ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle (2016)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12134

Systemic inflammation, body composition, and physical performance in old community-dwellers

Riccardo Calvani[1], Federico Marini[2], Matteo Cesari[3,4], Thomas W. Buford[5], Todd M. Manini[5], Marco Pahor[5], Christiaan Leeuwenburgh[5], Roberto Bernabei[1], Francesco Landi[1] & Emanuele Marzetti[1]*

[1]*Department of Geriatrics, Neurosciences, and Orthopaedics, Catholic University of the Sacred Heart, Rome, Italy;* [2]*Department of Chemistry, 'Sapienza' University of Rome, Rome, Italy;* [3]*Gérontopôle, Centre Hospitalier Universitaire de Toulouse, Toulouse, France;* [4]*Institut National de la Santé et de la Recherche Médicale (UMR1027), Université de Toulouse III Paul Sabatier, Toulouse, France;* [5]*Department of Aging and Geriatrics, University of Florida, Gainesville, FL, USA*

Abstract

Background Chronic inflammation, changes in body composition, and declining physical function are hallmarks of the ageing process. The aim of the present study was to provide a preliminary characterisation of the relationship among these age-related phenomena via multivariate modelling.

Methods Thirty-five old adults (OAs) and 17 young adults (YAs) were enrolled. The volume of skeletal muscle, subcutaneous adipose tissue (SAT), and intermuscular adipose tissue (IMAT) of the thigh was quantified by three-dimensional magnetic resonance imaging. Muscle strength was measured by knee extension strength testing. In OAs, physical performance was further assessed via the Short Physical Performance Battery (SPPB). Multi-block partial least squares-discriminant analysis (PLS-DA) was employed to explore the relationship among inflammatory profiles and functional and imaging parameters. Double cross-validation procedures were used to validate the predictive ability of the PLS-DA model.

Results The optimal complexity of the PLS-DA model was found to be two latent variables. The proportion of correct classification was 92.3% in calibration (94.1% in YAs and 91.4% in OAs), 84.6% in internal validation (95.3% in YAs and 78.5% in OAs), and 82.6% in external validation (94% in YAs and 76.9% in OAs). Relative to YAs, OAs were characterised by smaller muscle volume, greater IMAT volume, lower muscle strength, and higher levels of myeloperoxidase, P-selectin, soluble intercellular adhesion molecule 1, and vascular cell adhesion molecule 1. Compared with OAs with SPPB >8, those scoring ≤ 8 were characterised by smaller muscle volume, greater SAT volume, lower muscle strength, and higher levels of interleukin 1 beta, 6, 10, 12, 13, tumour necrosis factor alpha, and granulocyte-macrophage colony-stimulating factor.

Conclusions Multi-block PLS-DA identified distinct patterns of relationships among circulating cytokines and functional and imaging parameters in persons of different ages and varying levels of physical performance. The longitudinal implementation of such an innovative strategy could allow for the tracking of health status over time, the early detection of deviations in health trajectories, and the monitoring of response to treatments.

Keywords Ageing; Cytokines; Short Physical Performance Battery (SPPB); Multi-block partial least squares - discriminant analysis; Muscle strength; Inflammaging

Received: 23 March 2016; Revised: 1 June 2016; Accepted: 10 June 2016
**Correspondence to:* Emanuele Marzetti, Department of Geriatrics, Neurosciences, and Orthopaedics, Catholic University of the Sacred Heart School of Medicine, Teaching Hospital 'Agostino Gemelli', L.go A. Gemelli 8, Rome 00168, Italy. Tel: +39 (06) 3015-5559; Fax: + 39 (06) 3051-911, *Email*: emarzetti@live.com

Introduction

Chronic low-grade inflammation, changes in body composition, and declining physical performance are three interconnected phenomena that characterise the ageing process.[1–3] The 'perfect storm' triggered by such a triad eventually leads to adverse health outcomes, including disability, the loss of independence, morbidity, and mortality.[4]

The association between inflammation and body composition changes as well as physical function impairment has been known for many years.[5–7] Yet, no 'gold standard' inflammatory biomarker has been identified that is consistently associated with adverse outcomes in older adults.[8] Indeed, the effects of inflammation on health status have been inferred mostly through the analysis of single biomarkers. However, these mediators act in a complex and coordinated network involving multiple feedback mechanisms through which the function of individual factors may be modified, replaced, or modulated by others. Nevertheless, only a handful of studies have attempted to develop a comprehensive appraisal of inflammatory markers in relation to adverse health-related events in older adults.[3,7,9–12]

Notably, although muscle mass is an important determinant of muscle strength and physical function, a substantial divergence has been reported between changes in muscle quantity and function over the course of ageing.[13] Indeed, the annual decline in lower extremity strength is approximately three-fold greater than the loss of lean mass.[14] Remarkably, maintaining or even gaining muscle mass does not protect against declines in muscle strength during ageing.[14] These observations indicate that, in addition to muscle quantity, the quality of the remaining tissue impacts force production in advanced age.[14] For instance, qualitative alterations of muscle composition may disrupt tissue architecture and promote chronic local inflammation, which may further deteriorate muscle performance.[15,16]

In order to capture the complex interrelation linking humoral, functional, and body compositional factors during ageing, a new analytical approach is needed that moves from the study of single parameters to the development of multivariate/multidimensional modelling of a panel of complementary processes.[17] The present study was therefore undertaken to provide a preliminary characterisation of the relationship linking systemic inflammation, body composition, and physical function in old age. To address this research question, an array of inflammatory mediators was assayed in blood samples of community-living young and old persons. Three-dimensional magnetic resonance imaging (3D-MRI) was used to determine the tissue composition of the thigh. Muscle strength and function of the lower extremities were assessed through standard tests. Multivariate statistical models were constructed to explore the complex, multidirectional connections relating systemic inflammation, thigh tissue composition, and physical function.

Materials and methods

Participants

Sedentary young (18–35 years) and old (>70 years) community-living persons were enrolled under the coordination of the Recruitment Core of the University of Florida's Claude D. Pepper Older Americans Independence Centre. Recruitment strategies included media articles, direct mailings, newspaper announcements, and presentations to community groups. Following telephone interview, eligible persons were invited to attend a screening visit during which the purpose and procedures of the study were explained and informed consent was obtained.

A set of eligibility criteria was elaborated to minimise the possible confounding effects of co-morbid conditions, medications, or lifestyle habits on the relationship among inflammation, body composition, and physical performance.[1] Other selection criteria were utilised to ensure that candidate participants would understand the purpose of the investigation and comply with the study procedures.[1] The presence of any of the following characteristics was considered exclusionary: smoking in the prior 12 months; engagement in regular physical exercise (i.e. any kind of structured physical activity performed for more than 20 min per week in the previous two months); history of recreational drug use or alcohol abuse; active treatment for cancer or cancer in the past three years; heart failure New York Heart Association class III or IV; stroke with upper and/or lower extremity involvement; Parkinson's disease or other neurological disorders likely to interfere with physical function; major psychiatric illnesses; peripheral vascular disease Lériche-Fontaine stage 3 or 4; history of life-threatening cardiac arrhythmias; cognitive impairment (i.e. Mini Mental State Examination score ≤21); renal disease requiring dialysis; lung disease requiring chronic steroids; chronic viral diseases (e.g. hepatitis B and C, HIV); lower extremity amputation; severe knee or hip osteoarthritis limiting mobility; diabetes with visual, vascular, or neuropathic complications; inflammatory diseases (e.g. rheumatoid arthritis, vasculitis, autoimmune disorders, inflammatory bowel disease); use of growth hormone, oestrogen replacement, testosterone, steroids, or non-steroidal anti-inflammatory drugs on a regular basis; severe obesity [i.e. body mass index (BMI) ≥35]; being underweight (i.e. BMI ≤18.5); active weight loss >5 kg in prior three months; life-threatening illnesses with an estimated life expectancy <1 year. Persons on statin treatment were asked to refrain from drug administration one month prior to blood draw upon their general practitioner's approval. Eligible persons were excluded if they had contraindications to the acquisition of MRI (e.g. claustrophobia, heart pacemaker/defibrillator, metallic stents, aneurysm clips, metal implants or

DOI: 10.1002/jcsm.12134

prosthesis, neurostimulation systems, insulin pumps, or other infusion pumps). The study protocol was approved by the University of Florida's Institutional Review Board and all participants provided written informed consent before enrolment.

Three-dimensional magnetic resonance imaging for thigh composition analysis

T1-weighted MRI was employed to analyse the tissue composition of the thigh of the dominant side. Images were obtained using a 3.0-tesla magnet (Philips Medical Systems, Bothell, WA), as detailed elsewhere.[18] Muscle tissue, subcutaneous adipose tissue (SAT), and intermuscular adipose tissue (IMAT) were quantified volumetrically, as described by Buford *et al.*[1] Images were analysed using the freely available software package MIPAV 1.3 (Medical Image Processing, Analysis and Visualization; Center for Information Technology, National Institutes of Health, Bethesda, MD; http://mipav.cit.nih.gov).

Assessment of muscle strength and physical function

In all participants, muscle strength was assessed via knee extension strength testing. The test was set up to measure the maximal concentric isokinetic strength of knee extensors of the dominant side.[18] Briefly, participants were asked to exert their maximum force while extending the knee from 90° to 0° of flexion at 60° per second with a hip angle of 90–100°. The maximal peak torque achieved out of three test repetitions (N·m) was used for the analysis.

In older participants, physical function was further assessed through the Short Physical Performance Battery (SPPB).[19] The SPPB is composed of three timed subtests that evaluate standing balance, usual gait speed, and the ability to rise from a chair. For the standing balance test, participants were asked to stand in three increasingly challenging positions for 10 s each: standing with feet in side-by-side, semi-tandem, and full tandem positions. For the gait speed subtest, participants were asked to walk at their usual pace along a 4 m course, starting from a standing still position. The faster of two trials ($m \cdot s^{-1}$) was used for the calculation of the summary score. Finally, for the chair-stand subtest, participants were asked to rise from a chair and sit down five times as quickly as possible with arms folded across the chest. Each of the three SPPB subtasks was categorised into a five-level score according to predefined cut-points,[19] with 0 representing inability to do the test and 4 corresponding to the highest level of performance.

Multiplexed immunoassay for the quantification of inflammatory markers

A panel of 14 inflammatory markers, growth factors, and vascular adhesion molecules, related to systemic and/or vascular inflammation, was measured via a multiplex, magnetic bead-based immunoassay (MILLIPLEX® MAP; EMD Millipore, Billerica, MA), as detailed elsewhere.[3] Briefly, analytes were assayed in the serum by the MILLIPLEX® MAP High Sensitivity Human Cytokine Kit Multiplex Assay (Cat. # HSCYTMAG-60SK) and in the plasma by the MILLIPLEX® MAP Human Cardiovascular Disease Magnetic Bead Panel 2—Cardiovascular Disease Multiplex Assay (Cat. # HCVD2MAG-67K). The multiplex immunoassay panels were analysed on a MILLIPLEX® Analyzer 3.1 xPONENT System (Luminex® 200™), and data analysis was performed through the MILLIPLEX® Analyst software. The inter-assay coefficients of variation were $<5\%$ for the High Sensitivity Human Cytokine Kit Multiplex Assay and $<15\%$ for the Cardiovascular Disease Multiplex Assay.

Statistical analysis

All analyses were performed using in-house routines running under Matlab R2012b environment (The MathWorks, Natick, MA). Differences in demographic, anthropometric, clinical, functional, and body composition characteristics between the experimental groups were assessed via the Mann–Whitney *U* test and χ^2 test, for continuous and categorical variables, respectively. Both tests were two-sided, with statistical significance set at $P < 0.05$.

To explore the relationship among inflammation, thigh composition, and physical function, a classification approach based on multi-block partial least squares-discriminant analysis (PLS-DA) was adopted. PLS-DA is the classification analogue of PLS regression, a method that builds the linear relation between a set of responses Y and a matrix of predictors X by projecting the latter onto a low-dimensional space of latent (abstract) variables (LVs). LVs are characterised by having the highest covariance with the responses to be predicted. In order for the method to also be used in classification where the responses to be predicted are of a qualitative and not quantitative nature (in the present study, whether the participant is young or old), y is coded as a 'dummy' binary vector, assuming the value 1 if the participant is old and 0 if he/she is young. The classification of the individuals is then operated on the basis of the predicted value of y, adopting a threshold value of 0.5. If the predicted y is above that value, the individual is classified as old, while if it is below, he/she is predicted to be young.

In particular, in the present investigation, because the set of predictors originated from different experimental domains (i.e. blood biochemistry, imaging, and physical function), a multi-block approach was adopted to fuse the information

obtained from the physical performance/imaging tests and the multiplexed assay, in order to interpret the relationship between them. In multi-block PLS-DA, the two data matrices containing the blocks of predictors (X_{block1} and X_{block2}) are concatenated after a pre-processing stage called block-scaling (each matrix is divided by its variance, so that the two blocks contribute equally to the model) to form a single matrix X_{mb}:

$$X_{mb} = [X_{block1} X_{block2}].$$

X_{mb} has as many rows as the number of individuals and as many columns as the sum of the variables in the two blocks. A PLS-DA model is then built using the matrix X_{mb} as the predictors and the dummy vector containing the class information as the response.

The statistical reliability of the PLS-DA model was subsequently verified by a double cross-validation procedure and by means of randomisation tests. The double cross-validation is a variant of standard cross-validation and includes an outer and an inner loop. The first loop mimics an external test set to be used for the validation of a PLS-DA model the optimal complexity of which is chosen on the basis of the error in the inner loop samples. The randomisation test is used to obtain a non-parametric distribution of the figures of merit of the PLS-DA model under the null hypothesis to assess its statistical significance. Three figures of merit were considered in the present study: (i) the number of misclassifications (NMC); (ii) the area under the receiver operating characteristic (ROC) curve (AUROC); and (iii) the value of the discriminant Q^2 (DQ^2). NMC corresponds to the number of classification errors that occurred between the age groups (young vs. old). AUROC is a measure of a test's discriminatory power. Its values range between 1 (perfect classification) and 0 (no discrimination). DQ^2 is a modification of the standard Q^2 and was introduced to cope with the peculiarities of classification problems addressed by regression methods.[20] DQ^2 is especially suitable for discriminating between groups in which the biological responses can be subtle and highly variable among the individuals. As its regression analogue, DQ^2 assumes its highest values in the case of a perfect discrimination between classes. Different from standard Q^2, DQ^2 is not bound to the 0–1 range of values (i.e. it can also be negative).

Results

Descriptive characteristics of the study population

A total of 52 participants were recruited for the study, 17 young adults (YAs) and 35 older adults (OAs). Demographic, anthropometric, and functional characteristics, and thigh composition of the participants by age group are listed in Table 1. Relative to the YA group, OAs showed higher BMI and lower knee extensor strength. The thigh composition of OA participants was characterised by smaller muscle and SAT volumes, and greater IMAT volume compared with YAs. No differences between groups were observed with regard to gender or ethnicity distribution. The median concentrations of circulating inflammatory markers in the two age groups are reported in Table 2.

Integration of inflammatory, functional, and imaging parameters via multi-block partial least squares-discriminant analysis

To proceed with the multi-block PLS-DA analysis, data obtained from multiplexed assay, 3D-MRI, and functional tests were arranged in two matrices of dimension 52×14 and 52×4, respectively. Each matrix was first auto-scaled and then block-scaled prior to be concatenated in the final 52×18 matrix X_{mb} to be used in the multi-block PLS-DA modelling. To explore the relationship among systemic inflammation, thigh composition, and physical performance, X_{mb} was employed as the predictor matrix to build a classification model for the discrimination between YA and OA participants. The optimal complexity of the model as well as the validation of its predictive ability was assessed by means of a double cross-validation procedure, coupled with randomisation tests. Twenty runs of double cross-validation with six cancellation groups in the outer loop and five in the inner loop were carried out and the results compared with those of randomisation tests with 1000 permutations performed under the same conditions (*Figure* 1). The double cross-validation procedure provided information on both the model complexity and its reliability. In all iterations, the optimal model complexity was two LVs, which were then considered to build the final model.

As shown in *Figure* 1, the differences in cytokine, thigh composition, and physical performance profiles between age groups were statistically significant. Indeed, an average NMC of 4 was found in calibration, with 92.3% correct classification (94.1% in YAs and 91.4% in OAs). In the internal validation set, correct classification was achieved in 84.6% of study participants (95.3% in YAs and 78.5% in OAs). Finally, in the external validation set, the proportion of correct classification was 82.6% (94% in YAs and 76.9% in OAs). In *Figure* 1a, NMC in external validation is compared with its distribution under the null hypothesis. The NMC value obtained on the real dataset fell outside of the corresponding null hypothesis distribution, indicating that the profiles of YAs were significantly different from those of OAs ($P < 0.05$). These results are confirmed by the inspection of AUROC (*Figure* 1b) and DQ^2 plots (*Figure* 1c).

To explore the relationship between inflammatory mediators and functional and imaging parameters, scores and loadings plots for the two significant LVs in the optimal model

Table 1 Descriptive characteristics of the study sample according to the age group

	Young adults ($n = 17$)	Older adults ($n = 35$)	P
Age, years (mean ± SD)	23.4 ± 3.9	78.1 ± 5.9	<0.0001
Gender (female), *n* (%)	8 (47.1)	16 (45.7)	0.8376
Ethnicity (Caucasian), *n* (%)	15 (88.2)	35 (100)	0.1933
BMI, kg/m^2 (mean ± SD)	24.1 ± 4.9	27.4 ± 4.1	0.0153
Number of disease conditions* (mean ± SD)	0.2 ± 0.4	1.3 ± 1.2	<0.0001
Number of medications (mean ± SD)	2.1 ± 2.7	3.3 ± 3.0	0.07
Peak torque extension, N m (mean ± SD)	173.2 ± 38.4	110.2 ± 42.0	<0.0001
SPPB (mean ± SD)	—	9.2 ± 3.00	—
Thigh muscle volume, cm^3 (mean ± SD)	563.3 ± 121.8	407.6 ± 127.6	0.0001
Thigh IMAT volume, cm^3 (mean ± SD)	88.4 ± 20.9	115.3 ± 28.5	0.0012
Thigh SAT volume, cm^3 (mean ± SD)	447.4 ± 262.6	325.1 ± 155.4	0.0399

*Includes hypertension, coronary artery disease, prior stroke, peripheral vascular disease, diabetes, chronic obstructive pulmonary disease, and osteoarthritis.
BMI: body mass index; IMAT: intermuscular adipose tissue; SAT: subcutaneous adipose tissue; SD: standard deviation; SPPB: Short Physical Performance Battery.

were considered. The scores plot, which describes the coordinates of the participants onto the space spanned by the LVs, is depicted in *Figure* 2a. The plot shows that the separation between YA and OA participants occurs along a direction that passes approximately through the first and third quadrant of the graph. By comparing this finding with the loadings plot (*Figure* 2b), which describes the importance of the experimental variables in determining the projection (i.e. in the construction of the LVs), it is possible to interpret this difference in terms of the observed predictors. In particular, a systematic relation between greater SAT and lower IMAT volumes is evident, with higher values of IMAT being correlated with increased concentrations of P-selectin, myeloperoxidase (MPO), soluble intercellular adhesion molecule 1 (sICAM-1), and vascular cell adhesion molecule 1 (sVCAM-1). The scores plot further indicates that higher concentrations of those four cytokines and greater IMAT volumes (and correspondingly smaller SAT volume) differentiate OA from YA participants. Moreover, lower muscle volume and knee extensor strength are correlated with higher concentrations of a group of inflammatory cytokines [interleukin (IL) 1 beta, IL6, IL10, IL12, IL13, tumour necrosis factor alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF)], which also appear to be highly related to one another.

Finally, even though OAs were analysed as a single group, the PLS-DA model was able to separate those with SPPB >8 ('high-functioning'; $n = 21$) from participants scoring ≤8 ('low-functioning'; $n = 14$) (*Figure* 3). 'Low-functioning' OAs were characterised by lower muscle volume and knee extensor strength and correspondingly higher concentrations of IL1beta, IL6, IL10, IL12, IL13, TNF-α, and GM-CSF.

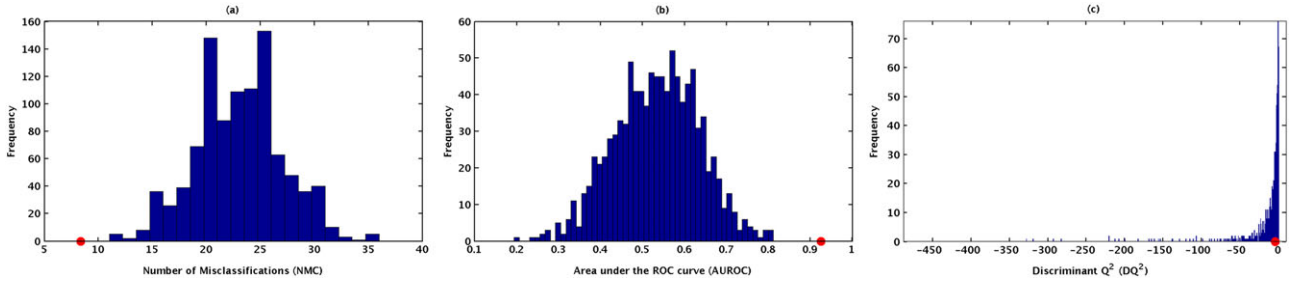
Table 2 Median concentrations of circulating inflammatory biomarkers according to the age group

	Young adults ($n = 17$) Median (IQR)	Older adults ($n = 35$) Median (IQR)
GM-CSF, pg·mL^{-1} *	1.02 (0.11–3.71)	0.32 (0.09–2.49)
IFN-γ, pg·mL^{-1} *	1.83 (0.35–12.43)	0.46 (0.09–8.19)
IL1β, pg·mL^{-1} *	0.28 (0.08–0.62)	0.13 (0.06–0.56)
IL5, pg·mL^{-1} *	0.10 (0.05–0.35)	0.20 (0.09–1.00)
IL6, pg·mL^{-1} *	0.83 (0.35–1.52)	2.01 (0.81–5.23)
IL8, pg·mL^{-1} *	3.06 (2.58–3.65)	3.59 (2.83–5.60)
IL10, pg·mL^{-1} *	15.59 (9.70–27.30)	18.86 (15.28–33.60)
IL12(p70), pg·mL^{-1} *	2.27 (0.05–6.40)	0.68 (0.05–3.60)
IL13, pg·mL^{-1} *	0.66 (0.15–1.99)	0.89 (0.10–4.48)
TNF-α, pg·mL^{-1} *	6.06 (4.34–8.53)	7.57 (4.35–11.29)
MPO, ng·mL^{-1} ¥	21.39 (16.81–34.56)	22.34 (16.59–34.78)
P-selectin, ng·mL^{-1} ¥	32.63 (19.95–46.24)	43.05 (30.24–51.95)
sICAM-1, ng·mL^{-1} ¥	56.72 (39.24–77.99)	67.67 (49.56–87.92)
sVCAM-1, ng·mL^{-1} ¥	686.0 (618.5–864.0)	896.5 (781.0–976.0)

IQR: interquartile range.
*Serum analyte.
¥Plasma analyte.
GM-CSF: granulocyte macrophage colony-stimulating factor; IFN-γ: interferon gamma; IL: interleukin; MPO: myeloperoxidase; sICAM-1; soluble intercellular adhesion molecule 1; sVCAM-1: soluble vascular cell adhesion molecule 1; TNF-α: tumour necrosis factor alpha.

DOI: 10.1002/jcsm.12134

Figure 1 Distribution of (a) number of misclassifications (NMC), (b) area under the receiver operating characteristic (ROC) curve (AUROC), and (c) discriminant Q^2 (DQ^2) values under their respective null hypothesis as estimated by permutation tests with 1000 randomisation (blue histograms) and the corresponding values obtained by the PLS-DA model on unpermuted data (red circles).



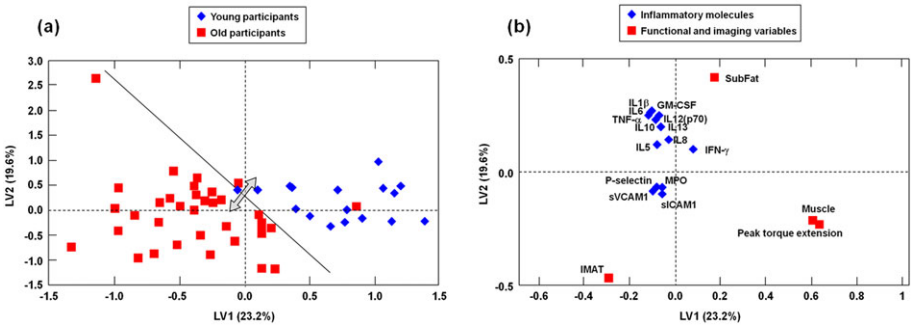
Discussion

In recent years, chronic systemic inflammation, changes in body composition, and declining physical performance have been among the most actively investigated topics in biogerontology. Indeed, each of them is intrinsic to the ageing process and is linked to a wide range of adverse health outcomes.[21] Although inflammation, body composition deterioration, and functional impairment are intimately interconnected, the literature is devoid of investigations that have examined the relationship linking those three hallmarks of ageing. Indeed, depending on the study design, one of these phenomena was typically considered as a confounding factor in the analysis of the other two components.

In this exploratory study, an innovative analytical approach based on multi-block PLS-DA was adopted to capture potentially relevant information that could otherwise remain concealed using traditional statistical methods. The most noticeable findings were the negative relation, determined both in YA and OA participants, between SAT and IMAT volumes, and the positive correlation of IMAT with circulating levels of P-selectin, MPO, sVCAM-1, and sICAM-1 (*Figure* 2b). This latter finding, while corroborating previous observations of increasing fat deposition in aged muscle,[22] adds interesting cues on the systemic inflammatory milieu in which the age-related changes in body composition occur. IMAT depots, besides disrupting myofibre orientation and, hence, force generation, can release several inflammatory mediators.[22] These, in turn, may contribute to local and systemic inflammation[23] as well as to muscle dysfunction.[24] On the other hand, chronic systemic inflammation promotes ectopic lipid deposition in non-adipose tissues, including muscle.[18] Our findings together with those of previous investigations suggest that a self-reinforcing mechanism could take place with advancing age, through which inflammation and alterations of body composition contribute to physical function impairment.

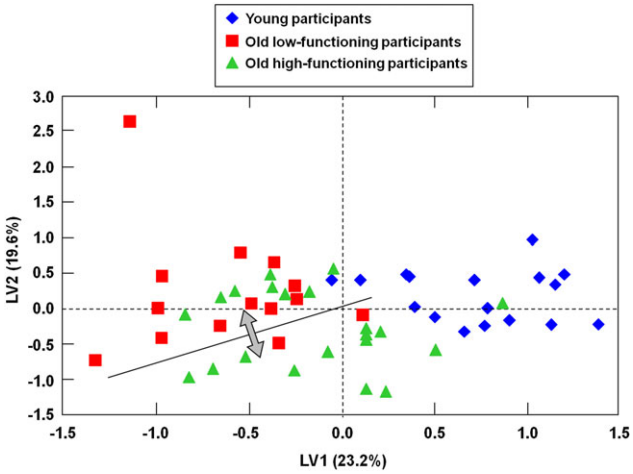
Although the study was not designed to address mechanistic hypotheses, it may be speculated that the relationship between IMAT and a specific cluster of inflammatory cytokines could reflect the involvement of endothelial activation in age-related muscle remodelling. Indeed, P-selectin, sICAM-1, and sVCAM-1 are all released by activated endothelial cells.[24] MPO, on the other hand, contributes to endothelial

Figure 2 Scores (a) and loadings (b) plots showing the relationships among inflammatory, functional, and thigh composition parameters in the space spanned by the two latent variables (LV1 and LV2), as determined by multi-block PLS-DA. In the scores plot, the diagonal line and the double-headed arrow (added to facilitate interpretation) indicate the boundary between age groups and the direction along which the separation occurs, respectively. The loadings plot shows a negative correlation between subcutaneous fat (SubFat) and intermuscular adipose tissue (IMAT) volumes, and a positive correlation between IMAT volume and circulating levels of P-selectin, MPO, sVCAM-1, and sICAM-1.



DOI: 10.1002/jcsm.12134

Figure 3 Scores plot showing the separation of participants according to inflammatory, functional, and thigh composition parameters in the space spanned by the two latent variables (LV1 and LV2), as determined by multi-block PLS-DA. The diagonal line (added to facilitate interpretation) corresponds to the boundary between older adults with SPPB >8 ('high-functioning') and those with SPPB ≤8 ('low-functioning'). The double-headed arrow indicates the direction along which the separation occurs.



dysfunction through several mechanisms, involving oxidative and nitrosative damage, nitric oxide catabolism, NADPH consumption, and complement-dependent cytotoxicity.[25]

Considerable variability in biomarker concentrations was observed within and between age groups; notwithstanding, consistent patterns were detected. In OA participants, circulating levels of a set of pro- and anti-inflammatory mediators (i.e. IL1beta, IL6, IL10, IL12, IL13, TNF-α, and GM-CSF) were found to be higher than in YAs. This cluster of cytokines, which were highly correlated with one another, was inversely correlated with muscle volume and knee extensor strength (*Figure* 2b). This finding is consistent with a recent report by Petersen *et al.*[7] who described similar age-dependent patterns of relationships between inflammatory mediators and physical performance measures. Moreover, a significant association between higher circulating levels of both pro- and anti-inflammatory cytokines (including IL6, IL10, and TNF-α) and SPPB scores has recently been reported in older Chinese persons with various degrees of disability.[12]

While a chronic increase in circulating levels of pro-inflammatory mediators is a well-recognised age-related phenomenon,[4] the co-occurrence of higher levels of IL10 and IL13 and their inverse relation with muscle volume and strength represent novel findings. Besides their well-known role in attenuating monocyte/macrophage function,[26] IL10 and IL13 may also influence muscle trophism, regeneration, and metabolism.[27–29] Thus, our findings suggest the existence of a generalised perturbation in the cytokine network, which could impact muscle health. Further studies are needed to fully dissect such processes, with the prospect of targeting inflammatory pathways to maintain or restore muscle mass and function in old age.

Remarkably, by inspecting the scores and prediction plots of the PLS-DA model (*Figure* 3), a separation can be appreciated between 'low-'and 'high-functioning' OA participants. Specifically, 'high-functioning' individuals show a profile of inflammatory, thigh composition, and muscle strength indices intermediate between YAs and 'low-functioning' participants. It is worth noting that that the profile of approximately one third of 'high-functioning' OAs is overlapping with that of YAs. The relationship pattern is not attributable to differences in age or co-morbidity burden, which were similar in the two OA groups (data not shown). This finding supports the idea that, in older persons, functional status is intimately linked with inflammatory and muscle-specific parameters. The longitudinal analysis of these profiles through multi-block PLS-DA models may allow the health status of an older person to be tracked over time, in order to timely identify possible deviations from the successful ageing trajectory.[17]

Although reporting novel findings, our study presents some limitations that need to be acknowledged. First, analyses were conducted in a relatively small group of participants and involved a vast array of experimental variables. However, the PLS-DA approach is particularly suited for such an experimental design because it allows analysing matrices in which (i) the number of variables is larger than the number of individuals; (ii) the variables are correlated with each other; and (iii) the differences in biological parameters could be subtle and highly variable among the individuals.[30] Moreover, the double cross-validation procedure confirmed the reliability of the PLS-DA model,[20,31] which adds value to our findings. Yet, because of the limited sample size, analyses could not be adjusted for possible confounders. Eligibility criteria were purposely restrictive to minimise the chances that the relationship among inflammation, thigh composition, and functional status would be confounded by disease conditions, medications, or lifestyle habits. At the same time, this choice does not allow for the results to be extended to severely ill, multimorbid older persons. In addition, the study sample was mostly comprised of Caucasian individuals, which impedes generalising the findings to other ethnic groups. Although enrolees were not engaged in structured physical exercise, the amount of physical activity was not objectively quantified. Therefore, the relationship between the variables analysed and the overall level of physical activity could not be established. The lack of information on food intake prevented the possible influence of diet on the variables of interest to be explored. The cross-sectional design of the study does not allow inference to be drawn on the time course of changes of the variables considered. Finally, although a large number of inflammatory markers were assayed, not all known mediators could be considered. Notably, C-reactive protein, one of the most popular inflammatory markers,

was not measurable with the multiplex assay kits employed in the present study.

In conclusion, multi-block PLS-DA revealed distinct patterns of relationships among circulating cytokines and functional and imaging parameters in persons of different ages and varying levels of physical performance. As opposed to conventional monodimensional approaches, the simultaneous evaluation of multiple factors belonging to different domains may be better suited to cope with the heterogeneity of complex age-related phenomena.[17] Indeed, the longitudinal implementation of such an innovative strategy could allow for the tracking of health status over time, the early detection of deviations in health trajectories, and the monitoring of response to treatments. Longitudinal analyses could also help simplify the proposed approach by identifying which variable or set of parameters shows the best ability to predict relevant health outcomes in late life, for future application in the clinical arena. Eventually, this knowledge would assist in developing more comprehensive and patient-tailored interventions.[32,33]

Acknowledgments

This study was supported by Innovative Medicines Initiative—Joint Undertaking (IMI-JU 115621; E.M., F.L., M.C., R.B., R.C.), the 'Centro Studi Achille e Linda Lorenzon' (E.M. and R.C.), grants from the Italian Ministry of Education, Universities, and Research (MIUR—linea D1 2012 and D3.2 2013; E.M., F.L.), and the Claude D. Pepper Older Americans Independence Center at the University of Florida's Institute on Aging (NIA 1P30AG028740; C.L., M. P., T.M.M., T.W.B.). The authors wish to thank Brian B. Bouverat and Marvin L.S. Dirain (University of Florida, Gainesville) for their technical assistance with the multiplex assay.

The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle.[34]

Conflict of interest

E.M., F.L., M.C., and R.C. are partners of the SPRINTT consortium, which is partly funded by the European Federation of Pharmaceutical Industries and Associations (EFPIA).

E.M. served as a consultant for Huron Consulting Group, Genactis, and Novartis. M.C. served as a consultant for and/or received honoraria for scientific presentations from Nestlé, Novartis, and Pfizer; he also received a research grant from Pfizer.

References

1. Buford TW, Lott DJ, Marzetti E, Wohlgemuth SE, Vandenborne K, Pahor M, et al. Age-related differences in lower extremity tissue compartments and associations with physical function in older adults. *Exp Gerontol* 2012;**47**:38–44.
2. Hughes VA, Frontera WR, Roubenoff R, Evans WJ, Singh MA. Longitudinal changes in body composition in older men and women: role of body weight change and physical activity. *Am J Clin Nutr* 2002;**76**:473–481.
3. Marzetti E, Landi F, Marini F, Cesari M, Buford TW, Manini TM, et al. Patterns of circulating inflammatory biomarkers in older persons with varying levels of physical performance: a Partial Least Squares-Discriminant Analysis (PLS-DA) approach. *Front Med* 2014;**1**:27.
4. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014;**69**:S4–S9.
5. Jo E, Lee SR, Park BS, Kim JS. Potential mechanisms underlying the role of chronic inflammation in age-related muscle wasting. *Aging Clin Exp Res* 2012;**24**:412–422.
6. De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol* 2006;**80**:219–227.
7. Peterson MJ, Thompson DK, Pieper CF, Morey MC, Kraus VB, Kraus WE, et al. A novel analytic technique to measure associations between circulating biomarkers and physical performance across the adult life span. *J Gerontol A Biol Sci Med Sci* 2016;**71**:196–202.
8. Roubenoff R. The "cytokine for gerontologists" has some company. *J Gerontol A Biol Sci Med Sci* 2014;**69**:163–164.
9. Hsu FC, Kritchevsky SB, Liu Y, Kanaya A, Newman AB, Perry SE, et al. Association between inflammatory components and physical function in the health, aging, and body composition study: a principal component analysis approach. *J Gerontol A Biol Sci Med Sci* 2009;**64**:581–589.
10. Bandeen-Roche K, Walston JD, Huang Y, Semba RD, Ferrucci L. Measuring systemic inflammatory regulation in older adults: evidence and utility. *Rejuvenation Res* 2009;**12**:403–410.
11. Varadhan R, Yao W, Matteini A, Beamer BA, Xue QL, Yang H, et al. Simple biologically informed inflammatory index of two serum cytokines predicts 10 year all-cause mortality in older adults. *J Gerontol A Biol Sci Med Sci* 2014;**69**:165–173.
12. Dong B, Sun B. Inflammatory markers and disability in Chinese older adults. *J Gerontol Geriatr Res* 2016;**5**:275.
13. Hughes VA, Frontera WR, Wood M, Evans WJ, Dallal GE, Roubenoff R, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. *J Gerontol A Biol Sci Med Sci* 2001;**56**:B209–B217.
14. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci* 2006;**61**:1059–1064.
15. Tuttle LJ, Sinacore DR, Mueller MJ. Intermuscular adipose tissue is muscle specific and associated with poor functional performance. *J Aging Res* 2012;**2012**:172957.
16. Addison O, Drummond MJ, LaStayo PC, Dibble LE, Wende AR, McClain DA, et al. Intramuscular fat and inflammation differ in older adults: the impact of frailty and inactivity. *J Nutr Health Aging* 2014;**18**:532–538.
17. Calvani R, Marini F, Cesari M, Tosato M, Anker SD, von Haehling S, et al. Biomarkers for physical frailty and sarcopenia: state of the science and future developments. *J Cachexia Sarcopenia Muscle* 2015;**6**:278–286.
18. Marzetti E, Lees HA, Manini TM, Buford TW, Aranda JM Jr, Calvani R, et al. Skeletal

muscle apoptotic signaling predicts thigh muscle volume and gait speed in community-dwelling older persons: an exploratory study. *PLoS One* 2012;**7**:e32829.
19. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;**49**:M85–M94.
20. Szymańska E, Saccenti E, Smilde AK, Westerhuis JA. Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* 2012;**8**:3–16.
21. Seals DR, Justice JN, LaRocca TJ. Physiological geroscience: targeting function to increase healthspan and achieve optimal longevity. *J Physiol* 2016;**594**:2001–2024.
22. Addison O, Marcus RL, Lastayo PC, Ryan AS. Intermuscular fat: a review of the consequences and causes. *Int J Endocrinol* 2014;**2014**:309570.
23. Zoico E, Rossi A, Di Francesco V, Sepe A, Olioso D, Pizzini F, et al. Adipose tissue infiltration in skeletal muscle of healthy elderly men: relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. *J Gerontol A Biol Sci Med Sci* 2010;**65**:295–299.
24. Liao JK. Linking endothelial dysfunction with endothelial cell activation. *J Clin Invest* 2013;**123**:540–541.
25. Lau D, Baldus S. Myeloperoxidase and its contributory role in inflammatory vascular disease. *Pharmacol Ther* 2006;**111**:16–26.
26. Opal SM, DePalo VA. Anti-inflammatory cytokines. *Chest* 2000;**117**:1162–1172.
27. Prokopchuk O, Liu Y, Wang L, Wirth K, Schmidtbleicher D, Steinacker JM. Skeletal muscle IL-4, IL-4Ralpha, IL-13 and IL-13Ralpha1 expression and response to strength training. *Exerc Immunol Rev* 2007;**13**:67–75.
28. Deng B, Wehling-Henricks M, Villalta SA, Wang Y, Tidball JG. IL-10 triggers changes in macrophage phenotype that promote muscle growth and regeneration. *J Immunol* 2012;**189**:3669–3680.
29. Jiang LQ, Franck N, Egan B, Sjögren RJ, Katayama M, Duque-Guimaraes D, et al. Autocrine role of interleukin-13 on skeletal muscle glucose metabolism in type 2 diabetic patients involves microRNA let-7. *Am J Physiol Endocrinol Metab* 2013;**305**:E1359–E1366.
30. Westerhuis JA, van Velzen EJJ, Hoefsloot HCJ, Smilde AK. Discriminant Q2 (DQ2) for improved discrimination in PLSDA models. *Metabolomics* 2008;**4**:293–296.
31. Westerhuis JA, Hoefsloot HCJ, Smit S, Vis DJ, Smilde AK, van Velzen EJJ, et al. Assessment of PLS-DA cross-validation. *Metabolomics* 2008;**4**:81–89.
32. Marzetti E, Sanna T, Calvani R, Bernabei R, Landi F, Cesari M. Brand new medicine for an older society. *J Am Med Dir Assoc* 2016;**17**:558–559.
33. Cesari M, Marzetti E, Thiem U, Pérez-Zepeda MU, Abellan Van Kan G, Landi F, et al. The geriatric management of frailty as paradigm of "The end of the disease era". *Eur J Intern Med* 2016;**31**:11–14.
34. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle. *J Cachexia Sarcopenia Muscle* 2015;**4**:315–316.