UNIVERSITY OF THESSALY

Effect of Inflammation on Muscle Protein Synthesis and Breakdown in the Aged Human Skeletal Muscle

by

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List of Abbreviations

ALM: Appendicular lean mass
CRP: C-reactive protein
***ECSI**: Elevated chronic systemic inflammation
***ESI**: Elevated systemic inflammation
FFM: Fat free mass
hs-CRP: High sensitive C-reactive protein
***HSI**: High Systemic Inflammation
IL-6: Interleukin 6
LBM: Lean body mass
LCSI: Low chronic systemic inflammation
LSI: Low systemic inflammation
MPS: Muscle protein synthesis
MUFA: Monounsaturated fatty acid
MVPA: Moderate-to-vigorous physical activity
NF-κB: Nuclear factor kappa beta
PA: Physical activity
PUFA: Polyunsaturated fatty acid
RDA: Recommended dietary allowance
ROS: Reactive oxygen species
SMI: Skeletal muscle index
SPPB: Short physical performance battery
TNF-α: Tumor Necrosis factor-a
UPS: Ubiquitin proteasome system
VM: Vector magnitude
WHO: World health organization
ΔΕΟΑ: Δραστικά είδη οξυγόνου και αζώτου
ΜΕ: Μέγιστη επανάληψη
ΜτΕ: Μέγεθος της επίδρασης
ΧΣΦ: Χρόνια συστημική φλεγμονή

**ECSI, ESI and HSI have all the same meaning (i.e. denote increased levels of chronic low-grade systemic inflammation*).

Candidate's responsibilities throughout the study

During the PhD programme the candidate had the following duties:

- Completion of the courses required for the accomplishment of 10 to 20 ECTS
- Submission of the required documents to receive ethics approval for the study
- Submission of applications for research grands/funds/studentships
- Implementation of the study
- Implementation of the biochemical and molecular analysis on biological samples
- Perform the data analysis as well as the relevant power and statistical analysis
- Preparation of scientific manuscripts and submission for publication
- Writing the doctoral thesis and public defense
- Participation in relevant training schools/conferences
- Presentation of scientific results at internal group meetings, workshops and conferences

Skills acquired during the PhD programme

Through the present study and being also actively involved in other research projects and clinical trials during the PhD programme, the candidate has gained considerable experience and advanced knowledge in the field of muscle physiology, metabolism and nutrition, reflected by the following acquired skills:

- Design and implement clinical trials
- Design and implement supplementation studies
- Evaluation of the physical performance and physical activity level in athletes as well as in young and older individuals
- Assessment of human body composition using advanced techniques and instrumentation such as DXA
- Coordination of muscle biopsy and blood sampling procedures in clinical trials
- Administration of D_2O in humans for the assessment of fractional synthesis rate (FSR)
- Application of advanced molecular methodologies, including immunohistochemistry, molecular signaling and enzymatic reactions, to assess changes in the protein level and phosphorylation state.
- Academic writing including scientific papers, research proposals and grands.

Abstract

Introduction: The development of chronic, low-grade systemic inflammation in the elderly (inflammaging) has been shown to increase the risk for chronic diseases and geriatric syndromes while it has been also associated with accelerated skeletal muscle wasting, strength loss and functional impairments.

Aim: The aim of the present thesis was i) to examine whether anabolic signaling, activation of amino acid transporters, ribosome biogenesis and proteasome activity in the aged skeletal muscle are affected by low-grade systemic inflammation in the fasted state and following exercise and protein feeding, and ii) to investigate differences in habitual physical activity, dietary intake, physical performance and blood indices of oxidative stress and antioxidant status among elderly men with low and elevated low-grade systemic inflammation.

Methods: Forty-four male adults aged 63-75 years who complied with the inclusion criteria were included in the study. Initially they were screened for systemic levels of hs-CRP and then had their anthropometric profile, body composition, sarcopenia status and functional performance assessed. Habitual physical activity and daily dietary intake were assessed over a 7-day period using accelerometry and diet recalls, respectively. Of these 44 individuals, 12 with high systemic inflammation (HSI: hs-CRP: > 1.0 mg/L) and 12 with low (LSI: hs-CRP: ≤ 1.0 mg/L) were included in the clinical trial. On the experimental day, participants performed 8 sets of 10 repetitions at 70% of 1RM on a knee-extension machine and immediately after they ingested a whey protein bolus. Muscle biopsy samples were collected before exercise (fasted state) and at 3h following protein ingestion. Blood samples were collected before exercise and every 30 minutes during the 3-hour postprandial period.

Results: In the fasted state, HSI exhibited higher chymotrypsin-like activity, protein carbonyl concentration and phosphorylated IKKα/β, by 40%, 47% and 37%, respectively, as compared to LSI ($P < 0.05$). No differences were detected among groups in terms of trypsin-like activity, protein expression of the proteasome β-subunits, nuclei levels of Nrf2 and 3-nitrotyrosine levels. In addition, a significant correlation ($P < 0.05$) was observed between hs-CRP levels and chymotrypsin like activity. At 3h following protein ingestion, chymotrypsin-like activity increased in HSI and LSI by 44% ($P < 0.05$) and 86% ($P < 0.05$), respectively (without group differences), while trypsin-like activity increased only LSI by 38% ($P < 0.05$). Protein carbonyls decreased in HSI by 31% ($P < 0.05$) while Nrf2 increased only LSI by 28% ($P < 0.05$). Protein expression of β and βi proteasome subunits as well as 3-nitrotyrosine levels and phosphorylation of IKKα/β remained unaltered in the postprandial period in both groups. Moreover, rpS6 phosphorylation increased only in LSI at 3h

after protein ingestion (by 1.5 fold, $P < 0.05$) and the rise was significantly higher compared to that in HSI ($P < 0.05$). Protein expression of amino acid transporters and ribosome biogenesis indices demonstrated no changes in either group. In addition, at baseline LSI was more physically active than HSI performing more steps by 30% ($P < 0.05$) and spending more time in MVPA by 42% ($P < 0.05$). Also, LSI demonstrated higher antioxidant vitamin intake ($P < 0.05$) that was accompanied by increased plasma antioxidant capacity (by 60%, $P < 0.05$), as compared to HSI.

Conclusions: The results of the present thesis indicate that inflammaging is characterized by a pro-oxidative environment and increased proteasome activity in the fasted state, and by reduced translation efficiency following protein feeding. Moreover, higher physical activity level and increased antioxidant consumption on a daily basis are discriminant factors of inflammaging and healthy aging and therefore, may considered as crucial, lifestyle-based factors that may prevent the development of low-grade systemic inflammation.

Περίληψη

Εισαγωγή: Η ανάπτυξη χρόνιας συστημικής φλεγμονής σε άτομα τρίτης ηλικίας (*inflammaging*), έχει δειχθεί ότι αυξάνει το κίνδυνο εμφάνισης χρόνιων ασθενειών και γηριατρικών συνδρόμων, ενώ έχει συσχετιστεί και με ταχύτερη απώλεια μυϊκής μάζας, δύναμης και λειτουργικής ικανότητας.

Σκοπός: Σκοπός της παρούσας διδακτορικής διατριβής ήταν α) να εξετάσει εάν η διέγερση του αναβολικού σηματοδοτικού μονοπατιού, η ενεργοποίηση των μεταφορέων αμινοξέων, η ριβοσωμική βιογένεση και η ενεργότητα του πρωτεασώματος στο σκελετικό μυ ηλικιωμένων ατόμων επηρεάζονται από τη χρόνια συστημική φλεγμονή σε κατάσταση νηστείας καθώς και μετά από συνδυασμό άσκησης και κατανάλωσης πρωτεΐνης, και β) να διερευνήσει τις πιθανές διαφορές στα επίπεδα φυσικής δραστηριότητας, στη διατροφική πρόσληψη, στη φυσική απόδοση καθώς και σε δείκτες οξειδωτικού στρες και αντιοξειδωτικής ικανότητας, μεταξύ ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή.

Μεθοδολογία: 44 άνδρες εθελοντές ηλικίας 63-75 ετών οι οποίοι πληρούσαν τα κριτήρια συμμετοχής, συμπεριλήφθηκαν στη μελέτη. Αρχικά, υποβλήθηκαν σε αιμοληψία για τον προσδιορισμό των επιπέδων hs-CRP στην κυκλοφορία και στη συνέχεια υποβλήθηκαν σε αξιολόγηση του σωματικού ύψους και βάρους, της σύστασης σώματος, του επιπέδου σαρκοπενίας και της φυσικής απόδοσης. Επιπλέον αξιολογήθηκαν η φυσική δραστηριότητα και η διατροφική πρόσληψη για 7 συνεχόμενες ημέρες με χρήση επιταχυνσιομέτρων και διατροφικών ημερολογίων, αντίστοιχα. Από τους 44 συμμετέχοντες, 12 με υψηλή χρόνια συστημική φλεγμονή (υψηλή ΧΣΦ: hs-CRP: > 1.0 mg/L) και 12 με χαμηλή (χαμηλή ΧΣΦ: hs-CRP: ≤ 1.0 mg/L) έλαβαν μέρος στην κλινική δοκιμασία. Την ημέρα της κλινικής δοκιμασίας, οι συμμετέχοντες εκτέλεσαν 8 σετ των 10 επαναλήψεων στο 70% της 1ΜΕ στο μηχάνημα εκτάσεις γονάτων και αμέσως μετά κατανάλωναν συμπλήρωμα πρωτεΐνης ορού γάλακτος, σε μία δόση. Δείγματα μυός (μυϊκές βιοψίες) συλλέχθηκαν πριν την άσκηση (σε κατάσταση νηστείας) καθώς και στις 3 ώρες μετά τη λήψη του συμπληρώματος πρωτεΐνης. Αιμοληψίες πραγματοποιήθηκαν πριν την άσκηση και κάθε 30 λεπτά για 3 ώρες μετά την κατανάλωση του συμπληρώματος πρωτεΐνης.

Αποτελέσματα: Στην κατάσταση νηστείας , η ομάδα υψηλής ΧΣΦ παρουσίασε υψηλότερη ενεργότητα χυμοθρυψίνης, μεγαλύτερη συγκέντρωση πρωτεϊνικών καρβονυλίων και μεγαλύτερη ποσότητα φωσφορυλιωμένης κινάσης ΙΚΚα/β, κατά 40%, 47% και 37%, αντίστοιχα, σε σύγκριση με την ομάδα χαμηλής ΧΣΦ ($P < 0.05$). Δεν παρατηρήθηκαν διαφορές μεταξύ των δύο ομάδων όσον αφορά την ενεργότητα τρυψίνης, την έκφραση των β υπομονάδων του πρωτεασώματος και του ανοσοπρωτεασώματος, τα επίπεδα του Nrf2 στον πυρήνα καθώς και τα επίπεδα 3-νιτροτυροσίνης. Επίσης, σημαντική συσχέτιση εντοπίστηκε μεταξύ των επιπέδων hs-CRP και της ενεργότητας

χυμοθρυψίνης (P < 0.05) σε κατάσταση νηστείας. Στις 3 ώρες μετά την κατανάλωση του συμπληρώματος πρωτεΐνης, η ενεργότητα χυμοθρυψίνης αυξήθηκε σημαντικά και στις δυο ομάδες (υψηλή ΧΣΦ: +44%, χαμηλή ΧΣΦ: +86%, P < 0.05), χωρίς να διαφέρουν μεταξύ τους, ενώ η ενεργότητα της τρυψίνης αυξήθηκε μόνο στην ομάδα χαμηλής ΧΣΦ κατά 38% (P < 0.05). Η συγκέντρωση πρωτεϊνικών καρβονυλίων μειώθηκε κατά 31% στην ομάδα υψηλής ΧΣΦ (P < 0.05), ενώ τα επίπεδα του Nrf2 αυξήθηκαν σημαντικά μόνο την ομάδα χαμηλής ΧΣΦ (+28%, P < 0.05). Η έκφραση των *β* υπομονάδων του πρωτεασώματος και των αντίστοιχων *βi* του ανοσοπρωτεασώματος καθώς και τα επίπεδα 3-νιτροτυροσίνης και φωσφορυλιωμένης κινάσης ΙΚΚα/β παρέμειναν αμετάβλητα μετά την κατανάλωση της πρωτεΐνης. Επιπλέον, η φωσφορυλίωση της ριβοσωμικής πρωτεΐνης rpS6 αυξήθηκε σημαντικά στις 3 ώρες μετά τη λήψη του συμπληρώματος πρωτεΐνης μόνο στην ομάδα χαμηλής ΧΣΦ (+ 1.5 φορά, P < 0.05) και τα επίπεδά της διέφεραν σημαντικά από εκείνα της ομάδας υψηλής ΧΣΦ (P < 0.05). Η έκφραση των μεταφορέων αμινοξέων αλλά και η ενεργοποίηση δεικτών ριβοσωμικής βιογένεσης δεν παρουσίασαν σημαντικές μεταβολές σε καμία απ' τις δυο ομάδες. Οι δυο ομάδες ωστόσο, διέφεραν σημαντικά μεταξύ τους όσον αφορά τα επίπεδα φυσικής δραστηριότητας, με την ομάδα χαμηλής ΧΣΦ να εκτελεί περισσότερα βήματα (+ 30%, P < 0.05) και να δαπανά περισσότερο χρόνο σε μέτρια-προς-υψηλής έντασης δραστηριότητες (+ 42%, P < 0.05) σε σύγκριση με την ομάδα υψηλής ΧΣΦ. Επίσης, η ομάδα χαμηλής ΧΣΦ παρουσίασε υψηλότερη πρόσληψη αντιοξειδωτικών βιταμινών σε σχέση με την ομάδα υψηλής ΧΣΦ (P < 0.05)η οποία συνοδευόταν και από υψηλότερη αντιοξειδωτική ικανότητα στο πλάσμα (+ 60%, P < 0.05).

Συμπεράσματα: Τα αποτελέσματα της παρούσας διατριβής υποδεικνύουν ότι τα ηλικιωμένα άτομα με υψηλή ΧΣΦ χαρακτηρίζονται από αυξημένα επίπεδα οξειδωμένων πρωτεϊνών και αυξημένη ενεργοποίηση του πρωτεολυτικού μηχανισμού του πρωτεασώματος στο μυ, σε κατάσταση νηστείας, καθώς επίσης και από μειωμένη μεταφραστική δραστηριότητα στο ριβόσωμα, στη μεταγευματική περίοδο. Επιπλέον, μεγαλύτερα επίπεδα φυσικής δραστηριότητας σε ημερήσια βάση και μεγαλύτερη πρόσληψη αντιοξειδωτικών βιταμινών μέσω της διατροφής φαίνεται ότι διαφοροποιούν τα άτομα με χαμηλή και υψηλή ΧΣΦ, και επομένως θα πρέπει θεωρούνται καθοριστικοί παράγοντες για την υγεία στα άτομα τρίτης ηλικίας, οι οποίοι μπορούν να επιδράσουν ανασταλτικά στην εκδήλωση ΧΣΦ.

CHAPTER 1

1.1 General Introduction

1.1.1 Inflammaging

Acute inflammation, is a fundamental and necessary response of the human immune system to harmful conditions such as infectious diseases and damaging agents, that in early life is beneficial and protective, ensuring survival [1]. On the other hand, the development of chronic inflammation, particularly in later life, has been associated with increased susceptibility to infectious diseases and age-related pathologies such as Alzheimer's disease, atherosclerosis, diabetes (Type 2) and osteoporosis [2, 3] and thus can be detrimental. Indeed, aging is associated with low-grade chronic systemic inflammation, characterized by a 2- to 3-fold elevation in plasma levels of pro-inflammatory cytokines and acute phase proteins, particularly that of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) [4, 5]. Since the beginning of 2000, when it was first introduced by Franceschi and his colleagues, the term "inflammaging" has been commonly adopted to describe this age-related chronic pro-inflammatory status [4]. In addition to IL-6, TNF-α and CRP, the pro-inflammatory cytokine interleukin-1 (IL-1) and the soluble receptors IL-1Ra, TNF receptor and soluble IL-6 receptor are also considered valuable markers of inflammaging being widely used for its identification [5-7]. According to many epidemiological and cross-sectional studies, inflammaging is associated with chronic diseases and geriatric syndromes such as cardiovascular diseases, atherosclerosis, metabolic syndrome, type 2 diabetes mellitus, neurodegenerative diseases, cancer, and chronic obstructive pulmonary disease [8-11] as well as with increased rate of frailty, morbidity, and mortality [1, 12, 13]. In addition, as indicated by large cohort studies increased levels of IL-6, TNF-α and CRP are good predictors of both physical and cognitive decline and the risk of mortality in the elderly [14, 15]. Therefore, being implicated in the pathogenesis of most age-related pathologies, inflammaging, represents a key determinant of successful aging and longevity and consequently a target perspective to counteract age-related pathologies.

1.1.2 Pathogenesis of inflammaging

Up to date, the underpinning mechanism that drives inflammaging remains unclear. Increasing amount of evidence suggests that inflammaging is a complex and multifactorial process, with different tissues, organs, and biological systems (i.e. muscle tissue, adipose tissue, liver, immune system, gut microbiota) contributing to its pathophysiology [16]. Thus, its origin cannot be simply attributed to a specific number of factors and mechanisms. However, it is believed that cumulative lifetime exposure to antigens as well as to chemical, physical, and nutritional stressors that the immune system has to cope with, in combination with the recent dramatic increase in life expectancy,

result in prolonged overstimulation of the immune system with advancing age and the propagation of inflammaging [1, 4].

This chronic stimulation of the immune system has been recognized to be triggered and sustained by a continuous interplay between oxidative stress and inflammation [17-20]. In fact, upon activation in response to pathogens, immune cells, and especially those of the innate immunity (i.e. neutrophils, macrophages and monocytes), release high amounts of reactive oxygen species (ROS) as part of their defensive action [20]. Although low levels of ROS are required for proper cell function and tissue homeostasis, overproduction of ROS in combination with the age-related reduction in antioxidant capacity, results in oxidative stress and oxidative damage of cell components such as DNA, proteins and lipids [18, 20]. ROS production though, not only triggers oxidative damage but also leads to pro-inflammatory cytokine production and promotes an inflammatory response through activation of the Toll-like-receptors (TLRs) and Nalp-3 inflammosome intracellular pathways [20] and stimulation of the redox-sensitive transcription factor NF-κB [19]. The consequent inflammatory response, characterized by increased cytokine secretion, elicits additional ROS production from immune cells (e.g., monocytes and macrophages) resulting in a vicious cycle that promotes a chronic pro-inflammatory phenotype [18] and provokes inflammaging.

1.1.3 Inflammaging, skeletal muscle loss and functional impairment

The concept of inflammaging has gained particular interest in the last few years, since beyond its strong association with chronic diseases and geriatric syndromes it has been also shown to be related with reduced skeletal muscle mass as well as with strength and physical performance impairments [21, 22]. Data form longitudinal studies provide compelling evidence that higher levels of inflammatory markers such IL-6, TNF-α and CRP are linked with greater risk of losing muscle mass [23], strength and physical function [24], though a cause-effect relationship remains to be established. In fact, data derived from 3075 older individuals (aged 70–79 years) in the Health, Aging, and Body Composition Study [25] indicate that those with elevated concentrations of IL-6 and TNF-a exhibited smaller skeletal muscle area, less appendicular muscle mass, and reduced strength. In addition, a 5-year follow-up study examining >2000 elderly individuals (aged 70–79 years) revealed that those with higher concentrations of TNF-α and its soluble receptor at baseline exhibited greater reductions in muscle mass and strength [26]. Likewise, raised IL-6 and CRP concentrations in the older adults have been related with a 2- to 3-fold greater risk of losing >40% of their muscle strength [27].

In vivo and *in vitro* studies indicate that increased systemic inflammation may hamper the Akt/mTOR signaling pathway in skeletal muscle and attenuate the muscle's anabolic potential

leading to ″anabolic resistance‟ [28-31] (Figure 1.1). In addition, elevated concentrations of pro-inflammatory cytokines such as TNF-α and IL-6 induce activation of the NF-κB pathway and subsequent activation of the ubiquitin-proteasome system (UPS) resulting in protein degradation and skeletal muscle loss [32-36] (Figure 1.1). However, the verification of these mechanisms in the aged, human skeletal muscle under conditions of chronic low-grade systemic inflammation is still missing.

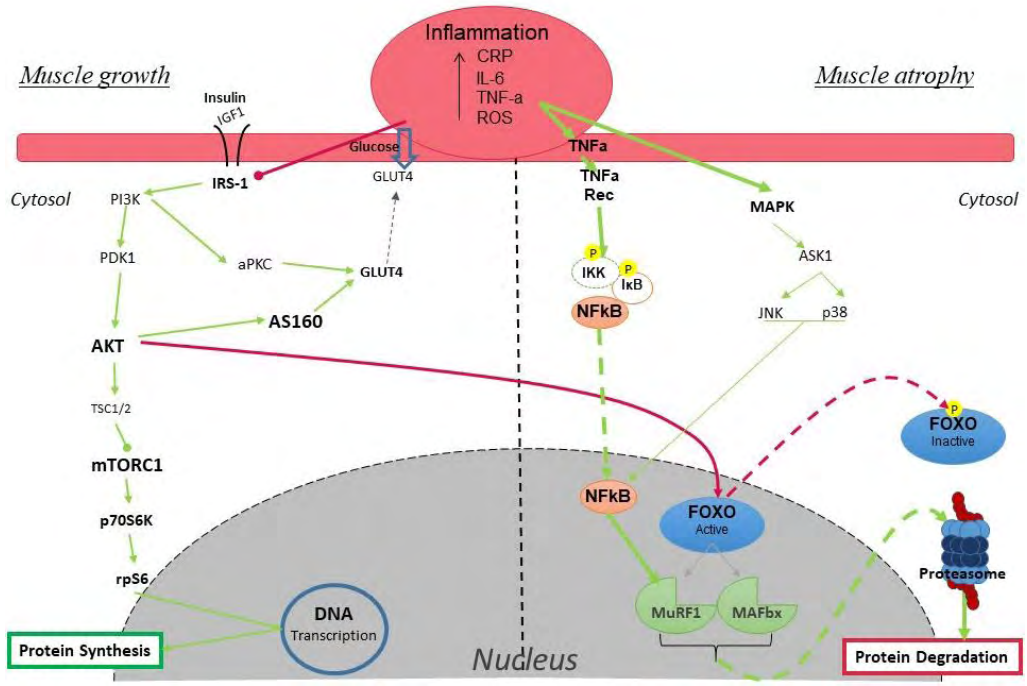

Figure 1.1. Schematic representation of the proposed molecular mechanisms through which chronic inflammation may lead to loss of skeletal muscle mass. Green arrows indicate activation; Red arrows indicate

Collectively, these data strongly suggest that inflammaging is associated with loss of skeletal muscle mass and function in the elderly and as such may predispose them to sarcopenia, significant functional limitations, frailty and hospitalization [22, 37]. The molecular mechanisms though, mediating the interaction between inflammaging, skeletal muscle loss and functional decline warrants further investigation. Moreover, the existing literature though, lacks a direct comparison of healthy elderly individuals characterized by low and elevated systemic inflammation in parameters related to strength and functional performance of the upper and lower body.

1.1.4 Physical activity and dietary intake as potential regulators of inflammaging

According to World Health Organization (WHO) physical activity (PA) is defined as any bodily movement produced by skeletal muscles (i.e. any form of recreational activities, walking, cycling, dancing, sports, activities around home etc.) and results in increased energy expenditure [38]. Regular participation in PA is strongly associated with health-related benefits and protects against

cardiovascular [39, 40] and metabolic diseases [39, 41], cognitive impairment [42], mental health disorders [40, 43], obesity [39, 44], frailty [39, 45, 46], sarcopenia [47] and osteoporosis [40, 48]. Moreover, evidence form observational, cross-sectional studies indicates that habitual PA is associated with lower levels of inflammatory mediators such as IL-6, CRP, and TNF-α and reduced incidence of chronic low-grade systemic inflammation in older adults [23, 49-60]. Actually, there is evidence of an inverse relationship between PA and disease-related (i.e. chronic obstructive pulmonary disease and obesity) systemic inflammation in middle-aged adults [55, 56]. In addition, studies that objectively assessed daily PA in healthy older individuals have revealed that the time spent in moderate-to-vigorous PA is negatively associated with markers of systemic inflammation [23, 59], suggesting that the beneficial effect of PA on inflammatory status may be intensity-dependent. However, observational studies are not designed to identify the underline mechanisms though which PA reduces inflammation, and thus, such an information is still missing. Furthermore, a direct comparison of objectively assessed daily PA, sedentary time and PA-related energy expenditure among healthy older individuals characterized by different inflammatory profile is still lacking. This comparison would shed light on the role of PA-related parameters (i.e. sedentary time, time in light, moderate and vigorous PA, total step count, energy expenditure) in the development of chronic low-grade systemic inflammation.

Nutrition, including dietary pattern, is also a lifestyle factor that play a critical role in immune function and have the potential to modulate chronic inflammation [61-63] by inducing either a pro-inflammatory or an anti-inflammatory effect [64]. For instance, high adherence to the Mediterranean diet which is mainly characterized by increased consumption of vegetables, fruit, legumes, whole-nuts and fish has been inversely correlated with markers of systemic inflammation in healthy adults [65], while diets rich in fatty acids and glucose result in increased postprandial glycaemia and lipaemia and have been proposed to trigger chronic inflammation [66]. The interaction though between diet and inflammation is primarily mediated by the macronutrient and micronutrient profile [64]. There is substantial amount of evidence suggesting that glucose and saturated fatty acids when consumed in high doses may stimulate a chronic pro-inflammatory response in insulin-sensitive tissues [64, 67] and propagate chronic systemic inflammation [16]. On the other hand, increased polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acid consumption has been reported to induce anti-inflammatory effects and protect against chronic inflammation and chronic inflammatory diseases [68]. Likewise, protein and amino acids derived from either plant- or dairy-based protein sources are considered anti-inflammatory nutrients as they have been suggested to reduce local and systemic inflammation by reducing the production of inflammatory cytokines and ROS, and also by enhancing the activity of antioxidant enzymes [64, 69]. Further, there is also

evidence indicating that dietary antioxidants and trace elements such as vitamins A, C, E and selenium may improve immune function and prevent the onset of chronic inflammatory conditions [63]. However, there is no evidence regarding the differences in daily dietary intake among elderly individuals with low and elevated systemic inflammation.

1.2 Thesis Objectives

The primary objective of the present thesis was to investigate whether the development of chronic, low-grade systemic inflammation in the elderly i) accelerates muscle protein breakdown through proteasome activation and ii) induces anabolic resistance. Secondly, this thesis aimed at examining whether elderly individuals with low and elevated systemic inflammation differ among themselves in terms of i) habitual physical activity level, ii) dietary intake profile, iii) strength and iv) functional performance as well as in systemic levels of v) oxidative stress and vi) immune system markers.

1.3 Research Design

A total number of 50 male, older adults aged 63-75 years were initially recruited. Forty-four individuals that complied with the study criteria, were then screened for systemic levels of hs-CRP and had their anthropometric profile, body composition (with DXA), sarcopenia status (according to criteria established by the European Working Group on Sarcopenia in Older People, EWGSOP), functional capacity (based on the Short Physical Performance Battery, SPPB) and the knee-extension one repetition maximum (1RM) assessed. Moreover, individuals' habitual physical activity level and daily dietary intake were also assessed using accelerometry (ActiGraph GT3X+, over a7-day period) and diet recalls (over a 7-day period), respectively. Of these 44 individuals, 12 with high systemic inflammation (hs-CRP: > 1.0 mg/L) and 12 with low (hs-CRP: ≤ 1.0 mg/L) were included in the clinical trial. In the experimental day (clinical trial), individuals arrived at the laboratory after an overnight fasted state and a baseline blood sample and a muscle biopsy from vastus lateralis muscle were collected. Immediately after, individuals performed 8 sets with 10 repetitions at 70% of 1RM and 2 min rest between each set, on a knee-extension machine. After exercise, they ingested 0.4 g whey protein isolate/kg body weight, as a single bolus, and then they remained in a sitting position over a 3-hour period. Blood samples were collected every 30min during the 3-hour postprandial period while a second muscle biopsy was obtained at 3h (Figure 1.2).

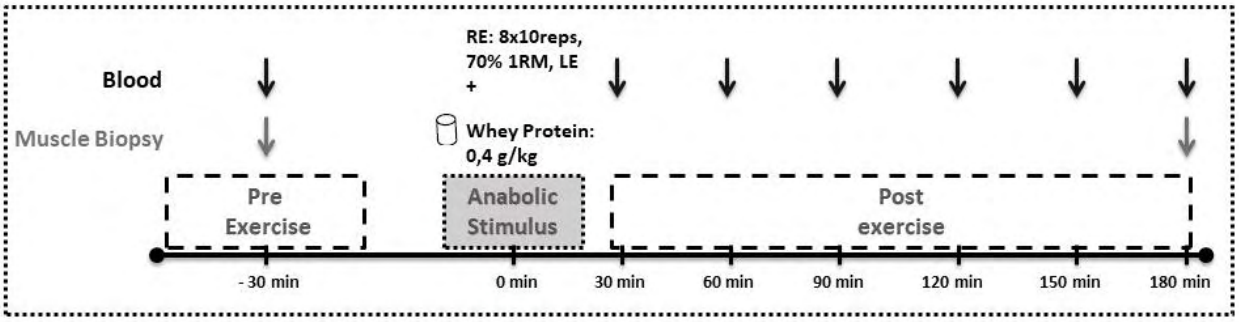

Figure 1.2. Schematic representation of the clinical trial. Twenty-four subjects participated in the clinical trial, including an acute resistance exercise protocol and whey protein ingestion. Blood samples and muscle biopsies were collected in the fasted state before exercise as well as in the postprandial period.

CHAPTER 2

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Inflammaging and Skeletal Muscle: Can Protein Intake Make a Difference?

Draganidis D, Karagounis LG, Athanailidis I, Chatzinikolaou A, Jamurtas AZ, Fatouros IG.

Abstract

Inflammaging is the chronic low-grade inflammatory state present in the elderly, characterized by increased systemic concentrations of proinflammatory cytokines. It has been shown that inflammaging increases the risk of pathologic conditions and age-related diseases, and that it also has been associated with increased skeletal muscle wasting, strength loss, and functional impairments. Experimental evidence suggests that the increased concentrations of proinflammatory cytokines and primary tumor necrosis factor a observed in chronic inflammation lead to protein degradation through proteasome activation and reduced skeletal muscle protein synthesis (MPS) via protein kinase B/Akt downregulation. Dairy and soy proteins contain all the essential amino acids, demonstrate sufficient absorption kinetics, and include other bioactive peptides that may offer nutritional benefits, in addition to those of stimulating MPS. Whey protein has antioxidative effects, primarily because of its ability to enhance the availability of reduced glutathione and the activity of the endogenous antioxidative enzyme system. Soy protein and isoflavone-enriched soy protein, meanwhile, may counteract chronic inflammation through regulation of the nuclear transcription factor kB signaling pathway and cytokine production. Although evidence suggests that whey protein, soy protein, and isoflavone enriched soy proteins may be promising nutritional interventions against the oxidative stress and chronic inflammation present in pathologic conditions and aging (inflammaging), there is a lack of information about the anabolic potential of dietary protein intake and protein supplementation in elderly people with increased systemic inflammation. The antioxidative and anti-inflammatory effects, as well as the anabolic potential of protein supplementation, should be further investigated in the future with well-designed clinical trials focusing on inflammaging and its associated skeletal muscle loss.

Keywords: inflammaging, oxidative stress, skeletal muscle loss, frailty, whey protein, soy protein

2.1. Introduction

The progressive loss of skeletal muscle mass and function (i.e., muscle strength and endurance and ability to perform daily physical activities) with advancing age is a well-documented process [70-72] that may lead to functional limitations, frailty, and hospitalization [37, 73]. Muscle mass is maintained by a constant equilibrium between the rates of muscle protein synthesis (MPS) and degradation, in which a net increase or a decrease occurs when the balance is disturbed. Nutritional-, hormonal-, neuropathic-, and inactivity-related factors all may contribute to deregulation of the molecular milieu of the aged muscle, resulting in muscle wasting and loss of independence [37]. In the elderly, the development of low-grade, chronic, systemic inflammation is often observed with age, characterized by a 2- to 3-fold elevation in circulating inflammatory mediators. This has been termed ‘‘inflammaging’’ (inflamm-aging) [5]. Proinflammatory cytokines are key components in this chronic inflammatory state; thus, the assessment of inflammaging primarily is based on the measurement of systemic concentrations of IL-6, IL-1, and TNF-a, their soluble receptors IL-1Ra, TNF receptor, and soluble IL-6 receptor, respectively, and that of the acute-phase C-reactive protein (CRP) [5-7]. Furthermore, inflammaging may be assessed at the skeletal muscle tissue level by the quantification of infiltrating macrophages, cytokine concentrations, and the examination of inflammatory pathways [6, 7]. Although the molecular mechanisms involved in the interaction between inflammaging and muscle loss is far from understood, research carried out in animal models revealed that augmented low-grade inflammation may favor muscle protein breakdown and inhibit protein synthesis [29, 74].

Although older adults exhibit anabolic resistance (i.e., a higher protein amount is required to maximally stimulate MPS than for young individuals) to protein intake, dietary protein is still the most potent anabolic stimulus in older adults, because it has been shown to efficiently activate the skeletal muscle anabolic response in the postprandial period, at rest, and after resistance exercise [75, 76]. A higher (>1.2 g/(kg body weight · d) compared with a lower (<1.0 g/(kg body weight · d) protein intake appears to preserve muscle quality in the aged with high levels of systemic inflammation [77], suggesting that adequate protein intake may preserve muscle function under chronic inflammatory conditions. Dairy proteins that include high amounts of branched-chain amino acids demonstrate fast (whey protein) and slow (casein) digestion and absorption kinetics and may efficiently stimulate MPS in healthy aged skeletal muscle [78-80]. Soy protein, on the other hand, which demonstrates somewhat slower kinetics than whey and faster digestion rates than casein [78], is also rich in branched-chain amino acids and able to upregulate MPS [78-80]. Whey and soy protein also possess antioxidant and anti-inflammatory properties [81, 82]. Thus, both animal and plant protein sources may represent efficient nutritional strategies to counteract inflammaging and its

detrimental effects on skeletal muscle. This review aims to provide evidence for an anti-inflammatory and anticatabolic role of protein supplementation in aged skeletal muscle by presenting molecular and physiologic data that link protein consumption and muscle wasting under proinflammatory conditions.

2.2. Inflammaging and its association with Frailty

Chung et al. [83] proposed the molecular inflammation theory, according to which the age-related increase in reactive oxygen and nitrogen species concentrations and redox balance disturbances may lead to a chronic low-grade inflammatory state by activating redox-sensitive transcriptional factors. The NF-kB pathway is the most important redox-sensitive signaling pathway through which oxidative stress may increase the expression of numerous pro-inflammatory molecules, especially cytokines such as TNF-a, IL-6, IL-1b, and CRP, stimulating inflammation [32, 84]. As the inflammatory response escalates, additional reactive nitrogen and oxygen species are released from immune cells (e.g., monocytes and macrophages) resulting in a propagation of cytokine production [7, 17]. Thus, a vicious cycle is propagated, driving a chronic systemic proinflammatory state that in the elderly has been termed inflammaging [4] (Figure 2.1).

Is inflammaging, however, associated with skeletal muscle wasting and strength loss? Data derived from 3075 men and women aged 70–79 y in the Health, Aging, and Body Composition Study [25] showed that those with high concentrations of IL-6 and TNF-a had smaller skeletal muscle area, less appendicular muscle mass, and reduced strength. Similarly, elderly subjects with elevated IL-6 and CRP concentrations demonstrated a 2- to 3-fold greater risk of losing >40% of their muscle strength [27]. Moreover, according to a 5-y follow-up study in 2177 men and women aged 70–79 y, increased baseline concentrations of TNF-a and its soluble receptor were linked to a greater decline in muscle mass and strength [26]. Although the underlying molecular pathway leading from inflammation to functional decline has not been clarified yet, increased IL-6 concentrations in the elderly contribute to the development of disability and functional dependence [85-89] via direct interactions with key growth factors in skeletal muscle [90, 91]. These findings accord well with the observation that orally administered cyclo-oxygenase inhibitors in older adults engaged in resistance exercise lead to increased skeletal muscle mass and strength gains by reducing the production of IL-6 and muscle ring-finger-1 in skeletal muscle [92]. Therefore, these studies provide compelling evidence of an association between inflammaging and a deterioration of skeletal muscle size and function.

In vivo and in vitro studies indicate that inflammaging-related muscle wasting may be attributed to a TNF-a mediated upregulation of the NF-kB pathway and the subsequent activation of the

ubiquitin–proteasome system (UPS) [32-34]. Increased concentrations of pro-inflammatory cytokines, i.e., TNF-a and/or IL-6, have been shown to activate the inhibitor of NF-kB (IkB) kinase, which phosphorylates the IkB complex and results in its degradation, thereby allowing the translocation of the NF-kB complex into the nucleus [35]. The 20S proteasome is the catalytic part of the UPS, performing the degradation and removal of abnormal, misfolded, and denatured proteins, and it may also remove healthy proteins under certain circumstances [93-97]. Under conditions of chronic inflammation, increased NF-kB expression activates the UPS, resulting in protein degradation by the 20S proteasome subunit and muscle wasting [32, 34, 36, 98]. In experimental animals, infusion or injection of TNF-a resulted in a pronounced loss of skeletal muscle and body mass [99, 100], probably in a concentration-dependent manner [101]. Moreover, infusion of IL-6 has been shown to alter amino acid turnover and decrease the phosphorylation of signaling proteins involved in the anabolic pathway, suggesting that increased IL-6 concentrations contribute to skeletal muscle atrophy [102, 103].

An interaction between the NF-kB-related proteolytic cascade and anabolic pathways has also been observed in human skeletal muscle. Older humans exhibited a blunted MPS response to feeding compared with their younger counterparts that was attributed to NF-kB overexpression in their skeletal muscles [28]. Later studies revealed that the increased TNF-a–dependent NF-kB expression attenuates the activation of anabolic signaling molecules such as Akt and S6K1, leading to reduced MPS and insulin resistance [30, 31]. Thus, chronic inflammation may not only lead to NF-kB–

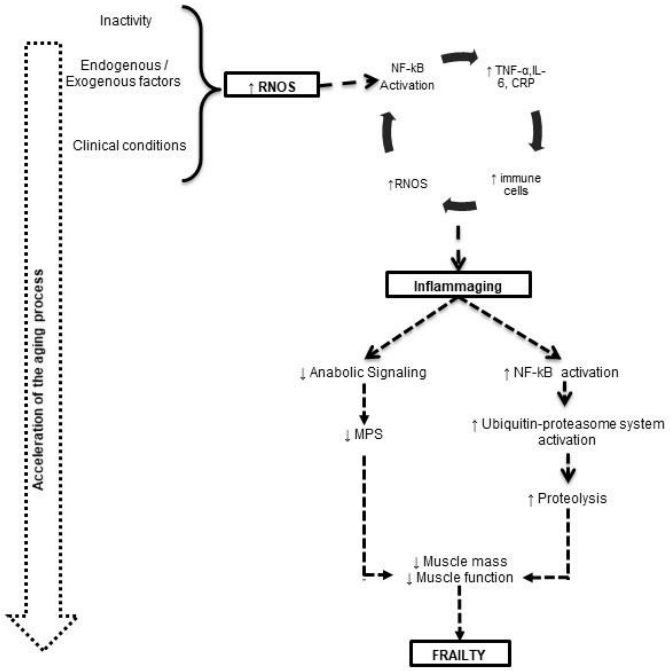

Figure 2.1. Schematic representation of the pathway linking inflammaging and frailty. CRP: C-reactive protein, MPS: Muscle protein synthesis, RNOS: Reactive oxygen and nitrogen species, ↓: decrease, ↑: increase.

related skeletal muscle wasting, but it also may hamper anabolic signaling pathways in skeletal muscle. Anti-inflammatory treatment with ibuprofen in aged rats reduced systemic inflammation, increased the rate of MPS and activation of anabolic intracellular signaling pathways, and significantly suppressed muscle protein breakdown [29]. Although the verification of this mechanism (Figure 2.1) in humans is still missing, this study clearly shows that inflammaging should be targeted by nutritional, exercise, and pharmaceutical interventions aimed at the limitation of sarcopenia and skeletal muscle loss. The molecular mechanisms regulating the TNF-a/NF-kB/ubiquitin– proteasome pathway and its crosstalk with Akt-related signaling in the skeletal muscle of aged adults warrants further investigation. Although exercise-induced inflammation has been shown to activate muscle satellite cell content as part of the regeneration or remodeling process [104, 105], there are limited data on the impact that age-related chronic inflammation has on satellite cells. Beenakker et al. [106] show that there is no association between chronic systemic inflammation and satellite cell number in patients with rheumatoid arthritis. Future investigations need to examine whether inflammaging affects satellite cell responses, and, if so, what the impact is of anabolic approaches, such as protein feeding and/or resistance exercise training on these responses.

2.3. A Rationale for Protein Supplementation for Inflammaging

Protein ingestion is a nutritional strategy that has been studied extensively as a means of attenuating age-dependent muscle loss and therefore maintain quality of life [107]. This mainly is due to the resulting postprandial aminoacidemia, which is known in the short term (hours) to stimulate MPS [108, 109], especially when combined with resistance-type exercise [47, 110, 111]. Evidence indicates that MPS is less sensitive to protein intake in elderly patients than it is in young individuals; thus, higher relative amounts of protein may be required in each meal to stimulate MPS maximally in the aged [110, 112, 113]. Although the RDA for protein intake in adults is 0.8 g/(kg body weight _ d), consumption of protein above the RDA has been proposed to more efficiently prevent muscle wasting and offer health benefits to the aged [76, 114, 115]. Higher protein intake in community-dwelling adults has been associated with an attenuation of skeletal muscle loss over a 3-y follow-up [116], whereas protein intake has been negatively associated with skeletal muscle strength loss in inflammaging [77]. Apart from the anabolic potential of protein, higher intake in the elderly also has been proposed in order to boost glutathione synthesis by providing greater availability of cysteine, which is a precursor amino acid [117]. Because the glutamate cysteine ligase Michaelis constant for cysteine is close to intracellular cysteine concentrations, increased cysteine intake from dietary protein or other cysteine-rich sources may lead to substantial glutathione synthesis, especially when intracellular concentrations of glutathione are relatively low [117].

Glutathione acts as a potent antioxidant in the intracellular environment, because it counteracts the produced reactive oxygen and nitrogen species and also downregulates signaling pathways mediating immune cell mobilization. Therefore, there is a potential link between protein intake and skeletal muscle health in older adults with low-grade inflammation. Recently, researchers have attempted to shed light on the antioxidant and anti-inflammatory properties of dairy and plant proteins by using both in vivo and in vitro experimental models. Therefore, the interaction between inflammaging and protein intake will be presented separately for each protein type in the following paragraphs. For each protein type, existing evidence will be reviewed in respect to the effect of proteins on 1) both systemic and local (skeletal muscle) anti-inflammatory and antioxidant potential, and 2) their ability to affect skeletal muscle loss and function.

Dairy proteins. Over the last 5 years there has been growing interest in the antioxidant and anti-inflammatory role of dairy proteins, primarily that of whey. In vitro models, although they use an artificial environment, have offered valuable insight in this area. When C2C12 myoblasts were incubated with whey protein (80.05 g/100 g) and various concentrations (0.1–0.4 g/L) of hydrogen peroxide, it was revealed that whey protein was able to prevent hydrogen peroxide–induced toxicity, reduce lipid peroxidation, and enhance the activity of several antioxidant enzymes [118]. Similarly, whey protein hydrolysates (WPHs; 100 mg/mL and 200 mg/mL pre- and postincubation, respectively) protected PC12 cells exposed to hydrogen peroxide from oxidative damage by reducing intracellular concentrations of Ca2+, suppressing mitochondrial apoptotic pathways (by 14%), and maintaining the membrane potential of the mitochondrial membrane, thereby improving mitochondrial function [119]. In line with the results from Xu et al. [118] in C2C12 myoblasts, WPH supplementation in PC12 cells [119] upregulated the activity of antioxidant enzymes, such as catalase and superoxide dismutase (SOD). The antioxidant properties of whey protein were illustrated further when C2C12 muscle cell lines were treated with sheep whey protein (0.78–6.24 mg) by increasing reduced glutathione concentrations and reducing TBARs and reactive oxygen species (ROS) [120]. Therefore, it appears that whey protein supplementation in the muscle and other cell lines prevents the onset of oxidative stress by enhancing the activity of endogenous antioxidant enzymes and increasing reduced glutathione availability, as well as maintaining mitochondria integrity. These results were corroborated in findings reported by studies that used rodent models [121-123].

Intraperitoneal [4 mg/(kg body weight · d)] or oral [8 mg/(kg body weight · d)] ingestion of WPH in albino mice with hepatonephrotoxicity attenuated the elevation of serum markers of oxidative damage, such as glutathione pyruvate transaminase, alkaline phosphatase, creatinine, and TBARs, upregulated the activities of antioxidant enzymes, and preserved serum urea nitrogen at normal

concentrations, suggesting that WPH also has the ability to enhance the endogenous antioxidant system in vivo under pathologic conditions [121]. When the antioxidant properties of diets containing various amounts of whey and casein protein (20% casein compared with 10% casein and 10% whey protein) were compared under conditions of elevated oxidative stress induced by iron overloading, it was shown that rats fed the diet including whey protein had greater levels of reduced glutathione and SOD activity in erythrocytes and reduced lipid peroxidation and DNA damage in leukocytes and colonocytes compared with those that received casein only, suggesting that whey was primarily responsible for the enhanced antioxidant defense [122]. Furthermore, diabetic rats supplemented with 100 mg whey protein/kg body weight exhibited considerable reductions in malondialdehyde, NO, and ROS concentrations and also preserved their glutathione concentrations [123]. These in vivo results, although derived from tissues other than skeletal muscle, are in agreement with those reported from in vitro studies [118-120] in which whey protein was systematically shown to possess antioxidant properties despite varying doses and supplementation protocols applied. This antioxidant profile of whey protein is attributed primarily to enhanced antioxidant enzyme activity and increased reduced glutathione concentrations.

Although human supplementation studies in inflammaging are lacking, human protein feeding studies under proinflammatory conditions offer valuable information. The anti-inflammatory role of protein supplementation in humans has been tested in the context of exercise [124-129], as well as in various clinical proinflammatory conditions, such as cystic fibrosis and obesity [130-134]. Exercise, especially eccentric or unaccustomed, has been associated with microtrauma of skeletal muscle fibers and an intense aseptic type of inflammation that is characterized by immune cell activation, excessive ROS generation, perturbation of redox status, and deterioration of muscle performance [135-137]. During a 6-d block of intense training, athletes receiving a daily supplement containing protein, leucine, carbohydrate, and fat at 20, 7.5, 89, and 22 g/h, respectively, for 1–3 h postexercise over 6 d demonstrated increased counts of circulating neutrophil and respiratory burst activity on day 6 compared with those receiving only a carbohydrate control beverage [124]. However, in this case, we could not determine whether the effect was attributable to leucine, protein, or a combination of the supplement's ingredients. In another study, well-trained cyclists performed 3 high-intensity ride sessions over 4 d (day 3 was a rest day), with supplementation on days 1 and 2 with a protein blend [whey protein isolate, calcium caseinate, and soy protein isolate (SPI)] at a dosage of 0.8 g protein/(kg fat-free mass · h) during a 4-h postexercise recovery period [125]. A protein effect was observed in the postexercise period, leading to reduced creatine kinase concentrations, but no significant alterations were observed for any of the oxidative stress and inflammatory markers measured [125]. Similarly, during a 9-wk weight-training period,

consumption of 33 g whey protein/d (3 servings of 11 g/d, in a bar form) did not prevent exercise-induced oxidative stress, whereas an equal amount of soy protein (33 g/d, 3 servings of 11 g/d, in a bar form) preserved postexercise antioxidative capacity, as evidenced by free-radical scavenging capacity and plasma myeloperoxidase response [126]. In contrast to these findings, acute anti-inflammatory and antioxidative properties have been attributed to whey protein after a cycling session to exhaustion [127, 128]. When whey protein [4 dosages of 0.28 g/(kg body weight · h)] was consumed immediately postexercise and daily during the recovery period after an exhaustive cycling trial that induced a marked inflammatory and oxidative stress response, an attenuation of IL-6, plasma TBARs, and CRP was observed during the first 4 h after exercise, whereas plasma total antioxidant capacity (TAC), protein carbonyls, and erythrocyte reduced glutathione and catalase concentrations remained unaltered [127, 128]. Similar findings (i.e., attenuated elevation of TBARs and protein carbonyls, and increased reduced glutathione availability) have been reported for whey protein in ultramarathon runners receiving daily 2 whey protein bars (14.3 g whey protein/100 g bar) for 2 mo [129]. Therefore, most of these human exercise studies support an anti-inflammatory and antioxidant role for whey protein. Nevertheless, these studies involved healthy young individuals, and data on skeletal muscle performance and molecular responses are lacking. We must mention, however, that one previous study suggested that antioxidant supplementation (i.e., vitamins C and E) may offset some positive adaptations induced by exercise training [138]. These findings were reported for young athletes, but, to our knowledge, no data exist for inflammaging.

To our knowledge, only a small number of studies examined the effects of a nutritional intervention of dairy-based protein diets in aged adults on chronic inflammation and oxidative stress. Either acute (a bolus of 45 g of protein) or chronic (54 g of protein/d for a 12-wk period) consumption of whey protein isolate did not alter the responses of circulating proinflammatory markers such as IL-6, TNF-a, and CRP in overweight postmenopausal women and overweight adults aged 18–65 y, respectively [130, 131]. Similarly, when obese individuals received a soy-based protein diet, after a wash-out period, no changes in inflammatory and oxidative stress markers were observed [132]. In contrast, when whey isolate and calcium caseinate (45 g of each protein in a crossover design) were consumed in combination with a fat-rich meal by obese, nondiabetic individuals in the context of an acute clinical trial, an acute suppression of markers of low-grade inflammation was observed [133]. The anti-inflammatory potential of whey protein also was evident in cystic fibrosis patients who consumed 20 g whey protein/d for 3 mo [134]. These few human studies provided valuable insight regarding the anti-inflammatory role of protein in the presence of low-grade inflammation and partly verify the in vitro and in vivo results described earlier. Both dairy proteins seem to have a protective effect against low-grade inflammation, with whey eliciting a slightly greater attenuation of

proinflammatory cytokines than does casein [133]. Thus, the rationale for using dairy proteins to counteract low-grade inflammation and oxidative stress and prevent sarcopenia may be valid, and future investigations should explore this prospect in human skeletal muscle in inflammaging.

Studies that investigated the effects of dairy protein on inflammatory and oxidative stress responses are presented in Table 2.1

Table 2.1. Evidence for the anti-inflammatory and anti-oxidative role of dairy proteins.

Reference	Cell/organism tested	Condition	Type of protein	Supplementation Protocol	Effects on Inflammation	Effects on Oxidative stress
Xu et al. (2011)	C_2C_{12} myoblasts	H_2O_2-induced toxicity	WP	0.1-0.4 mg/mL	N/A	↓ Lipid peroxidation ↓ DNA oxidative damage ↑Activity of SOD, CAT & GPx
Jin et al. (2013)	Rat pheochromocytoma line 12 cells (PC12)	H_2O_2-induced oxidative stress	WPH	100μg/mL WPH or 200μg/mL WPH for 2h and then other 100 or 200 μg/mL WPH for 24h	N/A	↓ ROS and Ca^{2+} levels ↓ Activity of caspase-3 ↑ Bcl-2 mitochondrial expression ↓ Bax mitochondrial expression ↑ Activity of CAT & SOD
Kerasioti et al. (2014)	Muscle C_2C_{12} Cells	Tert-butyl hydroperoxide (tBHP) – induced oxidative stress	Sheep WP	0.78 – 6.24 mg WP for 24 h	N/A	↓ ROS levels ↓ TBARS levels ↑ GSH availability
Athira et al. (2013)	Albino mice	Paracetamol-induced oxidative stress	WPH	4 mg/kg/day WPH intraperitoneally or 8 mg/kg/day WPH orally for 4 days	N/A	↓ Oxidative damage ↓ TBARS levels ↑ Activity of SOD, CAT & GPx
Kim et al. (2013)	8-week-old Sprague Dawley rats	Iron overload-induced oxidative stress	WP + Casein	10g WP + 10g casein / 100g diet for 6 weeks	N/A	↓ Lipid peroxidation ↓ DNA damage ↑ erythrocyte GSH levels ↑ Activity of SOD
Ebaid et al. (2011)	Adult diabetic rats	Wounded diabetic rats	WP	Orally 100mg/kg/day WP, for 30 days	Restored IL-1β, TNF-α, IL-6, IL-4 & neutrophil infiltration during wound healing	↓ MDA levels ↓ NO & ROS concentrations Preserved GSH levels
Nelson et al. (2013)	Male cyclists/triathletes (35 ± 10 years)	Exercise-induced inflammation	WP + Leucine	20g WP+7.5g leucine /h, for 3h post exercise, daily for 6 days	↔ IL-6 ↔ IL-10 ↑ neutrophil O_2^- (*on day 6*)	
Rowlands et al. (2008)	Male cyclists (34 ± 10 years)	Exercise-induced inflammation & oxidative stress	WPI + Calcium Caseinate (***Soy nuggets also included***)	0.8g of protein/kg FFM/h, for 4h post-exercise	↔ TNF-α ↔ IL-6 ↔ CRP	↔ MDA
Brown et al. (2004)	Male experienced weightlifters (19-25 years)	Exercise-induced oxidative stress	WP	33g/day WP (3 servings of 11g)	N/A	↓ Plasma radical scavenging capacity

Kerasioti et al. (2012)	Physically active men (28 years)	Exercise-induced oxidative stress	WP	4 doses of 0.28g WP/kg /h	N/A	↑ Myeloperoxidase ↓ TBARS levels ↔ Plasma TAC & Protein Carbonyls ↔ Erythrocyte GSH & CAT
Kerasioti et al. (2013)	Physically active men (28 years)	Exercise-induced inflammation	WP	4 doses of 0.28g WP/kg /h	↓ IL-6 ↓ CRP	N/A
Samaras et al. (2014)	Ultra-marathon runners (43 years)	Resting oxidative stress levels	WP	2 protein bars/day (*30,80g of protein per 100g of bar of which 14,3g WP) for two months	N/A	↓ TBARS levels ↓ Protein carbonyls ↑ GSH Availability ↔ TAC
Pal & Ellis (2011)	Overweight/Obese postmenopausal women (40-65 years)	Postprandial (*6 hours*) inflammatory markers	WPI	45g	↔ Plasma IL-6 ↔ Plasma TNF-α ↔ Plasma CRP	N/A
Pal & Ellis (2010)	Overweight/Obese individuals (18-65 years)	Systemic inflammation	WPI	54g WPI/day, for 12 weeks	↔ Plasma IL-6 ↔ Plasma TNF-α ↔ Plasma CRP	N/A
Zemel et al. (2010)	Overweight/Obese individuals (31±10 years)	Systemic inflammation & oxidative stress	Non fat dry milk	30g of protein (distributed in 3 doses/day) for 28 days	↓ TNF-α ↓IL-6 ↓ MCP-1	↓ Plasma MDA ↓ 8-isoprostane factor-α
Holmer-Jensen et al. (2011)	Obese non-diabetic individuals (40-68 years)	Postprandial low-grade inflammation	WPI	45g of WPI (*15E% of the meal*)	↑ CCL5/RANTES ↓ MCP-1	N/A
Holmer-Jensen et al. (2011)	Obese non-diabetic individuals (40-68 years)	Postprandial (4 hours) low-grade inflammation	Calcium caseinate	45g of protein (15E% of the meal)	↑ CCL5/RANTES ↓ MCP-1	N/A
Grey et al. (2003)	Cystic fibrosis patients (25 years)	GSH availability	WPI	20g of WPI/day (2 servings of 10g/day)	N/A	↑ Lymphocyte GSH levels

[1]**↑**: Indicates increase, [2]**↓**: Indicates decrease, [3]**↔**: Indicates no effect, [4]**≈**: Indicates maintenance, [5]**CAT**: Catalase, [6]**CCL/RANTES**: CC chemokine ligand-5, [7]**CRP**: C-reactive protein, [8]**FFM**: Fat free mass, [9]**GPx**: Glutathione peroxidase, [10]**IL-1β**: Interleukine-1β, [11]**IL-4**: Interleukine-4, [12]**IL-6**: Interleukine-6, [13]**IL-10**: Interleukine-10, [14]**MDA**: malondialdehyde, [15]**MCP-1**: Monocyte chemotactic protein-1, [16]**NO**: Nitric oxide, [17]**ROS**: Reactive oxygen species, [18]**SOD**: superoxide dismutase, [19]**TAC**: Total antioxidant capacity, [20]**TBARS**: thiobarbituric acid reactive substances, [21]**TNF-α**: tumor necrosis factor-α, [22]**WP**: Whey protein, [23]**WPH**: Whey protein hydrolysates, [24]**WPI**: Whey protein isolate.

Table 2.2. Evidence for the anti-inflammatory and anti-oxidative role of soy protein, isoflavone enriched soy protein and soy milk.

Reference	Cell/organism tested	Condition	Type of protein	Supplementation Protocol	Effects on Inflammation	Effects on Oxidative stress
Aoki et al. (2002)	Male Wistar rats (4 week old)	Paraquat-induced oxidative stress	SPI	20% of the daily diet (8g of food the 1st day and then increased by 0.5g each day), for 14 days	N/A	↓ Lipid peroxidation levels ≈ Glutathione levels
Takenaka et al. (2003)	Male Wistar rats (4 week old)	Paraquat-induced oxidative stress	SPI	20% of the daily diet (8g of food the 1st day and then increased by 0.5g each day), for 14 days	N/A	↓ TBARS levels ↓ GSSG/GSH ratio
Burris et al. (2014)	Apolipoprotein E knockout mice	Hyperlipidemia-induced chronic inflammation	SPI	3.9g /day SPI, for 5 weeks	↓ NF-κB activation ↓ Expression of TNF-α, IL-6 and IL-1β ↓Expression of VCAM-1 and MCP-1	N/A
Blum et al. (2003)	Postmenopausal women (55 years)	Vascular inflammation	SPI	25g/day SPI, for 6 weeks	↔ sIL-2 receptor ↔ E-selectin ↔ P-selectin ↔ VCAM-1 ↔ ICAM-1	N/A
Brown et al. (2004)	Male experienced weightlifters (19-25 years)	Exercise-induced oxidative stress	Soy (DrSoy® Bars)	33g/day (3 servings of 11g)	N/A	≈ Plasma radical scavenging capacity ≈ Myeloperoxidase
Hagen et al. (2009)	Male Wistar rats with myocardial infarction	Myocardial oxidative stress	SP + ISF	206 g/kg/day SP + 189 mg/100g of SP/day ISF, for 9 weeks	N/A	↑ SOD, CAT & GPx activity ↑ CAT activity ↓ Protein carbonyls ↓ Lipid peroxidation
Sreeja et al. (2014)	Adult male albino Wistar rats	Fructose induced oxidative stress and inflammation	SP + ISF	20g/kg/day SP for 8 weeks	↓ mRNA expression of IL-6, TNF-α & PAI-1 ↓ Activation of JNK & IKKβ ↓ nuclear NF-kB levels	↓ 4-HNE & 3-NT in liver

Greany et al. (2008)	Postmenopausal women (47-69 years)	Chronic inflammation	SP + ISF	26g/day SP + 44mg/day ISF, for 6 weeks	↔ CRP ↔ E-selectin ↔ VCAM-1 & ICAM-1	N/A
Tormala et al. (2008)	Postmenopausal women (57 years), Tribolone users	Vascular inflammation	SP + ISF	52g/day ISF + 112mg/day ISF, for 8 weeks	↔ CRP ↔ ICAM-1 ↑ VCAM-1	N/A
Archarjee et al. (2015)	Postmenopausal women (54 years), with or without MetS (*equol producers*)	Inflammatory markers related to CHD risk	SP + ISF (***soy nut***)	25g/day SP + 101mg/day ISF, for 8 weeks	↓ CRP ↓ sICAM-1	N/A
Azadbakht et al. (2007)	Postmenopausal women with MetS	Systemic inflammation	SP + ISF (***soy nut***)	37.5g/day SP + 340mg/day ISF, for 8 weeks,	↓ IL-18 ↓ CRP ↓ TNF-α ↓ E-selectin	N/A
Nasca et al. (2008)	Postmenopausal women (58,3 ± 6 years), hypertensive	Systemic inflammation	SP + ISF (***soy nut***)	25g/day SP + 101mg/day ISF, for 8 weeks	↓ sVCAM-1 ↓ CRP ↔ sICAM-1 ↔ IL-6 ↔ MMP-9	N/A
Fanti et al. (2006)	Adult ESRD patients (60 ± 3,4 years)	Chronic inflammation	SP + ISF	25g/day SP + 54 mg/day ISF, 3 times/week, & 11g/day SP + 26 mg/day ISF, 4 times/week, for 8 weeks	↓ CRP	N/A
Mangano et al. (2013)	Healthy women (> 70 years) with Baseline CRP: 5.24 pg/ml Baseline IL-6: 2.76 pg/ml	Systemic inflammation	SP + ISF	18g/day SP + 105mg/day ISF, for 1 year	↓ IL-6	N/A
Vega-Lopez et al. (2005)	Hypercholesterolemic individuals (> 50 years)	Antioxidant protection related to elevated LDL cholesterol	SP + ISF	17% of total energy SP + 1.25mg ISF / 1000kcal / day, for 42 days	N/A	↔ LDL oxidizability ↔ Urinary F2-isoprostanes ↔ MDA ↔ Protein carbonyls (native plasma)

						↓ Protein carbonyls (oxidized plasma)
Vega-Lopez et al. (2005)	Hypercholesterolemic individuals (> 50 years)	Antioxidant protection related to elevated LDL cholesterol	SP + ISF	16% of total energy SP + 46.21mg ISF / 1000kcal / day, for 42 days	N/A	↔ LDL oxidizability ↔ Urinary F2-isoprostanes ↔ MDA ↔ Protein carbonyls
Swain et al. (2002)	Postmenopausal women (41,9-61,6 years)	Menopause associated antioxidant status	SP + ISF	40g/day SP for 24 weeks (***ISF content not described***)	N/A	↓? Total antioxidant status
Beavers et al. (2009)	Postmenopausal women (40-60 years)	Systemic inflammation and oxidative stress	Soymilk	6g SP/serving, 3 servings/day, for 4 weeks	↔ TNF-α ↔ IL-6 ↔ IL-1β	↔ SOD activity ↔ GPx activity
Miraghajani et al. (2012)	Type 2 diabetic patients with nephropathy ()	Inflammation and oxidative stress	Soymilk	a bolus of 240mL soymilk/day, for 4-weeks	↔ TNF-α ↔ IL-6 ↔ hs-CRP	↔ MDA
Mitchell & Collins (1999)	Healthy adult men (20-50 years)	Oxidative DNA damage	Soymilk	1L soymilk/day, for 4 weeks	N/A	↓ DNA damage
Jenkins et al. (2002)	Hypercholesterolemic men postmenopausal women (62 years)	Inflammatory markers	Soy diet (included soymilk)	50g/day SP + 73mg/day ISF, for 1 month	↔ TNF-α ↔ CRP ↑ IL-6 (***in women only***)	
Jenkins et al. (2002)	Hypercholesterolemic men postmenopausal women (62 years)	Inflammatory markers	Soy diet (included soymilk)	52g/day SP + 10mg/day ISF, for 1 month	↔ TNF-α ↔ CRP	

[1]↑: Indicates increase, [2]↓: Indicates decrease, [3]↔: Indicates no effect, [4]≈ : Indicates maintenance, [5]**CAT**: Catalase, [6]**CHD**: Coronary heart disease, [7]**CRP**: C-reactive protein, [8]**GPx**: Glutathione peroxidase, [9]**hs-CRP**: High sensitivity C-reactive protein, [10]**4-HNE**: 4-hydroxy-2,3-nonenal, [11]**ICAM-1**: Intracellular adhesion molecule-1, [12]**IKKβ**: IκB Kinase, [13]**IL-1β**: Interleukine-1β, [14]**IL-6**: Interleukine-6, [15]**IL-18**: Interleukine-18, [16]**ISF:** Isoflavones, [17]**JNK**: c-Jun N-terminal kinase, [18]**MDA**: malondialdehyde, [19]**MCP-1**: Monocyte chemotactic protein-1, [20]**MMP-9**: Matrix metalloproteinase, [21]**3-NT**: 3-Nitrotyrosine, [22]**NF-κB:** nuclear factor kappa-B, [23]**PAI-1**: plasminogen-activator inhibitor-1, [24]**sICAM-1**: soluble intracellular adhesion molecule-1, [25]**sIL-2**: soluble interleukine-2 receptor**,** [26]**sVCAM-1**: soluble vascular cell adhesion molecule-1, [27]**SOD**: superoxide dismutase, [28]**SP:** Soy protein, [29]**SPI:** Soy protein isolate, [30]**TNF-α**: tumor necrosis factor-α, [31]**VCAM-1**: Vascular cell adhesion molecule-1.

Soy protein. Soy protein represents 35–40% of soybean content and is considered to be a protein source of high nutritional quality, because it contains all the essential amino acids and, in particular, it has less saturated fat than dairy foods and is cholesterol-free [139]. However, soy protein in its isolated form, to our knowledge, has been poorly investigated by researchers looking for protein supplements and protein-rich diets to counteract inflammation and oxidative stress.

When SPI (20% of the daily diet; 8 g of food on day 1, increased by 0.5 g each day for the remaining 13 d) was administered to rats exposed to paraquat-induced oxidative stress, an attenuation of lipid peroxidation and enhanced reduced glutathione concentrations was observed [140, 141]. A recent study showed that a 5-wk supplementation with SPI in hyperlipidemic mice counteracted the NF-kB–dependent inflammatory response manifested as reduced activation of NF-kB and expression of TNF-a, IL-6, IL-1b, vascular cell adhesion molecule 1 (VCAM-1), and monocyte chemoattractant protein 1 because of inhibition of IkB phosphorylation [81]. Therefore, given that in chronic inflammatory conditions the increased activation of the NF-kB signaling pathway leads to protein degradation through the 20S proteasome subunit [32, 34, 36, 98] soy protein may be a potent nutritional intervention against chronic inflammation and its associated skeletal muscle loss. In contrast to animal studies, in what is, as far as we know, the only human study that tested a 6-wk supplementation with SPI (25 g/d) in postmenopausal women, supplementation did not alter inflammatory markers such as soluble IL-2 receptor, E-selectin and P-selectin, VCAM-1, and intercellular adhesion molecule 1 [142]. However, the efficacy of SPI in inhibiting chronic inflammation should be further investigated in human clinical trials in order to come to a clear conclusion.

Soy foods also contain phytoestrogens named isoflavones that can be removed when these foods are washed with alcohol [139, 143]. Genistin, daidzin, and glycitein are the primary bioactive isoflavones in soybeans and soy foods [139]. Research on the antioxidative role of isoflavones has shown reduced oxidative stress levels, improved antioxidant enzyme activity, and attenuated oxidative damage in animal models [144-147], as well as in humans [148, 149]. Specifically, the incorporation of 206 g SPI/kg body weight (based on the AIN-93G diet), combined with 189 mg isoflavones/100 g SPI, in the daily diet for a 9-wk period attenuated myocardial oxidative stress levels in rats that suffered from myocardial infarction and underwent heart surgery [150, 151]. Another study investigated the antioxidative action of an isoflavone-enriched soy protein compared with a casein-based diet on fructose-induced oxidative and inflammatory responses in rats, suggesting that, in contrast to casein, soy protein is able not only to suppress oxidative stress, but also to elicit an anti-inflammatory response [152]. As previously reported for SPI [81], soy isoflavones and mainly genistein also have been shown to hamper the

activation of NF-kB and TNF-a in aged mdx mice [153]. Therefore, the effect of isoflavone-enriched soy protein on inflammation may be attributed to its isoflavone content and amino acid composition that seem to prevent the nuclear translocation and subsequent activation of NF-kB, which activates the expression of proinflammatory cytokines such as TNF-a and IL-6 [81, 152, 153].

In postmenopausal women, the administration of 26 g soy protein/d enriched with 44 mg isoflavones/d for 6 wk did not affect circulating concentrations of CRP and various adhesion molecules [154]. Tormala et al. [155] increased the amount of supplemented soy protein to 52 g/d and the amount of isoflavones to 112 mg/d, and also extended the supplementation period to 8 wk, but they did not observe any anti-inflammatory action either. In contrast, consumption of soy nuts containing either 25 g soy protein/d and 101 mg isoflavones/d or 37.5 g soy protein/d and 340 mg isoflavones/d for 8 wk led to significant reductions in blood CRP, soluble intercellular adhesion molecule 1, E-selectin, TNF-a, and IL-18 in postmenopausal women with metabolic syndrome [82, 156], as well as in VCAM-1 in hypertensive postmenopausal women [157]. However, discrepancies between these studies may be related to the different supplementation protocols applied, as well as to the clinical status of the participants (e.g., in the study by Tormala et al. [155], subjects were tibolone users). Moreover, the anti-inflammatory potential of soy nuts may be attributed to the fact that nuts contain all the bioactive compounds of a soybean, including soy protein, fat, and phytoestrogens, whereas supplemented proteins are isolated and in some cases are combined with isoflavones only [139, 158, 159]. Interestingly, in patients with end-stage renal disease, which is characterized by systemic inflammation (CRP > 10.0 mg/L), the administration of SPI that retained its isoflavone content led to a marked elevation of circulating isoflavone concentrations that was inversely correlated with inflammatory markers [160]. Moreover, in a 1-y clinical study, the intake of 18 g soy protein/d along with 105 mg isoflavone/d reduced blood concentrations of IL-6 in healthy older (>70 y of age) women with baseline CRP and IL-6 values of 5.24 pg/mL and 2.76 pg/mL, respectively [161]. To the best of our knowledge, this is the only human study that used a population with characteristics of inflammaging, and it suggested that a combination of sufficient quantities of soy protein and isoflavones may have the potential to prevent or alleviate inflammation. Although this study did not look into skeletal muscle responses, it supports a rationale for soy protein use as an anti-inflammatory intervention. Two studies that examined the antioxidant potential of soy proteins provided some positive evidence. In a crossover design, administration of either a soy protein (17% of total energy and 1.25 mg isoflavones/1000 kcal) or an isoflavone-enriched soy protein (16% of total energy and 46.21 mg isoflavones/1000 kcal) diet for 42 d had no significant impact on markers of oxidative

stress, but improved TAC by 10% [162]. Swain et al. [163] also reported that supplementation with soy protein in perimenopausal women improved TAC. Collectively, these studies suggest that soy protein with isoflavones may represent a potent anti-inflammatory and antioxidant agent, further supporting the rational for its use in proinflammatory conditions such as inflammaging. Although this data supports an anti-inflammatory role for soy protein, to our knowledge, no data exist regarding its effectiveness in promoting muscle mass and function in inflammaging. When dairy and soy protein were compared in healthy aged adults, the 2 types of proteins were equally effective in improving body composition and functionality, but the former was more effective in increasing muscle strength [164].

Studies that investigated the effects of soy protein and isoflavone-enriched soy protein on inflammatory and oxidative stress responses are presented in Table 2.2.

2.4. Conclusions

In conclusion, oxidative stress and inflammation interact in a vicious cycle, creating a chronic state of systemic inflammation that in the elderly is known as inflammaging. Many health-related dysfunctions and chronic diseases, as well as loss of muscle mass and consequently independence in the elderly, have been associated with inflammaging; therefore, it is crucial to develop nutritional, exercise-based, and pharmaceutical strategies to counteract its detrimental effects. Dairy and soy products contain high-quality proteins of high nutritional value because of their amino acid composition and absorption kinetics. Whey protein exhibits antioxidative properties that are attributed to its ability to increase glutathione availability and enhance the activity of the antioxidative enzymes SOD, catalase, and glutathione peroxidase. Evidence from animal models and cell lines indicate that whey protein may regulate multiple intracellular pathways related to ROS production. However, future studies should explore the TNF-a/NF-kB/ubiquitin–proteasome pathway and its crosstalk with Akt-related signaling in skeletal muscle in inflammaging in response to various protein feeding protocols. Whey administration may attenuate exercise-induced oxidative stress and inflammation, aswell as inflammation resulting from clinical complications and obesity. Soy protein is a promising nutritional strategy against chronic inflammation, with it having been shown that either in its isolated form or isoflavone-enriched, it is able to inhibit the activation of the NF-kB and subsequently the upregulation of proinflammatory cytokines, such as TNF-a, IL-6, and IL-1b, as well as other mediators, such as VCAM-1 and monocyte chemoattractant protein 1. Although this mechanism of action is evident in animal models only, soy protein supplementation has been associated with reduced concentrations of chronic low-grade inflammation in the elderly as well. There is a great need for

well-controlled experimental trials to determine whether an increase in protein consumption may aid MPS and muscle function in older adults with elevated systemic inflammation. Well-controlled randomized trials should compare dairy with plant protein feeding with or without an anabolic type of exercise in aged adults with a proinflammatory profile with the use of long-term supplementation protocols, as well as an assessment of muscle function and mass. A schematic representation of potential mechanisms through which protein supplementation may offset inflammation and boost muscle anabolism and performance in the elderly is presented in Figure 2.2.

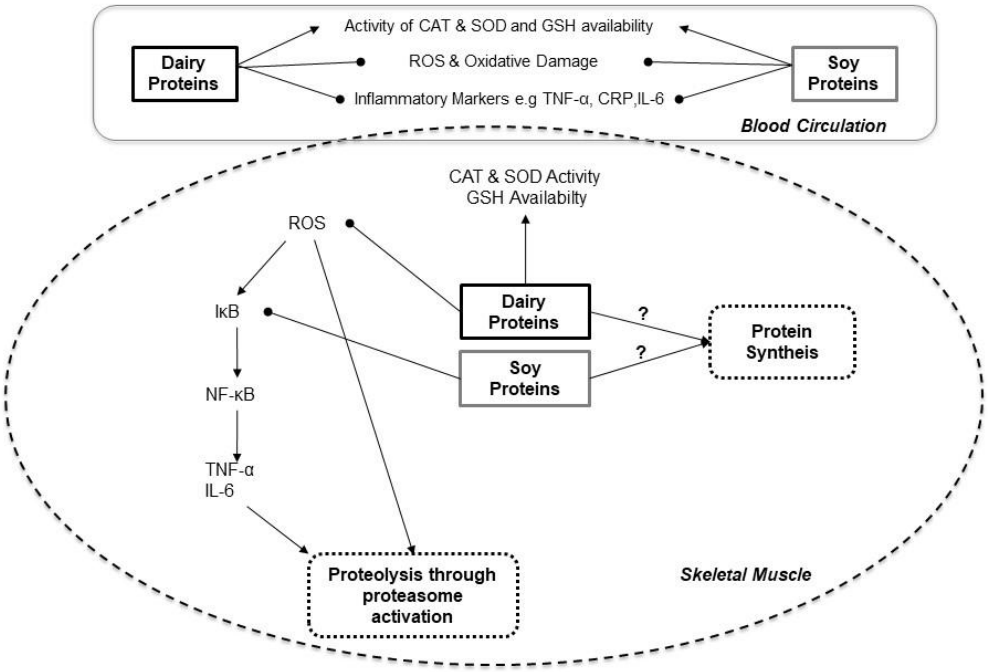

Figure 2.2. Mechanistic links between protein feeding and inflammaging. CAT: catalase, CRP: C-reactive protein, GSH: reduced glutathione, IkB: inhibitor of NF-κB, ROS: reactive oxygen species, SOD: superoxide dismutase, ?: lack of evidence regarding the ability of these proteins to stimulate muscle protein synthesis in inflamed elderly, →: increase or activation, ─●: decline or inhibition.

CHAPTER 3

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Disparate Habitual Physical Activity and Dietary Intake Profiles of Elderly Men with Low and Elevated Systemic Inflammation

Draganidis D, Jamurtas AZ, Stampoulis T, Laschou V, Deli CK, Georgakouli K, Papanikolaou K, Chatzinikolaou A, Michalopoulou M, Papadopoulos C, Tsimeas P, Chondrogianni N, Koutedakis Y, Karagounis LG, Fatouros IG

Abstract

The development of chronic, low-grade systemic inflammation in the elderly (inflammaging) has been associated with increased incidence of chronic diseases, geriatric syndromes, and functional impairments. The aim of this study was to examine differences in habitual physical activity (PA), dietary intake patterns, and musculoskeletal performance among community-dwelling elderly men with low and elevated systemic inflammation. Nonsarcopenic older men free of chronic diseases were grouped as 'low' (LSI: $N = 17$; 68.2 ± 2.6 years; hs-CRP: <1 mg/L) or 'elevated' (ESI: $N = 17$; 68.7 ± 3.0 years; hs-CRP: >1 mg/L) systemic inflammation according to their serum levels of high-sensitivity CRP (hs-CRP). All participants were assessed for body composition via Dual Emission X-ray Absorptiometry (DEXA), physical performance using the Short Physical Performance Battery (SPPB) and handgrip strength, daily PA using accelerometry, and daily macro- and micronutrient intake. ESI was characterized by a 2-fold greater hs-CRP value than LSI ($p < 0.01$). The two groups were comparable in terms of body composition, but LSI displayed higher physical performance ($p < 0.05$), daily PA (step count/day and time at moderate-to-vigorous PA (MVPA) were greater by 30% and 42%, respectively, $p < 0.05$), and daily intake of the antioxidant vitamins A (6590.7 vs. 4701.8 IU/day, $p < 0.05$), C (120.0 vs. 77.3 mg/day, $p < 0.05$), and E (10.0 vs. 7.5 mg/day, $p < 0.05$) compared to ESI. Moreover, daily intake of vitamin A was inversely correlated with levels of hs-CRP ($r = -0.39$, $p = 0.035$). These results provide evidence that elderly men characterized by low levels of systemic inflammation are more physically active, spend more time in MVPA, and receive higher amounts of antioxidant vitamins compared to those with increased systemic inflammation.

Keywords: aging, chronic low-grade systemic inflammation, physical activity, nutrition, physical performance, chronic diseases

3.1. Introduction

Chronic exposure to antigens as well as to chemical, physical, and nutritional stressors that the immune system has to cope with, in combination with the dramatic increase in life expectancy, result in the overstimulation of the immune system with advancing age and the development of a chronic and persistent pro-inflammatory state [1, 4]. This age-associated, low-grade, chronic inflammatory status has been termed as "inflammaging" [4] and is clinically assessed by measuring systemic concentrations of cytokines and acute-phase proteins, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) [5]. Inflammaging represents a significant risk factor for age-related frailty, morbidity, and mortality [1, 13] as many chronic diseases and geriatric syndromes such as cardiovascular diseases, atherosclerosis, metabolic syndrome, type 2 diabetes mellitus, neurodegenerative diseases, cancer, and chronic obstructive pulmonary disease have been associated with chronic inflammation [8-11]. Moreover, increased levels of IL-6, TNF-α, and CRP in the elderly have been associated with lower muscle mass and physical performance [25-27] as well as with increased risk for sarcopenia and osteoporosis [15, 22, 88]. Thus, the concept of inflammaging appears to be a key determinant of successful aging and longevity and as such a valuable tool to counteract age-related pathologies [1].

To date, inflammaging is defined as a complex and multifactorial process whose origin cannot be simply attributed to a specific number of factors/mechanisms, as a complete understanding of the extent to which different tissues, organs, and biological systems contribute to its pathophysiology is lacking [5, 16]. However, both physical activity (PA) and nutrition are considered powerful lifestyle factors that may, cooperatively or independently, influence both healthy aging and lifespan in humans [39, 40]. Specifically, being physically active substantially reduces the risk of developing cardiovascular [39, 40] and metabolic diseases [39, 41], obesity [39, 44], frailty [39, 45, 46], sarcopenia [47], osteoporosis [40, 48], cognitive impairment [42], and mental health disorders [40, 43] in a dose-response manner [165, 166]. Numerous studies reported that higher volume of habitual PA is related to lower levels of IL-6, CRP, and TNF-α in older adults [23, 49-60]. Most of these studies, though, are based on self-reported PA estimations [49-54, 57, 58, 60] that may result in increased risk of recall bias [167] and therefore do not provide an objective determination of different intensity levels (i.e., light, moderate, vigorous, or very vigorous PA). However, to our knowledge, four studies have utilized accelerometry to provide an objective assessment of PA [23, 55, 56, 59]. In two of them, an inverse relationship between PA and disease-related (chronic obstructive pulmonary disease and obesity) systemic inflammation was revealed in middle-aged adults [55, 56]. Similarly, two other studies reported

that time spent in MVPA is negatively associated with markers of systemic inflammation in the healthy elderly [23, 59]. Although these data clearly suggest that habitual PA is inversely associated with mediators of systemic inflammation in older adults, a direct comparison of objectively assessed PA, sedentary time, and PA-related energy expenditure among the elderly with low and increased systemic inflammation is still lacking.

Ideally, this comparison would be more conclusive by the concurrent examination of habitual PA/inactivity and dietary intake levels, since both factors may impact systemic inflammation. In fact, available data suggest that the role of nutrition and dietary pattern is pivotal for immune function and low-grade systemic inflammation [61-63]. Both macronutrient and micronutrient intake may interfere with immune responses, triggering either a pro-inflammatory or an anti-inflammatory effect [64]. Excessive consumption of glucose and saturated fatty acids (SFA) (particularly long-chain SFA) are reported to activate pro-inflammatory markers in insulin-sensitive tissues [64, 67] and may result in systemic inflammation [16], while high phospholipid consumption, especially that of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), elicit antiinflammatory properties and reduce the risk of chronic inflammation and its associated chronic diseases [68]. On the other hand, consumption of either plant- or dairy-based protein or amino acids may offer anti-inflammatory effects by reducing levels of inflammatory mediators [64, 69]. Furthermore, adequate intake of antioxidants and trace elements, particularly vitamins A, C, E, and selenium, also enhances immunity and elicits a protective effect against chronic inflammatory conditions [63]. However, to our knowledge, the literature lacks evidence regarding differences in dietary habits among older healthy adults with low and high systemic inflammation.

Given the pivotal role of both PA and macronutrient/micronutrient intake in mediating immunity and chronic inflammatory responses, a direct comparison of them among older adults exhibiting low and elevated systemic inflammation may identify which parameters of these lifestyle factors function as discriminants of healthy aging and inflammaging. Therefore, the aim of the present study was to compare levels of objectively assessed habitual PA and dietary macronutrient/micronutrient intake, among otherwise healthy elderly men of low and increased systemic inflammation.

3.2. Materials and Methods

3.2.1 Experimental Design and Participants

A total of fifty community-dwelling elderly men aged 65–75 years were recruited from the surrounding area of Thessaly (Greece) through postings, newspaper, and media advertisements. All volunteers completed a health history questionnaire and were also examined by a physician. In order to be included in the study, volunteers had to initially meet all of the following inclusion/exclusion criteria: (a) nonsmokers; (b) independently living; (c) absence of chronic disease (i.e., cancer, metabolic, cardiovascular, neurological, pulmonary, or kidney disease); (d) absence of inflammatory disease (i.e., osteoarthritis, rheumatoid arthritis); (e) absence of type 2 diabetes, and (f) no recent or current use of antibiotics or other medication that could affect inflammatory status (i.e., corticosteroids). Subsequently, those who fulfilled these criteria underwent assessment of body height, body weight, body composition, handgrip strength, and physical performance (via the SPPB) testing to estimate their weight status and stage of sarcopenia according to the European Working Group on Sarcopenia in Older People (EWGSOP) [168]. Volunteers who were characterized as presarcopenic/sarcopenic were excluded from the study at this stage, since substantial loss of skeletal muscle mass is accompanied by significant performance decline [168], resulting in lower levels of habitual PA [169]. Volunteers who were classified as obese were also excluded since obesity is linked to metaflammation, an adipose-tissue-mediated chronic inflammatory state that differs in terms of pathophysiology from inflammaging [8, 16]. Accordingly, thirty-four volunteers who fulfilled the eligibility criteria participated in the study. The determination of inflammatory status was based on two consecutive measurements of high-sensitivity CRP (hs-CRP) and participants were grouped as “low systemic inflammation” (LSI: hs-CRP <1 mg/L) or “elevated systemic inflammation” (ESI: hs-CRP >1 mg/L) according to a previous report [170]. Participants were then provided with accelerometers and food diaries to monitor their habitual PA and daily macronutrient/micronutrient intake, respectively, over a 7-day period. They were fully informed about the aim and the experimental procedures of the study, as well as about the benefits involved, before obtaining written consent. The Institutional Review Board of the University of Thessaly approved the study and all procedures were in accordance with the 1975 Declaration of Helsinki (as revised in 2000).

3.2.2. Body Composition

Standing body mass and height were measured on a beam balance with stadiometer (Beam Balance-Stadiometer, SECA, Vogel & Halke, Hamburg, Germany) with participants wearing light clothing and no shoes as described previously [171]. Body composition [including fat mass, fat-free mass (FFM), percent of fat, lean body mass (LBM)] was assessed by dual emission X-ray absorptiometry (DXA, GE Healthcare, Lunar DPX NT, Diegem, Belgium) with participants

in supine position as described before [172]. Appendicular lean mass (ALM) and skeletal muscle mass index (SMI) were calculated as the sum of muscle mass (kg) of the four limbs (based on DXA scan) and as ALM divided by height by meters squared (kg/m^2), respectively [168], while sarcopenia status was determined according to the criteria established by EWGSOP [168].

3.2.3. Physical Activity

Physical activity was monitored by using the accelerometers ActiGraph, GT3X+ (ActiGraph, Pensacola, FL, USA) over a 7-day period. Accelerometers were attached to elastic, adjustable belts and did not provide any feedback to the participants. Participants were taught how to wear the belt around the waist with the monitor placed on the right hip and they were asked to wear it throughout the day, except for bathing or swimming and sleep, for seven consecutive days. To be included in the analysis, participants had to have ≥four days with ≥10 wear hours/day (i.e., four valid days) [173]. Nonwear time was calculated using the algorithms developed by Choi et al. [174] for vector magnitude (VM) data and defined as periods of 90 consecutive minutes of zero counts per minute (cpm), including intervals with nonzero cpm that lasted up to 2 min and were followed by 30 consecutive minutes of zero cpm. Daily activity and sedentary time were estimated according to VM data and expressed as steps/day and time in sedentary (<199 cpm), light (200–2689 cpm), moderate (2690–6166 cpm), vigorous (6167–9642 cpm), and moderate-to-vigorous (≥2690 cpm) PA [175]. The manufacturer software ActiLife 6 was utilized to initialize accelerometers and download data using 60-s epoch length.

3.2.4. Dietary Assessment

Participants were taught by a registered dietitian how to estimate food servings and sizes of different food sources and how to complete food diaries. They were allowed to weigh out food servings, so that they could precisely report the amount of specific food portions, while they were also provided with colored photographs depicting different portion sizes that they could use to compare their food weights. Furthermore, complete instructions on how to describe portion sizes based on household measures or other standard units were also administered to our participants. Participants recorded their daily dietary intake for seven consecutive days, describing, in as much detail as possible all portions of food and drinks/water. For commercially available products, the name of the manufacturer, fat content (i.e., 1%. 2% etc), and other related information had to be noted. The Science Fit Diet 200 A (Science Technologies, Athens, Greece) dietary software was utilized to analyze diet recalls and data regarding total energy (kJ), protein (g/kg/day & g/day), leucine (g/day), branched chain amino acids (BCAA, g/day), carbohydrates (g/day), fat (g/day),

vitamin A (IU/day), vitamin C (mg/day), vitamin E (mg/day), selenium (μg/day), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA).

3.2.5. Systemic Inflammation

Blood samples were collected early in the morning between 07:00 and 09:00 am, after an overnight fasting. Participants were asked to avoid alcohol and abstain from intense physical activity for ≥48 h before blood sampling. Blood was drawn from an antecubital arm vein via a 10-gauge disposable needle equipped with a Vacutainer tube holder (Becton Dickinson) with participants seated. To separate serum, blood samples were allowed to clot at room temperature and then centrifuged (15,000 g, 15 min, 4 °C). The supernatant was dispensed in multiple aliquots (into Eppendorf tubes) and stored at −80 °C for later analysis of hs-CRP. Serum hs-CRP was quantitatively measured in duplicate using the C-Reactive Protein (Latex) High Sensitivity assay (CRP LX High Sensitive, Cobas®) on a Cobas Integra® 400 plus analyzer (Roche) with a detectable limit of 0.01 mg/dL and an inter-assay coefficient of one standard deviation (1 SD).

3.2.6. Statistical Analyses

All data are presented as means ± SD. The normality of data was examined using the Shapiro–Wilk test (N = 17/group). Because our data sets in most of our variables differed significantly from normal distribution, we rejected the hypothesis of normality and applied nonparametric tests. To test differences in body composition, daily PA-related parameters, and dietary macronutrient/micronutrient intake among the two groups (LSI vs. HSI) a Kruskal–Wallis test was applied. Pearson's correlation analysis was used to examine the relation of dietary antioxidant vitamins intake, number of steps, and time in MVPA per day with serum levels of hs-CRP. Correlation coefficients of $r < 0.2$, $0.2 < r < 0.7$ and $r > 0.7$ were defined as small, moderate, and high, respectively. Effect sizes (ES) and confidence intervals (CI) were also calculated for all dependent variables using the Hedge's g method corrected for bias. ES was interpreted as none, small, medium-sized, and large for values 0.00–0.19, 0.20–0.49, 0.50–0.79, and ≥0.8, respectively. The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed using the SPSS 20.0 software (IBM SPSS Statistics). The G * Power program (G * Power 3.0.10) was utilized to perform power analysis. With our sample size of 17/group we obtained a statistical power greater than 0.80 at an α error of 0.05.

3.3. Results

Participants' characteristics are presented in Table 3.1. Participants were healthy and had no pathological levels of hs-CRP. The two groups, though, differed significantly in respect to hs-

CRP values (ESI: 2.1 ± 0.8 vs. LSI: 0.7 ± 0.2 mg/dL, p = 0.00), with ESI displaying a 2-fold elevation in serum hs-CRP compared to LSI. Averaged BMI values in LSI and ESI were 27.3 ± 3.1 kg/m^2 and 27.9 ± 2.5 kg/m^2, respectively, which classifies them as nonobese according to the criteria established by the World Health Organization (WHO) [176]. Moreover, all participants were characterized as nonsarcopenic, since they exhibited SMI >7.26 kg/m^2, handgrip strength >30 kg, and physical performance score in SPPB >8. No differences were detected in respect to BMI, fat mass, percent of fat, FFM, LBM, ALM, SMI, and handgrip strength among groups. However, significant differences were observed in physical performance, with LSI achieving a higher SPPB score compared to ESI (LSI: 11.9 ± 0.2 vs. ESI: 11.2 ± 1.0; χ^2 = 6.436, p = 0.016; ES = 0.90; 95% CI = −1.63, −0.17).

Table 3.1. Participants' characteristics.

Parameter	LSI (n = 17)	ESI (n = 17)
Age (years)	68.2 ± 2.6	68.7 ± 3.0
Body Height (m)	1.71 ± 0.07	1.73 ± 0.04
Body Weight (kg)	82.3 ± 8.5	85.2 ± 7.5
BMI (kg/m^2)	27.3 ± 3.1	27.9 ± 2.5
Fat Mass (kg)	24.1 ± 7.0	26.3 ± 4.1
Fat (%)	29.5 ± 6.6	31.8 ± 2.1
Fat-Free Mass (kg)	56.3 ± 4.6	58.4 ± 5.2
Lean Body Mass (kg)	53.3 ± 4.5	55.3 ± 5.1
ALM (kg)	23.2 ± 2.4	24.4 ± 2.1
SMI (kg/m^2)	8.12 ± 0.7	8.13 ± 0.6
Grip Strength (kg)	34.3 ± 5.5	36.7 ± 6.6
SPPB (score)	11.9 ± 0.2	11.2 ± 1.0 [1]
Sarcopenia Status	Non-Sarcopenic	Non-Sarcopenic
hs-CRP (mg/L)	0.7 ± 0.2	2.1 ± 0.8 [2]

Data are presented as mean ± SD. ALM: Appendicular Lean Mass; SMI: Skeletal Muscle Mass Index; SPPB: Short Physical Performance Battery; hs-CRP: High-Sensitivity CRP. [1] significant difference between groups, $p < 0.05$, [2] significant difference between groups, $p < 0.01$.

Results comparing sedentary time and PA among groups are shown in Figure 3.1. The two groups were comparable in sedentary time throughout the day (LSI: 378.2 ± 98.7 vs. ESI: 370.5 ± 95.9 min/day; χ^2 = 0.008, p = 0.927) and in the time they spent in light PA/day (LSI: 342.9 ± 93.1 vs. ESI: 331.7 ± 98.2 min/day; χ^2 = 0.357, p = 0.550), while a trend for significantly more time spent in moderate PA/day by the LSI group was also observed (LSI: 59.5 ± 16.7 vs. ESI: 44.1 ± 18.2 min/day; χ^2 = 3.637, p = 0.057). Interpretation of the level of moderate PA by group means examined in relation to the PA guidelines adopted by the WHO revealed that both groups met the recommendation for at least 150 min of moderate-intensity PA throughout the week.

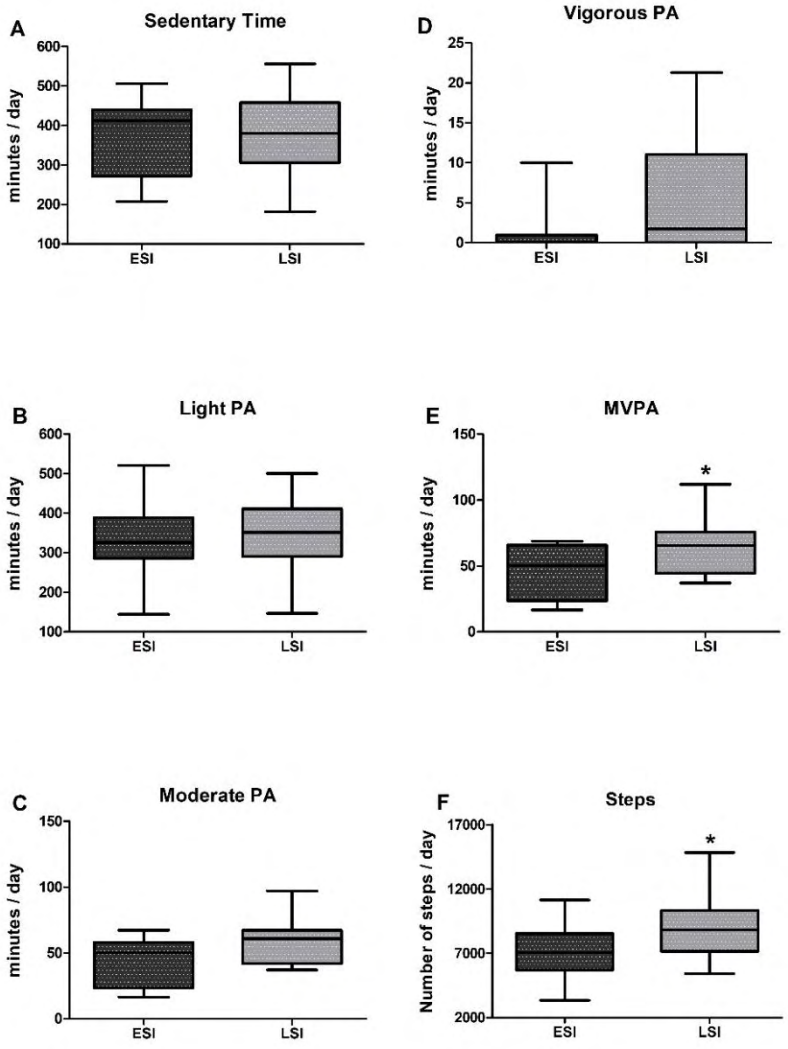


Figure 3.1. (**A**) Sedentary time, (**B**) time spent in light, (**C**) moderate, (**D**) vigorous, (**E**) moderate-to-vigorous (MVPA) PA, and (**F**) total step count throughout the day, in low (LSI) and elevated (ESI) systemic inflammation groups. Values are presented as mean ± SD. ***** denotes significant difference between groups at $p < 0.05$.

By performing an individual examination in both groups, we found that all participants in LSI and approximately 86% of participants in ESI met this criterion. Significant differences between LSI and ESI were observed in MVPA and daily step count, with LSI spending more time in MVPA throughout the day (LSI: 65.2 ± 21.5 vs. ESI: 45.9 ± 19.8 min/day; $\chi^2 = 3.997$, $p = 0.044$; ES = 0.91; 95% CI = −1.68, −0.13) and performing more steps (LSI: 9000.1 ± 2496 vs. ESI: 6968.3 ± 2075 steps/day; $\chi^2 = 4.087$, $p = 0.043$; ES = 0.86; 95% CI = −1.63, −0.08) than ESI, by 42% and 30%, respectively. The average step count/day for LSI was 9000.1 steps, which is close to the upper recommended limit for older adults (7100–10,000 steps/day) [177] while the ESI did not meet these recommendations, performing 6968.3 steps/day. Almost 86% of participants in the LSI group performed >7100 steps daily while slightly more than half (53%) of participants in

the ESI group did so. A longitudinal analysis combining both groups revealed a trend for an inverse correlation between hs-CRP level and daily step count (r = −0.37, p = 0.055). Time in vigorous PA/day did not differ among groups (LSI: 5.3 ± 6.9 vs. ESI: 1.0 ± 2.6 min/day; χ^2 = 2.315, p = 0.128), probably because of a high interindividual variability. Moreover, the two groups demonstrated similar PA-related energy expenditure throughout the day, as no differences observed in terms of kJ/day (LSI: 2554.3 ± 1033.5 vs. ESI: 2654.3 ± 1041.8 kJ/day, p = 0.798) and METs/day (LSI: 1.28 ± 0.1 vs. ESI: 1.23 ± 0.1 METs/day, p = 0.203) (Figure 3.2).

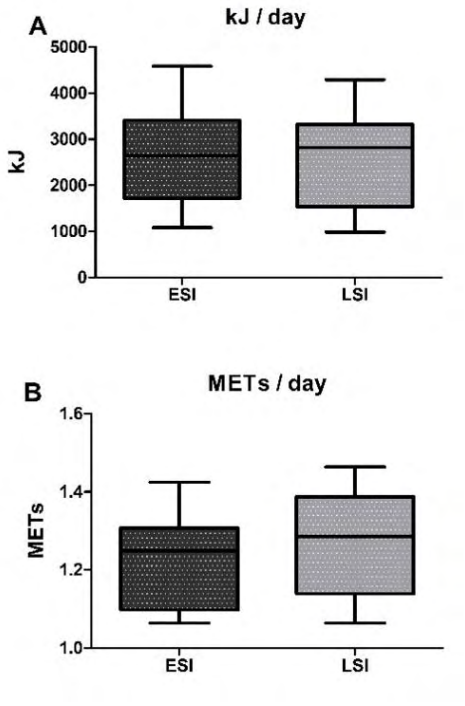


Figure 3.2. Daily PA-related energy expenditure expressed as (**A**) kJ and (**B**) METs in low (LSI) and elevated (ESI) systemic inflammation groups. Values are presented as mean ± SD.

LSI and ESI demonstrated similar total energy and macronutrient intake throughout the day (Table 3.2). The two groups had a daily energy intake of 6949.6–6794.8 kJ, constituted by 15–16% protein, 38% carbohydrate, and 42% fat. The mean protein intake in both groups was 0.8 g/kg body weight/day, which represents the recommended daily allowance (RDA) that meets 97.5% of the population [178]. However, approximately 46% of participants in both groups had a daily protein intake of 0.5–0.7 g/kg body weight/day. Separate analysis in leucine and BCAA intake revealed that both LSI and ESI received 0.6 g of leucine/kg body weight/day and 0.13–0.14 g of BCAAs/kg body weight/day, which meets the current recommendations for amino acid intake in adults [178]. The two groups, though, differed significantly in respect to daily antioxidant vitamin intake, with the LSI group receiving higher amounts of vitamin A (LSI:

6590.7 ± 2219 vs. ESI: 4701.8 ± 1552.6 IU/day; $\chi^2 = 5.616$, $p = 0.018$; ES = 0.95; 95% CI = 1.72, 0.18), vitamin C (LSI: 120.0 ± 55.5 vs. ESI: 77.3 ± 39.1 mg/day; $\chi^2 = 5.421$, $p = 0.020$; ES = 0.87; 95% CI = 1.63, 0.11), and vitamin E (LSI: 10.0 ± 2.9 vs. ESI: 7.5 ± 3.0 mg/day; $\chi^2 = 4.496$, $p = 0.034$; ES = 0.75; 95% CI = 1.50, 0.01) than ESI, by 37%, 59%, and 33%, respectively. Moreover, by performing a longitudinal analysis of both groups we observed that daily vitamin A intake was inversely correlated with levels of hs-CRP (r = −0.39, $p = 0.035$) (Figure 3.3). On the contrary, daily intake of selenium (LSI: 93.2 ± 29.8 vs. ESI: 96.1 ± 29.7 μg/day, $p = 0.793$), PUFA (LSI: 10.1 ± 2.4 vs. ESI: 8.9 ± 2.6 g/day, $p = 0.215$), and MUFA (LSI: 43.7 ± 10.8 vs. ESI: 37.9 ± 10.9 g/day, $p = 0.168$) was comparable in the two groups.

Table 3.2. Dietary macronutrient and micronutrient intake in LSI and ESI groups.

Parameter	LSI (n = 17)	ESI (n = 17)	p Value	χ^2
Total Energy (kJ/day)	6952.9 ± 1241.8	6797.8 ± 1136.8	0.771	0.085
Protein				
g/day	63.8 ± 20.3	66.9 ± 14.6	0.183	1.770
g/kg BM/day	0.8 ± 0.3	0.8 ± 0.2	0.817	0.054
% of total calories	15 ± 2.7	16 ± 3.0		
Leucine (g/day)	4.89 ± 1.7	5.13 ± 1.2	0.430	0.624
BCAAs (g/day)	11.38 ± 3.6	11.53 ± 2.4	0.533	0.389
Carbohydrates				
g/day	156.2 ± 37.6	154.9 ± 52.7	0.901	0.016
% of total calories	37.7 ± 6.9	37.5 ± 8.4		
Fat				
g/day	79.3 ± 12.5	73.7 ± 17.0	0.318	0.996
% of total calories	42.0 ± 4.0	41.7 ± 7.1		
PUFA (g/day)	10.1 ± 2.4	8.9 ± 2.6	0.275	1.191
MUFA (g/day)	43.7 ± 10.8	37.9 ± 10.9	0.359	0.840
Vitamin A (IU/day)	6590.7 ± 2219.6	4701.8 ± 1552.6 [1]	0.018	5.616
Vitamin C (mg/day)	120.0 ± 55.5	77.3 ± 39.1 [1]	0.020	5.421
Vitamin E (mg/day)	10.0 ± 2.9	7.5 ± 3.0 [1]	0.034	4.496
Selenium (μg/day)	93.2 ± 29.8	96.1 ± 29.7	0.589	0.292

Data are presented as mean ± SD. BM: Body mass; BCAA: Branched chain amino acids; PUFA: Polyunsaturated fatty acids; MUFA: Monounsaturated fatty acids. [1] Significant difference between groups.

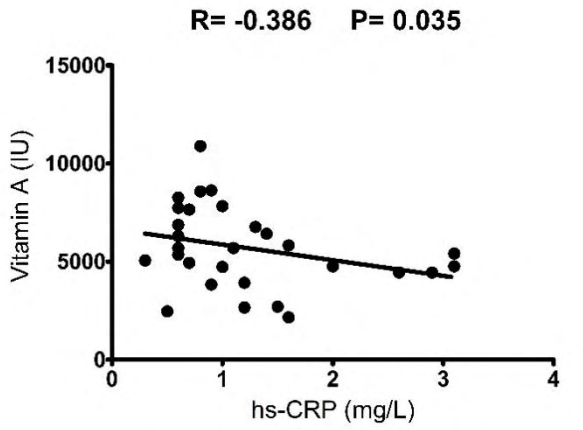


Figure 3.3. The relationship between serum hs-CRP level and daily dietary intake of Vitamin A.

3.4. Discussion

The present study is the first, to our knowledge, to compare the levels of habitual PA, sedentary time, and dietary intake between healthy elderly men with low and elevated low-grade systemic inflammation (inflammaging). Our findings suggest that older adults characterized by low levels of systemic inflammation perform more steps and spent more time in MVPA throughout the day and they receive higher amounts of dietary antioxidant vitamins (i.e., vitamins A, C, and E) on a daily basis compared to their counterparts with elevated systemic inflammation.

Participants were categorized as having either “low” or “elevated” low-grade systemic inflammation according to their serum levels of hs-CRP. This acute-phase protein is considered a valid and informative marker of inflammaging [179] and has been previously used as a single marker to identify levels of systemic inflammation in older adults [170]. The term inflammaging, first introduced by Franceschi and his colleagues [4], refers to the development of a chronic, low-grade inflammation phenotype with advancing age. However, the presence of obesity, either in young or older individuals, results in elevated systemic inflammation, which has been defined as metaflammation (metabolic inflammation) and is primarily mediated by the adipose tissue [8]. Although the underpinning mechanisms of inflammaging and metaflammation may be different, these two chronic inflammatory conditions may overlap [16]. Therefore, in an attempt to focus on inflammaging in this study, we included only nonobese elderly men (according to WHO criteria). Moreover, LSI and ESI groups were very homogeneous in terms of body composition, since they did not differ in body weight, fat mass, percent of fat, FFM, and LBM. All participants were also nonsarcopenic according to the criteria established by the EWGSOP [168], since the existence of sarcopenia could act as a covariate in our investigation, interfering with their ability to habitually perform PA [169].

Previous cross-sectional studies have investigated the association between habitual PA and inflammatory biomarkers in middle-aged and older adults [23, 49-52, 54-60]. However, only two utilized accelerometry to quantify not only the quantity but also the quality (intensity) of habitual PA in the otherwise healthy elderly with physiological and elevated chronic, low-grade systemic inflammation [23, 59]. This study attempted to extend the current literature by providing insights concerning the differences in PA and dietary intake profile among elderly men with low and elevated low-grade systemic inflammation. The use of accelerometry to objectively assess the quantity and intensity of habitual PA is a strength of our study, as most of the previously cited studies [49-52, 54, 57, 58, 60] are based on questionnaires, self-reports, or interviews. The use of accelerometers over a 7-day period to assess PA and sedentary time has been reported to be a valid and reproducible methodological approach in the elderly [180].

Although sedentary time and time spent in light- and moderate-intensity activities throughout the day were similar between LSI and ESI, we noted that overall the LSI group performed more steps and spent more time in MVPA on a daily basis. This suggests that not only the volume of habitual PA but also the intensity in which daily physical activities are performed may interfere with the development of chronic, low-grade systemic inflammation in older individuals. Our findings further build on previous reports that higher volume of habitual PA is associated with lower levels of pro-inflammatory mediators in healthy elderly individuals [50, 54, 57] and COPD patients [55]. Moreover, this inverse association between PA and inflammation is suggested to be dose-dependent, so that the more physically active an individual is, the lower the chronic inflammatory milieu [50, 52, 60]. Although only a trend ($r = -0.37$, $p = 0.055$) for an inverse correlation between hs-CRP level and daily step number was observed in our study, possibly because of an interindividual variability in daily step counts of our participants (we used accelerometers whereas questionnaires were utilized by others), these findings collectively suggest that habitual PA may be associated with inflammaging in an inverse, dose-response pattern. Furthermore, it has been recently reported that the impact of PA on chronic low-grade inflammation is not only dose-dependent but also intensity-dependent, as moderate-to-vigorous activities induce greater improvements in the inflammatory profile of older adults while light- or moderate-intensity physical activities are accompanied by no changes in inflammatory mediators [181]. Indeed, Wahlin-Larsson et al. [23] found that in recreationally active elderly women, the time spent in MVPA is inversely associated with serum levels of CRP, a finding also reported in younger individuals [182]. The mechanism/s through which PA reduces or prevents low-grade systemic inflammation in the elderly remains to be elucidated. Observational, cross-sectional studies are not designed to identify the mechanisms that underline the effects of systematic PA

on chronic inflammation and as such, more intervention studies are needed [167, 181]. Based on the fact that inflammaging is tightly regulated by the balance between pro- and anti-inflammatory mediators [183], a possible mechanism could be that PA, and especially MVPA, suppresses the production of pro-inflammatory cytokines and molecules that trigger the inflammatory milieu, and enhances the production of anti-inflammatory mediators [167, 181, 184]. Moreover, the process of inflammaging may be further affected by the age-associated increase in the production of reactive oxygen and nitrogen species (RONS) that lead to redox balance disturbances and subsequent activation of the redox-sensitive NF-κB signaling pathway that stimulates the expression of numerous pro-inflammatory mediators such as TNF-α, IL-6, IL-1β, and CRP [69, 83]. As such, a vicious cycle of RONS and pro-inflammatory molecule production is propagated, driving a chronic, systemic pro-inflammatory phenotype [17, 69]. Regular participation in moderate-to-vigorous intensity exercise has been shown to attenuate both basal and exercise-induced levels of oxidative damage, enhance the antioxidant capacity, and improve the DNA repair machinery in healthy, elderly individuals [185, 186]. Thus, it can be proposed that systematic MVPA may prevent the development of inflammaging by lowering the production of RONS and levels of oxidative damage in the elderly.

LSI and ESI also differed significantly in terms of physical performance. More specifically, LSI exhibited higher performance in the SPPB test compared to ESI and this observation is in line with previous findings reporting that older adults with elevated systemic inflammation demonstrate lower physical performance [24, 187]. Although the underlying mechanism leading from chronic inflammation to functional decline has not been clarified yet, it has been reported that systemic inflammation may impact physical performance by decreasing skeletal muscle mass [22, 69]. However, in this study, the two groups demonstrated similar LBM, ALM, and SMI, indicating that the observed difference in physical performance was not muscle-mass-dependent. A previous report, though, by Wahlin-Larsson and colleagues [23] provided evidence that increased systemic inflammation influences muscle regeneration by decreasing the proliferation rate of myoblasts. In addition, increased inflammation and cytokine production may also reduce the quiescent satellite cells pool and attenuate their differentiation capacity [22]. Therefore, it can be assumed that elevated systemic inflammation may contribute to physical performance deterioration by attenuating the regeneration potential of the aged skeletal muscle.

We also utilized 7-day recalls to perform a thorough screening of the dietary intake in the LSI and ESI groups, focusing on macronutrients and micronutrients that have been shown to elicit either a pro- or an anti-inflammatory effect, and could be therefore characterized as ‘key modifiers’ in the process of inflammaging. LSI and ESI demonstrated similar energy and

macronutrient intake, consuming 6794.8–6949.6 kJ/day composed of 15–16% protein, 38% carbohydrates, and 42% fat. Our group recently conducted a literature review suggesting that protein intake, especially that of whey protein and soy or isoflavone-enriched soy protein, may indirectly offer antioxidative and anti-inflammatory benefits beyond its ability to stimulate skeletal muscle protein synthesis [69]. Also, Zhou et al. [188] performed a meta-analysis on the effects of whey protein supplementation on levels of CRP, concluding that increased whey protein intake may induce favorable effects on individuals with elevated baseline CRP levels. However, in this study, we noted that daily protein intake was similar between LSI and ESI, with both groups receiving on average ~0.8 g/kg BM/day, which is in line with WHO RDA for protein [178]. BCAA and leucine intake were also compared among groups to provide a qualitative determination of daily protein intake. Although leucine is classified as a BCAA, we decided to present it separately because its role may differ from that of the other BCAAs, especially in the elderly where a higher amount of leucine should be consumed through diet to efficiently stimulate muscle protein synthesis and preserve muscle loss [189, 190]. In our present work, we observed that LSI and ESI had a similar daily intake of BCAAs and leucine, meeting the recommendations for amino acid intake in adults [178]. Daily carbohydrate intake was also similar among groups (154–156 g/day), indicating that it does not play a prominent role in the development of inflammaging. Previous reports have noted that only increased consumption of high glycemic index carbohydrates may be associated with increased levels of inflammation [191]. Unfortunately, the determination of glycemic index and glycemic load in our participants' daily diets was not feasible.

Similarly, no differences were observed in total fat consumption among groups, with LSI and ESI receiving 79 and 74 g/day, respectively, which corresponds in both groups to 42% of daily energy intake. Although previous reports have indicated that increased fat consumption is associated with elevated systemic markers of inflammation [191, 192], this was not the case here. High fat diets, and primarily SFA, have been reported to induce substantial alterations in the gut microbial flora (i.e., increases gut mucosa permeability, epithelial brier disruption) that result in enhanced translocation of lipolysaccharide (LPS) in the circulation, thus promoting the development of low-grade systemic inflammation [192, 193]. However, it should be highlighted here that not all SFA demonstrate equal properties and consumption of specific SFA (i.e., C14:0, C15:0, C17:0, CLA, and trans-palmitoleic) has been associated with positive effects on cardiovascular health [194]. On the other hand, increased intake of MUFA and/or PUFA has been proposed to counteract the pro-inflammatory cascade by reducing the translocation of LPS in the circulation [192] and suppressing the eicosanoid and PAF inflammatory pathways [68]. Indeed,

many studies have revealed an inverse association between higher intake of dietary PUFA and/or MUFA and levels of pro-inflammatory mediators such as hs-CRP and IL-6 [191]. In this study, although no statistically meaningful differences were observed in dietary MUFA and PUFA intake between groups, LSI displayed a higher intake of MUFA and PUFA, by 15% and 13.5%, respectively, compared to ESI.

Interestingly, we noted significant differences between LSI and ESI in terms of antioxidant vitamin intake. More specifically, daily dietary intake of vitamins A, C, and E in LSI was higher by 37%, 59%, and 33%, respectively, as compared to ESI. These vitamins play a major role in immune function, so that adequate intake enhances innate, cell-mediated, and humoral antibody immunity while deficiency promotes the opposite effects [63, 195]. With aging, the production of reactive oxygen and nitrogen species and that of pro-inflammatory cytokines rises significantly, propagating a vicious cycle of oxidative stress and inflammation that promotes a chronic low-grade inflammatory state [17, 69]. Vitamin A has been shown to promote a T-helper type 2 immune response by reducing the expression of pro-inflammatory cytokines (i.e., interferon-γ, TNF-α and IL-12) and adipocytokines (i.e., leptin) [63, 195] while it may also inhibit the activation of the redox-sensitive nuclear factor-kappa B (NF-κB) [63, 195], a principal mediator of the bidirectional interaction between oxidative stress and inflammation [69]. Moreover, the pivotal role of vitamin A in chronic inflammation is further supported by the fact that a deficit in vitamin A intake is associated with a pronounced pro-inflammatory state and inability to cope with pathogens, as well as with reduced phagocytic capacity of macrophages [63]. Vitamin C also reduces the production of pro-inflammatory cytokines through inhibition of the transcription factor NF-κB [63]. The anti-inflammatory effect of this micronutrient is further supported by a previous investigation where vitamin C intake was inversely associated with levels of CRP and tissue plasminogen activator (t-PA) antigen in elderly men [196]. Furthermore, vitamin C acts as a potent antioxidant, protecting cells from ROS-mediated oxidative damage, while it may also boost the synthesis of other antioxidants such as vitamin E [63]. Likewise, vitamin E is able to confer protection against oxidative stress by increasing the concentration of endogenous antioxidant enzymes, such as SOD, CAT, and GPX, and it also prevents oxidative damage in the cell membrane [63, 197]. Evidence based on human studies indicates that vitamin E supplementation in older adults improves immune function [63] and is associated with a lower concentration of pro-inflammatory mediators [198]. Collectively, these data corroborate the higher antioxidant vitamin intake observed in LSI in the present study, indicating that vitamins A, C, and E may contribute to the control of low-grade systemic inflammation in the elderly. By contrast, no differences were observed in selenium intake between LSI and ESI, although

selenium is also considered a micronutrient that may efficiently influence both innate and acquired immune function and may enhance the antioxidative defense system [63].

3.5. Conclusions

We found that elderly men with low levels of systemic inflammation are characterized by higher quality and quantity of habitual PA and ingested higher amounts of antioxidant vitamins A, C, and E through normal diet when compared to those with increased systemic inflammation. To the best of our knowledge, this is the first study to directly compare elderly men of low and increased low-grade systemic inflammation in respect to habitual PA and dietary profile. PA and antioxidant vitamin intake appear to be discriminant factors of inflammaging and healthy aging. Future research should further explore the cause and effect as well as the dose-response relationship between PA and/or antioxidant vitamins and inflammaging.

CHAPTER 4

Published as:

Δραγανίδης Δ, Πούλιος Α, Λάσχου Β, Τζατζάκης Θ, Παπανικολάου Κ, Κρητικός Σ, Δελή Χ, Γεωργακούλη Κ, Αυλωνίτη Α, Παππάς Α, Τζιαμούρτας Α, Φατούρος Ι. Σύγκριση ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή σε δείκτες δύναμης, οξειδωτικού στρες και φλεγμονής. *Αναζητήσεις στη Φυσική Αγωγή & τον Αθλητισμό*, 2018.

Comparison of elderly men with low and elevated chronic systemic inflammation in indices of strength, oxidative stress and inflammation

Draganidis D, Poulios A, Laschou V, Tzatzakis T, Papanikolaou K, Kritikos S, Deli CK, Georgakouli K, Avloniti A, Pappas A, Jamurtas AZ, Fatouros IG.

Abstract

The aim of the study was to examine differences among elderly men with low and elevated chronic systemic inflammation (CSI) in indices of the immune system, oxidative stress, antioxidant capacity and strength of the upper and lower body. A total of thirty-three, healthy, elderly men aged 65-75 years were included in the study and grouped as either ″low‟ CSI (LCSI: n=16; hs-CRP: < 1 mg/L) or ″elevated‟ CSI (ECSI: n=17; hs-CRP: > 1 mg/L) according to their serum levels of high-sensitivity CRP (hs-CRP). All participants were assessed for anthropometrics, body composition via Dual Emission X-ray Absorptiometry (DXA), handgrip strength and lower limb muscle strength on a leg extension machine. Blood samples were also collected for the determination of white blood cells (WBC), granulocytes (GRA), monocytes (MON) and lymphocytes (LYM) concentration as well as for the measurement of protein carbonyls (PC) and total antioxidant capacity (TAC) in serum. ECSI was characterized by almost a 4-fold greater hs-CRP value compared to LCSI (ECSI: hs-CRP = 0.6±0.1 mg/L vs LCSI: hs-CRP = 2.3±0.8 mg/L, p=0.00). ECSI and LCSI were comparable in terms of anthropometric characteristics, body mass index, fat percent, fat mass, fat free mass, lean body mass as well as in handgrip and lower limb muscle strength. Moreover, no differences were observed among groups in WBC, GRA, MON and LYM counts and in PC concentration. In contrast, significant differences observed between groups in TAC, with LCSI displaying a greater antioxidant capacity than ECSI by 60% ($p<0.05$). In conclusion, white blood cell counts and protein carbonyl concentration as well as muscle strength of the lower and upper body are not different among elderly men with ″low‟ CSI and ″elevated‟ CSI. However, those with low levels of CSI are characterized by a greater antioxidant capacity compared to their counterparts with elevated CSI.

Keywords: chronic systemic inflammation, white blood cells, protein carbonyl, muscle strength

Σύγκριση ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή σε δείκτες δύναμης, οξειδωτικού στρες και φλεγμονής

Δραγανίδης Δ, Πούλιος Α, Λάσχου Β, Τζατζάκης Θ, Παπανικολάου Κ, Κρητικός Σ, Δελή Χ, Γεωργακούλη Κ, Αυλωνίτη Α, Παππάς Α, Τζιαμούρτας Α, Φατούρος Ι

Περίληψη

Σκοπός της μελέτης ήταν να διερευνήσει την ύπαρξη διαφορών μεταξύ ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή (ΧΣΦ), σε δείκτες ανοσοποιητικού συστήματος, οξειδωτικού στρες, αντιοξειδωτικής ικανότητας και δύναμης άνω και κάτω άκρων. Στη μελέτη έλαβαν μέρος συνολικά 33 υγιείς, άνδρες εθελοντές ηλικίας 65-75 ετών, οι οποίοι κατηγοριοποιήθηκαν με βάση τα επίπεδα της C-αντιδρώσας πρωτεΐνης υψηλής ευαισθησίας (hs-CRP) σε δύο ομάδες: α) ομάδα χαμηλής ΧΣΦ (hs-CRP < 1 mg/L) και β) ομάδα υψηλής ΧΣΦ (hs-CRP > 1 mg/L). Όλοι οι συμμετέχοντες υποβλήθηκαν σε αξιολόγηση των σωματομετρικών τους χαρακτηριστικών, της σύστασης σώματος με μηχάνημα διπλής ενεργειακής απορρόφησης ακτινών Χ (DXA), της μέγιστης δύναμης χειρολαβής και των 10ΜΕ κάτω άκρων στο μηχάνημα εκτάσεις γονάτων. Επιπλέον υποβλήθηκαν σε αιμοληψία για τη βιοχημική αξιολόγηση των λευκοκυττάρων και των υποπληθυσμών τους, των πρωτεϊνικών καρβονυλίων, που είναι δείκτης οξείδωσης των πρωτεϊνών, και της συνολικής αντιοξειδωτικής ικανότητας στον ορό. Η μέση συγκέντρωση hs-CRP στην ομάδα υψηλής ΧΣΦ ήταν περίπου 4 φορές μεγαλύτερη από εκείνη στην ομάδα χαμηλής ΧΣΦ (υψηλής ΧΣΦ: hs-CRP=0.6±0.1 mg/L / χαμηλής ΧΣΦ: hs-CRP=2.3±0.8 mg/L, p=0.00). Οι δύο ομάδες δεν διέφεραν μεταξύ τους όσον αφορά τα σωματομετρικά χαρακτηριστικά, το δείκτη μάζας σώματος, το ποσοστό λίπους, τη λιπώδη μάζα, την άλιπη μάζα, τη μυϊκή μάζα καθώς και τη δύναμη χειρολαβής και κάτω άκρων. Επίσης δεν εντοπίστηκαν διαφορές μεταξύ των ομάδων στη συγκέντρωση λευκοκυττάρων και πρωτεϊνικών καρβονυλίων. Αντίθετα, σημαντικές διαφορές εντοπίστηκαν στη συνολική αντιοξειδωτική ικανότητα, με την ομάδα χαμηλής ΧΣΦ να παρουσιάζει κατά 60% υψηλότερη αντιοξειδωτική ικανότητα από την ομάδα υψηλής ΧΣΦ ($p<0.05$). Συμπερασματικά, η συγκέντρωση λευκοκυττάρων και πρωτεϊνικών καρβονυλίων αλλά και τα επίπεδα δύναμης δεν διαφοροποιούνται μεταξύ ηλικιωμένων ανδρών με χαμηλή ΧΣΦ και υψηλή ΧΣΦ. Ωστόσο, τα άτομα με χαμηλή ΧΣΦ χαρακτηρίζονται από υψηλότερη αντιοξειδωτική ικανότητα.

Λέξεις κλειδιά: χρόνια συστημική φλεγμονή, λευκοκύτταρα, πρωτεϊνικά καρβονύλια, μυϊκή δύναμη

4.1. Εισαγωγή

Σε υγιή άτομα τρίτης ηλικίας συχνά παρατηρείται η ανάπτυξη χρόνιας συστημικής φλεγμονής (ΧΣΦ), η οποία χαρακτηρίζεται από αυξημένα επίπεδα προ-φλεγμονώδη κυτταροκινών στην κυκλοφορία κατά 2 έως 3 φορές σε σχέση με τις φυσιολογικές τους τιμές [5]. Η εκδήλωση της ΧΣΦ αποδίδεται στη μακροχρόνια έκθεση του ανοσοποιητικού συστήματος σε αντιγόνα και στρεσογόνους παράγοντες (χημικούς, φυσικούς ή/και διατροφικούς) που σε συνδυασμό με την σημαντική αύξηση στο προσδόκιμο ζωής προάγουν την παρατεταμένη ενεργοποίηση του ανοσοποιητικού [1, 4]. Το φαινόμενο αυτό στη διεθνή βιβλιογραφία αναφέρεται ως ″inflammaging‟, βασισμένο στα δύο συνθετικά ″inflammation‟ (= φλεγμονή) και ″aging‟ (= γήρανση) [4], ενώ για τη διάγνωσή του οι κύριες μεταβλητές που αξιολογούνται είναι η συγκέντρωση των κυτταροκινών ιντερλευκίνη-6 (IL-6) και ιντερλευκίνη-1 (IL-1), του παράγοντα νέκρωσης όγκων-α (TNF-α) καθώς και η συγκέντρωση της C-αντιδρώσας πρωτεΐνης (CRP), στο πλάσμα [5]. Η ΧΣΦ αποτελεί μια απειλητική για την υγεία κατάσταση καθώς η ύπαρξή της έχει συνδεθεί με την παθογένεση αρκετών χρόνιων ασθενειών και γηριατρικών συνδρόμων όπως το μεταβολικό σύνδρομο, ο διαβήτης τύπου 2, η αθηροσκλήρωση, οι καρδιαγγειακές και νευροεκφυλιστικές παθήσεις και η χρόνια αποφρακτική πνευμονοπάθεια [8, 10, 11].

Σύμφωνα με τη θεωρεία της ″μοριακής φλεγμονής‟ στη γήρανση, η οποία αναπτύχθηκε στις αρχές της δεκαετίας του 2000 από τους Chung και συνεργάτες [83], τα κύτταρα του ανοσοποιητικού συνυπάρχουν σε έναν φαύλο κύκλο με δραστικά είδη οξυγόνου και αζώτου (ΔΕΟΑ) κατά τον οποίο μια σημαντική αύξηση στα τελευταία πυροδοτεί την αυξημένη κινητοποίηση στα πρώτα καταλήγοντας στην εμφάνιση της ΧΣΦ. Πιο συγκεκριμένα, η αυξημένη παραγωγή ΔΕΟΑ λόγω της γήρανσης σε συνδυασμό με τη μειωμένη αντιοξειδωτική ικανότητα στα άτομα αυτά, προκαλεί οξειδωτικό στρες και αυξημένο οξειδωτικό τραυματισμό σε μακρομόρια όπως το DNA, οι πρωτεΐνες και τα λιπίδια [7, 19]. Αυτό έχει ως αποτέλεσμα τη διαταραχή της οξειδοαναγωγικής ισορροπίας και την ενεργοποίηση του ρυθμιζόμενου από την οξειδοαναγωγική κατάσταση μεταγραφικού παράγοντα NF-κB (nuclear factor kappa B) μέσω του οποίου διεγείρεται η ενεργοποίηση προ-φλεγμονώδη μορίων της φλεγμονής, κυρίως των κυτταροκινών IL-6, IL-1, TNF-α και της CRP [32, 84]. Καθώς η διαδικασία της φλεγμονής εξελίσσεται, αυξάνεται η κινητοποίηση των μονοκυττάρων και μακροφάγων τα οποία με τη σειρά τους εκκρίνουν περαιτέρω ΔΕΟΑ παρατείνοντας με αυτόν τον τρόπο τον φαύλο αυτό

κύκλο [17]. Ωστόσο, η συγκεκριμένη θεωρεία βασίζεται κυρίως σε ερευνητικά δεδομένα προερχόμενα από μελέτες σε πειραματόζωα και κύτταρα, ενώ η διερεύνησή της σε ηλικιωμένα άτομα είναι ελλιπής. Επίσης άγνωστο παραμένει εάν τα ηλικιωμένα άτομα με υψηλή ΧΣΦ παρουσιάζουν διαφορετικά επίπεδα οξειδωτικού στρες, αντιοξειδωτικής ικανότητας και κυττάρων του ανοσοποιητικού από τα αντίστοιχα άτομα με χαμηλή ΧΣΦ.

Επιπλέον, ερευνητικά δεδομένα υποδεικνύουν ότι ηλικιωμένα άτομα με υψηλή ΧΣΦ παρουσιάζουν αυξημένο κίνδυνο για ταχύτερη απώλεια μυϊκής μάζας και δύναμης καθώς και για ανάπτυξη σαρκοπενίας [25-27, 69]. Συγκεκριμένα, σε μελέτη στην οποία συμμετείχαν συνολικά 986 άνδρες και γυναίκες με μέση ηλικία 74,6 έτη διαπιστώθηκε ότι όσοι χαρακτηρίζονταν από αυξημένα επίπεδα IL-6 και CRP παρουσίασαν κατά 2 έως 3 φορές μεγαλύτερο κίνδυνο απώλειας > 40% της μυϊκής τους μάζας τρία χρόνια αργότερα, συγκριτικά με εκείνους που χαρακτηρίζονταν από χαμηλές συγκεντρώσεις των συγκεκριμένων κυτταροκινών [27]. Παρομοίως, σε δείγμα 2177 ηλικιωμένων ανδρών και γυναικών (70-79 έτη) η υψηλότερη συγκέντρωση TNF-α στην αρχική μέτρηση συσχετίστηκε με μεγαλύτερη απώλεια μυϊκής μάζας και δύναμης πέντε χρόνια αργότερα [26]. Παρ' όλα αυτά, η σύγκριση μεταξύ ηλικιωμένων ατόμων με χαμηλή ΧΣΦ και υψηλή ΧΣΦ σε λειτουργικά τεστ δύναμης άνω και κάτω άκρων δεν έχει διερευνηθεί.

Συνεπώς, ο σκοπός της συγκεκριμένης μελέτης ήταν να συγκρίνει ηλικιωμένους άνδρες με χαμηλή ΧΣΦ και υψηλή ΧΣΦ: i) στους δείκτες του ανοσοποιητικού συστήματος: α) λευκοκύτταρα, β) κοκκιοκύτταρα, γ) μονοπύρηνα και δ) λεμφοκύτταρα, ii) στον δείκτη οξείδωσης των πρωτεϊνών: πρωτεϊνικά καρβονύλια, iii) στην συνολική αντιοξειδωτική ικανότητα του ορού, καθώς και iv) στη δύναμη άνω και κάτω άκρων μέσω αξιολόγησης της δύναμης χειρολαβής και της μέγιστης δύναμης στο μηχάνημα εκτάσεις γονάτων, αντίστοιχα.

4.2. Μέθοδος και Διαδικασία

4.2.1. Συμμετέχοντες

Για την εύρεση εθελοντών στην ευρύτερη περιοχή της Θεσσαλίας μοιράστηκαν ενημερωτικά φυλλάδια και πραγματοποιήθηκαν ενημερωτικές ομιλίες σε χώρους όπου συχνάζουν άτομα τρίτης ηλικίας (π.χ. Κ.Α.Π.Η., κέντρα άθλησης και αναψυχής). Η αρχική προϋπόθεση ήταν οι εθελοντές να είναι άνδρες ηλικίας 65-75 ετών. Περίπου 55-60 εθελοντές προσήλθαν στη Σ.Ε.Φ.Α.Α. του Π.Θ., στο χώρο του SMART Lab, όπου συμπλήρωσαν έναν έντυπο ιατρικού ιστορικού και εξετάστηκαν επίσης από ιατρό. Προκειμένου να συμπεριληφθούν στη μελέτη, οι εθελοντές έπρεπε να πληρούν τα παρακάτω κριτήρια: α) να είναι μη καπνιστές, β) να είναι ανεξάρτητοι στην καθημερινότητά τους, γ) να μην πάσχουν από οποιαδήποτε χρόνια πάθηση

(π.χ. μεταβολική, καρδιαγγειακή, νευρολογική, αναπνευστική ή νεφρική), δ) να μην πάσχουν από φλεγμονώδεις παθήσεις (π.χ. οστεοαρθρίτιδα, ρευματοειδής αρθρίτιδα), ε) να μην πάσχουν από διαβήτη τύπου 2 και ζ) να μην χρησιμοποιούν ή έχουν χρησιμοποιήσει πρόσφατα αντιβιοτικά ή άλλου είδους φαρμακευτικά σκευάσματα τα οποία θα μπορούσαν να επηρεάσουν τα επίπεδα συστημικής φλεγμονής. Ακολούθως, 33 εθελοντές οι οποίοι πληρούσαν τα παραπάνω κριτήρια συμπεριλήφθηκαν τελικά στη μελέτη και πραγματοποίησαν τρεις ακόμη επισκέψεις. Στην πρώτη επίσκεψη, υποβλήθηκαν σε αξιολόγηση των σωματομετρικών τους χαρακτηριστικών, της σύστασης σώματος και της δύναμης άνω και κάτω άκρων. Στις επόμενες δύο διαδοχικές επισκέψεις (μια εβδομάδα μετά την πρώτη επίσκεψη), οι εθελοντές προσέρχονταν νωρίς το πρωί, μετά από ολονύκτια νηστεία και υποβάλλονταν σε μια αιμοληψία (~8-10 ml) για τον προσδιορισμό των δεικτών του ανοσοποιητικού, του οξειδωτικού στρες, της αντιοξειδωτικής ικανότητας και του επιπέδου ΧΣΦ. Η ΧΣΦ αξιολογήθηκε με βάση τις τιμές της hs-CRP (high-sensitivity CRP), όπου ο μέσος όρος των τιμών της hs-CRP από τις δυο μετρήσεις χρησιμοποιήθηκε ως η τελική τιμή για το κάθε άτομο, και η κατηγοριοποίηση των εθελοντών σε ″χαμηλή" ΧΣΦ και ″υψηλή" ΧΣΦ έγινε ως εξής: ″χαμηλή" ΧΣΦ = hs-CRP < 1 mg/L και ″υψηλή‟ ΧΣΦ = hs-CRP > 1 mg/L, σύμφωνα με προηγούμενη μελέτη [199]. Οι εθελοντές ενημερώθηκαν πλήρως για τον σκοπό της συγκεκριμένης μελέτης αλλά και για τα πιθανά οφέλη από τη συμμετοχής τους και στη συνέχεια υπέγραψαν έντυπο συναίνεσης. Η μελέτη έλαβε έγκριση από την Εσωτερική Επιτροπή Δεοντολογίας του Πανεπιστημίου Θεσσαλίας.

4.2.2. Διαδικασία μέτρησης

Σωματομετρικά χαρακτηριστικά και σύσταση σώματος: Το σωματικό βάρος και ύψος των συμμετεχόντων μετρήθηκε με ακρίβεια μισού κιλού (0,5 kg) και μισού εκατοστού (0,5 cm) αντίστοιχα, σε μηχανικό ζυγό με αναστημόμετρο (Beam Balance-Stadiometer, SECA, Vogel & Halke, Hamburg, Germany). Οι εξεταζόμενοι φορώντας ελαφρύ ρουχισμό και χωρίς παπούτσια πραγματοποίησαν την μέτρηση από όρθια θέση στο κέντρο του ζυγού, έχοντας τις φτέρνες ενωμένες και τα πέλματα να σχηματίζουν γωνία περίπου 60^{o}, σύμφωνα με προηγούμενη μελέτη [171]. Η σύσταση σώματος αξιολογήθηκε σε μηχάνημα διπλής ενεργειακής απορρόφησης ακτινών Χ (DXA, GE Healthcare, Lunar DPX NT, Diegem, Belgium), με τον εξεταζόμενο σε ξαπλωτή θέση, όπως έχει περιγραφεί σε προηγούμενη μελέτη [172].

Δύναμη άνω και κάτω άκρων: Για την αξιολόγηση της δύναμης άνω άκρων μετρήθηκε η μέγιστη δύναμη χειρολαβής με τη χρήση φορητού υδραυλικού χειροδυναμόμετρου Jamar (Jamar 5030J1, Jamar Technologies, Horsham, Pennsylvania, USA). Οι δοκιμαζόμενοι εκτέλεσαν την μέτρηση από καθιστή θέση, με τον αγκώνα να σχηματίζει γωνία 90^{o} και τον καρπό σε ουδέτερη

θέση κρατώντας το δυναμόμετρο στη θέση 2 [200]. Από αυτή τη θέση πραγματοποιήθηκαν 3 προσπάθειες σε κάθε χέρι με διάλειμμα 60 δευτερολέπτων, όπου στην κάθε προσπάθεια εκτελέστηκε μέγιστη ισομετρική σύσπαση για 5 δευτερόλεπτα. Ως τελική επίδοση καταγράφηκε η μεγαλύτερη τιμή σε χιλιόγραμμα (kg). Η αξιολόγηση της δύναμης των κάτω άκρων εκτιμήθηκε μέσω της εκτέλεσης 10 μέγιστων επαναλήψεων (10 ΜΕ) στο μηχάνημα έκτασης γονάτων. Οι εξεταζόμενοι, μετά από κατάλληλη προθέρμανση εκτελούσαν το πρώτο σετ και σε περίπτωση που ήταν σε θέση να εκτελέσουν περισσότερες από 10 επαναλήψεις, ο εξεταστής τους σταματούσε και τους έδινε διάλειμμα 2 λεπτών. Έπειτα αυξάνονταν τα κιλά και επιχειρούσαν δεύτερη προσπάθεια. Αν και πάλι ήταν σε θέση να εκτελέσουν περισσότερες από 10 επαναλήψεις, ο εξεταστής επαναλάμβανε την ίδια διαδικασία. Η διαδικασία αυτή συνεχιζόταν μέχρι οι εξεταζόμενοι να εκτελέσουν 10 μέγιστες επαναλήψεις (περίπου 3-4 σετ).

Αιμοληψίες και διαχείριση των δειγμάτων: Σε κάθε αιμοληψία λαμβάνονταν 8-10 ml αρτηριοφλεβικού αίματος από τη μεσοβασιλική φλέβα και ενώ ο εξεταζόμενος βρισκόταν σε καθιστή θέση. Περίπου 2 ml διοχετεύονταν σε σωλήνα με αντιπηκτικό (EDTA) και στη συνέχεια τοποθετούνταν στον αυτόματο αναλυτή αίματος (Mythic 18, Orphee SA, Geneva, Switzerland) για να πραγματοποιηθεί η γενική ανάλυση αίματος. Τα υπόλοιπα 6-8 ml μοιράζονταν σε δυο σωληνάρια με κενό αέρος, τα οποία παρέμεναν για 20 λεπτά σε θερμοκρασία δωματίου και στη συνέχεια φυγοκεντρούνταν στις 3500 στροφές για 10 λεπτά στους 4^{o}C. Αμέσως μετά τη φυγοκέντρηση το υπερκείμενο υγρό (ορός) συλλέγονταν και μοιραζόταν σε σωληνάκια eppendorf και στη συνέχεια αποθηκεύονταν στους -80^{o}C, για την ανάλυση των πρωτεϊνικών καρβονυλίων, της συνολικής αντιοξειδωτικής ικανότητας και της hs-CRP.

Πρωτεϊνικά καρβονύλια: Για τη μέτρηση των πρωτεϊνικών καρβονυλίων πραγματοποιήθηκε η βιοχημική αντίδραση του κάθε δείγματος με 2,4- dinitrophenylhydrazine (DNPH) που επέφερε τη μετατροπή του σε 2,4-dinitrophenylhydrazone (DNP-hydrazone). Ο προσδιορισμός της συγκέντρωσης του 2,4- DNP-hydrazone, πραγματοποιήθηκε με τη μέτρηση της απορρόφησης του κάθε δείγματος στο φωτόμετρο, στα 375 nm. Η τελική συγκέντρωση των πρωτεϊνικών καρβονυλίων υπολογιζόταν σύμφωνα με την εξίσωση: PC (nmol/mL) = ($Abs_{δείγμα}$ – $Abs_{τυφλό}$) / 0,022 × 1000/50 [201].

Συνολική αντιοξειδωτική ικανότητα: Η συνολική αντιοξειδωτική ικανότητα του ορού αξιολογήθηκε μέσω της βιοχημικής αντίδρασης των αντιοξειδωτικών συστατικών του ορού με τη ρίζα 1,1-diphenyl-2-picrylhydrazyl (DPPH), κατά την οποία τα αντιοξειδωτικά δρουν ως δότες υδρογόνου προκαλώντας την αναγωγή της συγκεκριμένης ρίζας, μειώνοντας με αυτό τον τρόπο τη συγκέντρωσή της. Η τελική συγκέντρωση της ρίζας DPPH υπολογίστηκε μετρώντας την απορρόφηση του δείγματος στα 520 nm. Τα αποτελέσματα εκφράστηκαν ως μmol DPPH που

καθαρίστηκαν ανά ml ορού, χρησιμοποιώντας την εξίσωση: [(%A_{bs} μείωσης / 100) x 50 x 50] / 100 [201].

hs-CRP: Πραγματοποιήθηκε ποσοτική ανάλυση της hs-CRP στον ορό, με τη μέθοδο της θολοσιμετρικής ανάλυσης ενισχυμένης με τη χρήση σωματιδίων λάτεξ (CRP LX High Sensitive, Cobas®), σε αναλυτή Cobas Integra 400 plus (Roche). Το κατώτατο όριο ανίχνευσης της ανάλυσης ήταν 0,1 mg/L και ο συντελεστής διακύμανσης μεταξύ των αναλύσεων ήταν μία τυπική απόκλιση (1 SD) [199].

4.2.3. Στατιστική ανάλυση

Ο έλεγχος της κανονικότητας των δεδομένων πραγματοποιήθηκε με βάση το Shapiro-Wilk τεστ. Για τη διερεύνηση των διαφορών μεταξύ των δυο ομάδων (χαμηλή ΧΣΦ και υψηλή ΧΣΦ) στις εξαρτημένες μεταβλητές χρησιμοποιήθηκε το T-Test για ανεξάρτητα δείγματα. Το μέγεθος της επίδρασης (ΜτΕ) καθώς και τα διαστήματα εμπιστοσύνης (ΔΕ) για τις εξαρτημένες μεταβλητές υπολογίστηκαν με τη μέθοδο του Hedge's g. Το ΜτΕ χαρακτηρίστηκε ως κανένα, μικρό, μεσαίου μεγέθους και μεγάλο για τιμές 0.00-0.19, 0.20-0.49, 0.50-0.79 και ≥ 0.8, αντίστοιχα. Το επίπεδο σημαντικότητας ορίστηκε στο $p<0.05$. Όλα τα δεδομένα παρουσιάζονται ως μέσος όρος ± τυπική απόκλιση. Για τις στατιστικές αναλύσεις χρησιμοποιήθηκε το στατιστικό πακέτο SPSS 20.0 (IBM SPSS Statistics).

4.3. Αποτελέσματα

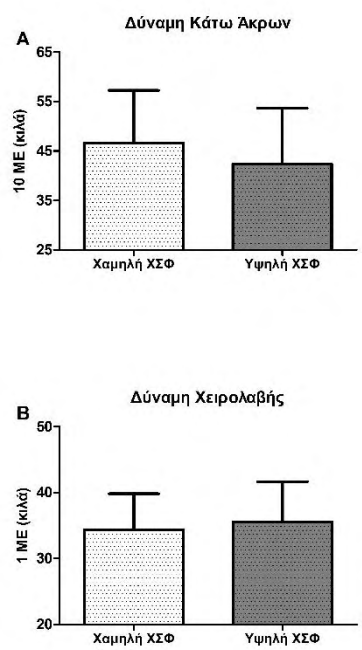
Τα περιγραφικά χαρακτηριστικά των δύο ομάδων παρουσιάζονται στον Πίνακα 4.1. Οι δύο ομάδες δεν διέφεραν μεταξύ τους στα σωματομετρικά χαρακτηριστικά και σε παραμέτρους της σύστασης σώματος. Ωστόσο, σημαντική ήταν η διαφορά τους στα επίπεδα ΧΣΦ με την ομάδα υψηλής ΧΣΦ να παρουσιάζει σχεδόν κατά 3 φορές υψηλότερες τιμές hs-CRP συγκριτικά με την ομάδα χαμηλής ΧΣΦ (χαμηλή ΧΣΦ: 0.6±0.1 / υψηλή ΧΣΦ: 2.3±0.8, $p=0.00$, ΜτΕ=2.86, ΔΕ=-3.84, -1.89).

Πίνακας 4.1. Περιγραφικά χαρακτηριστικά των δύο ομάδων.

Παράμετρος	**Χαμηλή ΧΣΦ** (N=16)	**Υψηλή ΧΣΦ** (N=17)
Ηλικία (έτη)	69.1 ± 2.8	68.8 ± 2.9
Σωματικό ύψος (μ)	1.70 ± 0.07	1.72 ± 0.05
Σωματικό βάρος (κιλά)	81.4 ± 7.6	84.7 ± 5.5
ΔΜΣ (κιλά/$μ^2$)	26.92 ± 2.77	27.86 ± 2.51
Λιπώδης μάζα (κιλά)	23.20 ± 6.26	25.54 ± 3.14
Λίπος (%)	29.41 ± 4.14	31.25 ± 1.72
Άλιπη μάζα (κιλά)	56.89 ± 4.26	57.70 ± 4.35
Μυϊκή μάζα (κιλά)	53.86 ± 4.13	55.12 ± 5.11
Μυϊκή μάζα άκρων (κιλά)	23.32 ± 2.43	24.15 ± 1.83
Δείκτης μυϊκής μάζας (κιλά/$μ^2$)	8.03 ± 0.78	8.11 ± 0.64
hs-CRP (mg/L)	0.6 ± 0.1	2.3 ± 0.8*****

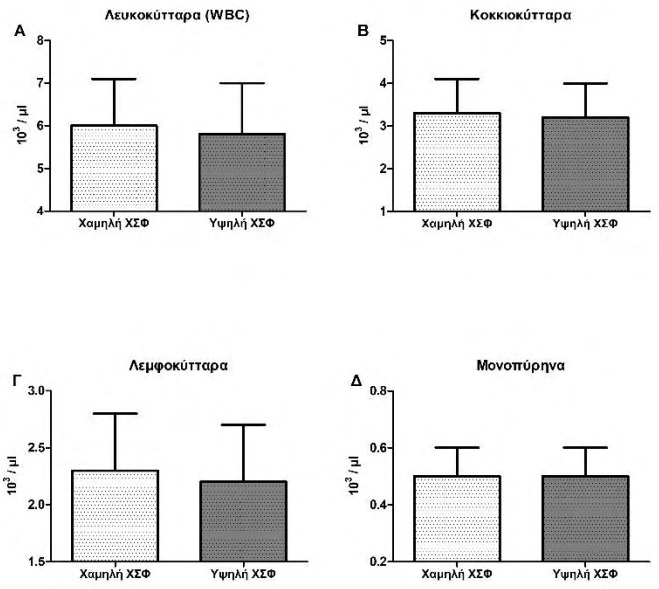
***** : Υποδηλώνει σημαντική διαφορά μεταξύ των ομάδων, $p < 0.01$.

Τα αποτελέσματα έδειξαν ότι στην αξιολόγηση της δύναμης, τόσο στη μέγιστη δύναμη χειρολαβής (t(30)=1.139, p>0.05) όσο και στις 10 ΜΕ κάτω άκρων στο μηχάνημα έκτασης γονάτων (t(30)=-0.526, p>0.05), δεν υπήρξαν στατιστικά σημαντικές διαφορές μεταξύ των δυο ομάδων (Σχήμα 4.1).



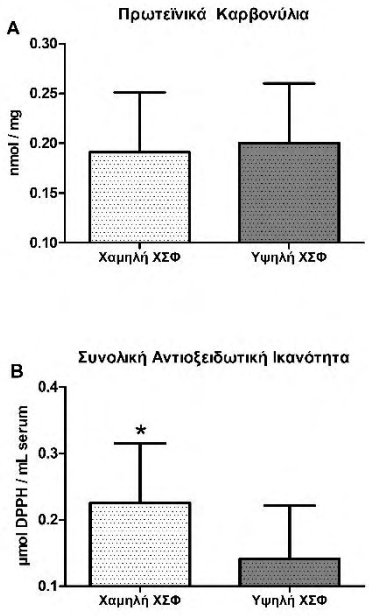
Σχήμα 4.1. 10ΜΕ κάτω άκρων στο μηχάνημα εκτάσεις γονάτων (1Α) και μέγιστη δύναμη χειρολαβής (1Β) στις δυο ομάδες.

Επίσης από τη σύγκριση των ομάδων χαμηλή ΧΣΦ και υψηλή ΧΣΦ ως προς του δείκτες του ανοσοποιητικού συστήματος διαπιστώθηκε ότι δεν υπήρχε στατιστικά σημαντική διαφορά μεταξύ τους στα επίπεδα λευκοκυττάρων (t(30)=-1.088, p>0.05), κοκκιοκυττάρων (t(30)=-0.963, p>0.05), μονοπύρηνων (t(30)=-0.916, p>0.05) και λεμφοκυττάρων (t(30)=-0.433, p>0.05) (Σχήμα 4.2).



Σχήμα 4.2. Συστημικά επίπεδα Λευκοκυττάρων (2Α), Κοκκιοκυττάρων (2Β), Λεμφοκυττάρων (2Γ) και Μονοπύρηνων (2Δ) στις δύο ομάδες.

Από τη σύγκριση των δύο ομάδων σε δείκτες οξειδωτικό στρες και αντιοξειδωτικής ικανότητας, προέκυψε ότι η συγκέντρωση πρωτεϊνικών καρβονυλίων δεν διέφερε μεταξύ των ομάδων ($t(28)=0.334$, $p>0.05$), ωστόσο, οι δυο ομάδες παρουσίασαν σημαντική διαφορά στη συνολική αντιοξειδωτική ικανότητα του ορού, με την ομάδα χαμηλή ΧΣΦ να παρουσιάζει υψηλότερη αντιοξειδωτική ικανότητα από τη ομάδα υψηλή ΧΣΦ ($t(28)=-2.275$, $p=0.03$, ΜτΕ=0.96, ΔΕ=0.24, 1.69) (Σχήμα 4.3).



Σχήμα 4.3. Συγκέντρωση πρωτεϊνικών καρβονυλίων (3Α) και συνολική αντιοξειδωτική ικανότητα (3Β) στις δυο ομάδες.

4.4. Συζήτηση

Στη συγκεκριμένη μελέτη εξετάστηκε η ύπαρξη διαφορών μεταξύ ηλικιωμένων ανδρών με χαμηλή και υψηλή ΧΣΦ, σε δείκτες του ανοσοποιητικού συστήματος, οξειδωτικού στρες, αντιοξειδωτικής ικανότητας και της δύναμης των άνω και κάτω άκρων. Για το σκοπό αυτό πραγματοποιήθηκε βιοχημική αξιολόγηση για τον προσδιορισμό της συγκέντρωσης των λευκοκυττάρων, των κοκκιοκυττάρων, των μονοπύρηνων και των λεμφοκυττάρων στην κυκλοφορία καθώς και της συγκέντρωσης πρωτεϊνικών καρβονυλίων και της συνολικής αντιοξειδωτικής ικανότητας στον ορό. Όσον αφορά τη δύναμη, αξιολογήθηκε η μέγιστη δύναμη χειρολαβής και οι 10ΜΕ στα κάτω άκρα στο μηχάνημα έκτασης γονάτων. Τα αποτελέσματα της μελέτης υποδεικνύουν ότι ηλικιωμένα άτομα με χαμηλή ΧΣΦ και υψηλή ΧΣΦ παρουσιάζουν παρόμοια συγκέντρωση λευκοκυττάρων, οξειδωτικού στρες και δύναμης, ωστόσο εκείνοι με χαμηλή ΧΣΦ χαρακτηρίζονται από υψηλότερη αντιοξειδωτική ικανότητα.

Οι συμμετέχοντες στη παρούσα μελέτη διαχωρίστηκαν στις δυο ομάδες, χαμηλή ΧΣΦ και υψηλή ΧΣΦ, σύμφωνα με τα επίπεδα της hs-CRP στον ορό. Ο συγκεκριμένος δείκτης συστημικής φλεγμονής θεωρείται ένας έγκυρος και αξιόπιστος δείκτης ΧΣΦ [179] και έχει χρησιμοποιηθεί σε αρκετές μελέτες προκειμένου να κατηγοριοποιήσει ηλικιωμένα άτομα με βάση τα επίπεδα φλεγμονής [170, 199]. Οι δυο ομάδες παρουσίασαν παρόμοια σωματομετρικά χαρακτηριστικά και σύσταση σώματος, γεγονός που υποδηλώνει ότι το δείγμα ήταν αρκετά ομοιογενές και διέφερε μόνο ως προς τα επίπεδα ΧΣΦ. Επιπλέον, σύμφωνα με τα κριτήρια του Παγκόσμιου Οργανισμού Υγείας (WHO) για την παχυσαρκία και με βάση τις τιμές του ΔΜΣ των συμμετεχόντων, τα άτομα των δυο ομάδων κατατάσσονται ως μη-παχύσαρκα, κάτι που είναι σημαντικό για την ερμηνεία των αποτελεσμάτων της συγκεκριμένης μελέτης. Η ύπαρξη παχυσαρκίας έχει συνδεθεί με την ανάπτυξη ΧΣΦ, η οποία όμως οφείλεται κατά κύριο λόγο στο λιπώδη ιστό (μεταβολική φλεγμονή) [8, 16], και όχι αποκλειστικά στη γήρανση που είναι και ο σκοπός της παρούσας μελέτης.

Μέχρι σήμερα, η αξιολόγηση της ύπαρξης ΧΣΦ αλλά και η σύγκριση των επιπέδων της μεταξύ ηλικιωμένων ατόμων βασίζεται κατά κύριο λόγο στη μέτρηση των κυτταροκινών IL-1, IL-6, TNF-α και της C-αντιδρώσας πρωτεΐνης [25-27, 170, 199]. Δεν υπάρχουν μελέτες που να εξετάζουν εάν η αύξηση στα επίπεδα των κυτταροκινών συνοδεύεται από αυξημένη συγκέντρωση λευκοκυττάρων και των υποπληθυσμών τους σε ηλικιωμένα άτομα με ΧΣΦ. Η κινητοποίηση των λευκοκυττάρων σε συνθήκες οξείας φλεγμονής, όπως η άσηπτη φλεγμονή που προκαλείται από την άσκηση, είναι άμεση και η συγκέντρωσή τους στην κυκλοφορία παραμένει αυξημένη για 24-48 ώρες [202]. Τα κοκκιοκύτταρα, τα οποία αποτελούνται από ουδετερόφιλα, βασεόφιλα και ηωσινόφιλα, αυξάνονται επίσης αμέσως μετά από άσκηση λόγω της ύπαρξης

άσηπτης φλεγμονής και παραμένουν αυξημένα έως και 48 ώρες μετά [203]. Ωστόσο, στην παρούσα μελέτη δεν παρατηρήθηκαν διαφορές στα λευκοκύτταρα και τους υποπληθυσμούς τους μεταξύ των ομάδων χαμηλή ΧΣΦ και υψηλή ΧΣΦ, υποδεικνύοντας ότι τα συγκεκριμένα κύτταρα του ανοσοποιητικού παρουσιάζουν διαφορετικό μοτίβο ενεργοποίησης σε συνθήκες οξείας φλεγμονής και ΧΣΦ.

Η εκδήλωση ΧΣΦ σε άτομα τρίτης ηλικίας έχει αποδοθεί στη μειωμένη αντιοξειδωτική ικανότητα και στην αυξημένη παραγωγή ΔΕΟΑ που έχουν ως αποτέλεσμα την πρόκληση οξειδωτικού στρες και οξειδωτικού τραυματισμού σε πρωτεΐνες, λιπίδια και DNA [7, 19]. Η παρατεταμένη αύξηση στα επίπεδα οξειδωτικού στρες οδηγεί σε διαταραχή της οξειδοαναγωγικής ισορροπίας και την επακόλουθη ενεργοποίηση του μεταγραφικού παράγοντα NF-κΒ ο οποίος με τη σειρά του διεγείρει την ενεργοποίηση προ-φλεγμονώδη κυτταροκινών και της CRP [32, 84]. Με αυτό τον τρόπο εξελίσσεται η διαδικασία της φλεγμονής, κατά την οποία τα μονοκύτταρα και μακροφάγα εκκρίνουν περαιτέρω ΔΕΟΑ δημιουργώντας έτσι έναν φαύλο κύκλο που έχει ως αποτέλεσμα την μακροχρόνια εκδήλωση φλεγμονής [17, 83]. Στη συγκεκριμένη μελέτη η συγκέντρωση πρωτεϊνικών καρβονυλίων, που αποτελεί δείκτη οξείδωσης των πρωτεϊνών, ήταν παρόμοια στα άτομα με χαμηλή ΧΣΦ και υψηλή ΧΣΦ. Ωστόσο, οι δυο ομάδες διέφεραν σημαντικά μεταξύ τους όσον αφορά τη συνολική αντιοξειδωτική ικανότητα, με την ομάδα χαμηλής ΧΣΦ να παρουσιάζει κατά 60% μεγαλύτερη αντιοξειδωτική ικανότητα συγκριτικά με την ομάδα υψηλής ΧΣΦ. Το συγκεκριμένο εύρημα είναι σε συμφωνία με πρόσφατη μελέτη η οποία έδειξε ότι τα ηλικιωμένα άτομα με χαμηλή ΧΣΦ προσλαμβάνουν μεγαλύτερες ποσότητες αντιοξειδωτικών βιταμινών μέσω της τροφής και είναι περισσότερο δραστήρια κατά τη διάρκεια της ημέρας, συγκριτικά με άτομα ίδιας ηλικίας με υψηλή ΧΣΦ [199]. Επομένως, τα παραπάνω ευρήματα αποδεικνύουν πως η διατήρηση της αντιοξειδωτικής ικανότητας, μέσω επαρκούς πρόσληψης διαιτητικών αντιοξειδωτικών και φυσικής δραστηριότητας, μπορεί να προλάβει την εκδήλωση ΧΣΦ στα άτομα τρίτης ηλικίας.

Προηγούμενες μελέτες έχουν συσχετίσει τα επίπεδα συστημικής φλεγμονής σε ηλικιωμένα άτομα με την απώλεια μυϊκής δύναμης [25-27]. Πιο συγκεκριμένα, άτομα με αυξημένα επίπεδα CRP ή/και προφλεγμονώδη κυτταροκινών όπως η IL-6 και ο TNF-α, παρουσίασαν κατά 2 έως 3 φορές μεγαλύτερη απώλεια δύναμης στα επόμενα 2-5 χρόνια, τόσο στη μέγιστη δύναμη χειρολαβής [27] όσο και στη δύναμη κάτω άκρων [25, 26]. Στην παρούσα μελέτη ωστόσο, συγκρίνοντας τη μέγιστη δύναμη χειρολαβής και τις 10ΜΕ κάτω άκρων μεταξύ των ομάδων χαμηλή ΧΣΦ και υψηλή ΧΣΦ, δεν εντοπίστηκαν στατιστικά σημαντικές διαφορές. Η απώλεια μυϊκής δύναμης ως αποτέλεσμα της ΧΣΦ φαίνεται πως επέρχεται μέσω της προκαλούμενης από τη ΧΣΦ απώλεια μυϊκής μάζας [25, 69]. Σύμφωνα με πρόσφατη ανασκοπική μελέτη, η ΧΣΦ

μπορεί να οδηγήσει σε απώλεια μυϊκής μάζας και λειτουργικής ικανότητας, είτε μέσω αύξησης της πρωτεόλυσης ενεργοποιώντας το σύμπλοκο του πρωτεασώματος, είτε μέσω μείωσης της δυνατότητας ενεργοποίησης των σηματοδοτικών πρωτεϊνών που επάγουν την πρωτεϊνοσύνθεση στο μυ [69]. Επομένως, το γεγονός ότι οι δυο ομάδες στη παρούσα μελέτη δεν διέφεραν ως προς τα επίπεδα δύναμης άνω και κάτω άκρων, μπορεί να αποδοθεί στο ότι δεν εντοπίστηκαν μεταξύ τους διαφορές στη μυϊκή μάζα.

4.5. Συμπεράσματα

Συμπερασματικά, τα ευρήματα της παρούσας μελέτης υποδεικνύουν ότι ηλικιωμένα άτομα με χαμηλή ΧΣΦ και υψηλή ΧΣΦ δεν διαφέρουν μεταξύ τους ως προς τα συστημικά επίπεδα λευκοκυττάρων και οξείδωσης των πρωτεϊνών, ωστόσο οι συμμετέχοντες με χαμηλή ΧΣΦ παρουσιάζουν μεγαλύτερη αντιοξειδωτική ικανότητα από εκείνους με υψηλή ΧΣΦ. Η συγκέντρωση των λευκοκυττάρων και των υποπληθυσμών τους στην κυκλοφορία φαίνεται ότι δεν επηρεάζεται από την ανάπτυξη ΧΣΦ. Αντίθετα, η καλύτερη αντιοξειδωτική ικανότητα εμφανίζεται ως αποτρεπτικός παράγοντας για την εκδήλωση ΧΣΦ. Επιπλέον, άτομα με χαμηλή και υψηλή ΧΣΦ δεν παρουσιάζουν διαφορές στη δύναμη άνω και κάτω άκρων εφόσον παρουσιάζουν παρόμοια επίπεδα μυϊκής μάζας.

4.6. Σημασία για την ποιότητα ζωής

Η εκδήλωση χρόνιας συστημικής φλεγμονής στα άτομα τρίτης ηλικίας είναι μια απειλητική για την υγεία κατάσταση καθότι συνδέεται άμεσα με την παθογένεση αρκετών χρόνιων ασθενειών και γηριατρικών συνδρόμων, αλλά και με την απώλεια μυϊκής μάζας και λειτουργικής ικανότητας. Όλα αυτά συνεπάγονται την επιδείνωση της ποιότητας ζωής αυτών των ατόμων έχοντας παράλληλα σοβαρό αντίκτυπο στην οικονομική τους κατάσταση καθότι αυξάνονται σημαντικά τα έξοδα νοσηλείας. Η διατήρηση της αντιοξειδωτικής ικανότητας με την αύξηση της ηλικίας είναι ένας σημαντικός παράγοντας που μπορεί να προλάβει την εκδήλωση χρόνιας συστημικής φλεγμονής και επιτυγχάνεται μέσω επαρκούς πρόσληψης διαιτητικών αντιοξειδωτικών και φυσικής δραστηριότητας.

CHAPTER 5

Unpublished data:

Results related to inflammaging, proteasome-mediated proteolysis and anabolic resistance

5.1. Inflammaging and proteolysis via the ubiquitin-proteasome system

5.1.1. Variables measured in skeletal muscle

Proteasome activity and protein levels: The proteasome is the main proteolytic system in skeletal muscle composed by 7 α- and 7 β-subunits. Three of the proteasome β-subunits, β1, β2 and β5, and the corresponding immunoproteasome βi-subunits, β1i, β2i and β5i, are responsible for the proteasome proteolytic activities caspase-like (C-L), trypsin-like (T-L) and chymotrypsin-like (CT-L), respectively [204]. The proteolytic activities T-L and CT-L were measured though a specific proteasome peptidase assay while protein expression levels of proteasome (β5) and immunoproteasome (β1i, β2i and β5i) subunits were measured using immunoblot analysis (western blotting) as described previously [172].

Protein oxidation: Oxidative protein modification was evaluated by measuring the formation of protein carbonyl and 3-nitrotyrosine groups in skeletal muscle. Protein carbonyl groups were detected via immunoblot analysis (western blotting) using the OxyBlot kit, whereas 3-nitrotyrosine groups were detected via immunohistochemistry.

Phosphorylated IKKα/β: The IκB kinase (IKK) is a protein complex composed by two catalytic subunits α and β (IKKα and IKKβ) and a regulatory subunit γ (IKKγ) [32]. Phosphorylated IKKα/β (activated) results in degradation of the inhibitors of NF-κB (IκBs) and activation of the NF-κB [32]. Phosphorylated levels of IKKα/β were detected using immunohistochemistry.

Nuclear-erythroid factor 2 (Nrf2): Nrf2 is a transcription factor that mediates adaptive responses to cellular stress and regulates the antioxidant response system protecting cells from oxidative stress [204]. Upon activation, Nrf2 is translocated into the nucleus and results in proteasome activation [204]. Nuclei levels of Nrf2 were detected using immunohistochemistry.

Heme Oxygenase 1 (HO1): HO1 is positively regulated by Nrf2 under conditions of oxidative stress, protecting against oxidative damage and ensuring cell survival [205]. Protein expression of HO1 was measured using immunoblotting (western blotting).

5.1.2. Results

Proteasome activity and protein levels: At baseline, the two groups differed significantly in CT-L proteasome activity with HSI eliciting higher activity than LSI by almost 40% (HSI: 256.9 ± 86.5 UF vs LSI: 185.6 ± 51.0 UF; $P = 0.044$; ES: 0.99; 95% CI: 0.00/1.97) (Figure 5.1A). Moreover, a Pearson correlation analysis revealed that baseline levels of CT-L activity are significantly correlated with levels of hs-CRP ($r = 0.576$; $P = 0.010$) (Figure 5.2A). At 3h

following protein supplementation, CT-L activity increased in both HSI (P = 0.015; ES: -1.18; 95% CI: -2.24/-0.11) and LSI (P = 0.001; ES: -1.74; 95% CI: -2.77/-0.71) by 44% and 86% respectively (Figure 5.1A). Although the rise in CT-L activity was greater in LSI by 42%, no statistically meaningful differences observed between groups. By performing a longitudinal analysis of both groups we observed that the rise in CT-L activity following exercise was inversely correlated with levels of CT-L activity at baseline (r = -0.616; P = 0.006) (Figure 5.2B).

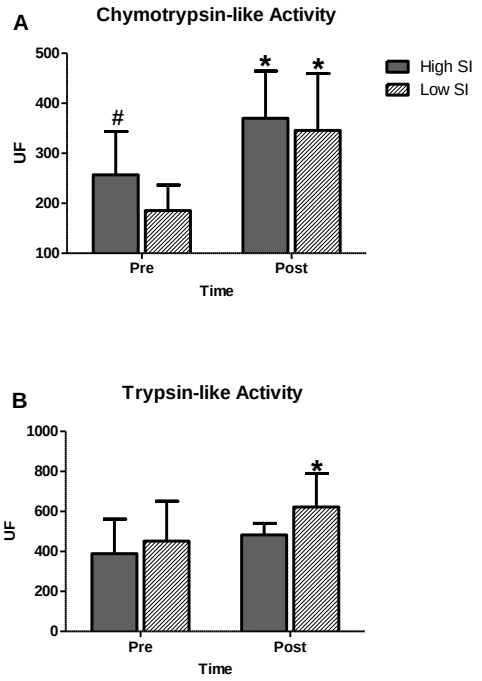


Figure 5.1. Changes in Chymotrypsin-like (**A**) and trypsin-like (**B**) proteasome activity in HIS and LSI.

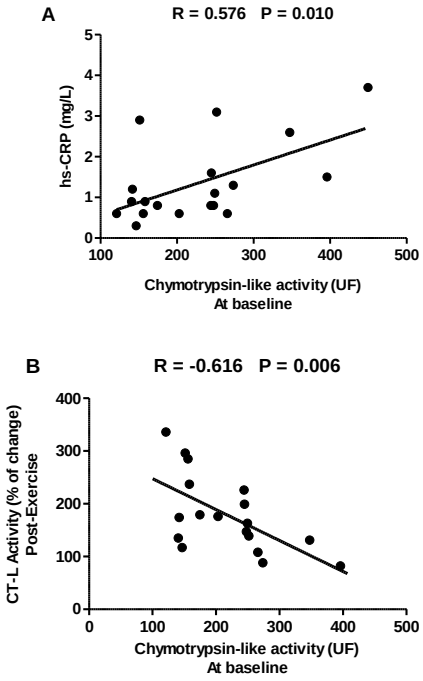


Figure 5.2. The correlation of baseline Chymotrypsin-like activity with hs-CRP levels (**A**) and percent change of chymotrypsin-like activity following protein ingestion (**B**).

No differences observed in T-L proteasome activity between HSI and LSI (HIS: 388.9 ± 173.0 UF vs LSI: 452.4 ± 198.8 UF) at baseline (Figure 5.1B). T-L activity increased by 24% in HSI and by 38% in LSI at 3h following protein supplementation, however only the rise observed in LSI was statistically meaningful (P = 0.049; ES: -0.88; 95% CI: -1.85/0.09) (Figure 5.1B).

Protein expression levels of the catalytic immunoproteasome subunits β1i, β2i and β5i, and the proteasomal β5 subunit remained unaltered following protein supplementation in both groups (Figure 5.3). No group differences observed at any time point.

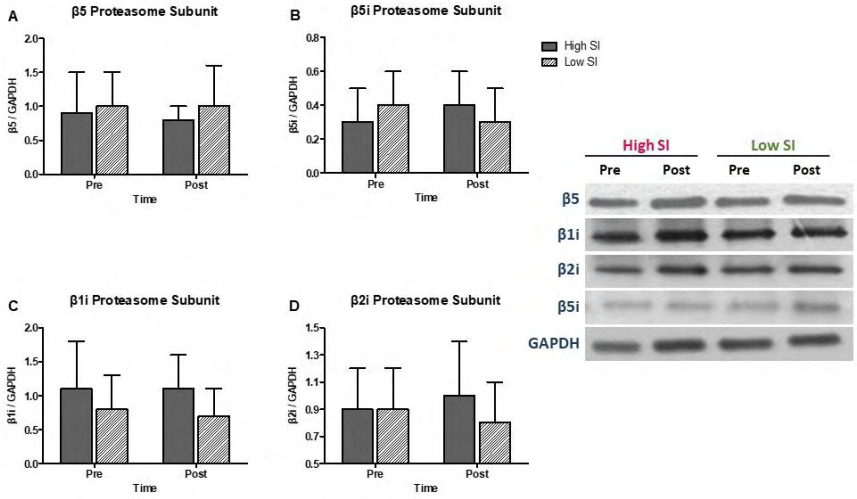


Figure 5.3. Changes in the expression of proteasome and immunoproteasome *β*-subunits in HSI and LSI.

Protein oxidation: Concentration of protein carbonyl groups at baseline was higher in HSI compared to LSI by 47% (HSI: 23.5 ± 6.2 vs LSI: 15.9 ± 7.6; *P* = 0.026; ES: 0.79; 95% CI: -0.20/1.78) (Figure 5.4). In HSI, protein carbonyl concentration declined by 31% at 3h following protein supplementation (*P* = 0.025; ES: 1.23; 95% CI: 0.16/2.29), whereas in LSI it remained unaltered (Figure 5.4). No differences observed among groups at 3h post-supplementation (HSI: 16.2 ± 4.3 vs LSI: 14.7 ± 4.0) (Figure 5.4).

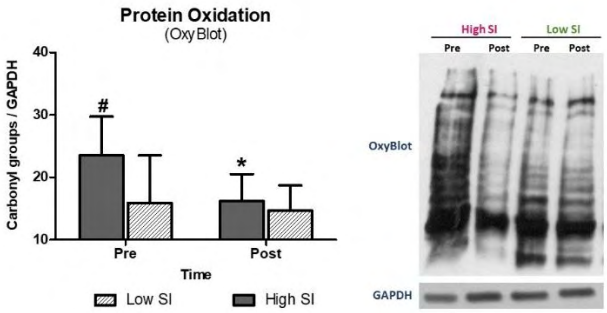


Figure 5.4. Changes in protein carbonyl groups in HSI and LSI. ***** Indicates significant difference from baseline, *p*<0.05, **#** Indicates significant difference between groups, *p*<0.05.

3-Nitrotyrosine levels were unchanged from basal in both groups, independent of muscle fiber type (Figure 5.5A-C). In addition, no group differences observed in 3-nitrotyrosine levels at any time point. However, in both groups, there was a significantly higher concentration of 3-nitrotyrosine in type II compared to type I muscle fibers both at baseline (HSI: *P* = 0.000; ES: -2.07; 95% CI: -3.29/-0.86 and LSI: *P* = 0.000; ES: -1.86; 95% CI: -2.91/-0.81) and 3 hours after

protein supplementation (HSI: *P* = 0.001; ES: -2.65; 95% CI: -4.08/-1.21 and LSI: *P* = 0.000; ES: -1.65; 95% CI: -2.67/-0.64) (Figure 5.5D).

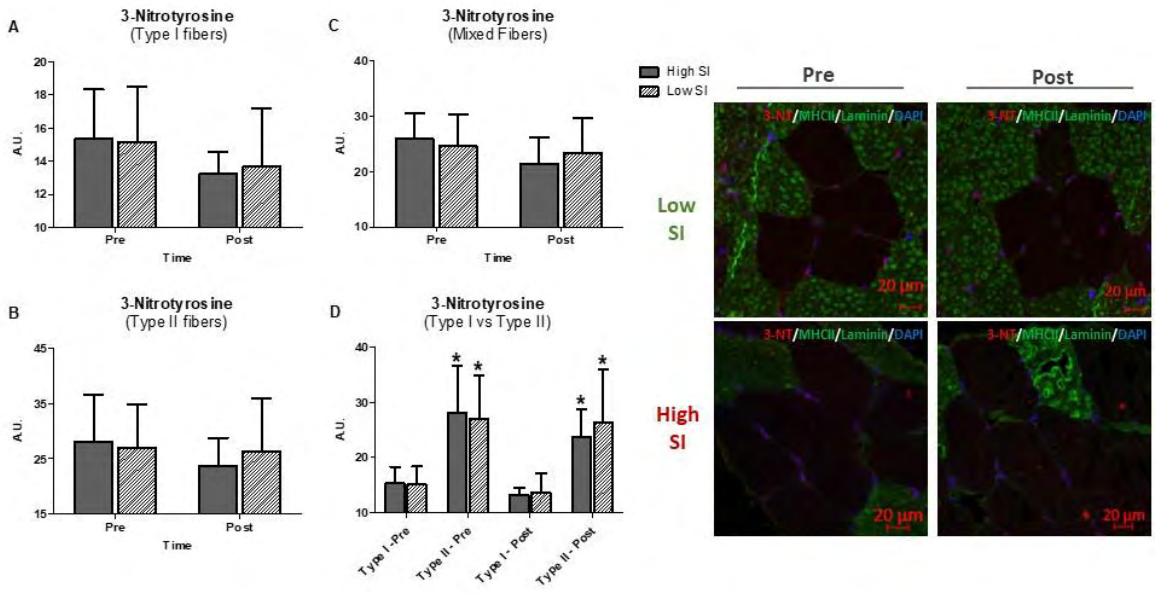


Figure 5.5. Changes in 3-Nitrotyrosine levels in HSI and LSI. 3-Nitrotyrosine levels in type I muscle fibers (**A**), type II muscle fibers (**B**), mixed fibers (**C**) and comparison of type I vs type II fibers (**D**) between HSI and LSI. ***** Indicates significant difference between type I and type II fibers, $p<0.05$.

Phosphorylated IKKα/β: At baseline, HSI demonstrated significantly higher amount of phosphorylated IKKα/β protein by 37% as compared to LSI (HSI: 14.69 ± 3.2 A.U. vs LSI: 10.70 ± 3.1 A.U.; *P* = 0.044; ES: 1.18; 95% CI: 0.01/2.36), whereas no group differences were detected at 3h post-supplementation (HSI: 11.77 ± 3.2 vs LSI: 10.20 ± 2.6) (Figure 5.6). Phosphorylation of IKKα/β following protein supplementation was not statistically changed from baseline in either group, despite a 20% reduction observed in HSI (Figure 5.6).

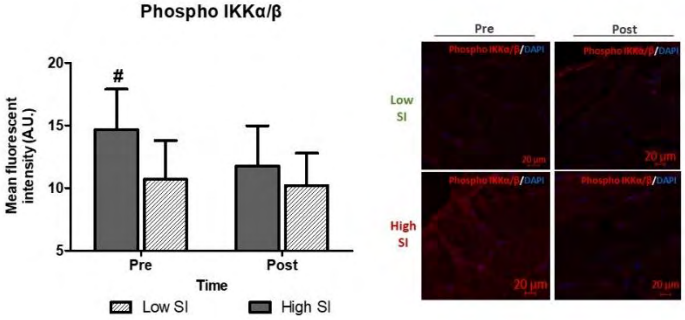


Figure 5.6. Changes in phosphorylated IKKα/β in HSI and LSI. # Indicates significant difference between groups, $p<0.05$.

Nuclear-erythroid factor 2 (Nrf2): Nuclei levels of Nrf2 were equal among groups in both the basal state (HSI: 42.44 ± 7.8 vs LSI: 38.90 ± 6.5) and following protein supplementation (HSI: 44.65 ± 5.9 vs LSI: 49.73 ± 10.6) (Figure 5.7). At 3h of the postprandial period, a significant rise of the Nrf2 nuclei levels was observed only in LSI by almost 28% (P = 0.012; ES: -1.15; 95% CI: -2.28/-0.02) (Figure 5.7).

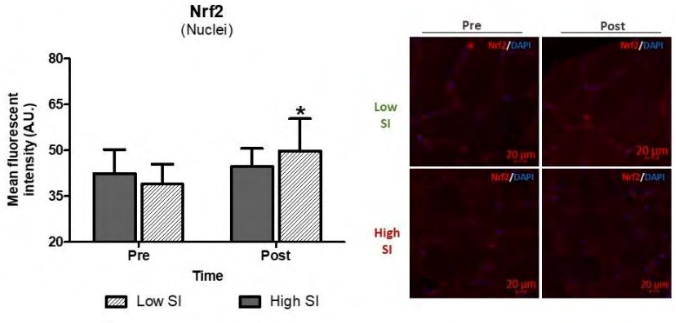


Figure 5.7. Changes in nuclei levels of Nrf2 in HSI and LSI. ***** Indicates significant difference from baseline, p<0.05.

Heme Oxygenase 1 (HO1): Heme Oxygenase 1 (HO1) protein was comparable among groups in both time points (**Pre:** HSI: 0.43 ± 0.18 vs LSI: 0.43 ± 0.19; **Post:** HSI: 0.44 ± 0.15 vs LSI: 0.42 ± 0.19) and remained unaltered from baseline independent of group (Figure 5.8).

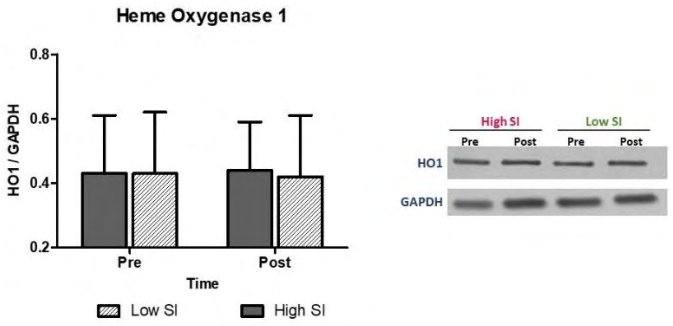


Figure 5.8. Changes in Heme Oxygenase 1 in HSI and LSI.

5.2. Inflammaging and anabolic resistance

5.2.1. Variables measured in skeletal muscle

Ribosomal protein S6 (rpS6): rpS6 is a downstream mediator of the Akt – mTOR signaling cascade that upon activation enhances translation initiation leading to increased protein synthesis [206]. We measured levels of phosphorylated rpS6 using immunoblotting (western blotting).

Ribosome biogenesis: Ribosome biogenesis is the process characterizing the synthesis of new ribosomes and regulates the translational capacity [206]. The expression of the upstream regulator

of ribosome biogenesis, c-Myc and phosphorylation state of the transcription initiation factor 1A (TIF-1A) were tested using immunoblotting (western blotting).

Amino acid transporters: The activity of amino acid transporters in skeletal muscle regulates the availability of amino acids and therefore is crucial for muscle protein synthesis [207]. Protein expression of the amino acid transporters, L-type amino acid transporter 1 (LAT1) and sodium-coupled neutral amino acid transporter 2 (SNAT2)/SLC38A2, was measured using immunoblotting (western blotting).

5.2.2. Results

Ribosomal protein S6 (rpS6): Phosphorylation of rpS6 was significantly increased only in LSI following exercise and protein ingestion by 1.5-fold (P = 0.001; ES: -1.44; 95% CI: -2.48/-0.41). Moreover, at 3h following protein ingestion phosphorylation of rpS6 was greater in LSI compared to HSI (HSI: 1.3 ± 0.4 fold change vs LSI: 2.5 ± 1.4 fold change; P = 0.030; ES: -1.08; 95% CI: -2.09/-0.06) (Figure 5.9).

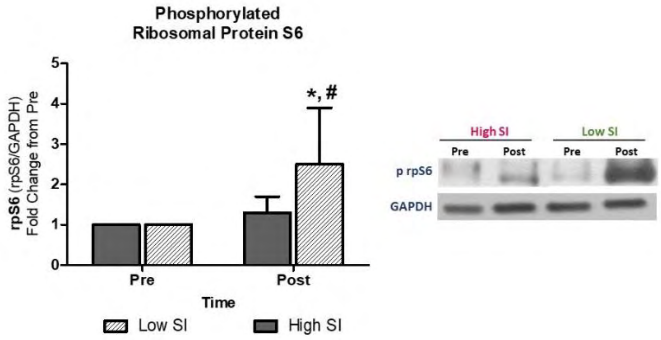


Figure 5.9. Changes in phosphorylated rpS6 in HSI and LSI.

Ribosome biogenesis: c-Myc protein expression remained unchanged in either group following exercise and protein ingestion (P > 0.05) and was similar among groups in both time-points (Figure 5.10A). Similarly, phosphorylation state of TIF-1A was comparable between LSI and HSI in both time points and no significant alteration (P > 0.05) was observed after protein ingestion for both groups (Figure 5.10B).

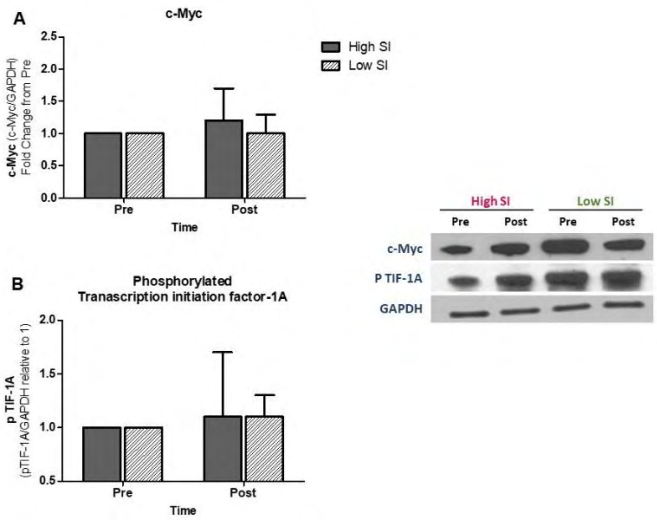


Figure 5.10. Changes in protein expression of c-Myc (A) and phosphorylation state of TIF-1A (B) in HSI and LSI.

Amino acid transporters: LAT1 protein expression was similar between LSI and HSI in both time-points and was not altered significantly ($P > 0.05$) after protein ingestion in either group (Figure 5.11A). Likewise, TIF-1A phosphorylation remained unaltered during the 3h postprandial period in both LSI and HSI ($P > 0.05$) and no differences were observed among groups across time (Figure 5.11B).

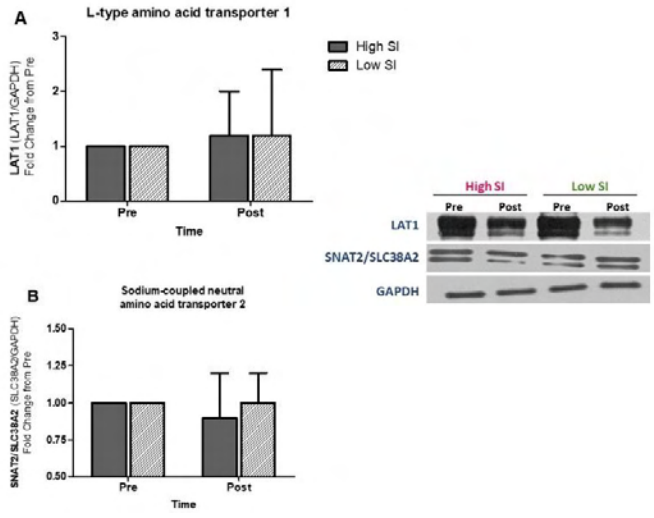


Figure 5.11. Changes in protein expression of LAT1 (A) and SNAT2/SLC38A2 (B) in HSI and LSI.

CHAPTER 6

General Conclusions

By integrating results from previous *in vivo* and *in vitro* studies, our literature review identified that the main mechanisms through which inflammaging may lead to loss of skeletal muscle mass and frailty is the reduced skeletal muscle's anabolic potential and the increased activation of the proteolytic machinery [69]. The former incorporates the reduced activation of the intracellular anabolic signaling pathway and the unresponsiveness of muscle protein synthesis to protein feeding, while the latter is characterized by increased activation of the transcriptional factor NF-κB and subsequent upregulation of proteasome activity, leading to proteolysis [69]. Our results corroborate these findings providing evidence for first time that the NF-κB/ubiquitin-proteasome pathway is activated while the translational efficiency is downregulated in the skeletal muscle of aged adults characterized by chronic low-grade systemic inflammation.

In the fasted state, we observed that older adults with increased systemic inflammation exhibited higher chymotrypsin-like proteasome activity accompanied by greater amount of phosphorylated IKKα/β and increased concentration of protein carbonyl groups in skeletal muscle. Upon activation, the IKKα/β phosphorylates and inactivates the inhibitor of the NF-κB, IkB complex, allowing the NF-κB to translocate into the nucleus [35] and activate the UPS [32, 34]. The UPS is also activated in response to increased oxidative stress and protein oxidation in order to maintain redox balance [208]. Under oxidative stress, the transcriptional factor Nrf2 is activated and translocate to the nucleus where it regulates the transcription of various protective genes such as HO1 and the expression of proteasome genes [208]. However, in the present thesis nuclear levels of Nrf2 and HO1 expression in the fasted state were equal among groups. Therefore, we suggest that during chronic low-grade systemic inflammation in the elderly, the elevated levels of pro-inflammatory mediators and the increased protein oxidation may increase proteasome activity in the fasted state, through the redox-sensitive transcriptional factor NF-κB (Figure 6.1). Protein expression of the catalytic β (proteasome) and βi (immunoproteasome) subunits was similar among groups, indicating that the observed difference in proteasome activity was dependent on activity per se and not on the increased amount of proteasome as well. Furthermore, we observed a significant correlation between hs-CRP levels and chymotrypsin-like activity, providing evidence that inflammaging affects proteasome activation in a dose-response manner.

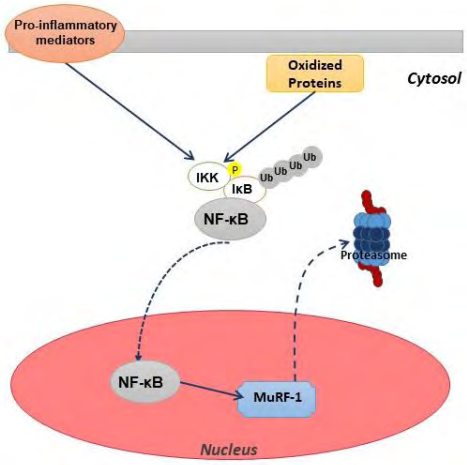


Figure 6.1. Schematic representation of the proposed molecular mechanism through which inflammaging leads to activation of the ubiquitin-proteasome system.

At 3h following exercise and protein ingestion, chymotrypsin-like proteasome activity increased equally in both groups, supporting previous evidence that following an acute bout of resistance exercise the UPS is activated in skeletal muscle of older adults, independently of protein ingestion [209, 210]. Protein expression of β and βi subunits remained unaltered in the postprandial period in both groups, indicating that acute resistance exercise alters proteasome activity but not the proteasome quantity in the aged skeletal muscle, and this effect in not dependent on the inflammatory status. Interestingly though, we observed that trypsin-like proteasome activity was elevated in the postprandial state only in the LSI group, and this elevation was accompanied by increased nuclear levels of Nrf2. Thus, unlike to chymotrypsin-like activity, inflammaging may differentially affect the trypsin-like activity following acute exercise and protein feeding, via the Nrf2 pathway. However, this observation is a preliminary finding that should be further investigated by future studies.

The concentration of 3-nitrotyrosine in skeletal muscle remained unaffected by exercise and protein feeding in both groups. Interestingly, type II skeletal muscle fibers were characterized by higher levels of 3-nitrotyrosine compared to type I fibers, independent of the inflammatory status and the time-point measured. Protein carbonyls declined significantly in the HSI group at 3h following protein ingestion as a response to the increased chymotrypsin-like activity, whereas their concentration in LSI group remained unaltered, probably because of the already low levels in the fasted state.

With regard to the skeletal muscle's anabolic potential, our data also suggest that inflammaging may negatively affect the anabolic signaling following exercise and protein ingestion. More specifically, we observed that phosphorylation of ribosomal protein S6 increased only in older adults characterized by low levels of systemic inflammation, whereas no changes

were observed in indices of ribosome biogenesis and amino acid transporters in either group. Therefore, we suggest that the previously reported reduced muscle protein synthetic response to protein feeding under conditions of chronic inflammation [28, 29], may be primarily driven by reduced translational efficiency via attenuated activation of the rpS6 and subsequently lower translation initiation rate. Collectively, in the present thesis we provide molecular evidence that older adults with increased low-grade systemic inflammation exhibit increased proteasomic activity in the fasted state and reduced translational efficiency in response to exercise and protein feeding, and as such may be more vulnerable to skeletal muscle wasting and functional impairments compared to their healthy counterparts.

We also investigated levels of habitual physical activity, daily dietary intake and physical performance as well as blood markers of oxidative stress and antioxidant status in older adults characterized by low and elevated levels of chronic low-grade systemic inflammation. Although no significant differences observed in lower and upper limb strength among groups, we noticed that elderly individuals with low systemic inflammation exhibited higher functional performance and were also more physically active, performing more steps and more intense activities throughout the day, as compared to those characterized by increased systemic inflammation. In addition, dietary intake of antioxidant vitamins (i.e. vitamins A, C and E) was also higher in those with low inflammation and accompanied by higher antioxidant capacity in plasma as well. Therefore, higher quality and quantity of habitual physical activity and increased antioxidant consumption through diet appear to be discriminant factors of inflammaging and successful aging, and thus may considered as promising lifestyle-based interventions to prevent the development of chronic low-grade systemic inflammation in the elderly.

Future studies should further invest on these primary findings by exploring the dose-response relationship between PA, antioxidant vitamin intake and inflammaging, in acute and long-term interventions. Moreover, there is also great need for well-controlled clinical trials to explore the response of the NF-κB/ubiquitin proteasome pathway and the Akt-related signaling pathway to chronic interventions aiming at reducing low-grade systemic inflammation in older adults, by utilizing various protein supplementation protocols and types of exercise. Plant and animal derived protein supplementation, especially that of whey and soy protein, may elicit antioxidative and anti-inflammatory properties and may therefore be considered as a promising nutritional strategy to offset the inflammaging associated skeletal muscle loss and frailty [69]. However, their effectiveness has been only tested in animal models so far, and thus, future studies should investigate whether increased consumption of plant- and dairy-based protein may aid muscle

protein synthesis and preserve muscle function in elderly individuals characterized by increased systemic inflammation.

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APPENDIX A

(Ethics approval)

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΦΥΣΙΚΗΣ ΑΓΩΓΗΣ ΚΑΙ ΑΘΛΗΤΙΣΜΟΥ

Εσωτερική Επιτροπή Δεοντολογίας

Τρίκαλα: 5/4/2017
Αριθμ. Πρωτ.: 1211

Έγκριση της πρότασης για διεξαγωγή Έρευνας με τίτλο: «Η επίδραση της φλεγμονής στην αναβολική ικανότητα και στον μηχανισμό πρωτεόλυσης του σκελετικού μυ ηλικιωμένων ατόμων»

Επιστημονικώς υπεύθυνη / επιβλέπουσα: Φατούρος Ιωάννης
Ιδιότητα: Αναπληρωτής Καθηγητής
Ίδρυμα: Πανεπιστήμιο Θεσσαλίας
Τμήμα: Επιστήμης Φυσικής Αγωγής και Αθλητισμού

Κύριος ερευνητής / φοιτητής: Δραγανίδης Δημήτριος
Πρόγραμμα Σπουδών: Διδακτορικό ΠΣ ΠΘ
Ίδρυμα: Πανεπιστήμιο Θεσσαλίας
Τμήμα: Επιστήμης Φυσικής Αγωγής και Αθλητισμού

Η προτεινόμενη έρευνα θα είναι : Διδακτορική διατριβή

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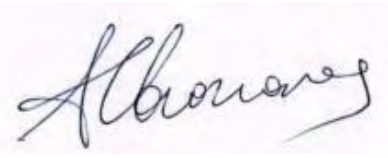
Η Εσωτερική Επιτροπή Δεοντολογίας του Τ.Ε.Φ.Α.Α., Πανεπιστημίου Θεσσαλίας μετά την υπ. Αριθμ. 3-1/5-4-2017 συνεδρίασή της εγκρίνει τη διεξαγωγή της προτεινόμενης έρευνας.

Ο Πρόεδρος της
Εσωτερικής Επιτροπής
Δεοντολογίας – ΤΕΦΑΑ



Τσιόκανος Αθανάσιος
Αναπληρωτής Καθηγητής

University of Thessaly
Department of Physical Education and Sport Science

Internal Ethics Committee

Trikala: 5/4/2017
Protocol Number: 1211

Approval of research entitled: "Effect of chronic inflammation on muscle protein synthesis and breakdown in the aged human skeletal muscle"

Scientist responsible – supervisor: Dr. Ioannis Fatouros
Associate Professor
School of Physical Education and Sport Science
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Main researcher: Draganidis Dimitrios
PhD Student
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The proposed research will be: PhD Thesis

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The Internal Ethics Committee (IEC) of the Department of PE and Sport Science (DPESS), University of Thessaly, examined the proposal in its 3-1/5-4-2017 meeting and approves the implementation of the proposed research.

The Chair of the IEC – DPESS

Athanasios Tsiokanos, PhD

APPENDIX B

(Publications included in the thesis)

The Journal of Nutrition. First published ahead of print August 31, 2016 as doi: 10.3945/jn.116.230912.

The Journal of Nutrition
Critical Review

Inflammaging and Skeletal Muscle: Can Protein Intake Make a Difference?[1,2]

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Abstract

Inflammaging is the chronic low-grade inflammatory state present in the elderly, characterized by increased systemic concentrations of proinflammatory cytokines. It has been shown that inflammaging increases the risk of pathologic conditions and age-related diseases, and that it also has been associated with increased skeletal muscle wasting, strength loss, and functional impairments. Experimental evidence suggests that the increased concentrations of proinflammatory cytokines and primary tumor necrosis factor α observed in chronic inflammation lead to protein degradation through proteasome activation and reduced skeletal muscle protein synthesis (MPS) via protein kinase B/Akt downregulation. Dairy and soy proteins contain all the essential amino acids, demonstrate sufficient absorption kinetics, and include other bioactive peptides that may offer nutritional benefits, in addition to those of stimulating MPS. Whey protein has antioxidative effects, primarily because of its ability to enhance the availability of reduced glutathione and the activity of the endogenous antioxidative enzyme system. Soy protein and isoflavone-enriched soy protein, meanwhile, may counteract chronic inflammation through regulation of the nuclear transcription factor κB signaling pathway and cytokine production. Although evidence suggests that whey protein, soy protein, and isoflavone-enriched soy proteins may be promising nutritional interventions against the oxidative stress and chronic inflammation present in pathologic conditions and aging (inflammaging), there is a lack of information about the anabolic potential of dietary protein intake and protein supplementation in elderly people with increased systemic inflammation. The antioxidative and anti-inflammatory effects, as well as the anabolic potential of protein supplementation, should be further investigated in the future with well-designed clinical trials focusing on inflammaging and its associated skeletal muscle loss. *J Nutr* doi: 10.3945/jn.116.230912.

Keywords: inflammaging, oxidative stress, skeletal muscle loss, frailty, whey protein, soy protein

Introduction

The progressive loss of skeletal muscle mass and function (i.e., muscle strength and endurance and ability to perform daily physical activities) with advancing age is a well-documented process (1–3) that may lead to functional limitations, frailty, and hospitalization (4, 5). Muscle mass is maintained by a constant equilibrium between the rates of muscle protein synthesis (MPS)[7] and degradation, in which a net increase or a decrease occurs when the balance is disturbed. Nutritional-, hormonal-, neuropathic-, and inactivity-related factors all may contribute to deregulation of the molecular milieu of the aged muscle, resulting in muscle wasting and loss of independence (5).

In the elderly, the development of low-grade, chronic, systemic inflammation is often observed with age, characterized by a 2- to 3-fold elevation in circulating inflammatory mediators. This has been termed "inflammaging" (inflamm-aging) (6). Proinflammatory cytokines are key components in this chronic inflammatory state; thus, the assessment of inflammaging primarily is based on the measurement of systemic concentrations of IL-6, IL-1, and TNF-α, their soluble receptors IL-1Ra, TNF receptor, and soluble IL-6 receptor, respectively, and that of the acute-phase C-reactive protein (CRP) (6–8). Furthermore, inflammaging may be assessed at the skeletal muscle tissue level by the quantification of infiltrating macrophages, cytokine concentrations, and the examination of inflammatory pathways (7, 8). Although the molecular mechanisms involved in the interaction between inflammaging and muscle loss is far from

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[2] Author disclosures: D Draganidis, LG Karagounis, I Athanailidis, A Chatzinikolaou, AZ Jamurtas, and IG Fatouros, no conflicts of interest.
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[7] Abbreviations used: Bax, BCL2 associated X, apoptosis regulator; Bcl-2, B-cell lymphoma 2; CRP, C-reactive protein; IκB, inhibitor of NF-κB; MPS, muscle protein synthesis; ROS, reactive oxygen species; SOD, superoxide dismutase; SPI, soy protein isolate; TAC, total antioxidant capacity; UPS, ubiquitin–proteasome system; VCAM-1, vascular cell adhesion molecule 1; WPH, whey protein hydrolysate.

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understood, research carried out in animal models revealed that augmented low-grade inflammation may favor muscle protein breakdown and inhibit protein synthesis (9, 10).

Although older adults exhibit anabolic resistance (i.e., a higher protein amount is required to maximally stimulate MPS than for young individuals) to protein intake, dietary protein is still the most potent anabolic stimulus in older adults, because it has been shown to efficiently activate the skeletal muscle anabolic response in the postprandial period, at rest, and after resistance exercise (11, 12). A higher (>1.2 g/(kg body weight · d) compared with a lower (<1.0 g/(kg body weight · d) protein intake appears to preserve muscle quality in the aged with high levels of systemic inflammation (13), suggesting that adequate protein intake may preserve muscle function under chronic inflammatory conditions. Dairy proteins that include high amounts of branched-chain amino acids demonstrate fast (whey protein) and slow (casein protein) digestion and absorption kinetics and may efficiently stimulate MPS in healthy aged skeletal muscle (14–16). Soy protein, on the other hand, which demonstrates somewhat slower kinetics than whey and faster digestion rates than casein (14), is also rich in branched-chain amino acids and able to upregulate MPS (14, 16). Whey and soy protein also possess antioxidant and anti-inflammatory properties (17, 18). Thus, both animal and plant protein sources may represent efficient nutritional strategies to counteract inflammaging and its detrimental effects on skeletal muscle. This review aims to provide evidence for an anti-inflammatory and anticatabolic role of protein supplementation in aged skeletal muscle by presenting molecular and physiologic data that link protein consumption and muscle wasting under proinflammatory conditions.

Inflammaging and Its Association with Frailty

Chung et al. (19) proposed the molecular inflammation theory, according to which the age-related increase in reactive oxygen and nitrogen species concentrations and redox balance disturbances may lead to a chronic low-grade inflammatory state by activating redox-sensitive transcriptional factors. The NF-κB pathway is the most important redox-sensitive signaling pathway through which oxidative stress may increase the expression of numerous proinflammatory molecules, especially cytokines such as TNF-α, IL-6, IL-1β, and CRP, stimulating inflammation (20, 21). As the inflammatory response escalates, additional reactive nitrogen and oxygen species are released from immune cells (e.g., monocytes and macrophages) resulting in a propagation of cytokine production (8, 22). Thus, a vicious cycle is propagated, driving a chronic systemic proinflammatory state that in the elderly has been termed inflammaging (23) (**Figure 1**).

Is inflammaging, however, associated with skeletal muscle wasting and strength loss? Data derived from 3075 men and women aged 70–79 y in the Health, Aging, and Body Composition Study (24) showed that those with high concentrations of IL-6 and TNF-α had smaller skeletal muscle area, less appendicular muscle mass, and reduced strength. Similarly, elderly subjects with elevated IL-6 and CRP concentrations demonstrated a 2- to 3-fold greater risk of losing >40% of their muscle strength (25). Moreover, according to a 5-y follow-up study in 2177 men and women aged 70–79 y, increased baseline concentrations of TNF-α and its soluble receptor were linked to a greater decline in muscle mass and strength (26). Although the underlying molecular pathway leading from inflammation to functional decline has not been clarified yet, increased IL-6 concentrations in the elderly contribute to the development of disability and functional dependence (27–31) via direct interactions with key growth factors in skeletal muscle (32, 33). These findings accord well with the observation that orally administered cyclo-oxygenase inhibitors in older adults engaged in resistance exercise lead to increased skeletal muscle mass and strength gains by reducing the production of IL-6 and muscle ring-finger-1 in skeletal muscle (34). Therefore, these studies provide compelling evidence of an association between inflammaging and a deterioration of skeletal muscle size and function.

In vivo and in vitro studies indicate that inflammaging-related muscle wasting may be attributed to a TNF-α mediated upregulation of the NF-κB pathway and the subsequent activation of the ubiquitin–proteasome system (UPS) (21, 35, 36). Increased concentrations of proinflammatory cytokines, i.e., TNF-α and/or IL-6, have been shown to activate the inhibitor of NF-κB (IκB) kinase, which phosphorylates the IκB complex and results in its degradation, thereby allowing the translocation of the NF-κB complex into the nucleus (37). The 20S proteasome is the catalytic part of the UPS, performing the degradation and removal of abnormal, misfolded, and denatured proteins, and it also may remove healthy proteins under certain circumstances (38–42). Under conditions of chronic inflammation, increased NF-κB expression activates the UPS, resulting in protein degradation by the 20S proteasome subunit and muscle wasting (21, 35, 43, 44). In experimental animals, infusion or injection of TNF-α resulted in a pronounced loss of skeletal muscle and body mass (45, 46), probably in a concentration-dependent manner (47). Moreover, infusion of IL-6 has been shown to alter amino acid turnover and decrease the phosphorylation of signaling proteins involved in the anabolic pathway, suggesting that increased IL-6 concentrations contribute to skeletal muscle atrophy (48, 49).

An interaction between the NF-κB-related proteolytic cascade and anabolic pathways also has been observed in human skeletal muscle. Older humans exhibited a blunted MPS response to feeding compared with their younger counterparts that was attributed to NF-κB overexpression in their skeletal muscles (50). Later studies revealed that the increased TNF-α–dependent NF-κB expression attenuates the activation of anabolic signaling molecules such as Akt and S6K1, leading to reduced MPS and insulin resistance (51, 52). Thus, chronic inflammation may not only lead to NF-κB–related skeletal muscle wasting, but it also may hamper anabolic signaling pathways in skeletal muscle. Anti-inflammatory treatment with ibuprofen in aged rats reduced systemic inflammation, increased the rate of MPS and activation of anabolic intracellular signaling pathways, and significantly suppressed muscle protein breakdown (10). Although the verification of this mechanism (Figure 1) in humans is still missing, this study clearly shows that inflammaging should be targeted by nutritional, exercise, and pharmaceutical interventions aimed at the limitation of sarcopenia and skeletal muscle loss. The molecular mechanisms regulating the TNF-α/NF-κB/ubiquitin–proteasome pathway and its crosstalk with Akt-related signaling in the skeletal muscle of aged adults warrants further investigation. Although exercise-induced inflammation has been shown to activate muscle satellite cell content as part of the regeneration or remodeling process (53, 54), there are limited data on the impact that age-related chronic inflammation has on satellite cells. Beenakker et al. (55) show that there is no association between chronic systemic inflammation and satellite cell number in patients with rheumatoid arthritis. Future investigations need to examine whether inflammaging affects satellite cell responses,

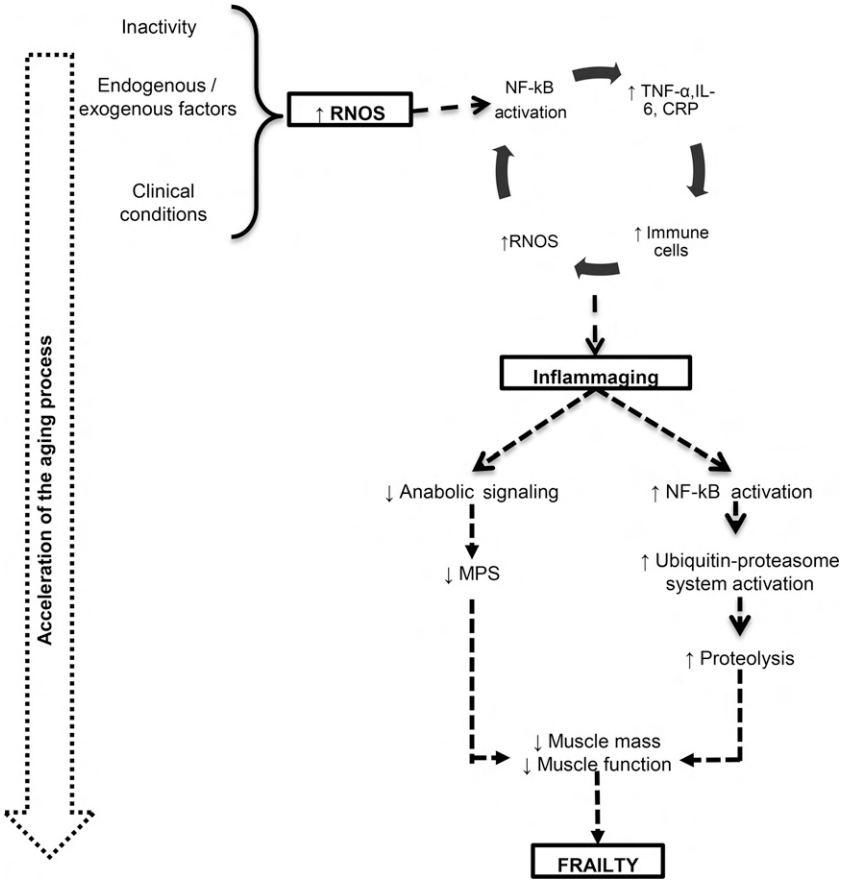

FIGURE 1 Potential pathway linking inflammaging and frailty. CRP, C-reactive protein; MPS, muscle protein synthesis; RNOS, reactive oxygen and nitrogen species; ↑, increase; ↓, decrease.

and, if so, what the impact is of anabolic approaches, such as protein feeding and/or resistance exercise training on these responses.

A Rationale for Protein Supplementation for Inflammaging

Protein ingestion is a nutritional strategy that has been studied extensively as a means of attenuating age-dependent muscle loss and therefore maintain quality of life (56). This mainly is due to the resulting postprandial aminoacidemia, which is known in the short term (hours) to stimulate MPS (57, 58), especially when combined with resistance-type exercise (59–61). Evidence indicates that MPS is less sensitive to protein intake in elderly patients than it is in young individuals; thus, higher relative amounts of protein may be required in each meal to stimulate MPS maximally in the aged (61–63).

Although the RDA for protein intake in adults is 0.8 g/(kg body weight · d), consumption of protein above the RDA has been proposed to more efficiently prevent muscle wasting and offer health benefits to the aged (12, 64, 65). Higher protein intake in community-dwelling adults has been associated with an attenuation of skeletal muscle loss over a 3-y follow-up (66), whereas protein intake has been negatively associated with skeletal muscle strength loss in inflammaging (13). Apart from the anabolic potential of protein, higher intake in the elderly also has been proposed in order to boost glutathione synthesis by providing greater availability of cysteine, which is a precursor amino acid (67). Because the glutamate cysteine ligase Michaelis constant for cysteine is close to intracellular cysteine concentrations, increased cysteine intake from dietary protein or other cysteine-rich sources may lead to substantial glutathione synthesis, especially when intracellular concentrations of glutathione are relatively low (67). Glutathione acts as a potent antioxidant in the intracellular environment, because it counteracts the produced reactive oxygen and nitrogen species and also downregulates signaling pathways mediating immune cell mobilization. Therefore, there is a potential link between protein intake and skeletal muscle health in older adults with low-grade inflammation. Recently, researchers have attempted to shed light on the antioxidant and anti-inflammatory properties of dairy and plant proteins by using both in vivo and in vitro experimental models. Therefore, the interaction between inflammaging and protein intake will be presented separately for each protein type in the following paragraphs. For each protein type, existing evidence will be reviewed in respect to the effect of proteins on *1*) both systemic and local (skeletal muscle) anti-inflammatory and antioxidant potential, and *2*) their ability to affect skeletal muscle loss and function.

Dairy proteins. Over the last 5 y there has been growing interest in the antioxidant and anti-inflammatory role of dairy proteins, primarily that of whey. In vitro models, although they use an artificial environment, have offered valuable insight in this area. When C_2C_{12} myoblasts were incubated with whey protein (80.05 g/100 g) and various concentrations (0.1–0.4 g/L) of hydrogen peroxide, it was revealed that whey protein was able to prevent hydrogen peroxide–induced toxicity, reduce lipid peroxidation, and enhance the activity of several antioxidant enzymes (68). Similarly, whey protein hydrolysates (WPHs; 100 μg/mL and 200 μg/mL pre- and postincubation, respectively) protected PC12 cells exposed to hydrogen peroxide from oxidative damage by reducing intracellular concentrations of Ca^{2+}, suppressing mitochondrial apoptotic pathways (by 14%),

and maintaining the membrane potential of the mitochondrial membrane, thereby improving mitochondrial function (69). In line with the results from Xu et al. (68) in C_2C_{12} myoblasts, WPH supplementation in PC12 cells (69) upregulated the activity of antioxidant enzymes, such as catalase and superoxide dismutase (SOD). The antioxidant properties of whey protein were illustrated further when C_2C_{12} muscle cell lines were treated with sheep whey protein (0.78–6.24 mg) by increasing reduced glutathione concentrations and reducing TBARs and reactive oxygen species (ROS) (70). Therefore, it appears that whey protein supplementation in the muscle and other cell lines prevents the onset of oxidative stress by enhancing the activity of endogenous antioxidant enzymes and increasing reduced glutathione availability, as well as maintaining mitochondria integrity. These results were corroborated in findings reported by studies that used rodent models (71–73).

Intraperitoneal [4 mg/(kg body weight · d)] or oral [8 mg/(kg body weight · d)] ingestion of WPH in albino mice with hepatonephrotoxicity attenuated the elevation of serum markers of oxidative damage, such as glutathione pyruvate transaminase, alkaline phosphatase, creatinine, and TBARs, upregulated the activities of antioxidant enzymes, and preserved serum urea nitrogen at normal concentrations, suggesting that WPH also has the ability to enhance the endogenous antioxidant system in vivo under pathologic conditions (71). When the antioxidant properties of diets containing various amounts of whey and casein protein (20% casein compared with 10% casein and 10% whey protein) were compared under conditions of elevated oxidative stress induced by iron overloading, it was shown that rats fed the diet including whey protein had greater levels of reduced glutathione and SOD activity in erythrocytes and reduced lipid peroxidation and DNA damage in leukocytes and colonocytes compared with those that received casein only, suggesting that whey was primarily responsible for the enhanced antioxidant defense (72). Furthermore, diabetic rats supplemented with 100 mg whey protein/kg body weight exhibited considerable reductions in malondialdehyde, NO, and ROS concentrations and also preserved their glutathione concentrations (73). These in vivo results, although derived from tissues other than skeletal muscle, are in agreement with those reported from in vitro studies (68–70) in which whey protein was systematically shown to possess antioxidant properties despite varying doses and supplementation protocols applied. This antioxidant profile of whey protein is attributed primarily to enhanced antioxidant enzyme activity and increased reduced glutathione concentrations.

Although human supplementation studies in inflammaging are lacking, human protein feeding studies under proinflammatory conditions offer valuable information. The anti-inflammatory role of protein supplementation in humans has been tested in the context of exercise (74–79), as well as in various clinical proinflammatory conditions, such as cystic fibrosis and obesity (80–84). Exercise, especially eccentric or unaccustomed, has been associated with microtrauma of skeletal muscle fibers and an intense aseptic type of inflammation that is characterized by immune cell activation, excessive ROS generation, perturbation of redox status, and deterioration of muscle performance (85–87). During a 6-d block of intense training, athletes receiving a daily supplement containing protein, leucine, carbohydrate, and fat at 20, 7.5, 89, and 22 g/h, respectively, for 1–3 h postexercise over 6 d demonstrated increased counts of circulating neutrophil and respiratory burst activity on day 6 compared with those receiving only a carbohydrate control beverage (74). However, in this case, we could not determine whether the effect was attributable to leucine, protein, or a combination of the supplement's ingredients. In another study, well-trained cyclists performed 3 high-intensity ride sessions over 4 d (day 3 was a rest day), with supplementation on days 1 and 2 with a protein blend [whey protein isolate, calcium caseinate, and soy protein isolate (SPI)] at a dosage of 0.8 g protein/(kg fat-free mass · h) during a 4-h postexercise recovery period (75). A protein effect was observed in the postexercise period, leading to reduced creatine kinase concentrations, but no significant alterations were observed for any of the oxidative stress and inflammatory markers measured (75). Similarly, during a 9-wk weight-training period, consumption of 33 g whey protein/d (3 servings of 11 g/d, in a bar form) did not prevent exercise-induced oxidative stress, whereas an equal amount of soy protein (33 g/d, 3 servings of 11 g/d, in a bar form) preserved postexercise antioxidative capacity, as evidenced by free-radical scavenging capacity and plasma myeloperoxidase response (76). In contrast to these findings, acute anti-inflammatory and antioxidative properties have been attributed to whey protein after a cycling session to exhaustion (77, 78). When whey protein [4 dosages of 0.28 g/(kg body weight · h)] was consumed immediately postexercise and daily during the recovery period after an exhaustive cycling trial that induced a marked inflammatory and oxidative stress response, an attenuation of IL-6, plasma TBARs, and CRP was observed during the first 4 h after exercise, whereas plasma total antioxidant capacity (TAC), protein carbonyls, and erythrocyte reduced glutathione and catalase concentrations remained unaltered (77, 78). Similar findings (i.e., attenuated elevation of TBARs and protein carbonyls, and increased reduced glutathione availability) have been reported for whey protein in ultramarathon runners receiving daily 2 whey protein bars (14.3 g whey protein/100 g bar) for 2 mo (79). Therefore, most of these human exercise studies support an anti-inflammatory and antioxidant role for whey protein. Nevertheless, these studies involved healthy young individuals, and data on skeletal muscle performance and molecular responses are lacking. We must mention, however, that one previous study suggested that antioxidant supplementation (i.e., vitamins C and E) may offset some positive adaptations induced by exercise training (88). These findings were reported for young athletes, but, to our knowledge, no data exist for inflammaging.

To our knowledge, only a small number of studies examined the effects of a nutritional intervention of dairy-based protein diets in aged adults on chronic inflammation and oxidative stress. Either acute (a bolus of 45 g of protein) or chronic (54 g of protein/d for a 12-wk period) consumption of whey protein isolate did not alter the responses of circulating proinflammatory markers such as IL-6, TNF-α, and CRP in overweight postmenopausal women and overweight adults aged 18–65 y, respectively (80, 81). Similarly, when obese individuals received a soy-based protein diet, after a wash-out period, no changes in inflammatory and oxidative stress markers were observed (82). In contrast, when whey isolate and calcium caseinate (45 g of each protein in a crossover design) were consumed in combination with a fat-rich meal by obese, nondiabetic individuals in the context of an acute clinical trial, an acute suppression of markers of low-grade inflammation was observed (83). The anti-inflammatory potential of whey protein also was evident in cystic fibrosis patients who consumed 20 g whey protein/d for 3 mo (84). These few human studies provided valuable insight regarding the anti-inflammatory role of protein in the presence of low-grade inflammation and partly verify the in vitro and in vivo results described earlier. Both dairy proteins seem to have a protective effect against low-grade inflammation, with whey eliciting a slightly greater attenuation of proinflammatory cytokines than does casein (83). Thus, the

TABLE 1 Evidence for the anti-inflammatory and antioxidative role of dairy proteins[1]

Reference	Cell or organism tested	Condition	Type of protein	Supplementation protocol	Effects on inflammation	Effects on oxidative stress
Xu et al. (68)	C_2C_{12} myoblasts	Hydrogen peroxide–induced toxicity	WP	0.1–0.4 g/L	NA	↓ Lipid peroxidation ↓ DNA oxidative damage ↑ Activity of SOD, catalase, and GPx
Jin et al. (69)	Rat pheochromocytoma line 12 cells	Hydrogen peroxide–induced oxidative stress	WPH	100 μg WPH/mL or 200 μg WPH/mL for 2 h and then another 100 or 200 μg WPH/mL for 24 h	NA	↓ ROS and Ca^{2+} concentrations ↓ Activity of caspase-3 ↑ Bcl-2 mitochondrial expression ↓ Bax mitochondrial expression ↑ Activity of catalase and SOD
Kerasioti et al. (70)	Muscle C_2C_{12} cells	Tert-butyl hydroperoxide–induced oxidative stress	Sheep WP	0.78–6.24 mg WP for 24 h	NA	↓ ROS concentrations ↓ TBAR concentrations ↑ Reduced glutathione availability
Athira et al. (71)	Albino mice	Acetaminophen-induced oxidative stress	WPH	4 mg WPH/(kg body weight · d) intraperitoneally or 8 mg WPH/(kg body weight · d) orally for 4 d	NA	↓ Oxidative damage ↓ TBAR concentrations ↑ Activity of SOD, catalase, and GPx
Kim et al. (72)	8-wk-old Sprague Dawley rats	Iron overload–induced oxidative stress	WP + casein	10 g WP + 10 g casein/100-g diet for 6 wk	NA	↓ Lipid peroxidation ↓ DNA damage ↑ Erythrocyte reduced glutathione concentrations ↑ Activity of SOD
Ebaid et al. (73)	Adult diabetic rats	Wounded diabetic rats	WP	100 mg WP/(kg body weight · d) orally for 30 d	Restored IL-1β, TNF-α, IL-6, IL-4, and neutrophil infiltration during wound healing	↓ Malondialdehyde concentrations ↓ NO and ROS concentrations Preserved reduced glutathione concentrations
Nelson et al. (74)	Male cyclists and triathletes (35 ± 10 y of age)	Exercise-induced inflammation	WP + leucine	20 g WP + 7.5 g leucine/h for 3 h postexercise daily for 6 d	↔ IL-6 ↔ IL-10 ↑ neutrophil O_2^- (on day 6)	
Rowlands et al. (75)	Male cyclists (34 ± 10 y of age)	Exercise-induced inflammation and oxidative stress	WPI + calcium caseinate (soy nuggets also included)	0.8 g protein/(kg FFM · h) for 4 h postexercise	↔ TNF-α ↔ IL-6 ↔ CRP	↔ Malondialdehyde
Brown et al. (76)	Male experienced weightlifters (19–25 y of age)	Exercise-induced oxidative stress	WP	33 g WP/d (3 servings of 11 g)	NA	↓ Plasma radical scavenging capacity ↑ Myeloperoxidase
Kerasioti et al. (77)	Physically active men (28 y of age)	Exercise-induced oxidative stress	WP	4 doses of 0.28 g WP/(kg body weight ·h)	NA	↓ TBAR concentrations ↔ Plasma TAC and protein carbonyls ↔ Erythrocyte reduced glutathione and catalase
Kerasioti et al. (78)	Physically active men (28 y of age)	Exercise-induced inflammation	WP	4 doses of 0.28 g WP/(kg body weight · h)	↓ IL-6 ↓ CRP	NA
Samaras et al. (79)	Ultramarathon runners (43 y of age)	Resting oxidative stress concentrations	WP	2 protein bars/d (30.80 g protein per 100-g bar, of which 14.3 g was WP) for 2 mo	NA	↓ TBAR concentrations ↓ Protein carbonyls ↑ Reduced glutathione availability ↔ TAC

(Continued)

TABLE 1 *Continued*

Reference	Cell or organism tested	Condition	Type of protein	Supplementation protocol	Effects on inflammation	Effects on oxidative stress
Pal and Ellis (81)	Overweight and obese postmenopausal women (40–65 y of age)	Postprandial (6-h) inflammatory markers	WPI	45 g	↔ Plasma IL-6 ↔ Plasma TNF-α ↔ Plasma CRP	NA
Pal and Ellis (80)	Overweight and obese individuals (18–65 y of age)	Systemic inflammation	WPI	54 g WPI/d for 12 wk	↔ Plasma IL-6 ↔ Plasma TNF-α ↔ Plasma CRP	NA
Zemel et al. (82)	Overweight and obese individuals (31 ± 10 y of age)	Systemic inflammation and oxidative stress	Nonfat dry milk	30 g protein (distributed in 3 doses/d) for 28 d	↓ TNF-α ↓ IL-6 ↓ MCP-1	↓ Plasma malondialdehyde ↓ 8-Isoprostane factor-α
Holmer-Jensen et al. (83)	Obese nondiabetic individuals (40–68 y of age)	Postprandial low-grade inflammation	WPI	45 g WPI (15 E% of the meal)	↑ CCL5/RANTES ↓ MCP-1	NA
Holmer-Jensen et al. (83)	Obese nondiabetic individuals (40–68 y of age)	Postprandial (4-h) low-grade inflammation	Calcium caseinate	45 g protein (15 E% of the meal)	↑ CCL5/RANTES ↓ MCP-1	NA
Grey et al. (84)	Cystic fibrosis patients (25 y of age)	Reduced glutathione availability	WPI	20 g WPI/d (2 servings of 10 g/d)	NA	↑ Lymphocyte reduced glutathione concentrations

[1] Bax, BCL2 associated X, apoptosis regulator; Bcl-2, B-cell lymphoma 2; CCL5/RANTES, CC chemokine ligand 5; CRP, C-reactive protein; E%, percentage of energy; FFM, fat-free mass; GPx, glutathione peroxidase; MCP-1, monocyte chemoattractant protein 1; NA, not applicable; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; WP, whey protein; WPH, whey protein hydrolysate; WPI, whey protein isolate; ↑, increase; ↓, decrease; ↔, no effect; ≈, maintenance.

rationale for using dairy proteins to counteract low-grade inflammation and oxidative stress and prevent sarcopenia may be valid, and future investigations should explore this prospect in human skeletal muscle in inflammaging.

Studies that investigated the effects of dairy protein on inflammatory and oxidative stress responses are presented in **Table 1**.

Soy protein. Soy protein represents 35–40% of soybean content and is considered to be a protein source of high nutritional quality, because it contains all the essential amino acids and, in particular, it has less saturated fat than dairy foods and is cholesterol-free (89). However, soy protein in its isolated form, to our knowledge, has been poorly investigated by researchers looking for protein supplements and protein-rich diets to counteract inflammation and oxidative stress.

When SPI (20% of the daily diet; 8 g of food on day 1, increased by 0.5 g each day for the remaining 13 d) was administered to rats exposed to paraquat-induced oxidative stress, an attenuation of lipid peroxidation and enhanced reduced glutathione concentrations was observed (90, 91). A recent study showed that a 5-wk supplementation with SPI in hyperlipidemic mice counteracted the NF-κB–dependent inflammatory response manifested as reduced activation of NF-κB and expression of TNF-α, IL-6, IL-1β, vascular cell adhesion molecule 1 (VCAM-1), and monocyte chemoattractant protein 1 because of inhibition of IκB phosphorylation (17). Therefore, given that in chronic inflammatory conditions the increased activation of the NF-κB signaling pathway leads to protein degradation through the 20S proteasome subunit (21, 35, 43, 44), soy protein may be a potent nutritional intervention against chronic inflammation and its associated skeletal muscle loss. In contrast to animal studies, in what is, as far as we know, the only human study that tested a 6-wk supplementation with SPI (25 g/d) in postmenopausal women, supplementation did not alter inflammatory markers such as soluble IL-2 receptor, E-selectin and P-selectin, VCAM-1, and intercellular adhesion molecule 1 (92). However, the efficacy of SPI in inhibiting chronic inflammation should be further investigated in human clinical trials in order to come to a clear conclusion.

Soy foods also contain phytoestrogens named isoflavones that can be removed when these foods are washed with alcohol (89, 93). Genistin, daidzin, and glycitein are the primary bioactive isoflavones in soybeans and soy foods (89). Research on the antioxidative role of isoflavones has shown reduced oxidative stress levels, improved antioxidant enzyme activity, and attenuated oxidative damage in animal models (94–97), as well as in humans (98, 99). Specifically, the incorporation of 206 g SPI/kg body weight (based on the AIN-93G diet), combined with 189 mg isoflavones/100 g SPI, in the daily diet for a 9-wk period attenuated myocardial oxidative stress levels in rats that suffered from myocardial infarction and underwent heart surgery (100, 101). Another study investigated the antioxidative action of an isoflavone-enriched soy protein compared with a casein-based diet on fructose-induced oxidative and inflammatory responses in rats, suggesting that, in contrast to casein, soy protein is able not only to suppress oxidative stress, but also to elicit an anti-inflammatory response (102). As previously reported for SPI (17), soy isoflavones and mainly genistein also have been shown to hamper the activation of NF-κB and TNF-α in aged mdx mice (103). Therefore, the effect of isoflavone-enriched soy protein on inflammation may be attributed to its isoflavone content and amino acid composition that seem to prevent the nuclear translocation and subsequent

TABLE 2 Evidence for the anti-inflammatory and anti-oxidative role of soy protein, isoflavone enriched soy protein and soy milk[1]

Reference	Cell or organism tested	Condition	Type of protein	Supplementation protocol	Effects on inflammation	Effects on oxidative stress
Aoki et al. (90)	Male Wistar rats (4 wk old)	Paraquat-induced oxidative stress	SPI	20% of the daily diet (8 g food on day 1, increased by 0.5 g each day) for 14 d	NA	↓ Lipid peroxidation concentrations ≈ Glutathione concentrations
Takenaka et al. (91)	Male Wistar rats (4 wk old)	Paraquat-induced oxidative stress	SPI	20% of the daily diet (8 g food on day 1, increased by 0.5 g each day) for 14 d	NA	↓ TBAR concentrations ↓ GSSG:GSH ratio
Burris et al. (17)	ApoE knockout mice	Hyperlipidemia-induced chronic inflammation	SPI	3.9 g SPI/d for 5 wk	↓ NF-κB activation ↓ Expression of TNF-α, IL-6, and IL-1β ↓ Expression of VCAM-1 and MCP-1	NA
Blum et al. (92)	Postmenopausal women (aged 55 y)	Vascular inflammation	SPI	25 g SPI/d for 6 wk	↔ Soluble IL-2 receptor ↔ E-selectin ↔ P-selectin ↔ VCAM-1 ↔ ICAM-1	NA
Brown et al. (76)	Male experienced weightlifters (19–25 y of age)	Exercise-induced oxidative stress	Soy (DrSoy Bars)	33 g/d (3 servings of 11 g)	NA	≈ Plasma radical scavenging capacity ≈ Myeloperoxidase
Hagen et al. (101)	Male Wistar rats with myocardial infarction	Myocardial oxidative stress	SP + ISF	206 g SP/(kg body weight · d) + 189 mg ISF/(100 g SP · d) for 9 wk	NA	↑ SOD, catalase, and GPx activity ↑ Catalase activity ↓ Protein carbonyls ↓ Lipid peroxidation
Sreeja et al. (102)	Adult male albino Wistar rats	Fructose-induced oxidative stress and inflammation	SP + ISF	20 g SP/(kg body weight · d) for 8 wk	↓ mRNA expression of IL-6, TNF-α, and PAI-1 ↓ Activation of JNK, and IKKβ ↓ NF-kB concentrations	↓ 4-HNE and 3-NT in liver
Greany et al. (104)	Postmenopausal women (47–69 y of age)	Chronic inflammation	SP + ISF	26 g SP/d + 44 mg ISF/d for 6 wk	↔ CRP ↔ E-selectin ↔ VCAM-1 and ICAM-1	NA
Törmälä et al. (105)	Postmenopausal women (57 y of age); tibolone users	Vascular inflammation	SP + ISF	52 g ISF/d + 112 mg ISF/d for 8 wk	↔ CRP ↔ ICAM-1 ↑ VCAM-1	NA
Archarjee et al. (106)	Postmenopausal women (54 y of age), with or without MetS (equol producers)	Inflammatory markers related to coronary artery disease risk	SP + ISF (soy nut)	25 g SP/d +101 mg ISF/d for 8 wk	↓ CRP ↓ Soluble ICAM-1	NA
Azadbakht et al. (18)	Postmenopausal women with MetS	Systemic inflammation	SP + ISF (soy nut)	37.5 g SP/d + 340 mg ISF/d for 8 wk	↓ IL-18 ↓ CRP ↓ TNF-α ↓ E-selectin	NA

(Continued)

TABLE 2 *Continued*

Reference	Cell or organism tested	Condition	Type of protein	Supplementation protocol	Effects on inflammation	Effects on oxidative stress
Nasca et al. (107)	Postmenopausal women (58.3 ± 6 y of age), hypertensive	Systemic inflammation	SP + ISF (soy nut)	25 g SP/d + 101 mg ISF/d for 8 wk	↓ Soluble VCAM-1 ↓ CRP ↔ Soluble ICAM-1 ↔ IL-6 ↔ MMP-9	NA
Fanti et al. (110)	Adult ESRD patients (60 ± 3.4 y of age)	Chronic inflammation	SP + ISF	25 g SP/d + 54 mg ISF/d, 3 times/wk, and 11 g SP/d + 26 mg ISF/d, 4 times/wk, for 8 wk	↓ CRP	NA
Mangano et al. (111)	Healthy women (>70 y of age) with baseline CRP 5.24 pg/mL and baseline IL-6 2.76 pg/mL	Systemic inflammation	SP + ISF	18 g SP/d + 105 mg ISF/d for 1 y	↓ IL-6	NA
Vega-López et al. (112)	Hypercholesterolemic individuals (>50 y of age)	Antioxidant protection related to elevated LDL cholesterol	SP + ISF	17% of total energy SP + 1.25 mg ISF/(1000 kcal · d) for 42 d	NA	↔ LDL oxidizability ↔ Urinary F2-isoprostanes ↔ Malondialdehyde ↔ Protein carbonyls (native plasma) ↓ Protein carbonyls (oxidized plasma)
Vega-López et al. (112)	Hypercholesterolemic individuals (>50 y of age)	Antioxidant protection related to elevated LDL cholesterol	SP + ISF	16% of total energy SP + 46.21 mg ISF/(1000 kcal · d) for 42 d	NA	↔ LDL oxidizability ↔ Urinary F2-isoprostanes ↔ Malondialdehyde ↔ Protein carbonyls
Swain et al. (113)	Postmenopausal women (41.9–61.6 y of age)	Menopause associated antioxidant status	SP + ISF	40 g SP/d for 24 wk (ISF content not described)	NA	↓? Total antioxidant status
Beavers et al. (115)	Postmenopausal women (40–60 y of age)	Systemic inflammation and oxidative stress	Soy milk	6 g SP/serving, 3 servings/d, for 4 wk	↔ TNF-α ↔ IL-6 ↔ IL-1β	↔ SOD activity ↔ GPx activity
Miraghajani et al. (116)	Type 2 diabetic patients with nephropathy (51 ± 10 y of age)	Inflammation and oxidative stress	Soy milk	Bolus of 240 mL soy milk/d for 4 wk	↔ TNF-α ↔ IL-6 ↔ hs-CRP	↔ Malondialdehyde
Mitchell and Collins (117)	Healthy adult men (20–50 y of age)	Oxidative DNA damage	Soy milk	1 L soy milk/d for 4 wk	NA	↓ DNA damage
Jenkins et al. (118)	Hypercholesterolemic men and postmenopausal women (62 y of age)	Inflammatory markers	Soy diet (including soy milk)	50 g SP/d + 73 mg ISF/d for 1 mo	↔ TNF-α ↔ CRP ↑ IL-6 (in women only)	
Jenkins et al. (118)	Hypercholesterolemic men and postmenopausal women (62 y of age)	Inflammatory markers	Soy diet (including soy milk)	52 g SP/d + 10 mg ISF/d for 1 mo	↔ TNF-α ↔ CRP	

[1] CRP, C-reactive protein; ESRD, end-stage renal disease; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intracellular adhesion molecule 1; IKKβ, inhibitor of NF-κB kinase β; ISF, isoflavone; JNK, c-Jun *N*-terminal kinase; MCP-1, monocyte chemotactic protein 1; MetS, metabolic syndrome; MMP-9, matrix metalloproteinase 9; NA, not applicable; PAI-1, plasminogen activator inhibitor 1; SOD, superoxide dismutase; SP, soy protein; SPI, soy protein isolate; VCAM-1, vascular cell adhesion molecule 1; 3-NT, 3-nitrotyrosine; 4-HNE, 4-hydroxy-2,3-nonenal; ↑, increase; ↓, decrease; ↔, no effect; ≈, maintenance.

activation of NF-κB, which activates the expression of proinflammatory cytokines such as TNF-α and IL-6 (17, 102, 103).

In postmenopausal women, the administration of 26 g soy protein/d enriched with 44 mg isoflavones/d for 6 wk did not affect circulating concentrations of CRP and various adhesion molecules (104). Törmälä et al. (105) increased the amount of supplemented soy protein to 52 g/d and the amount of isoflavones to 112 mg/d, and also extended the supplementation period to 8 wk, but they did not observe any anti-inflammatory action either. In contrast, consumption of soy nuts containing either 25 g soy protein/d and 101 mg isoflavones/d or 37.5 g soy protein/d and 340 mg isoflavones/d for 8 wk led to significant reductions in blood CRP, soluble intercellular adhesion molecule 1, E-selectin, TNF-α, and IL-18 in postmenopausal women with metabolic syndrome (18, 106), as well as in VCAM-1 in hypertensive postmenopausal women (107). However, discrepancies between these studies may be related to the different supplementation protocols applied, as well as to the clinical status of the participants [e.g., in the study by Törmälä et al. (105), subjects were tibolone users]. Moreover, the anti-inflammatory potential of soy nuts may be attributed to the fact that nuts contain all the bioactive compounds of a soybean, including soy protein, fat, and phytoestrogens, whereas supplemented proteins are isolated and in some cases are combined with isoflavones only (89, 108, 109). Interestingly, in patients with end-stage renal disease, which is characterized by systemic inflammation (CRP > 10.0 mg/L), the administration of SPI that retained its isoflavone content led to a marked elevation of circulating isoflavone concentrations that was inversely correlated with inflammatory markers (110). Moreover, in a 1-y clinical study, the intake of 18 g soy protein/d along with 105 mg isoflavone/d reduced blood concentrations of IL-6 in healthy older (>70 y of age) women with baseline CRP and IL-6 values of 5.24 pg/mL and 2.76 pg/mL, respectively (111). To the best of our knowledge, this is the only human study that used a population with characteristics of inflammaging, and it suggested that a combination of sufficient quantities of soy protein and isoflavones may have the potential to prevent or alleviate inflammation. Although this study did not look into skeletal muscle responses, it supports a rationale for soy protein use as an anti-inflammatory intervention. Two studies that examined the antioxidant potential of soy proteins provided some positive evidence. In a crossover design, administration of either a soy protein (17% of total energy and 1.25 mg isoflavones/1000 kcal) or an isoflavone-enriched soy protein (16% of total energy and 46.21 mg isoflavones/1000 kcal) diet for 42 d had no significant impact on markers of oxidative stress, but improved TAC by 10% (112). Swain et al. (113) also reported that supplementation with soy protein in perimenopausal women improved TAC. Collectively, these studies suggest that soy protein with isoflavones may represent a potent anti-inflammatory and antioxidant agent, further supporting the rational for its use in proinflammatory conditions such as inflammaging. Although this data supports an anti-inflammatory role for soy protein, to our knowledge, no data exist regarding its effectiveness in promoting muscle mass and function in inflammaging. When dairy and soy protein were compared in healthy aged adults, the 2 types of proteins were equally effective in improving body composition and functionality, but the former was more effective in increasing muscle strength (114).

Studies that investigated the effects of soy protein and isoflavone-enriched soy protein on inflammatory and oxidative stress responses are presented in **Table 2**.

Conclusions

In conclusion, oxidative stress and inflammation interact in a vicious cycle, creating a chronic state of systemic inflammation that in the elderly is known as inflammaging. Many health-related

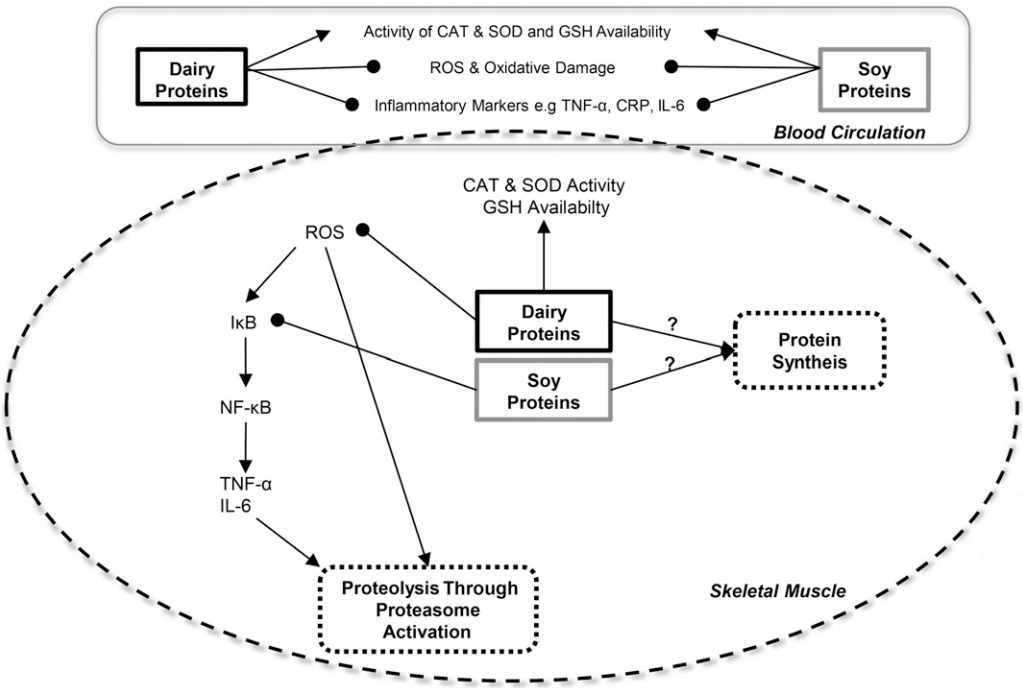

FIGURE 2 Mechanistic links between protein feeding and inflammaging. CAT, catalase; CRP, C-reactive protein; GSH, reduced glutathione; IκB, inhibitor of NF-κB; ROS, reactive oxygen species; SOD, superoxide dismutase; ?, lack of evidence regarding the ability of these proteins to stimulate muscle protein synthesis in inflamed elderly; →, increase or activation; —●, decline or inhibition.

dysfunctions and chronic diseases, as well as loss of muscle mass and consequently independence in the elderly, have been associated with inflammaging; therefore, it is crucial to develop nutritional, exercise-based, and pharmaceutical strategies to counteract its detrimental effects. Dairy and soy products contain high-quality proteins of high nutritional value because of their amino acid composition and absorption kinetics. Whey protein exhibits antioxidative properties that are attributed to its ability to increase glutathione availability and enhance the activity of the antioxidative enzymes SOD, catalase, and glutathione peroxidase. Evidence from animal models and cell lines indicate that whey protein may regulate multiple intracellular pathways related to ROS production. However, future studies should explore the TNF-α/NF-κB/ubiquitin–proteasome pathway and its crosstalk with Akt-related signaling in skeletal muscle in inflammaging in response to various protein feeding protocols. Whey administration may attenuate exercise-induced oxidative stress and inflammation, as well as inflammation resulting from clinical complications and obesity. Soy protein is a promising nutritional strategy against chronic inflammation, with it having been shown that either in its isolated form or isoflavone-enriched, it is able to inhibit the activation of the NF-κB and subsequently the upregulation of proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β, as well as other mediators, such as VCAM-1 and monocyte chemoattractant protein 1. Although this mechanism of action is evident in animal models only, soy protein supplementation has been associated with reduced concentrations of chronic low-grade inflammation in the elderly as well. There is a great need for well-controlled experimental trials to determine whether an increase in protein consumption may aid MPS and muscle function in older adults with elevated systemic inflammation. Well-controlled randomized trials should compare dairy with plant protein feeding with or without an anabolic type of exercise in aged adults with a proinflammatory profile with the use of long-term supplementation protocols, as well as an assessment of muscle function and mass. A schematic representation of potential mechanisms through which protein supplementation may offset inflammation and boost muscle anabolism and performance in the elderly is presented in **Figure 2**.

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Disparate Habitual Physical Activity and Dietary Intake Profiles of Elderly Men with Low and Elevated Systemic Inflammation

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Abstract: The development of chronic, low-grade systemic inflammation in the elderly (inflammaging) has been associated with increased incidence of chronic diseases, geriatric syndromes, and functional impairments. The aim of this study was to examine differences in habitual physical activity (PA), dietary intake patterns, and musculoskeletal performance among community-dwelling elderly men with low and elevated systemic inflammation. Nonsarcopenic older men free of chronic diseases were grouped as 'low' (LSI: $n = 17$; 68.2 ± 2.6 years; hs-CRP: <1 mg/L) or 'elevated' (ESI: $n = 17$; 68.7 ± 3.0 years; hs-CRP: >1 mg/L) systemic inflammation according to their serum levels of high-sensitivity CRP (hs-CRP). All participants were assessed for body composition via Dual Emission X-ray Absorptiometry (DEXA), physical performance using the Short Physical Performance Battery (SPPB) and handgrip strength, daily PA using accelerometry, and daily macro- and micronutrient intake. ESI was characterized by a 2-fold greater hs-CRP value than LSI ($p < 0.01$). The two groups were comparable in terms of body composition, but LSI displayed higher physical performance ($p < 0.05$), daily PA (step count/day and time at moderate-to-vigorous PA (MVPA) were greater by 30% and 42%, respectively, $p < 0.05$), and daily intake of the antioxidant vitamins A (6590.7 vs. 4701.8 IU/day, $p < 0.05$), C (120.0 vs. 77.3 mg/day, $p < 0.05$), and E (10.0 vs. 7.5 mg/day, $p < 0.05$) compared to ESI. Moreover, daily intake of vitamin A was inversely correlated with levels of hs-CRP ($r = -0.39$, $p = 0.035$). These results provide evidence that elderly men characterized by low levels of

systemic inflammation are more physically active, spend more time in MVPA, and receive higher amounts of antioxidant vitamins compared to those with increased systemic inflammation.

Keywords: aging; chronic low-grade systemic inflammation; physical activity; nutrition; physical performance; chronic diseases

1. Introduction

Chronic exposure to antigens as well as to chemical, physical, and nutritional stressors that the immune system has to cope with, in combination with the dramatic increase in life expectancy, result in the overstimulation of the immune system with advancing age and the development of a chronic and persistent pro-inflammatory state [1,2]. This age-associated, low-grade, chronic inflammatory status has been termed as "inflammaging" [1] and is clinically assessed by measuring systemic concentrations of cytokines and acute-phase proteins, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) [3]. Inflammaging represents a significant risk factor for age-related frailty, morbidity, and mortality [2,4] as many chronic diseases and geriatric syndromes such as cardiovascular diseases, atherosclerosis, metabolic syndrome, type 2 diabetes mellitus, neurodegenerative diseases, cancer, and chronic obstructive pulmonary disease have been associated with chronic inflammation [5–8]. Moreover, increased levels of IL-6, TNF-α, and CRP in the elderly have been associated with lower muscle mass and physical performance [9–11] as well as with increased risk for sarcopenia and osteoporosis [12–14]. Thus, the concept of inflammaging appears to be a key determinant of successful aging and longevity and as such a valuable tool to counteract age-related pathologies [2].

To date, inflammaging is defined as a complex and multifactorial process whose origin cannot be simply attributed to a specific number of factors/mechanisms, as a complete understanding of the extent to which different tissues, organs, and biological systems contribute to its pathophysiology is lacking [3,15]. However, both physical activity (PA) and nutrition are considered powerful lifestyle factors that may, cooperatively or independently, influence both healthy aging and lifespan in humans [16,17]. Specifically, being physically active substantially reduces the risk of developing cardiovascular [16,17] and metabolic diseases [16,18], obesity [16,19], frailty [16,20,21], sarcopenia [22], osteoporosis [17,23], cognitive impairment [24], and mental health disorders [17,25] in a dose-response manner [26,27]. Numerous studies reported that higher volume of habitual PA is related to lower levels of IL-6, CRP, and TNF-α in older adults [28–40]. Most of these studies, though, are based on self-reported PA estimations [28–33,36,37,40] that may result in increased risk of recall bias [41] and therefore do not provide an objective determination of different intensity levels (i.e., light, moderate, vigorous, or very vigorous PA). However, to our knowledge, four studies have utilized accelerometry to provide an objective assessment of PA [34,35,38,39]. In two of them, an inverse relationship between PA and disease-related (chronic obstructive pulmonary disease and obesity) systemic inflammation was revealed in middle-aged adults [34,35]. Similarly, two other studies reported that time spent in MVPA is negatively associated with markers of systemic inflammation in the healthy elderly [38,39]. Although these data clearly suggest that habitual PA is inversely associated with mediators of systemic inflammation in older adults, a direct comparison of objectively assessed PA, sedentary time, and PA-related energy expenditure among the elderly with low and increased systemic inflammation is still lacking.

Ideally, this comparison would be more conclusive by the concurrent examination of habitual PA/inactivity and dietary intake levels, since both factors may impact systemic inflammation. In fact, available data suggest that the role of nutrition and dietary pattern is pivotal for immune function and low-grade systemic inflammation [42–44]. Both macronutrient and micronutrient intake may interfere with immune responses, triggering either a pro-inflammatory or an anti-inflammatory effect [45].

Excessive consumption of glucose and saturated fatty acids (SFA) (particularly long-chain SFA) are reported to activate pro-inflammatory markers in insulin-sensitive tissues [45,46] and may result in systemic inflammation [15], while high phospholipid consumption, especially that of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), elicit antiinflammatory properties and reduce the risk of chronic inflammation and its associated chronic diseases [47]. On the other hand, consumption of either plant- or dairy-based protein or amino acids may offer antiinflammatory effects by reducing levels of inflammatory mediators [45,48]. Furthermore, adequate intake of antioxidants and trace elements, particularly vitamins A, C, E, and selenium, also enhances immunity and elicits a protective effect against chronic inflammatory conditions [44]. However, to our knowledge, the literature lacks evidence regarding differences in dietary habits among older healthy adults with low and high systemic inflammation.

Given the pivotal role of both PA and macronutrient/micronutrient intake in mediating immunity and chronic inflammatory responses, a direct comparison of them among older adults exhibiting low and elevated systemic inflammation may identify which parameters of these lifestyle factors function as discriminants of healthy aging and inflammaging. Therefore, the aim of the present study was to compare levels of objectively assessed habitual PA and dietary macronutrient/micronutrient intake, among otherwise healthy elderly men of low and increased systemic inflammation.

2. Materials and Methods

2.1. Experimental Design and Participants

A total of fifty community-dwelling elderly men aged 65–75 years were recruited from the surrounding area of Thessaly (Greece) through postings, newspaper, and media advertisements. All volunteers completed a health history questionnaire and were also examined by a physician. In order to be included in the study, volunteers had to initially meet all of the following inclusion/exclusion criteria: (a) nonsmokers; (b) independently living; (c) absence of chronic disease (i.e., cancer, metabolic, cardiovascular, neurological, pulmonary, or kidney disease); (d) absence of inflammatory disease (i.e., osteoarthritis, rheumatoid arthritis); (e) absence of type 2 diabetes, and (f) no recent or current use of antibiotics or other medication that could affect inflammatory status (i.e., corticosteroids). Subsequently, those who fulfilled these criteria underwent assessment of body height, body weight, body composition, handgrip strength, and physical performance (via the SPPB) testing to estimate their weight status and stage of sarcopenia according to the European Working Group on Sarcopenia in Older People (EWGSOP) [49]. Volunteers who were characterized as presarcopenic/sarcopenic were excluded from the study at this stage, since substantial loss of skeletal muscle mass is accompanied by significant performance decline [49], resulting in lower levels of habitual PA [50]. Volunteers who were classified as obese were also excluded since obesity is linked to metaflammation, an adipose-tissue-mediated chronic inflammatory state that differs in terms of pathophysiology from inflammaging [5,15]. Accordingly, thirty-four volunteers who fulfilled the eligibility criteria participated in the study. The determination of inflammatory status was based on two consecutive measurements of high-sensitivity CRP (hs-CRP) and participants were grouped as "low systemic inflammation" (LSI: hs-CRP < 1 mg/L) or "elevated systemic inflammation" (ESI: hs-CRP > 1 mg/L) according to a previous report [51]. Participants were then provided with accelerometers and food diaries to monitor their habitual PA and daily macronutrient/micronutrient intake, respectively, over a 7-day period. They were fully informed about the aim and the experimental procedures of the study, as well as about the benefits involved, before obtaining written consent. The Institutional Review Board of the University of Thessaly approved the study and all procedures were in accordance with the 1975 Declaration of Helsinki (as revised in 2000).

2.2. Body Composition

Standing body mass and height were measured on a beam balance with stadiometer (Beam Balance-Stadiometer, SECA, Vogel & Halke, Hamburg, Germany) with participants wearing light clothing and no shoes as described previously [52]. Body composition [including fat mass, fat-free mass (FFM), percent of fat, lean body mass (LBM)] was assessed by dual emission X-ray absorptiometry (DXA, GE Healthcare, Lunar DPX NT, Diegem, Belgium) with participants in supine position as described before [53]. Appendicular lean mass (ALM) and skeletal muscle mass index (SMI) were calculated as the sum of muscle mass (kg) of the four limbs (based on DXA scan) and as ALM divided by height by meters squared (kg/m^2), respectively [49], while sarcopenia status was determined according to the criteria established by EWGSOP [49].

2.3. Physical Activity

Physical activity was monitored by using the accelerometers ActiGraph, GT3X+ (ActiGraph, Pensacola, FL, USA) over a 7-day period. Accelerometers were attached to elastic, adjustable belts and did not provide any feedback to the participants. Participants were taught how to wear the belt around the waist with the monitor placed on the right hip and they were asked to wear it throughout the day, except for bathing or swimming and sleep, for seven consecutive days. To be included in the analysis, participants had to have ≥four days with ≥10 wear hours/day (i.e., four valid days) [54]. Nonwear time was calculated using the algorithms developed by Choi et al. [55] for vector magnitude (VM) data and defined as periods of 90 consecutive minutes of zero counts per minute (cpm), including intervals with nonzero cpm that lasted up to 2 min and were followed by 30 consecutive minutes of zero cpm. Daily activity and sedentary time were estimated according to VM data and expressed as steps/day and time in sedentary (<199 cpm), light (200–2689 cpm), moderate (2690–6166 cpm), vigorous (6167–9642 cpm), and moderate-to-vigorous (≥2690 cpm) PA [56]. The manufacturer software ActiLife 6 was utilized to initialize accelerometers and download data using 60-s epoch length.

2.4. Dietary Assessment

Participants were taught by a registered dietitian how to estimate food servings and sizes of different food sources and how to complete food diaries. They were allowed to weigh out food servings, so that they could precisely report the amount of specific food portions, while they were also provided with colored photographs depicting different portion sizes that they could use to compare their food weights. Furthermore, complete instructions on how to describe portion sizes based on household measures or other standard units were also administered to our participants. Participants recorded their daily dietary intake for seven consecutive days, describing, in as much detail as possible all portions of food and drinks/water. For commercially available products, the name of the manufacturer, fat content (i.e., 1%. 2%, etc.), and other related information had to be noted. The Science Fit Diet 200 A (Science Technologies, Athens, Greece) dietary software was utilized to analyze diet recalls and data regarding total energy (kJ), protein (g/kg/day & g/day), leucine (g/day), branched chain amino acids (BCAA, g/day), carbohydrates (g/day), fat (g/day), vitamin A (IU/day), vitamin C (mg/day), vitamin E (mg/day), selenium (μg/day), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA).

2.5. Systemic Inflammation

Blood samples were collected early in the morning between 07:00 and 09:00 am, after an overnight fasting. Participants were asked to avoid alcohol and abstain from intense physical activity for ≥48 h before blood sampling. Blood was drawn from an antecubital arm vein via a 10-gauge disposable needle equipped with a Vacutainer tube holder (Becton Dickinson) with participants seated. To separate serum, blood samples were allowed to clot at room temperature and then centrifuged (15,000× *g*, 15 min, 4 °C). The supernatant was dispensed in multiple aliquots (into Eppendorf tubes) and stored

at −80 °C for later analysis of hs-CRP. Serum hs-CRP was quantitatively measured in duplicate using the C-Reactive Protein (Latex) High Sensitivity assay (CRP LX High Sensitive, Cobas®) on a Cobas Integra® 400 plus analyzer (Roche) with a detectable limit of 0.01 mg/dL and an inter-assay coefficient of one standard deviation (1 SD).

2.6. Statistical Analyses

All data are presented as means ± SD. The normality of data was examined using the Shapiro–Wilk test (n = 17/group). Because our data sets in most of our variables differed significantly from normal distribution, we rejected the hypothesis of normality and applied nonparametric tests. To test differences in body composition, daily PA-related parameters, and dietary macronutrient/micronutrient intake among the two groups (LSI vs. HSI) a Kruskal–Wallis test was applied. Pearson's correlation analysis was used to examine the relation of dietary antioxidant vitamins intake, number of steps, and time in MVPA per day with serum levels of hs-CRP. Correlation coefficients of $r < 0.2$, $0.2 < r < 0.7$ and $r > 0.7$ were defined as small, moderate, and high, respectively. Effect sizes (ES) and confidence intervals (CI) were also calculated for all dependent variables using the Hedge's g method corrected for bias. ES was interpreted as none, small, medium-sized, and large for values 0.00–0.19, 0.20–0.49, 0.50–0.79, and ≥0.8, respectively. The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed using the SPSS 20.0 software (IBM SPSS Statistics). The G * Power program (G * Power 3.0.10) was utilized to perform power analysis. With our sample size of 17/group we obtained a statistical power greater than 0.80 at an α error of 0.05.

3. Results

Participants' characteristics are presented in Table 1. Participants were healthy and had no pathological levels of hs-CRP. The two groups, though, differed significantly in respect to hs-CRP values (ESI: 2.1 ± 0.8 vs. LSI: 0.7 ± 0.2 mg/dL, $p = 0.00$), with ESI displaying a 2-fold elevation in serum hs-CRP compared to LSI. Averaged BMI values in LSI and ESI were 27.3 ± 3.1 kg/m^2 and 27.9 ± 2.5 kg/m^2, respectively, which classifies them as nonobese according to the criteria established by the World Health Organization (WHO) [57]. Moreover, all participants were characterized as nonsarcopenic, since they exhibited SMI > 7.26 kg/m^2, handgrip strength > 30 kg, and physical performance score in SPPB > 8. No differences were detected in respect to BMI, fat mass, percent of fat, FFM, LBM, ALM, SMI, and handgrip strength among groups. However, significant differences were observed in physical performance, with LSI achieving a higher SPPB score compared to ESI (LSI: 11.9 ± 0.2 vs. ESI: 11.2 ± 1.0; $\chi^2 = 6.436$, $p = 0.016$; ES = 0.90; 95% CI = −1.63, −0.17).

Table 1. Participants' characteristics.

Parameter	LSI (n = 17)	ESI (n = 17)
Age (years)	68.2 ± 2.6	68.7 ± 3.0
Body Height (m)	1.71 ± 0.07	1.73 ± 0.04
Body Weight (kg)	82.3 ± 8.5	85.2 ± 7.5
BMI (kg/m^2)	27.3 ± 3.1	27.9 ± 2.5
Fat Mass (kg)	24.1 ± 7.0	26.3 ± 4.1
Fat (%)	29.5 ± 6.6	31.8 ± 2.1
Fat-Free Mass (kg)	56.3 ± 4.6	58.4 ± 5.2
Lean Body Mass (kg)	53.3 ± 4.5	55.3 ± 5.1
ALM (kg)	23.2 ± 2.4	24.4 ± 2.1
SMI (kg/m^2)	8.12 ± 0.7	8.13 ± 0.6
Grip Strength (kg)	34.3 ± 5.5	36.7 ± 6.6
SPPB (score)	11.9 ± 0.2	11.2 ± 1.0 [1]
Sarcopenia Status	Non-Sarcopenic	Non-Sarcopenic
hs-CRP (mg/L)	0.7 ± 0.2	2.1 ± 0.8 [2]

Data are presented as mean ± SD. ALM: Appendicular Lean Mass; SMI: Skeletal Muscle Mass Index; SPPB: Short Physical Performance Battery; hs-CRP: High-Sensitivity CRP. [1] significant difference between groups, $p < 0.05$, [2] significant difference between groups, $p < 0.01$.

Results comparing sedentary time and PA among groups are shown in Figure 1. The two groups were comparable in sedentary time throughout the day (LSI: 378.2 ± 98.7 vs. ESI: 370.5 ± 95.9 min/day; χ^2 = 0.008, p = 0.927) and in the time they spent in light PA/day (LSI: 342.9 ± 93.1 vs. ESI: 331.7 ± 98.2 min/day; χ^2 = 0.357, p = 0.550), while a trend for significantly more time spent in moderate PA/day by the LSI group was also observed (LSI: 59.5 ± 16.7 vs. ESI: 44.1 ± 18.2 min/day; χ^2 = 3.637, p = 0.057). Interpretation of the level of moderate PA by group means examined in relation to the PA guidelines adopted by the WHO revealed that both groups met the recommendation for at least 150 min of moderate-intensity PA throughout the week.

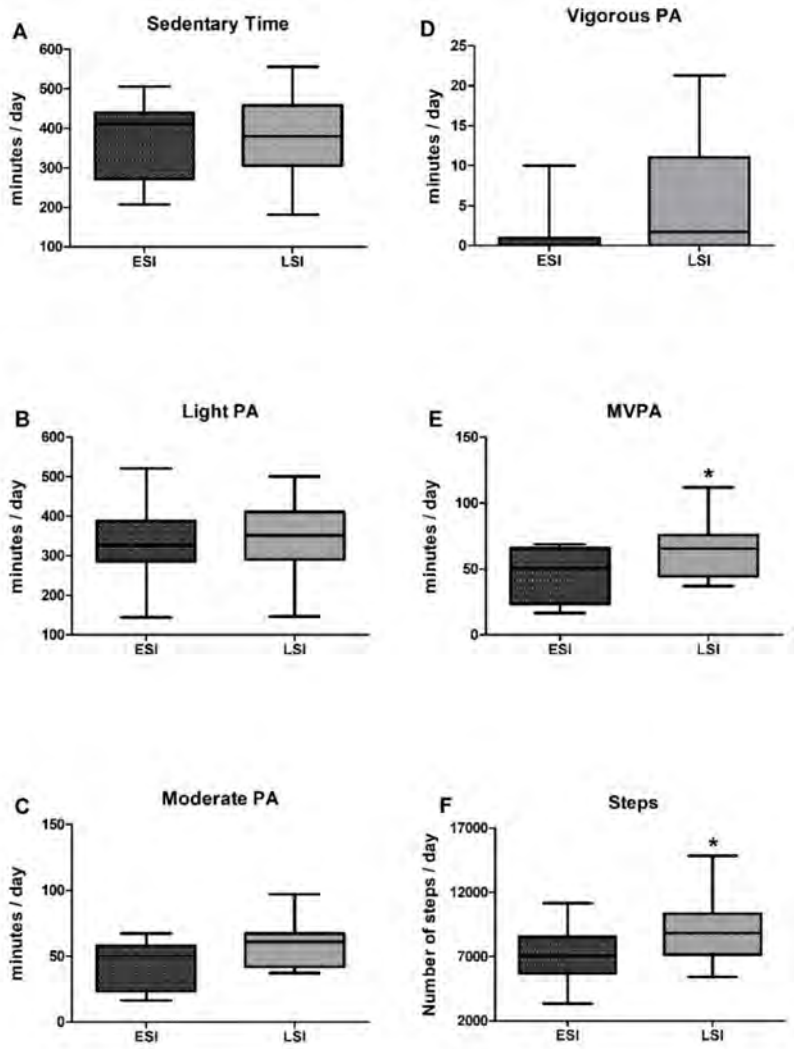

Figure 1. (**A**) Sedentary time, (**B**) time spent in light, (**C**) moderate, (**D**) vigorous, (**E**) moderate-to-vigorous (MVPA) PA, and (**F**) total step count throughout the day, in low (LSI) and elevated (ESI) systemic inflammation groups. Values are presented as mean ± SD. * denotes significant difference between groups at $p < 0.05$.

By performing an individual examination in both groups, we found that all participants in LSI and approximately 86% of participants in ESI met this criterion. Significant differences between LSI and ESI were observed in MVPA and daily step count, with LSI spending more time in MVPA throughout the day (LSI: 65.2 ± 21.5 vs. ESI: 45.9 ± 19.8 min/day; χ^2 = 3.997, p = 0.044; ES = 0.91; 95% CI = −1.68, −0.13) and performing more steps (LSI: 9000.1 ± 2496 vs. ESI: 6968.3 ± 2075 steps/day; χ^2 = 4.087, p = 0.043; ES = 0.86; 95% CI = −1.63, −0.08) than ESI, by 42% and 30%, respectively. The average step count/day for LSI was 9000.1 steps, which is close to the upper recommended limit for older

adults (7100–10,000 steps/day) [58] while the ESI did not meet these recommendations, performing 6968.3 steps/day. Almost 86% of participants in the LSI group performed >7100 steps daily while slightly more than half (53%) of participants in the ESI group did so. A longitudinal analysis combining both groups revealed a trend for an inverse correlation between hs-CRP level and daily step count ($r = -0.37$, $p = 0.055$). Time in vigorous PA/day did not differ among groups (LSI: 5.3 ± 6.9 vs. ESI: 1.0 ± 2.6 min/day; $\chi^2 = 2.315$, $p = 0.128$), probably because of a high interindividual variability. Moreover, the two groups demonstrated similar PA-related energy expenditure throughout the day, as no differences observed in terms of kJ/day (LSI: 2554.3 ± 1033.5 vs. ESI: 2654.3 ± 1041.8 kJ/day, $p = 0.798$) and METs/day (LSI: 1.28 ± 0.1 vs. ESI: 1.23 ± 0.1 METs/day, $p = 0.203$) (Figure 2).

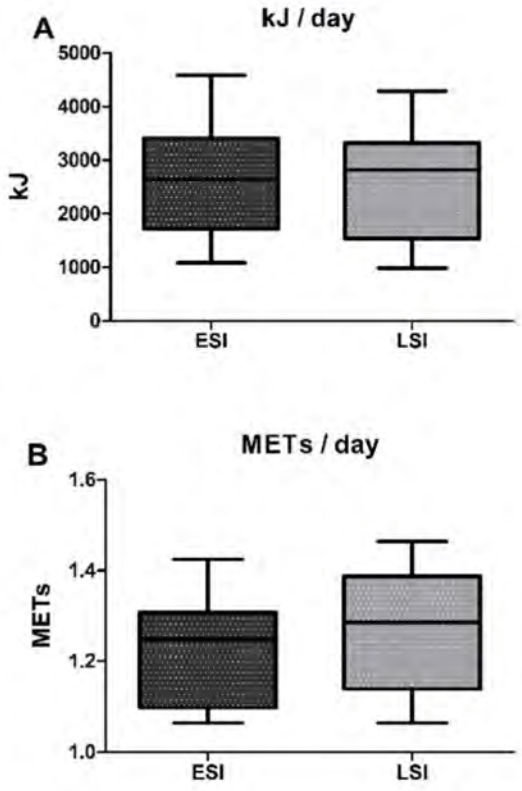

Figure 2. Daily PA-related energy expenditure expressed as (**A**) kJ and (**B**) METs in low (LSI) and elevated (ESI) systemic inflammation groups. Values are presented as mean ± SD.

LSI and ESI demonstrated similar total energy and macronutrient intake throughout the day (Table 2). The two groups had a daily energy intake of 6949.6–6794.8 kJ, constituted by 15–16% protein, 38% carbohydrate, and 42% fat. The mean protein intake in both groups was 0.8 g/kg body weight/day, which represents the recommended daily allowance (RDA) that meets 97.5% of the population [59]. However, approximately 46% of participants in both groups had a daily protein intake of 0.5–0.7 g/kg body weight/day. Separate analysis in leucine and BCAA intake revealed that both LSI and ESI received 0.6 g of leucine/kg body weight/day and 0.13–0.14 g of BCAAs/kg body weight/day, which meets the current recommendations for amino acid intake in adults [59]. The two groups, though, differed significantly in respect to daily antioxidant vitamin intake, with the LSI group receiving higher amounts of vitamin A (LSI: 6590.7 ± 2219 vs. ESI: 4701.8 ± 1552.6 IU/day; $\chi^2 = 5.616$, $p = 0.018$; ES = 0.95; 95% CI = 1.72, 0.18), vitamin C (LSI: 120.0 ± 55.5 vs. ESI: 77.3 ± 39.1 mg/day; $\chi^2 = 5.421$, $p = 0.020$; ES = 0.87; 95% CI = 1.63, 0.11), and vitamin E (LSI: 10.0 ± 2.9 vs. ESI: 7.5 ± 3.0 mg/day; $\chi^2 = 4.496$, $p = 0.034$; ES = 0.75; 95% CI = 1.50, 0.01) than ESI, by 37%, 59%, and 33%, respectively. Moreover, by performing a longitudinal analysis of both groups we observed that daily vitamin A intake was inversely correlated with levels of hs-CRP ($r = -0.39$, $p = 0.035$) (Figure 3). On the contrary,

daily intake of selenium (LSI: 93.2 ± 29.8 vs. ESI: 96.1 ± 29.7 μg/day, $p = 0.793$), PUFA (LSI: 10.1 ± 2.4 vs. ESI: 8.9 ± 2.6 g/day, $p = 0.215$), and MUFA (LSI: 43.7 ± 10.8 vs. ESI: 37.9 ± 10.9 g/day, $p = 0.168$) was comparable in the two groups.

Table 2. Dietary macronutrient and micronutrient intake in LSI and ESI groups.

Parameter	LSI (n = 17)	ESI (n = 17)	p Value	χ^2
Total Energy (kJ/day)	6952.9 ± 1241.8	6797.8 ± 1136.8	0.771	0.085
Protein				
g/day	63.8 ± 20.3	66.9 ± 14.6	0.183	1.770
g/kg BM/day	0.8 ± 0.3	0.8 ± 0.2	0.817	0.054
% of total calories	15 ± 2.7	16 ± 3.0		
Leucine (g/day)	4.89 ± 1.7	5.13 ± 1.2	0.430	0.624
BCAAs (g/day)	11.38 ± 3.6	11.53 ± 2.4	0.533	0.389
Carbohydrates				
g/day	156.2 ± 37.6	154.9 ± 52.7	0.901	0.016
% of total calories	37.7 ± 6.9	37.5 ± 8.4		
Fat				
g/day	79.3 ± 12.5	73.7 ± 17.0	0.318	0.996
% of total calories	42.0 ± 4.0	41.7 ± 7.1		
PUFA (g/day)	10.1 ± 2.4	8.9 ± 2.6	0.275	1.191
MUFA (g/day)	43.7 ± 10.8	37.9 ± 10.9	0.359	0.840
Vitamin A (IU/day)	6590.7 ± 2219.6	4701.8 ± 1552.6 [1]	0.018	5.616
Vitamin C (mg/day)	120.0 ± 55.5	77.3 ± 39.1 [1]	0.020	5.421
Vitamin E (mg/day)	10.0 ± 2.9	7.5 ± 3.0 [1]	0.034	4.496
Selenium (μg/day)	93.2 ± 29.8	96.1 ± 29.7	0.589	0.292

Data are presented as mean ± SD. BM: Body mass; BCAA: Branched chain amino acids; PUFA: Polyunsaturated fatty acids; MUFA: Monounsaturated fatty acids. [1] Significant difference between groups.

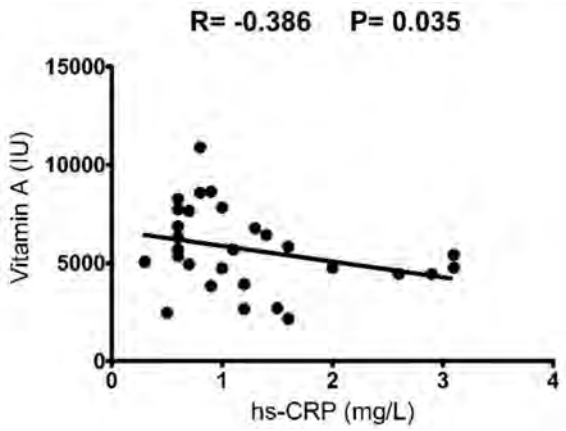

Figure 3. The relationship between serum hs-CRP level and daily dietary intake of Vitamin A.

4. Discussion

The present study is the first, to our knowledge, to compare the levels of habitual PA, sedentary time, and dietary intake between healthy elderly men with low and elevated low-grade systemic inflammation (inflammaging). Our findings suggest that older adults characterized by low levels of systemic inflammation perform more steps and spent more time in MVPA throughout the day and they receive higher amounts of dietary antioxidant vitamins (i.e., vitamins A, C, and E) on a daily basis compared to their counterparts with elevated systemic inflammation.

Participants were categorized as having either "low" or "elevated" low-grade systemic inflammation according to their serum levels of hs-CRP. This acute-phase protein is considered a valid and informative marker of inflammaging [60] and has been previously used as a single marker to identify levels of systemic inflammation in older adults [51]. The term inflammaging, first introduced

by Franceschi and his colleagues [1], refers to the development of a chronic, low-grade inflammation phenotype with advancing age. However, the presence of obesity, either in young or older individuals, results in elevated systemic inflammation, which has been defined as metaflammation (metabolic inflammation) and is primarily mediated by the adipose tissue [5]. Although the underpinning mechanisms of inflammaging and metaflammation may be different, these two chronic inflammatory conditions may overlap [15]. Therefore, in an attempt to focus on inflammaging in this study, we included only nonobese elderly men (according to WHO criteria). Moreover, LSI and ESI groups were very homogeneous in terms of body composition, since they did not differ in body weight, fat mass, percent of fat, FFM, and LBM. All participants were also nonsarcopenic according to the criteria established by the EWGSOP [49], since the existence of sarcopenia could act as a covariate in our investigation, interfering with their ability to habitually perform PA [50].

Previous cross-sectional studies have investigated the association between habitual PA and inflammatory biomarkers in middle-aged and older adults [28–31,33–40]. However, only two utilized accelerometry to quantify not only the quantity but also the quality (intensity) of habitual PA in the otherwise healthy elderly with physiological and elevated chronic, low-grade systemic inflammation [38,39]. This study attempted to extend the current literature by providing insights concerning the differences in PA and dietary intake profile among elderly men with low and elevated low-grade systemic inflammation. The use of accelerometry to objectively assess the quantity and intensity of habitual PA is a strength of our study, as most of the previously cited studies [28–31,33,36,37,40] are based on questionnaires, self-reports, or interviews. The use of accelerometers over a 7-day period to assess PA and sedentary time has been reported to be a valid and reproducible methodological approach in the elderly [61].

Although sedentary time and time spent in light- and moderate-intensity activities throughout the day were similar between LSI and ESI, we noted that overall the LSI group performed more steps and spent more time in MVPA on a daily basis. This suggests that not only the volume of habitual PA but also the intensity in which daily physical activities are performed may interfere with the development of chronic, low-grade systemic inflammation in older individuals. Our findings further build on previous reports that higher volume of habitual PA is associated with lower levels of pro-inflammatory mediators in healthy elderly individuals [29,33,36] and COPD patients [34]. Moreover, this inverse association between PA and inflammation is suggested to be dose-dependent, so that the more physically active an individual is, the lower the chronic inflammatory milieu [29,31,40]. Although only a trend ($r = -0.37$, $p = 0.055$) for an inverse correlation between hs-CRP level and daily step number was observed in our study, possibly because of an interindividual variability in daily step counts of our participants (we used accelerometers whereas questionnaires were utilized by others), these findings collectively suggest that habitual PA may be associated with inflammaging in an inverse, dose-response pattern. Furthermore, it has been recently reported that the impact of PA on chronic low-grade inflammation is not only dose-dependent but also intensity-dependent, as moderate-to-vigorous activities induce greater improvements in the inflammatory profile of older adults while light- or moderate-intensity physical activities are accompanied by no changes in inflammatory mediators [62]. Indeed, Wahlin-Larsson et al. [39] found that in recreationally active elderly women, the time spent in MVPA is inversely associated with serum levels of CRP, a finding also reported in younger individuals [63]. The mechanism/s through which PA reduces or prevents low-grade systemic inflammation in the elderly remains to be elucidated. Observational, cross-sectional studies are not designed to identify the mechanisms that underline the effects of systematic PA on chronic inflammation and as such, more intervention studies are needed [41,62]. Based on the fact that inflammaging is tightly regulated by the balance between pro- and anti-inflammatory mediators [64], a possible mechanism could be that PA, and especially MVPA, suppresses the production of pro-inflammatory cytokines and molecules that trigger the inflammatory milieu, and enhances the production of anti-inflammatory mediators [41,62,65]. Moreover, the process of inflammaging may be further affected by the age-associated increase in the

production of reactive oxygen and nitrogen species (RONS) that lead to redox balance disturbances and subsequent activation of the redox-sensitive NF-κB signaling pathway that stimulates the expression of numerous pro-inflammatory mediators such as TNF-α, IL-6, IL-1β, and CRP [48,66]. As such, a vicious cycle of RONS and pro-inflammatory molecule production is propagated, driving a chronic systemic pro-inflammatory phenotype [48,67]. Regular participation in moderate-to-vigorous intensity exercise has been shown to attenuate both basal and exercise-induced levels of oxidative damage, enhance the antioxidant capacity, and improve the DNA repair machinery in healthy, elderly individuals [68,69]. Thus, it can be proposed that systematic MVPA may prevent the development of inflammaging by lowering the production of RONS and levels of oxidative damage in the elderly.

LSI and ESI also differed significantly in terms of physical performance. More specifically, LSI exhibited higher performance in the SPPB test compared to ESI and this observation is in line with previous findings reporting that older adults with elevated systemic inflammation demonstrate lower physical performance [70,71]. Although the underlying mechanism leading from chronic inflammation to functional decline has not been clarified yet, it has been reported that systemic inflammation may impact physical performance by decreasing skeletal muscle mass [14,48]. However, in this study, the two groups demonstrated similar LBM, ALM, and SMI, indicating that the observed difference in physical performance was not muscle-mass-dependent. A previous report, though, by Wahlin-Larsson and colleagues [39] provided evidence that increased systemic inflammation influences muscle regeneration by decreasing the proliferation rate of myoblasts. In addition, increased inflammation and cytokine production may also reduce the quiescent satellite cells pool and attenuate their differentiation capacity [14]. Therefore, it can be assumed that elevated systemic inflammation may contribute to physical performance deterioration by attenuating the regeneration potential of the aged skeletal muscle.

We also utilized 7-day recalls to perform a thorough screening of the dietary intake in the LSI and ESI groups, focusing on macronutrients and micronutrients that have been shown to elicit either a pro- or an anti-inflammatory effect, and could be therefore characterized as 'key modifiers' in the process of inflammaging. LSI and ESI demonstrated similar energy and macronutrient intake, consuming 6794.8–6949.6 kJ/day composed of 15–16% protein, 38% carbohydrates, and 42% fat. Our group recently conducted a literature review suggesting that protein intake, especially that of whey protein and soy or isoflavone-enriched soy protein, may indirectly offer antioxidative and anti-inflammatory benefits beyond its ability to stimulate skeletal muscle protein synthesis [48]. Also, Zhou et al. [72] performed a meta-analysis on the effects of whey protein supplementation on levels of CRP, concluding that increased whey protein intake may induce favorable effects on individuals with elevated baseline CRP levels. However, in this study, we noted that daily protein intake was similar between LSI and ESI, with both groups receiving on average ~0.8 g/kg BM/day, which is in line with WHO RDA for protein [59]. BCAA and leucine intake were also compared among groups to provide a qualitative determination of daily protein intake. Although leucine is classified as a BCAA, we decided to present it separately because its role may differ from that of the other BCAAs, especially in the elderly where a higher amount of leucine should be consumed through diet to efficiently stimulate muscle protein synthesis and preserve muscle loss [73,74]. In our present work, we observed that LSI and ESI had a similar daily intake of BCAAs and leucine, meeting the recommendations for amino acid intake in adults [59]. Daily carbohydrate intake was also similar among groups (154–156 g/day), indicating that it does not play a prominent role in the development of inflammaging. Previous reports have noted that only increased consumption of high glycemic index carbohydrates may be associated with increased levels of inflammation [75]. Unfortunately, the determination of glycemic index and glycemic load in our participants' daily diets was not feasible.

Similarly, no differences were observed in total fat consumption among groups, with LSI and ESI receiving 79 and 74 g/day, respectively, which corresponds in both groups to 42% of daily energy intake. Although previous reports have indicated that increased fat consumption is associated with elevated systemic markers of inflammation [75,76], this was not the case here. High fat diets,

and primarily SFA, have been reported to induce substantial alterations in the gut microbial flora (i.e., increases gut mucosa permeability, epithelial brier disruption) that result in enhanced translocation of lipolysaccharide (LPS) in the circulation, thus promoting the development of low-grade systemic inflammation [76,77]. However, it should be highlighted here that not all SFA demonstrate equal properties and consumption of specific SFA (i.e., C14:0, C15:0, C17:0, CLA, and trans-palmitoleic) has been associated with positive effects on cardiovascular health [78]. On the other hand, increased intake of MUFA and/or PUFA has been proposed to counteract the pro-inflammatory cascade by reducing the translocation of LPS in the circulation [76] and suppressing the eicosanoid and PAF inflammatory pathways [47]. Indeed, many studies have revealed an inverse association between higher intake of dietary PUFA and/or MUFA and levels of pro-inflammatory mediators such as hs-CRP and IL-6 [75]. In this study, although no statistically meaningful differences were observed in dietary MUFA and PUFA intake between groups, LSI displayed a higher intake of MUFA and PUFA, by 15% and 13.5%, respectively, compared to ESI.

Interestingly, we noted significant differences between LSI and ESI in terms of antioxidant vitamin intake. More specifically, daily dietary intake of vitamins A, C, and E in LSI was higher by 37%, 59%, and 33%, respectively, as compared to ESI. These vitamins play a major role in immune function, so that adequate intake enhances innate, cell-mediated, and humoral antibody immunity while deficiency promotes the opposite effects [44,79]. With aging, the production of reactive oxygen and nitrogen species and that of pro-inflammatory cytokines rises significantly, propagating a vicious cycle of oxidative stress and inflammation that promotes a chronic low-grade inflammatory state [48,67]. Vitamin A has been shown to promote a T-helper type 2 immune response by reducing the expression of pro-inflammatory cytokines (i.e., interferon-γ, TNF-α and IL-12) and adipocytokines (i.e., leptin) [44,79] while it may also inhibit the activation of the redox-sensitive nuclear factor-kappa B (NF-κB) [44,79], a principal mediator of the bidirectional interaction between oxidative stress and inflammation [48]. Moreover, the pivotal role of vitamin A in chronic inflammation is further supported by the fact that a deficit in vitamin A intake is associated with a pronounced pro-inflammatory state and inability to cope with pathogens, as well as with reduced phagocytic capacity of macrophages [44]. Vitamin C also reduces the production of pro-inflammatory cytokines through inhibition of the transcription factor NF-κB [44]. The anti-inflammatory effect of this micronutrient is further supported by a previous investigation where vitamin C intake was inversely associated with levels of CRP and tissue plasminogen activator (t-PA) antigen in elderly men [80]. Furthermore, vitamin C acts as a potent antioxidant, protecting cells from ROS-mediated oxidative damage, while it may also boost the synthesis of other antioxidants such as vitamin E [44]. Likewise, vitamin E is able to confer protection against oxidative stress by increasing the concentration of endogenous antioxidant enzymes, such as SOD, CAT, and GPX, and it also prevents oxidative damage in the cell membrane [44,81]. Evidence based on human studies indicates that vitamin E supplementation in older adults improves immune function [44] and is associated with a lower concentration of pro-inflammatory mediators [82]. Collectively, these data corroborate the higher antioxidant vitamin intake observed in LSI in the present study, indicating that vitamins A, C, and E may contribute to the control of low-grade systemic inflammation in the elderly. By contrast, no differences were observed in selenium intake between LSI and ESI, although selenium is also considered a micronutrient that may efficiently influence both innate and acquired immune function and may enhance the antioxidative defense system [44].

5. Conclusions

We found that elderly men with low levels of systemic inflammation are characterized by higher quality and quantity of habitual PA and ingested higher amounts of antioxidant vitamins A, C, and E through normal diet when compared to those with increased systemic inflammation. To the best of our knowledge, this is the first study to directly compare elderly men of low and increased low-grade systemic inflammation in respect to habitual PA and dietary profile. PA and antioxidant vitamin intake appear to be discriminant factors of inflammaging and healthy aging. Future research should further

explore the cause and effect as well as the dose-response relationship between PA and/or antioxidant vitamins and inflammaging.

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Σύγκριση ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή σε δείκτες δύναμης, οξειδωτικού στρες και φλεγμονής

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Περίληψη

Σκοπός της μελέτης ήταν να διερευνήσει την ύπαρξη διαφορών μεταξύ ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή (ΧΣΦ), σε δείκτες ανοσοποιητικού συστήματος, οξειδωτικού στρες, αντιοξειδωτικής ικανότητας και δύναμης άνω και κάτω άκρων. Στη μελέτη έλαβαν μέρος συνολικά 33 υγιείς, άνδρες εθελοντές ηλικίας 65-75 ετών, οι οποίοι κατηγοριοποιήθηκαν με βάση τα επίπεδα της C-αντιδρώσας πρωτεΐνης υψηλής ευαισθησίας (hs-CRP) σε δύο ομάδες: α) ομάδα χαμηλής ΧΣΦ (hs-CRP < 1 mg/L) και β) ομάδα υψηλής ΧΣΦ (hs-CRP > 1 mg/L). Όλοι οι συμμετέχοντες υποβλήθηκαν σε αξιολόγηση των σωματομετρικών τους χαρακτηριστικών, της σύστασης σώματος με μηχάνημα διπλής ενεργειακής απορρόφησης ακτινών Χ (DXA), της μέγιστης δύναμης χειρολαβής και των 10ΜΕ κάτω άκρων στο μηχάνημα εκτάσεις γονάτων. Επιπλέον υποβλήθηκαν σε αιμοληψία για τη βιοχημική αξιολόγηση των λευκοκυττάρων και των υποπληθυσμών τους, των πρωτεϊνικών καρβονυλίων, που είναι δείκτης οξείδωσης των πρωτεϊνών, και της συνολικής αντιοξειδωτικής ικανότητας στον ορό. Η μέση συγκέντρωση hs-CRP στην ομάδα υψηλής ΧΣΦ ήταν περίπου 4 φορές μεγαλύτερη από εκείνη στην ομάδα χαμηλής ΧΣΦ (υψηλής ΧΣΦ: hs-CRP=0.6±0.1 mg/L / χαμηλής ΧΣΦ: hs-CRP=2.3±0.8 mg/L, p=0.00). Οι δύο ομάδες δεν διέφεραν μεταξύ τους όσον αφορά τα σωματομετρικά χαρακτηριστικά, το δείκτη μάζας σώματος, το ποσοστό λίπους, τη λιπώδη μάζα, την άλιπη μάζα, τη μυϊκή μάζα καθώς και τη δύναμη χειρολαβής και κάτω άκρων. Επίσης δεν εντοπίστηκαν διαφορές μεταξύ των ομάδων στη συγκέντρωση λευκοκυττάρων και πρωτεϊνικών καρβονυλίων. Αντίθετα, σημαντικές διαφορές εντοπίστηκαν στη συνολική αντιοξειδωτική ικανότητα, με την ομάδα χαμηλής ΧΣΦ να παρουσιάζει κατά 60% υψηλότερη αντιοξειδωτική ικανότητα από την ομάδα υψηλής ΧΣΦ ($p<0.05$). Συμπερασματικά, η συγκέντρωση λευκοκυττάρων και πρωτεϊνικών καρβονυλίων αλλά και τα επίπεδα δύναμης δεν διαφοροποιούνται μεταξύ ηλικιωμένων ανδρών με χαμηλή ΧΣΦ και υψηλή ΧΣΦ. Ωστόσο, τα άτομα με χαμηλή ΧΣΦ χαρακτηρίζονται από υψηλότερη αντιοξειδωτική ικανότητα.

Λέξεις κλειδιά: *χρόνια συστημική φλεγμονή, λευκοκύτταρα, πρωτεϊνικά καρβονύλια, μυϊκή δύναμη*

Comparison of elderly men with low and elevated chronic systemic inflammation in indices of strength, oxidative stress and inflammation

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Abstract

The aim of the study was to examine differences among elderly men with low and elevated chronic systemic inflammation (CSI) in indices of the immune system, oxidative stress, antioxidant capacity and strength of the upper and lower body. A total of thirty-three, healthy, elderly men aged 65-75 years were included in the study and grouped as either ″low‟ CSI (LCSI: n=16; hs-CRP: < 1 mg/L) or ″elevated‟ CSI (ECSI: n=17; hs-CRP: > 1 mg/L) according to their serum levels of high-sensitivity CRP (hs-CRP). All participants were assessed for anthropometrics, body composition via Dual Emission X-ray Absorptiometry (DXA), handgrip strength and lower limb muscle strength on a leg extension machine. Blood samples were also collected for the determination of white blood cells (WBC), granulocytes (GRA), monocytes (MON) and lymphocytes (LYM) concentration as well as for the measurement of protein carbonyls (PC) and total antioxidant capacity (TAC) in serum. ECSI was characterized by almost a 4-fold greater hs-CRP value compared to LCSI (ECSI: hs-CRP = 0.6±0.1 mg/L vs LCSI: hs-CRP = 2.3±0.8 mg/L, p=0.00). ECSI and LCSI were comparable in terms of anthropometric characteristics, body mass index, fat percent, fat mass, fat free mass, lean body mass as well as in handgrip and lower limb muscle strength. Moreover, no differences were observed among groups in WBC, GRA, MON and LYM counts and in PC concentration. In contrast, significant differences observed between groups in TAC, with LCSI displaying a greater antioxidant capacity than ECSI by 60% ($p<0.05$). In conclusion, white blood cell counts and protein carbonyl concentration as well as muscle strength of the lower and upper body are not different among elderly men with ″low‟ CSI and ″elevated‟ CSI. However, those with low levels of CSI are characterized by a greater antioxidant capacity compared to their counterparts with elevated CSI.

Keywords: *chronic systemic inflammation, white blood cells, protein carbonyl, muscle strength.*

Εισαγωγή

Σε υγιή άτομα τρίτης ηλικίας συχνά παρατηρείται η ανάπτυξη χρόνιας συστημικής φλεγμονής (ΧΣΦ), η οποία χαρακτηρίζεται από αυξημένα επίπεδα προ-φλεγμονώδη κυτταροκινών στην κυκλοφορία κατά 2 έως 3 φορές σε σχέση με τις φυσιολογικές τους τιμές (Calcada et al., 2014). Η εκδήλωση της ΧΣΦ αποδίδεται στη μακροχρόνια έκθεση του ανοσοποιητικού συστήματος σε αντιγόνα και στρεσογόνους παράγοντες (χημικούς, φυσικούς ή/και διατροφικούς) που σε συνδυασμό με την σημαντική αύξηση στο προσδόκιμο ζωής προάγουν την παρατεταμένη ενεργοποίηση του ανοσοποιητικού (Claudio Franceschi, 2007; C. Franceschi et al., 2000). Το φαινόμενο αυτό στη διεθνή βιβλιογραφία αναφέρεται ως ″inflammaging‟, βασισμένο στα δύο συνθετικά ″inflammation‟ (= φλεγμονή) και ″aging‟ (= γήρανση) (C. Franceschi et al., 2000), ενώ για τη διάγνωσή του οι κύριες μεταβλητές που αξιολογούνται είναι η συγκέντρωση των κυτταροκινών ιντερλευκίνη-6 (IL-6) και ιντερλευκίνη-1 (IL-1), του παράγοντα νέκρωσης όγκων-α (TNF-α) καθώς και η συγκέντρωση της C-αντιδρώσας πρωτεΐνης (CRP), στο πλάσμα (Calcada et al., 2014). Η ΧΣΦ αποτελεί μια απειλητική για την υγεία κατάσταση καθώς η ύπαρξή της έχει συνδεθεί με την παθογένεση αρκετών χρόνιων ασθενειών και γηριατρικών συνδρόμων όπως το μεταβολικό σύνδρομο, ο διαβήτης τύπου 2, η αθηροσκλήρωση, οι καρδιαγγειακές και νευροεκφυλιστικές παθήσεις και η χρόνια αποφρακτική πνευμονοπάθεια (De Martinis, Franceschi, Monti, & Ginaldi, 2005; C. Franceschi, Garagnani, Vitale, Capri, & Salvioli, 2016; Singh & Newman, 2011).

Σύμφωνα με τη θεωρεία της ″μοριακής φλεγμονής‟ στη γήρανση, η οποία αναπτύχθηκε στις αρχές της δεκαετίας του 2000 από τους Chung και συνεργάτες (Chung, Kim, Kim, & Yu, 2001), τα κύτταρα του ανοσοποιητικού συνυπάρχουν σε έναν φαύλο κύκλο με δραστικά είδη οξυγόνου και αζώτου (ΔΕΟΑ) κατά τον οποίο μια σημαντική αύξηση στα τελευταία πυροδοτεί την αυξημένη κινητοποίηση στα πρώτα καταλήγοντας στην εμφάνιση της ΧΣΦ. Πιο συγκεκριμένα, η αυξημένη παραγωγή ΔΕΟΑ λόγω της γήρανσης σε συνδυασμό με τη μειωμένη αντιοξειδωτική ικανότητα στα άτομα αυτά, προκαλεί οξειδωτικό στρες και αυξημένο οξειδωτικό τραυματισμό σε μακρομόρια όπως το DNA, οι πρωτεΐνες και τα λιπίδια (Kregel & Zhang, 2007; Meng & Yu, 2010). Αυτό έχει ως αποτέλεσμα τη διαταραχή της οξειδοαναγωγικής ισορροπίας και την ενεργοποίηση του ρυθμιζόμενου από την οξειδοαναγωγική κατάσταση μεταγραφικού παράγοντα NF-κB (nuclear factor kappa B) μέσω του οποίου διεγείρεται η ενεργοποίηση προ-φλεγμονώδη μορίων της φλεγμονής, κυρίως των κυτταροκινών IL-6, IL-1, TNF-α και της CRP (Li, Malhotra, & Kumar, 2008; Powers, Kavazis, & McClung, 2007). Καθώς η διαδικασία της φλεγμονής εξελίσσεται, αυξάνεται η κινητοποίηση των μονοκυττάρων και μακροφάγων τα οποία με τη σειρά τους εκκρίνουν περαιτέρω ΔΕΟΑ παρατείνοντας με αυτόν τον τρόπο τον φαύλο αυτό κύκλο (Baylis, Bartlett, Patel, & Roberts, 2013). Ωστόσο, η συγκεκριμένη θεωρεία βασίζεται κυρίως σε ερευνητικά δεδομένα προερχόμενα από μελέτες σε πειραματόζωα και κύτταρα, ενώ η διερεύνησή της σε ηλικιωμένα άτομα είναι ελλιπής. Επίσης άγνωστο παραμένει εάν τα ηλικιωμένα άτομα με υψηλή ΧΣΦ παρουσιάζουν διαφορετικά επίπεδα οξειδωτικού στρες, αντιοξειδωτικής ικανότητας και κυττάρων του ανοσοποιητικού από τα αντίστοιχα άτομα με χαμηλή ΧΣΦ.

Επιπλέον, ερευνητικά δεδομένα υποδεικνύουν ότι ηλικιωμένα άτομα με υψηλή ΧΣΦ παρουσιάζουν αυξημένο κίνδυνο για ταχύτερη απώλεια μυϊκής μάζας και δύναμης καθώς και για ανάπτυξη

σαρκοπενίας (Draganidis et al., 2016; Schaap et al., 2009; Schaap, Pluijm, Deeg, & Visser, 2006; Visser et al., 2002). Συγκεκριμένα, σε μελέτη στην οποία συμμετείχαν συνολικά 986 άνδρες και γυναίκες με μέση ηλικία 74,6 έτη διαπιστώθηκε ότι όσοι χαρακτηρίζονταν από αυξημένα επίπεδα IL-6 και CRP παρουσίασαν κατά 2 έως 3 φορές μεγαλύτερο κίνδυνο απώλειας > 40% της μυϊκής τους μάζας τρία χρόνια αργότερα, συγκριτικά με εκείνους που χαρακτηρίζονταν από χαμηλές συγκεντρώσεις των συγκεκριμένων κυτταροκινών (Schaap et al., 2006). Παρομοίως, σε δείγμα 2177 ηλικιωμένων ανδρών και γυναικών (70-79 έτη) η υψηλότερη συγκέντρωση TNF-α στην αρχική μέτρηση συσχετίστηκε με μεγαλύτερη απώλεια μυϊκής μάζας και δύναμης πέντε χρόνια αργότερα (Schaap et al., 2009). Παρ' όλα αυτά, η σύγκριση μεταξύ ηλικιωμένων ατόμων με χαμηλή ΧΣΦ και υψηλή ΧΣΦ σε λειτουργικά τεστ δύναμης άνω και κάτω άκρων δεν έχει διερευνηθεί.

Συνεπώς, ο σκοπός της συγκεκριμένης μελέτης ήταν να συγκρίνει ηλικιωμένους άνδρες με χαμηλή ΧΣΦ και υψηλή ΧΣΦ: i) στους δείκτες του ανοσοποιητικού συστήματος: α) λευκοκύτταρα, β) κοκκιοκύτταρα, γ) μονοπύρηνα και δ) λεμφοκύτταρα, ii) στον δείκτη οξείδωσης των πρωτεϊνών: πρωτεϊνικά καρβονύλια, iii) στην συνολική αντιοξειδωτική ικανότητα του ορού, καθώς και iv) στη δύναμη άνω και κάτω άκρων μέσω αξιολόγησης της δύναμης χειρολαβής και της μέγιστης δύναμης στο μηχάνημα εκτάσεις γονάτων, αντίστοιχα.

Μέθοδος και Διαδικασία

Συμμετέχοντες

Για την εύρεση εθελοντών στην ευρύτερη περιοχή της Θεσσαλίας μοιράστηκαν ενημερωτικά φυλλάδια και πραγματοποιήθηκαν ενημερωτικές ομιλίες σε χώρους όπου συχνάζουν άτομα τρίτης ηλικίας (π.χ. Κ.Α.Π.Η., κέντρα άθλησης και αναψυχής). Η αρχική προϋπόθεση ήταν οι εθελοντές να είναι άνδρες ηλικίας 65-75 ετών. Περίπου 55-60 εθελοντές προσήλθαν στη Σ.Ε.Φ.Α.Α. του Π.Θ., στο χώρο του SMART Lab, όπου συμπλήρωσαν έναν έντυπο ιατρικού ιστορικού και εξετάστηκαν επίσης από ιατρό. Προκειμένου να συμπεριληφθούν στη μελέτη, οι εθελοντές έπρεπε να πληρούν τα παρακάτω κριτήρια: α) να είναι μη καπνιστές, β) να είναι ανεξάρτητοι στην καθημερινότητά τους, γ) να μην πάσχουν από οποιαδήποτε χρόνια πάθηση (π.χ. μεταβολική, καρδιαγγειακή, νευρολογική, αναπνευστική ή νεφρική), δ) να μην πάσχουν από φλεγμονώδεις παθήσεις (π.χ. οστεοαρθρίτιδα, ρευματοειδής αρθρίτιδα), ε) να μην πάσχουν από διαβήτη τύπου 2 και ζ) να μην χρησιμοποιούν ή έχουν χρησιμοποιήσει πρόσφατα αντιβιοτικά ή άλλου είδους φαρμακευτικά σκευάσματα τα οποία θα μπορούσαν να επηρεάσουν τα επίπεδα συστημικής φλεγμονής. Ακολούθως, 33 εθελοντές οι οποίοι πληρούσαν τα παραπάνω κριτήρια συμπεριλήφθηκαν τελικά στη μελέτη και πραγματοποίησαν τρεις ακόμη επισκέψεις. Στην πρώτη επίσκεψη, υποβλήθηκαν σε αξιολόγηση των σωματομετρικών τους χαρακτηριστικών, της σύστασης σώματος και της δύναμης άνω και κάτω άκρων. Στις επόμενες δύο διαδοχικές επισκέψεις (μια εβδομάδα μετά την πρώτη επίσκεψη), οι εθελοντές προσέρχονταν νωρίς το πρωί, μετά από ολονύκτια νηστεία και υποβάλλονταν σε μια αιμοληψία (~8-10 ml) για τον προσδιορισμό των δεικτών του ανοσοποιητικού, του οξειδωτικού στρες, της αντιοξειδωτικής ικανότητας και του επιπέδου ΧΣΦ. Η ΧΣΦ αξιολογήθηκε με βάση τις τιμές της hs-CRP (high-sensitivity CRP), όπου ο μέσος όρος των τιμών της hs-CRP από τις δυο μετρήσεις χρησιμοποιήθηκε ως η τελική τιμή για το κάθε

άτομο, και η κατηγοριοποίηση των εθελοντών σε ″χαμηλή" ΧΣΦ και ″υψηλή" ΧΣΦ έγινε ως εξής: ″χαμηλή" ΧΣΦ = hs-CRP < 1 mg/L και ″υψηλή‟ ΧΣΦ = hs-CRP > 1 mg/L, σύμφωνα με προηγούμενη μελέτη (Draganidis et al., 2018). Οι εθελοντές ενημερώθηκαν πλήρως για τον σκοπό της συγκεκριμένης μελέτης αλλά και για τα πιθανά οφέλη από τη συμμετοχής τους και στη συνέχεια υπέγραψαν έντυπο συναίνεσης. Η μελέτη έλαβε έγκριση από την Εσωτερική Επιτροπή Δεοντολογίας του Πανεπιστημίου Θεσσαλίας.

Διαδικασία μέτρησης

Σωματομετρικά χαρακτηριστικά και σύσταση σώματος: Το σωματικό βάρος και ύψος των συμμετεχόντων μετρήθηκε με ακρίβεια μισού κιλού (0,5 kg) και μισού εκατοστού (0,5 cm) αντίστοιχα, σε μηχανικό ζυγό με αναστημόμετρο (Beam Balance-Stadiometer, SECA, Vogel & Halke, Hamburg, Germany). Οι εξεταζόμενοι φορώντας ελαφρύ ρουχισμό και χωρίς παπούτσια πραγματοποίησαν την μέτρηση από όρθια θέση στο κέντρο του ζυγού, έχοντας τις φτέρνες ενωμένες και τα πέλματα να σχηματίζουν γωνία περίπου 60^{o}, σύμφωνα με προηγούμενη μελέτη (Fatouros et al., 2010). Η σύσταση σώματος αξιολογήθηκε σε μηχάνημα διπλής ενεργειακής απορρόφησης ακτινών Χ (DXA, GE Healthcare, Lunar DPX NT, Diegem, Belgium), με τον εξεταζόμενο σε ξαπλωτή θέση, όπως έχει περιγραφεί σε προηγούμενη μελέτη (Draganidis et al., 2017).

Δύναμη άνω και κάτω άκρων: Για την αξιολόγηση της δύναμης άνω άκρων μετρήθηκε η μέγιστη δύναμη χειρολαβής με τη χρήση φορητού υδραυλικού χειροδυναμόμετρου Jamar (Jamar 5030J1, Jamar Technologies, Horsham, Pennsylvania, USA). Οι δοκιμαζόμενοι εκτέλεσαν την μέτρηση από καθιστή θέση, με τον αγκώνα να σχηματίζει γωνία 90^{o} και τον καρπό σε ουδέτερη θέση κρατώντας το δυναμόμετρο στη θέση 2 (Roberts et al., 2011). Από αυτή τη θέση πραγματοποιήθηκαν 3 προσπάθειες σε κάθε χέρι με διάλειμμα 60 δευτερολέπτων, όπου στην κάθε προσπάθεια εκτελέστηκε μέγιστη ισομετρική σύσπαση για 5 δευτερόλεπτα. Ως τελική επίδοση καταγράφηκε η μεγαλύτερη τιμή σε χιλιόγραμμα (kg). Η αξιολόγηση της δύναμης των κάτω άκρων εκτιμήθηκε μέσω της εκτέλεσης 10 μέγιστων επαναλήψεων (10 ΜΕ) στο μηχάνημα έκτασης γονάτων. Οι εξεταζόμενοι, μετά από κατάλληλη προθέρμανση εκτελούσαν το πρώτο σετ και σε περίπτωση που ήταν σε θέση να εκτελέσουν περισσότερες από 10 επαναλήψεις, ο εξεταστής τους σταματούσε και τους έδινε διάλειμμα 2 λεπτών. Έπειτα αυξάνονταν τα κιλά και επιχειρούσαν δεύτερη προσπάθεια. Αν και πάλι ήταν σε θέση να εκτελέσουν περισσότερες από 10 επαναλήψεις, ο εξεταστής επαναλάμβανε την ίδια διαδικασία. Η διαδικασία αυτή συνεχιζόταν μέχρι οι εξεταζόμενοι να εκτελέσουν 10 μέγιστες επαναλήψεις (περίπου 3-4 σετ).

Αιμοληψίες και διαχείριση των δειγμάτων: Σε κάθε αιμοληψία λαμβάνονταν 8-10 ml αρτηριοφλεβικού αίματος από τη μεσοβασιλική φλέβα και ενώ ο εξεταζόμενος βρισκόταν σε καθιστή θέση. Περίπου 2 ml διοχετεύονταν σε σωλήνα με αντιπηκτικό (EDTA) και στη συνέχεια τοποθετούνταν στον αυτόματο αναλυτή αίματος (Mythic 18, Orphee SA, Geneva, Switzerland) για να πραγματοποιηθεί η γενική ανάλυση αίματος. Τα υπόλοιπα 6-8 ml μοιράζονταν σε δυο σωληνάρια με κενό αέρος, τα οποία παρέμεναν για 20 λεπτά σε θερμοκρασία δωματίου και στη συνέχεια φυγοκεντρούνταν στις 3500 στροφές για 10 λεπτά στους 4^{o}C. Αμέσως μετά τη φυγοκέντρηση το υπερκείμενο υγρό (ορός)

συλλέγονταν και μοιραζόταν σε σωληνάκια eppendorf και στη συνέχεια αποθηκεύονταν στους -80^{o}C, για την ανάλυση των πρωτεϊνικών καρβονυλίων, της συνολικής αντιοξειδωτικής ικανότητας και της hs-CRP.

Πρωτεϊνικά καρβονύλια: Για τη μέτρηση των πρωτεϊνικών καρβονυλίων πραγματοποιήθηκε η βιοχημική αντίδραση του κάθε δείγματος με 2,4- dinitrophenylhydrazine (DNPH) που επέφερε τη μετατροπή του σε 2,4-dinitrophenylhydrazone (DNP-hydrazone). Ο προσδιορισμός της συγκέντρωσης του 2,4- DNP-hydrazone, πραγματοποιήθηκε με τη μέτρηση της απορρόφησης του κάθε δείγματος στο φωτόμετρο, στα 375 nm. Η τελική συγκέντρωση των πρωτεϊνικών καρβονυλίων υπολογιζόταν σύμφωνα με την εξίσωση: PC (nmol/mL) = ($Abs_{δείγμα}$ – $Abs_{τυφλό}$) / 0,022 × 1000/50 (Mohr et al., 2016).

Συνολική αντιοξειδωτική ικανότητα: Η συνολική αντιοξειδωτική ικανότητα του ορού αξιολογήθηκε μέσω της βιοχημικής αντίδρασης των αντιοξειδωτικών συστατικών του ορού με τη ρίζα 1,1-diphenyl-2-picrylhydrazyl (DPPH), κατά την οποία τα αντιοξειδωτικά δρουν ως δότες υδρογόνου προκαλώντας την αναγωγή της συγκεκριμένης ρίζας, μειώνοντας με αυτό τον τρόπο τη συγκέντρωσή της. Η τελική συγκέντρωση της ρίζας DPPH υπολογίστηκε μετρώντας την απορρόφηση του δείγματος στα 520 nm. Τα αποτελέσματα εκφράστηκαν ως μmol DPPH που καθαρίστηκαν ανά ml ορού, χρησιμοποιώντας την εξίσωση: [(%A_{bs} μείωσης / 100) x 50 x 50] / 100 (Mohr et al., 2016).

hs-CRP: Πραγματοποιήθηκε ποσοτική ανάλυση της hs-CRP στον ορό, με τη μέθοδο της θολοσιμετρικής ανάλυσης ενισχυμένης με τη χρήση σωματιδίων λάτεξ (CRP LX High Sensitive, Cobas®), σε αναλυτή Cobas Integra 400 plus (Roche). Το κατώτατο όριο ανίχνευσης της ανάλυσης ήταν 0,1 mg/L και ο συντελεστής διακύμανσης μεταξύ των αναλύσεων ήταν μία τυπική απόκλιση (1 SD) (Draganidis et al., 2018).

Στατιστική Ανάλυση

Ο έλεγχος της κανονικότητας των δεδομένων πραγματοποιήθηκε με βάση το Shapiro-Wilk τεστ. Για τη διερεύνηση των διαφορών μεταξύ των δυο ομάδων (χαμηλή ΧΣΦ και υψηλή ΧΣΦ) στις εξαρτημένες μεταβλητές χρησιμοποιήθηκε το T-Test για ανεξάρτητα δείγματα. Το μέγεθος της επίδρασης (ΜτΕ) καθώς και τα διαστήματα εμπιστοσύνης (ΔΕ) για τις εξαρτημένες μεταβλητές υπολογίστηκαν με τη μέθοδο του Hedge's g. Το ΜτΕ χαρακτηρίστηκε ως κανένα, μικρό, μεσαίου μεγέθους και μεγάλο για τιμές 0.00-0.19, 0.20-0.49, 0.50-0.79 και ≥ 0.8, αντίστοιχα. Το επίπεδο σημαντικότητας ορίστηκε στο $p<0.05$. Όλα τα δεδομένα παρουσιάζονται ως μέσος όρος ± τυπική απόκλιση. Για τις στατιστικές αναλύσεις χρησιμοποιήθηκε το στατιστικό πακέτο SPSS 20.0 (IBM SPSS Statistics).

Αποτελέσματα

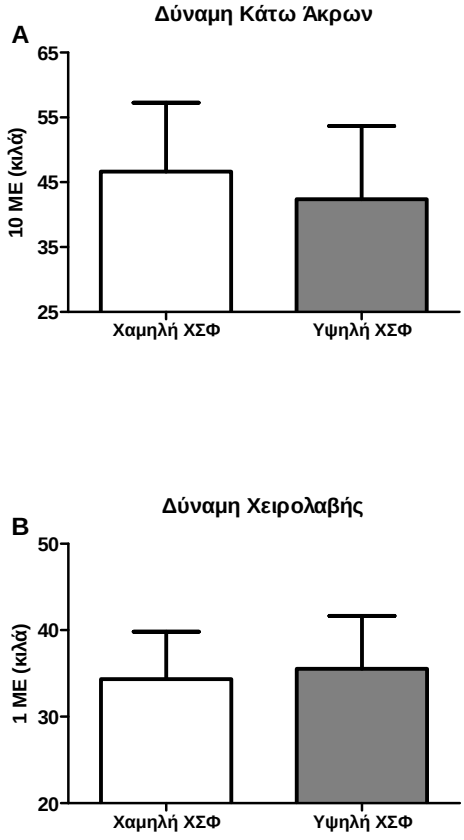
Τα περιγραφικά χαρακτηριστικά των δύο ομάδων παρουσιάζονται στον Πίνακα 1. Οι δύο ομάδες δεν διέφεραν μεταξύ τους στα σωματομετρικά χαρακτηριστικά και σε παραμέτρους της σύστασης σώματος. Ωστόσο, σημαντική ήταν η διαφορά τους στα επίπεδα ΧΣΦ με την ομάδα υψηλής ΧΣΦ να παρουσιάζει σχεδόν κατά 3 φορές υψηλότερες τιμές hs-CRP συγκριτικά με την ομάδα χαμηλής ΧΣΦ (χαμηλή ΧΣΦ: 0.6±0.1 / υψηλή ΧΣΦ: 2.3±0.8, $p=0.00$, ΜτΕ=2.86, ΔΕ=-3.84, -1.89).

Πίνακας 1. Περιγραφικά χαρακτηριστικά των δύο ομάδων.

Παράμετρος	**Χαμηλή ΧΣΦ** (N=16)	**Υψηλή ΧΣΦ** (N=17)
Ηλικία (έτη)	69.1 ± 2.8	68.8 ± 2.9
Σωματικό ύψος (μ)	1.70 ± 0.07	1.72 ± 0.05
Σωματικό βάρος (κιλά)	81.4 ± 7.6	84.7 ± 5.5
ΔΜΣ (κιλά/μ^2)	26.92 ± 2.77	27.86 ± 2.51
Λιπώδης μάζα (κιλά)	23.20 ± 6.26	25.54 ± 3.14
Λίπος (%)	29.41 ± 4.14	31.25 ± 1.72
Άλιπη μάζα (κιλά)	56.89 ± 4.26	57.70 ± 4.35
Μυϊκή μάζα (κιλά)	53.86 ± 4.13	55.12 ± 5.11
Μυϊκή μάζα άκρων (κιλά)	23.32 ± 2.43	24.15 ± 1.83
Δείκτης μυϊκής μάζας (κιλά/μ^2)	8.03 ± 0.78	8.11 ± 0.64
hs-CRP (mg/L)	0.6 ± 0.1	2.3 ± 0.8*****

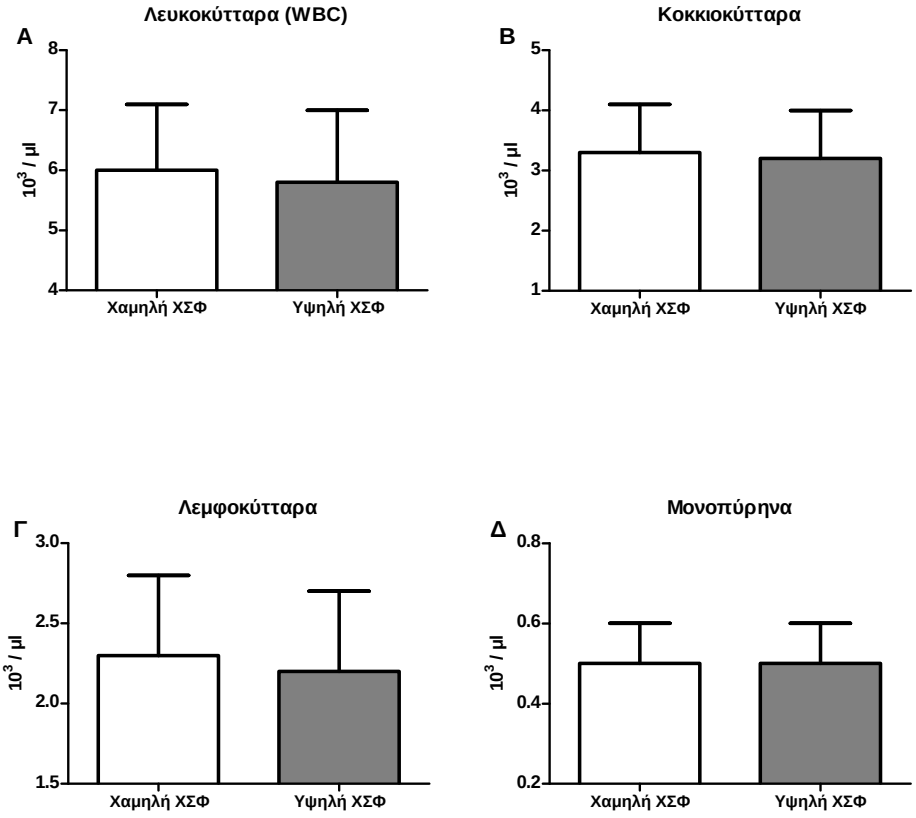
***** : Υποδηλώνει σημαντική διαφορά μεταξύ των ομάδων, $p < 0.01$.

Τα αποτελέσματα έδειξαν ότι στην αξιολόγηση της δύναμης, τόσο στη μέγιστη δύναμη χειρολαβής ($t(30)=1.139$, $p>0.05$) όσο και στις 10 ΜΕ κάτω άκρων στο μηχάνημα έκτασης γονάτων ($t(30)=-0.526$, $p>0.05$), δεν υπήρξαν στατιστικά σημαντικές διαφορές μεταξύ των δυο ομάδων (Σχήμα 1).



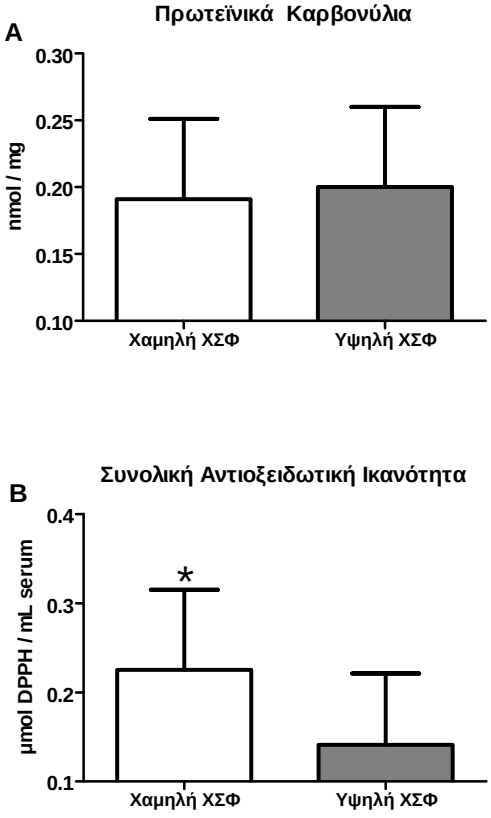
Σχήμα 1. 10ΜΕ κάτω άκρων στο μηχάνημα εκτάσεις γονάτων (1Α) και μέγιστη δύναμη χειρολαβής (1Β) στις δυο ομάδες.

Επίσης από τη σύγκριση των ομάδων χαμηλή ΧΣΦ και υψηλή ΧΣΦ ως προς του δείκτες του ανοσοποιητικού συστήματος διαπιστώθηκε ότι δεν υπήρχε στατιστικά σημαντική διαφορά μεταξύ τους στα επίπεδα λευκοκυττάρων ($t(30)=-1.088$, $p>0.05$), κοκκιοκυττάρων ($t(30)=-0.963$, $p>0.05$), μονοπύρηνων ($t(30)=-0.916$, $p>0.05$) και λεμφοκυττάρων ($t(30)=-0.433$, $p>0.05$) (Σχήμα 2).



Σχήμα 2. Συστημικά επίπεδα Λευκοκυττάρων (2Α), Κοκκιοκυττάρων (2Β), Λεμφοκυττάρων (2Γ) και Μονοπύρηνων (2Δ) στις δύο ομάδες.

Από τη σύγκριση των δύο ομάδων σε δείκτες οξειδωτικό στρες και αντιοξειδωτικής ικανότητας, προέκυψε ότι η συγκέντρωση πρωτεϊνικών καρβονυλίων δεν διέφερε μεταξύ των ομάδων ($t(28)=0.334$, $p>0.05$), ωστόσο, οι δυο ομάδες παρουσίασαν σημαντική διαφορά στη συνολική αντιοξειδωτική ικανότητα του ορού, με την ομάδα χαμηλή ΧΣΦ να παρουσιάζει υψηλότερη αντιοξειδωτική ικανότητα από τη ομάδα υψηλή ΧΣΦ ($t(28)=-2.275$, $p=0.03$, ΜτΕ=0.96, ΔΕ=0.24, 1.69) (Σχήμα 3).



Σχήμα 3. Συγκέντρωση πρωτεϊνικών καρβονυλίων (3Α) και συνολική αντιοξειδωτική ικανότητα (3Β) στις δυο ομάδες.

Συζήτηση

Στη συγκεκριμένη μελέτη εξετάστηκε η ύπαρξη διαφορών μεταξύ ηλικιωμένων ανδρών με χαμηλή και υψηλή ΧΣΦ, σε δείκτες του ανοσοποιητικού συστήματος, οξειδωτικού στρες, αντιοξειδωτικής ικανότητας και της δύναμης των άνω και κάτω άκρων. Για το σκοπό αυτό πραγματοποιήθηκε βιοχημική αξιολόγηση για τον προσδιορισμό της συγκέντρωσης των λευκοκυττάρων, των κοκκιοκυττάρων, των μονοπύρηνων και των λεμφοκυττάρων στην κυκλοφορία καθώς και της συγκέντρωσης πρωτεϊνικών καρβονυλίων και της συνολικής αντιοξειδωτικής ικανότητας στον ορό. Όσον αφορά τη δύναμη, αξιολογήθηκε η μέγιστη δύναμη χειρολαβής και οι 10ΜΕ στα κάτω άκρα στο μηχάνημα έκτασης γονάτων. Τα αποτελέσματα της μελέτης υποδεικνύουν ότι ηλικιωμένα άτομα με χαμηλή ΧΣΦ και υψηλή ΧΣΦ παρουσιάζουν παρόμοια συγκέντρωση λευκοκυττάρων, οξειδωτικού στρες και δύναμης, ωστόσο εκείνοι με χαμηλή ΧΣΦ χαρακτηρίζονται από υψηλότερη αντιοξειδωτική ικανότητα.

Οι συμμετέχοντες στη παρούσα μελέτη διαχωρίστηκαν στις δυο ομάδες, χαμηλή ΧΣΦ και υψηλή ΧΣΦ, σύμφωνα με τα επίπεδα της hs-CRP στον ορό. Ο συγκεκριμένος δείκτης συστημικής φλεγμονής θεωρείται ένας έγκυρος και αξιόπιστος δείκτης ΧΣΦ (Morrisette-Thomas et al., 2014) και έχει χρησιμοποιηθεί σε αρκετές μελέτες προκειμένου να κατηγοριοποιήσει ηλικιωμένα άτομα με βάση τα επίπεδα φλεγμονής (Draganidis et al., 2018; Labonte et al., 2014). Οι δυο ομάδες παρουσίασαν παρόμοια σωματομετρικά χαρακτηριστικά και σύσταση σώματος, γεγονός που υποδηλώνει ότι το δείγμα ήταν

αρκετά ομοιογενές και διέφερε μόνο ως προς τα επίπεδα ΧΣΦ. Επιπλέον, σύμφωνα με τα κριτήρια του Παγκόσμιου Οργανισμού Υγείας (WHO) για την παχυσαρκία και με βάση τις τιμές του ΔΜΣ των συμμετεχόντων, τα άτομα των δυο ομάδων κατατάσσονται ως μη-παχύσαρκα, κάτι που είναι σημαντικό για την ερμηνεία των αποτελεσμάτων της συγκεκριμένης μελέτης. Η ύπαρξη παχυσαρκίας έχει συνδεθεί με την ανάπτυξη ΧΣΦ, η οποία όμως οφείλεται κατά κύριο λόγο στο λιπώδη ιστό (μεταβολική φλεγμονή) (Cevenini, Monti, & Franceschi, 2013; C. Franceschi et al., 2016), , και όχι αποκλειστικά στη γήρανση που είναι και ο σκοπός της παρούσας μελέτης.

Μέχρι σήμερα, η αξιολόγηση της ύπαρξης ΧΣΦ αλλά και η σύγκριση των επιπέδων της μεταξύ ηλικιωμένων ατόμων βασίζεται κατά κύριο λόγο στη μέτρηση των κυτταροκινών IL-1, IL-6, TNF-α και της C-αντιδρώσας πρωτεΐνης (Draganidis et al., 2018; Labonte et al., 2014; Schaap et al., 2009; Schaap et al., 2006; Visser et al., 2002). Δεν υπάρχουν μελέτες που να εξετάζουν εάν η αύξηση στα επίπεδα των κυτταροκινών συνοδεύεται από αυξημένη συγκέντρωση λευκοκυττάρων και των υποπληθυσμών τους σε ηλικιωμένα άτομα με ΧΣΦ. Η κινητοποίηση των λευκοκυττάρων σε συνθήκες οξείας φλεγμονής, όπως η άσηπτη φλεγμονή που προκαλείται από την άσκηση, είναι άμεση και η συγκέντρωσή τους στην κυκλοφορία παραμένει αυξημένη για 24-48 ώρες (Sakelliou et al., 2016). Τα κοκκιοκύτταρα, τα οποία αποτελούνται από ουδετερόφιλα, βασεόφιλα και ηωσινόφιλα, αυξάνονται επίσης αμέσως μετά από άσκηση λόγω της ύπαρξης άσηπτης φλεγμονής και παραμένουν αυξημένα έως και 48 ώρες μετά (Poulios et al., 2018). Ωστόσο, στην παρούσα μελέτη δεν παρατηρήθηκαν διαφορές στα λευκοκύτταρα και τους υποπληθυσμούς τους μεταξύ των ομάδων χαμηλή ΧΣΦ και υψηλή ΧΣΦ, υποδεικνύοντας ότι τα συγκεκριμένα κύτταρα του ανοσοποιητικού παρουσιάζουν διαφορετικό μοτίβο ενεργοποίησης σε συνθήκες οξείας φλεγμονής και ΧΣΦ.

Η εκδήλωση ΧΣΦ σε άτομα τρίτης ηλικίας έχει αποδοθεί στη μειωμένη αντιοξειδωτική ικανότητα και στην αυξημένη παραγωγή ΔΕΟΑ που έχουν ως αποτέλεσμα την πρόκληση οξειδωτικού στρες και οξειδωτικού τραυματισμού σε πρωτεΐνες, λιπίδια και DNA (Kregel & Zhang, 2007; Meng & Yu, 2010). Η παρατεταμένη αύξηση στα επίπεδα οξειδωτικού στρες οδηγεί σε διαταραχή της οξειδοαναγωγικής ισορροπίας και την επακόλουθη ενεργοποίηση του μεταγραφικού παράγοντα NF-κΒ ο οποίος με τη σειρά του διεγείρει την ενεργοποίηση προ-φλεγμονώδη κυτταροκινών και της CRP (Li et al., 2008; Powers et al., 2007). Με αυτό τον τρόπο εξελίσσεται η διαδικασία της φλεγμονής, κατά την οποία τα μονοκύτταρα και μακροφάγα εκκρίνουν περαιτέρω ΔΕΟΑ δημιουργώντας έτσι έναν φαύλο κύκλο που έχει ως αποτέλεσμα την μακροχρόνια εκδήλωση φλεγμονής (Baylis et al., 2013; Chung et al., 2001). Στη συγκεκριμένη μελέτη η συγκέντρωση πρωτεϊνικών καρβονυλίων, που αποτελεί δείκτη οξείδωσης των πρωτεϊνών, ήταν παρόμοια στα άτομα με χαμηλή ΧΣΦ και υψηλή ΧΣΦ. Ωστόσο, οι δυο ομάδες διέφεραν σημαντικά μεταξύ τους όσον αφορά τη συνολική αντιοξειδωτική ικανότητα, με την ομάδα χαμηλής ΧΣΦ να παρουσιάζει κατά 60% μεγαλύτερη αντιοξειδωτική ικανότητα συγκριτικά με την ομάδα υψηλής ΧΣΦ. Το συγκεκριμένο εύρημα είναι σε συμφωνία με πρόσφατη μελέτη η οποία έδειξε ότι τα ηλικιωμένα άτομα με χαμηλή ΧΣΦ προσλαμβάνουν μεγαλύτερες ποσότητες αντιοξειδωτικών βιταμινών μέσω της τροφής και είναι περισσότερο δραστήρια κατά τη διάρκεια της ημέρας, συγκριτικά με άτομα ίδιας ηλικίας με υψηλή ΧΣΦ (Draganidis et al., 2018). Επομένως, τα παραπάνω ευρήματα αποδεικνύουν πως η διατήρηση της αντιοξειδωτικής ικανότητας, μέσω επαρκούς πρόσληψης

διαιτητικών αντιοξειδωτικών και φυσικής δραστηριότητας, μπορεί να προλάβει την εκδήλωση ΧΣΦ στα άτομα τρίτης ηλικίας.

Προηγούμενες μελέτες έχουν συσχετίσει τα επίπεδα συστημικής φλεγμονής σε ηλικιωμένα άτομα με την απώλεια μυϊκής δύναμης (Schaap et al., 2009; Schaap et al., 2006; Visser et al., 2002). Πιο συγκεκριμένα, άτομα με αυξημένα επίπεδα CRP ή/και προφλεγμονώδη κυτταροκινών όπως η IL-6 και ο TNF-α, παρουσίασαν κατά 2 έως 3 φορές μεγαλύτερη απώλεια δύναμης στα επόμενα 2-5 χρόνια, τόσο στη μέγιστη δύναμη χειρολαβής (Schaap et al., 2006) όσο και στη δύναμη κάτω άκρων (Schaap et al., 2009; Visser et al., 2002). Στην παρούσα μελέτη ωστόσο, συγκρίνοντας τη μέγιστη δύναμη χειρολαβής και τις 10ΜΕ κάτω άκρων μεταξύ των ομάδων χαμηλή ΧΣΦ και υψηλή ΧΣΦ, δεν εντοπίστηκαν στατιστικά σημαντικές διαφορές. Η απώλεια μυϊκής δύναμης ως αποτέλεσμα της ΧΣΦ φαίνεται πως επέρχεται μέσω της προκαλούμενης από τη ΧΣΦ απώλεια μυϊκής μάζας (Draganidis et al., 2016; Visser et al., 2002). Σύμφωνα με πρόσφατη ανασκοπική μελέτη, η ΧΣΦ μπορεί να οδηγήσει σε απώλεια μυϊκής μάζας και λειτουργικής ικανότητας, είτε μέσω αύξησης της πρωτεόλυσης ενεργοποιώντας το σύμπλοκο του πρωτεασώματος, είτε μέσω μείωσης της δυνατότητας ενεργοποίησης των σηματοδοτικών πρωτεϊνών που επάγουν την πρωτεϊνοσύνθεση στο μυ (Draganidis et al., 2016). Επομένως, το γεγονός ότι οι δυο ομάδες στη παρούσα μελέτη δεν διέφεραν ως προς τα επίπεδα δύναμης άνω και κάτω άκρων, μπορεί να αποδοθεί στο ότι δεν εντοπίστηκαν μεταξύ τους διαφορές στη μυϊκή μάζα.

Συμπερασματικά, τα ευρήματα της παρούσας μελέτης υποδεικνύουν ότι ηλικιωμένα άτομα με χαμηλή ΧΣΦ και υψηλή ΧΣΦ δεν διαφέρουν μεταξύ τους ως προς τα συστημικά επίπεδα λευκοκυττάρων και οξείδωσης των πρωτεϊνών, ωστόσο οι συμμετέχοντες με χαμηλή ΧΣΦ παρουσιάζουν μεγαλύτερη αντιοξειδωτική ικανότητα από εκείνους με υψηλή ΧΣΦ. Η συγκέντρωση των λευκοκυττάρων και των υποπληθυσμών τους στην κυκλοφορία φαίνεται ότι δεν επηρεάζεται από την ανάπτυξη ΧΣΦ. Αντίθετα, η καλύτερη αντιοξειδωτική ικανότητα εμφανίζεται ως αποτρεπτικός παράγοντας για την εκδήλωση ΧΣΦ. Επιπλέον, άτομα με χαμηλή και υψηλή ΧΣΦ δεν παρουσιάζουν διαφορές στη δύναμη άνω και κάτω άκρων εφόσον παρουσιάζουν παρόμοια επίπεδα μυϊκής μάζας.

Σημασία για την Ποιότητα Ζωής

Η εκδήλωση χρόνιας συστημικής φλεγμονής στα άτομα τρίτης ηλικίας είναι μια απειλητική για την υγεία κατάσταση καθότι συνδέεται άμεσα με την παθογένεση αρκετών χρόνιων ασθενειών και γηριατρικών συνδρόμων, αλλά και με την απώλεια μυϊκής μάζας και λειτουργικής ικανότητας. Όλα αυτά συνεπάγονται την επιδείνωση της ποιότητας ζωής αυτών των ατόμων έχοντας παράλληλα σοβαρό αντίκτυπο στην οικονομική τους κατάσταση καθότι αυξάνονται σημαντικά τα έξοδα νοσηλείας. Η διατήρηση της αντιοξειδωτικής ικανότητας με την αύξηση της ηλικίας είναι ένας σημαντικός παράγοντας που μπορεί να προλάβει την εκδήλωση χρόνιας συστημικής φλεγμονής και επιτυγχάνεται μέσω επαρκούς πρόσληψης διαιτητικών αντιοξειδωτικών και φυσικής δραστηριότητας.

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Αγαπητέ κ. Δραγανίδη,

Η εργασία σας με τίτλο «**Σύγκριση ηλικιωμένων ανδρών με χαμηλή και υψηλή χρόνια συστημική φλεγμονή σε δείκτες δύναμης, οξειδωτικού στρες και φλεγμονής**» η οποία έχει κατατεθεί για δημοσίευση στο περιοδικό «**ΑΝΑΖΗΤΗΣΕΙΣ ΣΤΗ ΦΥΣΙΚΗ ΑΓΩΓΗ & ΤΟΝ ΑΘΛΗΤΙΣΜΟ**» έχει ελεγχθεί από δύο κριτές, τα σχόλια των οποίων ακολουθούν, και από εμένα. Όλοι πιστεύουμε ότι η εργασία περιέχει ενδιαφέροντα στοιχεία τα οποία συνδέονται με το τους στόχους του περιοδικού. Η εργασία σας γίνεται **δεκτή προς δημοσίευση**. Παρακαλώ δώστε βάση στην παρατήρηση του κριτή 1, που αναφέρει ότι στην εργασία χρειάζονται να γίνουν ορισμένες γραμματικές και συντακτικές διορθώσεις, και στη συνέχεια καταθέστε την εργασία στην τελική της μορφή. Ελπίζω τα σχόλια των κριτών να ήταν εποικοδομητικά για τη δόμηση της εργασίας. Σας ευχαριστώ για την τιμή που κάνατε να επιλέξετε τις **«ΑΝΑΖΗΤΗΣΕΙΣ ΣΤΗ ΦΥΣΙΚΗ ΑΓΩΓΗ & ΤΟΝ ΑΘΛΗΤΙΣΜΟ»** να δημοσιεύσετε την εργασία σας.

Με τιμή,

Θανάσης Τζιαμούρτας, Ph.D., FACSM

Assistant Editor