, whereas higher levels of ACT decrease the risk of muscle strength loss in older men and women.

**Keywords:** Sarcopenia; Muscle strength; Interleukin-6; C-reactive protein;  $\alpha$ 1-Antichymotrypsin

## Introduction

Aging is associated with an increase in levels of pro-inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- $\alpha$ , which play a central role in the hepatic production of C-reactive protein (CRP),  $\alpha$ 1-antichymotrypsin (ACT), and other acute phase proteins.<sup>1</sup> There is strong evidence that increased levels of inflammatory markers, including IL-6 and CRP, are associated with medical conditions such as diabetes mellitus,<sup>2</sup> atherosclerosis,<sup>3,4</sup> and cardiovascular disease<sup>5,6</sup> in older persons. Furthermore, high levels of inflammatory markers are associated with increased mortality.<sup>7</sup> Increased levels of ACT have been shown to be associated with Alzheimer disease<sup>8</sup> and late-life depression.<sup>9</sup>

Higher levels of inflammatory markers also have been hypothesized to play a role in the functional decline of older persons. Both cross-sectional and longitudinal studies have shown associations of high levels of IL-6 and/or CRP with low physical performance and disability.<sup>10-14</sup> The causal pathway leading from inflammation to disability has not been fully explained, but it is suggested that inflammatory markers may cause a decline of physical functioning through the catabolic effects of inflammatory markers on muscle.<sup>15</sup> Experimental studies have shown that administration of IL-6 or TNF- in rats causes muscle breakdown.<sup>16,17</sup> These data suggest that inflammation may be associated with the loss of muscle mass (sarcopenia) and muscle strength with aging.

A few studies on the association of inflammatory markers with muscle strength and mass have been conducted. A recent cross-sectional study showed an association of high levels of IL-6 and TNF- with low muscle mass and strength.<sup>18</sup> Another study found an association of low levels of CRP with high grip strength; however, high baseline levels were not predictive of 7-year decrease in grip strength.<sup>12</sup> To our knowledge, no prospective studies on the association of inflammation and decline in muscle mass have been conducted.

The aim of this prospective study was to investigate whether high serum levels of IL-6, CRP, and ACT are associated with loss of muscle strength and loss of muscle mass during 3 years of follow-up in the Longitudinal Aging Study Amsterdam (LASA), a population-based sample of older persons.

## Methods

### Study Sample

Data for this study were collected in the LASA study, an ongoing interdisciplinary cohort study on predictors and consequences of changes in physical, cognitive, emotional, and social functioning in older persons.<sup>19</sup> The sampling and data-collection procedures and nonresponse have been described elsewhere in detail.<sup>20</sup> In summary, a random sample of men and women, aged 55 years and more, stratified by age, sex, urbanization, and expected 5-year mortality, was drawn from the population registers of 11 municipalities in 3 regions of the West, Northeast, and South of The Netherlands. In total, 3107 subjects were enrolled in the baseline examination (1992/1993) and were representative of the Dutch older population.<sup>21</sup> Follow-up measurements were done every 3 years, consisting of a face-to-face main interview, which was carried out at the respondent's home by specially trained interviewers. Two to 6 weeks later, the main interview was followed by a medical interview and a separate visit to the hospital or health care center. During this visit, blood samples were collected, and muscle mass was assessed with dual-X ray absorptiometry (DXA) (only respondents from the western part of The Netherlands).

For the present study, the baseline study sample included respondents who participated in the main interview and the medical interview of the second follow-up of LASA (1995/1996) and who were aged 65 years and older as of January 1, 1996 ( $n = 1509$ ). Figure 1 shows the selection of the respondents for the present study on the association of inflammatory markers with loss of muscle strength and muscle mass.

Informed consent was obtained from all respondents, and the study was approved by the ethical review board of the VU University Medical Center.

### Grip Strength

Handgrip strength (kilograms) was measured using a strain-gauged dynamometer (Takei TKK 5001, Takei Scientific Instruments Co. Ltd., Tokyo, Japan) at the participants' homes. Participants were asked to perform 2 maximum force trials with each hand. For the final scores, the maximum values of the right and the left hand were summed and divided by 2.<sup>22</sup> Relative change in muscle strength was calculated as the difference between baseline and follow-up strength, divided by baseline strength and multiplied by 100. Loss of muscle strength was defined as a loss of grip strength greater than 40% during follow-up, approximating the lowest 15% of the study sample, based on the study of Visser et al.<sup>23</sup>

### **Appendicular Skeletal Muscle Mass**

Appendicular skeletal muscle mass (kilograms) was assessed using DXA (Hologic QDR 2000 scanner, Hologic Inc., Waltham, Mass) in the enhanced array mode and software version V5.70A. We used the sum of fat-free, bone-free mass of the arms and legs as an indicator of appendicular skeletal muscle mass (ASMM). Relative change in ASMM was calculated as the difference in ASMM between baseline and follow-up examination, divided by baseline ASMM and multiplied by 100. We defined sarcopenia as a loss of ASMM greater than 3%, approximating the lowest 15% of the study sample, similar to our definition of loss of grip strength.<sup>23</sup>

### **Inflammatory Markers**

In 1995 and 1996 blood samples were collected in the morning. Participants were allowed to take tea and toast but no dairy products before blood sampling. The blood samples were centrifuged and serum was stored at  $-70^{\circ}\text{C}$  until processing in 2002 and 2003.

The serum levels of IL-6, CRP, and ACT were determined using regular immunoassays (enzyme-linked immunoassay [ELISA]) at Sanquin Research (Amsterdam, The Netherlands). The IL-6 ELISA was obtained from the Business Unit Immune Reagents of Sanquin Research and performed according to the manufacturer's instructions. CRP levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and a biotinylated monoclonal antibody against CRP (CLB anti-CRP-2) was used as the detecting antibody. ACT was measured with an ELISA in which specific monoclonal antibodies against ACT were used.<sup>24</sup> Recombinant IL-6, purified CRP, and pooled human plasma were used as standards in the respective assays. Results were expressed as micrograms/milliliter (CRP), picograms/milliliter (IL-6), and percentage of normal human plasma levels (ACT). The normal human pooled plasma (% NHP) for ACT was approximately 300  $\mu\text{g}/\text{mL}$ . The lower detection limits were 0.8  $\mu\text{g}/\text{mL}$  for CRP and 5  $\text{pg}/\text{mL}$  for IL-6. The inter-assay coefficient of variation was less than 5.2% for ACT, less than 4.2% for CRP, and less than 5% for IL-6 for levels above the detection limit. The intra-assay coefficient of variation was 4.1% for ACT, 3.2% for CRP, and 3.3% for IL-6. All values were measured in duplicate, with averages being used in statistical analyses.

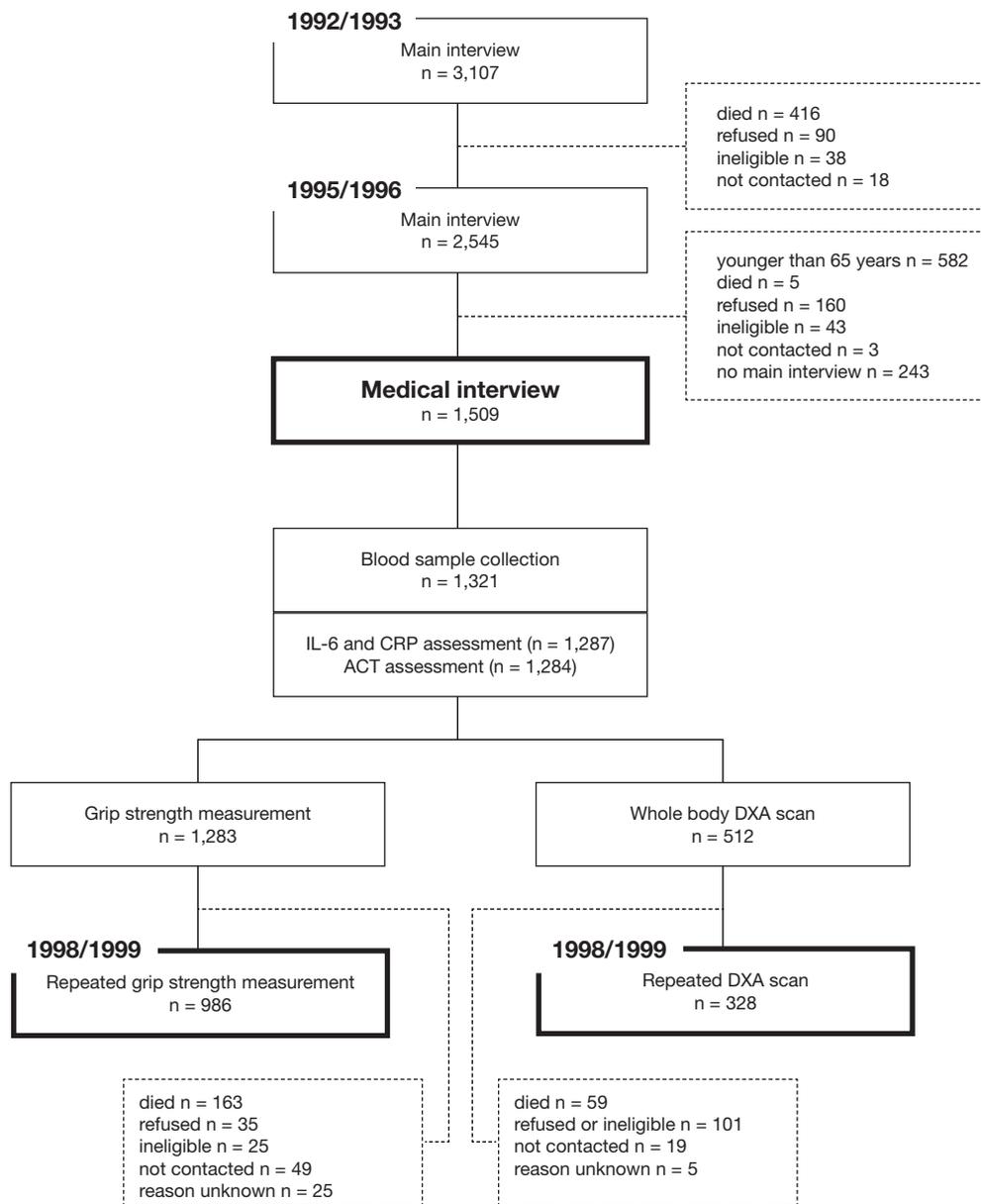


Figure 1

Study design

### **Potential Confounders**

Covariates included age, sex, level of education, body mass index or total body fat, smoking, alcohol use, physical activity, anti-inflammatory drug use, several chronic diseases, cognitive impairment, and depressive symptoms. These confounders are known to be associated with the levels of IL-6, CRP, or ACT, <sup>2-4,8,9,25-29</sup> as well as muscle strength and muscle mass. <sup>30-34</sup>

Data on age and sex were derived from the population registries at baseline. Education level was assessed by asking the respondent for the highest education level completed, ranging from incomplete elementary school (score 1) to university education (score 9). Body mass index was calculated as weight (kilograms)/height (meters).<sup>2</sup> Body weight was measured without clothes and shoes using a calibrated balance beam scale. Height was measured using a stadiometer. Total body fat was obtained in persons who participated in the DXA measurement. Smoking status was categorized as never, former, and current smoker. Alcohol use was expressed as the mean number of glasses of alcohol per week. Physical activity (minutes/day) was measured with the validated LASA Physical Activity Questionnaire.<sup>35</sup>

The LASA Physical Activity Questionnaire is a face-to-face questionnaire that covers the frequency and duration of walking outside, bicycling, light household activities, heavy household activities, and a maximum of 2 sport activities during the previous 2 weeks.

Anti-inflammatory drug use (nonsteroidal anti-inflammatory drugs and aspirin) was assessed by asking the respondents whether they used medication prescribed by a doctor. If so, interviewers examined all medication containers of prescribed drugs and registered what kind of medication, the dosage, and the period of use. All medications were coded according to the Anatomic, Therapeutic Chemical classification.

Chronic diseases were assessed by self-report during the main interview and included pulmonary disease (asthma or chronic obstructive pulmonary disease), cardiac disease, diabetes mellitus, arthritis (osteoarthritis and/or rheumatoid arthritis), cancer, stroke, and peripheral atherosclerosis.

Cognitive functioning was measured by means of the Mini-Mental State Examination (score range 0-30).<sup>36</sup> Depressive symptoms were assessed with the Center for Epidemiology Studies-Depression scale.<sup>37</sup> The cutoff points for cognitive impairment (Mini-Mental State Examination  $\leq 23$ ) and depressive symptoms (Center for Epidemiology Studies-Depression scale  $\geq 16$ ) were chosen at preestablished points.<sup>36,37</sup>

### **Statistical Analysis**

Analyses were performed using SPSS 11.0.1 (SPSS Inc, Chicago, Ill). Characteristics of persons with and without loss of strength and sarcopenia were compared using chi-square tests, Student *t* tests, or Mann-Whitney tests.

CRP and ACT were categorized into quartiles, with equal numbers across the categories. Because of the high number of respondents with levels below the detection limit, IL-6 was coded into 3 categories, with levels above the detection limit of 5 pg/mL in the highest category. Persons with lower levels were divided into 2 groups, based on the median level of IL-6 in this group. The category with the lowest levels of IL-6, CRP, or ACT was used as a reference group. Multiple linear regression analyses were performed, using relative change in muscle strength and muscle mass as continuous outcome variables. In addition, multiple logistic regression analyses were used to investigate the association of levels of inflammatory markers with the dichotomous outcomes of loss of strength and sarcopenia. In a first model, results were adjusted for age and sex. In a second model, results were additionally adjusted for education level, smoking status, number of chronic diseases, alcohol use, physical activity, anti-inflammatory drug use, body mass index (or total body fat when available), cognitive impairment, and depressive symptoms. We examined whether there was a linear trend across categories of IL-6, CRP, or ACT by including the categorical variables as an ordinal variable in the regression model. When the *P* value for trend was less than .05, indicating a linear association, IL-6, CRP, or ACT was put into the regression models as continuous variables. When the *P* value for trend was greater than .05, the association was considered nonlinear, and the categories of inflammatory markers were included as dummy variables. Associations of continuous measures of IL-6, CRP, and ACT are presented as change in muscle strength or mass per standard deviation (SD) of log-transformed IL-6, log-transformed CRP, and ACT (SD 0.93, 1.14, and 44.34, respectively). Potential sex differences were tested by using sex \* inflammatory marker product terms in additional analyses. To test whether there was significant interaction between the inflammatory markers, product terms defined as inflammatory marker \* inflammatory marker were included in additional analyses.

### **Results**

The sample consisted of 986 men and women (mean age 74.6 years [SD 6.2]; 52.7% were female) of the LASA study. The mean 3-year change in muscle strength was -12.9% (SD 25.0). Among the 986 participants with complete follow-up data, 134 participants experienced a loss of muscle strength greater than 40%, representing the lowest 15% of the study sample.

Table 1

Baseline characteristics according to 3-year change in muscle strength and muscle mass

Characteristic	3-year change in muscle strength (n = 986)			3-year change in muscle mass (n = 328)		
	Stable/Gain (n = 851)	Loss‡ (n = 139)	p value	Stable/Gain (n = 277)	Loss§ (n = 51)	p value
Age, y	74.3 ± 6.1*	76.9 ± 6.4	<.001	73.9 ± 6.0	74.8 ± 6.3	.31
Female, %	50.5	68.5	<.001	54.1	41.2	.09
Education level	3.6 ± 1.9	3.3 ± 2.0	.12	3.8 ± 2.1	3.7 ± 2.1	.72
Baseline grip strength, kg	58.6 ± 20.1	47.0 ± 23.7	<.001	56.6 ± 19.2	57.5 ± 17.0	.74
Baseline muscle mass, kg	27.0 ± 4.0	27.2 ± 4.5	.57	17.9 ± 4.3	19.0 ± 4.3	.09
Body mass index, kg/m <sup>2</sup>				26.9 ± 4.0	26.2 ± 3.8	.29
Total fat mass, kg				27.1 ± 9.2	24.8 ± 9.2	.10
Smoking, %						
Never	35.3	43.2		30.6	33.3	
Former	47.9	37.0		52.3	47.1	
Current	16.8	19.9	.05	17.1	19.6	.78
Alcoholic drinks per week	3.0 (0.5-13.0)†	3.0 (0.5-12.0)†	.73	6.0 (0.5-18.0)†	4.0 (1.0-14.0)†	.92
Physical activity, min/day	147 ± 94	142 ± 93	.49	145 ± 88	172 ± 127	.18
Anti-inflammatory agents use, %	25.5	22.6	.45	22.1	15.7	.30
Pulmonary disease, %	13.6	13.0	.86	11.4	11.8	.94
Cardiac disease, %	24.1	28.8	.22	22.4	13.7	.16
Diabetes mellitus, %	5.5	10.3	.03	6.4	5.9	.89
Arthritis, %	42.5	58.9	<.001	48.4	33.3	.05
Cancer, %	10.3	13.7	.22	10.0	13.7	.42
Stroke, %	5.6	11.0	.02	5.0	5.9	.79
Peripheral atherosclerosis, %	9.4	15.1	.04	9.6	13.7	.37
Number of chronic diseases	1 (0-2)†	1 (1-2)†	.001	1 (0-2)†	1 (0-2)†	.41
Cognitive impaired, %	6.5	15.1	<.001	4.0	3.9	.99
Depressive symptoms, %	11.5	23.7	<.001	12.6	11.8	.86
IL-6, pg/mL	1.7 (1.0-2.9)†	2.4 (1.6-3.7)†	<.001	2.5 (1.4-3.9)†	2.2 (1.3-3.2)†	.34
CRP, µg/mL	2.8 (1.4-6.0)†	4.0 (1.6-7.0)†	.03	2.9 (1.5-6.3)†	2.7 (1.1-6.8)†	.44
ACT, % of normal value	162.4 ± 41.5	160.2 ± 52.6	.58	153.4 ± 38.4	138.8 ± 38.4	.01

IL = interleukin; CRP = C-reactive protein; ACT = α1-antichymotrypsin.  
 \* Results presented as mean standard deviation, unless otherwise indicated.  
 † Results presented as median and interquartile range because of skewed distribution.  
 ‡ Loss of muscle strength 40%.  
 § Loss of muscle mass 3%.

The mean 3-year change in muscle mass ( $n = 328$ ) was +1.9% (SD 5.4%), and 51 participants experienced sarcopenia based on a loss of muscle mass greater than 3%. The Pearson correlation coefficients between the inflammatory markers were 0.36 ( $p < .01$ ) for IL-6 with CRP, 0.16 ( $p < .01$ ) for IL-6 with ACT, and 0.40 ( $p < .01$ ) for CRP with ACT.

Baseline characteristics for participants with and without loss of strength or sarcopenia are shown in Table 1. Participants who lost muscle strength were significantly ( $p < .05$ ) older, were more likely to be female, had a lower initial grip strength, were more likely to never have smoked, had a poorer health status (higher prevalence of diabetes mellitus, arthritis, stroke and peripheral atherosclerosis), and were more likely to be cognitive impaired and have depressive symptoms. Participants who lost muscle mass were more likely to be male

Table 2

Multiple regression analysis of inflammatory markers with relative change in muscle strength (%)

	N	Muscle strength			
		Model 1		Model 2	
		B (SE)	P value	B (SE)	P value
IL-6 (pg/mL)					
<1.7	443	Reference		Reference	
1.7-4.9	442	-6.54 (1.74)	<.001	-6.37 (1.75)	<.001
>4.9	100	-7.52 (2.87)	.009	-8.15 (2.88)	.005
		P for trend <.001		P for trend <.001	
CRP ( $\mu$ g/mL)					
<1.4	237	Reference		Reference	
1.4-2.8	259	-0.22 (2.35)	.93	-0.07 (2.39)	.98
2.9-6.1	249	-0.95 (2.37)	.69	-0.56 (2.46)	.82
>6.1	241	-2.46 (2.39)	.31	-2.00 (2.52)	.43
		P for trend .28		P for trend .41	
ACT (% NHP)					
<132	242	Reference		Reference	
132-155	242	0.86 (2.38)	.72	1.25 (2.39)	.60
156-181	253	4.00 (2.35)	.09	4.24 (2.36)	.07
>181	246	2.58 (2.36)	.27	2.50 (2.38)	.30
		P for trend .15		P for trend .17	

B = regression coefficient; SE = standard error; ACT =  $\alpha$ 1-antichymotrypsin; NHP = normal human pooled plasma; CRP = C-reactive protein; IL = interleukin.  
 Model 1: adjusted for age and sex.  
 Model 2: additionally adjusted for education level, smoking, number of chronic diseases, alcohol use, physical activity, anti-inflammatory drug use, body mass index, cognitive impairment, and depressive symptoms.

( $p = .09$ ), had a higher muscle mass ( $p = .09$ ), had lower total fat mass ( $p = .10$ ), and were less likely to have arthritis.

We investigated the association of inflammatory markers with relative change in grip strength (Table 2). Values in the second (1.7-4.9 pg/mL) and third (>4.9 pg/mL) categories of IL-6 levels had a greater decline in grip strength compared with the lowest category (<1.7 pg/mL), even after adjustment for potential confounders. The  $P$  value for trend was statistically significant, indicating a linear association of IL-6 with change in muscle strength. Therefore, continuous levels of log-transformed IL-6 (LN\_IL-6) per SD increase in LN\_IL-6 were also analyzed, showing a decline in muscle strength of 3.21 kg (standard error 0.81) per 0.93 pg/mL (SD) increase in LN\_IL-6 ( $p < .001$ ). Higher levels of CRP and ACT were not associated with relative change in muscle strength.

Next, we investigated the association of inflammatory markers with loss of muscle strength (Figure 2). High levels of IL-6 (>4.9 pg/mL) were associated with a 3-fold increased risk of strength loss compared with low (<1.7 pg/mL) IL-6 levels (odds ratio [OR] 3.65, 95% confidence interval [CI] 1.92-6.91). The  $P$  value for trend was less than .001. Analyses with LN\_IL-6 as a continuous measure showed that after adjustment, the risk of strength loss was 1.54 (95% CI 1.26-1.89) per 0.93 (SD) in LN\_IL-6 ( $p < .001$ ) (data not shown). High CRP levels (>6.1  $\mu\text{g/mL}$ ) were associated with a 2-fold increased risk of strength loss (OR 1.90, 95% CI 1.06-3.41) compared with low CRP levels (<1.4  $\mu\text{g/mL}$ ), with a significant trend across the quartiles ( $p = .04$ ). After adjustment for potential confounders, the OR for strength loss per SD increase (SD 1.14) in LN\_CRP was 1.24, 95% CI 1.01-1.53 ( $p = .04$ , data not shown).

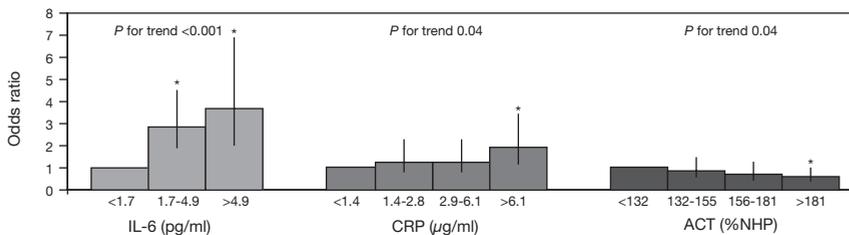


Figure 2

Odds ratios (with 95% CI) of 3 categories of IL-6 and quartiles of CRP and ACT with loss of muscle strength. Adjusted for age, sex, education level, smoking, number of chronic diseases, alcohol use, physical activity, anti-inflammatory drug use, body mass index, cognitive impairment, and depressive symptoms. \* $P < .05$  versus lowest category; see Methods for cut points categories; 95% CI expressed as vertical line. ACT =  $\alpha 1$ -antichymotrypsin; CI = confidence interval; CRP = C-reactive protein; IL = interleukin; NHP = normal human pooled plasma.

Table 3

Multiple regression analysis of inflammatory markers with relative change in muscle mass (%)

	N	Muscle mass			
		Model 1		Model 2	
		B (SE)	P value	B (SE)	P value
<b>IL-6 (pg/mL)</b>					
<1.7	94	Reference		Reference	
1.7-4.9	182	0.40 (0.74)	.59	-0.01 (0.74)	.99
>4.9	51	2.07 (1.02)	.04	1.32 (1.03)	.20
		<i>P</i> for trend .06		<i>P</i> for trend .27	
<b>CRP (µg/mL)</b>					
<1.4	79	Reference		Reference	
1.4-2.8	88	0.60 (0.92)	.71	-0.11 (0.92)	.90
2.9-6.1	78	1.74 (0.94)	.51	0.96 (0.95)	.32
>6.1	83	1.39 (0.93)	.07	0.08 (0.98)	.94
		<i>P</i> for trend .07		<i>P</i> for trend .69	
<b>ACT (% NHP)</b>					
<131	107	Reference		Reference	
132-155	86	0.87 (0.86)	.31	0.75 (0.87)	.39
156-181	83	1.04 (0.86)	.23	0.83 (0.86)	.34
>181	49	2.42 (1.02)	.02	1.81 (1.02)	.07
		<i>P</i> for trend .02		<i>P</i> for trend .09	

SE = standard error; IL = interleukin; CRP = C-reactive protein; ACT =  $\alpha$ 1-antichymotrypsin; NHP = normal human pooled plasma.  
 Model 1: adjusted for age and sex.  
 Model 2: additionally adjusted for education level, smoking, number of chronic diseases, alcohol use, physical activity, anti-inflammatory drugs, total body fat, cognition, and depression.

Finally, higher levels of ACT (>181% NHP) were associated with a decreased risk of strength loss compared with low ACT levels (<132% NHP) (OR 0.57, 95% CI 0.33-1.00).

Among the subgroup with repeated muscle mass measurements, after additional adjustment for potential confounders, no associations of IL-6 and CRP with relative change in muscle mass were observed (Table 3). However, higher ACT levels tended to be associated with a smaller decline in muscle mass ( $p = .07$ ). Finally, the association between inflammatory markers and sarcopenia (defined as loss of appendicular muscle mass >3%) was investigated. No associations with IL-6, CRP, or ACT were found (results not shown).

No gender interaction was observed for any of the associations ( $p > .10$ ). The interaction term IL-6 \* ACT was found to be significant in additional analyses with loss of muscle strength ( $p = .05$ ). Persons with IL-6 levels greater than 4.9 pg/mL and with ACT levels greater than 181% NHP had an increased risk of strength loss (OR 7.27, 95% CI 1.00-54.56).

## Discussion

The results of this study suggest that higher concentrations of serum IL-6 and CRP are associated with loss of muscle strength in older persons. A novel finding is that higher levels of ACT decrease the loss of muscle strength and tended to decrease sarcopenia. The associations were present after adjustment for sociodemographic factors, chronic diseases, and lifestyle factors, including physical held dynamometry and DXA), and we had the opportunity activity, and were consistent for men and women.

This study was performed in a longitudinal setting, as the changes in muscle strength and muscle mass were measured over a 3-year period in a selected subset of a populationbased sample of both men and women. Muscle strength and muscle mass were measured using direct measures (handheld dynamometry and DXA), and we had the opportunity to study the association of a proinflammatory cytokine (IL-6) and 2 acute phase proteins (CRP and ACT).

Higher levels of IL-6 and TNF- have been associated with lower muscle strength and lower muscle mass in a cross-sectional study.<sup>18</sup> Taaffe et al<sup>12</sup> found cross-sectional associations of high levels of IL-6 and CRP with low grip strength. However, in contrast with our results, these markers did not predict 7-year change in grip strength. In our study, we used a shorter follow-up period of 3 years. Because higher levels of IL-6 and CRP are associated with mortality,<sup>7</sup> it might be better to have a short follow-up; the selective dropout of less healthy persons will be smaller. The association between the inflammatory markers and loss of muscle strength remained similar, or was even stronger, when the analyses were restricted to those with high gripstrength values at baseline (defined as grip strength higher than the sex-specific median) (results not shown). This suggests that the observed association was not driven by ongoing muscle strength loss at the baseline of the study.

To our knowledge, this is the first study to investigate the association of ACT with change in muscle strength and mass. The association of high levels of ACT with decreased risk of strength loss seems surprising, because ACT is an acute phase protein, positively correlated with CRP. However, ACT is also a serum protease inhibitor, regulating the activity of proteolytic enzymes during inflammation.<sup>38</sup> It has been demonstrated that ACT is strongly

accumulated at normal human and rat neuromuscular junctions.<sup>39</sup> The role of ACT in neuromuscular junctions is unknown, but it suggests that ACT may play a role in inhibiting excessive or unwanted proteases in the region of the neuromuscular junction. In another study, an increased accumulation of ACT has been demonstrated in muscle fibers of patients with inclusion-body myositis, which is an inflammatory muscle disease characterized by progressive muscle weakness and wasting.<sup>40</sup> None of the control muscle biopsies contained these ACT accumulations. Possibly, during inflammation, muscle tissue might be protected from breakdown by high levels of ACT. However, as we found in this study, persons with high levels of both ACT and IL-6 seem to have an increased risk of muscle strength loss, suggesting that IL-6 is able to suppress or undo the protective role of ACT in muscle. Future studies may help further define this possible mechanism.

Although we did not find an association between inflammatory markers and muscle mass, results of experimental studies support a direct link between inflammatory markers and muscle mass. Administration of IL-6 or TNF- in rats increases skeletal muscle protein breakdown.<sup>16,17</sup> Studies in patients have shown that specific inflammatory diseases like chronic obstructive pulmonary disease,<sup>41</sup> rheumatoid arthritis,<sup>42</sup> and heart failure<sup>43</sup> are associated with lower muscle mass. An indirect relationship between inflammation and sarcopenia could also be hypothesized. It is suggested that levels of IL-6, CRP, and ACT are a marker of subclinical disease, with the disease being the cause of loss of muscle mass and strength. Although we adjusted for several prevalent inflammation-related chronic diseases, it is possible that other (subclinical) diseases may have been present in this study sample, which could still confound the associations observed. However, when we included 3-year weight change in all analyses as a possible marker of acute disease, this did not change the results (results not shown). The differential results for loss of muscle strength and loss of muscle mass may indicate that these 2 outcomes are based on different mechanisms. This was also supported by the poor relationship between the 2 study outcomes (Pearson correlation coefficient 0.20).

Some limitations of the study need to be addressed. First, because 3-year change in muscle mass was only determined in 25.5% of the study population ( $n = 328$ ), the power of the analyses regarding muscle mass loss was limited (IL-6: 0.19; CRP: 0.09; ACT: 0.50). Future studies need to include 700 respondents per category of IL-6, CRP, or ACT levels to detect a statistical significant trend in muscle mass loss with a power of 0.80 and an alpha of 0.05. Second, the persons who agreed to the DXA scans were healthier and more physically capable than those who did not, because they had to be able to visit the VU University Medical Center for additional examination. This selection may have affected our results. Also, persons with a more severe disease might have higher levels of inflammatory markers than

persons with less severe diseases, but we did not take the severity of the diseases reported by the participants into account in the analyses. A third limitation is the use of grip strength as a measure of overall body strength. Grip strength has been suggested to be a less reliable instrument to determine overall muscle strength, because it might not represent the muscle strength in the lower extremity. However, 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.<sup>44,45</sup> In a LASA subgroup (n = 422), the correlation coefficient of grip strength with leg extension strength (measured with a MicroFET handheld dynamometer (Hoggan Health Industries, West Jordan, Utah) was 0.45 (p.01). Other studies, using strength measures of specific upper and lower body muscle groups, are needed to confirm our results.

Finally, we used levels of inflammatory markers measured at baseline. In the future, it would be interesting to investigate whether change in inflammatory markers is associated with 3-year decline in muscle strength and muscle mass.

## **Conclusion**

The results of this prospective, population-based study show that high levels of IL-6 and CRP increase the risk of muscle strength loss in older persons. High levels of ACT, on the other hand, reduce this risk. These observations suggest an inflammatory-related component involved in the age-related loss of muscle strength.

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