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Inflammatory markers are associated with quality of life, physical activity, and gait speed but not sarcopenia in aged men (40–79 years)

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Abstract

Background Age-related chronic low-grade inflammation (inflammaging) is one of the proposed mechanisms behind sarcopenia. However, findings regarding inflammatory markers in sarcopenic older adults are conflicting. This study aimed to determine the association between inflammatory markers, prevalent as well as incident sarcopenia, sarcopenia-defining parameters, quality of life (QoL), and physical activity in middle-aged and older men.

Methods Men aged 40–79 years (mean 59.66 ± 11.00y) were recruited from population registers in eight European centres for participation in the European Male Aging study (EMAS). Subjects were assessed at baseline (2003–2005) and again after a median follow-up of 4.29 years. In 2577 participants, associations between baseline inflammatory markers [high-sensitive C-reactive protein (hs-CRP), white blood cell count (WBC), albumin] and baseline physical activity (PASE) and QoL (SF-36) were analysed. In the Leuven and Manchester cohort (n = 447), data were available on muscle mass (whole-body dual X-ray absorptiometry) and strength. In this subgroup, cross-sectional associations between baseline inflammatory markers and sarcopenia-defining parameters (handgrip strength, chair stand test, appendicular lean mass, and gait speed) and prevalent sarcopenia were examined. In a further subgroup (n = 277), associations with knee extensor strength were explored. Longitudinally, predictive value of baseline inflammation on functional decline, physical activity, QoL, and incident sarcopenia was examined. Subgroup analyses were performed in subgroups with chronic inflammation and stratified by age. Linear and logistic regressions were used, adjusted for age, body mass index, centre, and smoking.

Results At baseline, hs-CRP and WBC were negatively associated with PASE score (hs-CRP: $\beta = -7.920$, $P < 0.001$; and WBC: $\beta = -4.552$, $P < 0.001$) and the physical component score of SF-36 (hs-CRP: $\beta = -1.025$, $P < 0.001$; and WBC: $\beta = -0.364$, $P < 0.001$). Baseline WBC levels were negatively associated with gait speed ($\beta = -0.013$; $P = 0.025$), quadriceps isometric 90° ($\beta = -5.983$; $P = 0.035$) and isokinetic 60°/s peak torque/body weight ($\beta = -5.532$; $P = 0.027$). The prevalence of sarcopenia at baseline was 18.1% (n = 81). Of those without sarcopenia at baseline, 64 (18.6%) satisfied criteria for sarcopenia at follow-up. There were no significant associations between

baseline inflammatory markers and either prevalent or incident sarcopenia, or change in level of sarcopenia-defining parameters between baseline and follow-up.

Conclusions In middle-aged and older men, hs-CRP and WBC were negatively associated with QoL and PASE scores, while WBC was negatively associated with gait speed and knee strength. Associations with hs-CRP remained significant in all ages, whereas WBC levels were only associated with PASE, gait speed and knee strength in older adults (60–79 years). Baseline inflammatory markers (hs-CRP, WBC and albumin) did not predict functional decline, decline in physical activity, decreased QoL or incident sarcopenia.

Keywords Inflammation; Sarcopenia; Muscle strength; Older adult; Ageing; Inflammaging

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Introduction

Sarcopenia, the age-related loss of muscle mass and function (muscle strength and physical performance), is an important health challenge in the ageing individual.[1] It affects up to 29% of community-dwelling older adults and up to 33% of nursing home residents.[2] Loss of muscle mass and strength impact on an older person's independence and quality of life (QoL).[1] For example, almost 20% of women and almost 10% of men aged 65 years or older cannot lift a 4.5 kg weight or kneel down due to sarcopenia.[3]

The underlying pathophysiology of sarcopenia is complex and still remains, at least partly, unclear. One of the driving mechanisms is thought to be age-related chronic low-grade inflammation—the so-called *inflammaging*- with increased inflammatory markers and decreased anti-inflammatory markers.[4] However, findings regarding inflammatory markers in sarcopenic older adults are often conflicting. First, a recent meta-analysis found that sarcopenic persons had significantly higher levels of C-reactive protein (CRP), but levels of interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) were not significantly different.[5] In contrast, other studies do show associations between increased IL-6 levels and sarcopenia.[6,7] Furthermore, it was recently demonstrated that in geriatric outpatients (80.8 ± 6.7 years), lower levels of albumin are associated with lower gait speed and handgrip strength.[8] Albumin can be considered an inflammatory marker due to its properties as a negative acute phase protein—with decreasing levels of albumin in case of inflammation—but is also a nutritional biomarker with a strong association with body mass index (BMI) in older adults.[9,10]

During the last decade, the definition of sarcopenia has evolved to include muscle function (determined by muscle strength and physical performance) rather than muscle mass solely. Recently, the European Working Group on Sarcopenia in Older People revised the operational definition of sarcopenia (EWGSOP2),[11] placing muscle strength upfront as the principal determinant. These revised diagnostic criteria are increasingly being used in daily clinical practice. However, most previous studies regarding inflammatory status and sarcopenia used earlier diagnostic criteria which focused on muscle mass.

The way in which physical activity, an important component in sarcopenia treatment and prevention strategies, is affected by inflammation still remains unclear.[12,13] There are data suggesting that IL-6 has an inverse relationship with physical activity (measured with an accelerometer) in obese middle-aged and older adults (≥55 years), even after adjustment for BMI, whereas CRP only correlated with the physical activity energy expenditure and not with other physical activity parameters.[14] Similarly, the associations between inflammation and QoL are contradictory. Previous research suggests that the coexistence of a subclinical increase in CRP and IL-6 in middle-aged adults (45–69 years) is associated with QoL measurements, in particular Short Form (SF)-36 scores.[15] This might be due to the increased risk on functional decline and diseases, related to inflammation.[4,15] In contrast, another study did not find significant associations between circulating inflammatory markers (CRP, TNFα, and various interleukins) and some of the known QoL measurements like SF-36 and the personal wellbeing index.[16] Because QoL is affected by sarcopenia and inflammation contributes to the development of sarcopenia, it is worthwhile to explore the relation inflammation-QoL.

To conclude, there are some inconsistent data supporting an association between inflammatory markers and sarcopenia or sarcopenia-defining parameters (muscle mass, strength, and physical performance) as well as physical activity and QoL. Further clarification of these associations, both cross-sectional and longitudinal, is needed in well-described populations. In this analysis, we used data from the European Male Ageing Study (EMAS), a population cohort of middle-aged and older men, to determine (i) the cross-sectional association between inflammatory markers, muscle mass and muscle function, physical activity, and QoL; (ii) the incidence of sarcopenia; and (iii) the influence of baseline inflammatory markers on both incidence of sarcopenia and change in muscle mass and function, QoL, and physical activity. We hypothesized that markers of inflammation at baseline would be associated with prevalent sarcopenia or

its defining parameters, as well as QoL and physical activity and that they would predict both the development of sarcopenia and decline in muscle mass and function.

Methods

Subjects and study design

Men aged 40–79 years were recruited from population registers in eight European centres: Leuven (Belgium); Manchester (UK); Florence (Italy); Santiago de Compostela (Spain); Łódź (Poland); Szeged (Hungary); Tartu (Estonia); Malmö (Sweden) for participation in EMAS. Details of the methods have been published elsewhere.[17] In brief, community-dwelling men were invited (2003–2005) to participate by letter of invitation which included a postal questionnaire. Those who agreed to take part attended a local study centre for an interviewer-assisted questionnaire, as well as clinical and biological assessments. Between 2007 and 2010, participants were invited to take part in a repeat survey. Ethical approval for the study was obtained in accordance with local institutional requirements. All subjects provided written informed consent.

Study questionnaires and clinical assessments

The postal questionnaire included questions on lifestyle including smoking and previous medical history including heart diseases and high blood pressure. The interviewer-assisted questionnaire included questions about current medications. Height was measured to the nearest 1 mm using a stadiometer (Leicester Height Measure, SECA Ltd, UK) and body weight to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA Ltd, UK). Follow-up assessments were performed after a median interval of 4.29 years [interquartile range (IQR) = 4.14–4.45].

Quality of life and physical activity

The interviewer-assisted questionnaire included the SF-36, a generic health-related QoL instrument that includes eight health domains: physical functioning, role limitations due to physical health, role limitations due to emotional health, mental health, bodily pain, general health, vitality, and social functioning.[18] These items make use of a norm-based scoring method, combining these eight health domains into component summary scales scores for mental and physical QoL. Physical activity was assessed using the Physical Activity Scale for the Elderly (PASE).[19] PASE is a weighed score, based on a self-reported questionnaire, scoring occupational, household, and leisure activities items performed in the previous 7 days.

Inflammation

Fasting venous blood samples were taken at baseline and follow-up in all subjects. White blood cell count (WBC) and albumin were measured in the hospital laboratory of individual study centres. High-sensitive CRP (hs-CRP) was measured centrally in Santiago de Compostela, by using an immunoassay with intra-assay and inter-assay coefficients of variation of 2.8% and 3.1%, respectively (Immulite 2000 high-sensitivity assay, Diagnostics Products Corporation, Siemens, Deerfield, IL, USA) and a detection limit of 0.1 mg/L. Subjects were considered having chronic inflammation when both at baseline and follow-up the inflammatory marker was in the upper (hs-CRP and WBC) or lower quartile (albumin) in order to analyse the effects in subgroup analyses.

Sarcopenia-defining parameters

At baseline and follow-up, subjects of Leuven and Manchester underwent whole-body dual X-ray absorptiometry (DXA) scans on QDR 4500A Discovery scanners (Hologic Inc, Bedford, MA, USA), with measurement of appendicular lean mass (aLM). Scans were analysed using Hologic APEX 4.0 software. Whole body DXA allows to assess muscle mass by using the aLM and calculating the skeletal muscle mass index (SMI, defined as aLM/height^2 (units kg/m^2). The precision error of DXA measurements was 0.57% in Leuven (n = 20) and 0.56% in Manchester (n = 31).[20] Scanners were cross-calibrated with the European Spine Phantom. Muscle strength was evaluated by measuring grip strength with the Jamar 1 hand-held dynamometer (TEC Inc., Clifton, NJ, USA). Maximal grip strength was recorded as the highest of three measurements at both sides.[21] Lower extremity muscle strength was measured through the chair stand test,[11] in which the time that a participant needs to stand up and sit again for five consecutive times is measured. Physical performance was assessed by gait speed, expressed as meters per second and measured as a component of the Reuben's physical performance test (seconds taken to walk 50 ft or 15.24 m).[22]

Sarcopenia definition

To define sarcopenia, the recent EWGSOP2 diagnostic criteria were used.[11] A subject was considered having probable sarcopenia when maximal hand grip strength was $<$27 kg or when chair stand test was performed in $>$15 seconds. When also SMI was $<$7.0 kg/m^2, muscle mass was considered low and sarcopenia confirmed. If gait speed was $<$0.8 m/s on top of low muscle strength and muscle mass, sarcopenia was considered severe. Incident sarcopenia was defined as the presence of sarcopenia (probable/confirmed/severe

sarcopenia) at follow-up among subjects who did not have sarcopenia at baseline.

Knee extensor muscle strength assessment

In the Leuven cohort, isometric and isokinetic strength were evaluated in the knee extensors of the left leg.[23] Strength was measured using an isokinetic dynamometer (Cybex II, Lumex Inc., Ronkonkoma, NY, USA) according to standardized manufacturer's procedures. Maximum isometric strength was measured at 60° and 90°, the highest value of three measurements taken as maximum isometric strength for each position. Maximum isokinetic strength was measured at different angular velocities (60°/s and 90°/s) as the highest value of three attempts.[24] Results were presented as % peak torque/body weight (PT/BW). Validation of the short term-reproducibility of this assessment has been published previously.[23]

Statistical analysis

Descriptive statistics were used to summarize subject baseline characteristics. Prevalence of sarcopenia was determined using the EWGSOP2 criteria at both baseline and follow-up. Analyses with hs-CRP were performed with natural log transformed data due to its skewed distribution. Linear regression was used to determine associations between baseline inflammatory markers and each sarcopenia-defining parameter (aLM, SMI, grip strength, gait speed, and chair stand test). Linear regression was used to determine associations between baseline inflammatory markers and knee extension muscle strength in the Leuven cohort and to detect associations between inflammatory markers and PASE score, SF-36 physical and mental component score in the whole cohort. Comparison of sarcopenia-defining parameters between baseline and follow-up was performed with the Wilcoxon signed-rank test. Linear regression was used to detect associations between baseline inflammatory markers as continuous variables and annual % change in the sarcopenia-defining parameters, PASE or SF-36 scores between baseline and follow-up. Annual % change was calculated as [(follow-up minus baseline)/baseline]*100/time (years) between baseline and follow-up measurement. Logistic regression was used to determine the associations between baseline inflammatory markers and the risk of both prevalent and incident sarcopenia based on the EWGSOP2 definition with results expressed as odds ratios and 95% confidence interval. Longitudinal analyses were repeated in the subgroup of subjects with chronic inflammatory markers. Cross-sectional analyses were repeated in subgroups stratified by age (40–59 years vs. 60–79 years). Multivariable models were adjusted for potential confounders including age, centre, BMI, and smoking. Statistics were performed using STATA SE 16.1.

Results

Subjects

In total, 3369 middle-aged and older European men participated in EMAS. An overview of the different exclusions, according to the analysis performed, can be found in *Figure* 1. Regarding the cross-sectional analyses for associations between inflammatory markers and QoL (SF-36) and physical activity (PASE), 107 of the 3369 subjects were excluded due to use of anabolic steroid or corticosteroids at baseline. Moreover, 443 subjects had missing data on at least one of the inflammatory markers and 242 did not have data on PASE score or SF-36 questionnaire at baseline, leaving 2577 subjects for the cross-sectional analyses. After excluding 758 subjects with missing PASE or SF-36 data at follow-up, longitudinal analyses could be performed in 1819 subjects. In order to acquire as large as possible study populations for each longitudinal analysis with baseline inflammatory markers and annual % change of the sarcopenia-defining parameters, different sample groups were composed according to data availability (*Figure* 1).

For the cross-sectional analyses in the Leuven and Manchester cohort (n = 847), 32 subjects were excluded due to use of anabolic steroids or corticosteroids at baseline. Additionally, 25 subjects missing data on at least one of inflammatory markers and 343 subjects missing data on at least one of the sarcopenia-defining parameters at baseline were excluded, leaving 447 subjects included in these analyses. For the knee extensor muscle strength analyses, an additional 170 subjects with missing data on knee muscle strength measurements were excluded, leaving 277 subjects in the subgroup. For the longitudinal analyses in the Leuven and Manchester cohort (n = 411), 36 participants with at least one of the sarcopenia-defining parameters missing at follow-up were excluded from the cross-sectional cohort (n = 447). Finally, subgroup analyses with chronic inflammation were performed only in subjects within this cohort with complete follow-up data on all inflammatory markers and sarcopenia-defining parameters (n = 364). Baseline characteristics of the study groups, according to the analyses, can be found in *Table* 1.

Baseline inflammatory markers and PASE or SF-36

Of the participants of EMAS, 2577 had available data on inflammatory markers, PASE, and SF-36. Strong significant inverse associations were found between both hs-CRP and WBC and PASE score (hs-CRP β: −7.920, $P < 0.001$; WBC β:

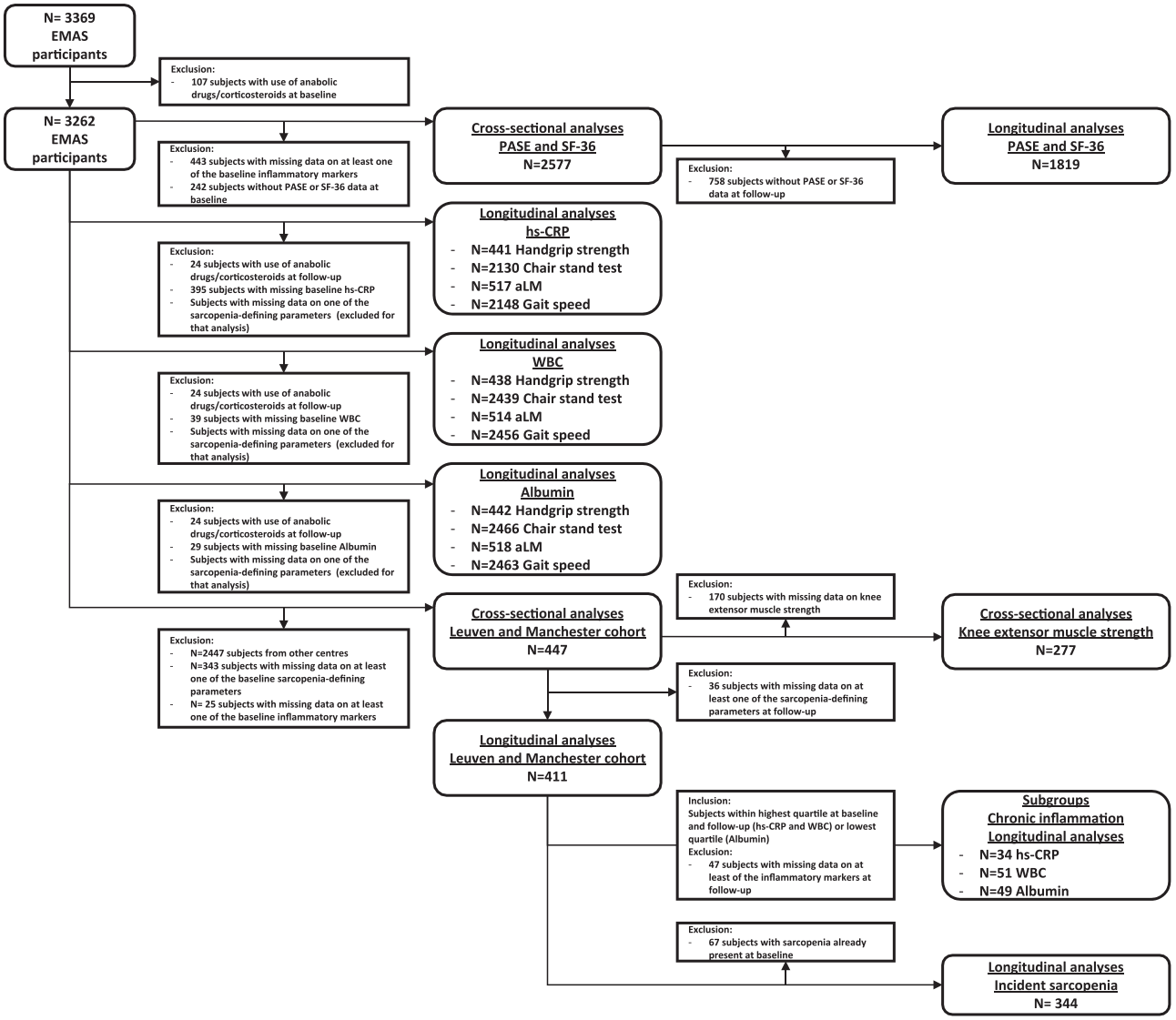

Figure 1 Overview of the different analyses and corresponding study population in this manuscript. EMAS, European Male Aging study; PASE, Physical Activity Scale for the Elderly

−2.963, $P < 0.001$), even after adjusting for age, BMI, centre, and smoking (*Table* 2). A positive association between albumin levels and total PASE was found, although this was not significant after adjustment for the same putative confounders. Regarding SF-36, hs-CRP and WBC showed strong significant inverse associations with the physical component score of SF-36 (hs-CRP β: −1.025, $P < 0.001$; WBC β:−0.364, $P < 0.001$), but not the mental component score. Albumin levels showed positive associations with both physical and mental component scores. However, after adjustment, these lost statistical significance. Longitudinally, we could not find associations between baseline inflammatory markers and change in PASE or SF-36 scores between baseline and follow-up (*Table* S1).

A stratified cross-sectional analysis according to age groups 40–59 years (middle-aged adults) and 60–79 years (older adults) was performed (*Figure* 2), demonstrating that hs-CRP was significantly inversely associated with SF-36 physical component scores in both middle-aged (β: −0.651; $P < 0.01$) and older adults (β: −1.387; $P < 0.01$, as well as with PASE score (middle-aged β: −5.866; $P = 0.01$ and older adults β: −10.567; $P < 0.01$), adjusted for age, BMI, centre, and smoking. Similarly, WBC levels were associated with SF-36 physical component scores in both age groups (middle-aged β: −0.285; $P = 0.01$ and older adults β: −0.460; $P < 0.01$). However, WBC levels were only significantly associated with PASE score in older adults (β: −4.558; $P < 0.01$), but this was no longer significant in middle-aged adults (β: −1.956; $P = 0.072$).

Table 1 Baseline characteristics

Baseline characteristics		
Variable	Mean/median	SD/IQR
n = 2577[a]		
Age (years)	59.66	(±11.00)
BMI (kg/m^2)	27.74	(±4.13)
Number of comorbidities	1	[0–2]
hs-CRP (mg/L)	0.231	[0.118–0.475]
WBC (×10^9/L)	6.2	(±2.1)
Albumin (g/dL)	4.51	(±0.31)
PASE score	198.79	(±92.05)
SF-36 physical component score	51.17	(±9.35)
SF-36 mental component score	49.87	(±8.22)
n = 447[b] (Manchester and Leuven cohort)		
Number of comorbidities	1	[0–1]
Handgrip strength (kg)	44.00	(±8.98)
Chair stand test (s)	12.4	(±3.1)
aLM (kg)	24.88	(±3.51)
SMI (kg/m^2)	8.10	(±0.96)
Gait speed (m/s)	1.15	(±0.17)
Sarcopenia (EWGSOP2)		
No sarcopenia	366	(81.88%)
Probable sarcopenia	71	(15.88%)
Confirmed sarcopenia	9	(2.01%)
Severe sarcopenia	1	(0.22%)
n = 277[c]		
Isometric 60° PT/BW (%)	206.9	(±70.3)
Isometric 90° PT/BW (%)	206.2	(±153.6)
Isokinetic 60°/s PT/BW (%)	145.4	(±57.4)
Isokinetic 90°/s PT/BW (%)	125.8	(±55.3)

BMI, body mass index; CI, confidence interval; hs-CRP, high-sensitive C-reactive protein; IQR, interquartile range; PT/BW, % peak torque/body weight; PASE, Physical Activity Scale for the Elderly; SD, standard deviation; WBC, white blood cell count

[a]Analyses with baseline inflammatory markers vs. physical activity (PASE) and quality of life (SF-36).

[b]Analyses with baseline inflammatory markers vs. baseline sarcopenia-defining parameters.

[c]Analyses with baseline inflammatory markers vs. knee extensor muscle strength.

Baseline inflammatory markers, sarcopenia-defining parameters, and prevalent sarcopenia

Using baseline data from the Leuven and Manchester participants (n = 447), an increase in WBC was negatively associated with gait speed at baseline (β: −0.013, P = 0.025), a result which remained significant after adjusting for age, BMI, smoking, and centre (*Figure* 3). A similar inverse association between an increase in hs-CRP and gait speed was found, although the result became non-significant after adjustment. Albumin was positively associated with grip strength, although non-significant after adjustment. None of the other parameters defining sarcopenia including chair stand test, grip strength and appendicular lean mass were significantly associated with WBC, hs-CRP or albumin level (*Figure* 3). Based on the EWGSOP2 criteria, 18.1% (n = 81) of subjects with data on baseline sarcopenia-defining parameters could be classified as being sarcopenic: 71 (15.9%) as

Table 2 Associations between baseline inflammatory markers and physical activity (PASE) and quality of life (SF-36)

Baseline inflammatory markers vs. physical activity (PASE) and quality of life (SF-36)						
Inflammatory markers n = 2577	PASE score		SF-36 mental component		SF-36 physical component	
	β-coefficient (95% CI)		β-coefficient (95% CI)		β-coefficient (95% CI)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
hs-CRP[b] (per unit)	−17.434 (−20.753; −14.114)***	−7.920 (−11.032; −4.808)***	−0.189 (−0.533; 0.154)	−0.297 (−0.656; 0.063)	−1.736 (−2.030; −1.441)***	−1.025 (−1.318; −0.732)***
WBC (per unit)	−3.006 (−4.672; −1.340)***	−2.963 (−4.552; −1.373)***	−0.127 (−0.296; 0.043)	−0.132 (−0.315; 0.051)	−0.276 (−0.425; −0.128)***	−0.364 (−0.514; −0.214)***
Albumin (per unit)	48.961 (37.548; 60.374)***	8.156 (−2.855; 19.168)	−1.792 (−2.964; −0.620)**	0.280 (−0.986; 1.547)	2.575 (1.548; 3.603)***	0.324 (−0.717; 1.366)

BMI, body mass index; CI, confidence interval; hs-CRP, high-sensitive C-reactive protein; IQR, interquartile range; PT/BW, % peak torque/body weight; PASE, Physical Activity Scale for the Elderly; SD, standard deviation; WBC, white blood cell count.
Linear regression
[a]Adjusted for age, BMI, centre and smoking.
[b]log-transformed variable.
**$P < 0.01$.
***$P < 0.001$.

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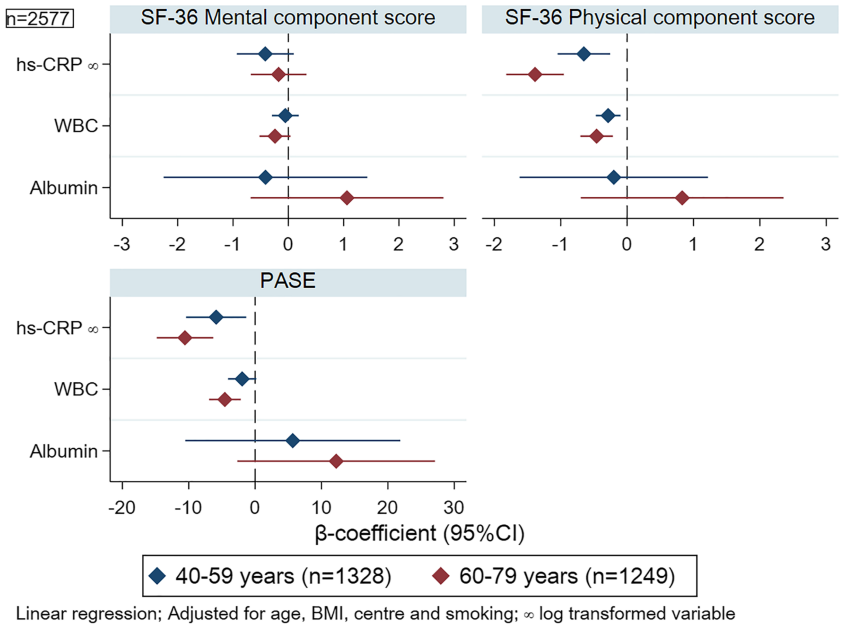

Figure 2 Subgroup analyses for associations between baseline inflammatory markers and baseline physical activity [Physical Activity Scale for the Elderly (PASE)] and quality of life (QoL) (SF-36), stratified by age. BMI, body mass index; CI, confidence interval.

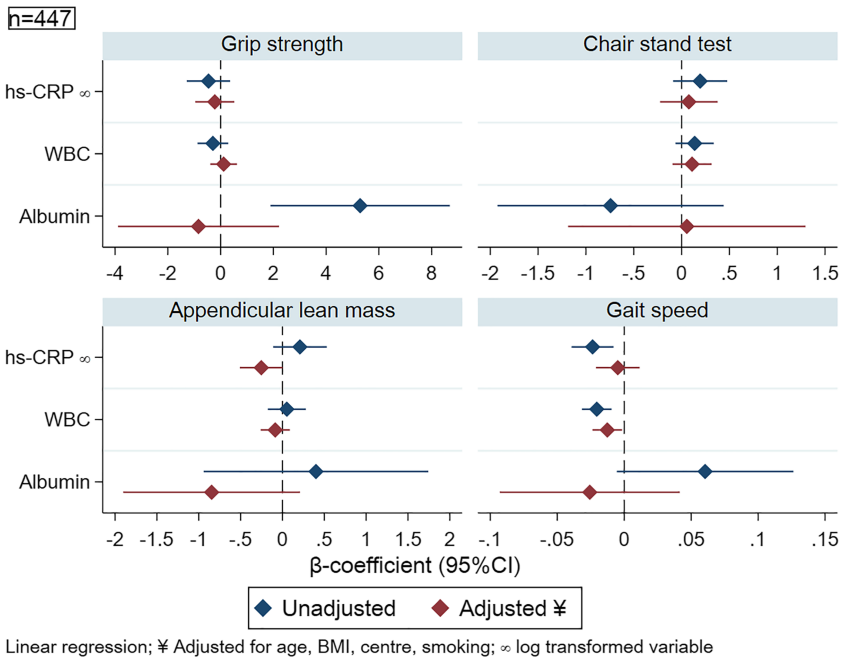

Figure 3 Associations between baseline inflammatory markers and baseline sarcopenia-defining parameters. BMI, body mass index; CI, confidence interval; hs-CRP, high-sensitive C-reactive protein; WBC, white blood cell count.

probable sarcopenia, 9 (2.0%) as confirmed sarcopenia, and 1 subject (0.2%) as severely sarcopenic. No associations between baseline inflammatory markers (hs-CRP, WBC and albumin) and prevalent sarcopenia were identified (*Table* S2).

Subgroup analyses according to age (*Table* S3) revealed that the found inverse association of WBC levels with gait speed was only significant in older adults (β: −0.017; P = 0.030) but not in middle-aged adults (β: −0.007; P = 0.376), adjusted for putative confounders.

Table 3 Associations between baseline inflammatory markers and change in sarcopenia-defining parameters

Associations between baseline inflammatory markers and change in sarcopenia-defining parameters								
	Annual % change in handgrip strength		Annual % change in chair stand test		Annual % change in aLM		Annual % change in Gait speed	
	β-coefficient (95% CI)		β-coefficient (95% CI)		β-coefficient (95% CI)		β-coefficient (95% CI)	
Inflammatory marker	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Sample size	$n = 441$		$n = 2130$		$n = 517$		$n = 2148$	
hs-CRP[b] (per unit)	−0.261 (−0.574; 0.053)	−0.135 (−0.473; 0.203)	0.040 (−0.366; 0.446)	−0.115 (−0.536; 0.306)	−0.042 (−0.134; 0.049)	0.007 (−0.093; 0.106)	−0.322 (−0.521; −0.123)**	0.017 (−0.152; 0.186)
Sample size	$n = 438$		$n = 2439$		$n = 514$		$n = 2456$	
WBC (per unit)	−0.079 (−0.303; 0.144)	−0.034 (−0.265; 0.197)	0.061 (−0.200; 0.322)	−0.126 (−0.430; 0.177)	−0.049 (−0.113; 0.016)	−0.035 (−0.102; 0.032)	−0.168 (−0.268; −0.069)**	0.006 (−0.088; 0.100)
Sample size	$n = 442$		$n = 2446$		$n = 518$		$n = 2463$	
Albumin (per unit)	1.544 (0.215; 2.873)*	0.228 (−1.196; 1.652)	−0.656 (−2.012; 0.700)	−1.485 (−3.253; 0.283)	0.233 (−0.155; 0.621)	−0.058 (−0.477; 0.361)	−0.706 (−1.227; −0.185)**	0.451 (−0.101; 1.003)

BMI, body mass index; CI, confidence interval; hs-CRP, high-sensitive C-reactive protein; IQR, interquartile range; PT/BW, % peak torque/body weight; PASE, Physical Activity Scale for the Elderly; SD, standard deviation; WBC, white blood cell count.
Linear regression.
[a]Adjusted for age, BMI, centre, and smoking.
[b]log-transformed variable.
*$P < 0.05$.
**$P < 0.01$.

Baseline inflammatory markers and change in sarcopenia-defining parameters

Between baseline and follow-up, EMAS participants of the Leuven and Manchester cohort had a mean annual % aLM loss of 0.37%/year (SD = 1.11). Mean annual % gait speed loss was 0.07%/year (SD = 4.85), whereas the mean % prolongation of chair stand test time was 2.26%/year (SD = 6.38). Grip strength did not differ significantly between both visits. All inflammatory markers showed a negative association with annual % change in gait speed (*Table* 3). However, after adjusting for age, BMI, centre, and smoking, these results became non-significant. Similarly, an association was found between albumin and annual % change in handgrip strength, although this was not—after adjustment for the same variables—significant. No other associations between baseline levels of inflammatory markers and annual % change in sarcopenia-defining parameters were found.

In subgroups with hs-CRP (n = 34) and WBC (n = 51) in the upper quartile, no significant associations were found between baseline inflammation level and change in sarcopenia-defining parameters (*Table* S4). Similarly, no associations were found between baseline albumin and functional decline in the subgroup (n = 49) with the lowest quartiles of albumin at baseline and follow-up.

Baseline inflammatory markers and incident sarcopenia

Of the 344 subjects without sarcopenia at baseline in the longitudinal analyses of the Leuven and Manchester cohort (n = 411), 64 (18.6%) were classified into one of the sarcopenia stages of EWGSOP2 definition at follow-up (*Table* 4). Of those 64 subjects with incident sarcopenia, 55 developed probable and 9 confirmed sarcopenia. Baseline inflammatory markers were not predictive for incident

Table 4 Number of subjects with sarcopenia at baseline and follow-up (Leuven and Manchester cohort)

Number of subjects with EWGSOP2 sarcopenia at baseline and follow-up							
		Follow-up					
	n = 411	No sarcopenia		Probable sarcopenia		Confirmed sarcopenia	
Baseline	No sarcopenia	280	(68.13%)	55	(13.38%)	9	(2.19%)
	Probable sarcopenia	30	(7.30%)	28	(6.81%)	2	(0.49%)
	Confirmed sarcopenia	3	(0.73%)	0	(0.00%)	4	(0.97%)

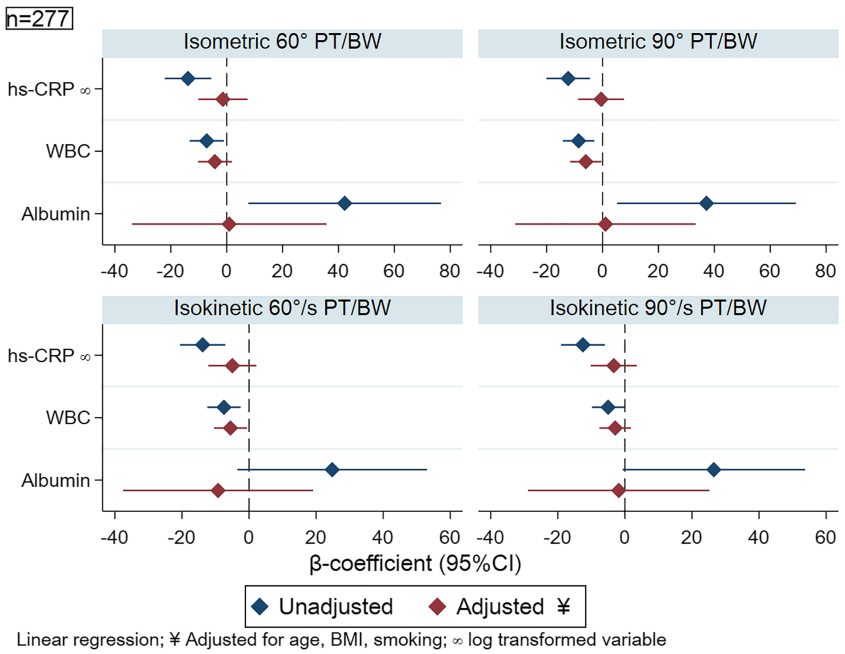

Figure 4 Associations between baseline inflammatory markers and baseline knee extensor muscle strength. BMI, body mass index; CI, confidence interval; PT/BW, % peak torque/body weight.

sarcopenia (*Table* S5). Likewise, there were no significant associations with incident sarcopenia in the subgroup analyses with chronic inflammation (*Table* S6).

Baseline inflammatory markers and knee extensor muscle strength

Among the 277 men with knee extensor muscle strength measurements, both hs-CRP and WBC were significantly inversely associated with isokinetic 60°/s PT/BW (*Figure* 4). After adjusting for age, BMI, and smoking, however, only WBC remained significantly associated (β: −5.532; $P = 0.027$). WBC levels had a weak inverse association with isometric 90° PT/BW after adjusting (β: −5.983; $P = 0.035$). hs-CRP was inversely associated with isometric 60° PT/BW and isokinetic 90°/s PT/BW, whereas WBC was inversely associated with isometric 60° PT/BW, however, after adjustments these associations also became non-significant. There were positive associations between albumin and isometric 60° PT/BW and isokinetic 90°/s PT/BW, although these too became non-significant after adjustment for putative confounders.

When examined according to age groups and after adjustment (age, BMI, and smoking) (*Figure* S1), the association of WBC levels with isometric 90° PT/BW only remained significant in older adults (β: −9.455; $P < 0.01$) but not in middle-aged adults (β: −1.493; $P = 0.75$). The association of WBC levels with isokinetic 60°/s PT/BW lost significance in both age groups, possibly due to the small sample sizes ($n = 135$ subjects aged 40–59 years and $n = 142$ subjects 60–79 years).

Discussion

In EMAS participants, WBC levels were inversely associated with gait speed, whereas no other significant associations between inflammatory markers and sarcopenia-defining parameters were found. In the analyses with knee extensor muscle strength, WBC levels were also inversely associated with isometric 90° and isokinetic 60°/S PT/BW. Interestingly, hs-CRP and WBC levels were inversely associated with both physical activity (PASE score) and the physical component score of the SF-36 questionnaire in a large cohort ($n = 2577$) of the EMAS study. Regarding the longitudinal analyses in this paper, baseline levels of hs-CRP, WBC and albumin were not associated with change in handgrip strength, chair stand test, aLM, gait speed, PASE, or SF-36 scores nor with incident sarcopenia, not even in subgroups with chronic inflammation.

Physical activity was measured in EMAS through PASE, a validated questionnaire for use in community-dwelling older adults.[19] We demonstrated a significant inverse association between hs-CRP and PASE score (β: −7.920, $P < 0.001$) as well as between WBC levels and PASE score (β: −2.963, $P < 0.001$) and thus confirmed the hypothesis of a cross-sectional association between inflammation and physical activity. This suggests that with increasing inflammation, physical activity declined in middle-aged and older men. The exact cause of the association inflammation-physical activity needs further targeted research, as this might be a "vicious circle" with inactivity causing inflammation and increased inflammation on its turn leading to fatigue and thus more inactivity.[14] Interestingly, no longitudinal associations between inflammation and PASE were found. Regarding physical activity and inflammation, previous research of the National Health and Nutrition Examination Survey (NHANES) shows that WBC levels are increased in sedentary adults or adults with less physical activity (mean age 43.1 years, 95% CI 42.3–43.9).[25] Similarly, a significant inverse association of CRP with physical activity expenditure was previously found in a predominantly female cohort of middle-aged and older adults (66.2 ± 6.4y).[14]

QoL was divided in physical and mental component scores of SF-36 in EMAS. This study confirmed significant inverse associations between inflammatory markers (hs-CRP and WBC) and the SF-36 physical component score (β: −1.025, $P < 0.001$ for hs-CRP and β: −0.364, $P < 0.001$ for WBC). We did not find a significant association with the mental component scores. These findings suggest that inflammation might influence health-related QoL, mainly the physical component. An explanation might lie in the positive association between QoL and physical activity.[26] Therefore, promoting physical activity might lead to benefits beyond physical health.[26] Thus, as inflammation may affect physical activity; it might also influence QoL. However, even after adjusting for physical activity by introducing PASE scores in the multivariable linear regression model, the associations found with the SF-36 physical component scores persisted (data not shown). This is suggestive for an effect of inflammation independent of the level of physical activity. Previous findings in literature regarding this topic are inconsistent. Poorer self-rated health is related with elevated inflammatory markers (IL-6 and CRP) in healthy older adults (63.8 ± 13.7 years).[27] In contrast, another study in 268 older adults (≥65 years) living in retirement communities could not confirm these associations.[16]

hs-CRP, *WBC*, and *albumin* were not significantly associated with prevalent or incident sarcopenia, nor change in the sarcopenia-defining parameters. Moreover, subgroup analyses in subjects with chronic inflammatory profiles did not reveal any significant association. These findings rejected the hypothesis that these inflammatory markers predict both the development of sarcopenia and the decline in muscle mass and function. Previous research suggests that increased TNFα levels are associated with a greater 5-year decline in handgrip strength in men.[28] Moreover, Schaap *et al*. found

in a 3 year follow-up study that higher levels of CRP increase the risk of muscle strength loss in older adults (74.6 ± 6.2 years).[29] The lack of findings in our study might have multiple causes. First, EMAS subjects might have been too young to suffer from age-related chronic low-grade inflammation. Puzianowska-Kuźnicka *et al*. demonstrated in a study with older adults (>65 years) that hs-CRP increased with age and more notably in the subjects aged 80+.[30] Moreover, when comparing our hs-CRP levels with those of Puzianowska-Kuźnicka *et al*., our median value is rather low (0.231 [0.118–0.475] mg/L in our study compared with 2.2 mg/L on average in adults aged 65–69 years).[30] This suggests that EMAS subjects were suffering less inflammation than an average person of similar age or are healthier. Furthermore, this study only examined three inflammatory markers, and therefore, a possible longitudinal association with other inflammatory markers (e.g. TNFα) is not to be excluded. Finally, besides inflammaging, many other factors might contribute to the development of sarcopenia (e.g. malnutrition and vitamin D deficiency).

Gait speed, a measurement for physical performance, was significantly and inversely associated with WBC levels at baseline. Besides this association, the hypothesis of a cross-sectional association between inflammatory markers and sarcopenia-defining parameters could not be confirmed, and no other associations between inflammatory markers and handgrip strength, chair stand test, or aLM were found. Possibly, EMAS subjects might have been too young to suffer from a loss of sarcopenia-defining parameters that is large enough to already detect significant associations.

Sarcopenia prevalence (probable/confirmed/severe sarcopenia) was 18.1% (*n* = 81) at baseline, when using the revised EWGSOP2 definition. The prevalence of sarcopenia in our population-based sample of middle-aged and older men is similar to the findings of Moreno-Gonzalez *et al*.[31] In their study with 1420 European community-dwelling older adults (>75 years), 22.1% of men could be classified into one of the sarcopenia categories of EWGSOP2.[31] Sarcopenia prevalence and incidence in EMAS, according to other definitions [International Working Group on Sarcopenia (IWGS), EWGSOP1, Baumgartner], have been described elsewhere.[20] When we apply the EWGSOP2 criteria in these EMAS analyses, prevalence and incidence were higher as compared with previous sarcopenia definitions. This might lie in the fact that EWGSOP2 places low muscle strength upfront, whereas most other sarcopenia definitions are primarily based on low muscle mass. During the follow-up period, 18.6% (*n* = 64) of subjects without sarcopenia at baseline (*n* = 344) developed EWGSOP2 sarcopenia (incident sarcopenia). In 55 of 64 subjects with incident sarcopenia, low muscle strength was diagnosed solely due to a prolongation of chair stand test time between baseline and follow-up, while handgrip strength was preserved. This is in line with sarcopenia affecting lower limbs earlier than upper limbs. Moreover, only nine subjects developed confirmed sarcopenia (low muscle strength and mass). This might explain the different incident rates, compared with previous sarcopenia definitions based on muscle mass.[20] However, diagnosing even probable sarcopenia is of the utmost importance, because this indicates a need for treatment in clinical practice according to the EWGSOP2 consensus.[11] Therefore, persons with probable sarcopenia were also included in present analyses.

Knee extensor muscle strength mainly represents quadriceps strength. Present study confirmed significant inverse associations between WBC levels and isometric 90° and isokinetic 60°/s PT/BW, even after adjusting for putative confounders. This implies a possible association between increased inflammation and knee muscle strength. Regarding the influence of inflammation on knee muscle strength, Custodero *et al*. found in a cohort of mobility limited older adults (≥70 years) a trend towards progressive reduction of isokinetic knee extensor strength 60°/s across increasing IL-6 tertiles.[32] Similarly, Zembron-Lacny *et al*. found in a sample of 33 older men (73.5 ± 6.3 years) and 22 young men (21.2 ± 1.3 years) an inverse association between some inflammatory markers (IL-1β, hs-CRP, and TNFα) and knee extensor isokinetic PT at 60°/S and 180°/s.[33] Recent data on age-related and sex-related decline of muscle strength suggest that isokinetic 60°/s knee extensor PT decline accelerates in men around the age of 66.7 years, whereas in female patients, this acceleration occurs much earlier (around age 49.3 years).[34] Isometric strength declines similarly in both genders, starting from the sixth decade.[34] Thus, the rather young age of EMAS participants might explain the limited number of associations found with knee muscle strength. This might also explain the loss of significance when analysing according to age group, with only the inverse association between WBC levels and isometric 90° PT/BW remaining significant in older adults.

Albumin did not have any significant associations with sarcopenia-defining parameters, knee extensor muscle strength, prevalent or incident sarcopenia, physical activity, or QoL that persisted after adjusting for confounders. Albumin is known to be influenced by factors other than inflammation (e.g. protein intake).[9,10] Moreover, the confounder adjustments in our study confirmed an important role for age and BMI as confounders in the analyses with albumin. Previous research by van Atteveld *et al*. suggested that lower albumin levels are significantly associated with lower gait speed and handgrip strength in geriatric outpatients.[8] However, these associations could not be confirmed in our study. This might be due to the differences in population (geriatric outpatients vs. middle-aged and older men), age (mean 80.8 years vs. 59.66 years) or confounder adjustments (adjustments for age, sex, and comorbidities vs. age, centre, BMI, and smoking).

Age is an important risk factor to develop sarcopenia. However, sarcopenia is not only present in older adults, but its development starts earlier in life. Peak handgrip strength

is reached around 29–39 years (men) or 26–42 years (women).[35] Afterwards, handgrip strength declines slowly with an acceleration to 1.5%/year in the sixth decade of life and up to 3%/year afterwards.[36] Therefore, a stratified analysis was performed according to age group, in order to examine differences between middle-aged (40–59 years) and older (60–79 years) men. WBC levels were associated with SF-36 physical component score and PASE score in older adults, but not in subjects aged 40–59 years. Similarly, the association between WBC levels and gait speed was only significant in older adults, but not in middle-aged men. On the contrary, significant negative associations between hs-CRP and PASE or the physical component of SF-36 were found in both middle-aged and older men. Possibly middle-aged subjects might be too young to be affected by inflammaging, which might explain the difference in significance compared with older adults. Moreover, gender differences of gait speed widen as age increases, with men being generally faster than women.[37] Overall, gait speed in EMAS participants was relatively high (mean of 1.15 ± 0.17 m/s compared with the EWGSOP2 cut-off for low gait speed of $<$0.80 m/s[11]). Additionally, the associations with PASE score in middle-aged subjects might have to be interpreted with caution because PASE is mainly validated for use in older adults ($>$65 years).[19] All these factors might explain the more modest findings in the middle-aged group.

Strengths of present study were the large sample sizes, as well as the multicentre prospective design of EMAS with standard methods and repeated measurements over a median follow up of 4.29 years. Longitudinal data on associations between inflammation and functional decline are scarce. To our knowledge, this study is the first to assess longitudinal associations between inflammation and incident sarcopenia, according to the latest EWGSOP2 definition. Moreover, by using hs-CRP, WBC and albumin, this study examined inflammatory markers that are clinically relevant and frequently used. Another strength is that EMAS consists of both middle-aged and older men with a similar distribution between the under 60 years group (n = 1328 [51.53%]) and the over 60 years group (n = 1249 [48.47%]) in the analyses with physical activity and QoL. Similar distribution was found in the cross-sectional analyses with sarcopenia-defining parameters, having n = 231 (51.68%) under 60 years and n = 216 (48.32%) over 60 years.

A limitation was the presence of only men in this study population. Therefore, conclusions of this study cannot be generalized to women. Age-related loss of muscle mass and function or sarcopenia is shown to be more prevalent in women than men.[38] Moreover, Payette *et al*. described that IL-6 (as inflammatory marker) is a significant predicator of sarcopenia, but only in women and not in men.[39] Thus, it is plausible that the associations between inflammatory markers and sarcopenia-defining parameters or sarcopenia would be different in a female cohort. Furthermore, no causality can be concluded from this type of association studies, as more targeted studies are required for the purpose of clarifying the pathways in which inflammaging alters muscle ageing. The limited amount of inflammatory markers assessed in the present study might also have restricted our findings. Future studies should include a wider variety of inflammatory markers that potentially plays a role in muscle wasting (e.g. IL-6, IL-10, and IL-13) in order to clarify these associations. Moreover, although the mean values of the inflammatory markers in this study are relatively low and narrowly distributed (*Tables* 1 and S7), undocumented but rare acute infections at the time of the study visit or in between visits cannot be entirely excluded. Also, data on exercise interventions or activity levels in between visits were not available and some muscle strength measurements might be subjected to the efforts and motivation of the participant at the moment of assessing, both potentially limiting our findings. We also opted not to adjust for comorbidities because this study intended to investigate the effects of inflammaging on various sarcopenia outcomes. Adjusting for multiple underlying comorbidities might conceal this inflammaging because this low-grade inflammaging might be caused by a wide range of comorbidities. Furthermore, the relatively young age of the subjects might also explain the lack of findings. Finally, although a follow-up interval of 4.29 years in EMAS can be regarded as substantial, considering the scarcity of longitudinal data on the topic, this might still be too short in order to detect significant findings on sarcopenia-related outcomes, especially in the middle-aged cohort.

In conclusion, this study confirmed some interesting cross-sectional associations between inflammatory markers and physical activity as well as the physical component of SF-36 in a cohort of middle-aged and older European men. Moreover, we confirmed an inverse cross-sectional association between WBC and physical performance measured through gait speed, as well as knee extensor strength. However, no significant associations of inflammatory markers with other sarcopenia-defining parameters, nor prevalent or incident sarcopenia were found.

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The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[40]

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Associations between baseline inflammatory markers and change in PASE and SF-36.

Table S2. Associations between baseline inflammatory markers and prevalent EWGSOP2 sarcopenia.
Table S3. Subgroup analyses for associations between baseline inflammatory markers and baseline sarcopenia-defining parameters, according to age.
Table S4. Subgroup analyses for associations between baseline inflammatory markers and annual % change in sarcopenia-defining parameters.
Table S5. Association between baseline inflammatory markers and incident sarcopenia.
Table S6. Subgroup analyses for associations between baseline inflammatory markers and incident sarcopenia.
Table S7. Distribution of inflammatory markers in subjects of the Leuven and Manchester cohort with complete data on inflammation and sarcopenia-defining parameters both at baseline and follow-up.

Figure S1. Subgroup analyses for associations baseline inflammatory markers and baseline knee extensor muscle strength, according to age.

Conflicts of interest

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