

Skeletal muscle mass and body fat in relation to successful ageing of older adults: the multi-national MEDIS study

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Abstract

Background: The determinants that promote successful ageing still remain unknown. The aim of the present work was to evaluate the role of skeletal muscle mass and body fat percentage (BF%), in the level of successful ageing. **Methods:** during 2005-2011, 2663 older (aged 65-100 years) from 21 Mediterranean islands and the rural Mani region (Peloponnesus) of Greece were voluntarily enrolled in the study. Appendicular skeletal muscle mass (ASM), skeletal muscle mass index (SMI) and BF% were calculated using population formulas. Dietary habits, energy intake, expenditure and energy balance were derived throughout standard procedures. A successful ageing index ranging from 0 to 10 was used. **Results:** The mean ASM mass was 24 ± 6.0 kg, the SMI was 0.84 ± 0.21 and the BF% was 44%. Females had lower SMI and higher BF% in comparison with males, respectively [(SMI: 0.66 ± 0.09 vs. 1.03 ± 0.11 ; BF%: 51% vs. 34%, ($p<0.001$)). High successful agers had better rates in ASM ($p=0.01$), SMI ($p<0.001$) and BF% ($p<0.001$), compared with the medium and low successful ones. Changes in SMI [b-coefficient (95% CI): 2.14 (1.57 to 2.71)] were positively associated with successful ageing, while changes in BF% [b-coefficient (95% CI): -0.04 (-0.05 to -0.03)] were inversely associated with successful ageing. Results from sensitivity analysis showed that the effects of variations on body composition were consistent, less pronounced in the positive energy balance group and more pronounced among the oldest old. **Conclusions:** Body composition changes seem to be associated with lower quality of life in the older adults, as measured through successful ageing.

Keywords: Successful ageing; appendicular skeletal muscle mass; body fat; energy balance; older adults

Introduction

It is well known that ageing is associated with various physiological changes. Changes in body composition and especially in muscle mass tissue as well as in the body fat are associated with advanced age [1]. It has been reported that the muscle mass loss is almost 2% for the middle aged populations while for the octogenarians this muscle tissue loss is around 50% in comparison with younger populations [2]. Skeletal muscle mass consists almost the half of body mass and has an important role in mobility as well as in various body's metabolic functions [3,4]. Taking into account its aforementioned role, any decline in the skeletal muscle mass has an inverse effect on human health. Muscle mass decline has been related with various disability patterns, with mental health disorders (i.e. cognitive problems) as well as with increased mortality [3,4,5]. The decline in muscle mass is often replaced by increase in the body fat mass [1]. Moreover it has been reported that not only the muscle mass loss but also the increase in body fat has been related with various co-morbidities, for the older adults. It is well known that high body fat (i.e. central obesity, excess waist circumference) is associated with various metabolic disorders such as, diabetes mellitus, hypertension, metabolic syndrome, cardiovascular diseases (CVDs), cancer and low quality of life [6].

Determinants that promote successful ageing still remain not well understood and appreciated. Clearly the process of ageing is quite complex and is associated with a variety of factors, not only with physical health. Successful ageing is a concept which is considered as low probability of disease and disability, high cognitive and physical capacity, active participation throughout various social activities and represents the

aforementioned complexity in the ageing process [7,8]. A variety of factors have been associated with successful ageing such as high waist circumference, alcohol consumption as well as various co-morbidities [8]. However until now, the role of skeletal muscle mass and BF% in the level of successful ageing has never been explored in the past.

Given the complexity of ageing pathway, the association of skeletal muscle mass and body fat with elder's health and with the ageing process, together with the lack of data among Mediterranean populations, the aim of the present work was to evaluate the role of skeletal muscle mass and BF%, in the level of successful ageing of a random sample of older adults living in the Mediterranean basin and who participated in the MEDIS (MEDiterranean ISlands) study. **Specifically, it was hypothesized that those with higher skeletal muscle mass and lower body fat percentage would be more likely to have higher successful ageing levels compared to those individuals with lower skeletal muscle mass and higher body fat percentage.** Additionally, the older Mediterranean's had rarely been studied in the past; a fact that makes this survey of major importance, as it included islands (i.e., Corfu and Crete, known from the historical Seven Countries Study and Ikaria, known from the Blue Zones) where others have previously reported determinants of healthy ageing and long-living [9].

Methods

The MEDIS study sample

During 2005-2011, a population-based, multi-national, convenience sampling was performed **to voluntarily enroll $n = 2512$ older people from 21 Mediterranean islands:** Republic of Cyprus ($n=300$), Malta ($n=250$), Sardinia ($n=60$), Sicily ($n=50$), Mallorca

and Menorca (n=111) and the Greek islands of Lesbos (n=142), Samothrace (n=100), Cephalonia (n=115), Crete (n=131), Corfu (n=149), Limnos (n=150), Ikaria (n=76), Syros (n=151), Naxos (n=145), Zakynthos (n=103), Salamina (n=147), Kassos (n=52), Rhodes and Karpathos (n=149), Tinos (n=129), as well as n=300 older adults from the rural region of Mani (a southern Greek peninsula). The sampling scheme anticipated a target sample size of 300 older people from Cyprus and Malta and at least 100 from each of the other islands; according to an a-priori power analysis, a sample of 2500 participants is adequate to test two-sided hypotheses of odds ratios equal to 1.20 achieving statistical power >80%. According to the study's protocol, individuals were not eligible for inclusion if they resided in assisted-living centers, had a clinical history of cardiovascular disease (CVD) or cancer, or had lived away from the island for a considerable period of time during their lives (i.e., >5 years); these exclusion criteria were applied because the study aimed to assess lifestyle habits that were not subject to modifications due to existing chronic health conditions or by environmental factors, other than living milieu. A group of health scientists (physicians, dietitians and nurses) with experience in field investigation collected all the required information using a quantitative questionnaire and standard procedures.

The study followed the ethical considerations provided by the World Medical Association (52nd WMA General Assembly, Edinburgh, Scotland, October 2000). The Institutional Ethics Board of Harokopio University approved the design and procedures of the study (reference No. 16/19-12-2006). Participants were informed about the aims and procedures of the study and gave their consent prior to being interviewed.

Evaluation of clinical and anthropometric characteristics

All the measurements taken in the different study centres were standardized and the questionnaires were translated in all the cohorts' languages following the World Health Organization (WHO) translation guidelines for tools assessment [10]. Weight, height and waist circumference were measured using a standard protocol; body mass index (BMI) was calculated as the ratio of weight by height squared (kg/m^2). Overweight was defined as BMI between 25 and $29.9 \text{ Kg}/\text{m}^2$ and obesity was defined as $\text{BMI} > 29.9 \text{ Kg}/\text{m}^2$. Moreover, waist circumference (WC) in cm was measured in the middle between the 12th rib and the iliac crest and hip circumference in cm was measured around the buttocks. Muscle mass was calculated throughout the appendicular skeletal muscle mass (ASM) based on the equation proposed by Lee et al [11]. Specifically, the equation was: $\text{ASM} = (0.244 * \text{weight}) + (7.8 * \text{height}) + (6.6 * \text{gender}) - (0.098 * \text{age}) + (\text{race} - 3.3)$. This indicator was further adjusted by BMI to create a skeletal muscle mass index (SMI) as the proportion of ASM/BMI [12]. The percentage of body fat (BF%) was calculated based on a sex specific equation using the waist circumference measurements [13]. Specifically for the males the equation was: $\text{BF}\% = (0.567 * \text{WC}) + (0.101 * \text{age}) - 31.8$ while for the females was: $\text{BF}\% = (0.439 * \text{WC}) + (0.221 * \text{age}) - 9.4$. Diabetes mellitus (type 2) was determined by fasting plasma glucose tests and was analyzed in accordance with the American Diabetes Association diagnostic criteria (glycated haemoglobin $\text{A1C} \geq 6.5$ or fasting blood glucose levels greater than $125 \text{ mg}/\text{dl}$ or 2-h plasma glucose $> 200 \text{ mg}/\text{dl}$ during an oral glucose tolerance test-OGTT- or a random plasma glucose $> 200 \text{ mg}/\text{dl}$, or by a prior diagnosis of diabetes). Participants who had blood pressure levels $\geq 140/90 \text{ mmHg}$ or used antihypertensive medications were classified as hypertensive. Fasting blood lipid levels (HDL-, LDL-cholesterol and triglycerides) were also recorded and

hypercholesterolemia was defined as total serum cholesterol levels >200 mg/dL or the use of lipid-lowering agents according to the NCEP ATPIII guidelines [14].

Evaluation of socio-demographic, dietary habits and other lifestyle characteristics of the participants

Basic socio-demographic characteristics, such as age, gender, years of school, financial status and lifestyle characteristics, such as smoking habits and physical activity status, were recorded. Regarding financial status, the participants were asked to report their mean income during the previous three years using a four-point scale (low, inadequate to cover daily expenses = 1, medium, trying hard to cover daily expenses = 2, good, adequate to cover daily expenses = 3, very good, very adequate to cover daily expenses = 4); this scale was decided upon because of the variety of the populations studied, as well as the common difficulty of accessing exact financial data. The participants that were in the upper category were classified as participants with high financial status while all the others were classified as low and medium financial status (high vs. low-medium financial status).

Physical activity was evaluated in MET-minutes per week, using the shortened, translated in all the cohort's languages and validated in Greek version of the self-reported International Physical Activity Questionnaire (IPAQ) [15]. As minimally active or "health-enhancing physical activity (HEPA) active" were classified individuals who reported at least 3 MET-minutes per week. Furthermore, the weekly frequency of physical activity was recorded. Dietary habits were assessed through a semi-quantitative, validated and reproducible food-frequency questionnaire [16]. To evaluate the level of adherence to the Mediterranean diet, the MedDietScore (theoretical range 0-55) was used

[17]. Higher values for this diet score indicate greater adherence to the Mediterranean diet. Energy intake was evaluated through the quantification of the portions of foods and beverages consumed, using food composition tables [18, 19]. Total daily energy expenditure (TEE) was estimated using the Schofield prediction equations, adopted by the 2004 FAO/WHO/UNU report [20], using age, weight and self-reported physical activity level (PAL) information (i.e. frequency and kind of physical activities as well as the duration of the activity). Moreover, the energy balance was calculated throughout the equation: Energy Balance = Energy intake – Total energy expenditure. Negative energy balance was considered when energy balance was < 0, while positive energy balance was considered when energy balance was > 0 [21]. Furthermore, consumption of various alcoholic beverages (i.e., wine, beer, whiskey, vodka, and the traditional ouzo, tsipouro and retsina) was measured in terms of wineglasses per day, adjusted for ethanol intake (i.e., one 100 ml glass was considered to have 12% ethanol) and classified for the present analyses, into 0 for no alcohol consumption and 1 for alcohol consumption of at least 1 glass/week. A similar dichotomized coding followed for the tea and coffee consumption.

Current smokers were defined as smokers at the time of the interview. Former smokers were defined as those who had previously smoked, but had not done so for a year or more. The remaining participants were defined as occasional or non-current smokers. Symptoms of depression during the previous month were assessed using the validated and locally adopted version of the shortened, self-report Geriatric Depression Scale (GDS) (range 0-20) [22]. Moreover, in order to evaluate the older adult's social participation, the weekly frequency of their social activities with their family, their friends as well as their yearly frequency of excursions were recorded.

Further details about the MEDIS study protocol may be found elsewhere [23].

Evaluated outcomes

Following the multi-dimensional approach of successful ageing already reported by several experts [24,25] as well as the MEDIS study group [8, 26, 27], 10 components (i.e., education as measured in years of school, financial status, physical activity status as classified using the IPAQ, body mass index, psychological level as measured using the GDS score, participation in social activities with friends, with family, yearly excursions, burden of CVD risk factors and dietary habits as evaluated using the MedDietScore) were incorporated for the measurement of successful ageing. The composed successful ageing index was represented as the cumulative score of the 10 components (theoretical range 0-10); specifically, individual ratings (from 0 to 1) in each of the 10 components were assigned, according to their positive or negative (i.e. reverse scoring) influence on successful ageing [8, 26, 27]. Furthermore, the tertiles of the successful ageing index, i.e. 1.91/10, 1.92.-3.08/10 and >3.09/10 were used as cutoffs to classify participants as low, moderate or high successful agers.

Statistical analysis

Continuous variables are presented as mean \pm standard deviation (SD) and categorical variables as frequencies. Comparisons of continuous variables between groups of study were performed using the independent samples t-test and the Analysis of Variance (for the normally distributed variables), or the Mann-Whitney U-test and the Kruskal-Wallis test (for the skewed variables). Associations between categorical variables were tested using the chi-square test. Spearman rho coefficient was applied to evaluate the correlation between continuous or ordinal variables, BF% and SMI. Linear

regression models were applied in order to evaluate the association between various socio-demographic, bio-clinical, nutritional factors (independent variables), the SMI, the BF% and the level of successful ageing (dependent outcome). Colinearity was tested using the Variance Inflation Factor criterion (VIF; values >4 suggested colinearity between independent variables and one of them was excluded from the model). The assumption of homoscedasity was tested by plotting the scatter plot of standardised residuals over the predicted score values. Results from linear regression models are presented as b-coefficients and their 95% Confidence Intervals (CI). All reported *p*-values were based on two-sided tests. SPSS software (version 20) was used for all calculations (IBM Statistics, Greece).

Results

In the entire sample, the mean ASM mass was 24 ± 6.0 kg, the mean SMI was 0.84 ± 0.21 and the mean BF% was 44%. The females had lower SMI and higher BF% comparing with the males, respectively (SMI: 0.66 ± 0.09 vs. 1.03 ± 0.11 , $p<0.001$; BF%: 51 vs. 34, $p<0.001$). Comparing participants living in rural and urban areas, there was no significant difference in the SMI and in the BF%. When the 6 geographical areas of the participants were taken into account (i.e., Aegean, Ionian islands, Crete, Cyprus, west Mediterranean islands and South Peloponnesus), the inhabitants of Crete had the highest percentage of body fat (i.e., 46%), followed by Aegean islands (i.e., 45%) and Cyprus (i.e., 44%) while the participants living in South Peloponnesus had the lowest ones (i.e., 42%) ($p<0.001$). Demographic, behavioral, clinical, anthropometric and lifestyle characteristics of the sample, by age group (<80 vs. >80 years old), are summarized in **Table 1**. Compared

with younger older adults, the octogenarians were rural residents ($p < 0.001$), had lower financial status ($p = 0.01$), were less physically active ($p = 0.004$) and ever smokers ($p = 0.02$), had lower prevalence of hypercholesterolemia ($p < 0.001$), lower rates of appendicular skeletal muscle mass (kg) ($p < 0.001$) and lower SMI rates (ASM kg/BMI) ($p < 0.001$), lower BMI levels ($p < 0.001$), lower education status ($p < 0.001$) and lower adherence to the Mediterranean diet ($p = 0.009$), while the most of them were living alone ($p < 0.001$). Moreover, no differences were observed between both age (<80 vs. >80 years old) with regards to BF%, the prevalence of hypertension, diabetes, and the level of successful ageing.

[Table 1]

In **Table 2** factors associated with the level of successful ageing among older Mediterranean individuals, are presented. Specifically, compared with low successful agers, the medium and high ones were not living alone ($p < 0.001$), were greater ever smokers ($p < 0.001$), had higher alcohol consumption ($p < 0.001$), while they had higher appendicular skeletal muscle mass (kg) ($p = 0.01$), higher rates of the SMI (ASM kg/BMI) ($p < 0.001$), lower BF% ($p < 0.001$) and lower waist circumference ($p < 0.001$).

[Table 2]

After adjusting for age, urban residence, sex, living alone, smoking habits, alcohol consumption, coffee, tea consumption, adherence to the Mediterranean diet, physical activity, hypertension, hypercholesterolemia and diabetes mellitus, it was found that the level of SMI (ASM kg/BMI) was positively associated with the successful ageing levels [b-coefficient (95% CI): 2.14 (1.57 to 2.71)] while the BF% [-0.04 (-0.05 to -0.03)] was inversely associated. When the analysis was stratified by age group (*models 2 and 3 of*

Table 3), in the oldest old, the increase in the SMI (ASM kg/BMI) had a greater impact in the level of successful ageing than in the group of the "younger" older participants [Older adults < 80yrs: 1.95 (1.29 to 2.60); Older adults \geq 80yrs: 2.3 (1.29 to 3.35)]. Moreover, the BF% was inversely related with the successful ageing score ($p < 0.001$) in both the age groups [i.e. b-coefficient (95% CI) for <80yrs vs. b (95% CI) for \geq 80yrs)] (Table 3). In addition when the analysis was stratified by gender, in males, the increase in the SMI (ASM kg/BMI) had a greater impact in their successful ageing score than in female individuals [Males: 2.5 (1.76 to 3.33); Females: 1.9 (1.03 to 2.77)]. Moreover the BF% was inversely related with the level of successful ageing in both the genders [Males: -0.04 (-0.05 to -0.02); Females: -0.05 (-0.06 to -0.04)].

Due to the high relation among under-nutrition with skeletal muscle mass and ageing status, the analysis was repeated separately for energy balance (positive energy balance vs. negative energy balance) and a consistent relationship was reported between the SMI (ASM kg/BMI) and level of successful ageing. Specifically, in the positive energy balance group, an increase in the SMI (ASM kg/BMI) was associated with a less pronounced increase in the level of successful ageing, than in the negative energy balance group [Positive energy balance: 1.50 (0.39 to 2.61); negative energy balance: 2.54 (1.79 to 3.29)]. Also a consistent inverse association between the BF% and the successful ageing score was reported ($p \leq 0.001$).

Concerning the macronutrients consumption, an inverse correlation was observed between carbohydrates (CHO) intake as an expression of daily energy intake (CHO/EI) and the level of SMI (ASM kg/BMI) ($\rho = -0.07$, $p < 0.006$) and a positive correlation was observed between protein (PRO) intake as an expression of daily energy intake (PRO/EI)

and SMI (ASM kg/BMI) ($\rho=0.11, p<0.001$). There was reported no correlation between dietary fat (FAT) intake as an expression of daily energy intake (FAT/EI) and SMI (ASM kg/BMI) ($\rho=0.04, p=0.07$), as well as among all the macronutrients intake (CHO, FAT and PRO) and the BF% (*p for all < 0.08*) (*data shown only in text*).

Discussion

In the present work it was revealed that skeletal muscle mass levels was positively associated with the successful ageing score of the older adults, irrespective of age, urban residence, sex, living alone, smoking habits, alcohol, and coffee and tea consumption. In addition, an inverse association among the BF% and the older adult's successful ageing level was reported. The aforementioned confirms the study's main hypothesis that higher skeletal muscle mass levels and lower BF% is related with better successful ageing among the older adults. The by-energy-balance group analysis reported that the later findings remained consistent among the positive and negative energy balance grouped participants. However, the changes in the SMI had greater effect among those in the group of the high energy intake. Moreover it was reported that the greater the alterations in the SMI, the greater the effect in the successful ageing level of the oldest old, in comparison with the younger participants. All of the aforementioned relationships, especially among elderly Mediterranean populations, have rarely been studied.

Despite the lack of previous findings regarding skeletal muscle mass and successful ageing among older populations, a number of studies have previously reported the role of skeletal muscle tissue on human health [3, 28]. Also in the literature it is reported that the oldest old facing greater declines in their muscle tissue [2]. Specifically,

Baumgartner et al., in the New Mexico Elder Health Survey reported that advanced age (>75 years old) was related to 3.28 and 2.28 higher odds of sarcopenia in males and females, respectively [2]. The later was also confirmed from the applied data analysis where the population ≥ 80 years old had lower rates in ASM and in the SMI. Within our unadjusted data analysis the greater the level of successful ageing the lower was the decline of skeletal muscle mass as well as the increase BF%. According to several researchers, the excess decrease in skeletal muscle mass was associated with higher disability and mortality rates as well as with various co-morbidities [3, 28]. In parallel the excess body fat, it is well known that is related with higher morbidity and mortality rates among the populations of various ages.[29]. In recent well documented multi-country study it was reported that sarcopenic obesity (a combination of low muscle mass with excess BF%) was associated with greater levels of disability [b-coefficient 3.01 (95%CI 1.14-4.88)] [30]. Also, throughout the unadjusted analysis, the older adults ever smoked had high degree of successful ageing. The later contradictory finding may have been altered due to the cross-sectional nature of the study, the potential survival bias, and/or lifestyle modifications due to doctor's consultations (probably older participants have modified their smoking habits not only because of age, but also because of known co-morbidities).

It has been reported that the process of muscle mass decline is multi-dynamic and is related with advanced age, various chronic diseases, endocrinal changes, inflammation and nutritional deficiencies [31]. The linear regression analysis confirmed the aforementioned and a positive association among the SMI and the level of a successful ageing was reported. Additionally, the stratified linear regression analysis by energy

balance group (i.e., positive vs. negative) was performed. According to the applied analysis, changes on SMI and on BF% were consistently related with the level of successful ageing among the energy balance groups. However, the aforementioned relationship between the SMI and the successful ageing level was less pronounced in the high energy balance group, a fact that indicates the possible interfering role of over-nutrition [32]. The maintenance of energy balance is a very complex procedure in the older adults and especially in the oldest old [33]. As has been reported in the literature, throughout the ageing process the decline in skeletal muscle tissue often is replaced from fat mass [34]. This process could be accelerated throughout the positive energy balance in relation with the low metabolic rate and the high physical inactivity of the elders [35]. Additionally well documented studies had proposed the role of macronutrients intake on skeletal muscle protein turnover [36]. Recently various studies were referred to positive balance between muscle protein synthesis and degradation after protein and amino acids dietary intake in the older adults [37, 38]. These findings, in combination with observations that were used in the present analysis (i.e., PRO/EI positively correlated with SMI and CHO/EI was inversely correlated with SMI), may explain the complex interrelations between the energy imbalance (positive or negative), dietary intake (macronutrients consumption), changes in body composition (i.e., SMI and BF) and successful ageing of older adults.

Taking into account the analysis by age group (i.e., older adults < 80 yrs vs. older adults > 80 yrs) the association between SMI, BF% and successful ageing were more pronounced among the oldest old individuals. Based on longitudinal data, BF% increases with age and appears a peak among the ages of 60 and 75 [39, 40], while, muscle mass

declines progressively for the age of 30s and over and is more accelerated after the age of 60 years [41]. Moreover, the relation between SMI and successful ageing was more prominent among males. According to several researchers, the aforementioned associations could be the result of specific biological and physiological pathways related to advanced age [1, 30] and to the well known gender-health paradox [42]. In contrast, BF% increase is related with declines in the physical activity that appeared in the older population as well as with the interfering factor of positive energy balance [43]. Previous results of the MEDIS study have shown that physical activity in comparison with sedentary life was related with almost 2 kg/m² (p≤0.001) decrease in the BMI levels of the older participants [44]. Moreover Tyrovolas et al., in a multi-country older adults study reported that low levels of physical activity compared to high levels were associated with higher odds for sarcopenia [OR 1.36 (95%CI 1.11-1.67)] and sarcopenic obesity [OR 1.80 (95%CI 1.23-2.64)] [45]. However, muscle mass loss is associated with testosterone and growth hormone declines due to the advanced age. Furthermore, in the older adults, the late response of the muscles on the insulin had been related with muscle mass declines. Moreover it had been reported that inflammation markers such as TNF- α , IL-6, and C - reactive protein, are negatively related with skeletal muscle tissue, while hormones such as adiponectin and leptin are reported to be also associated with muscle mass loss throughout a complex bio-molecular signaling [1]. These bio-physiological pathways could possibly explain the association of SMI and BF% with successful ageing as well as their different effect on the advanced age and for the population on energy imbalance. The progressive muscle mass loss in the older adults, if accompanied with muscle strength and gait speed decline could lead to sarcopenia [46], a condition related

with various health risk factors [47] and with high healthcare expenditures for the state [48]. These associations raise some concerns about the need for early measures (i.e., physical exercise and nutritional and health interventions and education etc.) in order to promote healthy and successful ageing throughout the prevention of body composition changes (i.e., muscle mass loss and increase of body fat %).

Strengths and limitations

The present study has several strengths. It is the first study that tried to evaluate the association of body composition parameters (i.e., skeletal muscle tissue and BF%), and the level of successful ageing of a large sample of 'healthy', independently-living older people in the Mediterranean basin. Among limitations, the fact that this is a cross-sectional study limits the potential for aetiological conclusions. Estimation of successful ageing among elders is a difficult task [8, 26, 27]. The calculation of BF% and ASM was based on equations that may under- or over- estimate the actual body composition rates. However, these formulas have been previously validated and present good agreement with the classical methods of bioelectrical impedance measurements and dual-energy X-ray absorptiometry (DXA) measurements [11, 13, 49]; recently large epidemiologic studies have used these formulas for the calculation of skeletal mass and body fat in diverse populations [45, 30]. **Another limitation is that the data analysis was not adjusted for menopause medication since the survey did not include a detailed medication intake assessment.** Finally, the cumulative successful ageing index that was used here by simply adding the presence of the common determinants of the individuals may not accurately estimate the successful ageing status of these individuals.

Conclusion

The present work investigated the role of body composition changes (BF% and SMI) on the successful ageing level of older Mediterranean people. It is of major interest nowadays to study the body composition transition of older people in order to understand the dynamics and the transforming nature of ageing. In the present study the successful agers had the better rates on ASM, SMI and BF%. Data analysis of the MEDIS study also revealed that major changes in body composition, like, the increase in the SMI and the BF%, might depict some of the major determinants of the successful ageing. Moreover the body composition changes were consistent among different sub-groups however the impact on successful ageing level was differentiated. Further exploration is needed in order to understand how these body composition factors interrelated and which are most important in the process of successful ageing. Taking into account the increased risk for the sarcopenia and sarcopenic obesity after the progressive loss of muscle mass tissue and the increase of fat mass [2], the prevention of skeletal muscle mass and body fat present an important goal for the public health authorities in order to maintain older population's healthy and successful ageing.

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Conflict of Interest Statement

Conflicts of interest: none

References

1. Sakuma K, Yamaguchi A (2013). Sarcopenic obesity and endocrinal adaptation with age. *Int J Endocrinol* 2013:204164.
2. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD (1998). Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 147:755–763
3. Cesari M, Pahor M, Lauretani F, Zamboni V, Bandinelli S, Bernabei R, Guralnik JM, Ferrucci L (2009). Skeletal muscle and mortality results from the InCHIANTI Study. *J Gerontol A Biol Sci Med Sci* 64:377–384.
4. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Rubin SM, Harris TB (2006). Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci*. 61:72–77
5. Hsu YH, Liang CK, Chou MY, Liao MC, Lin YT, Chen LK, Lo YK (2014). Association of cognitive impairment, depressive symptoms and sarcopenia among healthy older men in the veterans retirement community in southern Taiwan: a cross-sectional study. *Geriatr Gerontol Int* 14:102-8.
6. Tyrovolas S, Psaltopoulou T, Pounis G, Papairakleous N, Bountziouka V, Zeimbekis A, Gotsis E, Antonopoulou M, Metallinos G, Polychronopoulos E, Lionis C, Panagiotakos DB (2011). Nutrient intake in relation to central and overall obesity status among elderly people living in the Mediterranean islands: the MEDIS study. *Nutr Metab Cardiovasc Dis*;21:438-45.

7. Graham JE, Mitnitski AB, Mogilner AJ, Rockwood K (1999). The dynamics of cognitive aging: distinguishing functional age and disease from chronological age in a population. *Am J Epidemiol* 150:1045-1054
8. Tyrovolas S, Haro JM, Mariolis A, Piscopo S, Valacchi G, Tsakountakis N, Zeimbekis A, Tyrovola D, Bountziouka V, Gotsis E, Metallinos G, Tur JA, Matalas AL, Lionis C, Polychronopoulos E, Panagiotakos D (2014). Successful aging, dietary habits and health status of elderly individuals: A k-dimensional approach within the multi-national MEDIS study. *Exp Gerontol* 60:57-63.
9. Keys A, Menotti A, Aravanis C, et al (1984). The seven countries study: 2,289 deaths in 15 years. *Prev Med* 13:141-54.
10. World Health Organization. World Health Organization Translation Guidelines. http://www.who.int/substance_abuse/research_tools/translation/en/
Accessed 10 July, 2014
11. Lee RC, Wang Z, Heo M, Ross R, Janssen I, Heymsfield SB (2000). Total-body skeletal muscle mass: development and cross-validation of anthropometric prediction models. *Am J Clin Nutr* 72:796-803.
12. Cawthon P, Peters K, Shardell M, McLean RR, Dam TT, Kenny AM, Fragala MS, Harris TB, Kiel DP, Guralnik JM, Ferrucci L, Kritchevsky SB, Vassileva MT, Studenski SA, Alley DE (2014). Cut-points for low appendicular lean mass that identify older adults with clinically significant weakness. *J Gerontol A Biol Sci Med Sci* 69:567-75.

13. Santos Silva DA, Petroski EL, Peres MA (2012). Is high body fat estimated by body mass index and waist circumference a predictor of hypertension in adults? A population-based study. *Nutr J* 11:112.
14. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 285:2486-2497
15. Papathanasiou G, Georgoudis G, Papandreou M, Spyropoulos P, Georgakopoulos D, Kalfakakou V, Evangelou A (2009). Reliability measures of the short International Physical Activity Questionnaire (IPAQ) in Greek young adults. *Hellenic J Cardiol* 50:283-94.
16. Tyrovolas S, Pounis G, Bountziouka V, Polychronopoulos E, Panagiotakos D (2010). Repeatability and validation of a short, semi-quantitative food frequency questionnaire designed for older adults living in Mediterranean areas: the MEDIS-FFQ. *J Nutr Gerontol Geriatr* 29:311-24.
17. Panagiotakos D, Pitsavos C, Stefanadis C (2006). Dietary Patterns: A Mediterranean Diet Score and its Relation to CVD Risk and Markers. *Nutr Metab Cardiovasc Dis* 16:559-68.
18. U.S. Department of Agriculture ARS, USDA Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 22; 2009. <http://www.ars.usda.gov/nutrientdata>. Accessed 8 July, 2014
19. <http://nutrition.med.uoc.gr/GreekTables>, Accessed 6 July, 2014

20. FAO/WHO/UNU (2004). Human energy requirements: report of a joint FAO/WHO/UNU Expert Consultation. Rome: FAO.
21. Leibel RL, Rosenbaum M, Hirsch J (2009). Changes in energy expenditure resulting from altered body weight. *N Engl J Med* 332:621-8.
22. Fountoulakis KN, Tsolaki M, Iacovides A, Yesavage J, O'Hara R, Kazis A, Ierodiakonou C (1999). The validation of the short form of geriatric depression scale (GDS) in Greece. *Aging* 11:367-372.
23. Tyrovolas S, Haro JM, Polychronopoulos E, Mariolis A, Piscopo S, Valacchi G, Makri K, Zeimbekis A, Tyrovola D, Bountziouka V, Gotsis E, Metallinos G, Katsoulis Y, Tur JA, Matalas A, Lionis C, Panagiotakos D (2014). Factors associated with components of arterial pressure among older individuals (the multinational MEDIS study): the role of the Mediterranean diet and alcohol consumption. *J Clin Hypertens (Greenwich)*. 16:645-51.
24. Bowling A, Dieppe P (2005). What is successful ageing and who should define it? *BMJ* 331:1548–51.
25. Rowe JW, Kahn RL (1997). Successful aging. *Gerontologist* 37:433-40.
26. Tyrovolas S, Haro JM, Mariolis A, Piscopo S, Valacchi G, Makri K, Zeimbekis A, Tyrovola D, Bountziouka V, Gotsis E, Metallinos G, Tur JA, Matalas A, Lionis C, Polychronopoulos E, Panagiotakos D (2015). The Role of Energy Balance in Successful Aging Among Elderly Individuals: The Multinational MEDIS Study. *J Aging Health* 27:1375-91
27. Mariolis A, Foscolou A, Tyrovolas S, Piscopo S, Valacchi G, Tsakountakis N, Zeimbekis A, Bountziouka V, Gotsis E, Metallinos G, Tyrovola D, Tur JA,

- Matalas AL, Lionis C, Polychronopoulos E, Panagiotakos D; for the MEDIS study group (2015) Successful Aging among Elders Living in the Mani Continental Region vs. Insular Areas of the Mediterranean: the MEDIS Study[J]. *Aging Dis*, 10.14336/AD.2015.1002
28. Evans W (1997). Functional and metabolic consequences of sarcopenia. *J Nutr* 127:998S–1003S.
29. de Hollander EL, Bemelmans WJ, Boshuizen HC, Friedrich N, Wallaschofski H, Guallar-Castillón P, Walter S, Zillikens MC, Rosengren A, Lissner L, Bassett JK, Giles GG, Orsini N, Heim N, Visser M, de Groot LC; WC elderly collaborators (2012). The association between waist circumference and risk of mortality considering body mass index in 65- to 74-year-olds: A meta-analysis of 29 cohorts involving more than 58 000 elderly persons. *Int J Epidemiol* 41:805–817
30. Tyrovolas S, Koyanagi A, Olaya B, Ayuso-Mateos JL, Miret M, Chatterji S, Tobiasz-Adamczyk B, Koskinen S, Leonardi M, Haro JM (2015). The role of muscle mass and body fat on disability among older adults: A cross-national analysis. *Exp Gerontol.*;69:27-35.
31. Fielding RA, Vellas B, Evans WJ, et al (2011). International Working Group on Sarcopenia. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. *J Am Med Dir Assoc* 12:249–256.
32. Poehlman ET (1992). Energy expenditure and requirements in aging humans. *J Nutr* 122:2057-65.

33. Ritz P (2001). Factors affecting energy and macronutrient requirements in elderly people. *Public Health Nutr* 4:561-8.
34. St-Onge MP (2005). Relationship between body composition changes and changes in physical function and metabolic risk factors in aging. *Curr Opin Clin Nutr Metab Care* 8:523-8
35. Waters DL, Baumgartner RN (2011). Sarcopenia and obesity. *Clin Geriatr Med* 27:401-21
36. Nair KS (2005). Aging muscle. *Am J Clin Nutr* 81:953-963
37. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ (2005). Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 19:422-424
38. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR (2005). Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. *Am J Clin Nutr* 82:1065-1073
39. Rissanen A, Heliövaara M, Aromaa A (1988). Overweight and anthropometric changes in adulthood: a prospective study of 17,000 Finns. *Int J Obes* 12:391-401.
40. Droyvold WB, Nilsen TI, Kruger O, Holmen TL, Krokstad S, Midthjell K, Holmen J (2006). Change in height, weight and body mass index: Longitudinal data from the HUNT Study in Norway. *Int J Obes* 30:935-939.
41. Rantanen T, Masaki KT, Foley D, Izmirlian G, White L, Guralnik JM (1998). Grip strength changes over 27 yr in Japanese-American men. *J Appl Physiol* 85:2047-2053.

42. Sánchez-López M, Cuellar-Flores I, Dresch V (2012). The Impact of Gender Roles on Health. *Women & Health* 52:182–196
43. Bouchonville MF, Villareal DT (2013). Sarcopenic obesity: how do we treat it? *Curr Opin Endocrinol Diabetes Obes* 20:412-9.
44. Tyrovolas S, Bountziouka V, Papairakleous N, Zeimbekis A, Anastassiou F, Gotsis E, Metallinos G, Polychronopoulos E, Lionis C, Panagiotakos D (2009). Adherence to the Mediterranean diet is associated with lower prevalence of obesity among elderly people living in Mediterranean islands: the MEDIS study. *Int J Food Sci Nutr* 60:137-50.
45. Tyrovolas S, Koyanagi A, Olaya B, Ayuso-Mateos JL, Miret M, Chatterji S, Tobiasz-Adamczyk B, Koskinen S, Leonardi M, Haro JM (2015). Factors associated with skeletal muscle mass, sarcopenia, and sarcopenic obesity in older adults: a multi-continent study. *J Cachexia Sarcopenia Muscle*. DOI: 10.1002/jcsm.12076
46. Dam TT, Peters KW, Fragala M, Cawthon PM, Harris TB, McLean R, Shardell M, Alley DE, Kenny A, Ferrucci L, Guralnik J, Kiel DP, Kritchevsky S, Vassileva MT, Studenski S (2014). An evidence-based comparison of operational criteria for the presence of sarcopenia. *J Gerontol A Biol Sci Med Sci* 69:584-90.
47. Alexandre Tda S, Duarte YA, Santos JL, Wong R, Lebrão ML (2014). Prevalence and associated factors of sarcopenia among elderly in Brazil: findings from the SABE study. *J Nutr Health Aging* 18:284-90.

48. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R (2004) The healthcare costs of sarcopenia in the United States. *J Am Geriatr Soc* 52:80-85
49. Pagotto V, Silveira EA (2014). Applicability and agreement of different diagnostic criteria for sarcopenia estimation in the elderly. *Arch Gerontol Geriatr* 59:288-94

Table 1. Demographic, anthropometric, behavioral, clinical and lifestyle characteristics of the Multi-national MEDIS sample, by age group

	Older adults < 80yrs (n=1925)	Older adults > 80yrs (n=613)	P
Male sex (%)	48	55	0.005
Urban residence (%)	64	52	<0.001
BMI (kg/m ²)	28.7±4.8	27.3±4.3	<0.001
Education (years of school)	7.8±4.0	5.8±3.7	<0.001
High financial status (%)	20	15	0.01
Living alone (%)	23	34	<0.001
Ever smoking (%)	36	31	0.02
Physical activity (%)	44	37	0.004
MedDietScore (0-55)	32±4.8	31±5.2	0.009
Alcohol consumption (%)	49	46	0.15
Hypertension (%)	62	64	0.43
Diabetes Mellitus (%)	23	21	0.18
Hypercholesterolemia (%)	52	39	<0.001
ASM (kg)	24.3±5.9	22.1±5.9	<0.001
SMI (ASM/BMI)	0.86±0.2	0.82±0.2	<0.001
Body fat (%)	43.8	44	0.55
Successful ageing (0-10)	2.6±1.3	2.5±1.2	0.36

Abbreviations: BMI: Body mass index (kg/m²); ASM: Appendicular skeletal muscle mass; SMI: Skeletal muscle mass index

P-values derived from Student's t-test for normally distributed continuous data, chi-square test for categorical data and Mann-Whitney U-test for not-normally distributed continuous data.

Table 2: Demographic, anthropometric, and lifestyle characteristics of the Multi-national MEDIS sample, by successful ageing group.

	Degree of successful ageing			P
	Low (n=886)	Medium (n=826)	High (n=827)	
Age	75±7.1	74±7.6	73±7.9	0.002
Male sex (%)	36	53	61	<0.001
Urban residence (%)	62	56	63	0.004
Living alone (%)	31	24	20	<0.001
Waist circumference (cm)	106±12	98±14	97±14	<0.001
ASM (kg)	23±5.7	24±6.0	24±6.1	0.01
SMI (ASM/BMI)	0.77±0.18	0.86±0.21	0.92±0.22	<0.001
Body fat (%)	48	41	40	<0.001
Ever smoking (%)	24	37	46	<0.001
Alcohol consumption (%)	34	49	63	<0.001
Coffee consumption (%)	82	81	82	0.22
Tea consumption (%)	41	41	45	0.87

Abbreviations: ASM: Appendicular skeletal muscle mass; SMI: Skeletal muscle mass index.

P-values derived through one-way ANOVA for continuous variables (using the Bonferroni correction for the between groups comparisons) and the chi-square test for the categorical ones.

Table 3: Association of skeletal muscle mass and body fat with successful ageing.

	Model 1	Model 2	Model 3
	<i>n</i> =864	(< 80yrs, <i>n</i> =656)	(≥ 80yrs, <i>n</i> =208)
	b (95% CI)	b (95% CI)	b (95% CI)
SMI (ASM/BMI)	2.14 (1.57; 2.71)	1.95 (1.29; 2.60)	2.3 (1.29 to 3.35)
Body fat (%)	-0.04 (-0.05; -0.03)	-0.05 (-0.06; -0.04)	-0.03 (-0.05; -0.02)

Model 1, 2 and 3 adjusted for age, urban residence, sex, living alone, smoking habits, alcohol consumption, coffee and tea consumption, adherence to the Mediterranean diet, physical activity, hypertension, hypercholesterolemia and diabetes mellitus.

Abbreviations: ASM: Appendicular skeletal muscle mass; SMI: Skeletal muscle mass index