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U-Shaped Association between Dietary Acid Load and Risk of Osteoporotic Fractures in 2 Populations at High Cardiovascular Risk

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ABSTRACT

Background: Bone contributes to maintaining the acid-base balance as a buffering system for blood pH. Diet composition also affects acid-base balance. Several studies have linked an imbalance in the acid-base system to changes in the density and structure of bone mass, although some prospective studies and meta-analyses suggest that acid load has no deleterious effect on bone.

Objective: The aim of this study was to examine the associations between potential renal acid load (PRAL) and net endogenous acid production (NEAP) and the risk of osteoporotic fractures and bone mineral density (BMD) in 2 middle-aged and elderly Mediterranean populations.

Methods: We conducted a longitudinal analysis including 870 participants from the PREvención con Dieta MEDiterranea (PREDIMED) Study and a cross-sectional analysis including 1134 participants from the PREDIMED-Plus study. Participants were adults, aged 55–80 y, either at high cardiovascular risk (PREDIMED) or overweight/obese with metabolic syndrome (PREDIMED-Plus), as defined by the International Diabetes Federation, the American Heart Association, and the National Heart Association. PRAL and NEAP were calculated from validated food-frequency questionnaires. BMD was measured using DXA scans. Fracture information was obtained from medical records. The association between mean PRAL and NEAP and fracture risk was assessed using multivariable-adjusted Cox models. BMD differences between tertiles of baseline PRAL and NEAP were evaluated by means of ANCOVA.

Results: A total 114 new fracture events were documented in the PREDIMED study after a mean of 5.2 y of intervention and 8.9 y of total follow-up. Participants in the first and third PRAL and NEAP tertiles had a higher risk of osteoporotic fracture compared with the second tertile, showing a characteristically U-shaped association [HR (95% CI): 1.73 (1.03, 2.91) in tertile 1 and 1.91 (1.14, 3.19) in tertile 3 for PRAL, and 1.83 (1.08, 3.09) in tertile 1 and 1.87 (1.10, 3.17) in tertile 3 for NEAP]. Compared with the participants in tertile 1, the participants in the top PRAL and NEAP tertiles had lower BMD [PRAL: mean total femur BMD: 1.029 ± 0.007 and 1.007 ± 0.007 g/cm^2; $P = 0.006$ (tertiles 1 and 3); NEAP: mean total femur BMD: 1.032 ± 0.007 and 1.009 ± 0.007 g/cm^2; $P = 0.017$ (tertiles 1 and 3)].

Conclusions: The results of our study suggest that both high and low dietary acid are associated with a higher risk of osteoporotic fractures, although only high dietary acid was found to have a negative relation to BMD in senior adults with existing chronic health conditions. This trial was registered at http://www.isrctn.com/ as ISRCTN3573963 (PREDIMED) and ISRCTN89898870 (PREDIMED-Plus). *J Nutr* 2021;151:152–161.

Keywords: bone, fracture, dietary potential acid load, aging, bone mineral density

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Introduction

Loss of bone mineral density (BMD) and its associated complications affects several million people worldwide and has become a major economic burden on public health systems (1). There is, therefore, an urgent need to identify risk factors associated with the microstructure and health properties of bone. Aside from age-related progressive demineralization, loss of bone mass and osteoporosis can be exacerbated by other factors, including metabolic diseases, the use of certain medications, genetic factors, and modifiable lifestyle factors such as smoking and dietary habits, which jeopardize bone strength and increase the risk of fracture (1–4).

Several studies have linked an imbalance in the acid-base system to changes in the density and structure of bone mass (5–7). Mild systematic metabolic acidosis could release calcium from the bone matrix mediated for increasing osteoplastic resorption in an attempt to maintain homeostasis, weakening the bone and thus making it susceptible to fracturing (8, 9).

Diet composition affects the acid-base balance in the body. Foods from animal sources, such as cheese, fish, and meat, have more acid precursors, while fruit and vegetables are net alkalinizing in nature (8). Diet-induced low-grade acidosis could also affect bone health, reducing BMD and increasing its fragility (9). Cross-sectional studies suggest that bone could contribute to the neutralization of the dietary acid load with a detrimental effect on BMD (10, 11). Both potential renal acid load (PRAL) and net endogenous acid production (NEAP) are commonly used as theoretical models to estimate the dietary intake of certain nutrients in order to measure the acid-base load of a diet and estimate renal net acid excretion (8, 12). A prospective analysis conducted in youth found a positive association between urinary citrate, a biomarker that depends on both the diet and metabolism acid-base balance, and several parameters of bone quality and geometry. In addition, 15 y later, the authors reported that fracture risk was inversely associated with urinary citrate and positively associated with urinary PRAL in women but not in men (13). Under this hypothesis, a high-acid diet would contribute to an increased risk of osteoporosis and fractures due to the loss of bone mass, while the intake of base-producing foods could potentially prevent the acid-related loss of bone (11). In fact, it is possible that the diet of older people with altered renal function and renal acid-excretory ability as well as other diseases that can affect the acid-base balance may result in an imbalance capable of increasing bone demineralization (14). However, some observational studies and meta-analyses dispute the claim that acid load has a deleterious effect on bone in the general population, which opens the debate about the bone health benefits of base-forming diets (15–17). In 4672 elderly Dutch people, energy-adjusted NEAP was inversely associated with trabecular bone integrity but not with BMD (15). In a cross-sectional analysis conducted in the NHANES involving 1218 men >60 y old, lower femoral BMD was associated with a higher PRAL (7). In 8188 women and 6375 men aged 40–79 y from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, both higher PRAL and higher NEAP were associated with lower calcaneal broadband ultrasound attenuation (BUA) in women, but not with incident fractures (11). Therefore, the present study primarily aimed to determine whether there are any associations between PRAL and NEAP, 2 indicators of the dietary acid-base load, and osteoporotic fractures in a study conducted in a senior Mediterranean population at high cardiovascular risk. A secondary aim was to ascertain whether PRAL and NEAP are related to BMD in another independent study conducted in a senior Mediterranean population with overweight/obesity and metabolic syndrome.

Methods

Subjects and study design

Participants from 2 independent studies [PREvención con DIeta MEDiterranea (PREDIMED) and PREDIMED-Plus] were included in the present analysis.

Subjects and study design of the PREDIMED study

A longitudinal analysis of osteoporotic fractures was conducted for 870 participants in the PREDIMED study who were recruited in our center (**Supplemental Figure 1**). PREDIMED is a multicenter, randomized, and parallel-group trial conducted between 2003 and 2010 to assess the effectiveness of a Mediterranean diet in the prevention of cardiovascular diseases. An extended follow-up for clinical events ended in August 2015. A total of 56 participants were not randomly assigned and were assigned to the same intervention group because

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Supplemental Tables 1–10 and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: BMD, bone mineral density; eGFR, estimated glomerular filtration rate; FD, femoral diaphysis; FFQ, food-frequency questionnaires; FN, femur neck; ICD-CM, International Classification of Diseases, Clinical Modification; LS, lumbar spine; MET, metabolic equivalent of task; NEAP, net endogenous acid production; PRAL, potential renal acid load; PREDIMED, PREvención con DIeta MEDiterranea study; TF, total femur; TR, trochanter; T2D, type 2 diabetes.

they shared a household. The detailed protocol study is available at http://www.predimed.es and has been published previously (18). Briefly, participants were randomly assigned either to a Mediterranean diet group supplemented with extra-virgin olive oil, a Mediterranean diet group supplemented with nuts, or a low-fat diet group. Participants were women aged 60 to 80 y and men aged 55 to 80 y with a high risk of cardiovascular disease—that is, with either type 2 diabetes (T2D) or ≥3 of the following criteria: hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or antihypertensive drug use), hypercholesterolemia (high LDL cholesterol ≥160 mg/dL or medication), low HDL cholesterol (≤50 mg/dL in women or ≤40 mg/dL in men), overweight or obesity [BMI (in kg/m^2) ≥25], current smoker, or family history of premature coronary heart disease.

Subjects and study design of the PREDIMED-PLUS study

A cross-sectional analysis of bone density parameters was performed in the PREDIMED-Plus study, a multicenter, randomized, and parallel-group trial conducted in 6874 overweight or obese women and men aged 60 to 75 and aged 55 to 75 y, respectively (**Supplemental Figure 2**). From the total number of participants, only 1134 underwent a DXA scan and were included in the present analysis. The participants had a BMI ≥27 and <40 and metabolic syndrome defined in keeping with the updated harmonized criteria of the International Diabetes Federation, the American Heart Association, and the National Heart Association (19). The protocol and study information can be found at http://predimedplus.com.

Both studies are registered at http://www.isrctn.com/ (PREDIMED: ISRCTN3573963; PREDIMED-Plus: ISRCTN89898870). The protocols and procedures were conducted according to the ethical standards of the Declaration of Helsinki and approved by the relevant institutional ethics review boards at each study center. All participants in both trials provided written informed consent.

Bone assessment

For the purposes of this study, we considered osteoporotic fractures to be those that occurred at anatomic sites and under circumstances that most likely indicate osteoporosis according to the standards determined by Warriner et al. (19). These standards describe and assign scores for several fracture-risk groups according to age, sex, and race, with higher scores representing the most probable fractures due to osteoporosis. For this study, we included fractures with scores of >5. We did not include fractures located in the open clavicle [International Classification of Diseases, Clinical Modification (ICD-CM), 810.1–810.3], phalange (ICD-CM 816.1–816.13 and 826.0–826.1), tarsal/metatarsal (ICD-CM 825.0–825.39), scapula (ICD-CM 811.0–811.19), or skull/face (ICD-CM 800.00–804.99) in accordance with the ICD-CM. New cases of osteoporosis-related fractures were classified following a standardized systematic annual review of all medical records of each PREDIMED participant by qualified professionals and were confirmed by an independent researcher.

BMD (g/cm^2) at total femur (TF), lumbar spine (LS; from L1 to L4), femur neck (FN), trochanter (TR) and femoral diaphysis (FD) area sites was measured in PREDIMED-Plus participants using DXA scans (Lunar iDXA and DXA Lunar Prodigy Primo; GE Healthcare) administered by qualified radiology technicians. The T-scores for TF, LS, FN, and TR were estimated using the manufacturer's reference values for the Spanish adult population included in the DXA machine and BMD report. T-scores were estimated considering reference population, sex, age, weight, and height. The BMD of the femoral area sites was measured on the nondominant side.

Dietary assessment

Dietary intake was estimated using semi-quantitative food-frequency questionnaires (FFQs) (20). In the PREDIMED study, we gave a validated 137-item FFQ to all participants yearly from baseline to the end of the intervention period. In the PREDIMED-Plus we gave participants a 143-item FFQ. All dietary characteristics (i.e., mean PRAL/day, mean NEAP/day, mean energy intake/day, and the nutrients present in the analysis) were assessed from FFQs (administered in yearly intervals) and averaged for each subject over his or her own multi-annual intervention period according to the Spanish nutritional food-composition tables (21, 22). We have defined these variables as the yearly mean intake of each dietary characteristic. Both PRAL and NEAP are theoretical models based on dietary intakes of certain nutrients. PRAL predicts the renal net acid excretion considering the mineral and protein composition of foods, their intestinal absorption rates, sulfur metabolism, and the urinary excretion of organic acids (8). Estimated dietary NEAP is a reflection of the total amount of acid excreted, which is higher when the amount of anions exceeds that of cations during kidney filtration (23). PRAL was estimated with the algorithm defined by Remer and Manz (8): PRAL (mEq/d) = (0.49 · g protein intake/d) + (0.037 · mg phosphorus/d) − (0.021 · mg potassium/d) − (0.013 · mg calcium/d) − (0.026 · mg magnesium/d). NEAP was estimated using the equation developed by Frassetto et al. (12): NEAP (mEq/d) = 54.5 · (g protein/mEq potassium) − 10.2. Both estimations were calculated using individual nutritional values (i.e., protein, phosphorus, potassium, calcium, and magnesium) obtained from the FFQ. Negative PRAL values indicate that the diet tends to produce alkalinizing salts, while positive values indicate an acid-load diet. In the same way, lower NEAP values are associated with alkaline-load diets while higher values indicate acid-load diets.

Measurements

Weight and height were taken with calibrated scales and a wall-fixed stadiometer. BMI was calculated as weight (kilograms) divided by the square of height (meters squared). Trained staff collected yearly questionnaires from the participants to obtain information on lifestyle habits, medical conditions, and medication at baseline and at the end of the study for both interventions. The validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire was applied in the same time frame (24). Based on serum creatinine measurements, the glomerular filtration rate was estimated (eGFR) in mL/(min · 1.73 m^2) using the CKD-EPI (CKD Epidemiology Collaboration) equation for whites as a measure of kidney function (25).

Statistical analysis

General and dietary characteristics at baseline are presented as means ± SDs when variables were quantitative and had normal distribution or as medians (IQR) for non-normal distribution, and as percentages (*n*) for categorical variables. Differences between the 2 study populations were tested with 1-factor ANOVA and a chi-square test.

To evaluate the longitudinal association between PRAL or NEAP and the occurrence of osteoporotic fracture in the PREDIMED study, we divided the participants into tertiles for the yearly mean of PRAL or NEAP from the first FFQ at the baseline visit to the last FFQ before the fracture occurred, or until the end of follow-up or death in those who did not have osteoporotic fractures. Similarly, nutritional secondary adjusting variables (carbohydrate, fatty acids, fiber, and vitamin D consumption) were estimated as the yearly mean from the baseline visit to the final visit intervention, the last FFQ before the fracture occurred, or death. We analyzed the relation between the yearly mean PRAL and NEAP for tertiles and continuous variables and the risk of osteoporotic fracture with 3e multivariable Cox proportional regression models with robust variance estimators and considering intracluster correlations. For this purpose, the participants who shared a household were defined as clusters and were considered in the Cox models as a cluster variable. The first model was adjusted for age (years), sex, BMI (kg/m^2), leisure-time physical activity [metabolic equivalent of task (MET)-minutes/day], educational level (illiterate/primary education, secondary education, and academic/graduate), the intervention group, and smoking status (never, former, current smoker). The second model was also adjusted for the eGFR [mL/(min · 1.73 m^2)], the prevalence of diabetes (yes/no), the prevalence of previous fractures (yes/no), the prevalence of hypertension (yes/no), use of insulin (yes/no), use of estrogen drugs (yes/no), use of vitamin D and calcium supplements (yes/no), use of osteoporotic drugs

(yes/no), the total yearly mean intake of carbohydrates (grams/day), fatty acids (grams/day), fiber (grams/day), vitamin D (micrograms/day), and energy intake (kilocalories/day). Furthermore, we used a Cox regression analysis to assess the quadratic trend, assigning the median value of each tertile. Because the means of PRAL and NEAP showed a significant U-shape and the quadratic trend P values (P q-trend) were significant, the second tertile was established as the tertile of reference and the shape of the association was visually inspected using a restricted cubic spline with 3 knots placed at the 10th, 50th, and 90th percentiles of the PRAL score. The reference value was assigned to zero as a neutral value of PRAL for calculating HRs and 95% CIs. The results are presented as HRs and 95% CIs. The assumption of the Cox proportional hazard was checked with the Schoenfeld residuals using the "cox.zph" function in the "Survival" package ($P = 0.89$ for the most-adjusted PRAL model and $P = 0.91$ for the most-adjusted NEAP model).

To assess the cross-sectional associations between PRAL or NEAP and BMD in the PREDIMED-Plus study, we categorized participants into tertiles of baseline PRAL and NEAP values. We used ANOVA and ANCOVA for the crude and adjusted model, respectively. The latter was adjusted for sex, age (years), smoking status (never, current, former), leisure-time physical activity (MET/day), educational level (illiterate/primary education, secondary education, and academic/graduate), glycated hemoglobin, the prevalence of obesity (yes/no), the prevalence of osteoporosis (yes/no), eGFR [mL/(min · 1.73 m^2)], total energy intake (kilocalories/day), use of calcium and/or vitamin D supplements (yes/no), use of hormonal treatment (yes/no), use of osteoporotic drugs (yes/no), and use of insulin (yes/no). The assumptions of the ANCOVA models were checked using visual or quantitative methods. All graphs and tests (Shapiro-Wilk test and Levene's tests) yielded models that met the independence of observations, homogeneity of variance (all Levene's test P values >0.05), and normality of residuals (all Shapiro-Wilk test P values >0.05) criteria. Additionally, as described above, we assessed the quadratic trend, assigning the median score within each tertile, and using these scores as continuous variables in a multivariable linear regression model. We used the Tukey test to make multiple comparisons between tertiles. For this analysis, we used the official PREDIMED-Plus database updated on 13 June 2017. Possible interactions within sex × PRAL or sex × NEAP were evaluated using the likelihood ratio test. The fully adjusted models were compared with the same models plus interaction product terms. Because there were no effect modifications ($P > 0.05$), all of the analyses were performed with the complete study population in both trials. In addition, we conducted different sensitivity analyses. First, we repeated the PREDIMED-Plus database analyses using BMD T-scores (FD was not repeated for lack of T-score estimations). Second, we excluded participants with a history of fractures in both databases. Third, we excluded participants with fractures that occurred during the first year of intervention in the PREDIMED database. And finally, we excluded participants who take calcium and/or vitamin D supplements in both populations. The confounder variables included in the models were considered when significant differences were found across tertiles, based on known biological plausibility or considering their statistical influence on the present data. We considered statistically significant P values <0.05 for all tests with bilateral contrast. All statistical analyses were performed using the R software v 3.3.2 (www.r-project.org) (R Development Core Team, 2012), including the "Survival" v2.41–3 package for the Cox regression analysis and the "effects" v3.1–2 package for multivariable ANCOVA. The restricted cubic spline was constructed using Stata Statistical Software, release 14 (StataCorp LP, College Station, TX).

Results

A summary of the main sociodemographic, anthropometric, and clinical characteristics of the participants from the 2 studies is presented in **Table 1**. There were no significant differences in the percentage of sexes, BMI, physical activity expenditure, smoking habit, educational level, previous fractures, or use of drugs (except for calcium and vitamin D supplements) between the PRAL tertiles in the PREDIMED trial. Similarly, there were no significant differences in the percentage of sexes, prevalence of previous fractures, T2D, hypertension, or the use of drugs between the PRAL tertiles in the PREDIMED-Plus trial. However, BMI, physical activity, smoking status, and educational level were significantly different between the PRAL tertiles in the PREDIMED-Plus study participants, unlike in the PREDIMED study.

Table 2 shows the baseline dietary characteristics in both studies. The intake of proteins, total fatty acids, both SFAs and MUFAs, calcium, and phosphorus was significantly greater, whereas fiber and potassium intake was lower, in the participants in the highest tertile than those in the lower 2 tertiles in both study populations ($P < 0.05$). Furthermore, the intake of carbohydrates, vitamin D, and magnesium was higher in the third PRAL tertile in the PREDIMED-Plus participants ($P < 0.05$) but not in the PREDIMED group. Differences in the yearly mean nutrient intake according to PRAL tertiles are shown in **Supplemental Table 1**. As in the main tables, Supplemental Table 1 shows that total energy intake and the yearly mean intake of protein, total fatty acids, vitamin D, and phosphorus were significantly higher, and fiber and potassium intakes were lower, in the third PRAL tertile in the PREDIMED study population ($P < 0.05$).

Table 3 shows the association between PRAL and NEAP and the risk of osteoporotic fracture. A total of 114 new fracture events were documented in the PREDIMED study after a mean of 5.2 y of intervention and 8.9 y of total follow-up. The participants in the highest PRAL or NEAP tertiles had a 91% (HR: 1.91; 95% CI: 1.14, 3.19) or 87% (HR: 1.87; 95% CI: 1.10, 3.17) greater risk of osteoporotic fractures than those in the middle tertile, respectively. Likewise, the lowest tertile of the 2 scores showed a 73% (HR: 1.73; 95% CI: 1.03, 2.91) and 83% (HR: 1.83; 95% CI: 1.08, 3.09) higher risk of fracture compared with the second tertile, respectively, after adjusting for sociodemographic, anthropometric, and clinical variables. The restricted cubic spline analysis (**Figure 1**) suggests a nonlinear association between PRAL and fracture risk (P-nonlinearity = 0.007).

The comparative relations between BMD and PRAL are shown in **Table 4** and between BMD and NEAP in **Table 5**. The highest PRAL tertile was significantly associated with lower BMD at the TF, FN, TR, and FD but not at the LS. Similarly, the highest NEAP tertile was associated with lower BMD at the TF, FN, and TR but not at the LS and FD. **Supplemental Tables 2** and **3** show the analyses between T-scores and PRAL or NEAP. In the same trend, the highest PRAL and NEAP tertiles were significantly associated with a lower T-score at the TF, FN, and TR in the adjusted models.

None of the results of any of the sensitivity analyses differed from our principal findings (**Supplemental Tables 4–10**). When we excluded subjects with a previous history of fractures (151 participants in the PREDIMED, 10 participants in the PREDIMED-Plus), the fracture risk was significantly higher in the first tertile [HR (95% CI): 1.94 (1.09, 3.02) and 1.99 (1.12, 3.54) for PRAL and NEAP, respectively]. BMD was significantly lower in the third tertile for all locations except for the LS and FD. Similar findings were obtained when we excluded incident cases of osteoporosis-related fractures that occurred during the first year of follow-up in the PREDIMED study ($n = 7$) [HR (95% CI): 1.68 (1.00, 2.84) and 1.77 (1.03, 3.02) for PRAL and NEAP, respectively].

TABLE 1 Baseline characteristics of study participants according to tertiles of PRAL[1]

Variable	PREDIMED trial				PREDIMED-Plus trial			
	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	P^2	T1 ($n = 368$)	T2 ($n = 367$)	T3 ($n = 367$)	P^2
Age, y	67 ± 6	68 ± 6	67 ± 6	0.018	66 ± 5	65 ± 5	64 ± 5	<0.001
Women, % (*n*)	56 (163)	58 (169)	51 (148)	0.196	52 (190)	47 (174)	44 (163)	0.143
BMI, kg/m^2	29.6 ± 3.1	29.9 ± 3.3	29.4 ± 3.5	0.196	32.2 ± 3.2	32.7 ± 3.3	33.0 ± 3.5	0.006
Leisure-time energy expenditure in physical activity, MET-min/d	276 ± 274	250 ± 257	260 ± 259	0.486	431 ± 381	392 ± 352	343 ± 307	0.003
Smoking status, % (*n*)								
Never	60 (175)	68 (196)	58 (168)	0.200	46 (168)	44 (161)	36 (131)	0.015
Current	15 (42)	8 (24)	13 (39)		10 (38)	11 (39)	16 (60)	
Former	25 (73)	24 (70)	29 (83)		44 (162)	45 (168)	48 (176)	
Educational level, % (*n*)								
Illiterate/primary	78 (226)	76 (222)	74 (214)	0.776	56 (208)	54 (200)	45 (164)	0.019
Secondary	15 (42)	18 (52)	20 (59)		27 (99)	27 (101)	34 (126)	
Academic/graduate	7 (22)	6 (16)	6 (17)		17 (61)	18 (67)	21 (77)	
Previous fractures, % (*n*)	15 (42)	22 (63)	16 (46)	0.050	1 (5)	0 (1)	1 (4)	0.269
Diabetes, % (*n*)	46 (134)	52 (152)	57 (166)	0.029	25 (92)	19 (70)	22 (79)	0.143
Arterial hypertension, % (*n*)	90 (261)	85 (246)	82 (238)	0.022	83 (306)	83 (305)	81 (296)	0.624
eGFR, mL/(min · 1.73 m^2)	77.2 ± 17.4	75.5 ± 16.4	79.5 ± 17.1	0.021	84.6 ± 13.2	85.7 ± 12.7	85.0 ± 13.0	0.453
Medication use, % (*n*)								
Insulin	7 (19)	4 (12)	7 (20)	0.305	4 (14)	2 (7)	4 (14)	0.234
Calcium and vitamin D supplements	12 (35)	14 (41)	8 (22)	0.039	4 (16)	4 (15)	5 (17)	0.934
Oral anticoagulants	1 (2)	2 (5)	1 (2)	0.364	16 (59)	11 (42)	13 (48)	0.104
Oral antidiabetic drugs	32 (93)	33 (95)	39 (113)	0.157	19 (69)	14 (53)	15 (56)	0.236
Estrogens	2 (7)	2 (5)	3 (8)	0.604	2 (7)	2 (6)	3 (9)	0.655

[1]Values are means ± SDs or % (*n*). eGFR, estimated glomerular filtration rate; MET-min, metabolic equivalent of task minutes; PRAL, potential renal acid load; PREDIMED, PREvención con DIeta MEDiterranea; T, tertile.
[2] *P* values for comparisons of baseline parameters between 3 groups tested by 1-factor ANOVA for the continuous variable or Pearson's chi-square test for categorical variables.

Discussion

In the present study, we found a significant U-shaped association between PRAL and risk of osteoporotic fractures in a middle-aged and elderly population at high cardiovascular risk. Both high and low PRAL and NEAP values are associated with higher fracture risk. Nonetheless, the acid-forming potential of the diet was only associated with a lower BMD at different sites in a middle-aged and elderly population with metabolic syndrome.

To our knowledge, the U-shaped association between dietary acid load and osteoporotic fractures has never been reported before. Previous epidemiological studies have reported a U-shaped relation between dietary acid load and all-cause and cardiovascular mortality as well as T2D risk (26–28). Data from 2 prospective cohorts, the Swedish Mammography Cohort and the Cohort of Swedish Men, which included 81,697 men and women aged 45–84 y, showed that both excess diet alkalinity and acidity were associated with higher mortality risk after a

TABLE 2 Baseline dietary characteristics of study participants according to tertiles of PRAL[1]

Variable	PREDIMED trial				PREDIMED-Plus trial			
	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	P^2	T1 ($n = 368$)	T2 ($n = 368$)	T3 ($n = 367$)	P^2
Total energy intake, kcal/d	2260 ± 594	2260 ± 574	2405 ± 600	0.004	2400 ± 595	2370 ± 521	2610 ± 627	<0.001
Protein, g/d	90 ± 22	94 ± 21	101 ± 23	<0.001	92 ± 22	96 ± 20	108 ± 24	<0.001
Carbohydrates, g/d	234 ± 76	224 ± 69	236 ± 76	0.114	256 ± 74	236 ± 66	260 ± 85	<0.001
Total fatty acids, g/d	100 ± 29	104 ± 31	111 ± 31	<0.001	103 ± 30	106 ± 26	118 ± 30	<0.001
SFAs, g/d	25 ± 8	27 ± 9	30 ± 10	<0.001	24 ± 8	26 ± 8	32 ± 10	<0.001
MUFAs, g/d	51 ± 16	52 ± 16	55 ± 17	0.006	54 ± 16	54 ± 15	60 ± 16	<0.001
PUFAs, g/d	16 ± 6	16 ± 6	17 ± 6	0.251	17 ± 8	17 ± 7	18 ± 7	0.147
Alcohol, g/d	3 (0, 11)	2 (0, 11)	4 (0, 11)	0.914	5 (1, 14)	6 (1, 17)	4 (1, 15)	0.189
Fiber, g/d	25 ± 8	23 ± 7	22 ± 7	<0.001	30 ± 8	25 ± 8	24 ± 9	<0.001
Vitamin D, μg/d	6 ± 4	6 ± 3	6 ± 3	0.169	6 ± 3	6 ± 3	7 ± 3	0.005
Calcium, mg/d	987 ± 347	1030 ± 350	1070 ± 370	0.021	1020 ± 377	993 ± 306	1090 ± 376	<0.001
Potassium, mg/d	4460 ± 1160	4190 ± 1018	4080 ± 1017	<0.001	4940 ± 1083	4290 ± 902	4170 ± 983	<0.001
Phosphorus, mg/d	1620 ± 398	1690 ± 399	1820 ± 451	<0.001	1710 ± 444	1710 ± 392	1900 ± 466	<0.001
Magnesium, mg/d	372 ± 98	364 ± 95	370 ± 101	0.610	425 ± 105	391 ± 98	403 ± 108	<0.001
PRAL, mEq/d	−10 ± 14	−1 ± 2	9 ± 12	<0.001	−18 ± 9	−1 ± 3	13 ± 8	<0.001

[1]Values are means ± SDs or medians (IQRs). PRAL, potential renal acid load; PREDIMED, PREvención con DIeta MEDiterranea; T, tertile.
[2] *P* values for comparisons of baseline parameters between 3 groups tested by 1-factor ANOVA for variables with normal distribution or Kruskal-Wallis test for variables with non-normal distribution.

TABLE 3 Risk of osteoporotic fracture by tertiles of PRAL and NEAP in the PREDIMED trial[1]

	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	*P* q-trend
Follow-up: mean PRAL, mEq/d	−8.2 ± 5.2	1.1 ± 2.0	10.0 ± 4.7	
Fracture event, % (*n*)	14.5 (42)	10.3 (30)	14.5 (42)	
Crude model	1.46 (0.91, 2.32)	1 (Ref)	1.48 (0.93, 2.37)	0.068
Multivariable model[2]	1.65 (1.03, 2.63)	1 (Ref)	1.79 (1.11, 2.87)	0.013
Multivariable model[3]	1.73 (1.03, 2.91)	1 (Ref)	1.91 (1.14, 3.19)	0.007
Follow-up: mean NEAP, mEq/d	33.5 ± 2.8	38.7 ± 1.1	44.5 ± 2.9	
Fracture event, % (*n*)	14.5 (42)	10.3 (30)	14.5 (42)	
Crude model	1.83 (1.15, 2.94)	1 (Ref)	1.59 (0.98, 2.60)	0.014
Multivariable model[2]	1.82 (1.14, 2.91)	1 (Ref)	1.76 (1.07, 2.89)	0.008
Multivariable model[3]	1.83 (1.08, 3.09)	1 (Ref)	1.87 (1.10, 3.17)	0.007

[1] Values are means ± SDs, % (*n*), or HRs (95% CI). eGFR, estimated glomerular filtration rate; MET, metabolic equivalent of task; NEAP, net endogenous acid production; *P* q-trend, quadratic trend *P* value; PRAL, potential renal acid load; PREDIMED, PREvención con DIeta MEDiterranea; Ref, reference; T, tertile.
[2] Model adjusted for age (years), sex, BMI (kg/m^2), leisure-time physical activity (MET-minutes/day), educational level (illiterate/primary education, secondary education, and academic/graduate), intervention group, and smoking status (never, former, current smoker).
[3] Model additionally, adjusted for the eGFR [mL/(min · 1.73 m^2)], prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of previous fractures (yes/no), use of insulin (yes/no), use of estrogen drugs (yes/no), use of calcium and/or vitamin D supplementation (yes/no), total yearly mean carbohydrate intake (grams/day), total yearly mean fatty acid intake (grams/day), total yearly mean fiber intake (grams/day), total yearly mean vitamin D intake (micrograms/day), and total yearly mean energy intake (kilocalories/day).

15-y follow-up period (26). Similarly, a systematic review of 7 prospective cohort studies with 319,542 participants found a U-shaped association between PRAL and risk of T2D (27).

Acid-base balance is regulated within a tight interval in which any imbalance in blood pH or the bicarbonate buffer can affect health. The potential relation between the impact of diet-induced acid-base imbalance and several chronic diseases has attracted the attention of researchers for some time (26–30). Metabolic acidosis can also influence bone health in other ways, such as disrupting insulin sensitivity and glucose homeostasis, which can induce inflammation and oxidative stress (31, 32). Based on several measures of femoral BMD, our results showed that participants in the highest PRAL and NEAP tertiles had lower femoral BMD, with both scores within the range of values found in previous studies (11, 15, 16). Consistently high PRAL and NEAP values were associated with an increased risk of osteoporotic fracture, even after adjusting for major potential confounders such as sex, BMI, age, eGFR, and vitamin D and calcium supplementation. In vitro studies have shown that metabolic acidosis stimulates fibroblast growth factor 23

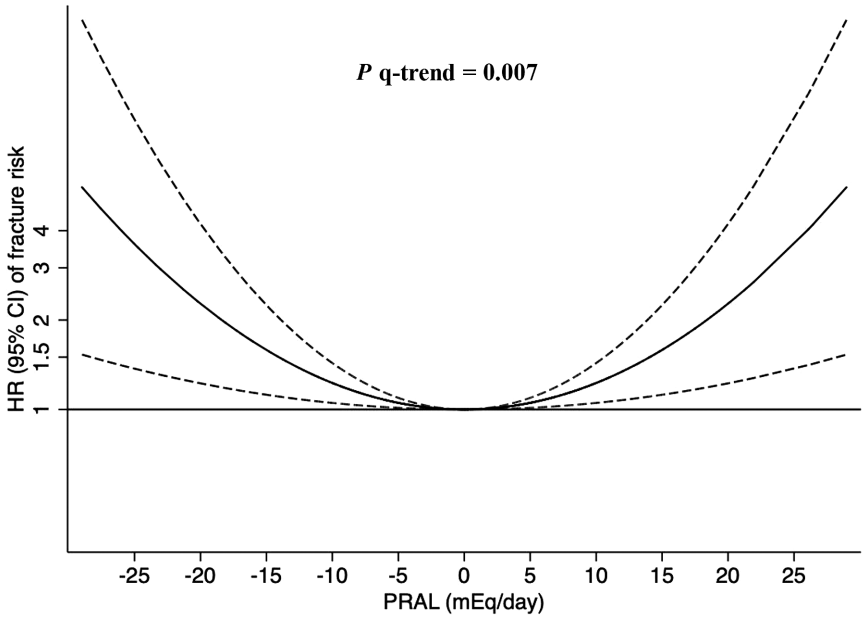

FIGURE 1 HRs (solid line) and 95% CIs for fracture risk associated with PRAL in the PREDIMED population ($n = 870$). The model was made with restricted cubic spline with 3 knots placed at the 10th, 50th, and 90th percentiles. A measure of 0 mEq/d was selected as the reference value. The model was adjusted for age (years), sex, BMI (kg/m^2), leisure-time physical activity (metabolic equivalent of task-minutes/day), educational level (illiterate/primary education, secondary education, and academic/graduate), intervention group, smoking status (never, former, current smoker), the eGFR [mL/(min · 1.73 m^2)], prevalence of diabetes (yes/no), prevalence of hypertension (yes/no), prevalence of previous fractures (yes/no), use of insulin (yes/no), use of estrogen drugs (yes/no), use of calcium and/or vitamin D supplementation (yes/no), total yearly mean carbohydrate intake (grams/day), total yearly mean fatty acid intake (grams/day), total yearly mean fiber intake (grams/day), total yearly mean vitamin D intake (micrograms/day), and total yearly mean energy intake (kilocalories/day). The curved solid line represents the HR, the horizontal solid line is the reference line, and dashed lines represent the 95% CIs. eGFR, estimated glomerular filtration rate; *P* q-trend, quadratic trend *P* value; PRAL, potential renal acid load; PREDIMED, PREvención con DIeta MEDiterranea.

TABLE 4 Comparisons of covariate-adjusted mean of BMD by tertiles of PRAL in the PREDIMED-Plus trial[1]

	T1 ($n = 368$)	T2 ($n = 367$)	T3 ($n = 367$)	*P*-Diff[2]	*P* q-trend[3]
Total femur, *n*	368	367	367		
PRAL, mEq/d	−17.61 ± 9.16	−1.38 ± 3.20	12.90 ± 7.76		
Adjusted BMD,[4,5] g/cm^2	1.030 ± 0.007	1.040 ± 0.007	1.010 ± 0.007	0.004	0.019
Lumbar spine: L1–L4, *n*	328	327	327		
PRAL, mEq/d	−17.23 ± 9.06	−1.45 ± 3.15	12.95 ± 7.73		
Adjusted BMD,[4] g/cm^2	1.170 ± 0.010	1.180 ± 0.010	1.160 ± 0.010	0.387	0.269
Femur neck, *n*	376	375	375		
PRAL, mEq/d	−17.68 ± 9.04	−1.49 ± 3.21	12.85 ± 7.72		
Adjusted BMD,[4,5] g/cm^2	0.930 ± 0.007	0.940 ± 0.007	0.910 ± 0.007	0.012	0.017
Trochanter, *n*	376	376	375		
PRAL, mEq/d	−17.76 ± 9.09	−1.48 ± 3.23	12.87 ± 7.72		
Adjusted BMD,[4,5,6] g/cm^2	0.870 ± 0.006	0.870 ± 0.006	0.840 ± 0.006	0.002	0.116
Femoral diaphysis, *n*	368	367	367		
PRAL, mEq/d	−17.61 ± 9.16	−1.38 ± 3.20	12.90 ± 7.76		
Adjusted BMD,[4,5] g/cm^2	1.240 ± 0.008	1.250 ± 0.008	1.210 ± 0.009	0.016	0.035

[1] Values are means ± SDs. BMD, bone mineral density; eGFR, estimated glomerular filtration rate; MET, metabolic equivalent of task; *P*-Diff, *P* value between tertiles; *P* q-trend, quadratic trend *P* value; PRAL, potential renal acid load; PREDIMED, PREvención con DIeta MEDiterranea; T, tertile.
[2] *P* value for comparisons between tertiles was tested by ANCOVA.
[3] *P* q-trend was tested by multivariable linear regression using the median of each PRAL tertile.
[4] Model adjusted for sex, age (years), smoking status (never, current, former), leisure-time physical activity (MET-minutes/day), educational level (illiterate/primary education, secondary education, and academic/graduate), glycated hemoglobin, prevalence of obesity (yes/no), prevalence of previous fractures (yes/no), eGFR [mL (min · 1.73 m^2)], total energy intake (kilocalories/day), use of calcium and/or vitamin D supplementation (yes/no), use of hormonal treatment (yes/no), and use of insulin (yes/no).
[5] $P < 0.05$ for comparisons between T2 and T3 with Tukey test.
[6] $P < 0.05$ for comparisons between T1 and T3 with Tukey test.

(FGF23) and osteoblastic cyclooxygenase 2 (COX2) activity, which are both important mediators of bone resorption and suppressors of serum concentrations of 1,25-dihydroxyvitamin D, which leads to weaker bone structure (33, 34).

Beyond the buffering capacity of bone, other mechanisms, including insulin resistance and inflammation, could contribute to explaining the association found between acidity and bone health. Low-grade metabolic acidosis has been related to reduced insulin secretion, higher insulin resistance (31), higher blood pressure, and increased hypertension risk (9), all risk factors for osteoporotic fractures. Moreover, in a recent study conducted with 172 elderly subjects, higher tertiles of NEAP were associated with lower concentrations of type I collagen cross-linked C-telopeptide (CTX-1) (35), but further research is necessary to determine whether dietary acid load could modulate bone cross-linkers.

A recent meta-analysis showed that alkaline supplementation had a protective effect on bone metabolism, reflected as a

TABLE 5 BMD according to tertiles of NEAP in the PREDIMED-Plus trial[1]

	T1	T2	T3	*P*-Diff[2]	*P*-trend[3]
Total femur, *n*	368	367	367		
NEAP, mEq/d	29.27 ± 3.73	37.02 ± 1.83	45.90 ± 5.07		
Adjusted BMD,[4,5,6] g/cm^2	1.030 ± 0.007	1.030 ± 0.007	1.010 ± 0.007	0.012	0.010
Lumbar spine: L1–L4, *n*	328	327	327		
NEAP, mEq/d	29.36 ± 3.76	36.98 ± 1.80	45.95 ± 5.14		
Adjusted BMD,[4] g/cm^2	1.170 ± 0.010	1.180 ± 0.010	1.160 ± 0.010	0.612	0.437
Femur neck, *n*	376	375	375		
NEAP, mEq/d	29.28 ± 3.69	36.98 ± 1.83	45.90 ± 5.07		
Adjusted BMD,[4,5] g/cm^2	0.930 ± 0.007	0.940 ± 0.007	0.910 ± 0.007	0.024	0.037
Trochanter, *n*	376	376	375		
NEAP, mEq/d	29.25 ± 3.70	36.97 ± 1.83	45.90 ± 5.07		
Adjusted BMD,[4,5,6] g/cm^2	0.870 ± 0.006	0.870 ± 0.006	0.840 ± 0.006	<0.001	<0.001
Femoral diaphysis, *n*	368	367	367		
NEAP, mEq/d	29.27 ± 3.73	37.02 ± 1.83	45.90 ± 5.07		
Adjusted BMD,[4] g/cm^2	1.240 ± 0.008	1.240 ± 0.008	1.220 ± 0.008	0.068	0.042

[1] Values are means ± SDs. BMD, bone mineral density; eGFR, estimated glomerular filtration rate; MET, metabolic equivalent of task; NEAP, net endogenous acid production; *P*-Diff, *P* value between tertiles; PREDIMED, PREvención con DIeta MEDiterranea; T, tertile.
[2] *P* values for comparisons between tertiles were tested by ANCOVA for the adjusted model.
[3] *P* trend was tested by multivariable lineal regression using the median of each NEAP tertile.
[4] Model adjusted for sex, age (years), smoking status (never, current, former), leisure-time physical activity (MET-minutes/day), educational level (illiterate/primary education, secondary education, and academic/graduate), glycated hemoglobin, prevalence of obesity (yes/no), prevalence of previous fractures (yes/no), eGFR [mL (min · 1.73 m^2)], total energy intake (kilocalories/day), use of calcium and/or vitamin D supplementation (yes/no), use of hormonal treatment (yes/no), and use of insulin (yes/no).
[5] $P < 0.05$ for comparisons between T2 and T3 with Tukey test.
[6] $P < 0.05$ for comparisons between T1 and T3 with Tukey test.

decrease in renal calcium excretion without an impact on either bone formation markers or BMD (36).

However, lower PRAL and NEAP dietary acid loads were associated with an increased risk of osteoporosis fractures in the PREDIMED study. These results were supported by 2 different sensitive analyses excluding subjects with a previous history of fractures and those who developed osteoporotic fractures during the first year of the study. Although it is not clear why the excess base-forming potential of the diet was associated with a higher risk of osteoporotic fractures, the difference in the quantity and quality of dietary protein intake could partly explain this association, although our data do not support this possible explanation (37). Protein intake has a positive effect on the release of several hormones, such as insulin-like growth factor I (IGF-I) and growth hormone, which stimulate bone formation and could reduce the impact of protein-related acidity (38). Discrepancies observed in the association between dietary acid load and osteoporotic fractures or densitometry parameters might be explained by differences in total leisure-time physical activity, which is substantially higher in the PREDIMED-Plus study subjects (39, 40). Although low BMD is an important conditioning factor for osteoporotic fractures, at equal BMD values, fractures occur more easily in some bones than others, implying that bone strength is also conditioned by bone geometry and microarchitecture (41). Further working hypotheses and research are necessary to determine whether the acid- or base-forming potential of diet could affect bone strength beyond differences in BMD.

This study benefits from 2 population-based prospective trials with detailed data on dietary intake and potential confounders, a large number of participants, long-term follow-up in the PREDIMED trial, and the use of yearly mean values for PRAL and NEAP in the prospective analysis, which allowed for a more accurate estimation of dietary changes during the follow-up and reduced the within-person variation. Other strengths are the use of a DXA scan for measuring BMD and an impartial score for recognizing fractures despite being based on fracture sets identified by diagnostic codes. The sensitive analysis findings also contribute to strengthening our results. Nevertheless, our results should be interpreted in the context of some limitations. We used 2 well-established, accepted, and validated algorithms to estimate PRAL and NEAP that have proven to be well correlated with renal net acid excretion and body acidity, but we do not have specific biomarkers in urine or plasma. Similarly, we did not have circulating biomarkers for vitamin D, glycated hemoglobin (only available for PREDIMED-Plus participants), or calcium, which would allow us to rule out their possible influence. As this is an observational analysis, and although we adjusted for major potential confounders, we cannot dismiss the role of residual confounding by unknown factors, such as mechanical loading, muscle area, sarcopenic obesity, or others, which makes it more difficult to draw causal conclusions. Since fractures were assessed in the context of a dietary intervention trial, we cannot completely rule out a potential residual effect of nutritional interventions on PRAL estimation. To decrease this potential bias as well as others related to the use of FFQs to estimate PRAL, we used the yearly mean for PRAL estimation along with the trial, and all of the analyses were adjusted for the intervention group. However, we cannot dismiss the possibility that the low PRAL values reflected a less bone-protective nutritional pattern unrelated to acid-base status. Additionally, to take a critical view of our work, our analyses are secondary objectives in the context of 2 cohort studies with well-defined objectives not related to bone metabolism. For this reason, we cannot rule out the possibility of an increased likelihood of false positives and type I errors after randomization. A final consideration is that the analyses were conducted with middle-aged and elderly subjects at high cardiovascular risk living in a Mediterranean country and might not be generalizable beyond this population.

In summary, the results of our study suggest that both high and low dietary acid loads are associated with a higher risk of osteoporotic fractures, although only high dietary acid showed an inverse relation with BMD in a senior population at high cardiovascular risk and metabolic syndrome. The potential mechanism that would explain the association between low dietary acid and fracture risk could also be mediated by mechanisms other than lower BMD and needs to be researched further.

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References

1. World Health Organization. Prevention and management of osteoporosis. World Health Organ Tech Rep Ser [Internet] 2003 [cited 2018 Sep 2];921:1–164. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15293701.
2. Schürer C, Wallaschofski H, Nauck M, Völzke H, Cummings SR, Melton LJ, Azagra R, López-Expósito F, Martin-Sánchez JC, Aguyé A, et al. Fracture Risk and Risk Factors for Osteoporosis. Dtsch Arztebl Int. [Internet] 2015;112:365–72, [cited 2015 Jan 17]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24322478.
3. Ezzati M, Riboli E. Behavioral and dietary risk factors for noncommunicable diseases. N Engl J Med [Internet] 2013 [cited 2018 Sep 3];369:954–64. Available from: http://www.nejm.org/doi/10.1056/NEJMra1203528.
4. Cauley JA, Cawthon PM, Peters KE, Cummings SR, Ensrud KE, Bauer DC, Taylor BC, Shikany JM, Hoffman AR, Lane NE, et al. Risk factors for hip fracture in older men: the Osteoporotic Fractures in Men Study (MrOS). J Bone Miner Res [Internet] 2016 [cited 2018 Sep 3];31:1810–9. Available from: http://doi.wiley.com/10.1002/jbmr.2836.
5. Lee DSH, Markwardt S, Goeres L, Lee CG, Eckstrom E, Williams C, Fu R, Orwoll E, Cawthon PM, Stefanick ML, et al. Statins and physical activity in older men: the Osteoporotic Fractures in Men Study. JAMA Intern Med 2014;174:1263–70.
6. Garcia AH, Franco OH, Voortman T, de Jonge EAL, Gordillo NG, Jaddoe VW V, Rivadeneira F, van den Hooven EH. Dietary acid load in early life and bone health in childhood: the Generation R Study. Am J

Clin Nutr. [Internet] 2015 [cited 2018 Sep 3];102:1595–603. Available from: https://academic.oup.com/ajcn/article/102/6/1595/4555178.
7. Mangano KM, Walsh SJ, Kenny AM, Insogna KL, Kerstetter JE. Dietary acid load is associated with lower bone mineral density in men with low intake of dietary calcium. J Bone Miner Res. [Internet] 2014 [cited 2018 Sep 3];29:500–6. Available from: http://doi.wiley.com/10.1002/jbmr.2053.
8. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. J Am Diet Assoc [Internet] 1995 [cited 2018 Sep 3];95:791–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7797810.
9. Carnauba R, Baptistella A, Paschoal V, Hübscher G. Diet-induced low-grade metabolic acidosis and clinical outcomes: a review. Nutrients [Internet] 2017 [cited 2018 Sep 3];9:538. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28587067.
10. Wynn E, Lanham-New SA, Krieg M-A, Whittamore DR, Burckhardt P. Low estimates of dietary acid load are positively associated with bone ultrasound in women older than 75 years of age with a lifetime fracture. J Nutr [Internet] 2008 [cited 2018 Sep 3];138:1349–54. Available from: https://academic.oup.com/jn/article/138/7/1349/4670210.
11. 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 [Internet] 2007 [cited 2018 Sep 3];85:1134–41. Available from: https://academic.oup.com/ajcn/article/85/4/1134/4648888.
12. Frassetto LA, Todd KM, Morris RC, Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr. [Internet] 1998 [cited 2018 Sep 3];68:576–83. Available from: https://academic.oup.com/ajcn/article/68/3/576/4648631.
13. Esche J, Johner S, Shi L, Schönau E, Remer T. Urinary citrate, an index of acid-base status, predicts bone strength in youths and fracture risk in adult females. J Clin Endocrinol Metab 2016;101:4914–21.
14. Frassetto L, Banerjee T, Powe N, Sebastian A. Acid balance, dietary acid load, and bone effects—a controversial subject. Nutrients [Internet] 2018 [cited 2018 Sep 10]. 10:517. Available from: http://www.mdpi.com/2072-6643/10/4/517.
15. de Jonge E, Koromani F, Hofman A, Uitterlinden AG, Franco OH, Rivadeneira F, Kiefte-de Jong JC. Dietary acid load, trabecular bone integrity, and mineral density in an ageing population: the Rotterdam study. Osteoporos Int 2017; [cited 2018 Sep 3]. 28:2357–65. Available from: http://link.springer.com/10.1007/s00198-017-4037-9.
16. McLean RR, Qiao N, Broe KE, Tucker KL, Casey V, Cupples LA, Kiel DP, Hannan MT. Dietary acid load is not associated with lower bone mineral density except in older men. J Nutr [Internet] 2011; [cited 2018 Sep 3]. 141:588–94. Available from: https://academic.oup.com/jn/article/141/4/588/4630600.
17. Fenton TR, Tough SC, Lyon AW, Eliasziw M, Hanley DA. Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. Nutr J [Internet]. 2011; [cited 2018 Sep 3]. 10:41. Available from: http://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-10-41.
18. Warriner AH, Patkar NM, Curtis JR, Delzell E, Gary L, Kilgore M, Saag K, Thomas-John M, Codd MB, Manne S,, et al., Which fractures are most attributable to osteoporosis?, J Clin Epidemiol [Internet]. 2011; [cited 2016 Jul 16]. 64:46–53. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0895435610002635.
19. Alberti K, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart J-C, James WPT, Loria CM, Smith SC, et al. Harmonizing the metabolic syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation [Internet] 2009 [cited 2014 Jul 9]. 120:1640–5. Available from: https://doi.org/10.1161/CIRCULATIONAHA.109.192644.
20. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, Perez-Bauer M, Martínez-González MA, Salas-Salvadó J, Martín-Moreno JM. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr [Internet] 2010 [cited 2015 Dec 6];103:1808–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20102675.
21. Mataix J. Tablas de Composición de Alimentos. 4th ed. Granada (Spain): Universidad de Granada; 2003.
22. Moreiras O, Carvajal A, Cabrera L , editors. Tablas de Composición de Alimentos [food-composition tables]. 9th ed. Madrid (Spain): Ediciones Pirámide; 2005.
23. Osuna-Padilla IA, Leal-Escobar G, Garza-García CA, Rodríguez-Castellanos FE. Carga ácida de la dieta; mecanismos y evidencia de sus repercusiones en la salud. Nefrología. [Internet] 2019[cited 2020 Jul 16];39:343–54. Available from: https://pubmed.ncbi.nlm.nih.gov/30737117/.
24. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol 1994 [cited 2016 Feb 13];139:1197–209. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8209878.
25. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, Kusek JW, Eggers P, Lente F V, Greene T, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.
26. Xu H, Åkesson A, Orsini N, Håkansson N, Wolk A, Carrero JJ. Modest U-shaped association between dietary acid load and risk of all-cause and cardiovascular mortality in adults. J Nutr 2016 [Internet] [cited 2018 Sep 3];146:1580–5. Available from: https://academic.oup.com/jn/article/146/8/1580/4630466.
27. Jayedi A, Shab-Bidar S. Dietary acid load and risk of type 2 diabetes: a systematic review and dose-response meta-analysis of prospective observational studies. Clin Nutr ESPEN 2018 [cited 2018 Sep 3];23:10–8. [Internet]. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2405457717304709.
28. Akter S, Nanri A, Mizoue T, Noda M, Sawada N, Sasazuki S, Tsugane S; Japan Public Health Center–based Prospective Study Group. Dietary acid load and mortality among Japanese men and women: the Japan Public Health Center-based Prospective Study. Am J Clin Nutr [Internet] 2017 [cited 2018 Sep 3];106:146–54. Available from: https://academic.oup.com/ajcn/article/106/1/146-154/4569818.
29. Della Guardia L, Roggi C, Cena H. Diet-induced acidosis and alkali supplementation. Int J Food Sci Nutr [Internet] 2016 [cited 2018 Sep 3];67:754–61. Available from: https://www.tandfonline.com/doi/full/10.1080/09637486.2016.1198889.
30. Fagherazzi G, Vilier A, Bonnet F, Lajous M, Balkau B, Boutron-Ruault M-C, Clavel-Chapelon F. Dietary acid load and risk of type 2 diabetes: the E3N-EPIC cohort study. Diabetologia [Internet] 2014 [cited 2018 Sep 3];57:313–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24232975.
31. Della Guardia L, Thomas MA, Cena H. Insulin sensitivity and glucose homeostasis can be influenced by metabolic acid load. Nutrients 2018 [cited 2018 Sep 3]. [Internet];10:618. Available from: http://www.mdpi.com/2072-6643/10/5/618.
32. Frassetto LA, Sebastian A. How metabolic acidosis and oxidative stress alone and interacting may increase the risk of fracture in diabetic subjects. Med Hypotheses [Internet] 2012 [cited 2018 Sep 3];79:189–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22583559.
33. Krieger NS, Bushinsky DA. Stimulation of fibroblast growth factor 23 by metabolic acidosis requires osteoblastic intracellular calcium signaling and prostaglandin synthesis. Am J Physiol Ren Physiol 2017;313:F882–6.
34. Krieger NS, Culbertson CD, Kyker-Snowman K, Bushinsky DA. Metabolic acidosis increases fibroblast growth factor 23 in neonatal mouse bone. Am J Physiol Ren Physiol 2012;303;F431.
35. Fatahi S, Rahmani J, Kord Varkaneh H. The relationship between dietary acid load and bone metabolism markers in Tehranian elderly. Razi J Med Sci [Internet] 2018 [cited 2018 Oct 11];25:58–68. Available from: http://rjms.iums.ac.ir/browse.php?a_code = A-10-3775-1&slc_lang = en&sid = 1.
36. Lambert H, Frassetto L, Moore JB, Torgerson D, Gannon R, Burckhardt P, Lanham-New S. The effect of supplementation with alkaline potassium salts on bone metabolism: a meta-analysis. Osteoporos Int 2015;26:1311–8.
37. Cao JJ. High dietary protein intake and protein-related acid load on bone health. Curr Osteoporos Rep [Internet] 2017 [cited 2018 Sep 3];15:571–6. Available from: http://link.springer.com/10.1007/s11914-017-0408-6.

38. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev [Internet] 2008 [cited 2018 Sep 3];29:535–59. Available from: https://academic.oup.com/edrv/article-lookup/doi/10.1210/er.2007-0036.
39. Ross M, Lithgow H, Hayes L, Florida-James G. Potential cellular and biochemical mechanisms of exercise and physical activity on the ageing process. Subcellular Biochemistry 2019;91:311–38.
40. Hart PD, Buck DJ. The effect of resistance training on health-related quality of life in older adults: systematic review and meta-analysis. Heal Promot Perspect 2019;9:1–12.
41. Njeh CF, Fuerst T, Diessel E, Genant HK. Is quantitative ultrasound dependent on bone structure? A reflection. Osteoporos Int [Internet] 2001 [cited 2018 Oct 11];12:1–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11305077.