Original Article

Dietary magnesium is positively associated with skeletal muscle power and indices of muscle mass and may attenuate the association between circulating C-reactive protein and muscle mass in women†

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Abstract Age-related loss of skeletal muscle mass and strength are risk factors for sarcopenia, osteoporosis, falls, fractures, frailty and mortality. Dietary magnesium (Mg) could play a role in prevention of age-related loss of skeletal muscle mass, power and strength directly through physiological mechanisms or indirectly through an impact on chronic low-grade inflammation, itself a risk factor for loss of skeletal muscle mass and strength. In a cross-sectional study of 2570 women aged 18-79 years we examined associations between intakes of Mg, estimated using an FFQ, DXA-derived measures of muscle mass (fat free mass as a percentage of body weight (FFM%), fat free mass index (FFMI, kg/m^2)), leg explosive power (LEP) and grip-strength (n=949 only). We also examined associations between circulating hs-CRP (C-reactive protein) and muscle mass and LEP, and explored the potential attenuation of these relationships by Mg. We compared our findings with those of age and protein intake. Endpoints were calculated by quintile of Mg and adjusted for relevant confounders. Significant positive associations were found between a higher Mg and indices of skeletal muscle mass and LEP, and also with hs-CRP, after adjustment for covariates. Contrasting extreme quintiles of Mg intake showed differences of 2.6% for FFM% (P trend <0.001), 0.4 kg/m^2 for FFMI (P trend=0.005), and 19.6 watts/kg for LEP (P trend<0.001). Compared to protein these positive associations were 7 times greater for FFM% and 2.5 times greater for LEP. We also found that higher hs-CRP was negatively associated with skeletal muscle mass and, in statistical modelling, that a higher dietary Mg attenuated this negative relationship by 6.5%, with greater attenuation in women aged over 50 years. No association was found between Mg and grip strength. Our results suggest that dietary magnesium may aid conservation of age-related loss of skeletal muscle mass and power in women of all ages.

Keywords: Aging, sarcopenia, DXA, cytokines, dietary magnesium

Introduction

Age-related loss of skeletal muscle, strength and power are important contributing risk factors to a number of conditions including sarcopenia (the loss of muscle mass and strength or physical performance), osteoporosis, falls, fractures, weakness, frailty and mortality (1-11). Maintenance of skeletal muscle mass and strength are also protective against osteoporosis (5-11). The prevalence of sarcopenia ranges from between 1% to 29%, in community dwelling populations over the age of 60 years, to 14% to 33% in those in long-term care (1, 12-16). The estimated health and social care costs of sarcopenia in the United States are $18.5 billion per year with costs of falls and fractures of £2.3 billion per year in the UK (and $17 billion in the US) (1, 7, 12-15, 17, 18).

Given the number and importance of the conditions associated with loss of skeletal muscle mass, strength, and function and the predicted increase in age profile of the world wide population (25% will be aged over 65 years by 2050) (19), prevention and treatment strategies are clearly needed. Nutrition can potentially play a role in prevention but the current evidence relating diet to aging of skeletal muscle is largely limited to protein (2). Dietary magnesium (Mg) is important to skeletal muscle due to its direct roles in muscle physiology, and metabolism, and indirect interaction with chronic low grade inflammation, which is also a risk factor for loss of skeletal muscle mass and strength (2, 16, 20-24). The physiological roles of Mg in skeletal muscle metabolism include synthesis of protein, ATP, oxygen uptake, glycogen breakdown, fat oxidation, and electrolyte balance (of K, Na and Ca) (25). The importance of Mg to these processes explains why 27% of Mg is stored in skeletal muscle, which is the largest store in the body (25).

Mg may also impact on muscle performance through energy metabolism, transmembrane transport and muscle contraction and relaxation, although the evidence in older populations has been limited to three studies, finding either a significant relationship between serum Mg concentrations or positive effects of Mg supplementation (26-30).

The chronic low grade inflammation, associated with ageing, is one mechanism responsible for loss of skeletal muscle mass, strength and power, and C-reactive protein (CRP) is an established marker of systemic inflammation (22, 23, 31). Increased circulation of inflammatory cytokines CRP (C-reactive protein), IL-6 and TNF-α have been associated with lower indices of skeletal muscle mass or physical performance, grip strength or disability, in a number of cross-sectional and longitudinal studies (20-24).

Dietary Mg could reduce the circulation of inflammatory cytokines and recent systematic review evidence found that a higher dietary intake of Mg was, in the main, inversely related to circulating CRP concentrations (32, 33). Previous intervention studies with Mg supplements found mixed effects on circulating hs-CRP but do not reflect the effects of diet, as supplements consist of single compounds with variable bioavailability (32, 34-36).

Despite the biological plausibility that greater intakes of dietary Mg would be associated with improved skeletal muscle mass, grip strength and power, to our knowledge there has only been one previous cross-sectional study which found a positive relationship between dietary Mg and appendicular lean mass but not grip strength (37). Furthermore, no previous population study has examined the possible mediating effects of dietary Mg on inflammation in addition to its association with skeletal muscle mass, strength and power.

This study was designed to investigate the hypothesis that dietary Mg would be associated with better indices of skeletal muscle mass, strength and power and would also attenuate a potential negative relationship between circulating hs-CRP and skeletal muscle mass and power. Using detailed data available for a large healthy adult population of female twins we aimed firstly to understand the relationship between dietary Mg and indices of skeletal muscle mass, strength and power, secondly to investigate the relationship between circulating hs-CRP and muscle mass, thirdly to understand the relationship between dietary Mg and circulating hs-CRP, and finally, to determine whether dietary Mg would attenuate the relationship between circulating hs-CRP and muscle mass or explosive leg power.

Methods

The women included in this study were from the TwinsUK registry which is an ongoing study of healthy adult twin volunteers who underwent clinical examinations for a number of characteristics associated with aging and is representative of adult singleton populations in the United Kingdom (38, 39). The women for this study were in two groups, group 1 selected as those participants who had completed a food frequency questionnaire (FFQ) and attended for dual-energy X-ray absorptiometry (DXA) measurements between 1996 and 2000 (referred to as the '*fat free mass group*' in this manuscript) included 2570 women. Within this 'fat free mass' group there were 1914 individuals with measures of leg explosive power and 1658 individuals who also had measures of high sensitivity C-reactive protein (hs-CRP). Within the fat free mass group 1189 individuals had both measurements of leg explosive power and hs-CRP. The '*grip strength group*' consisted of women who had completed an FFQ and had body composition and grip strength measurements measured between 2005 and 2008 and was aged 34 to 83 years at the time of the measurements. There were 512 individuals with full data available in both the muscle mass and grip strength groups.

Zygosity was ascertained by questionnaire and confirmed by multiplex-DNA fingerprinting (PE Applied Biosystems) (40). Information on lifestyle, medication use, menopausal status, and demographic variables were obtained using a standardised nurse-administered questionnaire. Physical activity was classified as heavy, moderate or inactive during work, home and leisure time using a questionnaire that has been strongly correlated with a more in depth assessment of physical activity in this cohort (41).

Ethical approval was obtained from the St. Thomas's Hospital Research Ethics Committee and informed consent acquired from all participants.

Measurement of dietary intake. Assessment of Mg, protein and energy intake were measured using a 131 item validated food frequency questionnaire (FFQ) with nutrients calculated using the UK Nutrient Database (42, 43). Individuals were excluded if answers to > 10 food items were left blank

or the ratio of estimated total energy intake to the estimated basal metabolic rate fell 2 SDs outside the mean ratio. The dietary intake data was estimated for the appropriate time period for the 'fat free mass' and 'grip strength' groups. To estimate potential dietary mis-reporting the ratio of reported energy intake (EI) to estimated energy expenditure (EER), the EI:EER ratio, was calculated and was included as a covariate for adjustment in the statistical analyses (44).

Assessment of body composition. Weight and height were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). Fat free mass (FFM) was measured by dual-X-ray absorptiometry (DXA) (Hologic QDR-2000 DXA scanner, Hologic Inc., Waltham, MA, USA) in kg. Fat free mass (FFM%) as a percentage of body weight was calculated as (FFM kg/total body weight) *100. The fat free mass index (FFMI) in kg/m^2 was calculated as (FFM kg/height in square meters) as this eliminates the proportional increase in fat free mass that occurs with increasing height (45).

Measurement of grip strength and leg explosive power. Isometric grip strength was assessed using a Jamar hand grip dynamometer (Sammons, Preston, UK) on the dominant arm with reproducibility assessed by repeated measurement on 24 individuals (CV of 11.4%) (46).

Leg explosive power (LEP) was used to measure physical fitness using the Nottingham power rig which measures the force and velocity of muscle contraction principally from the quadriceps (47). It is has been validated and is highly reliable (reliability coefficient 0.97, coefficient of variation 9.4%, over one week period in adults) (47). Values for LEP were divided by individual body weight and multiplied by the mean body weight of the population to obtain body-size adjusted values in watts/kg.

Measurement of C-reactive protein. Circulating high sensitivity C-reactive protein (hs-CRP) was measured by a highly sensitive automated microparticle capture enzyme immunoassay, standardised on the World Health Organisation International Reference Standard for CRP immunoassay, as previously described (48).

Statistical analyses

First set of analyses: We examined the relationship of dietary Mg, skeletal muscle mass and strength and hs-CRP in a three step process using regression techniques. Also, as data from members of twin pairs could not be treated as independent, we controlled for familial aggregation by using the robust regression cluster option in the Stata software. Firstly we estimated the independent association of dietary Mg with each of the outcome variables using multivariable regression adjusted analyses. Dietary Mg was divided into quintiles and statistical models run to calculate the unadjusted (model 1) and adjusted (model 2) values for FFM%, FFMI, grip strength, LEP and Hs-CRP. To account for the known associations between fat free mass, grip strength, LEP and Hs-CRP with age (years), physical activity (active, moderately active, inactive), smoking habit (never, former, current), protein intake (in quintiles) and potential mis-reporting (EI:EER) all models included these variables (2, 49, 50). Additionally as fat free mass increases with greater body size analyses for FFMI were also adjusted for total fat mass (kg) (45). For grip strength and LEP the models were also adjusted for menopausal status (pre-menopausal/ post-menopausal), the use of HRT (yes/no) and height (m) (51). As vitamin D has been related to muscle mass and function so we performed an additional model that included dietary vitamin D (52). The interaction between age and magnesium intake on grip strength was also tested. For hs-CRP model 2 also included anti-inflammatory (yes/no) and HRT medication (yes/no) (51). As the hs-CRP distribution was skewed natural long-transformed values were used in all models. P-trend values were calculated with regression using ANCOVA with the quintile categories used as a continuous variable. The values in Table 2 show the unadjusted means (model 1) and adjusted means and standard errors (model 2). In figure 2 the adjusted means for FFM% and FFMI are shown and in Figure 3 those for leg explosive power.

Second set of analyses: to compare the scale of our findings with dietary Mg with other factors known to influence indices of skeletal muscle mass we compared the associations with, of Mg and skeletal muscle mass (FFM% and FFMI) and LEP with those of age, and dietary protein.

The beta coefficients from the fully-adjusted regression model (model 2) were compared by including them in the equation:

(ß coefficient dietary Mg/ ß coefficient age or protein)*100.

For these analyses dietary Mg and protein were divided into quintiles, age into 10-year age groups and all other covariates as described above.

Third set of analyses - attenuation models. As we found an associations between dietary Mg and hs-CRP and with hs-CRP and skeletal muscle, to evaluate the potential attenuation of the relationship between hs-CRP and skeletal muscle by Mg we added Mg (in quintiles) to the fully adjusted hs-CRP regression model to assess the percent change of the beta coefficient for FFM% or FFMI. Attenuation as a percentage was calculated as $((\beta 2 - \beta 1)/ \beta 1)*100$. The significance of the attenuation was tested by dividing the attenuation coefficient by its standard error term to calculate a z score for the attenuation (53). A z score greater than 1.96 was considered significant at the 0.05 level.

In order to understand the whether the associations were different in older and younger women we repeated all analyses in the whole cohort stratified by age groups (<50 years or ≥50 years).

The contribution of different food groups to Mg intake was calculated.

All analyses were performed with Stata statistical software version 11.0 (Stata Corp, College Station, TX) and included the robust cluster regression option in STATA.

Results

The group of women in which grip strength was measured was older and had a higher BMI than the 'muscle mass group', as expected given the dates of measurement, Table 1. This 'grip strength group' had fewer women in the active and moderately active physical activity groups than the 'fat free mass group' (78.1% versus 60.5%).

Relationships between dietary Mg and indices of skeletal mass, strength and leg explosive power. Significant positive associations were found between dietary Mg and both FFM% (P trend <0.001)

and FFMI (P trend=0.005) after adjustment for covariates, with between extreme quintile differences of 2.6% for FFM% and 0.4 kg/m^2 for FFMI in the fat free mass group, Table 2. When stratified by age these trends were significant in both age groups with between quintile differences of 2.3% for FFM% (P=0.001) in those aged less than 50 years compared with 2.82% (P<0.001), in those over 50, see Figure 1. For FFMI there were stronger associations with dietary Mg in those aged less than 50 years compared with those aged 50 years or more; differences of 0.47 kg/m^2 (P=0.009) and 0.31 kg/m^2 (P=0.113), respectively, Figure 1.We found a strong positive association between LEP and dietary Mg both before and after adjustment for covariates with a difference of 19.6 watts/kg between quintile 1 and 5 (P trend <0.001) in the fully adjusted model, a 24.1% difference, Table 2. There was no association between grip strength and Mg intake in either the adjusted or unadjusted models in the grip strength group, Table 2. We also found no interaction between age and magnesium intake and grip strength (P=0.499). In analyses stratified by age these associations remained significant with between quintile differences of 14.2 watts/kg (P trend =0.001) and 14.9 watts/kg (P trend = 0.002) representing percentage differences of 15.1% and 21.2% in women under and over 50 years of age, respectively, see Figure 2. The addition of dietary vitamin D to the fully adjusted models did not alter the findings (data not shown).

Comparison with other known factors related to skeletal muscle mass. In comparison with other known factors related to skeletal muscle mass, in the multivariate adjusted regression model, the difference of FFM% per 10 years of age (ß coefficient) was -0.41% per 10 years (P<0.001 – CI -0.253 to -0.581), the association with protein as a percentage of energy was - was (ß coefficient) 0.08% per quintile (P=0.072 – CI -0.178 to 0.008) and the association of dietary Mg was 0.24% per quintile P<0.001 – CI 0.383 to 0.888). This indicates that the association between dietary Mg and FFM% was about 60% of that of age ((0.24%/0.41%)*100=58.5 %) (after adjustment for all covariates). Compared with the association with protein the association with Mg was three times greater than protein ((0.24%/0.08%) = 3). For FFMI the equivalent figures were 0.19kg/m^2 (P<0.001 – CI -0.012 to 0.027) for age and 0.094kg/m^2 (P=0.05 – CI 0.029 to 0.158) for Mg,

indicating that the association was around half that of age (((0.094/0.19)*100) = 49.5%). Similarly the association with dietary protein was 0.014 kg/m^2 (P=0.552 – CI -0.032 to 0.060) and compared with Mg was (0.094/0.014=6.7), indicating that the association with Mg was almost 7 times greater than with protein.

In analyses repeated with LEP the association with Mg compared with that of age was ((4.97 watts/kg 3.31 watts/kg)*100 = 150%) i.e. one and a half times that of age and for protein was ((4.98 watts/kg/1.96 watts/kg)*100 = 254%) i.e. 2.5 times that of protein.

Relationships between dietary Mg and hs-CRP. There was a inverse association between dietary Mg and hs-CRP in the adjusted model with a lower hs-CRP in the highest quintile of Mg intake (Q5) compared with Q1; an inter quintile difference of 0.59 mg/L (P trend = 0.011), equivalent to 28.9% of Q1, Table 2.

Relationships between indices of skeletal muscle and hs-CRP and the attenuation analyses. The unadjusted association between indices of muscle mass and CRP (per quintile of CRP) was -1.4 % (P trend = 0.001) for FFM% and 0.23 kg/m^2 (P trend = 0.001) for FFMI. When stratified by age group the figures for FFM% were -1.0% (P trend <0.001) in those aged less than 50 years and -1.4% (P trend <0.001) in those over 50 years of age. For FFMI the equivalent figures were 0.19 kg/m^2 (P trend <0.001) in those aged less than 50 years and -0.28 kg/m^2 (P trend <0.001) in those over 50 years of age.

In multivariable regression, with the covariates included in model 1, the association between FFM% and hs-CRP was -0.309% per quintile of hs-CRP (P trend =0.001). Inclusion of Mg additionally in the model (model 2) reduced this association of FFM% and hs-CRP to -0.289% per quintile of hs-CRP (P trend =0.001), Figure 3. When calculated as the percentage difference between model 1 and model 2 this calculated attenuation was 6.5% i.e. the association between hs-CRP and FFM% was attenuated by 6.5% when Mg was included in statistical model 2 (z score for attenuation = 2.7, p<0.05). After stratification for age, into those who were aged either more or less than 50 years, the equivalent coefficients were -0.285% (P=0.017) and 0.272% (P=0.038),

respectively (with Mg included in model 2), Figure 3. This represented attenuation by inclusion of Mg in the model of 4.6% in those aged less than 50 years (z score for attenuation = 1.7, p>0.05) compared with 5.8% in those aged 50 years and over (z score for attenuation = 1.8, p>0.05).

Repeating the statistical analyses with models 1 and 2 but including FFMI as the main outcome in the models, the association between hs-CRP in model 1 was -0.00253 kg/m^2 (P=0.83) and inclusion of Mg to model 2 reduced the association to -0.00015 kg/m^2 (P=0.99). The calculated percentage attenuation was for the whole age group was 0.06% and for those aged less than 50 years was 35%, and was 89% in those aged over 50 years. However, none of these associations were significant (P>0.5).

In our analyses with LEP and hs-CRP no association was found in the fully adjusted model - ß 0.024 watts per quintile of hs-CRP (P trend = 0.977), data not shown.

Contribution of foods to intake. The main foods that contributed to 81.1% of Mg intake in this population were fruit and vegetables (33.0 %), cereal foods (20.3%), dairy foods (14.8 %), potatoes (7.0 %) and meats and products (6.0%). Around a quarter of women (27.2%) had intakes of Mg below the UK Reference Nutrient Intake of 270 mg/d.

Discussion

To our knowledge this is the first study to assess comprehensively the associations between dietary Mg intake, skeletal muscle mass, power and strength, and an inflammatory biomarker, in the same well-characterised dataset. In our study we found that a higher dietary Mg intake was significantly associated in a beneficial direction with indices of skeletal muscle mass (FFM% and FFMI) and leg explosive power, and also with circulating CRP concentrations. These associations remained after adjustment for covariates including protein intake, age, physical activity and smoking habit. We also found that higher hs-CRP was negatively associated with lower indices of skeletal muscle mass and, in statistical modelling, that dietary Mg attenuated this negative relationship. Although our findings applied to women of all ages we found that the associations of dietary Mg with indices of skeletal muscle, and the attenuation by Mg, of the association between CRP and muscle, were

greater in women aged over 50 years. We found no associations between dietary Mg and grip strength or between LEP and hs-CRP.

In line with our positive finding of an association of dietary Mg with FFM% and FFMI the only previous study (in older men and women) found a positive relationship between Mg intake and appendicular lean mass, at baseline, of 0.07 kg per quartile of Mg intake (P=0.02) (37). This compares with the difference of 0.091kg/m^2 (P=0.05) of FFMI per quintile of Mg found in our cohort, although we adjusted for more covariates than those authors did. Our finding represents an interquartile absolute difference of 0.4kg/m^2 of FFMI which, expressed as a percentage of the lowest quintile, is equivalent to difference of 4.3% for FFMI. We also found a 24.2% greater LEP with the highest intake of Mg compared to the lowest. We found this difference with an intake of around 250 mg of Mg per day. This amount of Mg, administered as Mg oxide, in a recent intervention study of middle-aged overweight women, found an increase in percentage lean body mass (1.3%, P=0.03) over 8 weeks (29). In comparison our study found a cross-sectional difference of 2.6% of FFM% between extreme quintiles of Mg intake. To our knowledge our study is the first to investigate the association between dietary Mg and LEP (although a previous study investigated LEP and circulating Mg) (28). Moreover, our results with dietary Mg and indices of skeletal muscle mass and LEP were continuous across the whole range of distribution of intake, even though 27% of our cohort ate less than the UK reference nutrient intake. Mean intakes of Mg in our study were higher than in a recent UK population study, although we used an FFQ which produces higher intakes of certain nutrients than diary methods (54). Maintenance of circulating Mg is tightly regulated with concentrations of less than 0.7 mmol/L considered deficient and of 1.0 mmol/L considered to be in excess. Mg absorption varies according to intakes, and providing renal function is not compromised, it is unlikely that higher dietary intakes would have a detrimental effect but further research is needed (44).

To our knowledge only one other study has investigated the association between hs-CRP and leg power, finding a significant positive association with lower knee extensor power (55). The

lack of association that we found with dietary Mg and grip strength is in line with the only other previous study relating dietary Mg to knee muscle strength, although one study found a positive association between grip strength and circulating Mg (28, 37).

Similar to other previous studies we also found an association between a higher intake of Mg and circulating hs-CRP with an interquartile difference of 28.9%, Table 1 (20-22).

The calculated attenuation of dietary Mg on the negative relationship between hs-CRP and indices of muscle mass, of 6.5% for FFM%, demonstrates a significant effect of dietary Mg. This was greater in older than in younger women, indicating that dietary Mg may play a greater role in the conservation of the skeletal muscle in older women, through attenuation of circulating cytokines. Furthermore our findings are plausible, given the integral role Mg plays in skeletal muscle physiology, metabolism, composition and the previous research relating dietary and supplemental Mg to CRP (26-30, 34). Overall our results support the potential for dietary Mg to play a role in the conservation of skeletal muscle mass and power (26). The major dietary sources of Mg include green leafy vegetables and unprocessed grains and the difference between extreme quintiles of 256 mg found in our cohort could be supplied by a diet that included rich sources of Mg (for instance, almonds, spinach, bananas, fortified breakfast cereals and wholemeal bread), as well as by following the UK and USA dietary guidelines that include 5-portions of fruits and vegetables per day.

To place our study findings in context we compared the relationship with fat free mass and LEP and dietary Mg intake with those of either ten years of age (one decade) or of quintiles of percentage dietary protein intake, known factors that relate to skeletal muscle mass. The association per quintile of dietary Mg was between 3 and 7 times that of per quintile of protein, and between 50% and 60% of the equivalent of one decade of age. For LEP the associations were 2.5 times that of protein and 1.5 times that of age. This provides further support for the important potential for dietary Mg to play a role in conservation of skeletal muscle mass and power. Moreover leg

explosive power declines with age earlier, and more dramatically, than strength and has a greater impact on functional impairments and influences mobility more than leg strength (47, 56-58).

Sarcopenia is a particular issue for older adults, but muscle mass is lost from midlife onwards. Although the loss of strength as well as skeletal muscle mass does not entirely explain the onset of sarcopenia both remain important (59). Although we showed greater attenuation effects in those over 50 years for FFM% our results suggest that dietary Mg may also have benefits in mid-life, contributing to the preservation of both muscle mass and power, potentially attenuating the later development of sarcopenia. Thus our findings have relevance to the timing of future intervention studies. Moreover, unlike protein supplementation, Mg also holds promise for older populations.

The strengths of our study include the large population size and wide age distribution with DXA measurements of body composition and measures of LEP, grip strength and circulating hs-CRP and dietary intakes. One benefit of measuring dietary intake is that the results from this study are directly applicable for dietary advice. Our FFQ has also been validated using urinary excretion of potassium, and dietary potassium and Mg are highly correlated (42). We also measured total fat free mass which is highly correlated to appendicular lean mass, and used FFMI which takes into account differences in skeletal muscle with height (2).

One limitation of our study is that we were unable to investigate the association between hs-CRP and muscle strength due to the lack of availability of data but we were able to measure associations with LEP. Our study was also only in women and we do not know if the results would be the same in men, although low-grade inflammation is also a risk factor for loss of skeletal muscle mass and strength in men. Although we adjusted for all known confounders in our analyses we cannot discount the fact that dietary Mg may be a reflection of an overall healthy eating pattern. Moreover, as this was a cross-sectional study we cannot infer causation. Also, our statistical findings of the attenuation of dietary Mg on the association between indices of skeletal muscle mass and hs-CRP would need confirmation in an intervention trial.

In summary we found significant positive associations of dietary Mg with indices of skeletal muscle mass and leg explosive power. For skeletal muscle mass the scale of the associations with Mg were 2.6% for FFM% and 4.3% for FFMI which were three to seven times greater than the association that we found with protein. For LEP the scale of the association was 24.2 % and was 2.5 times greater than with protein. Furthermore dietary Mg was related to circulating CRP and, in statistical analyses, attenuated the negative association between hs-CRP and percentage of muscle mass by 6.5%, with the results being greater in older than in younger women. Our results suggest that dietary Mg may aid conservation of age-related loss of skeletal muscle mass and power, in women of all ages, and so requires further investigation.

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Table 1: Characteristics and dietary intakes of females aged 18-79 years

Characteristics	Fat free mass group (n=2570)	Grip strength group (n=949)
Age (years)	48.3 (12.7)	59.1 (9.3)
BMI (kg/m^2)	24.9 (4.1)	26.5 (4.7)
Weight (kg)	65.6 (11.2)	69.2 (12.5)
Height (m)	162 (6.1)	162 (5.9)
Fat mass (kg)	22.7 (7.9)	-
Fat free mass (%)	33.9 (7.2)	-
Fat free mass index (kg/m^2)	15.0 (1.7)	-
Hand grip strength (kg)	-	28.8 (5.9)
Leg explosive power [a] (watts)	89.8 (36.8)	-
Leg explosive power [a] (watts/kg)	90.9 (36.5)	-
hs-CRP [b] (mg/L)	2.49 (2.3)	-
Energy intake (kcal/d)	1979 (524)	1911 (635)
Magnesium (mg/d)	344 (92.3)	350 (110)
Protein (% energy)	16.6 (2.6)	17.7 (2.8)
Under-reporting (EI:EER, %)	87.4 (24.6)	89.2 (33.7)
Physical activity (active, %)	24.2% (622)	26.0% (247)
(moderately active, %)	53.9% (1385)	34.5% (327)
(inactive, %)	21.9% (563)	39.5% (375)
Smoking status (current, %)	18.2% (468)	9.7% (92)
Anti-inflammatory medication (yes, %)	6.2% (102) [2]	-
Menopausal status (post-menopausal, %)	-	89.8% (852)
Hormone replacement therapy (yes, %)	6.3% (105) [2]	9.5% (90)

Values are mean (SD) or % (n=)

EI:EER - ratio of reported energy intake to estimated energy requirements, expressed as a percentage

Values for a subset of [a]1914 and [b]1658 participants in the fat free mass group

Table 2: Percentage fat free mass, fat free mass index, grip strength and high sensitivity C-reactive protein by quintile of magnesium intake in 2570 females aged 18-79 years

	Model	Q1 (n=514)	Q2 (n=514)	Q3 (n=514)	Q4 (n=514)	Q5 (n=514)	P-trend	Q5-Q1	% of Q1
Magnesium (mg/d)		225 (31.6)	291 (14.0)	337 (12.6)	384 (15.9)	481 (60.8)	-	-	-
Fat free mass (%)	1	61.1 (0.31)	60.7 (0.30)	61.2 (0.31)	61.3 (0.31)	61.3 (0.32)	0.335	0.2	0.3
	2	60.0 (0.34)	60.3 (0.27)	61.1 (0.24)	61.5 (0.25)	62.6 (0.34)	<0.001	2.6	4.3
Fat free mass index (kg/m^2)	1	14.8 (0.09)	15.0 (0.08)	15.0 (0.08)	15.1 (0.08)	15.2 (0.08)	0.008	0.4	2.7
	2	14.8 (0.09)	15.0 (0.07)	15.1 (0.06)	15.1 (0.07)	15.2 (0.08)	0.005	0.4	2.7
Grip strength (kg)[a]	1	28.5 (0.44)	28.9 (0.49)	28.5 (0.44)	28.7 (0.47)	29.2 (0.44)	0.348	0.7	2.5
	2	28.7 (0.41)	28.8 (0.44)	28.4 (0.37)	29.1 (0.39)	28.7 (0.49)	0.847	0	0.0
Leg explosive power (watts/kg)[b]	1	88.9 (1.97)	88.9 (1.86)	89.2 (1.92)	94.1 (2.03)	93.1 (2.12)	0.032	4.2	4.7
	2	81.1 (2.30)	86.6 (1.85)	89.2 (1.76)	96.8 (1.94)	100.7 (2.47)	<0.001	19.6	24.2
Hs-CRP (mg/L)[c]	1	1.73 (1.54-1.94)	1.54 (1.38-1.72)	1.55 (1.38-1.73)	1.66 (1.48-1.87)	1.51 (1.36-1.69)	0.295	-0.22	-12.7
	2	1.94 (1.69-2.23)	1.57 (1.41-1.75)	1.58 (1.42-1.75)	1.60 (1.44-1.79)	1.35 (1.19-1.53)	0.011	-0.56	-28.9

Values for magnesium are mean (SD) and all other values are mean (SE). P-trend values were calculated using ANCOVA. Q5-Q1 is the value in Q5 minus the value in Q1. % of Q1 is the difference between Q5 and Q1 calculated as the percentage of the value in Q1.
Model 1 was unadjusted. Model 2 was adjusted for age, physical activity, smoking status, energy intake, protein intake and underreporting and fat free mass index was additionally adjusted for fat mass.

[a] Analysis for the grip strength group (n=949). Model 1 was unadjusted. Model 2 was adjusted for age, physical activity, smoking status, energy intake, protein intake, underreporting, menopausal

status, use of HRT and height. Participant numbers were Q1=192; Q2=190; Q3=191; Q4=190; Q5=186 and intakes of magnesium (mean ± SD) were as follows Q1=219 ± 32.9; Q2= 290 ± 14.6; Q3= 339 ± 14.7; Q4= 394 ± 17.7; Q5= 516 ± 97.2

[b] Subset analysis for 1914 participants in the fat free mass group. Model 1 was unadjusted. Model 2 was adjusted for age, physical activity, smoking status, energy intake, protein intake, underreporting, menopausal status, use of HRT and height. Participant numbers were Q1=383; Q2=383; Q3=383; Q4=383; Q5=382 and intakes of magnesium (mean ± SD) were as follows Q1=224 ± 32.4; Q2= 291 ± 13.8; Q3= 336 ± 12.9; Q4= 384 ± 15.4; Q5= 480 ± 59.3

[c] Subset analysis for 1658 participants in the fat free mass group. Values are geometric mean (95% CI). Model 1 was unadjusted. Model 2 was adjusted for age, BMI, physical activity, smoking status, energy intake, protein intake, underreporting, and use of anti-inflammatory medication and HRT. P-trend values were based on log concentrations using ANCOVA. Participant numbers were Q1=332; Q2=332; Q3=331; Q4=332; Q5=331 and intakes of magnesium (mean ± SD) were as follows Q1=232 ± 30.1; Q2= 293 ± 12.5; Q3= 336 ± 12.8; Q4= 384 ± 15.7; Q5= 480 ± 60.7

Figures:

Figure 1: Percentage fat free mass and fat free mass index by quintile of magnesium in 2570 females, stratified by age group

Bars represent the adjusted means; error bars represent the 95% confidence intervals. Means were adjusted for age, physical activity, smoking status, energy intake, protein intake and underreporting and fat free mass index was additionally adjusted for fat mass. *P-trend <0.05 calculated using ANCOVA.

Figure 2: Leg explosive power by quintile of magnesium in 1914 females, stratified by age group
Bars represent the adjusted means; error bars represent the 95% confidence intervals. Means were adjusted for age, physical activity, smoking status, energy intake, protein intake, underreporting, menopausal status, use of HRT and height. *P-trend <0.05 calculated using ANCOVA.

Figure 3: Percentage fat free mass by quintile of high sensitivity C-reactive protein and attenuation by magnesium in 1658 females, stratified by age group

Bars represent the percentage difference between β-coefficient 1 and 2. β-coefficient 1 is the association of fat free mass (%) per quintile of high sensitivity C-reactive protein adjusted for age, BMI, physical activity, smoking status, energy intake, protein intake, underreporting, and use of anti-inflammatory medication and HRT. β-coefficient 2 is the association in fat free mass (%) per quintile of high sensitivity C-reactive protein adjusted as model 1 and additionally for magnesium intake. Attenuation as a percentage was calculated as ((β2- β1)/ β1)*100

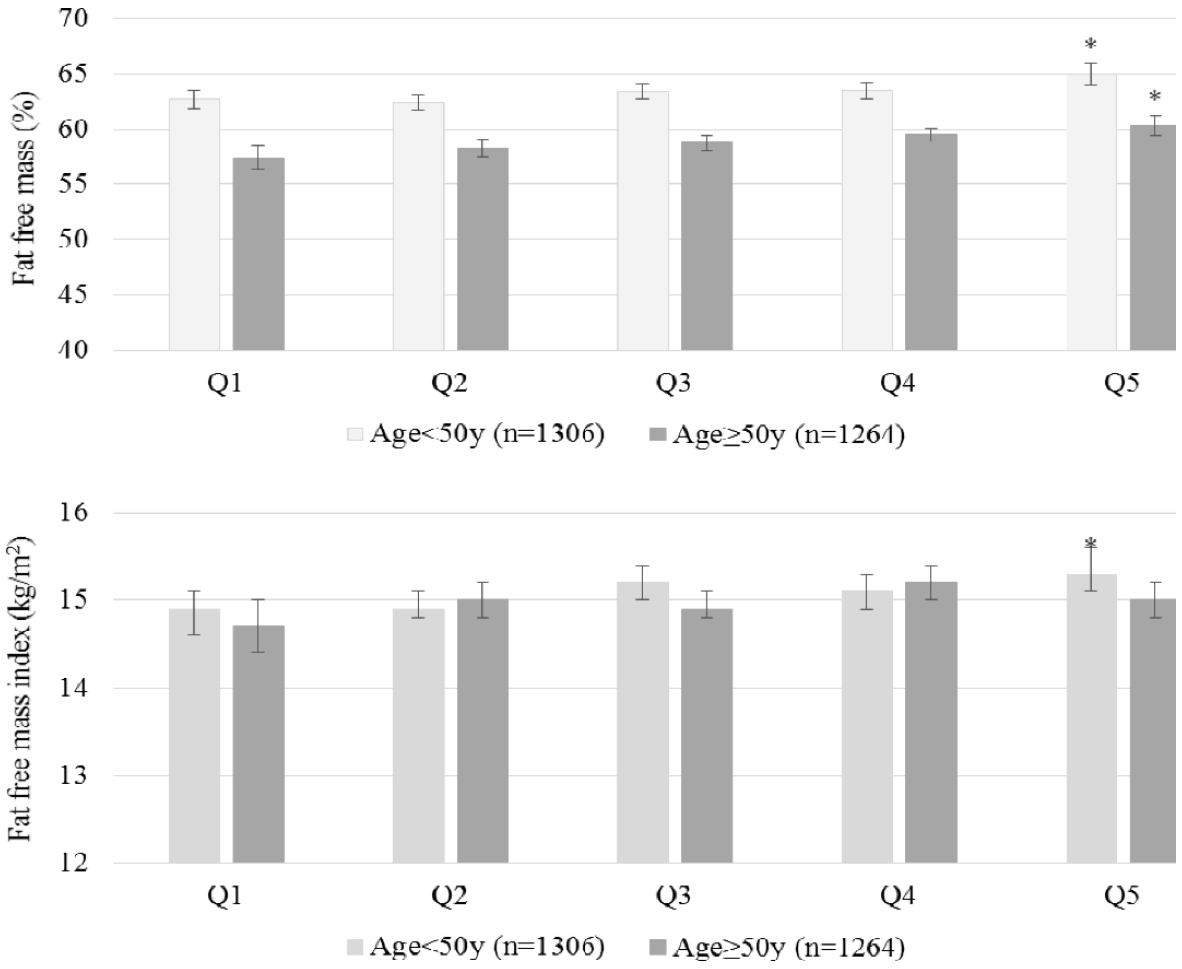


Figure 1

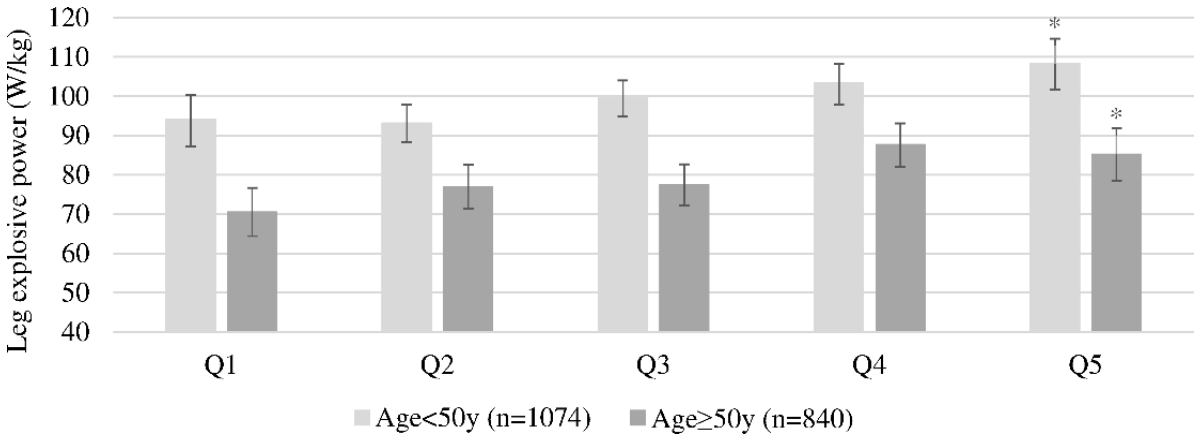
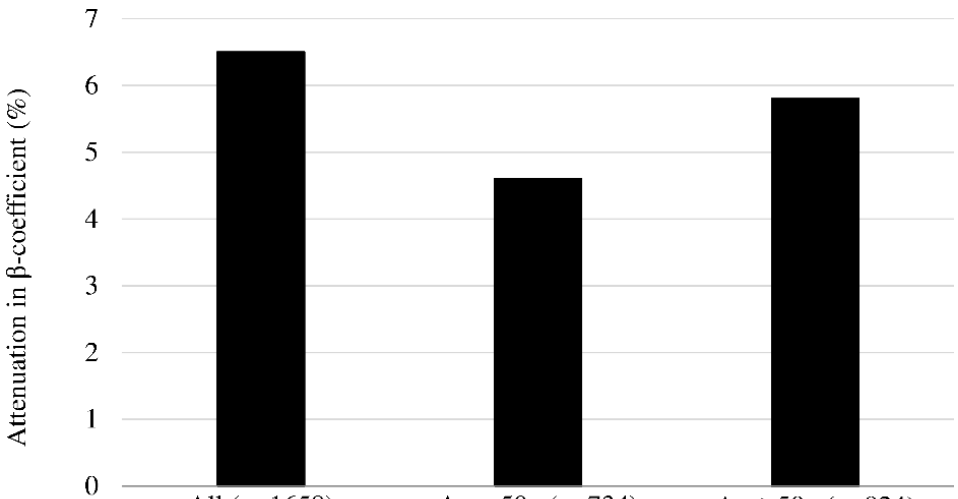


Figure 2



	All (n=1658)	Age<50y (n=734)	Age≥50y (n=924)
Attenuation (%)	6.5	4.6	5.8
β coefficient 1	-0.309	-0.285	-0.346
β coefficient 2	-0.289	-0.272	-0.326

Figure 3