AT: Article

Dietary acid-base load and its association with risk of osteoporotic fractures and low estimated skeletal muscle mass.

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ABSTRACT

Background/objectives: Age-related decline in skeletal muscle mass and strength, loss of bone density, and increased risk of osteoporotic fractures are important public health issues. Systemic acid-base balance is affected by dietary intake and may be relevant to these conditions. We therefore investigated associations of dietary acid-base load with skeletal muscle mass, bone density status, and fracture risk.

Subjects/methods: We analysed the European Prospective Investigation into Cancer and Nutrition-Norfolk cohort of >25,000 individuals, 39-79 years at baseline. Potential renal acid load (PRAL) was calculated from 7-day food diary data. As a proxy for skeletal muscle mass, we estimated fat free mass from bioelectrical impedance analysis and scaled this for BMI (FFM_{BMI}). Bone density status was assessed by heel-bone broadband ultrasound attenuation (BUA), and fracture rates were obtained from health-care records. Multivariable regression was used to test musculoskeletal outcomes across sex-specific quintiles of PRAL.

Results: PRAL in quintiles was negatively associated with FFM_{BMI} in men (n=6350, $p<0.001$) and women (n=7989, $p<0.001$), with quintile 5 vs 1 differences of -1.5% and -3.2% (both $p<0.001$). PRAL was also negatively associated with BUA in women (n=8312, $p=0.016$; quintile 5 vs 1 difference -1.5%, $p=0.024$). The combined hazard of hip, wrist, and spine fractures (mean±SD follow-up 17.9±4.9 years) was higher with increasing quintiles of PRAL in men (610 fractures; n=11,511; $p=0.013$) and women (1,583 fractures; n=13,927; $p=0.009$), with quintile 5 vs 1 hazard ratios of 1.33 (95% CI: 1.03-1.72, $p=0.029$) and 1.21 (95% CI: 1.03-1.42, $p=0.022$), but associations were not consistent for all fractures sites and age-groups tested.

Conclusions: This study provides strong evidence, albeit observational, for a negative association between PRAL and musculoskeletal health in middle to older age men and women, and thus supports the rationale for a less acidic dietary load.

INTRODUCTION

Sarcopenia, the decline in skeletal muscle mass and function with age[1], and osteoporosis, the loss of bone density and strength through an imbalance of bone resorption and bone formation[2], together present a significant public health concern for our ageing population. Musculoskeletal health is affected by nutrition across the life course; it is well established that there are particular nutrient requirements for attainment of optimal bone and skeletal muscle mass during growth, and the need for optimised nutrition later in life is now also becoming better understood[3]. Most previous research has studied associations between individual components of the diet and musculoskeletal health, but it is likely that the balance of dietary components is also important[4]. Potential Renal Acid Load (PRAL) is a means to quantify acid-base load of the diet as well as the effect of diet on systemic acid-base balance. Fruits and vegetables have a low PRAL and tend to promote systemic alkalinity due to the bicarbonate present, while hepatic oxidation of the sulphur-containing amino acids, cysteine and methionine found in meats, grains, and cheeses, generates hydrogen ions and thus has the opposite effect[5].

Bone is critical to maintaining acid-base balance and provides significant buffering capacity to control pH[6]. Experimental studies have suggested that metabolic acidosis is associated with bone resorption and previous EPIC-Norfolk data analyses showed a detrimental association between more acidic dietary intake, estimated from Food Frequency Questionnaires (FFQs), and bone density, but no effect on fractures was seen[7]. This is also supported by findings of the Aberdeen Prospective Osteoporosis Screening Study which showed lower estimated dietary acid load was associated with greater bone density in women[8], but other studies have shown mixed results[9, 10]. We also know that metabolic acidosis may be detrimental to skeletal muscle by decreasing protein synthesis and increasing

proteolysis and oxidation of amino acids, through actions of the ubiquitin proteasome pathway and insulin-like growth factor-1 signalling[11]. It has been associated with muscle wasting in patients with chronic renal failure[12], and in acidotic obese individuals undergoing very low calorie diets for weight-loss[13, 14]. Whilst this process is a useful adaptive response to acidosis resulting in release of amino acids in the blood as a substrate for synthesis of glutamine and in turn ammonia, which helps mop up excess hydrogen ions for excretion as ammonium ions and thus reduce the acidosis[15], it does nevertheless occur to the detriment of muscle. Some evidence exists from the TwinsUK study of healthy women which showed a positive association between a more alkaline diet and muscle mass indexes[16], but despite this mechanistic understanding, there are few population studies reporting effects of acidosis or dietary acid load on skeletal muscle in men as well as women[15, 17].

This study therefore aimed to address the shortcomings in previous research by providing an update to earlier EPIC-Norfolk analyses using 7-day food diary data, and an additional 10 years follow-up of fracture data, to investigate associations of dietary acid load with bone density status and longitudinal associations with fractures, and also carry out novel investigation of the association of dietary acid-base load and skeletal muscle mass in the EPIC-Norfolk cohort of 25,639 middle to older-aged men and women.

MATERIALS AND METHODS

Population cohort

EPIC-Norfolk is a UK cohort within the multicentre European Prospective Investigation into Cancer and Nutrition (EPIC) and has been described in detail elsewhere[18]. Between 1993

and 1997, 25,639 free-living men and women aged 39-79 years attended a baseline health-check. Of these, 17,304 individuals aged 42-82 years attended a second health-check between 1998 and 2000.

Data collection

Height and weight were recorded to the 1mm and 0.2kg, respectively, according to standard protocols[18]. Participants completed a health and lifestyle questionnaire which included detail on smoking status, family history of osteoporosis, menopausal status, hormone replacement therapy (HRT). Physical activity was also assessed by questionnaire which placed participants into categories, validated against heart-rate monitoring data[19]. A 7-day food diary was used to estimate dietary intake of each participant[20]. This process has been shown by validation studies to be more accurate in estimating dietary nutrient intake than food-frequency questionnaires (FFQ)[18, 21]. The data from the food diaries was recorded using custom designed software (Data Into Nutrients for Epidemiological Research; DINER)[22], and then further checking and translation of the data for nutrient analysis was carried out using a linked system, DINERMO[23]. The Vitamin and Mineral Supplement (ViMiS) database was used to quantify supplement contributions to nutrient intakes[24]. PRAL was calculated according to the following equation: PRAL (in mEq/d) = (mg phosphorus/d×0.0366) + (g protein/d×0.4888) − ((mg potassium/d×0.0205) + (mg calcium/d×0.0125) + (mg magnesium/d×0.0263))[7, 25].

Bone outcomes

Heel bone measurements of broadband ultrasound attenuation (BUA; dB/MHz) were taken using a CUBA (contact ultrasound bone analyser) device (McCue Ultrasonics, Winchester, UK) during the second health-check. Measurements for each foot were taken in duplicate and

the overall mean used for analysis. The five different CUBA devices used in the study were each calibrated daily with a specific imaging phantom and a roving phantom was used monthly to check calibration between devices. The coefficient of variation was 3.5%. Bone density assessment using CUBA has previously been shown effective at predicting fracture risk[26], and was a suitable alternative to the gold-standard of Dual X-ray absorptiometry (DXA) for use in a general practice environment. At each health check, fracture incidence data were collected by questionnaire and cross-checked with the East Norfolk Health Authority database (ENCORE) of hospital attendances[27]. International Classification of Diseases 9 and 10 diagnostic codes for the three most common osteoporotic fractures (hip, spine, or wrist)[28] were used to retrieve data linked to each participant's NHS number. All known osteoporotic fractures in the cohort, up to 31st March 2016, have been accounted for.

Skeletal muscle outcomes

Fat free mass, as a proxy measure of skeletal muscle mass, was derived using a Tanita TBF-531 bioelectrical impedance analysis (BIA) machine (Tanita Corp, Tokyo, Japan) as described previously for this cohort[29]. FFM standardised by BMI (FFM_{BMI}) was calculated as FFM divided by BMI[30], to compensate for differences in skeletal muscle mass with increasing BMI.

Statistical analysis

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 v.15 software[31]. Sex stratification was used in all our analyses, and we also analysed data split into two age groups (<65 years or ≥65 years) as prior study of this population has shown sex and age-related differences in bone density and fracture risk. P

values <0.05 were considered to be statistically significant.

Cross-sectional analyses were carried out using dietary data from the first health-check combined with ultrasound and BIA data from the second health-check. We used multivariable adjusted regression to test for differences in heel-bone BUA and FFM measures across sex-specific quintiles of PRAL, where the lowest PRAL quintile represents the most alkaline (least acidic) diet. We treated the median values for quintiles as a continuous variable in order to test for trends[32]. Where a significant trend was identified, we also tested for inter-quintile differences with ANCOVA, and calculated the percentage difference in the outcome measure for those that were significant. For example, a percentage difference between PRAL quintiles 5 and 1 for BUA was calculated by: ((quintile 1 BUA – quintile 5 BUA) ÷ quintile 1 BUA) × 100). We repeated analyses with the cohort split into <65 year olds and ≥65 year olds, using the same PRAL quintiles defined in the whole cohort to facilitate comparisons. All ultrasound and BIA models were adjusted for age, BMI (ultrasound model only), smoking status, physical activity, family history of osteoporosis (ultrasound model only), menopausal and HRT status in women, and steroid use[26, 33]. In additional analyses we added protein intake as a percentage of energy, and calcium (ultrasound model only)[34, 35]. Participants were excluded from analyses if data were not available for all the variables in the multivariable models, except for menopausal status, HRT use, and smoking status, which were recoded as described previously[36].

Longitudinal analyses were conducted with data from the first health-check plus hospital records of fractures (hip, spine, and wrist). Prentice-weighted Cox regression was used to investigate individual associations between incidence of hip, spine, or wrist fractures and sex-specific quintiles of PRAL, using the same covariates as in cross-sectional analyses. Total

fracture risk was also investigated using the first occurrence of a fracture at any site (hip, spine, or wrist). Follow-up time was calculated between the date of an individual's first health-check and the hospital record search, or death if earlier.

RESULTS

Relevant characteristics of cohort participants are summarised in **Table 1**. There were data for 6350 men and 7989 women in the muscle analysis group, 6490 and 8312 in the ultrasound analysis group, and 11511 and 13927 in the fracture analysis group. Details of the proportions of individuals in age-stratified analyses in each all-age PRAL quintile are shown in **Table 2**.

Skeletal muscle analyses

In all age analyses, PRAL in quintiles was negatively associated with FFM_{BMI} in both men (n=6350, p<0.001) and women (n=7989, p<0.001). A quintile 5 vs 1 difference of -1.5% (p<0.001) was seen for men, and -3.2% (p<0.001) for women, thus indicating that individuals in the most acidic dietary quintile (highest PRAL) had significantly lower BMI-corrected fat free mass than those in the least acidic quintile (lowest PRAL). In age-stratified analyses, the negative associations of PRAL with FFM_{BMI} were evident in both <65 year olds (n=3477 men, p=0.013; n=4887 women, p<0.001) and ≥65 year olds (n=2873 men, p=0.005; n=3102 women, p<0.001) (see **Figure 1** and **Appendix**). Addition of calcium and protein as a percentage of energy to the models caused a loss of statistical significance for associations in men, and women <65 years old, but did not alter the findings for women in all age and ≥65 year old groups.

Bone ultrasound analyses

In all age analyses, PRAL in quintiles was negatively associated with BUA in women (n=8312, p=0.016) and a maximal inter-quintile difference of -1.5% (p=0.024) was seen between quintiles 5 and 1 (see **Figure 2** and Appendix). In age-stratified analyses, the negative associations of PRAL with BUA were only evident in individuals <65 years old (n=5082, p<0.046) and not in those ≥65 years old (n=3230, p=0.187). No significant associations were seen for men. Addition of calcium and protein as a percentage of energy to the models did not alter these findings.

Fracture analyses

In all age analyses, the combined risk of hip, wrist, and spine fractures (mean±SD follow-up 17.9±4.9 years) was positively associated with quintiles of PRAL in both men (610 fractures; n=11,511; p=0.013) and women (1,583 fractures; n=13,927; p=0.009); quintile 5 vs 1 fracture hazard ratio was 1.33 for men (95% CI: 1.03-1.72, p=0.029) and 1.21 (95% CI: 1.03-1.42, p=0.022) for women (see **Figure 3** and Appendix). Risk of hip fracture alone was also positively associated with quintiles of PRAL in women (809 fractures; p=0.003; n=13,927). Risk of wrist or spine fractures alone was not associated with quintiles of PRAL. Risk of hip fracture in men was also significantly positively associated with quintiles of PRAL when calcium and protein as a percentage of energy were included in the model (269 fractures; n=11,511; p=0.037); all other trends were unchanged by the addition of these covariates.

In individuals <65 years old, combined risk of hip, wrist, and spine fractures was positively associated with quintiles of PRAL in men (297 fractures; n=7695; p=0.038), but not women (711 fractures; n=9703; p=0.348) (see **Figure 3** and Appendix). However, risk of hip fracture alone was positively associated with quintiles of PRAL in women (264 fractures; n=9703; p=0.028), as was risk of wrist (90 fractures; n=7695; p=0.049) and spine fractures (139

fractures; n=7695; p=0.033) in men. Trends were identical when calcium and protein as a percentage of energy were included in the models.

In individuals ≥65 years old, combined risk of hip, wrist, and spine fractures was positively associated with quintiles of PRAL in women (872 fractures; n=4224; p=0.035), but not men (313 fractures; n=3816; p=0.138) (see **Figure 3** and Appendix). However, risk of hip fracture alone was positively associated with quintiles of PRAL in both men (184 fractures; n=3816; p=0.022) and women (545 fractures; n=4224; p=0.039). The maximal hazard ratio for hip fracture in men was for quintile 4 vs 1 (1.85, 95% CI: 1.13-3.05, p=0.015). When calcium and protein as a percentage of energy were included in the models, there were no significant changes to the trends seen for men, but the associations seen in women became non-significant.

DISCUSSION

This study provides novel evidence of negative associations between Potential Renal Acid Load (PRAL) and musculoskeletal health. Higher PRAL signifies a more acidic diet and in the EPIC-Norfolk cohort was associated with significantly lower FFM_{BMI} (used as a proxy measure of skeletal muscle mass, scaled for BMI). These associations were evident in both men and women, and for both <65 and ≥65 year olds when analysed in stratified regression models. PRAL was also negatively associated with BUA in women, but trends were not apparent in ≥65 year olds when analysed alone. These cross-sectional analysis findings are greatly strengthened by the evidence from our longitudinal analyses of fracture risk. Although associations were not consistent for all fractures sites and age groups, our study showed that the combined hazard of hip, wrist, and spine fractures was higher with increasing quintiles of PRAL in both men and women. These are particularly novel findings for men, for whom there

has been limited previous evidence for associations of dietary acid-base load with skeletal muscle[17] and bone measures[7, 9, 10], and a dearth of evidence of a link with fracture risk[37, 38]. Overall, however, our findings are supported by evidence from previous smaller and single-sex studies[7-10]. A number of mechanisms, including bone buffering of acid-base balance and alteration in protein metabolism in muscle, have been proposed to explain how PRAL may influence skeletal muscle,[11-14] and bone health health[6, 39, 40]. However, these are not fully recognised and our significant findings reinforce the need for future studies to address this so we can better understand the reasons for the associations observed.

Dietary acid-base load is a balance between hydrogen ion generating foods such as meats, cereals, and dairy, and foods providing base precursors such as fruits and vegetables[5]. Healthy renal function allows excretion of excess dietary hydrogen ions, which is thus reflected in urine pH. For example, previous EPIC-Norfolk analyses have shown low PRAL, low meat consumption and high fruit and vegetable intake was associated with a more alkaline urine pH[41]. On average women in this cohort consumed a less acidic diet than the men, and thus based on our findings one might hypothesise that their musculoskeletal outcomes would be better. However, this was not enough to fully compensate for women's characteristically lower skeletal muscle mass and bone density, and greater fracture risk described in previous studies on this population[26, 29, 42]. Nevertheless, the magnitude of the effects seen here are noteworthy, especially given that decline in musculoskeletal health in the elderly is likely to be multifactorial. For example, we identified a maximal difference in FFM_{BMI} of 4.4% between quintiles 5 and quintiles 1 for women ≥65 years which is highly relevant in comparison to the previously published figure of an estimated 3.7% loss of muscle mass per decade[43]. Likewise, PRAL inter-quintile differences in BUA and fracture risk are also expected to be clinically relevant.

Our study has a number of strengths, but also some limitations. To our knowledge this is the first comprehensive study which has investigated in parallel the cross-sectional associations of dietary acid-base load with both skeletal muscle mass, and bone density status, and also longitudinal fracture risk in men and women. Indeed, the previous published study investigating associations between PRAL and bone measures in the EPIC-Norfolk cohort showed some effects on bone density, but only in women, did not analyse BIA data, and showed no effects on fractures[7]. The findings presented here, based on analysis of an additional 10 years of fracture data, and 7-day food diary nutrient data in place of FFQ data, are thus a significant advance. In fact, the quantitative 7-day food diaries developed for use in EPIC have been validated previously and in comparison to alternative methods, such as FFQ or 24-hour recall, provide more precise dietary intake figures and thus PRAL estimates for our analyses[23]. Indeed, previous analysis of urine pH and different dietary assessment methods in EPIC found the strongest relationship with 7-day food diary data[41]. It is important to note that our investigation has focused on PRAL contributed from foods in the typical diet of the EPIC cohort participants. We may therefore have underestimated the total nutrient contributions relevant to PRAL calculation, although contributions of supplements are known to be small in this cohort[24]. In addition, this means that our results should not be generalised to conclusions relating to artificially modifying acid-base load by non-dietary means.

Although we have used a BIA-derived fat free mass index as a proxy measure of skeletal muscle mass, and heel-bone BUA as a measure of bone density status, in place of more sophisticated measures such as dual-energy X-ray absorptiometry, computer tomography, or magnetic resonance imaging[44], these techniques provided safe, non-radiation exposing,

pragmatic methods for obtaining suitable data for our analyses, and have previously been shown to be relevant to clinical outcomes[45, 46]. We acknowledge, however, that these methods do have limitations, in particular the use of BIA in obese individuals[47], but we have been careful to analyse data only within their established limits of accuracy. We used hospital admission data to determine fracture incidence and it is possible that this method may underestimate incidence of fractures, particularly spine fractures, and could differ between sexes. Our conclusions are also limited by the inherent drawback of observational studies which precludes us from drawing any causal associations. However, we adjusted all our models for relevant potential confounders decided *a priori* from evidence of previous studies, which increases the likelihood that our observations are valid.

Conclusions

This study provides strong evidence for a negative association between more acidic PRAL and musculoskeletal health in middle to older age men and women. Our findings from a long-term population cohort thus add to the evidence that dietary balance of acidogenic meats, cereals, and dairy, and alkalinogenic fruits and vegetables is important and, with the caveat that these are observational data, provides support for moderation of dietary acid-base load for optimal musculoskeletal health.

DISCLOSURE

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Author Contributions AAW developed the research question with RPGH who analysed the data and drafted the manuscript. AAW also arranged data collection in conjunction with RNL, who implemented record linkage. AA assisted with presentation of the findings within the

manuscript. K-TK is principal investigator of the EPIC-Norfolk Study. All authors contributed to data interpretation, review of the manuscript and its approval.

Data availability: The EPIC datasets used in our analyses are available to other researchers by request to the EPIC team at Cambridge University. Details can be provided by the corresponding author.

Ethics: All procedures were approved by The Norfolk District Health Authority Ethics Committee and participants provided written informed consent according to the Declaration of Helsinki.

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Conflict of Interest: AW and RH presented preliminary findings from this study at the 3rd International Acid-Base Symposium in Smolenice, Slovak Republic, 24-28th June 2018. AW and RH's registration fees, and AW's travel were paid for by the conference organisers, NuOmix-Research k.s..

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Figure 1 – Adjusted FFM_{BMI} of 6350 men and 7989 women from the EPIC-Norfolk cohort according to PRAL quintiles, stratified by sex and age group.

Models adjusted for age, smoking status, physical activity, menopausal and HRT status in women, and steroid use.

Data plotted as multivariable regression adjusted mean ± SEM.

* = P value <0.05 vs quintile 1; ** = P value <0.01; *** = P value <0.001.

Figure 2 – Adjusted heel-bone BUA of 6490 men and 8312 women from the EPIC-Norfolk cohort according to PRAL quintiles, stratified by sex and age group.

Models adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, and steroid use.

Data plotted as multivariable regression adjusted mean ± SEM.

* = P value <0.05 vs quintile 1.

Figure 3 – Risk of hip, spine, and wrist fractures in 11511 men and 13927 women from the EPIC-Norfolk cohort at follow-up versus baseline, according to PRAL quintiles, stratified by sex and age group.

Models adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, and steroid use.

Data plotted as cox proportional hazard ratio ± SEM.

* = P value <0.05 vs quintile 1.

Table 1 – Relevant characteristics of the muscle analysis cohort (n=14339), the ultrasound cohort (n=14802), and the fracture cohort (n=25438) from EPIC-Norfolk, stratified by sex.

Selected Characteristics	**Muscle cohort**		**Ultrasound cohort**		**Fracture cohort**	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
	n=6350	**n=7989**	**n=6490**	**n=8312**	**n=11511**	**n=13927**
Age (years)	62.9 (9.0)	61.5 (9.0)	62.9	61.6 (9.0)	59.7 (9.3)	58.9 (9.3)
BMI (kg/m^2)	26.7 (3.0)	26.1 (3.7)	26.9	26.5 (4.4)	26.5 (3.3)	26.2 (4.3)
Fat free mass (kg)	61.6 (5.9)	40.6 (4.5)	--	--	--	--
BUA (dB/MHz)	--	--	90.1	72.1	--	--
PRAL (mEq/day)	0.6 (11.1)	-4.5 (9.8)	0.7	-4.4 (9.8)	1.1 (11.5)	-3.8
Calcium intake (mg/day)	942.7	785.6	942.2	784.5	919.5	766.1
Total energy intake (kcal/day)	2286.0	1735.2	2285.3	1731.0	2240.3	1694.1
Smoking (%)						
Current	8.5	8.7	8.6	8.7	12.8	12.1
Former	55.5	31.9	55.6	32.4	54.1	31.9
Never	36.0	59.4	35.8	58.9	33.1	55.9
Physical activity (%)						
Inactive	27.3	25.9	27.6	26.3	30.8	30.4
Moderately inactive	25.1	32.5	25.1	32.7	24.6	32.1
Moderately active	25.0	24.2	24.9	23.9	23.0	22.2
Active	22.5	17.4	22.4	17.1	21.5	15.3
Family history of osteoporosis						
No	97.4	93.7	97.3	93.7	97.3	94.2
Yes	2.6	6.3	2.7	6.3	2.7	5.8
Corticosteroid use (%)						
Current or former (>3 months)	4.2	5.1	4.2	5.1	3.0	3.4
Never (<3 months)	95.8	94.9	95.8	94.9	97.0	96.6
Menopausal status (%)						
Pre-menopausal	--	6.0	--	5.8	--	16.8
Peri-menopausal (<1 y)	--	3.3	--	3.3	--	5.4
Peri-menopausal (1-5 y)	--	17.5	--	17.6	--	17.9
Post-menopausal	--	73.2	--	73.3	--	59.9
HRT use (%)						
Current	--	21.3	--	21.2	--	20.3
Former	--	17.9	--	17.9	--	11.4
Never	--	60.8	--	60.9	--	68.4

Data presented as mean (SD), or % for categorical variables.

Table 2 – Proportions of <65 year olds and ≥65 year olds in each PRAL quintile for each analysis cohort, stratified by sex.

Men		**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**
Muscle cohort	Median PRAL (mEq/d)	-12.9	-4.2	1.0	6.0	13.6
	<65 year olds (%)	21.6	19.5	19.4	18.7	20.8
	≥65 year olds (%)	18.1	20.6	20.7	21.6	19.0
Ultrasound cohort	Median PRAL (mEq/d)	-12.8	-4.2	1.0	6.0	13.7
	<65 year olds (%)	21.6	19.3	19.4	18.8	20.9
	≥65 year olds (%)	18.0	20.9	20.8	21.4	18.9
Fracture cohort	Median PRAL (mEq/d)	-12.8	-4.2	1.0	6.0	13.7
	<65 year olds (%)	21.5	19.3	19.1	19.2	21.0
	≥65 year olds (%)	17.0	21.4	21.9	21.7	18.1
Women		**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**
Muscle cohort	Median PRAL (mEq/d)	-16.4	-8.6	-3.7	0.5	7.0
	<65 year olds (%)	21.9	20.8	20.1	18.6	18.6
	≥65 year olds (%)	17.0	18.8	19.8	22.2	22.2
Ultrasound cohort	Median PRAL (mEq/d)	-16.3	-8.5	-3.7	0.7	7.1
	<65 year olds (%)	22.0	20.6	20.1	18.7	18.6
	≥65 year olds (%)	16.9	19.0	19.9	22.0	22.1
Fracture cohort	Median PRAL (mEq/d)	-16.3	-8.5	-3.7	0.7	7.1
	<65 year olds (%)	22.7	20.6	19.3	19.1	18.3
	≥65 year olds (%)	13.9	18.7	21.5	22.2	23.8

Appendix

Table 1 – Adjusted FFM_{BMI} of 6350 men and 7989 women from the EPIC-Norfolk cohort according to PRAL quintiles, stratified by sex and age group. Models adjusted for age, smoking status, physical activity, menopausal and HRT status in women, and steroid use.

FFM_{BMI}		**Q1**		**Q2**		**Q3**		**Q4**		**Q5**		**Q5 vs Q1**
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	**%dif**
Men	**All ages**	2.348	0.007	2.327	0.007	2.321	0.007	2.317	0.007	2.313	0.007	-1.501
	<65y	2.384	0.009	2.361	0.011	2.356	0.010	2.361	0.010	2.349	0.010	-1.473
	≥65y	2.305	0.011	2.286	0.010	2.280	0.010	2.264	0.010	2.270	0.010	-1.560
Women	**All ages**	1.612	0.006	1.594	0.006	1.579	0.006	1.570	0.006	1.561	0.006	-3.191
	<65y	1.638	0.008	1.628	0.008	1.607	0.008	1.601	0.009	1.596	0.009	-2.550
	≥65y	1.574	0.010	1.540	0.010	1.538	0.010	1.521	0.010	1.505	0.010	-4.409

Table 2 – Adjusted heel-bone BUA of 6490 men and 8312 women from the EPIC-Norfolk cohort according to PRAL quintiles, stratified by sex and age group. Models adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, and steroid use.

BUA		**Q1**		**Q2**		**Q3**		**Q4**		**Q5**		**Q5 vs Q1**
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	**%dif**
Men	**All ages**	90.020	0.481	89.244	0.481	90.849	0.481	89.802	0.481	90.378	0.480	0.398
	<65y	91.186	0.610	90.008	0.645	91.489	0.644	90.534	0.653	91.677	0.621	0.539
	≥65y	88.620	0.774	88.288	0.718	90.052	0.720	88.873	0.709	88.729	0.755	0.123
Women	**All ages**	72.783	0.346	72.132	0.345	71.906	0.344	71.788	0.345	71.675	0.345	-1.523
	<65y	78.006	0.432	76.736	0.445	76.501	0.451	77.254	0.467	76.535	0.469	-1.886
	≥65y	64.470	0.575	64.861	0.540	64.663	0.528	63.359	0.503	64.008	0.502	-0.717

Table 3 – Risk of hip, spine, and wrist fractures in 11511 men and 13927 women from the EPIC-Norfolk cohort at follow-up versus baseline, according to PRAL quintiles, stratified by sex and age group. Models adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, and steroid use. Cox proportional hazard ratios are versus quintile 1.

Fracture risk			**Q2**			**Q3**			**Q4**			**Q5**		
			HR	CI lower	CI upper	HR	CI lower	CI upper	HR	CI lower	CI upper	HR	CI lower	CI upper
Men	**All ages**	**Total**	1.034	0.792	1.351	1.208	0.933	1.565	1.205	0.928	1.563	1.333	1.030	1.724
		Hip	1.158	0.774	1.732	1.082	0.789	1.630	1.423	0.964	2.100	1.333	0.892	1.991
		Wrist	1.089	0.594	1.997	1.750	1.006	3.044	1.039	0.558	1.933	1.675	0.957	2.933
		Spine	0.946	0.625	1.431	1.105	0.740	1.648	1.125	0.754	1.678	1.308	0.886	1.930
	<65 years	**Total**	1.076	0.735	1.575	1.171	0.808	1.698	1.115	0.765	1.624	1.456	1.027	2.063
		Hip	1.019	0.529	1.963	0.671	0.326	1.384	0.950	0.492	1.831	1.018	0.541	1.915
		Wrist	1.462	0.710	3.011	1.748	0.869	3.516	1.117	0.517	2.411	2.151	1.107	4.180
		Spine	1.002	0.554	1.811	1.346	0.778	2.329	1.262	0.723	2.204	1.668	0.993	2.800
	≥65 years	**Total**	1.001	0.668	1.457	1.238	0.861	1.781	1.305	0.907	1.878	1.204	0.821	1.767
		Hip	1.320	0.786	2.218	1.428	0.852	2.394	1.853	1.125	3.051	1.641	0.970	2.776
		Wrist	0.538	0.170	1.699	1.577	0.632	3.932	0.883	0.309	2.526	0.763	0.241	2.413
		Spine	0.863	0.483	1.540	0.863	0.480	1.552	0.985	0.555	1.750	0.914	0.498	1.679
Women	**All ages**	**Total**	1.047	0.888	1.235	0.952	0.806	1.126	1.149	0.978	1.350	1.206	1.028	1.416
		Hip	0.968	0.761	1.231	0.926	0.729	1.176	1.163	0.925	1.461	1.307	1.045	1.636
		Wrist	1.377	1.013	1.872	1.219	0.891	1.668	1.390	1.023	1.889	1.167	0.848	1.607
		Spine	0.903	0.687	1.187	0.787	0.594	1.043	0.914	0.696	1.199	1.163	0.898	1.507
	<65 years	**Total**	1.108	0.881	1.393	0.982	0.773	1.247	1.142	0.907	1.439	1.210	0.959	1.527
		Hip	0.922	0.617	1.377	1.073	0.727	1.583	1.103	0.750	1.624	1.485	1.025	2.150
		Wrist	1.682	1.130	2.504	1.322	0.865	2.020	1.620	1.077	2.438	1.399	0.910	2.151
		Spine	0.936	0.653	1.343	0.724	0.489	1.074	0.914	0.632	1.322	1.120	0.783	1.602
	≥65 years	**Total**	0.985	0.775	1.251	0.914	0.722	1.158	1.142	0.909	1.434	1.187	0.948	1.486
		Hip	0.985	0.728	1.333	0.847	0.625	1.148	1.178	0.885	1.568	1.233	0.929	1.636

Wrist	1.030	0.636	1.668	1.066	0.668	1.701	1.115	0.701	1.774	0.899	0.558	1.450
Spine	0.848	0.556	1.294	0.837	0.554	1.266	0.917	0.610	1.378	1.203	0.819	1.766

Figure 1

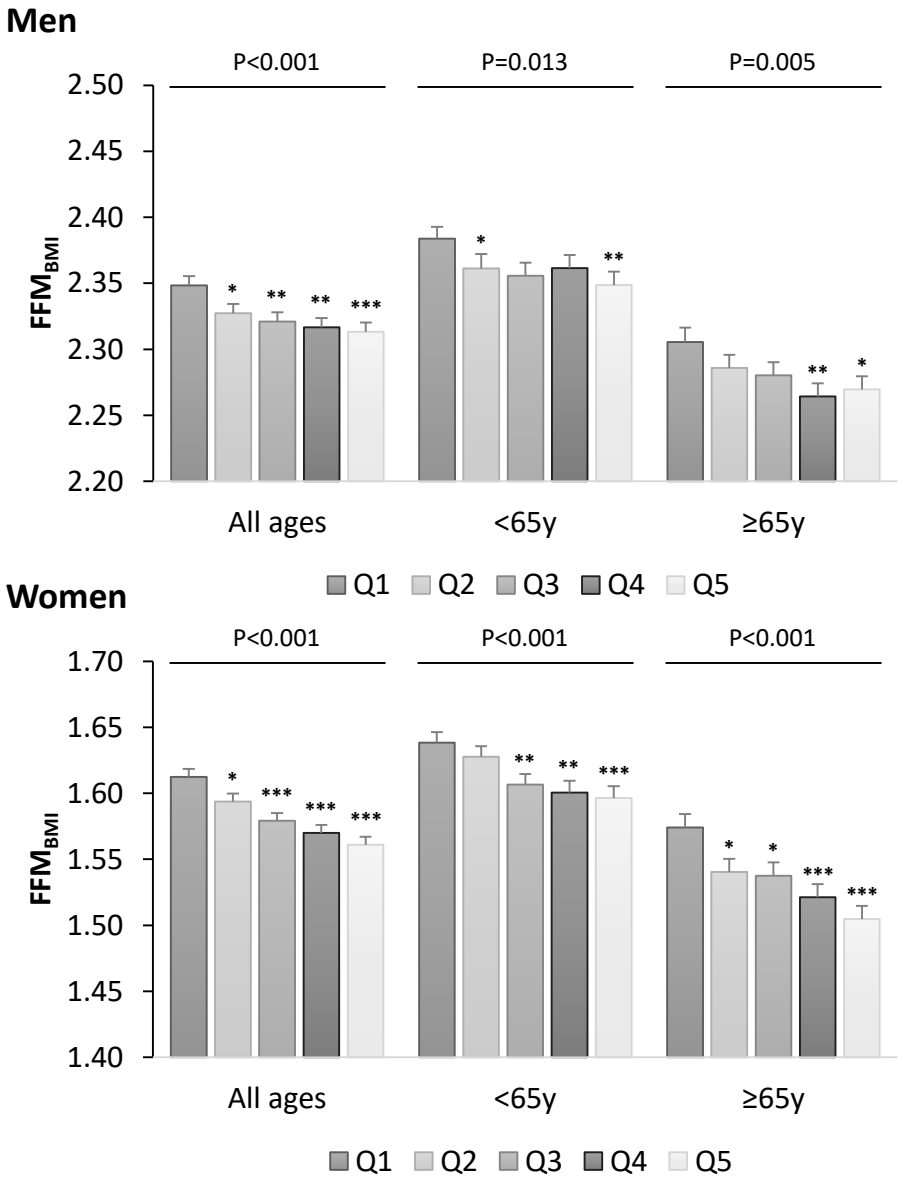

Men
P<0.001
P=0.013
P=0.005
2.50
2.45
2.40
2.35
2.30
2.25
2.20
FFM_BMI
All ages
<65y
≥65y
Q1 Q2 Q3 Q4 Q5
Women
P<0.001
P<0.001
P<0.001
1.70
1.65
1.60
1.55
1.50
1.45
1.40
FFM_BMI
All ages
<65y
≥65y
Q1 Q2 Q3 Q4 Q5

Figure 2

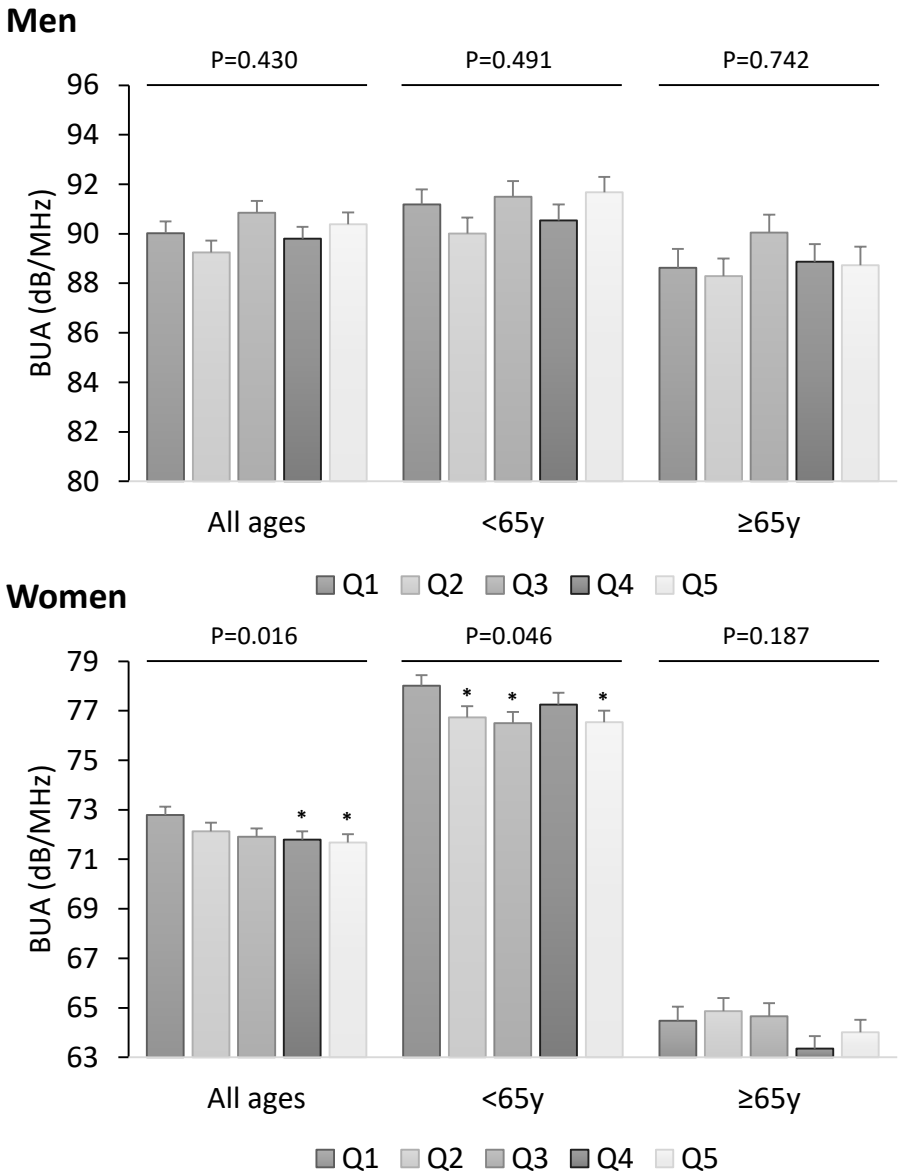
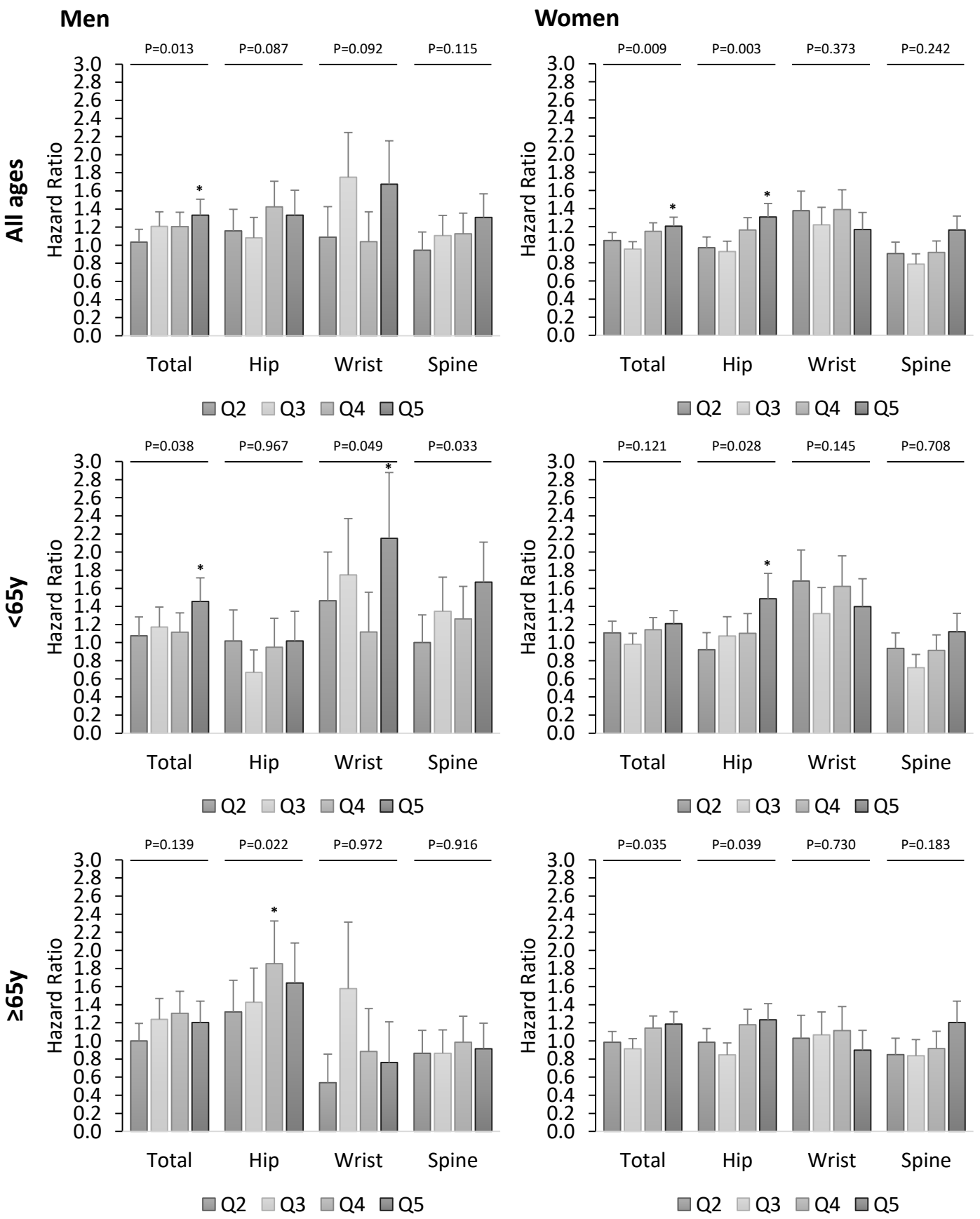

Men
P=0.430
P=0.491
P=0.742
96
94
92
90
88
86
84
82
80
BUA (dB/MHz)
All ages
<65y
≥65y
Q1 Q2 Q3 Q4 Q5
Women
P=0.016
P=0.046
P=0.187
79
77
75
73
71
69
67
65
63
BUA (dB/MHz)
*
All ages
<65y
≥65y
Q1 Q2 Q3 Q4 Q5

Figure 3


Men
Women
All ages
<65y
≥65y
Hazard Ratio
P=0.013
P=0.087
P=0.092
P=0.115
P=0.009
P=0.003
P=0.373
P=0.242
P=0.038
P=0.967
P=0.049
P=0.033
P=0.121
P=0.028
P=0.145
P=0.708
P=0.139
P=0.022
P=0.972
P=0.916
P=0.035
P=0.039
P=0.730
P=0.183
Total
Hip
Wrist
Spine
Q2
Q3
Q4
Q5