

Exercise, Sarcopenia and Immunosenescence

MARTIN KRAUSE

Bachelor Applied Science (Physiotherapy)

Master of Applied Science (Musculoskeletal Physiotherapy)

Graduate Certificate Health Science Education

Private Practitioner

Back in Business Physiotherapy

Suite 705, 107 Walker Street,

North Sydney, 2060

Australia

mkrause@acay.com.au

I acknowledge that I have no conflicts of interest in submitting this manuscript for consideration for publication by the Journal of Gerontology: Medical Science. I have not submitted this manuscript to another journal. This review manuscript has been prepared by me, and is my own original work.

Martin Krause
9/11/2003

Abstract

Sarcopenia is characterized by the reduction in muscle mass and may contribute to immunosenescence, as it is thought that muscle provides an important reservoir of heat shock proteins (HSP) and glutamine as well as act as a site for the action of insulin. HSPs are the cellular link, which activate T lymphocyte proliferation. Exercise can activate HSPs and may provide the 'danger signal' for T lymphocyte re-activation after a period of quiescence. Muscle glutamine appears to be important for inter-organ transport as well being a precursor for anti-oxidant activity. Dose specific exercise is thought to improve immune function through an improved ratio of TNF- α : IL-6, which may promote the anabolic effects of IGF-1 and insulin thereby possibly improving muscle mass, leading to enhanced mechanical loading tolerance. Additionally, older people with preserved muscle mass have the highest number of NK cells. The weight of evidence suggests that the preservation of muscle mass and/or reversal of sarcopenia through exercise could be a useful anabolic method to provide a protein reservoir for later use when the older person is exposed to infection, inflammation and/or severe trauma.

Introduction

Sarcopenia is characterized by reduced muscle mass [1]. This is significant as skeletal muscle contains 50-75% of all proteins in the human body and represents a store of energy and nitrogen which becomes a vital supply of fuel for the immune system, as well as a substrate for wound healing during malnutrition, injury and disease [2]. Loss of muscle mass may result in immunosenescence, which is characterized by impaired cellular immune function concomitant with increased inflammatory activity [3]. Little is known about the effects of exercise on the senescent immune system [3]. Moreover, exercise has been suggested as a prototype for studying the effects of stress factors on the cellular immune system, because several other physical stressors, such as burns, surgery, acute myocardial infarction and hyperthermia induce similar immune responses [3].

Muscle accounts for 90% of the cross sectional area in active young men, but only 30% of that area in frail older women [4]. The prevalence of clinically significant sarcopenia is estimated to range from 8.8% in 'young old' women to 17.5% in 'old old' men [1]. This review will entertain the notion that the loss of muscle mass due to sarcopenia attenuates the inflammatory-immune response due to the lack of protein stores (glutamine [5], heat shock protein [HSP] [6]). Cytokines such as Interleukin-6 (IL-6) could enhance the immune response through inhibition of Tumor Necrosis Factor alpha (TNF- α) production and insulin resistance [7]. TNF- α is considered to enhance protein degradation through the activation of HSP [8], whereas Insulin [9, 10] and Insulin like Growth Factor (IGF-1) [11] are thought to enhance protein synthesis. Additionally, it will be hypothesized that

dose specific exercise training can ameliorate some of the effects of sarcopenia as well as enhance immune function by balancing protein synthesis with proteolysis.

T lymphocyte immune responses

Cytokines are a group of low molecular weight regulatory proteins secreted by white blood cells and a variety of other cells which generally function as intercellular messenger molecules [12]. Proliferation of T lymphocytes is essential for the first step in an adaptive immune response [3]. Essentially there are 2 types of immune cells, the 'memory cells' and 'virgin cells'. With age, the percentage of memory cells increases with a corresponding decrease in virgin cells [3]. Consequently, a reduction in naïve cell responses, and a resulting shift to memory cell proliferative response has been shown in the older person [13]. Fortunately, with advancing age a progressive increase in natural killer (NK) cell number appears to compensate for a decreased number and function of T lymphocytes [14]. More importantly, these findings suggest that cells from older individuals do not suffer from a quantitative decline in cytokine production on a per cell basis [13]. Indeed, centenarians and nonagenarians who presented with the highest number of NK cells and best preserved cytolytic function, also had preserved integrity of muscle mass [14]. Therefore, this suggests that the maintenance of muscle cell mass could preserve the immune response to environmental stressors.

Muscle as a source of protein for immune function

Since muscle represents approximately 40% of body weight, it is thought to be an important reservoir of proteins [8], which may be called upon by the immune system in response to injury [10]. Even a 10% loss in lean body mass (LBM) corresponds with

impaired immune function [15] and a loss of approximately 30% of the body proteins can result in death [2]. During severe trauma, such as burns, the need for essential amino acids drives the catabolic loss of protein from skeletal muscle, which can be as high as 1% per day of illness [16]. Accelerated muscle proteolysis is the primary cause of this loss of lean body mass characteristic of many diseases [17]. Some peptides generated by the breakdown of cell proteins are transported to the cell surface where they are presented to cytotoxic lymphocytes, which destroy cells presenting foreign (eg viral) peptides [17]. This may partially explain why successful aging has been associated with the preservation of muscle mass [14] as this would endow these individuals to draw on their store of protein for inflammatory-immune responses.

Increased cytokine activity has been associated with aging and muscle weakness [18], possibly resulting in inactivity. This age associated progressive dysregulation of immune response [19], is seen in older women with high IL-6 serum levels who have a higher risk of developing physical disability and experience steeper declines in walking ability than those with lower levels [18]. However, the statistical interaction of IL-6 concentration with disability was non-linear and the progression of disability and IL-6 concentration over time was not investigated [18]. Therefore, it is difficult to conclude whether elevated levels of IL-6 are the cause or the effect of skeletal muscle weakness, as the progressive withdrawal of a number of anabolic stimuli, also causes skeletal muscle weakness [18]. Furthermore, high levels of TNF- α and IL-6 have been shown to be associated with classical risk factors such as smoking, physical inactivity and body mass index (BMI) [20]. It is difficult to conclude whether the high levels of cytokines lead to

inactivity or whether they are the result of reduced skeletal muscle loading capacity from inactivity. However a cycle of inactivity and chronic inflammatory-immune response is plausible.

Heat shock protein (HSP)

Numerous attempts to link exercise to meaningful alterations in immune function have been largely unconvincing [21]. It has been argued that it is local as opposed to the global immune system activation by HSPs, which is at the core of immune effects of exercise [21]. It is thought that HSPs on a cells surface activate T lymphocyte proliferation when accompanied by a co-stimulatory ‘danger signal’ [21]. These T lymphocytes can switch between the active and quiescent memory states, to be later re-activated in the presence of an antigen and co-stimulatory ‘danger signal’ [21].

Decreases in HSP72, a heat shock protein specifically related to skeletal muscle [22], is considered to promote apoptosis [23] possibly due to the lack of this secondary ‘danger signal’ [21]. Not surprisingly, apoptosis has been linked with sarcopenia [24]. HSP are involved in protein folding and sorting, in the assembly of protein complexes as well as binding of denatured proteins and are primarily induced in response to stress [6].

Impaired recovery from acute complications and the reduced renewal of damaged and toxic proteins are potential undesired consequences of low-protein turnover [25]. Indeed, critically ill patients have been shown to have low protein synthesis in skeletal muscle correlating with metabolic status and clinical indices of the severity of the disease [26]. Specific adaptation in muscle associated with enhanced proteolysis can occur [17], whereby HSPs provide a link between immune response to infection and autoimmunity

during fever [27]. In fact older people have been shown to have a reduced incidence of fever in response to injury [28]. Although, exercise induced HSP activity has been described [6, 22] it is uncertain what level of exercise can provide the ‘danger signal’ required to enhance immune function [21].

Exercise may provide a hormonal stimulus to regulate HSP proteolysis. Recently, rodent investigations have demonstrated that Insulin-Like Growth Factor 1 (IGF-1) inhibits both lysosomal and ubiquitin-proteasome dependent stress protein breakdown in skeletal muscle [11] thus suggesting a hormonal regulating mechanism. Increased IGF-1 concentrations have been demonstrated near the Z-bands in the elderly after resistance training regime [29]. In particular, eccentric exercises have been associated with damage to these Z-bands [30]. Therefore, IGF-1 production in muscle may be responsible for the regulation of protein synthesis after HSP induced proteolysis as a result of exercise induced trauma.

Muscle glutamine and inflammatory-immune response

Skeletal muscle may represent an important source of anti-oxidants. Alterations of respiration in mitochondria of muscle cells has been associated with aging and sarcopenia [10, 31-34]. Interestingly, the muscle protein glutamine is a substrate for glutathione, which acts as an endogenous scavenger with an ability to counteract oxidative injury from oxygen free radicals [16]. This may be particularly important, as the level of oxidative stress imposed on the aging muscle is influenced by two fundamental biological processes: the increased generation of reactive oxygen species (ROS) and age-associated

changes in antioxidant defense [33, 35]. Therefore, since glutamine accounts for nearly two thirds of the free intracellular amino acid pool and is abundant in skeletal muscle [16] it is likely to be an important source of anti-oxidants.

It is thought that TNF may mediate protein degradation in cachexia through a ubiquitin-proteasome pathway [8]. This skeletal muscle proteolysis seen in response to severe injury has been speculated to be for the provision of precursors for glutamine synthesis [16]. Glutamine is also thought to be a significant inter-organ nitrogen and carbon transporter as well as being important in glycogen metabolism and therefore can presumably affect mitochondrial oxidative responses [16]. Additionally, in the fasting state, an increase in glucocorticoids and reduction in insulin results in muscle proteolysis [17] presumably through an inter-organ mechanism. During acidosis, some amino acids from muscle protein are converted to glutamine, which is used by the kidneys in acid excretion and energy metabolism [17] (figure 1). Therefore, muscle may represent an important anti-oxidative organ during an inflammatory-immune response. Hence, in the presence of sarcopenia, reduced ability to counteract damage by oxidative radicals may be expected due to reduced glutamine stores.

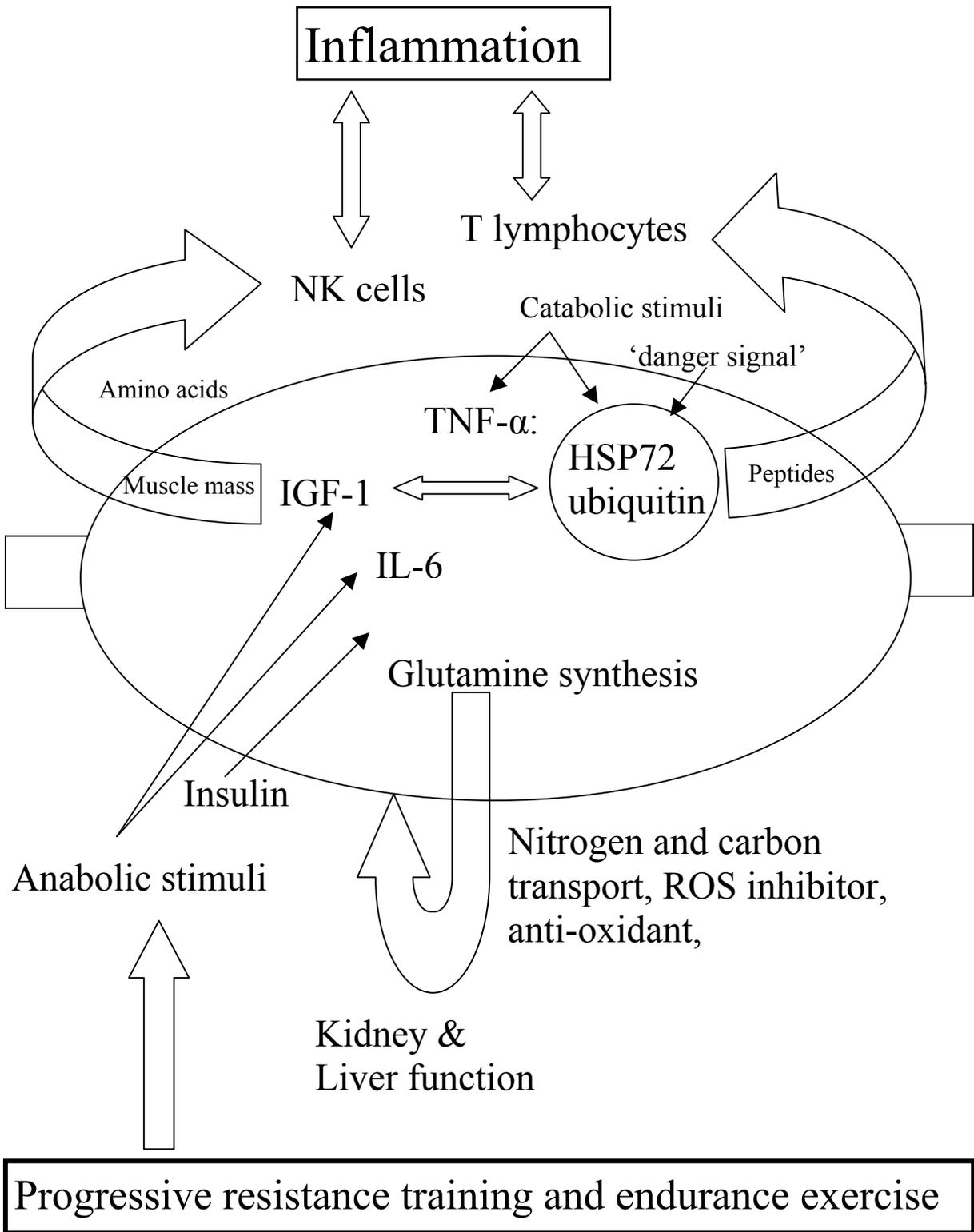


Figure 1: Interaction of catabolic cytokines, Natural killer (NK) cells, tumor necrosis factor (TNF), heat shock protein (HSP), glutamine during inflammation and the anabolic pathway of protein synthesis through Insulin-like Growth Factor (IGF-1), Interleukin (IL-6) and insulin through exercise

Implications for exercise training and immune function

Investigators have recently demonstrated that muscle contractions induce the production and release of IL-6 but not TNF- α into the circulation [7]. Contrary to the conclusions of Ferrucci et al (2002) [18], other authors suggested that muscle-derived IL-6 contributes to mediate the beneficial metabolic effects of exercise and may inhibit TNF- α production and insulin resistance [7]. Administration of insulin has been shown to promote protein synthesis [9]. Furthermore, resistance exercise training has been shown to attenuate the catabolic effect of TNF by suppressing skeletal muscle TNF- α expression [36].

However, a 12 week high intensity progressive resistance training program did not affect immune function in healthy older people or subjects with systemic inflammation [37].

Yet, eccentric exercise is associated with an increase in pro-inflammatory cytokines [3, 30]; whereas concentric exercise has been associated with inflammation from oxidative stress [32] and hydrogen ion accumulation due to hypoperfusion [5]. Furthermore, a recent review concluded that older people have a preserved ability to recruit T lymphocytes and NK cells in response to exercise [3]. However, the cells recruited had a replicative history suggesting they were memory cells. Additionally, a lack of investigations into circulating levels of pro-inflammatory cytokines during eccentric exercise in older versus young populations was lamented by these authors [3].

Nevertheless, it would appear that resistance training could be beneficial for IL-6 production as well as TNF- α suppression post exercise.

Although investigators have demonstrated enhanced muscle size with resistance training in older people [38], there is little evidence to suggest that this change in size is sufficient to improve immune function [3, 39]. Yet, resistance training has been shown to significantly increase lean body mass and strength in HIV associated muscle wasting [40], therefore indicating a role for exercise in the presence of protease inhibitors and retroviral activity. Additionally, improved lean body mass and reduced fat has been shown to enhance IGF-1 levels during endurance training [41]. Consensus indicates that ‘moderate exercise’ may enhance immune function and may reduce the incidence of infection while long term exhaustive exercise results in immuno-suppression and an increased susceptibility to infections [12, 42-45]. This is consistent with Ji [33] who suggest that the major benefit of non-exhaustive exercise is to induce a mild oxidative stress that stimulates the expression of antioxidant enzymes, as well as the induction of IGF-1 [11] seen in resistance training [29,46]. Additionally, resistance training accompanied by nutritional supplementation has been shown to result in significant muscle hypertrophy [47]. Biomechanical principles dictate that for the same force, the strain in a skeletal muscle is reduced proportional to the skeletal muscle’s cross sectional area [48]. Therefore, if contractile skeletal muscle mass is maintained or enhanced, then it is plausible that a greater spectrum of ‘moderate exercise’ can be entertained.

Highly conditioned elderly humans seem to have a better preserved immune system, although it is not possible to conclude if this is linked to training or other lifestyle-related factors [3]. Specifically, a bi-directional neuro-immune system has been implicated for exercise induced modulation of immune function through the autonomic nervous system

[49]. Importantly, the effect of exercise on immune function requires future investigation using dose specific criteria [39, 50] as over-training is characterized by reduced catecholamine levels, decreases in neutrophil function, serum and salivary immunoglobulin concentrations and NK cell numbers and possibly cytotoxic activity in peripheral blood, at least in younger individuals [51]. In particular, the 2-4 hours post exercise have been shown to demonstrate inflammation with concomitant suppression of immune function, especially after high intensity, eccentric exercise in young populations [5, 12]. If these findings can be extrapolated to older people then the periodization of training and careful selection of the type, volume, frequency, duration and intensity of exercise would appear to be important to gain the maximum anabolic and minimum catabolic effect. Additionally, if the perception of effort is too great the person is less likely to exercise [52]. Recently, increases in muscle mass of 1kg in frail older women and 2.2kg in men was demonstrated using a 3 month resistance training regime incorporating 8 exercises, commencing with 3 sessions/week, 1-2 sets/session, 6-8 repetitions/set at 65-75% 1RM, which was gradually increased to 3 sessions/week, 3 sets/session, 8-12 repetitions/set at 75-85% 1RM [53]. Whether, this anabolic effect on muscle mass loss concomitantly ameliorates immunosenescence requires further clinical research.

Conclusion

Sarcopenia is characterized by the reduction in muscle mass and may contribute to immunosenescence as muscle is thought to provide an important reservoir of heat shock proteins and glutamine as well as to represent a site for the action of insulin. HSPs are the cellular link, which activate T lymphocyte proliferation. Exercise can activate HSPs

and may provide the 'danger signal' for T lymphocyte re-activation after a period of quiescence. Muscle glutamine appears to be important for inter-organ transport as well being a precursor for anti-oxidant activity. Gathering evidence suggests that dose specific exercise could improve immune function through an improved ratio of TNF- α : IL-6, which promotes the anabolic effects of IGF-1 and insulin thereby improving muscle mass, which can lead to enhanced mechanical loading tolerance. Additionally, older people with preserved muscle mass have the highest number of NK cells. Taken together the evidence suggests that the preservation of muscle mass and/or reversal of sarcopenia through exercise could improve the protein reservoir for a person to draw upon when they are exposed to infection, inflammation and/or severe trauma.

Acknowledgement:

I would like thank Maria Antoinette Fiatarone Singh for her inspiration and comments during the preparation of this manuscript.

Maria Antoinette Fiatarone Singh
University of Sydney
Faculty of Health Sciences
The John Sutton Chair of Exercise and Sport Science
PO Box 170
East Street, 2141
Lidcombe, NSW
Australia

WORD COUNT: 2436 (not incl abstract)

ABSTRACT: 182

References:

1. Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS. Sarcopenia. *J Lab Clin Med.* 2001;137:231-243.
2. Rasmussen BB, Phillips SM. Contractile and Nutritional Regulation of Human Muscle Growth. *Exercise Sport Sci Rev.* 2003;31:127-131.
3. Bruunsgaard H, Pedersen BK. Effects of exercise on the immune system in the elderly population. *Immunol Cell Biol.* 2000;78:523-531.
4. Rosenberg IH, Roubenoff R. Stalking Sarcopenia. *Ann Intl Med.* 1995;23:727-728.
5. Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exercise.* 2000;32:317.
6. Puntchart A, Vogt M, Widmer HR, Hoppeler H, Billeter R. Hsp70 expression in human skeletal muscle after exercise. *Acta Physiol Scand.* 1996;157:411-417.
7. Pedersen BK, Bruunsgaard H. Possible beneficial role of exercise in modulating low-grade inflammation in the elderly. *Scand J Med Sci Sports.* 2003;13:56-62.
8. Kotler DP. Cachexia. *Ann Intern Med.* 2000;133:622-634.
9. Ferrando AA, Chinkes DL, Wolf SE, Matin S, Herndon DN, Wolfe R. A submaximal dose of insulin promotes net skeletal muscle protein synthesis in patients with severe burns. *Ann Surg.* 1999;229:11-18.
10. Shipman J, Guy J, Abumrad NN. Repair of metabolic processes. *Crit Care Med Healing Responses in Critical Illness.* 2003;31:S512-S517.
11. Fang C-H, Li B-G, Wray CJ, Hasselgren P-O. Insulin-Like Growth Factor-I inhibits Lysosomal and Proteasome-Dependent proteolysis in skeletal muscle after burn injury. *J Burn Care Rehabil.* 2002;23:318-325.
12. Pedersen BK, Rohde T, Ostrowski K. Recovery of the immune system after exercise. *Acta Physiol Scand.* 1998;162:325-332.
13. Sandmand M, Bruunsgaard H, Kemp K, Andersen-Ranberg K, Pedersen AN, Skinhoj P. Is ageing associated with a shift in the balance between Type 1 and Type 2 cytokines in humans? *Clin Exp Immunol.* 2002;127:107-114.

14. Mariani ., Ravaglia G, Forti P *et al.* Vitamin D, thyroid hormones and muscle mass influence natural killer (NK) innate immunity in healthy nonagenarians and centenarians. *Clin Exp Immunol.* 1999; 116: 19-27.
15. Demling RH, DeSanti L. Oxandrolone, an anabolic steroid, significantly increases the rate of weight gain in the recovery phase after major burns. *J Trauma Injury Infection & Critical Care.*1997;43:47-51.
16. Griffiths RD, Hinds CJ, Little RA. Manipulating the metabolic response to injury. *Br Med Bull Intensive Care Medicine.* 1999;55:181-195.
17. Mitch WE, Goldberg AL. Mechanisms of disease: mechanisms of muscle wasting -- the role of the Ubiquitin-Proteasome pathway. *N Engl J Med.* 1996;335:1897-1905.
18. Ferrucci L, Penninx BW, Volpato S *et al.* Change in muscle strength explains accelerated decline of physical function in older women with high Interleukin-6 serum levels. *J Am Geriatr Soc.* 2002; 50: 1947-1954.
19. Apovian CM. Nutrition and Aging. *Curr Opin Endocrinology & Diabetes.* 2000;7:231-235.
20. Bruunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. *Clin Exp Immunol.* 2003;132:24-31.
21. Moseley P. Exercise, stress, and the immune conversation. *Exercise Sport Sci Rev.* 2000;28:128-132.
22. Skidmore R, Gutierrez JA, Guerriero V, Kregel KC. HSP70 induction during exercise and heat stress in rats: role of internal temperature. *Am J Physiol.* 1995;268:R92-R97.
23. Joaquin AM, Gollapudi SP. Functional Decline in Aging and Disease: A Role for Apoptosis. *J Am Geriatr Soc.* 2001;49:1234-1240.
24. Roubenoff R. Catabolism of aging: is it an inflammatory process? *Curr Opin Clin Nutr Metab Care.* 2003;6:295-299.
25. Biolo G, Antonione R, Barazzoni R, Zanetti M, Guarnieri G. Mechanisms of altered protein turnover in chronic diseases: a review of human kinetic studies. *Curr Opin Clin Nutr Metab Care.* 2003;6:55-63.
26. Essen P, McNurlan MA, Gamrin L *et al.* Tissue protein synthesis rates in critically ill patients. *Crit Care Med.* 1998;26:92-100.

27. Huang YH, Haegerstrand A, Frostegard J. Effects of in vitro hyperthermia on proliferative responses and lymphocyte activity. *Clin Exp Immunol.* 1996;103:61-66.
28. Frankenfield DM, Cooney RN, Smith JS, Rowe W. Age-related differences in the metabolic response to injury. *J Trauma Injury Infection & Critical Care* 2000;48:49.
29. Urso, M.L., T.M.F. Manfredi, and M.A. Fiatarone, Skeletal muscle IGF-I receptor localization and quantitation following resistance training in the frail elderly. *Med Sci Sports Exercise.* 2001;33:S187
30. Fielding RA, Manfredi T, Parzick A, Fiatarone M, Evans W, Cannon JG. Eccentric exercise-induced muscle injury in humans. *Med Sci Sports Exercise* 1996;28:188.
31. Ji LL, Leeuwenburgh C, Leichtweis S, et al. Oxidative Stress and Aging: Role of Exercise and Its Influences on Antioxidant Systems. *Ann N Y Acad Sci.* 1998;854:102-117.
32. Ji LL. Exercise at old age: does it increase or alleviate oxidative stress? *Ann N Y Acad Sci.* 2001;928:236-247.
33. Ji LL. Exercise-induced modulation of antioxidant defense. *Ann N Y Acad Sci.* 2002;959:82-92.
34. Reid MB, Durham WJ. Generation of Reactive Oxygen and Nitrogen Species in Contracting Skeletal Muscle: Potential Impact on Aging. *Ann N Y Acad Sci.* 2002;959:108-116.
35. Wei Y.-H. Oxidative stress and mitochondrial DNA mutations in human aging. *Proceedings of the Society for Exp Biol Med (Maywood).* 1998;217:53-63.
36. Greiwe JS, Cheng BO, Rubin DC, Yarasheski KE, Semenkovich CF. Resistance exercise decreases skeletal muscle tumor necrosis factor [alpha] in frail elderly humans. *FASEB Journal.* 2001;15:475-482.
37. Rall LC, Roubenoff R, Cannon JG, Abad LW, Dinarello CA, Meydani SN. Effects of progressive resistance training on immune response in aging and chronic inflammation. *Med Sci Sports Exercise.* 1996;28:1356-1365.
38. Zinna EM, Yarasheski KE. Exercise treatment to counteract protein wasting of chronic diseases. *Curr Opin Clin Nutr Metab Care.* 2003;6:87-93.
39. Woods JA, Lowder TW, Keylock KT. Can Exercise Training Improve Immune Function in the Aged? *Ann N Y Acad Sci.* 2002;959:117-127.

40. Roubenoff R, McDermott A, Weiss L et al. Short-term progressive resistance training increases strength and lean body mass in adults infected with human immunodeficiency virus. *AIDS*. 1999;13:231-239.
41. Sun G, Gagnon J, Chagnon YC, Pérusse L et al. Association and linkage studies between the IGF-1 gene and body composition: the heritage family study. *Med Sci Sports Exercise*. 1998; 30:7.
42. Gleeson M, Overview: Exercise immunology. *Immunol Cell Biol*. 2000;78:483-484.
43. Woods JA, Lowder TW, Keylock KT. Exercise and cellular innate immune function. *Med Sci Sports Exercise*. 1999;31:57-66
44. Dressendorfer RH, Petersen SR, Moss Lovshin SE, Hannon JL, Lee SF, Bell GJ. Performance enhancement with maintenance of resting immune status after intensified cycle training. *Clin J Sport Med*. 2002;12:301-307.
45. Armstrong LE, VanHeest JL. The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. *Sports Med*. 2002;32:185-209.
46. Fiatarone Singh MA, Ding W, Manfredi TJ et al. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *Am J Physiol Endocrinol Metab*. 1999;277:E136-E143.
47. Fiatarone MA, O'Neill EF, Ryan ND et al. Exercise Training and Nutritional Supplementation for physical frailty in very elderly people. *N Engl J Med*. 1994;330:1769-1775.
48. Hunter SM, White M, Thompson M. Techniques to evaluate elderly human muscle function: a physiological basis. *J Geront Biol Sci*. 1998;53A:B204-B216.
49. Jonsdottir IH. Neuropeptides and their interaction with exercise and immune function. *Immunol Cell Biol*. 2000;78:562-570.
50. Krishnathasan DB, Vandervoort A. Eccentric Strength Training Prescription for Older Adults. *Topics in Geriatric Rehabilitation*. 2000;15:29-40.
51. Mackinnon LT. Overtraining effects on immunity and performance in athletes. *Immunol Cell Biol*. 2000;78:502-509.
52. Marcell TJ. Sarcopenia: causes, consequences, and preventions. *J Gerontol Med Sci*. 2003;58:911-916.
53. Yarasheski KE. Exercise, aging, and muscle protein metabolism. *J Gerontol Med Sci*. 2003;58:918-922.