Interaction of HDL cholesterol concentrations on the relationship between physical function and inflammation in community-dwelling older persons

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Abstract

Background: the existence of a relationship among inflammation, high-density lipoprotein cholesterol (HDL-C) and physical function has been suggested.

Objective: the aim of the study is to investigate the possible interaction of HDL-C on inflammation and physical function.

Design: cross-sectional study.

Setting: town of Tuscania (Italy).

Subjects: all the 329 community-dwelling older persons aged ≥75 years (mean age 79.8±5.2 years, women 56.2%).

Methods: HDL-C, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and interleukin-6 (IL-6) were measured. Activities of daily living (ADL), instrumental ADL (IADL) and 4-m walking speed were assessed. Linear regression models were performed.

Results: given the multiple significant interactions, models were stratified according to HDL-C concentrations. In participants with normal HDL-C concentrations, only IL-6 showed a significant association with IADL (β=−0.439, SE=0.176, P=0.01). In participants with low HDL-C concentrations, all three inflammatory biomarkers were significantly associated with 4-m walking speed and IADL. IL-6 was also significantly associated with ADL (β=−0.755, SE=0.259, P=0.006), whereas borderline significances were reported for CRP and ESR.

Conclusions: the association between inflammation and physical function is particularly enhanced in elders with low HDL-C concentrations. Though HDL-C may merely act as a wellbeing index, HDL-C concentrations should be considered in studies evaluating inflammation and physical function.

Keywords: HDL cholesterol, physical function, inflammation, older persons, elderly

Background

Chronic inflammation represents one of the most important biological features characterizing the ageing process [1]. The age-related increase of systemic inflammation has been associated with a wide spectrum of biological, clinical and functional outcomes, including frailty and mortality [2]. Moreover, inflammatory biomarkers are associated with measures of physical performance, physical function and muscle strength [3], and are predictive of physical disability in older persons [4]. A direct detrimental effect of inflammation on the ageing skeletal muscle has been indicated as a major contributor to the development of sarcopenia [5]. Notably, it has been shown that inflammation is also able to affect lipoprotein metabolism [6], which is reflected by a decrease in serum levels of high-density lipoprotein cholesterol (HDL-C) in the presence of inflammation. In this regard, Zuliani and colleagues [7] recently showed the existence of an inverse relationship between plasma interleukin-6 (IL-6) and HDL-C concentrations in communi-
ty-dwelling older persons. It is noteworthy that the relationship between inflammation and HDL-C is likely to be not univocal, but bidirectional. In fact, explaining the atheroprotective effects of HDL-C, a growing number of *in vitro* and *in vivo* studies have recently documented anti-inflammatory and antioxidant properties of HDL-C [8].

Lending support to the existence of a vicious cycle among low HDL-C concentration, inflammation and low physical function, it has recently been demonstrated that HDL-C concentrations are positively associated with physical function in older persons [9]. All this evidence, besides strengthening the hypothesis of a close relationship existing among HDL-C concentrations, inflammation and physical function, also suggests that HDL-C concentration might represent an important ‘marker of wellbeing ’ in older persons.

Our aim is to explore the existence of the relationship between HDL-C, inflammation and physical function. In particular, we hypothesize that the inverse relationship between inflammatory biomarkers and physical function might be particularly enhanced by the presence of low levels of HDL-C, given the anti-inflammatory and antioxidant properties suggested for this lipoprotein [8]. However, this hypothesis has never been specifically tested in literature.

In the present study, we conducted secondary analyses on a sample of community-dwelling older persons to investigate the possible interactions of HDL-C concentrations on the relationships between well-established inflammatory biomarkers (i.e. C-reactive protein [CRP], IL-6 and erythrocyte sedimentation rate [ESR]) and three measures of physical function (i.e. 4-m walking speed, activities of daily living [ADL] and instrumental activities of daily living [IADL]).

The present analyses were performed among 329 participants, after exclusion of 58 participants because of missing values for the study variables. Excluded participants had a significantly (*P*<0.05) lower cognitive performance and 4-m walking speed, and higher IADL dependency, compared to those considered for the present analyses.

### HDL cholesterol concentrations

HDL cholesterol concentrations were determined using commercial enzymatic tests (Roche Diagnostics, Mannheim, Germany). The inter-assay coefficient of variation was <5.0%. HDL cholesterol levels were categorized according to well-established gender-specific cut-points [11] that are <50mg/dL for women and <40mg/dL for men.

### Physical function measures

Walking speed was evaluated by measuring the participant’s usual gait speed (in metres per second) over a 4-m course. Participants repeated the test twice, and the best performance of the two was considered for the present analyses.

Functional status was assessed by self-reported ADL [12] and IADL [13]. The number of impaired ADLs was calculated considering the following six tasks: transferring from bed to chair, walking across a room, eating, bathing, using the toilet and personal hygiene. The assessment of impaired IADLs was based on the evaluation of the following eight tasks: use of the telephone, grocery shopping, preparation of meals, doing housework, doing laundry, use of transportations, managing medications and managing finances. For the present analyses, higher scores at the ADL (range 0–6) and IADL (range 0–8) scales indicate higher physical function.

### Inflammatory biomarkers

Phlebotomy was performed following a standardized protocol at the baseline visit. ESR was immediately measured using a standard Westergren tube. The rate (in millimetres per hour) at the first hour was recorded and used for the present analyses.

Blood samples were immediately centrifuged at 4°C. Aliquots of the serum and plasma were stored upon separation at −80°C until final analysis. Plasma concentrations of CRP were determined using a high sensitivity enzyme-linked immunoassorbent assay (ELISA) and colorimetric competitive immunoassay. The CRP intra-assay coefficient of variation was 5.0%. Plasma IL-6 concentrations were also determined using an ELISA kit (Quantikine HS, R&D Systems, Minneapolis, MN) with a 7% intra-assay coefficient of variation.

### Covariates

Covariates considered in the present analyses included socio-demographic characteristics (age, gender, living status, smoking habit and alcohol consumption), body mass index...
Table 1. Main characteristics of the study sample (n=329) according to HDL-C concentrations

<table>
<thead>
<tr>
<th></th>
<th>Normal HDL-C (n=263)</th>
<th>Low HDL-C (n=66)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>79.5±5.2</td>
<td>80.8±5.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Gender (women)</td>
<td>52.5</td>
<td>71.2</td>
<td>0.006</td>
</tr>
<tr>
<td>Current smoking</td>
<td>4.2</td>
<td>1.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Current alcohol consumption</td>
<td>72.6</td>
<td>60.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.2±4.8</td>
<td>29.3±4.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Abbreviated Mental Test</td>
<td>8.0±2.1</td>
<td>7.6±2.3</td>
<td>0.21</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>10.9±6.7</td>
<td>12.8±6.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Physical Activity for the Elderly scale</td>
<td>152.1±56.6</td>
<td>139.5±69.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Living alone</td>
<td>29.7</td>
<td>24.2</td>
<td>0.38</td>
</tr>
<tr>
<td>Number of clinical conditions</td>
<td>2.1±1.4</td>
<td>2.7±1.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-steroidal anti-inflammatory drugs</td>
<td>7.2</td>
<td>9.1</td>
<td>0.61</td>
</tr>
<tr>
<td>Cortisone</td>
<td>4.2</td>
<td>4.5</td>
<td>0.90</td>
</tr>
<tr>
<td>Statins</td>
<td>9.9</td>
<td>9.1</td>
<td>0.85</td>
</tr>
<tr>
<td>4-m walking speed (m/s)</td>
<td>1.08±0.48</td>
<td>0.78±0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADL</td>
<td>5.3±1.3</td>
<td>4.5±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IADL</td>
<td>6.4±2.4</td>
<td>4.9±3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.28 (0.15–0.58)</td>
<td>0.64 (0.24–1.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>0.83 (0.52–1.27)</td>
<td>1.50 (0.69–2.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>13.0 (6.0–22.0)</td>
<td>20.0 (12.0–35.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values expressed as mean±SD, percentage or median (inter-quartile range).

Table 2. Unadjusted and adjusted linear regression models predicting physical function measures from inflammatory biomarkers (log values) according to HDL-C concentrations. Results are expressed as beta coefficients (standard errors)

<table>
<thead>
<tr>
<th></th>
<th>Normal HDL-C (n=263)</th>
<th>Low HDL-C (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Model 1</td>
</tr>
<tr>
<td>4-m walking speed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>−0.037 (0.035)</td>
<td>−0.021 (0.032)</td>
</tr>
<tr>
<td>ADL</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>−0.103 (0.040)</td>
<td>−0.085 (0.037)</td>
</tr>
<tr>
<td>ESR</td>
<td>−0.171 (0.039)</td>
<td>−0.080 (0.040)</td>
</tr>
<tr>
<td>IADL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>−0.069 (0.086)</td>
<td>−0.030 (0.080)</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>−0.246 (0.099)</td>
<td>−0.157 (0.094)</td>
</tr>
<tr>
<td>ESR</td>
<td>−0.223 (0.095)</td>
<td>−0.129 (0.101)</td>
</tr>
</tbody>
</table>

Normal HDL-C concentrations: ≥50mg/dl for women and ≥40mg/dl for men; low HDL-C concentrations: <50mg/dl for women and <40mg/dl for men.

(BMI), physical activity level and clinical information (cognitive performance, depressive symptoms, number of clinical conditions). Participants who currently smoke cigarettes and drink alcoholic beverages (any kind) were defined as ‘current smokers ’ and ‘current alcohol drinkers,’ respectively. BMI was calculated as weight (in kilogrammes) divided by the square of height (in metres). Cognitive performance was assessed using the Abbreviated Mental Test [14]. Depressive symptoms were evaluated using the Geriatric Depression scale (30 items) [15]. Physical activity
was assessed using the Physical Activity Scale for the Elderly questionnaire [16].

Clinical diagnoses were obtained from participants’ general practitioners before the study visit, confirmed by study physicians and coded according to the International Classification of Diseases. The following clinical diagnoses were combined to create a global score of comorbidity (i.e. number of clinical conditions) to consider as potential confounder of the studied relationships: history of cancer, angina, myocardial infarction, arthritis, osteoporosis, gastric ulcer, renal failure, anaemia, history of stroke, peripheral artery disease, chronic obstructive pulmonary disease (COPD) and liver disease. Drugs were coded according to the Anatomical Therapeutic and Chemical codes.

Statistical analyses

Spearman’s correlation analyses were performed between HDL-C concentrations, inflammatory biomarker levels and physical function measures. Interactions of HDL-C concentrations on the relationships between physical function measures (dependent variables) and inflammatory biomarker levels (independent variables) were tested using linear regression models. Descriptive analyses were performed using chi-square and Student t-test statistics. Given the non-normal distribution of inflammatory biomarker concentrations, these variables were log transformed to make them normally distributed. Medians and inter-quartile ranges were provided for non-normally distributed variables, and Mann–Whitney tests were conducted to evaluate their possible differences across HDL cholesterol concentration groups. Unadjusted and adjusted linear regression models were performed to calculate the regression coefficients (and standard errors, SE) of the associations between physical function measures (dependent variable) and inflammatory biomarker levels (independent variable). Adjusted models considered as covariates age, gender and all those variables showing relevant differences (P<0.1) across HDL-C levels at the univariate analyses. An alpha value of 0.05 was set as statistical significance. All analyses were performed using SPSS software (version 16.0, SPSS Inc., Chicago, IL).

Results

The study sample (n=329) had a mean age of 79.8±5.2 years, and was composed of a slightly higher number of women than men (56.2% vs 43.8%). The mean number of clinical conditions per participant was 2.1±1.3. Arthritis (79.3%), gastric disease (30.4%), COPD (25.5%), osteoporosis (19.1%), angina (15.8%), cerebrovascular disease (12.8%) and anaemia (11.6%) were the most prevalent clinical conditions observed in the study sample.

Spearman’s correlation analyses among HDL-C concentrations, inflammatory biomarker levels and physical function measures were performed. The highest correlation coefficients were reported among the physical function measures (all r>0.500, all P values <0.001). Correlation coefficients among inflammatory biomarkers were 0.360 between CRP and IL-6, 0.303 between CRP and ESR and 0.238 between ESR and IL-6 (all P values <0.001). Statistically significant correlations were also observed between HDL-C and CRP, IL-6, ADL and IADL (absolute r values ranging from 0.212 to 0.138; all P values <0.05). The only non-statistically significant correlations were reported between HDL-C and ESR (r=−0.043, P=0.33), HDL-C and 4-m walking speed (r=0.083, P=0.18) and 4-m walking speed and CRP (r=−0.121, P=0.06).

Results showed statistically significant interactions of HDL-C concentrations on the following associations: ADL and CRP (P for interaction term <0.001); IADL and CRP (P for interaction term=0.001); 4-m walking speed and IL-6 (P for interaction term=0.03); and ADL and IL-6 (P for interaction term=0.006). Suggestive interactions of HDL-C were also reported for 4-m walking speed and CRP (P for interaction term=0.16) and IADL and IL-6 (P for interaction term=0.23). Non-significant interaction of HDL-C concentrations on the associations between physical function measures and ESR was observed (all P values for interaction terms >0.40). Given the multiple significant interactions, statistical models were then stratified according to HDL-C concentrations (i.e. normal HDL cholesterol ≥50mg/dL for women and ≥40mg/dL for men vs low HDL cholesterol 50mg/dL for women and <40mg/dL for men).

Table 1 presents the main socio-demographic, clinical and biological characteristics of the study sample according to HDL-C levels. Participants with low HDL-C concentrations (n=66) were more likely to be women, present more depressive symptoms at the Geriatric Depression Scale and have a higher number of clinical conditions (all P values <0.05) compared to those with normal HDL-C levels. Borderline significant differences between participants with low vs normal HDL-C concentrations were also found for age, alcohol consumption and BMI (all P values <0.10). Participants with low HDL-C had significantly worse physical function and higher inflammatory biomarker levels than participants with normal HDL-C concentrations (all P values <0.001).

Table 2 shows results from unadjusted and adjusted linear regression models performed between physical function measures (dependent variables) and inflammatory biomarker levels (independent variable) according to HDL-C concentrations. Among participants with normal HDL-C concentrations, no significant association was reported between CRP levels and physical function. Significant relationships of ESR with 4-m walking speed and ADL were reported at the unadjusted models (β=−0.171, SE 0.039, P<0.001 and β=−0.223, SE 0.095, P=0.02, respectively), but results were significantly weakened by the inclusion of potential confounders in the statistical models. IL-6 was the only biomarker of inflammation showing a statistically significant association with IADL, even in the adjusted models (Model 2 adjustment: β=−0.439, SE 0.176, P=0.01). On the other hand, analyses performed among participants with low HDL-C concentra-
M. Cesari et al.

relations showed overall stronger associations between physical function and inflammation. In fact, all three inflammatory biomarkers of interest (i.e. CRP, IL-6 and ESR) were significantly associated with 4-m walking speed and IADL, even after adjustment for potential confounders (all P values <0.05). IL-6 levels were also significantly associated with ADL (Model 2: \( \beta = -0.755, SE = 0.259, P = 0.006 \)), whereas borderline significances were reported for CRP and ESR (both P values <0.1).

Discussion

To our knowledge, this is the first study formally exploring the interaction of HDL-C on physical function and inflammation in older persons. In fact, recent studies separately explored the relationships existing among HDL-C, inflammatory biomarkers and physical function. Our results show significant interactions of HDL-C concentrations on the relationship between physical function and inflammation. Stratified analyses demonstrate that physical function measures are strongly associated with inflammatory biomarker levels in older persons with low HDL-C concentrations (i.e. <40mg/dL for men and <50mg/dL for women [11]). On the other hand, in those participants with normal HDL-C concentrations, only IL-6 showed a statistically significant association with IADL. No significant relationship was detected for CRP and ESR levels with physical function measures in participants with normal HDL-C concentrations.

The demonstration of a HDL-C interaction on the well-established link between inflammation and physical function has both clinical and research relevance. The stronger associations existing between inflammatory biomarkers and measures of physical function in participants with low HDL-C levels are in agreement with the antioxidant and anti-inflammatory properties attributed for HDL-C [8]. It is possible that participants with normal HDL-C concentrations may have an improved antioxidant and/or anti-inflammatory defence to prevent or delay the initiation of the disabling process.

The antioxidant properties of HDL are linked to its ability to secrete enzymes involved in the detoxification of lipid hydroperoxides such as paraoxonase-1 [17] and paraoxonase-3 [18]. Another mechanism through which HDLs may exert their antioxidant activities is by decreasing the production of superoxides and inactivating the neutrophil nicotinamide adenine dinucleotide phosphate oxidase (an enzyme complex involved in the production of reactive oxygen species) [8]. Moreover, HDLs have specifically shown to inhibit the oxidation of phospholipids within low-density lipoproteins [19]. Interestingly, HDL-C plays a major role in the degradation of lipid oxidation products [20], which have shown to be predictive of mobility disability in older persons [21].

The inflammatory and oxidative pathways are closely related. It is not surprising that the anti-inflammatory activity of HDL is linked to its ability to limit lipid peroxidation [22]. Moreover, HDLs have shown to down-regulate the production of inflammatory biomarkers [22,23]. Consequently, individuals with low protection due to the reduced HDL-C levels may be those who are more exposed to the detrimental effects of inflammation and oxidative stress. Interestingly, under certain conditions, the normally anti-inflammatory/antioxidant nature of HDL can be modified. In particular, the HDL may become pro-inflammatory and pro-oxidant in the presence of chronic systemic inflammation [24]. This means that the age-related increase of inflammatory biomarkers, when reaching a specific (hypothetical) threshold, may reverse the protective role played by HDL and activate with a positive feedback the enhancement of the inflammatory cascade (with its detrimental clinical and biological consequences). It has been suggested that the mechanisms by which HDL-C concentrations decrease in the presence of sustained inflammation are due to a reduction in cholesterol uptake by cells and an increase in their catabolism [25]. Moreover, several studies have shown that, in the presence of inflammatory stimuli or metabolic diseases (e.g. type 2 diabetes), HDLs can become dysfunctional, lose their ‘traditional’ protective role and even enhance the inflammatory/oxidative status [23].

Inflammation is not only responsible for lower HDL-C levels but may also concur in limiting physical function. In fact, inflammation and oxidative damage have been associated with several biological and clinical modifications (e.g. sarcopenia) playing a major role in the age-related physical function decline. Therefore, the activation of a vicious cycle can be hypothesized among (i) the reduction of the protective role played by HDL-C, (ii) the worsening of the inflammatory/oxidative status and (iii) the impairment of those subsystems necessary for physical functioning. In this context, a recent meta-analysis [26] showed that aerobic exercise training, the main clinical intervention against disability, is able to increase HDL-C levels. It is noteworthy that physical exercise has demonstrated antioxidant and anti-inflammatory effects [27].

Another explanation of the important defensive role played by HDL can also be found in those studies indicating this lipoprotein as part of the immune system [28]. Supporting this hypothesis, HDL has been shown to account for a relevant part of the antiviral activity of human plasma [29]. Interestingly, the close link between inflammation and immunosenescence is at the basis of well-established theories of ageing [1].

The main limitation of our study resides in its cross-sectional design. Given the study design, we could not investigate any cause–effect mechanism for the studied relationships. We cannot exclude the levels of HDL-C, which may merely act as a ‘wellbeing’ index so that the stronger associations reported in participants with low HDL-C levels may simply reflect their frailer health status. It is important to mention in this context the possibility that the higher physical function might simply be due to the lower inflammatory status characterizing participants with normal HDL-C. In fact, in our analyses, as expected, participants with normal HDL-C concentrations present lower inflammatory marker levels. However, our analyses were adjusted for several po-
ential confounders to reduce the risk of false-positive results. Our findings also suggest that IL-6 may represent a more sensible marker of inflammation compared to CRP and ESR, especially when evaluating inflammation in relationship with lipid profile and/or physical function. The stronger evidence found for IL-6 compared to other inflammatory biomarkers (including CRP) is in line with results from previous studies indicating this inflammatory cytokine as a marker of particular interest for the geriatric and gerontological research, even on different outcomes [3, 7, 30]. Another limitation of the present study is the use of three commonly adopted markers of inflammation that may still not be sufficient to describe the complex inflammatory pathway. Moreover, a measure of oxidative stress might have been helpful to corroborate our findings. Finally, our study population was composed by community-dwelling older persons living in the countryside and consuming a very healthful diet with lots of fruit and vegetables. The high dietary intake of antioxidants may have biased our results, potentially underestimating the strength of the studied relationships.

**Conclusion**

The present study reports the existence of a significant interaction of HDL-C on the relationship between inflammation and physical function. Older persons with low HDL-C levels present stronger associations between inflammatory biomarkers and physical function. HDL-C levels should be considered as a potential confounder in studies evaluating the link between inflammation and physical function.

**Key points**

- HDL cholesterol concentrations are associated with significant changes in the relationship between inflammation and physical function in older persons.
- In the presence of low levels of HDL cholesterol, stronger associations between inflammatory biomarkers and physical function are reported.
- Interleukin-6 is the inflammatory biomarker showing the most consistent results in the association between inflammation and physical function, independently of HDL cholesterol levels.

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**Conflicts of interest**

The authors declare that they have no conflict of interest.

**References**

Do older pedestrians have enough time to cross roads in Dublin? A critique of the Traffic Management Guidelines based on clinical research findings

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Abstract

Background: the safety of older pedestrians at urban intersections is a matter of gerontological concern. Many older pedestrians report inability to complete crossings in the time given by pedestrian lights. Standard times for pedestrian lights in Dublin pelican crossings are specified in the Traffic Management Guidelines (TMG). The Technology Research for Independent Living Centre is building a database of gait assessments of Irish community-dwelling older people using GAITRite™.

Objective: to compare the usual walking speed of our participants against that required by the TMG.

Design: cross-sectional observational study.

Setting: comprehensive geriatric assessment outpatient clinic.

Subjects: 355 community-dwelling older subjects aged ≥60 assessed between August 2007 and September 2008 (mean age 72.7, SD 7.2).

Methods: linear regression analysis between age and observed walking speed, followed by comparison of predicted walking speeds at four different ages (i.e. 60, 70, 80 and 89) against minimum walking speeds required to cross standard Irish roads when regulated by the pelican system.

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