## Treatment of obesity in older adults

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# Treatment of obesity in older adults

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door

**Amely Maria Verreijen**

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# Chapter 1

## General Introduction

# GENERAL INTRODUCTION



## Prevalence of obesity in older adults

Older adults represent the fastest growing population in the Netherlands. Within 10 years more than one third of our population is 55 years or older (1). The prevalence of obesity in older adults is high, especially among adults aged 55 to 65 years. In this age group 19% is obese, and this prevalence is expected to rise based on the increase in prevalence over the past 10 years (2). Moreover, in the older age groups the prevalence of obesity has increased by approximately 10% the past 4 decades, with 18% being obese in the 65 to 75 years age group, and 15% in the 75+ years age group (2). With the current prevalence of obesity among older adults, the total number of older adults with obesity will be more than one million in less than 10 years.

This trend is also visible in Western countries worldwide: the pace at which populations are aging is increasing rapidly and goes hand in hand with the increased prevalence of obesity (3-6), resulting in large numbers of obese older adults in the future.

## Health risks of obesity at older age based on epidemiological findings

Obesity at older age is related to several health risks. First, obesity at older age is associated with a higher mortality risk (7, 8). Although several studies have described that a raise in BMI may be protective in older adults (9, 10), this association is often confounded by underlying chronic disease and smoking (8, 11).

Second, obesity, even at old age, is associated with a higher risk for cardiovascular diseases and type 2 diabetes (12). Aging is related to a loss of muscle mass and an increase in fat mass, and a shift towards more visceral fat mass and ectopic fat infiltration (13). Obesity may further amplify these changes in fat mass and fat distribution (14). Increased visceral fat and ectopic fat is a risk factor for developing metabolic disorders, such as hypertension, dyslipidemia and insulin resistance that may lead to or worsen the course of cardiovascular disease (15, 16) and type 2 diabetes at older age (4, 17).

Third, obesity at older age is associated with musculoskeletal impairments and compromised physical functioning. Although a higher BMI at older age is associated with a higher bone mineral density, it does not correlate well with overall fracture risk and seems to be associated with specific sites: a lower fracture risk in the hip, pelvic and wrist, but a higher fracture risk in the ankle and upper arm (18). Obesity is strongly linked with other musculoskeletal impairments, such as osteoarthritis of the hip or knee (19), which is a major source of pain (20, 21). Both pain and obesity at older age are independently related to lower gait speed (21). Furthermore, although obese subjects appear stronger because obesity is related to a greater absolute maximum muscle strength, the maximum

strength per kilogram body weight is lower and therefore muscle performance in obesity seems lower (22). In line, other studies demonstrate that obesity in older age is related to compromised physical functioning (23, 24), and higher fall risk (25). Severe decrements in physical functioning because of obesity could translate into years of disability during an extended lifetime, and to loss of independence and placement in nursing homes (24, 26).

Finally, obesity at older age is associated with cancer (27), pulmonary abnormalities (28), cognitive dysfunction (29), obstructive sleep apnea (30) and impaired quality of life (31-33). These inverse associations of obesity and indicators of health and physical functioning are even more pronounced in obese older adults that also have low muscle mass and muscle function, also referred to as sarcopenic obesity (34-36).

## Voluntary weight loss in older adults: what are the benefits?

Many benefits of voluntary weight loss in older adults are described. Older subjects with obesity that follow a weight loss program that included caloric restriction are at reduced risk for long-term chronic medical conditions by a reduction of systolic and diastolic blood pressure, have an increase in HDL-cholesterol levels, a reduction of triglycerides, a reduction of ectopic fat, an improvement in insulin sensitivity and glucose regulation, a reduction in markers of inflammation (37-40) and a reduction in medication use (38). In addition, an improvement in physical performance (37, 41-43), a decline in osteoarthritis symptoms (44), an improved cardiorespiratory fitness, and fewer feelings of fatigue (45), a 15% reduction in all-cause mortality (46) and an increase in quality of life (43) are observed. These positive effects are described for weight loss trials in older adults in general. When an increase in exercise was part of the weight loss strategy positive effects are mostly more pronounced (37, 38, 43).

## Voluntary weight loss in older adults: what are the risks?

Although the described health risks of obesity, and the benefits of voluntary weight loss, clinicians often are reluctant to recommend weight loss interventions for older adults because of potential health risks (43). Caloric restriction during weight loss induces a catabolic state including catabolism of skeletal muscle tissue. (38, 47-49) A systematic review of weight loss trials in subjects aged 50 years and over demonstrates that 50% of the included study groups that received only caloric restriction (without exercise) lost ≥25% of their body weight as fat free mass (FFM) (48). A meta-analysis of weight loss trials that analyzed the effect of only caloric restriction (without exercise) on muscle strength parameters, concludes that diet-induced weight loss in overweight or obese adults has a potential adverse effect on muscle strength (50). Since older adults in general lose muscle mass and function during the aging process, referred to as the process of sarcopenia (51), a further loss of muscle mass and muscle strength may lead to an acceleration of this process and increases the risk of negative health outcomes such as falls, functional decline, frailty, and mortality (52).

Additionally, weight loss in general -whether intentional or not- decreases bone mineral density (18, 53, 54) and is associated with fracture risk (55). Furthermore, weight-loss interventions that include an exercise program, may induce the risk of musculoskeletal injuries in older adults (38, 56). Also the concern for risk of nutrient deficiency during caloric restriction without adequate dietary counselling is described (47). Finally, evidence suggests that the frequently observed weight regain after weight loss (57) presumably facilitates an increase in fat mass (and not lean mass) (58), which may negatively affect physical performance (59) and potentially increase the risk of sarcopenic obesity (47, 60, 61).

Negative effects of voluntary weight loss on muscle mass, muscle strength and bone mineral density are mostly described for weight loss interventions without exercise. When exercise was part of the weight loss strategy the loss of fat free mass (FFM) and bone mineral density were attenuated, and the effect on muscle strength were mostly reversed (37, 38, 43, 49). **Figure 1** summarizes risks and benefits of voluntary weight loss in obese older adults.

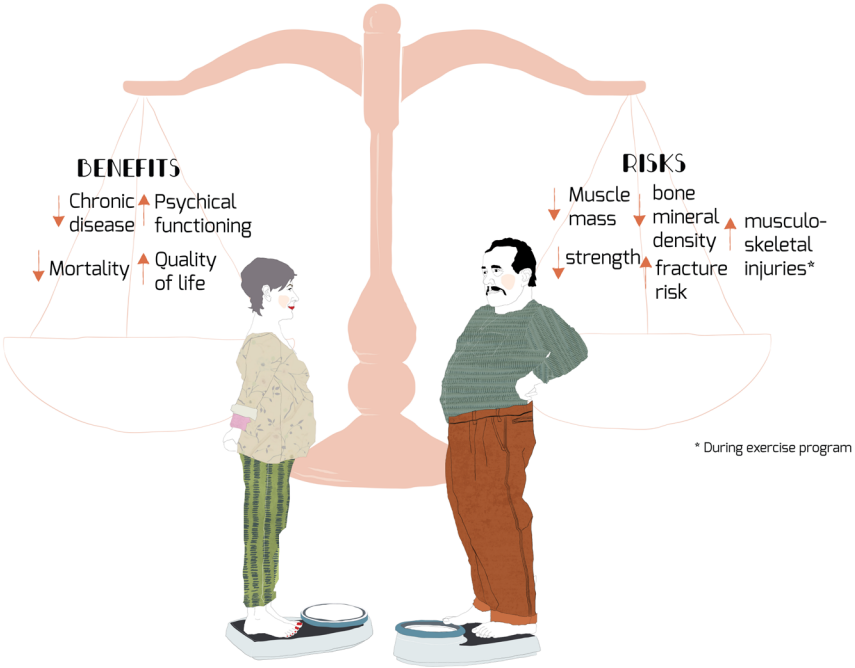


**Figure 1:** Risks and benefits of voluntary weight loss in older obese adults

## Role of dietary protein and exercise during voluntary weight loss

Strategies to preserve muscle mass during weight loss focus on the anabolic stimuli exercise (48) and sufficient intake of protein (62). Resistance exercise in older obese adults has shown to preserve muscle mass (63), and bone mineral density (64) during weight loss. In addition, since the amino acids from dietary protein not only act as building blocks for the muscle, but also stimulate muscle protein synthesis as seen from short-term metabolic studies (65), the consumption of adequate amounts of protein might preserve muscle mass during weight loss (62). However, not all studies found this muscle preserving effect of a higher intake of protein during energy restriction (66, 67), but those studies did not include an active exercise program. Protein supplementation possibly enhances the effect of resistance exercise on muscle mass and strength in older adults (68, 69). A higher protein diet combined with resistance exercise during a period of weight loss could therefore be a beneficial strategy to reduce muscle loss in older adults with obesity, although evidence is still limited (49).

## Current dietetic practice

In 2012 a survey was conducted to gain more insight in how Dutch dietitians treat older adults with obesity (70). Results of this survey indicated a great diversity in treatment characteristics among dietitians. In general, dietitians were cautious in the level of caloric restriction they advised (–150 to – 650 kcal of estimated needs). Approximately half of the dietitians estimated caloric needs by a predictive resting energy equation, and used various equations and different values for body weight in these equations (current body weight or body weight at specific BMI-levels). To our knowledge, there is no study that evaluated the accuracy of these predictive resting energy equations in an older population with obesity, including an evaluation of which body weight to use. Furthermore, two third of the dietitians in this survey indicated that resistance training and a high protein diet could be useful in the treatment of obesity in older adults, whereas only 15% indicated to advise resistance training and only 44% indicated to advise a high protein diet. This variety in treatment characteristics may both reflect the diversity of older adults with obesity and the need for treatment guidelines for this group, especially since the percentage of older adults with obesity visiting primary care dietitians is already high and expected to rise even further (1, 2, 71, 72). More knowledge is needed to optimize treatment options for weight loss in older adults with obesity in which benefits are increased and risks are minimized (49).

## Aim and thesis outline

Because of the increasing prevalence of obese older adults and its negative impact on health and physical functioning, it is of great importance to optimize the benefits of voluntary weight loss in obese older adults. This thesis covers several relevant aspects of the treatment of obesity in obese older adults and addresses four questions:

1. What are the caloric needs of older obese adults before and during weight loss?
2. What is an optimal protein intake for older adults?
3. What is the effect of a higher amount of protein (in combination with exercise) during a weight loss intervention on preservation of muscle mass?
4. What are optimal treatment options for obese older adults?

These four questions form the four parts of this thesis illustrated in **Figure 2**. In **part 1 'Estimating caloric needs'** we evaluate the validity of existing resting energy expenditure equations in an older obese population (*chapter 2*), and study the effect of weight loss on caloric needs and the presence of adaptive thermogenesis (*chapter 3*). In **part 2 'Optimal protein intake'** we study the relation between protein intake and the 5-year change in mid-thigh muscle area in older adults (*chapter 4*). In *chapter 5* we evaluate the association between the amount of protein intake at breakfast and lunch and total daily protein intake in older adults. In **part 3 'Muscle mass preservation during weight loss'** we study the effect of a high protein (and vitamin D) supplement on preservation of muscle mass during voluntary weight loss (*chapter 6*), and the effect of a high protein diet and/or exercise on preservation of fat free mass during voluntary weight loss (*chapter 7*) in overweight and obese older adults. **Part 4 'Towards optimal treatment'** focuses on strategies to prevent and treat sarcopenic obesity in older adults (*chapter 8*). Finally, in *chapter 9*, we discuss the main findings and methodological issues of the studies within each part in the light of the existing literature. This chapter concludes with directions for future research.


**Figure 2:** Outline of this thesis

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# Part 1

## Estimating caloric needs

# Chapter 2

## Which equations for estimating resting energy expenditure should we use for community dwelling older adults with obesity?

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Submitted

## ABSTRACT

**Background & Aim** Predictive equations for resting energy expenditure (REE) are used in the treatment of obesity, but their accuracy in obese older adults is unknown. This study evaluates which predictive REE equation is the best alternative to indirect calorimetry in obese older adults.

**Methods** A cross-sectional analysis was performed on data of 341 obese older adults (≥ 55 y, BMI ≥ 30 kg/m$^2$) from the Netherlands, Belgium and the USA. REE (by indirect calorimetry), body weight, body height, age, sex, and fat free and fat mass were measured. The measured REE was used as a reference and compared with 41 existing REE equations based on body weight or body composition (fat free mass and/or fat mass) parameters. The accuracy of the equations was evaluated by the percentage accurate prediction (within 10% of REE measured) for the total study sample as well as for the Dutch, Belgian, American black, and American white subgroups.

**Results** The total study population existed of 62% females, with a mean age ± SD of 65 ± 7 y, and mean BMI of 35 ± 4 kg/m$^2$. The highest percentage of accurate predictions for the total population was 61% by Harris & Benedict re-evaluated by Rosa et al.. REE equations performed differently in each subroup. The best equation in each subgroup gave 71-79% accurate predictions, which indicates that a higher accuracy is achieved by a population specific approach.

**Conclusions** No single REE equation performed best across all subgroups. The REE of over 70% of the obese older population could be accurately predicted by REE equations using a subgroup specific approach. Prediction suggestions per subgroup are provided when measurement of REE by indirect calorimetry is not feasible.

# INTRODUCTION

The prevalence of obesity among older adults is high: approximately 20% of the older European population (1) and 40 % of the older American population is obese (2), with increasing trends over time. Similar to younger age groups, obesity in old age dramatically increases the risk of hypertension, dyslipidemia and insulin resistance that may lead to cardiovascular disease and type 2 diabetes. Furthermore, obesity in older adults is associated with disability and worsening of chronic diseases including type 2 diabetes, cardiovascular disease, and osteoarthritis (3, 4). A recent systematic review shows that voluntary weight loss in older obese subjects improves cardiovascular parameters and physical functioning and concludes that old age alone should not be a contra-indication to intensive lifestyle intervention aimed at reducing body weight (4).



Weight loss can be achieved by a reduction of calorie intake (in combination with more physical activity) (5, 6). Dieticians need to estimate individual energy requirements to set a reachable goal for dietary intake during caloric restriction. This requires knowledge of individual energy requirements, which is based on the resting energy expenditure (REE) and the level of physical activity (6, 7). Because the measurement of REE by indirect calorimetry, considered the gold standard (8), is generally not feasible in dietetic practice, it is important to use an accurate equation to predict REE.

Predictive equations for REE have been evaluated for older adults in many validation studies (6, 9-19), but only one focused on obese older adults (18). In this study by Siervo et al. (18), only 29 subjects were included, highlighting the need for larger studies. Also, most predictive equations tested in these validation studies are based on body weight, body height, sex, and age, whereas equations using body composition information, such as fat free mass (FFM) or fat mass (FM), are often not included (19).

The aim of this study is to evaluate which predictive REE equation, based on body weight or body composition, is the best alternative to indirect calorimetry for the assessment of REE of obese older adults.

# SUBJECTS AND METHODS

***Study population***

A cross-sectional analysis was performed on three study samples of in total 341 community-dwelling obese older adults (age ≥ 55 years, BMI ≥ 30 kg/m$^2$) from the Netherlands, Belgium and the Unites States of America (USA).

Dutch sample

Baseline data of 194 obese (BMI ≥ 30 kg/m$^2$) older adults (≥ 55 years) participating in these three intervention studies at the Amsterdam Nutritional Assessment Center at Amsterdam University of Applied Sciences were included:

1. The 'muscle preservation study' (MPS) (20),
2. The 'weight loss with protein and exercise' (WelPrex) study (21), and
3. The PROBE study: a protein and lifestyle intervention to preserve muscle mass in obese older type 2 diabetes patients (22).

A full description of the eligibility criteria of these three studies is available online in the Dutch Trial Register (MPS: NTR2751; WelPrex: NTR4556; PROBE: NTR4497; http://www.trialregister.nl). These studies were approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam (2010/280, MPS), the Medical Ethics Committee Independent Review Board Nijmegen (NL43226.072.14, WelPrex) and the Medical Ethics Committee Assen (NL46790.056.14, PROBE), all in The Netherlands.

Belgian sample

Data of 72 obese older females from the department of Nutrition, Public Health Medicine and at the Obesity Clinic (University Hospital Gasthuisberg) were included. A more detailed description of the study population is provided elsewhere (23). All participants gave informed consent and all procedures were in accordance with ethical standards of the institution.

American sample

Data of 75 obese older subjects participating in an energy expenditure substudy of the Health, Aging, and Body Composition (Health ABC) study were included. A detailed description of the Health ABC study population is provided elsewhere (24). All participants gave written informed consent. All protocols were approved by the Human Subjects Review Boards at the University of Pittsburgh and the University of Tennessee at Memphis.

***Measurements***

**Table 1** describes the devices and the protocols for measuring REE, and devices for measuring body weight (BW), body height, FFM and FM, for the three study samples.

***Selection of predictive REE equations***

A systematic search was performed in PubMed for all available dates until November 2019 using the key words 'basal metabolism', 'indirect calorimetry', 'energy metabolism', 'resting energy expenditure', 'basal metabolic rate', 'predict*', 'equation', 'estim*', 'formula' in every possible combination. The search was limited to English language, adults (18+). More references were obtained by screening publications. Inclusion criteria were: 1)

equations developed for adults, 2) equations based on body weight, height, age, sex and/or FFM and FM. Exclusion criteria were: equations for specific patient groups (except for diabetes), equations for athletes, and equations including other body composition related variables like skinfold thickness of the chest, or total lean body mass (excluding bone mass), since these variables did not match the available data on body composition. Because most selected publications present more than one equation, the best performing equation for both with and without body composition was included based on the highest value for explained variance ($R^2$). When a publication presents equations both with and without body composition variables, then the equation for both with and without body composition variable with the highest $R^2$ was selected. In total, 41 equations were retrieved from the literature, of which 17 were based on body composition parameters FFM and/or FM (**Supplementary Table A**).



**Table 1:** Devices and protocols for measuring resting energy expenditure (REE), body weight, body height, fat free mass (FFM) and fat mass (FM) for the three study samples

| | Dutch data | Belgian data | USA data |
|---|---|---|---|
| **Measurement of REE by indirect calorimetry (ventilated hood system)** | | | |
| **Device indirect calorimetry** | Vmax Encore n29; Viasys Healthcare, Houten, Netherlands | Deltatrac II; Datex, Acertys Healthcare NV, Aartselaar, Belgium | Deltatrac II; Datex, Ohmeda Inc, Helsinki, Finland |
| **Protocol subject instructions** | – Fasted at least 5h before the measurement (48)<br>– At least 14h no exercise or sauna visit<br>– No smoking and alcohol on the day of the measurement | – Overnight fast<br>– Subjects had not been physically active before the measurement and the evening before<br>– Subjects rested for 30 min before the measurement | Overnight fast (no calorie containing beverages/food after 10 pm on the night before the visit) |
| **Protocol measurement** | – Calibrated for volume and with 2 standard gases every day before use<br>– Measurements took 30 min, and only steady state periods of measurement were selected<br>– The first 5 min of the measurements were discarded | – Calibrated for volume and with 2 standard gases every day before use<br>– Gas exchanges were measured for 40 min<br>– Data from the first 10 min were excluded, as were the data for 2 min after any movement or loss of wakefulness<br>– The remaining minute-by-minute data were averaged | – Calibrated for volume and with 2 standard gases every day before use<br>– Measurements took 30 min, and only steady state periods of measurement were selected according<br>– The first 5 min of the measurements were discarded |

| Measurement of BW, height, FM and FFM | | | |
|---|---|---|---|
| **Body weight** | Calibrated scale as part of the BODPOD system (BODPOD, Life Measurement Inc., Concord, CA) | Calibrated scale (SECA) | Standard balance beam scale |
| **Body height** | Wall-mounted Seca 222 stadiometer | Wall-mounted Harpenden stadiometer | Wall-mounted Harpenden stadiometer |
| **FM and FFM** | Air displacement plethysmography (BODPOD, Life Measurement Inc., Concord, CA) | Bio-impedance analysis (Bodystat 1500, Euromedix, Leuven, Belgium) | Dual-X-ray absorptiometry, (4500A, version 8.20a; Hologic, Waltham, MA) |

***Statistics***

The REE estimated by different predictive equations was compared to the measured REE by indirect calorimetry. The accuracy of the equations was evaluated by: 1) the percentage accurate predictions, which is defined as a predicted REE within 10% of the measured REE. An underprediction was defined as a predicted REE of more than 10% lower than the measured REE. An overestimation was defined as a predicted REE of more than 10% higher than the measured REE. 2) the mean difference or bias between the estimated REE and the measured REE (*Mean difference = Estimated REE – measured REE*) in kcal/d, 3) limits of agreement, calculated by *mean difference between calculated and measured REE ± (SD of this difference * 1.96)* in kcal/d and 4) the mean absolute error, which is presented to give insight in the average absolute deviation in kcal/day between the estimated and measured REE (25). The most accurate equation was selected based on the highest percentage of accurate predictions, since it is relevant in practice that the estimation is accurate for most (favorably all) subjects.

Subgroup analysis was performed for the Dutch, Belgian, American black and American white subgroups, since both scatterplot analysis (Y-axis measured REE, X-axis estimated REE) and One-Way ANOVA (post-hoc test Bonferroni) revealed that the bias of the 10 best equations based on the total study population was significantly different for the Dutch, Belgian and American subgroups, with differences in bias up to 300 kcal/day. The independent samples t-test showed that the bias of these 10 equations also differed between black vs. white Americans with differences in bias of more than 100 kcal/day. Other tested variables were sex (male versus female), BMI-category (BMI 30-34.9 kg/m$^2$, 35-39.9 kg/m$^2$, equal to or more than 40 kg/m$^2$), age category (55-69 y versus 70 y or older) and body height category (body height < 1.70m versus ≥ 1.70m). For these variables lower differences in bias were observed between the subgroups (0-100 kcal/day), except for age-category. Since differences by age-category overlapped with the study population

(the American subgroup was older) no specific subgroup analyses were performed by age category.

Additionally, the accuracy evaluation was also performed for the equations based on adjusted body weight at BMI 30.0 kg/m$^2$ (26) and at BMI 27.5 kg/m$^2$ (27) in the four subgroups, to investigate whether using adjusted body weight in the REE equations resulted in a better performance of the equation. The ratio of FFM to total body weight is lower in obese subjects compared to normal weight subjects. Using actual body weight could potentially overestimate REE as FFM has a higher metabolic rate than FM (28).



Finally, the characteristics of subjects whose REE was underestimated, accurately predicted, or overestimated by the most accurate REE equation were compared. The variables used for characterization were age, sex, ethnicity, body weight, height, BMI, FFM, FFM-index, FM an FM-percentage. Differences were tested by One-Way-ANOVA or Chi-square (for nominal variables).

Statistical analyses were performed using SPSS software (version 24.0, IBM). Statistical significance was defined as a two-tailed $P < 0.05$.

## RESULTS

***Subject characteristics***

In total 341 subjects were included for data-analysis: 194 Dutch, 72 Belgian and 75 American subjects, of whom 42 were black American and 33 white American. The total study population consisted of 62% females, with a mean age ± SD of 65 ± 7 y, and mean BMI of 35 ± 4 kg/m$^2$ (**Table 2**). Mean age was highest for the American subgroups and mean measured REE was lowest for the American black subgroup. The American black subgroup was the only population with black subjects and the Belgian subgroup consisted of females only.

**Table 2:** General characteristics of the total study population and the four subgroups Dutch, Belgium, American black and American white separately

| | Total | Dutch | Belgian | American black | American white |
|---|---|---|---|---|---|
| | n = 341 | n = 194 | n = 72 | n = 42 | n = 33 |
| REE (kcal/day) | 1685 ± 309 | 1814 ± 296 | 1607 ± 222 | 1373 ± 211 | 1497 ± 215 |
| REE (kcal/kg FFM/day) | 31.1 ± 4.9 | 32.7 ± 4.3 | 32.5 ± 3.5 | 24.3 ± 2.7 | 27.3 ± 2.9 |
| Age (y) | 65.4 ± 7.1 | 64.3 ± 5.7 | 59.1 ± 3.9 | 74.2 ± 3.1 | 74.5 ± 2.8 |
| Sex (% female) | 62% | 52% | 100% | 57% | 45% |
| Weight (kg) | 99.1 ± 13.6 | 101.0 ± 13.9 | 98.6 ± 13.7 | 94.8 ± 13.1 | 94.5 ± 9.9 |
| Height (m) | 1.67 ± 0.09 | 1.71 ± 0.09 | 1.61 ± 0.06 | 1.66 ± 0.09 | 1.66 ± 0.08 |
| BMI (kg/m$^2$) | 35.3 ± 4.4 | 34.6 ± 4.0 | 38.1 ± 5.0 | 34.5 ± 3.7 | 34.1 ± 2.9 |
| % BMI 30 – 35 (kg/m$^2$) | 56% | 62% | 31% | 64% | 67% |
| % BMI 35 – 40 (kg/m$^2$) | 31% | 30% | 42% | 21% | 27% |
| % BMI ≥ 40 (kg/m$^2$) | 13% | 8% | 28% | 14% | 6% |
| FFM (kg) males | 65.8 ± 7.0 | 66.5 ± 7.5 | - | 65.9 ± 5.1 | 62.4 ± 4.2 |
| FM (kg) males | 38.6 ± 8.6 | 40.5 ± 8.5 | - | 31.2 ± 7.4 | 35.9 ± 4.8 |
| FM (%) males | 36.6 ± 5.1 | 37.7 ± 5.0 | - | 31.7 ± 4.6 | 36.2 ± 3.0 |
| FFM (kg) females | 48.3 ± 5.7 | 47.2 ± 5.9 | 49.6 ± 5.1 | 50.2 ± 5.3 | 46.8 ± 5.8 |
| FM (kg) females | 47.3 ± 10.5 | 48.1 ± 10.7 | 48.9 ± 10.2 | 42.4 ± 10.2 | 42.2 ± 6.0 |
| FM (%) females | 49.1 ± 5.0 | 50.1 ± 5.5 | 49.3 ± 4.1 | 45.3 ± 4.4 | 47.2 ± 3.6 |
| Ethnicity (% black) | 12% | 0% | 0% | 100% | 0% |

***Accuracy of the predictive equations for REE***

**Table 3** presents the accuracy analysis based on percentage accurate predictions, bias, limits of agreement and mean absolute error in the total study population. The best performing equation for the total study population according to the highest percentage of accurate predictions was Harris & Benedict re-evaluated by Rosa et al. (29), with the REE of 61% of subjects being accurately predicted.

**Table 4** shows the ten most accurate equations per subgroup, according to the highest % of accurate predictions. In general, the best equation in the subgroups had a percentage of accurate prediction of at least 10%-points better than the best equation in the total study population. For the Dutch sample the Harris and Benedict equation re-evaluated by Roza et al. (29) performed best, with 71% accuracy. For the Belgium sample both Ganpule_FFM (30) and Ikeda (31) performed best, with 74% accurate predictions. Within the American sample, the REE of the black subgroup was best predicted by the Bernstein_FFM (32) equation, with 79% accuracy. For the American white sample the Mifflin_StJeor_FFM (33) equation performed best, with 73% accuracy. For the American samples equations based on body composition parameters gave 3-11% more accurate predictions than the best

equation without body composition parameters, indicating that prediction was better when equations based on body composition parameters were used. For the Dutch and Belgium sample, this difference was not observed.

**Figure 1** shows the percentage of accurate predictions using actual BMI, adjusted body weight at BMI 30, and adjusted BMI at 27.5 kg/m$^2$ for the equations based on body weight. Adjustment of the obese body weight leads to a lower percentage of accurate predictions in the Dutch and Belgian subgroups, indicating that prediction was best when actual body weight was used. In contrast, in the American subgroups a higher percentage of accurate predictions was achieved when adjusting the obese body weight, with generally better accuracy when using body weight at BMI 27.5 versus 30 kg/m$^2$. When body composition information is not available Livingston Kohlstadt using BW at BMI 27.5 kg/m$^2$ is suggested for the American black subgroup, and Mifflin_StJeor using BW at BMI 30 kg/m$^2$ is suggested for the American white subgroup.

**Table 3:** Evaluation of resting energy expenditure (REE) prediction equations with percentage accurate predictions, bias, limits of agreement and mean absolute error, in the total sample of 341 obese subjects aged 55 years and older

| **REE equation** | **Mean REE ± SD** (kcal/d) | **Under predictions**[1] (%) | **Accurate predictions**[2] (%) | **Over predictions**[3] (%) | **Bias**[4] (kcal/day) | **Limits of agreement**[5] (kcal/d) | **Mean absolute error**[6] (kcal/d) |
|---|---|---|---|---|---|---|---|
| *Measured REE* | *1685 ± 310* | | | | | | |
| Bernstein | 1387 ± 188 | 74 | 25 | 1 | -298 ± 213 | -716, 120 | 310 ± 196 |
| Bernstein_FFM | 1345 ± 196 | 81 | 18 | 1 | -340 ± 220 | -772, 91 | 351 ± 203 |
| Cunningham1980_FFM | 1689 ± 227 | 21 | 53 | 26 | 4 ± 237 | -461, 469 | 189 ± 143 |
| Cunningham1991_FFM | 1558 ± 227 | 45 | 40 | 14 | -128 ± 237 | -593, 338 | 221 ± 154 |
| EU | 1721 ± 222 | 14 | 55 | 30 | 36 ± 204 | -365, 436 | 166 ± 124 |
| FAO_WT | 1734 ± 211 | 13 | 55 | 32 | 49 ± 209 | -361, 459 | 172 ± 128 |
| FAO_WTHT | 1720 ± 198 | 14 | 55 | 31 | 35 ± 211 | -378, 449 | 173 ± 125 |
| Fredrix | 1784 ± 207 | 6 | 52 | 42 | 99 ± 202 | -297, 494 | 183 ± 130 |
| Ganpule | 1880 ± 197 | 4 | 40 | 56 | 195 ± 219 | -233, 624 | 243 ± 164 |
| Ganpule_FFM | 1669 ± 225 | 21 | 59 | 21 | -16 ± 211 | -429, 397 | 169 ± 127 |
| HB_1919 | 1714 ± 239 | 15 | 58 | 27 | 29 ± 200 | -364, 421 | 161 ± 123 |
| HB_1984 | 1708 ± 252 | 14 | 61 | 25 | 23 ± 202 | -373, 419 | 160 ± 125 |
| Henry_WT | 1714 ± 242 | 16 | 57 | 27 | 29 ± 210 | -382, 440 | 169 ± 128 |
| Henry_WTHT | 1672 ± 243 | 21 | 57 | 21 | -14 ± 208 | -422, 395 | 166 ± 126 |
| HorieWaitzberg_Gonzalez_FFM | 1872 ± 204 | 3 | 46 | 51 | 187 ± 221 | -247, 620 | 233 ± 171 |
| Huang | 1755 ± 268 | 11 | 56 | 33 | 70 ± 213 | -347, 487 | 178 ± 136 |
| Huang_FFM | 1696 ± 235 | 17 | 59 | 25 | 10 ± 210 | -402, 423 | 166 ± 129 |
| Ikeda | 1592 ± 167 | 35 | 51 | 14 | -93 ± 218 | -521, 335 | 187 ± 146 |
| Ikeda_FFM | 1592 ± 170 | 35 | 50 | 15 | -94 ± 215 | -515, 328 | 186 ± 143 |
| Johnstone_FFM | 1713 ± 227 | 15 | 57 | 27 | 27 ± 212 | -388, 443 | 170 ± 129 |
| Korth | 1774 ± 279 | 8 | 56 | 36 | 89 ± 209 | -320, 498 | 178 ± 141 |
| Korth_FFM | 1721 ± 264 | 18 | 53 | 30 | 36 ± 244 | -443, 515 | 193 ± 153 |
| Lazzer_2007 | 1820 ± 220 | 5 | 49 | 45 | 135 ± 212 | -280, 551 | 201 ± 150 |
| Lazzer_2007_FFM | 1773 ± 222 | 9 | 55 | 37 | 88 ± 210 | -324, 500 | 181 ± 137 |
| Lazzer_2010 | 1752 ± 229 | 11 | 55 | 33 | 66 ± 207 | -340, 472 | 173 ± 132 |
| Lazzer_2010_FFM | 1686 ± 200 | 21 | 55 | 25 | 1 ± 229 | -449, 451 | 184 ± 137 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Livingston_Kohlstadt | 1597 ± 194 | 32 | 55 | 13 | -88 ± 205 | -490, 314 | 175 ± 138 |
| Lorenzo | 1757 ± 230 | 9 | 57 | 35 | 72 ± 202 | -324, 467 | 171 ± 129 |
| Lührmann | 1770 ± 208 | 8 | 55 | 36 | 85 ± 209 | -325, 495 | 182 ± 134 |
| Lührmann_FFM | 1766 ± 216 | 10 | 54 | 36 | 81 ± 220 | -350, 512 | 185 ± 144 |
| Mifflin_StJeor | 1618 ± 227 | 28 | 59 | 13 | -67 ± 201 | -460, 326 | 167 ± 129 |
| Mifflin_StJeor_FFM | 1496 ± 207 | 56 | 34 | 10 | -189 ± 236 | -651, 273 | 250 ± 170 |
| Müller | 1745 ± 220 | 11 | 57 | 31 | 60 ± 207 | -345, 465 | 172 ± 129 |
| Müller_BMI | 1735 ± 237 | 13 | 57 | 30 | 50 ± 206 | -354, 455 | 168 ± 130 |
| Müller_FFM | 1716 ± 214 | 14 | 58 | 28 | 31 ± 206 | -373, 436 | 166 ± 126 |
| Müller_BMI_FFM | 1698 ± 220 | 16 | 59 | 25 | 13 ± 205 | -389, 415 | 164 ± 124 |
| Nelson_FFM | 1597 ± 268 | 38 | 47 | 15 | -88 ± 232 | -543, 366 | 203 ± 142 |
| Owen | 1659 ± 250 | 25 | 54 | 22 | -26 ± 227 | -471, 418 | 181 ± 139 |
| Owen_FFM | 1466 ± 264 | 62 | 29 | 9 | -220 ± 245 | -700, 261 | 276 ± 179 |
| Schofield_WT | 1674 ± 207 | 21 | 56 | 23 | -11 ± 211 | -424, 402 | 170 ± 125 |
| Schofield_WTHT | 1676 ± 218 | 19 | 57 | 23 | -10 ± 207 | -416, 397 | 169 ± 121 |

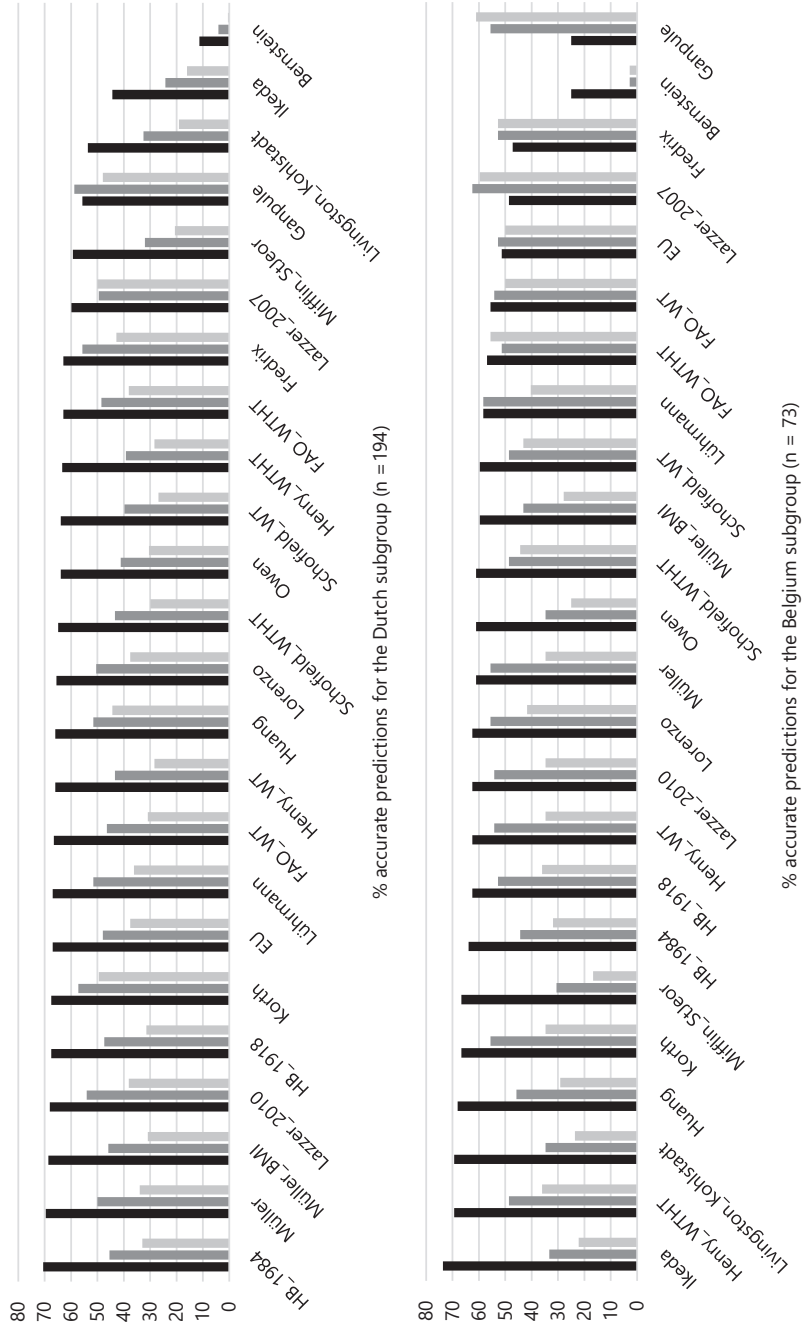
[1]The percentage of subjects by this predictive equation <10% of the measured value
[2]The percentage of subjects predicted by this predictive equation within 10% of the measured value
[3]The percentage of subjects predicted by this predictive equation >10% of the measured value
[4]Bias= mean (Estimated REE – measured REE)
[5]Calculated by (Estimated REE – measured REE) ± (SD of the bias * 1.96)
[6]Mean absolute error is the average absolute deviation in kcal/day between the estimated and measured REE

**Table 4:** Evaluation of the ten best performing[1] resting energy expenditure (REE) prediction equations with percentage accurate predictions, bias, limits of agreement and mean absolute error, in the four subgroups of obese subjects aged 55 years and older

| REE equation | Mean REE ± SD (kcal/d) | Under predictions[2] (%) | Accurate predictions[3] (%) | Over predictions[4] (%) | Mean Difference[5] (kcal/day) | Limits of agreement[6] (kcal/d) | Mean absolute error[7] (kcal/d) |
|---|---|---|---|---|---|---|---|
| **Dutch subgroup (n = 194)** | | | | | | | |
| *Measured REE* | *1814 ± 296* | | | | | | |
| HB_1984 ☆ | 1777 ± 274 | 18 | 71 | 11 | -37 ± 185 | -399, 325 | 145 ± 120 |
| Müller_FFM | 1764 ± 230 | 19 | 70 | 11 | -50 ± 176 | -395, 296 | 143 ± 114 |
| Müller | 1795 ± 234 | 16 | 70 | 15 | -19 ± 179 | -370, 333 | 142 ± 111 |
| Huang_FFM | 1746 ± 254 | 22 | 69 | 9 | -68 ± 176 | -412, 276 | 145 ± 119 |
| Müller_BMI | 1789 ± 251 | 17 | 69 | 15 | -24 ± 180 | -376, 328 | 141 ± 113 |
| Müller_BMI_FFM | 1747 ± 237 | 22 | 69 | 9 | -67 ± 176 | -412, 278 | 147 ± 117 |
| Lazzer_2010 | 1804 ± 243 | 16 | 68 | 17 | -10 ± 178 | -359, 339 | 140 ± 110 |
| Lührmann_FFM | 1801 ± 239 | 15 | 68 | 18 | -14 ± 182 | -369, 343 | 142 ± 144 |
| HB_1918 | 1779 ± 262 | 20 | 68 | 13 | -35 ± 185 | -398, 328 | 147 ± 118 |
| Korth | 1852 ± 292 | 9 | 68 | 23 | 38 ± 188 | -332, 407 | 149 ± 121 |
| **Belgian subgroup (n = 72)** | | | | | | | |
| *Measured REE* | *1607 ± 222* | | | | | | |
| Ganpule_FFM ☆ | 1586 ± 142 | 15 | 74 | 11 | -21 ± 165 | -345, 303 | 130 ± 104 |
| Ikeda ☆ | 1559 ± 138 | 17 | 74 | 10 | -48 ± 172 | -385, 289 | 135 ± 116 |
| Ikeda_FFM | 1549 ± 132 | 18 | 72 | 10 | -58 ± 197 | -390, 274 | 135 ± 117 |
| Henry_WTHT | 1575 ± 134 | 17 | 69 | 14 | -32 ± 180 | -386, 322 | 140 ± 117 |
| Livingston_Kohlstadt | 1527 ± 111 | 22 | 69 | 8 | -80 ± 176 | -425, 265 | 147 ± 125 |
| Huang | 1610 ± 150 | 13 | 68 | 19 | 3 ± 167 | -323, 329 | 127 ± 107 |
| Huang_FFM | 1591 ± 147 | 17 | 68 | 15 | -16 ± 167 | -343, 312 | 127 ± 108 |
| Cunningham1980_FFM | 1573 ± 111 | 19 | 68 | 13 | -34 ± 176 | -378, 310 | 140 ± 110 |
| Lazzer_2010_FFM | 1600 ± 101 | 15 | 67 | 18 | -7 ± 179 | -358, 343 | 143 ± 106 |
| Korth | 1640 ± 162 | 10 | 67 | 24 | 33 ± 168 | -296, 363 | 134 ± 106 |
| **Black American subgroup (n = 42)** | | | | | | | |
| *Measured REE* | *1373 ± 215* | | | | | | |
| Bernstein_FFM ☆ | 1344 ± 177 | 14 | 79 | 7 | -29 ± 136 | -295, 237 | 102 ± 93 |
| Bernstein | 1301 ± 157 | 26 | 67 | 7 | -73 ± 167 | -400, 255 | 130 ± 127 |
| Owen_FFM | 1510 ± 243 | 7 | 45 | 48 | 136 ± 167 | -191, 464 | 175 ± 126 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Mifflin_StJeor | 1529 ± 216 | 5 | 38 | 57 | 156 ± 162 | -162, 473 | 188 ± 121 |
| Mifflin_StJeor_FFM | 1534 ± 185 | 5 | 36 | 60 | 161 ± 139 | -112, 434 | 187 ± 101 |
| Ikeda | 1529 ± 158 | 2 | 33 | 64 | 156 ± 156 | -149, 461 | 192 ± 106 |
| Livingston_Kohlstadt ★ | 1524 ± 182 | 7 | 31 | 62 | 151 ± 148 | -139, 441 | 186 ± 99 |
| Ikeda_FFM | 1539 ± 158 | 2 | 29 | 69 | 166 ± 149 | -125, 457 | 196 ± 99 |
| Schofield_WTHT | 1589 ± 186 | 2 | 24 | 74 | 216 ± 155 | -89, 521 | 237 ± 120 |
| Cunningham1991_FFM | 1599 ± 203 | 0 | 24 | 76 | 226 ± 144 | -55, 507 | 242 ± 104 |
| **White American subgroup (n = 33)** | | | | | | | |
| *Measured REE* | *1497 ± 215* | | | | | | |
| Mifflin_StJeor_FFM ☆ | 1503 ± 183 | 9 | 73 | 18 | 5 ± 133 | -256, 266 | 107 ± 77 |
| Owen_FFM | 1488 ± 238 | 15 | 67 | 18 | -9 ± 156 | -315, 297 | 114 ± 105 |
| Nelson_FFM | 1584 ± 148 | 6 | 67 | 27 | 87 ± 147 | -201, 375 | 135 ± 103 |
| Ikeda | 1540 ± 143 | 6 | 64 | 30 | 43 ± 145 | -242, 328 | 124 ± 85 |
| Mifflin_StJeor ✯ | 1548 ± 205 | 9 | 64 | 27 | 50 ± 153 | -250, 350 | 123 ± 102 |
| Livingston_Kohlstadt | 1551 ± 184 | 9 | 61 | 30 | 54 ± 148 | -236, 343 | 115 ± 105 |
| Ikeda_FFM | 1543 ± 148 | 6 | 58 | 36 | 45 ± 141 | -232, 322 | 119 ± 86 |
| Cunningham1991_FFM | 1565 ± 201 | 9 | 58 | 33 | 67 ± 136 | -199, 334 | 122 ± 88 |
| Ganpule_FFM | 1631 ± 203 | 3 | 52 | 46 | 134 ± 139 | -138, 405 | 159 ± 108 |
| Johnstone_FFM | 1653 ± 196 | 0 | 52 | 49 | 156 ± 136 | -110, 423 | 171 ± 116 |
| Lazzer_2010_FFM | 1669 ± 180 | 0 | 49 | 52 | 172 ± 131 | -85, 428 | 185 ± 110 |
| Bernstein | 1313 ± 130 | 52 | 49 | 0 | -184 ± 156 | -489, 121 | 195 ± 141 |

[1] Best performing is defined as the formula with the highest percentage of accurate predictions
[2] The percentage of subjects by this predictive equation is <10% of the measured value
[3] The percentage of subjects predicted by this predictive equation is within 10% of the measured value
[4] The percentage of subjects predicted by this predictive equation is >10% of the measured value
[5] Mean difference = Estimated REE – measured REE
[6] Lower limit, upper limit. Calculated by (Estimated REE – measured REE) ± (SD of the bias * 1.96)
[7] Mean absolute error gives insight in the average absolute deviation in kcal/day between the estimated and measured REE
☆ Tentative suggested equation for this subgroup
★ Tentative suggested equation for this subgroup using BW at BMI 27.5 kg/m$^2$, when body composition information is not available
✯ Tentative suggested equation for this subgroup using BW at BMI 30.0 kg/m$^2$, when body composition information is not available


HB_1984
Muller
Muller_BMI
Lazzer_2010
HB_1918
Korth
EU
Uitnmann
FAO_WT
Henry_WT
Huang
Lorenzo
Schofield_WTHT
Owen
Schofield_WT
Henry_WTHT
FAO_WTHT
Fredrix
Lazzer_2007
Mffilin_Stjeor
Ganpule
Livingston_Kohlstadt
Ikeda
Bernstein
% accurate predictions for the Dutch subgroup (n =194)
0
10
20
30
40
50
60
70
80
Ikeda
Henry_WTHT
Livingston_Kohlstadt
Huang
Korth
Mffilin_Stjeor
HB_1984
HB_1918
Henry_WT
Lazzer_2010
Lorenzo
Muller
Owen
Schofield_WTHT
Muller_BMI
Schofield_WT
Uitnmann
FAO_WTHT
FAO_WT
EU
Lazzer_2007
Fredrix
Bernstein
Ganpule
% accurate predictions for the Belgium subgroup (n = 73)
0
10
20
30
40
50
60
70
80

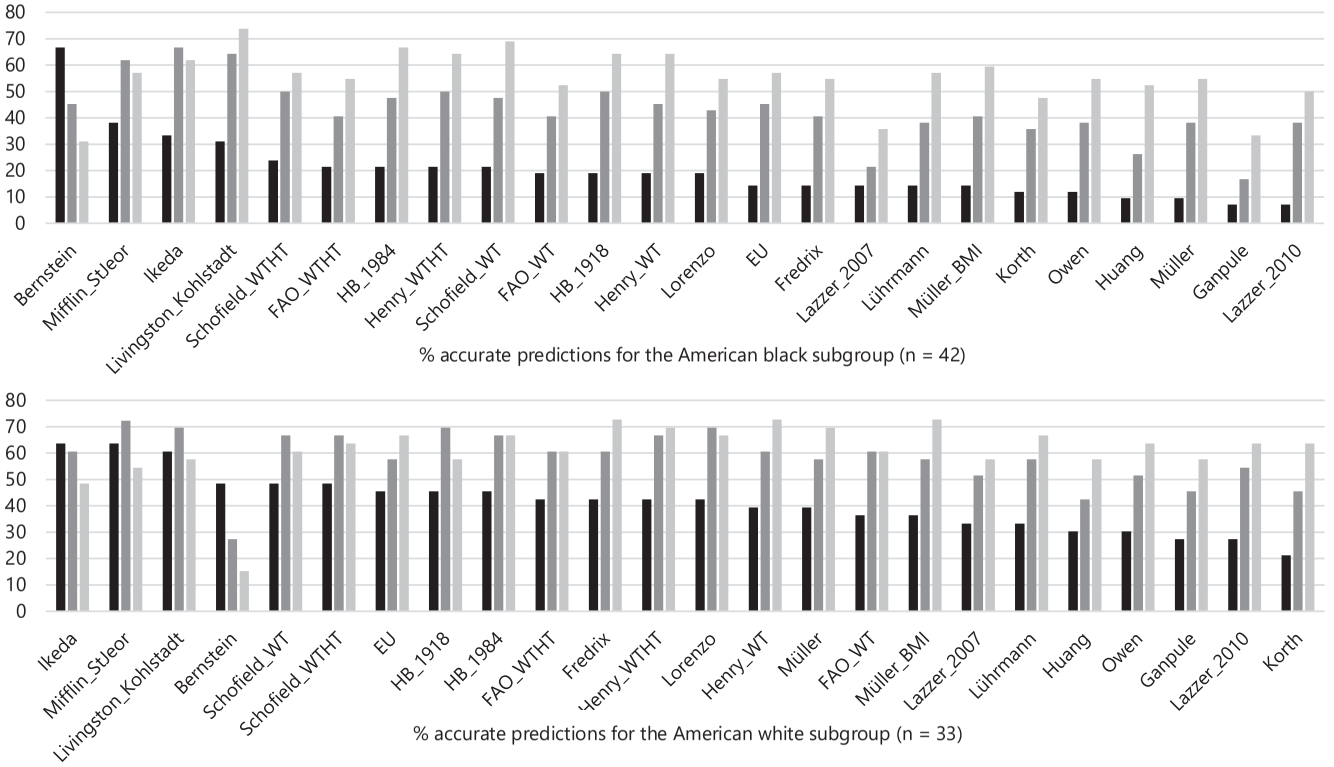



**Figure 1:** Percentage accurate predictions for resting energy equations based on body weight for four subgroups of obese older adults. Black bars are based on actual body weight, dark grey bars are based on adjusted body weight at BMI 30 kg/m$^2$, and light grey bars are based on adjusted body weight at BMI 27.5 kg/m$^2$

In general, no significant differences were observed between the underpredicted, accurately predicted and overpredicted subjects, with the exception of sex (**Supplementary Table B)**. The overpredicted group included less females (44%) than the accurately predicted and underpredicted groups ($P$ = 0.039). In summary, the underestimated, accurately estimated or overestimated subjects could not be clearly characterized.

## DISCUSSION

This study evaluates the accuracy of existing equations for REE in obese older adults and demonstrates that no single REE equation performed best across all subgroups. REE can be accurately predicted for more than 70% of the population using a subgroup-specific equation.

To our knowledge, only one previous study evaluated the validity of REE equations in an older obese sample (18). Using data on 29 obese (37 ± 5 kg/m$^2$) older Caucasian adults (66 ± 5 y) the equation of Fredrix (11) was the most valid out of 6 evaluated equations, with 66% accuracy. Fredrix (11) is an equation originally developed in 40 healthy Dutch older subjects with a BMI range of 21 – 31 kg/m$^2$. The equations of Huang (Australia) (34), Horie–Waitzberg & Gonzalez (Brazil) (35), Lazzer 2007 (Italy) (36, 37), Lazzer 2010 (Italy) (38) were specifically developed for a (severely) obese but not older population. In general these equations were not superior to the other equations evaluated in our study population of generally not severely obese older adults. Overall, over- and underprediction did not appear to be related to age or fat percentage of the subjects (Supplementary table B).

None of the evaluated equations were developed for obese older adults specifically. Obese older adults in general have less FFM per kg body weight than non-obese older adults (39) and obese younger adults (40, 41), which impacts REE (42). Our results indeed demonstrate that the best performing equation was an equation based on FFM in three of our four samples. Using an REE prediction equation based on FFM seems to be preferred in obese older adults.

For the Dutch and Belgian population the use of actual body weight gave a higher percentage of accurate predictions than the use of adjusted body weight, whereas for the American populations using adjusted body weight gave higher accuracy. Thus topping off of the independent variable body weight results in an average lower mean REE value, therefore closer to the mean American measured REE. For Dutch and Belgian group it replaced overpredictions with underpredictions with less accurate predictions. This might potentially be explained by the lower absolute REE measured in the American subgroup, which were also older compared to the Dutch and Belgian subgroups.

A remarkable finding is the low REE in the black American subpopulation. Literature describes that the lower REE in black subjects is possibly caused by a smaller mass of high metabolically active organs (43). Considering the much higher metabolic rate of organ tissue, only relatively small differences in mass may result in large differences in REE (42). Furthermore, other factors such as physical activity level and differences in the REE measurement protocols may have contributed to the observed difference in measured REE.



A strength of this study is the larger sample size then the previous study of Siervo et al. (18). The large sample size provides the opportunity to gain insight in the wide heterogeneity among REE estimates for obese older adults. This study has some limitations as well. A first limitation is that the Belgian subgroup consisted of females only, which makes the generalizability lower. There was no significant difference in the percentage of accurate predictions between males and females for the 10 best performing REE equations in the total study population, which indicates that the impact of this female-only subgroup on generalizability seems limited. A second limitation of this study is the smaller sample size in the American subgroups. Results are based on 44 black American and 33 white American subjects, and therefore our results should be confirmed in studies with larger sample sizes. A third limitation is the use of data samples using different protocols for measuring REE and body composition. Different methods for measuring body composition has been shown to result in only very small differences in predicted REE by Korth et al. (44). The difference in protocol for measuring REE may have amplified the observed differences in accuracy of the REE equations between the Dutch, Belgian and American populations.

***Conclusion and implications***

REE for the total population of obese older adults was best predicted by Harris & Benedict re-evaluated by Rosa et al. with 61% accuracy. A higher accuracy (71-79%) was achieved by a subgroup specific approach and for most subgroups an REE prediction equation based on FFM seems to be preferred. Tentative equations are suggested per subpopulation of obese older adults when measurement of REE by indirect calorimetry is not feasible.

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**Supplementary Table A:** Predictive equations for resting energy expenditure from literature

| Equation and year of publication | | Subject characteristics on which equation was originally developed | | | Predictive equations as used in this study |
|---|---|---|---|---|---|
| | | N | Age | BMI | |
| | | *Used method for body composition measurement* | | | |
| **Bernstein** (1) | 1983 | N = 202<br>48 M<br>154 F | M: Mean age<br>40.4 ± 12.6 y<br>F: Mean age<br>39.4 ± 12.0 y | Mean BMI:<br>37 kg/m$^2$ | M: 11.2×BW + 10.23×HT(cm) – 5.8×AGE – 1032 = kcal/d<br>F: 7.48×BW- 0.42×HT(cm) – 3.0×AGE + 844 = kcal/d |
| **Bernstein FFM** (1) | | *FFM: by TBW by $^3H_2O$ dilution* | | | 19.02×FFM + 3.72×FM – 1.55×AGE + 236.7 = kcal/d |
| **Cunningham FFM** (2) | 1980 | N = 223 of Harris and Benedict 1919 data were used (see below)<br>*FFM: estimated by the formula of Moore et al. 1963* | | | 501.6 + 21.6×FFM = kcal/d |
| **Cunningham FFM** (3) | 1991 | A generalized prediction equation is proposed based on a synthetic review of previous studies Cunningham 1980, Dore 1982, Bernstein 1983, Garrow & Webster 1985, Ravussin and Bogardus 1989, Owen 1988, Kashiwazaki 1988, Mifflin 1989. | | | 370 + 21.6×FFM = kcal/d |
| **EU** (4) | 1993 | Based on FAO/WHO/UNU & Schofield (both see below), except for older groups (≥ 60 y) selected data taken from Schofield, amplified by data collected on Scottish and Italian elderly subjects. | | | M 30-59 y: 11.6×BW + 879 = kcal/d<br>M 60-74 y: 11.9×BW + 700 = kcal/d<br>M ≥ 75 y: 8.4×BW + 819 = kcal/d<br>F 30-59 y: 8.7×BW + 829 = kcal/d<br>F 60-74 y: 9.2×BW + 688 = kcal/d<br>F ≥ 75 y: 9.8×BW + 624 = kcal/d |
| **FAO/WHO/UNU weight** (5) | 1985 | Based on Schofield equation<br>N ~ 11.000 | | | M 30-60 y: 11.6× BW + 879 = kcal/d<br>M >60 y: 13.5× BW + 487 = kcal/d<br>F 30-60 y: 8.7×BW + 829 = kcal/d<br>F >60 y: 10.5× BW + 596 = kcal/d |
| **FAO/WHO/UNU weight and height** (5) | | | | | M 30-60 y: 11.3×BW –16×HT(m) + 901 = kcal/d<br>M >60 y: 8.8×BW + 1128×HT(m) – 1071 = kcal/d<br>F 30-60 y: 8.7×BW – 25×HT(m) + 865 = kcal/d<br>F >60 y: 9.2×BW + 637×HT(m) - 302 = kcal/d |

| | | | | | |
|---|---|---|---|---|---|
| **Fredrix** (6) | 1990 | N = 40<br>18 M<br>22 F | Mean age:<br>65 ± 8 y<br>M: 63 ± 8 y<br>F: 66 ±7 y | Mean BMI:<br>25.9 ± 2.5 kg/m$^2$<br>M: 26.4 ± 2.4 kg/m$^2$<br>F: 25.5 ± 26 kg/m$^2$ | 1641 + 10.7×BW - 9.0×AGE – 203×SEX (1=male, 2=female) = kcal/d |
| **Ganpule** (7) | 2007 | N = 137 Japanese<br>71 M<br>66 F | M: mean age<br>36 ± 16 y<br>F: mean age<br>37 ±16 y | M: Mean BMI<br>23.4 ± 3.1 kg/m$^2$<br>F: Mean BMI<br>21.4 ± 3.3 kg/m$^2$ | 0.1238+0.048×BW+0.0234×HT(cm)–0.0138×AGE–0.05473×SEX (0=male, 1=female) = MJ/d |
| **Ganpule FFM** (7) | | *FFM and FM: by sum of 3 skinfolds. Tahara's equations (2002) and Brozek equation (1963) were used.* | | | 2.3958 + 0.0787×FFM - 0.0109×AGE + 0.0268×FM – 0.3314×SEX (0=male, 1=female) = MJ/d |
| **Harris and Benedict** (8) | 1918 | N = 239<br>136 M<br>103 F | M: 16-63 y<br>Mean age<br>27 ± 9 y<br>F: 15-74 y<br>Mean age<br>31 ± 14 y | Weight range<br>25.0-125 kg | M: 66.4730 + 13.7516×BW + 5.0033×HT(cm) – 6.7550×AGE= kcal/d<br>F: 655.0955 + 9.5634×BW+ 1.8496×HT(cm) – 4.6756×AGE= kcal/d |
| **Harris and Benedict re-evaluated by Roza et al.** (9) | 1984 | N = 337<br>168 M<br>169 F | M: Mean age<br>30 ± 14 y<br>F: Mean age<br>40 ± 22 y | Not reported | M: 88.362 + 4.799×HT(cm) + 13.397×BW – 5.677×AGE= kcal/d<br>F: 447.593 + 3.098×HT(cm) + 9.247×BW – 4.330×AGE = kcal/d |
| **Henry (Oxford) weight** (10) | 2005 | N= 10552<br>1544 M<br>4702 F<br>Based on 166 separate investigations (excluding Italians) | | | M 30-60 y: 0.0592×BW + 2.48 = MJ/d<br>M >60 y: 0.0563×BW + 2.15 = MJ/d<br>F 30-60 y: 0.0407×BW + 2.90 = MJ/d<br>F >60 y: 0.0424×BW + 2.38 = MJ/d |
| **Henry (Oxford) weight and height** (10) | | | | | M 30-60 y: 0.0476×BW + 2.26×HT(m) – 0.574 = MJ/d<br>M >60 y: 0.0478×BW + 2.26×HT(m) –1.07 = MJ/d<br>F 30-60 y: 0.0342×BW + 2.1×HT(m) – 0.0486 = MJ/d<br>F >60 y: 0.0356×BW + 1.76×HT(m) + 0.0448 = MJ/d |

| | | | | | |
|---|---|---|---|---|---|
| **Horie–Waitzberg, & Gonzalez FFM** (11) | 2011 | N=120<br>37 M<br>83 F | Mean age:<br>41.6 ± 11.6 y<br>M: 38.5 ± 11.7<br>F: 43.0 ± 11.3 y | Severely obese patients<br>Mean BMI:<br>46.9 ± 6.2 kg/m$^2$<br>M: 49.1 ± 6.7 kg/m$^2$<br>F: 45.5 ± 5.5 kg/m$^2$ | 560.43 + 5.39×BW + 14.14×FFM = kcal/d |
| **Huang** (12) | 2004 | N = 1038<br>279 M<br>759 F<br>(n=142 type 2 diabetics) | Mean age<br>44.9 ± 12.7 y | BMI ≥ 35 kg/m$^2$<br>Mean BMI<br>46.4 ± 8.4 kg/m$^2$ | 71.767 – 2.337×AGE + 257.293×SEX + 9.996×BW + 4.132×HT(cm) + 145.959×DM = kcal/d<br><br>If diabetic status is unknown (Belgium data):<br>60.655 - 1.440×AGE + 273.821×SEX) + 10.158×BW + 3.933×HT(cm) = kcal/d |
| **Huang FFM** (12) | | *FFM and FM: by bio-impedance analysis* | | | 14.118×FFM + 9.367×FM – 1.515×AGE + 220.863×SEX + 521.995 = kcal/d |
| **Ikeda** (13) | 2013 | Japanese type 1 and 2 diabetics, N=68<br>39 M<br>29 F | Mean age<br>59.8 ± 11.2 y<br>M: 58.3 ± 10.3 y<br>F: 61.8 ± 12.2 y | BMI: 24.0 ± 4.7 kg/m$^2$<br>M: 23.9 ± 5.3 kg/m$^2$<br>F: 24.2 ± 3.8 kg/m$^2$ | 10×BW – 3×AGE + 125×SEX + 750 = kcal/d |
| **Ikeda FFM** (13) | | *FFM and FM: by dual energy X-ray absorptiometry* | | | 691.6 + 11.6×FFM + 8.9×FM - 2.6×AGE + 106.7×SEX = kcal/d |
| **Johnstone FFM** (14) | 2006 | N = 150<br>43 M<br>107 F | 21-64 y<br>M: Mean age<br>47 ± 9.7 y<br>F: Mean age<br>42 ± 11.4 y | BMI 16.7-49.3 kg/m$^2$ | 1613 + 31.6×FM + 90.2×FFM – 12.2×AGE = kJ/d |
| | | *FFM and FM: by air displacement plethysmography* | | | |
| **Korth** (15) | 2007 | N = 104<br>50 M<br>54 F | 20-68y<br>Mean age<br>37.1 ± 15.1 | BMI 17.6-40.9 kg/m$^2$<br>Mean BMI<br>25.9 ± 4.1 kg/m$^2$ | 41.5×BW + 35.0×HT(cm) + 1107.4×SEX – 19.1×AGE – 1731.2 = kJ/d |
| **Korth FFM** (15) | | *FFM: by dual energy X-ray absorptiometry* | | | 105.1×FFM + 1422 = kJ/d |

| | | | | | |
|---|---|---|---|---|---|
| **Lazzer 2007** (16, 17) | 2007 | N = 346<br>164 M<br>182 F | M: 20-65 y<br>F:19-60 y | M: Mean BMI<br>45.4 kg/m$^2$<br>F: Mean BMI<br>45.6 kg/m$^2$ | M: 0.048×BW + 4.655×HT(m) – 0.020×AGE – 3.605 = MJ/d<br>F: 0.042×BW + 3.619×HT(m) – 2.678 = MJ/d* |
| **Lazzer 2007 FFM** (16, 17) | | *FFM and FM: by bio-impedance analysis* | | | M: 0.081×FFM + 0.049×FM - 0.019×AGE + 2.194 = MJ/d<br>F: 0.067×FFM + 0.046×FM + 1.568 = MJ/d |
| **Lazzer 2010** (18) | 2010 | N = 7368<br>2000 M<br>5368 F | 18-74 y<br>M: Mean age<br>46.3 ± 13.8 y<br>F: Mean age<br>47.8 ± 13.9 y | M: Mean BMI<br>41.6 ± 6.8 kg/m$^2$<br>F: Mean BMI<br>41.9 ± 6.5 kg/m$^2$ | 46×BW – 14×AGE + 1140×SEX + 3252 = kJ/d |
| **Lazzer 2010 FFM** (18) | | *FFM and FM by bio-impedance analysis* | | | 82×FFM – 10×AGE – 44×SEX + 3517 = KJ/d |
| **Livingston and Kohlstadt** (19) | 2005 | N = 670<br>Based on Harris-Benedict 1919 data (n=239), Owen data (n=104) and own measurements (n=327) | | | M: 293×BW$^{0.4330}$ – 5.92×AGE = kcal/d<br>F: 248×BW$^{0.4356}$ – 5.09×AGE = kcal/d |
| **De Lorenzo** (20) | 2001 | N = 320<br>127 M<br>193 F | 18-59 y<br>Mean age<br>41.1 ± 11.5 y | 18.6 ± 39.6 kg/m$^2$<br>Mean BMI<br>27.8 ± 5.1 kg/m$^2$ | M: 53.284×BW + 20.957×HT(cm) – 23.859×AGE + 487 = kJ/d<br>F: 46.322×BW + 15.744×HT(cm)–16.66×AGE + 944 = kJ/d |
| **Lührmann** (21) | 2002 | N = 286<br>107 M<br>179 F | 60-85 y<br>M: Mean age<br>66.9 ± 5.1 y<br>F: Mean age<br>67.8 ± 5.7 y | M: Mean BMI<br>26.3 ± 3.1 kg/m$^2$<br>F: Mean BMI<br>26.4 ± 3.7 kg/m$^2$ | 3169 + 50.0×BW – 15.3×AGE + 746×SEX = kJ/d |
| **Lührmann FFM** (21) | | *FFM and FM: by bio-impedance analysis* | | | 1556 + 86.9×FFM + 24.0×FM = kJ/d |
| **Mifflin – St Jeor** (22) | 1990 | N = 498<br>251 M<br>247 F | 19-78 y<br>M: Mean age<br>44.4 ± 14.3 y<br>F: Mean age<br>44.6 ± 14.0 y | M: 19-42 kg/m$^2$<br>Mean BMI<br>27.5 ± 4.1 kg/m$^2$<br>F: 17-42 kg/m$^2$<br>Mean BMI<br>26.2 ± 4.9 kg/m$^2$ | 9.99×BW + 6.25×HT(cm) – 4.92×AGE + 166×SEX - 161 = kcal/d |

| | | | | | |
|---|---|---|---|---|---|
| **Mifflin – St Jeor FFM** (22) | | FFM by sum of 3 skinfolds, Jackson-Pollock | | | 19.7×FFM + 413 = kcal/d |
| **Müller** (23)<br>**Müller BMI** (23) | 2004 | N = 1046<br>388 M<br>658 F<br>Multi-center study | 5-80 y | Mean BMI<br>27 kg/m$^2$ | 0.047×BW + 1.009×SEX - 0.01452×AGE + 3.21 = MJ/d<br>BMI ≥30: 0.05×BW + 1.103×SEX – 0.01586×AGE + 2.924 = MJ/d |
| **Müller FFM** (23)<br>**Müller BMI FFM** (23) | | *FFM and FM by bio-impedance analysis* | | | 0.05192×FFM + 0.04036×FM + 0.869×SEX – 0.01181×AGE + 2.992 = MJ/d<br>BMI ≥30: 0.05685×FFM + 0.04022×FM + 0.808×SEX – 0.01402×AGE + 2.818 = MJ/d |
| **Nelson FFM** (24) | 1992 | N=213 adults<br>86 M<br>127 F<br>Based on various published datasets combined with their own data.<br>N=81 non-obese and N=127 obese<br>*FFM and FM: by densitometry and anthropometry* | | | 108×FFM + 16.9×FM = kJ/d |
| **Owen** (25, 26) | 1 9 8 7 (M)<br>1 9 8 6 (F) | N=104<br>60 M<br>44 F | M: 18-82 y<br>Mean age<br>38 ± 15.6 y<br>F: 18-65 y<br>Mean age<br>35 ± 12.2 y | M: 20.4-58.7 kg/m$^2$<br>Mean BMI<br>28.2 ± 7.5 kg/m$^2$<br>F: 18.2-49.6 kg/m$^2$<br>Mean BMI<br>27.8 ± 8.6 kg/m$^2$ | M: 879 + 10.2×BW = kcal/d<br>F: 795 + 7.18×BW = kcal/d |
| **Owen FFM** (25, 26) | | *FFM by densitometry, underwater weighing* | | | M: 290 + 22.3×FFM = kcal/d<br>F: 334 + 19.7×FFM = kcal/d |

| | | | | | |
|---|---|---|---|---|---|
| **Schofield weight** (27) | 1985 | N=7173 of which 4814 ≥ 18 y | M: Mean age 42 y<br>F: Mean age 43 y | M: Mean BMI 23 kg/m$^2$<br>F: Mean age 25 kg/m$^2$ | Equations using weight<br>M 30-60 y: 0.048×BW + 3.653 = MJ/d<br>M >60 y: 0.049×BW + 2.459 = MJ/d<br>F 30-60 y: 0.034×BW + 3.538 = MJ/d<br>F >60 y: 0.038×BW + 2.755 = MJ/d |
| **Schofield weight and height** (27) | | | | | M 30-60 y: 0.048× BW – 0.011×HT(m) + 3.67 = MJ/d<br>M >60 y: 0.038× BW + 4.068×HT(m) – 3.491 = MJ/d<br>F 30-60 y: 0.034× BW + 0.006×HT(m) + 3.53 = MJ/d<br>F >60 y: 0.033×BW + 1.917×HT(m) + 0.074= MJ/d |

Data are displayed as mean ± standard deviation; M = male; F = female; D = diabetic; ND = non diabetic; BW = body weight in kg; HT(cm) = height in cm; HT(m) =height in m; SEX: M = 1 F = 0; AGE =age in years

* The Lazzer 2007 FFM equation is corrected: for males the equation was published with - 2.194 in the end instead of + 2.194.

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**Supplementary Table B:** Characteristics of subjects that are underpredicted, accurately predicted or overpredicted by the best performing equation per subgroup[1] for resting energy expenditure

| | Underpredicted[2] | Accurately predicted[3] | Overpredicted[4] | *P* value[5] |
|---|---|---|---|---|
| N (%) | 55 (16%) | 247 (72%) | 39 (11%) | |
| Study sample (% D/B/USAb/USAw)[6] | 64/22/11/6% | 55/21/13/10% | 57/21/12/10% | 0.691 |
| Gender (% female) | 67% | 64% | 44% | 0.039 |
| Ethnicity (% black) | 11% | 13% | 8% | 0.571 |
| Diabetes (% yes)[7] | 43% | 45% | 29% | 0.255 |
| REE (kcal/day) | 1937 ± 272 | 1657 ± 297 | 1510 ± 233 | <0.001 |
| REE (kcal/kg FFM/day) | 36.0 ± 4.3 | 30.7 ± 4.2 | 26.3 ± 3.4 | <0.001 |
| Age (y) | 65.4 ± 6.6 | 65.4 ± 7.2 | 65.8 ± 7.2 | 0.928 |
| Weight (kg) | 97.7 ± 11.8 | 98.9 ± 14.1 | 102.3 ± 12.7 | 0.256 |
| Height (m) | 1.67 ± 0.08 | 1.67 ± 0.09 | 1.71 ± 0.12 | 0.089 |
| BMI (kg/m$^2$) | 35.1 ± 4.2 | 35.3 ± 4.4 | 35.3 ± 4.7 | 0.971 |
| FFM (kg) | 54.4 ± 9.4 | 54.6 ± 10.7 | 58.1 ± 10.8 | 0.138 |
| FM (kg) | 43.2 ± 10.2 | 44.2 ± 10.8 | 44.0 ± 10.5 | 0.837 |
| FM (%) | 44.1 ± 8.0 | 44.6 ± 7.8 | 43.0 ± 8.5 | 0.459 |

[1]Samples: Dutch (n=194), Belgian (n=72), American black (n=42), American white (n=33). The best performing equation for the Dutch population is Harris & Benedict re-evaluated by Rosa et al. 1984, for the Belgium population Ganpule_FFM 2007, for the American black population Bernstein_FFM 1983, and for the American white population Mifflin_StJeor_FFM 1990, according to the highest % of accurate predictions.
[2]The percentage of subjects by this predictive equation is <10% of the measured value
[3]The percentage of subjects predicted by this predictive equation is within 10% of the measured value
[4]The percentage of subjects predicted by this predictive equation is >10% of the measured value
[5]P-value for difference between groups with Chi-Square for the nominal variables (gender. ethnicity and diabetes) and One-Way ANOVA for the other variables.
[6]Study samples: D=Dutch, B=Belgian, USAb=American black, USAw= American white
[7]Diabetes data not available for the Belgium population, therefore n=72 missing.

# Chapter 3

## Reduction in energy expenditure during weight loss is higher than predicted based on fat free mass and fat mass in older adults

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## ABSTRACT

**Background & Aim** The aim of this study was to describe a decrease in resting energy expenditure during weight loss that is larger than expected based on changes in body composition, called adaptive thermogenesis (AT), in overweight and obese older adults.

**Methods** Multiple studies were combined to assess AT in younger and older subjects. Body composition and resting energy expenditure (REE) were measured before and after weight loss. Baseline values were used to predict fat free mass and fat mass adjusted REE after weight loss. AT was defined as the difference between predicted and measured REE after weight loss. The median age of 55 y was used as a cutoff to compare between older with younger subjects. The relation between AT and age was investigated using linear regression analysis.

**Results** In this study 254 (M = 88, F = 166) overweight and obese subjects were included (BMI: 31.7 ± 4.4 kg/m$^2$, age: 51 ± 14 y). The AT was only significant for older subjects (64 ± 185 kcal/d, 95% CI [32, 96]), but not for younger subjects (19 ± 152 kcal/d, 95% CI [-9, 46]). The size of the AT was significantly higher for older compared to younger adults (β = 47, $P$ = 0.048), independent of gender and type and duration of the weight loss program.

**Conclusions** We conclude that adaptive thermogenesis is present only in older subjects, which might have implications for weight management in older adults. A reduced energy intake is advised to counteract the adaptive thermogenesis.

# INTRODUCTION

Adaptive thermogenesis is defined as the decrease in resting energy expenditure (REE) greater than expected based on changes in fat free mass and fat mass (1). Because the REE contributes for approximately 70% of the total daily energy expenditure (2), adaptive thermogenesis can have substantial impact on daily energy expenditure. As a result it might be more difficult to preserve energy balance to maintain weight, or to create an energy deficit to lose weight.

Adaptive thermogenesis has been described in different weight loss studies (3-5). It has been stated that the discussion is no longer about the existence of the phenomenon of adaptive thermogenesis, but about its magnitude and practical implication (6).



In addition to the ongoing discussion on the magnitude and practical implication of adaptive thermogenesis, it has been pointed out that the relation between age and adaptive thermogenesis is unclear (7, 8). Since the prevalence of overweight and obesity is increasing as well as aging, the number of older adults with overweight and obesity increases (9). We have been interested in providing adequate interventions for obese older adults that result in healthy weight loss, i.e. loss of fat mass with muscle mass preservation (10). Because adaptive thermogenesis might have an unfavorable effect on the energy balance, it potentially reduces the success of weight loss interventions and weight maintenance in older adults. Therefore, the aim of this study was to describe adaptive thermogenesis in overweight and obese older adults during weight loss. For this purpose, we accumulated data over a larger age range, which made it possible to compare older with younger overweight individuals.

# METHODS

## Subjects

The data from 9 different weight loss studies were combined for this investigation, e.g. (10). The studies were conducted at the Amsterdam Nutritional Assessment Center (ANAC) of the Amsterdam University of Applied Sciences between 2006 and 2014. Subjects were overweight or obese (body mass index (BMI) > 25 kg/m$^2$) but otherwise healthy. Written informed consent was obtained from all subjects.

## Design

All subjects followed a hypocaloric diet. In addition, in some studies a subgroup of participants completed an exercise program. The weight loss programs lasted between 8 and 13 weeks. Baseline and Post measurements of body composition (air displacement

plethysmography) and resting energy expenditure (indirect calorimetry) were performed, see below.

## Measurements

REE was measured by indirect calorimetry using a ventilated hood system (Vmax Encore n29, Viasys Healthcare, Houten, the Netherlands). Each day before the first use the system was calibrated with 2 different standard gases and 1 standard volume according to the manufacturer's description. All measurements were performed in a quiet, well-ventilated, thermo-neutral environment. The subjects remained lying down and regular checks prevented subjects from falling asleep. Oxygen consumption and carbon dioxide production were measured for 30 minutes. The first 5 minutes of the measurement were discarded. A steady state period was selected (CV < 10%) based on visual interpretation of the time series graph. The Weir formula (11) was used to calculate the REE. Measurements were excluded when the respiratory exchange ratio was below 0.7 or above 1.0 (12).

Body composition was measured in duplo using air displacement plethysmography (Bodpod, Life Measurement Inc., Concord (CA), USA). The Bodpod was calibrated for weight and volume before each measurement. Body weight was measured to the nearest 0.001 kg on the electronic scale which was part of the Bodpod system. Subjects were measured wearing tight swim clothes or underwear and a Lycra swim cap. The Siri equation (13) was used to estimate the percentage body fat from the measured density. Fat mass and fat free mass were calculated using percentage body fat and body weight. Height was measured using a stadiometer (Seca 222, Seca, Hamburg, Germany).

Subjects did not drink alcohol within 24 hour, eat within 5 hours, exercise within 3 hours and drink water within 1 hour prior to each visit (12). Subjects who were assigned to a diet and exercise program did not train on the day of the measurements. Baseline and Post weight loss measurements were performed by trained research assistants at the same time of the day for both measurements.

## Calculation of adaptive thermogenesis

A backward linear regression analysis with baseline data including fat free mass, fat mass, age, gender and the fat free mass*age interaction as independent variables and REE as dependent variable was performed to derive a prediction equation for REE. Assumptions of linearity and multicollinearity of the predictors were confirmed. Analysis of the residuals showed that the regression equation was not affected by outliers and influential cases. The prediction equation was used to calculate the predicted REE at Post. In accordance with previous research (14, 15), adaptive thermogenesis was quantified as the difference between measured and predicted REE at Post:

*Adaptive Thermogenesis (AT) = Post weight loss predicted REE - measured REE*

Additionally, AT was corrected for Pre weight loss measured versus predicted REE differences between young and old subjects:

*Corrected Adaptive Thermogenesis (ATcorr) = (Post weight loss predicted REE - Post weight loss measured REE) – (Pre weight loss predicted REE – Pre weight loss measured REE)*

### Statistical analysis

The median age of 55 years was used as cutoff for age groups, i.e. participants aged 55 and over were defined as older subjects. Differences within age groups were analyzed with a paired t-test. Differences between the age groups were checked using an independent samples t-test. The relation between adaptive thermogenesis and age was investigated using a linear regression analysis that included gender and the type (diet or diet+exercise) and duration of the weight loss program. All values reported in text and tables are means ± SD. Two-sided probability values below 0.05 were considered statistically significant. Statistical analyses were performed using SPSS 22 for Windows (IBM Corp., IBM SPSS Statistics for Windows, Version 22.0. Amonk, NY).

## RESULTS

### Subjects

In total, 254 subjects were included in this study (M=88, F=166). Baseline characteristics of the younger and older subjects are displayed in **Table 1**. On average, older subjects had a significant higher fat mass than younger subjects (39.7 ± 10.0 vs. 36.3 ± 10.0 kg, $P < 0.01$) and a lower REE (1755 ± 295 kcal/d vs. 1840 ± 340 kcal/d, $P < 0.04$).

On average body weight declined by -3.2 ± 3.0 kg (-3.4 ± 3.3 kg fat mass; +0.1 ± 1.8 kg fat free mass) in older and by -2.8 ± 3.3 kg (-3.0 ± 3.6 kg fat mass; +0.2 ± 2.4 kg fat free mass) in younger subjects. After the weight loss program the REE was on average reduced by -84 ± 202 kcal/d in older and -49 ± 168 kcal/d in younger adults (Table 1).

### Adaptive thermogenesis

The backward linear regression analysis revealed small and insignificant beta values for gender and the fat free mass*age interaction term. Therefore, these variables were excluded from the prediction equation. The resulting prediction equation including fat free mass, fat mass and age explained 70% of the variation in REE at baseline (SEE=176 kcal/d, p<0.001):

*REEpred (kcal/d) = 21.630*[fat free mass (kg)] + 8.945*[fat mass (kg)] – 2.599*[age (y)] + 288.024*

Averaged over all subjects, the measured REE after the weight loss program (1729 ± 326 kcal/d) was significantly lower than the REE derived from the prediction equation (1771 ± 268 kcal/d), resulting in an adaptive thermogenesis of 42 ± 171 kcal/d (95% CI [21, 63]).

The adaptive thermogenesis was significant for older subjects (64 ± 185 kcal/d, 95% CI [32, 96]) but not for younger subjects (19 ± 152 kcal/d, 95% CI [-9, 46]). Also, if corrected for Pre weight loss values adaptive thermogenesis (ATcorr) was significant for older (57 ± 196 kcal/d, 95% CI [23, 91] but not for younger subjects (26 ± 165 kcal/d, 95% CI [-4, 56] (**Figure 1**). The ratio of post weight loss measured to predicted REE was 0.962 ± 0.106 for older adults.

The linear regression analysis revealed that the size of the adaptive thermogenesis was significantly higher for older than for younger adults (0 = younger, 1 = older, β = 47, *P* = 0.048), independent of gender and type and duration of the weight loss program. Age was not a significant predictor if adaptive thermogenesis was corrected for baseline values (β = 48, *P* = 0.064).

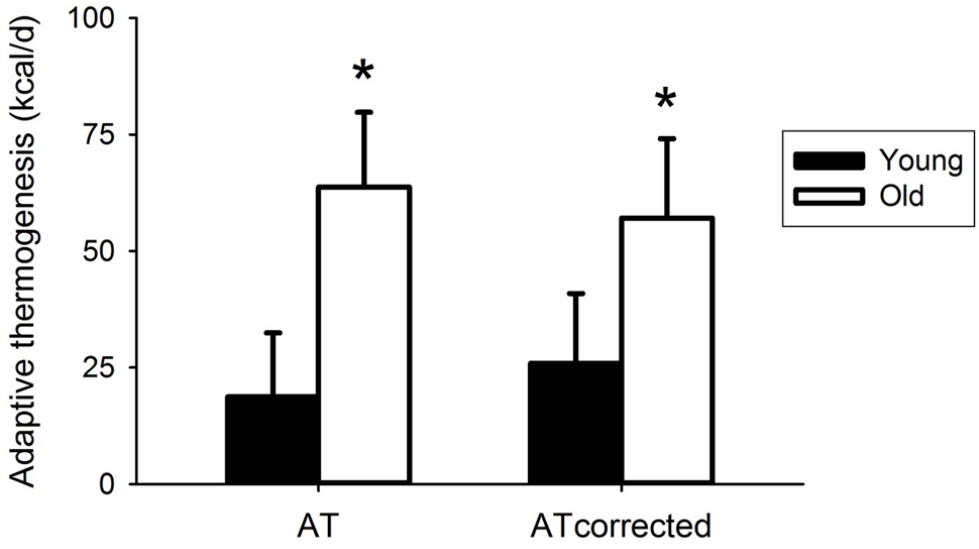


**Figure 1:** Values for adaptive thermogenesis (left) and corrected for Pre weight loss diffrences (right) for younger (55- y) and older (55+ y) subjects (means ± SEM).
* Indicates significant different from zero (*P* < 0.05).

**Table 1:** Characteristics at baseline and after the weight loss program for younger and older adults. Data are presented as mean ± SD.

| | **Younger** n = 122 | **Older** n = 132 | ***P* value** |
|---|---|---|---|
| Gender (m/f) | 36/86 | 52/80 | |
| Age (y) | 40 ± 9 | 62 ± 5 | <0.01 |
| BMI baseline (kg/m$^2$) | 31.0 ± 4.4 | 32.5 ± 4.3 | <0.01 |
| Weight (kg) | | | |
| *Pre* | 91.4 ± 17.3 | 93.1 ± 13.6 | 0.39 |
| *Post* | 88.6 ± 17.2 | 89.9 ± 13.4 | 0.52 |
| *Change* | -2.8 ± 3.3 * | -3.2 ± 3.0 * | 0.29 |
| Fat mass (kg) | | | |
| *Pre* | 35.9 ± 11.2 | 39.7 ± 10.0 | <0.01 |
| *Post* | 32.9 ± 11.1 | 36.3 ± 10.0 | <0.01 |
| *Change* | -3.0 ± 3.6 * | -3.4 ± 3.3 * | 0.38 |
| Fat free mass (kg) | | | |
| *Pre* | 55.5 ± 11.6 | 53.4 ± 11.3 | 0.14 |
| *Post* | 55.7 ± 12.0 | 53.5 ± 11.4 | 0.13 |
| *Change* | 0.2 ± 2.4 | 0.1 ± 1.8 | 0.86 |
| REEmeasured (kcal/d) | | | |
| *Pre* | 1840 ± 340 | 1755 ± 295 | 0.04 |
| *Post* | 1791 ± 327 | 1671 ± 315 | <0.01 |
| *Change* | -49 ± 168 * | -84 ± 202 * | 0.13 |
| REEpredicted (kcal/d) | | | |
| *Pre* | 1832 ± 285 | 1762 ± 245 | 0.04 |
| *Post* | 1810 ± 289 | 1735 ± 243 | 0.03 |
| Adaptive thermogenesis (kcal/d) | 19 ± 152 | 64 ± 185 * | 0.04 |

*= significant different from zero (p<0.05).
BMI=body mass index; REE=resting energy expenditure

## DISCUSSION

This study shows that adaptive thermogenesis could be quite significant in overweight and obese older adults during weight loss. Adaptive thermogenesis may have consequences for ongoing weight loss and weight maintenance in older adults.

## Age difference

It was shown in this study that age is related to the decrease in resting energy expenditure during weight loss that is larger than expected based on changes in body composition. Yet it should be noted that there was only a trend towards significance when AT was corrected for Pre weight loss values. Adaptive thermogenesis was shown in older but not in younger adults, meaning that older adults seem more 'protected' from losing body weight. It was shown before that REE decreases with increasing age, independent of changes in fat free mass and fat mass (16). Yet, subjects in that study gained weight and increased both fat free mass and fat mass throughout the 6-year follow-up period. So, it was stated that the relation between age and adaptive thermogenesis during weight loss remains to be established (17). To our knowledge our study is the first that shows the relation between age and adaptive thermogenesis during weight loss. The fat free mass was maintained in both age groups in our study, and only the fat mass decreased. This suggests a different alteration in the specific metabolic activity of the fat mass between younger and older adults. Another possible explanation for the difference in adaptive thermogenesis between the age groups might arise from differences in sex hormone levels. It was shown that weight loss resulted in increased sex hormone levels (18) and that these concentrations are related to resting energy expenditure (19). Since sex hormone concentrations are higher in younger than older adults, increased concentrations after weight loss - and accompanying increased resting energy expenditure - might be more applicable to younger adults. Perhaps this could partly counteract other weight loss induced physiological changes in younger but not in older adults.

## Magnitude

Different magnitudes of adaptive thermogenesis were reported in recent studies. Whereas 2 studies (5, 20) show a higher adaptive thermogenesis as compared to the older subjects in our study (64 ± 185 kcal/d), most studies show similar (4, 14, 21, 22) or lower (15) values. The adaptive thermogenesis in those studies is higher when compared to the total group average in our study (42 ± 171 kcal/d). Discrepancies may be explained by different duration (5), calculation methods (4, 20), intra-individual responses (6, 23) or measurement errors (24, 25). Also, differences in total weight loss might explain differences between studies. To illustrate, older subjects in our study lost 3.2 ± 3.0 kg body weight (3.4%), whereas this varied from 5% (15) up to 38% (5) in other studies. To put the weight loss in our studies in perspective, it should be noted that a weight loss of 3 kg body weight is regarded beneficial in elderly (9).

A strength of this investigation is the large sample size (N=254), while no more than 50 subjects were included in many other studies. (3, 5, 14, 20-22) Multiple studies were combined to realize this large sample size. We do not consider this a limitation, because

the data gathering was similar across studies, and the measurements were of high quality and in accordance with international standards (12).

### Practical relevance

Although the magnitude of the adaptive thermogenesis is different between studies, it has been pointed out that even a small structural change in the energy balance can have impact on the body composition (24). It was shown that the ratio between measured and predicted REE was 0.963 after an 8 week weight loss program (4). The adaptive thermogenesis was still present after 12 weeks (0.983) and 44 weeks (0.984) of weight maintenance, so it seems the effect of adaptive thermogenesis is long lasting. If the adaptive thermogenesis remains unalterably present, the unexplained reduced energy expenditure would be 23,360 kcal per year for older subjects in this study. This equals 2.5 kg body weight when applying 9441 kcal per kg body fat mass change (26). Although this is a rough estimation, compared to the average fat mass loss of 3.4 kg in this study it seems a significant reduction in energy expenditure. So, although large individual differences were observed, adaptive thermogenesis may be regarded as practically relevant for losing weight and obesity recidivism in older adults.



## CONCLUSION

We conclude that adaptive thermogenesis is present in older subjects, while it was not observed in younger subjects in this study. The adaptive thermogenesis in older subjects might have implications for weight loss and weight maintenance. Nutritionists and dietitians need to be aware of the role of adaptive thermogenesis in weight loss and weight maintenance, especially in older subjects. If necessary a reduced energy intake should be advised to counteract the adaptive thermogenesis.

### Statement of Authorship

Conception and design of the study: TH, PW. Acquisition of data: AV. Analysis and interpretation of data: TH, RM, MT, PW. Writing the manuscript: TH, AV, RM, MT, PW.

### Conflict of interest

The authors declare that there is no conflict of interests. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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# Part 2

## Optimal protein intake

# Chapter 4

## Dietary protein intake is not associated with 5-y change in mid-thigh muscle cross-sectional area by computed tomography in older adults: the Health, Aging, and Body Composition (Health ABC) Study

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## ABSTRACT

**Background** A higher protein intake is suggested to preserve muscle mass during aging and may therefore reduce the risk for sarcopenia.

**Objective** We explored whether the amount and type (animal or vegetable) of protein intake were associated with 5-y change in mid-thigh muscle cross-sectional area (CSA) in older adults (n=1561).

**Design** Protein intake was assessed at year 2 by a Block food-frequency questionnaire in participants (aged 70–79y) of the Health ABC study, a prospective cohort study. At year 1 and year 6 mid-thigh muscle CSA in square centimeters was measured by computed tomography. Multiple linear regression analysis was used to examine the association between energy adjusted protein residuals in grams per day (total, animal and vegetable protein) and muscle CSA at year 6, adjusted for muscle CSA at year 1 and potential confounders including prevalent health conditions, physical activity and 5-y change in fat mass.

**Results** Mean protein intake was 0.90 (95% CI: 0.88, 0.92) g/kg/d and mean 5-y change in muscle CSA was -9.8 (-10.6, -8.9) $cm^2$. No association was observed between energy adjusted total (ß=-0.00 (-0.06, 0.06) $cm^2$; P=0.982), animal (ß = -0.00 (-0.06, 0.05) $cm^2$; *P* = 0.923), or plant (ß= +0.07 (-0.06, 0.210) $cm^2$; *P* = 0.276) protein intake and muscle CSA at year 6, adjusted for baseline mid-thigh muscle CSA and potential confounders.

**Conclusions** This study suggests that a higher total, animal, or vegetable protein intake is not associated with 5-y change in mid-thigh muscle CSA in older adults. This conclusion contradicts some, but not all, previous research. This trial was registered at www.trialregister.nl as NTR6930.

## INTRODUCTION

Indicators of low muscle mass and in particular low strength have been associated with functional decline and disability in older adults (1, 2). Previous studies have indicated that dietary protein intake affects protein synthesis and net protein balance in older adults. Therefore, an adequate protein intake may help to slow the process of age-related muscle loss (3, 4).

Muscle loss in older adults is associated with loss of sensitivity of the skeletal muscle to protein ingestion (5), insulin resistance, and a higher extraction of amino acids by splanchnic tissue, which results in a lower availability of amino acids for muscle protein synthesis (6). Thus, a higher protein intake may be necessary to reduce the loss of muscle mass with aging. These findings support the recent suggestions (7) that the current recommended daily allowance for protein for older adults of 0.8 g/kg body weight/d (8) potentially underestimates the true requirement.



Until now, only few studies investigated the relation between dietary protein intake and longitudinal changes in lean mass in older adults. Houston et al. (9) studied the relation between protein intake and longitudinal changes in lean mass assessed by dual-energy X-ray absorptiometry (DXA) over a 3-y period. They demonstrated that older adults in the highest quintile of protein intake (mean intake 1.2 g/kg/d) lost nearly 40% less appendicular lean mass compared to those in the lowest quintile (mean intake 0.7 g/kg/d). This observation (9) is supported by cross-sectional studies in which higher protein intakes are associated with more lean body mass assessed by DXA in older persons (10, 11). However, Chan al. (12) did not find an association between total protein intake and change in appendicular muscle mass (assessed by DXA) in an older (65 y and older) Chinese population over a 4 year period.

Not only the total amount of protein intake, but protein source and amino acid composition might play a role in the age-related change in muscle mass. The essential amino acids (EAAs) deliver substrate for protein synthesis and are primarily responsible for its regulation. Of the EAAs leucine is recognized to have a specific stimulating role muscle protein synthesis (13, 14). However, the role of leucine in the age-related change in muscle mass remains unclear as prospective observational studies and supplementation trials provide conflicting results (15, 16).

To our knowledge, only a few longitudinal observational studies have investigated the association of protein intake, protein source (animal or protein) and leucine intake in older persons with lean mass change (9, 12, 15) with contrasting findings. Therefore, we investigated the association of the amount, type (animal/vegetable) and amino acid

composition of protein intake on 5-y change in mid-thigh muscle cross-sectional area (CSA) as measured by computed tomography (CT) in older adults.

# METHODS

## Study sample

Data from the Health, Aging, and Body Composition (Health ABC) study were used. The Health ABC study is a prospective cohort study and investigates the association among body composition, weight related health conditions, and functional limitations in older adults (for more information, see https://healthabc.nia.nih.gov/). Between April 1997 and June 1998, 3075 well-functioning black and white men and women aged 70-79 y were enrolled. Participants were recruited from a random sample of white Medicare-eligible residents and all of the black Medicare-eligible residents in the Pittsburgh, PA, and Memphis, TN, metropolitan areas. Subjects were eligible if they reported no difficulties in walking one-fourth of a mile, climbing up 10 steps, or performing basic activities of daily living; no history of active cancer in the 3 y prior to the study; planned to remain in the geographic area for ≥3 y; and were not enrolled in lifestyle intervention trials. All participants gave written informed consent. All protocols were approved by the Human Investigation and Review Boards at the University of Pittsburgh and the University of Tennessee at Memphis.

Participants were included in the data analyses (registered at www.trialregister.nl as NTR6930) if they had good quality CT data of the mid-thigh muscle both at the clinical visit at baseline (year 1) and year 6 (see below) (n = 1675) and completed the food-frequency questionnaire (FFQ) which was administered at the 12-mo follow-up clinic visit (year 2) (n = 2713). Participants were excluded if they had an FFQ with serious errors and/or reported energy intakes < 500 kcal/d or > 3500 kcal/d (women) or < 800 kcal/d or > 4000 kcal/d (men) (n = 116) (17). In total 1561 participants were included in the data-analyses (**Figure 1**).

## CT of mid-thigh muscle

The sum of the CSA (in $cm^2$) of muscle in both thighs was analyzed. Muscle area was measured by CT (Memphis clinic site: Somatom Plus 4, Siemens, Erlangen, Germany, or PQ 2000S, Marconi Medical Systems, Cleveland, OH, USA; Pittsburgh clinic site: 9800 Advantage, General Electric, Milwaukee, WI, USA). Year 1 and Year 6 mid-thigh CSA were measured with the same CT device for each subject. An anterior-posterior scout scan of the entire right femur was used to localize the mid-thigh position. The femoral length was measured in cranial-caudal dimension, and the midpoint was determined of the distance between the medial edge of the greater trochanter and the intercondyloid fossa.

A single, 10-mm-thick, axial image was then obtained at the femoral midpoint, making sure that the entire circumference of both thighs was included in the field of view. These scans were completed at 120 kVp, 200-250 mA. All CT scans of both sites were transferred to one reading center and were analyzed by a single observer on a SUN Workstation (SPARCstation II, Sun Microsystems). Skeletal muscle and adipose tissue areas of the mid-thigh were calculated from the axial CT images using IDL development software (RSI Systems). Muscle and adipose tissue areas were calculated by multiplying the number of pixels of a given tissue type by the pixel area. Density values were determined by averaging the pixel density values (defined on a Houndfield Unit scale) of the regions outlined on the images. The external contours of the thigh were determined using a threshold of 224 HU, and the external bone contours were derived at 150 HU. For each participant, the determination of soft tissue type was made using the bimodal image distribution histogram resulting from the distribution numbers in adipose tissue and muscle tissue. Intermuscular and visible intramuscular adipose tissue was separated from subcutaneous adipose tissue by manual drawing of contours around the deep fascial plane surrounding the thigh muscles. The total (left + right) mid-thigh CSA of nonadipose, nonbone tissue within the deep fascial plane was used as a measure of muscle mass. CT-scans were rated for quality based on scanning the same leg (right or left) at both points in time and a slice location on the femur within 20 mm of the first location. Data were included when CT-scans of the same leg was scanned at both points in time and slice location criteria were satisfied. Reproducibility of measuring muscle area at mid-thigh of both legs was assessed by reanalyzing a 5% convenience sample of the study cohort and showed a CV of 5% (18).



## Dietary assessment

Participants completed a 108-item interviewer-administered modified version of the Block FFQ (Block Dietary Data Systems) (19) to estimate usual nutrient intake over the previous year. This FFQ was developed specifically for Health ABC by Block Dietary Data Systems (Berkeley, CA) using the NHANES III 24-hour recall data for older (> 65 years) non-Hispanic white and black adults residing in the northeast or southern United States. Trained and certified interviewers used wood blocks, food models, standard kitchen measures, and flash cards to help participants estimate portion sizes for each food. Interviews were monitored once per month per certified interviewer throughout the study to ensure the quality and consistency of the data collection procedures. The Health ABC FFQ was analyzed for micro- and macronutrient content by Block Dietary Data Systems. Total energy and protein intake were calculated, as well as the source of protein (animal or vegetable) and the total amount of EAAs, branched chain amino acids (BCAAs) and leucine. Block et al. (19) evaluated the validity of the original FFQ, in which the food items and portion sizes are based on NHANES II instead of NHANES III. Correlations of this FFQ with a dietary food record yielded correlations >0.7 for energy and 17 selected nutrients including protein. Amino acids were not evaluated in this study, but a study of Ishihara et

al. (20) demonstrated that correlation coefficients for amino acids were similar to that for protein when comparing an FFQ with a 28-d weighted dietary record.

## Potential confounders

Demographic characteristics (age, sex, race, and study site), smoking status, alcohol consumption, and physical activity were established by an interviewer-administered questionnaire at baseline. Body mass index (BMI; kg/m$^2$) was calculated from measured body weight and body height. Body weight was measured using a standard balance beam scale. Body height was measured in with a wall-mounted Harpenden stadiometer. Alcohol drinking was categorized as ≤ 1 or > 1 alcoholic consumption per day. Smoking was categorized as never, former, or current. Physical activity was based on the estimated kcal per week spent on walking and exercise over the previous 7 d. The prevalence of diabetes, coronary heart disease, congestive heart failure, cerebrovascular disease, chronic obstructive pulmonary disease (COPD) and cancer (excluding skin) was determined at baseline by using algorithms based on self-report, medication use, blood values (fasting glucose and oral glucose tolerance test for diabetes), and measurements (pulmonary function testing for COPD). The use of oral steroids was determined from drug data coded by using the Iowa Drug Information System ingredient codes. Change in total body fat mass (in kg) over the 5-y follow-up was assessed by using the DXA whole body scan (DXA, 4500A, version 8.20a; Hologic) at year 1 and year 6. Interim hospitalizations, defined as an overnight stay, during the 5 y of follow-up were categorized as 0 or ≥ 1 hospitalizations.

## Statistical analysis

Baseline characteristics of participants were compared between quintiles of energy-adjusted protein intake by calculating a *P* value for trend. For the continuous variables this was done by using the median value in each quintile as a continuous variable in the linear regression model and for the dichotomous or categorical variables by using chi-square tests.

Multiple linear regression was used to examine the association between protein intake and mid-thigh muscle area at year 6 adjusted for mid-thigh muscle area at year 1 using IBM SPSS 22 (SPSS Inc.). Energy-adjusted protein residuals (continuous variable) were used as independent variable for protein intake and were calculated by regressing absolute protein intake on total energy intake. One unit protein residual higher is to be interpreted as a 1-g higher protein intake than expected based on energy intake. An advantage of this method is that it provides a measure of protein intake that is independent of total energy intake (21). The outcome variable was total mid-thigh muscle area (cm$^2$) at year 6 and all models adjusted for baseline total mid-thigh muscle area (year 1). Different models are presented adjusting for demographic characteristics and study site (model 1), making additional adjustment for health behavior (smoking and alcohol consumption), prevalent

health conditions (coronary heart disease, congestive heart failure, chronic obstructive pulmonary disease, cerebrovascular disease, diabetes, cancer and use of oral steroids), height and energy intake (model 2), and in addition for physical activity, hospitalizations (yes or no) and 5-y change in body fat mass (model 3). To investigate whether sex, age, race, study site or baseline thigh muscle area modified the association under study, the interaction terms sex x protein intake, age x protein intake, race x protein intake, study site x protein intake and baseline thigh muscle area x protein intake were tested but were not significant ($P > 0.20$); therefore, all analyses are presented in the total sample. For animal, vegetable protein and EAA intake similar statistical procedures were followed. Energy-adjusted animal or vegetable protein intake residuals were used. Models for animal protein were also adjusted for vegetable protein intake and vice versa. Total EAA, BCAA, and leucine intake were expressed as percentages of total protein intake.

The association between protein intake and change in thigh muscle area was also evaluated categorically by using sex-specific quintiles of energy-adjusted protein intake. Sex-specific quintiles were used to avoid the distribution of sex over the quintiles being skewed. Tests for linear trends across quintiles of protein intake were conducted by using the median value in each protein category as a continuous variable in the linear regression models. Outcome variable was 5-y change in thigh muscle area; adjustments were made for baseline thigh muscle area, age, sex, race and study site (model 1) and in addition for health behaviors, prevalent health conditions, height, energy intake, physical activity, hospitalizations, and 5-y change in body fat mass (model 3).

Finally we analyzed whether a higher protein intake (≥ 0.8 g/kg/d and ≥ 1.2 g/kg/d) was associated with a higher thigh muscle area at year 6 adjusted for baseline values and all potential confounders (model 3 as already described) as compared with a lower protein intake and we performed a sensitivity analysis including nondiabetic subjects only, since diabetes influences the decline in muscle mass (22).

## RESULTS

Of all 3075 participants included in the Health ABC study, 1561 were included from the data-analyses (Figure 1). Participants excluded from the analysis (n=1514) were slightly older, less physically active, had a higher protein intake in grams and grams per kg body weight, were more likely to be black and more likely to smoke ($P < 0.05$). No significant differences were observed for thigh muscle area at baseline, BMI, gender distribution and alcohol consumption (data not shown).

Mean (95% CI) age of the study sample was 73.4 (73.2, 73.5) y, with 52% being female and 33% being black. The mean (95% CI) protein intake was 66.0 (64.7, 67.2) g/d or 0.90 (0.88, 0.92) g/kg body weight/d. Mean (95% CI) 5-y decline in thigh muscle area was -9.8 (-10.6, -8.9) $cm^2$ or -4.0% (-4.4%, -3.6%). **Table 1** describes the characteristics of the study sample (n = 1561) by quintile of energy-adjusted total protein intake. Participants in the lower quintiles of protein intake were more likely to be black, less physically active, and less likely to have diabetes. No differences in baseline thigh muscle area were observed.

**Figure 2** displays the crude 5-y change in mid-thigh muscle area by quintiles of protein intake (in g/kg/day), showing no trend across the quintiles. The association between quintiles of sex-specific energy-adjusted total protein intake and thigh muscle area at year 6 adjusted for baseline thigh muscle area and potential confounders show similar results (**Figure 3**). **Table 2** shows no associations of total, animal and plant protein intake with thigh muscle area at year 6, adjusted for baseline thigh muscle area and potential confounders. In line with total protein intake, total EAA, BCAA and leucine intake were not significantly associated with change in thigh muscle area.

The mean difference (95% CI) in CT slice location between year 1 and 6 was 3.9 (3.5, 4.2) mm. When performing the analysis only on the leg on which the CT scout scan is performed, results were in line with the results in Table 2. The *ßs* (with 95% CIs) and *P* values of the fully adjusted model for energy-adjusted total protein intake residuals, energy-adjusted animal protein intake residuals, and energy-adjusted vegetable protein intake residuals were 0.004 (-0.025, 0.033), $P = 0.782$; 0.003 (-0.026, 0.032), $P = 0.821$; and 0.031 (-0.040, 0.101) $cm^2$, $P = 0.395$, respectively.

In an additional analysis we analyzed whether a higher protein intake (≥ 0.8 g/kg/d or ≥ 1.2 g/kg/d) was associated with a greater thigh muscle area at year 6 adjusted for baseline thigh muscle area and confounders. When categorizing protein intake of our study population into ≥ 0.8 g/kg/d compared with < 0.8 g/kg/d, analyses revealed no difference between the groups with regard to adjusted thigh muscle area at year 6 (*ß*: -1.08 $cm^2$; 95% CI: -3.09, 0.93 $cm^2$, $P = 0.291$). Categorizing protein intake into ≥1.2 g/kg compared with <1.2 gave similar results (*ß*: -1.17 $cm^2$; 95% CI: -3.65, 1.32 $cm^2$, $P = 0.358$). Protein intake in grams per kilogram per day as a continuous variable tended to be negatively associated with change in thigh muscle area (fully adjusted model *β*: -3.58 $cm^2$; 95% CI: -7.21, 0.06 $cm^2$, $P = 0.054$). Furthermore, a sensitivity analysis restricted to non-diabetic subjects (n=1205) showed no significant association between energy adjusted protein residuals and change in thigh muscle area (fully adjusted model *β:* -0.01 $cm^2$; 95% CI: -0.07, 0.05 $cm^2$, $P = 0.815$).

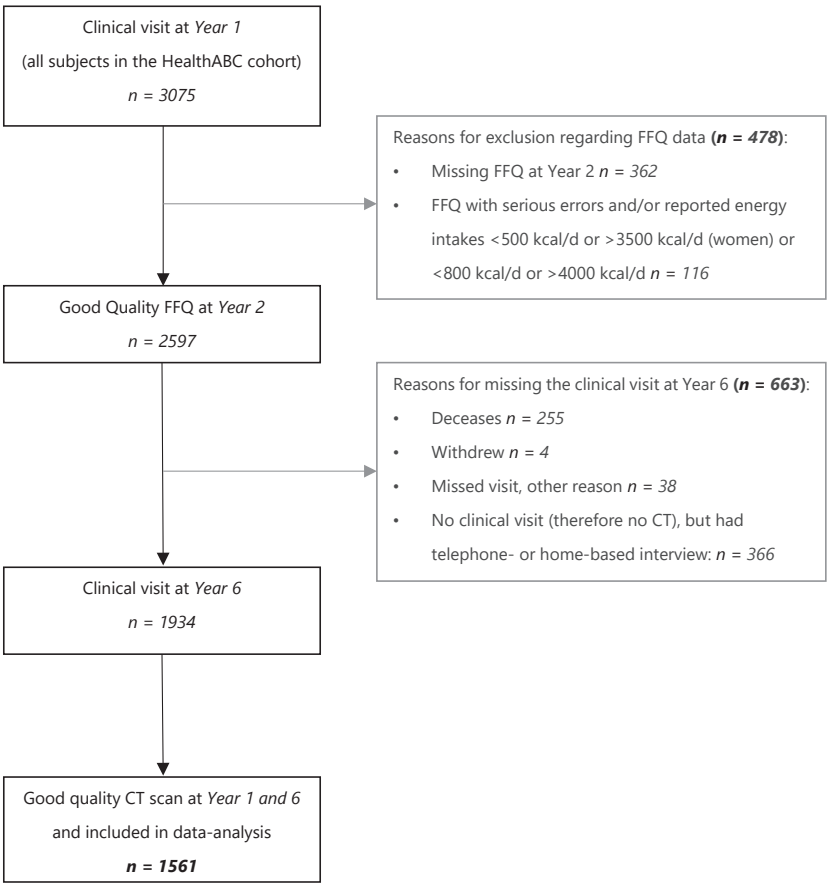


**Figure 1:** Flow chart for inclusion of participants of the Health ABC study in the data analyses. CT, computed tomography; FFQ, food-frequency questionnaire; Health ABC, Health, Aging, and Body Composition.

**Table 1:** Descriptive characteristics of Health ABC study participants at baseline by sex-specific quintiles (Q) of energy-adjusted total protein intake residuals[1]

| | **Q1** n = 312 | **Q2** n = 312 | **Q3** n = 313 | **Q4** n = 312 | **Q5** n = 312 | ***P*** [2] |
|---|---|---|---|---|---|---|
| Age, y | 73.4 (73.0, 73.7) | 73.4 (73.1, 73.7) | 73.4 (73.0, 73.7) | 73.5 (73.2, 73.8) | 73.2 (72.9, 73.6) | 0.631 |
| Female | 51.9 | 51.9 | 52.1 | 51.9 | 51.9 | 1.000 |
| Black | 42.3 | 33.0 | 29.7 | 29.8 | 31.1 | 0.002 |
| Current smoker | 7.4 | 7.4 | 6.4 | 7.7 | 4.8 | 0.291 |
| Alcohol consumption, > 1 unit/d | 11.5 | 6.1 | 6.1 | 7.7 | 8.0 | 0.295 |
| Walking and exercise, kcal/week | 892 (711, 1074) | 1066 (868, 1264) | 1176 (989, 1362) | 1350 (1066, 1634) | 1446 (1188, 1704) | <0.001 |
| **Prevalent health conditions** | | | | | | |
| Diabetes | 16.5 | 17.3 | 23.5 | 21.5 | 24.7 | 0.005 |
| Coronary heart disease | 20.0 | 18.2 | 17.6 | 18.5 | 20.8 | 0.788 |
| Congestive heart failure | 1.3 | 0.6 | 2.3 | 2.9 | 1.9 | 0.140 |
| Cerebrovascular disease | 6.8 | 6.8 | 4.5 | 6.1 | 6.5 | 0.792 |
| COPD | 13.3 | 20.1 | 17.1 | 17.1 | 15.2 | 0.932 |
| Cancer | 14.1 | 15.4 | 18.9 | 20.0 | 17.7 | 0.083 |
| Oral steroid use | 2.6 | 1.3 | 2.6 | 2.6 | 1.9 | 1.000 |
| BMI, kg/m$^2$ | 27.2 (26.7, 27.8) | 27.2 (26.7, 27.7) | 27.1 (26.6, 27.6) | 26.8 (26.3, 27.3) | 27.9 (27.4, 28.5) | 0.108 |
| Obese (BMI≥30 kg/m$^2$) | 24.4 | 21.5 | 19.8 | 26.0 | 28.8 | 0.701 |
| **Body composition** | | | | | | |
| FFM DXA, kg | 49.0 (47.8, 50.0) | 48.4 (47.3, 49.6) | 48.6 (47.4, 49.8) | 48.3 (47.0, 49.5) | 49.5 (48.4, 50.7) | 0.504 |
| FM DXA, kg | 27.0 (26.0, 27.9) | 26.8 (25.9, 27.8) | 26.4 (25.6, 27.4) | 26.1 (25.2, 27.0) | 27.5 (26.5, 28.5) | 0.600 |
| Thigh muscle area, cm$^2$ | 225 (219, 231) | 222 (216, 228) | 222 (216, 228) | 219 (213, 225) | 229 (223, 236) | 0.391 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Dietary intake** | | | | | | |
| Total energy, kcal | 2052 (1983, 2120) | 1700 (1634, 1766) | 1690 (1629, 1751) | 1714 (1649, 1780) | 2014 (1942, 2085) | 0.652 |
| Fat, % of energy | 34.8 (34.0, 35.6) | 33.2 (32.4, 34.0) | 33.6 (32.8, 34.4) | 32.0 (31.2, 32.8) | 32.0 (31.1, 32.9) | <0.001 |
| Carbohydrate, % of energy | 54.8 (53.9, 55.7) | 55.1 (54.2, 56.1) | 53.1 (52.2, 54.0) | 53.1 (52.3, 54.0) | 50.4 (49.4, 51.3) | <0.001 |
| Protein, % of energy | 10.9 (10.8, 11.0) | 12.8 (12.8, 12.9) | 14.3 (14.2, 14.3) | 16.0 (15.8, 16.1) | 18.7 (18.4, 19.0) | <0.001 |
| Protein, g/d | 56.2 (54.2, 58.2) | 54.5 (52.4, 56.7) | 59.7 (57.7, 61.8) | 67.2 (65.0, 69.3) | 92.2 (89.2, 95.3) | <0.001 |
| Protein, g/kg/d | 0.77 (0.74, 0.80) | 0.75 (0.72, 0.78) | 0.82 (0.79, 0.85) | 0.94 (0.91, 0.98) | 1.23 (1.19, 1.27) | <0.001 |
| Protein, g/kgFFM/d | 1.19 (1.14, 1.24) | 1.17 (1.12, 1.21) | 1.27 (1.22, 1.32) | 1.45 (1.40, 1.50) | 1.91 (1.85, 1.97) | <0.001 |
| Animal protein, g/d | 26.6 (25.4, 27.8) | 28.1 (26.8, 29.4) | 33.8 (32.5, 35.0) | 40.2 (38.8, 41.6) | 61.6 (59.3, 63.9) | <0.001 |
| Vegetable protein, g/d | 29.5 (28.3, 30.7) | 26.5 (25.3, 27.7) | 26.0 (24.9, 27.0) | 27.0 (25.8, 28.1) | 30.6 (29.2, 32.0) | 0.045 |

[1]Values are means (95% CIs) or percentages. COPD, chronic obstructive pulmonary disease; DXA, dual-energy X-ray absorptiometry; FFM, fat free mass; FM, fat mass; Health ABC, Health, Aging, and Body Composition; Q, quintile.
[2]*P* for trend across quintiles. For continuous variables: by using the median value in each quintile as a continuous variable in the linear regression model; for the dichotomous variables: by using the linear-by-linear association within chi-square tests.

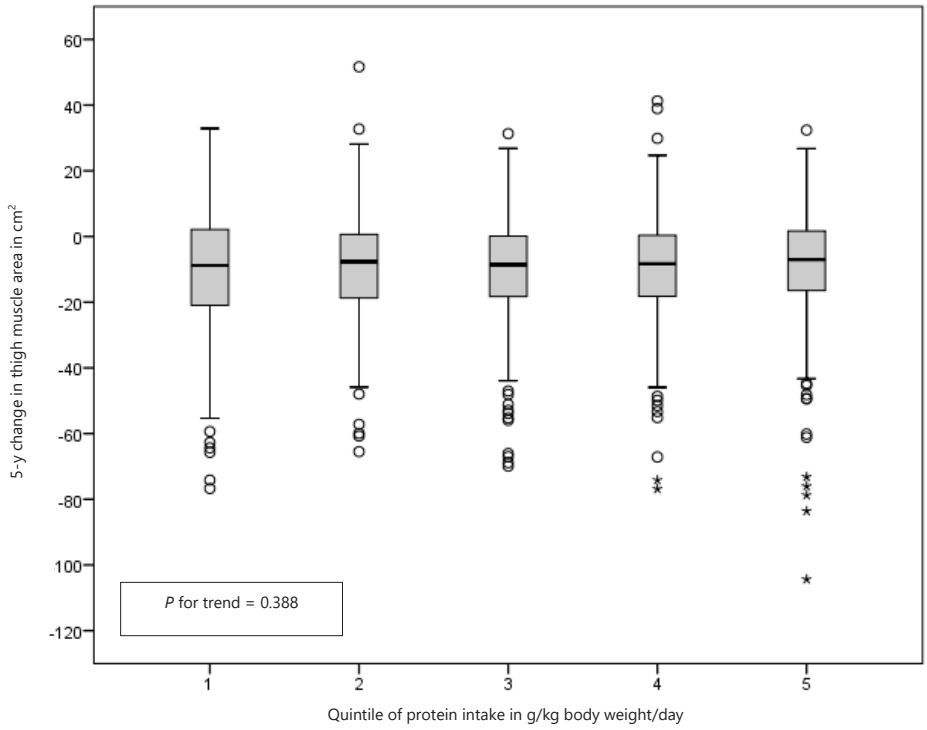


**Figure 2:** Five-year crude thigh muscle area change by quintile of protein intake in g/kg body weight/day in 1561 older participants of the Health ABC study. Median intakes of protein in g/kg/day were 0.50 for Q1, 0.68 for Q2, 0.85 for Q3, 1.03 for Q4 and 1.39 for Q5. Gray boxes represent the IQR (P25-P75), the black horizontal line within the grey box represents the median value (P50), whiskers display the lowest or highest value that is not an outlier or extreme, open dots represent outliers (>1.5 and ≤ 3 times the IQR) and asterisks represent extremes (>3 times the IQR). Tests for a linear trend across the quintiles were conducted by using the median value in each quintile as a continuous variable in the linear regression model. Health ABC, Health, Aging, and Body Composition; P, percentile; Q, quintile

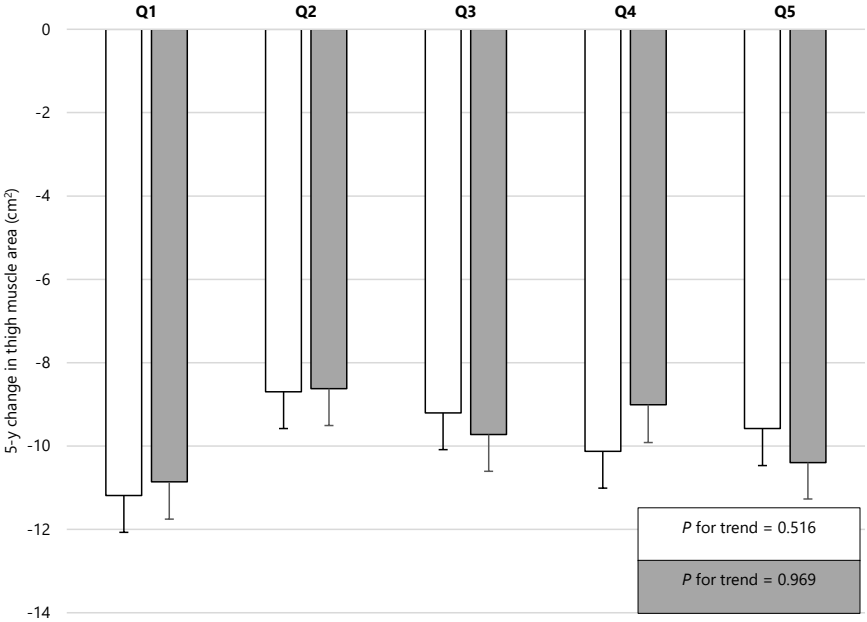


**Figure 3:** Five-year thigh muscle area loss by baseline sex-specific quintiles (Q) of energy-adjusted total protein intake residuals and adjusted for baseline thigh muscle area and potential confounders in 2 models (white and grey bars) in 1561 older participants of the HABC study. White bars represent estimated marginal means with SEs as calculated with general linear models of changes in thigh muscle area with adjustments for baseline thigh muscle, age, sex, race and study site. Gray bars represent estimated marginal means with SEs as calculated with general linear models of changes in thigh muscle area with additional adjustments for smoking, alcohol consumption, prevalent health conditions (coronary heart disease, congestive heart failure, chronic obstructive pulmonary disease, cerebrovascular disease, diabetes, cancer, use of oral steroids), height, energy intake, physical activity, interim hospitalization, change in fat mass. Tests for a linear trend across the quintiles were conducted by using the median value in each quintile as a continuous variable in the linear regression model. Health ABC, Health, Aging, and Body Composition; Q, quintile.

**Table 2:** Association between protein intake and thigh muscle area at year 6, adjusted for baseline thigh muscle area and potential confounders in 1561 older participants of the Health ABC study[1]

| | Thigh muscle area ($cm^2$) | |
|---|---|---|
| **Energy adjusted total protein intake residuals (g)[2]** | **β (95% CI)** | ***P*** |
| Model 1[3] | 0.011 (-0.045, 0.067) | 0.704 |
| Model 2[4] | 0.015 (-0.047, 0.077) | 0.636 |
| Model 3[5] | -0.001 (-0.056, 0.055) | 0.982 |
| **Energy adjusted animal protein intake residuals (g)[6]** | | |
| Model 1[3] | 0.007 (-0.050, 0.063) | 0.813 |
| Model 2[4] | 0.012 (-0.050, 0.074) | 0.702 |
| Model 3[5] | -0.003 (-0.058, 0.053) | 0.923 |
| **Energy adjusted vegetable protein intake residuals (g)[6]** | | |
| Model 1[3] | 0.132 (-0.002, 0.266) | 0.054 |
| Model 2[4] | 0.107 (-0.043, 0.256) | 0.162 |
| Model 3[5] | 0.075 (-0.060, 0.210) | 0.276 |

[1]Health ABC, Health, Aging, and Body Composition.
[2]Energy-adjusted total protein intake residuals in grams of protein (1 unit higher is to be interpreted as 1-g higher protein intake than expected based on energy intake).
[3]Model 1 is adjusted for baseline thigh muscle area, age, sex, race and study site.
[4]Model 2 is adjusted for determinants in model 1 and smoking, alcohol consumption, prevalent health conditions (coronary heart disease, congestive heart failure, chronic obstructive pulmonary disease, cerebrovascular disease, diabetes, cancer, use of oral steroids), height and energy intake.
[5]Model 3 is corrected for determinants in model 2 and physical activity, interim hospitalization and change in fat mass.
[6]All models for energy-adjusted animal protein residuals were also adjusted for energy-adjusted vegetable protein residuals and vice versa.

## DISCUSSION

This study is the first longitudinal study, to our knowledge, investigating the association between protein intake and thigh muscle area assessed by CT scan in a large study population of older adults. This study shows no association between total, animal or vegetable protein intake and EAA intake and change in thigh muscle area over a 5-y period. These results are contrary to our initial hypotheses. Since a higher protein intake has been shown to stimulate protein synthesis and a more positive protein balance, we expected that a higher protein intake and more specifically a higher animal protein and leucine intake would be associated with maintenance of muscle CSA (7, 23).

Like Houston et al. (9) we used data from the Health ABC study. In contrast to our findings, they did find an association between protein intake and change in lean mass. There are 2 potential explanations for this difference in results. First, their sample size was different

from our study (n=2066 compared with 1561) because of the difference in outcome measure (DXA compared with CT), a difference in follow-up period (3 vs. 5 years), and a difference in the time points of the used data (year 2 and year 5 as opposed to year 1 and year 6). The shorter follow-up period in their study resulted in less dropout due to mortality (188 compared with 255). Our study population therefore might have been somewhat healthier at baseline. A difference in health conditions might affect the association under study as, for example, the presence and severity of insulin resistance have been shown to affect the level of inhibition of the protein stimulating pathway mTOR (24, 25). The potential impact of health status should be explored in future studies. Second, we used a different outcome measure. Whereas Houston et al. (9) used (appendicular) lean mass assessed by DXA, we used thigh muscle area using the CT scan. One of the major advantages of using CT is the ability to measure fat infiltration into skeletal muscle, and therefore to measure actual muscle tissue area (26, 27). Furthermore, DXA probably also measures nonskeletal muscle tissues as lean mass (e.g., the fat free mass in adipose tissue) (28) and is therefore probably more confounded by body size than CT. Another advantage of CT is the ability to detect smaller changes in thigh muscle compared to DXA owing to smaller measurement errors (29). Thus, mid-thigh muscle area by CT is a more reliable method to assess actual muscle tissue and more sensitive to change over time. However, a potential draw-back of using the single slice CT muscle area is that it only assesses the muscle area at 1 location in the body and does not necessarily reflect whole body muscle mass (28).



Chan et al. (12) also studied the association between total protein intake and longitudinal change in appendicular lean mass (by DXA) over a 4-y period in 2726 Chinese elderly. In line with our results, they did not find an association. The intake of total protein however was relatively high in their study compared to ours with <0.9 g/kg/d in the lowest quartile vs. ≥ 1.6 g/kg/d in the highest quartile (vs. 0.77 g/kg/d in the lowest quintile and 1.23 g/kg/d in the highest quintile in our study). The authors argue that this high protein intake is one of the main reasons for finding no effect. They also studied the association between type of protein and muscle mass loss. In contrast to their expectations, higher vegetable, but not higher animal protein intake was associated with reduced muscle loss. Animal-based products generally contain more leucine. Since leucine has muscle protein synthesis-stimulating properties, one might expect that a higher animal protein intake would have a positive effect on muscle protein synthesis (13, 14). McDonald et al. (15) did demonstrate that a higher intake of leucine in the diet (~ 7 g leucine/d) in conjunction with a sufficient amount of protein among 79 older adults was associated with retention of lean mass assessed by bio-impedance analysis after 6 y. The protein intake in the lowest quartile of leucine intake was 0.61 g/kg/d compared with 1.26 g/kg/d in the highest quartile, with a slightly wider range than ours (0.77 g/kg/d and 1.23g/kg/d in the lowest and highest quintiles, respectively). They analyzed whether a higher leucine intake in conjunction with a higher protein intake was beneficial, therefore the effect of

leucine per se could not be determined in their study. In contrast to their findings, a meta-analysis of 8 supplementation trials showed that protein or amino acid supplementation did not increase muscle mass in older people (30). Two recent supplementation trials that were not yet included in this meta-analysis, used a whey protein, leucine and vitamin D-enriched supplement and showed an increase in appendicular lean mass (31, 32) and fractional synthesis rate (32) in older adults. These findings are in line with a recent 10-wk trial in which a diet providing 1.6 g/kg/d protein compared with 0.8 g/kg/d, on which older subjects lost appendicular lean mass, had a beneficial effect on lean body mass in older men (33). In summary, the relation between protein intake and amino acid composition of the diet and change in muscle mass over time in older adults remains unclear.

Several factors that potentially influence muscle mass change over time and are related to protein intake could not be taken into account in our analysis. First, older adults might require a higher threshold of protein per meal to raise muscle protein synthesis levels. Previous studies showed that a minimal amount of 25-30 g of high-quality protein per meal is needed to stimulate protein synthesis above baseline levels (4, 34) in the short term. Whether the distribution of protein intake affects muscle mass over the longer term remains to be elucidated (35, 36). Second, the effect of protein supplementation or a high protein diet on lean mass may be more pronounced in combination with resistance exercise (37-41). For future research, longitudinal studies are warranted that take into account the amount of protein ingested per meal, the distribution of protein intake over the day and the potential interaction of protein intake with resistance exercise.

Sample size of our study (n=1561) was sufficient, as ~500 subjects were needed to detect an expected difference (9) of 40% less decline in mid-thigh muscle area between the highest and lowest quintile of energy adjusted protein intake with a statistical power of 80% (9, 42). A limitation of this study is the use of a single FFQ at year 2 to estimate the usual intake of nutrients of the previous year. For the analysis presented here we assume that this intake did not change during the follow-up period. Eating habits, however, might change over time, including amongst other possibilities a decline in energy and protein intake because of the onset of chronic conditions or functional limitations. Another limitation of the FFQ in general is that it provides an imprecise means of estimating absolute amounts of nutrient intake including amino acids, but it can be used to rank nutrient intake (20, 43); also, underreporting is more present in subjects with a higher BMI (44). Furthermore, the time point of the CT measurement was in year 1, whereas the FFQ was filled in at year 2 which is methodologically less desirable. These limitations regarding the use of a single FFQ may have reduced the ability to detect an association between dietary protein intake and changes in thigh muscle area. A second limitation is that the scanning location of the mid-thigh muscle area by CT was based on a scout scan of a single leg. The same position on the same leg was used at follow-up. It is therefore unknown whether the scan

location on the opposite leg is the same between year 1 and year 6. However, when we repeated the analysis only using data of the leg on which the scout scan is performed, similar nonsignificant associations were observed. When selecting a subgroup of subjects with a follow-up slice location difference within 10 mm of the baseline location (n=1314), still no significant associations were observed between protein intake and thigh muscle area change. A third limitation is that no cross-calibration of the CT scan between the two sites was performed to assess possible differences to detect changes in mid-thigh muscle area over time, which potentially introduced measurement bias. However, when analyzing the sites separately, still no significant associations were observed between protein intake and thigh muscle area change. Finally, the level of exercise was also based on self-report, which gives an imprecise estimation of the level of exercise, and residual bias may be present.

In conclusion, this study suggests that a higher total, animal, vegetable protein or EAA intake is not associated with 5-y change in mid-thigh muscle CSA in older adults. This conclusion contradicts some, but not all, previous research. More research is required to determine the optimal protein intake for community-dwelling older adults.



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The authors' responsibilities were as follows: AV, MFE, PJMW, MV: analysis and interpretation of the data and drafting of the manuscript; DKH, TBH: study design and critical revision of the manuscript; IAB PMC, ABN, FAT: critical revision of the manuscript; MV: had primary responsibility for the final content; and all authors: read and approved the content of the final manuscript. None of the authors reported a conflict of interest related to the study.

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# Chapter 5

## A higher protein intake at breakfast and lunch is associated with a higher total daily protein intake in older adults: a post-hoc cross-sectional analysis of four randomised controlled trials

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## ABSTRACT

**Background**

A protein intake of 30 – 40 grams per meal is suggested to maximally stimulate muscle protein synthesis in older adults and could therefore contribute to the prevention of sarcopenia. Protein intake at breakfast and lunch is often low and offer a great opportunity to improve daily protein intake. Protein, however, is known for its satiating effects. Therefore, we explored the association between the amount of protein intake at breakfast and lunch and total daily protein intake in older adults.

**Methods**

Protein intake was assessed by a 3-day food record in 498 community dwelling older adults (≥ 55 years) participating different lifestyle interventions. Linear mixed model analysis was used to examine the association between protein intake at breakfast or lunch and total daily protein intake, adjusted for sex, age, body mass index, smoking status, study, and total energy intake.

**Results**

After adjustment for potential confounders, a 10 g higher protein intake at breakfast was associated with a 3.2 g higher total daily protein intake ($P = 0.008$) for males, and 4.9 g ($P < 0.001$) for females. A 10 g higher protein intake at lunch was associated with a 3.7 g higher total daily protein intake ($P < 0.001$) for males, and 5.8 g ($P < 0.001$) for females.

**Conclusions**

A higher protein intake at breakfast and lunch is associated with a higher total daily protein intake in community dwelling older adults. Stimulating a higher protein intake at breakfast and lunch might represent a promising nutritional strategy to optimize the amount of protein per meal without compromising total daily protein intake.

## INTRODUCTION

Our society is aging rapidly (1). Ageing is associated with loss of muscle mass, strength and performance, a process termed sarcopenia. Sarcopenia is associated with an increased risk of falls and fractures, morbidity and mortality. To prevent or even counteract sarcopenia is of major importance as it declines the risk for adverse health outcomes and health-related cost and improves quality of life (2). The cause of sarcopenia is multifactorial and includes physical inactivity and lower protein intakes (3). Increasing dietary protein intake has been suggested as important beneficial strategy to prevent and/or treat sarcopenia in older adults (4, 5).

Phillips et al. (6) suggested that a dietary protein intake per meal of 0.4 – 0.6 g/kg body weight (BW) or approximately 30 – 40 g is necessary to maximally stimulate skeletal muscle protein synthesis in older adults. Most community dwelling older adults in the Netherlands do not reach these suggested amounts of protein per meal, particularly at breakfast and lunch: mean (SD) protein intake is 11 ± 7 g at breakfast, and 18 ± 10 g at lunch (7). Multiple researchers suggest that an even distribution of proteins over the three meals (and therefore higher protein intakes at breakfast and lunch) with sufficient amounts of protein per meal, could translate in a higher anabolic response (8-11). Kim et al. (10) conclude that probably the most efficient way to maximize the anabolic response is to increase dietary protein intake at breakfast and lunch, without reducing protein intake at dinner (for consumption patterns with the hot meal in the evening). Because protein intake at breakfast and lunch in older adults is low (7), these meals offer great potential to increase daily protein intakes (12), aiming to stimulate muscle protein synthesis and optimise muscle maintenance (13, 14).

Proteins, however, have a strong satiating effect (15). Increasing the intake in one meal may result in compensation of protein intakes and other nutrients and energy at other meals (16). This compensation may be influenced by ageing because ageing affects hunger and satiety hormone secretion, as well as feelings of hunger and fullness (17). However, the relation between protein at breakfast or lunch and total daily protein intake in older adults is unclear (18, 19). Therefore, the present study aimed to explore the association between the amount of protein intake at breakfast and at lunch and total daily protein intake in community dwelling older adults.

# METHODS

## Study design and study population

A cross-sectional analysis was performed on baseline data of older adults (≥55 years) participating one of four different lifestyle interventions at the Amsterdam Nutritional Assessment Center at Amsterdam University of Applied Sciences. The four lifestyle interventions were:

1. The MPS (Muscle Preservation Study) (20): a randomised controlled trial in which the effect of a high whey protein-, leucine-, and vitamin D-enriched supplement was tested during a 13-week weight loss program including resistance exercise on preservation of muscle mass in an older (≥ 55 years) obese adults. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m$^2$ or as a BMI ≥ 28 in kg/m$^2$ with waist circumference > 88 cm (women) or > 102 cm (men).
2. The WelPrex (Weight Loss with Protein and Exercise) study (21): a randomised controlled trial in which the effect of a high protein diet and/or three times per week resistance exercise was tested during a 10-week weight loss program in older (≥ 55 years) overweight and obese adults. Overweight was defined as a BMI ≥ 28 kg/m$^2$ or as a BMI > 25 kg/m$^2$ with waist circumference > 88 cm (women) or > 102 cm (men).
3. The PROBE (protein and lifestyle intervention to preserve muscle mass in obese older type 2 diabetes patients) study (22): is a randomised controlled trial comparable to the MPS, a 13-week weight loss trial including resistance training in which the effect of the same supplement was tested, although this population was a diabetic older (≥ 55 years) population with obesity. Obesity was defined as a BMI ≥ 30 kg/m$^2$ or as a BMI ≥ 27 kg/m$^2$ with waist circumference > 88 cm (women) or > 102 cm (men).
4. The VITAMIN (VITal AMsterdam older adults IN the city) study (23): a randomised controlled trial that evaluated the effectiveness of a digitally supported home-based exercise training program, as well as the additional value of dietary protein on physical performance, in community dwelling older adults aged ≥ 55 years.

A full description of the eligibility criteria is available online in the Dutch Trial Register (MPS: NL2623; WelPrex: NL4434; PROBE: NL4357; VITAMINE: NL5472; http://www.trialregister.nl). Written informed consent was obtained from all subjects and the studies were performed in accordance with the Helsinki Declaration. These studies took place from March 2011 through September 2018 at the Amsterdam Nutritional Assessment Center at the Amsterdam University of Applied Sciences Amsterdam, The Netherlands.

## Assessment of dietary intake

Baseline dietary intake was assessed by a three-day food record at 2 week days and 1 weekend day. Food records were prestructured for the following eating moments: breakfast, in between breakfast and lunch, lunch, in between lunch and dinner, dinner, and in the evening. Subjects were asked to report their food intake as specific as possible and to report amounts of their intake in standard household measures (for example 3 slices of whole grain bread) or to weigh their food items on a kitchen weighing scale. Food records were checked for completeness during study visits by trained fourth grade students Nutrition and Dietetics under supervision of the study dietician. Additional information about unclear items or amounts was obtained and recorded. Food record data of the four studies were collected and verified in accordance with the standard operating procedures of our lab. The food items were coded and the nutritional intake data file was coupled to the computerized Dutch Food Composition Table (24, 25) to calculate total energy and macronutrient intakes. The dietician or coordinating investigator performed an additional verification and consistency check after the coding process. Subjects with completed dietary records on at least 2 days, and with average reported energy intake of at least 800 kcal/day were included for analysis. The outcome variable total daily protein intake was calculated in g, g/kg BW and g/kg fat free mass (FFM). Protein intake in g/kg BW was also adjusted for body weight for subjects with a BMI ≥ 30 kg/m$^2$ using body weight at BMI 27.5 kg/m$^2$ (26) and for subjects with a BMI < 22 kg/m$^2$ using body weight at BMI 22 kg/m$^2$ (27). This adjustment of body weight is applied to make it more comparable to true protein needs and to make it more comparable to what is often used in dietetic practice because body composition parameters are not always available. FFM in obese subjects is low relative to their body weight and therefore using actual body weight would probably overestimate protein needs. The opposite is the case for subjects with a low BMI: then, FFM is relatively high for their body weight, and using actual body weight would probably underestimate true needs.



## Assessment of general characteristics and potential confounders

Body composition, including fat mass (FM) and FFM, was determined using air displacement plethysmography (BODPOD, Life Measurement Inc., Concord, CA, USA). Body weight was measured on the calibrated scale as part of the BODPOD system. Body height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer (Seca 222; Seca, Hamburg, Germany). Waist circumference was measured in a standing position halfway between the anterior superior iliac spine and the lower rib after normal expiration (Seca 201; Seca). General characteristics (gender, age and smoking status (current smoker yes or no)) were self-reported at baseline.

### Statistical analysis

Linear mixed model analysis was used to examine the association of protein intake at breakfast (g) and protein intake at lunch (g) with total daily protein intake (g, g/kg BW, g/kg adjusted BW, g/kg FFM) at 2 or 3 days, with a random intercept for subject and a random slope for protein intake at breakfast. The random intercept takes into account that subjects provide dietary intake data from multiple days. The random slope is a variance parameter that is estimated from the different slopes, which is included in the model. These models are adjusted for sex, age, BMI, smoking status, study and total energy intake (kcal/d). Additionally, the association of protein intake at breakfast and protein intake at lunch (g) with protein intake during the rest of the day [total daily protein intake minus protein intake at breakfast or lunch in (g)] and protein intake during subsequent meals was studied. Finally, the association of intake of protein source (animal or plant) at breakfast and lunch with total daily protein intake was studied using the same mixed model analysis, with models for animal protein additionally adjusted for plant protein and vice versa.

Effect modification by sex, age, BMI and study was tested for the association between protein intake at breakfast (g) or protein intake at lunch (g) and total daily protein intake (g, g/kg BW, g/kg adjusted BW, g/kg FFM). For most associations sex was an effect modifier; therefore, all analyses were stratified for sex. All analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

### Subjects

In total, 498 participants were included into this analysis. **Figure 1** displays the number of participants originally included in each study (20-23) and the number of food records days used for this analysis. In total 1477 food record days were included in the analysis. Mean (SD) age of the study population was 67.7 (7.3) y, 42% were male, mean BMI was 30.0 (5.6) kg/m$^2$ and 21% were normal weight (BMI 20-25 kg/m$^2$), 30% were overweight (BMI 25-30 kg/m$^2$) and 49% were obese (BMI≥ 30 kg/m$^2$). The general characteristics of the study population are presented in **Table 1**.

### Dietary intake

Mean (SD) energy intake for the total study population was 1898 (526) kcal, with a protein intake of 82 (24) g or 0.97 (0.30) g/kg BW. Absolute intake of energy and protein was higher for males than for females, whereas protein intake in g/kg BW/day and in g/kg FFM/day was higher in females (**Table 2**). In total 70% of the study population reached a protein intake of 0.8 g/kg BW and 19% reached a protein intake of 1.2 g/kg BW. Only 1% (n = 4)

reached the suggested amount of 0.4 g/kg BW protein (28) at breakfast, 8% at lunch and 51% at dinner. These percentages are higher using adjusted body weight for subjects with a BMI ≥ 30 kg/m$^2$ or < 22 kg/m$^2$, and all percentages were higher for females compared to males (Table 2). **Figure 2** shows the protein and other macronutrient intakes at all eating moments during the day for the total study population. For males and females the distribution of protein intake over the day was comparable. For males, mean (SD) protein intake was 15.2 (8.2) g at breakfast, 19.9 (10.3) g at lunch and 38.3 (15.5) g at diner. For females, the intakes were 13.0 (6.2), 18.2 (8.7) and 33.9 (13.0) g, respectively.

The within-subject coefficient of variation was 23% for total daily protein intake (g), 32% for protein intake at breakfast (g) and 46% for protein intake at lunch (g).

## Association of protein intake at breakfast and lunch with total daily protein intake

**Table 3** shows the association of protein intake at breakfast and lunch with total daily protein intake, as well as with protein intake during the rest of the day, adjusted for sex, age, BMI, smoking status, study and total energy intake.



After adjustment for these potential confounders, a 10 g higher protein intake at breakfast was associated with a 3.2 g higher total daily protein intake ($P = 0.007$) corresponding to a 0.02 g/kg BW ($P = 0.048$) or 0.03 g/kg adjusted BW ($P = 0.045$) higher total daily protein intake for males. These associations were stronger for females: a 10 g higher protein intake at breakfast was associated with a 4.9 g higher total daily protein intake ($P < 0.001$) corresponding to a higher total daily protein intake of 0.06 g/kg BW ($P < 0.001$) or 0.07 g/kg adjusted BW ($P < 0.001$) (Table 3). However, after adjustment for potential confounders, protein intake at breakfast was significantly negatively associated with protein intake during the rest of the day (total daily protein intake minus protein intake at breakfast): a 10 g higher protein at breakfast was associated with a 6.8 g and 5.1 g lower protein intake during the rest of the day for males and females, respectively. Thus, a 10 g higher protein intake at breakfast did not translate in a 10 g higher total daily protein intake, instead translating into a 3.2 g (males) and 4.9 g (females) higher total intake and therefore a 6.8 g (males) and 5.2 g (females) lower protein intake during the rest of the day (Table 3). A higher protein intake at breakfast was negatively associated with the protein intake at lunch only for males (Table 3). For protein intake at lunch these associations are in line with the associations for breakfast (Table 3).

When analysing the association of intake of protein source (animal or plant) at breakfast and lunch with total daily protein intake, it appears that this association for plant and animal protein is different. A 10 g higher animal protein intake at breakfast is associated with a 5.6 g (95% CI= 2.7 – 8.5 g, $P < 0.001$) higher total daily protein intake for males and

7.6 g (5.2 – 10 g, $P < 0.001$) for females. A 10 g higher plant protein intake at breakfast, however, is associated with a non-significant 0.9 g (-2.6 – 4.3 g, $P = 0.631$) lower total daily protein intake for males, and 2.7 g (-1.0 – 6.5 g, $P = 0.156$) lower intake for females, as well as a significant lower protein intake during the rest of the day, including lunch and dinner. Associations for the source of protein intake at lunch with total daily protein intake, and with protein intake during the rest of the day were in line with the associations described for breakfast.

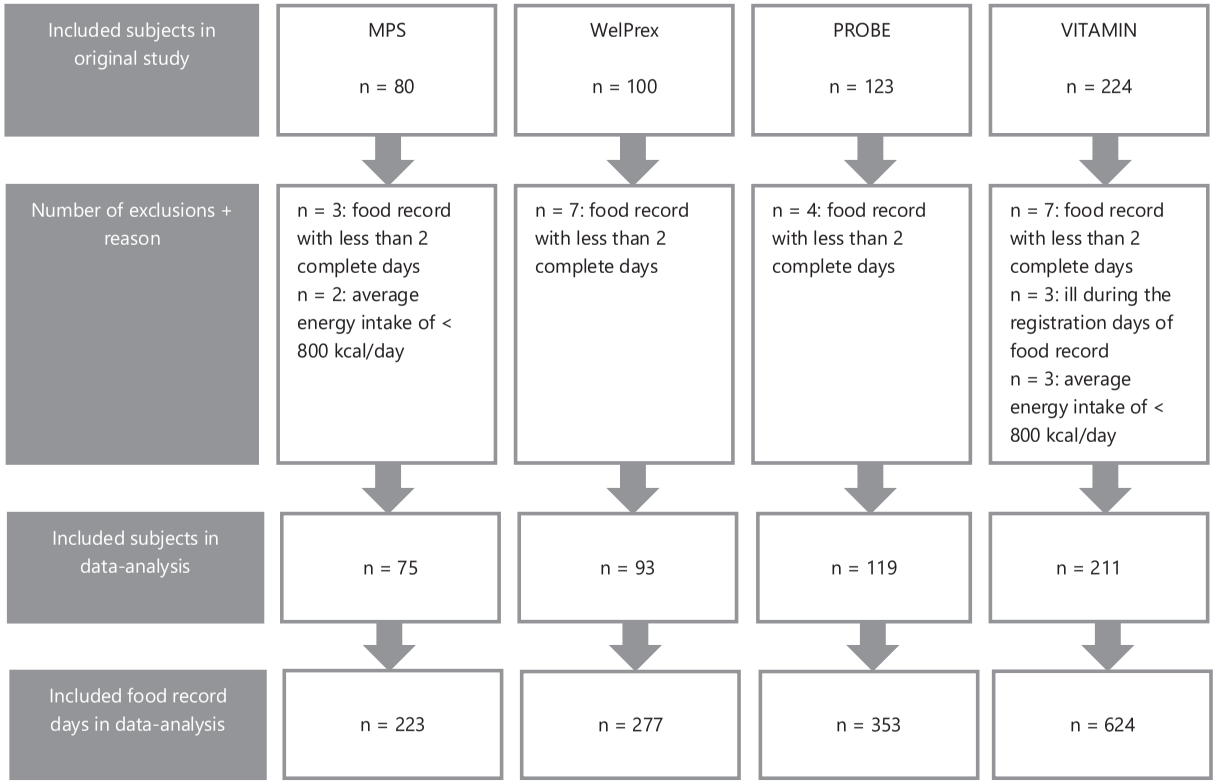


**Figure 1:** Flow chart for inclusion of baseline data of older adults (N=498) participating in lifestyle interventions at the Amsterdam Nutritional Assessment Center in the data analysis.

**Table 1:** Baseline characteristics of older adults participating in lifestyle interventions at the Amsterdam Nutritional Assessment Center

| | **Total study population** n = 498 | | **MPS**[1] n = 75 | **WelPrex**[1] n = 93 | **PROBE**[1] n = 119 | **VITAMIN**[1] n = 211 | ***P* value**[2] |
|---|---|---|---|---|---|---|---|
| | **Mean ± SD / %** | **Range**[3] | **Mean ± SD / %** | **Mean ± SD / %** | **Mean ± SD / %** | **Mean ± SD / %** | |
| **Age (years)** | 67.7 ± 7.3 | 55 – 91 | 63 ± 6 | 63 ± 5 | 67 ± 6 | 72 ± 6 | <0.001 |
| **% females** | 58.2% | | 60.0% | 62.4% | 33.6% | 69.7% | <0.001 |
| **Body weight (kg)** | 86.9 ± 18.5 | 46.0 – 146.3 | 95.4 ± 13.9 | 92.3 ± 14.5 | 100.6 ± 15.7 | 73.7 ± 13.9 | <0.001 |
| **Height (m)** | 1.70 ± 0.09 | 1.50 – 1.94 | 1.69 ± 0.09 | 1.69 ± 0.09 | 1.73 ± 0.09 | 1.68 ± 0.09 | <0.001 |
| **BMI (kg/m²)** | 30.0 ± 5.6 | 17.5 - 54.6 | 33.2 ± 4.4 | 32.1 ± 4.3 | 33.6 ± 4.4 | 25.9 ± 4.2 | <0.001 |
| **% overweight**[3] | 30.3% | | 24.0% | 33.3% | 18.5% | 37.9% | 0.001 |
| **% obese**[3] | 48.8% | | 76.0% | 65.6% | 81.5% | 13.3% | <0.001 |
| **Waist circumference (cm)** | 103 ± 15[4] | 66 – 146 | 111 ± 11 | 108 ± 12[6] | 115 ± 10[7] | 90 ± 11 | <0.001 |
| **Fat free mass (kg)** | 51.5 ± 11.9[4] | 28.2 – 85.3 | 54.0 ± 10.8[5] | 52.4 ± 12.1[6] | 58.5 ± 11.0 | 46.0 ± 10.0[8] | <0.001 |
| **Fat mass (kg)** | 35.2 ± 12.2[4] | 9.5 – 91.3 | 41.1 ± 10.9[5] | 39.8 ± 9.8[6] | 40.6 ± 11.6 | 27.7 ± 10.0[8] | <0.001 |
| **Body fat percentage (%)** | 40.0 ± 9.1[4] | 12.6 – 66.1 | 43.1 ± 8.6[5] | 43.3 ± 8.4[6] | 40.2 ± 8.2 | 37.2 ± 9.3[8] | <0.001 |
| **% smoking** | 7.3%[4] | | 9.5% | 8.6% | 10.1% | 4.3% | 0.180 |

[1]The four lifestyle interventions with trialregister numbers are the MPS (Muscle Preservation Study): NL2623; the WelPrex (Weight Loss with Protein and Exercise) study: NL4434; the PROBE (protein and lifestyle intervention to preserve muscle mass in obese older type 2 diabetes patients) study: NL4357 and the VITAMIN (VITal AMsterdam older adults IN the city): NL5472 (http://www.trialregister.nl)

[2]*P* value for differences between the four lifestyle interventions. For nominal variables Pearson's Chi-square test is used, for continuous variables One-Way ANOVA is used.

[3]Range is presented as minimum to maximum value.

[3]Overweight= BMI ≥ 25 and < 30 kg/m², obese = BMI ≥ 30 kg/m².

[4]*n* waist circumference and *n* smoking status = 495, *n* fat free mass, fat mass and body fat percentage = 479.

[5]Muscle Preservation study: *n* fat free mass, fat mass and body fat percentage = 70, *n* smoking status = 74.

[6]WelPrex study: *n* fat free mass, fat mass and body fat percentage and waist circumference = 92.

[7] PROBE study: *n* waist circumference = 117.

[8] VITAMIN study: *n* fat free mass, fat mass and body fat percentage = 198, *n* smoking status = 209.

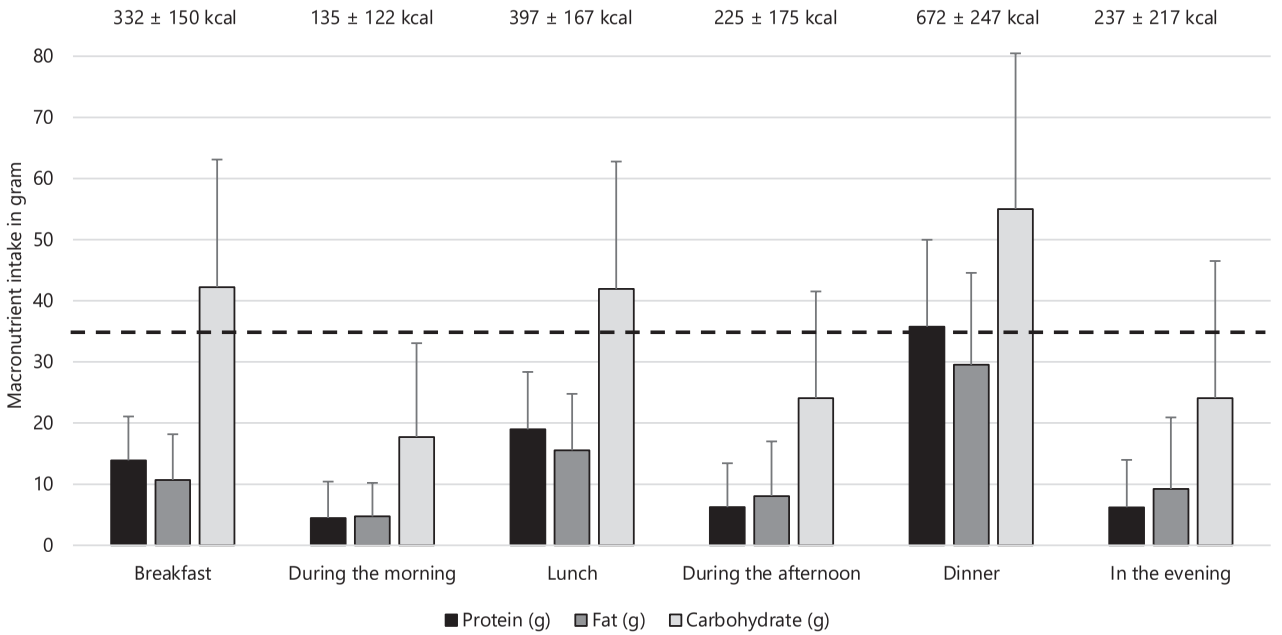


**Figure 2:** Macronutrient intake per meal. The bars represent an average macronutrient intake per eating moment over the 3-day food records (n = 498). The dashed line represents the amount of protein per meal which is suggested to stimulate protein synthesis (28) calculated using the average body weight of the study population.

**Table 2**: Average dietary intake per day[1] of older adults participating in lifestyle interventions at the Amsterdam Nutritional Assessment Center

| | **Total study population** n = 498 | | **Males** n = 208 | **Females** n = 290 |
|---|---|---|---|---|
| | **Mean ± SD, or %** | **Range[2]** | **Mean ± SD** | **Mean ± SD** |
| Energy (kcal) | 1898 ± 526 | 800 – 4069 | 2021 ± 521 | 1810 ± 512 |
| Energy (kJ) | 7958 ± 2200 | 3356 – 17073 | 8473 ± 2181 | 7589 ± 2142 |
| Total protein intake (g/day) | 82 ± 24 | 25 – 215 | 88 ± 27 | 77 ± 23 |
| Plant protein intake (g/day) | 29 ± 10 | 8 – 72 | 31 ± 11 | 28 ± 9 |
| Animal protein intake (g/day) | 52 ± 20 | 5 – 155 | 56 ± 20 | 50 ± 20 |
| Fat intake (g/day) | 74 ± 28 | 15 – 196 | 78 ± 27 | 71 ± 29 |
| Carbohydrate intake (g/day) | 195 ± 62 | 51 – 443 | 206 ± 64 | 186 ± 59 |
| Protein intake energy% | 17.6 ± 3.6 | 8.6 – 33.4 | 17.7 ± 3.4 | 17.5 ± 3.8 |
| Fat intake energy% | 34.6 ± 6.8 | 13.3 – 59.0 | 34.4 ± 6.5 | 34.8 ± 7.0 |
| Carbohydrate intake energy% | 41.2 ± 7.3 | 19.0 – 75.6 | 40.9 ± 6.9 | 41.5 ± 7.6 |
| Protein intake (g/kg BW/day) | 0.97 ± 0.30 | 0.30 – 2.33 | 0.93 ± 0.27 | 0.99 ± 0.31 |
| Protein intake (g/kg adj[3] BW/day) | 1.07 ± 0.31 | 0.37 – 2.40 | 1.04 ± 0.28 | 1.09 ± 0.32 |
| Protein intake (g/kg FFM[4]/day) | 1.64 ± 0.52 | 0.55 – 4.29 | 1.41 ± 0.37 | 1.81 ± 0.54 |
| % with intake ≥ 0.8 g/kg BW/day | 70% | | 67% | 73% |
| % with intake ≥ 1.2 g/kg BW/day | 19% | | 15% | 21% |
| % with intake ≥ 0.8 g/kg adj[3] BW/day | 83% | | 81% | 85% |
| % with intake ≥ 1.2 g/kg adj[3] BW/day | 29% | | 27% | 31% |
| % consuming ≥ 0.4 g/kg BW at breakfast | 1% | | 0% | 1% |
| % consuming ≥ 0.4 g/kg BW at lunch | 8% | | 7% | 10% |
| % consuming ≥ 0.4 g/kg BW at dinner | 51% | | 46% | 56% |
| % consuming ≥ 0.4 g/kg adj[3] BW at breakfast | 2% | | 1% | 2% |
| % consuming ≥ 0.4 g/kg adj[3] BW at lunch | 10% | | 9% | 10% |
| % consuming ≥ 0.4 g/kg adj[3] BW at dinner | 63% | | 60% | 65% |

[1]Average dietary intake is calculated from the mean intake per day of each subject (*n* = 498)
[2]Range is presented in minimum to maximum value.
[3]Using adjusted body weight for obese subjects (using body weight at BMI 27.5 kg/m$^2$) (26) and for subjects with a BMI < 22 kg/m$^2$ (using body weight at BMI 22 kg/m$^2$) (27)
[4]fat free mass (FFM) assessed using air displacement plethysmography (BODPOD, Life Measurement Inc.,), *n* total study population = 479, *n* female = 277, *n* male = 202

**Table 3:** Associations[1] of protein intake at breakfast and lunch in g/day with total daily protein intake, and with protein intake during the rest of the day[2] and subsequent meals[3] in older adults

| | Males n = 208 | | | Females n = 290 | | |
|---|---|---|---|---|---|---|
| | **Beta** | **95% CI[4]** | ***P* value** | **Beta** | **95% CI[4]** | ***P* value** |
| **Associations of protein intake at breakfast in g/day (independent variable)** | | | | | | |
| **Total protein intake (g/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.90 | 0.59– 1.20 | <0.001 | 1.09 | 0.82– 1.36 | <0.001 |
| Adjusted model[5]: | 0.32 | 0.09 – 0.56 | 0.007 | 0.49 | 0.27 – 0.70 | <0.001 |
| **Total protein intake (g/kg body weight/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.007 | 0.004– 0.010 | <0.001 | 0.010 | 0.007– 0.013 | <0.001 |
| Adjusted model[5]: | 0.002 | 0.000 – 0.005 | 0.048 | 0.006 | 0.003 – 0.009 | <0.001 |
| **Total protein intake (g/kg adjusted body weight/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.009 | 0.005 – 0.012 | <0.001 | 0.015 | 0.011 – 0.018 | <0.001 |
| Adjusted model[5]: | 0.003 | 0.000 – 0.006 | 0.045 | 0.007 | 0.004 – 0.010 | <0.001 |
| **Total protein intake (g/kg FFM[6]/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.012 | 0.007 – 0.016 | <0.001 | 0.021 | 0.014 – 0.028 | <0.001 |
| Adjusted model[5]: | 0.004 | -0.000 – 0.007 | 0.068 | 0.011 | 0.005 – 0.016 | <0.001 |
| **Protein intake during the rest of the day (g/day)[2] (dependent variable)** | | | | | | |
| Crude model[5]: | -0.10 | -0.41 – 0.20 | 0.497 | 0.09 | -0.18 – 0.36 | 0.496 |
| Adjusted model[5]: | -0.68 | -0.91 – -0.45 | <0.001 | -0.51 | -0.73 – -0.30 | <0.001 |
| **Protein intake at lunch (g/day) (dependent variable)** | | | | | | |
| Crude model[5]: | -0.08 | -0.19 – 0.04 | 0.191 | -0.00 | -0.13 – 0.13 | 0.952 |
| Adjusted model[5]: | -0.19 | -0.30 – -0.08 | 0.001 | -0.06 | -0.19 – 0.08 | 0.412 |
| **Protein intake at dinner (g/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.12 | -0.15 – 0.39 | 0.397 | 0.21 | 0.00 – 0.41 | 0.048 |
| Adjusted model[5]: | -0.08 | -0.28 – 0.13 | 0.462 | -0.12 | -0.30 – 0.07 | 0.228 |
| **Associations of protein intake at lunch in g/day (independent variable)** | | | | | | |
| **Total protein intake (g/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.78 | 0.60– 0.96 | <0.001 | 0.98 | 0.81– 1.15 | <0.001 |
| Adjusted model[5]: | 0.37 | 0.24 – 0.51 | <0.001 | 0.58 | 0.46 – 0.70 | <0.001 |
| **Total protein intake (g/kg body weight/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.007 | 0.005 – 0.009 | <0.001 | 0.012 | 0.010 – 0.015 | <0.001 |
| Adjusted model[5]: | 0.003 | 0.002 – 0.005 | <0.001 | 0.008 | 0.006 – 0.009 | <0.001 |
| **Total protein intake (g/kg adjusted body weight/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.009 | 0.006 – 0.011 | <0.001 | 0.013 | 0.011 – 0.016 | <0.001 |
| Adjusted model[5]: | 0.004 | 0.002 – 0.006 | <0.001 | 0.008 | 0.006 – 0.009 | <0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Total protein intake (g/kg FFM[6]/day) (dependent variable)** | | | | | | |
| Crude model[5]: | 0.011 | 0.008 – 0.014 | <0.001 | 0.023 | 0.019 – 0.028 | <0.001 |
| Adjusted model[5]: | 0.005 | 0.003 – 0.007 | <0.001 | 0.014 | 0.011 – 0.016 | <0.001 |
| **Protein intake during the rest of the day (g/day)[2] (dependent variable)** | | | | | | |
| Crude model[5]: | -0.22 | -0.40 – -0.04 | 0.020 | -0.02 | -0.19 – 0.15 | 0.817 |
| Adjusted model[5]: | -0.63 | -0.76 – -0.49 | <0.001 | -0.42 | -0.54 – -0.30 | <0.001 |
| **Protein intake at dinner (g/day) (dependent variable)** | | | | | | |
| Crude model[5]: | -0.00 | -0.13 – 0.13 | 0.968 | 0.14 | -0.00 – 0.28 | 0.054 |
| Adjusted model[5]: | -0.19 | -0.32 – -0.06 | 0.005 | -0.10 | -0.20 – 0.01 | 0.074 |

[1]For associations with independent variable protein intake at breakfast: analysed with linear mixed models with a random intercept for subject and a random slope for protein intake at breakfast, *n* = 1477 food record days; For associations with independent variable protein intake at lunch: analysed with linear mixed models with a random intercept for subject and a random slope for protein intake at lunch, *n* = 1477 food record days

[2]For associations with independent variable protein intake at breakfast: protein during the rest of the day (g) = daily protein intake (g) - protein intake at breakfast (g); for associations with independent variable protein intake at lunch: protein during the rest of the day (g) = daily protein intake (g) - protein intake at lunch (g).

[3]For associations with independent variable protein intake at breakfast: subsequent meals are lunch and dinner; for associations with independent variable protein intake at lunch: subsequent meal is dinner.

[4]CI, confidence interval

[5]The crude model is the model without adjustments; the adjusted model adjusted for sex, age, BMI, smoking status (current smoker yes/no), study and total energy intake.

[6]Fat free mass (FFM) is assessed using air displacement plethysmography (BODPOD, Life Measurement Inc., Concord, CA), *n* = 1420 food record days.

## DISCUSSION

The present study investigated the association between protein intake at breakfast and lunch with the total daily protein intake among older adults and demonstrates that a higher protein intake at breakfast and lunch is associated with a lower protein intake during the rest of the day (total daily protein intake minus breakfast), but overall with a higher total daily protein intake.

In our study population less than 30% met the suggested recommendation of 1.2 g protein/kg BW (29, 30) using adjusted body weight (26, 27). Having a higher protein intake at breakfast (≥30 g) was associated with more subjects reaching 1.2 g protein/kg BW: 52% vs. 28% of the subjects. For lunch, these percentages were 61% vs. 25% of the subjects. These findings are in line with a study of Tieland et al. (12), in which an even protein distribution over the day, with more protein at breakfast and lunch, was associated a higher percentage of subjects achieving the recommended daily allowance of 0.8 g/kg BW/day.

Because the present study has a cross-sectional design, no suggestions for a causal relation can be made. The study, however, does give an indication that a higher protein intake at breakfast and lunch might have a satiating effect because protein intake at both breakfast and lunch was negatively associated with protein intake during the rest of the day. The total daily protein intake, however, was not compromised and a higher protein intake at breakfast and lunch was still related to a higher total protein intake. However, a higher plant protein intake at breakfast and lunch was not associated with a higher total daily protein intake, in contrast to animal protein. This might suggest that plant protein sources have a stronger satiating effect, although this proposal should be considered with caution because other factors such as the food form play a role. For example, animal protein might be consumed in more liquid forms (e.g. milk or yoghurt), which probably suppresses appetite less compared to solid forms (31), although this requires further study. Lonnie et al. (31) reported that a higher consumption of plant proteins found in whole food also increases dietary fiber, which might amplify satiety. Data regarding the effects of plant proteins on appetite in older adults, however, are very limited and should be investigated in future studies, in addition to the food groups, food form and matrix (31).

To our knowledge, this study is the first to investigate the association between regular protein intake at breakfast and lunch and total daily protein intake. Hengeveld et al. (18) demonstrated that older adults (>70 years) with an adequate protein intake (≥ 0.8 g/kg) had higher protein intakes at all eating occasions, including breakfast and lunch, which is in line with our findings. Several other studies demonstrate that the use of protein enriched meals or foods does not limit and mostly increase the amount of protein per meal and the total daily protein intake in older adults (32-34). This indicates that satiating effects of higher protein meals or foods are limited in older adults (34). Giezenaar et al. (35) showed that although gastric emptying was slower in older compared to younger men, which gives a prolonged postprandial satiety, the acute administration of whey protein drinks before a meal suppressed subsequent energy intake in young, but not in healthy older men. These findings are substantiated by Clegg et al. (36).

Only 2% and 10% of our subjects reached the suggested amount of 0.4 g/kg protein (28) at breakfast and lunch, which suggests that the habitual protein intake of during breakfast and lunch in general is low. The range of habitual protein intake at breakfast and lunch in our study, however, is large and achieved with regular food products. This shows that a higher protein intake at breakfast is achievable for some older adults and also demonstrates potential for improvement. A higher protein intake at breakfast and lunch may lead to a higher number of eating occasions that reach the suggested anabolic threshold for optimal muscle protein synthesis (28). Regardless of the total daily protein intake, this is already a potential gain, which might impact subsequent muscle maintenance

or accretion (6) and is important with respect to preventing or counteracting sarcopenia. However, this has not yet been substantiated by long-term dietary intervention trials.

A limitation of our study is the high percentage of obese older adults (almost 50%) in our study population. Obese adults have a higher prevalence of carrying the specific single nucleotide polymorphisms in the fat mass and obesity-associated gene (FTO) (37). FTO might facilitate weight gain by decreasing the release of the satiety hormone leptin and increasing the release of hunger-promoting hormone ghrelin (38). Therefore, the satiating effect of a meal might be less in obese subjects. The representativeness of the study population to the general older population may thus be low. In our study, however, we did not observe differences in the association of protein intake at breakfast and lunch with total daily protein intake between obese and non-obese subjects. This suggests that potential differences in the release of hunger and satiety hormones for obese versus non-obese subjects do not appear to translate into differences in the relation between protein intake at breakfast or lunch and protein intake during the rest of the day. A lower protein intake at breakfast, however, was related to a lower BMI: the 10% of the participants with the lowest protein intake at breakfast had a significantly lower BMI than subjects with a higher protein intake at breakfast [28.3 (4.8) versus 30.2 ± 5.6 kg/m$^2$]. Because BMI was also related to the primary outcome total daily protein intake, all models were adjusted for BMI. Another limitation regards the reported energy intake in our study, which is comparable to Dutch older adults in general (18) whereas nearly half of our study population was obese. We, therefore, expected a higher energy intake in our study population. Based on previous research (39), overweight people tend to underestimate their dietary intake more often than normal-weight people, and therefore true energy and protein intake could be underestimated in our study. Park et al. (40) demonstrated that a dietary food record has advantages compared to a food frequency questionnaire: less underreporting of energy and nutrients. In both the overweight and the obese subjects, protein intake with a dietary record was less underreported than energy intake. A third limitation is that we did not adjust for the potential confounding factors education-level and income (41) because these variables were not available for all included studies. A final limitation is that our study population had a wide age range, from 55 to over 90 y. Although age was no effect modifier in the relation between protein intake at breakfast or lunch and total daily protein intake, the dietary intake of food groups and the dietary pattern may change during the ageing process due to a wide variety of factors (42).

This study also has some strengths. We used a 3-day dietary food record to assess protein intake, which probably gives a more realistic estimate of dietary intake than a recall-method in this older population, because it is likely to be less prone to short term memory loss. In addition, we used linear mixed model analysis that took into account the within

subject day-by-day variation of dietary intake, which gives a more sensitive analysis than using an average dietary intake per subject.

## Conclusions and Implications

In conclusion, a higher protein intake at breakfast and lunch is associated with a higher total daily protein intake in community dwelling older adults. This association holds true for animal protein, although not for plant protein for which no association was observed. In sum, stimulating a higher protein intake at breakfast and lunch might represent a promising nutritional strategy for optimising the amount of protein per meal without compromising total daily protein intake.


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## Conflics of interest, source of funding and authorship

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JvdH, CvD, RGM and AMV conducted the included studies (hands-on conduct of the experiments and data collection). AMV, MTS, IR, DH, MT and PJMW analysed and/or checked the statistical analysis. AMV, JvdH, MTS, IR, DH, MT, MFE, MV and PJMW wrote and/or revised the manuscript. PJMW had primary responsibility for the final content. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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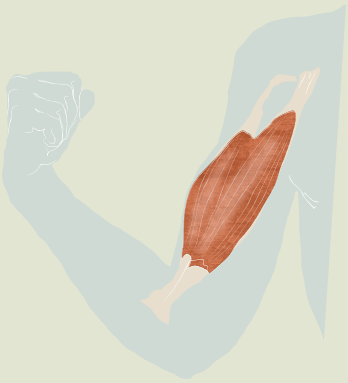

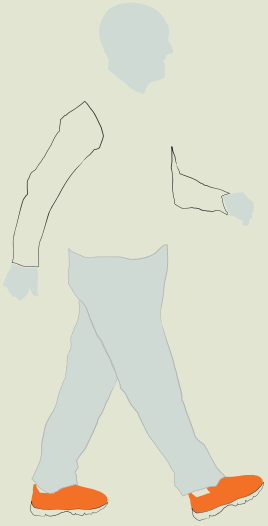

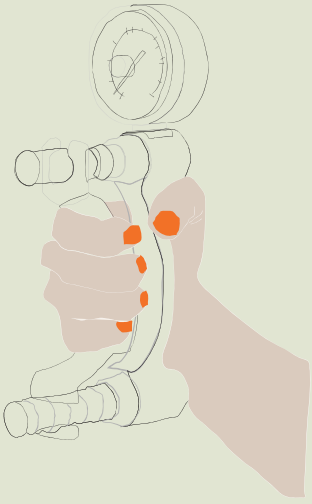

# Part 3

## Muscle mass preservation during weight loss

# Chapter 6

## A high whey protein-, leucine-, and vitamin D-enriched supplement preserves muscle mass during intentional weight loss in obese older adults: a double-blind randomized controlled trial

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## ABSTRACT

**Background** Intentional weight loss in obese older adults is a risk factor for muscle loss and sarcopenia.

**Objective** The objective was to examine the effect of a high whey protein-, leucine-, and vitamin D-enriched supplement on muscle mass preservation during intentional weight loss in obese older adults.

**Design** We included 80 obese older adults in a double-blind randomized controlled trial. During a 13-wk weight loss program, all subjects followed a hypocaloric diet (-600 kcal/d) and performed resistance training 3x/wk. Subjects were randomly allocated to a high whey protein-, leucine- and vitamin D-enriched supplement including a mix of other macro- and micronutrients (150 kcal, 21 g protein; 10x/wk, intervention group) or an isocaloric control. Primary outcome was change in appendicular muscle mass. Secondary outcomes were body composition, handgrip strength and physical performance. Data were analyzed by using ANCOVA and mixed linear models with sex and baseline value as co-variates.

**Results** At baseline, mean ± SD age was 63 ± 5.6 y, and body mass index (in kg/m$^2$) was 33 ± 4.4. During the trial protein intake was 1.11 ± 0.28 g/kg body weight/d in the intervention group compared with 0.85 ± 0.24 in the control group ($P < 0.001$). Both intervention and control groups decreased in body weight (-3.4 ± 3.6 kg and -2.8 ± 2.8 kg; both $P < 0.001$) and fat mass (-3.2 ± 3.1 kg and -2.5 ± 2.4 kg; both $P < 0.001$), with no differences between groups. The 13-wk change in appendicular muscle mass, however, was different in the intervention and control groups (+0.4 ± 1.2 kg and -0.5 ± 2.1 kg, respectively; ß = 0.95 kg (95%CI: 0.09; 1.81), $P = 0.03$). Muscle strength and function improved over time without significant differences between groups.

**Conclusions** A high whey protein-, leucine- and vitamin D-enriched supplement compared with isocaloric control preserves appendicular muscle mass in obese older adults during a hypocaloric diet and resistance exercise program and might therefore reduce the risk for sarcopenia. This trial was registered at the Dutch Trial Register (http://www.trialregister.nl) as NTR2751.

## INTRODUCTION

The prevalence of obesity among older adults is increasing rapidly (1). Obesity is related to insulin resistance, high blood pressure and dyslipidemia, which are metabolic risk factors for cardiovascular diseases and diabetes mellitus. In addition, obesity plays an important role in non-fatal physical disability in older adults (2). Weight loss leads to metabolic and functional benefits (3). However, a potential drawback of weight loss in older adults is the accompanying loss of skeletal muscle mass (4), which eventually may accelerate the development of sarcopenia (5, 6). Reduction in muscle mass and strength impairs physical function and activities of daily living and is associated with an increased risk of falling and physical disabilities (5, 6). Thus, although obese older adults may benefit from weight loss, therapy should focus on minimizing loss of muscle mass to preserve independence and quality of life (5).

Weight loss can be achieved by reduction of calorie intake and stimulation of physical activity. Strategies to preserve muscle mass during weight loss focus on resistance exercise and sufficient intake of high-quality protein (7-9). Resistance training is known to stimulate muscle protein synthesis in older adults, which supports muscle mass preservation and muscle function (10). High dietary protein intake, strategically timed at each meal, has also been shown to stimulate muscle protein synthesis and is another potent strategy to overcome the well-known muscle anabolic resistance in older aged individuals (1, 11, 12). Whey protein is a high quality protein, which has shown superiority in enhancing muscle protein synthesis compared to other protein sources in older adults (13, 14). This effect of whey was likely attributed to the faster digestion and absorption and the high content of essential amino acids, including leucine (15). Leucine is a powerful stimulator of muscle protein synthesis, and it was recently shown that leucine coingestion with a bolus of protein could further improve muscle protein synthesis (16).

The combination of a high intake of fast-digesting, high-quality protein and resistance exercise is suggested to have a synergistic effect on muscle mass preservation during weight loss (1, 17, 18), but data in obese older adults are limited (19). In addition, several studies suggest a positive effect of vitamin D on muscle protein metabolism (20, 21), and therefore vitamin D (800 IU) might have a potential beneficial effect on muscle mass preservation.

We therefore compared the effects of a high whey protein-, leucine- and vitamin D-enriched nutritional supplement to an isocaloric control during a 13-wk weight loss program consisting of a hypocaloric diet and resistance exercise training on appendicular muscle mass preservation in obese older adults.

## SUBJECTS AND METHODS

### Subjects

Obese men and women (aged ≥ 55 y) were recruited from the Dutch population through local flyers and advertisements. Obesity was defined as a BMI (in kg/m$^2$) > 30, or as a BMI > 28 with waist circumference > 88 cm (women) or > 102 cm (men). Potential subjects were excluded if they had any malignant diseases during the past five y, if they had participated in any weight loss program 3 mo before screening, if participation in the resistance training program was considered unsafe according to a physiotherapist, or when they were not able to comply with the full study protocol. A full description of the eligibility criteria is available online in the Dutch Trial Register (NTR2751; http://www.trialregister.nl). The study was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam (2010/280), and written informed consent was obtained from all subjects. The study took place from March 2011 until June 2012 at the Amsterdam University of Applied Sciences in The Netherlands.

### Design and randomization procedures

We performed a 13-wk randomized, controlled, double-blind, parallel group trial (i.e., Muscle Preservation Study). Eligible subjects were randomly allocated (1 : 1) to consume a high whey protein-, leucine-, and vitamin D-enriched supplement (intervention group) or an isocaloric control product (control group) by means of randomization envelopes with 4 different randomization codes stratified by gender. The randomization codes were generated by an independent statistician who was not involved in the conduct of the study. Body composition, including appendicular muscle mass, was assessed at baseline and after 13 weeks of intervention. Body weight, BMI, waist circumference, muscle strength and, physical functioning were measured at baseline, after 7 and 13 wk of intervention.

### Hypocaloric diet

All subjects followed a hypocaloric diet of 600 kcal below estimated energy needs according to the Dutch guideline (22). Energy needs were estimated by multiplying measured resting energy expenditure by indirect calorimetry (Vmax Encore n29; Viasys Healthcare), with physical activity level estimated by a 3-d physical activity record. This hypocaloric advice included the caloric content of 1 serving of the study products. The second serving, given only after training sessions, was provided in addition to the daily diet. At baseline, subjects received a standardized dietary plan according to the Dutch guideline (22) with a list of variation options. In the first week, subjects were called to check for compliance. Every 2 wk, subjects followed dietary counseling sessions in groups of 8-12 subjects in which experiences were shared and nutrition-related topics were discussed. Dietary intake was assessed by a 3-d food record at baseline and after 7 and 13 wk of intervention. Food records were checked for completeness during study visits, and

additional information was obtained about unclear items or amounts. Total energy and macronutrient intakes were calculated using a computerized Dutch Food Composition Table (23).

## Resistance exercise program

All subjects participated in the resistance exercise program, which was performed 3x/wk for 1 h under supervision of a qualified trainer for 13 wk. The training started with a 10-min warmup on a bicycle ergometer followed by 3 sets of 20 repetitions of the following 10 exercises: lateral pull down, arm curl, high row, shoulder press, leg curl, horizontal row, chest press, arm extension, leg extension and leg press. The number of repetitions was stepwise reduced to 12 repetitions, and the weights were increased to the ability of the participants. The training ended with 5-min cool-down on a bicycle ergometer.

## Study products

Study products were provided by Nutricia Research. The composition of the study products is displayed in **Table 1**. Both products were similar in taste and appearance and provided an energetic value of 150 kcal per serving in a volume of 150 mL. Subjects were asked to consume 10 servings of the study product per week throughout the 13-wk intervention period. Subjects consumed 1 serving daily, just before breakfast, whereas 3 servings were consumed immediately after exercise training (3x/wk). Study products had to be consumed as a single bolus within 5-10 min. Subjects were asked to record product intake in a diary to check compliance.



## Measurement of body composition, muscle strength and physical performance

Body composition including appendicular muscle mass (primary outcome), was measured with dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy / DPX-NT; GE Healthcare). To limit within-subject variation, we performed dual-energy X-ray absorptiometry scans at the same time of the day during both visits. Appendicular muscle mass was defined as the sum of lean mass (without bone) of both arms and legs. Skeletal muscle mass index was calculated dividing the appendicular skeletal muscle mass (kg) by height squared ($m^2$). Body weight was measured on a calibrated scale (Life Measurement). Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Seca 222; Seca).

For muscle strength, hand grip strength was measured with an isometric hand grip dynamometer (JAMAR 5030J1, Sammons Preston Rolyan) while the subject was in a sitting position. Three consecutive measures of hand grip strength (kg) for both hands were recorded to the nearest 0.1 kg, and all values were averaged. Physical performance was assessed with a 400-m walking test, a 4-m gait speed test, and a chair stand test (24).

### Statistical analysis

Double-data entry was performed and discrepancies were solved. Treatment codes were broken after locking the database. Statistical analyses were performed with appendicular muscle mass as primary outcome. In the analyses, we included all available post-baseline data for all participants independent of the level of compliance. Sample size for the present study was estimated using data from a pilot study combining a high-protein diet with resistance exercise training in older adults with fat-free mass as a proxy for appendicular muscle mass (unpublished internal data) because no other relevant published data were available. A sample size 40 per arm provided 80% power to detect an absolute difference of 2.0 kg fat-free mass with an SD of 1.7 kg and $P<0.05$ (2-sided), assuming a drop-out rate of 35%.

Subject characteristics and dietary intake at baseline were compared between groups using an independent-samples *t* test or the Fisher's exact test. Between-group differences on outcome variables that were measured at baseline and after 13 wk were analyzed with an ANCOVA by using the sex and baseline value as covariates. Between-group differences on outcome variables that were measured at baseline, 7 wk and 13 wk were analyzed by using a mixed linear model, including time (differentiating week 7 from week 13), intervention (differentiating the intervention group from the control group), and the time X intervention interaction as fixed factors; subject as random factor; and sex and baseline value as covariates. Intervention effect ß is the estimate for the difference between the intervention and the control group at 13 wk after correction for baseline and sex. Within-group differences were estimated using a paired-samples *t* test (for variables that were measured at baseline and after 13 wk) or the mixed linear model (for variables that were measured at baseline and after 7 wk and 13 wk).

SAS Enterprise Guide 4.3 for Window (SAS Institute) software was used for all statistical analyses. Data in text and tables are expressed as means ± SDs. Statistical significance was defined as a 2-tailed $P < 0.05$.

## RESULTS

### Subjects and compliance

We enrolled 80 subjects in the trial. The number of subjects screened, excluded and randomized is shown in **Figure 1**. Fifteen subjects dropped out during the study because of adverse events ($n = 6$) and personal reasons ($n = 9$), all not related to study product intake. There were no relevant differences in subject's characteristics between the groups (**Table 2**). Compliance to study product intake was comparable between groups: consumption of at least 7 study products per week by 91% in the intervention group and 97% in the

control group ($P = 0.61$). Adherence to the exercise program was comparable between groups: training on average more than 2 times per week by 72% in the intervention group and 88% in the control group ($P = 0.21$).

## Dietary intake

Baseline energy needs were calculated using the measured resting energy expenditure and the estimated baseline level of physical activity. No differences were observed between groups (intervention: 2621 ± 437 kcal/day; control: 2473 ± 636 kcal/day, $P = 0.33$). Self-reported mean dietary intake at baseline was 2072 ± 587 kcal/day in the intervention group and slightly higher compared to the 1775 ± 574 kcal/day in the control group ($P = 0.05$). Energy intake (including supplement) at week 13 of the study was not different between groups (**Table 3**, $P = 0.76$), although both groups significantly reduced their energy intake during the trial [change: -315 ± 499 kcal/d for intervention ($P = 0.005$) and -91 ± 504 kcal/d for control ($P = 0.01$)]. Protein intake at week 13 expressed as g/kg BW/day was 1.11 ± 0.28 in the intervention group compared to 0.85 ± 0.24 in the control group ($P < 0.001$), which corresponded to a higher dietary protein intake during intervention of 27.6 ± 24.9 g/d in the intervention group compared with the control group ($P < 0.001$). Contribution of carbohydrates to the total dietary intake energy percentage was higher in the control group than in the intervention group ($P < 0.001$) and there were no differences in the contribution of fat to the total dietary intake ($P = 0.92$).

## Body weight, BMI, waist circumference and body composition

The 13-wk weight loss intervention resulted in a significantly decreased body weight and fat mass in the intervention and control groups [-3.4 ± 3.6 kg and -2.8 ± 2.8 kg (both $P < 0.001$) and -3.2 ± 3.1 kg and -2.5 ± 2.4 kg (both $P < 0.001$), respectively] without significant differences between the groups (**Table 4**). Waist circumference and BMI also decreased over time (both $P < 0.001$), with no significant differences between groups (Table 4).

## Muscle mass, muscle strength and muscle function

After the 13-wk weight loss intervention the change in appendicular muscle mass was different in the intervention compared to the control group [+0.4 ± 1.2 kg and -0.5 ± 2.1 kg, respectively; ß = 0.95 kg (95% CI: 0.09, 1.81); $P = 0.03$) (**Figure 2**). No differences were observed in appendicular muscle mass for the intervention and control groups over time ($P = 0.15$ and $P = 0.11$, respectively). The 13-wk change in leg muscle mass was also different between the intervention and control groups [+0.3 ± 1.2 kg and -0.6 ± 1.8 kg, respectively; ß = 0.97 kg (95% CI: 0.24, 1.70); $P = 0.01$). Leg muscle mass was not different over time in the intervention group ($P = 0.08$) and showed a trend for a decline in the control group ($P = 0.06$) (Figure 2).

When appendicular muscle mass was adjusted for height, the skeletal muscle index still showed a significant change between intervention and control groups [+0.1 ± 0.4 kg/m$^2$ and -0.2 ± 0.7 kg/m$^2$, respectively; ß = 0.30 kg/m$^2$ (95% CI: 0.01, 0.59); $P$ = 0.04]. Muscle strength and muscle function improved over time without differences between groups (Table 4).

**Table 1**: Composition of the study products used in the double-blind, randomized, placebo-controlled trial of a high whey protein, leucine and vitamin D enriched supplement on preservation of muscle mass during a weight loss trial in obese older adults[1]

| Component | Intervention | Control |
|---|---|---|
| Energy | | |
| kcal | 150 | 150 |
| kJ | 635 | 635 |
| Protein, % | 55 | - |
| Carbohydrates, % | 25 | 82 |
| Fat, % | 18 | 18 |
| Fibre, % | 2 | - |
| Total protein, g | 20.7 | - |
| Total leucine,[2] g | 2.8 | - |
| Total EAA,[2] g | 10.6 | - |
| Carbohydrates | | |
| Total, g | 9.4 | 31.4 |
| Sugars, g | 4.2 | 2.6 |
| Total fat, g | 3.0 | 3.0 |
| Fibre | | |
| Total, g | 1.3 | - |
| Soluble, g | 1.3 | - |
| Minerals[3] | | |
| Sodium, mg | 150 | 142 |
| Potassium, mg | 279 | 176 |
| Chloride, mg | 70 | 344 |
| Vitamin $D_3$,[3] mg | 20 | - |

[1]per serving of 150 mL. BCAA, branched-chain amino acids (Leu, Ile, Val); EAA, essential amino acids (Leu, Ile, Val, Phe, Met, His, Trp, Thr, Lys).
[2]Provided by protein and free BCAA.
[3]Intervention product also contains micronutrients: calcium (500 mg), phosphorus (250 mg), magnesium (37 mg), iron (2.4 mg), zinc (2.2 mg), copper (270 μg), manganese (0.50 mg), fluoride (0.15 mg), molybdenum (15 μg), selenium (15 μg), chromium (7.5 μg), iodine (20 mcg), vitamine A (152 μg retinol equivalents), vitamin E (7.5 mg α-tocopherol equivalents), vitamin K-1 (12 μg), vitamin B-1 (0.23 mg), vitamin B-2 (0.25 mg), niacin (8.8 mg niacin equivalents), pantothenic acid (0.81 mg), vitamin B-6 (0.76 mg), folic acid (203 μg), vitamin B-12 (3.0 μg), biotin (6.1 μg), vitamin C (32 mg), carotenoids (0.30 mg) and choline (56 mg).

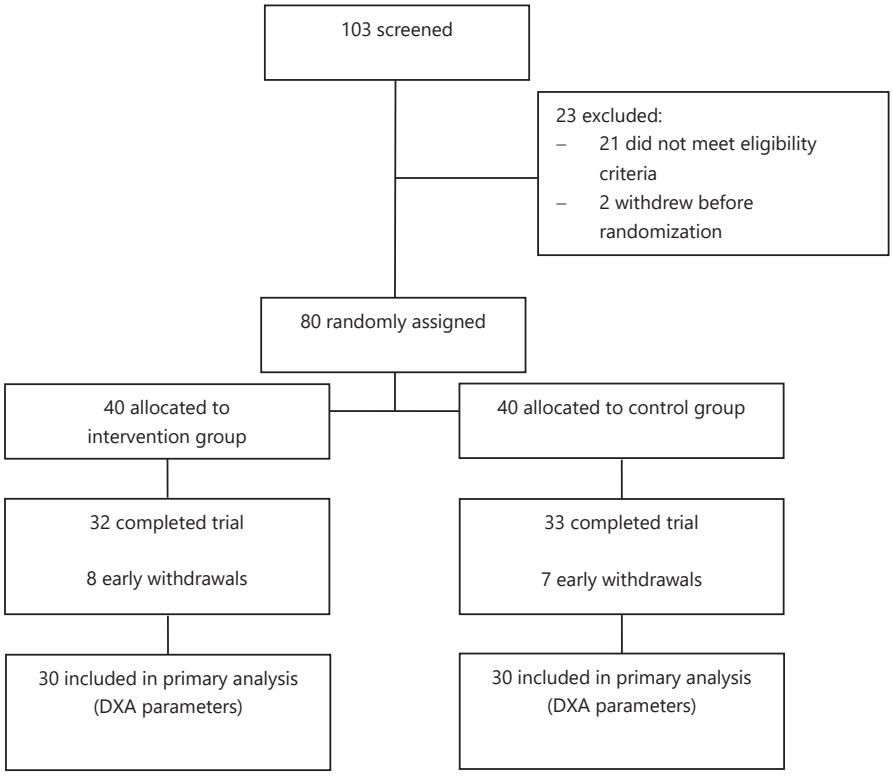


**Figure 1**: Flow chart of a double-blind, randomized, placebo-controlled trial of a high whey protein-, leucine-, and vitamin D-enriched supplement on preservation of muscle mass during a weight loss trial in obese older adults. DXA, dual-energy X-ray absorptiometry.

**Table 2**: Baseline characteristics of obese older subjects of the Muscle Preservation Study with both baseline and 13-wk measurement of primary outcome variable, by treatment[1]

| Characteristic | Intervention group n = 30 | Control group n = 30 | *P* value[2] |
|---|---|---|---|
| Male sex, n (%) | 14 (47) | 14 (47) | 1.00 |
| Origin, % Caucasian | 90 | 87 | 1.00 |
| Age, y | 63.7 ± 6.0 | 63.0 ± 6.0 | 0.61 |
| Height, m | 1.71 ± 0.10 | 1.68 ± 0.07 | 0.15 |
| Body weight, kg | 95.9 ± 11.9 | 94.1 ± 14.2 | 0.60 |
| BMI, kg/m$^2$ | 32.7 ± 3.1 | 33.3 ± 4.3 | 0.54 |
| BMI <30 kg/m$^2$, n (%) | 7 (23) | 6 (20) | 1.00 |
| BMI ≥ 30 kg/m$^2$, n (%) | 23 (77) | 24 (80) | |
| Waist circumference, cm | 111 ± 10 | 110 ± 11 | 0.85 |
| Fat mass, % | 40.8 ± 7.4 | 41.4 ± 7.7 | 0.75 |
| Appendicular muscle mass, kg | 23.2 ± 4.9 | 22.6 ± 4.9 | 0.64 |
| Skeletal muscle index, kg/m$^2$ | 7.83 ± 1.18 | 7.92 ± 1.21 | 0.77 |
| Handgrip strength,[3] kg | 30.9 ± 9.8 | 29.6 ± 10.1 | 0.63 |
| 400-m walk speed,[3] m/s | 1.36 ± 0.19 | 1.33 ± 0.16 | 0.53 |
| Time to complete 5 stands, s | 15.9 ± 4.7 | 13.5 ± 3.7 | 0.04 |
| Gait speed, m/s | 1.12 ± 0.26 | 1.07 ± 0.21 | 0.37 |
| Current smoker,[3] n (%) | 1 (3) | 4 (13) | 0.35 |
| Alcohol abstainers,[3] n (%) | 8 (28) | 7 (23) | 0.77 |
| Alcohol consumption among users,[4] servings/d | 1.7 ± 1.1 | 1.5 ± 0.8 | 0.44 |

[1]Values are means ± SD unless otherwise indicated.
[2]Significance level (2-sided P value) for comparison between groups by using independent Student's t test or Fisher's exact test (sex, origin, BMI group, current smoker, and alcohol abstainers).
[3]Intervention group, n = 29.
[4]Intervention group, n = 21; control group, n = 23.

**Table 3**: Dietary intake in intervention and control group during intervention (including supplements)[1]

| | Intervention group n = 30 | Control group n = 32 | *P* value [2] |
|---|---|---|---|
| Energy intake, kcal/d | 1823 ± 566 | 1662 ± 357 | 0.76 |
| Protein, g/d | 103 ± 29.0 | 75.4 ± 19.9 | <0.001 |
| Protein, g/kg BW/d | 1.11 ± 0.28 | 0.85 ± 0.24 | <0.001 |
| Protein, % of energy | 22.9 ± 3.4 | 18.3 ± 3.8 | <0.001 |
| Carbohydrate, % of energy | 42.0 ± 6.2 | 47.8 ± 5.0 | <0.001 |
| Fat, % of energy | 29.2 ± 4.0 | 29.3 ± 4.6 | 0.92 |

[1]Values are means 6 SDs; intake data at week 13. BW, body weight.
[2]Significance level of differences between groups by using mixed linear models with covariates sex and baseline value.

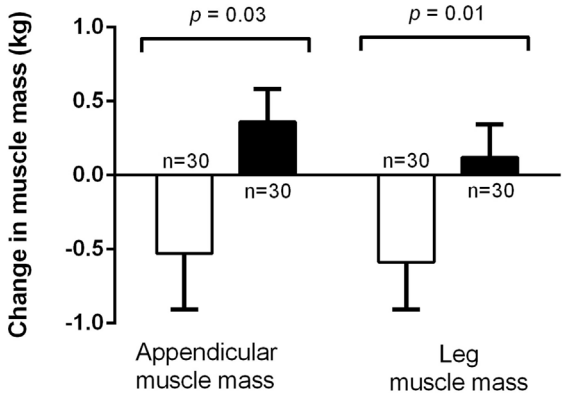


**Figure 2**: Change in appendicular muscle mass in intervention and control groups. Data represent mean changes over 13 wk with SEM. Intervention effect and significance level are based on ANCOVA with covariates sex and baseline value. White bars represent the control group; black bars represent the intervention group.

**Table 4**: Outcome measures for intervention and control group with intervention effect[1]

| | Intervention group | | | Control group | | | Intervention effect | |
|---|---|---|---|---|---|---|---|---|
| | Baseline (n) | Change (n) | *P* value | Baseline (n) | Change (n) | *P* value | Beta (95% CI) | *P* value |
| Body weight, kg | 96.7 ± 11.9 (32) | -3.4 ± 3.6 (32) | <0.001[2] | 93.2 ± 14.6 (33) | -2.8 ± 2.8 (33) | <0.001[2] | -0.37 (-1.68;0.94)[3] | 0.57[4] |
| BMI, kg/m$^2$ | 32.8 ± 3.1 (32) | -1.2 ± 1.3 (32) | <0.001[2] | 33.1 ± 4.3 (33) | -1.0 ± 0.9 (33) | <0.001[2] | -0.16 (-0.61;0.29)[3] | 0.49[4] |
| Waist circumference, cm | 111 ± 9.8 (32) | -4.4 ± 4.0 (32) | <0.001[2] | 109 ± 11 (33) | -3.7 ± 5.1 (33) | <0.001[2] | -0.69 (-2.72;1.34)[3] | 0.50[4] |
| Fat mass, kg | 38.6 ± 7.6 (30) | -3.2 ± 3.1 (30) | <0.001[5] | 38.5 ± 9.3 (30) | -2.5 ± 2.4 (30) | <0.001[5] | -0.70 (-2.09;0.69)[6] | 0.32[7] |
| Fat percentage | 40.8 ± 7.4 (30) | -2.3 ± 2.3 (30) | <0.001[5] | 41.4 ± 7.7 (30) | -1.6 ± 1.9 (30) | <0.001[5] | -0.62 (-1.64;0.40)[6] | 0.23[7] |
| Handgrip strength, kg | 31.3 ± 9.9 (31) | 2.0 ± 4.6 (31) | <0.001[2] | 29.1 ± 10.1 (32) | 2.2 ± 4.1 (32) | <0.001[2] | -0.01 (-1.7;1.68)[3] | 0.99[4] |
| 4-m gait speed, m/s | 1.12 ± 0.26 (29) | 0.11 ± 0.25 (29) | 0.003[2] | 1.04 ± 0.22 (32) | 0.11 ± 0.21 (32) | 0.007[2] | 0.02 (-0.09;0,12)[3] | 0.77[4] |
| 400-m walk speed, m/s | 1.37 ± 0.18 (27) | 0.04 ± 0.1 (27) | 0.007[2] | 1.33 ± 0.14 (31) | 0.05 ± 0.11 (31) | 0.002[2] | -0.004 (-0.057;0.049)[3] | 0.89[4] |
| Chair stand, s | 15.9 ± 4.7 (31) | -2.4 ± 4.0 (31) | <0.001[2] | 13.6 ± 3.8 (32) | -1.4 ± 3.1 (32) | <0.001[2] | 0.21 (-1.21;1.64)[3] | 0.76[4] |

[1]Values are means ± SDs.
[2]Significance level of estimate of change at week 13 by using mixed linear models with covariates sex and baseline value.
[3]Estimate of intervention effect at week 13 by using mixed linear models with covariates sex and baseline value.
[4]Significance level of estimate of group difference at week 13 by using mixed linear models with covariates sex and baseline value.
[5]Significance level of estimate of change at week 13 by using a paired *t* test.
[6]Estimate of intervention effect at week 13 by using ANCOVA with sex and baseline value as covariates.
[7]Significance level of estimate of group difference at week 13 by using ANCOVA with sex and baseline value as covariates.

## DISCUSSION

This trial is the first to show that use of a high whey protein–, leucine-, and vitamin D–enriched supplement preserves muscle mass during intentional weight loss by a hypocaloric diet combined with resistance exercise in obese older adults.

Weight loss treatment in older adults is still under discussion, due to the potential risk for permanent loss of muscle mass potentially impacting activities of daily life. Although data to support guidelines for weight loss treatment in older adults are limited, one of the main targets identified was the preservation of muscle mass by incorporating resistance exercise and increased protein consumption (1). At present the Recommended Dietary Allowance (RDA) for protein is 0.8 g/kg for all adults (25). Current expert opinion on protein requirements in the older adult or elderly population ranges from 1.0-1.2 g protein/kg BW/d (26). This implies that the intake of 0.8 g/kg BW/d during a hypocaloric diet is too low for maintenance of body protein mass (27). For overweight adults it has been shown that preservation of fat-free mass was more effective with a high-protein (1.2 g/kg BW/d) compared to a normal-protein (0.8 g/kg BW/d) diet (28). A recent guideline for treatment of obese elderly suggests that ingestion of 1.0 g/kg BW/d high-quality protein strategically timed at meals during a hypocaloric diet might be an approach to prevent major loss of muscle mass (1). We show preservation of skeletal muscle mass in obese older adults with an intake of 1.11 g protein/kg BW/d, thus supporting the recommendation described in this guideline.



Besides the total amount of protein intake per day, the amount of protein in 1 meal, as well as the quality of the protein in the meal seem relevant for muscle protein synthesis (8) and might explain our findings on muscle preservation in the intervention group. Several recent studies indicate that older adults are muscle anabolic resistant, which implies a blunted post prandial response to the anabolic stimuli from protein or amino acids compared with young adults (13, 29). However, providing older adults with a sufficient amount of protein or amino acid equivalent could still stimulate muscle protein synthesis (29, 30). Breen and Phillips (29) showed that the ingestion of at least 20 grams protein at once leads to a significant increase of muscle protein synthesis in older adults. In addition, protein quality has major effects on the efficacy to stimulate muscle protein synthesis. It has been shown that 20 g whey protein is more effective in stimulating postprandial muscle protein accretion than casein, casein hydrolysate, or soy protein in older men (14, 15). The whey-stimulating effects on muscle protein synthesis have been ascribed to its fast digestion, delivering amino acids in the circulation available for protein synthesis (31) and its high content of leucine, which is considered the most potent amino acid to stimulate muscle protein synthesis (32). The effect of leucine was corroborated by Wall et al. (16), showing that leucine co-ingestion with protein could further improve muscle

protein synthesis in older adults. In this study we therefore used a high whey protein-, leucine-enriched supplement to increase daily protein intake. The supplement was hypothesized to stimulate muscle protein synthesis in the older adult which could tip the balance towards preservation of muscle mass compared to the usual loss of muscle mass during intentional weight loss (33).

The intervention supplement used in this study also contained 800 IU of vitamin D. A low vitamin D status has been associated with impaired muscle mass and function in older adults (34), and vitamin D has also been suggested to have a positive impact on muscle protein metabolism (20, 21). Supplementing with vitamin D might therefore facilitate muscle mass preservation. However, the mechanism by which vitamin D positively affects muscle protein synthesis is not yet fully elucidated. The control supplement used in our study was matched for calories and not for specific nutrients, meaning that the observed effects should be attributed to the entire supplement, and effects of individual subcomponents cannot be determined.

Resistance exercise is a well-known facilitator that sensitizes the muscle, stimulates muscle protein synthesis, and promotes muscle hypertrophy in the older adult when performed frequently over time. Therefore, the combination of protein ingestion and resistance training enhancing muscle protein synthesis would be ideal to attenuate the loss of muscle mass (8, 35). Although in a different target group, a study with protein supplementation during a 24-wk progressive resistance exercise program in (pre) frail elderly indeed significantly increased lean mass compared to a control group (30).

Taken together there appears to be sufficient support to emphasize additional high-quality protein supplementation in combination with resistance exercise during a weight loss program to preserve muscle mass in older adults.

A limitation of this study was the high number of subjects (25%) not available for the analysis of the primary outcome, which could bias the results compared to an intention-to-treat analysis. Baseline characteristics of the dropouts were comparable to those subjects included in the final analysis, and dropout rate was equal in both groups. It is unknown to what extent this has influenced our findings.

Although the participants lost weight, the magnitude was below of what we expected. We advised a 600-kcal/day reduction in energy intake, which was not achieved based on the analyses of the 3-d food records. In addition, the accuracy of the 3-d food record in this study seems poor, because we observed large differences between baseline estimated energy need and baseline 3-d food records, which is not unknown and has been reported earlier (36). Our findings show that it is very difficult to reach and track -600-kcal/day

restriction in this target group. Dietary adherence seems strongly dependent on the counseling time with the dietician or the research setting available. Of several previous successful weight loss trials in overweight older adults (9, 37-41), 5 had weekly group sessions with a dietitian, and in 1 trial all meals were provided. In our study, the subjects visited the dietician only bi-weekly, which may have resulted in the limited weight loss observed.

Despite a muscle preserving effect of the supplement we did not observe differences between groups in muscle strength and physical performance. Overall, parameters for physical performance improved in both groups. Consistent with our findings, Tieland et al. (30) showed in their randomized controlled trial that protein supplementation in (pre) frail elderly increased muscle mass during resistance-type exercise without increasing physical functioning. Generally, during the first months of a resistance training program, a steep increase in muscle strength is seen as a result of improvements in neuromuscular activation and increases in muscle quality (42, 43). Furthermore, a study of Villareal et al. —a 1-y, randomized controlled trial, in which the independent and combined effects of weight loss and exercise were studied in obese older adults— showed that physical performance of older obese adults significantly improved in the weight-loss group (without exercise training), losing 9.7 kg over 1 y, even though lean body mass was lost (3.2 kg) (37). Interaction between weight loss and exercise training provided the largest improvement in physical functioning. The potential effect of preserved muscle mass attributable to the high whey-, leucine- and vitamin D-enriched supplement on physical function might therefore be masked by the effect of training and weight loss. We speculate that the effect of preserved skeletal muscle mass will likely contribute to improve strength and functioning as time progresses.



In conclusion, a high whey protein-, leucine-, and vitamin D-enriched supplement compared with an isocaloric control supplement as part of an intentional weight loss program, including a hypocaloric diet and resistance exercise, preserves skeletal muscle mass in obese older adults. These findings support the current advice to increase protein intake of high quality and sufficient quantity during a weight loss program in obese older adults to aid in the prevention of weight loss-induced sarcopenia.

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# Chapter 7

## Effect of a high protein diet and/or resistance exercise on the preservation of fat free mass during weight loss in overweight and obese older adults: a randomized controlled trial

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## ABSTRACT

**Background**

Intentional weight loss in obese older adults is a risk factor for accelerated muscle mass loss. We investigated whether a high protein diet and/or resistance exercise preserves fat free mass (FFM) during weight loss in overweight and obese older adults.

**Methods**

We included 100 overweight and obese adults (55–80 year) in a randomized controlled trial (RCT) with a 2 × 2 factorial design and intention-to-treat analysis. During a 10-week weight loss program all subjects followed a hypocaloric diet. Subjects were randomly allocated to either a high protein (1.3 g/kg body weight) or normal protein diet (0.8 g/kg), with or without a resistance exercise program 3 times/week. FFM was assessed by air displacement plethysmography.

**Results**

At baseline, mean (±SD) BMI was 32 ± 4 kg/m$^2$. During intervention, protein intake was 1.13 ± 0.35 g/kg in the high protein groups vs. 0.98 ± 0.29 in the normal protein groups, which reflects a 16.3 ± 5.2 g/d higher protein intake in the high protein groups. Both high protein diet and exercise did not significantly affect change in body weight, FFM and fat mass (FM). No significant protein*exercise interaction effect was observed for FFM. However, within-group analysis showed that high protein in combination with exercise significantly increased FFM (+0.6 ± 1.3 kg, $P = 0.011$).

**Conclusion**

A high protein diet, though lower than targeted, did not significantly affect changes in FFM during modest weight loss in older overweight and obese adults. There was no significant interaction between the high protein diet and resistance exercise for change in FFM. However, only the group with the combined intervention of high protein diet and resistance exercise significantly increased in FFM.

## BACKGROUND

Older adults represent the fastest growing population in Europe, but also in the rest of the world (1). The prevalence of obesity among this age group is 20–30% which has dramatically increased in the past decades (1). Obesity in older adults is a serious health problem related with multiple chronic health conditions and plays an important role in non-fatal disability (2), which in turn may contribute to lower quality of life (2).

Weight loss leads to metabolic and functional benefits (3). However, a potential drawback of weight loss in older adults is the accompanying loss of skeletal muscle mass (4), which in turn might accelerate the development of sarcopenia (5). Strategies to reduce the loss of skeletal muscle mass during weight loss include resistance exercise and sufficient intake of high quality protein (6, 7). Resistance exercise stimulates muscle protein synthesis, which in turn supports muscle mass preservation and muscle function (8). In addition, high dietary protein intake has been shown to stimulate muscle protein synthesis in older adults (1, 9-11). Several studies indicate that, in contrast to young adults, older adults might be resistant to anabolic stimuli from protein, which implies a blunted post prandial response (12, 13).

The number of weight loss trials in overweight or obese older adults is limited, and trials combining resistance exercise with a high protein diet are scarce (14). We previously studied the effect of a high whey protein-, leucine- and vitamin D-enriched supplement on muscle mass preservation during a 13-week weight loss program including 3 times/week resistance exercise in obese older adults (15). Subjects in the intervention group received a supplement containing 21 g whey protein (10 servings/wk), whereas the control group received an isocaloric control supplement. This study showed that the intervention group significantly preserved their muscle mass compared to the control group with an effect size of 0.95 kg (95% CI: 0.09;1.81).

Generally, dieticians give dietary advice regarding weight loss treatment based on regular foods, not including any specific supplements. Porter-Starr et al. (16) recently evaluated the effect of a high protein hypocaloric diet using meal-based protein foods in obese older adults over a 6-month period. They found a positive effect on physical performance, but no significant effect on fat free mass (FFM). No studies so far have evaluated the effects of a high protein diet using regular foods with or without resistance exercise on the preservation of FFM during weight loss in older overweight and obese subjects. In the present study we therefore evaluated the effects of a high protein diet and/or resistance training on preservation of FFM, fat mass (FM) loss, waist circumference loss and improvement of handgrip strength and physical performance during a 10-week weight loss trial in overweight and obese adults aged 55 years and over.

# METHODS

## Subjects

Overweight and obese men and women (≥55 y) with BMI ≥ 28 kg/m$^2$, or BMI>25 kg/m$^2$ with waist circumference > 88 cm (women) or > 102 cm (men), were recruited from the Amsterdam area through local flyers and advertisements. Potential subjects were excluded when they had participated in any weight loss program three months prior to screening; when participation in the resistance training program was considered unsafe according to a physiotherapist; or when they were not able to comply with the full study protocol. All women were postmenopausal and did not use hormone replacement therapy. A full description of the eligibility criteria is online available in the Dutch Trial Register (NTR4556, www.trialregister.nl). The study was approved by the Medical Ethics Committee Independent Review Board Nijmegen, Netherlands (NL43226.072.14) and written informed consent was obtained from all subjects. The study took place from May 2014 through December 2014 at the Amsterdam University of Applied Sciences in The Netherlands.

## Design and randomization procedures

We performed a 10-week randomized controlled trial with a 2-by-2 factorial design combining the factors 'high protein diet' and 'resistance exercise'. Eligible subjects were randomly allocated to either the control group (C) receiving a hypocaloric normal protein dietary advice, the high protein diet group (Pr) receiving a hypocaloric high protein dietary advice, the exercise group (Ex) receiving a hypocaloric normal protein dietary advice with an exercise program, or to the high protein diet and exercise group (PrEx) receiving both a hypocaloric high protein dietary advice and an exercise program. Randomization envelopes with four different codes stratified by gender were generated using a random number generator by the study coordinator. Body composition, waist circumference, handgrip strength and physical performance were assessed at study baseline and after 5 and 10 weeks of intervention.

## Hypocaloric diet and protein advice

All subjects followed a hypocaloric diet of 600 kcal below estimated energy needs (17). Energy needs were estimated by multiplying measured resting energy expenditure using indirect calorimetry (Vmax Encore n29; Viasys Healthcare, Houten, the Netherlands) with the estimated physical activity level using a 3-day physical activity record. Prescribed dietary protein intake was 0.8 g/kg body weight (BW) for the normal protein dietary advice, and 1.3 g/kg for the high protein dietary advice (using current BW for BMI < 30 kg/m$^2$ or using BW at BMI 27.5 kg/m$^2$ for those with a BMI ≥ 30 kg/m$^2$) (18). For each subject the amount of energy (kcal) and protein (g) was calculated and incorporated in the dietary advice, which was given at study baseline, together with a specific food variation list for

either the high protein or the normal protein diet. Foods were not provided. During intervention, subjects of all groups received five dietary consultations; two times during a face-to-face visit at week 5 and 9, and three times by telephone in week 2, 4 and 7. Dietary intake was assessed by a 3-day food record at baseline, after 5 and 10 weeks of intervention. Intake after 5 and 10 weeks was used to evaluate compliance to the prescribed diet. Food records were checked for completeness during study visits and additional information about unclear items or amounts was obtained. Total energy and macronutrient intakes were calculated using a computerized Dutch Food Composition Table (19).

## Exercise program

The exercise program involved resistance training 3 days a week for 1-h sessions. The training started with a 10-min warming up followed by two sets of 50 s of the following exercises: squats, lunges, chest press, shoulder press, biceps curls, triceps extensions, standing rows, step-ups and crunches. During the 10-week period the number of sets was gradually increased from 2 – 3 set for all exercises, the time to perform the exercises increased from 50 –75 s, and resistance was increased by using dumbbells, elastic bands, medicine balls and a step bench. The training ended with 5-min cooling down. The exercise program was developed by certified trainers and a physiotherapist and training sessions were supervised by certified trainers. Attendance to the training sessions was recorded by the trainer.

## Measurement of body composition, waist circumference, handgrip strength and physical performance

Body composition including FFM (primary outcome) and FM was determined using air displacement plethysmography (BODPOD, Life Measurement Inc., Concord, CA). BW was measured on the calibrated scale as part of the BODPOD system. Waist circumference was measured in a standing position halfway between the anterior superior iliac spine and the lower rib after normal expiration. Handgrip strength was measured with an isometric handgrip dynamometer (JAMAR 5030 J1, Sammons Preston Rolyan, Bollingbrook, CA) while the subject was seated with the elbow flexed at 90°. Three consecutive measures of handgrip strength (kg) at both hands were recorded to the nearest 0.1 kg and the sum of the maximum value of left and right hand was calculated. Physical performance was assessed with a 400-m gait speed test (m/s) (20), a 4-m gait speed test (fastest of 2 repetitions of usual gait speed, (m/s)), and a chair stand test (s) (21).

## Statistical analysis

Double-data entry was performed and discrepancies were checked and adjusted. Statistical analyses were performed with FFM change as primary outcome. A sample size of n = 21 per study group, n = 84 in total, provided 80% power to detect an absolute difference of 0.5 kg FFM with SD 0.4 kg and $P < 0.05$ (2-sided) (22, 23).

Subject characteristics and dietary intake at baseline were compared between groups using an independent samples t-test or the Fisher Exact test. Intention-to-treat analysis was performed using last observations carried forward for subjects with missing week 5 and/or week 10 measurements. Between group differences on outcome variables were analysed using a mixed linear model including time, protein (high/normal), exercise (yes/no) as fixed factors, subject as random factor and sex and baseline value of the outcome variable as covariates. For all outcome variables the interaction for protein*exercise was tested. This interaction tested whether the effect in the exercise groups is dependent on whether the subjects received the high or the normal protein diet (and vice versa). Within group changes over 10 weeks were estimated using a paired t-test.

Statistical analyses were performed using SPSS software (version 22.0, IBM). Data in text and tables are expressed as means with SD, unless stated otherwise. Statistical significance was defined as a two-tailed $P < 0.05$.

# RESULTS

## Subjects

We randomized 122 subjects into the four study groups. Before the baseline visits 22 subjects declined study participation for personal reasons. The number of subjects screened, excluded, randomized, and included in the analysis is shown in **Figure 1**. Mean age of the study population was 62.4 ± 5.4 y, 36% was male, mean BMI was 32.2 ± 4.3 kg/m$^2$ and 66% was obese. There were no relevant differences in subject's characteristics between the study groups at study baseline (**Table 1**). Of the 100 subjects with a baseline visit, 32 subjects dropped-out during the study because of adverse events not related to the study (n = 7), adverse events related to the study (n = 1, lash), personal reasons (n = 14), or unknown reasons (n = 10).

## Dietary intake and adherence to exercise program

There were no differences between groups in selfreported mean dietary intake at baseline and the energy reduction during treatment (**Table 2**). Protein intake during the trial was 1.13 ± 0.35 g/kg/d in the high protein groups. Protein intake was on average 87% of the protein target of 1.3 g/kg/d, with 29% of the subjects reaching this target. In the normal protein groups the protein intake during the trial was 0.98 ± 0.29 g/kg/d, which was on average 123% of the protein target, with 78% of the subjects reaching the protein target of 0.8 g/kg/d. The high protein groups had on average a 16.3 ± 5.2 g/d higher protein intake during intervention ($P = 0.002$) compared to the normal protein groups. With respect to the exercise program, mean adherence was 2.8 ± 0.3 times/week.

## Effects on body weight, waist circumference, FFM and FM

The 10-week weight loss trial resulted in a significantly decreased BW, waist circumference and FM in all groups. Overall loss in BW was -2.1 ± 2.6 kg, without significant effects of protein and exercise. Comparable results were observed for changes in waist circumference, where a mean decrease of -4 ± 4 cm was observed with no significant effects of protein and exercise (**Table 3**). **Figure 2** shows that the intervention did not significantly affect change in FFM, with exception of the high protein-exercise group which showed a significant increase in FFM of 0.6 ± 1.3 kg ($P = 0.011$). There was no significant effect of high protein and exercise on change in FFM and FM, but exercise significantly decreased body fat percentage with 0.8% ($P = 0.048$). There was no significant protein*exercise interaction for FM and FFM.

## Effects on handgrip strength and physical performance

No significant change in handgrip strength was observed over time, whereas all physical performance tests improved over time. However, no significant effects of protein and exercise on handgrip strength and physical performance tests were observed (Table 3). There was a significant interaction for protein*exercise for handgrip strength ($P = 0.030$) and 4-m gait speed ($P = 0.045$), indicating that combining a high protein diet with exercise had greater positive effects on handgrip strength and 4-m gait speed than high protein diet or exercise only (Table 3).

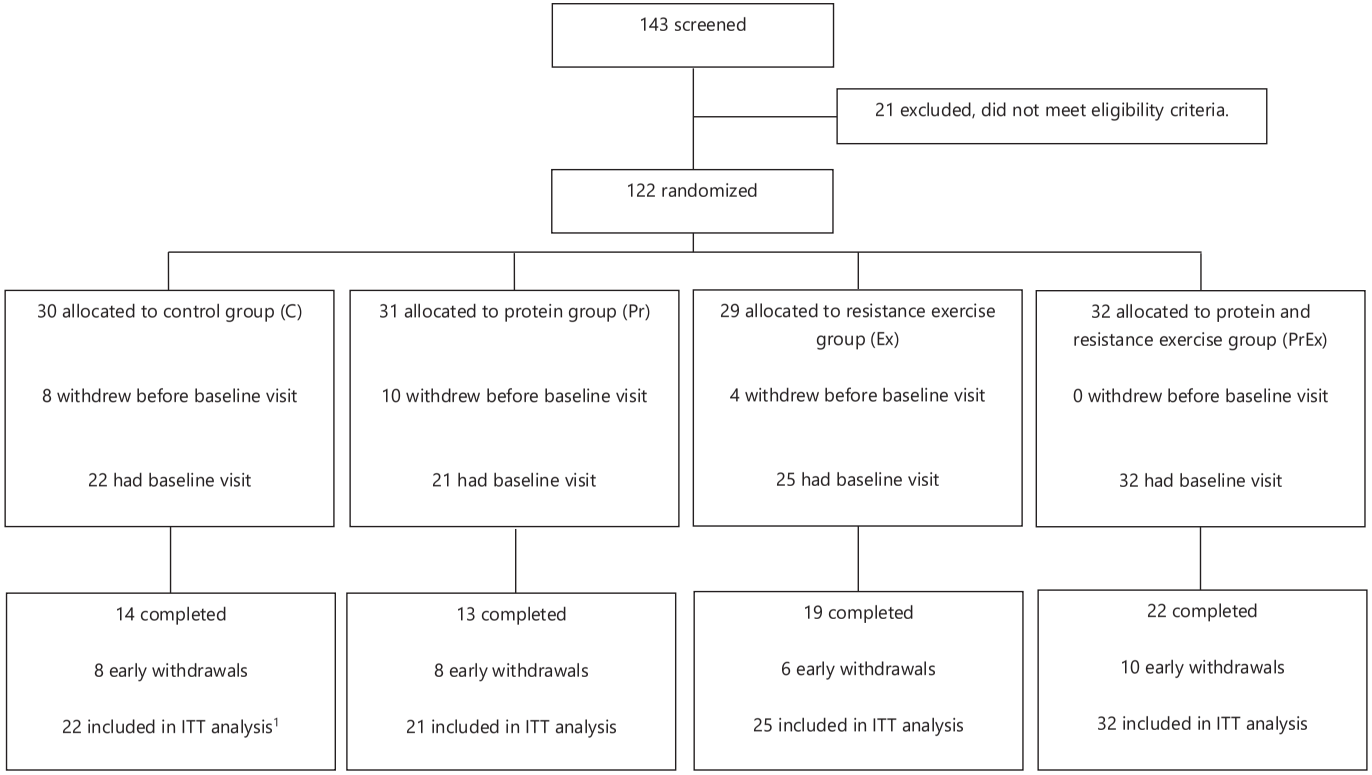


**Figure 1**: Flow chart of number of subjects screened, randomized, completed intervention and included in the analysis. 1 For one subject no body composition data were available at baseline, therefore n = 21 subjects were included in the intention to treat (ITT) analysis for the primary outcome fat free mass

**Table 1:** Baseline characteristics of 100 obese older subjects by treatment[a]

| Characteristic | Control n = 22 | Protein n = 21 | Exercise n = 25 | Protein + Exercise n = 32 | *P* value[b] |
|---|---|---|---|---|---|
| Sex, n (% male) | 6 (27%) | 8 (38%) | 9 (36%) | 13 (41%) | 0.786 |
| Origin, % Caucasian | 82% | 86% | 68% | 84% | 0.418 |
| Age, y | 63.4 ± 4.3 | 61.9 ± 6.1 | 63.1 ± 6.0 | 61.5 ± 5.1 | 0.529 |
| Body weight, kg | 92.7 ± 5.1 | 93.0 ±15.3 | 90.7 ± 14.7 | 93.5 ± 14.4 | 0.912 |
| BMI, kg/m$^2$ | 33.2 ± 4.8 | 32.1 ± 4.6 | 32.2 ± 4.7 | 31.6 ± 3.4 | 0.584 |
| BMI ≥ 30 kg/m$^2$, n (%) | 16 (73%) | 13 (62%) | 16 (64%) | 21 (66%) | 0.886 |
| Waist circumference, cm[c] | 110 ± 13 | 110 ± 12 | 107 ± 13 | 107 ± 9 | 0.761 |
| Fat mass, %[d] | 45.3 ± 8.2 | 44.7 ± 8.5 | 43.2 ± 8.7 | 41.6 ± 7.8 | 0.383 |
| Fat free mass, kg[d] | 51.0 ± 13.1 | 51.2 ± 10.4 | 51.5 ± 11.5 | 54.8 ± 12.7 | 0.584 |
| Handgrip strength, kg[e] | 62.2 ± 22.0 | 65.2 ± 17.4 | 70.1 ± 21.8 | 73.9 ± 24.3 | 0.234 |
| 4-m gait speed, m/s[f] | 1.17 ± 0.33 | 1.32 ± 0.28 | 1.28 ± 0.22 | 1.25 ± 0.18 | 0.284 |
| 400-m gait speed, m/s[f] | 1.40 ± 0.17 | 1.42 ± 0.20 | 1.49 ± 0.21 | 1.51 ± 0.22 | 0.133 |
| Time to complete 5 stands, s[f] | 13.5 ± 3.2 | 12.6 ± 3.1 | 11.1 ± 3.0 | 11.7 ± 3.4 | 0.058 |

[a]Data are presented as means ± SD or as number (percentage); [b]Significance level (two-sided *P*-value) for comparison between groups using One-Way ANOVA or Chi-square test (sex, origin and BMI group); [c]n protein and exercise group = 31; [d]n control group = 21; [e]Sum of maximum of left and right hand; n protein group = 20; [f]n exercise group = 24

**Table 2:** Dietary intake in the study groups at baseline and during intervention[a]

| | | Control | Protein | Exercise | Protein + Exercise | P-value[b] | Protein groups | Control groups | *P* value[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | n = 20 | n = 21 | n = 25 | n = 31 | | n = 52 | n =45 | |
| Energy, kcal/d | baseline | 1928 ± 849 | 1932 ± 539 | 1877 ± 522 | 2061 ± 621 | 0.730 | 2009 ± 587 | 1900 ± 678 | 0.397 |
| | during intervention | 1650 ± 531 | 1726 ± 449 | 1569 ± 463 | 1784 ± 579 | 0.452 | 1761 ± 526 | 1605 ± 490 | 0.137 |
| Protein, g/day | baseline | 85.7 ± 31.0 | 82.6 ± 21.4 | 82.6 ± 23.4 | 93.2 ± 31.2 | 0.425 | 88.9 ± 27.9 | 83.9 ± 26.7 | 0.372 |
| | during intervention | 76.6 ± 21.1 | 89.3 ± 22.6 | 73.9 ± 22.4 | 92.8 ± 32.9 | 0.025 | 91.4 ± 29.0 | 75.1 ± 21.6 | 0.002 |
| Protein, g/kg/day | baseline | 0.95 ± 0.36 | 0.92 ± 0.34 | 0.93 ± 0.30 | 1.00 ± 0.31 | 0.825 | 0.97 ± 0.32 | 0.94 ± 0.33 | 0.662 |
| | during intervention | 0.87 ± 0.29 | 1.02 ± 0.36 | 0.84 ± 0.23 | 1.02 ± 0.35 | 0.081 | 1.02 ± 0.35 | 0.86 ± 0.26 | 0.008 |
| Protein, g/adj_kg/day[d] | baseline | 1.12 ± 0.45 | 1.04 ± 0.30 | 1.08 ± 0.36 | 1.14 ± 0.39 | 0.820 | 1.10 ± 0.35 | 1.10 ± 0.39 | 0.972 |
| | during intervention | 1.00 ± 0.27 | 1.13 ± 0.33 | 0.97 ± 0.32 | 1.13 ± 0.37 | 0.177 | 1.13 ± 0.35 | 0.98 ± 0.29 | 0.027 |
| Protein, en%[e] | baseline | 18.5 ± 3.3 | 17.6 ± 3.4 | 17.9 ± 3.7 | 18.3 ± 3.6 | 0.838 | 18.0 ± 3.5 | 18.2 ± 3.5 | 0.814 |
| | during intervention | 18.9 ± 2.2 | 21.1 ± 3.5 | 18.9 ± 3.0 | 21.2 ± 4.6 | 0.033 | 21.1 ± 4.1 | 19.0 ± 2.6 | 0.002 |
| Carbohydrate, en% | baseline | 44.2 ± 5.2 | 43.3 ± 6.5 | 45.3 ± 6.0 | 43.7 ± 8.6 | 0.760 | 43.5 ± 7.7 | 44.8 ± 5.7 | 0.343 |
| | during intervention | 46.2 ± 5.8 | 40.4 ± 6.1 | 47.1 ± 6.4 | 43.3 ± 6.2 | 0.002 | 42.2 ± 6.3 | 46.7 ± 6.1 | 0.001 |
| Fat, en% | baseline | 33.1 ± 6.3 | 34.1 ± 5.6 | 32.3 ± 6.9 | 32.8 ± 7.7 | 0.832 | 33.3 ± 6.9 | 32.6 ± 6.6 | 0.625 |
| | during intervention | 30.9 ± 5.7 | 33.3 ± 5.7 | 29.2 ± 6.9 | 30.6 ± 7.1 | 0.214 | 31.7 ± 6.7 | 30.0 ± 6.4 | 0.204 |

[a]Data represent means ± SD using last observations carried forward; Intake during intervention is mean intake data at week 5 and 10; [b]Significance level of differences between four groups using One-Way ANOVA; [c]Significance level of differences between protein and non-protein groups using the t-test; [d] Protein in g/kg/day with adjusted weight using current weight for BMI < 30 kg/m$^2$ or using weight at BMI 27.5 kg/m$^2$ for BMI ≥ 30 kg/m$^2$, to make it comparable to the protein target; [e] en% stands for % of energy intake

**Table 3**: Change in outcome measures at 10 weeks of intervention with protein and exercise effects[a]

| | Control n = 22 | Protein n = 21 | Exercise n = 25 | Protein + Exercise n = 32 | Protein effect | | Exercise effect | | Protein * Exercise interaction | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Beta (95% CI)[b] | *P*[c] | Beta (95% CI)[b] | *P*[c] | Beta (95% CI)[d] | *P*[e] |
| Body weight, kg | -1.7 ± 1.8* | -2.1 ± 3.6* | -2.6 ± 2.9* | -2.0 ± 2.2* | +0.1 (-0.7;1.0) | 0.763 | -0.3 (-1.1;0.5) | 0.472 | NS | |
| BMI, kg/m$^2$ | -0.6 ± 0.6* | -0.8 ± 1.1* | -1.0 ± 1.0* | -0.9 ± 0.9* | -0.0 (-0.3;0.3) | 0.924 | -0.2 (-0.5;0.1) | 0.213 | NS | |
| Waist circumference, cm | -3 ± 4* | -3 ± 4* | -4 ± 4* | -3 ± 3*[f] | +0.3 (-0.9;1.4) | 0.673 | -0.4 (-1.6;0.8) | 0.555 | NS | |
| Fat mass, kg | -1.5 ± 2.5*[g] | -2.1 ± 3.4* | -2.8 ± 3.7* | -2.6 ± 2.4* | -0.0 (-1.0;0.9) | 0.946 | -0.8 (-1.7;0.2) | 0.124 | NS | |
| Fat percentage, % | -1.0 ± 2.1*[g] | -1.3 ± 2.2* | -1.9 ± 3.3* | -2.1 ± 2.0* | -0.1 (-0.9;0.6) | 0.736 | -0.8 (-1.6;-0.0) | 0.048 | NS | |
| Fat free mass, kg | -0.0 ± 1.4[g] | 0.0 ± 1.5 | +0.2 ± 2.3 | +0.6 ± 1.3* | +0.1 (-0.4;0.7) | 0.666 | +0.3 (-0.2;0.9) | 0.233 | NS | |
| Handgrip strength, kg[h] | +1.8 ± 6.6 | -1.7 ± 6.5 | -1.8 ± 11.6 | +2.0 ± 6.0 | -2.2 (-6.4;2.1) | 0.311 | -1.9 (-6.0;2.1) | 0.346 | 6.2 (0.6;11.8) | 0.030 |
| 4-m gait speed, m/s | +0.13 ± 0.24* | +0.08 ± 0.26 | +0.08 ± 0.13*[9] | +0.20 ± 0.24* | -0.04 (-0.15;0.06) | 0.440 | -0.04 (-0.14;0.06) | 0.476 | 0.14 (0.00;0.28) | 0.045 |
| 400-m gait speed, m/s | +0.04 ± 0.15 | +0.07 ± 0.10* | +0.07 ± 0.07*[9] | +0.08 ± 0.15* | +0.02 (-0.03;0.06) | 0.445 | +0.01 (-0.03;0.06) | 0.554 | NS | |
| Repeated chair stands, s | -1.6 ± 2.1* | -1.6 ± 1.7* | -1.0 ± 2.7[9] | -1.4 ± 2.7* | -0.1 (-0.9;0.6) | 0.703 | -0.2 (-0.9;0.6) | 0.643 | NS | |

[a]Data represent means ± SD using last observations carried forward ; [b]Estimate of protein or exercise effect at week 10 using mixed linear model including time, sex, protein (high/normal), exercise (yes/no) and baseline value; [c]Significance level of estimate of protein or exercise effect at week 10 using mixed linear model; [d]Estimate of interaction effect of protein*exercise at week 10 using mixed linear model including time, sex, protein (high/normal), exercise (yes/no), protein*exercise and baseline value only presented when the interaction effect was significant (P < 0.1). The effect of the combined protein-exercise intervention can be calculated by summating the beta's of the protein effect, the exercise effect and the interaction protein*exercise effect; [e]Significance level of the interaction effect of protein*exercise at week 10 using mixed linear model; [f]n = 31; [g]n = 21; [h]Sum of maximum of left and right hand, n protein group = 20; [9] n = 24; * Significant mean change at week 10 within group using a paired t-test; NS not significant ($P \geq 0.1$)

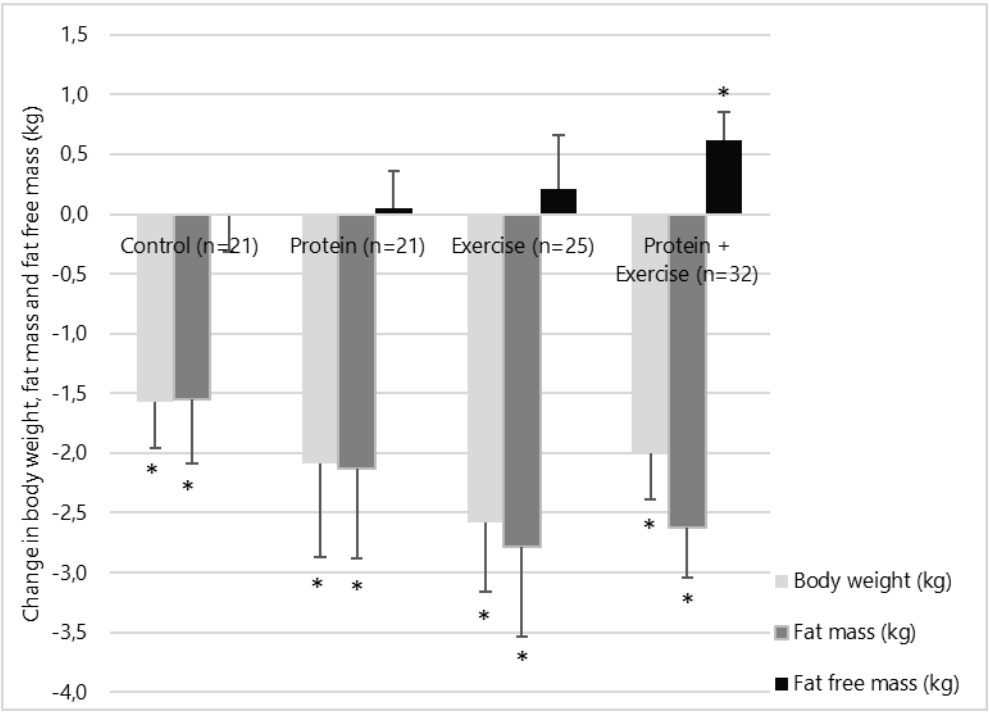


**Figure 2**: Change in body weight, fat mass and fat free mass in the four study groups. Data represent mean changes over 10 weeks with SEM using last observations carried forward for subjects with missing week 5 and/or week 10 measurements. * indicates within group change using a paired t-test

## DISCUSSION

In the present randomized controlled trial in overweight and obese older adults during weight loss, we observed no significant effect of the high protein diet (although at a lower level than targeted) and resistance exercise on FFM preservation and no statistically significant interaction between high protein and resistance exercise. However, only in the group with the combined intervention of high protein diet and resistance exercise program, FFM significantly increased.

The recommended dietary allowance (RDA) for protein is 0.8 g/kg/d and is age-independent (24). However, the recent expert opinion on protein requirements of older adults is higher, and recommended protein intake ranges from 1.0 – 1.2 g/kg/d (25). Specific recommendations for obese older adults during weight loss do not exist. Weijs et al. (26) showed that protein requirements under the challenged conditions of weight loss

may be substantially higher than 0.8 g/kg/d, and are probably even higher than 1.2 g/kg/d in order to preserve muscle mass.

In this study we demonstrated that it is difficult to reach a 1.3 g/kg/d protein intake using a hypocaloric high protein diet based on regular food products (mean intake was 1.13 g/kg/d).

Although subjects in the high protein groups had a 16 g per day higher protein intake compared to the normal protein groups (mean intake was 0.98 g/kg/d), the difference in protein intake might have been too small in order to detect an effect on preservation in FFM. Previously, we studied the effect of a high-whey protein, leucine and vitamin D supplement during weight loss on muscle mass preservation in older obese adults (15). In that study, the difference in protein intake was 28 g/d with an intake of 1.11 g/kg/d in the intervention group and 0.85 g/kg/d in the control group. This difference resulted in a muscle preserving effect of 0.95 kg. However, besides the difference in protein intake, also other components of the supplement, including leucine, vitamin D and other micronutrients might explain the effect on preservation of FFM in that study.

Two other possible explanations for the absence of a high-protein effect on FFM preservation in the present study should be considered. Firstly, older adults might require a minimum threshold of protein with one eating moment to raise muscle protein synthesis levels. Previous studies showed that a minimal amount of 20 g of high quality protein per meal is needed to stimulate protein synthesis above baseline levels (27). In our former study, the protein supplement was, ten times per week, supplied as 21 g protein at once (15). In the current study, only 39% of the subjects in the high protein groups had in total at least one eating moment with ≥ 20 g protein over the recorded days during intervention (week 5 and 10).



A second explanation for the absence of a high-protein effect on FFM preservation is the protein composition of the diet. Whey protein has been shown to be very effective in stimulating postprandial muscle protein accretion in older men (28, 29), which has been ascribed to its fast digestion and to the high leucine content. Since we did not focus on specific types of proteins during dietary counseling it is likely that the amount of leucine known to stimulate muscle protein synthesis (at least 2 g per meal (12)) for older adults was not reached for most subjects in our study.

We observed no overall exercise effect, except for relative fat mass (Table 3). However, when analysing the interaction between gender and exercise a significant interaction for FFM with beta +1.1 kg (95%–CI: –0,0;2,3) was shown, indicating that FFM in males responds

stronger to the exercise program than FFM in females. This is in line with expectations based on literature (30).

We observed a significant improvement in physical performance during 10-weeks intervention in all groups. We did, however, not observe an additional improvement in physical performance as a results of higher protein intake or resistance training. A suggested explanation could be that the observed FM loss overruled the possible effects of improvements in physical functioning due to high protein and exercise (31).

Previous studies have shown that on average 25–30% of weight loss is lean mass in older obese adults (14). In our study, all groups including the control group preserved their FFM. It could be speculated that subjects in the control group increased their level of physical activities and sports activities themselves to compensate for the fact that they were not allowed to participate in the exercise group training sessions. A slight increase in physical activity level during intervention was observed for all groups, and this was not different between the groups, which could partly explain the FFM preservation even in the control group. Another explanation could be the relatively high intake of protein in the control groups (average was 0.98 g/kg), which further reduced the protein intake contrast between groups and might have been beneficial for FFM preservation.

A limitation of this study is the unequally distributed number of subjects that withdrew from participation in the study groups before the baseline measurements. Group allocation could be a reason for declining further participation. Another limitation was the lower than expected magnitude of weight loss, which can be partly explained by the preservation of (C, Pr, Ex groups) or gain (PrEx group) in FFM. Furthermore, we advised a -600 kcal/d reduction in energy intake, which was not achieved based on the analyses of the 3-d food records. Most of previous successful weight loss trials in overweight older adults (14) had weekly group sessions with a dietitian. In our study, the subjects had a bi-weekly consultation, which may also have resulted in the limited weight loss observed. Since the amount of weight loss is modest, the change in FFM is also small. Additionally, the duration of the study might have been too short to achieve sufficient weight loss for group differences in FFM preservation due to protein intake to manifest. Finally, our study was designed and powered to find an effect of protein on FFM with a 0.5 g/kg/d difference between groups; however, only a 0.15 g/kg/d difference in protein intake was achieved, therefore making it difficult to draw firm conclusions regarding a higher versus control protein intake during weight loss with or without resistance exercise.

In conclusion, the lower than targeted protein intake of 1.13 g/kg/d obtained by consuming regular protein rich foods did not significantly affect FFM and FM change during modest weight loss in older overweight and obese subjects. There was no significant interaction

between the high protein diet and resistance exercise for FFM. However, only the group with the combined intervention of the high protein diet and the resistance exercise program significantly increased in FFM. This suggests that combining protein with resistance exercise is beneficial for FFM preservation during weight loss in older adults, which should be confirmed by future studies using a larger protein contrast.


## Acknowledgments

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## Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## Authors' contributions

The authors' contributions were as follows: PJMW and AMV: designed the research (project conception, development of overall research plan, and study oversight); SEvdP, AMV, RGM: conducted the research (hands-on conduct of the experiments and data collection); AMV, MFE, PJMW analyzed the data or performed the statistical analysis; AMV, MFE, RGM, MV, and PJMW wrote the manuscript; and PJMW: had primary responsibility for the final content. AMV, MFE, RGM, SEvdP, MV and PJMW had no conflict of interest. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

## Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee Independent Review Board Nijmegen, Netherlands (NL43226.072.14) and written informed consent was obtained from all subjects.

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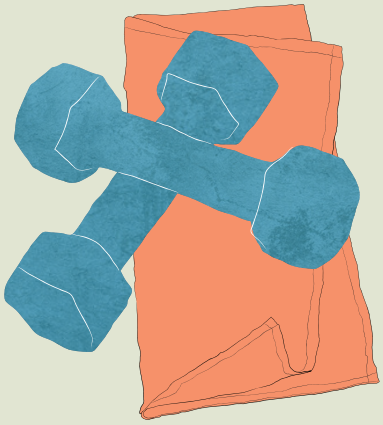

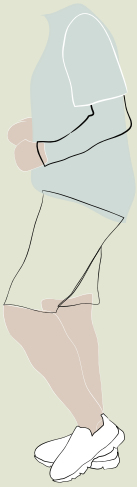

# Part 4

## Towards optimal treatment

# Chapter 8

*Review*

## Exercise and Nutrition Strategies to Counteract Sarcopenic Obesity

Inez Trouwborst, Amely Verreijen, Robert Memelink, Pablo Massanet, Yves Boirie, Peter Weijs and Michael Tieland

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## ABSTRACT

As the population is aging rapidly, there is a strong increase in the number of individuals with chronic disease and physical limitations. The decrease in skeletal muscle mass and function (sarcopenia) and the increase in fat mass (obesity) are important contributors to the development of physical limitations, which aggravates chronic diseases prognosis. The combination of the two conditions, as referred to as sarcopenic obesity, amplifies the risk for these negative health outcomes, demonstrating the importance of preventing or counteracting sarcopenic obesity. One of the main challenges is the preservation of skeletal muscle mass and function, while simultaneously reducing fat mass in this population. Exercise and nutrition are two key components in the development, but also prevention and treatment of sarcopenic obesity. The main aim of this narrative review is to summarize the different, both separate and combined, exercise and nutrition strategies to prevent and/or counteract sarcopenic obesity. This review therefore provides a current update of various exercise and nutritional strategies to improve contrasting body composition changes and physical functioning in sarcopenic obese individuals.

## INTRODUCTION

The world population is aging rapidly. In 2050, it is predicted that about 22% of the total population will be older than 60 years, and around 5% will be older than 80 years (1). In line with the aging of society, the prevalence of different health problems rapidly increases. In the western world, about 42% of older adults (≥ 55 years) have problems with performing activities of daily living and approximately 85-90% need medication for chronic diseases, such as type 2 diabetes, high blood pressure or cardiovascular disease (2, 3). As a result, the risk of falls, institutionalization, loss of independence and premature death has gradually increased in the last decennia. In addition, these burdens increase the demand on our health care system and result in a tremendous health care cost.

The contributing factors to physical limitations with increasing age are highly multifactorial. One major contributor, however, is the reduced skeletal muscle mass with age, which is accompanied by the impaired skeletal muscle function, which is referred to as sarcopenia (4). Obesity, or the increase in fat mass, is another major contributor to physical limitations in older adults (5). The coexistence of sarcopenia and obesity, known as sarcopenic obesity, has an even more detrimental effect on physical limitation, as they act in a synergistic manner (6, 7). Furthermore, sarcopenic obesity has also been reported to increase the risk for metabolic disturbances, which is detrimental for several cardio-metabolic chronic diseases (8). Prevention and/or treatment of sarcopenic obesity is therefore of major relevance for public health and individual healthy aging. Exercise strategies have been largely linked to improvements in the parameters of sarcopenia and obesity. Additionally, nutritional strategies have the potential to improve body composition parameters in sarcopenic and obese older adults. The main aim of this narrative review is to provide a current update of various exercise and nutritional strategies to prevent and/or counteract sarcopenic obesity in older adults.

## METHODS NARRATIVE REVIEW

PubMed and Google Scholar were searched in order to identify relevant articles. The search was performed between June 2017 and March 2018. The search strategy consisted of the Boolean operator "AND" to combine the following concepts:

- Sarcopenic obesity, body composition, and aging
- Nutrition and diet
- Exercise and physical activity

All of the relevant keyword variations that were used for these main concepts in the search strategy used the Boolean operator "OR". Search results were limited to human nutrition and/or exercise intervention studies, which were aimed at improving the body composition and physical performance parameters related to sarcopenic obesity. Acute (one-day) studies were not included. Studies with older adults were included, which were defined as individuals above the age of 55 years. Both English and Dutch articles were included.

## DEFINING SARCOPENIC OBESITY

Sarcopenic obesity has been defined as a combination of low skeletal muscle mass and high fat mass or bodyweight, which is also illustrated in the definitions that are used for both of the conditions (9). Different methods are used to characterize both sarcopenia and obesity. For instance, obesity has been defined using body mass index (BMI), skinfold measurements, or fat mass (10-14), and sarcopenia has been defined using skeletal muscle mass (10, 15, 16) or skeletal muscle mass, combined with force and/or performance (4, 17).

Difficulties with defining sarcopenic obesity have been mainly as a result of contrasting body composition change. While body weight and BMI remain relatively unchanged with increasing age, absolute skeletal muscle mass is decreased and the (visceral) fat mass is increased (4). The sole use of weight or BMI for diagnoses of sarcopenic obesity can therefore lead to a misinterpretation of the condition (18). Potentially, misaligned treatment methods may be applied, which may lead to a worsening of the condition. Importantly, defining sarcopenic obesity should always include a combination of methods, including measuring body fat, skeletal muscle mass, and ideally also muscle strength. Sarcopenic obesity is an age-related disease and will therefore, in this review, be defined as present in older adults, which includes individuals at the age of 55 and older.

The prevalence of sarcopenic obesity varies according to the definitions and the methods used for this definition. Using the dual-energy X-ray absorptiometry (DXA), the prevalence of sarcopenic obesity was 2% between individuals 60-69 years of age, and 10% for individuals over 80 years of age (10). If using the Relative Skeletal Muscle Index (RSMI), 8.9% of men and 7.1% of women were sarcopenic obese (19), but others using appendicular lean mass (ALM)/BMI found an even higher variable prevalence in the US population from 16% to 40% (20).

These difference in defining sarcopenic obesity lead to difficulties in comparing effectiveness of strategies that target the sarcopenic obesity. Therefore, this review focuses primarily on improvements in skeletal muscle mass, muscle strength, physical

performance, fat mass and waist circumference, which are parameters that are considered important when studying sarcopenic obesity (6).

## ETIOLOGY OF SARCOPENIC OBESITY

### Age-related changes in body composition

### Skeletal muscle tissue

Aging is strongly related to changes in body composition. Both the loss of skeletal muscle mass as well as the increase in adipose tissue are common features with aging (10, 21). The gradual decline in skeletal muscle mass is accompanied with the loss of muscle strength and physical performance, which is also described as sarcopenia (22). The development of sarcopenia is partially a result of low levels of physical activity and inadequate nutritional intake (4). Furthermore, several age-related changes, such as endocrine disturbances, mitochondrial dysfunction and neuro-degenerative diseases, can contribute to the development of sarcopenia with age (4).

Already at the age of 30, skeletal muscle mass is starting to decline with more significant losses after the age of 65 and 80 years (23). A quantitative review has shown that the median decline in skeletal muscle mass throughout the lifespan is 0.37% and 0.47% per year in women and men, respectively, with even higher rates in people aged 75 or over (24). Different studies have reported a substantial decrease in the muscle fiber size in the older adults, which accompanies this loss in the skeletal muscle mass (25-27). Emerging evidence, however, shows that the decline in skeletal muscle mass is not the sole contributor to the decline in physical performance. A variety of factors such as disturbances in motor coordination, excitation-contraction coupling, energetics, skeletal muscle integrity, fat infiltration, and decreased skeletal muscle aerobic capacity, are important for physical performance in the older adults (28, 29).

### Adipose tissue

A peak in adipose tissue mass is generally observed around the age of 65 and is mainly characterized by an increase in visceral fat mass (30, 31), which is highly associated with the development of obesity (32). Increased visceral fat is an important risk factor for many health conditions, such as type 2 diabetes, ischemic heart disease, hypertension and certain cancers, all contributing to decreased quality of life and premature mortality (33).

It was estimated that the prevalence of obesity in Europe in adults, aged 60 years or older, ranged from 20 to 30 percent in 2015 (34). Obesity is caused by an imbalance between energy intake and energy expenditure. High caloric intake and low levels of physical activity could, therefore, largely contribute to the development of obesity (35).

However, the reduction in oxidative capacity due to loss of skeletal muscle or to reduced mitochondrial function, may also contribute to the fat accumulation in the body and also within the skeletal muscle, that is, ectopic lipid deposition (36). In addition, hormonal changes, such as a decrease in growth hormone and testosterone secretion, reduced thyroid hormone responsiveness, and leptin resistance, are commonly seen with age and could, via different mechanisms, contribute to the development of obesity (37).

## Causes and consequences of sarcopenic obesity

Since older adults are at risk for both the development of sarcopenia as well as the development of obesity, a double burden exists. This is a condition that is defined as sarcopenic obesity (38). Since, by definition, sarcopenic obesity is a combination of two conditions, the consequences of sarcopenic obesity largely overlap with both sarcopenic and obesity. A few, but not all, consequences include an increased risk for physical limitations, hypertension, cardiovascular disease, type 2 diabetes, and dyslipidemia (39).

Interestingly, older adults with low skeletal muscle mass and strength are 1.95 to 2.62 times at risk of being obese, compared with older adults with normal skeletal muscle mass (40). This could be explained by the risk factors for the development of sarcopenia and obesity, as they are often similar (8, 40). This makes it not very surprising that the two conditions often coexist. Additionally, it is often thought that sarcopenia and obesity work in a synergistic manner, as the consequences of sarcopenic obesity are often more severe than for sarcopenia or obesity alone (6, 7).

An important risk factor for both sarcopenia and obesity is the lower rate of energy expenditure with age, which is a result of lower physical activity, as well as a fat free mass related-lower basal metabolic rate, which is often seen with older age (35, 41). Furthermore, the physiological factors associated with age, such as changes in hormone levels, vascular changes, low grade inflammation, and immunological factors, could contribute to the development of both sarcopenia and obesity (42). Therefore, it is not very surprising that the two conditions often coexist.

Not only do sarcopenia and obesity have similar pathological causes, but the physiological consequences of obesity are also risk factors for the development of sarcopenia (43). To illustrate this, obesity may cause the resistance to anabolic stimuli such as, growth factors, hormones, amino acids, and exercise, a phenomenon called 'anabolic resistance' (44). The increase in intramuscular fat is an important factor leading to anabolic resistance, by affecting signaling pathways that are involved in muscle protein synthesis, and thereby increasing the risk for sarcopenia (45-47). Furthermore, free fatty acids (48) and insulin resistance are metabolic reasons for anabolic resistance in the muscle of older subjects (49).

In addition, obesity is responsible for causing a systemic low-grade inflammation, particularly by visceral fat, which excretes several different pro-inflammatory cytokines, such as interleukin-6 (IL-6) and TNF-α (50, 51). Obesity-induced low-grade inflammation also has the potential to contribute to anabolic resistance, leading to several cardiovascular and metabolic complications, such as insulin resistance (52, 53). Finally, inflammation and insulin resistance have differential influences on muscle metabolism, both inhibiting protein synthesis. However, the activation of proteolysis is mostly stimulated by inflammation (54). Finally, obesity-induced muscular fat infiltration does not only accelerate anabolic resistance in obese older adults, but also affects the muscle quality. The storage of adipose tissue in the muscle leads to increased stiffness of the muscle, affecting the shortening and expansion capacity of the muscle fiber (55). This leads to a decrease in the force per unit of skeletal muscle tissue, and thus a decrease in muscle quality (56). In summary, there are multiple causes and consequences for sarcopenic obesity that are interrelated (**Figure 1**). Although some causes and consequences are not completely elucidated, effective nutritional and exercise strategies to counteract sarcopenic obesity are certainly needed.

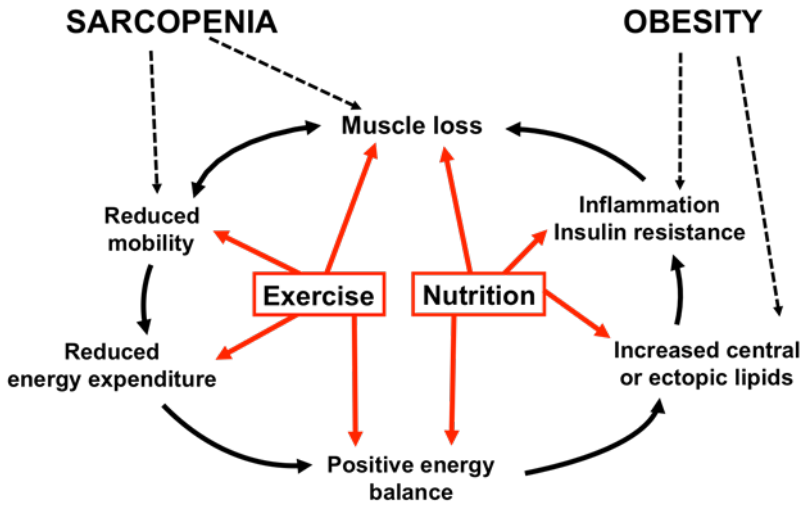


**Figure 1**: Pathophysiology and interventions in sarcopenic obesity. Black arrows indicate the pathophysiology of sarcopenic obesity. Red arrows indicate potential targets of nutritional and exercise interventions to counteract sarcopenic obesity.

## EXERCISE STRATEGIES

Exercise is found to be an effective strategy for treating different conditions in various populations, such as cardiovascular disease, pulmonary disease, diabetes, and several cancers (57). In addition, exercise as a strategy to prevent or treat obesity has often been proposed in the literature and has been widely studied in human intervention studies (58). In addition, exercise is a widely used strategy to improve muscle mass, muscle strength, and physical performance in (sarcopenic) older adults (59).

The potential mechanisms by which exercise can induce improvement in sarcopenia and obesity parameters are multi-factorial. Firstly, exercise has an important role in regulating energy balance. The energy costs that are accompanied with exercise could, in combination with a hypocaloric diet, contribute to a lower energy balance. Consequently, exercise is often a component of strategies targeting the loss of fat mass in obese older adults (60-64). Furthermore, exercise can improve physical functioning parameters such as hand-grip strength, gait speed, balance, and aerobic capacity, in both sarcopenic as well as in obese populations (65). Finally, exercise is, together with nutritional intake, the main anabolic stimulus leading to a muscle protein synthetic response (66). Although muscle protein breakdown is also stimulated with exercise, in the fed state, the net balance of the protein breakdown and synthesis is increased after exercise, which leads to muscle protein gain and thus muscle hypertrophy (67, 68). It should be noted that the main goal is to improve mobility and autonomy of the obese sarcopenic patients, by improving elasticity, strength, and muscular endurance. Exercise prescription should take into account the intensity, volume, frequency, and progression of training. Since exercise is an effective strategy to improve body composition parameters in both sarcopenia and obesity, exercise, as a strategy to counteract sarcopenic obesity, is extensively discussed in the literature. Below, the effect of different types of exercise on sarcopenic obesity parameters will be discussed, namely, resistance, eccentric, aerobic, concurrent, and electro exercise. **Table 1** provides an overview of different studies that have investigated the effect of various exercise modalities in sarcopenic obese individuals.

### Resistance exercise

Resistance exercise is currently seen as the most effective exercise strategy to elicit muscle hypertrophy and to improve muscle function and strength in older adults (69-72). The majority of the studies have been performed among older, non sarcopenic obese adults. For example, a meta-analysis of 49 studies, with in total 1328 participants of 50 years and over, showed that, on average, skeletal muscle mass was increased by 1.1 kg (95%CI: 0.9–1.2, $P < 0.01$) after an average of 20.5 weeks of resistance training two to three times a week (71). Another meta-analysis of Peterson and colleagues also illustrates the effect of resistance exercise on muscle strength in a total of 1079 older adults, where an average of

17.6 weeks led to muscle strength improvements up to 33% (SE: 2%, $P < 0.01$), depending on the type of muscle (70). As such, resistance exercise is a powerful strategy to counteract sarcopenia in older adults.

In a sarcopenic obese population, the effect of resistance exercise on body composition and skeletal muscle function is less established. Vasconcelos et al. (73) showed that a 10 week resistance exercise program was not effective to improve physical function (-0.14, 95%CI: -1.04-0.76), strength (-6J/kg, 95%CI: -0.90-12), or power (-13w/kg, 95%CI: -1.4-28) in older women with sarcopenic obesity, compared with the non-exercising control group. The relatively short duration of the intervention and the small sample size may have contributed to this result. Gadelha et al. (74), however, demonstrated improvements in both strength (12.42 N, SE: 1.18, $P < 0.001$) and skeletal muscle mass (0.29 kg, SE: 0.11, $P < 0.001$) compared to the control group after 24 weeks of traditional resistance training. Furthermore, a recent 12 week intervention-study examined the effect of elastic band resistance exercise in sarcopenic obese older women, and showed that skeletal muscle mass (0.73 kg, 95%CI: 0.08-1.39, $P < 0.05$), muscle quality (2.63 kg/kg, 95%CI: 1.21-4.05, $P < 0.01$), and physical capacity (8.58, 95%CI: 4.79-12.36, $P < 0.001$) were significantly improved compared to the no exercise group (75). Similar results were found in another program that involved elastic band training for 12 weeks in sarcopenic obese older women, which, in addition also demonstrated a significant decrease in fat mass (-0.58, $P = 0.035$), compared to the no exercise group (76). Furthermore, another recent study reported that 8 week resistance exercise in 60 sarcopenic obese older adults resulted in maintenance of skeletal muscle mass (0.1 kg, $P < 0.05$), decreased fat mass (-1.0 kg, $P < 0.05$), and increased grip strength (3.5N/kg, $P < 0.05$), compared to the non exercise group (77). Overall, the majority of the mentioned studies showed that resistance exercise is an effective strategy to improve body composition in sarcopenic obesity, and that it has the potential to improve physical performance.

## Eccentric exercise

Eccentric exercise is a different approach of the concentric contraction of the skeletal muscle, where the muscle contracts while stretching itself (for instance during stairs descent). This type of muscular work has the advantage of increasing muscle strength (78) with a reduced energy consumption in comparison with concentric contraction (79). This strategy has already been used in the rehabilitation of various groups of patients, such as diabetics, neurological diseases and groups of patients with cardiorespiratory pathologies (80). A study done in a geriatric population (14 women and 14 men, mean age 80 years, without sarcopenic obesity), compared a resistance exercise and a surplus exercise strategy. Patients were followed for 12 weeks, with body composition assessment (DXA) and muscle biopsies done before and after the 12 weeks of training. The eccentric exercise improved strength (1.3 N/kg $P < 0.05$), and body composition (fat reduction of -3.1% vs.

-0.2% [$P < 0.05$]) in comparison to concentric strategy (81). Another study compared two strategies of exercise, namely, a strategy with a combination of aerobic and eccentric exercise (A/E) with a purely aerobic strategy (A), for 16 weeks, in a population of type 2 diabetic patients. The BMI average in the A/E group was 35 kg/m$^2$, but individuals did not per se have sarcopenia. Both of the strategies improved glycaemic control, intramuscular fat, and physical performance (6 min walk test). The A/E group had a more marked decrease in BMI (-1.9 kg/m$^2$, 95%CI: -3.2—0.6, $P < 0.05$), with an increase in lean body mass (15.1 cm$^2$, 95%CI: 7.6-22.5, $P < 0.05$) (82). The listed studies did not include sarcopenic obese individuals, nonetheless, eccentric exercise could potentially be an effective strategy for this population, as is simultaneously targets fat mass and skeletal muscle mass.

## Aerobic exercise

Aerobic exercise may be an important strategy to improve muscle function, by enhancing muscle aerobic capacity in older adults (83, 84). Aerobic exercise has the potential to improve aerobic capacity by initiating mitochondrial adaptation (85), enhancing cardiovascular function (e.g., increased stroke volume capacity) (86) and increased capillary density of muscle tissue (87). Moreover, aerobic exercise training could have beneficial effects on body fat mass, especially in combination with a dietary intervention, and is therefore also seen as an effective strategy to counteract the development of obesity (88, 89).

The separate effects of aerobic exercise on muscle aerobic capacity in sarcopenic older adults and its effect on fat mass in obese older adults (although small (90)), are largely supported by the literature. This leads to the expectation that aerobic exercise could also improve these parameters in sarcopenic obese. However, to our knowledge, only one study investigated the effect of aerobic exercise specifically in a sarcopenic obese population. This 8 week randomized controlled trial included 60 sarcopenic obese older adults (aged 65-75), and found that aerobic exercise significantly led to improvements in body fat mass (-0.7 kg, $P < 0.05$) and visceral fat (-6 cm$^2$, $P < 0.05$), and maintained skeletal muscle mass (+0.1 kg, $P < 0.05$), compared to the control group (77), making it a promising strategy.

Although there is only limited evidence available on the effects on aerobic exercise on sarcopenic obesity, aerobic exercise seems like an effective tool for losing excess fat mass and improving muscle performance in sarcopenic obese older adults. Nonetheless, aerobic exercise in combination with other strategies, such as resistance exercise or a nutritional strategy, could potentially be more effective in targeting sarcopenic obesity.

## Concurrent exercise

Concurrent exercise is the combination of both resistance exercise and aerobic exercise. Both resistance exercise as well as aerobic exercise have potentially positive effects on

several body composition parameters in sarcopenic obesity, and could improve muscle function. Furthermore, in addition to this effect, the combination of these strategies, called concurrent exercise, can also promote the loss of fat mass. Concurrent exercise may therefore be an important strategy to improve skeletal muscle mass and function, and simultaneously support the loss of fat mass in sarcopenic obese older adults.

A randomized controlled trial with 45 healthy adults found that concurrent training six days a week for 12 weeks, resulted in similar improvements in maximal oxygen consumption ($VO_2$max) compared to aerobic exercise alone, but the improvement in knee extension one repetition maximum (1 RM) was lower than in the resistance exercise group alone (10.0 kg vs. 8.2 kg, $P < 0.05$) (91), and similar results were found in elderly men (n = 60) (92). These results indicate that aerobic exercise potentially leads to a blunted hypertrophic response to resistance exercise in non-sarcopenic obese individuals. Villareal et al. (64), however, showed that the combination of both aerobic and resistance exercise during a weight management program was more effective in improving the functional status of obese older adults, than resistance or aerobic exercise alone. In this clinical trial with 160 obese older adults, the Physical Performance Test (PTT) score improved more in the concurrent exercise group than in the separate exercise groups (21% increase versus 14% in both aerobic and resistance group ($P < 0.05$)). Additionally, muscle mass was best preserved after combined exercise (-3%) and resistance exercise (-2%) compared to aerobic exercise (-5%) ($P < 0.05$). There was one study available that was studying the effect of concurrent exercise in sarcopenic obese individuals, and it reported findings in line with Villareal et al. (64). In this randomized controlled trial with 139 sarcopenic obese women, it was reported that 3 months of bi-weekly 60-min concurrent exercise resulted in a 17.8% (SE: 4.2, $P = 0.119$) increase in knee extension strength (N), a significant increase in arm (1.8%, SE: 0.6, $P < 0.05$) and leg muscle mass (2.2, SE: 0.7, $P < 0.05$), and a decrease in total body fat mass (-5.5%, SE: 0.9, $P < 0.05$) compared to the control group (no exercise) (93). Although limited evidence is available, these results illustrate the potential beneficial effect of concurrent exercise on sarcopenic obesity.

## Electro stimulation

Electrostimulation has been recently become a popular technic to simulate skeletal muscular contraction. This method may be an alternative way for obese and sarcopenic patients to perform physical activity. A study in women older than 70 years compared electrostimulation, with and without protein treatment, and a control group (94). This feasibility study showed that electrostimulation could be applied in this population, because it had a positive effect on waist circumference and improved blood pressure, the latter being important for improving the metabolic syndrome. Specifically in a sarcopenic obese population, electrostimulation was compared with a control group without intervention. It was demonstrated that the electrostimulation had a stronger decrease

in the total body fat (-2.05%, 95%CI: -1.40—2.68, $P < 0.001$) and increase in the muscular strength (as measured by handgrip) (1.90 kg, 95%CI: 0.99-2.82, $P < 0.001$) compared to the control group (95). No adverse effect in patients treated with electrostimulation was detected, meaning that it was well tolerated. So the question as to know whether electrostimulation is a suitable method for working with patients who are not able to do physical activity, is still debated.

**Table 1**: Exercise and nutrition strategies to improve body composition and physical performance in sarcopenic obesity

| | N* | Age mean | Sarcopenic obesity definition | Type of intervention | Intervention effect** |
|---|---|---|---|---|---|
| Exercise strategies | | | | | |
| Vasconcelos et al. (73) | 28 | 72 | BMI and HGS | 10-wk RE 2/wk or no exercise | No sig. difference in SPPB (points) or muscle strength (kg) |
| Gadelha et al. (74) | 133 | 67 | BMI, FFM and PT | 24-wk RE 3/wk or no exercise | SMM (kg): +0.29, SE: 0.11, $P < 0.001$<br>Strength (kg): +12.42, SE: 1.18, $P < 0.001$ |
| Liao et al. (75) | 46 | 67 | BF% and SMI | 12-wk elastic RE or no exercise | SMM (kg): +0.73, 95%CI: 0.08-1.39, $P < 0.05$<br>Muscle strength (kg): +2.63, 95%CI: 1.21-4.05, $P < 0.01$<br>Physical capacity: +8.58, 95%CI: 4.79-12.36, $P < 0.001$ |
| Huang et al. (76) | 35 | >60 | BF% and SMI | 12-wk elastic RE 3/wk or no exercise | FM (kg): -0.58, P = 0.035<br>SMI (FMM/m$^2$): no sig. difference |
| Chen et al. (77) | 60 | 69 | BMI, VFA and SMI | 8-wk RE, AE, RE+AE or no exercise | FM (kg): RE: -1., AE: -0.7, RE+EA: -1.1, all with $P < 0.05$<br>SMM (kg): RE: +0.1, AE: +0.1, RE+EA: +0.2, all with $P < 0.05$<br>HGS (kg): RE: +3.5, $P < 0.05$, AE and RE+EA: no sig. difference |
| Kim et al. (93) | 139 | 81 | BF%, SMI, gait speed and HGS | 3-mo CE or no exercise | Arm muscle mass (kg): +1.8, SE: 0.6, $P < 0.05$<br>Leg muscle mass (kg): +2.2, SE: 0.7, $P < 0.05$<br>FM (kg): -5.5%, SE: 0.9, $P < 0.05$<br>Strength (kg): +17.8%, SE: 4.2, $P = 0.119$ |
| Kemmler et al. (95) | 100 | 77 | BMI, SMI and HGS | 16-wk electrostimulation or no exercise | FM (kg): -2.05%, 95%CI: -1.40—2.68, $P < 0.001$<br>HGS (kg): +1.90, 95%CI: 0.99-2.82, $P < 0.001$ |
| Nutrition strategy | | | | | |
| Muscariello et al. (96) | 104 | 67 | BMI and SMI | 3-mo hypocaloric diet with high (1.2 g/kg/bw) or low (0.8 g/kg/bw)(control diet) protein | FM (kg): no sig. difference<br>SMI (FMM/m$^2$): 0.2, SE: NP, $P < 0.01$ |

| | | | | | |
|---|---|---|---|---|---|
| Combined exercise and nutrition strategies | | | | | |
| Frimel et al. (97) | 30 | 69 | BMI and PPT | 6-mo hypocaloric diet with or without (control) RE | FM (kg): no sig. difference<br>FFM (kg): 1.8, SE: 1.5, $P < 0.05$<br>Strength (kg): up to 43%, SE: 45, $P < 0.05$ |
| Villareal et al. (63) | 107 | 70 | BMI and PPT | 1-y concurrent exercise with or without (control) hypocaloric diet | FFM (kg): -1.8, SE: 1.7 kg vs. -3.2, SE: 2.0 kg (control), $P < 0.001$<br>PPT (points): 5.4, SE: 2.4, $P = 0.04$ |
| Villareal et al. (64) | 160 | 70 | BMI and PPT | Hypocaloric with RE, AE or RE+AE or isocaloric with no exercise (control) | FFM (kg): RE: -2.7, SE: 0.3, $P < 0.01$, AE: -2.7, SE: 0.3, $P < 0.001$, RE+AE: 1.7, SE: 0.3, $P < 0.001$<br>PPT (points): RE: 3.9, SE: 0.4, AE: 0.9, SE: 0.4, RE+AE: 5.5, SE: 0.4, all with $P < 0.001$ |
| Kim et al. (93) | 139 | 81 | BF%, SMI and HGS | 3-mths CE with or without 3 gr supplementation of EAA or control | FM (kg): CE with EAA: -5.5 SE: 0.9, $P = 0.036$, CE without EAA: no sig. difference<br>FFM (kg): no sig. differences |

* Total number of subjects in studies is displayed. ** Intervention effects are displayed if significant difference is reported compared to the control group. BMI—Body mass index, SMM—skeletal muscle mass, BF%—body fat percentage, SMI—skeletal muscle index, VFA—visceral fat area, RE—resistance exercise, AE—aerobic exercise, CE—concurrent exercise, SPPB—short physical performance battery, HGS—handgrip strength, PT—peak torque, PPT—physical performance test, EAA—essential amino acids, SE—standard error, NP—not provided.

## NUTRITIONAL STRATEGIES

Nutrition is a key factor in the development of both sarcopenia and obesity. The mechanisms by which nutrition affects sarcopenia and obesity are, however, different. Sarcopenia is associated with an inadequate nutritional intake, whereas obesity is a result of an excess consumption of energy, leading to an imbalance between energy intake and energy expenditure (40). Designing nutritional strategies for sarcopenic obesity should target both an optimal nutrient intake, so as to increase skeletal muscle mass or prevent muscle mass loss, as well as an optimal nutrient and energy intake to decrease excess fat mass. The key question is how we can preserve muscle anabolism in a situation of energy deficit, in order to avoid a high proportion of weight loss as fat free mass in this population that is prone to muscle loss.

### Hypocaloric diets

A hypocaloric diet is an energy restriction diet that aims at losing body weight. The most optimal and safe range of energy restriction for sarcopenic obese older adults is an energy deficit of about 200-700 kcal per day (98). There is ample data available that hypocaloric diets are very effective in losing weight in obese older adults (99-101). However, although an energy restricted diet in obese older adults leads to the loss of fat mass, this is often accompanied with the loss of skeletal muscle mass (102-104). For instance, a study of Villareal et al. (63) reported an average fat mass loss of 7.1 kg (SE: 3.9, $P < 0.001$) in obese older adults following a hypocaloric diet (-500 to -750 kcal/day) for 52 weeks. However, this fat mass loss was accompanied with an additional 3.2 kg (SE: 2.0, $P < 0.001$) skeletal muscle mass. It is estimated that about 25 percent of the weight loss achieved with hypocaloric diets in obese older adults is skeletal muscle mass (34, 105-107). Especially for sarcopenic obese older adults, the loss of skeletal muscle mass is highly detrimental for retaining the ability to walk or climb stairs. Apart from the accompanied skeletal muscle mass loss with hypocaloric diets, solely focusing on losing weight can also have harmful effects for micronutrient status (108) and bone mineral density (109), and is therefore highly undesirable. A weight loss diet in this population should therefore always focus on preservation of muscle mass and could be combined with a high protein diet and/or micronutrient supplementation.

### Protein intake

It is well established that the intake of dietary amino acids, and especially essential amino acids (EAA), has a positive regulatory effect on the muscle protein synthesis in the muscle (110-112). However, the protein synthetic response to anabolic stimuli such as dietary protein intake, is blunted in older adults (113, 114) and in obese individuals (44). As a result, obese older adults may have higher protein needs compared to younger lean people in order to optimally promote muscle protein synthesis to maintain or to regain

muscle proteins (115). As a result, the recommended intake of 0.8 g/kg body weight (BW) for healthy adults may not be sufficient to meet the protein needs in older (obese) adults (116). To maintain and regain muscle mass and function in the long term in older people (>65 years), it is recommended to have a dietary protein intake of 1.0 to 1.2 g/kg BW with even a higher intake (1.2-1.5 g/kg BW), especially for individuals that suffer from chronic diseases (117). A dietary intervention study with 104 sarcopenic obese older adults (>65) showed that a 3-month hypocaloric diet high in proteins (1.2 g/kg BW) led to a small increase in muscle mass index (0.2, SE: unknown, $P < 0.01$), whereas a hypocaloric diet low in protein (0.8 g/kg) led to a significant decrease in muscle mass index (-0.2, SE: unknown, $P < 0.01$) (96). Although long-term intervention studies investigating the optimal protein intake for sarcopenic obese individuals are lacking, a minimal intake of 1.0-1.2 g/kg BW, seems essential to maintain muscle mass in this population to compensate for the anabolic resistance as is present in sarcopenic obesity, especially in periods of energy deficit.

The type of protein and the amino acid composition are also suggested to be relevant for muscle mass preservation or gain during weight loss. Whey protein, a milk derived protein, has been shown to be very effective in stimulating postprandial muscle protein accretion in older men (118, 119), which has been ascribed to its fast digestion and absorption kinetics, and the high leucine content (115, 120). In addition, intake of about 2.0-2.5 g/day leucine, mainly derived from animal sources, improves post-prandial muscle protein synthesis in elderly men (121). Furthermore, although it has been suggested by some, the differential effect of the different protein sources (plant or animal) on fat mass loss or gain, has not been fully confirmed (122). Overall, dietary protein that is derived from animal source products, rather than from plant-based sources, seems most effective in eliciting muscle protein synthesis (123). Although it has not yet been confirmed in sarcopenic obese individuals, higher intakes of animal source protein might contribute to improved muscle mass in this population.

Furthermore, the timing of protein intake seems important to optimally promote muscle protein synthesis. A study in which dietary intake of in total 1279 elderly was analysed, showed that about 80 percent of the dietary protein is consumed during the three main meals, of which most during dinner (124), also referred to as the 'pulse diet' (125). Interestingly, a more evenly distribution of dietary protein intake, that is, every 3 to 4 h (the 'spread diet'), led to higher protein synthesis rates (25%, $P = 0.003$) (126), and is associated with higher muscle strength, physical performance and skeletal muscle mass in older adults (127-129). Additionally, although only preliminary data is present, increasing the number of meals per day, may stimulate overall satiety, preventing excess food intake, and therefore potentially reducing the obesity risk (130). Thus, merely focusing on total amount of dietary protein may not be most optimal for improving the parameters

of sarcopenic obesity, as a more spread protein intake during the day may also be an important factor that augment the effect of its intake.

Another strategy would be to combine several anabolic nutrients such as protein, amino acids, vitamin D and omega 3. Indeed, recent studies have shown that combining whey protein, which has been enriched with leucine and with vitamin D, could increase protein synthesis and finally promote muscle mass gain in older adults (131, 132). It was reported that this combination was also effective in adults (35-65 years) (118) and in obese older adults (133).

In summary, the sufficient intake of dietary proteins is essential in sarcopenic obese older adults due to the blunted anabolic responses. The anabolic response to the dietary intake may be amplified by the relatively high intakes of animal source protein, which contain the amino acid leucine, in each meal and may be amplified by a more evenly distributed dietary protein intake over the day (spread diet). Strategies that aim to increase the skeletal muscle mass and function, by optimizing muscle protein synthesis in sarcopenic obesity, should therefore take these factors into account.

## Micronutrients

The low intake and low status of several micronutrients have been linked to the development of sarcopenia in older adults. Although mainly observational studies are available, minerals, and particularly, magnesium, selenium, and calcium seem to be most promising to counteract or prevent sarcopenia (134). Furthermore, low 25-hydroxy-vitamin D status is associated with the development of sarcopenia (135). In addition, a systematic review of Muir and Montero-Odasso (136) showed that the supplementation of vitamin D, with daily doses of 800 to 1000 IU, improves several sarcopenic parameters in older adults. Overall, micronutrient deficiencies predict the development of frailty and sarcopenia in older adults (137). Individuals that are at risk for micronutrient deficiencies, should therefore focus on improving micronutrient status, in order to potentially prevent to development of sarcopenia.



Especially in obese adults, the risk of developing micronutrient deficiencies is relatively high. Low concentrations of vitamin B6, vitamin C, 25-hydroxy-vitamin D, vitamin E, selenium, magnesium and zinc are found in several obese populations compared to normal-weight adults (138-140). In addition, obese individuals following a weight loss diet are especially at risk for micronutrient deficiencies (108). Although causal evidence is lacking, obesity related deficiencies in several micronutrients are also linked to a decline in muscle mass, strength, and physical performance (141), and thereby potentially worsening sarcopenic outcomes. To achieve adequate micronutrient intake during low caloric intake, nutrient dense food products or micronutrients supplements may be warranted.

### Hypocaloric diet with high protein intake

Each of the strategies discussed above are individually not effective in targeting all sarcopenic obesity parameters simultaneously. A hypocaloric diet induces fat mass loss, but this is accompanied with the loss of skeletal muscle mass, and protein intake promotes muscle protein synthesis, but is not effective in addressing obesity parameters. An optimal strategy should therefore combine different strategies to optimize its effect and target both fat mass, as well as skeletal muscle mass, muscle strength and physical performance. Below, the combination of a hypocaloric diet with high protein intake will be discussed as a potentially effective strategy.

In a meta-analysis, Kim et al. (142) analyzed the effects of protein intake (<25% vs. ≥ 25% of energy intake or ≥ 1.0 g/kg/d) during a hypocaloric diet on changes in body mass, skeletal muscle mass, and fat mass in older adults. They demonstrated that older adults preserved more skeletal muscle mass (0.83 kg, 95%CI: 0.47–1.19, P: unknown) and lost more fat mass (-0.53 kg, 95%CI: −1.08–0.03, P: unknown) during weight loss when consuming higher protein diets (intake ≥ 1.0 g/kg/d), compared with a lower protein diet. Although this seems a promising result for the treatment of sarcopenic obesity, inconsistent results were found in obese and physically limited older adults, where the effect of a high protein hypocaloric diet using meal-based protein foods over a 6 month period was evaluated (143). The intervention group received individual hypocaloric dietary advice (500 kcal below needs) incorporating ≥ 30 g protein in each meal (mainly animal protein), which resulted in a protein intake of 1.2 g/kg/d. They found a positive effect on physical performance, but no significant effect on skeletal muscle mass. Furthermore, Backx et al. (144) randomized overweight and obese older adults into either a high protein diet group (1.7 g/kg/d) or into a normal protein diet group (0.9 g/kg/d) during a 12 week hypocaloric diet. No preservation of muscle mass (-1.8 kg, SE: 2.2) or muscle strength (-8.8 kg, SE: 14.0) was observed in those consuming a high protein diet during caloric restriction (with no differences between diets for muscle mass: $P = 0.213$ and muscle strength: $P = 0.689$). Thus, although the combination hypocaloric high-protein diet seems to be effective in the prevention of sarcopenic obesity, this strategy does not seem to be effective for the treatment of sarcopenic obesity.

## COMBINED NUTRITION AND EXERCISE STRATEGIES

A one component strategy, whether it is an exercise or a nutritional strategy, may not be most effective in countering sarcopenic obesity. The combination of strategies, in order to target all aspects of sarcopenic obesity, seems to be the most appropriate. Different studies that use a combination of strategies so as to prevent or treat sarcopenic obesity will be discussed below.

## Hypocaloric diet and exercise

A hypocaloric diet in obese older adults is often accompanied with the loss of skeletal muscle mass. Strategies that preserve muscle mass while simultaneously stimulate the loss of fat mass, are therefore essential in the treatment of sarcopenic obesity. Adding exercise to a hypocaloric diet in sarcopenic obese older adults could potentially prevent the loss of skeletal muscle mass, by its muscle protein stimulating response. Furthermore, not only muscle mass, also muscle quality (strength and performance) can be improved following exercise. A hypocaloric diet in combination with exercise, in order to counteract sarcopenic obesity has therefore been largely discussed in the literature (63, 107, 145, 146).

To illustrate this, the effect of progressive resistance exercise added to an energy restriction diet for 6 months was studied in 30 frail obese older adults (70 ± 5 years), compared to only an energy restriction diet (97). They found that total fat mass was reduced in both of the groups, but the loss of skeletal muscle mass was reduced in the diet and exercise group, compared to the diet group (-1.8, SE: 1.5 vs. -3.5, SE: 2.1 kg, $P < 0.05$). In addition, the diet and exercise group had up to a 43% increase in strength (SE: 45, $P < 0.05$), whereas the diet group maintained strength. Similar results were found in a study of Villareal et al. (63) with 107 frail obese older adults. The authors evaluated the effect of a 1 year multi-component exercise program with and without hypocaloric diet (-500 to 750 kcal per day). The multi-component exercise program, consisting of aerobic, resistance, balance, and flexibility exercises, in combination with the hypocaloric diet, led to greater improvements in physical function assessed by the PPT score (5.4, SE: 2.4, $P = 0.04$) and it led to a significant improved ($P<0.001$) preservation of skeletal muscle mass (-1.8, SE: 1.7 kg), compared to the diet group (-3.2, SE: 2.0 kg). This finding is in line with a systematic review which reported that the addition of exercise to energy restriction in obese older adults attenuated loss of skeletal muscle mass on average from 24% to 11% (107).

A very recent and key study of Villareal et al. (64) evaluated the separate and combined effects of resistance and aerobic training, in combination with a hypocaloric diet, in 160 frail obese older adults. After 6 months, body weight decreased by 9% (SE: 0.9 kg, $P < 0.001$) in all exercise groups, and did not change significantly in the control group (no weight-management or exercise program). Skeletal muscle mass (SMM) decreased less and strength increased more in the combination and resistance groups (SMM: -3% [$P = 0.047$] and -2% [$P < 0.001$], respectively, strength: +18% [$P < 0.001$] and +19% [$P < 0.001$], respectively) than in the aerobic group (SMM: -5%, strength: +4%), whereas peak oxygen consumption increased more in the combination and aerobic groups (+17%, $P < 0.001$ and +18%, $P < 0.001$, respectively) than in the resistance group (+8%). The PPT score increased more in the combination group (+21%) than in the aerobic (+14%, $P = 0.002$) and resistance groups (+14%, $P = 0.004$). The authors conclude that combined

aerobic and resistance exercise was the most effective in improving functional status of obese older adults, in combination with a hypocaloric diet. However, although exercise in combination with a hypocaloric diet is more effective that either of the intervention alone, skeletal muscle mass is not completely preserved. Especially in the sarcopenic obese older adults, it is highly important that skeletal muscle mass is preserved, and ideally, increased.

## Protein intake and exercise

It is widely studied that the combination of high protein intake or protein supplementation with resistance exercise is an effective strategy to improve muscle mass, muscle strength, and physical performance in sarcopenic older adults (147-149). To our knowledge, there is only one study that investigated the role of exercise and protein, without including a hypocaloric diet, in body composition parameters in sarcopenic obese population (93). This randomized controlled trial included a total of 139 sarcopenic obese older adults who either followed a 60 min concurrent exercise with bi-daily supplementation of 3 g EAA, of which approximately 1.3 g was leucine, or only followed the exercise intervention. Improvement were observed for the exercise with EAA supplementation in fat mass (with EAA: -5.5 kg SE: 0.9, $P = 0.36$) compared to the control group (health education). No significant improvement in skeletal muscle mass and physical functioning were observed for both the exercise with and without EAA. The relative low amount of amino acid supplementation could be an explanation for the absence of effects. However, based on the individual effects of protein intake and exercise on sarcopenic obesity parameters, it was expected that changes in body composition would be found. Future studies should investigate the effect of a high protein diet combined with exercise to counteract sarcopenic obesity.

## Hypocaloric diet, protein intake and exercise

In a randomized controlled trial, the effect of a high whey protein-, leucine- and vitamin D-enriched supplement on muscle mass preservation was studied during a 13 week weight loss program including 3 times/week resistance exercise in obese older adults (150). Subjects in the intervention group received a supplement containing 21 g whey protein (10 servings/wk), whereas the control group received an isocaloric control supplement. In this study, the high whey protein-, leucine- and vitamin D-enriched supplement resulted in a muscle preserving effect of 0.95 kg (+0.4, SE: 1.2 and −0.5, SE: 2.1, respectively, $P = 0.03$), compared to control. In another study comparing a high whey protein diet (1.2 g/kg/d) with a control diet (0.9 g/kg/d maltodextrin), during caloric restriction and flexibility/aerobic exercise training, it was found that increasing protein intake during weight loss can counteract the deleterious effects on muscle mass by maintaining more muscle, relative to the weight lost (151). Other interesting results on the combination between high protein intake and exercise was found in a randomized controlled trial, including 100 overweight and obese adult who followed a hypocaloric diet, either combined with high protein,

resistance exercise, or both. The high protein diet (1.13 g/kg/d) and resistance exercise group alone did not significantly affect changes in fat free mass, whereas the combination of a high protein intake with resistance exercise resulted in a significant increase in fat free mass of 0.6 kg (SE: 1.3, $P = 0.011$) (152). Both Verreijen et al. (150), Mojtahedi et al. (151) and Verreijen et al. (152) included a training component in their studies, which might explain the muscle preserving effect of protein in these studies in comparison to the studies of Porter Starr et al. (143) and Backx et al. (144) as described in the paragraph 'Concurrent exercise'. However, it should be noted that these studies were not performed in a sarcopenic obese population. In summary, weight loss trials in obese older adults are effective in reducing body weight and fat mass, but may also reduce skeletal muscle mass. Higher protein intakes could help to prevent muscle mass loss especially when combined with an exercise intervention. Studies including sarcopenic obese individuals are highly warranted.

## FINAL REMARKS AND CONCLUSIONS

To date, many different effective strategies have been developed to counteract either sarcopenia or obesity. However, only a limited number of studies have focused on the combination of both conditions, sarcopenia and obesity. The aim to simultaneously increase skeletal muscle mass and decrease fat mass may be challenging for developing effective strategies. As sarcopenic obesity has a synergistic detrimental effect on physical functioning and overall health, effective strategies that counteract sarcopenic obesity are highly warranted. The present review aimed to summarize these effective strategies, however, combining the results in a meta-analysis was not possible as outcome measures and designs among studies differs tremendously, which may be seen as a limitation of this review. Additionally, because different methods are used to define sarcopenic obesity, it is difficult to compare the effectiveness of studies. Nevertheless, this review shows that sarcopenic obesity is a highly multi-factorial condition, which requires a multi-targeted approach. This review provides the latest overview of both exercise and nutrition interventions targeting both body composition and physical functioning in sarcopenic obese individuals.



In line with this aim, this review shows that a combination of a moderate weight loss diet, with concurrent exercise and high protein intake (≥1.2 g/kg/d), which is relatively high in animal protein, and spread throughout the day, has the highest potential in improving different parameters of sarcopenic obesity. However, further research is needed to better understand the optimal rate of weight loss, the type, intensity and frequency of the exercise, the combined effects of the different individual strategies (exercise and nutritional) on body composition and physical functioning parameters in sarcopenic

obese older adults. Finally, as new interventional technics as well as bariatric surgery are spreading out for the treatment of obesity in adults, we may have to consider the long term impact of bariatric surgery on muscle preservation after surgery, and the optimal strategy to maintain mobility in these aging patients in the future (153).

## Author Contributions

I.T. wrote the manuscript; M.T. conceptualized the manuscript. All authors read, reviewed, edited, and approved the manuscript.


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## Conflicts of Interest

The authors declare no conflict of interest.

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# Chapter 9

## General Discussion

# General Discussion

This thesis focusses on aspects that optimize the benefits and minimize the risks of weight loss in obese older adults. The four main research questions related to the treatment of obesity in older adults are covered in the four parts of this thesis and in this discussion. Each part provides the research question that is posed, followed by a discussion of the main findings of the studies in the light of the existing literature and existing recommendations. This discussion is concluded with directions for future research.

## Part 1: Estimating caloric needs

***'What are the caloric needs of obese older adults before and during weight loss?'***

A dietary plan with caloric restriction is usually the starting point for the treatment of obesity. This requires knowledge of individual caloric needs, which is often based on the resting energy expenditure (REE) and the level of physical activity (1, 2). Chapter 2 evaluates the accuracy of REE equations in obese older adults, and chapter 3 evaluates the presence of adaptive thermogenesis during a period of weight loss. These chapters are discussed below.

***Caloric needs of obese older adults before a period of weight loss***

The ESPEN guideline recommends a fixed factor for estimating total energy requirements of 30 kcal/kg body weight per day (kcal/kg/d for older adults in general, with close monitoring of body weight and adapting nutritional intake when needed (recommendation 1) (3). The scientific basis of this 30 kcal/kg/d is the estimate of the energy requirements in rest, which is proposed to be 20 kcal/kg/d based on findings in older persons with and without disease (4-6). However, these findings are not specifically based on obese older adults. Obese older adults in general have a higher fat mass (FM) and lower fat free mass (FFM) per kg body weight compared to non-obese older adults (7), which might result in lower REE requirements per kg body weight (8). This is in line with the observed average REE of 17 kcal/kg/d for the obese older adults included in our study (Chapter 2). The ESPEN guideline also indicates that the usual PAL level ranges from 1.2 to 1.8 in an older population. This leads to the estimation of a total energy expenditure of 24 to 36 kcal/kg/d or roughly 30 kcal/kg/d by using the mid-range PAL level of 1.5. However, average PAL levels in obese older adults are possibly lower than 1.5. Previous research showed an inverse association between BMI and PAL level in middle-aged and older adults (9), and in studies of our lab we estimated average PAL values of 1.2 to 1.5 for overweight or obese older adults using a 3-day activity log (10, 11) or an accelerometer (12). The ESPEN recommendation of 30 kcal/kg/d might therefore lead to an overestimation of the total caloric needs of obese older adults and thus may negatively influence weight loss success. In any case, close monitoring of changes in body weight and composition during the weight loss trajectory is advised.

A wide variety of treatment plans for achieving caloric restriction exists in published weight loss trials for obese older adults (13, 14). Some studies used a prefixed amount of calories (1200-1500 kcal) (15, 16), or prefixed amounts of calories based on initial body weight (1200-1800 kcal) (17-19) or body height (20). Most weight loss trials reported that their dietary plan is based on the estimation of total daily energy expenditure minus a fixed amount of calories to create an energy deficit. Daily energy expenditure is often estimated by an estimation of REE and an activity factor. Some studies reported the use of the Harris & Benedict 1919 equation (21-24), or the Schofield equation (25), whereas others measured the REE (10, 11, 26-28). Also, the applied PAL is different across studies and ranges from 1.2 to 1.6 (22-25, 29-31), or is estimated individually based on self-reported daily activity (10, 11, 26). This broad range in applied PAL level partly reflects the variation in activity level in this population. Some studies used equations for estimating total energy requirements of the Institute of Medicine & National Academy of Science (32, 33). Other studies (34, 35) did not estimate caloric needs, but based their dietary plan on a reduction in amount of calories from reported habitual intake. In addition, several weight loss interventions did not start with a dietary plan, but focused on nutritional and behavioral counselling to change nutritional habits and related caloric intake (32, 36-39). In summary, many different approaches exist as starting point to achieve caloric restriction in obese older adults: that estimation of caloric needs is often, but not always, chosen as starting point for a weight loss intervention.

If the approach of estimating energy needs is used as starting point for an energy restricted dietary plan, then the question arises: which REE equation is best to use? When looking at the Dutch study population presented in chapter 2, the average level of under- or overestimation of the REE with different equations ranges from minus 440 kcal to plus 111 kcal, which is amplified when multiplying estimated REE with the PAL. This leads to potential differences of more than 5000 kcal/week, which might result in a too rapid weight loss (including accelerated loss of muscle and bone mineral density) or no weight loss at all. Therefore, it is important to choose a prediction equation that performs reasonably well. Chapter 2 provides preliminary suggestions for equations, but several considerations need to be taken into account. First, if an equation performs well at group level, it does not necessarily translate into an accurate individual prediction. Even the most accurate equations in each subgroup did not accurately predict REE in 20 to 30% of the individuals. Second, chapter 2 demonstrates that the accuracy of the equations varied greatly per subgroup. Therefore, the translation of these results to other subpopulations should be done very cautiously, since these suggested equations do not guarantee a comparable accuracy in another population. If a prediction equation is used, it is suggested to select a well performing equation for one of the subgroups presented in chapter 2 that matches the population of interest best. Third, an accurate prediction of the PAL is also important given the wide variety of PAL among obese older adults. Finally, and most importantly, is

to closely monitor weight loss, preferably weekly in the first few months of weight loss, and when necessary to adjust the dietary plan, which is done in most successful weight loss interventions in obese older adults (27, 40-46).

***Adaptive thermogenesis during a period of weight loss***

During the period of weight loss energy requirements also change. To what extent these requirements change, depends on the amount of weight lost (47), the composition of the weight lost (48), the type of diet (49), and adaptive thermogenesis (50) during the weight loss program. Adaptive thermogenesis is the decrease in energy expenditure beyond what would be expected from the changes in FM and FFM during weight loss (51). Often adaptive thermogenesis refers to the greater than expected decrease in REE. This adaptive thermogenesis may be explained by lower circulating levels of leptin and thyroid hormones during and after weight loss, which may lower energy expenditure (52). Recently, a systematic review (50) evaluated the presence of adaptive thermogenesis in REE and demonstrated that 23 out of 29 studies found significant levels of adaptive thermogenesis, which is in line with our findings presented in chapter 3. However, this review also revealed that studies using more accurate methods of weight loss composition by MRI reported lower or non-significant values of adaptive thermogenesis. Most studies, like our study in chapter 3, used a 2-compartment model in which a standard density and hydration of FFM is presumed. Since FFM is composed of water, proteins, mineral and glycogen with different densities, any change in its composition during weight loss will alter the density of FFM (53). Especially in the initial phase of weight loss, water is lost, which changes the density of FFM. Therefore, estimated changes in FFM and FM using a 2-compartment model might be less accurate and this may also impact estimates of adaptive thermogenesis (50). Using a 2-compartment model to assess FM and FFM presents some limitations for the prediction of REE when comparing individuals before and after weight loss (54). The variation in levels of adaptive thermogenesis might therefore also depend on the method of estimating body composition (50). Another limitation that needs to be addressed is that in our study we did not include an active weight stable period after the weight loss period. Nunes et al. (50) conclude that adaptive thermogenesis seems to be attenuated, or even not present, after a period of weight stabilization or neutral energy balance. Therefore our findings might overestimate the presence of adaptive thermogenesis. In general, the levels of adaptive thermogenesis are relatively small compared to levels of error in estimating energy needs. We estimated an adaptive thermogenesis in our older population of 64 kcal per day, and therefore the impact on weight change is relatively small. Dietitians need to be aware of the potential existence of adaptive thermogenesis. The potential presence of adaptive thermogenesis further underlines the needs for closely monitoring of body weight during the weight loss intervention. The evidence for a lower than predicted REE in weight loss maintainers after a period of weight loss is still inconclusive (55).

## Part 2: Optimal protein intake

***'What is an optimal protein intake for older adults?'***

This part covers optimal protein intake for older adults in general, not during a period of weight loss. The current protein recommendation for older adult is 0.83 g/kg/d. (56) Expert groups, however, have advised higher daily protein intakes of at least 1 g/kg/d for older adults, primarily to support the preservation of muscle mass and function (3, 57, 58). Short term metabolic studies observed a small impairments in dietary absorption and digestion kinetics in in older adults compared to younger adults (59). Additionally, the blood flow to the muscle, the muscle uptake of dietary amino acids and the anabolic signaling for protein synthesis may decline during aging (60), and therefore a higher amount of protein per meal is needed in older adults to generate the same anabolic stimulus as in younger adults (61, 62). Based on acute studies, a higher protein intake is suggested to stimulate muscle protein synthesis and to preserve lean mass during the aging process and it is hypothesized that a balanced distribution of total protein intake with the consumption of at least 30–40 g of high-quality protein per meal would stimulate muscle protein synthesis most effectively. This could potentially, if practiced over months and years, slow the progression of sarcopenia (61).

***Current recommendations for protein intake in older adults***

The current European recommendation for protein is 0.83 g/kg/d for adults of all ages, including older adults (56). These recommendations are based on nitrogen balance studies in which subjects are fed a series of diets for approximately 10-15 days with in general different levels of protein while measuring nitrogen excretion by collecting and analyzing urinary and fecal excretion after an adaptation period. These data are then used to interpolate to a zero nitrogen balance, from which the average daily requirements are estimated. However, limited nitrogen balance studies are conducted in older individuals. Rand et al. (63) observed in their meta-analysis of nitrogen balance studies a non-significantly lower efficiency of nitrogen utilization in older adults, and non-significantly higher median nitrogen requirements. These results were based on data of only 14 older adults from one study, therefore this meta-analysis provides no strong evidence for higher protein recommendations for older versus younger adults. A more recent nitrogen balance study in 19 older and 23 younger adults did not show a difference in nitrogen requirements (64). With regard to the method of estimating protein needs for older adults, it is suggested that these short term nitrogen balance studies are insufficient to measure the impact of protein intake on the muscle over a prolonged period (65).

***Total daily protein intake and changes in muscle in older adults***

The often suggested higher daily protein requirements for older adults (57, 58, 66) are mostly based on short term experimental studies and findings from prospective cohort studies (67, 68). Our prospective cohort study in chapter 4 (69) focused on the association

of protein intake with change in muscle area by a highly accurate CT scan instead of other methods like DXA (67, 70) and predictions by BIA (71). We investigated the association of protein intake and 5-year change in mid-thigh muscle cross-sectional area in community dwelling older adults with mean protein intakes ranging from 0.77 g/kg BW/d protein in the lowest quintile of energy adjusted protein intake to 1.23 g/kg/d in the highest quintile. In all quintiles mid-thigh muscle cross-sectional area decreased over 5 years, but there was no difference in this decrease over the five quintiles of protein intake. This finding was robust, since all sub analyses and different levels of adjustment for potential confounders did not alter this finding. Since our study was a prospective cohort study, no cause and effect conclusions can be drawn. Two meta-analyses of protein supplementation trials also observed no effect on muscle mass, strength or function in older adults (72, 73). The Health Council of the Netherlands (NHC) recently revised the protein recommendations (74), and performed a systematic review specifically on the protein needs in older adults because of new available evidence from randomized controlled trials (RCTs) (75). They found a beneficial effect of increased protein intake on lean body mass in 7 of 18 included RCTs. Based on these 18 RCTs they concluded that increased protein intake has a possible beneficial effect on lean body mass in older participants with a habitual protein intake up to 1.05 g/kg/day and a total protein intake up to 1.7 g/kg/day. The effect on lean body mass appeared to be the same with or without physical exercise. Furthermore, based on improvements in muscle strength in 3 out of 8 RCTs, they concluded that protein supplementation also had a possible beneficial effect on muscle strength, but only in combination with exercise. Regarding physical functioning they concluded based on 12 RCTs that protein supplementation has likely no effect on physical function. Overall, the NHC concluded that the evidence was insufficient to derive a higher recommended protein intake for older adults.

These recommendations of EFSA (56) and NHC (75) of 0.83 g/kg/d apply to healthy older adults. However, more than half of older adults in Europe report at least one chronic disease (76), and the prevalence of malnutrition among community dwelling older adults is relatively high (5-10%) compared to younger adults (77). The amount of 0.83 g/kg for these groups might not be sufficient and true needs are potentially higher. Protein recommendations for these groups should be individually adapted according to nutritional status and disease status (3). The presence of obesity might also influence protein needs, since obesity is associated with low-grade inflammation (78), although this should be examined in future research.

***Protein intake per meal***

Not only total daily amount of protein for older adults is discussed (79), also the amount of protein per meal (61, 80, 81) and related distribution of protein intake over the day (82, 83), the protein source (59, 84-86), and timing with regard to exercise (87, 88) are

potentially relevant to overcome the lower anabolic response to protein and to preserve muscle mass. (59, 85, 86, 89). These findings are mainly based on short term metabolic studies or observational studies and evidence from longer term RCTs are scarce (84, 90-92). In chapter 5 we investigated the association between protein intake at breakfast and lunch and the total daily protein intake in older adults. Breakfast and lunch are often relatively low in protein (for food cultures with the main meal in the evening), thus protein intake during breakfast and lunch could be increased to extra stimulate muscle protein synthesis. However, since protein may have higher satiating properties compared to fat and carbohydrates, a higher protein intake at breakfast or lunch might not end up in a higher total protein intake (93). A recent systematic review demonstrated that protein supplementation in healthy older adults during a test meal suppressed energy intake during that ad libitum meal, but overall protein supplementation did not compromise total daily energy intake (94). In our study, we demonstrated that a higher protein intake at breakfast and lunch is associated with a lower protein intake during the rest of the day but, overall, with a higher total daily protein intake. Thus, improving protein intake at breakfast and lunch seems a good strategy to improve both the amount of protein per meal and to increase daily protein intake (95). Whether this strategy may help to improve muscle mass, strength and function needs further study (91, 96, 97). While the effect of larger protein meals on muscle protein synthesis seems clear, smaller protein meals still affect whole body protein synthesis (98). Therefore, in practice the higher amounts of protein in each main meal may be advised within the limits of appetite, but total daily protein intake of at least 0.83 g/kg/d remains the main advice.

## Part 3: Muscle mass preservation during weight loss

***'What is the effect of a higher amount of protein (in combination with exercise) during a weight loss intervention on preservation of muscle mass?'***

In chapter 6 we observed that a higher protein intake with a high-whey (leucine and vitamin D enriched) protein supplement combined with resistance exercise preserved on average approximately 1 kg appendicular lean mass during weight loss compared to an isocaloric control supplement. In both groups physical functioning improved, but no effect of the high-whey (leucine and vitamin D enriched) supplement was observed. In chapter 7 we observed no significant effect of the higher protein diet (at a lower than targeted intake of 1.1 g/kg/d) or resistance exercise on FFM. Also no statistically significant interaction on FFM between high protein and resistance exercise was observed. Only in the group with the combined intervention of high protein diet and resistance exercise the FFM significantly increased. The interaction between high protein and resistance exercise was significant for hand grip strength and 4-m gait speed, indicating that combining a high protein diet with exercise had greater positive effects on functional outcomes handgrip strength and 4-m gait speed than high protein diet or exercise alone.

***Relation muscle mass and physical functioning during a period of weight loss***

Especially during a period of weight loss, the relation between muscle mass and muscle strength and physical functioning in older adults is less clear. Villareal et al. demonstrated that during a period of weight loss, subjects lost lean mass, but gained strength and physical functioning (99, 100). Loss of fat mass may facilitate functioning, and previous research has shown that fat mass loss was a better predictor for improvements in physical functioning than lean mass loss during voluntary weight loss in older adults (101). Therefore, loss of fat mass may have overruled the possible effects of exercise and/or a higher protein intake. Likely also an improved muscle quality is of importance. Straight et al. (102) demonstrated that improved muscle quality was more important than the loss of body weight for improving physical functioning during a period of exercise and weight loss in overweight and obese older women. Thus, both the loss in fat mass and an improved muscle quality may both blur the relation between the amount of muscle mass and muscle strength and physical functioning during a period of weight loss in older adults.

***Amount of protein during weight loss***

Regarding protein intake during weight loss, our results from the RCT in chapter 6 are in line with other weight loss trials that demonstrate a lean mass preserving effect of a higher protein intake during a metabolically challenging period of weight loss (12, 103). A previous study explored the protein needs during weight loss based on our trial in chapter 6 by performing a receiver operating characteristic (ROC) curves analysis to estimate the optimal protein intake level to preserve muscle mass, and provided a protein intake level per day of 1.2 g/kg and 1.9 g/kg FFM as optimal protein intake for the accretion of muscle mass during weight loss in older adults (104). Four other RCTs studied the effects of a higher protein intake compared to normal protein intake of 0.8-0.9 g/kg/d on lean mass and physical function during weight loss (25, 105-107). Three out of four studies that examined the higher protein intake of approximately 1.2 g/kg/d concluded that a higher protein intake during caloric restriction preserved muscle mass (105, 106) or improved physical functioning (107). One other RCT (25) that examined the effect of 1.7 g/kg/d vs. 0.9 g/kg/d, however, did not observe any effect on lean mass preservation nor improvements of physical function. Therefore, older adults during a period of caloric restriction, should at least remain at the habitual level of protein intake. Although a recommendation for protein needs during weight loss is difficult to establish it is likely increased to 1.0-1.2 g/kg/d (104, 108, 109).

Only few weight loss studies have investigated the effect of distribution of protein over the day and the amount per meal on muscle protein synthesis or muscle mass in older adults. In a study of Murphy et al. (110) a hypocaloric diet was advised for 4 weeks, with 1.3 g/kg protein per day either equally balanced over 4 meals with each meal providing ≥

30 g protein, or skewed towards more protein during the evening meal. They measured synthesis rates of numerous skeletal muscle proteins, and did not observe an effect of the distribution on protein synthesis rates. Another weight loss study had a total duration of 6 months (107). They compared a high protein diet (1.2 g/kg/d) with at least 30 g protein per meal to a control diet with 0.8 g/kg/d protein. This study demonstrated a positive effect of the high protein diet on physical functioning (short physical performance battery score) during weight loss. However, this study design did not allow to determine the effect of protein distribution over the day on muscle outcomes. Since the evidence of a more balanced protein distribution on muscle outcomes during weight loss is very limited, no conclusion can be drawn on the effect of the distribution of protein over the day on muscle outcomes.

***Exercise training during weight loss***

A systematic review (111) that studied the effect of resistance training during caloric restriction in obese older adults based on 6 RCT's concluded that resistance exercise is able to almost fully prevent muscle loss induced by caloric restriction. An RCT comparing different types of exercise during weight loss in obese older adults revealed that although resistance exercise had the most lean mass preserving effect, the combination of aerobic and resistance exercise was significantly more favorable regarding physical functioning (112). The combination of aerobic and resistance exercise-type training seems favorable to elicit improvements in both muscle mass and physical functioning during a weight loss program. Furthermore, exercise in general is important for further improving cardiometabolic health (113), and potentially improves mental well-being (114), and may help to stay weight stable after a period of weight loss (115). Besides the effect on the preservation of muscle mass, resistance exercise during a period of weight loss has also been shown to preserve bone mineral density (41, 116, 117). Mention should be made on possible risks of exercise training in older adults: some cases of injuries associated with resistance training have been reported in older individuals. These injuries occur mainly in non-experienced older adults and are related to the workload, unfavorable positioning or incorrect technique, and exercise selection (118). In general, older adults taking up exercise are not at increased risk of injury compared to younger individuals (119, 120), but older adults should be well instructed and a training plan should be individualized (120).

***The interaction of a higher protein intake and exercise training during weight loss***

In our RCT presented in chapter 7 we studied the effect of a high protein diet, exercise and the combination of both on preservation of FFM during weight loss. Although we found no significant interaction between exercise and protein on FFM, three other RCTs in older obese adults without (121) or with diabetes (12, 122) did find an additional beneficial effect of a higher protein intake on top of resistance exercise: they demonstrated more favorable changes in body composition with more FM loss and more FFM preservation,

which is in line with our RCT presented in chapter 6. In our study presented in chapter 7 the difference in protein intake between the high protein and the normal protein group was only (lower than targeted) 0.15 g/kg or 16 g/day, which was a substantially smaller difference in protein intake compared to other studies (12, 121, 122), which potentially explains why we did not observe a significant interaction effect of protein and exercise on preservation of FFM.

In summary, to preserve muscle mass during weight loss, resistance exercise favorably in combination with aerobic exercise is recommended and based on the evidence discussed above, intake of approximately 1.0 - 1.2 g/kg BW/day protein is suggested for older adults during a period of voluntary weight loss.

## Part 4: Towards optimal treatment

***'What are optimal treatment options for obese older adults?'***

Chapter 8 focuses on exercise and nutrition strategies to prevention and treat sarcopenic obesity in older adults. Many aspects that are discussed in part 1 and 3 of the discussion, cover aspects of optimal treatment options and are also presented in chapter 8. There are two more issues that needs to be addressed in more detail in this section: the level of energy restriction during weight loss, and the monitoring of body weight and body composition during weight loss, since these are relevant aspects of the dietetic treatment of obesity in older adults and not fully covered yet in previous parts.

***Level of energy restriction during a period of weight loss***

The ESPEN guideline recommends only moderate energy restriction when weight loss is considered in obese older adults, in order to achieve a slow weight reduction and preserve muscle mass (recommendation 56) (3). More specifically, approximately 500 kcal/day less than estimated needs is recommended with a minimum intake of 1000-1200 kcal to assure appropriate intakes of macro- en micronutrients. In a recent systematic review of weight loss trials in older adults most weight loss interventions had a caloric deficit of -250 to -750 kcal per day (108). It also included two very low calorie diets (VLCD) with energy intakes around 800-1000 kcal/day through meal replacements in older adults (65-85y) without functional impairment or frailty at baseline (21, 27). These VLCDs were tested in combination with an exercise program and were compared with moderate caloric restriction. In both studies the weight loss in the VLCD group was greatest, but no differences in physical functioning was observed (21, 27). The absolute loss of lean mass and bone mineral density in one of the studies was also greater in the VLCD group, but the percentage of body weight that existed of lean mass increased most in the VLDL group (21). In the other study the VLDL group had a mean loss of 1.7 kg lean mass more, compared to the moderate caloric restriction group, but this was not significantly different. Since the number of subjects in that study was low, the power to

detect differences was probably insufficient (27). In line, an additional recent RCT (123) in postmenopausal women (without exercise) showed a stronger decrease in absolute amount of lean mass, and a decrease of bone mineral density in the hip in the VLCD group compared to the moderate caloric restriction group, but it had no greater adverse effect on relative whole-body lean mass. Although a VLCD achieves more weight loss, and the amount of lean mass relative to body weight seems not adversely effected, the VLCD seems to have a more detrimental effect on bone mineral density, and no effect on physical functioning is observed, although the power of these studies was potentially too low to detect differences in physical functioning. Results of these studies do not justify changes to the recommendation for moderate caloric restriction of the current ESPEN guideline (108). Related to the level of energy restriction is the nutritional quality of the dietary advice, since it is important to cover micronutrient needs with a lower amount of calories. Lowenstein (124) has suggested a minimal intake of 1500 kcal/day to cover the nutrient needs for older adults, but of course, it is important to check whether the individual dietary plan is nutritionally adequate.

***Monitoring body weight and body composition during weight loss***

We previously concluded that weekly monitoring on weight loss is important to be able to adjust the dietary plan. It is often posed that a moderate caloric restriction of 500 kcal per day in general leads to approximately 0.5 kg weight loss per week, based on the assumptions that for 1 kg of weight loss roughly a 7000 kcal deficit is needed (125). However, weight loss usually is more dynamic with a faster rate of weight loss in the first week (due to loss of glycogen and sodium accompanied by water loss) (49), and as body weight decreases, energy requirements also decline, and the pace of weight loss generally slows down (47). According to the dynamic weight loss model presented by Hall et al. (47), a caloric deficit of around 500 kcal per day leads to a weight loss of approximately 1-2 kg in the first week, but in the weeks thereafter weight loss levels off at approximately 0.2-0.5 kg/week. Thereafter –if the dietary plan is not adjusted– weight loss may completely level off. Then further adjustments should be made to the dietary advice or to the exercise regime or both, to stimulate further weight loss. Weight loss of 5%-10% of initial body weight in 6 month or more is suggested to improve obesity-related medical complications and physical functioning (3, 126).

Since weight loss is a poor predictor for change in lean mass, tracking of body composition changes is valuable. However, valid and precise methods to detect changes in body composition like dual X-ray absorptiometry (DXA) or densitometry are usually not feasible for dietetic practice, and, therefore, often bio-impedance analysis (BIA) is used (127). However, at individual level, changes measured by DXA do not always reflect changes predicted by BIA (128), and therefore results should be interpreted and communicated with caution (129). Monitoring changes in physical performance seems relevant, since

an improvement is expected and a decrease would be a signal to adjust the treatment. However, the type of test to use, at what frequency and the practical feasibility of such a test requires further study.

## Future directions for research

This section covers four issues that needs to be addressed in future research: first, the protein recommendations expressed in g/kg body weight. Second, the need for weight loss trials in an older (more sarcopenic) population (>70 years). Third, stimulating behavioral change during weight loss and fourth, the shift towards an ecological sustainable diet with more plant protein.

***Protein recommendation in g/kg body weight***

The recommended daily intake for protein is expressed in grams protein per kg body weight, which might not be suitable in case of obesity, and especially for obese older adults, since FFM is relatively low compared to their body weight (7, 130). Assuming that FFM is the driver for true protein needs, using actual body weight would probably overestimate protein needs (131). Therefore, it is suggested to use an adjusted body weight for obese older adults at a lower BMI-level, pragmatically chosen at BMI 27 kg/m$^2$ (132) or to use measured or estimated FFM (133). However, estimations of protein needs per kg FFM are not well established and are only based on one study with 23 hospitalized patients (age range 16-81 years, BMI information not available). In their study 0.8 g/kg actual BW was equal to 1.1 g/kg FFM, 1.0 g/kg actual BW with 1.5 g/kg FFM, and 1.3 g/kg actual BW with 1.9 g/kg FFM (134). The relevance of this issue is clarified by an example in **box 1**. In dietetic practice these different methods to calculate protein needs are used interchangeably, and the amount of protein advised may differ substantially depending on the method of calculation used, which highlights the relevance to address this issue in future research.

***Box 1: protein needs using different calculation methods, an example***

*A woman with a height of 1.60m, and a body weight of 90 kg (BMI 35 kg/m$^2$), with 50 kg FFM, and 40 kg FM, would need an estimated amount of:*

- ***72 g** protein using current body weight,*
- ***57 g** with adjusted body weight to a BMI of 27 kg/m2, and*
- ***55 g** using 1.1 g/kg FFM.*

*This a difference of **15-17** grams per day, which equals the amount of protein eaten during an average Dutch lunch.*

***Weight loss trials in an older population (>70 years)***

In our trials in chapter 6 and 7 we included subjects in the age of 55 years and older, and with a mean age of around 62 years. Our studies, and also other published weight loss trials in older adults (108), include relatively few adults of 75 years and older and most subjects were not physically impaired. As concluded previously (108), there is still a considerable lack of studies elucidating the risks and benefits of weight reduction in obese older adults above 70 years of age, especially in those with functional limitations and health impairments. Therefore, results of our weight loss trials (10, 11) and also other weight loss trials in older adults that were included in a recent review (108) may not be applicable to an older population with functional limitations and impaired health. Future research is needed to verify whether the observed beneficial effects of lifestyle interventions for weight loss also apply to a population older than 70, especially with functional limitations and health impairments.

***Stimulating behavioral change during weight loss in older adults***

Besides a healthy diet and exercise, as mentioned previously, older adults should be optimally supported to change their health behavior. Not only during the period of weight loss, but especially after a period of weight loss to maintain the weight loss. Effects of behaviour change interventions to facilitate weight loss, and weight loss maintenance, are modest (135). Factors that disturb self-management of health behavior are still poorly understood, but recently suggestions have been published how to improve the skills for self-management of energy balance behavior (136). The use of eHealth may offer possibilities for improving self-management of health behavior. Findings from a qualitative study -although not specifically in older adults- suggest that the combination of behavior change techniques and persuasive system design could stimulate motivation and adherence to support healthy behaviors (137). A dietary intervention trial from our lab demonstrated that the use of persuasive technology was well received in an older population to stimulate exercise training and protein intake (138). Factors that could improve the self-management of energy balance behavior (dietary intake and physical activity), including the support of eHealth, should be evaluated in future weight loss trials in which also the long term effect of weight loss is measured (139).

***Shift towards more plant protein***

The final aspect that is important to address is the concern for the ecological sustainability of diets in general and high protein diets in particular. Especially animal based protein place a burden on our planet, regarding the relatively high greenhouse gas emission, high land use, water use, and loss of biodiversity (140, 141). The EAT-Lancet commission calculated that a shift towards more plant-based and less animal based nutrition is needed to decrease the risk of irreversible and potentially catastrophic shifts in the Earth system (140). Furthermore, a more plant-based diet is associated with lower mortality

and lower risk of cardiovascular disease and cancer (142, 143). However, animal proteins are known for their high protein quality: their amino acid composition matches the human needs better, and the digestibility is higher compared to plant based protein (85, 86, 144, 145). Also short term experimental research demonstrate that the muscle synthetic response of plant protein for the same amount of whey protein in general is lower (145, 146). For older adults often high quality protein sources are advised, which are mostly animal proteins (109). Combining plant protein sources to optimize protein quality may be a potential strategy to improve the effect on muscle protein synthesis and potentially on muscle health (89, 147). In an unpublished pilot survey (2020) among 54 dietitians in the Netherlands, dietitians indicated their concern regarding amongst others the lower protein quality of more plant based diets and the potential effect on muscle health in older adults in general, and older obese adults during a period of weight loss specifically. To address this concern, future RCTs are warranted that study the effects of an ecologically sustainable diet, with more plant protein on muscle outcomes and overall health compared to a more animal based diet (usual diet) in obese older adults during a period of weight loss. During weight loss, muscle mass is generally lost, so potential differences in muscle outcomes between more plant protein and more animal protein can therefore probably be better detected.

To conclude, this thesis covers relevant aspects of the treatment of obesity in older adults, and aims to contribute to the optimization of this treatment in which risks of voluntary weight loss in obese older adults are minimized. In the summary of this thesis 7 recommendations are presented for dietetic practice to improve the treatment of obesity in older adults.

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# Chapter 10

Summary
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# Summary

The pace at which Western populations are aging is increasing and coincides with an increased prevalence of obesity, resulting in large numbers of obese older adults in the future. Obesity at older age is related to many health risks. Although prevention of obesity should be prioritized, obesity among older adults is still a reality. Described benefits of weight loss for obese older adults are a reduced risk of chronic disease, an improvement in physical functioning and quality of life, and a decreased mortality. Despite the described health risks of obesity, and the benefits of voluntary weight loss, clinicians often are reluctant to recommend weight loss interventions for older adults because of the potential risks of weight loss. These risks include the loss of muscle mass and strength, the reduction of bone mineral density and increased fracture risk. More knowledge is needed to optimize treatment options for weight loss in older adults with obesity in which these risks are minimized.

This thesis covers several relevant aspects of the treatment of obesity in obese older adults and addresses these four questions, which are covered in the four parts of this thesis:

1. What are the caloric needs of older obese adults before and during weight loss?
2. What is an optimal protein intake for older adults?
3. What is the effect of a higher amount of protein (in combination with exercise) during a weight loss intervention on preservation of muscle mass?
4. What are optimal treatment options for obese older adults?

## Part 1: Estimating caloric needs

With respect to research question 1 we evaluated the validity of 41 existing resting energy expenditure (REE) equations in an older population with obesity. We included data of 341 obese older adults from the Netherlands, Belgium and the USA and compared the estimated REE from 41 predictive equations to the measured REE with indirect calorimetry. These analyses were performed in the total study sample, and in the Dutch, Belgian, American black, and American white subgroups. The results, which are presented in *chapter 2* demonstrate that not one single equation was accurate for the total study sample, but up to 70-80% of the individuals could be predicted accurately within the subgroups. Cautious suggestions are provided for REE equations per subgroup. For the caloric needs during weight loss, we studied the presence of adaptive thermogenesis during weight loss in older overweight and obese adults compared to younger adults in *chapter 3*. Adaptive thermogenesis is the decrease in energy expenditure beyond what would be expected from the changes in fat free mass (FFM) and fat mass (FM) during weight loss. This study included 122 younger and 132 obese older adults. Before and after weight loss body composition and REE were measured. Baseline values of FFM and FM were used to generate a prediction equation for REE. This equation is also used to predict

REE after a period of weight loss. Predicted values of REE were compared with measured REE after a period of weight loss, and revealed the presence of adaptive thermogenesis in our older population of 64 kcal per day. Dietitians need to be aware of the role adaptive thermogenesis might play during weight loss.

## Part 2: Optimal protein intake

Regarding research question 2 we performed two observational studies. In *chapter 4* we investigated whether the amount and type of protein (animal or vegetable) intake were associated with the 5-year change in mid-thigh muscle cross-sectional area (CSA) assessed by computed tomography in older adults (n = 1561). Overall the mean (95% CI) loss in muscle CSA over the 5 year period was −9.8 (−10.6, −8.9) $cm^2$. No association of higher total, animal, or vegetable protein intake with 5-year change in mid-thigh muscle CSA was observed. In *chapter 5* we explored the association between the amount of protein intake at breakfast and lunch and total daily protein intake in older adults, since protein may have higher satiating properties compared to fat and carbohydrates. Protein intake was assessed by a 3-day food record in 498 community dwelling older adults. A higher protein intake at breakfast and lunch was associated with a higher total daily protein intake. Stimulating a higher protein intake at breakfast and lunch might represent a promising nutritional strategy for optimizing the amount of protein per meal without compromising total daily protein intake.

## Part 3: Muscle mass preservation during weight loss

Chapter 6 and 7 present two weight loss intervention studies in overweight and obese older adults to address research question 3. In our randomized controlled trial (RCT) in *chapter 6* we studied the effect of a high-whey (leucine and vitamin D enriched) protein supplement, combined with resistance exercise during a 3 month weight loss period with caloric restriction, on muscle mass preservation. We included 80 overweight or obese older adults (55 years and older). All subjects followed a hypocaloric diet and performed resistance training 3 times per week. Subjects were randomly allocated to a high-whey (leucine and vitamin D enriched) protein supplement or to an iso-caloric control supplement with no protein. The primary outcome, appendicular lean mass, was measured by dual-X-ray absorptiometry. A high whey (leucine and vitamin D enriched) protein supplement preserved 0.95 (95% CI: 0.09, 1.81) kg appendicular muscle mass compared to the isocaloric control supplement. In the second RCT (*chapter 7*) we studied the effect of a high protein diet and/or resistance exercise on FFM preservation during weight loss. We included 100 overweight and obese older adults. All subjects followed a 10-week weight loss program with a hypocaloric diet. Subjects were randomly allocated to either a high protein (1.3 g/kg/d) or normal protein diet (0.8 g/kg/d), with or without a resistance exercise program 3 times/week (2-by-2 factorial design). FFM was assessed by air displacement plethysmography. We observed no significant effect of the higher

protein diet and resistance exercise on FFM preservation. Also, no statistically significant interaction between high protein and resistance exercise was observed. However, the difference in protein intake between the high protein and the normal protein group was only 0.15 g/kg or 16 g/d, which was lower than targeted. Based on these results (chapter 6 and 7) a high-whey (leucine and vitamin D enriched) protein supplement helped to preserve muscle mass during a period of weight loss with resistance exercise. Whether a high protein diet (no supplement) combined with resistance exercise could be beneficial for FFM preservation during weight loss in older adults, should be confirmed by future studies using a larger protein contrast.

## Part 4: Towards optimal treatment

Chapter 8 covers research question 4 and provides a narrative review of the literature. The main aim of this review was to provide a current update of the various exercise and nutritional strategies to prevent and/or counteract sarcopenic obesity in older adults. This review concludes that a combination of a moderate weight loss diet, with resistance exercise in combination with aerobic training, and a higher protein intake of minimal 1.0-1.2 g/kg/d, which is relatively high in animal protein and a spread of protein intake over the main meals, has the highest potential in improving different parameters of sarcopenic obesity.

Chapter 9 discusses the main findings of the studies presented in this thesis in the light of the existing literature. The following recommendations, based on this thesis and related literature, are presented for dietetic practice to improve the treatment of obesity in older adults.

***Recommendations for dietetic practice:***

1. *If estimating energy needs is the starting point for the energy restricted dietary plan, select the best performing prediction equation for resting energy expenditure as suggested for one of the subgroups that matches your population best (Chapter 2).*
2. *It is important to closely monitor changes in body weight during weight loss, and adjust the dietary advice when necessary, preferably on a weekly basis in the first few months of the weight loss intervention.*
3. *Based on current evidence, a protein intake of approximately 1.0-1.2 g/kg/day is recommended for obese older adults during a weight loss intervention.*
4. *Caloric restriction to achieve weight loss should always be accompanied by exercise training: aerobic and resistance training combined seems to give best results regarding physical functioning. To preserve muscle mass and bone mineral density during weight loss, at least resistance exercise should be advised.*

5. *During the weight loss program the dietitian is advised to cooperate with a physiotherapist to optimize the exercise program, minimize the risk for musculoskeletal injuries and to select alternative exercises when the person has mobility limitations.*
6. *Based on current evidence, moderate caloric restriction of approximately 500 kcal below estimated needs during weight loss is advised, with close monitoring on body weight. Aiming at a weight loss of approximately 5-10% of initial body in 6 months or more is advised.*
7. *Monitoring body composition is advised, at lower frequency compared to body weight, for example once per month. Body composition changes measured with bio-electrical impedance analysis should be interpreted and communicated with caution.*

# Samenvatting

De vergrijzing in westerse landen neemt toe. Deze vergrijzing gaat gepaard met een toename van obesitas. Preventie van obesitas is van groot belang vanwege de vele gezondheidsrisico's die obesitas op latere leeftijd met zich meebrengt. De realiteit is echter dat er veel ouderen zijn die obesitas hebben. Gewichtsverlies bij obese ouderen heeft veel gezondheidsvoordelen, zoals een lager risico op chronische ziekten, of een vermindering van de ernst ervan, een verbetering van het lichamelijk functioneren, een verbetering van de kwaliteit van leven en een verminderde mortaliteit. Ondanks deze voordelen van gewichtsverlies, zijn behandelaars vaak terughoudend om gewichtsverlies bij ouderen te adviseren vanwege mogelijke risico's die het met zich meebrengt. Deze risico's zijn het verlies van spiermassa, spierkracht en botmineraaldichtheid. Er is meer kennis nodig om de behandeling van obesitas bij ouderen te optimaliseren waarbij deze risico's geminimaliseerd worden.

In dit proefschrift worden relevante aspecten van de behandeling van obesitas bij ouderen behandeld aan de hand van onderstaande vier vragen:

1. Wat is de energiebehoefte van obese ouderen voorafgaand aan en gedurende een periode van gewichtsverlies?
2. Wat is een optimale eiwitinname voor ouderen?
3. Wat is het effect van een hogere eiwitinname (gecombineerd met lichamelijke training) gedurende een periode van gewichtsverlies op het behoud van spiermassa?
4. Welke behandelopties optimaliseren de behandeling van obesitas bij ouderen?

## Deel 1: Schatten van de energiebehoefte

Voor het schatten van de energiebehoefte van obese ouderen hebben we in *hoofdstuk 2* de validiteit van 41 predictieformules voor het energieverbruik in rust (REE) geëvalueerd in een oudere populatie met obesitas. We hebben data van 341 obese ouderen uit Nederland, België en de Verenigde Staten (USA) geïncludeerd. De geschatte REE met de 41 formules zijn vergeleken met de gemeten REE (met indirecte calorimetrie). Deze analyses zijn uitgevoerd in de totale onderzoekspopulatie en ook in de Nederlandse, Belgische, Afro-Amerikaanse en Caucasische USA subgroepen. De resultaten laten zien dat er geen enkele formule geschikt was voor de gehele onderzoekspopulatie, maar dat binnen de subgroepen 70-80% van de populatie accuraat geschat kon worden. Voorzichtige aanbevelingen voor REE predictieformules per subgroep staan weergegeven in *hoofdstuk 2*. Met betrekking tot de energiebehoefte van obese ouderen tijdens een periode van gewichtsverlies hebben we in *hoofdstuk 3* de aanwezigheid van adaptieve thermogenese tijdens een periode van gewichtsverlies bestudeerd. Adaptieve thermogenese is de sterkere afname van de REE dan je zou verwachten op basis van de afname in vetvrije massa en vetmassa. In deze studie zijn 132 ouderen met obesitas vergeleken 122 jongere

personen met obesitas. Voorafgaand aan, en na een periode van gewichtsverlies zijn de lichaamssamenstelling en de REE bij alle deelnemers gemeten. Op basis van de vetvrije massa en de vetmassa op baseline is een predictieformule ontwikkeld om de REE te schatten. Deze formule is ook gebruikt om de REE te schatten na een periode van gewichtsverlies. Deze geschatte waarden na een periode van gewichtsverlies zijn vergeleken met de gemeten waarden na een periode van gewichtsverlies. Hieruit bleek dat adaptieve thermogenese aanwezig was bij ouderen met obesitas, met 64 kcal per dag. Voor diëtisten is het belangrijk om ervan bewust te zijn dat adaptieve thermogenese een rol kan spelen tijdens gewichtsverlies.

## Deel 2: Optimale eiwitinname

Om onderzoeksvraag 2 te bestuderen hebben we twee observationele studies uitgevoerd. In *hoofdstuk 4* hebben we onderzocht of de hoeveelheid en het type eiwit (dierlijk of plantaardig) samenhing met de 5-jaars verandering in het oppervlak van de spieren in het midden van het dijbeen, gemeten met computertomografie (CT-scan). Dit is onderzocht in 1561 ouderen. Gemiddeld (95% betrouwbaarheidsinterval) verloren de ouderen in 5 jaar een spieroppervlakte van −9.8 (−10.6, −8.9) $cm^2$. Een hogere inname van zowel de totale hoeveelheid eiwit als de hoeveelheid dierlijk of plantaardig eiwit bleek niet samen te hangen met een verandering in de spieroppervlakte. In *hoofdstu*k 5 hebben we samenhang tussen de hoeveelheid eiwit tijdens het ontbijt en de lunch en de totale inname van eiwit bij ouderen bestudeerd. Dit is relevant omdat eiwitten meer verzadiging kunnen geven in vergelijking met vetten en koolhydraten. De inname van eiwit is geschat met een 3-daags eetdagboek in 498 ouderen. Een hogere eiwitinname tijdens het ontbijt en tijdens de lunch hing samen met een hogere totale dagelijkse inname van eiwit. Het stimuleren van een hogere eiwitinname tijdens het ontbijt en de lunch zou gunstig kunnen zijn om zo de eiwitinname tijdens maaltijden te optimaliseren zonder dat dit ten koste gaat van de totale eiwitinname.

## Deel 3: Spiermassabehoud tijdens gewichtsverlies

Voor het beantwoorden van onderzoeksvraag 3 zijn twee gewichtsverlies studies beschreven (*hoofdstuk 6 en 7*) bij ouderen (55 jaar en ouder) met overgewicht of obesitas. In de gerandomiseerde gecontroleerde trial (RCT) in hoofdstuk 6 hebben we het effect van een wei-eiwit supplement (verrijkt met leucine en vitamine D) in vergelijking met een isocalorisch controlesupplement bestudeerd tijdens 3 maanden gewichtsverlies en krachttraining. Tachtig ouderen zijn via loting toegewezen aan de groep met het wei-eiwit supplement of aan de groep met het controlesupplement. Alle deelnemers kregen een energiebeperkt dieet voorgeschreven en namen deel aan 3 krachttrainingssessies per week. De primaire uitkomstmaat, de appendiculaire spiermassa (spiermassa van de armen en benen), is gemeten met dual-X-ray absorptiometry (DXA). De groep die het wei-

eiwit supplement kreeg, behield significant appendiculaire spiermassa met 0.95 (95% CI: 0.09, 1.81) kg in vergelijking met de controlegroep.

In de tweede RCT (hoofdstuk 7) hebben we het effect bestudeerd van een hogere hoeveelheid eiwit in de voeding en weerstandstraining op behoud van vetvrije massa tijdens gewichtsverlies. In deze studie zijn 100 ouderen geïncludeerd. Ze volgden een gewichtsverliesprogramma van 10 weken met een energiebeperkt dieet. De onderzoeksopzet was een 2-bij-2 factorieel design. Hierbij zijn de deelnemers op basis van toeval toegewezen zijn aan ofwel een hoog eiwit dieet (1.3 g/kg/d), of een normaal eiwit dieet (0.8 g/kg/d), met of zonder een weerstandstrainingsprogramma 3 keer per week. De vetvrije massa is gemeten met luchtverplaatsingsplethysmografie. In deze studie vonden we geen significant effect van zowel het hogere eiwitdieet als de weerstandstraining op het behoud van vetvrije massa tijdens een periode van gewichtsverlies. We vonden ook geen significante interactie tussen het hoge eiwit dieet en weerstandstraining op het behoud van vetvrije massa. Het verschil in eiwitinname tussen het hoge eiwit dieet en het normale eiwitdieet was echter lager dan verwacht en bedroeg slechts 0.15 g/kg of 16 g/d.

Gebaseerd op de resultaten van deze twee RCT's kan geconcludeerd worden dat een wei-eiwit supplement (verrijkt met leucine en vitamine D) bijdroeg aan spiermassabehoud gedurende een periode van gewichtsverlies met krachttraining. Of een hoog eiwitdieet (zonder supplementen) gecombineerd met weerstandstraining kan helpen om de spiermassa te behouden behoeft verder onderzoek, met een groter eiwitcontrast tussen de groepen.

## Deel 4: Naar een optimale behandeling

Hoofdstuk 8 behandelt onderzoeksvraag 4 en geeft een samenvatting van de literatuur. Het doel hiervan was om een update te geven van verschillende voedings- en trainingsstrategieën om sarcopene obesitas in ouderen te voorkomen en/of de ernst ervan te verminderen. Op basis van deze review concluderen we dat een combinatie van gematigd gewichtsverlies met krachttraining gecombineerd met aerobe training en een hogere eiwitinname van minimaal 1.0-1.2 g/kg/d het hoogste potentieel heeft om sarcopene obesitas te voorkomen of de ernst ervan te verminderen. Hierbij is de samenstelling van de eiwitinname relatief hoog in dierlijk eiwit en is de spreiding van de eiwitinname gelijk verdeeld over de hoofdmaaltijden.

Hoofdstuk 9 bediscussieert de belangrijkste bevindingen van de studies in dit proefschrift in het licht van de bestaande wetenschappelijke literatuur. De volgende aanbevelingen, gebaseerd op dit proefschrift en gerelateerde literatuur, worden gepresenteerd om de diëtetiek praktijk te helpen om de behandeling van oudere met obesitas te optimaliseren.

***Aanbevelingen voor de diëtetiek:***

1. *Als het schatten van de energiebehoefte het startpunt is van een energiebeperkt voedingsadvies: selecteer dan de meest accurate formule voor het schatten van het energieverbruik in rust, gebaseerd op de studiepopulatie die het beste overeenkomt met jouw cliënten (Hoofdstuk 2).*
2. *Het is belangrijk om de veranderingen in lichaamsgewicht goed te monitoren, en om het voedingsadvies zo nodig aan te passen, bij voorkeur wekelijks in de eerste maanden van gewichtsverlies.*
3. *Op basis van de beschikbare evidence wordt een eiwitinname van ongeveer 1.0-1.2 g/kg/d geadviseerd tijdens een periode van gewichtsverlies.*
4. *Calorische restrictie om gewichtsverlies te bereiken dient altijd samen te gaan met training: een combinatie van aerobe training en weerstandstraining lijkt het beste om het lichamelijk functioneren te verbeteren. Om spiermassaverlies tegen te gaan en botmineraaldichtheid te behouden gedurende gewichtsverlies dient in elk geval weerstandstraining geadviseerd te worden.*
5. *De diëtist wordt geadviseerd om samen te werken met een fysiotherapeut om het trainingsprogramma te optimaliseren, het risico op blessures te minimaliseren en om alternatieve oefeningen te selecteren wanneer de cliënt door beperkingen bepaalde oefeningen niet kan uitvoeren.*
6. *Op basis van de beschikbare evidence wordt voor gewichtsverlies een calorische restrictie van ongeveer 500 kcal onder de geschatte energiebehoefte geadviseerd, waarbij het belangrijk is om het lichaamsgewicht goed te monitoren. Gewichtsverlies van ongeveer 5-10% van het initiële lichaamsgewicht in 6 maanden of langer wordt hierbij geadviseerd.*
7. *Geadviseerd wordt om ook de lichaamssamenstelling te monitoren, op een lagere frequentie dan het gewicht, bijvoorbeeld één keer per maand. Veranderingen in lichaamssamenstelling gemeten met bio-elektrische impedantie analyse dienen met enige voorzichtigheid geïnterpreteerd en gecommuniceerd te worden.*

# Dankwoord

Na ruim 10 jaar is er een einde gekomen aan het promotie-tijdperk. Het was inspirerend, leerzaam, veeleisend, ontzettend leuk, soms ook niet leuk, spannend, super leerzaam en heel erg mooi en waardevol om te doen. Soms vergeleek ik het wel eens met een marathon lopen, daar zitten ook vergelijkbare fases in. En ook dat kun je niet alleen. Zonder de hulp van mijn lieve collega's, vrienden en familie, studenten en deelnemers was dit proefschrift niet tot stand gekomen.

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## About the author

Amely Verreijen was born on October 3rd, 1978 in Diessen, The Netherlands. After completing secondary school at the Gymnasium of the Koning Willem II college in Tilburg, she studied Nutrition and Health at the Wageningen University. She obtained her MSc. degree in 2001, and became an assistant lecturer in courses statistics and epidemiology at the division of Human Nutrition at the Wageningen University, where she discovered her love for working with students. Therefore, she started the Master of Education in Biology at the HU University of Applied Sciences Utrecht and obtained her MEd. degree in 2006.

In 2004 she was appointed as a lecturer at the Amsterdam University of Applied Sciences (AUAS), Bachelor program Nutrition and Dietetics, where she was responsible for courses in nutrition, research and the supervision of graduation students. Under supervision of Prof. Peter Weijs she started research projects together with students in the 'Voedingslab' (now the Amsterdam Nutritional Assessment Center or ANAC, research group Nutrition and Exercise). In close collaboration with more than 60 students and colleagues, Amely coordinated two randomised controlled trials focusing on muscle mass preservation during a period of weight loss in obese older adults. In 2011 she was awarded with 'HvA Research Battle' award for best researcher of the Amsterdam University of Applied Sciences. In 2010, 2011 en 2013 she was elected by students for lecturer of the year of this Bachelor program Nutrition and Dietetics. From 2018 she was one of the designers of the new curriculum of this Bachelor program.

In 2014 Amely obtained a NWO Doctoral Grant for Teachers for her research on the treatment of obesity in older adults, under supervision of Prof. Peter Weijs and Prof. Marjolein Visser. In 2015 she was awarded with the NASO (Netherlands Association for the Study of Obesity) publication award, and in 2018 she was nominated for the ESPEN (European Society for Clinical Nutrition and Metabolism) 'best abstract award'. Since 2021 Amely works as a postdoctoral researcher on ecological sustainable -more plant based- nutrition in the treatment of obesity in older adults.