FOOD SOURCES OF PROTEIN AND SARCOPENIC OBESITY IN OLDER WOMEN

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ABSTRACT

Sarcopenic obesity is a condition in which sarcopenia and obesity occur simultaneously. In sarcopenic obesity, the muscle mass and the muscle strength of the body are low while the body fat is high. This condition has been linked to frailty, loss of independence, lower physical performance, cardiovascular problems and increased mortality risk.

Prior studies have revealed that a diet higher in protein and a healthy diet can impede the onset of sarcopenia and reduce obesity in older age. The primary objective of this study is to investigate the association between food sources of protein and sarcopenic obesity in elderly women of Kuopio.

For this cross-sectional study, a sample of older women (n=555 and aged 65-72 years) was extracted from the OSTPRE-FPS. Their baseline characteristics, including handgrip strength, walking speed, body fat, muscle mass and bone mass density were measured by trained professionals and their dietary intake was recorded for 3 consecutive days. They were also given questionnaires to fill out information about mobility status, physical activity, time from menopause, smoking status, alcohol use and hormone therapy. To measure sarcopenic obesity, the EWGSOP sarcopenia criteria were used for sarcopenia and a 40% body fat cut-off point was used for obesity. The baseline characteristics and the food sources were analysed according to the sarcopenic obesity status.

The results, after adjusting them with confounding variables such as energy, revealed that among the animal-based food sources of protein only total dairy intake was associated with sarcopenic obesity as they were consumed the most in the sarcopenic obese group compared to sarcopenic, obese and referent group. The study further showed that plant-based food sources of protein were not associated with sarcopenic obesity. It also revealed that the sarcopenic obese women had a higher body mass index, weight and body fat percentage than the referent group while they had a lower gait speed, muscle mass percentage and mobility than the referent group.

Thus, the study concludes that in a given population sarcopenic obesity is not associated with plant and animal food sources of protein, except for total dairy products, where sarcopenic obesity is positively associated with it in a cross-sectional setting.

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ABBREVIATIONS

ALM	Appendicular lean mass
AWGSP	Asian Working Group for Sarcopenia
BF	Body fat
BF%	Body fat percentage
BMI	Body mass index
BMR	Basal metabolic rate
BW	Body weight
CT	Computed tomography
DALY	Disability-adjusted life-year
DXA	Dual X-ray absorptiometry
EWGSOP	European Working Group on Sarcopenia in Older Persons
E%	Energy percentage
FICSIT	Frailty and injuries: Cooperative studies of intervention techniques
FFM	Fat-free mass
FFQ	Food frequency questionnaire
FM	Fat mass
FNIH	Foundation for the National Institutes of Health
GH	Growth hormone
IL	Interleukin
IGF1	Insulin-like growth factor
IWGSP	International Working Group for the Study of Sarcopenia
LCPUFA	Long-chain polyunsaturated fatty acids
MAMA	Mean arm muscle area
MM	Muscle mass
MS	Muscle strength
NNR	Nordic Nutrition Recommendation
OR	Odds ratio
OSTPRE	Osteoporosis Risk Factor and Prevention Study
PF	Physical function
RSMI	Relative skeletal muscle index

SFT	Senior fitness test
SM	Skeletal muscle
SMI	Appendicular skeletal mass index
SO	Sarcopenic obesity
TNF	Tumour necrosis factor
US	United States of America
WHO	World Health Organization

Contents

1. INTRODUCTION

The world population is aging, and it is expected that by 2050, 22% of the population will be above 60 years of age and approximately 5% would be above 80 years (United Nations 2013). Increasing age is linked with a proliferation of health problems. Roughly 42% of adults above the age of 55 face some kind of difficulty while performing their daily activities, leading to higher risk of falls and injuries, loss of independence and resulting therefrom institutionalization (Trouwborst et al. 2018).

There are multiple factors that can hinder physical performance of a person with increasing age, such as sarcopenia and obesity. Sarcopenia is the loss of skeletal muscle mass (MM) with age accompanied by the reduced skeletal muscular function (Cruz-Jentoft et al. 2010) while obesity is the increase in fat mass (FM) (Kelly et al. 2008). A condition known as sarcopenic obesity (SO) arises when obesity and sarcopenia occur simultaneously, limiting the physical function of an individual, with both adverse effects of sarcopenia and obesity occurring together (Cauley 2015).

Sarcopenic obesity is an increasing burden on the health care systems, with more people getting hospitalized every year because of its consequences (Prado et al. 2012). It mostly occurs in older age individuals, affecting their body composition as well as physical and metabolic functions (Prado et al. 2012). Supervised dietary modification and physical activity have shown to be a promising approach for the management of SO as it reduces obesity and promotes muscle growth (Goisser et al. 2015).

There is strong evidence that suggest an intake of higher amount protein being important for preserving MM and physical function in older adults (Backx et al. 2016, Kim et al. 2016, Porter Starr et al. 2016, Nordic Nutrition Recommendation 2012). The recommended protein intake, discussed later in the thesis, is higher in older adults in comparison with younger cohorts, because the response to anabolic stimuli gets slower with increasing age (Guillet et al. 2004).

The research undertaken in this study aims to reveal the association of the intake of food sources of protein with SO, taking the older female population of Kuopio, Finland as study subjects.

Covariates, for example, physical function (PF), were also used in this study to understand their relevance to SO.

2. LITERATURE REVIEW

2.1. Obesity

World Health Organization (WHO) defines obesity as "a condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired" (WHO 2000). It is a complex disease and when combined with overweight, it affects one-third of the world population (Ng et al. 2014). The trends for obesity and overweight are on the rise worldwide, in both developed and developing countries for all age groups (Wang et al. 2008). For example in United States of America (US), if the current trend continues, 85% of the adult population will become overweight or obese by 2030 (Wang et al. 2008).

There are different ways of measuring obesity, for example through BMI and body fat percentage (BF%). BMI is the indicator of body fat used by WHO. As per the WHO guidelines, a BMI of less than 18.5 kg/m^2 is underweight, 18.5-24.9 kg/m^2 is normal, 25-29.9 kg/m^2 is overweight and ≥ 30 kg/m^2 is obese (WHO 2000). Other definitions that include BF% suggest that BMI can potentially be misleading (Johnson et al. 2017), because BMI and weight do not take into account the fact that the body fat (BF) increases and MM decreases with age, resulting in the change of BF% while the BMI may or may not change (Cruz-Jentoft et al. 2010).

The main cause of obesity is the imbalance of energy between the calories consumed and the calories required (imbalance in energy intake and expenditure). As a result, an energy surplus is created leading to an excess of BF (Schrauwen et al. 2010). Other causes of obesity can include a reduced oxidative capacity related to the decline in MM, resulting in ectopic lipid deposition in the muscles (Schrauwen et al. 2010).

Obesity has an impact on global mortality and disability rates. An estimated 2.8 million people die every year because of being obese or overweight and 35.8 million (2.3%) of global disability-adjusted life-years (DALYs) are caused by obesity or overweight (Villareal et al. 2005). The increase in the number of people living longer with a disability is also a determinant of the rising public health problem, because medical costs for the care provided to these patients also increase with time (Yang & Hall 2008, Edwards 2012).

2.2. Sarcopenia

The term sarcopenia is derived from Greek words *sarx*, meaning flesh and *penia*, meaning loss (Rosenberg 1997). Sarcopenia has been broadly defined as a reduction in MM that occurs with the progression of age and is associated with a decline in MS and an increased risk of limited mobility, disability and functional limitation in daily living activities (Berger & Doherty 2010). Different working groups and committees working on sarcopenia have defined a set of criteria that have to be observed in order to diagnose the condition (Table 1). For example, the International Working Group for the Study of Sarcopenia (IWGS) describes the condition as a combination of low appendicular or whole-body MM and compromised PF (e.g., gait speed of <0.8 m/s) (Fielding et al. 2011). The European Working Group on Sarcopenia in Older Persons (EWGSOP2) defines sarcopenia through the following components: low MS, low MM and low PF (Cruz-Jentoft et al. 2019). The EWGSOP2 uses dual X-ray absorptiometry (DXA) for measuring MM and handgrip dynamometer for grip strength. Although MM can also be measured via computed tomography (CT) and magnetic resonance imaging (MRI), these tools are rarely used in clinical practice due to lack of portability, high equipment cost and the requirement of trained personnel (Cruz-Jentoft et al. 2019). The Foundation for the National Institutes of Health (FNIH) Sarcopenia Project used dual X-ray absorptiometry (DXA) scan for measuring the MM and handgrip strength for muscle function while suggesting a sex-specific cut-off points that could be adjusted for BMI (Studenski et al. 2014). The Asian Working Group for Sarcopenia (AWGS) focused on subjects of Asian descent. As per their definition, gait speed and handgrip strength were used for initial testing and/or screening followed by the EWGSOP method for strength, MM measurement and physical performance with different, lower, cut-points (Chen et al. 2014).

Table 1. Different measuring tools for sarcopenia

Study Group	Measurement Tool	Sarcopenia Measurement
IWGSP	DXA	MM, Gait Speed
FNIH	Handgrip dynamometer	Handgrip strength
AWGS	DXA, Bioelectric impedance, Handgrip dynamometer	MM, Gait Speed, Handgrip strength
EWGSOP	DXA, Bioelectric impedance, Handgrip dynamometer	MM, Gait speed, Handgrip strength
EWGSOP2	DXA, Bioelectric impedance, Handgrip dynamometer	MM, Gait speed, Handgrip strength

Source: Adapted from (Batsis & Villareal 2018).

DXA = Dual X-ray Absorptiometry; EWGSOP= European Working Group on Sarcopenia in Older Persons (Cruz-Jentoft et al. 2010); EWGSOP2 = European Working Group on Sarcopenia in Older Persons (Cruz-Jentoft et al. 2019);FNIH = Foundation for the National Institutes of Health; IWGSP = International Working Group for the Study of Sarcopenia; MM = Muscle Mass.

The most commonly used definition of sarcopenia in epidemiological studies is the one given by EWGSOP whereby it is understood as "a loss of muscle mass in combination with a loss of muscle strength or physical performance" (Cruz-Jentoft et al. 2010). The cut-off values for sarcopenia were defined as: slow walking speed of ≤ 0.8 m/s, appendicular lean mass (ALM)/height^2 of ≤ 7.23 kg/m2 for men and ≤ 5.67 kg/m2 for women, and a grip strength of <30 kg for men and <20 kg for women (Cruz-Jentoft et al. 2010). However in 2019, EWGSOP agreed on redefining the cut-off values for sarcopenia and hence according to the EWGSOP2 criteria, the following are the new cut-off values: slow walking speed of ≤ 0.8 m/s, ALM/height^2 of ≤ 7.0 kg/m^2 for men and ≤ 5.5 kg/m^2 for women, grip strength of <27 kg for men and <16 kg for women (Cruz-Jentoft et al. 2019).

Sarcopenia has formally been recognized as a muscle disease with an ICD-10-MC Diagnosis Code (ICD10Data.com) (Vellas et al. 2018) and its prevalence is 5-13% in adults aged 60-70 years and up to 50% in the age group 80 and above (Fielding et al. 2011). The processes involved in the development and progression of sarcopenia are multifactorial and result in a disproportionate production and degradation of proteins in muscles. They can be partly explained by a reduction in anabolic response to the daily food intake (Remond et al. 2015).

The physiological and morphological changes occurring in a muscle with advancing age result in the infiltration of adipose tissue into the skeletal muscle as well as the decrease in the number and size of skeletal muscle fibers (Lexell 1995). Besides the muscular changes, environmental causes, disease triggers, inflammatory pathway activation, mitochondrial abnormalities, hormonal changes and loss of neuromuscular junctions could all contribute to sarcopenia (Walston 2012).

Health-wise, sarcopenia affects the person by increasing the risk of falls, becoming prone to weakness, frailty and a loss of independence to perform daily tasks (Mijnarends et al. 2018). Additionally, sarcopenic people require optimal care as the condition can lead to personal, social and economic burdens if not treated (Mijnarends et al. 2018).

Despite the link between sarcopenia and frailty in terms of function and independence, there is still no consensus on whether sarcopenia is a component of frailty or whether these two entities should be considered as separate geriatric conditions (Bauer & Sieber 2008, Cesari et al. 2014). While sarcopenia is a state of reduced MM and MS, frailty is a generalized state of increased sensitivity and vulnerability towards externally induced stress in the older age, associated with poor recovery following a stressful event, which leads to a higher risk of disability (Clegg et al. 2013). Sarcopenia and frailty share high relevance in lieu of their prevalence in older people, association with negative health outcomes, reversibility and easy evaluation in clinical practice as well as in regard to functional independence in the elderly (Bauer & Sieber 2008).

2.3. Sarcopenic obesity

Sarcopenic obesity is the co-existence of sarcopenia and obesity (Cauley 2015). The definition of SO is dependent on the criteria used for obesity and sarcopenia. Different definitions use different methods for determining the components of SO (Batsis & Villareal 2018). For example, if BMI is used in the definition of SO, it can be potentially misleading (Johnson Stoklossa et al. 2017), because BMI and weight do not take into account the BF% which is an essential part of measuring obesity and it relatively changes with age as the BF increases and MM decreases (Cruz-Jentoft et al. 2010). As seen in Table 2, different methods and cut-offs are used to measure sarcopenia and obesity, for instance, MS, gait speed, ALM divided by height squared, BMI and BF%.

Table 2. Different methods and cut-off points for sarcopenia and obesity

Study and Year	Sarcopenia diagnosis method	Measurement with cut-off points	Obesity cut-off point
Newman et al. 2003	ALM divided by height squared	DXA (men <7.23 kg/m^2; women <5.67 kg/m^2)	BMI (≥ 30 kg/m^2)
	ALM divided by height and FM	DXA (lowest twentieth percentile of DXA of individuals [sex-specific])	BMI (≥ 30 kg/m^2)
Villareal et al. 2005	ALM divided by height squared	ALM (<5.45 kg/m^2)	BMI (≥ 30 kg/m^2)
Vasconcelos et al. 2016	Muscle Strength	Handgrip Strength ≤ 21 kg	BMI (≥ 30 kg/m^2)
Liao et al. 2017	SMI Handgrip strength Gait speed	SMI < 7.1 kg/m^2 (women) Handgrip Strength <14.3 kg (or) Gait speed <1m/sec or both	BF% >30%

Source: Modified from (Batsis & Villareal 2018).

ALM = Appendicular lean mass; BF = Body Fat; BMI = Body Mass Index; DXA = Dual X-ray absorptiometry; FM = Fat Mass; SMI = Appendicular skeletal mass index.

2.3.1. Prevalence of sarcopenic obesity

The prevalence of SO is dependent on the criteria used to define it (Batsis and Villareal 2018), but also on ethnicity, age and sex (Du et al. 2018). Newman et al. (2003) found a prevalence of 8.9% in men and 7.1% in women for SO using relative skeletal muscle index (RSMI) in the US. Another study done on the subjects from the National Health and Nutrition Survey (NHANES,1999-2004) found that prevalence of SO was the highest in Hispanics and the lowest in non-Hispanic black Americans (Du et al. 2018).

2.3.2. Consequences of sarcopenic obesity

Obesity and sarcopenia are both associated with metabolic disorders and are important causes of morbidity, mortality and disability (Zamboni et al. 2008). Baumgartner et al. (2004) conducted a study on 451 elderly men and women and found a positive association of SO and disability. Another study done on 4000 older men, aged 60-79 years, over the course of 6 years showed that

SO men had 55% higher mortality risk than non-sarcopenic, non-obese men (Wannamethee et al. 2007). Similarly, a study done on 3366 older men and women (≥ 65 years), found that cardiovascular disease risk was 23% higher in SO group than sarcopenic and obese group alone (Stephen & Janssen 2009). Other consequences of SO include functional decline (Yang et al. 2015), postural instability (Ochi et al. 2010), increased risk of dyslipidemia (Baek et al. 2014), osteoarthritis (Lee et al. 2012) and depression (Hamer et al. 2015).

2.3.3. Aeteiology of sarcopenic obesity

The aetiological factors leading to SO, which are subsequently explained in the following paragraphs, are shown in Figure 1.

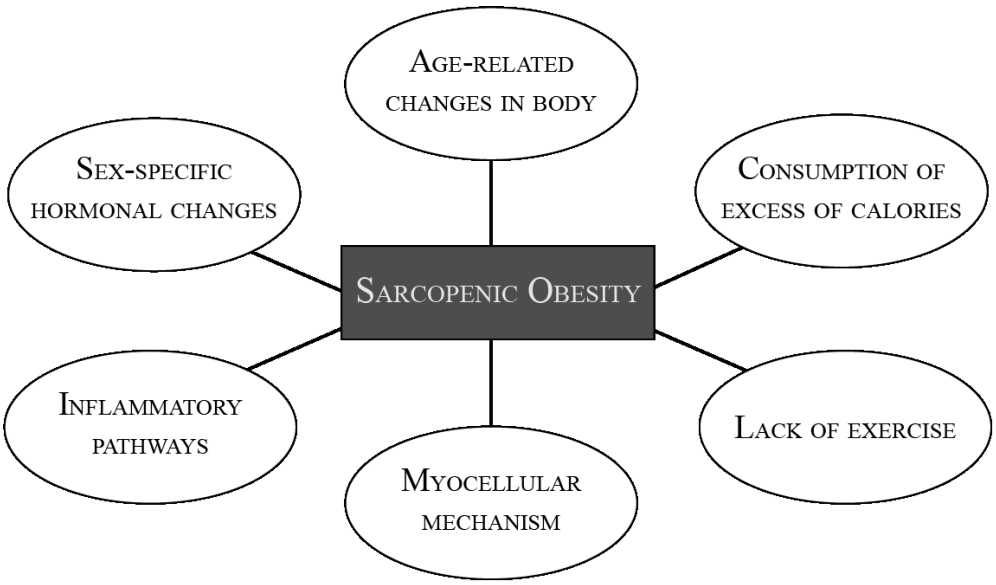

Figure 1. Aetiology and consequences of sarcopenic obesity.
Adapted from Batsis & Villareal (2018)

Changes in bone mass, body fat and myocellular mechanism with age

Ageing can lead to sarcopenia because of the infiltration of the adipose cells in muscles, which leads to lower muscle strength, quality and resistance to insulin (Stenholm et al. 2008). Besides infiltration, the age-related neuromuscular changes include reduced number, size, and strength of the muscle fibers. The loss in MM starts from the age of 30, increases with time and is the most

intensive during the age span of 65-80 years (Janssen et al. 2000). A study done by Mitchell et al. (2012) revealed that starting from the age of 30, the MM deteriorates by 0.37% per year in women and 0.47% per year in men with the rates getting higher once the subjects are 75 years and older. Neuromuscular changes associated with higher BF can lead to a systemic low-level inflammatory process and oxidative stress, which can impart further deterioration of musculoskeletal system resulting in sarcopenia, osteoporosis and frailty (Stenholm et al. 2008).

Hormonal changes and SO

The age-related changes affect the testosterone and estrogen in males and females, respectively. Hormonal changes, such as lower testosterone, estrogen and growth hormone (GH) secretion, lower thyroid hormone sensitivity and leptin resistance (all associated with aging), could potentially lead to obesity (Villareal et al. 2005). Furthermore, a study done by Feldman et al. (2002) on a cohort of 40-70 years old men showed a decline in testosterone by 0.8% per year which can have a negative effect on the MM and fat distribution (Yeap 2009). In women, the FM and body weight increases after menopause while the MM decreases. The increase in FM is in the visceral areas which make up for 15-20% of total fat stores (Trémollieres et al. 1996), thus resulting in an increase in waist circumference and a reduction in the proportional MM (Sowers et al. 2007).

Inflammatory pathways and anabolic resistance to protein synthesis

Different inflammatory pathways are common to muscle and visceral fat (Batsis & Villareal 2018). These inflammatory pathways potentially leading to inflammation can cause complications, such as anabolic resistance to protein synthesis, proteolysis, insulin resistance and metabolic complications (Mraz & Haluzik 2014). The insulin resistance, having a positive effect on muscle breakdown, promotes FM gain and ultimately result in a loss of MM (Schrager et al. 2007).

Similarly, the low-grade inflammation associated with obesity is caused by the activation of macrophages, T-lymphocytes and mast cells. This low-grade inflammation further leads to the secretion of tumor necrosis factor (TNF), GH and adipokines such as leptin that can promote obesity (Tam et al. 2010). Leptin upregulates pro-inflammatory cytokines TNF and interleukin (IL-6), resulting in the decrease of anabolic actions of insulin-like growth factor 1 (IGF1)

(Hamrick 2017). This decrease further leads to an age-related reduction in testosterone which can result in truncal obesity, sarcopenia, and frailty (Kadi 2008, Yeap 2009). Elevated TNF also blocks adiponectin, which is inversely related to obesity and leptin (Wang et al. 2014), thus impeding muscle protein synthesis and mitochondrial processes (Cartwright et al. 2007).

Exercise and SO

Exercise can have an important role in maintaining functional fitness (Brach et al. 2003). A study concluded by Brach et al. (2003) on 229 older women over the course of 14 years demonstrated a significant association between physical activity and maintenance of functional ability. If elderly people do not indulge in a physically active lifestyle, they can be prone to reducing their MM and joint mobility by 40% and 10-40% respectively, depending on the body part. They can also experience up to 30% MS loss which is related to the loss in MM (Zamboni et al. 2008).

Diet and SO

Diet can have an impact on the development of sarcopenia and obesity (Trouwborst et al. 2018). The mechanism by which dietary intake can affect sarcopenia is an imbalanced diet (Trouwborst et al. 2018) as older people tend to have less protein in their diet (Morley 1997) thus impairing the muscle turnover. The mechanism by which obesity is affected is the excessive consumption of food, which can lead to energy imbalance between energy intake and expenditure (Stenholm et al. 2008).

2.4. Protein

Proteins are nitrogen-containing substances that are formed by amino acids and are an important part of the muscle and other tissues (Hoffman & Falvo 2004). The body uses amino acids, hence proteins, from the dietary intake to build up muscles (Hoffman & Falvo 2004). Proteins can also be used as an energy source; however, it is not the first choice in the metabolism process (Nordic Nutrition Recommendations 2012). Proteins make up 15-20% of the human body, which corresponds to almost 12 kg in a person weighing 70 kg (Hoffman & Falvo 2004).

The body uses proteins for metabolism in its simplest form, i.e. amino acid. There are 20 amino acids that are needed for human metabolism and growth (Hoffman & Falvo 2004). The amino acids are further divided into essential and non-essential amino acids. Non-essential are the ones

that the body can produce by itself while essential are the ones that should be consumed from an external source, i.e. through diet (Hoffman & Falvo 2004).

Essential amino acids also have a positive regulatory effect on muscle protein synthesis in the muscle (Paddon-Jones et al. 2004). However, in older and obese people the response to anabolic stimuli gets slower (Guillet et al. 2004), resulting in a higher protein requirement than for younger adults in order to optimally promote muscle protein synthesis for maintaining or regaining MM (Breen & Phillips 2011).

2.4.1. Recommended protein intake and Food sources of protein

The recommended daily intake for protein in older adults varies in the scholarly literature. The PROT-AGE Study Group recommends a dietary protein intake of 1.0-1.2 g/kg of body weight (BW) per day in healthy older adults ≥ 65 years (Bauer et al. 2013). The Institute of Medicine recommends a dietary allowance of 0.8 g/kg of BW per day (Trumbo et al. 2002), however, it might not be sufficient to preserve MM and PF in older adults (Lemieux et al. 2014, Volpi et al. 2013). According to Nordic Nutrition Recommendation 2012 (NNR), the recommended amount of protein for older adults ≥ 65 years of age is 15-20% of the total energy intake and 1.1-1.3 g/kg of BW per day. Other literature suggests a dietary protein intake in the range of 1.0-1.5 g/kg of BW per day, as it may provide health benefits beyond simply meeting the minimum requirements (Paddon-Jones et al. 2015, Volpi et al. 2013).

Protein availability comes in a variety of plant and animal food sources. Some of the animal-based food sources of protein include eggs, milk, meat, fish and poultry while the common plant-based protein foods are cereals, vegetables, fruits, legumes and nuts (Hoffman & Falvo 2004).

2.4.2. Food sources of protein and body composition and physical function

The food source of protein can influence the odds of having sarcopenia in older age (Chan et al. 2016). For example a higher intake of plant based food sources of protein is associated with lower odds of developing sarcopenia (Chan et al. 2016), reduced risk of disability, decline in physical performance (Perälä et al. 2016) and a lower risk of reduced walking speed (Talegawakar et al. 2012). A study done by Kojima et al. (2015) found that after a 4-year follow up, the decline in MS in older women was low in people that frequently consumed soy and

yellow and green vegetables while the animal food sources of protein for example fish, meat and eggs were not associated with MS.

However, studies in contrast to the aforementioned ones have shown a significant association between animal food sources of protein and MM and MS (Houston et al. 2008, Robinson et al. 2008, Sahni et al. 2015, Alexandrov et al. 2018). For example, higher MS was associated with fish consumption in a cross-sectional study done on 2983 older adults i.e. 59-73 years (Robinson et al. 2008). Alexandrov et al. (2018) showed that AP from fish, egg and meat was positively associated with preserving MM. Another study concluded on 2066 older men and women (70-79 years) showed that AP was positively associated with MM and ALM (Houston et al. 2008). At the same time, Sahni et al. (2015) showed that AP was associated with increased MM of legs while PP was associated with increased quadriceps strength.

Table 3 shows studies on food sources of protein and its impact on MM, MS, sarcopenia and physical function.

Table 3. Studies on the association of food sources of protein with muscle mass, muscle strength, physical performance, and sarcopenia

Study	Design	Subjects	Length	Method	Result
Alexandrov et al. 2018	Cross-sectional study	31,278 men and 45,355 women with an age range of 18-91 years	-	FFQ was used to measure the protein intake and the estimated MM was measured by a 24-hour urine creatinine excretion.	Protein intake from fish, meat and egg was significantly associated with overall MM preservance.
Chan et al. 2016	Longitudinal Study	3957 Chinese older adults (aged ≥ 65 years) for cross-sectional analysis and 2948 for prospective analysis	5 years follow up	FFQ was used for dietary intake assessment while sarcopenia was defined according to the AWGS criteria.	Higher intake of vegetables, fruits and dairy was associated with the lower odds of prevalent sarcopenia in men.
Helsinki Birth Cohort Study, Finland (Perälä et al. 2016)	Longitudinal Study	1072 men and women with a mean age of 61.3 ± 0.2 years	10 years follow up	Food assessed with FFQ while physical performance assessed by the validated SFT battery.	Higher intake of cereals, fruits and berries and low intake of red and processed meat are related to a better overall physical performance and can reduce the risk of disability in old age.
Kojima et al. 2015	Longitudinal Study	575 older women between 75 to 85 years in 2008 and 78 to 89 years in 2012	4 years	Food intake was measured by asking closed-ended questions about frequencies of 10 food groups. For muscle strength, isometric knee extension was measured in the dominant leg using a dynamometer.	The decline in muscle strength with age was lower in people that consumed soy and green and yellow vegetables frequently.

Sahni et al. 2015	Cross-sectional Study	Men (n=1166) and women (n=1509) aged 59 ± 9 years	-	FFQ was used for food assessment. DXA scan was used for MM of legs while quadricep strength was calculated by dynamometer.	PP was associated with increased quadriceps strength. AP was associated with increased MM of legs.
Houston et al. 2008	Prospective Cohort Study	2066 older men and women aged 70-79 years	3 years	FFQ was used for food assessment. DXA scan was used for MM and aLM measurement.	Positive significant association was found between AP and MM and aLM while there was no significant association between PP and MM and aLM.
Robinson et al. 2008	Cross-Sectional Study	2983 older adults from 59 to 73 years of age	-	FFQ was used for food assessment while grip strength was calculated by dynamometer.	Each additional portion of fatty fish consumption/week was associated with higher grip strength (0.43 kg) in men and (0.48 kg) in women ($p<0.001$).
The Boston FICSIT Study, USA (Bernstein et al. 2002)	Cross-Sectional Study	98 older adults ≥ 70 years	-	Food intake was calculated by a 3-day dietary food record and the mean arm muscle area (MAMA) and the thigh muscle area was calculated	High fruit and vegetable intake score was associated with a higher MAMA ($p \leq 0.03$). No significant association in the thigh muscle area.
Haub et al. 2002	Randomized Controlled Trial	Men (n=21) 65 ± 5 years	12 weeks	FFQ was used for food assessment. Resistive training under the supervision of a trainer and body composition assessment measured by plethysmography. Cross-	Resistive training-induced hypertrophy was not significantly different in the group consuming a diet with beef as the main protein source than the LOV diet

				sectional muscle area of mid-thigh measured with GE CT scanner (General Electric, Milwaukee).	group with soy as the main protein source.
Campbell et al. 1999	Longitudinal Study	Men (n=19) 51-69 years	12 weeks	FFQ was used for food assessment. Body weight and height were measured indoors. Whole body-density measured by using hydrostatic weighing.	Omnivorous diet group experienced an increase in FFM and SM size compared to the LOV diet group.

AWGS = Asian Working Group for Sarcopenia; aLM = Appendicular lean mass; AP = Animal protein; CT = Computed Tomography; DXA = Dual X-ray Absorptiometry; EWGSOP = European Working Group on Sarcopenia; FFM = Fat free mass; FFQ = Food frequency questionnaire; FICSIT = Frailty and injuries: Cooperative studies of intervention techniques; LM = Lean Mass; LOV = Lactovovegetarian; MAMA = Mean arm muscle area ; MS = Muscle strength; SFT = Senior Fitness Test ; SM = skeletal muscle

2.4.3. Food sources of protein and body weight

Studies differ in their outcomes regarding the association of food sources of protein with body weight (Berryman et al. 2016, Bujnowski et al. 2011, Halkjær et al. 2011, Lin et al. 2011, Park et al. 2018). A study conducted by Park et al. in 2018, showed that although protein intake was negatively associated with BMI and waist circumference (WC), the difference between associations of plant-based and animal-based protein food sources with obesity was not significant (Park et al. 2018). Similarly, Berryman et al. (2016) also have not found any difference in plant- and animal-based protein food sources association with BMI, WC and body weight. A study done by Halkjaer et al. (2011) found that a higher intake of protein was not associated with weight gain, however, animal-based food protein groups were associated with long-term weight gain (Halkjær et al. 2011). Similarly, Bujnowski et al. (2011), in his study conducted on American men, revealed that animal-based food protein was positively associated with body mass and overweight/obesity while an inverse relationship was observed with plant-based food protein (Bujnowski et al. 2011). Table 4 presents a summary of studies on the association of different food sources of protein with body weight.

Table 4. Studies on the association of food sources of protein with body weight

Study	Design	Subjects	Length	Method/Diet/Supplementation	Result
Park et al. 2018	Cross-sectional study	2549 men and women aged ≥ 60 years		Health examination for example comorbidity, socioeconomic status, periodic health examination and behaviour surveys, and nutrition surveys for example FFQ, 24-hour dietary recall.	Protein intake was negatively associated with BMI and WC. There was no difference in the association of plant and animal protein with obesity.
Berryman et al. 2016	Cross-sectional study	11,111 men and women ≥ 19 years		Total dietary intake was calculated via the use of a 24-hour recall data.	Diets higher in animal and plant protein foods were associated with lower BMI, WC and body weight.
Bujnowski et al. 2011	Longitudinal Study	1730 white men, aged 40-55 years	7 years	FFQ was used for diet assessment which included the time and place of meals, weekday/weekend food pattern, special diets and changes in eating habits.	Animal protein intake was positively related to a higher body mass and overweight/ obesity. Vegetable protein was inversely related to body mass and overweight/obesity.
Halkjær et al. 2011	Observational study	89,432 men and women. The participants data was taken from six cohorts within five countries participating in the EPIC study.	6.5 years (mean)	Dietary assessment done via FFQ and anthropometric measures for example weight, waist circumference and height were taken at baseline and then during subsequent follow-ups.	A high intake of protein was not associated with lower weight or waist gain. Animal protein food groups were positively associated with long-term weight gain.

Lin et al. 2011	Cross-sectional study	3083 Belgian individuals aged ≥ 15 years	-	Dietary assessment was done via two non-consecutive 24-hour dietary recall while the anthropometric measures were self-reported except WC which was measured by a trained dietitian.	Plant protein food sources were associated with lower BMI and WC.

BMI = Body Mass Index; BW = Body weight; FFQ = Food frequency questionnaire; E% = Energy percentage; EPIC = European Prospective Investigation into Cancer and Nutrition; WC = Waist Circumference

2.5. Physical activity

Physical activity and exercise are commonly described as a healthy activity and has been found as an effective approach to reduce obesity (Vissers et al. 2013) and as a line of intervention to improve PF, MM and MS in older sarcopenic adults (Montero-Fernández & Serra-Rexach 2013).

Physical activity helps in energy regulation and in fat loss when combined with a hypocaloric diet, thus resulting in a lower energy balance, ultimately leading to a reduction in FM in obese older adults (Stoner et al. 2016). It can have a positive impact on the physical functioning parameters, for example, hand-grip strength, walking speed, balance and aerobic capacity, in obese and sarcopenic subjects (Cadore et al. 2014). A study done on older men aged 70-92 years old, showed a negative association between SO and physical activity. The men were asked to report their habitual physical activity levels, timings and intensity. The results revealed an association of increased physical activity with a reduced risk of sarcopenia and SO while sedentary behaviour was associated with an increased risk of SO (Aggio et al. 2016).

Likewise, exercise coupled with the high protein dietary intake is the key stimulus leading to a muscle protein synthetic response (Koopman & van Loon 2009); for example, in the fed state, muscle hypertrophy occurs after exercise. This is because the process of protein breakdown and formation is higher, leading to muscle hypertrophy and the gain of protein (Phillips et al. 2002).

The exercise regime followed by an individual should consider the volume, intensity, progression, and frequency of training while keeping in view the main goal of improving the MS, endurance, and elasticity in the SO adults leading to the autonomy as well as the improvement in mobility among them (Trouwborst et al. 2018). Different exercise regimes include resistance exercise, eccentric and aerobic exercises.

Resistance exercise is an effective way to improve MS and to cause muscle hypertrophy in the elderly (Liu & Latham 2009, Peterson et al. 2011). For instance, a meta-analysis of 49 studies done by Peterson et al. (2011), which was comprised of 1328 participants ≥ 50 years, revealed that by following a resistance training for 2-3 times a week for 20.5 weeks, an increase of 1.1 kg of MM in the subjects occurred. Another study consisting of a total of twenty-eight older women with SO exercising (resistance exercise) group and a non-exercising control group over a period

of 10 weeks showed that the resistance exercise did not improve the power, strength or physical function of the SO exercising women (Vasconcelos et al. 2016).

Eccentric exercise is another form of exercise in which the muscle contracts while stretching itself, for example, while going down the stairs (Trouwborst et al. 2018). Eccentric exercise is advantageous because it increases the strength of the muscles (Franchi et al. 2017) while lowering energy consumption as compared to the concentric contraction (Hoppeler 2016). A study done in 2011, comprising of 14 men and 14 women with a mean age of 80 years and without SO, who followed a 12-week exercise, aimed to compare the eccentric and resistance exercise. DXA and muscle biopsies taken before and after the training revealed that eccentric exercise enhanced the body composition by reducing the FM and improved MS (Mueller et al. 2011).

Similarly, aerobic exercise could also potentially be used for improving the muscle function in older adults (Forbes et al. 2012) and to counteract obesity (Willis et al. 2012). Aerobic exercise in combination with dietary intervention might reduce BF (Bouaziz et al. 2015). A randomized controlled trial done by Chen et al. (2017) for eight weeks revealed that aerobic exercise led to a decrease in BF mass while maintaining the MM in a group of 60 sarcopenic obese adults between the age of 65 and 75.

3. STUDY AIMS

The aim of the study was to investigate the association of dietary protein intake and its sources with SO in elderly women in Kuopio, Finland. The hypothesis was that plant-based food sources of protein are negatively associated with SO. In order to verify the hypothesis, a comparison of the baseline characteristics and food consumption among women with or without sarcopenia and obesity, or both was undertaken.

4. METHODOLOGY

4.1. Study design and population

The Osteoporosis Risk Factor and Prevention Study (OSTPRE) is an ongoing observational study that started in the Kuopio region, Finland in 1989 with the latest follow-up in 2018. The data used in this thesis were excerpted from OSTPRE-FPS, a sub-study of OSTPRE, which is a 3-year intervention study to investigate the effectiveness of calcium (1000 mg/d) and vitamin D (800 IU/d) supplementation in the prevention of fractures and falls in postmenopausal women (Kärkkäinen et al. 2010). The inclusion criteria were: residency in Kuopio, age of 65 or older at the end of November 2002, willingness to participate and no prior participation in former trials or bone densitometry measurements of the OSTPRE.

This study was a secondary analysis of subjects from the OSTPRE-FPS. Out of the 3,432 women at the baseline, a subsample of 750 women was randomly selected to take part in the detailed examination which included body composition, and clinical, physical and laboratory tests (intervention, n=375, and control group, n=375) (Kärkkäinen et al. 2010). There was no statistical power analysis for this study, and the original calculation was conducted by Kärkkäinen et al. (2010) prior to intervention. At the end of baseline measurements, 610 women successfully underwent full dual x-ray energy absorptiometry (DXA), of which 554 women returned a valid 3-day food-record. These women shaped the final analytical data for this study. All the clinical measurements were performed as part of OSTPRE-FPS at the Kuopio Musculoskeletal Research Unit of the Clinical Research Centre of the University of Kuopio with written consent and permission provided by the participants. The original study was approved by the ethical committee of Kuopio University Hospital in October 2001 and is registered in clinicaltrials.gov by the identification NCT00592917. The subject's measurements used in this master's thesis analysis are from the secondary analysis on the OSTPRE-FPS 3-year fracture prevention trial started in November of 2002 and the design of the study is cross-sectional.

4.2. Dietary intakes

A 3-day food record was used to collect data on food consumption in the form of a questionnaire. The participants received the instructions for the questionnaire beforehand while the responses were returned upon their visit to the research site. The questionnaire was to be filled for three

consecutive days with the inclusion of two weekdays and one day at the weekend (Saturday or Sunday). The participants were asked to follow instructions in the questionnaire while estimating the consumed amounts of food using household measures. The records were later checked by a nutritionist and in case of uncertainties, the participants were called and asked for clarification over the phone (Järvinen et al. 2012). Intake of nutrients and food consumption was calculated using the Nutrica program (Version 2.5, Finnish Social Insurance Institute, Turku, Finland) (Järvinen et al. 2012). The collected dietary data provided details on sources of food as well as the amount of proteins present in them. Table 5 illustrates the food sources of protein used in the study.

Table 5. Sources of protein used in the study

Animal protein food sources	Meat	The total meat in grams/day, as well as the protein intake from meat in grams/day.
	Dairy	Dairy, as well as the protein from dairy, was also calculated as grams/day. The variable was further divided into cheese, sour milk, milk, and other dairy products, for example, ice cream.
	Fish	Fish intake was included as grams/day. The protein intake from fish was also calculated as grams/day.
	Eggs	Egg consumption was also included as grams/day along with its protein intake.
Plant protein food sources	Vegetables	All vegetable intake and the protein intake from vegetables, root vegetables, legumes, nuts, mushrooms and vegetable products per day was included in the study as grams/day.
	Fruits and Berries	The fruit and berries intake was calculated together as grams per day as well as the protein from it.
	Cereals	The cereals intake and the protein from it was also calculated as grams per day. The cereal intake comprised of whole grain cereals, white bread and other cereal products.

4.3. Self-reported questionnaires

A self-administered postal questionnaire was used to gather the lifestyle-related information. The questionnaire provided data on age, time since menopause (year), hormone therapy use (used or never used), mobility status, alcohol use, smoking status (never, previous, and current smokers), and income per month which was used as a proxy for socio-economic status. For alcohol use, the frequency of consumption of portions of alcohol, with one portion being equal to 12g alcohol, was inquired in the self-reported questionnaire.

The level of mobility was self-reported by choosing 1 of 6 answers to the question "What is your current moving ability?". The answer choices were "full ambulatory status", "capable of walking but not running", "capable of not walking more than 1 km", "capable of not walking more than 100 m", "capable of moving only indoors" or "unable to move" (Sirola et al. 2010). The responses by the subjects were re-categorized into two groups, i.e. normal mobility and restricted mobility. The normal mobility status category included subjects that answered, "full ambulatory status" and "capable of walking but not running", while the restricted mobility category included women who were not able to walk more than 1km, 100m, only able to move indoors and immobile. The physical activity by the study subjects was also self-reported as hours per week including the type and intensity of the physical activity for each month of the year. The categories that constituted physical activity were " walking and hiking", "jogging-running-tracking", "cross country skiing", "cycling", "swimming", "aerobics", "ball games", "gardening and snow cleaning", "hunting/picking up berries and mushrooms", "fishing", "handicrafts making", "rowing with boat", "wood work", "downhill skiing", "ice skating", "gym", "dancing", "bowling", "housekeeping" and "other sports". The physical activity per week was then calculated by adding all the variables and calculating the average per week.

4.4. Anthropometric measures and body composition

The anthropometric measurements, height and weight, were measured in light indoor clothing without shoes followed by the calculation of BMI in kg/m^2. Specialized trained nurses performed DXA scans for the measurement of body composition using the same Lunar Prodigy following the imaging and analysis protocols provided by the manufacturer (Lunar Co., Madison, WI, USA). DXA provided distinctive measurements of total body FM, MM and bone mass (BM).

DXA is a commonly used tool to evaluate body composition and is proven to be more precise than bioimpedance for estimating body composition (Aandstad et al. 2014, Lohman et al. 2009). The sum of non-fat, non-bone skeletal MM in arms and legs was used to calculate the appendicular lean mass (aLM). The relative skeletal muscle index (RSMI) was calculated by dividing the aLM by the square of height in meter (m^2) (Poggiogalle et al. 2019). The indicators used have been reported in earlier studies (Isanejad et al. 2016, Mangano et al. 2017, Poggiogalle et al. 2019).

4.5. Physical function

Trained nurses assessed the physical function of the subjects. The assessment consisted of handgrip strength (PSI) and a 10-meter walking speed (gait speed) in meters per second (m/s).

For the grip strength measurement, three attempts were recorded with an approximate of 30 s resting time between each test. The non-dominant hand was used while being seated on a bench with the forearm flexed from the elbow at a 90° angle, near the torso. Attention was paid to make the attempts similar in fixed posture (JAMARTM handgrip dynamometer; 55 Sammons Preston, Bolingbrook, IL). The maximal result was recorded as the one that was the best attempt out of the three (Rikkonen et al. 2012). The intraclass correlation coefficient for grip strength measurements was 0.93 and grip strength was further expressed as a ratio to body mass to standardize it.

Walking speed was measured by asking the women to walk the 10 m distance. The time of the walk was recorded and the walking speed was calculated as m/s.

4.6. Sarcopenic obesity ascertainment

To define SO, we checked if both the conditions of being obese and sarcopenic were met. First, we defined sarcopenia according to the EWGSOP criteria (Cruz-Jentoft et al. 2010) the use of which has been reported in earlier studies (Isanejad et al. 2016). Women were categorized according to their RSMI values into quartiles: (quartile 1) 5.3–6.3 kg/m^2, (quartile 2) 6.3–6.7 kg/m^2, (quartile 3) 6.7–7.2 kg/m^2 and (quartile 4) 7.2–9.3 kg/m^2. A 5.45 kg/m^2 sarcopenia cut-off point was reported by Baumgartner et al. (2000), which was calculated as two standard deviations below the mean in the young reference population. In our study, however, only six

women had RSMI less than 5.45 kg/m^2. Therefore, we decided to use the lowest quartile below 6.3 kg/m^2 as a cut-off in the present study, and those women received a score of 1.

For the physical function test, hand grip strength and walking speed were used to define sarcopenia. The population was divided into quartiles also for their grip strength: (quartile 1) < 22.2 kg, (quartile 2) 22.3–25.6 kg, (quartile 3) 25.7–28.6 kg and (quartile 4) >28.7 kg and the lowest grip strength then was given a score of 1. For gait speed 10 m, we categorized women into quartiles according to their gait speed: (quartile 1) <1.42 m/s, (quartile 2) 1.42–1.63 m/s, (quartile 3) 1.64–1.85 m/s and (quartile 4) >1.85 m/s. The women who were unable to walk were also allocated in the lowest quartile and received a score of 1. A woman was classified as sarcopenic if she belonged to the lowest quartile of RSMI and had the lowest quartile of either walking speed or grip strength or both.

For obesity, Baumgartner et al. (2004) used 40% body fat as a cut-off point and the women who fall into this category were in the 60th percentile of the study. In our study, the 60th percentile was 40.9% of BF, therefore we similarly used 40% BF as a cut-off point for obesity.

4.7. Statistical analysis

Statistical analysis was performed using the SPSS statistics for Windows software version 25. Differences between groups were considered statistically significant if the p-value was <0.05. The variables were checked to be normally distributed. The variables that were not normally distributed were log transformed and those variables were used in statistical testing. One-way ANOVA was used to compare means and standard deviations (SD) of continuous variables, i.e. the baseline characteristics and food sources were compared with respect to the category of sarcopenia, obesity, sarcopenic obesity and referent group. For categorical variables, the Chi-square test was used to test the statistical significance and the numbers and percentages were reported accordingly.

Furthermore, the effect of covariates, i.e. energy intake, was also evaluated by running a univariate general linear model and the adjusted p-value was reported.

5. RESULTS

5.1. Baseline characteristics of the participants

The total study population comprised of 555 elderly women. The baseline characteristics are presented in Table 6. The subjects were 65-72 years old with a mean age of 67.8 ± 1.9 years, while the mean BMI was 28.6 ± 4.7 kg/m^2. Using a 40% BF% cut-off, 225 women were obese and as per the EWGSOP criteria, 30 women were sarcopenic and 31 were SO. The women's weight (p-value <0.001) in the sarcopenic group was the lowest (59.3 ± 6.4kg), followed by referent (65.9 ± 8.1kg), SO (70.9 ± 6.9kg) and the highest in the obese group (81.4 ± 10.9kg). The BMI was highest in the obese group, i.e. 32.2 kg/m^2 followed by SO 29.2 kg/m^2, referent 26.1 kg/m^2 and the lowest in the sarcopenic group i.e. 23.9 kg/m^2.The normal mobility percentage (p-value <0.001) among the subjects was the lowest in the obese group (88.5%), followed by SO group (89.6%), sarcopenic (95.8%) and referent (97.5%), while the mean gait speed was lowest in the sarcopenic group being 1.4 m/s, followed by SO 1.51 m/s, obese 1.54 m/s and the highest, 1.7 m/s, in the referent group (p-value <0.001). Handgrip strength was the highest in the referent group, 26.9 kg, followed by obese 25.6 kg, SO 22.8 kg and the lowest in the sarcopenic group, i.e. 19.8 kg (p-value <0.001).

The analysis of body composition revealed that MM and BF in kg were the lowest in the sarcopenic group being 35.8 kg and 20.8 kg respectively, while the obese group had the highest MM, i.e. 41.5 kg, and BF 36.3 kg, compared to the rest of the groups (p-value <0.001). The BF% was the lowest in the referent group being 34.4% followed by sarcopenic 34.7%, SO 44.2% and the highest in the obese group, i.e. 44.5% (p-value <0.001). The MM% was the lowest in the SO group being 50.8%, followed by obese 51.3%, sarcopenic 60.8% and the highest in the referent group, i.e. 61.3% (p-value <0.001).

The RSMI was the lowest in the sarcopenic group being 5.9 kg/m^2 and the highest in the obese group, i.e. 6.9 kg/m^2 (p-value <0.001). While the BMD was also the lowest in the sarcopenic group being 0.995 g/cm^2 and the highest in the SO group, i.e. 1.109 g/cm^2 (p-value <0.001).

Table 6. Baseline characteristics according to sarcopenic obesity status

Baseline Characteristics	Referent (n=269)	Sarcopenic (n=30)	Sarcopenic Obese (n=31)	Obese (n=225)	Total (n=555)	Significance
Age (years)	67.8 ± 1.9	68.3 ± 2.1	68.0 ± 2.1	67.8 ± 1.9	67.8 ± 1.9	0.561
Weight (kg)	65.9 ± 8.1	59.3 ± 6.4	70.9 ± 6.9	81.4 ± 10.9	72.1 ± 12.2	<0.001
Height (m)	1.59 ± 0.05	1.57 ± 0.06	1.56 ± 0.04	1.59 ± 0.05	1.59 ± 0.05	0.009
BMI (kg/m^2)	26.1 ± 3.0	23.9 ± 1.9	29.2 ± 2.9	32.2 ± 4.4	28.6 ± 4.7	<0.001
Physical activity (h/week)[a]	12.1 ± 16.2	9.6 ± 9.4	7.7 ± 9.4	9.3 ± 11.7	10.6 ± 14.0	0.921
Normal mobility n (%)	239 (97.5)	23 (95.8)	26 (89.6)	178 (88.5)	466 (92.7)	0.001
Gait speed 10m (m/s)	1.7 ± 0.3	1.4 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	<0.001
Handgrip (kg)	26.9 ± 4.1	19.8 ± 3.9	22.8 ± 12.8	25.6 ± 4.7	25.7 ± 5.4	<0.001
Married n (%)	131 (60.9)	34 (64.1)	19 (59.3)	129 (57.3)	313 (59.6)	0.139
Income (€/month)	879 ± 283	986 ± 291	807 ± 412	835 ± 296	863 ± 298	0.532
Current smoking n (%)	10 (4.0)	0 (0)	1 (3.4)	13 (6.4)	24 (4.7)	0.666
Alcohol use n (%)	122 (51.9)	14 (51.8)	11 (44.0)	112 (56.5)	253 (53.0)	0.589
Time from menopause (years)	18.5 ± 4.8	17.8 ± 4.5	18.2 ± 5.6	18.6 ± 5.3	18.5 ± 5.1	0.888
Hormone therapy use n (%)	121 (48.5)	15 (57.6)	20 (66.6)	101 (49.0)	257 (50.2)	0.336

Baseline Characteristics	Referent (n=269)	Sarcopenic (n=30)	Sarcopenic Obese (n=31)	Obese (n=225)	Total (n=555)	Significance
Body Composition						
Body fat (kg)	22.9 ± 5.1	20.8 ± 4.3	31.4 ± 4.7	36.3 ± 6.4	28.7 ± 8.7	<0.001
Body fat (%)	34.4 ± 4.6	34.7 ± 4.6	44.2 ± 3.1	44.5 ± 3.0	39.1 ± 6.4	<0.001
Lean mass (kg)	40.1 ± 3.9	35.8 ± 2.8	35.9 ± 2.7	41.5 ± 4.5	40.2 ± 4.4	<0.001
Lean mass (%)	61.3 ± 4.8	60.8 ± 4.5	50.8 ± 3.2	51.3 ± 4.0	56.6 ± 6.6	<0.001
RSMI (kg/m^2)	6.8 ± 0.6	5.9 ± 0.3	6.0 ± 0.2	6.9 ± 0.7	6.8 ± 0.7	<0.001
Total body BMD (g/cm^2)	1.071 ± 0.093	0.995 ± 0.092	1.109 ± 0.068	1.097 ± 0.091	1.075 ± 0.095	<0.001

aLM = Appendicular Lean Mass; BMD = bone mineral density; BMI = Bone Mass Index; RSMI = Relative Skeletal Mass Index (aLM/height2); Adjusted p-value = adjusted values for energy intake.

a = variable not normally distributed. Normal distribution is done via a log-transformation.

ANOVA was used to calculate means ± SD and p-value for continuous variables.

Chi-Square test was used for categorical values to find out the p-value.

5.2. Food consumption and protein intake from food sources according to sarcopenic obesity status

The food consumption, both from animal and plant sources, was analysed for different groups within the population, according to their SO status. The results are presented in Table 7. Among the animal food sources of protein according to SO status, the total dairy products intake was statistically different with sarcopenic group having the lowest amount of total dairy products 445 g/day, followed by obese, i.e. 490 g/day, referent 514 g/day and the SO group having the highest amount of total dairy products, i.e. 597 g/day (adjusted p-value = 0.006).

Among the plant food sources of protein, the SO group consumed the least amount of white bread, i.e. 4 g/day, while the obese group had the highest intake, i.e. 7 g/day. After adjusting the white bread intake with energy intake, its significance was lost (adjusted p-value = 0.055). Similarly, the total cereal intake was also the lowest in the SO group, i.e. 196 g/day and was the highest in the sarcopenic group, i.e. 225 g/day. The significance was again lost after adjusting it with energy intake (adjusted p-value = 0.906). The all-vegetable intake was the lowest in the SO group being 199 g/day, while it was the highest in the referent group, i.e. 231 g/day, but after adjusting it with energy intake, it was no longer statistically different (p-value = 0.325). The protein from cereal, vegetables and total plant protein was also the lowest in the SO group being 15, 3 and 20 g/day respectively compared to the sarcopenic, referent and obese groups in the respective variables, however, the significance was lost after adjusting it with energy intake.

Table 7. Food consumption and protein intake from food sources according to sarcopenic obesity status

Baseline Characteristics	Referent (n=253)	Sarcopenic (n=24)	Sarcopenic Obese (n=29)	Obese (n=206)	Total (n=512)	Significance	Adjusted p-value
Animal Food Sources							
Milk[a] (g/d)	318 ± 231	294 ± 235	410 ± 262	320 ± 246	323 ± 240	0.336	0.121
Sour milk[a] (g/d)	148 ± 150	106 ± 145	141 ± 137	126 ± 135	137 ± 143	0.159	0.971
Cheese[a] (g/d)	31 ± 28	24 ± 27	24 ± 22	26 ± 23	28 ± 26	0.712	0.781
Other milk products[a] (g/d)	17 ± 20	20 ± 27	23 ± 24	17 ± 22	18 ± 21	0.072	0.456
Total dairy products[a] (g/d)	514 ± 249	445 ± 285	597 ± 239	490 ± 259	505 ± 255	0.381	0.006
Eggs[a] (g/d)	18 ± 15	19 ± 17	18 ± 15	18 ± 15	18 ± 15	0.698	0.552
Total meat[a] (g/d)	76 ± 43	80 ± 69	73 ± 35	79 ± 50	77 ± 47	0.944	0.402
Fish[a] (g/d)	40 ± 43	53 ± 42	35 ± 32	42 ± 39	41 ± 41	0.547	0.324
Plant Food Sources							
Whole grain cereal (g/d)	115 ± 52	129 ± 48	105 ± 53	114 ± 54	114 ± 53	0.011	0.734
White bread[a] (g/d)	5 ± 12	6 ± 13	4 ± 13	7 ± 12	6 ± 12	0.358	0.055
Other cereal products (g/d)	94 ± 51	91 ± 47	87 ± 45	86 ± 41	90 ± 47	0.695	0.691
Total cereals (g/d)	215 ± 69	225 ± 63	196 ± 70	206 ± 66	211 ± 68	0.022	0.906
All vegetables (g/d)	231 ± 93	216 ± 81	199 ± 88	225 ± 95	226 ± 93	0.051	0.325
Fruit and berries[a] (g/d)	170 ± 118	188 ± 128	173 ± 144	163 ± 117	169 ± 120	0.442	0.907

Baseline Characteristics	Referent (n=253)	Sarcopenic (n=24)	Sarcopenic Obese (n=29)	Obese (n=206)	Total (n=512)	Significance	Adjusted p-value
Protein from food sources							
Dairy (g/d)	24 ± 12	23 ± 15	25 ± 10	22 ± 12	23 ± 12	0.500	0.383
Egg[a] (g/d)	2 ± 2	2 ± 3	1 ± 2	2 ± 3	2 ± 2	0.203	0.401
Fish[a] (g/d)	7 ± 12	12 ± 13	6 ± 12	7 ± 12	7 ± 12	0.566	0.598
Meat[a] (g/d)	12 ± 11	12 ± 14	12 ± 12	14 ± 13	13 ± 12	0.639	0.085
Animal protein (g/d)	46 ± 15	45 ± 21	45 ± 13	45 ± 15	45 ± 15	0.259	0.368
Cereal (g/d)	17 ± 6	19 ± 5	15 ± 5	16 ± 6	17 ± 6	0.020	0.849
Vegetables[a] (g/d)	4 ± 3	3 ± 2	3 ± 2	3 ± 2	4 ± 3	0.021	0.394
Fruits and berries[a] (g/d)	1.1 ± 0.0	1.1 ± 1.1	1.1 ± 1.0	0.9 ± 0.8	1.0 ± 1.0	0.173	0.633
Plant protein (g/d)	22 ± 6	23 ± 6	20 ± 6	21 ± 6	22 ± 6	<0.001	0.604
Other dietary intake values							
Carbohydrate (g/d)	198 ± 48	209 ± 52	192 ± 46	187 ± 48	194 ± 48	<0.001	0.492
Protein (g/d)	70 ± 17	70 ± 23	66 ± 17	67 ± 18	68 ± 18	0.017	0.425
Fat (g/d)	55 ± 17	63 ± 30	50 ± 15	52 ± 17	54 ± 18	0.003	0.323
Energy (kcal/d)	1608 ± 342	1709 ± 520	1498 ± 351	1514 ± 369	1568 ± 367	<0.001	-

a = variable not normally distributed. Normal distribution is done via log-transformation.

Adjusted p-value = p-value adjusted for energy intake ANOVA was used to calculate means ± SD and p-value for continuous variables.

6. DISCUSSION

This master's thesis focused on the association of food sources of protein with sarcopenic obesity in elderly women. The study design was cross-sectional and the study population was Kuopio-based elderly women. To our knowledge, it is the first study of its kind to focus on the association of food sources of protein with SO among elderly women in Kuopio.

6.1. Sarcopenic obesity and food sources of protein

Previous studies looking into dietary food sources, plant and animal, and their association with sarcopenia and obesity have shown conflicting results regarding the positive or negative association of the source with the parameters of SO (Sahni et al. 2015, Chen et al. 2014, Talegawkar et al. 2012, Volpi et al. 2003).

Studies done by Chen et al. (2014) and Crichton & Alkerwi (2014) have shown a negative association between dairy products and risk of sarcopenia and obesity. As per the literature, a possible explanation of the negative association of dairy products with sarcopenic obesity could be the relationship of the dairy components, for example whey/casein, calcium, branched chain amino acids and peptides, with the parameters of SO. For instance, casein has been associated with a reduction in body weight (Gilbert et al. 2011), because a slow gut emptying rate (compared to soy for example) can possibly affect the satiety and hunger, thus prolonging the fed state of an individual (Gilbert et al. 2011). Another mechanism could be the association of dairy with higher dietary thermogenesis, which can result in greater energy expenditure and less fat storage (Bendtsen et al. 2013, Gillbert et al. 2011). Similarly, dairy has leucine which can trigger muscle synthesis (Batsis & Villareal 2018). Calcium, which is also a component of dairy, has been associated with increased faecal excretion of fatty acids, promoting energy loss that might impact adiposity thus affecting body composition (Lorenzen & Astrup 2011). However, our study showed a positive association of SO with dairy products and the total dairy intake among the sarcopenic obese subjects was the highest compared to the referent group, obese group and the sarcopenic group which is in contrast with the aforementioned studies. Given the cross-sectional nature of this study, it is difficult to know the causative association of dairy products and sarcopenic obesity or whether being sarcopenic obese might have changed the dairy intake over time.

Similarly, studies done by Alexandrov et al. (2018), Morris & Jacques (2013) and Rondanelli et al. (2015) revealed a positive association between meat protein and MM. They explained that a possible mechanism on how meat protein and SO are related could be that meat protein contains higher leucine compared to plant protein and is thus more digestible, which can help in MM preservation (Rondanelli et al. 2015). Besides leucine, meat protein also contains iron and cobalamine which have been linked to post-excercise myofibriller protein synthetic response, which is a marker of muscle mass (Burd et al. 2015). However, our study did not find any significant association between meat protein and SO, which is in contrast with these studies.

Sarcopenia has also been negatively associated with fatty fish consumption. Prior studies have revealed that n-3 fatty acid, which is found in fish, acts as anti-inflammatory agent to prevent sarcopenia (Robinson et al. 2018) and obesity (Lee et al. 2018). Nevertheless, in our study the fish consumption did not show any statistically significant relation with SO.

As SO women experience a low grade systemic inflammation (Tam et al. 2010), we thought that a possible explanation for it could be a smaller intake of plant protein food sources (Tam et al. 2010), for example vegetables, berries, fruits and cereals, as plant food sources can have an anti-inflammatory and an antioxidant effect (Robinson et al. 2018). However, our study revealed no significant association with plant protein food sources. Robinson et al. (2018) also concluded that a greater amount of whole grain cereals, fruits and vegetables could be important for muscle strength and overall health (Robinson et al. 2018). Our study did not show any significant difference for whole grain cereals, fruits and vegetables in the SO group.

6.2. Strengths and limitations

There are several strengths and limitations in the study. Foremost, the study is comprised of healthy elderly Finnish population which is predominantly homogenous; therefore, the application of the results to a global elderly population needs to be done with caution. The study is also an observational study, because of which we cannot establish a causal relationship between variables.

The study used a single 3-day dietary record, which might not be the most reliable method to predict the long-term food intake. FFQ along with biomarker levels could have been a better alternative (Shim et al. 2014) because the use of FFQ alone has also been questioned (Freedman

et al. 2007, Kristal et al. 2005, Willett & Hu 2007). The day-to-day variability might have been reduced because of the food records being collected on consecutive days rather than non-consecutive days. However, a 3-day dietary record has been labeled as a suitable method for calculating energy and protein intake in elderly subjects (Lührmann et al. 1999).

Moreover, DXA measurement for the FM and MM were used in this study. The DXA measurements were carried out using the same Lunar Prodigy, following the protocols of imaging and analysis specified by the manufacturer (Lunar Co.) (Kärkkäinen et al. 2010). DXA has proven to be more reliable than bio impedance and is a commonly used tool in measuring the ratio between muscle, fat and bone (Aandstad et al. 2014). The physical function component of the study involves dynamometric grip strength measurement, which is an important indicator of upper and lower limb strength, thus adding strength to this study.

Other short comings observed were in relation to defining the key concepts used in this study. The EWGSOP criteria to define sarcopenia, which have been previously applied by other researchers (Cruz-Jentoft et al. 2010), were utilised also for this study. The sarcopenia cut-off point (RSMI <5.45 kg/m^2) was taken using Baumgartner et al. (2000) as a reference. It was calculated as two standard deviations below the mean in the young reference population. This value was not suitable for our study as only six participants had an RSMI lower than 5.45 kg/m^2. Therefore, we decided to use the lowest quartile below 6.3 kg/m^2 as the cut-off in this study. The arbitrariness of the cut-off point should be acknowledged when interpreting the results. Also, the updated criteria for sarcopenia by the EWGSOP2 (Cruz-Jentoft et al. 2019) were not applied to our studies because only 1 woman qualified as sarcopenic if the EWGSOP2 cut-off points were to be followed.

The obesity cut-off point was taken using Baumgartner et al. (2004) as a reference. Baumgartner used 40% body fat as a cut-off point for obesity and the women that fall into this category were in the 60^{th} percentile of the study. In our study, the 60^{th} percentile was 40.9% of BF, therefore we used 40% BF as a cut-off point for obesity. Once again, the arbitrariness of the cut-off point should be acknowledged when interpreting the results.

Several known and relevant confounders to sarcopenia and obesity were measured. However, lifestyle factors such as health status, development of disease, some unnoticed habits related to

physical activity level and/or dietary habits in the participants might have affected the observed results. For example, the lower protein intake and concordance to healthy dietary patterns might be linked to the compromised health of a participant and since we do not have information on the participants' earlier health status and eating patterns, there is a possibility of reverse causality.

7. CONCLUSION

The study undertaken in the master's thesis concludes that contrary to the initial hypothesis, plant-based protein sources are not associated with sarcopenic obesity in elderly women that participated in OSTPRE-FPS study in Kuopio, Finland. Sarcopenic obesity is also not associated with animal food sources, except for total dairy products, with which sarcopenic obesity has a positive association: sarcopenic obese women consumed more dairy products compared to other groups. Also, total protein intake was not associated with sarcopenic obesity.

The study further revealed that the sarcopenic obese women among the OSTPRE-FPS participants had higher body mass index, weight and body fat percentage than the referent group while they had a lower gait speed, muscle mass percentage and mobility than the referent group.

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