Nutrition, Aging, and Chronic Low-Grade Systemic Inflammation in Relation to Osteoporosis and Sarcopenia

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Abstract

Aging is accompanied by a chronic low-grade systemic inflammation state, characterized by an increase in circulating levels in inflammatory mediators, that has been strongly implicated in the pathophysiology of common chronic diseases, including osteoporosis and fractures, sarcopenia, and disability. While a range of genetic, hormonal, environmental, and lifestyle factors have been reported to contribute to increased levels of inflammation, various dietary patterns, foods, and nutrients have also been reported to have anti-inflammatory effects, particularly in people with chronic diseases characterized by increased inflammation such as cardiovascular disease, type 2 diabetes, and cancer. With regard to musculoskeletal and functional outcomes, the findings from cross-sectional and prospective studies and randomized controlled trials on the effects of dietary/supplemental calcium, vitamin D, protein, vitamin K, omega-3 fatty acids, or their combination or food products such as dairy on markers of inflammation are mixed. Currently there is little or no evidence that these nutrients or foods attenuate circulating inflammatory cytokines in healthy middle-aged and older adults. In contrast, in people with chronic disease and/or increased inflammation, including those with osteoporosis and sarcopenia, a limited number of human intervention trials, mostly conducted over 12–16 weeks, have reported that calcium-vitamin D supplementation, high-dairy diets, and increased dietary protein, vitamin K, or omega-3 fatty acids alone or in combination with resistance training can produce modest reductions in inflammation. Whether these short-term reductions in inflammatory markers are clinically important and translate into positive effects on muscle and bone health and function or reduced disability remains unknown. Further randomized controlled trials in older

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adults and the elderly with or at increased risk of chronic disease are needed to evaluate the long-term efficacy of different nutrients or combination of nutrients and dietary interventions on markers of inflammation and their relation to musculoskeletal health outcomes.

Keywords
Inflammation • Osteoporosis • Fracture • Sarcopenia • Calcium • Vitamin D • Protein • Vitamin K • Omega-3 fatty acids • Aging • Cytokines

Introduction

It is well established that inflammation is part of the normal immune response to injury or infection. This rapid and acute process typically lasts 2–3 days and is tightly regulated to promote healing and restore homeostasis at damaged or infected sites. However, it is now recognized that aging per se is associated with changes in, and a dysregulation of, the immune system (termed *immunosenscence*), including its inflammatory components [1, 2]. Indeed, there is considerable evidence that aging is associated with a persistent increase in proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 (IL-6), IL-one-beta (IL-1β), and acute phase reactants [C-reactive protein (CRP)], and a reduction in anti-inflammatory cytokines, particularly IL-10 [3–6]. This age-related systemic, chronic, but low-grade inflammation has been termed *inflammaging* [7] and is typically characterized by a two- to fourfold increase in circulating inflammatory mediators (cytokines and acute phase proteins) in older people [8]. Although this inflammatory response is just one aspect of the multiple of changes occurring in the immune system with aging, there is consistent evidence that this chronic low-grade systemic inflammation contributes to the pathophysiology of many common chronic diseases, including cardiovascular disease, diabetes, arthritis, cancer, dementia, Alzheimer's disease, metabolic syndrome, as well as osteoporosis, sarcopenia, frailty, disability, and mortality [2, 9]. While many factors have been reported to contribute to *inflammaging*, including genetic, hormonal, lifestyle, and environmental factors, as well as the natural aging process itself, there has been considerable interest in identifying both pharmacological and non-pharmacological strategies to combat inflammation given its link to many common chronic diseases.

Regular physical activity and exercise is recognized as one modifiable safe and effective non-pharmacological approach to reduce systemic inflammation in older people [10, 11]. There is also a growing body of evidence supporting an anti-inflammatory effect of various diets, nutrient, and micronutrients, including calcium, vitamin D, protein, vitamin K, and omega-3 fatty acids, all of which can play an important role in optimizing bone and/or muscle health. The aim of this chapter is to provide an overview of the current evidence on the role of nutritional strategies for targeting inflammation and their putative effects on slowing bone loss or preventing osteoporosis and related fractures and age-related changes in muscle mass and function in older people.

Origins of Chronic, Low-Grade Systemic Inflammation

At present the underlying mechanism(s) contributing to the age-related "proinflammatory" state remains to be determined, but it is generally accepted that it has multiple origins. It has been hypothesized that it may be due to an impairment of the mechanisms that induce the inflammatory response [4] or a failure of anti-inflammatory mechanisms to neutralize inflammatory responses that are continuously triggered throughout life [1, 12]. Others have reported that a range of lifestyle and hormonal factors contribute to the age-related

increase in inflammatory markers, including smoking, obesity, inactivity, excessive alcohol intake, malnutrition, infection, stress, anxiety and depression, and decreased sex steroids [1, 2, 4, 13–15]. Oxidative stress, which represents an imbalance in oxidant and antioxidant levels, has also been reported to induce an increase in inflammatory markers [16, 17]. While it is beyond the scope of this chapter to review the role of oxidative stress on inflammation and disease, it is important to note that increases in inflammation and oxidative stress are closely linked, with the latter strongly implicated in the etiology of a range of musculoskeletal and age-related diseases [9, 17]. This chapter will focus predominantly on the effects of nutrition on common inflammatory mediators, including CRP, IL-6, TNF-α, IL-1β, and IL-10, and their link to bone and muscle health.

Since some inflammatory mediators are used as markers of disease risk, it has been suggested that the proinflammatory state in the elderly may be largely due to the presence of comorbidities [1, 4]. Currently there is still ongoing debate about whether chronic low-grade, systemic inflammation is a cause or an effect of age-related diseases and/or the aging process per se. In a cross-sectional study of 1,327 community-dwelling adults aged 20–85+ years, Ferrucci et al. [4] reported that a battery of inflammatory markers (serum IL-6, sIL-6, IL-1ra, IL-18, CRP, and fibrinogen) increased significantly with age in both men and women (Fig. 1.1, panels a and b). However, adjusting for cardiovascular risk factors and morbidity substantially reduced the effects of age of many of the markers (Fig. 1.1, panels c and d), indicating that part of the proinflammatory state in older persons is likely to be related to the presence of risk factors for disease. Despite these findings, there are reports that increased circulating inflammatory mediators are associated with the development of various diseases, including osteoporosis, sarcopenia, and reduced muscle function, independent of comorbidities [13, 15]. The following sections will provide a brief overview of the effects of low-grade systemic inflammation on age-related bone and muscle loss, osteoporosis, sarcopenia, falls, and fractures.

Chronic Low-Grade Systemic Inflammation, Sarcopenia, and Falls Risk

Sarcopenia is a term used to describe the age-related loss in muscle mass, strength, and function, all of which have been associated with an increased risk of falls, fractures, and frailty which can lead to a loss of independence and quality of life [18, 19]. While the precise mechanism(s) underlying the age-associated changes in muscle remains to be fully determined, it has been reported that chronic inflammation can exert a catabolic effect on muscle tissue. Indeed, numerous cross-sectional studies have found that elevated levels of various circulating inflammatory cytokines, including IL-6, TNF-α, and CRP, are associated with low muscle mass, size, strength, and power [20–22], reduced physical performance [21] and increased disability in older adults [23]. Several prospective studies conducted over 2–5 years have also reported that higher levels of inflammatory markers and their soluble receptors, which may be more representative of prolonged and severe inflammation, are associated with accelerated losses in muscle strength and lean mass [24–27] and a greater decline in function as measured by walking speed [28]. However, these findings are not consistent, and there is evidence that the association between higher levels of serum IL-6, CRP, and TNF-α with muscle loss is attenuated after adjusting for changes in weight [26]. This suggests that weight-associated changes (most likely fat mass) may play an important role in mediating age-related muscle loss.

Adipose tissue, particularly visceral fat, acts as an active endocrine organ that can secrete a large number of proinflammatory cytokines (termed *adipokines*) as well as attenuate the release of anti-inflammatory markers, such as adiponectin [29]. Thus, it has been hypothesized that the loss of muscle mass with age may be related to concurrent increases in visceral and intermuscular fat, mediated by increases in circulating levels of inflammatory markers and/or insulin resistance (Fig. 1.2) [30, 31]. That is, increased fat mass may result in a systemic "spillover" of inflammatory mediators to other organs/tissues and/or local

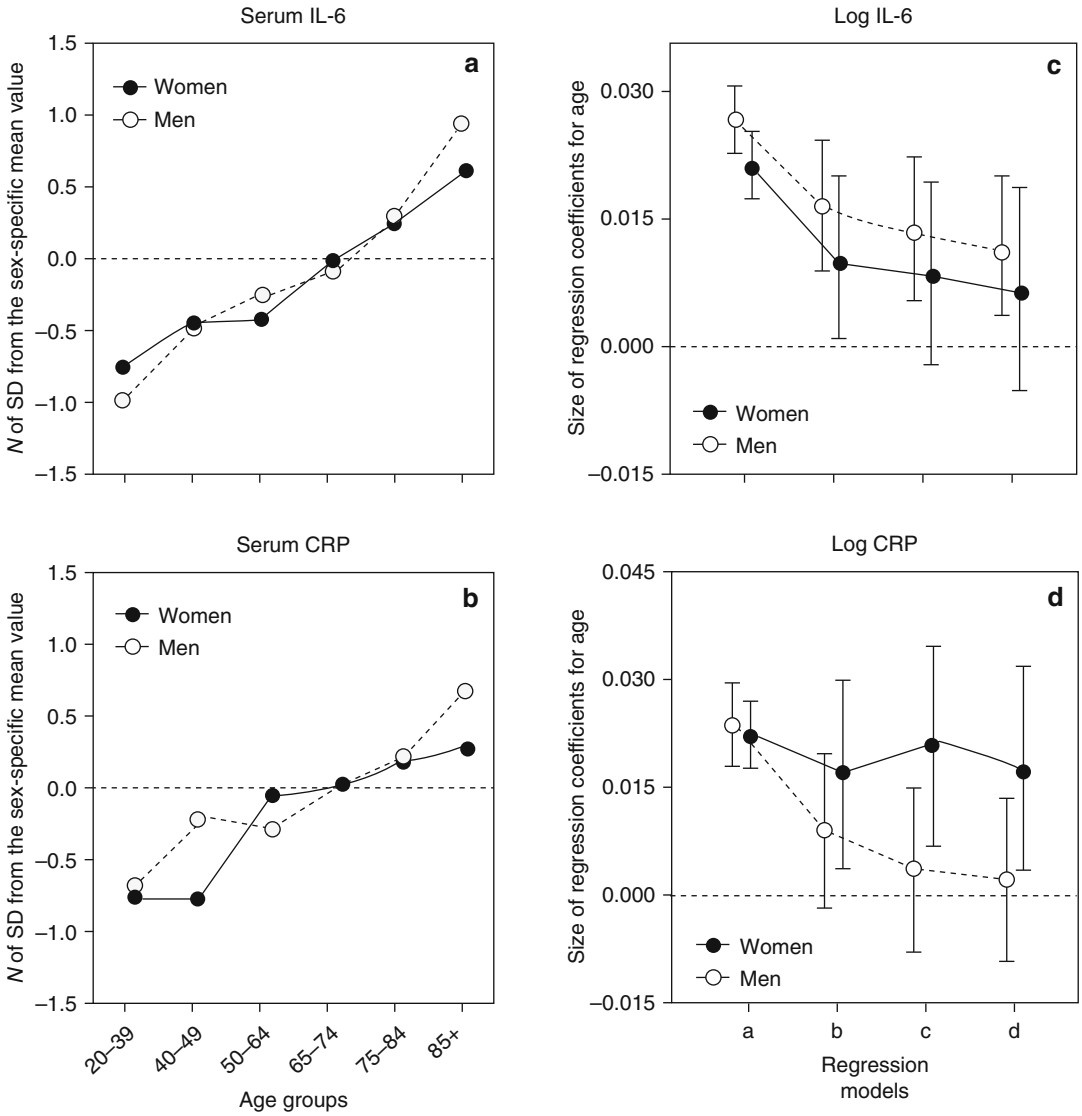

Fig. 1.1 Panels (**a**) and (**b**) show the mean serum IL-6 and CRP levels by sex and age groups expressed as number of standard deviations from the population mean. Panels (**c**) and (**d**) show the age regression coefficients and their 95 % confidence intervals estimated from linear regression models predicting level of IL-6 and CRP. Model "a" estimates the crude effect of age, model "b" is adjusted for cardiovascular risk factors, model "c" also adjusts for subclinical cardiovascular diseases, and model "d" is adjusted for major chronic diseases (CHD, CHF, stroke, PAD, COPD, diabetes, hypertension, osteoporosis, CRF, cancer, dementia, and depression) (Based on data from Ref. [4])

inflammatory reactions within the muscle itself that could have a catabolic effect by impairing muscle protein synthesis [9, 31, 32]. In addition, excess fat can also reduce the anabolic effects of insulin in stimulating muscle protein synthesis [31, 33]. Findings from the Health, Aging, and Body Composition study (Health ABC) involving 2,307 men and women aged 70–79 years showed that greater fat mass at baseline was associated with significantly greater loss in leg lean mass in both men and women over 7 years of follow-up [34]. However, the inclusion of various cytokines

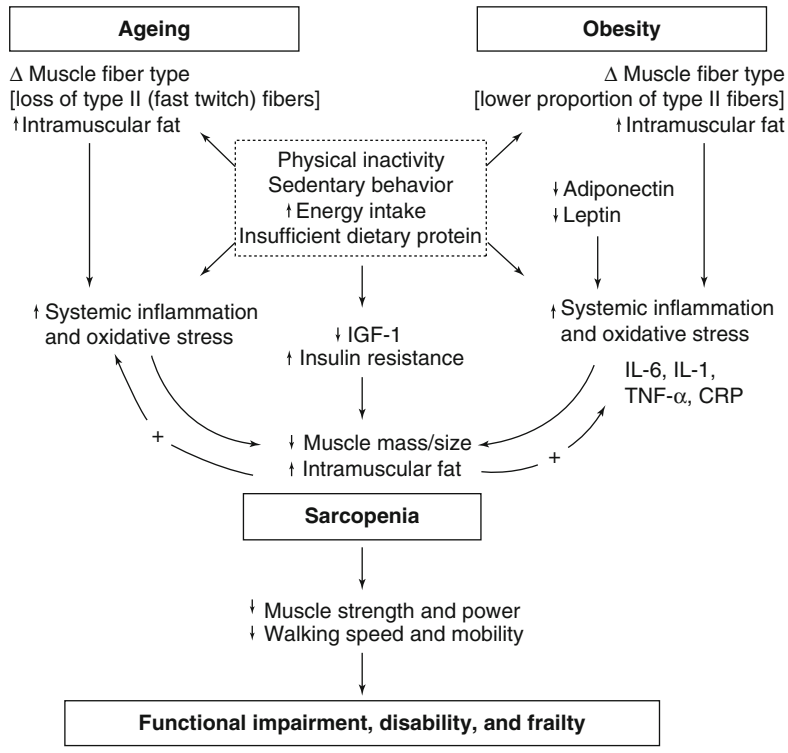

Fig. 1.2 A proposed model illustrating the key factors contributing to sarcopenia in obese people and older adults (*IGF-1* insulin-like growth factor-1, *IL-6* interleukin-6, *IL-1* interleukin-1, *TNF-α* tumor necrosis factor-α, *CRP* C-reactive protein). The symbol + denotes a positive effect (Adapted from Vincent et al. [31]. With permission from Elsevier)

or insulin resistance in the model did not change the results. This suggests that the accelerated loss of leg lean mass with greater fat mass was not related to higher levels of adipokines or insulin resistance, but further research is still needed to disentangle this interrelationship between muscle, fat, and inflammation.

It is widely recognized that multiple lifestyle, nutritional, and hormonal factors can contribute to the development of sarcopenia and its consequences, including decreased physical activity, inadequate energy intake and dietary protein, neurological changes, as well as age-related reductions in serum concentrations of sex steroids, growth factors [IGF-1], and circulating 25-hydroxyvitamin D [25(OH)D] levels [18, 19]. While it is difficult to ascertain the relative influence of each of these factors on age-related changes in muscle since they are often interrelated, a recent study examined the influence of both catabolic (serum IL-6, IL-1RA, TNF-α, CRP) and anabolic [serum insulin-like growth factor -1 (IGF-1), bioavailable testosterone, dehydroepiandrosterone sulfate (DHEA-S)] biomarkers on muscle loss after 6 years of follow-up in 716 men and women aged ≥65 years [35]. The main finding from this study was that having a greater number of elevated inflammatory (catabolic) markers was associated with a greater rate of decline in muscle strength than just having an increase in one inflammatory marker. In contrast, simultaneous reductions in several anabolic hormones were not associated with a greater strength decline than deficiency in just one marker alone [35]. Based on these findings, the authors concluded that a catabolic dysregulation is a major factor underlying age-related loss in muscle strength. However, as will be discussed in more detail below, there may be a synergy between certain anabolic and catabolic markers [20, 36]. For instance, women with low circulating IGF-1 levels and high IL-6 concentrations have been shown to be at higher risk of developing walking limitations and mobility disability than those with high IGF-1 and low IL-6 levels [36].

Chronic Low-Grade Systemic Inflammation, Osteoporosis, and Fracture Risk

Elevated circulating and local levels of proinflammatory cytokines have also been implicated in the etiology of age- and menopause-related bone loss, osteoporosis, and fractures. In vitro and experimental studies in animals have shown that various hormones and proinflammatory cytokines, including IL-6, TNF-α, IL-1, and IL-11, can interact to alter the bone remodeling balance [37–39]. More detailed information about the role of various proinflammatory and inhibitory cytokines on regulating osteoblast and osteoclast differentiation and activity is provided in several excellent reviews [37–39]. Briefly, the accelerated loss of bone associated with menopause and subsequent estrogen withdrawal has been related to an upregulation of proinflammatory cytokines by bone marrow and bone cells which can exert a catabolic effect on bone by stimulating osteoclastogenesis while simultaneously inhibiting osteoblast function through the regulation of the RANKL/RANK/OPG pathway [37–39]. It is important to highlight that these findings reflect the influence of cytokines at the local bone microenvironment, whereas most human studies measure circulating cytokine concentrations and relate these to BMD or fracture risk.

To date, the vast majority of human studies investigating the link between inflammation and bone have been cross-sectional, conducted in postmenopausal and older women and focused predominantly on the circulating cytokines CRP, IL-6, and TNF-α. While several of these studies reported that higher levels of high-sensitivity (hs)-CRP and IL-6 were associated with lower BMD in middle-aged and older adults [40, 41], others have failed to detect any significant association [42, 43] or differences in various cytokines between osteoporotic women and age-matched controls [44]. However, several recent prospective studies have reported that higher levels of circulating inflammatory cytokines were associated with increased bone loss [45–47]. In a study involving 168 healthy community-dwelling men and women aged 50–79 years who were followed for a mean of 2.9 years, Ding et al. [45] found that various inflammatory markers at baseline and their changes over time, particularly IL-6, were significantly associated with total body, lumbar spine, and hip bone loss. Others have reported that polymorphisms in cytokine genes [IL-1 receptor antagonist (IL-1ra), 174 GG polymorphism in IL-6], which alter the expression of a given cytokines, were associated with lower BMD, increased bone turnover, and fracture risk [48–50]. In addition, studies using antibodies against specific cytokines and/or their receptors or animals that do not express IL-6 provide further evidence implicating inflammation in bone loss [51–53].

Chronic, low-grade systemic inflammation has also been associated with an increased risk of osteoporotic fracture. In the Health ABC study involving 2,985 well-functioning white and black women and men aged 70–79 years, increased serum levels of inflammatory markers, particularly high receptor levels of proinflammatory cytokines, predicted a higher incident of fractures over 5.8 years of follow-up, independent of known risk factors including BMD. The finding that cytokine soluble receptors strongly predicted fracture risk is important because they may be more representative of a prolonged and severe underlying inflammatory state compared to the markers themselves which have a short half-life and often change transiently [54]. Another important finding from this study was that the risk of fracture was even greater in participants with two or more inflammatory markers in the highest quartile. This suggests that measuring several inflammatory markers may improve risk assessment compared to a single marker alone. While this study was limited to an assessment of all nontraumatic fractures, similar findings were observed in a recent nested case-control study with hip fracture as the outcome [55]. In this study, women aged 50–79 years with elevated levels (highest quartile) of inflammatory markers for all three cytokine soluble receptors (IL-6 SR, TNF SR1, and TNF SR2) had a 2.4- to 2.8-fold increased risk of incident hip fracture over a median of 7.1 years of follow-up compared to women with zero or one high inflammatory marker

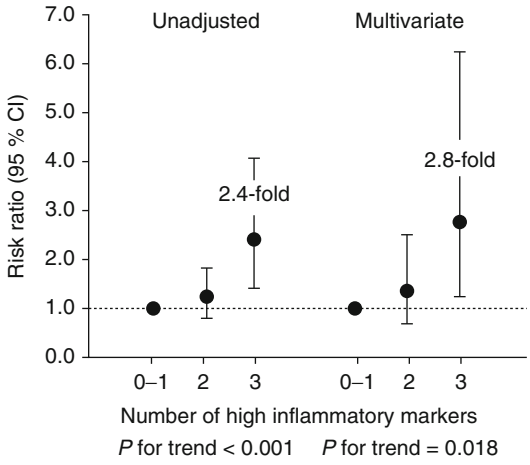

Fig. 1.3 Risk ratios with 95 % confidence intervals (CI) of hip fracture, according to the number of high inflammatory markers in the top quartile based on the distribution of cytokine soluble receptor concentrations among the controls (Based on data from Ref. [55])

(Fig. 1.3). Together, these findings highlight that identifying strategies to reduce inflammation may represent an important approach to reduce bone loss and fracture risk in the elderly.

Nutritional Strategies to Combat Inflammation and Musculoskeletal Disease

Exercise, weight loss, and caloric restriction are recognized to play an important role in reducing chronic systemic inflammation, but maintaining a healthy diet has also been associated with lower circulating concentrations of inflammatory markers. As discussed in a comprehensive review on the influence of dietary factors on low-grade inflammation [1], there is sound evidence to support an anti-inflammatory role of healthy eating patterns, such as the Mediterranean diet and vegetarian diets, and specific dietary factors, including whole grains, fruit and vegetables, fish and omega-3 fatty acids, and certain vitamins. From a disease-specific perspective, the vast majority of research into the role of diet on inflammation has been conducted in people with cardiovascular disease, type 2 diabetes, and cancer, all of which are characterized by increased levels of inflammation. This chapter will provide an overview of the evidence on the influence of nutrients specific to musculoskeletal health, such as calcium and dairy foods, vitamin D, protein, vitamin K, omega-3 fatty acids, or a combination of these factors, on inflammatory markers and their association to bone and muscle health.

Calcium and Dairy Foods

It has been reported that an increased intake of dietary calcium or a high-dairy diet may reduce inflammation (and oxidative stress), particularly in overweight and obese people, by promoting lipid metabolism and a loss of body fat [56–58]. Laboratory studies in rodents have shown that dietary calcium can inhibit lipogenesis and induce lipolysis by reducing 1,25-dihydroxyvitamin D-induced calcium signaling in adipocytes [59, 60]. While others contend that the effects of dietary calcium on lipolysis and/or lipogenesis are equivocal, there is consistent evidence that dietary calcium can promote modest weight loss through increased fecal-fat excretion [61]. Since obesity and related disorders are associated with increased inflammation, it has been proposed that calcium may play a key role in modulating adipose tissue cytokine production [56, 62]. This is supported by research in mice which has shown that high-calcium diets can decrease the expression of proinflammatory factors, such as TNF-α and IL-6, in visceral fat, adipocytes, and plasma and stimulate the expression of the anti-inflammatory marker IL-15 as well as adiponectin [56, 62]. In the same rodent model, it was also reported that a high-dairy diet (nonfat dry milk) may be more effective for reducing inflammation and oxidative stress than supplements (calcium carbonate) [62]. Together, these findings provide evidence that increased calcium or a high-dairy diet can modulate adipocyte cytokine production in a mouse model of obesity, but less is known about the influence of dietary and supplemental calcium or a high-dairy diet on inflammation in humans.

It is well known that the beneficial effects of calcium supplementation on maintaining or slowing bone loss and preventing osteoporotic fractures

are associated with suppression of parathyroid hormone (PTH). Since PTH can regulate circulating levels of IL-6 and TNF-α, which stimulate production of CRP [63], it has been suggested that the benefits of calcium supplements on bone could be mediated in part by a reduction in inflammation. To our knowledge, only one study has investigated the anti-inflammatory effects of calcium supplementation alone without other therapies. In a 12-month randomized controlled trial in healthy postmenopausal women that were part of a larger study of calcium supplementation on fracture incidence, Grey et al. [64] reported that supplementation with 1,000 mg/day of calcium citrate had no effect on CRP levels. Several intervention trials which have evaluated the effects of calcium plus vitamin D supplementation on inflammation in relatively healthy older adults also observed no improvements in inflammatory markers. For example, Gannage-Yared et al. [65] observed no effect of calcium (1,000 mg/day) plus vitamin D (800 IU/day) supplementation on circulating IL-6, TNF-α, or CRP concentrations in 47 healthy postmenopausal women in a 12-week randomized controlled trial. Similarly, secondary analysis of a 3-year randomized, double-blinded, placebo-controlled trial in adults aged ≥65 years showed that calcium (500 mg/day) plus vitamin D (700 IU/day) supplementation had no effect on either circulating CRP or IL-6 levels, despite beneficial effects on BMD [66]. In contrast, the findings from a recent pilot, randomized, double-blind, placebo-controlled, 2×2 factorial clinical trial in a high-risk patient group [colorectal adenoma patients (n=92)] showed that supplementation with calcium (2,000 mg/day), vitamin D (800 IU/day), or the combination for 6 months led to a 8–50 % nonsignificant reduction in various proinflammatory markers compared to placebo controls [67]. While the lack of a statistically significant effect is likely due to the small sample size, when they examined the effects of calcium and/or vitamin D on a combined inflammatory marker z-score that included all six measured cytokines, they found that the z-score decreased by 77 % in the vitamin D-only group (P=0.003); the change in the calcium (48 %, P=0.18) or combined group (33 %, P=0.40) was not significant. These preliminary findings provide some evidence that calcium and/or vitamin D supplementation may help to ameliorate inflammation in "high-risk" individuals with diseases known to have an inflammatory pathogenesis, but further intervention trials with adequate sample sizes are needed to test this hypothesis.

Since dairy foods contain additional factors that may have anti-inflammatory properties, such as angiotensin-converting enzyme inhibitors, vitamin D, protein, and related bioactive peptides, a number of studies have investigated the acute and long-term effects of a high-dairy diet on inflammation (and oxidative stress). In a retrospective analysis of archival samples from two clinical trials, Zemel and Sun [62] reported that high-dairy (~1,100–1,200 mg/day of calcium) compared to low-dairy (~400–500 mg/day of calcium) diets were effective in suppressing CRP levels and increasing adiponectin during weight loss and maintenance in obese adults. However, these findings must be interpreted with caution because of the concomitant reductions in adiposity. In a subsequent acute (28-day) blinded, randomized, crossover trial comparing a high-dairy (three daily serves, calcium 1,200–1,400 mg/day) versus soy-based placebo diet (calcium 500–600 mg/day) in overweight and mildly obese adults, circulating TNF-α and IL-6 levels decreased (as well as markers of oxidative stress) and adiponectin increased in the high-dairy group; the opposite effect was observed in the soy-protein group [58]. Given the crossover design and the lack of any changes in body composition in this study, these findings provide some confirmation that an increase in dairy food intake, even over a short period, may represent an effective strategy to reduce inflammation (and oxidative stress) in overweight and obese adults. Consistent with these findings, the results from a 12-week randomized controlled trial comparing an adequate-dairy (~3.5 daily serves) versus low-dairy (<0.5 daily servings) weight maintenance diet showed that the adequate-dairy diet significantly attenuated markers of inflammatory and oxidative stress in adults with metabolic syndrome [68]. Similarly, 12-weeks of supplementation with a vitamin D or calcium-vitamin D-enriched yoghurt drink reduced various

inflammatory markers (IL-1β, IL-6, fibrinogen, hs-CRP) and increased adiponectin in adults aged 30–60 years with type 2 diabetes [69]. In contrast, a 6-month trial in middle-aged and older adults with metabolic syndrome reported no effect of increased dairy intake (3–5 portions of dairy products daily) on markers of inflammation, adiponectin, or oxidative stress [70]. However, in this study the baseline calcium intake in the dairy group was 815 mg/day, which may have been too high to observe any effects from the additional dairy products. Taken together, these findings support previous work indicating that high-calcium or high-dairy diets may be most effective for attenuating inflammation in individuals with an inflammatory-related chronic disease(s).

To date, few studies have examined the anti-inflammatory effects of a high-dairy or calcium-vitamin D-enriched diet in relatively healthy older adults. In an 18-month, factorial 2 × 2 design randomized controlled trial in healthy middle-aged and elderly men which was designed to examine the independent and combined effects of exercise and calcium-vitamin D-fortified milk on bone and muscle health, we previously reported that serum IL-6 concentrations tended to increase in men who received low-fat, calcium (1,000 mg/day)-vitamin D_3 (800 IU/day)-fortified milk and decrease in those assigned to the exercise training [71]. However, these between-group differences did not persist after adjusting for changes in fat mass; there was no effect of the fortified milk or exercise on serum TNF-α or CRP. While the lack of an effect in this study may be explained in part by the high baseline calcium (~900 mg) and serum 25(OH)D levels (~85 nmol/L) in the men, the finding that changes in circulating IL-6 concentrations were largely dependent on changes in fat mass provides further evidence that calcium and/or vitamin D may be most effective for reducing inflammation in overweight or obese individuals or those with other chronic diseases.

Vitamin D

Vitamin D is recognized to have immunomodulatory effects with 1,25-dihydroxyvitamin D_3, the biological active form of vitamin D, shown to influence the differentiation and function of both innate and adaptive immune cell types and to modulate cytokine production (for a recent review refer to Hewison [72]). While the precise mechanism(s) by which vitamin D might attenuate inflammation is not clear, in vitro data suggests that the anti-inflammatory effects of vitamin D may be mediated by 1,25-dihydroxyvitamin D_3 coupling to the vitamin D receptor, which is present in many immune cells, to downregulate or transrepress inflammatory cytokines; various immune cells also have the capacity to regulate the activity of 1-α-hydroxylase, which converts 25(OH)D to 1,25-dihydroxyvitamin D_3 [69, 73]. Data from human epidemiological studies also supports a relationship between vitamin D status and various autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes [72]. However, the findings from observational and epidemiological studies examining the association between serum 25(OH)D concentrations and various inflammatory cytokines in asymptomatic adults or those with common chronic disease(s) are mixed [74–78].

In relatively healthy adults, it has been proposed that any association between vitamin D and inflammation may only exist at low serum 25(OH)D levels. To test this hypothesis, Amer and Qayyum [74] examined the relationship between circulating 25(OH)D and CRP concentrations in 15,167 asymptomatic adults aged ≥18 years involved in the NHANES survey from 2001 to 2006. Using linear spline regression analysis, with a single knot at the median serum 25(OH)D concentration of 21 ng/mL (52.5 nmol/L), they found that there was an inverse relation between serum 25(OH)D at levels <52.5 nmol/L and CRP in both the univariate and multivariate analyses adjusting for traditional cardiovascular risk factors. However, in the multivariate analysis, they also observed a positive relationship between CRP and 25(OH)D at levels above 52.5 nmol/L, suggesting that higher concentrations of 25(OH)D may be proinflammatory. While it is difficult to explain this latter finding, a limitation of this study is that only a single inflammatory marker was assessed and there was no adjustment for

geographic location or time of year. Furthermore, since this was a cross-sectional study, causality cannot be inferred, and thus randomized controlled trials are needed to determine the effects of vitamin D on inflammation.

To date, randomized controlled trials examining the effects of vitamin D supplementation on markers of low-grade inflammation in both healthy young and older adults and those with various chronic diseases have produced inconsistent findings. Most of the studies which have reported a positive effect of vitamin D supplementation on pro- and/or anti-inflammatory markers have been conducted in people with chronic disease, including chronic heart failure [79], chronic kidney disease [80], type 2 diabetes [81], multiple sclerosis [82], those with prolonged critical illness [83], and osteoporosis [84]. In the study in postmenopausal women ($n = 70$) with osteoporosis, 6 months of supplementation with 0.5 μg/day of calcitriol and 1,000 mg/day of calcium significantly increased lumbar spine, trochanteric, and intertrochanteric BMD and reduced serum IL-1 and TNF-α levels; there were no changes in the calcium-alone group [84]. It is difficult to compare these findings with other trials since the active vitamin D metabolite (calcitriol) was used. However, in the study by Pittas et al. [66] reported earlier, combined calcium (500 mg/day) plus cholecalciferol (700 IU/day) supplementation for 3 years had no effect on circulating CRP or IL-6 levels in subjects with normal or impaired fasting glucose, despite improvements in BMD. Based on these limited findings, it is difficult to make any conclusion as to whether the beneficial effects of vitamin D (or calcium plus vitamin D) on bone health and fractures are mediated, at least in part, by their effects on inflammation.

In recent years a number of intervention trials have investigated the effects of different doses of vitamin D on inflammatory markers in healthy adults and those who are overweight or obese. In a 6-month weight loss trial in healthy overweight adults with low 25(OH)D concentrations (mean 30 nmol/L), supplementation with 83 μg/day (3,332 IU/day) of cholecalciferol resulted in a more pronounced reduction in serum TNF-α, but not IL-6 or CRP, compared to weight loss alone [85]. In contrast, supplementation with 4,000 IU/day for 12 weeks in healthy overweight and obese adults with serum 25(OH)D levels around 50 nmol/L participating in a resistance training program had no effect on circulating inflammatory markers [86]. Similarly, incremental doses of 5, 10, and 15 μg/day of vitamin D (200, 400, and 600 IU/day) for 22 weeks throughout winter did not alter cytokine concentrations in either healthy young (aged 20–40 years) or free-living older adults (aged ≥64years) [87]. There are several likely explanations for this finding: (1) the baseline median 25(OH)D concentrations were 71 and 55 nmol/L in the young and older adults, respectively, which many consider to be sufficient; (2) the vitamin D dosing regimen did not improve serum 25(OH)D levels in the young adults, and the 400–600 IU doses only increased levels to ~70 nmol/L in the older adults; and (3) the baseline cytokine levels were already very low prior to the intervention, providing little scope for improvement.

It has been suggested that serum 25(OH)D concentrations as high as 80–100 nmol/L may be required for optimal immune function [88]. Thus, a recent study investigated the effects of high-dose vitamin D supplementation (40,000 or 20,000 IU of vitamin D_3 per week or a placebo) on markers of inflammation in 437 healthy overweight adults aged 21–70 years with a mean serum 25(OH)D concentration of 56 nmol/L [76]. Despite marked increases in serum 25(OH)D levels in the supplemented groups [median 25(OH)D levels at follow-up were 141 and 98 nmol/L for the 40,000 and 20,000 IU dose groups, respectively], there were no between-group differences for the change in a panel of 11 inflammatory cytokines after 12 months. These findings are consistent with previous research suggesting that the immunomodulatory effects of vitamin D (and other nutrients) may only be seen when the immune system is stimulated, as is evident in people with a chronic disease(s), and/or when circulating 25(OH)D levels are insufficient. Since many questions still remain as to the optimal level of serum 25(OH)D needed for health benefits, further long-term clinical trials should

also investigate if there is a concentration of 25(OH)D and dose of vitamin D that might be effective for improving immune function.

Protein

Adequate dietary protein is considered to be essential for the maintenance of both bone and muscle health, which has been largely attributed to the positive effect of protein on IGF-1 levels [89]. However, it has also been suggested that increased dietary protein may have an indirect effect on muscle and bone via a reduction in inflammation mediated by an increase in IGF-1; high levels of IL-6 decrease circulating levels of IGF-1, and low levels of IGF-1 stimulate IL-6, indicating that IL-6 may oppose the effect of IGF-1 on muscle and bone [20, 90]. There is also evidence that TNF-α can interfere with IGF-1 signaling and inhibit the signaling pathways downstream of the IGF-1 receptor and thus decrease muscle protein synthesis [91].

The loss of muscle with advancing age results from an imbalance between muscle protein synthesis and muscle protein breakdown. In older adults and the elderly, it has been reported that the stimulatory effect of an anabolic stimuli such as protein (amino acids) on muscle protein synthesis is blunted, an effect that has been coined *anabolic resistance* [92, 93]. To stimulate muscle protein synthesis and promote a positive net protein balance to optimize muscle health, it has been suggested that older adults require a higher daily dietary protein intake, particularly when undertaking progressive resistance training [92, 93]. A comprehensive review on the type, dose, and timing of protein needed to enhance muscle protein synthesis and increase or maintain muscle mass in the elderly is provided in several recent reviews [92, 93]. While the precise cause(s) of anabolic resistance in aging muscle remains unknown, studies in both rodents and humans have reported that an increase in proinflammatory markers is associated with a decrease in muscle protein synthesis [94, 95]. By blocking low-grade inflammation using a nonsteroidal anti-inflammatory drug (ibuprofen), Rieu et al. [96] found that the anabolic effects of food intake on muscle protein metabolism were maintained in older rats resulting in a decrease in muscle mass loss. Together, these findings support the results from several human prospective studies discussed earlier which showed that higher levels of inflammation were associated with accelerated losses in muscle mass and strength in older adults [24–28].

To our knowledge, few human studies have investigated the interactive effects of IGF-1 and inflammation on muscle or bone health. Data from a population-based study involving 526 adults aged 20–102 years found that there was a reciprocal relationship between IGF-1 and IL-6 on muscle strength and power [20]. In the analysis stratified according to tertiles of IL-6, serum IGF-1 was positively related to both muscle strength and power only in those in the lowest IL-6 tertile. Cappola et al. [36] also reported that a combination of low IGF-1 and high IL-6 levels was associated with an increased risk for incident walking limitation, mobility disability, disability in activities of daily living, and death in older women. To examine whether a synergistic relationship exists between dietary protein, inflammation, and changes in muscle strength in the elderly, Bartali et al. [97] followed 598 men and women aged 65 years and over for 3 years. Interestingly, dietary protein intake at baseline was not associated with changes in muscle strength, but there was a significant interaction between protein intake and serum CRP, IL-6, and TNF-α on changes in muscle strength. That is, in those with high levels of inflammatory markers, a lower protein intake was associated with a greater decline in muscle strength, independent of the presence of chronic conditions. These findings indicate that chronic inflammation can alter protein metabolism and may reduce the efficiency of protein on muscle, but this needs to be confirmed in a clinical trial.

There are few intervention studies which have examined whether a high-protein diet or protein supplementation can reduce inflammation, either directly or indirectly via an increase in IGF-1, and whether any subsequent changes in cytokine concentrations are related to changes in muscle mass,

strength, and/or function. A recent systematic review and meta-analysis on the effects of higher- versus lower-protein diets on a range of health outcomes reported no significant effect of higher-protein diets on circulating CRP levels [98]. However, the results from an 18-month randomized, open-label, crossover study in 41 elderly outpatients with sarcopenia found that nutritional supplementation with amino acids (8 g of essential amino acids twice daily) resulted in significant gains in lean mass with a parallel increase in IGF-1 and a reduction in TNF-α compared to those assigned to the placebo control group [99]. In a recent, as yet unpublished study, we found that daily consumption of lean red meat for 4 months, equivalent to a protein intake of ~1.3 g/kg/day, with progressive resistance training (PRT) led to a greater reduction in the proinflammatory marker IL-6 and significantly greater increases in serum IGF-1, lean mass, and muscle strength compared to elderly women assigned to PRT alone (Daly R unpublished observation). Similarly, in overweight and obese premenopausal women involved in a 16-week trial, consumption of diets higher in protein with an emphasis on dairy foods during a diet- and exercise-induced weight loss program improved markers of bone turnover as well as adipokine levels (adiponectin and leptin), serum osteoprotegerin (OPG), and RANKL, important regulators of bone formation/resorption that are influenced of cytokines, compared to those assigned to a low-dairy diet [100]. While there is currently inconclusive evidence with regard to the effects of dietary protein on bone health and fracture risk [101], the above findings provide some evidence that diets higher in protein, dairy foods, and dietary calcium, when combined with exercise, represent an effective approach to reduce inflammation via an increase in IGF-1 levels and thereby enhance both muscle and bone health.

Others have suggested that soy protein and other dairy proteins and peptides may also have anti-inflammatory properties [1]. However, a review on the effects of soy foods and soy isoflavones on inflammation found that there was no consistent evidence for an effect on the cytokines IL-6 and TNF-α [102]. Similarly, the findings from several human trials have shown that specific milk/dairy proteins (whey or casein protein or milk peptides) have no effect on inflammatory markers in overweight adults [103], mildly hypertensive people [104], or postmenopausal women [105]. Whether other specific branched chained amino acids, particularly leucine, which has a strong stimulatory effect on muscle protein synthesis, have anti-inflammatory properties and can modulate skeletal muscle health remains to be determined [106].

Vitamin K

Vitamin K has been strongly implicated in bone health due to its function as a cofactor in the post-translational γ-carboxylation of several vitamin K-dependent proteins, including osteocalcin, which plays an important role in bone mineralization [107]. While it is beyond the scope of this chapter to provide a comprehensive review on the effects of vitamin K supplementation on BMD and fracture risk, the findings from several recent reviews and meta-analyses of clinical trials indicate that supplementation with either form of vitamin K (phylloquinone, vitamin K_1; menaquinone, vitamin K_2), particularly at higher doses (phylloquinone >1,000 μg/day or menaquinone >45 mg/day), in combination with calcium and vitamin D, can improve indices of hip bone strength and protect against fractures [108, 109]. Interestingly however, the effects of vitamin K treatment on BMD are equivocal [107, 110], which has been attributed to the fact that there is considerable between study heterogeneity and publication bias in the studies that have been conducted [110].

If vitamin K does play a role in reducing fracture risk, many questions still remain as to the underlying mechanism(s) of action. One theory is that vitamin K may reduce circulating concentrations of proinflammatory cytokines. This is supported by data from in vitro studies which have shown that treatment with vitamin K was associated with a decrease in proinflammatory markers [111, 112]. Similarly, the findings from several human cross-sectional studies in middle-aged and older adults have shown that plasma phylloquinone

concentrations, which represent a marker of vitamin K status, and/or dietary phylloquinone intake were inversely associated with various inflammatory markers, including CRP and IL-6 [78, 113]. Unfortunately, a follow-up intervention study examining the effects of 3 years of supplementation with vitamin K (500 μg phylloquinone), together with additional calcium and vitamin D, failed to detect any significant effect on circulating levels of IL-6 or CRP [113] or BMD [114] in healthy older men and women. It is possible that the lack of a cytokine response in this study might be related to the supplemental dose (500 μg) of phylloquinone used [113]. In a previous trial, 1,000 μg/day of phylloquinone was found to be effective for slowing bone loss in postmenopausal women [115]. Nevertheless, the above findings are consistent with the results from calcium and/or vitamin D supplementation trials which generally observed no effects on inflammation in healthy adults.

Omega-3 Fatty Acids

It has been suggested that diets high in omega-3 ($n-3$) fatty acids or with a lower omega-6 ($n-6$) to $n-3$ ratio may have beneficial effects on both bone and muscle health, which might be mediated, at least in part, by a decrease in proinflammatory cytokines [116–119]. There is considerable evidence to support an anti-inflammatory effect of a high dietary intake of omega-3 fatty acids, particularly in those with chronic disease (for a review refer to Calder et al. [1]) However, the findings from human studies on the effects of omega-3 fatty acids or the $n-6$ to $n-3$ ratio ($n-6$ tends to increase inflammation) on BMD and fracture risk are mixed, with some studies reporting a protective effect of omega-3 fatty acids against bone loss [116, 120] and fractures [121], while others have observed no significant effect or even an increased fracture risk [117, 122, 123]. This is supported by the findings from a systematic review which found that only one of four randomized controlled trials with BMD as the primary outcomes showed significant improvements or maintenance of BMD following 18 months of omega-3 supplementation [primrose oil (high in linoleic acid) and fish oil] with calcium versus a placebo control of coconut oil and calcium [117]. Interestingly, this study was conducted in elderly women with osteopenia or osteoporosis, a group which may have chronic systemic inflammation, whereas the others trials which observed no effect involved healthy pre- and/or postmenopausal women or healthy older men. In a recent systemic review, Rangel-Huerta et al. [118] reported that omega-3 fatty acid supplementation was associated with lower inflammation in patients with acute and chronic diseases, but not in healthy subjects. While there is currently no consensus on the type or dose required to exert an anti-inflammatory effect and/or improve bone health [118], it is possible that any beneficial effect of omega-3 fatty acids on bone might be limited to those with osteoporosis, inflammatory disease(s), or chronic low-grade systemic inflammation. However, this needs to be confirmed in future intervention trials.

In recent years there has been some interest in the role of omega-3 fatty acids and fish oil supplementation alone or in combination with progressive resistance training as a strategy to treat and prevent sarcopenia. Data from an epidemiological study in adults aged 59–73 years revealed that fatty fish consumption was a strong positive predictor of grip strength [124]. In elderly women, combining fish oil supplementation with progressive resistance training led to greater increases in muscle strength and function than resistance training alone [125]. While no studies appear to have examined the association between omega-3 fatty acids and muscle mass, the findings from an 8-week trial in older adults revealed that omega-3 supplementation enhanced the rate of muscle protein synthesis in response to amino acid feeding; there was no effect on basal muscle protein synthesis rate [126]. This suggests that omega-3 fatty acids may attenuate the "anabolic resistance" commonly seen in the elderly, that is, enhance the sensitivity of muscle to anabolic stimuli such as amino acids. Importantly, they also found that increased omega-3 fatty acid intake was not associated with a decrease in inflammatory cytokines but did result in an increase in muscle anabolic signaling activity. While this suggests that omega-3 fatty acids may

have a positive effect on muscle via a mechanism independent of their anti-inflammatory effects, it is important to note that the participants in this study were healthy older adults with low circulating inflammatory markers. Again, further long-term trials are warranted in the elderly and at risk groups which should include measures of lower limb muscle mass or size.

Conclusion

Aging is accompanied by a chronic low-grade systemic inflammation state (termed *inflammaging*), characterized by higher circulating levels in inflammatory mediators, that has been strongly implicated in the pathophysiology of many chronic diseases, including osteoporosis and fractures, sarcopenia, and disability. While a wide range of factors have been reported to contribute to *inflammaging*, including genetic, hormonal, environmental, and lifestyle factors, various dietary patterns, foods, and nutrients have been reported to have anti-inflammatory effects, particularly in people with chronic diseases characterized by increased inflammation such as cardiovascular disease, type 2 diabetes, and cancer. With regard to musculoskeletal health and function, there are conflicting findings from cross-sectional and prospective studies and randomized controlled trials on the effects of nutrients such as dietary calcium, vitamin D, protein, vitamin K, omega-3 fatty acids, or their combination or food products such as dairy, on markers of inflammation. In healthy middle-aged and older adults, there is little or no evidence that these nutrients or foods attenuate circulatory inflammatory cytokines. In contrast, there is evidence from a limited number of human intervention trials, mostly conducted over 12–16 weeks, indicating that calcium-vitamin D supplementation, high-dairy diets, and increased dietary protein, vitamin K, or omega-3 fatty acids alone or in combination with exercise (resistance training) can produce modest reductions in inflammation in high-risk groups with chronic disease and/or increased inflammation, including people with osteoporosis and sarcopenia. Whether these short-term reductions in inflammatory markers are clinically important and translate into positive effects on muscle and bone health, improved muscle function, or reduced disability remains unknown. Further randomized controlled trials in older adults and the elderly with or at increased risk of chronic disease are needed to evaluate the long-term efficacy of different nutrients or combination of nutrients and dietary interventions on markers of inflammation and their relation to musculoskeletal health outcomes.

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