1 **Cross-sectional associations of dietary and circulating magnesium with skeletal muscle mass in**

2 **the EPIC-Norfolk cohort.**

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17 *Abbreviations:* BIA (Bioelectrical impedance analyser); DINER (Data Into Nutrients for

18 Epidemiological Research); EAR (Estimated Average Requirement); EPIC (European Prospective

19 Investigation into Cancer and Nutrition); FFM (Fat Free Mass), FFM% (Percentage Fat Free Mass),

20 FFM_{BMI} (Fat Free Mass standardised by Body Mass Index), FFQ (Food-Frequency Questionnaire);

21 HLQ (Health and Lifestyle Questionnaire); HRT (Hormone Replacement Therapy); RNI (Reference

22 Nutrient Intake).

23 This paper should be read with the accompanying supplemental material.

24

25 **ABSTRACT**

26 **Background:** Maintenance of skeletal muscle in older age is critical to reducing frailty and the risk of

27 falls and fractures. Nutrition has established importance for muscle health in general, but less research

28 has looked at associations of dietary intake of specific micronutrients on skeletal muscle mass in older

29 adults.

30 **Aims:** This study aimed to investigate the influence of dietary and circulating magnesium on skeletal

31 muscle mass in a UK population of 14,340 middle to older-aged men and women participating in the

32 EPIC-Norfolk cohort study.

33 **Methods:** Dietary nutrient intakes were estimated from 7-day food diaries and fat-free mass (FFM) by

34 bioelectrical impedance analysis. Multivariable regression was used to investigate associations of

35 FFM-based indices of muscle mass with quintiles of dietary magnesium intake or serum magnesium

36 concentration groups. All analyses were stratified by sex, and regression models were adjusted for

37 relevant covariates.

38 **Results:** Significant positive trends in FFM measures were evident across magnesium dietary intake

39 quintiles for both sexes (all $p < 0.001$; n=6350 men; n=7990 women) and both <60 and ≥60 year olds,

40 with all-age quintile 5 *versus* quintile 1 maximal differences of 4.6% in men and 6.3% in women;

41 highly relevant compared to the estimated 1% decline per year after 40 years of age. These

42 observations were not reflected in serum magnesium analyses, where no consistent trends were found

43 across the skeletal muscle mass indices tested.

44 **Conclusion:** Further investigation will be required to improve our understanding of the relationship

45 between serum magnesium concentration and skeletal muscle mass. However, this study has

46 demonstrated strong associations between dietary magnesium intake and indices of skeletal muscle

47 mass in a UK population of middle to older-aged adults, highlighting the likely importance of dietary

48 magnesium for optimal muscle health in this population.

49

50 **Keywords:** Sarcopenia, skeletal muscle, ageing, nutrition, general population studies.

INTRODUCTION

Sarcopenia is a syndrome characterised by a progressive and generalised loss of skeletal muscle mass and function with age (1). Significant reduction in skeletal muscle mass and strength impairs static and dynamic balance, which may increase risk of falls and thus the risk of resultant fractures (2). Indirectly, the maintenance of skeletal muscle is important in protecting against osteoporosis since the mechanical force of muscle contractions stimulates bone modelling and remodelling, which increases bone strength and mass (3). Previous research has also shown skeletal muscle mass to be positively correlated with both bone mineral content and density (2). Sarcopenia can therefore have significant implications for affected individuals, placing them at risk of adverse outcomes including physical frailty and falls, and resulting in an increased need for health and social care services (4). Muscle tissue also has a metabolic role in the body and thus loss of muscle mass may result in other detrimental outcomes, including change to metabolic rate, insulin resistance, and increased risk of hypertension (5).

Sarcopenia is a complex condition, with many contributory factors including hormonal changes, decreased protein synthesis, low-grade inflammation, oxidative stress, mitochondrial dysfunction, and neuromuscular ageing. Nevertheless, interventions targeting modifiable lifestyle behaviours, such as physical activity or diet, provide a potential strategy to reduce severity (4). It is recognised that appropriate nutrition is critical for normal muscle metabolism, but influences of specific dietary nutrients on sarcopenia are less well defined. Dietary protein has received most attention in the past (6), but more recently the importance of other dietary components, including vitamin D (7) and antioxidant micronutrients vitamins C (8) and E (9,10), has been suggested. Likewise, the mineral magnesium has drawn some attention. Older individuals may be particularly susceptible to developing low magnesium status due to physiological decline in function of the gastrointestinal and renal systems causing a reduction in absorption of dietary magnesium and an increase in urinary excretion (11). Second only to bone, skeletal muscle acts as a major store of magnesium where it is important

77 for energy metabolism, transmembrane transport, and muscle contraction and relaxation (12).

78 Magnesium supplementation has been shown to increase the muscle strength young adults gained

79 through exercise (13) and improve physical performance in older individuals (14). Epidemiological

80 studies have shown higher dietary magnesium intakes associated with greater skeletal muscle mass

81 and function (15) (16,17), and a significant positive association of serum magnesium concentration

82 with muscle performance in older adults (18). However, a comprehensive population cohort analysis

83 of dietary and circulating magnesium associations with skeletal muscle measures in both men and

84 women is currently lacking. This study therefore aims to address this by exploring the potential

85 associations of dietary magnesium intake and serum magnesium concentration with bio-impedance

86 estimated fat free mass (as a measure of skeletal muscle mass), in a mixed-sex UK population of

87 middle to older-aged individuals.

88

89 **MATERIALS AND METHODS**

90 Data analysed in this cross-sectional cohort study were from the Norfolk component of the European

91 Prospective Investigation into Cancer and Nutrition (EPIC). Written informed consent was provided

92 by participants according to the Declaration of Helsinki, and all procedures were approved by The

93 Norfolk District Health Authority Ethics Committee. Full details of recruitment to this cohort and the

94 procedures involved have been described previously (19). In summary, 25,639 men and women aged

95 40-79 years old living in the general community in Norfolk, UK, were recruited to the study and

96 participated in a baseline health-check between 1993 and 1997. Of these, 15,028 participants aged 42-

97 82 years had further data recorded at a second health-check between 1997 and 2000, when

98 bioelectrical impedance analysis (BIA) was undertaken.

99

100 At both health checks, height and weight were measured according to standard protocols (19). Height

101 was recorded to the nearest millimetre using a free-standing stadiometer and weight to the nearest 0.1

102 kilograms using calibrated digital scales with the participant wearing light clothing and no shoes. BMI

103 was calculated from these measurements (kg/m^2). BIA was carried out using a previously validated

104 (20,21) standard technique (Bodystat, Isle of Man, UK). The Tanita TBF-531 BIA analyser calculated

105 body density (BD) from total weight (Wt) in kg, height (Ht) in cm, and impedance (Z) in ohms, using

106 the following standard regression formulae for adults: BD in men = $1.100455 - 0.109766 \times Wt \times Z \div$

107 $Ht^2 + 0.000174 \times Z$; BD in women = $1.090343 - 0.108941 \times Wt \times Z \div Ht^2 + 0.00013 \times Z$. From this,

108 fat free mass (FFM) in kg was calculated: FFM = $Wt - ((4.57 \div BD - 4.142) \times Wt)$. This estimates the

109 total mass of non-fat compartments of the body, i.e. metabolic tissue, intra- and extra-cellular water,

110 and bone tissue. As a further index for assessment, percentage FFM (FFM%) was calculated as FFM

111 divided by total weight multiplied by 100, and in order to scale for differences in skeletal muscle mass

112 with increasing body weight or stature, FFM standardised by BMI (FFM_{BMI}) was calculated as FFM

113 divided by BMI (22).

114

115 Health and lifestyle questionnaires, as previously described (19), were completed by all participants to

116 gather data including age, physical activity, social class, smoking status, menopausal status and HRT

117 use, and corticosteroid use. Each participant's physical activity status was categorised, according to a

118 heart-rate data validated method (19,23), as *inactive*, *moderately inactive*, *moderately active*, or *active*.

119 Dietary intakes were assessed using 7 day food diaries completed by each participant detailing all

120 food and drink consumed, together with the portion sizes (24). DINER (Data Into Nutrients for

121 Epidemiological Research) software was used to enter the dietary information provided by the diaries

122 (25), which was then checked and processed by nutritionists to obtain nutrient data, using DINERMO

123 (26). Serum magnesium concentration was determined using blood sampled by peripheral

124 venepuncture during the baseline health check. Samples were prepared, using a technique optimised

125 for use in EPIC, and stored in liquid nitrogen at -196ºC until analysed by Quotient Bioresearch,

126 Fordham, UK, using an Olympus AU640 Chemistry Immuno Analyser to perform a xylidyl blue

127 based colorimetric assay (Beckman Coulter, USA). Measurements below 0.2 mmol/L or above 3.3

128 mmol/L were considered invalid and excluded from analyses.

129

The High Performance Computing Cluster supported by the Research and Specialist Computing Support service at the University of East Anglia was used for statistical data analysis with STATA software (v.13; Stata Corp., Texas). All analyses were stratified by sex since significant differences in body composition and skeletal muscle mass exist between men and women. Any p values <0.05 were considered to be statistically significant in individual analyses. Multivariable regression with ANCOVA was used to investigate differences in skeletal muscle measures across sex-specific quintiles of dietary magnesium intake. An adjusted model was tested, correcting for the potential effects of physiological (age, menopausal status, HRT status, corticosteroid use, statin use), lifestyle (smoking status, physical activity, social class) and dietary factors (total energy intake, and the percentage of total energy from protein); also included was the energy intake to estimated energy requirement ratio (EI:EER) as a percentage, to help correct for dietary misreporting (27). Likewise, differences in skeletal muscle measures across sex-specific groups of serum magnesium concentration were investigated using the same covariates, but excluding dietary factors in the adjusted model. Serum magnesium concentration in healthy individuals is kept under tight homeostatic control; published guidance suggests 0.7-1.0 mmol/L should be used as a reference range for healthy individuals (28). Serum magnesium concentration groups were therefore categorised as <0.7 mmol/L (group 1), 0.7-0.8 mmol/L (group 2), 0.8-0.9 mmol/L (group 3), 0.9-1.0 mmol/L (group 4), and >1.0 mmol/L (group 5). Group 2 has been used as the reference category for inter-group analyses. Participants were excluded from analyses if they had missing or extreme (<300 or >1000 ohms (29)) BIA impedance values (n=228 and n=22), FFM < 25kg (n=13), BMI $\geq$ 36 kg/m^2 (n=337), or had missing values for any covariates included in the multivariable model (n=88 for diet analyses, and n=48 for serum analyses). Analyses were repeated after stratifying for age (<60 and $\geq$60 years). Correlation between dietary and serum continuous scale magnesium variables was assessed by Pearson correlation coefficient.

154

RESULTS

Selected characteristics of participants are summarised in **Table 1** stratified by sex. All variables were significantly different in men and women except for corticosteroid and statin use. The UK Reference Nutrient Intake (RNI) for magnesium is 300 mg per day for men over the age of 18 years and 270 mg per day for women, while the Estimated Average Requirements (EARs) for men and women are 250 mg and 200 mg (30). In this cohort, mean dietary magnesium intakes were 332 ± 90 mg for men and 275 ± 73 mg for women. The largest contribution of magnesium in the diet of both men and women in this cohort came from cereals and cereal foods (33.7% of total dietary magnesium in men; 32.4% in women). Fruits and vegetables accounted for a further 11.5% in men and 15.0% in women, while hot beverages provided 10.1% in men and 11.4% in women. Further detail of the contribution of foods to magnesium intake is provided in **Supplemental Figure 1**. Prevalence of inadequate intakes estimated using the EAR cut-point method (31) was 14.3%, with a greater number of men with inadequate intakes than women (16.1% *vs.* 12.9%; p<0.001; n=14340). No correlation was evident between magnesium dietary intake and serum concentration (Pearson's r=0.007, p=0.646, n=4611 men; r=-0.030, p=0.020, n=5972 women).

In dietary model analyses, there were significant positive trends across magnesium intake quintiles in adjusted FFM, FFM%, and FFM_{BMI} for both men and women (all p<0.001; n=6350 men; n=7990 women) (see **Supplemental Table 1**). These trends were evident in both <60 (n=2366 men; n=3535 women) and ≥60 year olds (n=3984 men; n=4455 women) (see **Figure 1**). The largest all-age inter-quintile differences were apparent in women where adjusted FFM for those in quintile 5 was 2.9% greater than in quintile 1, FFM% was 4.2% greater, and FFM_{BMI} was 6.3% greater (all p<0.001; n=3196); quintile 5 *vs.* 1 differences in men were 2.0% for FFM, 2.4% for FFM%, and 4.6% for FFM_{BMI} (all p<0.001; n=2540). For women under 60 years of age, adjusted FFM in quintile 5 was 3.4% greater than in quintile 1, FFM% was 4.6% greater, and FFM_{BMI} was 7.2% greater (all p<0.001; n=1394); in men the differences were 2.2% for FFM, 2.2% for FFM%, and 4.8% for FFM_{BMI} (all

181   p<0.001; n=940). For women 60 years or over, adjusted FFM in quintile 5 was 2.8% greater than in

182   quintile 1, FFM% was 4.6% greater, and FFM_{BMI} was 6.8% greater (all p<0.001; n=1802); in men the

183   differences were 1.8% for FFM, 2.8% for FFM%, and 5.0% for FFM_{BMI} (all p<0.001; n=1600).

184

185   In all-age serum model analyses (see **Supplemental Table 2**) no linear trends were apparent between

186   magnesium serum concentration groups and FFM, FFM%, or FFM_{BMI}; likewise, no significant

187   differences were identified between muscle mass measures in the low normal concentration group

188   (group 2) *vs.* other groups. However, stratifying the serum data by age highlighted some significant

189   differences (see **Figure 2**). In individuals ≥60 years old, FFM was significantly lower in magnesium

190   concentration group 4 *vs.* group 2 in both men (p=0.031; n=1131) and women (p=0.020; n=1311), and

191   group 5 *vs.* group 2 in women only (p=0.029; n=928).

192

193   **DISCUSSION**

194   This study, using data from a large population cohort, has shown that associations between dietary

195   magnesium and indices of skeletal muscle mass exist in both men and women. Significant positive

196   trends in FFM, FFM% and FFM_{BMI} were evident across increasing quintiles of dietary magnesium

197   intake for both sexes, which remained after adjustment for important biological, lifestyle and other

198   dietary covariates. These results corroborate previous smaller-scale studies including the positive

199   relationship between magnesium intake and dual-energy X-ray absorptiometry-assessed appendicular

200   lean mass in individuals aged 50 to 79 years in the Tasmanian Older Adult Cohort Study (15), the

201   greater FFM seen with higher intakes of dietary magnesium in a UK study of women aged 34 to 83

202   years from the TwinsUK cohort (16), and the more recent large-scale cross-sectional analysis of

203   FFM% and FFM_{BMI} using UK Biobank data (17). Associations between serum magnesium

204   concentration groups and skeletal muscle mass indices are less clear, although this is unsurprising

205   considering the tight homeostatic control of circulating magnesium and the fact that less than 1% of

206   total body magnesium is present in the blood (32). This homeostatic control makes it less likely that a

207 serum magnesium concentration outside the normal range represents an extreme dietary intake of

208 magnesium, and more likely that it is the result of a pathological problem (e.g. abnormal renal

209 wasting) or diuretic medication (32). Indeed, our results showed correlation of magnesium serum

210 concentration with dietary intake in the EPIC-Norfolk cohort was negligible. Previous studies have

211 demonstrated that despite presenting with normal serum magnesium concentration, some individuals

212 may be severely magnesium deficient, with low concentrations in bone and muscle due to long-term

213 compensatory release of magnesium to maintain serum concentration when dietary intake has been

214 low for a long period of time (33). This may partly explain the inconsistent associations between

215 serum magnesium concentrations and skeletal muscle mass indices apparent in this study.

216

217 It is important to appreciate the magnitude of the differences seen with the dietary analyses. Indeed,

218 considering that the effect of age on skeletal muscle mass is already well-established (34), and

219 confirmed in this dataset where FFM_{BMI} was 5.4% lower in those ≥60 years *versus* those <60 years

220 (data not shown), the differences seen according to magnesium intake are highly relevant. For

221 example, the difference in adjusted FFM_{BMI} between magnesium quintile 5 and quintile 1 for women

222 was 6.3%. Furthermore, the difference in median daily dietary intake between quintiles 1 and 5 for

223 women was 173 mg, a difference which should be achievable as part of a normal diet (for example, by

224 ½ cup boiled spinach, ¼ cup roasted peanuts, and a medium-sized banana (35)). However, since a

225 typical Western diet containing a high proportion of processed foods and limited whole grains and

226 green vegetables is often deficient in magnesium (36), more needs to be done to promote an adequate

227 diet and avoid adverse musculoskeletal consequences.

228

229 Although sarcopenia is a particular issue in individuals aged 60 years old or older, loss of skeletal

230 muscle mass has been documented to progress from the age of 30 years onwards (4). Age

231 stratification of our dietary magnesium analyses demonstrated largely similar effects for those less

232 than 60 years of age, and those 60 years or older, albeit with lower values for muscle mass indices in

233   the older age group. This highlights the potential benefits of dietary magnesium for musculoskeletal

234   health in all ages of this cohort, and raises the possibility that optimal dietary magnesium intake could

235   help preserve skeletal muscle before sarcopenia becomes problematic later in life.

236

237   While previous research has demonstrated magnesium status to be strongly correlated with muscle

238   performance in both young and old individuals (13,14), the mechanisms by which magnesium may

239   influence muscle are not yet fully understood. Magnesium is critical for basic mitochondrial function:

240   cell-culture and animal studies have demonstrated that magnesium depletion can cause structural

241   damage to muscle cells due to oxidative stress and disrupted calcium homeostasis (37). In addition,

242   magnesium also has a postulated role in protecting against the chronic low-grade inflammation

243   associated with ageing and a known risk factor for sarcopenia (4). Indeed, circulating concentrations

244   of inflammatory cytokines, including C-reactive protein (CRP), IL-6, and TNF-α, have been

245   negatively associated with skeletal muscle measures of both mass and function in a number of studies

246   (16,38-40), and systematic review evidence indicates that dietary magnesium intake is inversely

247   associated with serum CRP concentration (41).

248

249   It is interesting to consider how results for the alternative skeletal muscle indices translate into clinical

250   importance for sarcopenia. FFM_{BMI} may provide a more useful measure than unstandardised FFM or

251   FFM% to assess change in skeletal muscle mass while correcting for differences attributable to body

252   size. This index has recently been used to define cutpoints in the Foundation for the National Institutes

253   of Health Biomarkers Consortium Sarcopenia Project (42), and as it is used in more studies of

254   different populations we will gain a greater understanding of how it describes body composition in

255   both normal and sarcopenic individuals.

256

257   This is the first study to have investigated associations between both dietary intake and circulating

258   magnesium, and measures of skeletal muscle in a mixed-sex UK cohort of older adults. However, we

259 recognise there are a number of limitations. These include the observational and cross-sectional study

260 design which precludes us from attributing causal links between magnesium dietary intake or serum

261 concentration and skeletal muscle measures, and reliance on self-reported measures for diet and

262 physical activity data. Nevertheless, the quantitative 7-day food diaries developed for use in EPIC

263 have been validated previously and are expected to provide more precise dietary intake figures

264 compared to alternative FFQ or 24-hour recall methods (26). Magnesium dietary data analysed here

265 were derived from food intake only, and therefore may underestimate total nutrient intakes. However,

266 the supplements consumed by this cohort provide a relatively small contribution to total magnesium

267 intake and thus are likely to have a negligible effect on our results (43). We assessed magnesium

268 status using serum magnesium concentrations which may not be the most reliable marker of Mg status

269 as discussed earlier. However, the preferred alternative of timed 24 hour urine collection which

270 linearly reflects dietary intake may be even less reliable for older individuals due to problems with

271 urine collection and complications of diuretic use (11). Magnetic resonance measurement of ionised

272 magnesium within skeletal muscle could provide useful data (44), but this method was not practical

273 for our large population sample, and thus serum magnesium measurement remains a useful indicator

274 of magnesium status for this type of study (45). Indices of skeletal muscle mass were calculated from

275 weight, height, and bioelectrical-impedance measurements, rather than the potentially more accurate

276 and precise methods of dual-energy X-ray absorptiometry, computer tomography, or magnetic

277 resonance imaging (46). However, bio-electrical impedance assessment has the advantage of avoiding

278 accessibility issues, costs, and radiation, associated with other methods. Consequences of loss of

279 skeletal muscle mass may extend beyond a reduction in strength and function due to the metabolic

280 role of muscle, and may include changes to metabolic rate, insulin resistance, and increased risk of

281 hypertension (5). While in this study it has not been possible to analyse functional muscle measures in

282 relation to magnesium we believe it is important to have considered the fundamental body

283 composition information provided by BIA data.

284

Conclusions

This study has highlighted strong positive associations between dietary magnesium intake and indices of skeletal muscle mass in both men and women of the EPIC-Norfolk cohort, with the scale of effects highly relevant in comparison to age-related losses. The results for circulating magnesium are less patent, potentially due to the tight homeostatic control of blood magnesium concentrations. These findings, which being observational in nature require confirmation by clinical trial, support a hypothesis that dietary magnesium is beneficial to skeletal muscle health in older individuals.

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422

423 **TABLES AND FIGURES**

424

425 **Table 1** – Selected characteristics of the EPIC-Norfolk cohort population stratified by sex for the diet

426 analysis group (n=14,340) and the serum analysis group (n=10,611).

427

Selected Characteristics	Diet analysis group			Serum analysis group		
	Men	**Women**		**Men**	**Women**	
	n=6350	**n=7990**	**P^1**	**n=4628**	**n=5983**	**P**
Age (years)	62.9 ± 9.0^2	61.5 ± 9.0	*<0.001*	62.9 ± 8.7^2	61.6 ± 8.9	*<0.001*
BMI (kg/m^2)	26.7 ± 3.0	26.1 ± 3.7	*<0.001*	26.7 ± 3.0	26.0 ± 3.7	*<0.001*
Magnesium intake (mg/day)	332 ± 90	275 ± 73	*<0.001*	--	--	
Total energy intake (kcal/day)	2286 ± 500	1735 ± 378	*<0.001*	--	--	
Protein % of energy	14.8 ± 2.4	15.5 ± 2.8	*<0.001*	--	--	
Serum [Mg] (mmol/L)	--	--		0.82 ± 0.11	0.80 ± 0.12	*<0.001*
FFM (kg)	61.6 ± 5.9	40.6 ± 4.5	*<0.001*	61.7 ± 5.9	40.6 ± 4.5	*<0.001*
FFM%	76.7 ± 5.8	60.9 ± 8.3	*<0.001*	76.8 ± 5.8	61.1 ± 8.1	*<0.001*
FFM$_{BMI}$	2.33 ± 0.26	1.58 ± 0.26	*<0.001*	2.33 ± 0.26	1.59 ± 0.26	*<0.001*
EI/EER%	91.1 ± 20.7	93.7 ± 21.8	*<0.001*	--	--	
Smoking			*<0.001*			*<0.001*
Current	542 (8.5)	696 (8.7)		375 (8.1)	489 (8.2)	
Former	3524 (55.5)	2551 (31.9)		2552 (55.1)	1909 (31.9)	
Never	2284 (36.0)	4743 (59.4)		1701 (36.8)	3585 (59.9)	
Physical activity			*<0.001*			*<0.001*
Inactive	1736 (27.3)	2070 (25.9)		1236 (26.7)	1537 (25.7)	
Moderately inactive	1595 (25.1)	2600 (32.5)		1164 (25.2)	1927 (32.2)	
Moderately active	1590 (25.0)	1933 (24.2)		1160 (25.1)	1445 (24.2)	
Active	1429 (22.5)	1387 (17.4)		1068 (23.1)	1074 (18.0)	
Corticosteroid use			*0.391*			*0.391*
Never (<3 months)	6086 (95.8)	7583 (94.9)		4444 (96.0)	5698 (95.2)	
Current or former (>3 months)	264 (4.2)	407 (5.1)		184 (4.0)	285 (4.8)	
Statin use			*0.391*			*0.391*
No	6003 (94.5)	7700 (96.4)		4389 (94.8)	5769 (96.4)	
Yes	347 (5.5)	290 (3.6)		239 (5.2)	214 (3.6)	
Menopausal status						
Pre-menopausal	--	475 (5.9)		--	475 (5.9)	
Peri-menopausal (<1 y)	--	266 (3.3)		--	266 (3.3)	
Peri-menopausal (1-5 y)	--	1400 (17.5)		--	1400 (17.5)	
Post-menopausal	--	5849 (73.2)		--	5849 (73.2)	
HRT						
Current	--	1704 (21.3)		--	1704 (21.3)	

			P value[1]			P value[1]
Former	--	1432 (17.9)		--	1432 (17.9)	
Never	--	4854 (60.8)		--	4854 (60.8)	
Social Class			*<0.001*			*<0.001*
Professional	523 (8.2)	547 (6.8)		385 (8.3)	401 (6.7)	
Managerial	2587 (40.7)	2950 (36.9)		1917 (41.4)	2226 (37.2)	
Skilled non-manual	797 (12.6)	1554 (19.4)		567 (12.3)	1180 (19.7)	
Skilled manual	1422 (22.4)	1577 (19.7)		1055 (22.8)	1190 (19.9)	
Semi-skilled	781 (12.3)	950 (11.9)		537 (11.6)	688 (11.5)	
Non-skilled	149 (2.3)	267 (3.3)		99 (2.1)	197 (3.3)	
Un-coded	91 (1.4)	145 (1.8)		68 (1.5)	101 (1.7)	

428 [1]P values are for differences between men and women, according to t-test for continuous or chi-square

429 for categorical variables. [2]Values are mean ± SD or frequency (percentage).

430 **Figure 1** – Adjusted skeletal muscle measures for individuals of the EPIC-Norfolk cohort stratified by

431 sex, age group, and quintiles of dietary magnesium intake (n=14,340).

432

433 [*] $p<0.05$; [**] $p<0.01$; [***] $p<0.001$ *versus* quintile 1, according to ANCOVA.

434 Adjusted for: age, menopausal status, HRT status, corticosteroid use, statin use, smoking status,

435 physical activity, social class, total energy intake, percentage of total energy from protein, and

436 EI:EER.

437 Values are presented as mean ± SE.

438 **Mg intake (mean ± SD; mg/day) by Mg quintiles (Q)**. *Men ≤60 years:* Mean, 350 ± 92; Q1, 226 ±

439 30; Q2, 283 ± 12; Q3, 323 ± 11; Q4, 368 ± 15; Q5, 470 ± 73. *Men >60 years:* Mean, 322 ± 87; Q1,

440 223 ± 31; Q2, 282 ± 12; Q3, 322 ± 11; Q4, 366 ± 16; Q5, 465 ± 71. *Women ≤60 years:* Mean, 285 ±

441 75; Q1, 187 ± 27; Q2, 235 ± 10; Q3, 268 ± 10; Q4, 305 ± 12; Q5, 385 ± 62. *Women >60 years:* Mean,

442 268 ± 71; Q1, 186 ± 26; Q2, 234 ± 10; Q3, 267 ± 10; Q4, 304 ± 13; Q5, 381 ± 56.

443

444

445 **Figure 2** – Adjusted skeletal muscle measures for individuals of the EPIC-Norfolk cohort stratified by

446 sex, age group, and serum concentration groups (n=10,611).

447

448 [*] $p<0.05$; [**] $p<0.01$; [***] $p<0.001$ *versus* group 2, according to ANCOVA.

449 Adjusted for: age, menopausal status, HRT status, corticosteroid use, statin use, smoking status,

450 physical activity, and social class.

451 Values are presented as mean ± SE.

452 **Serum Mg concentration groups:** <0.7 mmol/L (group 1), 0.7-0.8 mmol/L (group 2), 0.8-0.9

453 mmol/L (group 3), 0.9-1.0 mmol/L (group 4), and >1.0 mmol/L (group 5).
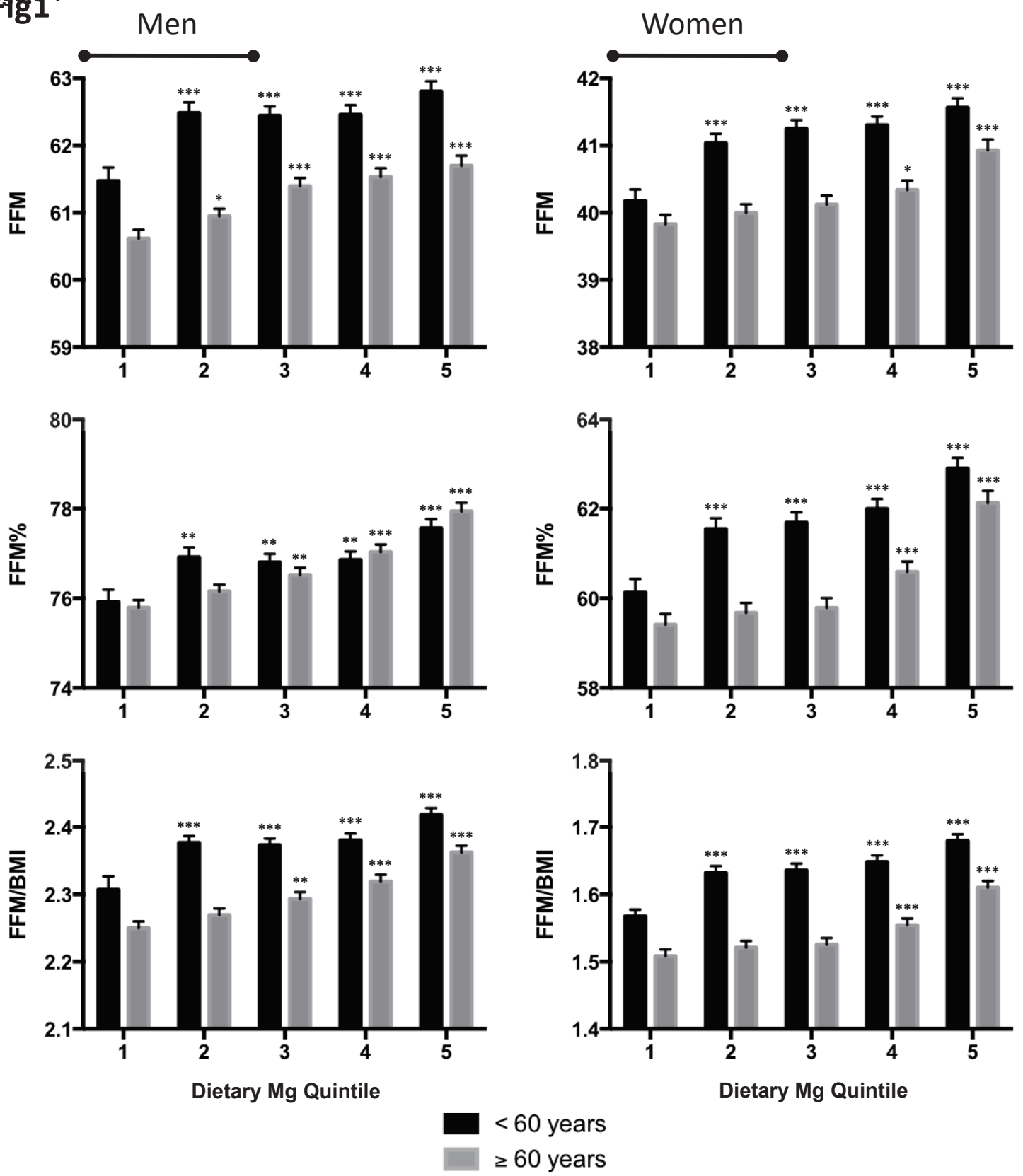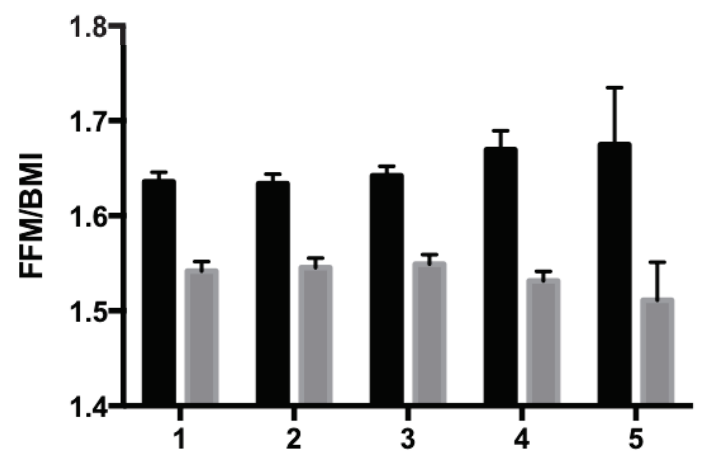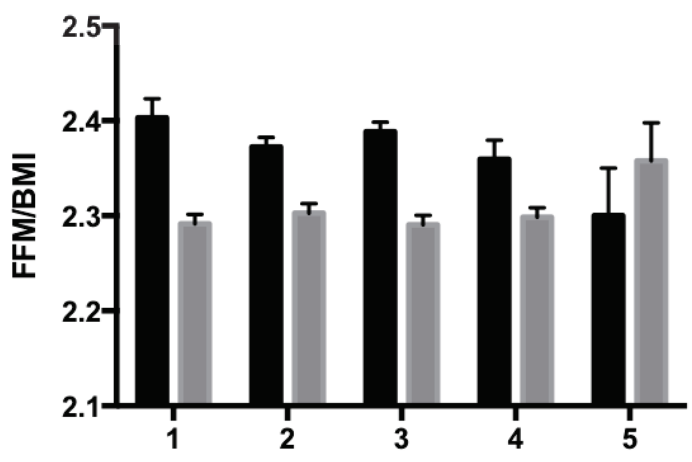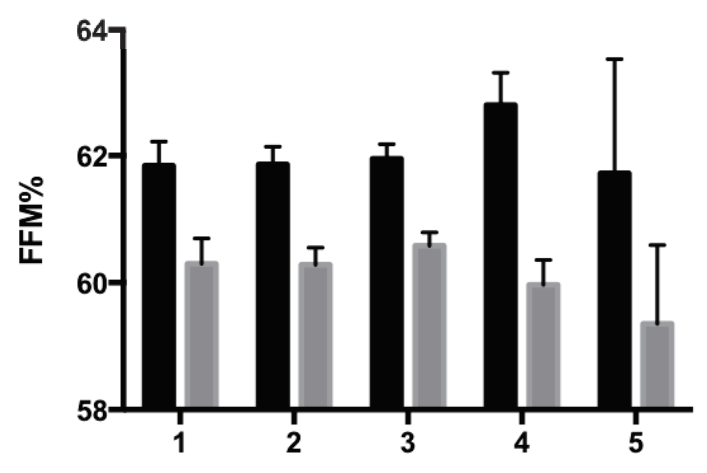
Figure 1

Fig1

Figure 2

Fig2
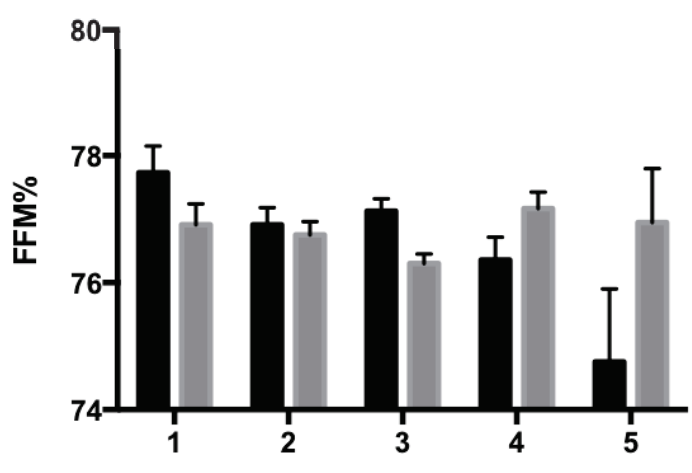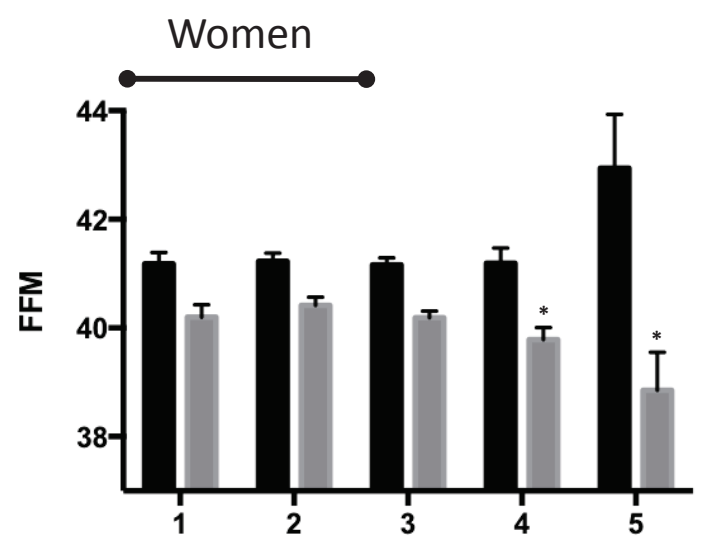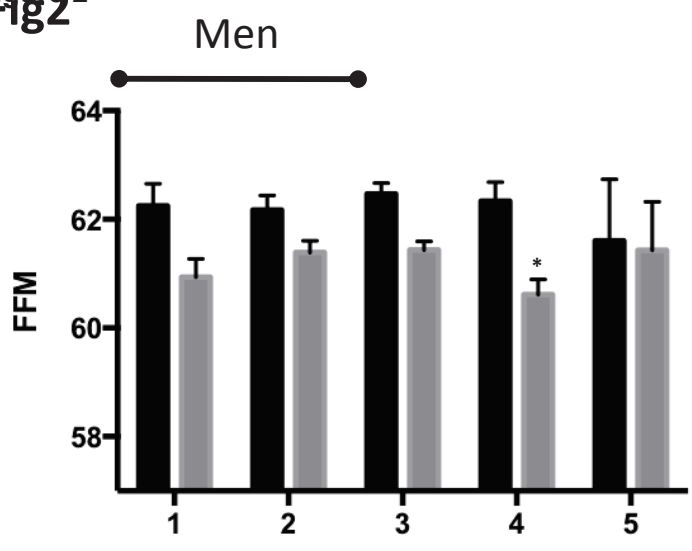
Supplemental Figure 1 – Percentage contribution of different foods to the dietary magnesium intake of EPIC-Norfolk cohort participants, stratified by sex (n=25,507).
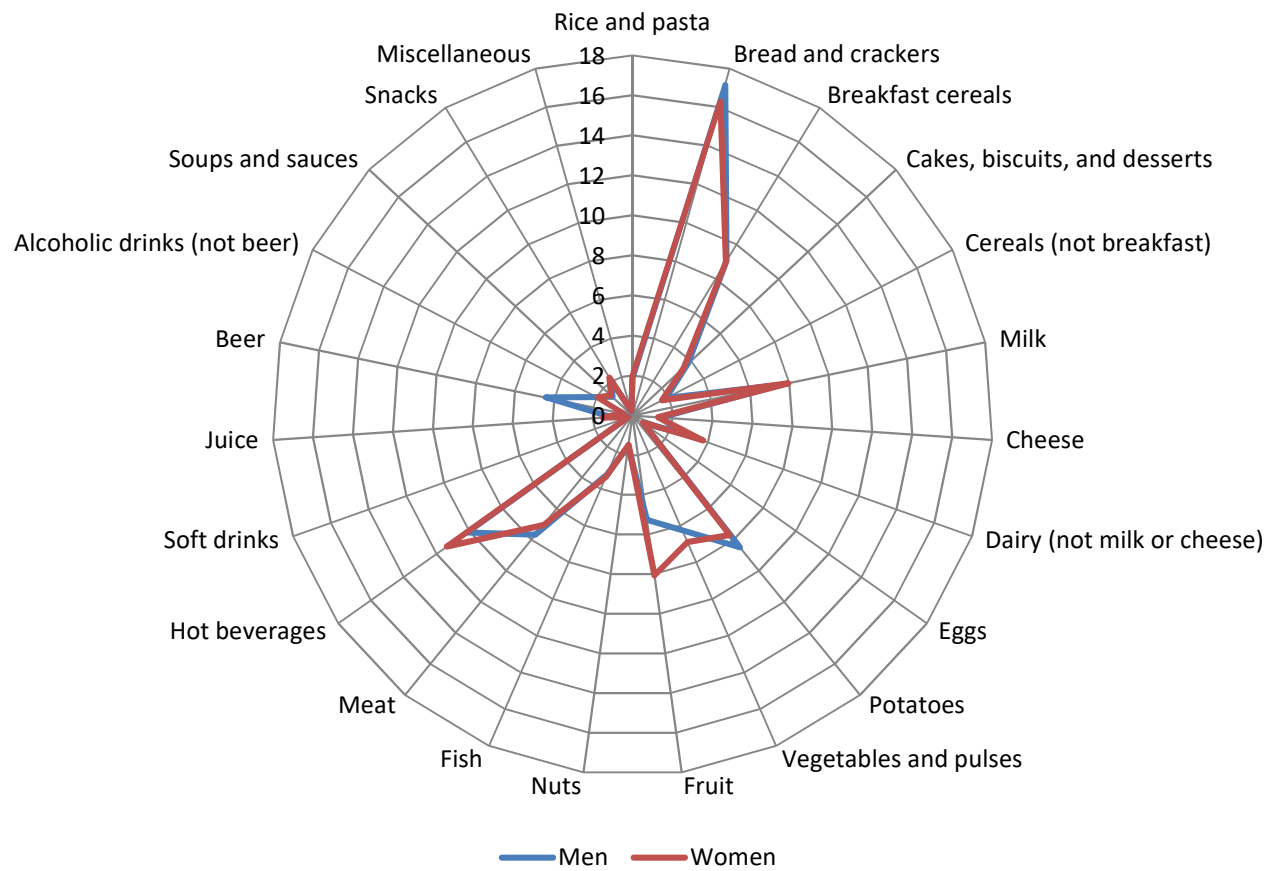
Rice and pasta 1.8% men, 2.0% women; bread and crackers 17.2% men, 16.3% women; breakfast cereals 9.0% men, 9.0% women; cakes, biscuits, and desserts 3.8% men, 3.5% women; cereals (not breakfast) 1.9% men, 1.7 women; milk 7.5% men, 8.0 women; cheese 1.4% men, 1.3% women; dairy

(not milk or cheese) 2.7% men, 3.7 women; eggs 0.7% men, 0.6% women; potatoes 8.5% men, 7.7% women; vegetables and pulses 6.3% men, 6.9% women; fruit 5.3% men, 8.1% women; nuts 1.6% men 1.5% women; fish 3.3% men, 3.4% women; meat 7.7% men, 7.1% women; hot beverages 10.1% men, 11.4% women; soft drinks 0.2% men, 0.2% women; juice 1.1% men 1.5% women; beer 4.4% men, 0.5% women; alcoholic drinks (not beer) 2.0% men, 2.0 women; soups and sauces 1.4% men, 1.5 women; snacks 2.1% men, 2.2% women; miscellaneous 0.2% men, 0.2% women.

Supplemental Table 1 – Adjusted skeletal muscle measures for individuals of the EPIC-Norfolk cohort stratified by sex and quintiles of dietary magnesium intake (n=14,340).

	Dietary magnesium intake												
	Total			**Quintile 1**		**Quintile 2**		**Quintile 3**		**Quintile 4**		**Quintile 5**		
	Mean	*SE*		*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	**P trend**
Men	**n=6350**			**n=1270**		**n=1270**		**n=1270**		**n=1270**		**n=1270**		
FFM (kg)	61.64	0.04		60.88	0.11	61.52***	0.10	61.82***	0.09	61.90***	0.10	62.10***	0.11	<0.001
FFM%	76.72	0.06		75.94	0.14	76.36*	0.13	76.56**	0.12	76.92***	0.13	77.80***	0.14	<0.001
FFM_{BMI}	2.33	0.003		2.27	0.008	2.31***	0.007	2.32***	0.007	2.34***	0.007	2.38***	0.008	<0.001
Women	**n=7990**			**n=1598**		**n=1598**		**n=1598**		**n=1598**		**n=1598**		
FFM (kg)	40.61	0.04		40.01	0.11	40.45***	0.09	40.64***	0.09	40.78***	0.10	41.19***	0.11	<0.001
FFM%	60.91	0.07		59.90	0.19	60.46*	0.16	60.58**	0.16	61.15***	0.16	62.45***	0.18	<0.001
FFM_{BMI}	1.58	0.003		1.54	0.007	1.57***	0.006	1.57***	0.006	1.59***	0.006	1.64***	0.007	<0.001

* $p<0.05$; ** $p<0.01$; *** $p<0.001$ *versus* quintile 1.

Adjusted for: age, menopausal status, HRT status, corticosteroid use, statin use, smoking status, physical activity, social class, total energy intake, percentage of total energy from protein, and EI:EER.

Mg intake (mean ± SD; mg/day) by Mg quintiles (Q). *Men:* Mean, 332 ± 90; Q1, 224 ± 31; Q2, 282 ± 12; Q3, 322 ± 11; Q4, 367 ± 16; Q5, 467 ± 72. Wo*men:* Mean, 275 ± 73; Q1, 186 ± 25; Q2, 235 ± 10; Q3, 268 ± 10; Q4, 304 ± 13; Q5, 383 ± 73.

Supplemental Table 2 – Adjusted skeletal muscle measures for individuals of the EPIC-Norfolk cohort stratified by sex and serum concentration

groups (n=10,611).

	Serum magnesium concentration group											
	Total		**Group 1**		**Group 2**		**Group 3**		**Group 4**		**Group 5**	
	Mean	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Men	**n=4628**		**n=480**		**n=1128**		**n=2242**		**n=710**		**n=68**	
FFM (kg)	61.67	0.09	61.43	0.26	61.64	0.17	61.84	0.12	61.27	0.22	61.52	0.70
FFM%	76.76	0.08	77.18	0.26	76.83	0.17	76.61	0.12	76.89	0.21	76.17	0.69
FFM_{BMI}	2.33	0.004	2.33	0.011	2.33	0.007	2.33	0.005	2.32	0.009	2.34	0.030
Women	**n=5983**		**n=845**		**n=1694**		**n=2721**		**n=661**		**n=62**	
FFM (kg)	40.64	0.06	40.62	0.15	40.77	0.11	40.62	0.09	40.38	0.17	40.32	0.57
FFM%	61.09	0.10	60.99	0.28	61.04	0.20	61.17	0.15	61.05	0.31	60.42	1.02
FFM_{BMI}	1.59	0.003	1.58	0.009	1.59	0.006	1.59	0.005	1.59	0.010	1.58	0.032

Adjusted for: age, menopausal status, HRT status, corticosteroid use, statin use, smoking status, physical activity, and social class.

Serum Mg concentration groups: <0.7 mmol/L (group 1), 0.7-0.8 mmol/L (group 2), 0.8-0.9 mmol/L (group 3), 0.9-1.0 mmol/L (group 4), and >1.0

mmol/L (group 5).