The biomarkers of sarcopenia in elderly people

Kishiko Ogawa

Laboratory of Nutritional Physiology, School of Food and Nutrition Sciences, The University of Shizuoka, 52-1 Yoda, Suruga-ku, Shizuoka-shi, Shizuoka 422-8526, Japan

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Abstract  Sarcopenia is a very prevalent age-related decline in skeletal muscle mass and muscle strength, and it has a biologic basis and is a distinct clinical syndrome in elderly people that is associated with high risk for adverse health outcomes. Currently, many parameters can potentially be used to track age-related skeletal muscle decline; among these parameters, biochemical markers for sarcopenia may be particularly useful for developing different aspects of therapeutic intervention. Evidence indicates that extracellular heat shock protein 72 in plasma may be defined, as a potential biomarker of sarcopenia. Extracellular adenosine triphosphate in plasma may trigger the release of heat shock protein 72 during a bout of exercise. Muscle hypertrophy in elderly women was caused by 12-week resistance exercise training, and this hypertrophy induced a reduction in extracellular heat shock protein 72 and pro-inflammatory cytokines and insulin like growth factor-I. The phenomenon in circulating levels of biomarkers may result from the lack of understanding of the mechanisms in exercise or muscle anabolic and catabolic systems, or secreted cell stress proteins are part of an extracellular homeostatic signaling system. Using two or more biomarkers in combination to clearly identify one condition may be one of multiple solutions. Using biomarkers combinatorially may allow clinicians to identify different domains of the sarcopenia syndrome and give them useful insights about the pathophysiological process underlying the phenomenon.

Keywords: low-grade inflammation, moonlighting protein, biochemical markers

Introduction

Sarcopenia is a geriatric syndrome in which there is a decrease in muscle mass and strength with aging\(^1\). The prevalence of sarcopenia has been estimated to be 5-13% for elderly people aged 60-70 years and the percentages increase to 11-50% for those aged 80 or above\(^2\). Sarcopenia is a normal part of ageing, but if unchecked it can lead to weakness, disability, falls, loss of independence, and frailty\(^3\). The European Working Group on Sarcopenia in Older People developed a definition of and diagnostic system for sarcopenia\(^4\), introduced quantitative assessments of muscle mass, muscle strength, and physical performance, and established cut-off points based on measurement of muscle mass, grip strength, and gait speed. The International Working Group on Sarcopenia has also proposed criteria for diagnosing sarcopenia\(^5\); these include a gait speed and muscle mass. Unfortunately, because those cut-off points are based on data from specific ethnic groups, the utility of the cut-off points or criteria could be limited. For definitions of sarcopenia, many reliable investigators often highlighted measurements of muscle mass or fat-free mass using dual energy X-ray absorptiometry (DXA), computed tomography (CT), bioelectrical impedance analysis, or magnetic resonance imaging. Although discussions of these methodological biomarkers are extremely important, the present review will focus on biochemical markers in blood of sarcopenia in elderly people when we assess the data of muscle mass, grip strength, and gait speed with regard to the definition of sarcopenia. Investigation of biochemical markers could improve diagnoses of, treatments for, and our understanding of the disablement process in sarcopenia in elderly people.

Biochemical markers for sarcopenia

Sarcopenia has many causative factors, including lifestyle, neurological, hormonal, nutritional, and immunological determinants\(^6\). Sarcopenic changes in the muscles include decreases in muscle fiber quality and quantity, α-motor neuron numbers, protein synthesis rates, and anabolic and sex hormone production\(^7\). Many different hypotheses about the processes causing the aforementioned sarcopenic alterations have been proposed, but the overall etiology is still incompletely understood\(^8\). Elderly, even when considered healthy, frequently have systemic low-grade inflammation\(^9\). Moreover, the levels of C-reactive protein (CRP), interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in elderly people are
related to severe muscle wasting and cachexia because these inflammatory cytokines are involved in the muscle catabolic processes that are associated with inflammation. In elderly Japanese women, muscle hypertrophy was induced by 12-week resistance-exercise training and was associated with reduction of CRP and TNF-α; these findings indicate that inflammatory markers and cytokines may be potential biomarkers for sarcopenia and useful as criteria when developing interventions and treatments. Nutritional vitamin D have recently received recognition as a potential intervention strategy for sarcopenia. Serum 25-hydroxyvitamin D (25(OH)D) levels are often measured as indicators of vitamin D status in the elderly, and 25(OH)D levels have been proposed as a parameter of physical performance. Vitamin D stimulates muscle cell proliferation and growth. Vitamin D deficiency is an important risk factor for osteoporosis and is associated with body sway and falls in the elderly. Proximal muscle weakness is a prominent feature of the clinical syndrome of vitamin D deficiency, and muscle weakness is a major risk factor for falls in the elderly. However, results of investigations into interventions for sarcopenia that involved vitamin D supplements were contradictory. Often, sunlight exposure, skin color, and region (sunny or not) influence the status of serum vitamin D level more than a supplement. Low serum levels of albumin or cholesterol reduce body mass with reduced intake of protein and energy, inducing sarcopenia. Hemoglobin and creatinine are also associated with sarcopenia. A 12-week resistance exercise training program increased hemoglobin levels (data not shown). Exercise in the elderly may induce increases in food intake and decreases in malnutrition and anemia; therefore, exercise may lead not only to improvements in muscle mass and physical performance in the elderly but also to improved nutritional status.

Hormones, including dehydroepiandrosterone, testosterone/procollagen type III N-terminal peptide (P3NP), insulin-like growth factor-1 (IGF-1)/growth hormone (GH), are potential biomarkers for sarcopenia. Age-related declines in sex hormones and growth factors are linked to age-related reductions in protein synthesis and muscle-cell function. Aging is associated with decreased expression of hormonal factors that promote protein synthesis and increased expression of both endocrine and inflammatory factors that negatively affect the protein balance by increasing protein degradation. Moreover, some biomarkers that are specifically linked to the neuromuscular junction may have an interesting role in evaluations of skeletal muscle modifications.

Recent findings indicate that heat shock protein (Hsp72 in plasma (i.e., extracellular Hsp (eHsp)) is a predictor of sarcopenia in community-dwelling people. Higher levels of eHsp72 were independently associated with lower skeletal muscle mass, grip strength, and walking speed (Fig. 1A-C); specifically, the odds ratios for skeletal muscle

Fig. 1  Odds ratio of risks for muscle mass (A), hand grip strength (B), walking speed (C), and plasma levels of Hsp72 (eHsp72). Subjects were grouped based on their tertiles of muscle mass, hand grip strength, walking speed and on their eHsp72 levels. All data were sex- and age-adjusted. L, bottom tertile; M, middle tertile; H, top tertile. The sample included 665 participants aged 65-96 years that lived in a community setting. The sex ratio distribution of the participants was significantly different from a random distribution (male, n=264; female, n=356, P<0.001), but there were no significant differences in the mean ages between the sexes (mean ± standard deviation: male, 73.5 ± 6.0 years; female, 73.4 ± 6.3 years).
Extracellular heat shock protein 72 and exercise

Hsps are highly conserved proteins that are expressed both constitutively and under stressful conditions. The major role of Hsps appears to be the protection of the proteome via the Hsp molecular chaperone function. Hsps recognize damaged proteins, and they channel the damaged proteins either into the repair-refolding pathways or into the proteolytic pathway. In terms of cell survival, Hsps allow cells to respond to damage and begin the processes required to resolve cellular insults, thereby allowing cells to survive. Among the Hsp families, the Hsp70 family is intrinsic to cellular life because these proteins permit other proteins to perform essential enzymatic reactions, signaling, and/or structural functions within the tightly packed milieu of the cell and because they work to avert the catastrophe of protein aggregation during stress. Hps70s, along with a cohort of other Hsps, are induced to extremely high levels by stress through powerful transcriptional activation, mRNA stabilization, and preferential translation.

Hsp72, which is a member of the Hsp70 family, circulates in the blood; this circulating Hsp72 is referred to as an eHsp. Levels of eHsp72 increased in response to pathological conditions in patients with peripheral and renal vascular diseases, Behçet’s disease, HIV infection, or cancer; in contrast, lower levels of eHsp72 lead to arteriosclerosis or psychological conditions of negative mood and/or stress. Clearly, eHsps play a role as pro-inflammatory immune effectors. Geriatric syndromes have a biological basis and are considered to be highly prevalent and associated with high risk for adverse health outcomes. Hsp72 decreases with aging, but centenarians are an exception among the elderly in that they have decreased eHsp72. A possible contributing cause of these aging adaptations may be that aging changes systemic eHsp72 activity. Aging is associated with the degeneration of Hsp expression with time and the loss of resistance to cellular oxidants. The effects of heat shock factor (Hsf1) and Hsp on longevity appear to be particularly mediated through their ability to protect motor neurons. For example, the presence of eHsp72 can have a protective effect against necrotic cell death of smooth muscle cells and against apoptosis of motor neurons. Because eHsp72 has a protective effect against apoptosis of motor neurons, we reasoned that inflammatory cytokines and eHsp72 might be independently associated with the prevalence of sarcopenia.

We found that the highest tertile levels of eHsp72 in a elderly population were associated with several age-related phenomena, including shrinking (as indicated by older and smaller bodies), weakness (indicated by lower grip strengths), slowness (indicated by low walking speeds), poor nutritional status (indicated by lower hemoglobin), and higher concentrations of inflammatory markers such as TNF-α and β2-microglobuline (MG). However, eHsp72 levels were not associated with biomedical parameters, including cholesterol, HbA1c, and creatinine, or psychological factors as measured with the GDS (Geriatric Depression Scale), self-rated health scale, or MMSE (Mini Mental State Examination). These results indicate that Hsp72 in plasma may be specifically linked to sarcopenia in elderly populations.

Biomarker for frailty

Many previous reviews have proposed the trajectory associated with sarcopenia and with frailty. Frailty and sarcopenia overlap, thus most frail people exhibit sarcopenia, and some older people with sarcopenia are also frail. However, the concept of frailty goes beyond physical factors and encompasses psychological and social dimensions. Diseases are also a trigger of sarcopenia and thus sarcopenia and any consequent lower physical activity can induce disease; conversely, diseases are a strong risk factor for sarcopenia.

Fried et al. have defined frailty as a clinical syndrome in which 5 criteria were present: 1. Shrinking (unintentional weight loss), 2. Self-reported exhaustion, 3. Weakness (grip strength), 4. Slow walking speed, and 5. Low physical activity. Based on this definition, together IL-6 and β2-MG were proposed a combinatorial biomarker for assessing frailty (Fig. 2). The odds ratio of highest tertiles of the IL-6 and β2-MG combination was 5-fold higher than that of the lowest tertiles of the IL-6 and β2-MG combination (Fig. 2). However, eHsp72 was independent of the IL-6 and β2-MG combination.
levels)10). These results show that currently we do not induce a reduction of eHsp72 in blood (i.e. extracellular repair of injured muscle; nevertheless, exercise training Hsp72 clearly protects muscle from injury and stimulates cytokines such as IL-6, TNF-α, and CRP. Intracellular markers are sometimes independent of one another. For instance, nobody doubts that IGF-I plays a crucial role in muscle hypertrophy as a promoter of protein synthesis in skeletal muscle. However, 12-weeks of resistance exercise induced a reduction of IGF-I in serum (i.e. extra-cellular levels)10), as well reductions of inflammatory markers play the same role for muscle anabolism and catabolism. However, the circulating levels of biochemical markers are sometimes independent of one another. For instance, nobody doubts that IGF-I plays a crucial role in muscle hypertrophy as a promoter of protein synthesis in skeletal muscle. However, 12-weeks of resistance exercise induced a reduction of IGF-I in serum (i.e. extracellular levels)10), as well reductions of inflammatory cytokines such as IL-6, TNF-α, and CRP. Intracellular Hsp72 clearly protects muscle from injury and stimulates repair of injured muscle; nevertheless, exercise training induces a reduction of eHsp72 in blood (i.e. extracellular levels)10). These results show that currently we do not completely understand the relationships between turnover of skeletal muscle and aging and that these relationships interact with multiple systems and produce a constellation of signs and symptoms. Many proteins are known to have more than one catalytic/molecular function and protein structure, respectively, or to physiological role and organ/body system, respectively. Proteins that have multiple activities are referred to as moonlighting protein55), and Hsps are considered moonlighting proteins, and different evolutionary mechanisms have been proposed to explain the presence of multiple activities in single-domain proteins. Henderson et al. are proposing a hypothesis, specifically, that secreted proteins are part of an extracellular homeostatic signaling system50. We must emphasize that the entire system must be understand in order to comprehend the role(s) of each individual protein in muscle anabolic and catabolic systems. In addition, just we have demonstrated that only in combination are IL-6 and β2-MG effective biomarkers for frailty, we may find that no one single protein can act as an accurate biomarker for sarcopenia. These biomarkers that were found in the seroepidemiological analysis will contribute to the understanding of the disablement process and to the diagnosis and treatment of sarcopenia in elderly people.

Perspective

Many studies have focused on the mechanisms of muscle hypertrophy. Based on basic investigations, we hypothesize that the circulating levels of biochemical markers play the same role for muscle anabolism and catabolism. However, the circulating levels of biochemical markers are sometimes independent of one another. For instance, nobody doubts that IGF-I plays a crucial role in muscle hypertrophy as a promoter of protein synthesis in skeletal muscle. However, 12-weeks of resistance exercise induced a reduction of IGF-I in serum (i.e. extracellular levels)10), as well reductions of inflammatory cytokines such as IL-6, TNF-α, and CRP. Intracellular Hsp72 clearly protects muscle from injury and stimulates repair of injured muscle; nevertheless, exercise training induces a reduction of eHsp72 in blood (i.e. extracellular levels)10). These results show that currently we do not completely understand the relationships between turnover of skeletal muscle and aging and that these relationships interact with multiple systems and produce a constellation of signs and symptoms. Many proteins are known to have more than one catalytic/molecular function and protein structure, respectively, or to physiological role and organ/body system, respectively. Proteins that have multiple activities are referred to as moonlighting protein55), and Hsps are considered moonlighting proteins, and different evolutionary mechanisms have been proposed to explain the presence of multiple activities in single-domain proteins. Henderson et al. are proposing a hypothesis, specifically, that secreted proteins are part of an extracellular homeostatic signaling system50. We must emphasize that the entire system must be understand in order to comprehend the role(s) of each individual protein in muscle anabolic and catabolic systems. In addition, just we have demonstrated that only in combination are IL-6 and β2-MG effective biomarkers for frailty, we may find that no one single protein can act as an accurate biomarker for sarcopenia. These biomarkers that were found in the seroepidemiological analysis will contribute to the understanding of the disablement process and to the diagnosis and treatment of sarcopenia in elderly people.

Fig. 2 Odds ratio of risks for frailty: combined tertiles of IL-6 and β2-microglobuline.

Subjects were grouped based on their tertiles of IL-6 and β2-microglobuline levels; each number in the figure was odds ratio of risks for frailty, and (95% confidence interval (CI)). Frailty was defined and assessed based on the “Kihon-check List”; the cut-off point was 5 points on the list; this cut-off was estimated to be valid based on the definition of Fried et al. (AUC:0.799, 95%CI: 0.696-0.903). All data were sex- and age-adjusted.

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