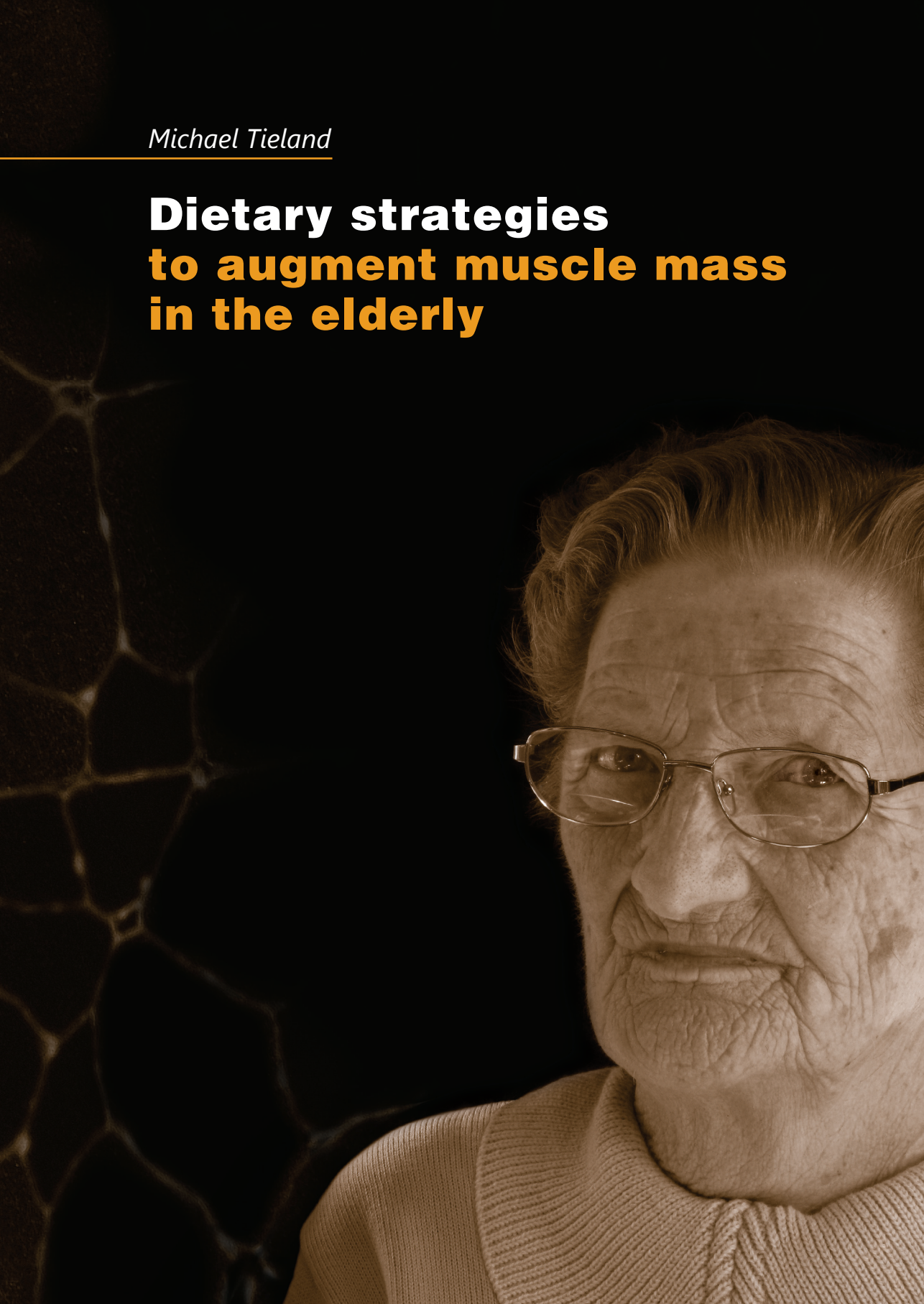
Michael Tieland

Dietary strategies to augment muscle mass in the elderly



Dietary strategies to augment muscle mass in the elderly

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Dietary strategies to augment muscle mass in the elderly

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Thesis

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ABSTRACT

Background: The world population is aging rapidly. This growth of the aging population is accompanied by an increased number of frail elderly people who are at risk of adverse health outcomes such as disability, co-morbidity and mortality. A dominant feature of frailty is the age-related loss of muscle mass, strength and performance, also called sarcopenia. Resistance-type exercise training and dietary protein supplementation are considered promising strategies to reverse sarcopenia and subsequent frailty. However, strong evidence for the impact of protein supplementation with or without resistance exercise in frail elderly people is scarce. Well-designed intervention studies in frail elderly people are needed to define new leads for the development of nutritional and exercise interventions to effectively prevent or treat the progressive loss of muscle mass, strength and physical performance with aging. Therefore, the aims of this thesis are to study 1) the impact of protein supplementation and 2) the impact of protein supplementation during prolonged resistance-type exercise training on muscle mass, strength and physical performance in frail elderly people.

Methods: First, we studied various characteristics of dietary protein intake, including the distribution of dietary protein intake throughout the day, and the use of protein-containing food sources in various elderly populations. With this knowledge, we designed two large intervention trials to study the impact of dietary protein supplementation with or without prolonged resistance-type exercise training on muscle mass, strength and physical performance in frail elderly people. In addition, we assessed the usefulness of handgrip strength as a measure of post exercise strength differences and studied the association of vitamin D status and vitamin D intake on muscle mass, strength and physical performance in a frail elderly population.

Results: Dietary protein intake in frail and institutionalized elderly people were especially low at breakfast and lunch. Supplementing protein at breakfast and lunch did not increase muscle mass but improved physical performance in frail elderly people. Resistance-type exercise training improved muscle leg strength and physical performance, but not handgrip strength. Supplementing protein at breakfast and lunch was required to significantly increase muscle mass during prolonged resistance-type exercise training in frail elderly people. Furthermore, low vitamin D status and vitamin D intake were associated with impaired physical performance.

Conclusions: Although dietary protein supplementation does not increase muscle mass, it represents a promising strategy to improve physical performance in frail elderly people. Prolonged resistance-type exercise training represents an effective strategy to improve strength and physical performance, but dietary protein supplementation is required to allow muscle mass gain during exercise training in frail elderly people.

Contents

1

Introduction

Aging

The world population is aging rapidly. Since 1980, the number of people aged 60 y and over has doubled to approximately 810 million. The elderly population will continue to grow to approximately 2 billion in 2050. It has been predicted that 22% of the total population will be older than 60 y and 4.4% will be older than 80 y in 2050[1]. In the Netherlands, the rise in the aging population will be even more pronounced as the number elderly people above 65 y will grow to 4.4 million or 25% of the total population in 2060[2]. This immense growth of the aging population is due to the post-world war II baby boom as well as the higher life expectancy for males and females. In 2060, it is expected that life expectancy will be 84.5 y for males and 87.4 y for females. This higher life expectancy is mainly attributed to education, hygiene, less environmental threats and better healthcare[3].

As society ages, the incidence of physical disability will increase as well. Approximately 30% of the population 55 y and older are confronted with moderate or severe physical disabilities[4]. This physical disability decreases quality of life, increases the risk of institutionalization and hospitalization and even of premature death[5]. In addition, the higher age-related prevalence of physical disability will increase the demand on our health care system. Prevention of disability or even treatment for disability is therefore relevant for public health. The Dutch Ministry of Health, Welfare and Sport emphasizes the importance of prevention and treatment of disability in order to age healthy and stay physically active and independent as long as possible[2].

Frailty

Frailty is a relatively new concept that is described as a geriatric syndrome of decreased reserves and resistance to stressors, which increases the risk of adverse outcomes such as the onset of disability, institutionalization and premature death[6,7]. The prevalence of frailty increases with age. A recent systematic review of 21 community-based studies involving 61500 elderly people reported a prevalence of frailty between 4–59%[8]. On average, 10.7% of the community-dwelling older persons are frail and 41.6% are pre-frail. After the age of 85 y, the average prevalence of community-based frailty increases enormously to almost 26.1%[8]. In the latter age category, the prevalence of frailty is even more pronounced in institutionalized elderly and is estimated to be 40%[9]. The reported prevalences differ substantially which is attributed to the definition of frailty used. The majority of definitions of frailty used are based upon physical function. The most commonly used frailty criteria

are those of Fried et al.[10]. These include unintentional weight loss, weakness, self-reported exhaustion, slow walking speed, and low physical activity. Subjects with one or two criteria present are defined as pre-frail and subjects with three or more criteria present are defined as frail[10]. It has been suggested, however, that frailty is much more broader than the physical criteria and should also include cognitive status, mood, social resources, number of comorbidities and nutritional status[6,11-13]. This multidimensional nature of frailty might be the cause of no uniformly and broadly accepted definition of frailty. Despite no clear consensus, more researchers acknowledge frailty as one of the most important geriatric conditions. Frailty has been described as a reversible condition[11] and therefore efficacious interventions need to be developed and tested to prevent pre-frailty and/or even reverse the frailty state.

Sarcopenia

An important determinant of frailty is the age-related loss of muscle mass, strength and performance also referred to as sarcopenia. The term sarcopenia was first introduced in the late eighties by Rosenberg and stems from the Greek. It literally means poverty (penia) of flesh (sarc)[14]. As the term sarcopenia reflects, muscle mass changes throughout the lifespan (**Figure 1.1**). From birth, the amount of muscle mass increases rapidly. At the age of approximately 35 y, muscle mass and strength start to decline[15-19]. A recent quantitative review showed that the median decline in muscle mass throughout the lifespan is 0.37% per year in women and 0.47% per year in men[20]. According to longitudinal studies in

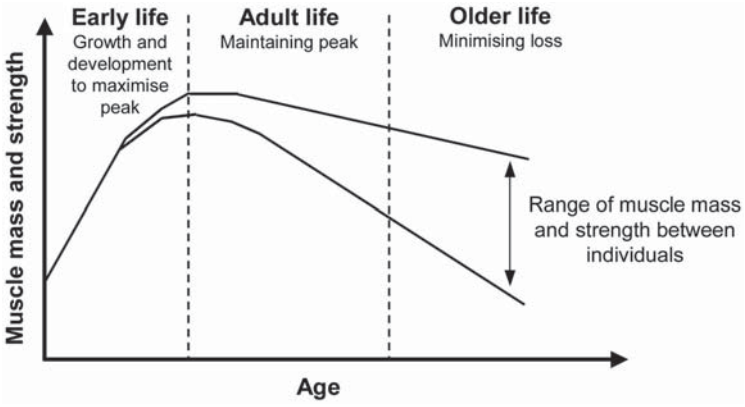

Figure 1.1 Changes of muscle mass and strength throughout the lifespan. Adapted from Sayer et al.[17].

people aged 75 y or over[20], muscle mass is lost at a rate of 0.64–0.70% per year in women and 0.80–0.98% per year in men. Strength is lost more rapidly. At the age of 75 y, strength is lost at a rate of 3–4% per year in men and 2.5–3% per year in women[20].

Baumgartner et al. operationalized sarcopenia as the amount of appendicular lean mass (sum of lean mass tissue of arms and legs) 2 SDs lower than the gender specific mean of appendicular lean mass of a young and healthy population[21]. Later Janssen et al. operationalized sarcopenia with a classification of severity[22]. Class 1 sarcopenia was considered present when the muscle mass index (total muscle mass/ total body weight) was between 1 and 2 SDs lower than a gender specific mean of a young population. Class 2 sarcopenia was considered present when the muscle mass index was more than 2 SDs lower than a gender specific mean of a young population. Subsequently, loss of muscle strength and physical performance became part of the definition of sarcopenia[23,24]. In 2010, the latest consensus definition was presented by the European Working Group on Sarcopenia in Older People[25]. They proposed a diagnosis of sarcopenia to require low muscle mass accompanied by either low muscle strength or low physical performance. Pre-sarcopenia was defined as low muscle mass according to the Baumgartner criteria, sarcopenia was determined as pre-sarcopenia with either loss of strength (lowest quartile of handgrip strength in sample distribution), or physical performance (gait speed ≤0.8 m/s) and severe sarcopenia was defined when all 3 aspects were present. Depending on the criteria, the prevalence of sarcopenia is approximately 6–24% of people above 60 y and increases with age to 40–50% of people above 80 y[23,26,27]. Although a global consensus definition is not yet reached, sarcopenia is now recognized as an important geriatric condition and represents a key factor in the development of frailty[6,11,23,28-30]. The progressive loss of skeletal muscle mass, strength, and physical performance results in an increased risk for adverse outcomes such as onset of disability, morbidity, institutionalization, and mortality[6]. As such, sarcopenia imposes a heavy burden on our health care system. In the US, the direct cost of sarcopenia in 2000 has been estimated to be 1.5% of the total health care cost, representing 18.5 billion dollar[31]. Furthermore, it should be noted that skeletal muscle mass is an important metabolic organ. Muscle mass is not only a major depot for glucose storage, it also plays an important role in glucose, fat and protein metabolism as well as in energy metabolism[32,33]. Consequently, it has been suggested that sarcopenia increases the risk of developing diabetes, cardiovascular diseases and obesity[33,3]4. Hence, the healthcare cost related to sarcopenia is believed to be far beyond the previously presented 18.5 billion dollar.

Causes of sarcopenia

The decline in skeletal muscle mass with aging is attributed to a disruption in the regulation of skeletal muscle protein turnover, leading to a structural imbalance between muscle protein synthesis and muscle protein breakdown[33,35]. Factors that impair muscle protein synthesis or factors that stimulate muscle protein breakdown are playing a key role in the development of sarcopenia[23]. In addition, skeletal muscle satellite cells (SC) have been suggested to be involved in the development of sarcopenia[36-38] as these muscle stems cells are essential for muscle fiber repair, maintenance and growth. The process of sarcopenia occurs over a prolonged period of time and is acknowledged to be multifactorial[34,39]. The major factors considered to be involved include inflammation, hormonal changes, neurological factors, physical inactivity, and inadequate nutritional intake[23,34].

Inflammation

Epidemiological data have shown that inflammatory cytokines such as interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) are elevated in elderly people[40]. These elevated levels of IL-6 of and TNF-α, also known as low-grade chronic inflammation, were associated with a decline in muscle mass and strength[41,42]. Animal studies have demonstrated that higher TNF-α concentrations in rats lead to significant loss of muscle mass[43-45], which is likely to occur via the activation of the ubiquitin–proteasome pathway and apoptosis, and perhaps via reduced basal muscle protein synthesis rates[46]. In addition, it is suggested that TNF-α also negatively affects the muscle regenerating capacity by destabilizing MyoD and myogenin[47]. These muscle specific transcription factors are involved in the transition from proliferation to differentiation of satellite cells[48]. In elderly people, the relatively high levels of inflammatory cytokines over many years inhibit differentiation of satellite cells, and hence maintenance of the muscle, resulting in a slow but progressive loss of muscle mass and subsequent sarcopenia.

Hormonal changes

Aging results in a significant decline in plasma testosterone, growth hormone (GH) and insulin like growth factor-1 (IGF-1) concentrations[49]. These reduced levels have been associated with sarcopenia. Both testosterone and GH are powerful anabolic agents that promote muscle protein synthesis and subsequent muscle mass accretion[24,50,51]. Circulating IGF-1 plays an active role in regulating GH secretion through a negative feedback mechanism and thereby influencing muscle mass. Furthermore, IGF-1 is produced locally in the muscle, where it stimulates the phosphorylation of mammalian target of rapamycin

(mTOR), one of the key regulators of muscle protein synthesis[52]. Although a decline in hormone status may contribute to the loss of muscle mass with aging, it remains unclear whether this decline is inherent to changes in lifestyle associated with aging[49].

Neurological factors

Age-related changes in the neuromuscular system play an important role in the onset of sarcopenia. The number and function of motor neurons decline with age[53]. These motor neurons are responsible for sending signals from the brain to the muscle to initiate movement. Motor units consist of a motor neuron and all of the muscle fibers innervated by that neuron. Fast twitch motor units are bigger and result in faster muscle contraction, producing much more force when compared to slow twitch motor units. It has been suggested that aging leads to accelerating denervation rates of fast twitch, large motor units[54], explaining the loss of type II muscle fibers in elderly people[55]. Although the loss of fast twitch motor units contributes to the loss of muscle, it mainly contributes to the age-associated loss of muscle strength[54]. Interestingly, the loss of muscle strength, also referred to dynapenia, occurs much more rapid than the concomitant loss of muscle mass[16]. The latter suggests that intervention studies should not only target muscle mass but should also focus on muscle strength and physical performance.

Physical inactivity

There is ample evidence that physical inactivity has a large impact on the average life expectancy as well as on quality of life. In fact, physical inactivity is one of the strongest predictors of physical disability in elderly people[56,57]. Epidemiological data have shown that low levels of physical activity relate to an accelerated decline in muscle mass, strength and physical performance[58-60]. Indeed, physical activity is considered the main anabolic stimulus, responsible for stimulating muscle protein synthesis and, as such, preserving muscle mass as protein turns over continually[61]. Evidence from bed rest and/or lower limb immobilization studies have shown that muscle mass and performance is lost very rapidly after acute inactivity[56,62]. Kortebein et al. observed a 0.95 kg loss of lean leg mass after just 10 d of bed rest in healthy elderly people[56]. The latter muscle loss was accompanied with a 12% loss of muscle strength and 14% loss of physical performance. These findings suggest that physical inactivity plays a key role in the development of sarcopenia, frailty and subsequent physical disability. In turn, sarcopenia, frailty and disability decrease the level of physical activity and thereby maintain the negative vicious circle as depicted in **Figure 1.2**[63].

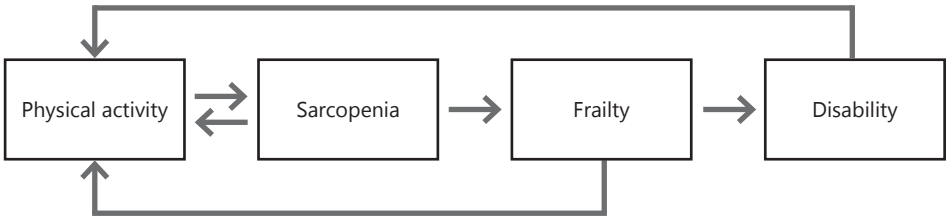

Figure 1.2 The role of physical activity in the development of sarcopenia, frailty and disability and vice versa, adapted from Freiberger et al.[63].

Inadequate nutritional intake

The aging process is associated with a decline in appetite and food intake known as anorexia of aging[64]. The prevalence of anorexia of aging amounts to 21% in elderly above 65 y and is more prevalent in frail and institutionalized elderly[65]. Anorexia and subsequent weight loss have been associated with adverse health outcomes such as falls, immobility and sarcopenia. In fact, recent epidemiological data from the ilSIRENTE study showed an 88% higher risk of sarcopenia in elderly suffering from anorexia compared with non-anorexic elderly people[66]. Anorexia of aging is related to a decline in the intake of various nutrients such as dietary protein and vitamin D. These nutrients are suggested to play an important role in the development of sarcopenia[67,68].

Dietary protein

In the absence of dietary protein intake, i.e. during fasting conditions, muscle protein breakdown exceeds muscle protein synthesis which creates a negative muscle protein balance, and, after a prolonged period of time, results in muscle loss. Following protein intake, i.e. in the postprandial phase, digestion and absorption of dietary protein increase the availability of plasma amino acids[69-71]. A rapid increase in postprandial plasma amino acid concentrations strongly increases muscle protein synthesis rates, reduces muscle protein breakdown rates, resulting in a positive protein balance. However, with the ingestion of small, meal-like amounts of dietary protein attenuation of the postprandial skeletal muscle protein synthetic response concurs in elderly people[72,73]. Such an attenuated postprandial muscle protein synthetic response would result in a negative muscle protein balance, and subsequent loss of muscle mass. The latter anabolic resistance to meal-like protein intakes might become of great clinical relevance over the course of many years. In fact, it has been proposed that anabolic resistance represents one of

the key-factors responsible in the development of sarcopenia and frailty[33]. To overcome the anabolic resistance to small protein intakes, it has been suggested that 25 to 30 g of dietary protein per meal is required to allow an appropriate stimulation of postprandial muscle protein synthesis. The suggested increase of dietary protein per meal would increase the daily protein intake to 1.2–1.5 g per kg bodyweight (g/kg-bw). It has been reported that this daily protein intake substantially attenuates muscle loss (i.e. 40%) when compared with a daily protein intake of 0.8 g/kg-bw, i.e., the current recommended dietary allowance (RDA)[74]. These results suggest that dietary protein supplementation might be a promising nutritional strategy aiming to postpone and/or treat sarcopenia in elderly people.

Vitamin D

Another important nutrient that might relate to sarcopenia is vitamin D. The reduction in endogenous vitamin D synthesis together with low vitamin D intakes result in a high prevalence of vitamin D deficiency among elderly people. The estimated prevalence of vitamin D deficiency among elderly people is between 45 and 57%[75-78] and among compromised geriatric patients vitamin D deficiency is even more pronounced[79,80]. Low vitamin D status has been associated with poor muscle mass and impaired physical performance in community-dwelling elderly people[81-85]. Mechanistically, it is suggested that the activation of the vitamin D receptor (VDR) in skeletal muscle tissue plays an important role in muscle protein turnover[86]. The activation of the VDR might stimulate skeletal muscle protein synthesis[86,87] and might prevent type II muscle fiber atrophy[88]. In addition, it has been suggested that 1,25-dihydroxycholecalciferol, the active form of 25-hydroxyvitamin D (25(OH)D), regulates muscle calcium concentrations by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma[81]. Alterations in intracellular calcium concentrations regulate the contraction and relaxation of the muscle, which may impact physical performance. Unfortunately, the role of vitamin D intake and vitamin D status in the development, prevention and treatment of sarcopenia and frailty is still unclear.

Interventions to counteract sarcopenia

The majority of intervention studies that target sarcopenia or frailty have focused on the above mentioned causes of sarcopenia. The most promising lifestyle interventions that stimulate anabolic and/ or inhibit catabolic pathways in elderly people are exercise and nutritional interventions.

Resistance-type exercise

Resistance-type exercise training is currently the most effective intervention to slow down the decline of muscle mass and muscle strength. Resistance-type exercise training involves planned, structured and repetitive small number of muscle contractions against heavy loads[35]. Whereas endurance-type exercise training mainly increases oxidative capacity[89], resistance-type exercise training enhances muscle mass and strength in healthy and frail elderly people[90-93]. A single session of resistance-type exercise increases both muscle protein synthesis and breakdown rates, albeit the latter to a lesser extent[61,94]. This results in an increased muscle net muscle protein balance that persists up to 48h in the young[61]. In the absence of food intake, however, the net muscle protein balance remains negative and muscle hypertrophy cannot occur. Furthermore, ample studies show that resistance-type exercise training improves functional outcomes such as balance, gait speed, chair rise and stair climbing power[92,95-97]. The aforementioned benefits are party due to an increase in the amount of contractile protein and the improvements in neural function[98-100].

Protein intake

It has been well-established that dietary protein ingestion stimulates skeletal muscle protein synthesis[69,94,101-103], and inhibits protein breakdown, resulting in a more positive muscle protein balance[94,103]. So far, evidence from long-term intervention studies showed no clear benefit of dietary protein supplementation on skeletal muscle mass in elderly people. Whereas some reveal an increase in skeletal muscle mass[104,105], others have failed to report any measurable impact of long-term dietary protein supplementation on skeletal muscle mass in elderly subjects[106-112]. This apparent discrepancy might be attributed to the selection of healthy versus more frail elderly subjects. Most studies investigating the impact of long-term protein supplementation have generally included healthy elderly people[106,109-112]. Long-term intervention studies investigating the efficacy of protein supplementation on skeletal muscle mass in frail elderly people, however, are scarce and show discrepant findings[104,107,108]. More research is warranted to investigate the impact of protein supplementation on skeletal muscle mass and physical performance in a frail elderly population. The latter research might provide evidence that protein supplementation is a promising nutritional strategy to prevent or even counteract sarcopenia and frailty[23,113].

Resistance-type exercise and protein intake

As mentioned previously, exercise improves muscle protein balance, but the net muscle protein balance will remain negative in the absence of food intake. Protein ingestion prior to or after exercise is required to further augment post-exercise muscle protein synthesis rates and inhibit protein breakdown[70,94,114,115], resulting in a positive protein balance and, as such, net muscle protein accretion[116-119]. Consequently, it has been proposed that dietary protein supplementation is required to maximize skeletal muscle mass gain during prolonged resistance-type exercise training and, as such, to more effectively counteract sarcopenia and frailty[23,113]. Indeed, a recent meta-analysis reported beneficial effects of protein supplementation during long-term resistance-type exercise training on muscle mass and strength in healthy elderly people[120]. In contrast, studies investigating the impact of protein supplementation during prolonged exercise intervention in frail elderly are scarce and report discrepant findings[107,108,121]. Although protein supplementation and resistance-type exercise training might be very promising strategies to attenuate or even reverse sarcopenia, in frail elderly people more research is warranted.

Rationale and outline of thesis

In summary, the growth of the aging population has increased the focus on the importance of maintaining independent, and delaying frailty and subsequent disability. A major cause of frailty and disability is sarcopenia. The cause of sarcopenia is multi-factorial and includes a sedentary lifestyle and inadequate protein intake. Resistance-type exercise training and dietary protein supplementation might be promising strategies to reverse the age-related loss of muscle mass, strength and performance and subsequent frailty. However, strong evidence of the impact of protein supplementation with or without resistance exercise in frail elderly people is scarce. Well-designed human randomized double-blind placebo-controlled intervention trials are needed to define new leads for the development of nutritional and exercise interventions in order to prevent or treat the progressive loss of muscle mass, strength and physical performance with aging. Therefore, the aims of this thesis are 1) to study the impact of protein supplementation on muscle mass, strength and physical performance in frail elderly people and 2) to study the impact of protein supplementation during prolonged resistance-type exercise training on muscle mass, strength and physical performance in frail elderly people.

To answer these research questions, we performed a set of studies that are described below. We started to study the protein intakes of community-dwelling, frail, and institutionalized elderly people (**chapter 2**). In this study, we assessed daily protein intake, distribution of

protein intake throughout the day, and the use of protein-containing food sources. With this knowledge, we designed intervention trials aiming to study the impact of protein supplementation on muscle mass, strength and physical performance in frail elderly people in the absence (**chapter 3**) or presence of a resistance-type exercise training program (**chapter 4**). Furthermore, we assessed the usefulness of handgrip strength as a measure for post exercise strength differences (**chapter 5**). **Chapter 6** presents the association of 25(OH)D status and vitamin D intake with muscle mass, strength and physical performance in a frail elderly population. And finally, in **chapter 7**, we discuss the main findings of the studies and provide general conclusions and directions for future research.

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2

Dietary protein intake in community-dwelling, frail, and institutionalized elderly people; scope for improvement

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Abstract

Purpose: Adequate dietary protein intake is required to postpone and treat sarcopenia in elderly people. Insight in dietary protein intake in this heterogeneous population segment is needed to locate dietary inadequacies and to identify target populations and feeding strategies for dietary interventions. Therefore, we assessed dietary protein intake, distribution of protein intake throughout the day, and the use of protein containing food sources in community-dwelling, frail, and institutionalized elderly people in the Netherlands.

Methods: Secondary analyses were carried out, using dietary data collected from studies among community-dwelling, frail, and institutionalized elderly people, to evaluate protein intake characteristics.

Results: Dietary protein intake averaged 1.1±0.3 g/kg-bw/d in community-dwelling, 1.0±0.3 g/kg-bw/d in frail, and 0.8±0.3 g/kg-bw/d in institutionalized elderly men. Similar protein intakes were found in women. Ten percent of the community-dwelling and frail elderly and 35% of the institutionalized elderly people showed a protein intake below the estimated average requirement (0.7 g/kg-bw/d). Protein intake was particularly low at breakfast in community-dwelling (10±10 g), frail (8±5 g), and institutionalized elderly people (12±6 g) with bread and dairy products as predominant protein sources.

Conclusions: Whereas daily protein intake is generally well above the recommended dietary allowance in community-dwelling and frail elderly people, a significant proportion of institutionalized elderly showed an intake below the current protein requirement, making them an important target population for dietary interventions. Particularly at breakfast, there is scope for improving protein intake.

Introduction

Sarcopenia, the age-related loss of skeletal muscle mass and strength, is accompanied by a decline in functional ability that affects many aspects of life[1]. Sarcopenia is a process caused by a combination of factors, which include a sedentary lifestyle and an inadequate dietary protein intake[2,3]. In both young and elderly people, dietary protein intake stimulates skeletal muscle protein synthesis and inhibits protein breakdown, resulting in a positive protein balance[4-6] and net muscle protein accretion[7,8]. Although results of acute studies show anabolic properties of dietary protein, so far, most dietary intervention studies that supplemented dietary protein for several months have failed to observe measurable gains in skeletal muscle mass in elderly people[9-13]. The absence of any apparent benefits of long-term protein supplementation might be attributed to a less than optimal feeding regimen. Various dietary protein intake strategies have been proposed. It has been suggested that the total amount of protein ingested is of importance to maintain skeletal muscle mass in elderly people. It has been reported that elderly people lose substantially less lean and appendicular lean body mass over time when consuming 1.2 g dietary protein per kg bodyweight per day (g/kg-bw/d) when compared with a dietary protein intake of 0.8 g/kg-bw/d, i.e. the current recommended dietary allowance (RDA)[14]. Besides the importance of total daily protein intake, the amount of protein ingested at each meal is of relevance[15]. Previous studies show similar post-prandial skeletal muscle protein synthetic responses between young and older individuals after ingesting a large bolus of dietary protein[16]. Smaller amounts of dietary protein, however, have revealed an attenuated post-prandial skeletal muscle protein synthetic response in elderly people when compared with young individuals[17,18]. In addition, it has been suggested that protein intake distribution throughout the day might be of importance for daily net protein balance. For example, Arnal and co-workers suggested that feeding dietary protein in pulses improves nitrogen balance more than feeding dietary protein spread over a variety of small meals throughout the day does[19]. Finally, evidence has emerged to suggest that the type, i.e. source, of protein consumed may modulate the skeletal muscle anabolic response to food intake[20-22]. Besides the fact that daily amount, distribution and/or source of dietary protein in nutritional intervention trials might not have been optimal to observe measurable gains in skeletal muscle mass in healthy elderly people, it has been suggested that the proposed positive effects of prolonged dietary protein supplementation are confined to specific elderly subpopulations, e.g. frail or institutionalized elderly people[13]. Unfortunately, detailed dietary protein intake characteristics among various elderly subpopulations are still lacking. Insight in these dietary protein characteristics is important to locate dietary

protein inadequacies in this heterogeneous population segment and, as such, to define more effective countermeasures.

The present study was performed to assess daily protein intake, protein intake distribution, and the specific protein sources that are consumed in community-dwelling, frail, and institutionalized elderly people in the Netherlands. This study aims to locate dietary protein inadequacies and to identify target populations and feeding strategies needed to define more effective dietary interventions to postpone and treat sarcopenia in elderly people.

Methods

Data collection

We used data from four previously performed studies among community-dwelling, frail, and institutionalized elderly people. Data of apparently healthy community-dwelling elderly people were derived from the most recent Dutch National Food Consumption Survey (DNFCS) conducted in 1998[23]. A total of 707 elderly men and women, who lived independently, were stratified into two age groups: 65–74 y and 75–97 y. Dietary intake data were randomly collected during the wk using 2-d food records. Data of frail independently living elderly people (n=194) came from baseline data of a randomized placebo-controlled trial, conducted in 1997, aiming to improve physical and mental health[24,25]. Criteria for frailty in this study were: age ≥70 y, requiring healthcare, physical inactivity and self-reported body mass index (BMI) of ≤25 kg/m^2 or recent involuntary weight loss. Dietary intake data were obtained by trained dieticians using 3-d food records collected on two non-consecutive weekdays and one weekend day. Data of the institutionalized elderly people were derived from baseline dietary assessments of two intervention studies. The first study, INST-1 (n=60), investigated the effect of supplementation on nutritional status and physical performance[26]. The second study, INST-2 (n=216), was designed to investigate the effect of ambiance during mealtimes in Dutch nursing homes[27]. The latter study selected elderly people who were housed in somatic wards. Dietary intake data were collected using 2-d food records in the INST-1 study and 3-d food records in the INST-2 study. In addition, subject characteristics including age, sex, physical activity, ADL performance, cognitive function, body weight, and BMI were used if available. Physical activity in the frail elderly population was measured using the validated Physical Activity Scale for Elderly (PASE)[28]. The PASE, with a score ranging from 0 to 400, is designed to assess activities commonly engaged by elderly people. In the elderly

people of the INST-1 study, the activities of daily living (ADL) were analyzed according to the Barthel index[29]. The Barthel index is developed to measure the performance of ADL and uses a scale from 0 to 100. A higher score indicates better functional capacity. In addition, cognitive function was measured in the INST-1 study using the Dutch revision of the Alzheimer's Disease Assessment Scale (ADAS). ADAS consists of a non-cognitive part and a cognitive part. The latter part is used in this study and referred to as ADAS-cog, consisting of 12 items with a total score ranging from 0 (no impairment) to 75 (severe impairment)[30,31]. Furthermore, Mini-Mental State Examination (MMSE) scores (0–30) were re-analyzed in INST-1 study[32].

Calculation of dietary protein intake

Dietary intake data were coded (food intake, amount, and mealtime) and cross-checked by dieticians. Portion sizes were documented in household measures, whereby frequently used household measures were checked in all the studies. Energy and protein intakes were calculated with a computerized Dutch food consumption table. The DNFCS and the INST-1 study used the Dutch food composition table of 1996 and the FRAIL and INST-2 study used the Dutch food composition tables of 1997 and 2001. Dietary protein intake was calculated as: 1) total protein intake (g/d), 2) protein intake per kilogram body weight (g/kg-bw/d) and 3) percentage of energy from protein (en%). Furthermore, protein intakes (g) per mealtime moment, i.e. breakfast, lunch, dinner, and between meals (snacks) were calculated and protein intake from specific food sources was assessed. Percentage of inadequate dietary protein intake in the community-dwelling, frail, and institutionalized elderly people was estimated using the cut point method[33] based on the protein estimated average requirement (EAR) of 0.7 g/kg-bw/d.

Statistical analysis

Data analyses were performed using the SPSS statistical software package (version 15.0). Descriptives were used to derive the mean and standard deviations of baseline characteristics. One-way ANOVA was used to compare differences in energy and protein intake between community-dwelling, frail, and institutionalized elderly people. In case of a significant difference ($P<0.05$) in energy and protein intake, Bonferroni's post-hoc test was applied to locate these differences.

Results

Characteristics of the participants

Descriptive characteristics of the study populations are presented in **Table 2.1**. In the community-dwelling, frail, and institutionalized elderly groups, the majority were women (58–75%). According to the PASE, low average physical activity levels were found in frail men (65±39) and women (63±30). The Barthel index score was 71±26 for both men and women in the institutionalized elderly people (INST-1) reflecting a reasonable level of independency in activities of daily living. Average ADAS-cog score was 18±12 and average MMSE score was 21±6 in institutionalized elderly people (INST-1).

Dietary intake

Lowest energy intakes were reported in institutionalized elderly people (5.8±1.5 to 8.2±1.6 MJ/d in men; 5.9±1.6 to 6.2±1.5 MJ/d in women) whereas community-dwelling elderly people showed the highest energy intakes (9.4±2.4 to 9.5±2.5 MJ/d in men; 7.5±1.9 MJ/d in women).

Table 2.1 Baseline characteristics of community-dwelling, frail, and institutionalized elderly people

	COMMUNITY-DWELLING		FRAIL	INSTITUTIONAL	
	DNFCS 65–74 y (n=400)	DNFCS 75–97 y (n=307)	FRAIL (n=194)	INST-1 (n=60)	INST-2 (n=216)
Age (y)					
Male	69.1±2.8	78.3±3.1	79.3±5.9	80.3±7.6	78.7±7.1
Female	69.4±2.9	78.5±3.9	77.8±5.3	80.2±6.5	81.1±7.8
Weight (kg)					
Male	78.5±10.4	77.5±10.6	73.2±8.3	78.1±7.6	75.9±13.4
Female	72.4±13.3	70.1± 1.7	63.7±8.7	64.4±10.5	71.6±17.4
BMI (kg/m^2)					
Male	25.4±3.0	25.5±3.1	24.3±2.1	27.1±3.9	22.7±9.7
Female	26.8±4.6	25.8±3.9	24.5±2.9	25.6±3.8	27.2±9.2

DNFCS: Dutch national food consumption survey. INST-1: intervention 1 among institutionalized elderly people. INST-2: intervention 2 among institutionalized elderly people. Values are means ± SD.

Lowest dietary protein intakes were observed in institutionalized elderly people showing a mean intake of 56±17 g/d for men and 55±15 g/d for women in the INST-2 study. The highest protein intakes, which averaged 85.9±23.9 g/d, were reported in community-dwelling elderly men (Table 2.2). Dietary protein intake, expressed as g/kg-bw/d, was 0.8±0.3 g/kg-bw/d in institutionalized elderly people, 1.0±0.3 g/kg-bw/d in frail elderly people, and 1.1±0.3 g/kg-bw/d in community-dwelling elderly people. Dietary protein intake of the institutionalized elderly people was significantly lower than the protein intake of community-dwelling elderly people ($P<0.001$) (**Table 2.2**). Furthermore, 21% of elderly people in the INST-1 study and 35% of the elderly people in the INST-2 study had a protein intake below the estimated average requirement (EAR), whereas 10% of the community-dwelling and frail elderly people had an intake below this reference.

The distribution of protein intake across breakfast, lunch, dinner, and snacks times (i.e. in between meals) is presented in **Figure 2.1**. Dietary protein intake at breakfast was 10±10 g

Table 2.2 Energy and protein intake in community-dwelling, frail, and institutionalized elderly people

	COMMUNITY-DWELLING		FRAIL	INSTITUTIONAL	
	DNFCS 65–74 y (n=400)	DNFCS 75–97 y (n=307)	FRAIL (n=194)	INST-1 (n=60)	INST-2 (n=216)
Energy intake (MJ/d)					
Male	9.4±2.4^{a}	9.2±2.5^{a}	8.7±2.0^{a}	8.2±1.6^{a}	5.8±1.5^{b}
Female	7.5±1.9^{a}	7.5±1.8^{ab}	7.0±1.5^{bc}	6.2±1.5^{cd}	5.9±1.6^{d}
Protein intake (g/d)					
Male	85.9±23.9^{a}	81.9±25.2^{ab}	75.4±21.3^{b}	66.9±18.8^{bc}	56.3±17.1^{c}
Female	72.9±18.2^{a}	71.6±18.8^{a}	62.4±14.9^{b}	54.0±12.9^{c}	55.5±15.4^{c}
Protein intake (g/kg-bw/d)					
Male	1.11±0.31^{a}	1.07±0.35^{ab}	1.04±0.29^{ab}	0.86±0.22^{bc}	0.78±0.28^{c}
Female	1.03±0.35^{a}	1.05±0.32^{a}	1.00±0.27^{a}	0.85±0.20^{b}	0.81±0.29^{b}
Protein intake (en%)					
Male	15.8±3.5^{ab}	15.3±3.2^{ab}	14.8±2.9^{a}	13.9±2.8^{a}	16.4±2.6^{b}
Female	16.9±3.9^{a}	16.5±3.5^{ab}	15.3±2.7^{c}	15.0±3.0^{bc}	16.3±2.5^{ac}

DNFCS: Dutch national food consumption survey. INST-1: intervention 1 among institutionalized elderly people. INST-2: intervention 2 among institutionalized elderly people. Values are means ± SD. Values with different superscript letters indicate significant differences in energy and protein intake of the elderly populations according to Bonferroni post hoc test ($P<0.05$).

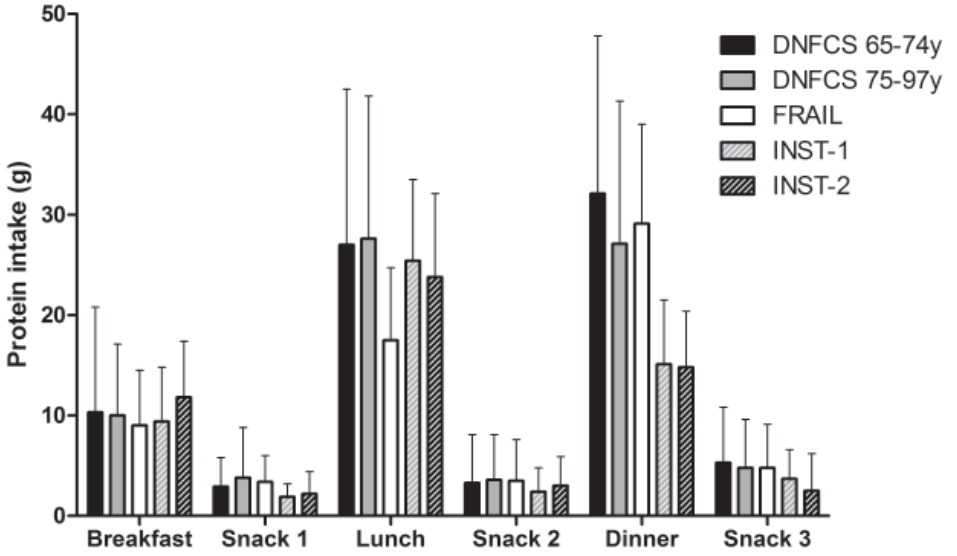

Figure 2.1 Daily protein intake in g distributed per mealtime in community-dwelling, frail, and institutionalized elderly people. DNFCS: Dutch national food consumption survey. INST-1: intervention 1 among institutionalized elderly people. INST-2: intervention 2 among institutionalized elderly people. Values are means ± SD. Snack 1 represents: Protein intake between breakfast and lunch. Snack 2 represents: Protein intake between lunch and dinner. Snack 3 represents: Protein intake after dinner.

in community-dwelling, 8±5 g frail, and 12±6 g in institutionalized elderly people. During lunch the community-dwelling elderly people consumed on average 27±15 g protein per meal. Seventy percent of the community-dwelling elderly people consumed a bread containing meal, which contained 19±9 g protein per meal during lunchtime (*data not shown*). When a hot meal was used during lunch, the average protein intake was 39±16 g and 35±9 g in elderly from the DNFC 65–74 y and DNFC 75–97 y study, respectively. Frail elderly people consumed on average 18±7 g of protein during lunchtime. In the institutionalized elderly people, the hot meal was consumed during lunchtime resulting in 24±8 g and 25±8 g protein for the elderly people in respectively the INST-2 and INST-1 study. During dinnertime the lowest protein intakes were found in the institutionalized elderly people because of the consumption of a bread meal.

During the day, mostly animal proteins (65%), especially from meat and dairy products, contributed to dietary protein intake (**Table 2.3**). During breakfast, 50% of the protein

Table 2.3 The 5 main food groups contributing to daily protein intake and protein intake during breakfast for community-dwelling, frail, and institutionalized elderly people

	COMMUNITY-DWELLING				FRAIL		INSTITUTIONAL			
	DNFCS 65–74 y		DNFCS 75–97 y		FRAIL		INST-1		INST-2	
	Food group	%	Food group	%	Food group	%	Food group	%	Food group	%
Daily protein intake	Meat	29	Meat	30	Meat	24	Dairy	24	Dairy	30
	Dairy	18	Dairy	19	Dairy	21	Meat	18	Meat	27
	Bread	14	Bread	15	Bread	15	Bread	13	Bread	12
	Cheese	9	Cheese	8	Cheese	10	Fish	8	Cheese	6
	Fish	5	Fish	5	Fish	4	Cheese	8	Vegetables	3
	Other	25	Other	23	Other	26	Other	29	Other	22
Protein intake during breakfast	Bread	41	Bread	42	Bread	43	Dairy	37	Dairy	40
	Cheese	21	Cheese	21	Cheese	23	Bread	30	Bread	26
	Dairy	15	Dairy	14	Dairy	18	Cheese	20	Cheese	15
	Meat	9	Meat	8	Cereals	3	Meat	6	Meat	9
	Eggs	4	Eggs	5	Eggs	3	Pastry	2	Eggs	5
	Other	10	Other	10	Other	10	Other	5	Other	5

DNFCS: Dutch National Food Consumption Survey. INST-1: intervention 1 among institutionalized elderly people. INST-2: intervention 2 among institutionalized elderly people. Values expressed in % of daily protein intake and protein intake during breakfast. Meat represents: Meat, meat products, and poultry. Dairy represents: Milk and milk products with the exception of cheese. Other represents: All other food groups contributing to protein combined.

intake was derived from vegetable proteins in the community-dwelling elderly with bread as predominant source (41%). In the institutionalized elderly people, dietary protein was mostly derived from dairy products during breakfast (37% in the INST-1 study and 40% in the INST-2 study). During the hot meal, either served at lunchtime or at dinnertime, meat and dairy products prevailed. There were no gender differences in the distribution of protein intake and in the contribution of specific food sources to dietary protein intake.

Discussion

This study provides detailed information on dietary protein intake, the distribution of protein intake throughout the day, and intake of protein containing food sources in community-dwelling, frail, and institutionalized elderly people. Dietary protein intakes are well above the RDA in community-dwelling and frail elderly people. In institutionalized elderly people, a significant proportion showed an intake below the average protein requirement, which makes them an important target population for dietary interventions. Dietary protein intake was particularly low at breakfast with bread and dairy as main protein sources.

A major strength of the present analysis is that dietary intake data were collected from well characterized elderly population groups differing in health status. The community-dwelling elderly subpopulation represents apparently healthy, independently nationwide living elderly people. This group was stratified into two different age groups in order to allow comparisons with frail and institutionalized elderly people, similar in age but with a different health status. As compared to community-dwelling elderly people, frail elderly people had a worse health profile and a lower physical activity level (PASE score of 85 vs. 64)[34]. Considering and reflecting on the current widely used Fried criteria, we feel confident to have properly classified the population as being frail[35-37]. Institutionalized elderly people were described either as a borderline-demented, or as a somatic disordered population[27].

For comparative purposes, the use of the same methodology is important. In our study the same dietary assessment method, the dietary record, was used across studies. This method has been described as a suitable instrument for assessing energy and protein intake in elderly people[38,39]. The latter has also been validated against urinary nitrogen studies in both community-dwelling and institutionalized elderly people[38]. Despite the similarity in dietary assessment method, a possible limitation might be the difference in number of days (2-d food records and 3-d food records). Additional analysis, however, showed no differences in the level of dietary protein intake between a 2- or 3-d assessment in both

frail and institutionalized elderly (INST-2). Furthermore, variances of the protein intake in the different elderly subpopulations were equal indicating a limited effect of the one day difference. Another limitation might be the use of different food composition tables across studies. As a result of updating food composition tables, composition of several products might have been changed. However, comparison between the food composition tables showed similar protein content of the various food products.

In our study, we observed the lowest average protein intake in the institutionalized elderly (0.8±0.3 g/kg-bw/d). Thirty-five percent of this population reported a daily protein intake below the estimated average protein requirement of 0.7 g/kg-bw/d[40]. Yet on average, the protein intake equals the RDA of 0.8 g/kg-bw/d. It has been discussed that though the RDA for daily protein intake might be adequate to prevent deficiency in young adults, it may be insufficient to maintain health, including the preservation of skeletal muscle loss at a more advanced age[15,41,42]. Several experimental studies suggest greater needs for elderly people when compared with young individuals[43,44]. Moreover, a prospective study among 2066 community-dwelling elderly people suggests higher requirements, as a protein intake of 1.2 g/kg-bw/d was significantly associated with approximately 40% less loss of lean body mass and appendicular lean body mass when compared with a protein intake of 0.8 g kg-bw/d after a 3 y period[14]. In view of these considerations, institutionalized elderly people, with an average protein intake of 0.8 g/kg-bw/d, would be an important target population for dietary interventions aiming to slow down or counteract sarcopenia.

In addition to daily protein intake, dietary protein intake with each meal might be important to maintain skeletal muscle mass in elderly people. Paddon-Jones et al. suggested that 25–30 g of dietary protein per meal is required to maximally stimulate skeletal muscle protein synthesis[15]. Ingestion of smaller, meal-like amounts of dietary protein, i.e. less than 20 g, attenuated the skeletal muscle protein synthetic response in elderly people when compared with young individuals[17]. In our study, we observed average protein intakes less than 12 g at breakfast. The latter protein intake is substantially below the proposed minimum of 20 g. Therefore, increasing the amount of dietary protein at breakfast to at least 20 g might represent a promising dietary strategy to enhance the skeletal muscle protein synthetic response in elderly people.

Finally, the intake of specific protein containing food sources might be of importance to modulate the muscle protein synthetic response[20-22]. In our study, 65% of daily protein intake was derived from animal products in all elderly subpopulations. Also breakfast was relatively rich in animal protein sources, especially dairy (including cheese), egg, and meat

sources. Though it is evident that the amount of protein during breakfast is too low to attain a maximal post-prandial muscle protein synthetic response[45], more work is needed to define the preferred protein source(s) that should be used to optimize post-prandial muscle protein synthetic response in elderly people.

In summary, institutionalized elderly people are an important target population for dietary interventions since a significant proportion of institutionalized elderly showed an intake below the average protein requirement. Improving dietary protein intake in the morning might represent an interesting strategy for dietary interventions aiming to postpone and treat sarcopenia in elderly people.

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3

Protein supplementation improves physical performance in frail elderly people; a randomized, double-blind, placebo-controlled trial

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Abstract

Objectives: Protein supplementation has been proposed as an effective dietary strategy to increase skeletal muscle mass and improve physical performance in frail elderly people. Our objective was to assess the impact of 24 wks dietary protein supplementation on muscle mass, strength, and physical performance in frail elderly people.

Design/setting/participants: A total of 65 frail elderly subjects were included and randomly allocated to either daily protein or placebo supplementation (15 g protein at breakfast and lunch).

Measurements: Skeletal muscle mass (DXA), muscle fiber size (muscle biopsy), strength (1-RM) and physical performance (SPPB) were assessed at baseline, after 12, and 24 wks of dietary intervention.

Results: Skeletal muscle mass did not change in the protein (from 45.8±1.7 to 45.8±1.7 kg) or placebo supplemented group (from 46.7±1.7 to 46.6±1.7 kg) following 24 wks of intervention ($P>0.05$). In accordance, type I and II muscle fiber size did not change over time ($P>0.05$). Muscle strength increased significantly in both groups ($P<0.01$), with leg extension strength tending to increase to a greater extent in the protein (57±5 to 68±5 kg) compared with the placebo group (57±5 to 63±5 kg) (treatment x time interaction effect: $P=0.059$). Physical performance improved significantly from 8.9±0.6 to 10.0±0.6 points in the protein group and did not change in the placebo group (from 7.8±0.6 to 7.9±0.6 points) (treatment x time interaction effect: $P=0.02$).

Conclusions: Dietary protein supplementation improves physical performance, but does not increase skeletal muscle mass in frail elderly people.

Introduction

Frailty is a geriatric syndrome of decreased reserves and resistance to stressors which results in an increased risk for adverse outcomes such as onset of disability, morbidity, institutionalization, and mortality[1]. An important and fundamental component of frailty is sarcopenia[2]. Sarcopenia is an age-related process characterized by the progressive loss of skeletal muscle mass, strength, and physical performance. The cause of sarcopenia is multi-factorial and includes a sedentary lifestyle and inadequate dietary protein intake[3-5]. It has been well-established that dietary protein ingestion stimulates skeletal muscle protein synthesis[6-10], and inhibits protein breakdown, resulting in a positive protein balance[9,10] and net muscle protein accretion[11-13]. Consequently, it has been proposed that increasing dietary protein intake represents an effective strategy to increase skeletal muscle mass and strength and, as such, counteract sarcopenia and frailty[14,15].

So far, evidence from long-term intervention studies shows no clear benefit of dietary protein supplementation on skeletal muscle mass in elderly people. Whereas some reveal an increase in skeletal muscle mass[16,17], others have failed to report any measurable impact of long-term dietary protein supplementation on skeletal muscle mass in elderly subjects[18-24]. This apparent discrepancy might be attributed to the selection of healthy versus more frail elderly subjects. Most studies investigating the impact of long-term protein supplementation have generally included healthy elderly people[18,21-24]. Long-term intervention studies investigating the efficacy of protein supplementation on skeletal muscle mass in frail elderly people, however, are scarce and show discrepant findings[16,19,20]. Whereas some report no effect of dietary protein supplementation on skeletal muscle mass and physical performance[19,20], others have reported a significant increase in muscle power and a tendency for an increase in skeletal muscle mass after protein supplementation[16]. To underpin the possible benefits of protein supplementation on skeletal muscle mass and physical performance in a frail elderly population, more evidence from well-designed long-term intervention trials is needed. Therefore, we investigated, in a randomized, double-blind, placebo-controlled manner, the impact of 24 wks dietary protein supplementation (15 g dairy protein, twice daily) on skeletal muscle mass, muscle fiber type characteristics, strength, and physical performance in a large group (n=65) of frail elderly men and women.

Methods

Subjects

Subjects with an age ≥65 y were recruited from an existing database of subjects, through distribution of information flyers, and by local information meetings organized between December 2009 and October 2010. Potentially eligible people were screened for pre-frailty and frailty using the Fried criteria[25]. These criteria were: [1] unintentional weight loss, [2] weakness, [3] self-reported exhaustion, [4] slow walking speed, and [5] low physical activity. Subjects were considered pre-frail when 1 or 2 criteria were applicable and frail when 3 or more criteria were present. After screening for frailty, the medical history of the subjects was evaluated, and subjects who were diagnosed with cancer or COPD were excluded. In addition, a fasted blood sample was collected to screen for type 2 diabetes and renal insufficiency. Subjects with type 2 diabetes, according to plasma glucose concentrations ≥7 mmol/L[26], and subjects with renal insufficiency, according to an estimated global filtration rate (eGFR) <60 mL/min/1.73 m^2 [27], were excluded. None of the subjects had a history of participating in any structured exercise training program over the past 2 y. In total, 65 pre-frail and frail elderly men and women were included in the 24 wks supplementation trial. The Wageningen University Medical Ethical Committee approved the study and subjects gave their written informed consent.

Study design

After inclusion, subjects were randomly allocated to either the protein or the placebo supplemented group. An independent person randomized the subjects by means of computer-generated random numbers in stratified permuted blocks of size 4, stratified by gender. Primary outcome measure was lean mass measured by dual-energy X-ray absorptiometry (DXA). Secondary outcome measures included muscle fiber cross sectional area (CSA, muscle biopsy), strength (one repetition maximum; 1-RM, handgrip strength), and physical performance (short physical performance battery; SPPB). Furthermore, blood samples were collected to determine plasma glucose, insulin, and markers of renal function and 3-d food records were collected to assess habitual dietary intake. All measurements were assessed at baseline (0 wks), after 12 wks, and after 24 wks of intervention. Sample size was calculated based on a lean mass difference of 1.14 kg between the protein group and placebo group[17]. With a SD of 1.4 kg, based on previously collected DXA data, a minimum of 24 subjects per treatment group would be required to detect a difference (power=80%, α=0.05). With an expected drop-out rate of 25%, a sample size of 30 subjects per treatment group was considered adequate.

Protein supplementation

Twice daily, subjects received either a 250 mL beverage containing 15 g protein (milk protein concentrate (MPC80), 7.1 g lactose, 0.5 g fat, and 0.4 g calcium, or a matching 250 mL placebo beverage containing no protein, 7.1 g lactose, and 0.4 g calcium (FrieslandCampina Consumer Products Europe, Wageningen, The Netherlands). The subjects consumed one beverage after breakfast and one beverage after lunch. All beverages were provided in non-transparent packages and were vanilla flavored to mask the contents of the drinks.

Anthropometrics and body composition

Height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Body weight was measured in the fasted state to the nearest 0.1 kg with a calibrated digital scale (ED-6-T; Berkel, Rotterdam, The Netherlands). In the fasted state, body composition and bone mineral density were measured by DXA (Lunar Prodigy Advance; GE Health Care, Madision, WI). DXA quality-assurance measurements were performed to ensure scanner reliability, and identical patient scan protocols were performed for all subjects.

Maximum strength and physical performance

Maximum strength was assessed by one repetition maximum (1-RM) strength tests on leg press and leg extension machines (Technogym, Rotterdam, the Netherlands). During a first familiarization session, the proper lifting technique was demonstrated and practiced, after which maximum strength was estimated using the multiple repetitions testing procedure for leg press and leg extension. In a second exercise session, ≥1 wk after the first strength estimation, the subjects' 1-RM strength was determined[5]. Handgrip strength was measured using a hydraulic hand dynamometer (Jamar, Jackson, MI, USA,). Three consecutive measures of handgrip strength at both hands were recorded to the nearest 0.5 kg with subjects sitting in an upward position and the arm in a 90 degrees angle position. The maximum strength effort was reported. Physical performance was assessed by the short physical performance battery (SPPB), which consists of three components: balance, gait speed, and chair rise ability[28]. Scores of 1 to 4 were based on categories of performance in the balance tests, on the time necessary to complete the walk, and on the time needed to perform the chair rise test. If subjects were unable to perform a test, they received a score of 0. A summary performance score of 0 to 12 was calculated by summing the scores of the 3 tests.

Blood sampling

After an overnight fast, blood samples were collected in EDTA-containing tubes and in serum tubes. EDTA-containing tubes were centrifuged at 1000g at 4˚C for 10 min and serum tubes were centrifuged 90 min after the blood collection at 1000g at 20˚C for 15 min. Aliquots of plasma and serum were frozen in liquid nitrogen and stored at -80˚C. Plasma samples were analyzed to determine glucose and insulin concentrations and serum samples were analyzed to determine creatinine concentrations to assess the estimated global filtration rate (eGFR)[29]. Plasma glucose concentrations were measured with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland). Insulin was measured by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO). Serum creatinine was measured by using Roche Modular System P (Roche Diagnostics GmbH, Mannheim, Germany).

Muscle biopsy sampling and immunohistochemistry

After local anesthesia, percutaneous needle biopsy samples (50–80 mg) were collected following an overnight fast from the *vastus lateralis* muscle, ~15 cm above the patella[30]. Any visible non-muscle tissue was removed immediately, and biopsy samples were embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands), frozen in liquid nitrogen-cooled isopentane, and stored at -80°C until further analyses.

From all biopsies, 5 µm thick cryosections were cut at -20˚C. Muscle biopsies were stained for muscle fiber typing as described in detail previously[31]. In short, the slides were incubated with primary antibodies against MHC-I (A4.840, Developmental Studies Hybridoma Bank, Iowa City, IA) and laminin (polyclonal laminin, Sigma, Zwijndrecht, the Netherlands). After washing, appropriate secondary antibodies were applied (goat anti-mouse IgM AlexaFluor555 and goat anti-rabbit IgG AlexaFluor647, respectively; Molecular Probes, Invitrogen, Breda, the Netherlands). Images were visualized and automatically captured at 10x magnification with a fluorescent microscope equipped with an automatic stage (IX81 motorised inverted microscope, Olympus, Hamburg, Germany). Muscle fiber type (fiber%) and fiber size were measured for each separate muscle fiber. All image recordings and analyses were performed by an investigator blinded to subject coding.

Dietary intake

The subjects recorded their food intake for 3 d. The days of recording were randomly assigned so that all days of the week, including weekend days, were equally represented.

Trained dieticians gave oral and written instructions about recording type of foods and estimating portion sizes in household measures. At a second visit, dieticians checked the food records for completeness, obtained additional information about unclear items or amounts, and used examples household measures to improve the estimation of portion sizes. Dietary intake data were coded (type of food, time of intake, and amount) and energy and macronutrient intakes were calculated using food calculation system (BAS nutrition software 2004, Arnhem, The Netherlands) in which the Dutch food composition database 2006 was included.

Health status

Overall health status of the subjects was assessed using the 12-Item Short Form Health Survey (SF-12). This is a short form of the widely used SF-36. The SF-12 generates a physical composite score and mental composite score, which are well-validated measures of general physical and mental health, respectively. Higher physical and mental composite scores indicate better health[32].

Blood pressure

After 10 min of supine rest, 4 blood pressure measurements with 2 min intervals were performed in the morning following an overnight fast, using a validated automatic blood pressure device (Omron HEM-907, Lake Forest, IL, USA). The first measurement was discarded and the subsequent 3 measurements were averaged.

Cognitive function

The Mini-Mental State Examination (MMSE) was used to screen for possible cognitive disorders. The score ranges from 0 to 30. A score >25 was used as a cut-off value for the absence of cognitive disorders[33].

Statistics

Data analysis was performed by the intention to treat principle and according to a predefined data analysis plan. Data are expressed as means±SEM. Baseline characteristics were compared between treatment groups using an independent student T-test. Differences between treatments over time were analyzed using mixed linear models with Toeplitz covariance structure. Time, treatment, and their interaction were defined as fixed factors and subject was defined as a random factor. Muscle fiber type characteristics

were analyzed by adding an additional within-subjects factor (fiber type) in the model. All statistical analyses were performed using SPSS Statistics v19. An α-level of 0.05 was used to determine statistical significance.

Results

Subjects

Between December 2009 and October 2010, 734 subjects were invited to participate in the study of which 165 subjects were screened. A total of 65 subjects fulfilled the frailty criteria and were found eligible to include into the study. In total, 8 subjects withdrew from the study, 4 in each group. For the intention to treat analyses, 4 dropouts were willing to have final assessments. Since there were no differences in primary and secondary outcome measures between the intention to treat analyses and the per protocol analysis, all data were analyzed according to the intention to treat principle. Baseline characteristics are presented in **Table 3.1** and showed no significant differences between groups (P>0.05).

Table 3.1 Subjects' characteristics

Variable	Placebo (n=31)	Protein (n=34)
Age (y)	81±1	78±1
Female / Male (#)	16/15	20/14
Weight (kg)	73.8±2.2	73.9±2.4
Height (m)	1.67±0.02	1.65±0.02
BMI (kg/m^2)	26.2±0.6	27.0±0.6
MMSE (points)	27.4±0.4	27.6±0.5
PCS12 (points)	42.7±1.8	43.9±1.7
MCS12 (points)	54.9±1.4	54.0±1.3
Glucose (mmol/L)	5.3±0.1	5.2±0.1
Insulin (mU/L)	18.0±1.2	18.0±1.2
eGFR (mL/min/1.73 m^2)	77.6±3.2	84.4±3.1
Systolic BP (mmHg)	150±4	152±4
Diastolic BP (mmHg)	75±2	76±2

Data represent means±SEM. BMI: Body Mass Index. MMSE: Mini Mental State Examination. PCS12: Physical component score SF12. MCS12: Mental component score SF12. eGFR: estimated Globular Filtration Rate. BP: Blood pressure. No differences between groups (P>0.05).

The average MMSE score was 27.4±0.4 and 27.6±0.5 points in the placebo and protein group, respectively, indicating an absence of cognitive disorders. The average adherence to the treatment based on ticked calendars and non-consumed beverages was ≥92% and did not differ between groups.

Body composition

No significant time, treatment, or treatment x time interaction effects were observed on any of the body composition parameters (**Table 3.2**: P>0.05). Lean body mass did not increase in the protein (from 45.8±1.7 to 45.8±1.7 kg) or placebo group (from 46.7±1.7 to 46.6±1.7 kg; **Figure 3.1**). In accordance, type I and II muscle fiber size did not change during the intervention in both the placebo and protein supplemented group (P>0.05) Type II muscle fiber size was smaller compared with type I muscle fiber CSA (P<0.05).

Muscle strength

Muscle strength parameters are presented in **Table 3.3**. At baseline, muscle strength did not differ between the protein and placebo group (P>0.05). After 24 wks, handgrip strength in both groups had not improved (P>0.05). Muscle strength, assessed at the leg press, had significantly increased from 124±9 to 139±9 kg in the placebo group and from

Table 3.2 Body composition at baseline (0 wks), after 12 wks, and after 24 wks of intervention in the placebo and protein group

	Placebo			Protein		
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks
Body weight (kg)[a]	73.8±2.2	74.1±2.3	73.3±2.3	73.9±2.4	74.5±2.3	74.3 ± 2.2
Fat mass (kg)[b]	23.9±1.5	24.0±1.5	23.4±1.5	25.3±1.5	25.2±1.5	25.3±1.5
ALM (kg)[b]	19.5±0.8	19.4±0.8	19.6±0.8	19.2±0.8	19.4±0.8	19.3±0.8
Type I muscle fiber CSA (mm^2)[c]	4.6±0.3	4.2±0.3	4.1±0.3	4.6±0.3	5.4±0.3	5.1±0.3
Type II muscle fiber CSA (mm^2)[c]	2.9±0.3	2.8±0.3	3.0±0.3	3.4±0.3	4.1±0.3	3.3±0.3
BMC (kg)[b]	2.6±0.1	2.6±0.1	2.6±0.1	2.5±0.1	2.5±0.1	2.5±0.1

Data represent means±SEM. ALM: Appendicular lean mass. CSA: Cross sectional area. BMC: Bone Mineral Content. Intention to treat data were analyzed using a mixed linear model ([a] n=65; [b] n=62, [c] n=46). No significant treatment x time interaction or main effects were observed (P>0.05).

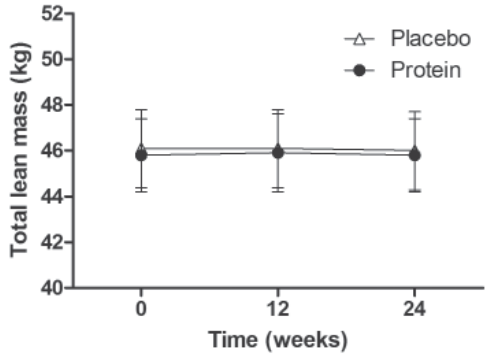

Figure 3.1 Intention to treat analysis on total lean mass in the placebo and protein group (n=62). Data represents means±SEM. There was a no significant treatment x time interaction effect or main effects (P>0.05).

Table 3.3 Muscle strength and physical performance at baseline (0 wks), after 12 wks, and after 24 wks of intervention in the placebo and protein group

	Placebo			Protein		
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks
Leg press strength (kg)[a]	124±9	129±9	139±9	118±8	121±8	136±8
Leg extension strength (kg)[a]	57±5	59±5	63±5	57±5	64±5	68±5
Handgrip strength (kg)[b]	26±2	27±2	26±2	26±2	25±2	26±2
Gait speed (sec)[c]	6.1±0.6	6.4±0.6	6.3±0.6	5.5±0.6	5.6±0.6	5.6±0.6
Chair rise (sec)[d]	11.9±1.1	13.7±1.1	14.1±1.2	13.2±1.0	11.7±1.0	11.1±1.1

Data represents means±SEM. Intention to treat data were analyzed using a mixed linear model ([a] n=50; [b] n=65, [c] n=61, [d] n=48). Leg press data showed a significant time effect (P<0.001) and no treatment x time interaction effect (P>0.05). Leg extension data showed a trend treatment x time interaction effect (P=0.059) and a significant time effect (P<0.0001). Chair rise data showed a trend treatment x time interaction effect (P=0.055). Handgrip and gait speed data showed no interaction or main effects (P>0.05).

118±8 to 136±8 kg in the protein group, with no significant treatment x time interaction effect (P>0.05). Muscle strength assessed at the leg extension, however, did reveal a trend treatment x time interaction effect (P=0.059), reflecting a borderline significantly greater increase in muscle strength in the protein group (57±5 to 68±5 kg) compared with the placebo group (57±5 to 63±5 kg).

Physical performance

Physical performance (SPPB) data are presented in **Figure 3.2**. At baseline, no significant difference in total SPPB score was observed between the placebo and protein group (P>0.05). After 24 wks, a significant treatment x time interaction effect was found (P=0.02). Physical performance improved significantly from 8.9±0.6 to 10.0±0.6 points in the protein group and showed no improvements in the placebo group (from 7.8±0.6 to 7.9±0.6 points). Of the 3 components of the SPPB, chair rise ability showed the most pronounced difference between the protein and placebo group (**Table 3.3**). After 24 wks, the ability to stand up from a chair tended to be faster in the protein group (13.7±1.0 to 11.1±1.1 sec) compared with the placebo group (11.9±1.1 to 14.1±1.2 sec; P-value for treatment x time interaction = 0.055).

Blood measurements

Baseline plasma glucose and insulin concentrations are presented in **Table 3.1**. Glucose and insulin concentrations did not differ at baseline and did not change over time in either group (*data not shown*). The estimated globular filtration rates (eGFR) did not differ between groups at baseline (**Table 3.1**) and did not change over time (*data not shown*).

Dietary intake

Dietary intake data are presented in **Table 3.4**. Baseline daily protein intake was 1.0 g/kg-bw/d and did not change significantly overtime in either group (P>0.05). Daily

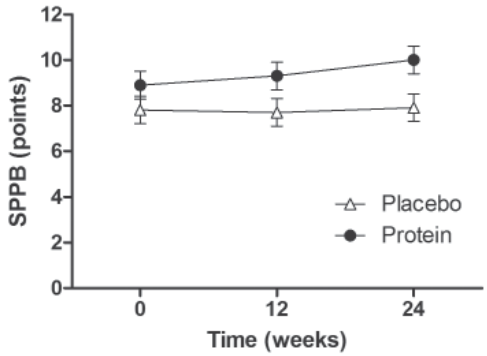

Figure 3.2 Intention to treat analysis on physical performance (SPPB) in the placebo and protein group (n=61). Data represents means±SEM. There was a significant treatment x time interaction effect (P=0.02).

Table 3.4 Habitual dietary intake (without protein supplements)

	Placebo			Protein		
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks
Energy intake (MJ/d)	8.1±0.4	8.1±0.4	7.8±0.4	8.1±0.4	7.3±0.4	7.5±0.4
Protein intake (g/d)	74±4	74±4	74±4	78±4	71±4	71±4
Protein (g/kg-bw/d)	1.0±0.0	1.1±0.1	1.0±0.1	1.0±0.0	1.1±0.1	1.0±0.1
Protein at breakfast (g)	11±1	11±1	12±1	13±1	12±1	12±1
Protein at lunch (g)	19±2	17±2	18±2	20±2	18±2	17±2
Protein at dinner (g)	32±2	35±2	33±2	32±2	29±2	29±2
Protein (en%)	16±1	16±1	17±1	16±1	17±1	16±1
Fat (en%)	33±1	35±1	34±1	36±1	36±1	35±1
Carbohydrate (en%)	48±1	46±1	46±1	44±1	44±1	44±1

Data represent means±SEM. en%: energy percentage. Intention to treat data were analyzed using a mixed linear model (n=65). Energy intake data showed no treatment x time interaction effect (P=0.60). A significant time effect was observed (P=0.04). There was no significant treatment, time, or treatment x time interaction effect of any of the other variables.

protein intake at breakfast, lunch, and dinner did not differ between groups prior to the intervention and did not change over time. With the addition of the protein supplements, 15 g protein at breakfast and 15 g protein at lunchtime, the daily protein intake increased from 1.0 to 1.4 g/kg-bw/d in the protein group during the intervention. No significant baseline differences in daily energy and macronutrient intake were observed between groups (P>0.05). After 24 wks, daily energy intake was significantly reduced (P=0.04), with no significant differences over time between groups (P>0.05). Fat and carbohydrate intake did not differ between the groups and did not change over time (P>0.05).

Health status, blood pressure and cognitive function

Health status (SF-12 scores), blood pressure and cognitive function (MMSE) parameters did not differ between groups at baseline (**Table 3.1**) and did not change over time in either group (*data not shown*).

Discussion

The present study shows that 24 wks of dietary protein supplementation did not augment skeletal muscle mass in frail elderly people. In contrast, physical performance had improved significantly following dietary protein supplementation.

In the present study, we aimed to investigate the impact of long-term dietary protein supplementation on skeletal muscle mass, strength, and physical performance in frail elderly men and women. To include an adequate sample of frail elderly subjects, a large group of elderly people were informed (n=734) and screened (n=165) to select 65 subjects that met the frailty criteria described by Fried et al.[25]. These criteria have been reported to be highly predictive for falls, hospitalization, disability, and mortality[25]. In agreement, the selected subjects were characterized by low baseline physical performance (**Figure 3.2**) and poor handgrip strength (**Table 3.3**), which confirms their frailty.

Compliance of the subjects to the dietary intervention, providing either protein (15 g) or placebo twice daily, was excellent. After 24 wks of dietary intervention, ≥92% of the provided drinks were consumed in both the protein and placebo supplemented group, showing that the consumption of 2 supplements per day was well tolerated. Although, we observed a reduction in daily energy intake over time, we observed no reduction in daily protein intake during the intervention (**Table 3.4**). Consequently, daily protein intake increased from 1.0±0.1 towards 1.4±0.1 g/kg-bw/d following protein supplementation, whereas in the placebo group daily protein intake did not change over time (1.0±0.1 g/kg-bw/d). Previous assessment of dietary protein intake in elderly subpopulations in the Netherlands has shown that daily protein intake is not equally distributed over the various main meals, and that breakfast and lunch are particularly low in protein[34]. In agreement, the frail elderly subjects consumed 13±1, 20±2 and 32±2 g protein with breakfast, lunch, and dinner, respectively, prior to intervention. By supplementing the subjects in the protein group with 15 g protein twice daily, protein intake increased to more than 25 g of protein with each main meal. In contrast, in the control group, the subjects ingested relative small amounts of dietary protein at breakfast (11±1 g) and lunch (17±2 g) during the intervention[34].

It has been suggested that 20-25 g of dietary protein per meal is required to allow an appropriate stimulation of post-prandial muscle protein synthesis[8,35-37]. Ingestion of smaller amounts of dietary protein has been reported to attenuate the skeletal muscle protein synthetic response in elderly people[38]. We hypothesized that increasing dietary protein intake at breakfast and lunch would stimulate post-prandial muscle protein synthesis and decrease muscle protein breakdown, resulting in a more positive protein balance

after each meal, resulting in net skeletal muscle protein accretion following 24 wks of intervention[34,36,39]. Despite the greater protein intake at both breakfast and lunch in the protein group, no measurable gains in skeletal muscle mass were detected on a whole-body or muscle fiber level. Our data seem to be in line with most previous publications showing no measurable effect of protein supplementation on skeletal muscle mass in elderly people[18-24,40]. We anticipated a 1.14 kg increase in muscle mass following 24 wks of protein supplementation. With a population size of 65, a significance level of 0.05, and a power of 0.8, the limit for a statistically detectable change in skeletal muscle mass would have been 1.0±1.4 kg, which is easily detected by DXA scanning (with a CV for lean tissue <0.5%). However, it should be noted that differences smaller than 1.0 kg would not have been detectable, but could still be of considerable clinical benefit over the course of years.

Despite the absence of a measurable gain in skeletal muscle mass following prolonged dietary protein supplementation in frail elderly people, we observed significant improvements in physical performance in the protein group (**Figure 3.2**). The SPPB score increased from 8.9±0.6 to 10.0±0.6 points in the protein group, whereas in the placebo the SPPB remained unchanged (7.8±0.6 to 7.9±0.6 points). Such an increase in physical performance is of substantial clinical relevance[41,42] and translates to a 30% relative risk reduction for disability[43] and a reduced risk for institutionalization and mortality[28]. In agreement with the SPPB, we observed a strong tendency (P=0.059) of greater gains in leg extension strength in the protein supplemented group when compared with the placebo group. Our findings tend to be in line with previous data showing that protein supplementation improves physical performance in frail elderly people in the absence of measurable increases in skeletal muscle mass[16]. The improvements in physical performance without a significant increase in skeletal muscle mass may be attributed to the absence of a linear relationship between skeletal muscle mass, strength, and physical performance[44,45]. In fact, changes in muscle strength and/or physical performance are generally observed before measurable changes in skeletal muscle mass become apparent[46]. The latter observation suggests that clinically relevant increases in strength and physical performance can be achieved without measurable increases in whole-body or appendicular lean mass. Furthermore, it could be speculated that such improvements in physical performance in the protein supplemented group are due to improvements in neuromuscular action and/or skeletal muscle quality[47]. More research is warranted to study the impact of greater dietary protein provision on muscle strength and physical performance in frail elderly people.

In the present study, we show that protein supplementation improves physical performance. Moreover, the increase in daily protein ingestion from 1.0 to 1.4 g/kg-

bw/d achieved by the supplements was not accompanied by any health complaints or side effects and did not affect renal function throughout the intervention period. These results and others[16] point towards the application of dietary protein supplementation in frail elderly people as a promising nutritional strategy to improve physical performance, attenuate the progression of frailty, and delay the onset of disability.

We concluded that long-term dietary protein supplementation (15 g dairy protein, twice daily) improves physical performance, but does not increase skeletal muscle mass in frail elderly people.

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Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people; a randomized, double-blind, placebo-controlled trial

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Abstract

Background: Protein supplementation has been proposed as an effective dietary strategy to augment the skeletal muscle adaptive response to prolonged resistance-type exercise training in elderly people. Our objective was to assess the impact of protein supplementation on muscle mass, strength, and physical performance during prolonged resistance-type exercise training in frail elderly men and women.

Methods: We performed a randomized, double-blind, placebo-controlled trial with 2 arms in parallel among 62 frail elderly subjects (78±1 y). These elderly subjects participated in a progressive resistance-type exercise training program (2 sessions per wk for 24 wks) during which they were supplemented twice daily with either protein (2 * 15 g) or a placebo. Lean body mass (DXA), strength (1-RM) and physical performance (SPPB) were assessed at baseline, after 12, and 24 wks of intervention.

Results: Lean body mass increased from 47.2 kg (95% CI, 43.5–50.9) to 48.5 kg (95% CI, 44.8–52.1) in the protein group and did not change in the placebo group (from 45.7 kg, 95% CI, 42.1–49.2 to 45.4 kg, 95% CI, 41.8–48.9) following the intervention (P-value for treatment x time interaction = 0.006). Strength and physical performance improved significantly in both groups (P=0.000) with no interaction effect of dietary protein supplementation.

Conclusions: Prolonged resistance-type exercise training represents an effective strategy to improve strength and physical performance in frail elderly people. Dietary protein supplementation is required to allow muscle mass gain during exercise training in frail elderly people.

Introduction

Frailty is a geriatric syndrome of decreased reserves and resistance to stressors, which increases the risk for adverse outcomes such as the onset of disability, morbidity, and institutionalization[1,2]. An important and fundamental component of frailty is sarcopenia[3]. Sarcopenia is characterized by a progressive loss of skeletal muscle mass, strength, and physical performance[4]. The cause of sarcopenia is multi-factorial and includes a sedentary lifestyle and inadequate protein intake[5-7]. A single session of resistance-type exercise increases both muscle protein synthesis and breakdown rates, albeit the latter to a lesser extent[8,9]. Although exercise improves muscle protein balance, net balance will remain negative in the absence of food intake. Protein ingestion prior to or after exercise is required to further augment post-exercise muscle protein synthesis rates and inhibit protein breakdown[8,10-12], resulting in a positive protein balance and, as such, net muscle protein accretion[13-16]. Consequently, it has been proposed that dietary protein supplementation is required to maximize skeletal muscle mass gain during prolonged resistance-type exercise training and, as such, to more effectively counteract sarcopenia and frailty[17,18].

So far, there is ample evidence reporting beneficial effects of long-term resistance-type exercise training on muscle mass and performance in healthy elderly people[19-25]. In contrast, studies investigating the impact of such prolonged exercise interventions in frail elderly are scarce and report discrepant findings[17,18,26]. Whereas Fiatarone et al. showed a significant increase in muscle mass after 10 wks of resistance-type exercise training[26], others have failed to detect measurable increases in muscle mass and/or physical performance in frail elders[27,28]. We hypothesized that dietary protein supplementation is needed to increase muscle mass, strength, and physical performance during prolonged resistance-type exercise training in frail elderly people. Therefore, 62 frail elderly men and women were selected to participate in a 24 wk supervised resistance-type exercise training program during which they were supplemented with or without additional dietary protein (2 * 15 g daily) in a randomized, double-blind, placebo-controlled, manner.

Methods

Subjects

Elderly subjects (≥65 y) were recruited from an existing database, through distribution of flyers, and by local information meetings between December 2009 and September

2010. Potentially eligible elderly people were screened for pre-frailty and frailty using the Fried criteria[2]. These criteria were: [1] unintentional weight loss, [2] weakness, [3] self-reported exhaustion, [4] slow walking speed, and [5] low physical activity. Pre-frailty was classified when one or two criteria were present and frailty was defined when three or more criteria were present. Medical history of all subjects was evaluated. Subjects who were diagnosed with cancer, COPD, muscle disease or who were unable to perform the exercise regimen were excluded. Subjects with type 2 diabetes (≥ 7 mmol/L)[29] and renal insufficiency (eGFR <60 mL/min/1.73 m^2)[30] were excluded. A resting electrocardiogram was performed to exclude silent ischemia. The Wageningen University Medical Ethical Committee approved the study and subjects gave their written informed consent.

Study design

After inclusion, subjects were randomly allocated to either protein or placebo supplementation. Both groups were included in a 24 wk resistance-type exercise training program. An independent person randomized subjects by means of computer-generated random numbers in stratified permuted blocks of size four, stratified by gender. Primary outcome measure was lean body mass measured by dual-energy X-ray absorptiometry (DXA). Secondary outcome measures included maximum strength (one repetition maximum; 1-RM, handgrip strength), and physical performance (short physical performance battery; SPPB). In addition, blood samples were collected to determine plasma glucose and insulin concentrations and markers for renal functional decline. Furthermore, 3-d food records were collected to define habitual dietary intake. All measures were collected prior to and after 12 and 24 wks of intervention.

Resistance-type exercise training program

The resistance-type exercise training was performed 2 times per wk under personal supervision for a 24 wk period. The sessions were performed in the morning and afternoon with at least 72 h between sessions. The training consisted of a 5 min warm-up on a cycle ergometer, followed by 4 sets on the leg press and leg extension machines and 3 sets on chest press, lat pulldown, pec-dec, and vertical row machines (Technogym, Rotterdam, the Netherlands). The workload started at 50% of 1-RM (10–15 repetitions per set) and was increased to 75% of 1-RM (8–10 repetitions) to stimulate muscle hypertrophy. Resting periods of 1 min were allowed between sets and 2 min between exercises. To evaluate changes in muscle strength, 1-RM was repeated after 4, 8, 12, 16, and 20 wks of training. Workload intensity was adjusted based on the 1-RM outcomes.

Protein supplementation

Twice daily, the subjects received either a 250 mL protein supplemented beverage containing 15 g protein (MPC80; milk protein concentrate), 7.1 g lactose, 0.5 g fat, and 0.4 g calcium, or a matching placebo supplement containing no protein, 7.1 g lactose, and 0.4 g calcium (FrieslandCampina Consumer Products Europe, the Netherlands). All beverages were vanilla flavored to mask the contents of the drinks and packages were non-transparent. The subjects consumed 1 beverage directly after breakfast and 1 beverage directly after lunch. Staff members and subjects were blinded towards treatment allocation until completion of data analysis.

Body composition

Height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Body weight was measured in the fasted state to the nearest 0.1 kg with a calibrated digital scale (ED-6-T; Berkel, Rotterdam, The Netherlands). In the fasted state, lean body mass, fat mass and bone mineral density were measured by DXA (Lunar Prodigy Advance; GE Health Care, Madision, WI, USA).

Maximum strength and physical performance

Maximum strength was assessed by 1-RM strength tests on leg press and leg extension machines (Technogym, Rotterdam, the Netherlands). During a first familiarization session, the proper lifting technique was practiced, after which maximum strength was estimated. In a second session, 1-RM strength was determined[10]. Handgrip strength was measured using a hydraulic hand dynamometer (Jamar, Jackson, MI, USA). Three consecutive measures of handgrip strength (kg) at both hands were recorded to the nearest 0.5 kg with subjects sitting in an upward position and the arm in a 90 degree angle. Physical performance was assessed by the SPPB, which consists of 3 components: balance, gait speed, and chair rise ability[31]. Scores of 1 to 4 were based on categories of performance in the balance tests, on the time necessary to complete the walk and on the time needed to perform the chair-rise test. A summary performance score of 0 to 12 was calculated by summing the scores of the tests.

Blood sampling

Following an overnight fast, blood samples were collected in EDTA-containing and serum tubes. EDTA-containing tubes were centrifuged at 1000g at 4˚C for 10 min and serum tubes were centrifuged 90 min after the blood collection at 1000g at 20˚C for 15 min.

Aliquots of plasma and serum were frozen in liquid nitrogen and stored at -80˚C. Plasma glucose concentrations were measured with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland). Plasma insulin concentrations were measured by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO, USA). Serum creatinine concentrations were measured by using Roche Modular System P (Roche Diagnostics GmbH, Mannheim, Germany).

Dietary intake

The subjects recorded their food intake for 3 d. The days of recording were randomly assigned so that all days of the week, including weekend days, were equally represented. Trained dieticians gave oral and written instructions about recording type of foods and estimating portion sizes in household measures. During a second visit, dieticians checked the food records for completeness, obtained additional information about unclear items or amounts, and used examples of household measures to improve the estimation of portion sizes. Dietary intake data were coded (type of food, time of intake, and amount) and energy and macronutrient intakes were calculated using a food calculation system (BAS nutrition software 2004, Arnhem, The Netherlands) in which the Dutch food composition database 2006 was included.

Health status

Overall health status of the subjects was assessed using the 12-Item Short Form Health Survey (SF-12). The SF-12 generates a physical composite score (PCS12) and mental composite score (MCS12). Higher physical and mental composite scores indicate better health[32].

Blood pressure

After 10 min of supine rest, 4 blood pressure measurements with 2 min intervals were performed in the morning following an overnight fast, using a validated automatic blood pressure device (Omron HEM-907, Lake Forest, IL, USA). The first measurement was discarded and the subsequent 3 measurements were averaged.

Cognitive function

The Mini-Mental State Examination (MMSE) was used to assess cognitive function[33]. The score ranges from 0 to 30. A higher score represents a better cognitive function.

Statistical analysis

Sample size was calculated based on an expected difference in lean body mass of 1.1 kg between groups[34]. With an SD of 1.4 kg, a minimum of 24 subjects per treatment group would be required to detect a difference (power=80%, α=0.05). With an expected drop-out rate of 25%[35,36], a sample size of 30 subjects per treatment group was considered adequate. Data analysis was performed by the intention to treat principle and according to a predefined data analysis plan. Means for baseline and follow-up data were expressed with SD, SEM or 95% confidence intervals (95% CI). Baseline characteristics were compared between treatment groups using an independent student T-test. Differences between treatments over time were analyzed using mixed linear models with Toeplitz covariance structure. Time, treatment, and their interaction were defined as fixed factors and subject was defined as a random factor. All statistical analyses were performed using SPSS Statistics v19. An α-level of 0.05 was used to determine statistical significance.

Results

Between December 2009 and October 2010, 686 subjects were invited to participate in the study, 233 subjects were screened, and 62 subjects were included in the study. In total, 11 subjects withdrew from the study: 5 from the protein and 6 from the placebo group. Ten subjects gave various non-study related medical complications as reasons for their withdrawal and 1 subject gave heavy burden of the study as reason for withdrawal. For the intention to treat analyses, 4 dropouts were willing to have final assessments (**Figure 4.1**). The average adherence to the treatment, based on ticked calendars and non-consumed returned beverages, was ≥98% and did not differ between groups (P>0.05). Baseline characteristics are presented in **Table 4.1** and showed no baseline differences between groups (P>0.05).

Body composition

Lean body mass increased from 47.2 (95% CI, 43.5–50.9) to 48.5 kg (95% CI, 44.8–52.1) in the protein group, and did not change in the placebo group (from 45.7 kg, 95% CI, 42.1–49.2 to 45.4 kg, 95% CI, 41.8–48.9) following 24 wks of intervention (**Figure 4.2**: P-value for treatment x time interaction = 0.006) The most apparent increase in lean mass in the protein group was in the extremities. Appendicular lean mass increased from 20.1 (95% CI, 18.3–21.8) to 21.0 kg (95% CI, 19.2–22.7) in the protein group only (**Table 4.2**: P-value for treatment x time interaction <0.001). Fat mass increased from 27.8 (95% CI,

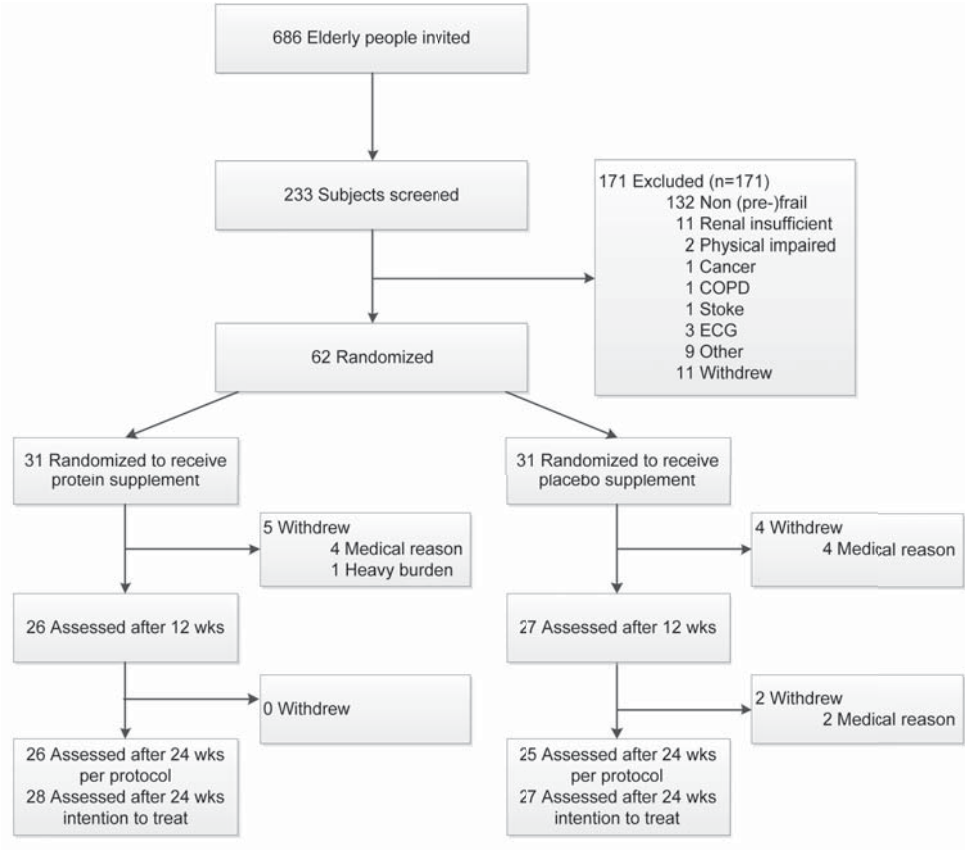

Figure 4.1 Participant flow.

23.8–31.4) to 28.5 kg (95% CI, 24.7–32.3) in the protein group and did not increase in the placebo group (from 28.4 kg, 95% CI, 24.8–32.1 to 27.9 kg, 95% CI, 24.2–31.6; P-value for treatment x time interaction <0.001).

Strength and physical performance

Strength and physical performance data are presented in **Table 4.3**. Leg press and leg extension strength improved over time in both the protein and placebo group (P<0.001) with no significant treatment x time interaction effect. In accordance, physical performance (SPPB) improved significantly from 8.0 (95% CI, 7.2–8.9) to 9.5 points (95% CI, 8.6–10.3) in the protein group and from 7.9 (95% CI, 7.0–8.8) to 9.2 points (95% CI, 8.3–10.1) in the placebo group with no treatment x time interaction (P>0.05).

Table 4.1 Baseline characteristics by treatment group

Variable	Placebo (n=31)	Protein (n=31)
Age, mean (SD), y	79 (6)	78 (9)
Women, No. (%)	21 (68)	20 (65)
Weight, mean (SD), kg	77.4 (13.2)	79.5 (15.6)
Height, mean (SD), m	1.66 (0.08)	1.66 (0.09)
BMI, mean (SD), kg/m^2	28.2 (4.6)	28.7 (4.5)
MMSE, mean (SD), points	28.1 (1.8)	27.6 (1.8)
PCS12, mean (SD), points	42.8 (9.8)	42.6 (6.4)
MCS12, mean (SD), points	56.6 (8.2)	56.6 (7.2)
Glucose, mean (SD), mmol/L	5.2 (0.5)	5.4 (0.5)
Insulin, mean (SD), mU/L	18.1 (6.7)	19.6 (6.9)
eGFR, mean (SD), mL/min/1.73 m^2	79.3 (19.9)	80.6 (14.1)
Systolic BP, mean (SD), mmHg	143 (20)	142 (19)
Diastolic BP, mean (SD), mmHg	73 (10)	74 (8)

BMI: Body Mass Index. MMSE: Mini Mental State Examination. PCS12: Physical component score SF12. MCS12: Mental component score SF12. eGFR: estimated Glomerular Filtration Rate. BP: Blood pressure.

Blood measurements and renal function

Baseline plasma glucose and insulin concentrations are presented in **Table 4.1**. Glucose and insulin concentrations did not change over time in either group (*data not shown*). The estimated glomerular filtration rates (eGFR) did not differ between groups at baseline (**Table 4.1**) and did not significantly changed from 80.6 (95% CI, 75.6–85.6) to 81.6 mL/min/1.73 m^2 (95% CI, 76.5–86.7) in the protein group with no significant treatment x time interaction effect (P>0.05).

Dietary intake

Dietary intake data are presented in **Table 4.4**. Baseline habitual protein intake was 1.0 (95% CI, 0.9–1.1) g/kg-bw/d and did not change over time in either group (P>0.05). When including the dietary protein supplements (i.e. 30 g/d), daily protein intake increased from 1.0 (95% CI, 0.9–1.1) to 1.3 (95% CI, 1.1–1.5) g/kg-bw/d in the protein group. Fat and carbohydrate intake did not differ between groups and remained similar over time. Habitual energy intake did not change significantly over time in either group (P>0.05).

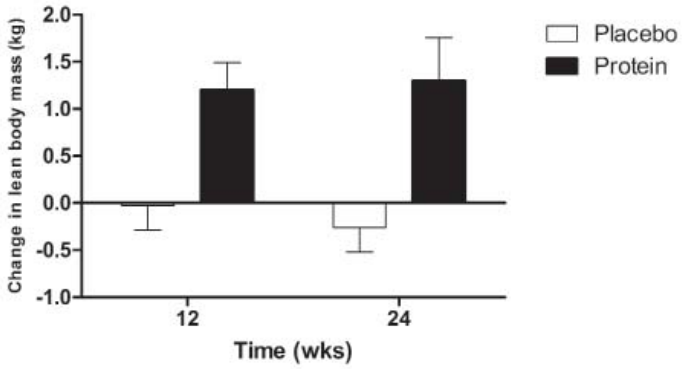

Figure 4.2 Intention to treat analysis on total lean body mass in the placebo and protein group (n=62). Data represent mean change in total lean mass after 12 and 24 wks relative to baseline (means ± SEM). P-value for treatment x time interaction = 0.006.

Health status, blood pressure and cognitive function

Health status (SF-12 scores), blood pressure and cognitive function (MMSE) parameters did not differ between groups at baseline (**Table 4.1**) and did not change over time in either group (*data not shown*).

Discussion

The present study showed that 24 wks of resistance-type exercise training improved muscle strength and functional performance in frail elderly men and women. However, dietary protein supplementation was shown to be required during resistance-type exercise training to allow an increase in skeletal muscle mass in this frail elderly population.

A large number of potential participants were recruited and screened to allow inclusion of an adequate sample of frail elderly subjects (**Figure 4.1**). The latter resulted in the selection of 62 elderly subjects who met the defined frailty criteria[37]. These criteria have been reported to be highly predictive for falls, hospitalization, disability, and mortality[37]. Confirming their frailty, the selected subjects showed a low baseline physical performance[38], poor leg[25], and handgrip strength[39] (**Table 4.3**).

Resistance-type exercise training has been established as an effective interventional strategy to counteract the age-related loss of muscle strength and performance in healthy and frail elderly people[19,20,23-26]. In agreement, we observed a substantial 43±4 and

Table 4.2 Body composition

	Placebo			Protein			Treatment X time interaction	Treatment effect	Time effect
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks	P-value	P-value	P-value
Body weight, kg, mean (95% CI)[a]	77.4 (72.2–82.7)	77.7 (72.4–82.9)	76.9 (71.6–82.2)	79.5 (74.2–84.8)	80.3 (75.1–85.6)	81.3 (76.0–86.6)	0.009	0.415	0.145
Lean mass, kg, mean (95% CI)[b]	45.7 (42.1–49.2)	45.6 (42.1–49.2)	45.4 (41.8–48.9)	47.2 (43.5–50.9)	48.4 (44.7–52.1)	48.5 (44.8–52.1)	0.006	0.34	0.01
Appendicular lean mass, kg, mean (95% CI)[b]	19.3 (17.6–20.9)	19.3 (19.7–21.0)	19.1 (17.5–20.8)	20.1 (18.3–21.8)	20.4 (18.6–22.1)	21.0 (19.2–22.7)	<0.001	0.31	0.02
Fat mass, kg, mean (95% CI)[b]	28.4 (24.8–32.1)	28.5 (24.8–32.1)	27.9 (24.2–31.6)	27.8 (24.0–31.6)	27.6 (23.8–31.4)	28.5 (24.7–32.3)	0.001	0.92	0.60
Bone mineral content, kg, mean (95% CI)[b]	2.5 (2.3–2.7)	2.5 (2.3–2.8)	2.5 (2.3–2.8)	2.5 (2.3–2.7)	2.5 (2.2–2.7)	2.5 (2.3–2.7)	0.25	0.80	0.22

CI indicates Confidence Interval. Intention to treat data were analyzed using a mixed linear model ([a] n=62; [b] n=56).

Table 4.3 Muscle strength and physical performance

	Placebo			Protein			Treatment X time interaction	Treatment effect	Time effect
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks	P-value	P-value	P-value
Leg press strength, kg, mean (95% CI)[a]	116 (101–130)	148 (132–162)	162 (147–178)	124 (109–139)	156 (141–171)	169 (154–184)	0.96	0.41	<0.001
Leg extension strength, kg, mean (95% CI)[a]	58.3 (51.7–64.9)	74.1 (66.8–81.4)	79.3 (72.2–86.4)	56 (49.5–62.7)	70.0 (62.7–77.3)	76.8 (69.8–83.9)	0.83	0.53	<0.001
Handgrip strength, kg, mean (95% CI)[a]	26.7 (23.1–30.3)	26.7 (23.1–30.3)	27.1 (23.5–30.8)	25.9 (22.3–29.5)	27.2 (23.6–30.9)	28.1 (24.5–31.7)	0.35	0.92	0.12
SPPB, points, mean (95% CI)[a]	7.9 (7.0–8.8)	8.3 (7.3–9.1)	9.2 (8.3–10.1)	8.0 (7.2–8.9)	9.2 (8.3–10.1)	9.5 (8.6–10.3)	0.23	0.46	<0.001
Gait speed, sec, mean (95% CI)[a]	5.4 (4.8–6.1)	5.6 (5.0–6.3)	5.3 (4.6–5.9)	5.3 (4.6–6.0)	5.3 (4.6–6.0)	5.2 (4.5–5.9)	0.41	0.72	0.18
Chair rise, sec, mean (95% CI)[b]	17.3 (14.8–19.9)	16.4 (13.9–19.0)	13.2 (10.5–15.9)	15.6 (13.0–18.1)	13.6 (10.9–16.3)	13.5 (10.7–16.2)	0.30	0.34	0.01

CI indicates Confidence Interval. SPPB indicates Short Physical Performance Battery. Intention to treat data were analyzed using a mixed linear model ([a] n=62, [b] n=57).

37±3% increases in leg strength and 1.3±0.3 and 1.5±0.3 point improvements in physical performance (SPPB) in the placebo and protein supplemented group, respectively (**Table 4.3**). The improvements in physical performance were mainly attributed to a decline in the time required to rise from a chair following 24 wks of training. These findings are consistent with previous results from shorter exercise training interventions among various elderly populations[25,26] and translate to a reduced risk for disability[38], institutionalization[31], and mortality[31]. The subjects attended 83±2% of the scheduled training sessions and performed on average 65±1% of their 1-RM in 4 sets on the leg press and leg extension machines. The excellent adherence confirms the feasibility of such an intense, supervised resistance-type exercise training program for the frail elders. Government and healthcare workers should be stimulated to facilitate the implementation of resistance-type exercise training in such frail elderly population.

We provided a dietary protein supplement immediately after breakfast and lunch with the intention to further augment the skeletal muscle adaptive response to resistance-type exercise training. By supplementing 15 g protein twice daily, protein intake increased to more than 25 g with each main meal in the protein supplemented group[40]. In the placebo group, the subjects continued to ingest relative small amounts of dietary protein at breakfast (13 g) and lunch (20 g) during the entire intervention period (**Table 4.4**). It has been reported that these relative small amounts of protein are insufficient to allow a proper increase in post-prandial muscle protein synthesis rates in elderly subjects[41], thereby compromising muscle mass maintenance. We hypothesized that increasing dietary protein intake at breakfast and lunch would stimulate muscle protein synthesis[42] and augment net muscle protein accretion during 24 wks of resistance-type exercise training. Confirming our hypothesis, we observed a significant 1.3±0.4 kg increase in lean body mass in the protein supplemented group. In contrast, no net increase in lean body mass was observed in the placebo group.

The muscle mass gain observed in the protein supplemented group entirely offset the decline in muscle mass that is generally reported in elderly people[43,44]. Instead of the annual loss of 0.5–1.0 kg muscle tissue[42,43], we observed a net 1.3 kg increase in lean mass in the protein supplemented group. In the placebo group, the exercise intervention prevented a measurable decline in lean muscle mass, but in contrast to the protein group no net gain in lean mass was observed. Nonetheless, the preservation of muscle tissue will reduce the risk of developing chronic metabolic diseases such as obesity and type 2 diabetes[45].

Despite a greater increase in muscle mass, protein supplementation did not further augment the increase in muscle strength and physical performance following 24 wks

Table 4.4 Habitual dietary intake

	Placebo			Protein			Treatment X time interaction	Treatment effect	Time effect
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks	P-value	P-value	P-value
Energy intake, MJ, mean (95% CI)[a]	7.8 (7.0–8.6)	7.5 (6.7–8.4)	7.8 (7.0–8.7)	8.6 (7.8–9.4)	8.0 (7.2–8.8)	8.2 (7.4–9.1)	0.70	0.29	0.21
Protein intake, g, mean (95% CI)[a]	76.4 (68.3–84.5)	70.0 (61.8–78.3)	77.7 (69.2–86.2)	77.7 (69.5–85.9)	74.2 (65.9–82.5)	83.5 (75.2–91.9)	0.61	0.47	0.004
Protein intake, g/kg-bw/d, mean (95% CI)[a]	1.0 (0.9–1.1)	0.9 (0.8–1.0)	0.9 (0.8–1.1)	1.0 (0.9–1.1)	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.79	0.96	0.64
Protein intake including supplement, g/kg-bw/d, mean (95% CI)[a]	1.0 (0.9–1.1)	0.9 (0.8–1.0)	0.9 (0.8–1.1)	1.0 (0.9–1.1)	1.3 (1.2–1.5)	1.3 (1.1–1.5)	0.00	0.00	0.01
Protein at breakfast, g, mean (95% CI)[a]	13.2 (10.8–15.6)	11.9 (9.4.–14.3)	11.9 (9.4–14.5)	11.8 (9.3–14.2)	13.0 (10.5–15.5)	12.8 (10.3–15.3)	0.08	0.92	0.99
Protein at lunch, g, mean (95% CI)[b]	17.7 (14.9–20.4)	20.3 (17.7–23.0)	16.2 (13.3–19.0)	18.9 (16.2–21.6)	22.1 (19.4–24.8)	20.9 (18.2–23.6)	0.10	0.12	0.003
Protein at dinner, g, mean (95% CI)[a]	33.5 (28.0–39.0)	32.5 (26.9–38.0)	36.6 (30.8–42.4)	34.8 (29.2–40.3)	35.4 (29.8–41.0)	40.0 (34.4–45.7)	0.82	0.46	0.04
Protein intake, en%, mean (95% CI)[a]	17.0 (15.9–18.1)	16.0 (14.8–17.1)	16.9 (15.7–18.1)	15.7 (14.6–16.9)	16.3 (15.2–17.5)	17.5 (16.3–18.7)	0.07	0.89	0.04
Fat intake, en%, mean (95% CI)[a]	35.0 (32.8–37.2)	35.3 (33.0–37.5)	34.1 (31.8–36.5)	32.7 (30.5–35.0)	31.9 (29.6–34.1)	33.1 (30.8–35.3)	0.38	0.10	0.93
Carbohydrate intake, en%, mean (95% CI)[a]	44.6 (41.8–47.4)	45.6 (42.8–48.4)	44.5 (41.6–47.4)	47.5 (44.6–50.3)	48.0 (45.2–50.9)	46.2 (43.3–49.0)	0.74	0.21	0.15

CI indicates Confidence Interval. En% indicates energy percentage. Intention to treat data were analyzed using a mixed linear model ([a] n=61, [b] n=60).

of resistance-type exercise training. The latter is not surprising, as a disproportionate increase in muscle strength generally occurs during the first few months of resistance-type exercise training. This increase in muscle strength was primarily attributed to changes in neuromuscular activation (i.e. motor unit recruitment) and/or increases in muscle quality[46]. The increase in skeletal muscle mass in the protein as opposed to the placebo supplemented group, will likely allow a further increase in muscle strength and performance as time progresses. This would translate in a greater training efficiency over a more prolonged training duration.

In the present study, we showed that dietary protein supplementation was required to gain muscle mass during prolonged exercise intervention in a frail elderly population. This protein supplementation (30 g/d) increased the habitual protein intake from 1.0 to 1.4 g/kg-bw/d and did not result in a reduction in habitual energy intake (**Table 4.4**). Furthermore, the greater protein intake was not accompanied by any health complaints and also did not seem to affect renal function throughout the intervention period. Our present findings strongly advocate the ingestion of more protein during resistance-type exercise training in frail elderly people as a means to attenuate or even reverse the loss of muscle mass with aging and, as such, prevent the progression of frailty and functional decline.

We conclude that resistance-type exercise training represents an effective and feasible strategy to improve strength and physical performance in frail elderly people. Daily dietary protein supplementation (15 g protein, twice daily) is required to allow muscle mass gain during prolonged resistance-type exercise training in frail elderly men and women.

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5

Handgrip strength does not represent an appropriate measure to evaluate changes in muscle strength during an exercise intervention program in frail elderly people

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Submitted for publication

Abstract

Background: Although handgrip strength is considered a strong predictor of negative health outcomes, it is unclear whether handgrip strength represents a useful measure to evaluate changes in muscle strength following resistance-type exercise training in elderly people.

Objective: To assess whether measuring handgrip strength provides proper insight in the efficacy of prolonged resistance-type exercise training to increase muscle mass, strength and physical performance in frail elderly people.

Design: Cross-sectional and prospective, parallel-group, intervention study.

Setting: University research center.

Participants: A total of 127 pre-frail and frail elderly people (≥65 y).

Measurements: Before, during, and after 24 wks of whole-body resistance-type exercise training handgrip strength (JAMAR), lean body mass (DXA), one-repetition maximum leg strength (1-RM), and physical performance (SPPB) were assessed in 127 frail elderly people.

Results: Handgrip strength correlated strongly with appendicular lean mass ($r=0.68$; $P<0.001$) and leg extension strength ($r=0.70$; $P<0.001$). After 24 wks of whole-body resistance-type exercise training, 1-RM leg extension strength improved significantly better when compared with the control group (from 57±2 to 78±3 kg vs 57±3 to 65±3 kg: $P<0.001$). In agreement, SPPB improved significantly more in the exercise group (from 8.0±0.4 to 9.3±0.4 points) when compared with the control group (from 8.3±0.4 to 8.9±0.4 points: $P<0.05$). These positive changes were not accompanied with any significant changes in handgrip strength (26.3±1.2 to 27.6±1.2 kg in the exercise group vs 26.6±1.2 to 26.3±1.3 kg in the control group: $P=0.71$).

Conclusion: Although handgrip strength strongly correlates with measures of muscle mass and leg strength in frail elderly people, handgrip strength does not provide a reliable means to evaluate the efficacy of exercise intervention programs to increase muscle mass or strength in an elderly population.

Introduction

Population demographics show that the number of elderly people aged 65 y and over will rise by approximately 50% over the next 30 years. This growth of the aging population is accompanied with an increased number of frail elderly people who are at risk of adverse health outcomes such as disability, co-morbidity and mortality. A dominant feature of frailty is the age-related loss of muscle mass and muscle strength, also called sarcopenia[1,2]. The latter is associated with physical impairment[3,4], disability[4,5] and loss of independence[6]. Interestingly, Goodpaster et al. showed that the decline in muscle strength occurs much more rapid than the concomitant loss of muscle mass[7]. Therefore, muscle strength has been identified as an important predictor for physical impairment, disability and institutionalization[8]. The importance of muscle strength for daily function necessitates the development of reliable and valid procedures to quantify muscle strength and evaluate the benefits of intervention programs.

There are numerous ways to assess muscle strength. A non-invasive, in-expensive and widely used measure to evaluate muscle strength is handgrip strength. This measure reflects the maximum isometric strength of the hand and forearm muscles, assessed with a dynamometer in a standing or sitting position. Ample epidemiological studies have shown that lower handgrip strength is strongly associated with health decline in elderly people, predominantly describing its association with physical disability[9-16] and mortality[13,17]. Although handgrip strength seems to represent a strong predictor of negative health outcomes, it is less clear whether handgrip strength correlates with measures of sarcopenia, such as muscle mass, leg muscle strength, and physical performance in frail elderly people.

Resistance-type exercise training has been shown to be a feasible and effective strategy to counteract sarcopenia. It has been well-established that resistance-type exercise training increases muscle mass and improves leg muscle strength in elderly people[4,5,18]. As such, resistance-type exercise training is now widely used to attenuate muscle mass loss, increase muscle strength and improve physical performance. As handgrip strength is non-invasive and an in-expensive assessment, we questioned whether measuring handgrip strength provides relevant insight in the efficacy of prolonged resistance-type exercise training to increase muscle mass, leg strength, and physical performance in an elderly population. We hypothesized that although handgrip strength strongly correlates with muscle mass, strength, and physical performance, handgrip strength does not respond to prolonged whole-body resistance-type exercise training in frail elderly people.

Methods

Subjects

A total of 127 pre-frail and frail (according to the Fried criteria[2]) elderly subjects (≥65 y) were included in the present study. Subjects who were diagnosed with any form of cancer, chronic obstructive pulmonary disease (COPD), diabetes (basal plasma glucose ≥7 mmol/L)[19], renal insufficiency (eGFR <60 mL/min/1.73 m^2)[20] were excluded from participation. None of the subjects had participated in a resistance-type exercise training program over the past 2 years. The inclusion of the elderly people and the design of the original studies are described in detail elsewhere[21,22] as the present study is part of a larger project. This project investigated the impact of protein supplementation with or without a resistance-type exercise training program on muscle mass, strength and physical performance in frail elderly people. After inclusion, 127 subjects underwent the same series of measurements at baseline, after 12 and 24 wks (outlined below) to assess handgrip strength (JAMAR), muscle mass (DXA), leg strength (1-RM) and physical performance (SPPB)[21,22]. Sixty-two of these subjects were included in a 24 wk whole-body resistance-type exercise training program (exercise group) and 65 subjects did not receive any exercise training (control group). The Wageningen University Medical Ethical Committee approved the studies and subjects gave their written informed consent.

Whole-body resistance-type exercise training program

Whole-body resistance-type exercise training was performed 2 times per wk under personal supervision for 24 wks. The sessions were performed in the morning and afternoon with at least 72 h between sessions. The training consisted of a 5 min warm-up on a cycle ergometer, followed by 4 sets on the leg press and leg extension machines and 3 sets on chest press, lat pulldown, pec-dec, and vertical row machines (Technogym, Rotterdam, the Netherlands). The workload started at 50% of 1-RM (10–15 repetitions per set) and increased to 75% of 1-RM (8–10 repetitions per set) to stimulate muscle hypertrophy. Resting periods of 1 min were allowed between sets and 2 min between exercises. To evaluate changes in muscle strength, 1-RM was repeated after 4, 8, 12, 16, and 20 wks of training. Workload intensity was adjusted based on the 1-RM outcomes.

Handgrip strength

Handgrip strength was measured using a hydraulic hand dynamometer (Jamar, Jackson, MI, USA). Three consecutive measures of dominant and non-dominant handgrip strength were recorded to the nearest 0.5 kg with subjects sitting in an upward position with the arm in a 90-degree angle position. The maximum strength effort was reported.

Body composition

Height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Body weight was measured in the fasted state to the nearest 0.1 kg with a calibrated digital scale (ED-6-T; Berkel, Rotterdam, The Netherlands). In the fasted state, whole-body, appendicular, and leg lean mass were measured by DXA (Lunar Prodigy Advance; GE Health Care, Madision, WI, USA).

Maximum leg strength

Maximum leg strength was assessed by 1-RM strength tests on leg press and leg extension machines (Technogym, Rotterdam, the Netherlands). During a first familiarization session, the proper lifting technique was demonstrated and practiced, after which maximum strength was estimated using the multiple repetitions testing procedure for leg press and leg extension. In a second exercise session, 1 wk or more after the first strength estimation, the subjects' 1-RM strength was determined[21,22].

Physical performance

Physical performance was assessed with the short physical performance battery (SPPB) that comprised 3 components, i.e. standing balance, gait speed and chair stands[23]. Scores of 1 to 4 were based on categories of performance in the balance tests, on the time necessary to complete the walk, and on the time needed to perform the chair rise test. When subjects were unable to perform a test, a score of 0 was allocated. A summary SPPB score between 0 and 12 was obtained through summation of the scores obtained in the 3 individual tests.

Statistical analysis

Characteristics of the study population were reported as the mean ± standard deviation (SD), or as percentage. Independent sample T-tests for continuous variables and a Chi-squared test for categorical variables were performed to compare participants from the

control and exercise group. Pearson's correlation coefficients were calculated to assess the relation of baseline handgrip strength with lean mass, leg strength, physical performance and post intervention handgrip strength. Differences between treatments (exercise and control) over time were analyzed using mixed linear models with an unstructured covariance matrix. Time, treatment, and their interaction were defined as fixed factors and subject was defined as a random factor. Data analysis was performed by the intention to treat principle and all statistical analyses were performed using SPSS Statistics v19. An α-level of 0.05 was used to determine statistical significance.

Results

Between December 2009 and October 2010, 1420 elderly people were approached, 398 were screened and 127 participants were included into the studies. In total, 19 subjects withdrew from the studies, 8 from the control and 11 from the exercise trained group. For the intention to treat analyses, 8 dropouts, 4 in each group, were willing to have final assessments. The average adherence to the exercise protocol was 83±2%. Baseline characteristics are presented in **Table 5.1** and showed no differences between groups (P>0.05).

Table 5.1 Characteristics of participants

Variable	Total group n=127	Control n=65	Exercise n=62
Age (y)	79.0±0.7	79.5±1.0	78.4±1.0
Women (%)	61	55	66
Height (m)	1.66±0.01	1.66±0.01	1.66±0.01
Weight (kg)	76.2±1.2	74.0±1.6[a]	78.5±1.8
Dominant handgrip strength (kg)	26.1±0.8	25.6±1.2	26.3±1.2[a]
Non-dominant handgrip strength (kg)	25.0±0.8	24.8±1.2	24.9±1.2[a]
Total lean mass (kg)	46.3±0.9	46.1±1.2[b]	46.1±1.2[c]
Appendicular lean mass (kg)	19.5±0.4	19.3±0.5[b]	19.3±0.5[c]
1-RM leg press (kg)	120±3	120±5[d]	120±4
1-RM leg extension (kg)	57±2	57±3[e]	57±2[a]
SPPB (points)	8.2±0.3	8.3±0.4[b]	8.0±0.4

Values are expressed as a mean±SEM or percentage. Superscript indicate missing values: [a] 1 missing value, [b] 4 missing values, [c] 6 missing values, [d] 16 missing values, [e] 17 missing values. There were no significant differences between the control and exercise group (P>0.05).

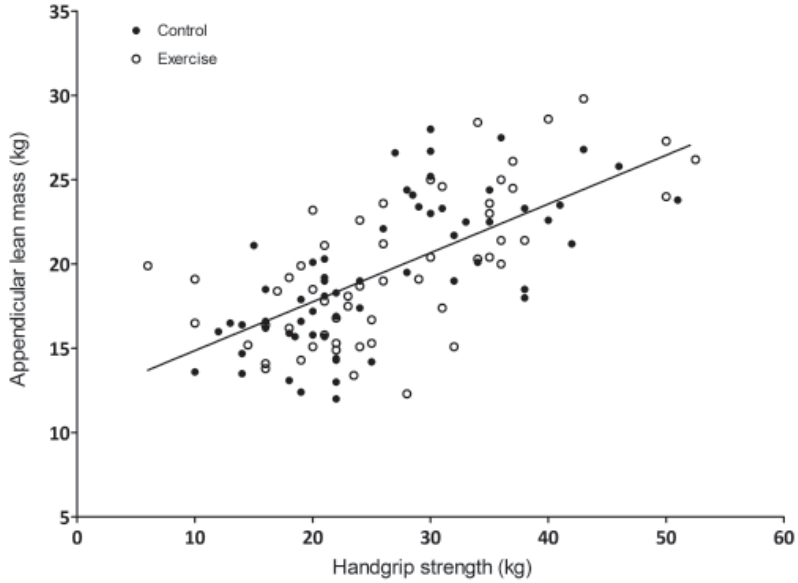

Figure 5.1 Baseline correlation between handgrip strength and appendicular lean mass in control (filled circles) and exercise (open circles) group (n=116). Handgrip strength correlated significantly with appendicular lean mass (r=0.68; P<0.001).

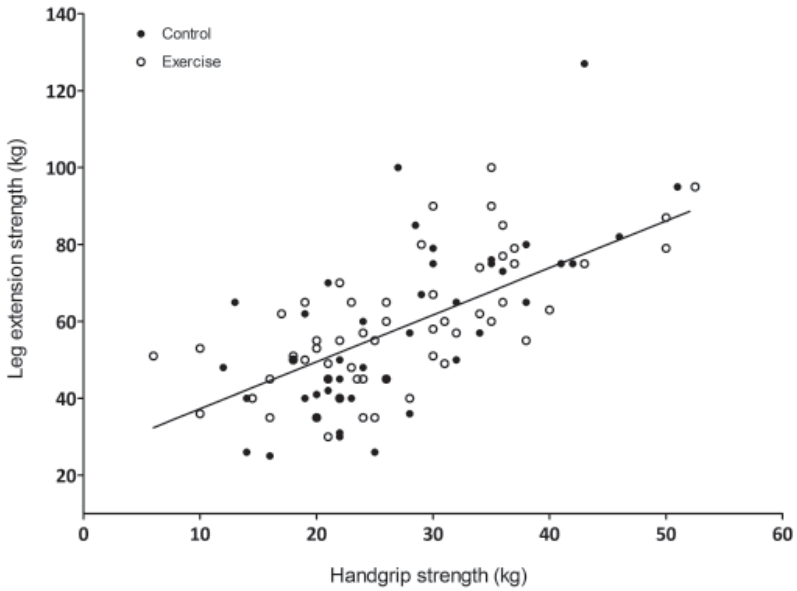

Figure 5.2 Baseline correlation between handgrip strength and leg extension strength in control (filled circles) and exercise (open circles) group (n=108). Handgrip strength correlated significantly with leg extension strength (r=0.69; P<0.001).

Table 5.2 Impact of control and exercise intervention on handgrip strength, lean mass, leg strength and physical performance

	Control			Exercise			Treatment X time	Treatment effect	Time effect
Variable	0 wks	12 wks	24 wks	0 wks	12 wks	24 wks			
Dominant handgrip strength (kg)	25.6±1.2	26.1±1.2	26.3±1.3	26.3±1.2	26.9±1.2	27.6±1.3	0.71	0.58	0.04
Non-dominant handgrip strength (kg)	24.8±1.2	25.1±1.2	25.9±1.2	24.9±1.2	25.7±1.3	26.2±1.2	0.77	0.81	0.01
Lean mass (kg)[a]	46.1±1.2	46.3±1.2	46.2±1.2	46.4±1.2	47.0±1.2	46.9±1.2	0.38	0.74	0.02
Appendicular lean mass (kg)[a]	19.3±0.5	19.4±0.6	19.4±0.6	19.7±0.6	19.8±0.6	20.0±0.6	0.21	0.57	0.05
1-RM leg press strength (kg)[b]	120±5	125±6	137±7	120±4	151±5	165±6	0.00	0.02	0.00
1-RM leg extension strength (kg)[b]	57±3	61±3	65±3	57±2	72±3	78±3	0.00	0.62	0.00
SPPB (points)[c]	8.3±0.4	8.5±0.4	8.9±0.4	8.0±0.4	8.7±0.4	9.3±0.4	0.03	0.90	0.00

Data represent means±SEM. Intention to treat data were analyzed using a mixed linear model ([a] n=118; [b] n=112, [c] n=123). 1-RM: one-repetition maximum, SPPB: short physical performance battery.

Correlations

Handgrip strength of the dominant hand correlated with total lean mass (r=0.70; P<0.001) and appendicular lean mass (r=0.68; P<0.001, **Figure 5.1**). Furthermore, handgrip strength correlated with 1-RM leg press (r=0.59; P<0.001), and 1-RM leg extension strength (r=0.69; P<0.001: **Figure 5.2**). Handgrip strength correlated poorly with SPPB (r=0.23; P=0.10). Correlations of non-dominant handgrip strength and the above mentioned outcome measures were similar (*data not shown*).

Whole-body resistance-type exercise

The impact of whole-body resistance-type exercise training on handgrip strength, muscle mass, leg strength and physical performance are presented in **Table 5.2**. After 24 wks, dominant handgrip strength had not changed significantly over time between the exercise (26.3±1.2 to 27.6±1.2) and control (25.6±1.2 to 26.3±1.3) group (P=0.71). However, a significant time effect was observed (P<0.05) indicating an increase in handgrip strength in both groups. Likewise for non-dominant handgrip strength, there was no significant treatment x time interaction (P=0.77), but a significant time effect

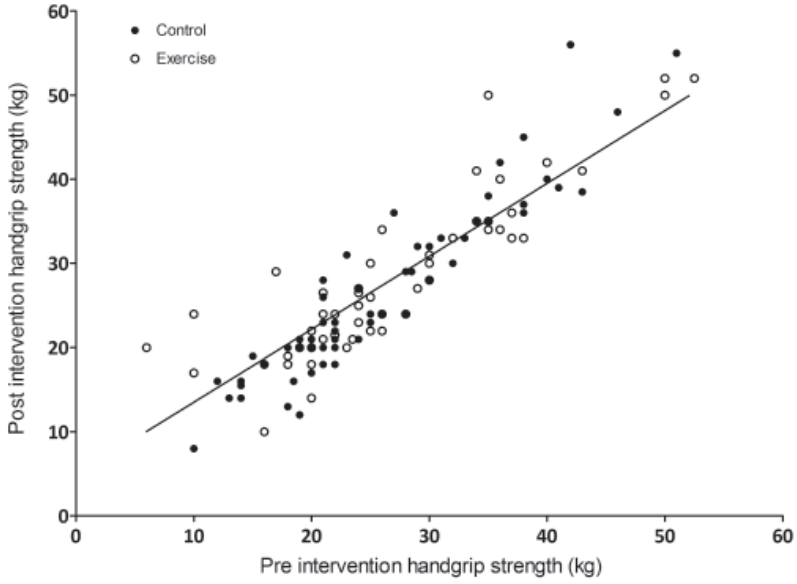

Figure 5.3 Correlation between pre and post intervention handgrip strength (n=114). Pre intervention handgrip strength correlated significantly with post intervention handgrip strength (r=0.88; P<0.001).

(P<0.05). In line, a significant intraclass correlation coefficient (ICC) of 0.91 (P<0.05) and a significant correlation of handgrip strength prior and post exercise intervention (r=0.88; P<0.001: **Figure 5.3**) was observed. In contrast with handgrip strength, a significant time x treatment interaction was observed for leg extension strength (P<0.001). The increase in leg extension strength was significantly larger in the exercise group when compared with the control group (from 57±2 to 78±3 kg vs 57±3 to 65±3 kg; **Figure 5.4**). Likewise,

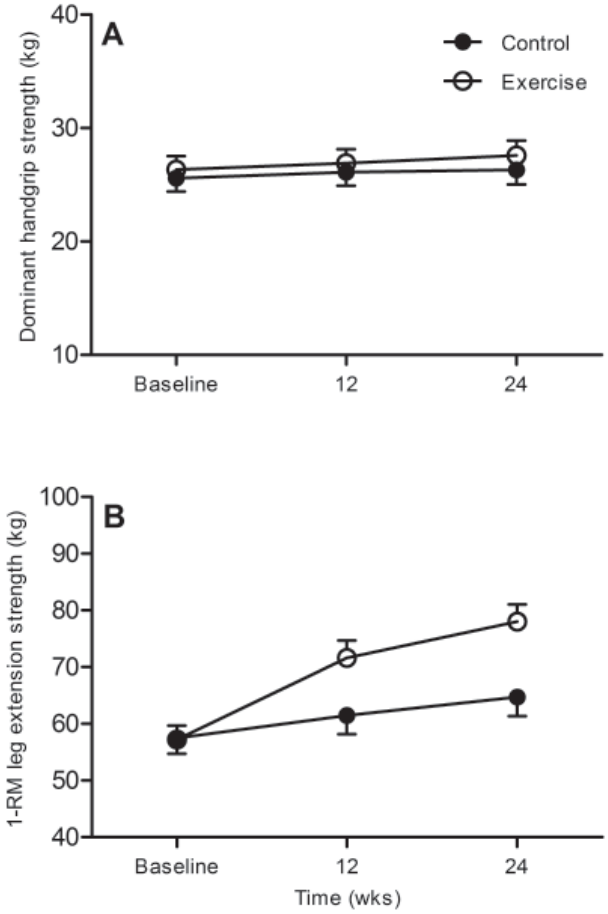

Figure 5.4 Intention to treat analysis on dominant handgrip strength (A: n=127) and 1-RM leg extension strength (B: n=112) in the control (filled circles) and exercise group (open circles). Data represents means±SEM. There was a no significant treatment x time interaction effect for the dominant handgrip strength (P=0.71), but significant treatment x time interaction effect for 1-RM leg extension strength (P<0.001).

the increase in leg press strength was significantly larger in the exercise group when compared with the control group (P<0.001). In agreement, SPPB improved significantly more in the exercise group (from 8.0±0.4 to 9.3±0.4 points) when compared with the control group (from 8.3±0.4 to 8.9±0.4 points: P<0.05).

Discussion

Prolonged whole-body resistance-type exercise training improves leg muscle strength and physical performance, but without measurable changes in handgrip strength even though handgrip strength correlates with appendicular lean mass and leg strength. Clearly, handgrip strength does not provide an appropriate means to evaluate the efficacy of an exercise intervention program to augment muscle mass, muscle strength, and/or physical performance in frail elderly people.

Handgrip strength is a non-invasive, in-expensive measurement and has been widely used in both clinical and epidemiologic settings. Although there is no clear consensus for the procedure of measurement of handgrip strength, a standardized protocol for measurements of handgrip strength has been established for older people which is based on the American Society of Hand Therapists (ASHT)[24]. The ASHT recommends subjects to be seated, with the shoulder adducted and elbow flexed at 90˚ and using a JAMAR dynamometer[24] to perform the test. Using the latter standardized protocol, we show that handgrip strength strongly correlates with muscle mass (**Figure 5.1**) and strength (**Figure 5.2**) in a frail elderly population. A number of other studies have reported on similar associations between handgrip strength and functional ability in healthy elderly populations[10-12], but none have studied the relation of handgrip strength with muscle mass, strength, and physical performance in a frail elderly population[9-16]. Our data support the general belief that handgrip strength can be used as a predictor for the progressive decline in muscle mass and strength with aging.

Using the JAMAR dynamometer, test–retest reliability of handgrip strength measurements has been confirmed in numerous studies, reporting intraclass correlation coefficient (ICC) values between 0.80–0.98[25]. Among community-dwelling elderly people, a more prolonged study reported an ICC of 0.95 for left handgrip strength and an ICC of 0.91 for right handgrip strength[26]. In agreement, we observed an ICC of 0.91 for both the dominant hand and non-dominant hand and a significant correlation of handgrip strength prior and post exercise intervention (**Figure 5.3**), confirming the reliability and reproducibility of the handgrip strength test in a frail elderly population.

Even though handgrip strength seems to be a reliable, non-invasive, in-expensive measurement and a strong predictor for the progressive decline in muscle mass and leg strength, handgrip strength does not represent a valid measure to evaluate changes in muscle strength (**Figure 5.4B**) and physical performance (**Table 5.2**) in response to prolonged whole-body resistance-type exercise training programs. Prolonged whole-body resistance-type exercise training has been well-established as an effective interventional strategy to counteract the age-related loss of muscle strength and performance in healthy and frail elderly people[27-32]. A recent meta-analysis studying the impact of resistance-type exercise training on muscle strength in elderly people showed a 29±2% increase in 1-RM leg press strength and a 33±2% increase in 1-RM leg extension strength[33]. In agreement, we observed a substantial 40±4% increase in leg press and 36±3% increase in leg extension strength following 24 wks of intervention. Importantly, the increase in leg muscle strength translated to a substantial 1.3±0.2 points improvement in physical performance as determined by the SPBB. Despite the whole-body resistance-type exercise program and the substantial improvements observed in leg muscle strength and physical performance, we failed to detect any significant changes in handgrip strength, even following 24 wks of resistance-type exercise training. Whereas some have reported minor increases in handgrip strength[34-37], the majority of studies have not been able to detect significant changes in handgrip strength in response to traditional whole-body resistance-type exercise training programs in elderly people[38-45]. Nonetheless, in such whole-body resistance-type exercise training programs, handgrip strength is often used as a parameter to assess overall muscle strength and/or functional improvements. The present study clearly shows that handgrip strength does not represent a clinically relevant and/or valid measure to evaluate changes in muscle strength and physical performance in response to prolonged whole-body resistance-type exercise training programs. The substantial increases in leg strength as much as 40% would have been overseen when only handgrip strength would have been selected as a parameter to evaluate the efficacy of a whole-body exercise training program. Therefore, care should be taken when interpreting data on handgrip strength in relation to changes in muscle strength and function over time.

We conclude that although handgrip strength correlates well with measures of muscle mass and leg strength in frail elderly people, handgrip strength does not provide a reliable means to evaluate the efficacy of exercise intervention programs to increase muscle mass, strength, and/or improve physical performance in elderly people.

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6

Compromised vitamin D status in frail elderly people is associated with reduced muscle mass and impaired physical performance

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Abstract

Background/Objectives: Serum 25-hydroxyvitamin D (25(OH)D) status has been associated with muscle mass, strength and physical performance in healthy elderly people. Yet, in pre-frail and frail elderly people this association has not been studied. The objective of this study was to explore the association between vitamin D intake and serum 25(OH)D status with muscle mass, strength and physical performance in a pre-frail and frail elderly population.

Subjects/Methods: This cross-sectional study included 127 pre-frail and frail elderly people in the Netherlands. Whole-body and appendicular lean mass (DXA), leg strength (1-RM), handgrip strength, and physical performance (SPPB) were measured and blood samples were collected for the assessment of serum 25(OH)D status (LC-MS/MS). In addition, habitual dietary intake (3-d food records) and physical activity data (accelerometers) were collected.

Results: In total, 53% of the participants had a serum 25(OH)D level below 50 nmol/L. After adjustment for confounding factors, 25(OH)D status was associated with appendicular lean mass ($\beta=0.012$, $P=0.05$), and with physical performance ($\beta=0.020$, $P<0.05$). Vitamin D intake was associated with physical performance ($\beta=0.18$, $P<0.05$), but not with appendicular lean mass ($P>0.05$).

Conclusion: In this elderly population, 25(OH)D status is compromised and is associated with reduced appendicular lean mass and impaired physical performance. In addition, also vitamin D intake is associated with impaired physical performance. Our findings highlight the need for well-designed intervention trials to assess the impact of vitamin D supplementation on 25(OH)D status, muscle mass and physical performance in pre-frail and frail elderly people.

Introduction

Frailty is a geriatric syndrome of decreased reserves and resistance to stressors, which increases the risk for falls, disability, morbidity, and institutionalization[1,2]. An important and fundamental component of frailty is sarcopenia[3]. Sarcopenia is characterized by a progressive loss of skeletal muscle mass and physical performance[4]. The cause of this loss in muscle mass and performance is multifactorial and might include vitamin D deficiency[5,6]. The Institute of Medicine currently considers a serum 25(OH)D level below 50 nmol/L as being insufficient[7]. The estimated prevalence of vitamin D deficiency among healthy elderly people is between 45 and 57%[8-10] and among compromised geriatric patients vitamin D deficiency is even more pronounced[11,12]. Compromised 25-hydroxyvitamin D (25(OH)D) status has been associated with poor muscle mass and impaired physical performance in community-dwelling elderly people[5,6,13-15]. Although significant associations between inadequate 25(OH)D status and reduced muscle mass, strength, and physical performance have been well-established in a healthy elderly population[5,6,13-15], there are few data available on such associations in more compromised, frail elderly subpopulations. Studying the association between 25(OH)D level and muscle mass, strength and physical performance in a frail elderly population is important, since a compromised 25(OH) D status might be more pronounced within this population together with decreased muscle mass, impaired physical performance[16], and their risk for falls and fractures[1,2]. A compromised 25(OH)D status among frail elderly may predispose to the development of muscle mass loss and impairments in strength and physical performance resulting in more frequent falls and fractures[17-20]. Therefore, in the present study, we examined the association between 25(OH)D level and vitamin D intake with muscle mass, strength and physical performance in a pre-frail and frail elderly population.

Methods

Study sample

For two RCTs[21,22], community-dwelling elderly participants, ≥65 y, were recruited between December 2009 and September 2010. Potentially eligible subjects were screened for pre-frailty and frailty using the Fried criteria[2]. These criteria were: [1] unintentional weight loss, [2] weakness, [3] self-reported exhaustion, [4] slow walking speed, and [5] low physical activity. Pre-frailty was classified when one or two criteria were present and frailty was defined when three or more criteria were present. Furthermore, subjects who were diagnosed with any form of cancer, chronic obstructive pulmonary disease (COPD),

diabetes type 1 and 2 (≥7 mmol/L)[23], or renal insufficiency (eGFR <60 mL/min/1.73 m^2)[24] were excluded. None of the subjects had participated in a resistance-type exercise program over the past 2 years. The baseline dataset of 127 pre-frail and frail elderly subjects was used for the current analysis. The Wageningen University Medical Ethical Committee approved the study and subjects gave their written informed consent.

Blood sampling and analysis

After an overnight fast, blood samples were collected in EDTA-containing tubes and in serum tubes. The EDTA-containing tubes were centrifuged at 1000g at 4˚C for 10 min and serum tubes were centrifuged 90 min after the blood collection at 1000g at 20˚C for 15 min. Aliquots of plasma and serum were snap frozen in liquid nitrogen and stored at -80˚C. The plasma samples were used to determine subjects' glucose and insulin concentrations and the serum samples for creatinine and 25(OH)D concentrations. Plasma glucose concentrations were measured with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland). Insulin was measured by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO, USA). Serum creatinine was measured by using Roche Modular System P (Roche Diagnostics GmbH, Mannheim, Germany). Serum 25(OH)D levels were measured using isotope dilution – online solid phase extraction liquid chromatography – tandem mass spectrometry (ID-XLC-MS/MS) by the Endocrine Laboratory of the VU University Medical Center, the Netherlands. 25(OH)D was released from its binding protein(s) and a deuterated internal standard (IS: 25(OH)D3-d6), was added. Samples were extracted and analyzed by XLC-MS/MS (a Symbiosis online SPE system; Spark Holland, Emmen, the Netherlands) coupled to a Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, MA, USA). The intra-assay and inter-assay coefficient of variance for this analysis were <6% and <8%, respectively, when using 3 concentrations between 25 and 180 nmol/L and the Limit of Quantification was 4.0 nmol/L.

Dietary intake

Dietary intake data, including vitamin D intake, was obtained by means of 3-d food records. The 3 d of recording were randomly assigned to ensure that all days of the wk, including weekend days, were equally represented. Vitamin D supplement use was not assessed. Dietary data were coded (type of food, time of intake, and estimated portion size) and calculated using a food calculation system (BAS nutrition software 2004, Arnhem, The Netherlands) in which the Dutch food composition database of 2006 was included.

Body composition, maximum strength and physical performance

Assessment of body composition, maximum strength and physical performance were performed within 4 wks following blood sampling. Lean body mass (LBM), appendicular lean mass (ALM), leg lean mass (LLM) and bone mineral content (BMC) were measured with Dual Energy X-ray Absorptiometry (DXA) scan (Lunar Prodigy Advance; GE Health Care, Madison, WI, USA). Maximum strength was assessed by one repetition maximum (1-RM) strength tests on leg press and leg extension machines (Technogym, Rotterdam, the Netherlands). During a familiarization session, the proper lifting technique was demonstrated and practiced, after which maximum strength was estimated. In a second session, ≥1 wk after the first strength estimation, the subjects' 1-RM strength was determined[25]. Handgrip strength was measured using a hydraulic hand dynamometer (Jamar, Jackson, MI, USA). Three consecutive measures of handgrip strength (kg) exerted by both hands were recorded to the nearest 0.5 kg with subjects sitting in an upward position with the arm in a 90 degree angle. The maximum strength effort was reported.

Physical performance was assessed with the short physical performance battery (SPPB) that comprised of 3 components i.e. standing balance, gait speed and chair stands[26]. Scores of 1 to 4 were based on categories of performance in the balance tests, on the time necessary to complete the walk, and on the time needed to perform the chair rise test. When subjects were unable to perform a test, a score of 0 was allocated. A summary SPPB score between 0 and 12 was obtained through summation of the scores obtained in the 3 individual tests.

Potential confounders

The following potential confounders were included in the statistical analysis: age, gender, height, body weight, alcohol (none, <1 consumption/d, >1 consumption/d), habitual physical activity, season of data collection, education (low, medium, high), serum creatinine, smoking, energy and protein intake. Height was measured with a wall-mounted stadiometer and body weight with a calibrated digital scale (ED-6-T; Berkel, Rotterdam, the Netherlands). Habitual physical activity data were quantified using a tri-axial accelerometer (ActiGraph GTX3, 2009, Pensacola, FL, USA) worn on the hip for 1 wk. Change of acceleration per second and epochs of 60 s were used. After 7 d, data were uploaded for analysis and analyzed using the MAH/UFFE analyzer, version 1.9.0.3 (MRC Epidemiology Unit, Cambridge, UK). Data files that did not meet 10 h of monitoring per day on at least 5 d as well as files that included periods of >100 min without activity were excluded from the analysis. Calcium intake was not included in the model because this did not change the β substantially.

Statistics

Characteristics of the study population were reported as the mean ± standard deviation (SD), as percentage or as medians (25–75 percentile). Participants were grouped according to published 25(OH)D status cut points: <50 nmol/L and ≥50 nmol/L[6,27]. Chi-squared tests for categorical variables and independent sample T-tests for continuous variables were performed to compare participants with 25(OH)D levels below and above 50 nmol/L. Multiple linear regression analysis was used to investigate the association of 25(OH)D status and vitamin D intake with the outcome variables, adjusted for age, gender, height, body weight, alcohol, physical activity, education, smoking, creatinine (model 2) and energy and protein intake (model 3). Serum creatinine was not included as a confounder in any of the models investigating the association of vitamin D intake with the dependent variables. The statistical analysis was carried out using SPSS version 19 (SPSS, Chicago, IL, USA). A P-value ≤0.05 was considered as statistically significant.

Results

General characteristics of the study population are presented in **Table 6.1**. In total, 53% of the elderly in this study were vitamin D insufficient, as reflected by the number of persons with serum 25(OH)D levels below 50 nmol/L. In addition, 94% had a vitamin D intake below the estimated average requirement (EAR) of 10 μg/d[7], with an average vitamin D intake of 4.6±3.0 μg/d. Participants with sufficient 25(OH)D levels were more likely to be younger (P=0.01) and more physically active (P<0.001), compared to those with insufficient levels. Furthermore, crude data, as presented in **Table 6.1**, suggest better physical performance with higher serum 25(OH)D levels. Participants with 25(OH)D levels ≥50 nmol/L scored 2 points higher on the SPPB when compared to participants with levels of 25(OH)D <50 nmol/L (P<0.001). Analyzing the different components of the SPPB showed a similar trend. Gait speed and chair rise were performed significantly faster and also balance scores were higher among those with sufficient 25(OH)D levels, 4.8 vs. 6.3 s (P=0.01), 13.6 vs. 16.6 s (P=0.02) and 3.5 vs. 2.8 points on a 4 point scale (P=0.01), respectively. Vitamin D intake did not correlated with serum 25(OH)D levels, r=0.09 (P=0.30).

Associations between serum 25(OH)D status and vitamin D intake with measures of body composition are shown in **Table 6.2**. Serum 25(OH)D status appeared to be positively associated with ALM, β=0.012 (P=0.05) and showed a tendency for a positive association with LLM, β=0.009 (P=0.08). There was no association between vitamin D intake and measures of body composition.

Table 6.1 Subject characteristics according to their 25(OH)D status

Variable	Total sample n=127	25(OH)D <50 nmol/L n=67	25(OH)D ≥50 nmol/L n=60	P-value
25(OH)D (nmol/L) (median ± lower/upper quartile)	47 (33–73)	35 (24–40)	73.5 (65–89.5)	0.00
Vitamin D intake (µg/d)	4.6±3.0	4.2±3.2	5.0±2.8	0.17
Age (y)	79.0±7.8	80.8±7.5	77.0±7.6	0.01
Women (%)	61	55	67	0.19
Weight (kg)[a]	76.2±13.8	77±14	75±14	0.38
Height (m)	1.66±0.09	1.66±0.09	1.66±0.09	0.86
BMI (kg/m^2)[a]	27.5±4.3	27.8±4.3	27.2±4.4	0.55
Alcohol (%)				0.67
None	28	30	25	
<1 consumption/ d	32	34	31	
≥1 consumption/ d	40	36	44	
Education level (%)				0.89
Low	5.5	6.0	5.0	
Middle	59.6	61.2	58.3	
High	34.6	32.8	36.7	
Smoking (%)[b]	6.5	6.2	6.9	0.87
Total lean mass (kg)[c]	46.3±9.3	46.8±9.9	45.7±8.5	0.50
Appendicular lean mass (kg)[c]	19.5±4.3	19.7±4.7	19.3±3.9	0.32
Lean leg mass (kg)[c]	14.7±3.2	14.8±3.5	14.5±2.8	0.23
Fat mass (kg)[c]	26.2±9.0	26.5±8.8	25.9±9.2	0.69
Bone mineral content (kg)[c]	2.5±0.6	2.6±0.7	2.5±0.6	0.64
1-RM leg press (kg)[d]	120±34	120±35	120±34	0.99
1-RM leg extension (kg)[e]	57±19	57±19	58±19	0.85
Handgrip strength (kg)[a]	26.1±9.2	26.0±8.6	26.2±10.1	0.95
SPPB (points)[b]	8.2±2.8	7.1±2.9	9.2±2.3	0.00
Balance (points)[b]	3.1+1.1	2.8±1.3	3.5±0.8	0.01
Gait speed (sec)[b]	5.6±2.7	6.3±3.2	4.8±1.7	0.01
Chair rise (sec)[f]	15.1±6.2	16.6±6.0	13.6±6.0	0.02
Physical activity (counts/min)[g]	139.5±94.1	99.6±66.7	181±101	0.00
Energy intake (MJ/d)[a]	8.2±2.2	8.0±2.2	8.4±2.1	0.36
Calcium intake (mg)	1032±395	990±347	1078±440	0.21
Protein intake (g/d)[a]	76.4±21.6	75.8±20.6	77.0±22.9	0.75
Glucose (mmol/L)[h]	5.3±0.5	5.3±0.5	5.2±0.4	0.57
Insulin (mU/L)[h]	18.5±7.0	18.6±6.4	18.3±7.6	0.85
Creatinine (mmol/L)	72.8±14.8	75.0±14.4	70.4±13.8	0.07
Season (%)				0.32
Summer	48	52	43	
Fall	52	48	57	

Values are expressed as a mean ± SD, median with upper and lower quartile or percentage. Superscript indicate missing values: [a] 1 missing value, [b] 4 missing values, [c] 10 missing values, [d] 16 missing values, [e] 18 missing values, [f] 28 missing values, [g] 21 missing values, [h] 17 missing values.

Table 6.2 Association of 25(OH)D status and vitamin D intake with body composition

	25(OH)D status				Vitamin D intake			
Variable	β	95% CI	P-value	n	β	95% CI	P-value	n
Total lean mass (kg)								
Model 1	0.002	-0.061 – 0.065	0.958	117	0.265	-0.287 – 0.818	0.343	117
Model 2	0.015	-0.010 – 0.039	0.234	96	0.028	-0.176 – 0.232	0.783	96
Model 3	0.018	-0.006 – 0.041	0.139	95	-0.096	-0.319 – 0.127	0.394	95
Appendicular lean mass (kg)								
Model 1	0.007	-0.022 – 0.036	0.631	117	0.114	-0.143 – 0.371	0.382	117
Model 2	0.011	-0.002 – 0.024	0.093	96	-0.007	-0.116 – 0.102	0.899	96
Model 3	0.012	0.000 – 0.025	0.050	95	-0.037	-0.157 – 0.084	0.546	95
Leg lean mass (kg)								
Model 1	0.005	-0.016 – 0.027	0.624	117	0.088	-0.101 - 0.277	0.359	117
Model 2	0.008	-0.002 – 0.019	0.124	96	-0.005	-0.093 – 0.083	0.910	96
Model 3	0.009	-0.001 – 0.019	0.079	95	-0.014	-0.112 – 0.083	0.773	95
Fat mass (kg)								
Model 1	-0.048	-0.108 – 0.013	0.124	117	0.210	-0.329 – 0.749	0.442	117
Model 2	-0.016	-0.039 – 0.008	0.198	96	-0.128	-0.325 – 0.070	0.202	96
Model 3	-0.019	-0.041 – 0.004	0.103	95	-0.025	-0.240 – 0.191	0.820	95
Fat percentage (%)								
Model 1	-0.047	-0.106 – 0.013	0.126	117	0.069	-0.462 – 0.600	0.798	117
Model 2	-0.023	-0.058 – 0.013	0.202	96	-0.115	-0.410 – 0.181	0.443	96
Model 3	-0.027	-0.061 – 0.007	0.115	95	0.024	-0.298 – 0.346	0.884	95

Model 1: unadjusted model. Model 2: adjusted for age, gender, height, body weight, alcohol, physical activity, education, smoking, creatinine. Model 3: additionally adjusted for energy and protein intake. Creatinine was not included as a confounder in any of the models investigating the association of vitamin D intake with the dependent variables.

Table 6.3 presents the associations of 25(OH)D status and vitamin D intakes with measures of maximum strength and physical performance. After full adjustment, significant associations were observed for 25(OH)D status and vitamin D intake with SPPB (β=0.020 (P=0.035) and β=0.180 (P=0.038), respectively).

Discussion

In total, 53% of the frail elderly had a compromised 25(OH)D status (<50 nmol/L). This compromised 25(OH)D status was associated with reduced appendicular lean mass and impaired physical performance. Low vitamin D intake was associated with impaired physical performance, but not with reduced appendicular lean mass.

The present study is the first cross sectional study investigating the association between 25(OH)D status and muscle mass in a pre-frail and frail elderly population. To allow the inclusion of an adequate sample of frail elderly subjects, 1420 elderly people were approached, 398 were screened and finally 127 participants met the frailty criteria described by Fried et al.[2]. These criteria have been reported to be highly predictive for falls, hospitalization, disability, and mortality[2]. In agreement, the selected subjects were characterized by low baseline physical performance, strength and poor handgrip strength (**Table 6.1**). Moreover, this study revealed a high prevalence of elderly people with an insufficient 25(OH)D level. Since our blood sampling took place during summer and fall, this reported prevalence may be an underestimation of the 25(OH)D status for winter and early spring. During the winter and early spring, 25(OH)D levels have been reported to be substantially lower due to low sunlight exposure[28], suggesting an even higher prevalence of frail elderly people with inadequate 25(OH)D levels.

In our study, a significant association between 25(OH)D status and ALM in a frail elderly population was found. This association of 25(OH)D status and ALM is supported by some[5,6], but not all epidemiological studies[29,30]. Mechanistically, it is suggested that the activation of the vitamin D receptor (VDR) in skeletal muscle tissue plays an important role in the balance of muscle protein turnover[31]. The activation of the VDR might stimulate skeletal muscle protein synthesis[31,32] and might prevent type 2 muscle fiber atrophy[33]. However, most work has been done *in vitro* and more research is needed in humans to understand the underlying mechanisms that support our findings on appendicular lean mass.

Our results indicated that 25(OH)D status and vitamin D intake are positively associated with physical performance measures in frail elderly people. We found that participants with 25(OH)D levels ≥50 nmol/L scored 2 points higher on the SPPB when compared to

Table 6.3 Association of 25(OH)D status and vitamin D intake with physical performance

	25(OH)D status				Vitamin D intake			
Variable	β	95% CI	P-value	n	β	95% CI	P-value	n
1-RM leg press (kg)								
Model 1	0.040	-0.193 – 0.274	0.732	111	2.497	0.426 – 4.567	0.019	111
Model 2	0.141	-0.079 – 0.361	0.205	95	1.463	-0.358 – 3.284	0.114	95
Model 3	0.141	-0.076 – 0.357	0.200	94	1.646	-0.383 – 3.676	0.110	94
1-RM leg extension (kg)								
Model 1	0.055	-0.074 – 0.185	0.397	109	0.945	-0.235 – 2.126	0.115	109
Model 2	0.062	-0.049 – 0.174	0.268	93	0.203	-0.746 – 1.153	0.671	93
Model 3	0.063	-0.050 – 0.176	0.269	92	0.235	-0.842 – 1.312	0.665	92
Handgrip strength (kg)								
Model 1	0.004	-0.057 – 0.065	0.902	126	0.533	0.002 – 1.065	0.049	126
Model 2	-0.016	0.068 – 0.035	0.527	103	0.008	-0.411 – 0.427	0.970	103
Model 3	-0.013	-0.064 – 0.039	0.620	102	-0.061	-0.529 – 0.408	0.797	102
SPPB (points)								
Model 1	0.033	0.016 – 0.051	0.000	123	0.160	-0.004 – 0.324	0.056	113
Model 2	0.022	0.003 – 0.040	0.020	102	0.064	-0.093 – 0.220	0.442	102
Model 3	0.020	0.001 – 0.038	0.035	101	0.180	0.010 – 0.350	0.038	101
Gait speed (m/s)								
Model 1	-0.028	-0.045 – -0.011	0.001	123	-0.147	-0.302 – 0.007	0.062	123
Model 2	-0.016	-0.033 – 0.001	0.059	102	-0.054	-0.196 – 0.088	0.448	102
Model 3	-0.014	-0.031 – 0.003	0.095	101	-0.160	-0.313 – -0.007	0.041	101
Chair rise (s)								
Model 1	-0.068	-0.115 – 0.022	0.004	99	0.102	-0.289 – 0.493	0.607	99
Model 2	-0.048	-0.106 – 0.010	0.103	82	0.211	-0.215 – 0.638	0.326	82
Model 3	-0.043	-0.100 – 0.015	0.145	81	-0.021	-0.505 – 0464	0.993	81
Balance score (points)								
Model 1	0.010	0.003 - 0.017	0.005	123	0.035	-0.029 – 0.099	0.278	123
Model 2	0.004	-0.003 – 0.012	0.268	102	0.011	-0.056 – 0.078	0.749	102
Model 3	0.004	-0.004 – 0.012	0.311	101	0.042	-0.033 – 0.117	0.267	101

Model 1: unadjusted model. Model 2: adjusted for age, gender, height, body weight, alcohol, physical activity, education, smoking, creatinine. Model 3: additionally adjusted for energy and protein intake. Creatinine was not included as a confounder in any of the models investigating the association of vitamin D intake with the dependent variables.

participants with levels of 25(OH)D <50 nmol/L (P<0.001). In accordance, the SPPB score significantly improved with 0.02 points per 1 nmol/L increase in 25(OH)D and 0.18 points per 1 µg increase in vitamin D intake, indicating a clinically relevant improvement[34]. These findings are in line with the majority[5,15,35-39], but not with all observational studies[29,40]. Our findings are in line with randomized, placebo-controlled trials, showing an improvement of physical performance after vitamin D supplementation in community-dwelling elderly people[14,41,42]. This improvement of physical performance might be attributed to the role of 1,25-dihydroxyvitamin D (1,25(OH)2D), the active form of 25(OH)D, in muscle. It has been suggested that 1,25(OH)2D regulates muscle calcium concentrations by modulating the activity of calcium pumps in sarcoplasmic reticulum and sarcolemma[13]. Alterations in intracellular calcium concentrations regulate the contraction and relaxation of muscle, which may impact physical performance. The latter underpins the importance of an adequate vitamin D intake and 25(OH)D status to improve or maintain physical performance.

A possible limitation might be the appearance of reverse causation due to the cross-sectional design of the study. It may be that participants with the highest physical activity level and physical performance score are the ones that are the most likely to go outside and consequently have a higher 25(OH)D status, and not *vice versa*. However, a causal relationship seems plausible because of biologic mechanisms[31] and evidence obtained from randomized, placebo-controlled trials[33,42,43] that confirm the causality of 25(OH)D status and physical performance.

Despite the association of 25(OH)D status with appendicular lean mass, we found no significant association between vitamin D intake and appendicular lean mass. The latter finding might be explained by the lack of correlation between vitamin D intake and 25(OH)D status. In our study, vitamin D intake was 4.6±3.0 µg/d which is in line with our expectations as food fortification in the Netherlands is not broadly practiced and vitamin D rich products are often not part of the daily diet. The lack of association between vitamin D intake and 25(OH)D status might be attributed by that low and narrow range of vitamin D intake. Despite the lack of correlation between vitamin D intake and 25(OH)D status in our study, a recent meta-regression analysis did show a significant association between vitamin D intake and 25(OH)D status[44]. Moreover, ample evidence presented an increase in 25(OH)D status after vitamin D supplementation[12,45-47], suggesting that vitamin D supplementation represents an effective strategy to improve 25(OH)D status. Especially among frail elderly people there is a greater need to take a vitamin D supplements because endogenous vitamin D synthesis decreases with age. This deceased vitamin D synthesis might be explained by a low outdoor habitual physical activity[48] and thus a low sunlight exposure as well as the reduced capacity to synthesize vitamin D in the skin[49]. More well-designed interventions

studies are warranted to investigate the impact of vitamin D supplementation on 25(OH)D status and its impact on muscle mass and physical performance in a frail elderly population.

In conclusion, vitamin D deficiency is highly prevalent in a frail elderly population, which is associated with reduced ALM and impaired physical performance. In addition, also vitamin D intake is associated with impaired physical performance.

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7

Discussion

The age-related loss of muscle mass and strength is currently recognized as one of the major determinants affecting physical frailty and disability. Lifestyle interventions that include physical activity and adequate nutrition might be promising strategies to attenuate or treat sarcopenia. In this thesis, we evaluated the daily protein intake of various elderly subpopulations, including that of frail elderly people. Based on this study, we designed intervention studies aiming to investigate the impact of protein supplementation with or without resistance-type exercise training on muscle mass, strength and physical performance in frail elderly people. These studies show that dietary protein supplementation did not increase muscle mass without a resistance-type exercise training program, but improved physical performance. Prolonged resistance-type exercise training improved strength and physical performance, but an adequate dietary protein is required to allow muscle mass gain during exercise training in frail elderly people.

Dietary protein intake

It has been suggested that adequate dietary protein intake is required to attenuate and treat sarcopenia in elderly people. In **chapter 3**, we observed that dietary protein supplementation did improved physical performance in frail elderly people. These findings tend to be in line with the majority of studies showing that protein supplementation improves physical performance in (frail) elderly people (**Table 7.1**). However, intervention studies are limited, the heterogeneity among physical performance measures is high and the underlying mechanisms are unknown. Therefore, more research is warranted to study the impact of dietary protein supplementation on physical performance in the elderly. Although these data suggest that protein supplementation may improve physical performance in frail elderly people, simply increasing protein intake will not lead to substantial muscle mass gain (**Table 7.1**). So far, the most effective strategy to augment muscle mass and improve muscle strength and physical performance, is through stimulating physical activity.

Physical activity

Resistance-type exercise training is currently the most effective intervention to elicit improvements in muscle hypertrophy, muscle strength and physical performance[14-19]. Indeed, a recent meta-analysis, that included 49 studies, showed that after an average of 20.5 wks of resistance-type exercise training, elderly people gained 1.1 kg (CI: 0.9–1.2) lean body mass[20]. Furthermore, an additional meta-analysis showed that elderly people

Table 7.1 Intervention studies on the impact of protein and/or amino acids on muscle mass, strength and physical performance in elderly people

Author, year	N	Population	Duration	Muscle mass	Muscle strength	Physical performance
Aquilani, 2008[1]	38	Heart failure patients	8 wks	↑		↑
Bjorkman, 2012[2]	106	Institutionalized	24 wks	→	→	
Carlsson, 2011[3]	84	Institutionalized	24 wks	→		
Dal Negro, 2010[4]	32	Sarcopenic	12 wks	↑		
Dillon, 2009[5]	14	Healthy	12 wks	↑	→	
Flakoll, 2004[6]	50	Healthy	12 wks	→	↑	↑
Kim, 2012[7]	74	Sarcopenic	12 wks	→	→	→
Leenders, 2011[8]	57	Diabetic	24 wks	→	→	
Rondanelli, 2011[9]	41	Institutionalized	8 wks		↑	↑
Scognamiglio, 2005[10]	95	Frail	12 wks		↑	↑
Solerte, 2008[11]	41	Sarcopenic	16 wks	→		
Tieland, 2012[12]	65	Frail	24 wks	→	→	↑
Verhoeven, 2009[13]	30	Healthy	12 wks	→	→	

Wks: weeks. ↑ indicate significance increase. → indicate no significant increase.

improved 1-RM leg press strength by 29±2% and 1-RM leg extension strength by 33±2% after an average of 18 wks of resistance-type exercise training[21]. In agreement, we observed a substantial 40±4% increase in leg press and 36±3% increase in leg extension strength following 24 wks of intervention. Importantly, the increase in leg muscle strength translated to a substantial 1.3±0.2 points improvement in physical performance as determined by the SPBB. Despite, the benefits of resistance-type exercise training on muscle strength and physical performance, we failed to observe changes in muscle mass after 24 wks of exercise training without protein supplementation. It has been suggested that exercised muscles become more sensitive for nutrients, allowing more of the available amino acids to be synthesized into muscle protein. In sedentary elderly subjects, however, sensitivity of skeletal muscle tissue to anabolic stimuli such as physical activity might be reduced[22,23]. As such, it could be speculated that a more sedentary lifestyle is responsible for the anabolic resistance to physical activity and protein intake in frail elderly people[22]. In agreement, our frail elderly subjects had a sedentary lifestyle and we did not find a significant effect of resistance-type exercise training without protein supplementation on muscle mass. The combination of resistance-type exercise training and protein supplementation, however, did result in an increase in muscle mass, suggesting that dietary protein is required to overcome anabolic resistance in frail elderly people.

Dietary protein intake and physical activity

Dietary protein intake following a single bout of resistance-type exercise increases muscle protein synthesis rates and inhibits muscle protein breakdown, thereby allowing net muscle protein accretion[24,25]. As such, it is often suggested that dietary protein supplementation can maximize the skeletal muscle adaptive response to prolonged resistance-type exercise training. There has been much discrepancy in the literature on the proposed surplus benefits of dietary protein supplementation during prolonged resistance-type exercise training. Whereas some studies report greater gains in muscle mass and strength when dietary protein is supplemented during prolonged resistance-type exercise training[26,27], most studies[28-35] have failed to confirm these findings. In an attempt to explain the apparent discrepancy, Cermak et al. conducted a meta-analysis that investigated the impact of dietary protein supplementation during prolonged resistance-type exercise training in elderly people[36]. This meta-analysis, that included studies published prior to May, 2011, showed that dietary protein supplementation during ~12 wks of resistance-type exercise training significantly resulted in a gain of 0.78 kg lean mass when compared with resistance-type exercise training without a protein based nutritional intervention[36].

Notably, when studies were examined individually, only one study reported a statistical significant surplus benefit of protein supplementation during resistance-type exercise training on muscle mass gain when compared with a placebo. Though not statistically significant, the majority of studies, including the latest studies of Leenders et al.[29], and Chale et al.[35], did show a small effect of dietary protein supplementation on muscle mass during prolonged resistance-type exercise training. Although these small differences, i.e. 0.78 kg lean mass, are not easily uncovered, over the course of many years these small differences may have a huge impact on muscle mass, strength, and physical performance. According to longitudinal studies in people aged 75 y or over[37], muscle mass is lost at a rate of 0.64–0.98% per year. The latter reduction might be translated to an average 0.3–0.5 kg loss of muscle mass for an average elderly individual (~50 kg lean mass). Our data show an increase of 1.2 kg lean mass after 12 wks and a total increase of 1.3 kg lean mass after 24 wks of intervention. When we extrapolate these data, one year of dietary protein supplementation and resistance-type exercise training would result in a further increase of some 0.2 kg lean body mass. Given the otherwise annual loss of 0.3–0.5 kg muscle mass, resistance-type exercise training and protein supplementation appear to prevent the age-related muscle loss and even augment muscle mass gain (i.e. 1.8–2.0 kg difference). In addition, muscle strength is lost more rapidly. At the age of 75 y, strength is lost at a rate of 3% per year[37]. We observed 30–40% increase in muscle strength after 24 wks of intervention, which might be further improved over the course of many years. Although these data are highly speculative and long-term interventions, i.e. one to five years, are warranted, our data confirm the important role of adequate dietary protein ingestion and resistance-type exercise training to attenuate and even treat sarcopenia in the elderly.

Dietary protein strategies to further augment muscle mass

As resistance-type exercise training is currently the most effective intervention to stimulate muscle hypertrophy and as dietary protein further seems to stimulate these benefits, the combination of resistance-type exercise training and dietary protein supplementation is recommended as preferred approach to attenuate and/or treat sarcopenia. The small benefits of dietary co-intervention on the adaptive response to prolonged resistance-type exercise training might be enlarged by optimizing the dietary protein supplementation regimen. Various dietary protein intake strategies have been proposed, including the amount, timing and source of dietary protein. In addition, the population studied might be of importance to uncover the benefits of dietary protein.

How much protein do we need

The recommended daily allowance (RDA) for protein for adults older than 18 y is 0.83 g/kg-bw/d[38], which is an estimate of the amount of protein necessary to avoid loss of lean body mass. Several experimental studies, however, suggest that the RDA for protein may not be adequate for older people to maintain skeletal muscle[21]. Accordingly, a daily protein intake between 1.2 and 1.5 g/kg-bw/d has been recommended to reduce the risk for adverse health outcomes, such as sarcopenia and frailty[39-47]. The latter is supported by data from our studies showing beneficial effects of protein supplementation in frail elderly people (**chapter 3 and 4**). Based on emerging evidence, we suggest that the RDA for protein for the elderly should be reconsidered. With a modification of the RDA for protein to 1.2–1.5 g/kg-bw/d, the protein intake of an average weighted individual (75 kg) would be 90–110 g per d. This amount of protein intake would allow to increase the protein intake of all main meals to 25–30 g, which showed to improve physical performance and supported the benefits of a resistance-type exercise training program in frail elderly people.

Timing of protein intake

Our observational data show that daily protein intake is not equally distributed over the various main meals, and that breakfast and lunch are particularly low in protein (**chapter 2**). Interestingly, evidence suggests that the post-prandial muscle protein synthetic response to smaller, meal-like amounts of amino acids is attenuated in older subjects when compared with young controls[23,48], resulting in a more negative net muscle protein balance and subsequent the degradation of muscle tissue. The attenuated muscle protein synthetic response to meal-like protein intakes will become of great clinical relevance over the course of many years. Consequently, it has been suggested that 25–30 g of dietary protein per main meal is required to allow an appropriate stimulation of post-prandial muscle protein synthesis and to overcome the attenuated protein synthetic response[49-52] and, as such, muscle mass accretion. In addition, ingesting ample protein prior to or post resistance-type exercise training has been shown to further improve post-exercise muscle protein synthesis[53]. In **chapter 4**, subjects consumed at least 25 g of protein prior to and post exercise training, allowing available amino acids to be synthesized into muscle protein, and as such, an 1.3±0.4 kg increase in muscle mass after 24 wks of intervention.

What source of protein

An increase in essential amino acid (EAA) availability represents the main anabolic signal responsible for stimulating postprandial muscle protein synthesis rates[54]. Therefore, sources rich in EAA, i.e. milk, whey, casein, meat, egg, fish, as well as some vegetable protein sources, are potent in stimulating muscle protein synthesis in elderly people. However, the effect on postprandial muscle protein synthesis rates differs among these food sources, despite a relatively high proportion of EAA. Previous work suggests that ingestion of whey protein results in greater postprandial protein retention when compared with ingestion of casein[51,55,56]. The greater anabolic properties of whey versus casein protein have been attributed to the faster digestion and absorption kinetics of whey, resulting in a greater increase in postprandial plasma amino acid availability, thereby further stimulating muscle protein synthesis in elderly people[51,55,57]. Furthermore, whey protein has a high content of leucine, an essential amino acid. Earlier studies showed that a leucine-enriched mix of EAA increase muscle protein synthesis to a greater extent than other forms of protein[58-60]. Consequently, it is suggested that increasing the leucine content of a meal represents an effective strategy to enhance muscle hypertrophy in elderly people. In long-term intervention studies, however, we were unable to confirm that leucine co-ingestion with each main meal increases net muscle mass gain in elderly people[8,13]. These trials suggest that a complete mix of EAA, i.e. protein fractions or food products, is more likely to be beneficial than one single amino acid. Milk protein, i.e. 80% casein and 20% whey protein, is suggested to be a very potent protein source to stimulate muscle mass gain[61]. Previous studies showed that milk stimulated protein accretion to a greater extent than an isonitrogenous quantity of soy proteins[62-64] did. In the presence of resistance-type exercise training, milk stimulated muscle protein synthesis[65,66] and increased lean body mass in young subjects[67,68]. Our studies confirm these results in frail elderly people showing a significant increase in lean body mass after 24 wks of milk protein supplementation during prolonged resistance-type exercise training (**chapter 4**). Thus, dairy based proteins are very potent dietary protein sources to stimulate muscle synthesis and to enhance the benefits of resistance-type exercise training, and as such, elicit improvements in muscle hypertrophy in the elderly.

Population

The loss of muscle mass with aging is associated with a more sedentary lifestyle and a less than adequate dietary protein intake. Therefore, it could be speculated that the surplus benefits of dietary protein supplementation during prolonged resistance-type

exercise training are more evident in compromised frail elderly people. Though we can only speculate on the various factors that might explain the differences in the efficacy by which protein supplementation modulates the gain in muscle mass and physical performance between the healthy and frail elderly population, it might be that this disparity is attributed to differences in muscle mass, performance, inflammatory status, hormones, insulin resistance, the level of habitual physical activity, as well as dietary protein intake. Indeed, 21% of our population were sarcopenic[69] and had significant lower muscle strength and physical performance scores compared with a healthy elderly population[29]. Consequently, the low muscle mass and impaired physical performance in frail elderly people would allow a window to reveal the surplus benefits of dietary protein supplementation during prolonged resistance-type exercise training. Furthermore, >90% of our frail elderly subjects had a sedentary lifestyle (Tieland et al., unpublished), which might reduce the sensitivity of skeletal muscle tissue to anabolic stimuli such as physical activity[22,23]. In fact, we observed no improvement in lean body mass in the placebo group, despite resistance-type exercise being a well-known intervention to augment muscle mass in healthy older adults[20]. Furthermore, frail elderly people tend to have higher plasma IL-6 and TNF-α concentrations[70], lower testosterone levels[71] and tend to be more insulin resistant[72]. It has been suggested that these factors play an important role in the anabolic resistance to physical activity and dietary intake in frail elderly people[72]. It could be speculated that the combination of an adequate dietary protein provision and resistance-type exercise training would allow frail elderly subjects to overcome the anabolic resistance, and as such, elicit improvements in muscle hypertrophy. Although, the latter hypothesis needs to be verified in more mechanistic studies, our data strongly support the benefits of dietary protein supplementation during resistance-type exercise training to augment muscle mass in a frail elderly population.

Future research strategies to augment muscle mass

As extensively discussed, dietary protein has been identified as the main dietary factor stimulating muscle protein synthesis and increasing muscle mass accretion during exercise training. However, other macronutrients may modulate the post-prandial muscle protein synthetic response to protein ingestion and therefore might play an important role in the development or treatment of sarcopenia and frailty.

Previous work suggests that carbohydrate co-ingestion with protein stimulates post-prandial muscle protein synthesis rates[73-78]. This stimulation might be attributed to the greater post-prandial insulin release following carbohydrate co-ingestion. In agreement,

higher insulin concentrations have been reported to stimulate muscle protein synthesis rates when ample amino acids are available[74]. However, recent studies failed to observe surplus benefits of carbohydrate co-ingestion on post-prandial muscle protein synthetic response when ample protein is ingested in young[77] and elderly subjects[79,80]. The latter results might be attributed to the population studied, since healthy subjects are generally not resistant to insulin. In frail elderly people, however, the high prevalence of insulin resistance might play an important role in the anabolic resistance to smaller, meal-like protein intakes. Therefore, it can be hypothesized that higher post-prandial insulin concentrations are required to maximize the muscle protein synthetic response to protein intake in a frail elderly population. Carbohydrate co-ingestion might induce greater post-prandial insulin release, allowing to overcome the anabolic resistance, and as such, stimulate the post-prandial muscle protein synthetic response to protein ingestion in frail elderly people.

Some evidence suggests that omega-3 fatty acids i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are useful nutrients to prevent and/or treat sarcopenia. In cross-sectional studies, omega-3 fatty acids have been associated with leg strength[81] and handgrip strength[82] as well as with physical performance[81,83]. Furthermore, omega-3 fatty acids stimulated the post-prandial muscle protein synthetic response post amino acid and insulin infusion in older adults[84]. The latter muscle protein synthetic response is suggested to be mediated via increased activation of the mTOR pathway[84]. Furthermore, omega-3 fatty acids have anti-inflammatory properties[85], which may also help to overcome the anabolic resistance to physical activity and/or protein ingestion in older adults. Yet, the impact of omega-3 fatty acids on muscle metabolism are poorly understood and its long-term effects on muscle mass and physical performance in elderly people are still unknown.

Vitamin D has been described as an important nutrient related to sarcopenia[86-90]. In **chapter 6**, we found that vitamin D status is compromised in frail elderly people. The 25(OH)D status was associated with poor muscle mass and impaired physical performance. However, care should be taken interpreting these results since reverse causation might be apparent due to the cross-sectional design of the study. To investigate causality, several controlled intervention studies investigated the impact of vitamin D on muscle mass[91,92], strength[93] and physical performance[93,91,94] in elderly people. A recent meta-analysis showed no beneficial effect of vitamin D treatment on handgrip strength, leg press and leg extension strength in community-dwelling elderly people[93]. In institutionalized elderly people that started with 25(OH)D status below 25 nmol/L, however, vitamin D supplementation did improve leg extension and proximal lower limb muscle strength[91,94], suggesting that a more compromised elderly population might benefit from vitamin

D supplementation to enhance muscle strength. Although data from the latter studies present promising findings, evidence of the impact of vitamin D on muscle mass is scarce and present discrepant findings. This discrepancy is expected, given the different study populations, initial degrees of insufficiency, and doses of vitamin D and co-interventions tested. Therefore, randomized placebo-controlled trials are needed to elucidate the impact of vitamin D on the development of sarcopenia and frailty.

Though, carbohydrate, omega-fatty acids and vitamin D are all potential agents to augment muscle mass and physical performance, a combination of those nutrients embedded in a supplement or with the use of (enriched) foods might be most promising. A multi-nutrient supplement with at least 15 g of protein provided at breakfast and lunch during resistance-type exercise training in a compromised elderly population might be a very promising strategy to augment muscle mass and improve physical performance.

Public health implications

In a growing elderly population, the prevention or treatment of sarcopenia might reduce the risk for disability, dependence, institutionalization, hospitalization and mortality[95-99]. Our data clearly demonstrate that dietary protein supplementation and prolonged resistance-type exercise training augmented muscle mass and physical performance. Therefore, dietary protein and resistance-type exercise training play an important role in the prevention and/or treatment of sarcopenia. Other macronutrients and vitamin D might even further support the anabolic properties of dietary protein and exercise training, suggesting that sarcopenia is reversible with an adequate lifestyle intervention. The ability to treat sarcopenia not only reduces the estimated cost of sarcopenia[36], but also decreases the risk of disability, dependence and frailty[95-99]. Furthermore, increasing muscle mass might reduce the risk for various diseases including diabetes and obesity[38,98], increase survival during cancer treatments and accelerate post-operative recovery and subsequent hospital discharge. As such, our results and future research directions are not only important for the preservation and/or treatment of sarcopenia and frailty, but may have a much broader health impact.

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Samenvatting

Het aantal ouderen in de wereld neemt sterk toe. Vanaf 1950 tot 2000 is het aantal ouderen op de wereld verdrievoudigd tot 600 miljoen. Nu wordt het aantal ouderen op de wereld in 2050 zelfs geschat op 2 miljard. Het aantal Nederlanders boven de 65 in het jaar 2060 wordt nu op 25% van de totale bevolking geschat. Meer dan 40% van die ouderen zal dan 80 jaar of ouder zijn. Deze verwachte stijging van het aantal ouderen zal ook tot gevolg hebben dat meer ouderen fysieke beperkingen ondervinden bij het uitvoeren van hun dagelijkse activiteiten. Op dit moment heeft ongeveer 30% van de mensen boven de 55 jaar een fysieke beperking. Fysieke beperkingen kunnen gepaard gaan met verlies van eigen onafhankelijkheid, een verhoogde kans op opname in een verzorgingshuis, chronisch metabole ziekten en zelfs leiden tot vervroegd overlijden.

Het Ministerie van Volksgezondheid, Welzijn en Sport benadrukt dan ook het belang van preventie en adequate behandeling van ouderen met fysieke beperkingen, zodat zij zo lang mogelijk fysiek actief en onafhankelijk kunnen blijven en op die manier gezond ouder kunnen worden.

Eén van de verklaringen voor het ontstaan van fysieke beperkingen gedurende het ouder worden is de progressieve afname van skeletspiermassa en spierkracht. Dit proces wordt ook wel sarcopenie genoemd. Sarcopenie gaat gepaard met een verminderde spiereiwitsynthese en een verhoogde spiereiwitafbraak, hetgeen resulteert in een negatieve spiereiwitbalans en afbraak van spiermassa. Factoren die hier mogelijk aan ten grondslag liggen zijn verhoogde inflammatie, veranderingen in hormoonspiegels, neurologische veranderingen, fysieke inactiviteit en inadequate voedselinname.

Om sarcopenie te voorkomen of tegen te gaan zijn krachttraining en een adequate eiwitinname belangrijk. Onderzoek wijst uit dat krachttraining de belangrijkste stimulus is voor de spiereiwitsynthese. Inspanning verhoogt ook de spiereiwitafbraak, maar inspanning verhoogt de spiereiwitsynthese sterker dan de spiereiwitafbraak, waardoor een minder negatieve spiereiwitbalans ontstaat. Echter, zonder de inname van eiwitten blijft de balans tussen spiereiwitsynthese en spiereiwitafbraak negatief. Indien eiwitten worden ingenomen voor, tijdens of na een krachttraining wordt de balans wel positief en wordt er meer spier opgebouwd.

Epidemiologisch onderzoek wijst uit dat de eiwitinname van gezonde ouderen zo rond de 1,1 gram per kg lichaamsgewicht per dag ligt. Bij fragiele ouderen ligt de inname iets lager en bij geïnstitutionaliseerde ouderen ligt de dagelijkse eiwitinname rond de 0,8 gram per kg lichaamsgewicht per dag (hoofdstuk 2). We hebben ook de eiwitinname per maaltijdmoment bestudeerd. Bij fragiele ouderen bedraagt de eiwitinname bij het ontbijt gemiddeld 11 gram en bij de lunch 16 gram. Dit lijkt onvoldoende te zijn voor een

maximale spiereiwitsynthese. Eerder onderzoek toont aan dat bij een eiwitinname van 15–20 gram de eiwitsynthese bij ouderen significant lager is dan bij jongere deelnemers. Er wordt gesuggereerd dat minimaal 25 gram eiwit per hoofdmaaltijd nodig is voor een meetbare toename in spiereiwitsynthese na een maaltijd. Uitgaande van het gegeven dat fragiele ouderen een (te) lage eiwitinname hebben bij het ontbijt en bij de lunch en het feit dat er nog maar weinig onderzoek is gedaan in deze ouderenpopulatie zijn we twee lange-termijn-interventiestudies gestart.

De eerste interventiestudie staat beschreven in hoofdstuk 3. In deze studie zijn de effecten van 24 weken eiwitsuppletie op spiermassa, spierkracht en fysiek functioneren van 65 fragiele ouderen onderzocht. De deelnemers hebben 24 weken lang twee keer per dag, bij het ontbijt en bij de lunch, een supplement met 15 gram eiwit of een placebosupplement gekregen. Na 24 weken interventie is de hoeveelheid spiermassa in beide groepen gelijk gebleven. Wel is het fysiek functioneren na 24 weken eiwitsuppletie significant verbeterd ($P<0,05$). In de controlegroep daarentegen is het fysiek functioneren onveranderd gebleven. We concluderen dat extra eiwitinname bij het ontbijt en bij de lunch het fysiek functioneren van fragiele ouderen significant verbetert.

De tweede interventiestudie staat beschreven in hoofdstuk 4. In deze studie zijn gedurende 24 weken de effecten van eiwitsuppletie én krachttraining op spiermassa, spierkracht en fysiek functioneren van 62 fragiele ouderen bestudeerd. De deelnemers hebben 24 weken lang twee keer per dag, bij het ontbijt en bij de lunch, een supplement met 15 gram eiwit of een placebosupplement gekregen. Zowel in de eiwitgroep als in de placebogroep hebben de deelnemers een krachttrainingsprogramma gevolgd. In zowel de eiwitgroep als in de placebogroep zijn spierkracht en fysiek functioneren significant toegenomen. Zo is de spierkracht na 24 weken met 40% toegenomen in beide groepen. Eiwitsuppletie is echter nodig om de spiermassa te doen vergroten. Na 24 weken krachttraining en eiwitsuppletie is de spiermassa met 1,3 kg significant toegenomen ($P<0,05$). In de controle groep daarentegen, is de hoeveelheid spiermassa onveranderd gebleven.

Naast deze twee interventiestudies is in hoofdstuk 5 de relatie tussen handknijpkracht en beenspierkracht en de impact van krachttraining op handknijpkracht van fragiele ouderen bestudeerd. Uit ons onderzoek is handknijpkracht geen goede maat gebleken om de lange-termijneffecten van krachttraining op spierkracht te meten bij oudere deelnemers.

In hoofdstuk 6 is de relatie tussen vitamine D status en spiermassa, kracht en fysiek functioneren bestudeerd bij fragiele ouderen. In deze observationele studie hebben wij een relatie tussen een lage vitamine D status en een verminderd fysiek functioneren gevonden. Ook is een verband tussen een lage vitamine D inname en een verminderd fysiek func-

tioneren aangetoond. Onze studie suggereert dat vitamine D een belangrijke rol speelt bij het fysiek functioneren van fragiele ouderen. Er zijn echter meer interventiestudies nodig om de impact van vitamine D op de spierfunctie van fragiele ouderen aan te tonen.

Onze bevindingen kunnen mogelijk een grote impact hebben op de gezondheid en kwaliteit van leven van ouderen. Ondanks dat eiwitsuppletie de spiermassa niet vergroot, lijkt eiwitsuppletie wel een veelbelovende strategie te zijn om fysiek functioneren van fragiele ouderen te verbeteren. Daarnaast toont dit proefschrift duidelijk aan dat langdurige krachttraining een effectieve methode is om spierkracht en fysiek functioneren van fragiele ouderen te verbeteren. Op basis van onze bevindingen is hierbij extra eiwitinname nodig om de spiermassa van fragiele ouderen te vergroten. Langdurige krachttraining en eiwitsuppletie kunnen een belangrijke bijdrage leveren om sarcopenie te beperken en wellicht ook te voorkomen.

Dankwoord

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Mike

About the author

Curriculum vitae

Michael Tieland was born on March 20, 1979 in Apeldoorn. He completed secondary school at the Sprengeloo college in 1995 and Executive Hotel Management at the Pascal College in 1999 in Apeldoorn. Thereafter he started his bachelor Nutrition and Management at the HAN in Nijmegen. After completing his bachelor thesis at the University of the Western Cape in South Africa and receiving his BSc degree in 2003, Michael Tieland started a MSc program at the division of Human Nutrition at the Wageningen University. He focused on nutritional physiology with a special interest for physical activity. During an internship at NUMICO research he worked on muscular function and metabolism during cancer therapy. He obtained his MSc degree in Nutritional Physiology in 2007 after performing his final thesis at Human Movement science group of professor Luc van Loon at the Maastricht University. During this period, Michael performed several human intervention studies focussed on protein ingestion prior to exercise to stimulate muscle protein synthesis.

Shortly after receiving his MSc degree, Michael started his PhD project entitled 'Dietary strategies to augment muscle mass and function in elderly people' at the Top Institute Food and Nutrition (TIFN) and at the division of Human Nutrition of Wageningen University. Under the supervision of professor Lisette de Groot and professor Luc van Loon, he performed long term intervention studies to investigate the impact of protein supplementation with and without resistance-type exercise training on muscle mass and physical performance in frail elderly people. At the annual TIFN conference in 2012, Michael received a poster prize and at the same conference in 2013, Michael was nominated for the 2012 publication prize. During his PhD project, Michael was also involved in teaching and joined various committees and was selected for the European Nutrition Leadership Programme in 2013.

Since April 2012, Michael was appointed as a post-doctoral fellow on the TIFN projects 'Weight management' and 'Muscle health and function' at the same division. In these projects, the impact of protein but also vitamin D supplementation on muscle mass and performance in elderly people will be studied. With the latter projects, the interest of nutritional physiology and muscle metabolism continues.

Publications

1. Beelen M, Tieland M, Gijsen AP, Vandereyt H, Kies AK, Kuipers H, Saris WH, Koopman R, van Loon LJ. Coingestion of carbohydrate and protein hydrolysate stimulates muscle protein synthesis during exercise in young men, with no further increase during subsequent overnight recovery. *J Nutr*. Nov 2008;138(11):2198-2204.
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8. van de Rest O, van der Zwaluw N, Tieland M, Adam JJ, Hiddink G, van Loon LJ, de Groot LC. Effect of resistance-type exercise training with or without protein supplementation on cognitive functioning in frail and pre-frail elderly. *Submitted*. 2013.
9. van der Zwaluw N, van de Rest O, Tieland M, Adam JJ, Hiddink G, van Loon LJ, de Groot LC. The impact of 24 weeks protein supplementation on cognitive performance in frail elderly. *Submitted*. 2013.

10. Brouwer-Brolsma EM & van de Rest O, Tieland M, van der Zwaluw N, Steegenga WT, Adam JJ, van Loon LJ, Feskens EJ, de Groot LC. Serum 25-hydroxyvitamin D is associated with executive function in Dutch frail elderly. *Submitted*. 2013.

11. Tieland M, Brouwer-Brolsma EM, Nienaber-Rousseau C, van Loon LJ, de Groot LC. Compromised vitamin D status in frail elderly people is associated with reduced muscle mass and impaired physical performance. *Submitted*. 2013.

12. Tieland M, Verdijk LB, de Groot LC, van Loon LJ. Handgrip strength does not represent an appropriate measure to evaluate changes in muscle strength during an exercise intervention program in frail elderly people. *Submitted*. 2013.

Overview of completed training activities

Name of the course	Graduate school/institute	Year
Discipline specific activities		
Evidence–based Nutrition: From Requirements to Recommendations and Policies	VLAG	2008
Stable isotope course Maastricht	Maastricht University	2010
Good clinical practice, wet- en regelgeving voor klinisch onderzoek	Ziekenhuis Gelderse vallei	2011
Advanced Topics in Clinical Trials	NIHES	2011
Conferences and meetings		
Successful aging through diet and healthy lifestyle conference, Warsaw, Poland, 2008		2008
IANA conference, Albuquerque, USA 2008		2008
Wageningen Nutritional science conference, 'Too much too little, Arnhem, The Netherlands, 2009		2009
Annual meeting NWO nutrition, Deurne, The Netherlands, 2009		2009
ESPEN conference, Vienna, Austria, 2009		2009
IANA conference, Toulouse, France, 2010		2010
Food valley expo conference, Ede, The Netherlands, 2010 (oral presentation)		2010
ESPEN conference, Gothenburg, Sweden, 2011 (poster presentation)		2011
ECSS conference, Brugge, Belgium, 2012 (poster presentation)		2012
Food for thought, Ede, The Netherlands, 2012 (oral presentation)		2012
Danish Gerontology Society and the Danish ESPEN, Copenhagen, Denmark, 2012 (oral presentation)		2012
National Gerontologie conference, Ede, The Netherlands, 2012 (poster presentation)		2012
Annual TIFN nutrition conference, Arnhem, The Netherlands, 2012 (poster presentation)		2012
Food valley expo conference, Arnhem, The Netherlands 2012 (oral presentation)		2012
General courses		
VLAG PhD week	VLAG	2008
Project- and time management	WGS	2008
Scientific writing	WGS	2011
Presentation skills	TIFN	2010
Multilevel analysis	VLAG	2011
Mixed linear models	PE&RC	2011
PhD Competence assessment	VLAG	2008
People, politics and power	NWO	2009

Overview of completed training activities continues on next page.

Overview of completed training activities *Continued*

Name of the course	Graduate school/institute	Year
Basis IP for researchers	TIFN	2012
Negotiation	NWO	2009
ENLP	ENLP	2013
Optionals (participation in discussion groups, PhD excursions, etc)		
Oldsmobiles	WUR	2008-2010
Analytical Epidemiology	WUR	2008
Preparing PhD research proposal	VLAG	2008
Journal club	WUR	2008-2010
Four month visit at Maastricht University, department of Human Movement Science	Maastricht University	2011
TIFN Scientific meetings (every 6 weeks including presentations)	TIFN	2008-2012
PhD tour (poster presentation and oral presentation) 2009	WUR	2009

nederlandse zuivel organisatie

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