Dietary magnesium and potassium intakes and circulating magnesium are associated with heel bone ultrasound attenuation and osteoporotic fracture risk in the EPIC-Norfolk cohort study.

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Abbreviations: BMD (Bone Mineral Density); BUA (Broadband Ultrasound Attenuation); CUBA (Contact Ultrasound Bone Analyser); DINER (Data Into Nutrients for Epidemiological Research); DXA (Dual X-ray Absorptiometry); EPIC (European Prospective Investigation into Cancer); FFQ (Food-Frequency Questionnaire); HLQ (Health and Lifestyle Questionnaire); HRT (Hormone Replacement Therapy); RNI (Reference Nutrient Intake).

ABSTRACT

Background: In our ageing population, maintenance of bone health is critical to reduce the risk of osteoporosis and potentially debilitating consequences of fractures in older individuals. Amongst modifiable lifestyle and dietary factors, dietary magnesium and potassium intake are postulated to influence bone quality and osteoporosis, principally via calcium-dependent alteration of bone structure and turnover.

Objective: To investigate the influence of dietary magnesium and potassium intakes, and circulating magnesium, on bone density status and fracture risk in a UK adult population.

Design: A random subset of 4000 individuals from the EPIC-Norfolk cohort of 25,639 men and women with baseline data was used for bone density cross-sectional analyses, and combined with fracture cases (n=1502) for fracture case-cohort longitudinal analyses (mean follow-up 13.4 years). Relevant biological, lifestyle, and dietary covariates were used in multivariate regression analyses to determine associations between dietary magnesium and potassium intakes and calcaneal broadband ultrasound attenuation (BUA), and in Prentice-weighted Cox regression to determine associated risk of fracture. Separate analyses, excluding dietary covariates, investigated associations of BUA and fractures with serum magnesium concentration.

Results: Significant positive trends in calcaneal BUA for women (n=1360), but not men (n=968), were apparent across increasing quintiles of Mg+K z-score intake (p=0.03), or potassium intake alone (p=0.04). Reduced hip fracture risk in both men (n=1958) and women (n=2755) was evident for individuals in specific Mg+K z-score intake quintiles *versus* the lowest. Significant trends in fracture risk in men across serum magnesium concentration groups were apparent for spine fractures (p=0.02), and total hip, spine, and wrist fractures (p=0.02). None of these individual significant associations remained after adjusting for multiple-testing.

Conclusions: These findings enhance the limited literature studying the association of magnesium and potassium with bone density and demonstrate that further investigation is warranted into the mechanisms involved and the potential protective role against osteoporosis.

INTRODUCTION

A multitude of factors are known to influence bone health, including modifiable factors such as diet, physical activity, and smoking, but also age, sex, and genetics (1). Osteoporosis, characterised by bone loss due to deterioration of bone microarchitecture and consequent increased risk of fracture, is significantly associated with age and thus represents a major public health concern for our ageing population (2). Calcium and vitamin D have traditionally been the primary nutritional candidates for osteoporosis prevention and maintenance of bone health (3), but more recently magnesium intake has also been linked with bone mass, and magnesium deficiency with osteoporosis (4-8). Magnesium is a major component of bone, with 67% of total body magnesium found there (9). Animal studies have suggested a number of mechanisms for involvement of magnesium in bone metabolism including: nitric oxide dependent effects on osteoblast activity and osteoclast number (10); influence of magnesium on hydroxyapatite crystal formation and consequent bone stiffness (11); regulation of calcium homeostasis through parathyroid hormone, 1,25-dihydroxyvitamin D, and magnesium-dependent calcium channels (9); and altered inflammatory cytokine release (12). Similarly, recent epidemiological studies have associated lower dietary potassium intake with poorer bone density (5, 6, 8, 13). Increasing potassium intake increases urinary retention, reducing loss of calcium and thus creating a more positive calcium balance and inhibiting bone resorption; urinary loss of phosphorus also decreases, which inhibits renal synthesis of 1,25-dihydroxyvitamin D and cuts intestinal absorption of calcium, stopping the positive calcium balance persisting (14). Occurrence of this stabilisation has recently been disputed, although potassium source differences may be the cause of the discrepancy between studies (15).

When considering the dietary association of magnesium and potassium with bone health it is most appropriate to study these minerals concurrently as they are frequently consumed

54 together from intact or moderately altered plant or animal tissues (16). Metabolism of
55 magnesium and potassium are linked as magnesium is required for effective Na^+/K^+-ATPase
56 pump function (17), magnesium and potassium have additive effects in preventing increase in
57 the endogenous sodium potassium pump inhibitor (16), and both have direct and indirect
58 effects on calcium homeostasis (9, 18). Previous studies of the association of dietary
59 magnesium and potassium with bone health have had limited generalisability due to their
60 focus on discrete population groups, such as narrow age-range groups of relatively old (5, 19)
61 or young individuals (20), restrictions to pre- (8, 20) or post-menopausal women only (13),
62 and non-UK residents (5, 7, 20). Indeed, the most recent and comprehensive study, with a
63 large cohort size and longitudinal analysis of fracture risk, was also limited to women only (7).
64 The current study therefore aimed to explore potential associations of dietary magnesium and
65 potassium intakes and circulating magnesium with bone density status and risk of incident
66 osteoporotic fractures in a general population of men and women in the UK, using a measure
67 of broadband ultrasound attenuation of the calcaneus and records of incident fractures of the
68 hip, spine, and wrist.

SUBJECTS AND METHODS

The EPIC-Norfolk cohort analysed in this study is part of the European Prospective Investigation into Cancer (EPIC), a global collaboration involving ten countries developed primarily to examine association between diet and cancer, with additional health outcomes also examined in EPIC-Norfolk. This cohort has been described in detail previously (21), but in brief the Norfolk cohort consisted of 25,639 men and women aged 40-79 years old living in the general community who participated in a baseline health-check between 1993 and 1997. A second health-check was attended by 15,786 participants, aged 42-82 years between 1997 and 2000, when quantitative ultrasound measurements of the calcaneus (heel bone) were performed according to standardised protocols using a CUBA (contact ultrasound bone analyser) device (McCue Ultrasonics, Winchester, United Kingdom). Quantitative ultrasound represents a cheaper, more rapid, and easier method of assessing bone density status in general practice compared to the gold-standard of Dual X-ray absorptiometry (DXA), and has been shown capable of predicting fracture risk (22). Measurements of broadband ultrasound attenuation (BUA; dB/MHz) from each foot were taken at least in duplicate and the mean of both feet was recorded, as described previously (22).

The dataset analysed here includes 4000 randomly selected participants with baseline health-check data, plus a group of 1502 participants with fractures, representing all hip, spine, and wrist fracture cases in the cohort up to 31st March 2009. Some overlap exists between the random subcohort and the fracture cases and thus the fracture case-cohort contains 5319 unique individuals (4713 participants had complete data for diet and fracture analyses; 3469 for serum and fracture analyses). Ultrasound data was available for 2341 individuals (2328 participants had complete data for diet and ultrasound analyses; 1726 for serum and ultrasound analyses).

The Norfolk District Health Authority Ethics Committee approved all procedures involving human subjects and written informed consent was provided by all participants according to the Declaration of Helsinki.

Height and weight were measured according to standard protocols (21) at both health checks, conducted either at a clinic or the participant's GP surgery. Height was determined to the nearest millimetre using a free-standing stadiometer. Weight was recorded to the nearest 0.2 kilograms with the participant wearing light clothing and no shoes. BMI was calculated from these measurements (kg/m^2).

Participants also completed a self-administered health and lifestyle questionnaire (HLQ) at both health checks. This included smoking status categorised as *current*, *former* or *never*; family history of osteoporosis categorised as *yes* or *no*; menopausal status (women only) categorised as *pre-menopausal*, *peri-menopausal (<1 year)*, *peri-menopausal (1-5 years)*, or *post-menopausal*; and HRT status (women only) categorised as *current*, *former*, or *never* users. A short physical activity questionnaire was used to assess typical physical activity over the previous 12 months. Physical activity levels were then categorised into *inactive*, *moderately inactive*, *moderately active*, and *active* categories by a method validated against heart-rate monitoring data (21, 23).

Dietary intake of each participant was assessed by using a 7 day food diary (24), with each participant recording all food and drink consumed within a 7 day period, as well as the portion sizes. This method has previously been validated, proving more accurate in estimating dietary nutrient intake than food-frequency questionnaires (FFQ) (21, 25). Detail of the

DINER (Data Into Nutrients for Epidemiological Research) software used to record and translate the dietary information provided by the 7-day food diaries into nutrient quantities is reported elsewhere (26). All data entries were checked by nutritionists trained in use of the system (27).

Serum magnesium concentration was determined using blood sampled by peripheral venepuncture during the baseline health check. Samples were stored in liquid nitrogen at -196ºC until analysed by Quotient Bioresearch, Fordham, UK, using an Olympus AU640 Chemistry Immuno Analyser to perform a xylidyl blue based colorimetric assay.

Fracture incidence data were collected by questionnaire at baseline and follow-up health checks. In addition the East Norfolk Health Authority database (ENCORE), which records all hospital contact Norfolk residents have in England and Wales, was available to EPIC researchers for data linkage (28). This enabled the incidence of osteoporotic fractures occurring in the cohort, up to the end of March 2009, to be determined by retrieving data using the NHS numbers of EPIC participants and the International Classification of Diseases (ICD) 9 and 10 diagnostic codes for osteoporotic fractures by site (hip, spine, and wrist).

Statistical analyses

Statistical analyses were performed using STATA statistical software (version 12; Stata Corp., College Station, Tx). All analyses were stratified by sex since significant differences in age-related changes in bone between men and women have previously been reported for this population, with a much greater magnitude of deterioration evident in women (22). Hypotheses and covariates included in regression models were well defined *a priori* using evidence from previous research and thus p-values ≤ 0.05 were considered to be statistically

144 significant in individual analyses. The individual hypotheses tested in this study have been
145 grouped into families of tests (**Supplemental Table 1**), allowing the significance of
146 individual p values to be determined in comparison to a Bonferroni-generated family-wise
147 critical p value.
148
149 Due to the high degree of collinearity between magnesium and potassium dietary intakes
150 (Pearson r=0.84 and r=0.82 in men and women, both $p<0.001$) and thus the potential for
151 statistical issues, and any independent effects to be diminished, a summation of magnesium
152 and potassium intake was used as the main exposure; however, since the amounts of each
153 mineral consumed varies widely, both minerals were standardised before summation,
154 resulting in a Mg+K z-score intake variable (5).
155
156 Univariate linear regression was used to estimate the association of selected biological,
157 lifestyle and dietary factors with sex-specific quintiles of dietary magnesium, potassium, or
158 Mg+K z-score intake. Multivariable regression with ANCOVA was used to investigate
159 differences in calcaneal BUA across sex-specific quintiles of dietary magnesium, potassium,
160 or Mg+K z-score intake. An adjusted model was tested, correcting for the potential effects of
161 biological (age, BMI, family history of osteoporosis, menopausal status, HRT status,
162 corticosteroid use), lifestyle (smoking status, and physical activity) and dietary factors
163 (calcium intake (29, 30), total energy intake (31), and calcium and vitamin D supplement use,
164 previously shown to influence bone ultrasound measurements in this population (22, 32).
165 Participants were excluded from analyses if they had missing values for any variables
166 included in the multivariate model (n=1672, 41.8%). In a similar way, differences in calcaneal
167 BUA across sex-specific groups of serum magnesium concentration were investigated using
168 the same covariates, but excluding dietary factors in the adjusted model. Published guidance

suggests 0.7-1.0 mmol/L should be used as a normal reference range (33). Concentration groups were categorised as <0.7 mmol/L (group 1, deficient), 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, excess).

Prentice-weighted Cox regression was used to investigate associations between incidence of fractures and sex-specific quintiles of dietary magnesium, potassium, or Mg+K z-score intake. An unadjusted model without covariates was tested followed by a model adjusting for the aforementioned variables. The full case-cohort dataset described above, including the random subset and all fracture cases, was used for these analyses. Participants were excluded from analyses if they had missing values for any variables included in the adjusted model. For analysis of specific-site fracture risk (hip, spine, or wrist) other fracture data were excluded from the analysis unless contained in the subcohort, in order to retain a distinct control group. Total risk of hip spine or wrist fracture was calculated as the risk of an individual having one of these types of fracture. This total does not include multiple fractures and therefore the specific-site fracture incidences described may not sum to the total. The association between incidence of fractures and sex-specific groups of serum magnesium concentration was investigated, using the same covariates, but excluding dietary factors in the adjusted model.

RESULTS

Descriptive statistics

Selected characteristics are summarised in **Table 1**, for men and women, as mean values ± SD, or frequency and percentage for categorical variables. There were 968 men and 1360 women in the ultrasound cohort population with information for all selected variables; in the fracture case-cohort there were data for 1958 men and 2755 women. The mean age was 63.0 ± 9.3 for men and 61.7 ± 9.2 for women in the ultrasound cohort; in the fracture case-cohort mean age at baseline was 59.7 ± 9.6 for men and 59.8 ± 9.5 for women. Mean BMI was 26.9 ± 3.4 kg/m^2 for men and 26.5 ± 4.4 kg/m^2 for women in the ultrasound cohort; in the fracture case-cohort mean BMI at baseline was 26.5 ± 3.3 kg/m^2 for men and 26.2 ± 4.3 kg/m^2 for women. Mean total daily energy intake was 2263 ± 478 kcal for men and 1732 ± 374 kcal for women in the ultrasound cohort; in the fracture case-cohort mean intake at baseline was 2239 ± 514 kcal for men and 1683 ± 385 kcal for women. Mean magnesium intake was 329 ± 92 mg/day for men and 277 ± 72 mg/day for women in the ultrasound cohort; in the fracture case-cohort mean intake at baseline was 321 ± 92 mg/day for men and 265 ± 73 mg/day for women; these values are slightly higher than the UK Reference Nutrient Intake (RNI) of 300 mg and 270 mg (34), respectively. Mean calcium intake was 925 ± 282 mg/day for men and 782 ± 247 mg/day for women in the ultrasound cohort; in the fracture case-cohort mean intake at baseline was 914 ± 296 mg/day for men and 762 ± 253 mg/day for women; these values are also higher than the UK RNI of 700 mg for all adults over 19 years old (34). Calcium supplements were used by 1.5% of men and 7.2% of women in the ultrasound cohort, and by 1.3% of men and 5.6% of women in the fracture case-cohort. Mean potassium intake was 3525 ± 803 mg/day for men and 3070 ± 662 mg/day for women in the ultrasound cohort; in the fracture case-cohort mean intake at baseline was 3445 ± 815 mg/day for men and 2969 ± 690 mg/day for women. Potassium intake for women in this cohort is therefore lower than the

UK RNI of 3500 mg for all adults over 18 years old (34). Mean serum magnesium concentration was 0.81 ± 0.12 mmol/L for men (n=1006) and 0.79 ± 0.13 mmol/L for women (n=720). Vitamin D supplements were used by 23.6% of men and 34.6% of women in the ultrasound cohort, and by 22.0% of men and 31.8% of women in the fracture case-cohort.

Current smokers represented 7.9% of men and 9.8% of women in the ultrasound cohort and the proportion of never smokers was higher for women than men (58.6% *vs.* 36.6%); in the fracture case-cohort current smokers and never smokers represented 12.2% and 32.6% of men, and 12.5% and 55.2% of women, respectively. There was a broad spread of physical activity levels across the four categories (inactive, moderately inactive, moderately active, or active) for both men and women, although there was a higher proportion of women classified as inactive or moderately inactive than men (59.1 *vs.* 52.8% ultrasound cohort; 64.8 *vs.* 55.5% fracture case-cohort). Family history of osteoporosis in the ultrasound cohort was 3.2% in men and 6.1% in women; in the fracture case-cohort it was 3.0% in men and 5.6% in women. The majority (72.1% ultrasound cohort; 64.1% fracture case-cohort) of women were post-menopausal and 37.5% in ultrasound cohort and 28.9% in the fracture case-cohort were current or former users of hormone replacement therapy (HRT). Current or former users of corticosteroids for 3 months or more accounted for 4.4% of men and 5.2% of women in the ultrasound cohort; in the fracture case-cohort it was 2.6% of men and 3.5% of women.

Associations between dietary magnesium and potassium intake and bone density

Mean calcaneal BUA values stratified by quintiles of dietary magnesium, potassium, or Mg+K z-score intake, are shown in **Figure 1** stratified by sex. Data are presented for the fully adjusted model. In men, no linear trends in fully adjusted BUA were apparent across quintiles of magnesium, potassium or Mg+K z-score intake. In women significant linear trends were

apparent across quintiles of potassium and Mg+K z-score intake, but not magnesium intake alone, for fully adjusted BUA (p=0.04, p=0.03 and p=0.15, respectively). Individual significant differences in fully adjusted BUA were also identified for women between quintile 5 and quintile 1 for Mg+K z-score intake (74.6 ± 16.1 dB/MHz, n=272 *vs.* 70.8 ± 16.3 dB/MHz, n=272; a 5.3% difference; p=0.02), but not potassium (74.0 ± 16.2 dB/MHz, n=272 *vs.* 71.0 ± 16.3 dB/MHz, n=272; a 4.2% difference; p=0.05) or magnesium alone (73.9 ± 15.8 dB/MHz, n=272 *vs.* 71.6 ± 16.2 dB/MHz, n=272; a 3.3% difference; p=0.11) (see Figure 1). No p values were below the Bonferroni-adjusted family-wise critical value (Supplementary Table 1).

Associations between serum magnesium groups and bone density

Analysis of bone density measures according to serum magnesium concentration groups, adjusting for all covariates previously described, with the exception of dietary factors, showed no significant differences in BUA in either men or women (see Figure 1 and Supplemental Table 1). Furthermore, no correlation was apparent between dietary magnesium intake and serum magnesium concentration for either men (r=0.01, p=0.87, n=717) or women (r=-0.04, p=0.25, n=1006).

Associations between dietary magnesium and potassium intake and fracture risk

Between baseline and follow-up, the percentage of men with one or more hip, spine, or wrist fractures was 23.4% lower in quintile 5 *versus* quintile 1 for magnesium intake quintiles, 18.1% for potassium quintiles, and 10.2% for Mg+K z-score quintiles. In women these figures were 35.9%, 32.1% and 30.8%. Risk of hip fracture in men was significantly lower in Mg+K z-score quintiles 2 and 5 than quintile 1 in the fully adjusted model (p=0.03 and p=0.02) (**Figure 2** and **Supplemental Table 2**). The lowest risk of hip fracture in men was

evident in Mg+K z-score quintile 5 (0.35 (95% CI: 0.14, 0.85)). In women, a significantly reduced risk of hip fracture was evident in Mg+K z-score quintile 4 *versus* quintile 1 in the fully adjusted model (0.59 (95% CI: 0.36, 0.97), p=0.04). A reduced risk of spinal fracture in women was evident for dietary magnesium quintile 3 *versus* quintile 1 (0.49 (95% CI: 0.25, 0.97), p=0.04) (Figure 2 and **Supplemental Table 3**), but not Mg+K z-score or potassium quintiles (Figure 2, Supplementary Table 2 and **Supplemental Table 4**). No p values were below the Bonferroni-adjusted family-wise critical value (Supplementary Table 1).

Analysis of risk of fracture according to concentration groups of serum magnesium showed a number of significant associations (Figure 2 and **Supplemental Table 5**). In men there were significant trends in fracture risk across serum concentration groups for spine fractures (p=0.02), and total hip, spine, and wrist fractures (p=0.02), but not for hip (p=0.06) or wrist fractures alone (p=0.38). Hip fracture risk was significantly lower in groups 2 (p=0.03) and 3 (p<0.01) than group 1 in the fully adjusted model, with the lowest risk in group 3 (0.34 (95% CI: 0.17, 0.70)). Spinal fracture risk was significantly lower (0.20 (95% CI: 0.05, 0.75), p=0.02) in group 4 than group 1; total risk of hip, spine, and wrist fractures was significantly lower in groups 2 (p=0.03), 3 (p=0.03), and 4 (p<0.01) than group 1, with the lowest risk in group 4 (0.41 (95% CI: 0.22, 0.77)). In women there were no significant trends for fracture risk across groups of magnesium serum concentration, nor between specific groups compared to group 1. No p values were below the Bonferroni-adjusted family-wise critical value (Supplementary Table 1).

DISCUSSION

This study has shown significant associations between combined dietary magnesium and potassium intake and a quantitative measure of bone density, with significantly higher calcaneal BUA evident in women in the highest *versus* lowest Mg+K z-score intake quintiles of these micronutrients, after adjustment for important biological, lifestyle and other dietary covariates. Furthermore, risk of hip fracture in both women and men was significantly reduced in specific higher Mg+K intake quintiles compared to the lowest. We believe this study is also the first to show lower total risk of hip, spine, or wrist fracture for men with a clinically normal serum magnesium concentration compared to those classed as deficient. However, while each of the described associations was significant individually, no significant associations were evident after adjusting for multiple-testing.

The mechanisms by which magnesium and potassium may influence bone metabolism are not fully understood, although a number of theories have been proposed. Insufficient magnesium results in an increased rate of hydroxyapatite formation, resulting in larger crystals and thus lower bone mass and brittle bones which may be unable to support normal loads. Magnesium also has an effect on osteoblast activity and osteoclast number through a nitric oxide dependent mechanism (10), and both magnesium and potassium affect bone metabolism through altered calcium homeostasis via influences on calcium transport and urinary retention (9, 10, 14). A number of other studies investigating associations between magnesium and potassium and bone health, either individually or in combination, have demonstrated some degree of improvement with higher intake (4-7, 13, 19, 20), and thus the results presented here largely corroborate these findings. However, a recent USA study (7) of post-menopausal women found no difference in relative risk of hip and total fractures across quintiles of magnesium intake. Conversely, high magnesium intake (≥422.5 mg/day) was associated with

307 increased falls and wrist or lower-arm fractures (7). By contrast, our analyses show significant
308 reduction in hip fracture risk with moderately high (206-442 mg/day; quintile 4) combined
309 magnesium and potassium intakes, and no significant increases in risk of wrist fracture in
310 either men or women in fully adjusted models, although it is acknowledged that the 95%
311 confidence intervals for wrist fracture risk are wide. Differences between the population
312 groups in the two studies with respect to genetics, demographic lifestyle, the range of
313 magnesium intakes, and dietary analysis methods (Orchard *et al* (7) used FFQs) may explain
314 the discrepancy (27, 35). Also the Orchard study (7) did not present their results adjusted for
315 potassium and energy, although they stated that potassium did not modify the associations
316 between magnesium and fracture risk.
317
318 The magnitude of the differences seen here is similar to data published by other authors. For
319 example, fully adjusted BUA was 5.3% greater (+3.8 dB/MHz) in Mg+K z-score quintile 5
320 *versus* quintile 1 for women. This compares to 3.5% and 3.8% increases in lumbar spine
321 BMD for premenopausal women quartile 4 *versus* quartile 1 of dietary magnesium and
322 potassium intakes, respectively (8). Also similar are results from Ryder *et al* (19) and Orchard
323 *et al* (7) showing whole body BMD was 4.0% greater and 3.0% greater, respectively, for
324 women in magnesium quintile 5 *versus* quintile 1. Tucker *et al* (5) show larger differences in
325 BMD across quartiles of combined magnesium and potassium: quartile 4 *versus* quartile 1 for
326 women had 12.8% greater lumbar spine BMD, although the relatively old age and limited
327 number in this group (562 women, 69-97 years old) could explain the greater differences seen.
328 In terms of the implications of the magnitude of change seen in the current study, previous
329 published data for this cohort showed a 5 dB/MHz greater BUA was associated with HRT use,
330 and that a 20 dB/MHz decline in BUA approximately doubled fracture risk (36), thus
331 demonstrating the relevance of our observations.

Our findings showed no correlation between dietary magnesium intake and serum magnesium concentration for either men or women. Although supplementation studies with magnesium have demonstrated that serum is a suitable biomarker for diet, other studies like ours found no relationship between dietary and serum magnesium; this is likely a reflection of the tight homeostatic control of this cation in the circulation (37-39). However, while serum magnesium concentration was not associated with calcaneal BUA, nor risk of hip, spine, or wrist fracture in women, a number of significant associations with fracture risk were evident in men, with those in the healthy normal clinical range, 0.7-1.0 mmol/L (33), showing significantly reduced risk compared to those with sub-optimal concentrations.

Strengths and Limitations

In the UK, dietary intake of magnesium is mainly provided by fruit and vegetables, cereals, and beverages; potassium is provided by dietary fruit and vegetables, meat, potato, and savoury snacks (40). Accurate estimation of dietary nutrient intake is critical to the findings of this type of study. The methodology used here of quantitative 7-day food diaries has been validated previously and is expected to have provided more precise dietary intake figures compared to FFQs or 24-hour recall methods (27). Indeed previous UK EPIC analyses have shown correlations between potassium intake estimated from food diary data and 24 hour potassium excretion were significantly greater than for FFQ or 24-hour recall (41). It is reasonable to assume that this validity would also translate to magnesium. The strong collinearity between dietary intake of magnesium and potassium, a likely consequence of magnesium rich food typically also being rich in potassium, makes it difficult to differentiate individual effects of these nutrients on bone density. Other studies have considered this to varying degrees, but an appropriate compromise is achieved by presenting data using

357 standardised magnesium and potassium intakes which have been combined and re-
358 standardised (5), thus the inclusion of this data analysis is a strength of this work. Previous
359 use of this methodology was confined to analyses of BMD measures alone (5), making our
360 additional longitudinal analysis of fracture risk valuable. Hospital admission data was used to
361 determine fracture incidence and it is acknowledged this may underestimate incidence,
362 particularly for spine fractures, and could differ between sexes. We used a subset of the EPIC-
363 Norfolk dietary data and, in order to reduce the potential for bias, included randomly selected
364 participants from the cohort. Magnesium and potassium dietary data were derived from food
365 intake only, and therefore may underestimate total nutrient intakes, although supplements
366 consumed by this cohort provide a relatively small contribution to mineral intakes (42); we
367 included calcium and vitamin D supplement use in our models nevertheless. We acknowledge
368 that mineral contributions of drinking and bottled water may be imprecise due to varying
369 concentrations not detailed sufficiently in food composition tables. Although this
370 observational study cannot show causality in effects, this report is, to our knowledge, the first
371 to provide analysis of bone quality and fracture risk by magnesium serum concentration
372 groups in addition to dietary intake in a general population of both men and women.
373
374 **Conclusions**
375 This study has positively associated dietary magnesium and potassium intake with a
376 quantitative ultrasound measure of bone density status and reduced fracture risk in a mixed
377 UK population group of men and pre- and post-menopausal women. These results thus
378 support policies to promote a good quality diet with sufficient magnesium and potassium
379 intake. Clinically normal serum magnesium concentration, compared to suboptimal
380 concentration, has also been shown to be associated with reduced risk of incident fracture in
381 men. Further study will be required to determine how generalisable the results of these

analyses are, and to fully understand the relationship between intake of these micronutrients, bone health, and osteoporosis.

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References

1. Martini LA. Magnesium supplementation and bone turnover. Nutr Rev 1999;57(7):227-9.
2. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet 2011;377(9773):1276-87. doi: 10.1016/S0140-6736(10)62349-5.
3. Gennari C. Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutr 2001;4(2B):547-59.
4. Rondanelli M, Opizzi A, Perna S, Faliva MA. Update on nutrients involved in maintaining healthy bone. Endocrinol Nutr 2013;60(4):197-210. doi: 10.1016/j.endonu.2012.09.006.
5. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel DP. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 1999;69(4):727-36.
6. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 2000;71(1):142-51.
7. Orchard TS, Larson JC, Alghothani N, Bout-Tabaku S, Cauley JA, Chen Z, Lacroix AZ, Wactawski-Wende J, Jackson RD. Magnesium intake, bone mineral density, and fractures: results from the Women's Health Initiative Observational Study. Am J Clin Nutr 2014;99(4):926-33. doi: 10.3945/ajcn.113.067488.
8. New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 1997;65(6):1831-9.

9. Wu L, Luthringer BJ, Feyerabend F, Schilling AF, Willumeit R. Effects of extracellular magnesium on the differentiation and function of human osteoclasts. Acta Biomater 2014;10(6):2843-54. doi: 10.1016/j.actbio.2014.02.010.

10. Castiglioni S, Cazzaniga A, Albisetti W, Maier JA. Magnesium and osteoporosis: current state of knowledge and future research directions. Nutrients 2013;5(8):3022-33. doi: 10.3390/nu5083022.

11. Zheng J, Mao X, Ling J, He Q, Quan J, Jiang H. Association Between Serum Level of Magnesium and Postmenopausal Osteoporosis: A Meta-analysis. Biol Trace Elem Res 2014;159(1-3):8-14. doi: 10.1007/s12011-014-9961-3.

12. Rude RK, Gruber HE, Norton HJ, Wei LY, Frausto A, Kilburn J. Reduction of dietary magnesium by only 50% in the rat disrupts bone and mineral metabolism. Osteoporos Int 2006;17(7):1022-32. doi: 10.1007/s00198-006-0104-3.

13. Farrell VA, Harris M, Lohman TG, Going SB, Thomson CA, Weber JL, Houtkooper LB. Comparison between dietary assessment methods for determining associations between nutrient intakes and bone mineral density in postmenopausal women. J Am Diet Assoc 2009;109(5):899-904. doi: 10.1016/j.jada.2009.02.008.

14. Lemann J, Jr., Pleuss JA, Gray RW. Potassium causes calcium retention in healthy adults. J Nutr 1993;123(9):1623-6.

15. Moseley KF, Weaver CM, Appel L, Sebastian A, Sellmeyer DE. Potassium citrate supplementation results in sustained improvement in calcium balance in older men and women. J Bone Miner Res 2013;28(3):497-504. doi: 10.1002/jbmr.1764.

16. Demigne C, Sabboh H, Remesy C, Meneton P. Protective effects of high dietary potassium: nutritional and metabolic aspects. J Nutr 2004;134(11):2903-6.

17. Gonzalez W, Altieri PI, Alvarado S, Banchs HL, Escobales N, Crespo M, Borges W. Magnesium: the forgotten electrolyte. Bol Asoc Med P R 2013;105(3):17-20.

18. Weaver CM. Potassium and health. Adv Nutr 2013;4(3):368S-77S. doi: 10.3945/an.112.003533.

19. Ryder KM, Shorr RI, Bush AJ, Kritchevsky SB, Harris T, Stone K, Cauley J, Tylavsky FA. Magnesium intake from food and supplements is associated with bone mineral density in healthy older white subjects. J Am Geriatr Soc 2005;53(11):1875-80. doi: 10.1111/j.1532-5415.2005.53561.x.

20. Kim MH, Yeon JY, Choi MK, Bae YJ. Evaluation of magnesium intake and its relation with bone quality in healthy young Korean women. Biol Trace Elem Res 2011;144(1-3):109-17. doi: 10.1007/s12011-011-9044-7.

21. Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, Wareham N. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer 1999;80 Suppl 1:95-103.

22. Welch A, Camus J, Dalzell N, Oakes S, Reeve J, Khaw KT. Broadband ultrasound attenuation (BUA) of the heel bone and its correlates in men and women in the EPIC-Norfolk cohort: a cross-sectional population-based study. Osteoporos Int 2004;15(3):217-25. doi: 10.1007/s00198-003-1410-7.

23. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003;6(4):407-13. doi: 10.1079/PHN2002439.

24. Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, Lubin R, Thurnham DI, Key TJ, Roe L, et al. Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium

and serum vitamin C and carotenoids as biomarkers. Int J Epidemiol 1997;26 Suppl 1:S137-51.

25. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5(6B):1113-24. doi: 10.1079/PHN2002394.

26. Welch AA, McTaggart A, Mulligan AA, Luben R, Walker N, Khaw KT, Day NE, Bingham SA. DINER (Data Into Nutrients for Epidemiological Research) - a new data-entry program for nutritional analysis in the EPIC-Norfolk cohort and the 7-day diary method. Public Health Nutr 2001;4(6):1253-65. doi: S136898000100115X [pii].

27. Lentjes MA, McTaggart A, Mulligan AA, Powell NA, Parry-Smith D, Luben RN, Bhaniani A, Welch AA, Khaw KT. Dietary intake measurement using 7 d diet diaries in British men and women in the European Prospective Investigation into Cancer-Norfolk study: a focus on methodological issues. Br J Nutr 2013:1-11. doi: 10.1017/S0007114513002754.

28. Moayyeri A, Kaptoge S, Dalzell N, Bingham S, Luben RN, Wareham NJ, Reeve J, Khaw KT. Is QUS or DXA better for predicting the 10-year absolute risk of fracture? J Bone Miner Res 2009;24(7):1319-25. doi: 10.1359/jbmr.090212.

29. Flynn A. The role of dietary calcium in bone health. Proc Nutr Soc 2003;62(4):851-8. doi: 10.1079/PNS2003301.

30. Heaney RP. Calcium, dairy products and osteoporosis. J Am Coll Nutr 2000;19(2 Suppl):83S-99S.

31. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65(4 Suppl):1220S-8S; discussion 9S-31S.

32. Jakes RW, Khaw K, Day NE, Bingham S, Welch A, Oakes S, Luben R, Dalzell N, Reeve J, Wareham NJ. Patterns of physical activity and ultrasound attenuation by heel bone among Norfolk cohort of European Prospective Investigation of Cancer (EPIC Norfolk): population based study. BMJ 2001;322(7279):140.

33. Ayuk J, Gittoes NJ. Contemporary view of the clinical relevance of magnesium homeostasis. Ann Clin Biochem 2014;51(Pt 2):179-88. doi: 10.1177/0004563213517628.

34. Department of Health. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects No 41. London: HSMO, 1991.

35. Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R, Oakes S, Khaw KT, Wareham N, Day NE. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. Public Health Nutr 2001;4(3):847-58. doi: S1368980001000817 [pii].

36. Khaw KT, Reeve J, Luben R, Bingham S, Welch A, Wareham N, Oakes S, Day N. Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study. Lancet 2004;363(9404):197-202. doi: 10.1016/S0140-6736(03)15325-1.

37. Gamez C, Artacho R, Ruiz-Lopez MD, Navarro M, Puerta A, Lopez MC. Serum concentration and dietary intake of Mg and Ca in institutionalized elderly people. Sci Total Environ 1997;203(3):245-51.

38. Witkowski M, Hubert J, Mazur A. Methods of assessment of magnesium status in humans: a systematic review. Magnes Res 2011;24(4):163-80. doi: 10.1684/mrh.2011.0292.

39. Jahnen-Dechent W, Ketteler M. Magnesium basics. Clin Kidney J 2012;5(Suppl 1):i3-i14. doi: 10.1093/ndtplus/sfr163.

40. Welch AA, Bingham SA, Reeve J, Khaw KT. More acidic dietary acid-base load is associated with reduced calcaneal broadband ultrasound attenuation in women but not in men: results from the EPIC-Norfolk cohort study. Am J Clin Nutr 2007;85(4):1134-41.

41. Bingham SA, Day NE. Using biochemical markers to assess the validity of prospective dietary assessment methods and the effect of energy adjustment. Am J Clin Nutr 1997;65(4 Suppl):1130S-7S.

42. Lentjes MA, Bhaniani A, Mulligan AA, Khaw KT, Welch AA. Developing a database of vitamin and mineral supplements (ViMiS) for the Norfolk arm of the European Prospective Investigation into Cancer (EPIC-Norfolk). Public Health Nutr 2011;14(3):459-71. doi: 10.1017/S1368980010002867.

TABLES AND FIGURES

Table 1 – Selected characteristics of the EPIC-Norfolk cohort population stratified by sex for the ultrasound cohort group (n=2328) and the fracture case-cohort group (n=4713).

Selected Characteristics	**Ultrasound cohort**[1]			**Fracture case-cohort**[2]		
	Men	**Women**		**Men**	**Women**	
	n=968	**n=1360**	***P***[3]	**n=1958**	**n=2755**	***P***
Age (years)	63.0 ± 9.3[4]	61.7 ± 9.2	*<0.001*	59.7 ± 9.6	59.8 ± 9.5	*0.809*
BMI (kg/m^2)	26.9 ± 3.4	26.5 ± 4.4	*0.039*	26.5 ± 3.3	26.2 ± 4.3	*0.004*
Magnesium intake (mg/day)	329 ± 92	277 ± 72	*<0.001*	321 ± 92	265 ± 73	*<0.001*
Potassium intake (mg/day)	3525 ± 803	3070 ± 662	*<0.001*	3445 ± 815	2969 ± 690	*<0.001*
Calcium intake (mg/day)	925 ± 282	782 ± 247	*<0.001*	914 ± 296	762 ± 253	*<0.001*
Calcium supplement use	14 (1.5)	98 (7.2)	*<0.001*	25 (1.3)	155 (5.6)	*<0.001*
Vitamin D supplement use	228 (23.6)	471 (34.6)	*<0.001*	430 (22.0)	875 (31.8)	*<0.001*
Total energy intake (kcal/day)	2263 ± 478	1732 ± 374	*<0.001*	2239 ± 514	1683 ± 385	*<0.001*
Serum [Mg] (mmol/L)	0.81 ± 0.12[5]	0.79 ± 0.13[6]	*0.003*	0.81 ± 0.12[7]	0.79 ± 0.13[8]	*0.001*
BUA (dB/MHz)	89.6 ± 17.4	72.1 ± 16.5	*<0.001*	--	--	
Smoking			*<0.001*			*<0.001*
Current	76 (7.9)	133 (9.8)		238 (12.2)	343 (12.5)	
Former	538 (55.6)	430 (31.6)		1082 (55.3)	890 (32.3)	
Never	354 (36.6)	797 (58.6)		638 (32.6)	1522 (55.2)	
Physical activity			*<0.001*			*<0.001*
Inactive	275 (28.4)	342 (25.1)		614 (31.4)	908 (33.0)	
Moderately inactive	236 (24.4)	462 (34.0)		472 (24.1)	877 (31.8)	
Moderately active	248 (25.6)	333 (24.5)		436 (22.3)	577 (20.9)	
Active	209 (21.6)	223 (16.4)		436 (22.3)	393 (14.3)	

Family history of osteoporosis			*0.001*		*<0.001*
No	937 (96.8)	1277 (93.9)		1900 (97.0)	2601 (94.4)	
Yes	31 (3.2)	83 (6.1)		58 (3.0)	154 (5.6)	
Corticosteroid use			*0.391*		*0.243*
Current or former (>3 months)	43 (4.4)	71 (5.2)		50 (2.6)	97 (3.5)	
Never (<3 months)	925 (95.6)	1289 (94.8)		1908 (97.5)	2658 (96.5)	
Menopausal status						
Pre-menopausal	--	86 (6.3)		--	414 (15.0)	
Peri-menopausal (<1 y)	--	47 (3.5)		--	127 (4.6)	
Peri-menopausal (1-5 y)	--	246 (18.1)		--	448 (16.3)	
Post-menopausal	--	981 (72.1)		--	1766 (64.1)	
HRT						
Current	--	288 (21.2)		--	472 (17.1)	
Former	--	222 (16.3)		--	324 (11.8)	
Never	--	850 (62.5)		--	1959 (71.1)	

[1]Ultrasound group characteristics at 2nd health-check (time of ultrasound).

[2]Fracture group characteristics at 1st health-check or time of consent.

[3]P values are for differences between men and women for each applicable variable, according to t-test for continuous or chi-square for categorical variables.

[4]Values are mean ± SD or frequency (percentage).

[5]n=720. [6]n=1006. [7]n=1460. [8]n=2009

Figure 1 – Fully adjusted[1] calcaneal Broadband Ultrasound Attenuation (BUA) of the EPIC-Norfolk cohort population (968 men and 1360 women) stratified by sex and quintiles of Magnesium[2] or Potassium[3] dietary intake, z-score quintiles of dietary Magnesium+Potassium[4] intake, or serum Magnesium concentration groups[5] (720 men and 1006 women).

[*] $p \leq 0.05$ *versus* quintile 1, according to ANCOVA (not significant after multiple testing adjustment).

[1]Adjusted for: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, calcium intake and supplement use (excluding serum Mg model), vitamin D supplement use (excluding serum Mg model), and total energy intake (excluding serum Mg model). Values are presented as mean ± SD.

[2]**Mg intake (mean ± SD; mg/day) by Mg quintiles (Q)**. *Men:* mean, 329 ± 32; Q1, 218 ± 31; Q2, 277 ± 12; Q3, 319 ± 13; Q4, 366 ± 16; Q5, 466 ± 73. *Women:* mean, 277 ± 72; Q1, 189 ± 26; Q2, 237 ± 10; Q3, 270 ± 10; Q4, 307 ± 12; Q5, 383 ± 58.

[3]**K intake (mean ± SD; mg/day) by K quintiles**. *Men:* mean, 3525 ± 803; Q1, 2505 ± 344; Q2, 3099 ± 125; Q3, 3478 ± 101; Q4, 3854 ± 122; Q5, 4697 ± 603. *Women:* mean, 3070 ± 662; Q1, 2196 ± 287; Q2, 2721 ± 99; Q3, 3038 ± 90; Q4, 3367 ± 106; Q5, 4030 ± 429.

[4]**Mg intake (mean ± SD; mg/day) by Mg+K z-score quintiles**. *Men:* mean 329 ± 92; Q1, 221 ± 35; Q2, 279 ± 22; Q3, 321 ± 29; Q4, 364 ± 29; Q5, 460 ± 78. *Women:* mean 277 ± 72; Q1, 192 ± 29; Q2, 238 ± 19; Q3, 271 ± 21; Q4, 306 ± 24; Q5, 378 ± 61. **K intake (mean ± SD; mg/day) by Mg+K z-score quintiles**. *Men:* mean 3525 ± 803; Q1, 2539 ± 375; Q2, 3117 ± 218; Q3, 3489 ± 229; Q4, 3857 ± 270; Q5, 4630 ± 668. *Women:* mean 3070 ± 662; Q1, 2217 ± 309; Q2, 2753 ± 177; Q3, 3047 ± 205; Q4, 3351 ± 230; Q5, 3983 ± 479.

[5]**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).

Figure 2 – Risk[1] of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population (1958 men and 2755 women) at follow-up *versus* baseline stratified by sex and quintile of Magnesium[2] or Potassium[3] dietary intake, z-score quintiles of dietary Magnesium+Potassium[4] intake, or serum Magnesium concentration groups[5] (1460 men and 2009 women). (Prentice-weighted Cox proportional hazard ratio and 95% CI of quintiles or groups, quintile or group 1 as reference).

[*] $p \leq 0.05$ *versus* quintile 1, according to ANCOVA; [**] $p \leq 0.01$ (not significant after multiple testing adjustment). Insufficient data was available in the highest serum Mg concentration group for some hazard ratio calculations.

[1]Adjusted for: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, calcium intake and supplement use (excluding serum Mg model), vitamin D supplement use (excluding serum Mg model), and total energy intake (excluding serum Mg model). Values are presented as mean ± SD.

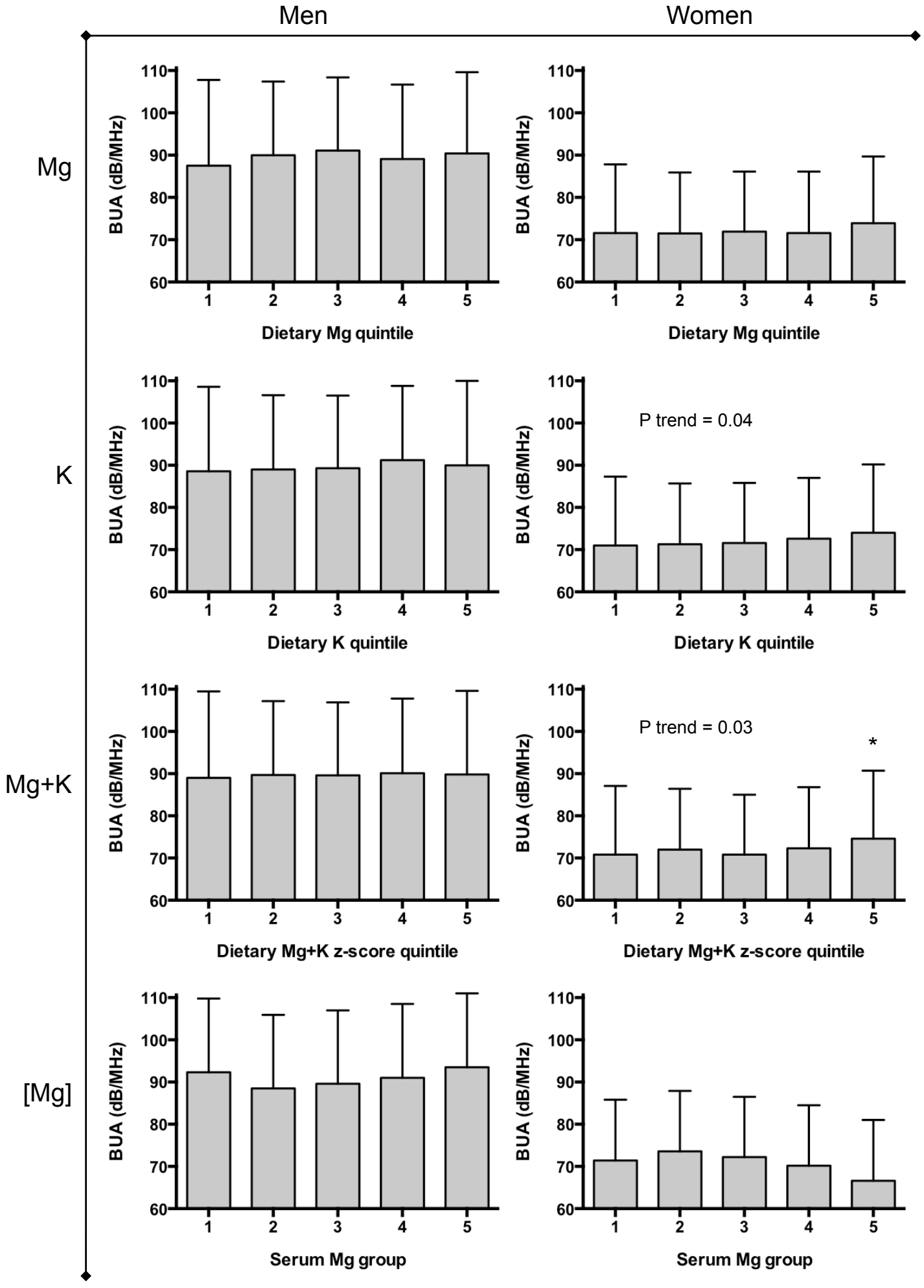
[2]**Mg intake (mean ± SD; mg/day) by Mg quintiles (Q)**. *Men:* mean, 321 ± 93; Q1, 209 ± 31; Q2, 268 ± 12; Q3, 312 ± 13; Q4, 358 ± 15; Q5, 460 ± 75. *Women:* Mean, 265 ± 73; Q1, 175 ± 25; Q2, 223 ± 10; Q3, 257 ± 9; Q4, 294 ± 13; Q5, 373 ± 59.

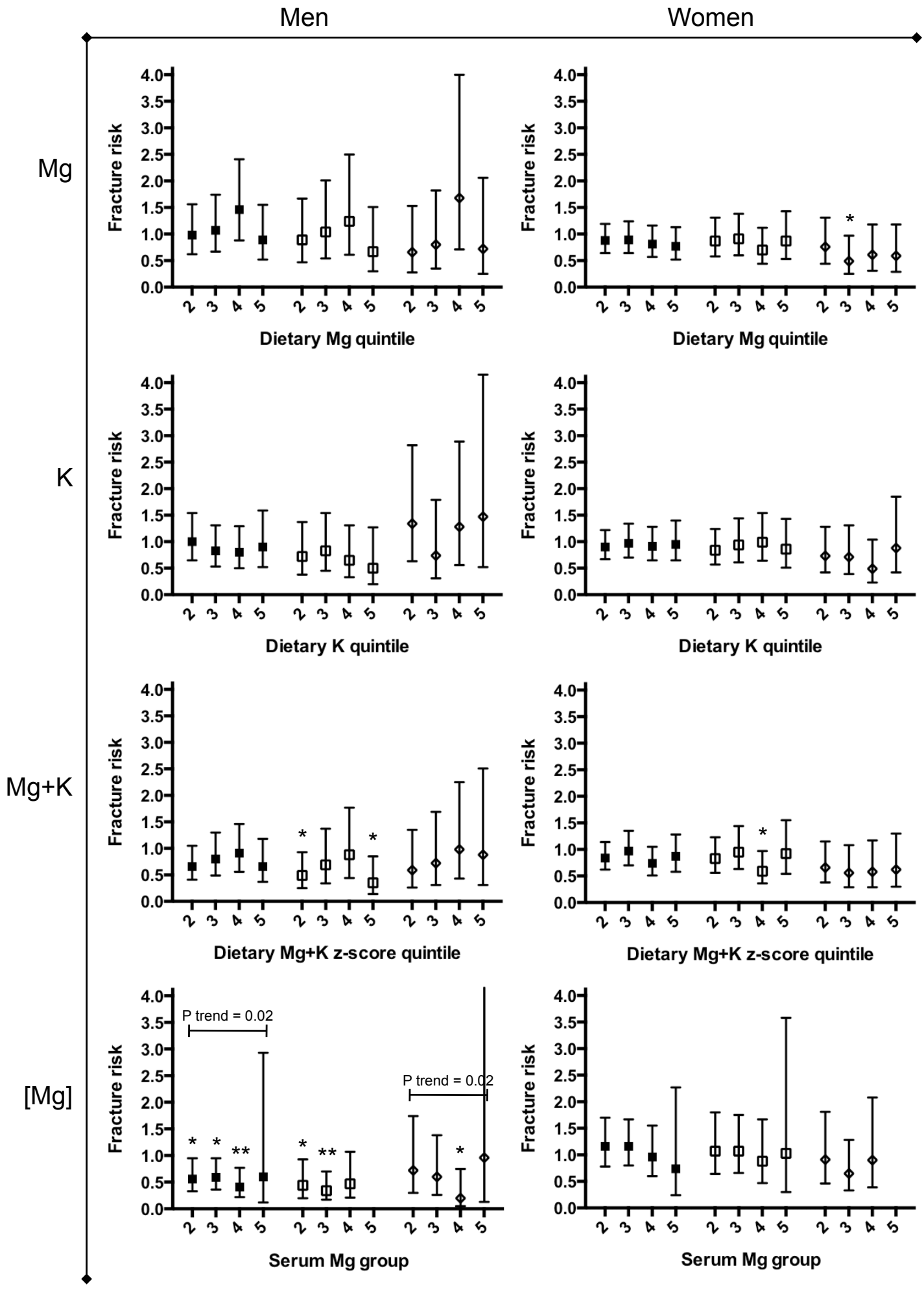
[3]**K intake (mean ± SD; mg/day) by K quintiles**. *Men:* mean, 3449 ± 821; Q1, 2390 ± 356; Q2, 3019 ± 119; Q3, 3405 ± 111; Q4, 3797 ± 126; Q5, 4635 ± 607. *Women:* mean, 2964 ± 689; Q1, 2065 ± 285; Q2, 2595 ± 102; Q3, 2921 ± 92; Q4, 3268 ± 113; Q5, 3974 ± 448.

[4]**Mg intake (mean ± SD; mg/day) by Mg+K z-score quintiles**. *Men:* mean 321 ± 93; Q1, 212 ± 35; Q2, 271 ± 23; Q3, 314 ± 28; Q4, 357 ± 28; Q5, 454 ± 80. *Women:* mean 265 ± 73; Q1, 178 ± 29; Q2, 225 ± 19; Q3, 257 ± 20; Q4, 294 ± 24; Q5, 368 ± 63. **K intake (mean ± SD; mg/day) by Mg+K z-score quintiles**. *Men:* mean 3449 ± 821; Q1, 2422 ± 386; Q2, 3040 ± 212; Q3, 3419 ± 245; Q4, 3788 ± 263; Q5, 4577 ± 663. *Women:* mean 2964 ± 687; Q1, 2087 ± 307; Q2, 2618 ± 183; Q3, 2925 ± 189; Q4, 3257 ± 223; Q5, 3935 ± 490.

[5]**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).


Men
Women
Mg
K
Mg+K
[Mg]
BUA (dB/MHz)
110
100
90
80
70
60
1
2
3
4
5
Dietary Mg quintile
Dietary K quintile
Dietary Mg+K z-score quintile
Serum Mg group
P trend = 0.04
P trend = 0.03
*


Men
Women
Mg
K
Mg+K
[Mg]
Fracture risk
Dietary Mg quintile
Dietary K quintile
Dietary Mg+K z-score quintile
Serum Mg group
P trend = 0.02
P trend = 0.02
Total
Hip
Spine

ONLINE SUPPLEMENTAL MATERIAL

Supplemental Table 1 – Families of tests and their hypotheses included in this study of the EPIC-Norfolk cohort population.

Experiment/family hypothesis		Mg					K					Mg+K					Family-wise critical p
		Trend	Q2 vs. Q1	Q3 vs. Q1	Q4 vs. Q1	Q5 vs. Q1	Trend	Q2 vs. Q1	Q3 vs. Q1	Q4 vs. Q1	Q5 vs. Q1	Trend	Q2 vs. Q1	Q3 vs. Q1	Q4 vs. Q1	Q5 vs. Q1	
Dietary intake of Mg and/or K has no association with BUA in men		0.44	0.19	0.07	0.43	0.21	0.31	0.87	0.72	0.21	0.55	0.70	0.69	0.76	0.57	0.72	P < 0.0033 *(0.05/15)*
Dietary intake of Mg and/or K has no association with BUA in women		0.15	0.95	0.77	0.98	0.11	0.04	0.81	0.63	0.24	0.05	0.03	0.36	0.97	0.29	0.02	P < 0.0033 *(0.05/15)*
Serum Mg concentration has no association with BUA in men		0.97	0.10	0.21	0.61	0.83	--	--	--	--	--	--	--	--	--	--	P < 0.01 *(0.05/5)*
Serum Mg concentration has no association with BUA in women		0.24	0.12	0.54	0.51	0.28	--	--	--	--	--	--	--	--	--	--	P < 0.01 *(0.05/5)*
Dietary intake of Mg and/or K has no association with risk of fracture in men	Total	0.69	0.94	0.77	0.15	0.69	0.46	0.99	0.42	0.37	0.75	0.56	0.08	0.37	0.69	0.16	P < 0.00083 *(0.05/60)*
	Hip	0.73	0.71	0.91	0.55	0.34	0.16	0.32	0.56	0.23	0.14	0.25	0.03	0.29	0.73	0.02	
	Spine	0.60	0.33	0.59	0.24	0.54	0.57	0.45	0.51	0.56	0.47	0.76	0.21	0.45	0.97	0.81	
	Wrist	0.21	0.13	0.10	0.06	0.16	0.43	0.39	0.46	0.46	0.28	0.51	0.34	0.22	0.21	0.42	
Dietary intake of Mg and/or K has no association with risk of fracture in women	Total	0.18	0.40	0.50	0.45	0.18	0.82	0.50	0.85	0.60	0.80	0.36	0.27	0.87	0.09	0.47	P < 0.00083 *(0.05/60)*
	Hip	0.37	0.51	0.65	0.14	0.57	0.84	0.39	0.76	0.98	0.56	0.42	0.35	0.81	0.04	0.75	
	Spine	0.11	0.32	0.04	0.14	0.14	0.45	0.27	0.28	0.06	0.73	0.21	0.14	0.08	0.13	0.21	
	Wrist	0.34	0.47	0.72	1.00	0.14	0.75	0.54	0.99	0.96	0.51	0.77	0.73	0.61	0.95	0.56	
Serum Mg concentration has no association with risk of fracture in men	Total	0.02	0.03	0.03	0.005	0.53	--	--	--	--	--	--	--	--	--	--	P < 0.0025 *(0.05/20)*
	Hip	0.06	0.03	0.003	0.07	--	--	--	--	--	--	--	--	--	--	--	
	Spine	0.02	0.47	0.23	0.02	0.97	--	--	--	--	--	--	--	--	--	--	
	Wrist	0.38	0.08	0.41	0.05	0.96	--	--	--	--	--	--	--	--	--	--	
Serum Mg concentration has no association with risk of fracture in women	Total	0.78	0.46	0.44	0.87	0.60	--	--	--	--	--	--	--	--	--	--	P < 0.0025 *(0.05/20)*
	Hip	0.76	0.79	0.79	0.70	0.96	--	--	--	--	--	--	--	--	--	--	
	Spine	0.22	0.80	0.21	0.80	--	--	--	--	--	--	--	--	--	--	--	
	Wrist	0.18	0.72	0.75	0.17	0.44	--	--	--	--	--	--	--	--	--	--	

ONLINE SUPPLEMENTAL MATERIAL

P values are quoted to 3 decimal places when less than 0.01, otherwise 2 decimal places are used.

Supplemental Table 2 – Risk of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population (1958 men and 2755 women) at follow-up *versus* baseline, stratified by z score quintiles of dietary Magnesium+Potassium intake (Prentice-weighted Cox proportional hazard ratio and 95% CI).

Men		**Dietary Magnesium+Potassium Intake**					
		Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	**P for trend**
Total[1]	*248/1958*	*60/392*	*46/392*	*48/391*	*54/392*	*40/391*	
	Full Model[2]	1.0 (ref)	0.66 (0.41-1.05)	0.80 (0.49-1.30)	0.91 (0.56-1.46)	0.66 (0.37-1.18)	0.56
Hip	*112/1843*	*37/369*	*21/369*	*21/368*	*24/369*	*9/368*	
	Full Model	1.0 (ref)	0.49 (0.25-0.93)*	0.69 (0.34-1.37)	0.88 (0.44-1.77)	0.35 (0.14-0.85)*	0.25
Spine	*78/1809*	*19/362*	*13/362*	*13/362*	*18/362*	*15/361*	
	Full Model	1.0 (ref)	0.59 (0.26-1.35)	0.72 (0.31-1.69)	0.98 (0.43-2.25)	0.88 (0.31-2.51)	0.76
Wrist	*70/1807*	*7/362*	*13/361*	*16/362*	*17/361*	*17/361*	
	Full Model	1.0 (ref)	1.60 (0.61-4.16)	1.79 (0.71-4.50)	1.76 (0.72-4.28)	1.49 (0.57-3.91)	0.51
Women							**P for trend**
Total	*616/2755*	*156/551*	*127/551*	*126/551*	*99/551*	*108/551*	
	Full Model	1.0 (ref)	0.84 (0.62-1.14)	0.97 (0.70-1.35)	0.74 (0.51-1.05)	0.87 (0.58-1.28)	0.36
Hip	*339/2526*	*92/506*	*73/505*	*70/505*	*44/505*	*60/505*	
	Full Model	1.0 (ref)	0.83 (0.56-1.23)	0.95 (0.63-1.44)	0.59 (0.36-0.97)*	0.92 (0.54-1.55)	0.42
Spine	*124/2335*	*38/467*	*26/467*	*19/467*	*19/467*	*22/467*	
	Full Model	1.0 (ref)	0.66 (0.38-1.15)	0.56 (0.29-1.08)	0.58 (0.29-1.17)	0.62 (0.30-1.30)	0.21
Wrist	*218/2410*	*49/482*	*43/482*	*49/482*	*42/482*	*35/482*	
	Full Model	1.0 (ref)	0.92 (0.58-1.46)	1.13 (0.71-1.81)	1.02 (0.61-1.70)	0.85 (0.48-1.48)	0.77

[1] Total risk is for an individual having a hip, spine, or wrist fracture, thus specific fracture incidences may not sum to total.

[2] Full model: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, calcium intake and supplement use, vitamin D supplement use, and total energy intake. * $p \leq 0.05$ *versus* quintile 1 (not significant after multiple testing adjustment).

ONLINE SUPPLEMENTAL MATERIAL

Supplemental Table 3 – Risk of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population (1958 men and 2755 women) at follow-up *versus* baseline, stratified by quintiles of dietary Magnesium intake (Prentice-weighted Cox proportional hazard ratio and 95% CI).

Men		**Dietary Magnesium Intake**					
		Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	**P for trend**
Total[1]	*248/1958*	*51/392*	*49/392*	*51/391*	*58/392*	*39/391*	
	Full Model[2]	1.0 (ref)	0.98 (0.62-1.56)	1.07 (0.67-1.74)	1.46 (0.88-2.41)	0.89 (0.52-1.55)	0.69
Hip	*112/1843*	*31/369*	*24/369*	*24/368*	*21/369*	*12/368*	
	Full Model	1.0 (ref)	0.89 (0.47-1.67)	1.04 (0.54-2.01)	1.24 (0.61-2.50)	0.67 (0.30-1.51)	0.73
Spine	*78/1809*	*17/362*	*12/362*	*14/362*	*24/362*	*11/361*	
	Full Model	1.0 (ref)	0.66 (0.28-1.53)	0.80 (0.35-1.82)	1.68 (0.71-4.00)	0.72 (0.25-2.06)	0.60
Wrist	*70/1807*	*5/362*	*14/361*	*16/362*	*18/361*	*17/361*	
	Full Model	1.0 (ref)	2.34 (0.78-7.06)	2.51 (0.85-7.43)	2.79 (0.94-8.26)	2.26 (0.72-7.03)	0.21
Women							**P for trend**
Total	*616/2755*	*159/551*	*124/551*	*120/551*	*111/551*	*102/551*	
	Full Model	1.0 (ref)	0.88 (0.64-1.19)	0.89 (0.64-1.24)	0.81 (0.57-1.16)	0.77 (0.52-1.13)	0.18
Hip	*339/2526*	*93/506*	*70/505*	*66/505*	*52/505*	*58/505*	
	Full Model	1.0 (ref)	0.87 (0.58-1.31)	0.91 (0.60-1.38)	0.70 (0.44-1.12)	0.87 (0.53-1.43)	0.37
Spine	*124/2335*	*39/467*	*27/467*	*16/467*	*21/467*	*21/467*	
	Full Model	1.0 (ref)	0.76 (0.44-1.31)	0.49 (0.25-0.97)*	0.61 (0.31-1.18)	0.59 (0.29-1.18)	0.11
Wrist	*218/2410*	*53/482*	*42/482*	*43/482*	*48/482*	*32/482*	
	Full Model	1.0 (ref)	0.84 (0.53-1.33)	0.91 (0.56-1.49)	1.00 (0.61-1.64)	0.64 (0.35-1.16)	0.34

[1] Total risk is for an individual having a hip, spine, or wrist fracture, thus specific fracture incidences may not sum to total.

[2] Full model: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, calcium intake and supplement use, vitamin D supplement use, and total energy intake. * $p \leq 0.05$ *versus* quintile 1 (not significant after multiple testing adjustment).

ONLINE SUPPLEMENTAL MATERIAL

Supplemental Table 4 – Risk of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population (1958 men and 2755 women) at follow-up *versus* baseline, stratified by quintiles of dietary Potassium intake (Prentice-weighted Cox proportional hazard ratio and 95% CI).

Men		**Dietary Potassium Intake**					
		Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	**P for trend**
Total[1]	*248/1958*	*55/392*	*56/392*	*48/391*	*44/392*	*45/391*	
	Full Model[2]	1.0 (ref)	1.00 (0.65-1.54)	0.83 (0.53-1.31)	0.80 (0.50-1.29)	0.91 (0.52-1.59)	0.46
Hip	*112/1843*	*34/369*	*24/369*	*26/368*	*18/369*	*10/368*	
	Full Model	1.0 (ref)	0.72 (0.38-1.37)	0.83 (0.45-1.54)	0.65 (0.33-1.31)	0.50 (0.20-1.27)	0.16
Spine	*78/1809*	*15/362*	*19/362*	*11/362*	*17/362*	*16/361*	
	Full Model	1.0 (ref)	1.34 (0.63-2.82)	0.74 (0.31-1.79)	1.28 (0.56-2.89)	1.47 (0.52-4.15)	0.57
Wrist	*70/1807*	*8/362*	*13/361*	*14/362*	*15/361*	*20/361*	
	Full Model	1.0 (ref)	1.49 (0.60-3.67)	1.39 (0.58-3.35)	1.40 (0.57-3.45)	1.66 (0.66-4.20)	0.43
Women							**P for trend**
Total	*616/2755*	*156/551*	*125/551*	*120/551*	*109/551*	*106/551*	
	Full Model	1.0 (ref)	0.90 (0.67-1.22)	0.97 (0.70-1.34)	0.91 (0.65-1.28)	0.95 (0.65-1.40)	0.82
Hip	*339/2526*	*93/506*	*68/505*	*64/505*	*62/505*	*52/505*	
	Full Model	1.0 (ref)	0.84 (0.57-1.24)	0.94 (0.61-1.44)	0.99 (0.64-1.54)	0.86 (0.51-1.43)	0.84
Spine	*124/2335*	*38/467*	*25/467*	*21/467*	*14/467*	*26/467*	
	Full Model	1.0 (ref)	0.73 (0.42-1.28)	0.71 (0.39-1.31)	0.49 (0.23-1.04)	0.88 (0.42-1.85)	0.45
Wrist	*218/2410*	*53/482*	*41/482*	*45/482*	*43/482*	*36/482*	
	Full Model	1.0 (ref)	0.87 (0.56-1.36)	1.00 (0.63-1.59)	0.99 (0.61-1.61)	0.84 (0.49-1.42)	0.75

[1] Total risk is for an individual having a hip, spine, or wrist fracture, thus specific fracture incidences may not sum to total.

[2] Full model: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, calcium intake and supplement use, vitamin D supplement use, and total energy intake.

ONLINE SUPPLEMENTAL MATERIAL

Supplemental Table 5 – Risk of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population (1460 men and 2009 women) at follow-up *versus* baseline, stratified by groups defined by serum Magnesium concentration (Prentice-weighted Cox proportional hazard ratio and 95% CI).

Men		**Serum Magnesium Concentration**					
		Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**P for trend**
Total[1]	*183/1460*	*30/183*	*45/365*	*86/689*	*20/206*	*2/17*	
	Full Model[2]	1.0 (ref)	0.56 (0.33-0.95)*	0.59 (0.36-0.95)*	0.41 (0.22-0.77)**	0.60 (0.12-2.93)	0.02
Hip	*82/1374*	*16/171*	*21/346*	*31/641*	*14/201*	*0/15*	
	Full Model	1.0 (ref)	0.44 (0.20-0.93)*	0.34 (0.17-0.70)**	0.47 (0.21-1.07)	--	0.06
Spine	*56/1348*	*8/164*	*17/340*	*27/635*	*3/193*	*1/16*	
	Full Model	1.0 (ref)	0.72 (0.30-1.74)	0.60 (0.26-1.38)	0.20 (0.05-0.75)*	0.96 (0.13-7.30)	0.02
Wrist	*52/1352*	*9/165*	*9/335*	*29/642*	*4/194*	*1/16*	
	Full Model	1.0 (ref)	0.42 (0.16-1.11)	0.71 (0.32-1.60)	0.30 (0.09-1.01)	1.06 (0.12-9.21)	0.38
Women							**P for trend**
Total	*445/2009*	*53/285*	*131/603*	*209/881*	*47/215*	*5/25*	
	Full Model	1.0 (ref)	1.16 (0.78-1.70)	1.16 (0.80-1.67)	0.96 (0.60-1.55)	0.74 (0.24-2.27)	0.78
Hip	*249/1848*	*27/265*	*69/553*	*121/808*	*28/198*	*4/24*	
	Full Model	1.0 (ref)	1.07 (0.64-1.80)	1.07 (0.66-1.75)	0.88 (0.47-1.67)	1.03 (0.30-3.58)	0.76
Spine	*90/1704*	*14/251*	*29/513*	*34/733*	*13/187*	*0/20*	
	Full Model	1.0 (ref)	0.91 (0.46-1.81)	0.65 (0.33-1.28)	0.90 (0.39-2.08)	--	0.22
Wrist	*218/1757*	*19/254*	*48/528*	*78/767*	*11/187*	*1/21*	
	Full Model	1.0 (ref)	1.11 (0.63-1.94)	1.09 (0.64-1.85)	0.58 (0.27-1.26)	0.44 (0.05-3.53)	0.18

[1] Total risk is for an individual having a hip, spine, or wrist fracture, thus specific fracture incidences may not sum to total.

[2] Full model: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, and corticosteroid use. * $p \leq 0.05$ *versus* quintile 1; ** $p \leq 0.01$ (not significant after multiple testing adjustment).