Inflammatory Markers and Physical Performance in Older Persons: The InCHIANTI Study

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Background. Some studies have proposed chronic inflammation as an underlying biological mechanism responsible for physical function decline in elderly people. The aim of this study is to evaluate the relationship between several inflammatory markers and physical performance in an older population.

Methods. This study is part of the “Invecchiare in Chianti” (InCHIANTI) study, a prospective population-based study of older people, aimed at identifying risk factors for late-life disability. The study sample consisted of 1020 participants aged 65 years and older living in the Chianti area of Italy. Physical performance was assessed using walking speed, the chair-stand test, and the standing balance test. Hand-grip strength was assessed using a hand-held dynamometer. Serum levels of C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), IL-10, IL-1β, IL-6sR, and IL-1RA were determined. Linear regression analyses were used to assess the multivariate relationship of inflammatory marker levels with physical performance, scored as a continuous variable from 0 to 3, and hand-grip strength after adjustment for demographics, chronic conditions, medication use, and other biological variables.

Results. CRP, IL-6, and IL1RA were significantly correlated with physical performance ($r = -0.162, r = -0.251,$ and $r = -0.127,$ respectively). Significant correlations with hand-grip strength were found for CRP and IL-6 ($r = -0.081$ and $r = -0.089,$ respectively). After adjustment for covariates, high levels of IL-6 and IL-1RA continued to be strongly associated with worse physical performance ($p < .001$ and $p = 0.004,$ respectively). High levels of CRP ($p < .001$) and IL-6 ($p < .001$) were associated with low hand-grip strength. Mean adjusted physical performance scores ranged from 2.21 in the CRP < 0.59 mg/dl group to 2.07 in the CRP > 0.60 mg/dl group ($p$ for trend = .004), and from 2.25 in the lowest IL-6 quartile to 2.08 in the highest IL-6 quartile ($p$ for trend < .001). This trend was also reflected in mean adjusted hand-grip strength, with a range from 28.8 kg for the CRP < 0.59 mg/dl group to 26.0 kg for the CRP > 0.60 mg/dl group ($p$ for trend = .001), and from 27.4 kg for the lowest IL-6 quartile to 25.1 kg for the highest IL-6 quartile ($p$ for trend = .001).

Conclusions. Inflammation, measured as high levels of IL-6, CRP, and IL-1RA, is significantly associated with poor physical performance and muscle strength in older persons. These data also support the biological face validity of physical performance measures. The assessment of inflammatory markers may represent a useful screening test and perhaps a potential target of intervention.

AGING is associated with a decline in physical function and performance that negatively impacts quality of life and may compromise independence (1,2). The assessment of physical function is a critical component of the assessment of older persons and some performance measures, such as the summary performance score and hand-grip strength, have been shown to be useful in the prediction of institutionalization, disability, and mortality (3–5). A biological mechanism recently proposed to underlie the decline in physical function is chronic inflammation (6,7). Inflammation is the body’s integrated reaction and defense against disturbances of homeostasis, particularly infections and injuries. This response is initially characterized by a local release of cytokines, soluble polypeptides responsible for the amplification and regulation of the inflammatory cascade (8). Cytokines are also involved in numerous physiological functions, such as muscle and bone tissues turnover, immunoregulation, and hematopoiesis (9,10), and their circulating levels have been related to several disease processes, primarily atherosclerosis and cardiovascular disease (11–13).

Inflammation has been associated with increased morbidity and mortality in elderly persons (14,15). Moreover, it has been proposed that a chronic inflammatory state may be detrimental by accelerating the progression of medical conditions that result in functional decline and disability (7,16). Finally, a direct role of inflammation in the development of disability can be hypothesized based on the catabolic effects that proinflammatory cytokines may have on muscles. In fact, an accelerated decline of muscle mass and strength with aging is probably one of the major causes of disability in late life (3,14).

Even though some inflammatory markers, such as C-reactive protein (CRP) and interleukin (IL)-6, have been studied before (7,16–18), there is still a lack of knowledge about many others. Tumor necrosis factor-alpha (TNF-α) is a first-line factor in promoting and developing the inflammation pathway (19). IL-10 has important regulatory
Effects on immunological and inflammatory responses because of its capacity to inhibit the production of proinflammatory cytokines by monocytes (20). IL-1β is one of two forms of IL-1, and its main biological activity consists of the stimulation of T-helper cells, which are induced to secrete IL-2, a cytokine able to promote inflammatory cells proliferation (21). IL-6sR is critically involved in the transition between the acute and sustained state of inflammation and in the perpetuation of chronic inflammatory diseases (22). If inflammation is part of the pathway to physical decline and disability, it will be interesting to expand research to new inflammatory markers in order to better understand mechanisms underlying age-related physical performance and strength loss.

The aim of our study was to evaluate the potential association between inflammation and physical performance. In our study, we explored the relationship between blood levels of several inflammatory markers (such as CRP, IL-6, IL-10, TNF-α, and IL-1β), and cytokine-soluble receptors (such as IL-6sR and IL-1RA) with physical performance (assessed through measures of lower extremity performance and hand-grip strength), in an elderly community-dwelling population.

Methods

The present study is part of the “Invecchiare in Chianti” (Aging in the Chianti Area, InCHIANTI) study, a prospective population-based study of older people, designed by the Laboratory of Clinical Epidemiology of the Italian National Research Council of Aging (INRCA), Florence, Italy. The aims of the InCHIANTI study are to identify risk factors for the onset of disability in older persons, to study physiologic subsystems critical for walking, and to define critical ranges for tests that evaluate the integrity of the physiologic subsystems that are important for walking. The study population for these analyses included 1156 participants aged between 65 and 102 years, randomly selected from residents in two towns of the Chianti geographic area (Greve in Chianti and Bagno a Ripoli, Tuscany, Italy), using a multistage stratified sampling method (23). The data collection started in September 1998 and was completed in March 2000. A detailed description of the sampling procedure and data collection method has been previously published (23). The INRCA Ethical Committee approved the entire study protocol.

The present analyses were performed on 1020 participants. We excluded participants in whom inflammatory markers, physical performance tests, or hand-grip strength were not tested ($n = 136$). Excluded participants were almost similar, except for having an older age (mean 80.2 years, $SE$ [standard error] $\pm 0.8$), with respect to sociodemographic characteristics, to those eligible for the present analyses.

Physical Performance Tests

Performance of the lower extremities was assessed using three tests of walking speed, ability to stand from a chair, and maintain balance in progressively more challenging positions. Walking speed was defined as the best performance (time in seconds) of two 4-meter walks at a usual pace along a corridor. For the chair-stand test, the participant was asked to rise and sit down 5 times as quickly as possible with hands folded across the chest. The performance was expressed as total time (in seconds) to complete the test. For the standing balance test, participants were asked to stand in three progressively more difficult positions for 10 seconds each: a side-by-side-feet standing position, a semitandem position, and a full tandem position.

An arithmetic summary performance score was calculated to obtain a continuous measure (24). The timed scores of the performance tests were rescaled to values ranging from 0 to 1, where 1 indicated the best performance and 0 the worst performance. Worst performers were participants who were unable to complete the task or who had a performance above the 99th percentile (walking speed: 15.5 s; chair-stand test: 27.3 s; standing balance test: 30 s). The following formulas were applied to rescale measures: 1) walking speed: $1 - \frac{\text{time in s}}{15.5}$; 2) chair-stand test: $1 - \frac{\text{time in s}}{27.3}$; 3) standing balance test: time in s/30. A summary physical performance score ranging from 0 to 3 was calculated by adding these three rescaled scores.

The hand-grip strength test was measured with a handheld dynamometer (hydraulic hand “BASELINE”; Smith & Nephew, Agrate Brianza, Milan, Italy). Participants were asked to perform the task twice with each hand. The average of the best results obtained at each side was used for the present analyses.

Covariates

Covariates included sociodemographic variables (age, gender, site, smoking habit, Mini-Mental State Examination [MMSE] score, education), comorbidity (self-reported diagnoses of hypertension, angina, myocardial infarction, stroke, cancer, diabetes, congestive heart failure, chronic obstructive pulmonary disease [COPD]), as well as biological parameters (body mass index [BMI] computed as weight in kg/height in meters squared, creatinine, total cholesterol, high-density lipoprotein [HDL] cholesterol, and albumin levels). Serum lipids were measured from fresh samples drawn after an overnight fast. Commercial enzymatic tests (Roche Diagnostics, Mannheim, Germany) were used for determining serum total and HDL cholesterol concentrations. The interassay coefficient of variation was less than 3.8% for total cholesterol and less than 5.0% for HDL cholesterol. Serum creatinine was detected by a standard creatinine Jaffe’s method (Roche Diagnostics); the interassay coefficient was less than 2.5%. Serum albumin (%) was detected by electrophoresis (mean interassay coefficient, 0.8%) and its concentration was calculated from serum total proteins (the interassay coefficient was lower than 1%).

Participants were also asked to report any medication taken in the last 2 weeks. Drugs were coded according to the Anatomical Therapeutic and Chemical codes (25).
hemolysis or activation. After having been aliquoted, sera were frozen and stored at –80°C until enzyme-linked immunosorbent assay (ELISA) tests were performed. Serums did not undergo any freezing and defrosting cycle. All cytokine assays were done at the INRCA central laboratory. IL-6, IL-6sR, and TNF-α were quantified with immunoassay kits (BioSource Cytoscreen human IL-6, human IL-6sR, and human TNF-α UltraSensitive kits; BioSource International, Inc., Camarillo, CA). The minimum detectable concentrations were 0.10 pg/ml for IL-6, 8 pg/ml for IL-6sR, and 0.09 pg/ml for TNF-α. The interassay coefficient of variation was 7% for all three kits. Serum IL-1RA level was detected by ELISA method using commercial kits (EASATM ELISA Human IL-1RA, BioSource International Inc., Camarillo, CA, USA); the minimum detectable concentration was 0.00 pg/ml and the mean interassay coefficient of variation was 8.2%. Serum IL-10 was detected by human IL-10 Cyto-SETS ELISA kits (BioSource International Inc.). The minimum detectable concentration was 1.00 pg/ml. CRP concentrations were measured with an immunoturbidimetric assay (Roche Diagnostics). The lower detection was 0.3 mg/dl.

All assays were done in duplicate for all cytokine measures (except IL-10) and were repeated if the second measure was more than 10% greater or less than the first. The average of the two measures was used in the analyses.

Statistical Analyses
Since plasma levels of inflammatory markers were not normally distributed, analyses were performed using their log values. Spearman’s correlation tests were used to evaluate correlations of inflammatory markers with physical performance and strength. Linear regression analyses were used to identify regression coefficients per standard deviation increase in (log) plasma inflammatory markers for physical performance and hand-grip strength. Analyses were adjusted for age, site, smoking habit, education, hypertension, angina/myocardial infarction, congestive heart failure, diabetes, stroke, cancer, COPD, creatinine, total cholesterol, HDL cholesterol, albumin, angiotensin-converting enzyme (ACE) inhibitor, statin, and nonsteroidal antiinflammatory drug (NSAID) use. Gender interactions were assessed by testing the interaction term added to the adjusted model as a covariate. Since plasma cytokine levels may be especially important in combination with high or low levels of their receptors, analyses were also conducted to evaluate the association of cytokine/cytokine-soluble receptor ratios with physical performance and strength. To assess the adjusted summary physical performance score and hand-grip strength, multivariate analyses of covariance were performed for inflammatory markers showing significant correlations in the previous adjusted analyses. Because the CRP distribution did not permit us to use quartiles, we used the following cutoff to identify 4 CRP groups: CRP < 0.59 mg/dl (11.6%); CRP = 0.59 mg/dl (15.0%); CRP = 0.60 mg/dl (48.5%); CRP > 0.60 mg/dl (24.9%).

RESULTS
The main characteristics of the participants considered in the present study are shown in Table 1. The mean age of the sample population was 75.4 years (SE = 0.2), and 56.2% were female. The most common diagnoses reported by participants were hypertension (44.6%), diabetes (11.4%), cancer (10.1%), and angina/myocardial infarction (7.5%). NSAID and ACE inhibitor use was reported by 21.7% and 13.4%, respectively.

Spearman’s correlations between inflammatory markers and physical performance are shown in Table 2. CRP, IL-6,
and IL-1RA were highly correlated with the summary physical performance score (\(r = -0.162, r = -0.251,\) and \(r = -0.127,\) respectively) and with each single test. Significant correlations were also found for CRP and IL-6 with hand-grip strength (\(r = -0.081\) and \(r = -0.089,\) respectively).

Regression coefficients for the association of inflammatory markers with summary performance score and hand-grip strength (adjusted for sociodemographic variables alone and then for sociodemographic variables, comorbidity, biological markers, and medication use) are shown in Table 3. High levels of IL-6 and IL-1RA were associated with a lower physical performance score (\(p < .001\) and \(p = .004,\) respectively). Stratification of the analyses according to specific inflammatory markers with summary performance score and hand-grip strength was performed. Significant gender interaction was found in the relationships between inflammatory markers and summary performance score.

Significant associations between higher levels of CRP (\(p < .001\)) and IL-6 (\(p < .001\)) were also found for lower hand-grip strength. Significant gender interactions for hand-grip strength were found for CRP (\(p = .014\)) and IL-6 (\(p < .001\)). Stratified analyses for gender showed that men were more likely to decrease their hand-grip strength at increasing levels of CRP (men: \(\beta = -1.697, SE = 0.471, p < .001\); women: \(\beta = -0.446, SE = 0.277, p = .108\)) and IL-6 (men: \(\beta = -2.030, SE = 0.466, p < .001\); women: \(\beta = -0.646, SE = 0.323, p = .046\)) than women.

When we tested the ratios IL-6/IL-6sR and IL-1β/IL-1RA, the first one only showed a significant value of association with summary physical performance score and hand-grip strength. However, the similar \(R^2\) explained variance found in the model that included the ratio term did not differ from the model that included the IL-6 level, indicating that the ratio term between IL-6 and IL-6sR did not improve prediction of the model.

We examined which factors were associated with both the physical performance measures and the inflammatory markers to identify the strongest confounders in our association. Age seemed to be the most important confounder of the link between inflammatory markers and physical performance outcomes.

We also performed restricted analyses for summary performance score and hand-grip strength in participants without comorbidity (\(n = 660\), after having excluded those with at least one of the following diseases: angina/myocardial infarction, congestive heart failure, COPD, stroke, cancer, and diabetes. Results were consistent with previous findings. Finally, we calculated the mean adjusted physical performance

### Table 2. Spearman’s Correlation Between Inflammatory Markers and Physical Performance Measures

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>CRP</th>
<th>IL-6</th>
<th>IL-6sR</th>
<th>IL-10</th>
<th>TNF-α</th>
<th>IL-1RA</th>
<th>IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary performance score</td>
<td>−0.162(^t)</td>
<td>−0.251(^t)</td>
<td>0.005</td>
<td>−0.021</td>
<td>−0.053</td>
<td>−0.127(^t)</td>
<td>0.034</td>
</tr>
<tr>
<td>Walking test</td>
<td>−0.143(^t)</td>
<td>−0.254(^t)</td>
<td>−0.010</td>
<td>−0.020</td>
<td>−0.070(^t)</td>
<td>−0.133(^t)</td>
<td>0.000</td>
</tr>
<tr>
<td>Chair-stand test</td>
<td>−0.150(^t)</td>
<td>−0.210(^t)</td>
<td>−0.001</td>
<td>−0.040</td>
<td>−0.013</td>
<td>−0.102(^t)</td>
<td>0.030</td>
</tr>
<tr>
<td>Standing balance test</td>
<td>−0.132(^t)</td>
<td>−0.262(^t)</td>
<td>0.046</td>
<td>0.014</td>
<td>−0.085(^t)</td>
<td>−0.105(^t)</td>
<td>0.015</td>
</tr>
<tr>
<td>Hand-grip</td>
<td>−0.081(^t)</td>
<td>−0.089(^t)</td>
<td>−0.046</td>
<td>−0.035</td>
<td>0.029</td>
<td>−0.059</td>
<td>−0.031</td>
</tr>
</tbody>
</table>

**Notes:** *p value < .05.
\(^t\)p value < .01.
CRP = C-reactive protein; IL = interleukin; TNF-α = tumor necrosis factor-alpha.

### Table 3. Adjusted Regression Coefficients (With SE) Per SD Increase in (log) Plasma Inflammatory Markers in Relation to Summary Performance Score and Hand Grip Strength

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Summary Performance Score</th>
<th>Hand-Grip Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted for Sociodemographic Variables*</td>
<td>Fully Adjusted**</td>
</tr>
<tr>
<td></td>
<td>Regression Coefficients (SE)</td>
<td>(p) Value</td>
</tr>
<tr>
<td>CRP</td>
<td>−0.026 (0.015)</td>
<td>.291</td>
</tr>
<tr>
<td>IL-6</td>
<td>−0.090 (0.016)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL-6sR</td>
<td>−0.001 (0.016)</td>
<td>.940</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.008 (0.015)</td>
<td>.606</td>
</tr>
<tr>
<td>TNF-α</td>
<td>−0.020 (0.015)</td>
<td>.183</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>−0.060 (0.015)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL-1β</td>
<td>−0.029 (0.015)</td>
<td>.050</td>
</tr>
</tbody>
</table>

**Notes:** *Adjusted for age, gender, site, education, adjusted MMSE, smoking.
**Adjusted for age, gender, site, education, adjusted MMSE, smoking, BMI, hypertension, angina/myocardial infarction, congestive heart failure, diabetes, stroke, cancer, COPD, creatinine, total cholesterol, HDL cholesterol, albumin, ACE inhibitor, statins, and NSAID use.
\(^1\)Interaction term for gender: Regression Coefficient (SE) = 1.071 (0.433); \(p = .014\).
\(^2\)Interaction term for gender: Regression Coefficient (SE) = 2.231 (0.584); \(p < .001\).
SE = standard error; CRP = C-reactive protein; IL = interleukin; TNF = tumor necrosis factor; MMSE = Mini-Mental State Exam; BMI = body mass index; COPD = chronic obstructive pulmonary disease; HDL = high-density lipoprotein; ACE = angiotension-converting enzyme; NSAID = nonsteroidal antiinflammatory drug; SD = standard deviation.
score and hand-grip strength across inflammatory marker levels using analyses of covariance. As shown in Figure 1, adjusted physical performance scores decreased from 2.21 (28th percentile) in the CRP < 0.59 mg/dl group to 2.07 (21st percentile) in the CRP > 0.60 mg/dl (p = .004) group, and from 2.25 (31st percentile) in the lowest IL-6 quartile to 2.08 (22nd percentile) in the highest quartile (p < .001, p for trend < .001). A decrease in the adjusted hand-grip strength for increasing levels of CRP and IL-6 was also found. A decrease in mean adjusted hand-grip strength from 28.8 kg (61st percentile) to 26.0 kg (54th percentile) was reported from the lowest to the highest CRP level groups (p = .001), and from 27.4 kg (57th percentile) to 25.1 kg (50th percentile) from the lowest to the highest IL-6 quartile (p = .002, p for trend = .001). No other inflammatory marker showed a significant trend or association with mean adjusted physical performance score or hand-grip strength.

DISCUSSION

The present study evaluated the relationship between inflammation and the results of objective tests of physical performance. Several previous studies have suggested an effect of inflammation on disability and muscular strength (7,8,14,16,26). In this study, we were able to test the simultaneous effect of a large number of cytokines, acute phase proteins, and soluble receptors. Our findings show that high CRP, IL-6, and IL-1RA levels are significantly and independently associated with poorer physical performance and muscle strength in elderly people. In our study, we included various inflammatory markers (e.g., IL-6sR, IL-10, IL-1β, IL-1RA) that have not been examined in the context of physical performance in older persons.

In line with our findings, higher levels of IL-6 have been associated with lower muscle mass and lower muscle strength in elderly people (7,14,16,27), and increased IL-6 and CRP levels have been associated with increased mortality (7,8). Recently, a longitudinal study also showed that nondisabled persons with increased levels of IL-6 were more likely to develop disability in the next 4 years (16). The magnitude of the associations between IL-6 and CRP with physical performance and grip strength reported in the study by Taaffe was comparable to our associations (7).

A unique finding of this study was the strong, independent association between IL-1RA and physical performance. The IL-1RA is a pure antagonist of the proinflammatory cytokines IL-1α and IL-1β, and, as such, plays an important role in regulating the inflammatory process (28–30). In fact, IL-1β is an early-acting inducer of the inflammation cascade, and its influence on systemic levels of inflammatory markers, such as CRP, has been demonstrated (31). IL1-RA acts as an antiinflammatory substance and its inhibition activities on inflammation have been demonstrated in several diseases, such as rheumatoid arthritis (32) and hemorrhagic shock (33). IL-1RA levels, as those of other cytokine receptors, are increased in the presence of inflammation and tend to remain at higher levels even longer than cytokines (34,35).

Several possible mechanisms may explain the inverse association between inflammatory marker levels and physical performance. In fact, cytokines have such a wide spectrum of activities in the context of immunoregulation, tissue homeostasis, hematoeosis, and the inflammatory cascade (9,10) that is difficult to indicate a single possible mechanism that is able to explain their potential effects on physical performance. More likely, inflammation influences performance by its effect on body composition, namely by accelerating changes that are typical of the aging process (16). Higher levels of CRP have been associated with obesity and insulin resistance (36–39). These findings could be explained through the inverse association found between physical activity and markers of inflammation (26). A direct influence of cytokine levels on muscle mass has also been
demonstrated in humans (40) as well as in animal models (41–43). Some studies suggest that chronic inflammation can lead to hypermetabolism and relative anorexia (44). Moreover, reduced lean tissue mass has been associated with IL-6 and TNF-α levels in patients with congestive heart failure or COPD (40,45), as well as in healthy older adults (14). Furthermore, it has been shown that some treatments, such as short-term thalidomide in patients infected with the human immunodeficiency virus, inhibit cytokine production, leading to weight gain and lean tissue anabolism (19).

Our study has several limitations. The cross-sectional design does not allow us to evaluate the cause–effect association between inflammation and physical decline. Further investigation aimed at assessing the predictive value of inflammatory marker levels on strength and physical decline are needed. Another limitation may be found in the circadian variability characteristic of cytokines. However, most of the blood sampling was done at the same time in the morning, limiting this potential source of bias. The cytokine circadian variability during the day could be an explanation to the lack of findings in our study for TNF-α, IL-10, IL-1β, and IL6-sR. Further studies aimed at assessing the role of these inflammatory markers are needed.

Our findings support the idea that inflammatory markers and, in this particular study, IL-6, CRP, and IL-1RA, are inversely associated with physical performance. Furthermore, our data provide biological face validity for the use of physical performance measures, increasingly applied in research and clinical geriatric assessment settings. Recently, Kuller discussed whether inflammatory markers or elevated acute-phase proteins are part of the pathway to the development of disability or just nonspecific markers of subclinical disease associated with inflammation and disability (6). However, the assessment of inflammatory markers may represent a useful screening test and perhaps a potential target of intervention. In the effort to better understand the physio-pathological pathway leading to the onset of disability in elderly people, experimental clinical trials looking at physical decline are needed, especially among healthier older people with increased inflammatory markers.

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