The Journal of Nutrition
Critical Review

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

Dimitrios Draganidis,[3] Leonidas G Karagounis,[3,6] Ioannis Athanailidis,[4] Athanasios Chatzinikolaou,[4] Athanasios Z Jamurtas,[3,5] and Ioannis G Fatouros[3]*

[3]School of Physical Education and Sports Science, University of Thessaly, Trikala, Greece; [4]School of Physical Education and Sports Science, Democritus University of Thrace, Komotini, Greece; [5]Institute of Human Performance and Rehabilitation, Centre for Research and Technology—Thessaly, Trikala, Greece; and [6]Department of Nutrition and Health Research, Nestle Research Centre, Lausanne, Switzerland

Abstract

Inflammaging is the chronic low-grade inflammatory state present in the elderly, characterized by increased systemic concentrations of proinflammatory cytokines. It has been shown that inflammaging increases the risk of pathologic conditions and age-related diseases, and that it also has been associated with increased skeletal muscle wasting, strength loss, and functional impairments. Experimental evidence suggests that the increased concentrations of proinflammatory cytokines and primary tumor necrosis factor α observed in chronic inflammation lead to protein degradation through proteasome activation and reduced skeletal muscle protein synthesis (MPS) via protein kinase B/Akt downregulation. Dairy and soy proteins contain all the essential amino acids, demonstrate sufficient absorption kinetics, and include other bioactive peptides that may offer nutritional benefits, in addition to those of stimulating MPS. Whey protein has antioxidative effects, primarily because of its ability to enhance the availability of reduced glutathione and the activity of the endogenous antioxidative enzyme system. Soy protein and isoflavone-enriched soy protein, meanwhile, may counteract chronic inflammation through regulation of the nuclear transcription factor κB signaling pathway and cytokine production. Although evidence suggests that whey protein, soy protein, and isoflavone-enriched soy proteins may be promising nutritional interventions against the oxidative stress and chronic inflammation present in pathologic conditions and aging (inflammaging), there is a lack of information about the anabolic potential of dietary protein intake and protein supplementation in elderly people with increased systemic inflammation. The antioxidative and anti-inflammatory effects, as well as the anabolic potential of protein supplementation, should be further investigated in the future with well-designed clinical trials focusing on inflammaging and its associated skeletal muscle loss. *J Nutr* 2016;146:1940–52.

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

Introduction

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

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

[1] The authors report no funding received for this study.
[2] Author disclosures: D Draganidis, LG Karagounis, I Athanailidis, A Chatzinikolaou, AZ Jamurtas, and IG Fatouros, no conflicts of interest.
*To whom correspondence should be addressed. E-mail: ifatouros@pe.uth.gr.
[7] Abbreviations used: Bax, BCL2 associated X, apoptosis regulator; Bcl-2, B-cell lymphoma 2; CRP, C-reactive protein; IκB, inhibitor of NF-κB; MPS, muscle protein synthesis; ROS, reactive oxygen species; SOD, superoxide dismutase; SPI, soy protein isolate; TAC, total antioxidant capacity; UPS, ubiquitin–proteasome system; VCAM-1, vascular cell adhesion molecule 1; WPH, whey protein hydrolysate.

Manuscript received February 4, 2016. Initial review completed February 23, 2016. Revision accepted July 18, 2016.
First published online August 31, 2016; doi:10.3945/jn.116.230912.

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

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

Inflammaging and Its Association with Frailty

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

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

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

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

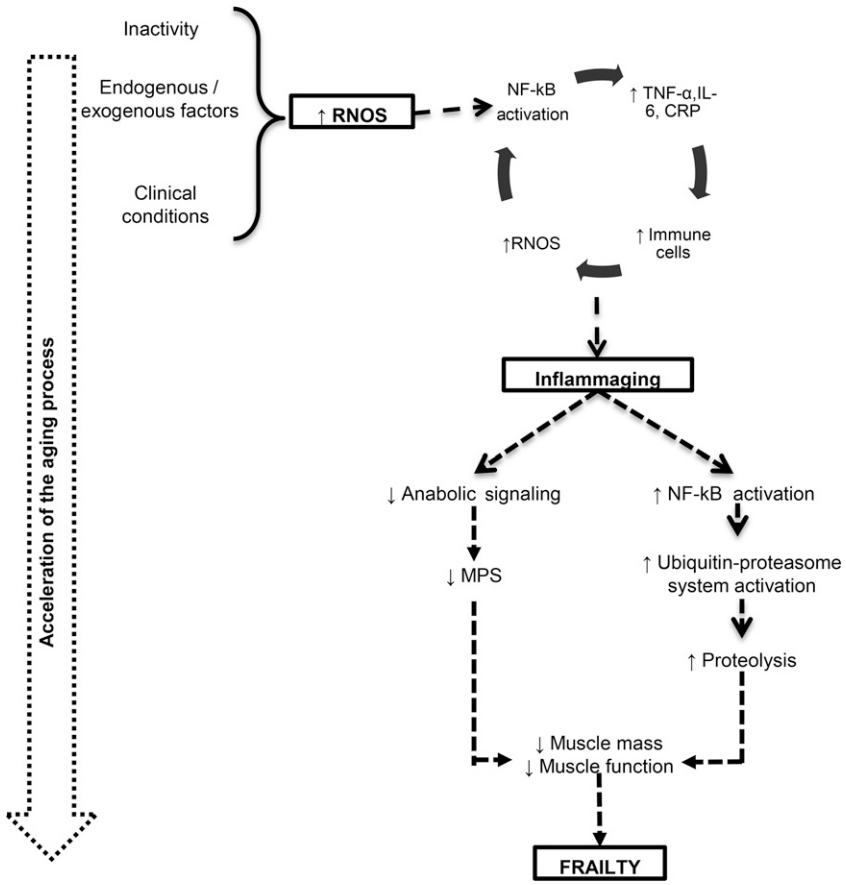

FIGURE 1 Potential pathway linking inflammaging and frailty. CRP, C-reactive protein; MPS, muscle protein synthesis; RNOS, reactive oxygen and nitrogen species; ↑, increase; ↓, decrease.

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

A Rationale for Protein Supplementation for Inflammaging

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

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

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

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

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

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

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

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

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

(Continued)

TABLE 1 *Continued*

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

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

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

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

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

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

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

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

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

(Continued)

TABLE 2 *Continued*

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

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

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

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

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

Conclusions

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

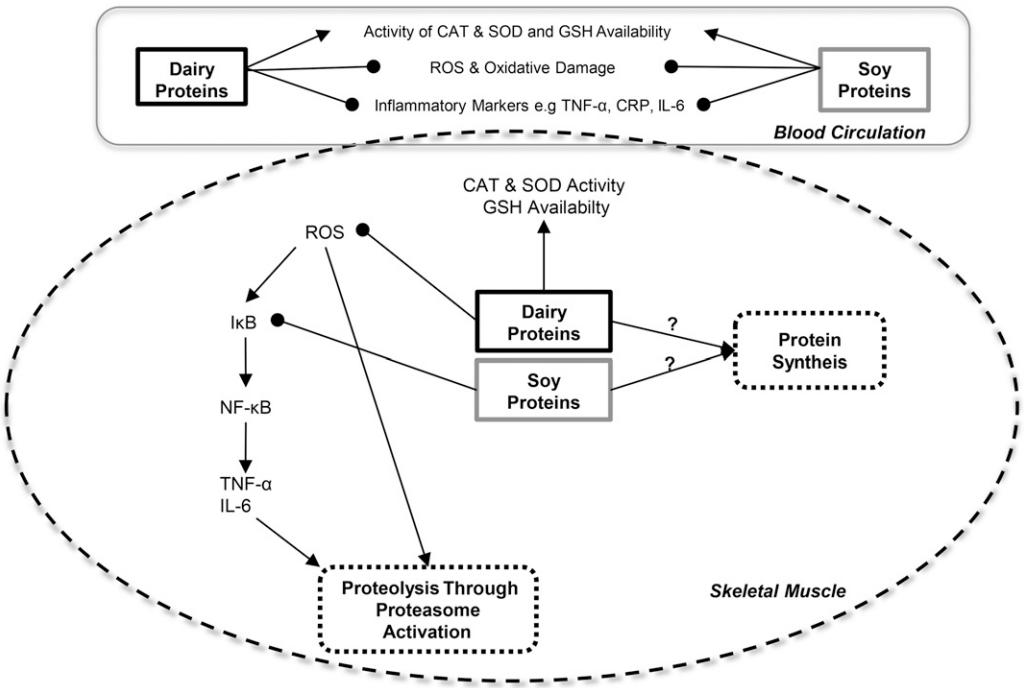

FIGURE 2 Mechanistic links between protein feeding and inflammaging. CAT, catalase; CRP, C-reactive protein; GSH, reduced glutathione; IκB, inhibitor of NF-κB; ROS, reactive oxygen species; SOD, superoxide dismutase; ?, lack of evidence regarding the ability of these proteins to stimulate muscle protein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

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

Acknowledgments

We thank Alexandra Avloniti and Theodoros Stamboulis for their valuable assistance with the preparation of this manuscript. DD, LGK, IA, AC, AZJ, and IGF wrote parts of the manuscript; and DD, LGK, and IGF had primary responsibility for the final content. All authors read and approved the final manuscript.

References

1. Waters DL, Baumqartner RN, Garry PJ, Vellas B. Advantages of dietary, exercise-related, and therapeutic interventions to prevent and treat sarcopenia in adult patients: an update. Clin Interv Aging 2010;5:259–70.
2. Morley JE, Abbatecola AM, Argiles JM, Baracos V, Bauer J, Bhasin S, Cederholm T, Coats AJ, Cummings SR, Evans WJ, et al. Sarcopenia with limited mobility: an international consensus. J Am Med Dir Assoc 2011;12:403–9.
3. Rennie MJ, Selby A, Atherton P, Smith K, Kumar V, Glover EL, Philips SM. Facts, noise and wishful thinking: muscle protein turnover in aging and human disuse atrophy. Scand J Med Sci Sports 2010;20:5–9.
4. McLean RR, Kiel DP. Developing consensus criteria for sarcopenia: an update. J Bone Miner Res 2015;30:588–92.
5. Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. Br Med Bull 2010;95:139–59.
6. Calçada D, Vianello D, Giampieri E, Sala C, Castellani G, de Graaf A, Kremer B, van Ommen B, Feskens E, Santoro A, et al. The role of low-grade inflammation and metabolic flexibility in aging and nutritional modulation thereof: a systems biology approach. Mech Ageing Dev 2014;136–137:138–47.
7. Beyer I, Mets T, Bautmans I. Chronic low-grade inflammation and age-related sarcopenia. Curr Opin Clin Nutr Metab Care 2012;15:12–22.
8. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007;292:R18–36.
9. Balage M, Averous J, Remond D, Bos C, Pujos-Guillot E, Papet I, Mosoni L, Combaret L, Dardevet D. Presence of low grade inflammation impaired postprandial stimulation of muscle protein synthesis in old rats. J Nutr Biochem 2010;21:325–31.
10. Rieu I, Magne H, Savary-Auzeloux I, Averous J, Bos C, Payron MA, Combaret L, Dardevet D. Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats. J Physiol 2009;587:5483–92.
11. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78:250–8.
12. Wolfe RR, Miller S, Miller K. Optimal protein intake in the elderly. Clin Nutr 2008;27:675–84.
13. Bartali B, Frongillo EA, Stipanuk MH, Bandinelli S, Salvini S, Palli D, Morais JA, Volpato S, Guralnik JM, Ferrucci L. Protein intake and muscle strength in older persons: does inflammation matter? J Am Geriatr Soc 2012;60:480–4.
14. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 2009;107:987–92.
15. West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, Moore DR, Stellingwerff T, Phillips SM. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. Am J Clin Nutr 2011;94:795–803.
16. Burd NA, Yang Y, Moore DR, Tang JE, Tarnopolsky MA, Phillips SM. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. Br J Nutr 2012;108:958–62.
17. Burris RL, Ng HP, Nagarajan S. Soy protein inhibits inflammation-induced VCAM-1 and inflammatory cytokine induction by inhibiting the NF-kB and AKT signaling pathway in apolipoprotein E-deficient mice. Eur J Nutr 2014;53:135–48.
18. Azadbakht L, Kimiagar M, Mehrabi Y, Esmaillzadeh A, Hu FB, Willett WC. Soy consumption, markers of inflammation, and endothelial function: a cross-over study in postmenopausal women with the metabolic syndrome. Diabetes Care 2007;30:967–73.
19. Chung HY, Kim HJ, Kim JW, Yu BP. The inflammation hypothesis of aging: molecular modulation by calorie restriction. Ann N Y Acad Sci 2001;928:327–35.
20. Powers SK, Kavazis AN, McClung JM. Oxidative stress and disuse muscle atrophy. J Appl Physiol 2007;102:2389–97.
21. Li H, Malhorta S, Kumar A. Nuclear factor-kappa B signaling in skeletal muscle atrophy. J Mol Med (Berl) 2008;86:1113–26.
22. Baylis D, Bartlett DB, Patel HP, Roberts HC. Understanding how we age: insights into inflammaging. Longev Healthspan 2013;2:8.
23. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000;908:244–54.
24. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M, Harris TB. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J Gerontol A Biol Sci Med Sci 2002;57:M326–32.
25. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med 2006;119:526.e9–17.
26. Schaap LA, Pluijm SM, Deeg DJ, Harris TB, Kritchevsky SB, Newman AB, Colbert LH, Pahor M, Rubin SM, Tylavsky FA, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. J Gerontol A Biol Sci Med Sci 2009;64:1183–9.

27. Alemán H, Esparza J, Ramirez FA, Astiazaran H, Payette H. Longitudinal evidence on the association between interleukin-6 and C-reactive protein with the loss of total appendicular skeletal muscle in free-living older men and women. Age Ageing 2011;40:469–75.
28. Payette H, Roubenoff R, Jacques PF, Dinarello CA, Wilson PW, Abad LW, Harris T. Insulin-like growth factor-1 and interleukin 6 predict sarcopenia in very old community-living men and women: the Framingham heart study. J Am Geriatr Soc 2003;51:1237–43.
29. Rohleder N, Kudielka BM, Hellhammer DH, Wolf JM, Kirschbaum C. Age and sex steroid-related changes in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. J Neuroimmunol 2002;126:69–77.
30. Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti MC, Cohen HJ, Penninx B, Pahor M, Wallace R, Havlik RJ. Serum IL-6 level and the development of disability in older persons. J Am Geriatr Soc 1999;47:639–46.
31. de Gonzalo-Calvo D, de Luxan-Delgado B, Rodriguez-Gonzalez S, Garcia-Macia M, Suarez FM, Solano JJ, Rodriguez-Colunga MJ, Coto-Montes A. Interleukin 6, soluble tumor necrosis factor receptor I and red blood cell distribution width as biological markers of functional dependence in an elderly population: a translational approach. Cytokine 2012;58:193–8.
32. Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C, Guralnik JM, et al. Chronic inflammation and the effect of IGF-1 on muscle strength and power in older persons. Am J Physiol Endocrinol Metab 2003;284:E481–7.
33. Bodell PW, Kodesh E, Haddad F, Zaldivar FP, Cooper DM, Adams GR. Skeletal muscle growth in young rats is inhibited by chronic exposure to IL-6 but preserved by concurrent voluntary endurance exercise. J Appl Physiol 2009;106:443–53.
34. Trappe TA, Liu SZ. Effects of prostaglandins and COX-inhibiting drugs on skeletal muscle adaptations to exercise. J Appl Physiol 2013;115:909–19.
35. Li YP, Reid MB. NF-kappa B mediates the protein loss induced by TNFalpha in differentiated skeletal muscle myotubes. Am J Physiol Regul Integr Comp Physiol 2000;279:R1165–70.
36. Saini A, Al-Shanti N, Stewart CE. Waste management – cytokines, growth factors and cachexia. Cytokine Growth Factor Rev 2006;17:475–86. Corrected and republished from: Cytokine Growth Factor Rev 2007;18(3–4):345.
37. Peterson JM, Bakkar N, Guttridge DC. NF-κB signaling in skeletal muscle health and disease. Curr Top Dev Biol 2011;96:85–119.
38. Chondrogianni N, Fragoulis EG, Gonos ES. Protein degradation during aging: the lysosome- and the proteasome-dependent cellular proteolytic systems. Biogerontology 2002;3:121–3.
39. Chondrogianni N, Stratford FL, Trougakos IP, Friguet B, Rivett AJ, Gonos ES. Central role of the proteasome in senesce human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation. J Biol Chem 2003;278:28026–37.
40. Chondrogianni N, Gonos ES. Proteasome inhibition induces a senescence-like phenotype in primary human fibroblasts cultures. Biogerontology 2004;5:55–61.
41. Tanaka K, Kasahara M. The MHC class I ligand-generating system: roles of immunoproteasomes and the interferon-gamma inducible proteasome activator PA28. Immunol Rev 1998;163:161–76.
42. Voges D, Zwickl P, Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 1999;68:1015–68.
43. Sandri M. Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. Int J Biochem Cell Biol 2013;45:2121–9.
44. Löw P. The role of ubiquitin-proteasome system in ageing. Gen Comp Endocrinol 2011;172:39–43.
45. Llovera M, Lopez-Soriano FJ, Argiles JM. Effects of tumor necrosis factoralpha on muscle-protein turnover in female Wistar rats. J Natl Cancer Inst 1993;85:1334–9.
46. Ling PR, Schwartz JH, Bistrian BR. Mechanisms of host wasting induced by administration of cytokines in rats. Am J Physiol 1997;272:E333–9.
47. Mayot G, Breuille D, Jarret AR, Obled C, Papet I. Systemic low-grade inflammation does not decrease skeletal muscle mass and protein synthesis in old rats. J Musculoskelet Neuronal Interact 2008;8:410–7.
48. Haddad F, Zaldivar F, Cooper DM, Adams GR. IL-6-induced skeletal muscle atrophy. J Appl Physiol 2005;98:911–7.
49. van Hall G, Steensberg A, Fischer C, Keller C, Moller K, Moseley P, Pedersen BK. Interleukin-6 markedly decreases skeletal muscle protein turnover and increases nonmuscle amino acid utilization in healthy individuals. J Clin Endocrinol Metab 2008;93:2851–8.
50. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underline amino acid resistance of wasting, aging muscle. FASEB J 2005;19:422–4.
51. Rivas DA, Morris EP, Haran PH, Pasha EP, Morais Mda S, Dolnikowski GG, Phillips EM, Fielding RA. Increased ceramide content and NFκB signaling may contribute to the attenuation of anabolic signaling after resistance exercise in aged males. J Appl Physiol 2012;113:1727–36.
52. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-α induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. Diabetes 2005;54:2939–45.
53. Hyldahl RD, Olson T, Welling T, Groscost L, Parcell AC. Satellite cell activity is differentially affected by contraction mode in human muscle following a work-matched bout of exercise. Front Physiol 2014;5:485.
54. Farup J, Rahbek SK, Knudsen IS, de Paoli F, Mackey AL, Vissing K. Whey protein supplementation accelerates satellite cell proliferation during recovery from eccentric exercise. Amino Acids 2014;46:2503–16.
55. Beenakker KG, Duijnisveld BJ, Van Der Lindeen HM, Visser CP, Westendorp RG, Butler-Brown G, Nelissen RG, Maier AB. Muscle characteristics in patients with chronic inflammation. Muscle Nerve 2012;46:204–9.
56. Wolfe RR. The role of dietary protein in optimizing muscle mass, function and health outcomes in older individuals. Br J Nutr 2012;108:S88–93.
57. Pennings B, Groen B, de Lange A, Gijsen AP, Zorenc AH, Senden JMG, van Loon LJC. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. Am J Physiol Endocrinol Metab 2012;302:E992–9.
58. Tipton KD, Rasmussen BB, Miller SL, Wolfe SE, Owens-Stovall SK, Petrini BE, Wolfe RR. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. Am J Physiol Endocrinol Metab 2001;281:E197–206.
59. Koopman R, Gleeson BG, Gijsen AP, Groen B, Senden JM, Rennie MJ, van Loon LJ. Post-exercise protein synthesis rates are only marginally higher in type I compared with type II muscle fibers following resistance-type exercise. Eur J Appl Physiol 2011;111:1871–8.
60. Moore DR. Keeping older muscle "young" through dietary protein and physical activity. Adv Nutr 2014;5:599S–607S.
61. Morley JE, Argiles JM, Evans WJ, Bhasin S, Cella D, Deutz NE, Doehner W, Fearon KC, Ferrucci L, Hellerstein MK, et al. Nutritional recommendations for the management of sarcopenia. J Am Med Dir Assoc 2010;11:391–6.
62. Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, Phillips SM. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. J Gerontol A Biol Sci Med Sci 2015;70:57–62.
63. Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BB, Verdijk LB, van Loon LJ. Aging is accompanied by a blunted protein synthetic response to protein ingestion. PLoS One 2015;11:e0140903.
64. Morley JE. Sarcopenia: diagnosis and treatment. J Nutr Health Aging 2008;12:452–6.
65. Volpi E, Campbell WW, Dwyer JT, Johnson MA, Jensen GL, Morley JE, Wolfe RR. Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? J Gerontol A Biol Sci Med Sci 2013;68:677–81.
66. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 2008;87:150–5.
67. McCarty MF, DiNicolantonio JJ. An increased need for dietary cysteine in support of glutathione synthesis may underline the increased risk for mortality associated with low protein intake in the elderly. Age (Dordr) 2015;37:96.

68. Xu R, Liu N, Xu X, Kong B. Antioxidant effects of whey protein on peroxide-induced cytotoxicity. J Dairy Sci 2011;94:3739–46.
69. Jin MM, Zhang L, Yu HX, Meng J, Sun Z, Lu RR. Protective effect of whey protein hydrolysates on H_2O_2-induced PC12 cells oxidative stress via a mitochondrial-mediated pathway. Food Chem 2013;141:847–52.
70. Kerasioti E, Stagos D, Priftis A, Aivazidis S, Tsatsakis AM, Hayes AW, Kouretas D. Antioxidant effects of whey protein on muscle C2C12 cells. Food Chem 2014;155:271–8.
71. Athira S, Mann B, Sharma R, Kumar R. Ameliorative potential of whey protein hydrolysate against paracetamol-induced oxidative stress. J Dairy Sci 2013;96:1431–7.
72. Kim J, Paik HD, Yoon YC, Park E. Whey protein inhibits iron overload-induced oxidative stress in rats. J Nutr Sci Vitaminol (Tokyo) 2013;59:198–205.
73. Ebaid H, Salem A, Sayed A, Metwalli A. Whey protein enhances normal inflammatory responses during cutaneous wound healing in diabetic rats. Lipids Health Dis 2011;10:235.
74. Nelson AR, Jackson L, Clarke J, Stellingwerff T, Broadbent S, Rowlands DS. Effect of post-exercise protein-leucine feeding on neutrophil function, immunomodulatory plasma metabolites and cortisol during a 6-day block of intense cycling. Eur J Appl Physiol 2013;113:2211–22.
75. Rowlands DS, Rossler K, Thorp RM, Graham DF, Timmons BW, Stannard SR, Tarnopolsky MA. Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. Appl Physiol Nutr Metab 2008;33:39–51.
76. Brown EC, DiSilvestro RA, Babaknia A, Devor ST. Soy versus whey protein bars: effects on exercise training impact on lean body mass and antioxidant status. Nutr J 2004;3:22.
77. Kerasioti E, Kiskini A, Veskoukis A, Jamurtas A, Tsitsimpikou C, Tsatsakis AM, Koutedakis Y, Stagos D, Kouretas D, Karathanos V. Effect of a special carbohydrate-protein cake on oxidative stress markers after exhaustive cycling in humans. Food Chem Toxicol 2012;50:2805–10.
78. Kerasioti E, Stagos D, Jamurtas A, Kiskini A, Koutedakis Y, Goutzoulas N, Pournaras S, Tsatsakis AM, Kouretas D. Anti-inflammatory effects of a special carbohydrate-whey protein cake after exhaustive cycling in humans. Food Chem Toxicol 2013;61:42–6.
79. Samaras A, Tsarouhas K, Paschalidis E, Giamouzis G, Triposkiadis F, Tsitsimpikou C, Becker AT, Goutzourelas N, Kouretas D. Effect of a special carbohydrate-protein bar and tomato juice supplementation on oxidative stress markers and vascular endothelial dynamics in ultra-marathon runners. Food Chem Toxicol 2014;69:231–6.
80. Pal S, Ellis V. The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. Obesity (Silver Spring) 2010;18:1354–9.
81. Pal S, Ellis V. Acute effects of whey protein isolate on blood pressure, vascular function and inflammatory markers in overweight postmenopausal women. Br J Nutr 2011;105:1512–9.
82. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. Am J Clin Nutr 2010;91:16–22.
83. Holmer-Jensen J, Karhu T, Mortensen LS, Pedersen SB, Herzig KH, Hermansen K. Differential effects of dietary protein sources on postprandial low-grade inflammation after a single high fat meal in obese non-diabetic subjects. Nutr J 2011;10:115.
84. Grey V, Mohammed SR, Smountas AA, Bahlool R, Lands LC. Improved glutathione status in young adult patients with cystic fibrosis supplemented with whey protein. J Cyst Fibros 2003;2:195–8.
85. Michailidis Y, Karagounis LG, Terzis G, Jamurtas AZ, Spengos K, Tsoukas D, Chatzinikolaou A, Mandalidis D, Stefanetti RJ, Papassotiriou I, et al. Thiol-based antioxidant supplementation alters human skeletal muscle signaling and attenuates its inflammatory response and recovery after intense eccentric exercise. Am J Clin Nutr 2013;98:233–45.
86. Chatzinikolaou A, Christoforidis C, Avloniti A, Draganidis D, Jamurtas AZ, Stampoulis T, Ermidis G, Sovatzidis A, Papassotiriou I, Kambas A, et al. A microcycle of inflammation following a team handball game. J Strength Cond Res 2014;28:1981–94.
87. Barbas I, Fatouros IG, Douroudos II, Chatzinikolaou A, Michailidis Y, Draganidis D, Jamurtas AZ, Nikolaidis MG, Parotsidis C, Theodorou AA, et al. Physiological and performance adaptations of elite Greco-Roman wrestlers during a one-day tournament. Eur J Appl Physiol 2011;111:1421–36.
88. Morrison D, Hughes J, Della Gatta PA, Mason S, Lamon S, Russell AP, Wadley GD. Vitamin C and E supplementation prevents some of the cellular adaptations to endurance-training in humans. Free Radic Biol Med 2015;89:852–62.
89. Xiao CW. Health effects of soy protein and isoflavones in humans. J Nutr 2008;138:1244S–9S.
90. Aoki H, Otaka Y, Igarashi K, Takenaka A. Soy protein reduces paraquat-induced oxidative stress in rats. J Nutr 2002;132:2258–62.
91. Takenaka A, Annaka H, Kimura Y, Aoki H, Igarashi I. Reduction of paraquat-induced oxidative stress in rats by dietary soy peptide. Biosci Biotechnol Biochem 2003;67:278–83.
92. Blum A, Lang N, Peleg A, Vigder F, Israeli P, Gumanovsky M, Lupovitz S, Elgazi A, Ben-Ami M. Effects of oral soy protein on markers of inflammation in postmenopausal women with mild hypercholesterolemia. Am Heart J 2003;145:e7.
93. Anthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996;126:43–50.
94. Yoon GA, Park S. Antioxidant action of soy isoflavones on oxidative stress and antioxidant enzyme activities in exercised rats. Nutr Res Pract 2014;8:618–24.
95. Sankar P, Zachariah B, Vickneshwaran V, Jacob SE, Sridhar MG. Amelioration of oxidative stress and insulin resistance by soy isoflavones (from Glycine max) in ovariectomized Wistar rats fed with high fat diet: the molecular mechanisms. Exp Gerontol 2015;63:67–75.
96. Park E, Shin JI, Park OJ, Kang MH. Soy isoflavone supplementation alleviates oxidative stress and improves systolic blood pressure in male spontaneously hypertensive rats. J Nutr Sci Vitaminol (Tokyo) 2005;51:254–9.
97. Hsieh HM, Wu WM, Hu ML. Soy isoflavones attenuate oxidative stress and improve parameters related to aging and Alzheimer's disease in C57BL/6J mice treated with D-galactose. Food Chem Toxicol 2009;47:625–32.
98. Djuric Z, Chen G, Doerge DR, Heilbrum LK, Kucuk O. Effect of soy isoflavone supplementation on markers of oxidative stress in men and women. Cancer Lett 2001;172:1–6.
99. Ryan-Borchers TA, Park JS, Chew BP, McGuire MK, Fournier LR, Beerman KA. Soy isoflavones modulate immune function in healthy postmenopausal women. Am J Clin Nutr 2006;83:1118–25.
100. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76 rodent diet. J Nutr 1993;123:1939e51.
101. Hagen MK, Lehenbauer-Luke AR, Paludo AC, Schenkel P, Goncalves L, Fernandes TG, Caron R, Llesuy S, Mill JG, Bello-Klein A. Diet with isolated soy protein reduces oxidative stress and preserves ventricular function in rats with myocardial infarction. Nutr Metab Cardiovasc Dis 2009;19:91–7.
102. Sreeja S, Geetha R, Priyadarshini E, Bhavani K, Anuradha CV. Substitution of soy protein for casein prevents oxidative modification and inflammatory response induced in rats fed high fructose diet. ISRN Inflamm 2014;2014:641096.
103. Messina S, Bitto A, Aguennouz M, Vita GL, Polito F, Irrena N, Altavilla D, Marini H, Migliorato A, Sguadrito F, et al. The soy isoflavone genistein blunts nuclear factor kappa-B, MAPKs and TNF-α activation and ameliorates muscle function and morphology in mdx mice. Neuromuscul Disord 2011;21:579–89.
104. Greany KA, Nettleton JA, Wangen KE, Thomas W, Kurzer MS. Consumption of isoflavone-rich soy protein does not alter homocysteine or markers of inflammation in postmenopausal women. Eur J Clin Nutr 2008;62:1419–25.
105. Törmälä R, Appt S, Clarkson TB, Mueck AO, Seeger H, Mikkola TS, Ylikorkala O. Impact of soy supplementation on sex steroids and vascular inflammation markers in postmenopausal women using tibolone: role of equol production capability. Climacteric 2008;11:409–15.
106. Acharjee S, Zhou JR, Elajami TK, Welty FK. Effect of soy nuts and equol status on blood pressure, lipids and inflammation in postmenopausal women stratified by metabolic syndrome status. Metabolism 2015;64:236–43.
107. Nasca MM, Zhou JR, Welty FK. Effect of soy nuts on adhesion molecules and markers of inflammation in hypertensive and normotensive postmenopausal women. Am J Cardiol 2008;102:84–6.

108. Steinberg FM, Guthrie NL, Villablanca AC, Kumar K, Murray MJ. Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. Am J Clin Nutr 2003;78:123–30.
109. Sirtori CR, Lovati MR. Soy proteins and cardiovascular disease. Curr Atheroscler Rep 2001;3:47–53.
110. Fanti P, Asmis R, Stephenson TJ, Sawaya BP, Franke AA. Positive effect of dietary soy in ESRD patients with systemic inflammation–correlation between blood levels of the soy isoflavones and the acute-phase reactants. Nephrol Dial Transplant 2006;21:2239–46.
111. Mangano KM, Hutchins-Wiese HL, Kenny AM, Walsh SJ, Abourizk RH, Bruno RS, Lipcius R, Fall P, Kleppinger A, Kenyon-Pesce L, et al. Soy proteins and isoflavones reduce interleukin-6 but not serum lipids in older women: a randomized controlled trial. Nutr Res 2013;33:1026–33.
112. Vega-López S, Yeum KJ, Lecker JL, Ausman LM, Johnson EJ, Devaraj S, Jialal I, Lichtenstein AH. Plasma antioxidant capacity in response to diets high in soy or animal protein with or without isoflavones. Am J Clin Nutr 2005;81:43–9.
113. Swain JH, Alekel DL, Dent SB, Peterson CT, Reddy MB. Iron indexes and total antioxidant status in response to soy protein intake in perimenopausal women. Am J Clin Nutr 2002;76:165–71.
114. Thomson RL, Brinkworth GD, Noakes M, Buckley JD. Muscle strength gains during resistance exercise training are attenuated with soy compared with dairy or usual protein intake in older adults: A randomized controlled trial. Clin Nutr 2016;35:27–33.
115. Beavers KM, Serra MC, Beavers DP, Cooke MB, Willoughby DS. Soymilk supplementation does not alter plasma markers of inflammation and oxidative stress in postmenopausal women. Nutr Res 2009;29:616–22.
116. Miraghajani MS, Esmaillzadeh A, Najafabadi MM, Mirlohi M, Azadbakht L. Soy milk consumption, inflammation, coagulation, and oxidative stress among type 2 diabetic patients with nephropathy. Diabetes Care 2012;35:1981–5.
117. Mitchell JH, Collins AR. Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men-a pilot study. Eur J Nutr 1999;38:143–8.
118. Jenkins DJ, Kendall CW, Connelly PW, Jackson CJ, Parker T, Faulkner D, Vidgen E. Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. Metabolism 2002;51:919–24.