The Relation of Inflammaging With Skeletal Muscle Properties in Elderly Men

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Abstract
Aging is associated with a progressive decline of muscle mass and/or the qualitative impairment of the muscle tissue. There is growing evidence of the prominent role of low-grade chronic inflammation in age-related changes in the neuromuscular system. The purpose of the study was to identify the inflammatory mediators responsible for deficit in functional fitness and to explain whether inflammation is related to changes in body composition and the decline of muscle strength in older men. Thirty-three old-aged males (73.5 ± 6.3 years) and twenty young-aged males (21.2 ± 1.3 years) participated in the study. The body composition (bioelectrical impedance analysis), functional capacity (6-min walking test) and knee extension strength (isokinetic test) were estimated. In serum, circulating inflammatory markers H₂O₂, IL-1β, TNFα, and hsCRP as well as growth factors IGF-I and PDGFBB concentrations were determined (immunoenzymatic methods). The concentrations of H₂O₂, IL-1β, TNFα, and hsCRP were significantly higher in older than young men. The growth factors IGF-I and PDGFBB were twofold lower and related to high levels of IL-1β and TNFα in the elderly. The changes in cytokines and growth factors levels were correlated with age and peak torque (TQ at 60°/s and 180°/s) in the knee extension. The result of the 6-min walking test was inversely correlated with fat mass index (FMI, r = −.983; p < .001). The generation of inflammatory mediators in older men was related to changes in body composition, maximum strength muscle, and age-related changes in skeletal muscle properties responsible for deficit in functional fitness.

Keywords
body composition, cytokines, functional fitness, growth factors, inflammation

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Skeletal muscle contractions empower human body movements and are essential for maintaining stability. Skeletal muscle tissue accounts for almost half of the human body mass and, in addition to its power-generating role, is a crucial factor in maintaining homeostasis. Given its central role in human mobility and metabolic function, any deterioration in the contractile, material, and metabolic properties of skeletal muscle has an extremely important effect on human health (Zembron-Lacny, Dziubek, Rogowski, Skorupka, & Dąbrowska, 2014).

Aging is associated with a progressive decline of muscle mass and/or the qualitative impairment of the muscle tissue. There is growing evidence of the prominent role of low-grade chronic inflammation in age-related changes in the neuromuscular system (Salminen, Kaarniranta, & Kauppinen, 2012). Multiple causes may contribute to inflamingaging, such as pro-inflammatory tissue damages, a
dysfunctional immune system (Deeks, 2011), pro-inflammatory cytokines secreted by senescent cells, enhanced NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation, and a defective autophagy response system (Salminen et al., 2012). These factors enhance the activation of inflammatory pathways such as the Nalp-3 inflammasome, and then induce the production of cytokines such as interleukin 1β (IL-1β), tumor necrosis factor (TNFα), and reactive oxygen species (ROS) system (Cannizzo, Clement, Sahu, Follo, & Santambrogio, 2011; Green, Galluzzi, & Kroemer, 2011; Salminen et al., 2012). Inflammatory mediators, particularly TNFα, are potent stimulants of proteolysis through the ubiquitin–proteasome-dependent system. A significant negative relationship between myosin heavy chain protein synthesis rates and circulating markers of immune response has been observed (Toth, Ades, Tischler, Tracy, & LeWinter, 2006). Visser et al. (2002) demonstrated that for each increase in standard deviation in the TNFα value, a 1.2–1.3 kg reduction is seen in hand grip strength. Reactive oxygen species (ROS) appear to function as second messengers for TNFα in skeletal muscles increased mitochondrial apoptotic susceptibility, and reduced mitochondrial biogenesis (Chabi et al., 2008). The mitochondria constitute the major source of ROS, including superoxide radicals and hydrogen peroxide, which can cause oxidative damage to surrounding structures; particularly vulnerable is the mitochondrial DNA (mtDNA), which is in close proximity to the primary site of ROS production. Oxidation by ROS results in the synthesis of faulty proteins, oxidized lipids, and mtDNA mutations, which may lead to cellular and mitochondrial dysfunction as well as may accelerate apoptosis muscle stem cells, called satellite cells (SCs; Peterson, Johannsen, & Ravussin, 2012).

During the past decade, skeletal muscles have been identified as a secretory organ that produces molecules, such as insulin growth factor I (IGF-1) and platelet-derived growth factors BB (PDGFBB; Pedersen & Febbraio, 2012). The growth factors play an important role in muscle regeneration due to their ability to stimulate the activation, proliferation, and differentiation of SCs, and also synthesize muscle proteins. The reduction of the number of SCs is associated with the loss muscle mass and strength (Pallafacchina, Blauw, & Schiaffino, 2013). The level of factors affecting satellite cells activity and muscle function in older adults is unknown. The purpose of the study was threefold: (a) to identify the age-related changes in skeletal muscle properties responsible for deficits in functional status, (b) to establish the level of inflammation in young and older adults, and (c) to explain whether the changes in body composition and the decline of muscle strength are related to inflammatory mediators.

### Table 1. Anthropometric and Body Composition Data in Young and Older Men (Mean ± SD).

| Age, years | Young (n = 20) | Elders (n = 33) | ANOVA HSD Tukey |
|------------|---------------|----------------|-----------------|
| Young      | 21.2 ± 1.3    | 73.5 ± 6.3     | p < .001        |
| Height, cm | 181.8 ± 7.6   | 169.3 ± 6.0    | p < .001        |
| Weight, kg | 74.0 ± 6.1    | 76.3 ± 10.3    | p > .05         |
| BMI, kg/m² | 22.5 ± 2.4    | 26.6 ± 3.1     | p < .01         |
| FFM, kg    | 60.0 ± 4.7    | 53.8 ± 7.1     | p < .001        |
| FFMI, kg/m²| 18.2 ± 1.9    | 18.7 ± 2.0     | p > .05         |
| FM, kg     | 14.0 ± 2.6    | 22.5 ± 5.5     | p < .001        |
| %FM        | 18.9 ± 8.2    | 29.3 ± 5.1     | p < .001        |
| FMI, kg/m² | 4.3 ± 0.8     | 7.8 ± 1.9      | p < .001        |

Note. ANOVA = one-way analysis of variance; BMI = body mass index; FFM = fat-free mass; FFMI = fat-free mass index; FM = fat mass; FMI = fat mass index.

### Methods

#### Subjects

Thirty-three healthy males between 60 and 88 years old and twenty males between 20 and 24 years old participated in the study (Table 1). The oldest subjects were recruited from the University of the Third Age (U3A) Wroclaw and did not report absolutely any chronic diseases, that is, they represented successful aging (Geard, Reaburn, Rebar, & Dionigi, 2016). Inclusion criteria were: age 60–90 years for older men and age 20–30 years for young men, signed informed consent. Exclusion criteria were: acute infectious disease and chronic systemic diseases (cardiovascular disease, chronic diseases of liver and kidneys, diabetes mellitus, oncologic disease or other serious disease, based on the assessment of the responsible physician, and investigator) and participation in professional sport activities. The current health status and lifestyle of the subjects were estimated by Doctor of Medicine by using of the health history questionnaire (Ååstrand, 1956; Dursine & Moore, 2003).

All subjects were informed of the aim of the study and gave their written consent for participation in the project. The Bioethics Commission at Regional Medical Chamber Zielona Gora, Poland (No. 4/59/2015), in accordance with the Helsinki Declaration, approved the protocol of the study.

#### Body Composition

Body mass (BM) and body composition (fat-free mass [FFM] and fat mass [FM]) were estimated by a bioelectrical impedance (BIA) method using Tanita Body Composition Analyser MC-980 (Japan) calibrated prior to each test session in accordance to the manufacturer’s guidelines. Duplicate measures were taken with the participant in a
standing position; the average value was used for the final analysis. The recurrence of measurement was 98%. The measurements were taken between 7:00 a.m. and 9:00 a.m., before blood sampling. FFM and FM indexes were calculated according to the definition by VanItallie, Yang, Heymsfield, Funk, and Boileau (1990): \( \text{FFMI} = \frac{\text{FFM}}{\text{height}^2} \) and \( \text{FMI} = \frac{\text{FM}}{\text{height}^2} \). Note that, mathematically, BMI (kg/m\(^2\)) = FFMI + FMI. Thus, measured FFMI, FMI, and %FM values falling below the values for a BMI of 18.5 kg/m\(^2\) were defined as low; measured FFMI, FMI, and %FM values falling in the range for BMI between 18.5 kg/m\(^2\) and 25.0 kg/m\(^2\) were considered normal, and values above that range were considered high (Bahadori et al., 2006).

**Functional Fitness**

The 6-min walking test is part of the Senior Fitness Test Protocol (Rikli & Jones, 2013), and is designed to test the functional fitness of older adults. The walking course is laid out in a 50-m rectangular area (dimensions 20 m × 5 m), with cones placed at regular intervals to indicate distance walked. The aim of the test is to walk as quickly as possible for 6 min covering as much ground as possible. Subjects set their own pace (a preliminary trial is useful to practice pacing), and are able to stop for a rest if they desire. Physical activity level, age, and gender were predictive factors for the result of the 6-min walking test (6-min walking distance [6MWD]) of older adults according to the equation by Enright and Sherrill (1998) for males:

\[
6\text{MWD} = \left( \frac{74.31 \times \text{height}_{\text{cm}}}{5.02 \times \text{age} - 1.76 \times \text{weight}_{\text{kg}}} \right) - 309
\]

**Isokinetic Testing**

Peak torque (peak TQ) in the knee joint was assessed using the isokinetic digital dynamometer, Multi Joint 3 Biodex System; both lower limbs were tested. Each time the patient was informed how to perform the task. The test relies on flexion and extension of legs at the knee joint with maximal force and measures maximal flexion and extension peak torque for two arc speeds: 60°/s and 180°/s (five movements for each speed and 1-min rest between each set). According to Hoffman (2009), peak TQ has been previously used as an indicator of muscle function.

**Blood Sampling**

Blood samples were taken from the median cubital vein between 7.00 a.m. and 9.00 a.m. using S-Monovette-EDTA tubes (Sarstedt, Austria). Within 20 min, they were centrifuged at 1,000 × g and +4 °C for 10 min. Aliquots of plasma were stored at −80 °C.

**Inflammatory Markers**

The serum hydrogen peroxide (H\(_2\)O\(_2\)) concentration was determined in duplicate by the colorimetric method using the Oxis Research kit (USA). H\(_2\)O\(_2\) detection limit was 6.25 µmol/L. The intra-assay coefficient of variation (CV) for the H\(_2\)O\(_2\) kit was <10%. Serum interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) levels were determined in duplicate by enzyme immunoassay methods using Commercial kits R&D Systems (USA). Detection limits for IL-1β and TNFα were 0.023 pg/ml and 0.038 pg/ml, respectively, and CV for both cytokines was <8.0%. C-reactive protein (hsCRP) concentration was determined in duplicate by DRG ELISA kit (USA). Detection limit was 0.001 mg/L, and CV for the hsCRP kit was <3%.

**Growth Factors**

Serum insulin-like growth factor (IGF-I) and muscle isoform of platelet-derived growth factor (PDGFBβ) were evaluated in duplicate by R&D Systems ELISA kits (USA). Detection limits were 0.026 ng/ml and 20 pg/ml, respectively. The CV for the growth factors kits was <5%.

**Statistical Analysis**

Statistical calculations were performed using the statistical software Statistica 13.1 (StatSoft Inc., Tulsa, OK, USA). All data were tested for distribution normality using the Shapiro–Wilk test. The values of W for inflammatory markers and growth factors were closed to the one-value; therefore statistical significances were assessed using one-way analysis of variance (ANOVA) and post-hoc test (HSD Tukey). Associations among measured parameters were analyzed using Pearson’s linear regression (r coefficient). Statistical significance was set at \( p < .05 \). Results are expressed as mean and standard deviation (\( x \pm SD \)).

**Results**

The study comprised 53 healthy older and younger men. Over 90% of the elderly were 65 years or older. The generation of inflammatory mediators was related to changes in body composition, maximum strength muscle, and age-related changes in skeletal muscle properties responsible for deficits in functional fitness.
Body Composition

Among the older adults, BMI ranged from 19.9 to 32.7 (Table 1). About 12% of investigated seniors were classified as obese and 61% as overweight, 27% had normal weight. In the younger group, 85% of the subjects had a normal BMI. Normal FFMIs were from 16.0 kg/m² to 11.3 kg/m² in the older males, and from 12.4 kg/m² to 23.0 kg/m² in the older males. The FMI values were categorized as obese and overweight (FMI ≥ 18 kg/m²) were distinguished by low functional status in seniors from U3A. The functional status of knee extensors as measured by 6MWD showed good functional status in seniors from U3A. The result of the 6MWD equation was 199 ± 26 m, which shows that muscle functionality is, on average, 50% lower in the elderly (Table 2). Knee extension and flexion peak TQ at 60°/s and 180°/s in younger men were significantly different in older men compared to younger men. All observed inflammatory markers, that is, IL-1β, TNFα, hsCRP, as well as IGF-I and PDGFBB in Younger and Older Men (Mean ± SD).

![Table 2. Results of Peak Torque (Peak TQ), Peak TQ/BW, Total Work, Average Power, and Agonist/Antagonist Ratio in the Knee Joint at 60°/s and 180°/s (Mean ± SD).](image)

![Table 3. Serum Hydrogen Peroxide H₂O₂, Cytokines IL-1β and TNFα, hsCRP, as well as IGF-I and PDGFBB in Younger and Older Men (Mean ± SD).](image)

Functional Fitness

The result of the 6MWD equation was 199 ± 49, which shows good functional status in seniors from U3A. The older men classified as obese and overweight (FMI ≥ 8 kg/m²) were distinguished by low functional status (6MWD <150).

Isokinetic Testing

The analysis of mean values for parameters showed that muscle functionality is, on average, 50% lower in the elderly (Table 2). Knee extension and flexion peak TQ, peak TQ/BW (peak torque/body weight), total work and agonist/antagonist ratio measurements obtained at 60°/s and 180°/s in older men were significantly different from young males. The older men with high fat content demonstrated low values of knee extension peaks TQ at 60°/s and 180°/s.

Inflammatory Markers

There were significant age-related increases in inflammatory molecules (Table 3). H₂O₂, IL-1β, and TNFα levels were twofold higher while TNFα level was sixfold higher in the elderly. hsCRP was fourfold higher in older men compared to younger men. All observed inflammatory markers inversely correlated with age (for IGF-I: r = −0.690, and for PDGFBB r = −0.812; p < .001). The changes in IGF-I and PDGFBB concentrations were also related to the knee extension peak TQ at 60°/s.
Table 4. Relationships (Correlation Coefficients; *p < .001*) Between Peak TQ, Inflammatory Markers $\text{H}_2\text{O}_2$, $\text{IL-1}\beta$, TNF$\alpha$, and hsCRP as well as IGF-I and PDGF$^{BB}$

|                  | $\text{H}_2\text{O}_2$, $\mu\text{mol/L}$ | $\text{IL-1}\beta$, pg/ml | TNF$\alpha$, pg/ml | hsCRP, mg/L | IGF-I, ng/ml | PDGF$^{BB}$, pg/ml |
|------------------|------------------------------------------|---------------------------|-------------------|-------------|--------------|------------------|
| Peak TQ/ER at 60°/s, Nm | -.539                                    | -.654                     | -.717             | -.852       | .713          | .785             |
| Peak TQ/ER at 180°/s, Nm | -.489                                    | -.637                     | -.704             | -.799       | .656          | .700             |

Note. ER = extensors of right knee; IGF-I = insulin growth factor; PDGF = platelet-derived growth factor.

Table 5. Relationships (Correlation Coefficients; *p < .001*) Between Pro-Inflammatory Mediators (IL-$\beta$, TNF$\alpha$, and hsCRP) and IGF-I and PDGF$^{BB}$

|                  | IGF-I, ng/ml | PDGF$^{BB}$, pg/ml |
|------------------|-------------|-------------------|
| $\text{IL-1}\beta$, pg/ml | -.582       | -.550             |
| TNF$\alpha$, pg/ml | -.544       | -.683             |
| hsCRP, mg/L      | -.664       | -.778             |

Note. IGF-I = insulin growth factor; PDGF = platelet-derived growth factor.

and 180°/s (Table 4). Levels of IGF-I and PDGF$^{BB}$ were significantly reduced by high levels of IL-$\beta$, TNF$\alpha$ and hsCRP (Table 5).

Discussion

Age-related muscle changes are characterized by a gradual loss of spinal motor neurons due to apoptosis, reduced growth factors signaling and protein uptake, elevated amounts of circulating cytokines and ROS generation and so forth. Some denervated muscle fibers are reinnervated through collateral sprouting of nearby surviving motor axons or motor end plates, which results in the formation of enlarged motor units. Consequently, the age-related loss of spinal motor neurons leads to a decline in muscle fiber number and size, resulting in impaired mechanical muscle performance (reduced maximal muscle strength, power, and rate of force development) that translates into a reduced functional capacity during everyday tasks (Aagaard et al., 2001; Zembron-Lacny et al., 2014). These age-related changes in skeletal muscle system are the result of chronic activation of macrophages, which leads to an increase in pro-inflammatory cytokines (Franceschi & Campisi, 2014). The inflammatory mediators such as $\text{H}_2\text{O}_2$, IL-$\beta$, and TNF$\alpha$ are induced by various stimuli such as bacteria, viruses, and tissue damages (Cannizzo et al., 2011). The increase in pro-inflammatory factors is far less than that seen in acute infection; thus the ageing effects on pro-inflammatory cytokine expression are considered to be a chronic low-grade state (Hansen, Baptiste, Fjeldborg, & Horohov, 2015).

In the study, the levels of $\text{H}_2\text{O}_2$, IL-$\beta$, TNF$\alpha$, and hsCRP were several times higher in the elderly, which underlie the low-grade inflammatory status. Meng et al. (2015) demonstrated that the circulating systemic inflammatory markers are associated with less muscle mass, lower muscle strength, slower walking speed, poorer balance, and lower self-reported functional ability.

The high concentration of hsCRP strongly reduces muscle function and growth factors levels in older men (Tables 4 and 5). While the exact biological actions of hsCRP are not established, its levels predict risk of mobility/disability and accelerated decline in muscle strength and physical performance in older adults (Meng et al., 2015; Verghese, Holtzer, Lipton, & Wang, 2012). The observed fourfold increase in hsCRP concentration in older men proves the presence of low-grade inflammation during ageing. Inflammaging is considered a predictor of fragility and this condition is currently accepted as a pathogenic factor in the development of several age-related diseases and increased mortality risk. The precise etiology of inflammaging and its potential causal role in contributing to adverse health outcomes remain largely unknown. The identification of pathways that control age-related inflammation across multiple systems is therefore important in order to understand whether treatments that modulate inflammaging may be beneficial in the elderly population (Franceschi & Campisi, 2014; Lohr et al., 2014).

One of the possibility catabolic actions of inflammatory mediators on skeletal muscle is the inhibition of protein synthesis and myogenesis in myoblasts. Earlier studies demonstrated that after IL-$\beta$ stimulation, the total protein level did not increase, but rather synthesis of the acute phase proteins was favored (Weissman, 1990). Studies indicate that TNF$\alpha$ can interfere with muscle growth and regeneration in part by disrupting growth factors signaling pathways (Strle et al., 2004). TNF$\alpha$ also is responsible for triggering the death receptor-mediated apoptosis, which plays a significant role in atrophy of muscle fibers, especially type II fibers (fast), by a decreased number of motor units (Marzetti et al., 2010).

The cytokines IL-$\beta$ and TNF$\alpha$ are involved, not only in muscle mass decrease, but also in increase in fat mass. At the extreme, these two processes lead to a condition known as “sarcopenic obesity.” Studies have highlighted that pro-inflammatory cytokines produced by adipose tissue accelerate muscle catabolism, and thus contribute to the vicious circle that initiates and sustains sarcopenia (Schrager et al., 2007). The study confirmed that fat tissue...
can be source of inflammatory mediators, which enhance age-related muscle loss. Older men with high FMI demonstrated high concentrations of IL-1β and TNFα.

The increased level of pro-inflammatory cytokines negatively impacted concentrations of investigated growth factors IGF-I and PDGFBB, which play a central role in myofiber hypertrophy and atrophy, and this balance is of critical importance for muscle wasting in ageing. Grounds, Radley, Gebski, and Shavlakadze (2008) suggested that the effects of IL-1β and TNFα on muscle atrophy may be mediated in part via interference with IGF-I signaling and inhibition of the anabolic signaling cascade downstream of the IGF-I receptor.

The long-term increased concentrations of TNFα and IL-1β may induce muscle resistance to IGF-I (O’Connor et al., 2008). Additionally, TNFα may abolish anti-apoptotic effects of IGF-I, reducing the survival of differentiated myoblasts (Grounds et al., 2008). Lohr et al. (2014) demonstrated in large size of the sample that increased inflammatory markers, such as hsCRP with low IGF-I may even increase mortality in young and older men and women.

Among many of the stimulatory growth factors, PDGFBB plays a crucial role in myogenic proliferation and differentiation (Pallafacchina et al., 2013). Despite the fact that knowledge about the level of circulating IGF-I in the elderly is well developed, in case of PDGFBB it is quite enigmatic. In general, there are few data describing the influence of ageing on the level of circulating PDGFBB. In addition to confirming that the level of circulating PDGFBB is lower in the elderly, this study has indicated that both IL-1β and TNFα have a negative influence on this molecule. There is only one dataset concerning the level of circulating PDGFBB in the muscles of the elderly, especially its interaction with IL-1β or TNFα (Banerjee et al., 2011; Bentzinger, von Maltzahn, & Rudnicki, 2010). Banerjee et al. (2011) have reported that the inflammatory processes can contribute to the PDGFBB profile in the elderly. These results suggest that mechanisms responsible for negative correlations between investigated pro-inflammatory cytokines and PDGFBB are similar to that for IGF-I.

IGF-I and PDGFBB are important downstream mediators of the anabolic effects of growth hormone and their serum levels are inversely correlated with age. Kaplan et al. (2008) demonstrated that the total IGF-I level is associated with strength, mobility, and mortality. Although there is evidence that ageing muscle retains the ability to synthesize IGF-I, ageing may also be associated with attenuation of the ability of exercise to induce an isoform of IGF-I that promotes satellite cell proliferation (Owino, Yang, & Goldspink, 2001). These results indicate an age-related decrease in systemic derived growth factors, which may be responsible, at least in part, for the age-related decline in muscle function. A large number of studies have suggested the implications of cross-talk between pro-inflammatory cytokines and growth factors in skeletal muscle, which is likely the underlying mechanism of sarcopenia (Meng et al., 2015).

The age-related shifts in body composition toward more fat mass, especially the accumulation of more internalized fat storage, and the loss of muscle mass and function increase the risk of injury from sudden falls and developing a wide range of chronic disorders. FFM and FM indexes have been useful for the clinical evaluation of a deficit in fat-free mass with or without excess fat mass for a given age category, complementing the classical concept of BMI in a more qualitative manner. In the present study, an increased fat storage specifically affected FMI but not FFMI in older men as compared to younger men, which corresponds well with studies done by Bahadori et al. (2006) and VanItallie et al. (1990). The majority of seniors demonstrated very good results in the 6-min walking test which corresponds very well with studies done by Enright and Sherrill (1998). Their good functional status could be related to their participation in various physical and health educational forms at the University of the Third Age (U3A). According to Zielinska-Wieczorkowska, Kedziora-Kornatowska, and Ciennoczołowski (2011), the high life quality of the U3A students significantly denotes the level of their knowledge concerning illnesses, afflictions, depression, and the health benefits of physical activity.

Conclusions

In the current study, the strong dominance of inflammatory mediators H₂O₂, IL-1β, TNFα, and hsCRP over the anabolic factors IGF-I and PDGFBB in the older men were observed, whereas in young men the reverse situation was detected. The enhancement of pro-inflammatory state with age was responsible for deficits in maximum strength muscle and functional capacity. However, it is too early to draw a clear conclusion on a clinically relevant relationship between inflammation and skeletal muscle properties due to the small sample size.

Declaration of Conflicting Interests

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