

# Relationship of Interleukin-6 and Tumor Necrosis Factor- $\alpha$ With Muscle Mass and Muscle Strength in Elderly Men and Women: The Health ABC Study

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**Background.** A decline in muscle mass and muscle strength characterizes normal aging. As clinical and animal studies show a relationship between higher cytokine levels and low muscle mass, the aim of this study was to investigate whether markers of inflammation are associated with muscle mass and strength in well-functioning elderly persons.

**Methods.** We used baseline data (1997–1998) of the Health, Aging, and Body Composition (Health ABC) Study on 3075 black and white men and women aged 70–79 years. Midthigh muscle cross-sectional area (computed tomography), appendicular muscle mass (dual-energy x-ray absorptiometry), isokinetic knee extensor strength (KinCom), and isometric grip strength were measured. Plasma levels of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were assessed by enzyme-linked immunosorbent assay (ELISA).

**Results.** Higher cytokine levels were generally associated with lower muscle mass and lower muscle strength. The most consistent relationship across the gender and race groups was observed for IL-6 and grip strength: per *SD* increase in IL-6, grip strength was 1.1 to 2.4 kg lower ( $p < .05$ ) after adjustment for age, clinic site, health status, medications, physical activity, smoking, height, and body fat. An overall measure of elevated cytokine level was created by combining the levels of IL-6 and TNF- $\alpha$ . With the exception of white men, elderly persons having high levels of IL-6 ( $>1.80$  pg/ml) as well as high levels of TNF- $\alpha$  ( $>3.20$  pg/ml) had a smaller muscle area, less appendicular muscle mass, a lower knee extensor strength, and a lower grip strength compared to those with low levels of both cytokines.

**Conclusions.** Higher plasma concentrations of IL-6 and TNF- $\alpha$  are associated with lower muscle mass and lower muscle strength in well-functioning older men and women. Higher cytokine levels, as often observed in healthy older persons, may contribute to the loss of muscle mass and strength that accompanies aging.

HIGH levels of inflammatory markers have been associated with increased morbidity and mortality in older persons (1–3). In addition, higher interleukin-6 (IL-6) levels in community-dwelling elderly persons have been associated with physical disability (4). This association was recently confirmed by a longitudinal study reporting that older, nondisabled persons with higher plasma IL-6 levels were more likely to develop disability in the next 4 years (5). These data suggest an important role for inflammation in the development of disability in old age.

Whether elevated proinflammatory cytokine levels are on the causal pathway to the development of disability or a marker of underlying disease associated with disability is still unclear (6). Medical conditions such as diabetes mellitus (7), cancer, (subclinical) atherosclerosis (8), congestive heart failure and rheumatoid arthritis (9), and lifestyle factors such as smoking (10) all have been associated with higher levels of proinflammatory cytokines and may contribute to

future disability. However, a direct role for these cytokines on the development of disability can also be hypothesized. Proinflammatory cytokines may have a catabolic effect on muscle mass and muscle strength, both important determinants of disability and functional performance (11,12).

Several studies in humans have shown a relationship between proinflammatory cytokine levels and muscle mass. In rheumatoid arthritis patients, lean body mass, a crude indicator of skeletal muscle mass, was inversely related to tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) production by circulating mononuclear cells (13). Among patients with chronic heart failure or chronic obstructive pulmonary disease, IL-6 and TNF- $\alpha$  levels were correlated with reduced lean tissue mass (14,15). Moreover, treatment of patients with human immunodeficiency virus (HIV) with thalidomide, a potent anticytokine agent, resulted in an increase of lean body mass (16). The presence of a direct link between proinflammatory cytokines and muscle mass is also supported by the results

from experimental studies. Administration of IL-6 or TNF- $\alpha$  in rats increases skeletal muscle protein breakdown, decreases the rate of protein synthesis, reduces the total skeletal muscle amino acid concentration, and causes muscle wasting (17–22).

Whether the association of proinflammatory cytokine levels with muscle mass or muscle strength is also present in the general population of older adults remains unclear. The aim of the study was to investigate whether higher plasma levels of the proinflammatory cytokines IL-6 and TNF- $\alpha$  are associated with lower muscle mass and lower muscle strength in well-functioning black and white men and women aged 70–79 years.

## METHODS

### Study Population

The Health, Aging, and Body Composition (Health ABC) Study cohort includes 3075 black and white men and women. Whites were recruited from a random sample of Medicare beneficiaries residing in zipcodes from the metropolitan areas surrounding Pittsburgh, Pa. and Memphis, Tenn. Blacks were recruited from all age-eligible residents in these geographic areas. All potential participants received a mailing, followed by a telephone eligibility screen. Eligibility criteria included age 70–79 years in the recruitment period from March 1997 to July 1998, self-report of no difficulty walking one quarter of a mile or climbing 10 steps without resting, no difficulty performing basic activities of daily living, no reported use of a cane, walker, crutches, or other special equipment to get around, no history of active treatment for cancer in the prior 3 years, and no plan to move out of the area in the next 3 years. Participants with missing data on both proinflammatory cytokines ( $n = 37$ ), on any body composition measure ( $n = 250$ ), or on grip strength ( $n = 42$ ) were excluded, leaving 2746 participants (850 white men, 494 black men, 764 white women, 638 black women) available for the statistical analyses (89.3% of original cohort). Compared with the 2746 participants included in the statistical analyses, those who were excluded were older (74.0 vs 73.6 y), had a greater body mass index (BMI) (28.7 vs 27.2 kg/m<sup>2</sup>), lower strength (95.9 vs 107.0 Nm for knee extensor strength and 58.0 vs 61.9 kg for grip strength), higher cytokine levels [0.77 vs 0.66 pg/ml for log(IL-6) and 1.21 vs 1.15 pg/ml for log(TNF- $\alpha$ )], and were more likely to have heart disease (27.7% vs 22.0%) and current symptomatic osteoarthritis (24.7% vs 13.2%) (all  $p$  values < .05).

### Proinflammatory Cytokines

The plasma concentrations of the proinflammatory cytokines IL-6 and TNF- $\alpha$  were determined. Blood samples were collected at the clinic in the morning following an overnight fast of at least 8 hours. Specimens were processed according to standardized protocols by the Laboratory of Clinical Biochemistry at the University of Vermont. Plasma IL-6 concentration was measured in duplicate by means of a commercial ELISA High Sensitivity HS600 Quantikine kit (R&D Systems Inc., Minneapolis, MN). The lower detection limit was 0.10 pg/ml, the detection range was 0.156–17.0 pg/ml, and the interassay coefficient of variation was

7%. This method has a high degree of reliability and reproducibility, and a single IL-6 measurement has been shown in elderly persons to be representative of an individual's IL-6 level over an extended period of time (23). Plasma TNF- $\alpha$  concentration was measured in duplicate using the ELISA High Sensitivity HSTA50 kit (R&D Systems Inc., Minneapolis, MN). The lower detection limit was 0.18 pg/ml, and the detection range was 0.5–32 pg/ml.

### Muscle Mass

Two indicators of muscle mass were used in the study: appendicular skeletal muscle mass by dual-energy x-ray absorptiometry (DXA) and midthigh cross-sectional muscle area by computed tomography (CT).

Appendicular skeletal muscle mass was measured using fan beam DXA (Hologic QDR4500A, software version 8.21, Hologic, Waltham, MA) and was calculated as the sum of nonfat, nonbone tissue of both arms and legs.

The cross-sectional area of muscle in both thighs was measured by using CT (Memphis clinic site: Siemens Somatom Plus 4 [Siemens, Erlangen, Germany] and Picker PQ 2000S [Marconi Medical Systems, Cleveland, OH], at Pittsburgh clinic site: GE 9800 Advantage [General Electric, Milwaukee, WI]). To locate the midthigh scan position, an AP scout including the entire femur was obtained. The femoral length was measured in cranial-caudal dimension, and the scan position was determined as the midpoint of the distance between the medial edge of the greater trochanter and the intercondyloid fossa of the right leg. A single, 10-mm thick axial image (120 kVp, 200–250 mAs) of both thighs was obtained. All CT scans were transferred to a reading center and were analyzed by a single observer on a SUN Workstation (SPARCstation II, Sun Microsystems, Mountain View, CA) using IDL development software (RSI Systems, Boulder, CO). Areas were calculated by multiplying the number of pixels of a given tissue type by the pixel area. Density values were determined by averaging the CT number (pixel density) values of the regions outlined on the images. CT numbers were defined on a Hounsfield Unit (HU) scale where 0 equals the HU of water and –1000 equals the HU of air. The external contours of the thigh were determined using a threshold of –224 HU, and the external bone contours were derived at 150 HU. For each participant, the determination of soft tissue type was made using the bimodal image distribution histogram resulting from the distribution numbers in adipose tissue and muscle tissue (24). Intramuscular and visible intramuscular adipose tissue was separated from subcutaneous adipose tissue by drawing a line along the deep fascial plane surrounding the thigh muscles. The total area of nonadipose, nonbone tissue within the deep fascial plane was used as a measure of muscle area. Reproducibility of muscle area was assessed by reanalyzing a 5% convenience sample of the study cohort and showed a coefficient of variation <5%.

### Muscle Strength

Two indicators of muscle strength were obtained: isometric grip strength and isokinetic knee extensor strength.

Grip strength was measured using a hand-held dynamometer (Jamar, TEC, Clifton, NJ). The dynamometer was indi-

vidually adjusted for hand size, and two trials were performed for each hand. The sum of maximum strength of each hand was used as a measure of grip strength.

The maximal isokinetic strength of the knee extensors was assessed by a Kin-Com 125 AP Dynamometer (Chattanooga, TN) at 60° per second and was calculated from the average of three reproducible and acceptable trials out of a maximum of six trials. Participants with a systolic blood pressure  $\geq 200$  mm Hg, diastolic blood pressure  $\geq 110$  mm Hg, or who reported a history of cerebral aneurysm or cerebral bleeding, bilateral total knee replacement, or severe bilateral knee pain were excluded from the test. Due to these stringent exclusion criteria, data from 2456 participants were available for the statistical analyses of knee extensor strength (79.9% of original cohort).

### Potential Confounders

Covariates included clinic site, age, body height, total body fat, physical activity, health status, use of antiinflammatory drugs, and smoking. Body height was measured to the nearest mm using a wall-mounted stadiometer. Because taller persons have more muscle and greater strength, height was included in the regression models. Total body fat was assessed using fan beam DXA (Hologic QDR4500, software version 8.21). Physical activity of the past 7 days was assessed by questionnaire during a home interview. The time spent on climbing stairs, walking for exercise, walking for other purposes, aerobics, weight or circuit training, high-intensity exercise activities, and moderate-intensity exercise activities was obtained as well as information on the intensity level at which each activity was carried out. A metabolic equivalent value was assigned to each activity/intensity combination and was used to calculate the number of kilocalories per week per kilogram of body weight spent on that activity (25). For each participant, the scores of all performed activities were summed and multiplied by body weight to create an overall physical activity score in kilocalories per week. Previous or current presence of chronic conditions was determined using self-reported physician-diagnosed disease information, clinic data, and medication use. The inflammation-related diseases included in this study were heart disease, lung disease, current symptomatic osteoarthritis, and diabetes mellitus. Daily use of antiinflammatory drugs (nonsteroidal antiinflammatory drugs [NSAIDs], salicylates, and other antiinflammatory agents) was determined from drug data coded using the Iowa Drug Information System (IDIS) ingredient codes (26). Smoking was defined as a continuous variable assessing lifetime cigarette use in pack-years.

### Statistical Analyses

Analyses were performed stratified by gender and race using SAS software (SAS Institute, Inc., Cary, NC). Reported correlations were Pearson's product-moment correlations. Multiple linear regression analysis was used to test the association of the proinflammatory cytokine levels with muscle mass or muscle strength. Muscle mass and muscle strength were used as continuous dependent variables in the regression models. IL-6 and TNF- $\alpha$  concentrations were log transformed to derive a near-normal distribution and were

used as continuous variables in the regression models (Figure 1). To facilitate interpretation of the results, regression coefficients (with standard error) were expressed per population standard deviation of log(IL-6) ( $=0.6$  pg/ml) or log(TNF- $\alpha$ ) ( $=0.4$  pg/ml). Potential racial differences in the relationship of cytokine levels with muscle mass and muscle strength were assessed in stratified analyses and were tested by using product-terms in additional analyses stratified by gender only. We also combined the levels of both cytokines to create an overall measure of cytokine status with higher specificity. High proinflammatory cytokine status was defined as IL-6  $> 1.80$  pg/ml and TNF- $\alpha > 3.20$  pg/ml. Low proinflammatory cytokine status was defined as IL-6  $< 1.80$  pg/ml and TNF- $\alpha < 2.40$  pg/ml. The intermediate group included persons with all other possible combinations of cytokine levels. To test for trend, the three categories were entered in the model as an ordinal variable. All analyses were adjusted for clinic site, height, age, heart disease, lung disease, diabetes mellitus, current symptomatic osteoarthritis, use of antiinflammatory drugs, physical activity, and smoking. When investigating the relationship between proinflammatory cytokine levels and muscle strength, we additionally adjusted for muscle mass to study the independent effect of cytokine levels on muscle strength. Previous studies from our group and other groups have shown that high body fat or high BMI is associated with greater muscle area, greater strength, and higher in-

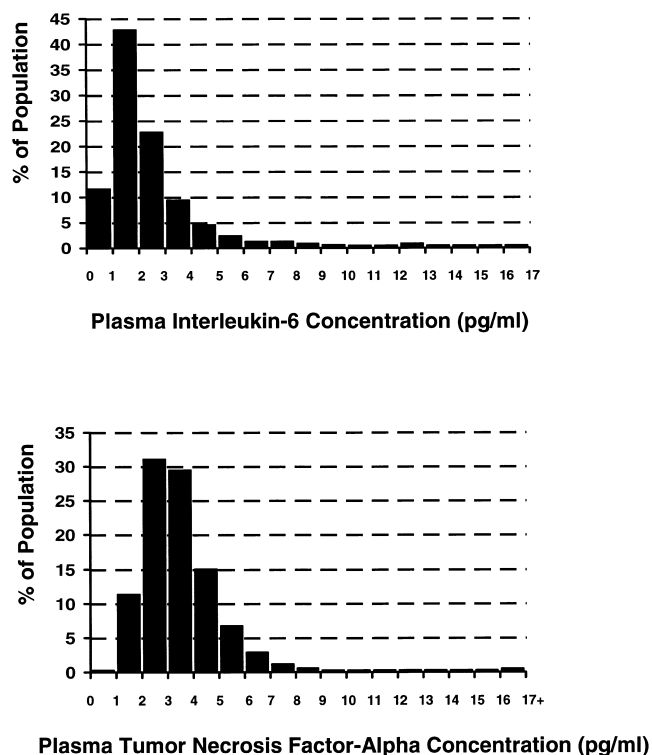


Figure 1. Distribution of plasma concentration of interleukin-6 and tumor necrosis factor- $\alpha$  in well-functioning men and women aged 70 to 79 years, participants of the Health, Aging, and Body Composition Study.

flammation levels (2,27–29). Body fat was therefore considered as an important confounder of the investigated associations and was adjusted for in all analyses.

## RESULTS

Characteristics of the well-functioning study population are shown in Table 1. In both men and women, blacks had a greater muscle area and greater strength compared to whites. Heart disease, diabetes mellitus, and arthritis—diseases that are inflammation related—were more prevalent in blacks compared to whites. Blacks had higher IL-6 and lower TNF- $\alpha$  levels compared to whites. The racial difference in TNF- $\alpha$  levels remained after differences in body fat, physical activity, smoking, medication use, and health status were taken into account. Of the total population, 95% had IL-6 levels below 6.8 pg/ml and TNF- $\alpha$  levels below 6.1 pg/ml (Figure 1).

Because the analyses using quartiles did not suggest the presence of nonlinear associations (not shown), we investigated the associations of log(IL-6) and log(TNF- $\alpha$ ) as continuous variables with muscle mass and muscle strength (Table 2). To facilitate the interpretation of the results, we expressed muscle area and muscle strength per standard deviation increase in IL-6 or TNF- $\alpha$ . Consistent across race and gender, a standard deviation increase in IL-6 was associated with a 1.1 to 2.4 kg decrease in grip strength. Similarly, in women, a standard deviation increase in TNF- $\alpha$  was associated with a decrease in grip strength of 1.2–1.3 kg. In black men, muscle mass and muscle strength were associated with both proinflammatory cytokines, while for black women, muscle mass was decreased in those with higher TNF- $\alpha$ .

We also investigated whether the relationship of cytokine levels with muscle area or muscle strength was different between blacks and whites. A race interaction with both cytokines was observed for thigh muscle area in men, with the relationship being stronger in blacks than whites. For each standard deviation increase, black men had a greater decrement in thigh muscle area compared with white men. No race interaction for thigh muscle area was observed among women and for appendicular muscle mass in men and women. With regard to muscle strength, no race interactions were observed, except for the association between TNF- $\alpha$  and knee extensor strength in women ( $p = .04$ ). Per standard deviation increase in TNF- $\alpha$ , black women had a greater decrement in knee extensor strength than white women.

By combining the levels of IL-6 and TNF- $\alpha$ , we created an overall measure of proinflammatory cytokine status with high specificity. The prevalence of a high cytokine status according to our definition was 31.2% and 28.5% in white and black men, respectively, and 24.1% and 22.4% in white and black women, respectively. We investigated the association of this overall marker of cytokine status with muscle mass and muscle strength (Table 3). With the exception of white men, elderly persons having high levels of IL-6 ( $>1.80$  pg/ml) as well as high levels of TNF- $\alpha$  ( $>3.20$  pg/ml) had less appendicular muscle mass, a smaller muscle area, a lower grip strength, and a lower knee extensor strength compared with those with low levels of both cytokines. For example, those with high cytokine status had a 3.3% to 6.5% smaller muscle area and a 5.5% to 8.8% lower knee extensor strength compared with those with low cytokine status. No statistical interaction was observed between

Table 1. Subject Characteristics

Characteristic	White Men ( <i>n</i> = 850)	Black Men ( <i>n</i> = 494)	White Women ( <i>n</i> = 764)	Black Women ( <i>n</i> = 638)
Age (y)	73.9 (2.9)*	73.5 (2.8) <sup>§</sup>	73.5 (2.8)	73.3 (3.0)
Height (m)	1.73 (0.06)	1.73 (0.07)	1.60 (0.06)	1.60 (0.06)
Weight (kg)	81.4 (12.4)	81.3 (14.3)	66.0 (12.1)	74.9 (15.4) <sup>§</sup>
BMI (kg/m <sup>2</sup> )	26.9 (3.7)	27.1 (4.2)	25.9 (4.5)	29.4 (5.8) <sup>§</sup>
Total body fat (kg)	21.5 (6.6)	19.9 (7.2) <sup>§</sup>	24.7 (7.6)	28.8 (9.6) <sup>§</sup>
Physical activity (kcal/wk)**	1946 (3518)	1180 (2139) <sup>§</sup>	965 (1538) <sup>§</sup>	639 (965) <sup>§</sup>
Smoking (pack-years)	17 (0–42.0)	15 (0–39.0) <sup>§</sup>	0 (0–15.5)	0 (0–15.4)
Appendicular muscle mass (kg)	24.5 (3.4)	26.5 (4.2) <sup>§</sup>	16.1 (2.5)	19.2 (3.4) <sup>§</sup>
Thigh muscle area (cm <sup>2</sup> )	254.6 (38.5)	277.2 (50.1) <sup>§</sup>	170.6 (27.6)	202.2 (33.5) <sup>§</sup>
Grip strength (kg)	75.0 (15.0)	80.8 (17.9) <sup>§</sup>	44.8 (10.1)	50.0 (12.4) <sup>§</sup>
Knee extensor strength (Nm) <sup>†</sup>	130.9 (33.4)	135.9 (37.4) <sup>§</sup>	78.5 (19.5)	86.2 (23.6) <sup>§</sup>
Memphis study site (%)	50.8	49.0	55.2	47.3 <sup>§</sup>
Heart disease (%)	29.7	25.9	13.7	18.7 <sup>§</sup>
Lung disease (%)	3.9	4.9	4.4	4.6
Diabetes mellitus (%)	13.9	21.9 <sup>§</sup>	7.5	19.9 <sup>§</sup>
Arthritis (%)	10.5	5.8 <sup>§</sup>	20.7	13.3 <sup>§</sup>
Use antiinflammatory drugs (%) <sup>‡</sup>	50.6	45.9 <sup>§</sup>	44.7	43.0
Interleukin-6 (pg/ml)	1.84 (1.31–2.83)	2.06 (1.35–3.09) <sup>§</sup>	1.63 (1.10–2.47)	1.92 (1.29–3.00) <sup>§</sup>
Tumor necrosis factor- $\alpha$ (pg/ml)	3.36 (2.64–4.30)	3.03 (2.31–3.89) <sup>§</sup>	3.20 (2.49–4.06)	2.90 (2.20–3.86) <sup>§</sup>

Note: BMI = body mass index; NSAIDs = nonsteroidal antiinflammatory drugs.

\*Values are expressed as mean (standard deviation), percentage of population, or median (interquartile range).

\*\*Walking and exercise.

<sup>†</sup>*n* = 786 white men, *n* = 426 black men, *n* = 691 white women, *n* = 553 black women.

<sup>‡</sup>Daily use of NSAIDs, salicylates, or other antiinflammatory drugs.

<sup>§</sup>*p* < .05 blacks vs whites within gender, Student's *t* test for differences of means, chi-square test for proportions, and Wilcoxon rank-sum test for comparison of medians.

Table 2. Adjusted<sup>†</sup> Regression Coefficients (With Standard Error) per Standard Deviation<sup>‡</sup> Increase in (log) Plasma Interleukin-6 (IL-6) and (log) Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) in Relation to Muscle Mass and Muscle Strength in Black and White Men and Women

Parameter	White Men	Black Men	White Women	Black Women
<b>IL-6</b>				
Appendicular muscle mass (kg)	-0.02 (0.08)	-0.28 (0.14)*	-0.10 (0.07)	+0.02 (0.08)
Thigh muscle area (cm <sup>2</sup> )	-0.12 (1.18)	-5.74 (2.04)**	-1.10 (0.88)	+0.19 (1.15)
Grip strength (kg)	-1.29 (0.49)**	-2.37 (0.82)**	-1.20 (0.35)**	-1.12 (0.44)*
Knee extensor strength (Nm)	-1.38 (1.16)	-4.61 (1.92)*	-0.94 (0.72)	-2.17 (0.98)*
<b>TNF-<math>\alpha</math></b>				
Appendicular muscle mass (kg)	+0.05 (0.09)	-0.29 (0.14)*	-0.08 (0.07)	-0.20 (0.08)*
Thigh muscle area (cm <sup>2</sup> )	+0.96 (2.08)	-6.59 (2.08)**	-1.21 (1.00)	-2.77 (1.13)*
Grip strength (kg)	-0.27 (0.54)	-1.24 (0.84)	-1.21 (0.39)**	-1.33 (0.45)**
Knee extensor strength (Nm)	-1.01 (1.25)	-6.05 (2.00)**	-1.57 (0.80)*	-1.84 (1.00)

<sup>†</sup>Adjusted for study site, age, height, total body fat, smoking, antiinflammatory drugs, heart disease, lung disease, diabetes mellitus, arthritis, and physical activity.

<sup>‡</sup>Based on total population: 0.6 for log(IL-6) and 0.4 for log(TNF- $\alpha$ ).

\* $p < .05$ ; \*\* $p < .01$ .

cytokine status and antiinflammatory drug use ( $p > .2$ ) in all gender and race groups. The results for grip strength were similar after additional adjustment for appendicular muscle mass ( $p$  value for trend .0007 and .2 for black and white men, and .0008 and .0006 for black and white women) or additional adjustment for muscle area ( $p$  value for trend .002 and .2 for black and white men, and .0008 and .001 for black and white women). However, the results for knee extensor strength were attenuated after additional adjustment for muscle mass. For example, after adjustment for appendicular muscle mass, the  $p$  values for trend were .2 in black and white men, and .07 and .08 in black and white women.

## DISCUSSION

The results of this study show that, except for white men, higher IL-6 levels and higher TNF- $\alpha$  levels are associated with lower muscle mass and lower muscle strength in well-functioning elderly persons. It is the first large-scale study investigating this association using accurate methodology to assess muscle mass and muscle strength.

The combined measure of IL-6 and TNF- $\alpha$  showed the strongest association with muscle mass and muscle strength. Persons with IL-6 levels above the median as well as TNF- $\alpha$  levels above the median had lower muscle mass and lower muscle strength compared to those who had cytokine levels below the median. The log transformed concentrations of IL-6 and TNF- $\alpha$  were positively correlated ( $r = +0.28$ ,  $p = .0001$ ). The use of a combined measure is likely to increase the specificity for proinflammatory cytokine status, that is the probability that persons with low levels of both IL-6 and TNF- $\alpha$  have no ongoing subclinical inflammation, and will reduce nondifferential misclassification. This may explain the stronger associations observed for the combined measure as compared to the associations for each individual cytokine.

The relationship between cytokine levels and muscle mass/strength was observed after adjustment for several inflammation-relation diseases including heart disease, lung disease, current symptomatic osteoarthritis, and diabetes mellitus. These findings support the presence of a direct relationship between cytokine levels and muscle mass. This

relationship has already been suggested by the results of several (experimental) studies in humans (13–16) and animals (17–22). Several smaller studies suggest that IL-6 levels may increase with aging, even in healthy persons (30,31). Recently, higher muscle TNF- $\alpha$  protein levels were observed among frail elderly compared to young healthy individuals (32). The higher cytokine levels in older persons may predispose to sarcopenia, thereby increasing the risk

Table 3. Adjusted\* Means (With Standard Error) of Muscle Mass and Muscle Strength According to Low, Intermediate, and High Proinflammatory Cytokine Status in Black and White Men and Women

Parameter	White Men	Black Men	White Women	Black Women
<b>Appendicular Muscle Mass (kg)</b>				
Low**	24.3 (0.2)	26.8 (0.3)	16.2 (0.1)	19.7 (0.2)
Intermediate	24.9 (0.1)	27.0 (0.2)	16.2 (0.1)	19.1 (0.1) <sup>‡</sup>
High	24.4 (0.2) <sup>†</sup>	26.0 (0.3) <sup>†,‡</sup>	15.9 (0.1) <sup>†</sup>	18.9 (0.2) <sup>‡</sup>
	.6 <sup>§</sup>	.05	.1	.003
<b>Thigh Muscle Area (cm<sup>2</sup>)</b>				
Low	251.9 (2.3)	282.6 (3.9)	172.2 (1.5)	206.4 (2.1)
Intermediate	258.2 (1.9)	285.0 (3.2)	171.5 (1.4)	203.6 (1.9)
High	254.5 (2.2)	264.2 (4.1) <sup>†,‡</sup>	166.6 (1.8) <sup>†,‡</sup>	197.5 (2.6) <sup>‡</sup>
	.5	.002	.03	.01
<b>Grip Strength (kg)</b>				
Low	75.0 (1.0)	83.1 (1.5)	46.8 (0.6)	52.1 (0.8)
Intermediate	75.7 (0.8)	82.5 (1.3)	44.4 (0.5) <sup>‡</sup>	50.0 (0.7) <sup>‡</sup>
High	73.7 (0.9)	74.1 (1.6) <sup>†,‡</sup>	43.3 (0.7) <sup>‡</sup>	47.1 (1.0) <sup>†,‡</sup>
	.3	.0001	.0002	.0002
<b>Knee Extensor Strength (Nm)</b>				
Low	130.7 (2.3)	139.0 (3.5)	79.8 (1.2)	89.3 (1.8)
Intermediate	134.3 (1.9)	138.5 (3.1)	80.0 (1.1)	86.7 (1.6)
High	126.9 (2.2) <sup>†</sup>	128.8 (3.8) <sup>†</sup>	75.1 (1.5) <sup>†,‡</sup>	81.4 (2.3) <sup>‡</sup>
	.2	.07	.03	.01

\*Adjusted for study site, age, height, total body fat, smoking, antiinflammatory drugs, heart disease, lung disease, diabetes mellitus, arthritis, and physical activity.

\*\*Low cytokine status = IL-6 < 1.80 pg/ml and TNF- $\alpha$  < 3.20 pg/ml, High cytokine status = IL-6 > 1.80 pg/ml and TNF- $\alpha$  > 3.20 pg/ml, Intermediate cytokine status = all other combinations.

<sup>†</sup> $p < .05$  vs intermediate group.

<sup>‡</sup> $p < .05$  vs low group.

<sup>§</sup> $p$  value for trend.

for functional decline and disability. Future studies should focus on proinflammatory cytokine levels in relation to loss of muscle mass and muscle strength.

Cytokine levels were also inversely related to muscle strength. The associations with grip strength were generally stronger than those with knee extensor strength. Due to the stringent exclusion criteria of the KinCom test, 290 persons were excluded from that test. These persons were older, had a lower grip strength, and had a smaller thigh muscle area compared with those who were not excluded from this test. These frailer persons were, however, included in the grip strength test. This difference may explain the stronger associations for grip strength compared to knee extensor strength. After additional adjustment for muscle mass, the relationship between cytokine levels and knee extensor strength was reduced. This suggests that proinflammatory cytokine levels may partly influence muscle strength through their effect on muscle mass.

Except for white men, participants with a high proinflammatory cytokine status had a 1.9% to 4.1% smaller appendicular muscle mass, a 3.3% to 6.5% smaller muscle area, a 7.5% to 10.8% lower grip strength, and a 5.5% to 8.8% lower knee extensor strength compared with those with a low cytokine status. These results suggest that a high cytokine status may be critical for those persons at the thresholds for maintaining physical function and independence. However, our results were based on cross-sectional data and should be confirmed in prospective studies. The associations were observed in a well-functioning population that excluded the oldest old. Persons with low muscle mass and low muscle strength were more likely to be excluded from the Health ABC study. The cohort may therefore have limited our ability to observe associations between higher proinflammatory cytokine levels and low muscle mass/strength. Another limitation of the study may be that information was used on the presence of chronic disease but not on disease severity. The severity of the disease may be related to the proinflammatory cytokine levels, and residual confounding due to disease severity cannot be excluded. In addition, potential confounding due to other factors, including type of physical activity and sex steroids, factors that also may be different between gender and race groups, cannot be excluded.

Total body fat was included as a potential confounder of the relationship of IL-6 and TNF- $\alpha$  with muscle mass and muscle strength. As previously stated, high body fat and high body mass index have been associated with greater muscle area, greater strength, and higher levels of inflammation (2,27–29). Indeed, in the present study, body fat was positively correlated with cytokine levels, especially in women who have relatively more body fat (IL-6:  $r = +0.23$ – $0.26$ , TNF- $\alpha$ :  $r = +0.09$ – $0.20$ ). In addition, body fat was positively correlated with muscle area ( $r = +0.42$ – $0.50$ ) and appendicular muscle mass ( $r = +0.50$ – $0.71$ ), justifying our decision. However, if body fat is considered the main source of cytokine production (33,34), the following pathway could be hypothesized: body fat  $\rightarrow$  cytokines  $\rightarrow$  muscle mass. In this case, body fat should not be considered a confounder of the relation between cytokines and muscle mass. Because increases in body fat are paralleled by in-

creases in lean body mass (35), indicating a direct relationship between body fat and muscle mass that can be explained by the training effect of weight bearing and support of a larger body mass, we still consider adjustment for body fat appropriate.

In conclusion, higher plasma concentrations of IL-6 and TNF- $\alpha$  are associated with lower muscle mass and lower muscle strength in well-functioning older men and women. Higher cytokine levels may contribute to the loss of muscle mass and strength with aging, also called sarcopenia.

#### ACKNOWLEDGMENTS

Dr. Visser's research has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences. Dr. Pahor is supported by the Claude D. Pepper Older Americans Independence Center Grant NIA P60-AG-10484. Dr. Goodpaster is supported by NIA K01-AG-00851. This study was supported by NIA NO1-AG-6-2101, NO1-AG-6-2103, and NO1-AG-6-2106, the American Heart Association Grant 9970066N, and the Ministero dell'Università e delle Ricerche Scientifica e Tecnologica, Italy.

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#### REFERENCES

1. Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol.* 1997;17:1121–1127.
2. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med.* 1999;106:506–512.
3. Strandberg TE, Tilvis RS. C-reactive protein, cardiovascular risk factors, and mortality in a prospective study in the elderly. *Arterioscler Thromb Vasc Biol.* 2000;20:1057–1060.
4. Cohen HJ, Pieper CF, Harris T, Rao KMK, Currie MS. The association of plasma IL-6 levels with functional disability in community-dwelling elderly. *J Gerontol Med Sci.* 1997;52A:M201–M208.
5. Ferrucci L, Harris TB, Guralnik JM, et al. Serum IL-6 level and the development of disability in older persons. *J Am Geriatr Soc.* 1999;47:639–646.
6. Kuller LH. Serum levels of IL-6 and development of disability in older persons. *J Am Geriatr Soc.* 1999;47:755–756.
7. Pickup JC, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci.* 2000;67:291–300.
8. Erren M, Reinecke H, Junker R, et al. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. *Arterioscler Thromb Vasc Biol.* 1999;19:2355–2363.
9. Badolato R, Oppenheim JJ. Role of cytokines, acute-phase proteins, and chemokines in the progression of rheumatoid arthritis. *Semin Arthritis Rheum.* 1996;26:526–538.
10. Tracy RP, Psaty BM, Bovill EG, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vasc Biol.* 1997;17:2167–2176.
11. Rantanen T, Guralnik JM, Foley D, et al. Midlife hand grip strength as a predictor of old age disability. *JAMA.* 1999;281:558–560.
12. Visser M, Newman AB, Nevitt MC, et al., for the Health, Aging and Body Composition Study. Muscle quantity and composition in relation to lower-extremity performance in elderly men and women: the Health ABC study. *J Am Geriatr Soc.* In press.
13. Roubenoff R, Roubenoff RA, Cannon JG, et al. Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation. *J Clin Invest.* 1994;93:2379–2386.
14. Schols AM, Buurman WA, Staal van den Brekel AJ, Dentener MA, Wouters EF. Evidence for a relation between metabolic derangements and increased levels of inflammatory mediators in a subgroup of patients with chronic obstructive pulmonary disease. *Thorax.* 1996;51:819–824.

15. Anker SD, Ponikowski PP, Clark AL, et al. Cytokines and neurohormones relating to body composition alterations in the wasting syndrome of chronic heart failure. *Eur Heart J*. 1999;20:683–693.
16. Haslett P, Hempstead M, Seidman C, et al. The metabolic and immunologic effects of short-term thalidomide treatment of patients infected with the human immunodeficiency virus. *AIDS Res Hum Retroviruses*. 1997;13:1047–1054.
17. Charters Y, Grimble RF. Effect of recombinant human tumour necrosis factor alpha on protein synthesis in liver, skeletal muscle and skin of rats. *Biochem J*. 1989;258:493–497.
18. Hoshino E, Pichard C, Greenwood CE, et al. Body composition and metabolic rate in rat during a continuous infusion of cachectin. *Am J Physiol*. 1991;260:E27–E36.
19. Garcia-Martinez C, Lopez-Soriano FJ, Argiles JM. Acute treatment with tumour necrosis factor-alpha induces changes in protein metabolism in rat skeletal muscle. *Mol Cell Biochem*. 1993;125:11–18.
20. Goodman MN. Tumor necrosis factor induces skeletal muscle protein breakdown in rats. *Am J Physiol*. 1991;260:E727–E730.
21. Goodman MN. Interleukin-6 induces skeletal muscle protein breakdown in rats. *Proc Soc Exp Biol Med*. 1994;205:182–185.
22. Tayek JA. Effects of tumor necrosis factor alpha on skeletal muscle amino acid metabolism studies in-vivo. *J Am Coll Nutr*. 1996;15:164–168.
23. Rao KMK, Pieper CS, Currie MS, Cohen HJ. Variability of plasma IL-6 and crosslinked fibrin dimers over time in community dwelling elderly subjects. *Am J Clin Pathol*. 1994;102:802–805.
24. Seidell JC, Oosterlee A, Thijssen MA, et al. Assessment of intra-abdominal and subcutaneous abdominal fat: relation between anthropometry and computed tomography. *Am J Clin Nutr*. 1987;45:7–13.
25. Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc*. 1993;25:71–80.
26. Pahor M, Chrischilles EA, Guralnik JM, Brown SL, Wallace RB, Carbone P. Drug data coding and analysis in epidemiologic studies. *Eur J Epidemiol*. 1994;10:405–411.
27. Visser M, Harris TB, Langlois J, et al. Body fat and skeletal muscle mass in relation to physical disability in very old men and women of the Framingham Heart Study. *J Gerontol Med Sci*. 1998;53A:M214–M221.
28. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA*. 1999;282:2131–2135.
29. Rantanen T, Penninx BW, Masaki K, Lintunen T, Foley D, Guralnik JM. Depressed mood and body mass index as predictors of muscle strength decline in old men. *J Am Geriatr Soc*. 2000;48:613–617.
30. Wei J, Xu H, Davies JL, Hemmings GP. Increase in plasma IL-6 concentration with age in healthy subjects. *Life Sci*. 1992;51:1953–1956.
31. Hager K, Machein U, Krieger S, Platt D, Seefried G, Bauer J. Interleukin-6 and selected plasma proteins in healthy persons of different ages. *Neurobiol Aging*. 1994;15:771–772.
32. Greiwe JS, Cheng B, Rubin DC, Yaraheski KE, Semenkovich CF. Resistance exercise decreases skeletal muscle tumor necrosis factor  $\alpha$  in frail elderly humans. *FASEB J*. 2001;15:475–482.
33. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- $\alpha$ , in vivo. *J Clin Endocrinol Metab*. 1997;82:4196–4200.
34. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab*. 2000;85:3338–3342.
35. Forbes GB. Longitudinal changes in adult fat-free mass: influence of body weight. *Am J Clin Nutr*. 1999;70:1025–1031.

Received October 3, 2001

Accepted January 14, 2002