Essential amino acids supplementation and its effects on age related loss of muscle mass and function - A systematic review

Master Thesis in Medicine

Marcus Schober

Supervisor Elisabet Rothenberg

Food and Meal Science, Kristianstad University

UNIVERSITY OF GOTHENBURG

Programme in Medicine

Gothenburg, Sweden 2015

Contents

Abstract ... 3
Abbreviations ... 4
Introduction ... 5
Background ... 5
Sarcopenia definition and diagnosis ... 5
Patophysiology ... 6
Current potential treatments ... 7
Essential amino acids ... 9
Research question ... 9
Methods ... 11
Inclusion & exclusion criteria ... 11
Study selection ... 11
Quality assessment of studies ... 12
Ethics ... 12
Results ... 13
Study characteristics ... 13
Intervention results ... 20
Study quality according to GRADE ... 21
Evidence strength of effect ... 21
Disscussion ... 22
Summary of evidence ... 22
Limitations ... 24
Conclusions and implications ... 25
Acknowledgements ... 26
Populärvetenskaplig sammanfattning ... 29
Reference list ... 29
Appendix: Review templates ... 33

Abstract

Background: Loss of muscle mass, strength and/or function are common in the increasing elderly population. A conceptual and diagnostic term often used for these age related alterations of muscle mass is sarcopenia. Strategies to maintain functional ability and health are therefore of interest. Using dietary supplements, essential amino acids have shown promise to prevent this muscle wasting.

Aim: To evaluate currently published data investigating the use of essential amino acids in prevention of age related muscle loss in individuals with or at risk of sarcopenia over the age of 65. Outcomes will be lean body mass, strength and functional tests.

Methods: The electronic databases PubMed and Scopus were searched. Inclusion criteria were experimental studies in English from 1994-2015, using essential amino acid supplementation and subjects above 65 years. Search terms conducted were "essential amino acids" and "sarcopenia". A manual search for studies in found articles was also performed.

Results: Eight studies meeting the predetermined inclusion criteria were analyzed. The studies all indicated that intake of essential amino acids could maintain or increase lean body mass and muscle strength/function. The highest effect was seen in those subjects with sarcopenia.

Conclusions: Supplementation with essential amino acids seem to be effective in individuals above 65 years of age with low muscle mass, low strength or impaired function for maintenance or increased lean body mass and muscle strength/function. Optimal dose, intervention period and adequate combination of amino acids remain to be determined

Keywords: Essential amino acids, sarcopenia, leucine.

Abbreviations

1RM	One-repetition max
25OHD	25-hydroxy-vitamin D
AA	Amino acids
ADL	Activities of daily life
BIA	Bioelectrical impedance analysis
BMI	Body mass index
COPD	Chronic obstructive pulmonary disease
DXA	Dual-energy X-ray absorptiometry
EAA	Essential amino acids
EFSA	European food safety authority
DHEA	Dehydroepiandrosterone
GH	Growth Hormone
HMB	Beta-Hydroxy beta-methylbutyric acid
IGF-1	Insulin-like growth factor 1
LBM	Lean body mass
Leu	Leucine
MIMS	Maximal isometric muscle strength
RCT	Randomized controlled trial
SD	Standard deviation
SMI	Skeletal muscle mass index
SPPB	Short physical performance battery
VDR	intracellular vitamin D receptor

Introduction

Background

With an increasing elderly population, conditions that deteriorate the health of those of old age will become more prevalent (1). The progressive loss of muscle mass and muscle function lowers mobility and quality of life (2). The current conceptual and diagnostic definition of this condition is sarcopenia. It is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength. Old age is considered as the primary cause if no other specific cause or disorder can be found (2).

Sarcopenia leads to varying degrees of immobility and loss in quality of life (1). From the fifth decade there is a loss of 1-2 % of lean body mass per decade and after the age of 60 there is potentially a 3 % loss of muscle strength per decade. It is estimated that 5-13% of people aged 60-70 are affected by sarcopenia and 11-50% affected above the age of 80 (3). When in 2015 comparing different definitions of sarcopenia in 308 elderly (mean age 81) individuals the prevalence varied between 0-15% in healthy individuals and 2-34% in geriatric outpatients (4). Worth noting is that the relationship between muscle mass and muscle strength is not linear (5). Thus an evaluation of muscle function is often as important.

This study is a systematic review intending to evaluate the effect of an EAA supplement on muscle mass, strength and function in individuals over the age of 65. Muscle wasting is common in individuals above the age of 65, so this age was used as a cut-off (3). Since sarcopenia is not yet a universally accepted and used diagnosis, articles with subjects fitting the chosen inclusion criteria but not diagnosed with sarcopenia is included in the analysis of data.. The subjects analyzed in the studies that are not diagnosed are referred to as at risk of sarcopenia.

Sarcopenia definition and diagnosis

There are currently three different definitions in wider use, a problem when aiming to design studies, diagnose and evaluate treatments (1, 6, 7). The diagnosis of sarcopenia currently in the widest use in Europe is defined in the 2010 report “Sarcopenia: European consensus on

definition and diagnosis" (1). In summary the definition is having low muscle mass and one or both conditions of low muscle strength or poor physical performance **(d**etailed methods and cut-off points for the diagnosis based on body mass index (BMI) is explained in the article). For measurement of muscle mass the preferred method is Dual-energy X-ray absorptiometry (DXA) and the cut-off point is a skeletal muscle mass index (SMI) 2 standard deviations (SD) below the mean of young adults of the same gender. For measurement of muscle strength a preferred method is measuring handgrip strength and <30kg for males and <20kg for females is a cut-off point. Performance could be measured using short physical performance battery test (SPPB) which tests balance, gait speed and chair stand. Each test is graded 0-4 with 4 being the highest. So 10-12 is considered high performance. The cut-off score for the diagnostic criteria is <8 for both genders. Even though different definitions on the diagnoses exist, they share many similarities and international working groups are at the moment trying to create a universal definition.

Pathophysiology

Adapted from **A.J. Cruz-Jentoft et al 2010 (1)**

Sarcopenia is a multifactorial condition with multiple potential underlying mechanisms (8) (as illustrated in Figure 1). The primary cause is age related and behavioral e.g. a more sedentary life style, decreasing energy intake and age related physiological changes such as altered sensitivity and synthesis of anabolic hormone and impaired protein metabolism (2). Both these factors contribute to lowering the general nutrition intake, especially the dietary protein intake and its quality (1).

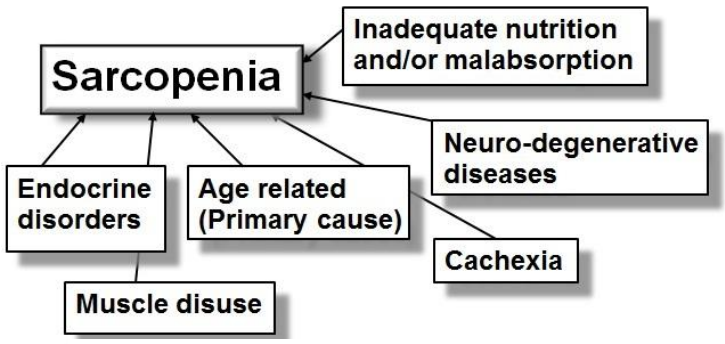

Figure 1. Mechanisms of sarcopenia.

A decreased level of growth hormone/insulin-like growth factor 1 (GH/IGF1) is often seen in parallel to changes in body composition with increased visceral fat mass and decreased lean body mass (LBM) (9). Of all the hormonal pathways the cortisol-GH axis has been proposed as the most influential factor for changes in body composition (10).

Low grade inflammation is associated with a variety of conditions such as insulin resistance, osteoporosis and reduced protein synthesis (2). Loss of LBM including muscle mass

according to ageing does not necessarily imply weight loss, suggesting that a corresponding accumulation of body fat occurs (11). Abdominal fat accumulation is in itself associated with low grade inflammation caused by higher levels of circulating pro-inflammatory cytokines as TNF, IL1 and IL6 (2). This may negatively affect protein synthesis and metabolism.

The quality and quantity of protein intake is also a contributor to muscle wasting. The recommended intake for the general population is 0.8 g protein/kg body weight/day and the European food safety authority (EFSA) published in 2011 a report on their scientific opinion on daily protein intake. They found that the daily protein intake in elderly 65 years + in the United Kingdom for males was mean 71.5 g (SD 17.0 g) and for females mean 56.0 g (SD 13.4 g). Most European countries had comparable protein intake levels (12). An American study observing 2066 subjects aged 70-79 showed that about 40 % did not even meet the minimum 0.8 g protein/kg body weight/day in intake (13).

A higher protein intake might be motivated since it is connected to an impaired response of protein metabolism to nutrition (14). This phenomena could partly be attributed to that is seems elderly have an altered function of specific amino acid (AA) transporters (15). This mechanism could explain some of their dietary AA resistance. A study compared the accretion of muscle protein in healthy elderly compared to healthy young subjects. The protein uptake was less in the elderly suggesting that a higher dose is needed for optimal protein synthesis (16). Impaired protein metabolism seem to be age related even though the physiological mechanisms is poorly understood. Forced inactivity such as in hospitalization leads to loss of muscle mass (17). This is an increased risk for loss of function in the elderly. One study illustrate that inactivity in itself impair different anabolic signaling pathways, especially $mTORC_{1.}$ This effect is more significant in older individuals compared to younger (18). Hence inactivity and old age in itself may blunt the anabolic stimulation of EAA.

As shown, the protein intake in elderly is often insufficient. Low protein intake in combination with other factors mentioned such as impaired protein metabolism and sensitivity might be justify to increase the recommended intake. A position paper from the PROT-AGE study group recommends 1.2g protein/kg body weight/day to maintain or regain lean body mass (19). Another report from 2008 even suggest the optimal intake for elderly might be as high as 1.5g protein/kg body weight/day (20). A level that could be difficult to reach by

normal food intake in frail or sick older adults, illustrating the need for a different approach to reach adequate intake. En excess protein intake might also have negative consequences. There is potential risks with bones mineral loss and negative impact on renal function (21).

These factor mentioned highlights the complicated and multifactorial mechanisms of sarcopenia, both intrinsic changes in the muscles, general changes such as inflammation, impaired metabolism and lifestyle/behavioral factors contribute to the condition.

Current potential treatments

There are several diverse suggested treatment approaches to different mechanisms (3). Some results have been obtained with resistance training, hormonal treatment, metabolic treatments such as vitamin D and nutritional interventions such as high caloric supplements and essential amino acids (22).

Resistance training in combination with nutrition has been shown beneficial for muscle synthesis (23, 24). However many older adults suffer from physical disabilities and hospitalizations may lead to forced inactivity. Physical therapy is not always an option and other effective treatment methods to ameliorate muscle loss while inactive is needed. Since peak muscle mass is often achieved earlier in life, effort to prevent sarcopenia trough diet and nutrition throughout the individuals lifetime might be a key to achieve physical capability in old age (25).

Hormonal treatment attempts with growth hormone (GH) has been tried, but no increased muscle strength was found in elderly (26). Administering GH does increase LBM but was also associated with adverse effects, mainly diabetes and glucose intolerance (27). A review from 2007 on GH administration to healthy elderly concluded that GH did show a small effect on LBM but increased to rate of adverse events to much to be recommended as a potential therapy (28). A study conducted on testosterone for men and dehydroepiandrosterone (DHEA) for women concluded: *"Neither DHEA nor low-dose testosterone replacement in elderly people has physiologically relevant beneficial effects on body composition, physical performance, insulin sensitivity, or quality of life"* (29).

25-hydroxy-vitamin D (25OHD) is a vitamin as well as a pro-hormone obtained through diet and synthesized by the skin from the exposure of ultraviolet light. It primarily promotes bone resorption and intestinal calcium absorption, increasing serum calcium concentration. But it also seem to have other effects through the intracellular vitamin D receptor (VDR) that has been found in both cardiac and muscle cells (30). Its association to sarcopenia was examined in a prospective study from 2010 (31). 686 community dwelling elderly individuals were followed for a mean of 2.6 ± 0,4 years. Serum levels of 25OHD were repeatedly measured using immunoassays and their sun exposure was measured using questionnaires. Muscle mass and muscle strength were measured using DXA and strength dynamometers. The study showed that serum 25OHD concentrations ≥50 nm were associated with a higher muscle mass and muscle function. However since this was a prospective study and not a controlled trial there is a lot of methodological limitations and unexplored confounding factors.

Omega-3 fatty acids are polyunsaturated fatty acids. They cannot be synthesized by the body and has to be supplied through the diet. Omega-3 acids contribute to normal metabolism through several different pathways. Though supplementation does not appear to decrease all-cause mortality on a population based level (32). Its suggested positive anabolic effect was reviewed in a study from 2014 (33) and even thou the mechanism is not fully understood Omega-3 seem to lower the elderly's anabolic resistance to AA. Th area should be explored further and maybe Omega-3 could potentially be administered in conjunction with AA to increase the anabolic response.

Other pharmacological strategies besides hormones treatments have not yet been developed. But an observational study from 2002 showed that elderly subject treated with angiotensin converting inhibitors a clear reduction in the decline in muscle strength was observed compared to other anti-hypertensive agents (34). Thus there seem to be a possible future potential to combine nutritional and pharmacological approaches in prevention and treatment.

Essential amino acids

Protein supplements alone do not show a positive effect on LBM compared placebo in a diverse elderly population, according to a meta-analysis (35). The efficiency of essential amino acids (EAA) for protein synthesis is more than twice that of whey protein on a g/g

basis (36). EAA has a great advantage compared to other protein supplementation since it increases the protein synthesis significantly more per calorie consumed. This Results in less satiety, an important factor in the elderly who already struggle to consume enough calories. A systematic review and meta-analysis of leucine (Leu), and EAA concluded that is suggested that "Leu supplementation is useful to address the age-related decline in muscle mass in elderly individuals, as it increases the muscle protein fractional synthetic rate" (37). Showing the potential of EAA supplementation in promoting protein synthesis and thus aid in maintaining or regaining lean body mass in elderly.

Research question

Can EAA supplementation maintain or increase LBM, strength and function in elderly individuals (≥65 years) with or at risk of sarcopenia.

Methods

Inclusion & exclusion criteria

Searches were conducted in PubMed and Scopus databases. Selected studies were limited to those in the English language with a 20-year span ranging from 1994-2014. Search terms conducted for all three databases were "essential amino acids sarcopenia". A manual search for studies in each selected studies and earlier systematic reviews found from this search was also done. Only studies eligible according to the inclusion and exclusion criteria were included. The searches were conducted in January 2015.

Table 1. Inclusion & exclusion criteria

Inclusion criteria	Exclusion criteria
English language	In-vitro studies
Published studies, year span 1994-2014	Case reports
Experimental studies	Review articles
Clinical subjects, age >65 years	Observational studies
EAA supplementation	
Full texts found	

Study Selection

One person conducted the searches and the studies were selected according to the inclusion and exclusion criteria.

Of the 134 studies screened 125 were excluded for not being eligible according to the inclusion/exclusion criteria. One study fitted all the initial criteria but was excluded from the final analysis since a full text was not obtained. It was a study by

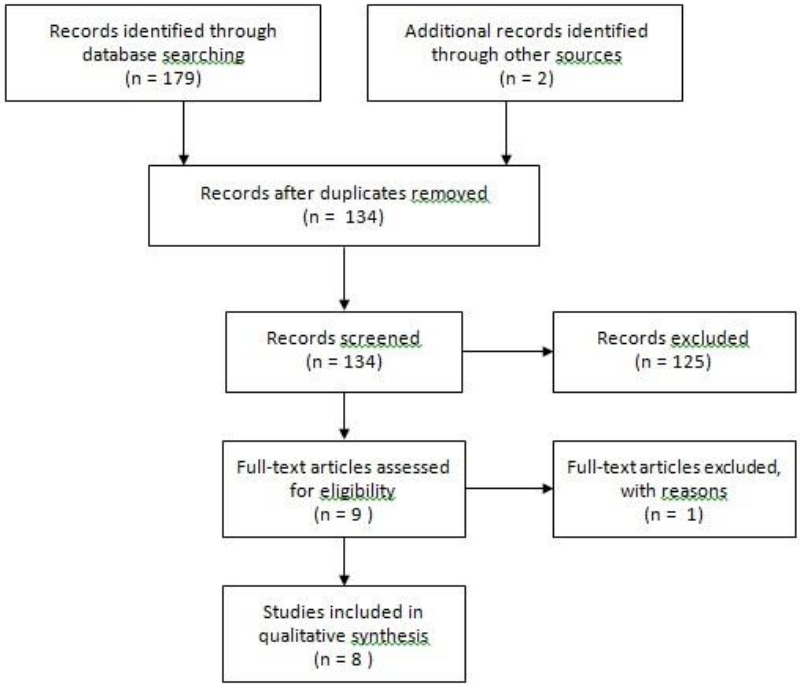

Figure 2. Flow chart

Scognamilio et al 2004 (38). In accordance to the research question and the inclusion & exclusion criteria studies with subject both diagnosed and not diagnosed with sarcopenia were included. Figure 2 shows a flowchart for this study selection process.

Quality assessment of studies

Quality analysis and grading of studies were performed according to suggested guidelines defined in the GRADE system. Review templates were acquired from HTA-centrum from the Sahlgrenska Academy (39). According to GRADE, there are three different grades of evidence when grading the quality of the studies: low, moderate and high. Further, there are four different grades when grading the evidence strength of chosen endpoints: very low, low, moderate and high.

Endpoints analyzed were LBM, muscle strength and muscle function. Muscle function is of crucial importance for the elderly in the ability of managing basic needs in an everyday life. There is no universal definition of muscle function. Effects chosen in the present studies were mostly MIMS hand dynamometers and different walking tests. Both functions have a large impact on autonomy and the overall quality of life.

Ethics

This systematic review is based on earlier published work and therefore was ethical establishment not necessary. All individual articles in this review had ethical approval.

Results

Study descriptions from eight studies are summarized in table 2 spanning from 2005 to 2012 (40-47). The intervention results are summarized in table 3.

Study characteristics

The studies involved a total of 454 subjects (273 female, 140 male, 41 subjects in one study had not their gender specified). The aim was to examine studies with subjects 65 years and older. In one study the inclusion age was >40 years (41). However, with mean age presented as 75 ± 7, most subjects were ≥65 years and therefore the study was included in this review. All analyzed studies were clinical trials, six were RCTs, one study had only one intervention group and one was an open label cross-over study.

They all had different study protocols and used somewhat different variables, such as blood lactate, glucose, cytokines etc. Most of these were considered not relevant for this systematic review. The most relevant variables for the present study were LBM and muscle function/strength. LBM was measured using DXA except in the two Dal Negro et al studies that used bioelectrical impedance analysis (BIA). Strength/function was measured using different functional methods in the studies.

Scognamiglio et al 2005

The aim of this study was to assess the effect of 12g/day oral EEA supplementation on muscle strength and muscle function. 100 eldery subjects (> 65 years) with reduced physical capacity were randomized non blinded into either the EAA group or a placebo group for 3 months. The authors did not look at lean body mass but evaluated muscle strength using a MIMS hand dynamometer and function was evaluated using standardized walking tests.

All measured outcomes improved compared to baseline in the EAA group but not the placebo group. The improvements in ambulatory capacity and handgrip strength were greater. This can partly be explained by the fact that the subjects were encountered in everyday practice during the study. Even though subjects in intervention group and placebo group participated in the same exercise routine, the large increase was seen only in the intervention group.

Børsheim et al 2008

The aim of this study was to determine if a regular intake of EAA would improve LBM and muscle function in elderly glucose intolerant subjects (defined as a plasma glucose concentration >180 mg/dl at 1 h or >140 mg/dl at 2 h after oral intake of 75 g glucose). The authors of the study reasoned that since insulin resistance is common in the elderly population they wanted the subjects to have the same insulin sensitivity so the positive interaction between insulin and AA would result in a difference in anabolic response.

This was an intervention study with only one intervention group. 12 subjects were included (67.0 ±5.6 SD years, 7 females, 5 males) and they ingested 11g EAA + arginine for 16 weeks. They were outpatients and their regular diet or activity was not modified. The subjects underwent a full body DXA at week 0, 4, 8, 12, and 16 to determine LBM. Muscle strength and function was determined with 1RM max tests in weight machines and different functional tests such as walking speed, timed floor transfer and static balance test.

After 16 weeks there was a significant improvement in LBM, muscle strength and most of the physical muscle function tests. This occurred despite no other changes in physical activity or dietary changes.

The study did not include a placebo group. The authors assumed that no improvement would occur in the subjects without the intervention based on. Subjects' baseline values were used as a control for comparison.

Solerte et al 2008

The aim of this study was to examine the effect of orally administered EAA in elderly subjects (age range 66–84 years) with reduced LBM and diagnosed with sarcopenia. Subjects were outpatients living independently. The protocol was a randomized open-label crossover with 41 subjects, no information were given on the gender. Main outcome was lean body mass which was measured using a DXA and the intervention consisted of 16g EAA (8g morning and evening) daily.

This cross-over study had different phases. First a run in period of 30 days and then the baseline values was established. The subjects were then divided into 2 groups. One received

placebo and the other the EAA intervention for 4 months. Once this was done both groups had a 15 day washout period and then the groups switched who got the placebo and who got the EAA for 4 months again. After the crossover periods both groups received EAA for 8 months, something the authors call a "maintenance period". The parameters (except LBM) were examined at baseline and after 4, 6, 8, and 16 months. The result does support the assumption that the gain of using EAA supplementation is greatest the first months and then stabilizes.

This study lacked both information about the gender and the LBM results of the intervention compared to placebo during the different study periods. So the results impact on this review was unfortunately limited.

Dillon et al 2009

The aim of this study was to examine if EAA supplementation improves LBM, maximum muscle strength, and the metabolic outcomes muscle protein fractional synthesis rate and the IGF-I muscle protein receptor expression. It was a double-blind RCT with 7 elderly (68 ± 2 years) women in each group. They were all living autonomously at home. One group received placebo and the other 15g EAA daily. The intervention spanned 3 months with one measurement day 1 and at the end of the study period. Subjects collected their capsules once a month. LBM was measured using DXA. Muscle strength was assessed using One-repetition max (1RM) in traditional weight machines. Different metabolic outcomes were not included in the result of this review.

The results was that the ingestion of EAA increased LBM 3,9 % in the intervention group but in the placebo group they only increased 0,7 %. Strength remained unchanged in both group so they were not statistically significant and the final data was not shown.

Ferrando et al 2009

Since prolonged inactivity is common in hospitalization the aim of this study was to evaluate the effect of EAA supplementation in elderly (mean 68 ± 5 years) that was subject to a 10 day bed rest protocol. The subjects were divided into two groups with 11 subjects in each group completing the study. They were given either a placebo or 15 g of EAA three times a day during the bed rest period. LBM and different tests of muscle function/strength was chosen as end points. As seen in table 3 both groups lost LBM and muscle strength but the EAA group

lost significantly less. Methods used in measuring were muscle protein synthesis rate over 24h and DXA was used for lean and fat body mass.

This study had a different study design from the other studies that primarily examined free living patients. The study convincingly demonstrated that EAA ameliorate muscle loss without affecting satiety even when in forced inactivity. Intervention period was only 10 days but should reflect a typical hospitalization.

Dal Negro et al 2010

The aim of this study was to examine if 8g of EAA supplementation compared to placebo in chronic obstructive pulmonary disease (COPD) patients could improve body composition, muscle metabolism, physical activity, cognitive function, and health status. COPD is a common condition and often associated with weight loss. The study is an RCT with a double blind protocol. 32 out-patients (25 males) were randomized into two groups, 16 each. One received 4g EAA and the other a placebo. Measures were done at baseline, after 4 weeks and at the end which was 12 weeks. Muscles mass was evaluated using BIA.

The EAA supplemented group improved in all measured outcome variables compared to the placebo group. Most impressive was the 6 kg weight increase, were 3.6 kg of this was measured as fat free mass increase.

Subjects in this study were diagnosed with sarcopenia as well as COPD. Their high catabolic state compared to many subjects in the other studies is most likely the reason for the high positive results.

Dal Negro et al 2012

This study is similar to the previous one with the same main author. The aim of this study was to evaluate 8g EAA compared to placebo in 88 COPD out-patients with a BMI < 23. Subjects were randomized to receive placebo (n=44) or EAAs (n=44) for 12 weeks, it was a non-blinded RCT. Subjects were not formally diagnosed with sarcopenia but had many of the characteristics of the diagnosis, such as low muscle mass. Primary outcome measures were changes in both physical activities in daily life and quality of life. Measurements were done using questionnaires and “Sense wear armbands”. They measured muscle function with hand dynamometers, walking speed and muscle mass using BIA which is reported in table 3. There

was a positive effect on muscle mass in both groups but only the EAA supplemented group increased muscle function, measured as steps per day and hand grip strength.

Kyung Kim et al 2012

The aim of this study was to use a RCT protocol and evaluate the effect of four different methods for increasing strength and muscle mass in community dwelling Japanese elderly (75 years and older) women. The four different interventions were 6g EAA supplementation, exercise, exercise + 6g EAA and only health education. Out of 155 women enrolled into the study 144 completed the three month intervention. The conclusion was that EAA + exercise was the best for increasing strength and muscle mass. Compared to only health education the EAA group showed more significant improvements. So EAA in itself without exercise was beneficial for these sarcopenic women.

This study differed somewhat from the others in having four different intervention groups. When trying to compare these results with the other studies the EAA group was treated as the intervention group and the health education group was considered the placebo group.

Table 2. Study descriptions

Author, year	Study Design	Intervention	n Subjects	Subject status	Mean age years	BMI
Scognamiglio et al 2005	*Blinded RCT* *Duration: 12 weeks*	*AA mixture (All EAA + tyrosine and cystine) 12 g/day* *12 weeks*	*95 subjects* *42 male* *53 female*	*Subjects not complicated with sarcopenia, otherwise healthy elderly subjects with reduced physical activity*	*I = 74* P = 74 ± 5	*I = 27.4* ± 3.6 *P = 27.3* ± 3.7
Børsheim et al 2008	*Clinical trial, one intervention group* *Duration: 16 weeks*	*10 g of EAA + 1.1g arginine two times a day (total 22 g EAA in a day)* *16 weeks*	*12 subjects* *5 male* *7 female*	*Subjects not complicated with sarcopenia, defined as elderly impaired glucose tolerant subjects*	*67 ± 5.6*	*Non given, but weight was 74.3 ± 19.7 kg*
Solerte et al 2008	*A randomized, open-label, crossover study* *Duration: 48 weeks of supplementation*	*16 g/day of EAA* *78 weeks*	*41, no information about gender*	*Elderly subjects complicated with sarcopenia and reduced whole-body lean mass*	*No mean age given, Age span was 66–84*	*19 - 23*
Dillon et al 2009	*Double blind RCT* *Duration: 12 weeks*	*15 g/day EAA* *12 weeks*	*14 female subjects*	*Subjects not complicated with sarcopenia, otherwise healthy living independently at home*	*I = 67* ± 1 P = 69 ± 3	*I = 26.8* *P = 26.7*
Ferrando et al 2009	*Non blinded RCT* *Duration: 10 days of bed rest*	*15 g EAA three times a day (total 45 g EE a day)* *10 days*	*22 subjects* *7 male* *15 female*	*Healthy elderly subjects, subjected to a 10 day bed rest protocol*	*I = 71 ± 6* *P = 68 ± 5*	*Non given, but weight was in P = 83 ± 19 kg and I = 72 ± 8 kg*

Author, year	Study Design	Intervention	n Subjects	Subject status	Mean age years	BMI
Dal Negro et al 2010	*Double blind RCT* *Duration: 12 weeks*	*4 g EAA two times a day (total 8 g EAA a day)* *12 weeks*	*32 subjects* *25 male* *7 female*	*Subjects complicated with sarcopenia suffering from chronic obstructive pulmonary disease*	*I = 75 ± 7* *P = 75 ± 7*	*I = 20.2* ± 1.4 P = 20.2 ± 1.8
Dal Negro et al 2012	*Non blinded RCT* *Duration: 12 weeks*	*4 g EAA two times a day (total 8 g EAA a day)* *12 weeks*	*88 subjects* *61 male* *27 female*	*Outpatients with stable, severe COPD*	*I = 75 ± 5* *P = 73 ± 8*	*I = 19.95 ± 1.63* *P = 20.1 ± 2*
Kyung Kim et al 2012	*Non blinded RCT* *Duration: 3 months.*	*3 g Leu rich EAA two times a day (total 6 g EAA a day)* *12 weeks*	*150 subjects, all female. Assigned to 4 different groups. N = 37 in the EAA group.*	*Free living, defined as sarcopenic*	*75 years and older*	*< 22*

I = Intervention group.

P = Placebo group.

n = Number of subjects.

Intervention results

The results in selected and relevant measurements are displayed in two tables. Table 3 displays baseline value, end value and its % change.

Table 3. Results after intervention compared to baseline in accordance to selected endpoints.

Author, year	Endpoint	Baseline	End	% change
Scognamiglio et al 2005	MIMS right hand, kg	Week 0: I: 14.6 ± 2.2 P: 14.4 ± 2.4	Week 12: I: 20.2 ± 2.0 P: 14.0 ± 2.8	I: + 38.3 % P: - 2.8 %
	6-min walking distance, m	Week 0: I: 212.5 ± 34 P: 212.0 ± 36	Week 12: I: 268.8 ± 34.9 P: 212.0 ± 40	I: + 26.5 % P: ± 0 %
Børsheim et al 2008	Lean body mass, kg	Week 0: 47.97 ± 3.0	Week 16: 48.57 ± 3.0	I: + 1.3 %
	Lean leg mass, kg	Week 0: 14.98 ± 1.0	Week 16: 15.31 ± 1.08	I: + 2.2 %
	Leg 1 repetition max	Week 0: 127.5 ± 21.8	Week 16: 145.6 ± 19.2	I: + 14.2 %
	Walking speed m/s	Week 0: 1.26 ± 0.05	Week 16: 1.34 ± 0.05	I: + 6.3 %
Solerte et al 2008	Lean body mass, kg	Week 0: I_1: 47.5 I_2: 46.5	Week 78: I_1: 50.0 I_2: 49.0	I_1: + 5.3 % I_2: + 5.4 %
Dillon et al 2009	Lean body mass, kg	Week 0: I: 43.5 ± 2.8 P: 40.7 ± 2.4	Week 12: I: 45.2 ± 3.0 P: 41.0 ± 2.8	I: + 3.9 % P: + 0.7 %
	Upper/lower body Strength tests	Week 0: NS	Week 12: NS	-
Ferrando et al 2009	Lean body mass, kg	Day 0: I: 43.0 ± 0.2 P: 46,8 ± 0.3	Day 10: I: 42.1 ± 0.2 P: 45.3 ± 0.3	I: - 2.1 % P: - 3.2 %
	Stair ascent power (Nm/s)	Day 0: I: 293.5 ± 37.2 P: 407.2 ± 69.9	Day 10: I: 284.2 ± 43.0 P: 336.5 ± 47.6	I: - 3.2 % P: - 17.4 %
	Standing plantar flexion (rep/30 s)	Day 0: I: 21.8 ± 1.4 P: 21.8 ± 3.4	Day 10: I: 21.4 ± 2.5 P: 20.9 ± 2.8	I: - 1.4 % P: - 4.1 %
Dal Negro et al 2010	Lean body mass, kg	Week 0: I: 40.4 ± 4.0 P: 39.9 ± 4.8	Week 12: I: 44.0 ± 4.5 P: 39.7 ± 2.8	I: + 8.9 % P: - 0.5 %
	Steps per day	Week 0: I: 638.8 ± 661.8 P: 609.8 ± 454.7	Week 12: I: 1140.5 ± 524.4 P: 562.9 ± 601.9	I: + 78.5 % P: - 7.7 %
Dal Negro et al 2012	Lean body mass, kg	Week 0: I: 40.2 ± 4.23 P: 35.80 ± 5	Week 12: I: 43.86 ± 4.76 P: 39.6 ± 2.5	I: + 9.1 % P: + 10.6 %

	MIMS right hand, kg	Week 0: I: 21.6 ± 1.36 P: 22.1 ± 1.9	Week 12: I: 23.2 ± 1.6 P: 21.5 ± 1.7	 I: + 7.4 % P: - 2.7 %
	Steps per day	Week 0: I: 638.68 ± 662.1 P: 609.70 ± 454.8	Week 12: I: 1140.33 ± 524 P: 562.77 ± 601.9	 I: + 78.7 % P: - 7.7 %
Kyung Kim et al 2012	Lean body mass, kg	Month 0: I: 26.25 ± 1.81 P: 27.48 ± 2.04	Month 3: I: 26.53 ± 2.10 P: 27.66 ± 2.23	 I: + 1.0 % P: + 0.6 %
	Maximum walking speed, m/s	Month 0: I: 1.71 ± 0.28 P: 1.57 ± 0.31	Month 3: I: 1.92 ± 0.27 P: 1.64 ± 0.31	 I: + 12.3 % P: + 4.5 %
	Knee extension strength, Nm/kg	Month 0: I: 1.15 ± 0.25 P: 1.14 ± 0.26	Month 3: I: 1.14 ± 0.25 P: 1.00 ± 0.26	 I: - 0.9 % P: - 12.3 %

I = Intervention group.

P = Placebo/control group.

NS = No significant difference so data wasn't further reported.

MIMS = maximal isometric muscle strength (handgrip dynamometer)

Study quality according to GRADE

Table 4. Study quality according to GRADE

Author, year	Study quality according to GRADE
Scognamiglio et al 2005	Moderate
Børsheim et al 2008	Moderate
Solerte et al 2008	Low
Dillon et al 2009	High
Ferrando et al 2009	Moderate
Dal Negro et al 2010	High
Dal Negro et al 2012	High
Kyung Kim et al 2012	Moderate

Evidence strength of effect:

Lean body mass: Moderate (+++)

Muscle Strength/function: High (++++)

Discussion

Summary of evidence

Even though the eight different intervention studies included in this systematic review were mutually different in study quality, dosage and length of the intervention they all showed a significant positive effect of EAA on selected outcome variables compared to placebo or at baseline. Studies included a total of 454 subjects. Which was often sufficient to get a statistical significant result? Still it's a limited number of subjects to generalize the positive results to a wider diverse population of older adults.

Dose EAA given varied between 6.7 – 45g/day (mean 15g). The study which spanned 16 weeks and with a dose of EAA in the higher span, 22g (40) did not show greater effect on outcome variables than those with lower dose, between 8 - 15 g (LBM only + 1,3%). The dose-response in the analyzed studies is not linear. 22g EAA a day does not seem to be more efficient then 8-15g a day. The most efficient dosage is therefore hard to determine based on present results. There have been attempts to determine the optimal AA dose for protein synthesis in elderly but it has proven methodologically difficult since it's both hard to measure reliable and apply pre-clinical laboratory results to a clinical realistic setting. Studies on muscle protein synthesis often use young men as test subjects which is far from representative of elderly sarcopenic individuals (48). Further research on optimal dosage for sarcopenic individuals is needed but supplementation with 15g EAA daily seems a warranted dosage to aim for based on the present scientific material.

The duration of intervention varied between studies even though the majority of studies were 12 weeks and seem it could be representative for a longer period of impaired mobilization. Long term follow after supplementation was discontinued is also up is missing. It is therefore not possible to say anything about the long term effect of the intervention. Having a positive effect during this short but critical period should theoretically be sufficient to maintain enough muscle mass and function to again being able to be self-sufficient in activities of daily life (ADL).

When examining individual EAA for further optimization of the supplement, Leu seem to be the EAA which have the most positive effect on protein synthesis and alone has been suggested as a so called pharmaconutrient to prevent and treat sarcopenia (49). Leu metabolizes in the body to the active metabolite Beta-hydroxy-beta-methylbutyrate (HMB) which compared to a energetically equivalent dose Leu further stimulate muscle protein synthesis trough the mTOR–p70S6K1 pathway (50). Therefore there are expectations that HMB is an even more energetically efficient promoter of muscle synthesis than AA or EAA. This was evalutated in a recent study (51). This study had 19 subjects confined to a complete bed rest for ten days and followed by eight weeks of resistance training. One group was randomized to receive 1,5g HMB twice daily starting five days before the bed rest and ended at the end of the exercise period. The control group got a placebo powder. DXA was used to evaluate body composition. There was a significant difference in preservation of muscle mass, but difference in muscle function could not be observed between groups. Authors concluded sample size was probably too small to show effect on muscle function, with a larger sample size they theorize an effect probably would be observed (51). These results are early and experimental but are however promising for further investigation.

Physiological changes occurring during aging are factors that need to be considered according to evaluating optimal dosage and timing for supplementation with EAA. Changes such at that AA transporters gets up regulated differently in muscle in young compared to old men after resistance exercise and AA ingestion (15). There is also indications that elderly have a diminished muscle accretion of muscle protein when ingesting a bolus EEA compared to young individuals (16). The muscle tissue itself seems to change when aging. Muscle size and muscle strength are not proportional throughout life (5). This can be explained by how many connections and how effective they are. It has been shown that nerve conduction is slower and that connections are not as numerous and effective for elderly < 65 years as in younger individuals. A term that some use of for this phenomenon is dynapenia, referring to these impaired neuromuscular connections (52). Again showing that activation of muscles throughout life can also maintain this neuromuscular connection regardless of LBM and that future studies on protein synthesis, function and EEA needs to be done on elderly test subjects.

Resistance training as mentioned in the introduction has shown to be beneficial for muscle function and syntheses in older adults (23, 24). Since disease and disability is more frequent

in the elderly population, resistance training is not always an alternative. Frequent episodes of physical inactivity (and inflammation) such as hospital stays could also make resistance training and ADL hard to maintain. Therefore nutritional supplement strategies for periods of forced inactivity are still needed. Still when it is possible, nutrition together with resistance training are in most cases complementary treatment strategies that has shown a synergistic anabolic effect (23)..

Still many questions remain unsolved according to optimal intake of EAA, other AA, HMB and dietary protein intake in elderly sarcopenic individuals for maintenance of muscle mass and function. Question such as what is an optimal dosage for maximum muscle protein synthesis? Should supplementation be administered as a bolus dose or spread out? Should a combination of EAA be used? Should other micronutrients mentioned earlier in the review be added? To hopefully resolve some of these questions a large RCT should be performed on elderly geriatric subjects defined as sarcopenic. Suggesting, intervention for at least 16 weeks comparing different interventions such as only EAA and combinations with other suggested micronutrients such as omega-3 and HMB, compared with placebo. The test subjects should have the sarcopenia diagnosis defined in the "European consensus report" (1) is recommended to be used. All subjects should have a follow up on the pre-defined endpoints a few months after supplementation is discontinued to see if the potential difference in outcomes persist and actually effect individuals health and quality of life. The reasoning being that if EAA supplementation can maintain or increase a certain level of LBM this could keep the individuals active enough to keep their strength and function and not deteriorate further. Even a short period of impaired mobilization in the elderly can be enough to lead to a permanent impaired physical function and lack of autonomy.

Sarcopenia as well as other forms of malnutrition is generally underdiagnosed, and needs to be recognized to get more attendance in the healthcare system (3). If the condition is not systematically diagnosed, appropriate interventions to treat and ameliorate sarcopenia will be difficult. Currently there is a lack of treatment options besides physical therapy which is not possible for everyone. So when treatments, such as perhaps EAA or similar dietary supplementation interventions become more available and established clinicians might be more aware and prone to properly diagnose and treat sarcopenia when they also have treatment options.

Limitations

Systematic reviews have their methodological limitations, further reviewed in (53). Some of these limitations include that inclusion and exclusion criteria are pre-defined, by the author. The selection of inclusion/exclusion criteria can lead to inclusion-bias. For example choosing a certain cut-off age on the subjects that might affect the intervention effect. It is important to consider that criterias often are selected based on the author's current knowledge of the field reviewed. The risk of publication bias also needs to be considered, studies that yield positive results tend to be published more often than those presenting negative results. The selection of studies will therefore not be a random sample from the literature. The analysis of each study is also performed by one or a few persons and even though standardized forms were used to grade the studies there is a risk of some subjectivity.

A further limitation with the present systematic review is the current lack of a worldwide consensus on the definition of sarcopenia. Even though there is some overlap of the definitions on the subjects included in the studies and they all fit the inclusion and exclusion criteria they were slightly different in study population, length of intervention (10 days to 48 weeks, 12 weeks were used in five out of eight studies), with no long term follow up and dosage of EAA used (6.7 – 45g/day, mean 15g). The subjects in the studies were all above 65 years of age but some of their baseline values considering muscle mass and comorbidities differed. Only the population in three of the eight studies was officially diagnosed with sarcopenia. But the others resembled the sarcopenic definition, with low LBM, low strength and having low SPBB. Still the lack of consistence both with regard to the criteria for inclusions, methods for evaluating effect, type of EAA supplementation, dosage and time for administration together constitutes the main limitation in applying the results to a wider clinical practice.

Conclusions and implication

To summarize results from the eight heterogenic studies all showed effect of EAA supplementation either for maintaining or increasing LBM, strength and function. It seems that the more frail subjects also gained the best clinical effect. It supports the theory that the more untrained muscle also gain the best effect from strength training. The conclusion is

therefore that short term supplementation with EAA to individuals over 65 years of age and with or at risk of sarcopenia is beneficial both for ameliorating/gaining muscle mass and strength/function. Based on current research there is potential for clinical use of EAA supplementation in the future. Theoretically, based on present studies it seems to be most effective in the short term, for example to administer EAA supplement during and a few weeks after the hospital stay, as the long term effect of continued supplementation is not yet studied. So still many questions remain regarding mechanisms, optimal EAA, dose and duration of therapy.

Another aspect when prescribing supplementations to older adults is the frequent prevalence of polypharmacy. Therefore adding more tablets would perhaps be too demanding resulting in low compliance to prescribed medications/treatments. Instead other forms of EAA administration could be considered. EAA fortified sip-feeds or other forms of EAA liquid fortification or food fortification would also be possible. An advantage with sip feed is that they also contain energy and other nutrients. However, compliance to sip feed may also vary considerably. A problem with food fortification is that if done improperly it will negatively affect sensory aspects and therefore affect compliance since many elderly show impaired appetite and altered or lowered taste and smell perception (54). In summary prescription of EAA must be made not only with regard to individual needs but also considering the specific conditions and preferences of the individual patient for good compliance.

Sarcopenia as a nutrition related disorder also needs to be clearly defined, both for research purpose and clinical work. One could argue that it is a natural consequence of aging but the multifactorial etiology and individual variations in its progression warrants further research into the pathophysiology and potential treatments such as EAA supplementation. New knowledge within these fields may open up for innovative new treatment strategies and targeting mechanisms to ameliorate or reverse progression associated with the sarcopenic syndrome.

Acknowledgements

I would first like to thank assistant professor Elisabet Rothenberg for your excellent supervision and professional support during this long project. I also highly appreciate the

opportunity given to participate with a poster in the ESPEN congress 5-8 September in Lisbon where I had several rewarding discussions on the subject covered in this essay. I also extend my gratitude for feedback and many interesting scientific discussions to my dear friend Dr Sofia Tapani.

Populärvetenskaplig sammanfattning

Förlust av muskelmassa, styrka och funktion är vanligt förekommande bland äldre. Förekomsten ökar med stigande ålder och sambandet med sjukdom är starkt. Fysisk inaktivitet, i synnerhet ofrivillig inaktivitet som att vara sängliggande under en sjukhusvistelse bidrar till förlusten. Detta fenomen benämns ofta i litteraturen som ”sarkopeni”. Orsakerna till sarkopeniutvecklingen är flera, men fysiologiska förändringar relaterad till hög ålder anses vara den främsta orsaken om ingen annan specifik anledning kan hittas. Hur sarkopeni ska beskrivas och diagnosticeras finns publicerat (1).

I denna systematiska litteraturöversikt sammanställs åtta studier som studerat effekten av en extra tillförsel essentiella (livsnödvändiga) aminosyror till individer över 65 år. Man har för att utvärdera effekten mätt muskelmassa och muskelstyrka/funktion. Studierna skilde sig något åt avseende doser av essentiella aminosyror och tiden för behandling, men man gav i medel 15g och de flesta studier pågick 12 veckor. Samtliga studier bedömdes som relevanta för att kunna besvara den aktuella frågeställningen.

Eftersom behovet av fördjupad kunskap om sarkopeni och lindrande behandlingar vid detta tillstånd är stort är litteraturöversikter som denna viktiga för att summera den kunskap som finns.

Slutsatsen är att tillskott av essentiella aminosyror till äldre över 65 år som är diagnostiserade med sarkopeni eller i riskzonen för att utveckla detta tillstånd ger en positiv effekt på muskelmassa styrka och funktion. Essentiella aminosyror stimulerar uppbyggnad av muskelprotein betydligt mer än motsvarande mängd energi genom andra proteinkällor.

Mer forskning inom området behövs dock för att kunna använda tillskottet praktiskt i klinisk vardag.

Reference list

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis. Age and Ageing. 2010;39(4):412-23.
2. Boirie Y. Physiopathological mechanism of sarcopenia. Journal of Nutrition, Health and Aging. 2009;13(8):717-23.
3. von Haehling S, Morley J, Anker S. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. J Cachexia Sarcopenia Muscle. 2010;1(2):129-33.
4. Reijnierse EM, Trappenburg MC, Leter MJ, Blauw GJ, Sipilä S, Sillanpää E, et al. The Impact of Different Diagnostic Criteria on the Prevalence of Sarcopenia in Healthy Elderly Participants and Geriatric Outpatients. Gerontology. 2015.
5. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2006;61(10):1059-64.
6. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, et al. Sarcopenia: An Undiagnosed Condition in Older Adults. Current Consensus Definition: Prevalence, Etiology, and Consequences. International Working Group on Sarcopenia. Journal of the American Medical Directors Association. 2011;12(4):249-56.
7. Studenski SA, Peters KW, Alley DE, Cawthon PM, McLean RR, Harris TB, et al. The FNIH sarcopenia project: Rationale, study description, conference recommendations, and final estimates. Journals of Gerontology - Series A Biological Sciences and Medical Sciences. 2014;69 A(5):547-58.
8. Walrand S, Guillet C, Salles J, Cano N, Boirie Y. Physiopathological mechanism of sarcopenia. Clinics in Geriatric Medicine. 2011;27(3):365-85.
9. Zadik Z, Chalew SA, McCarter Jr RJ, Meistas M, Kowarski AA. The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. Journal of Clinical Endocrinology and Metabolism. 1985;60(3):513-6.
10. Nass R, Thorner MO. Impact of the GH-cortisol ratio on the age-dependent changes in body composition. Growth Hormone and IGF Research. 2002;12(3):147-61.
11. Hughes VA, Frontera WR, Roubenoff R, Evans WJ, Fiatarone Singh MA. Longitudinal changes in body composition in older men and women: Role of body weight change and physical activity. American Journal of Clinical Nutrition. 2002;76(2):473-81.
12. EFSA Panel on Dietetic Products NaAN. Scientific Opinion on Dietary Reference Values for protein. . EFSA Journal 2012;10(2):2557 [66 pp].
13. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: The Health, Aging, and Body Composition (Health ABC) study. American Journal of Clinical Nutrition. 2008;87(1):150-5.
14. Short KR, Nair KS. The effect of age on protein metabolism. Current Opinion in Clinical Nutrition and Metabolic Care. 2000;3(1):39-44.
15. Dickinson JM, Drummond MJ, Coben JR, Volpi E, Rasmussen BB. Aging differentially affects human skeletal muscle amino acid transporter expression when essential amino acids are ingested after exercise. Clinical nutrition (Edinburgh, Scotland). 2013;32(2):273-80.
16. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. American Journal of Clinical Nutrition. 2005;82(5):1065-73.

17. Kortebein P, Symons TB, Ferrando A, Paddon-Jones D, Ronsen O, Protas E, et al. Functional impact of 10 days of bed rest in healthy older adults. Journals of Gerontology - Series A Biological Sciences and Medical Sciences. 2008;63(10):1076-81.
18. Drummond MJ, Dickinson JM, Fry CS, Walker DK, Gundermann DM, Reidy PT, et al. Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults. American Journal of Physiology - Endocrinology and Metabolism. 2012;302(9):E1113-E22.
19. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-Based Recommendations for Optimal Dietary Protein Intake in Older People: A Position Paper From the PROT-AGE Study Group. Journal of the American Medical Directors Association. 2013;14(8):542-59.
20. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. Clinical nutrition (Edinburgh, Scotland). 2008;27(5):675-84.
21. Pedersen AN, Cederholm T. Health effects of protein intake in healthy elderly populations: A systematic literature review. Food and Nutrition Research. 2014;58.
22. Cruz-Jentoft AJ, Landi F, Schneider SM, Zúñiga C, Arai H, Boirie Y, et al. Prevalence of and interventions for sarcopenia in ageing adults: A systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). Age and Ageing. 2014;43(6):48-759.
23. Timmerman KL, Dhanani S, Glynn EL, Fry CS, Drummond MJ, Jennings K, et al. A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults. American Journal of Clinical Nutrition. 2012;95(6):1403-12.
24. Liu CJ, Latham NK. Progressive resistance strength training for improving physical function in older adults. Cochrane Database of Systematic Reviews. 2009(3).
25. Robinson S, Cooper C, Aihie Sayer A. Nutrition and sarcopenia: A review of the evidence and implications for preventive strategies. Journal of Aging Research. 2012;2012.
26. Rudman D, Rudman IW, Cohn L, Feller A, Lalitha PY, Gergans GA, et al. Effects of human growth hormone in men over 60 years old (Reply I). New England Journal of Medicine. 1990;323(22):1562-3.
27. Blackman MR, Sorkin JD, Münzer T, Bellantoni MF, Busby-Whitehead J, Stevens TE, et al. Growth hormone and sex steroid administration in healthy aged women and men: A randomized controlled trial. Journal of the American Medical Association. 2002;288(18):2282-92.
28. Liu H, Bravata DM, Olkin I, Nayak S, Roberts B, Garber AM, et al. Systematic Review: The Safety and Efficacy of Growth Hormone in the Healthy Elderly. Annals of Internal Medicine. 2007;146(2):104-15.
29. Nair KS, Rizza RA, O'Brien P, Dhatariya K, Short KR, Nehra A, et al. DHEA in elderly women and DHEA or testosterone in elderly men. New England Journal of Medicine. 2006;355(16):1647-59.
30. Lamberg-Allardt C, Brustad M, Meyer HE, Steingrimsdottir L. Vitamin D - a systematic literature review for the 5th edition of the Nordic Nutrition Recommendations. Food & nutrition research. 2013;57.
31. Scott D, Blizzard L, Fell J, Ding C, Winzenberg T, Jones G. A prospective study of the associations between 25-hydroxy-vitamin D, sarcopenia progression and physical activity in older adults. Clinical Endocrinology. 2010;73(5):581-7.
32. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: A systematic review and meta-analysis. JAMA. 2012;308(10):1024-33.

33. Di Girolamo FG, Situlin R, Mazzucco S, Valentini R, Toigo G, Biolo G. Omega-3 fatty acids and protein metabolism: Enhancement of anabolic interventions for sarcopenia. Current Opinion in Clinical Nutrition and Metabolic Care. 2014;17(2):145-50.
34. Onder G, Penninx BW, Balkrishnan R, Fried LP, Chaves PH, Williamson J, et al. Relation between use of angiotensin-converting enzyme inhibitors and muscle strength and physical function in older women: an observational study. Lancet. 2002;359(9310):926-30.
35. Xu Z-r, Tan Z-j, Zhang Q, Gui Q-f, Yang Y-m. Clinical Effectiveness of Protein and Amino Acid Supplementation on Building Muscle Mass in Elderly People: A Meta-Analysis. PLoS ONE. 2014;9(9):e109141.
36. Paddon-Jones D, Sheffield-Moore M, Katsanos CS, Zhang XJ, Wolfe RR. Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. Experimental Gerontology. 2006;41(2):215-9.
37. Xu ZR, Tan ZJ, Zhang Q, Gui QF, Yang YM. The effectiveness of leucine on muscle protein synthesis, lean body mass and leg lean mass accretion in older people: a systematic review and meta-analysis. British Journal of Nutrition. 2014.
38. Scognamiglio R, Avogaro A, Negut C, Piccolotto R, Vigili de Kreutzenberg S, Tiengo A. The effects of oral amino acid intake on ambulatory capacity in elderly subjects. Aging Clinical and Experimental Research. 2004;16(6):443-7.
39. from HTA-centrum. Outcome tables [2015 january]. Available from:, https://www2.sahlgrenska.se/sv/SU/Forskning/HTA-centrum/Hogerkolumn-undersidor/Hjalpmedel-under-projektet/
40. Børsheim E, Bui QUT, Tissier S, Kobayashi H, Ferrando AA, Wolfe RR. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clinical Nutrition. 2008;27(2):189-95.
41. Dal Negro RW, Aquilani R, Bertacco S, Boschi F, Micheletto C, Tognellai S. Comprehensive effects of supplemented essential amino acids in patients with severe COPD and sarcopenia. Monaldi Archives for Chest Disease - Pulmonary Series. 2010;73(1):25-33.
42. Dal Negro RW, Testa A, Aquilani R, Tognella S, Pasini E, Barbieri A, et al. Essential amino acid supplementation in patients with severe COPD: A step towards home rehabilitation. Monaldi Archives for Chest Disease - Pulmonary Series. 2012;77(2):67-75.
43. Dillon EL, Sheffield-Moore M, Paddon-Jones D, Gilkison C, Sanford AP, Casperson SL, et al. Amino acid supplementation increases lean body mass, basal muscle protein synthesis, and insulin-like growth factor-I expression in older women. Journal of Clinical Endocrinology and Metabolism. 2009;94(5):1630-7.
44. Ferrando AA, Paddon-Jones D, Hays NP, Kortebein P, Ronsen O, Williams RH, et al. EAA supplementation to increase nitrogen intake improves muscle function during bed rest in the elderly. Clinical Nutrition. 2010;29(1):18-23.
45. Kim HK, Suzuki T, Saito K, Yoshida H, Kobayashi H, Kato H, et al. Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese sarcopenic women: A randomized controlled trial. Journal of the American Geriatrics Society. 2012;60(1):16-23.
46. Scognamiglio R, Piccolotto R, Negut C, Tiengo A, Avogaro A. Oral amino acids in elderly subjects: Effect on myocardial function and walking capacity. Gerontology. 2005;51(5):302-8.
47. Solerte SB, Gazzaruso C, Bonacasa R, Rondanelli M, Zamboni M, Basso C, et al. Nutritional Supplements with Oral Amino Acid Mixtures Increases Whole-Body Lean Mass and Insulin Sensitivity in Elderly Subjects with Sarcopenia. American Journal of Cardiology. 2008;101(11 SUPPL.):S69-S77.

48. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. The American journal of physiology. 1998;275(1 Pt 1):E73-8.
49. Leenders M, van Loon LJ. Leucine as a pharmaconutrient to prevent and treat sarcopenia and type 2 diabetes. Nutrition Reviews. 2011;69(11):675-89.
50. Wilkinson DJ, Hossain T, Hill DS, Phillips BE, Crossland H, Williams J, et al. Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. The Journal of Physiology. 2013;591(11):2911-23.
51. Deutz NEP, Pereira SL, Hays NP, Oliver JS, Edens NK, Evans CM, et al. Effect of β-hydroxy-β-methylbutyrate (HMB) on lean body mass during 10 days of bed rest in older adults. Clinical Nutrition. 2013;32(5):704-12.
52. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. Frontiers in physiology. 2012;3:260.
53. Bartolucci AA, Hillegass WB. Overview, strengths, and limitations of systematic reviews and meta-analyses. Evidence-Based Practice: Toward Optimizing Clinical Outcomes: Springer; 2010. p. 17-33.
54. Schiffman SS, Graham BG. Taste and smell perception affect appetite and immunity in the elderly. European journal of clinical nutrition. 2000;54 Suppl 3:S54-63.

Appendix: Review templates

Review template 1.

Granskningsmall för randomiserad kontrollerad prövning – modifierad från SBU mall

Författare, år alternativt SBU:s identifikationsnummer:

Anvisningar:

- Alternativet ”oklart” används när uppgiften inte går att få fram från texten
- Alternativet ”ej tillämpligt” väljs när frågan inte är relevant.
- Det finns förtydligande kommentarer till vissa delfrågor. Dessa anges med en fotnot.

	Ja	Nej	Oklart	Ej tillämpligt
Extern validitet - (Directness)				
1. Studiepopulation				
a) Framgår det hur många personer som exkluderades före randomiseringen?	☐	☐	☐	☐
b) Är redovisningen av personer som inte randomiserades, trots att de var valbara, adekvat?	☐	☐	☐	☐
c) Är den population som deltagarna togs från tydligt beskriven och relevant	☐	☐	☐	☐
d) Är sättet att rekrytera deltagare acceptabelt?	☐	☐	☐	☐
e) Är studiens inklusionskriterier adekvata?	☐	☐	☐	☐
f) Är studiens exklusionskriterier adekvata?	☐	☐	☐	☐
Intern validitet (Risk of bias – Study limitations)				
2. Tilldelning av åtgärd/intervention/behandling				
a) Användes en randomiseringsmetod som på ett acceptabelt sätt minimerar risken för manipulation?	☐	☐	☐	☐
b) Utfördes randomiseringen så att fördelningen blev oförutsägbar och slumpmässig? [1]	☐	☐	☐	☐
c) Påbörjade samtliga deltagare, som randomiserades, behandlingen? [2]	☐	☐	☐	☐
3. Gruppernas jämförbarhet				

	Ja	Nej	Oklart	Ej tillämpligt
a) Var grupperna vid baseline rimligt lika avseende egenskaper som kan påverka resultatet (t ex ålder, kön, sjukdoms svårighetsgrad)?	☐	☐	☐	☐
4. Blindning (maskering) [3]				
Blindades följande på tillfredsställande sätt:				
a) Patienter	☐	☐	☐	☐
b) Prövare/behandlare	☐	☐	☐	☐
c) Utvärderare av resultat	☐	☐	☐	☐

5.Studielängd				
a) Är studiens längd adekvat?	☐	☐	☐	☐
b) Är uppföljningstiden adekvat?	☐	☐	☐	☐

	Ja	Nej	Oklart	Ej tillämpligt
6. Bortfall (antalet randomiserade deltagare som inte har följts upp enligt studieprotokollet) [4]				
a) Går det att följa deltagarnas väg genom studien t ex i ett flödesschema?	☐	☐	☐	☐
b) Är storleken på bortfallet efter randomisering acceptabelt?	☐	☐	☐	☐
c) Är orsakerna till bortfallet acceptabla?	☐	☐	☐	☐
7. Följsamhet (”compliance, adherence, concordance”) [5]				
a) Framgår det i vilken utsträckning deltagarna fullföljde behandlingen?	☐	☐	☐	☐
b) Var andelen som fullföljde behandlingen acceptabel?	☐	☐	☐	☐
8. Rapportering av effektmått och biverkningar				
a) Var det primära effektmåttet definierat i förväg och adekvat rapporterat?	☐	☐	☐	☐
b) Var de sekundära effektmåtten definierade i förväg och adekvat rapporterade?	☐	☐	☐	☐
c) Baserades slutsatserna på enbart i förväg definierade effektmått och subgruppsanalyser? [6]	☐	☐	☐	☐
d) Har utfallen av samtliga viktiga effektmått redovisats på ett adekvat sätt? [7]	☐	☐	☐	☐
e) Rapporterades biverkningar/komplikationer på ett tillfredsställande sätt?	☐	☐	☐	☐

Precision

a) Redovisas resultaten på ett adekvat sätt? [8]	☐	☐	☐	☐
b) Har resultaten beräknats med lämplig analysmetod? [9]	☐	☐	☐	☐
c) Var den minsta kliniskt relevanta effekten definierad på förhand?	☐	☐	☐	☐
d) Är den valda minsta kliniskt relevanta effekten av rimlig storlek?	☐	☐	☐	☐
e) Har man använt acceptabla metoder för att mäta effekterna?	☐	☐	☐	☐
f) Mättes observatörsöverensstämmelsen på ett acceptabelt sätt? [10]	☐	☐	☐	☐
g) Är de överväganden och beräkningar som ligger till grund för antal deltagare acceptabla (”power”-analys)? [11]	☐	☐	☐	☐

Bindningar och jäv				
a) Anges eventuella bindningar och jäv (”declaration of interest ”)?	☐	☐	☐	☐
b) Bedömer du att studiens resultat inte påverkats av intressekonflikter?	☐	☐	☐	☐

Review template 2.

Granskningsmall för kohortstudier med kontrollgrupp – modifierad från SBU mall

Författare, år alternativt SBU:s identifikationsnummer:

Anvisningar:

- Alternativet ”oklart” används när uppgiften inte går att få fram från texten.
- Alternativet ”ej tillämpligt” väljs när frågan inte är relevant.
- Det finns förtydligande kommentarer till vissa delfrågor. Dessa anges med en fotnot.

	Ja	**Nej**	**Oklart**	**Ej tillämpligt**
Extern validitet (Directness)				
1. Jämförbarhet				
a) Är kontrollgruppen eller grupperna adekvat valda? [1]	☐	☐	☐	☐
b) Är det en kliniskt relevant kontrollgrupp?	☐	☐	☐	☐
c) Är det sannolikt att interventions- och kontrollgruppen valdes ut och diagnostiserades på ett likartat sätt? [2]	☐	☐	☐	☐

Intern validitet (Risk of bias – Study limitations)				
2.1. Förväxlingsfaktorer (”confounders”)				
a) Har författarna identifierat alla viktiga förväxlingsfaktorer och tagit hänsyn till dem i analyserna? [3]	☐	☐	☐	☐
b) Var skillnaderna mellan grupperna i baslinjedata små? [3]	☐	☐	☐	☐
c) Är risken för selektions- eller indikationsbias liten? [4]	☐	☐	☐	☐
2.1. Följsamhet, bortfall				
2.1.1 Följsamhet (”compliance, adherence”)				
a) Framgår det i vilken utsträckning deltagarna fullföljde behandling?	☐	☐	☐	☐
b) Var andelen som fullföljde behandlingen acceptabel?	☐	☐	☐	☐
2.1.2 Bortfall (antalet deltagare som inte har följts upp enligt studieprotokollet)				
a) Redovisas hur stort bortfallet är? [5]	☐	☐	☐	☐
b) Anges orsakerna till bortfallet? [5]	☐	☐	☐	☐
c) Är bortfallet acceptabelt? [5]	☐	☐	☐	☐
3. Blindning				
Var de som bedömde utfallen omedvetna om deltagare tillhörde interventions- eller kontrollgruppen? [6]	☐	☐	☐	☐
4. Analys				
a) Har den statistiska analysen av osäkerhet hanterats på ett adekvat sätt? [8]	☐	☐	☐	☐
b) Försökte författarna statistiskt korrigera för obalanser mellan grupperna med avseende på förväxlingsfaktorer/”confounders”? [9]	☐	☐	☐	☐
5. Biverkningar				
Mättes biverkningar/komplikationer på ett tillfredsställande sätt?	☐	☐	☐	☐

Precision				
a) Är de överväganden och beräkningar som ligger till grund för urvalsstorleken (”sample size”) tydligt beskrivna? [7]	☐	☐	☐	☐

b) Är den statistiska styrkan (”power”) tillfredsställande hög? [7]	☐	☐	☐	☐

Bindningar och jäv				
a) Finns en förteckning över eventuella bindningar och jäv (”declaration of interest”)?	☐	☐	☐	☐
b) Bedömer du att studiens resultat inte påverkats av intressekonflikter?	☐	☐	☐	☐

Review template 3

Work sheet –Certainty of evidence (GRADE)

Disease/ disorder:
Intervention/ method vs. control:
Outcome variable:
Included studies RCT, No……. SR, No…… Cohort studies, No…….
Number of patients:

Assessment of risk of bias (“internal validity”) Assessment of “external validity”

Study	**Random sequence generation**	**Allocation concealment**	**Blinding of participants and**	**Blinding of outcome assessment**	**Incomplete outcome data**	**Selective reporting**	**Other bias**	**Summary Risk of Bias**	**Directness**	**Precision** and precision

+ = low risk/good ? = unclear risk – = high

Study limitations (Randomisation, blinding, follow-up, drop-out/withdrawals, intention-to-treat)	Mark with cross-sign	
No serious limitations	0	
Some limitations (*but not enough to downgrade)*)	0?	
Serious limitations (*downgrade one step*)	-1	
Very serious limitations (*downgrade two steps*)	-2	
Comment limitations or reasons to downgrade:		

Consistency (Estimate of relative effect, same magnitude and direction across studies? overlapping confidence intervals?)	Mark with cross-sign	
☐ Based on meta-analysis? Statistical analysis of heterogeneity: ☐ Chi^2 ☐ I^2		
No serious inconsistency	0	
Some inconsistency (*but not enough to downgrade*)	0?	
Serious inconsistency (*downgrade one step*)	-1	
Very serious inconsistency (*downgrade two steps*)	-2	
Comment limitations or reasons to downgrade:		

Directness (study population – external validity, specificity of intervention, relevance of the comparator to the intervention, clinical setting, adequate time of follow-up)	Mark with cross-sign	
No uncertainty	0	
Some uncertainty (*but not enough to downgrade*)	0?	
Serious indirectness (*downgrade one step*)	-1	
Very serious indirectness (*downgrade two steps*)	-2	
Comment limitations or reasons to downgrade:		

Precision (Few events, wide confidence intervals that also include possible unfavourable effects)	Mark with cross-sign	
No imprecision	0	
Uncertain precision (*but not enough to downgrade*)	0?	
Serious imprecision (*downgrade one step*)	-1	
Very serious imprecision (*downgrade two steps*)	-2	
Comment limitations or reasons to downgrade:		

Publication bias (Few and small studies from one research group or company which all show the same type of results, well.known unpublished studies)	Mark with cross-sign	
Unlikely	0	
Uncertainty (*but not enough to downgrade*)	0?	
Likely (*downgrade one step*)	-1	
Very likely (*downgrade two steps*)	-2	
Comment limitations or reasons to downgrade:		

Magnitude of effect	Mark with cross-sign	
Not relevant	0	
Large effect (RR<0,5 or >2) (*upgrade one step*)	+1	
Very large effect (RR<0,2 or >5) (*upgrade two steps*)	+2	
Comment limitations or reasons to downgrade:		

Comments on other important aspects of the level of evidence (clear dose-response gradient that may allow upgrading?, confounders that clearly reduce the magnitude of the effect?)

Is the sum of uncertainties (?) enough to motivate downgrading with one step?	Mark with cross-sign	
Yes	- 1	
No	0	

Certainty of evidence	Mark with plus-signs	
High	⊕⊕⊕⊕	
Moderate	⊕⊕⊕◯	
Low	⊕⊕◯◯	
Very low	⊕◯◯◯	

Namn. Datum: