BRIEF REPORTS

Muscle Strength After Resistance Training Is Inversely Correlated with Baseline Levels of Soluble Tumor Necrosis Factor Receptors in the Oldest Old

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OBJECTIVES: To test the hypothesis that physical exercise induces an antiinflammatory response that is associated with reduced chronic activation of the tumor necrosis factor (TNF)-alpha system in frail elders and that the increase in muscle strength after resistance training is limited by systemic low-grade inflammation.

DESIGN: A 12-week controlled resistance-training study.

SETTING: Nursing homes in Copenhagen, Denmark.

PARTICIPANTS: Twenty-one frail nursing home residents aged 86 to 95 completed the study.

INTERVENTION: Ten participants were randomized to a program of resistance training of knee extensors and flexors three times a week for 12 weeks; the remaining 11 participants served as a control group who joined social activities supervised by an occupation therapist.

MEASUREMENTS: Muscle strength, plasma levels of TNF-α, soluble TNF receptor (sTNFR)-1, and interleukin (IL)-6 were measured before and at the end of the intervention period.

RESULTS: The training program improved muscle strength but did not affect plasma levels of TNF-α and sTNFR-I or IL-6. However, plasma levels of sTNFR-I at baseline were inversely correlated with the increase in muscle strength.

CONCLUSION: Low-grade activation of the TNF system could limit the increase in muscle strength after resistance training in the oldest old. Furthermore, data suggest that the antiinflammatory response induced by 12 weeks of resistance training is not sufficient to reduce chronic activation of the TNF system, but the small sample size limited this interpretation. J Am Geriatr Soc 52:237–241, 2004.

Key words: resistance exercise; low-grade inflammation; TNF-α; IL-6; antiinflammatory activity

Aging is associated with systemic low-grade inflammation, and elevations in circulating levels of tumor necrosis factor (TNF-) and interleukin (IL)-6 have been associated with age-related inflammatory diseases such as dementia, cardiovascular diseases, and type 2 diabetes mellitus in cross-sectional epidemiological studies.1 Moreover, circulating levels of inflammatory markers are strong predictors of mortality risk in old populations.2–5 It has also been suggested that the syndrome of frailty is a metabolic imbalance caused by overproduction of catabolic cytokines such as TNF-α and by reduced availability or action of anabolic hormones, resulting from aging itself and the presence of associated chronic conditions.6 Consistent with this, high plasma concentrations of TNF-α and IL-6 were associated with low muscle mass and lower muscle strength in well-functioning older people,7 which may partly explain why older nondisabled persons with high IL-6 were more prone to developing functional disability in the next 4 years.8 Nevertheless, it is still unclear whether associations between TNF-α, IL-6, sarcopenia, and functional disability represent causal pathways or whether levels of cytokines simply act as markers of underlying medical disorders that are associated with sarcopenia and functional disability. Furthermore, it is possible that IL-6 acts partly as a surrogate marker for TNF-α in many epidemiological studies because the production of TNF-α and IL-6 is tightly linked (e.g., TNF-α stimulates the production of IL-6, and in return, IL-6 inhibits the transcription of TNF-α9 and stimulates the production of antiinflammatory cytokines and the shedding of TNF receptors (TNFRs) that bind...
TNF-α with high affinity). This illustrates that, although IL-6 has often been classified as a proinflammatory cytokine, it also has important antiinflammatory properties.10 Soluble TNFRs (sTNFRs) and TNF-α in the plasma are strongly correlated, and it has been suggested that sTNFRs are long-term markers of TNF-α.11,12

Progressive resistance training of healthy and frail elderly people appears to be an effective way of improving muscle strength that may result in functional and metabolic benefits in the oldest old.13 Muscle contractions induce production and release of IL-6 into the circulation.14 In contrast, TNF-α is briefly expressed locally in the contracting muscle, but it does not escape into the plasma in detectable amounts.14 It has been suggested that muscle-derived IL-6 contributes to the beneficial metabolic effects of exercise, and this may partly be mediated through strong antiinflammatory activities, resulting in decreased production of TNF-α.14 In theory, elderly people may benefit from exercise-induced antiinflammatory activities, providing a possible pathway to reduce systemic low-grade inflammation.15 In accordance with this hypothesis, TNF-α levels were higher in skeletal muscles of frail, elderly people than in young, healthy controls, but TNF-α levels in muscles decreased after resistance exercise for 3 months in the elderly group.16 To the authors’ knowledge, whether this phenomenon is also accompanied by a decrease in systemic low-grade activation of the TNF system has not been tested, but chronic low-grade inflammation may cause an irreversible state of cachexia that limit training-induced improvement in muscle strength in old-old people.

The purpose of the present study was to test the hypothesis that 12 weeks of resistance training induced an antiinflammatory response that was associated with reduced chronic activation of the TNF system and that systemic low-grade inflammation limited the increase in muscle strength after resistance training in frail, 90-year-old nursing home residents.

METHODS

Participants

The present study was a part of a larger survey of old-old nursing-home residents in Copenhagen with the purpose of testing the functional effect of resistance training for 12 weeks. Participants were randomly assigned to a training group that performed a program of resistance training of extensors/flexors of the knee or to a control group that participated in a program of social/occupational activities. A complete medical examination was performed before inclusion. Exclusion criteria were acute illness, hypertension, severe cardiovascular disease, moderate/severe cognitive impairment, severe impairment of motor function, and neurological disorder. The local ethical committee approved the protocol.

The study included a subgroup of participants who also gave blood samples for examining inflammatory markers. Blood samples were received from 39 nursing home residents at baseline, but only 21 subjects (two men and 19 women) aged 86 to 95 completed the full intervention period; they provided the basis for the present study. Ten participants were randomized to the program of resistance

<table>
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<tr>
<th>Table 1. Clinical Characteristics at Baseline</th>
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<td>Characteristic</td>
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<tr>
<td>Age, mean (range)</td>
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<tr>
<td>Men/women</td>
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<tr>
<td>Cardiovascular disease, n</td>
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<td>Respiratory disorder, n</td>
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<td>Bone/joint disorder, n</td>
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<td>Gastrointestinal disorder, n</td>
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<td>Use of nonsteroidal antiinflammatory drugs,</td>
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Note: Training = 12 weeks of resistance training; control = 12 weeks of social/occupational activities.

training (9 women and 1 man) and 11 participants (10 women and 1 man) were allocated to the control group. Clinical characteristics are presented in Table 1.

Resistance Training

The training protocol from a previous study of old-old people was used.17 The training program included three exercise sessions per week for 12 weeks. The principle of the exercise program was low repetitions with a high weight resistance. The duration of each exercise session was approximately 45 minutes. Participants were seated upright on a training chair (Quadriiceps Exercise Table, Kebo Care A/S, Hvidovre, Denmark). When knee extensors were trained, the subject started from a position of 90° knee flexion, and the leg was then extended against a well-padded lever arm to near-full extension. A second lever arm (0.5 m) with attached adjustable weights was connected to the rotary axis of the lever arm. The maximum amount of weight that could be lifted (1-repetition maximum (1-RM)) was tested every week for each leg and at the completion of the training program. Subjects had at least three trials to be certain that true 1-RM was measured. Each training session consisted of three sets of eight knee extensions with a load equivalent to 50% to 80% of the actual 1-RM and 2 minutes of breaks between the three sets. Each extension lasted 6 seconds and was followed by an interval of 2 to 3 seconds before the next extension in the series of eight repetitions. The same principle was used for training of knee flexors. Participants started from a position of full knee extension, and the leg was flexed against a weight resistance to a position of 90° knee flexion. A 1-RM of the strongest leg was used in all statistical analyses as an estimate of the muscle strength. A physiotherapist supervised exercise sessions.

The Older Control Group

An occupational therapist supervised social activities, including group gatherings, twice a week for 12 weeks. This intervention did not include any physical training.

Plasma Levels of Cytokines

Cytokines were measured in ethylenediamine tetraacetic acid plasma supplemented with Trasylol (24.5 μg crystalline aprotinin/mL), which is an unspecific protease inhibitor
designed to stabilize circulating cytokines. Plasma was stored at −80°C until analyzed using enzyme-linked immunosorbent assay (ELISA) kits (HSTA50, HS600, and DRT100, R&D Systems, Minneapolis, MN). Samples were analyzed before and after the 12 weeks of resistance training in the same assay mixed with samples from the older control group to eliminate the influence of intra-assay and interassay variation. Detection limits were 0.2 pg/mL, 0.1 pg/mL, and 2 ng/mL for TNF-α, IL-6, and sTNFR-I, respectively. The TNF-α and IL-6 immunoassays measure the total amount of free TNF-α or IL-6 plus the amount bound to soluble receptors. The sTNFR-I immunoassay measures the total amount of free receptors plus the total amount of receptors bound to TNF. All samples and standards were analyzed as duplicates, and the mean of duplicates was used in the statistical analyses. The actual coefficient of variation within plates was 15.7%, 8.7%, and 8.2%, for TNF-α, IL-6, and sTNFR-I, respectively. With regard to the actual interassay precision, the coefficient of variation was 32.6% for TNF-α and 31.6% for IL-6.

Statistics
Statistical data analysis was performed using SYSTAT software 8.0 (SYSTAT, Evanston, IL). P < .05 was considered significant in all analyses. A two-tailed t test was used to compare the two elderly groups at baseline. An analysis of variance (ANOVA) for repeated measurements was performed including the intervention group (training vs social activity) as an independent variable to evaluate potential exercise-induced changes in inflammatory markers and muscle strength (dependent variables) during the 12 weeks of intervention. Models were checked using standard plots. Cytokine data were log transformed to fulfill the assumption of normality and equal standard deviations. Associations between continuous parameters were evaluated using Pearson correlation coefficient.

RESULTS
There was no difference in the muscle strength or in circulating levels of cytokines in the training group compared with those of the controls at baseline (Figure 1).

In the training group, the average participation rate was 84% (range 72–97%) of the planned sessions. In the control group, the average participation rate was 97% (range 79–100%). There was no correlation between muscle strength at baseline and circulating levels of TNF-α, sTNFR-I, or IL-6 (data not shown).

Muscle strength before and after the 12 weeks of intervention is shown in Figure 1. Twelve weeks of resistance training resulted in increased strength of flexor (P < .0005) and extensor muscles (P < .0005) of the knee. A small increase in muscle strength was also detected in the control group (flexors: P = .02; extensors: P = .04), but the improvement was significantly more pronounced in the training group (interaction in ANOVA: P < .0005). Resistance training did not affect circulating levels of TNF-α, sTNFR-I, or IL-6 (Figure 1).

It was also investigated whether cytokines at baseline were correlated with muscle strength after training to test the hypothesis that low-grade inflammation in the old-old reflects a catabolic state that may limit the training effect. The sTNFR-I at baseline was negatively correlated with the strength of the flexor and extensor muscles and the combined muscle strength of extensors and flexors after 12 weeks of resistance training (Figure 2). The baseline level of sTNFR-I also tended to be inversely correlated with the absolute increase (delta) in muscle strength calculated as the difference between the combined muscle strength at baseline and after 12 weeks of training, but this association was not significant (delta combined muscle strength: r = −0.53, n = 9, P = .1). There were no similar correlations in the control group or with regard to TNF-α (P > .1). Moreover, in the training group, there were no correlations between delta combined muscle strength and delta TNF-α (r = −0.47, n = 9, P = .2), delta sTNFR-I (r = 0.14, n = 9, P = .7), and delta IL-6 (r = 0.22, n = 9, P = .6).

All statistical analyses were also performed without the two men, leaving nine women in the exercise group and 10 women in the control group. This exclusion did not affect results.
in a wide range of chronic disorders, including rheumatoid arthritis, human immunodeficiency virus (HIV) infection, heart failure, and leukemia, but sTNFRs are stronger markers of severity and clinical outcome than TNF itself, and accordingly, it has been concluded that additional measurements of sTNFRs are essential for evaluation of the TNF system in chronic disorders.\textsuperscript{12}

The inverse correlation between circulating levels of sTNFR-I at baseline and muscle strength after the completion of the exercise program in the present study may reflect muscle catabolic activities related to the TNF system. Studies of cultured muscle cells have indicated that TNF-\(\alpha\) disrupts the differentiation process and is able to promote catabolism in mature cells, but the molecular mechanisms that regulate this response are only beginning to be understood.\textsuperscript{19} TNF-\(\alpha\) is known to cause increased basal energy expenditure, anorexia, and loss of muscle and bone mass in vivo\textsuperscript{19,20} and has been associated with wasting/ cachexia in chronic inflammatory disorders.\textsuperscript{20–22} Consistent with this, muscle protein synthesis was inversely related to local levels of TNF-\(\alpha\) protein in muscles in a previous study of frail, old-old humans who also performed resistance training.\textsuperscript{16} Associations between muscle strength and circulating levels of TNF-\(\alpha\) and IL-6 have been reported in 3,075 Americans aged 70 to 79.\textsuperscript{7} No similar relations at baseline were found in the present study, but this discrepancy is probably due to the size of the present study. Considering the high systemic TNF-\(\alpha\) levels in the oldest old, it is likely that the catabolic activity of TNF-\(\alpha\) plays a clinical role in this population, but the design of the present study did not allow us to conclude whether the inverse correlation between baseline levels of sTNFR-I and final muscle strength represent a causal pathway or whether levels simply act as markers of underlying medical disorders that are associated with sarcopenia.

The present study was not able to confirm the hypothesis that exercise reduced systemic low-grade elevations in the TNF system. The power of the study may be too small to detect an exercise-induced change in plasma levels of cytokines. It is also possible that the duration of the exercise program was too short to reduce chronic activation of the TNF system, but this time period was sufficient to increase the muscle strength by 100\% in the training group. It is possible that a more global training program would be able to reduce systemic low-grade inflammation in the old. Training of knee muscles was chosen because these muscles are important in functional activities and more severely affected by aging than muscles of the upper extremities.\textsuperscript{23,24} Furthermore, the strength of extensor muscles in the knee is a predictor of dependency and survival,\textsuperscript{25,26} demonstrating the strong clinical relevance of this particular muscle group. To the authors’ knowledge, the present study is the first to test the hypothesis that antiinflammatory activity induced by resistance training is able to modulate circulating levels of inflammatory markers in the oldest old, but consistent with the present data, it has been demonstrated that resistance training was not associated with decreased in vitro stimulated production of TNF-\(\alpha\) or IL-6 in healthy elderly people.\textsuperscript{27} In contrast, muscle TNF-\(\alpha\) messenger ribonucleoprotein and TNF-\(\alpha\) protein decreased in response to resistance training in frail elderly humans,\textsuperscript{16} supporting the hypothesis that exercise has local antiinflammatory

**DISCUSSION**

This study demonstrated that baseline levels of sTNFR-I were inversely correlated with muscle strength after the completion of a 12-week-long resistance exercise program in frail, old-old people (\(P = .03\)), indicating that the gain of muscle strength was negatively influenced by activities in the TNF system that may provide a marker of frailty in the oldest old. There was no relationship between exercise-induced increase in muscle strength and changes in plasma levels of TNF-\(\alpha\), sTNFR, and IL-6.

sTNFRs bind TNF-\(\alpha\) with high affinity and may act as inhibitors or carriers of TNF-\(\alpha\), prolonging its biological effects.\textsuperscript{18} Plasma levels of sTNFR-I are strongly linked to local and systemic production of TNF-\(\alpha\), but sTNFRs are more stable in the plasma and accordingly are often a more consistent marker of activity in the TNF system than circulating levels of TNF-\(\alpha\).\textsuperscript{18} For instance, TNF is considered to play an important pathophysiological role

![Figure 2. Correlations between plasma levels of soluble tumor necrosis factor type 1 receptor (sTNFR-1) at baseline and the muscle strength after 12 weeks of resistance training.](image)
effects. Studies of protein release across contracting and noncontracting limbs have failed to demonstrate release of TNF-α at rest or after exercise in healthy humans or in patients with type 2 diabetes mellitus. Accordingly, it is unlikely that muscle-derived TNF-α has biological effects outside the muscle. In contrast, IL-6 is released in large amounts from the muscle to the plasma in response to muscle contractions but not during rest, suggesting that muscle-derived IL-6 plays a role during physical activities but not in relation to systemic low-grade inflammation in chronic diseases.

The control group in the present study participated in social activities that did not include physical training, but this group demonstrated small increases in muscle strength during the intervention period. This finding may reflect that frail old-old nursing home residents are often completely inactive and that participation in social events or increased motivation may provide sufficient stimulus to increase their muscle strength, although it is far from the gains observed in the training group. Furthermore, a learning effect cannot be excluded.

In conclusion, a resistance-training program of knee flexors and extensors did not influence the level of low-grade inflammatory activity in the oldest old, whereas plasma levels of sTNFR-I at baseline were inversely related to muscle strength at the end of the training program. This finding indicates that low-grade activation of the TNF system, which may provide a marker of frailty, limited the increase in muscle strength.

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REFERENCES