Associations between age-related changes in bone microstructure and strength and dietary acid load in a cohort of community-dwelling, healthy men and postmenopausal women

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ABSTRACT

Background: The importance of dietary acid load (DAL) in the pathogenesis of osteoporosis is still debated. Age-related changes in bone microstructure and strength in relation to DAL remain largely unexplored.

Objectives: We investigated the associations between changes in areal and volumetric bone mineral density (BMD), bone microstructure and strength, fracture risk, and DAL in a prospective cohort of 65-y-old healthy men and postmenopausal women.

Methods: Potential renal acid load (PRAL; mEq/d) was calculated as a DAL proxy to characterize participants' diet as alkaline (Alk-D; PRAL < -5), neutral (Neut-D; $-5 \leq$ PRAL ≤ 5), or acidic (Acid-D; PRAL >5). We measured areal BMD (aBMD) by DXA, and distal radius and tibia bone microstructure using high-resolution peripheral quantitative computed tomography, at baseline ($n = 853$) and after 6.1 ± 1.4 y ($n = 708$). Bone strength was estimated using finite element analyses at baseline and after 3.0 ± 0.5 y ($n = 613$). Prevalent and incident fractures were recorded.

Results: The majority of the participants (59%) had an Alk-D, while 23% had a Neut-D, and 18% an Acid-D. Baseline aBMD and bone microstructure and strength did differ or were slightly better in women or men with an Acid-D versus those consuming an Alk-D or Neut-D. Indeed, women with an Acid-D had higher trabecular number ($P = 0.010$ vs. Alk-D; $P = 0.001$ vs. Neut-D), while men had higher hip and radius aBMD ($P = 0.008$ and 0.024 vs. Neut-D, respectively) and radius strength ($P = 0.026$ vs. Neut-D). Over the follow-up, women in the Acid-D group experienced lower cortical and endocortical bone loss at the radius than did the Alk-D and Neut-D groups (cortical thickness, $P = 0.008$ and < 0.001; trabecular area, $P = 0.001$ and < 0.001, respectively). No association between fractures and PRAL was observed.

Conclusions: These null or favourable associations between baseline values or changes in aBMD, bone microstructure and strength, and DAL in this cohort of 65-y-old healthy individuals do not support adverse DAL-mediated effects on bone. This trial was registered at http://www.isrctn.com as ISRCTN11865958. *Am J Clin Nutr* 2020;112:1120–1131.

Keywords: dietary acid load, acid-ash theory, osteoporosis, bone microstructure, bone mineral density, bone strength, fractures

Introduction

The "acid-ash hypothesis" postulates that dietary acid load (DAL) may be a risk factor for osteoporosis (1–4). Foods with acid-generating potential, because of their sulfur and phosphate

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Supplemental Tables 1 and 2 and Supplemental Figures 1–3 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/ajcn/.

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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Abbreviations used: Acid-D, acidic diet; aBMD, areal bone mineral density; Alk-D, alkaline diet; ALM, appendicular lean mass; BMD, bone mineral density; Ct, cortical; Ct.Ar, cortical area; Ct.BMD, cortical volumetric BMD; DAL, dietary acid load; FRAX, fracture risk assessment tool; GERICO, Geneva Retirees Cohort; HR-pQCT, high-resolution peripheral quantitative computed tomography; IGF-1, insulin-like growth factor 1; NEAP, net endogenous acid production; Neut-D, neutral diet; P1NP, procollagen type 1 amino-terminal propeptide; pQCT, peripheral quantitative computed tomography; PRAL, potential renal acid load; PTH, parathyroid hormone; Tb, trabecular; Tb.Ar, trabecular area; Tb.BMD, trabecular volumetric BMD; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Sp.SD, Tb separation SD;Tb.Th, trabecular thickness; Tt, total; Tt.Ar, total area; Tt.BMD, total volumetric BMD; vBMD, volumetric BMD; β-CTX, β-carboxyterminal cross-linked telopeptide of type I collagen.

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content (animal protein and grains), have been suggested to cause chronic, low-grade metabolic acidosis, unless this effect is counterbalanced by alkali salts of organic acids (e.g., citrate and bicarbonate) provided by fruit and vegetable consumption (5). Among other mechanisms (i.e., renal, intra- and extracellular buffers) to correct acid-base imbalances, bone mineral might be mobilized to neutralize DAL, with potentially negative consequences for bone health over time (3, 6). Mechanistically, bone demineralization in response to acute metabolic acidosis has been linked to impaired osteoblastic function, enhanced bone resorption (3, 7), and increased calcium excretion, which is attributed by some (7, 8), but not others (9, 10), to calcium release from bone. Over the past decades, the "acid-ash hypothesis" of osteoporosis has been a subject of reviews and debates (3, 4, 6, 8, 11–13). This has caused confusion for the general public and poses challenges for dietary advice for bone health, particularly with respect to the benefits of dietary proteins on bone and muscle (6, 12–16).

In epidemiological studies, DAL has been estimated by potential renal net acid load (PRAL) and net endogenous acid production (NEAP) algorithms based on nutritional data. PRAL considers the nutrient ionic balance and intestinal absorption rates of major nutrients with acid-forming (protein, phosphorous) and base-forming (potassium, magnesium, and calcium) potential (17). NEAP consists of a simpler expression of DAL based on the ratio of protein to potassium intakes (18). Several observational studies have shown inverse (19–24) or no (25–27) associations between PRAL and/or NEAP and areal bone mineral density (aBMD). These studies are, however, limited by their cross-sectional design and the assessment of aBMD using DXA or calcaneal ultrasound, while neither of these techniques allow a reliable distinction of cortical (Ct) and trabecular (Tb) bone or the evaluation of bone strength. Several prospective studies largely do not support an association between PRAL/NEAP and fracture risk (20, 25, 28) or bone loss as assessed by DXA or peripheral quantitative computed tomography (pQCT) (29, 30). Furthermore, 2 large meta-analyses by Fenton et al. concluded that there was insufficient evidence to suggest that DAL contributes to changes in calcium balance (9) or bone health status (14). Given these discordant results and the scarcity of longitudinal data, it is critical to investigate more refined bone phenotypes such as bone microstructure (i.e., bone geometry, mineral density, microarchitecture) and bone strength. These, albeit critical determinants of fracture risk (31), have never been assessed in relation to DAL using state-of-the-art technologies such as high-resolution pQCT (HR-pQCT) and microfinite element analyses. Such data obtained in a well-characterized and homogenous cohort of healthy older people may identify microstructural defects not captured by aBMD (32, 33), and could thus provide potential mechanisms for a relation, if any, between bone fragility and DAL.

Therefore, we investigated the associations between baseline values and changes of bone microstructure and strength, as well as fracture risk, and DAL in a population-based longitudinal cohort of healthy postmenopausal women and men.

Methods

Study population

The present study is a cross-sectional and longitudinal analysis of the Geneva Retirees Cohort (GERICO; http://www.isrctn.com/, ISRCTN11865958). The objectives, design, and inclusion/exclusion criteria of the original study have been detailed previously (34). In brief, GERICO is a prospective cohort designed to determine genetic, musculoskeletal, and lifestyle factors associated with bone microstructure and fracture risk in newly retired men and women (mean age: 65 ± 1 y) living in the canton of Geneva, Switzerland. Healthy, mostly Caucasian, community-based individuals aged 63–67 y were recruited around the time of their retirement during the period 2008–2011 through advertisements in the local press, the Geneva University Hospitals, or local workplaces. Exclusion criteria were major comorbidities that may affect skeletal health (34). The primary outcomes of the study were changes in total volumetric bone mineral density (Tt.BMD) and bone strength (failure load) at the distal radius. Indeed, these parameters were identified as the strongest predictors of fracture risk in this cohort (31). Other bone microstructural and strength parameters at the distal radius or tibia, and DXA-derived aBMD at the hip, spine, and radius, were evaluated as secondary outcomes. Participants were invited to attend 2 follow-up visits at intervals of ∼3 y during the periods 2012–2014 (first follow-up visit) and 2015–2018 (second follow-up visit), during which incident fractures were recorded and changes in musculoskeletal bone traits were assessed. For the cross-sectional analyses, we included postmenopausal women and men with available nutritional and bone data at baseline (n = 853). Participants included in the cross-sectional analyses were further included in the longitudinal analyses if they attended at least one of the 2 follow-up visits (mean ± SD: 6.1 ± 1.4 y; n = 708) (**Supplemental Figure 1**). For the participants who attended both follow-up visits, the data from their latest visit were considered in the longitudinal analysis. The protocol of the original study was approved by the Geneva University Hospitals' Ethics Committee and participants provided a written informed consent prior to their involvement in any study procedure.

Assessment of DAL

To assess habitual dietary intake at baseline, participants were asked to record the amount of all food and beverages they consumed over a 3-d period (2 weekdays and 1 weekend day). All participants were given a small booklet with visual and written guidance on how to accurately describe food items and beverages [including (brand) name of the food, preparation methods, ingredient list for recipes] and estimated portion sizes. Food records were reviewed by a dietitian or trained research staff during a face-to-face interview with the participants who were asked to complete and clarify all entries. Estimates of energy and nutrient intakes were based on the Centre d'Information sur la Qualité des Aliments (Information Center on Food Quality; CIQUAL) food-composition tables (Ed. Tec&Doc, Lavoisier available online at https://ciqual.anses.fr/, and INRA, Maison Alfort, France, 1995) and the Swiss Food Code and Nutrient Database using the nutritional software PRODI® version 5.3 (Nutri-Science GmbH, Freiburg, Germany). The following dietary variables were extracted: total energy intake (kilocalories per day), total protein intake (grams per day) and micronutrient intake (calcium, phosphorus, potassium, and magnesium in milligrams per day).

The PRAL and NEAP were calculated as measures of dietary acid-base load based on participants' 3-d food records at baseline. PRAL was estimated using the formula developed by Remer and

Manz (5): PRAL (mEq/d) = [0.4888 × protein (g/d)] + [0.0366 phosphorus (mg/d)] − [0.0205 × potassium (mg/d)] − [0.0125 × calcium (mg/d)] − [0.0263 × magnesium (mg/d)]. NEAP was estimated based on the equation provided by Frassetto et al. (18): NEAP (mEq/d) = [54.5 protein (g/d)/potassium (mEq/d)] − 10.2.

Three DAL groups were defined according to PRAL: alkaline diet (Alk-D; PRAL < -5 mEq/d), neutral diet (Neut-D; $-5 \leq$ PRAL ≤ 5 mEq/d), and acidic diet (Acid-D; PRAL > 5 mEq/d).

Assessment of aBMD and body composition

aBMD at the lumbar spine, proximal femur, and distal radius was measured at baseline and follow-up visits by DXA (Hologic QDR Discovery Instrument; Hologic, Inc.). The CV of repeated bone mineral density (BMD) measurements ranged from 1.0% to 1.6% (35). Body composition including total and appendicular lean mass (ALM) and fat mass was assessed using the same equipment at the same time points. According to the updated recommendations of the European Working Group on Sarcopenia in Older People (EWGSOP2), ALM/(height)^2 was defined as low, if it was < 7 kg/m^2 in men and < 5.5 kg/m^2 in women (36).

Assessment of bone microstructure and bone strength

At baseline and follow-up, volumetric BMD (vBMD) and microstructural parameters for total (Tt), Ct, and Tb bone compartments were assessed by HR-pQCT (XtremCT scanner; Scanco Medical, Brüttisellen, Switzerland) at the distal radius and tibia (for a detailed description of these measurements, please refer to references 33 and 35). Changes in vBMD and microstructural parameters over the follow-up period were assessed following a cross-sectional area-based registration to ensure common regions of interest on 2 successive HR-pQCT images of the same participant and were expressed as annual percentage changes from baseline. For this purpose, the baseline HR-pQCT variables were recalculated in a common region that was considered for the follow-up measurement. This region represented (mean ± SD) 92% ± 7% for the radius and 95% ± 4% for the tibia of the baseline area used in the cross-sectional analysis. Recorded variables were as follows: Tt, Ct, and Tb vBMD (Tt.BMD, Ct.BMD, and Tb.BMD; mg hydroxyapatite /cm^3) and areas (Tt.Ar, Ct.Ar, and Tb.Ar; mm^2); Tb number (Tb.N; mm^{-1}), thickness (Tb.Th; mm), and separation (Tb.Sp; mm); Tb.Sp.SD (mm), an estimate of the heterogeneity of the Tb structure; Ct thickness (Ct.Th; mm); and Ct porosity (Ct.Po) quantified with an automatic segmentation of the Ct compartment using a dual threshold technique (37). Failure load, a proxy of bone strength, was estimated by applying finite element analysis on segmented HR-pQCT images using the finite element (FE) solver integrated in the Image Processing Language (IPL) software (version 1.15; Scanco Medical AG) (11). Changes in failure load were assessed in 613 participants after a follow-up of 3.0 ± 0.5 y, without registration in order to avoid variations in results due to differences in stack height, as previously reported (38).

Incident fractures during follow-up

Prevalent fractures were recorded at baseline during an interview, in which all subjects were asked if they had suffered a fracture in adult age, details about the fracture site, age at time of fracture, type and intensity of trauma associated with the fracture, and modalities of fracture treatment. During the follow-up period, subjects participated in structured interviews scheduled at follow-up visits and were asked to report incident fractures, the bone site, and the circumstances that led to the injury. Fractures were further confirmed by medical/surgical, radiology reports, or records of hospitalization. For the present analysis, we considered only low-trauma clinical fractures (i.e., fractures that resulted from falls from a standing height or lower, and with the exclusion of skull, finger, and toe fractures).

Assessment of bone turnover markers, bone-related hormones, and renal function

Blood samples were drawn in the morning following an overnight fast at the end of the 3-d dietary assessment period to reflect the biological profile concomitant with the assessment of DAL. Serum and plasma samples were isolated and stored at −80°C until batch analyses. Serum procollagen type 1 amino-terminal propeptide (P1NP), β-carboxyterminal cross-linked telopeptide of type I collagen (β-CTX), total 25-hydroxyvitamin D, and parathyroid hormone (PTH) were assessed on a Cobas-6000 analyzer using Elecsys reagents (Roche Diagnostics, Rotkreuz, Switzerland) (39). Insulin-like growth factor 1 (IGF-1) was determined using an automated chemiluminescence-based immunoassay (Immunodiagnostic Systems IDS-iSYS Nordic) (39). Creatinine was determined by ultraviolet-visible absorption spectrophotometry on a routine procedure analyzer. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate glomerular filtration rate (40).

Assessment of covariates

Height (measured using a Harpenden Stadiometer; Holtain Ltd.) and weight (measured to the nearest 0.1 kg using standard electronic scales) were used to BMI (kg/m^2). Physical activity was evaluated by a face-to-face questionnaire that uses an inventory of 45 activities to estimate the time spent on usual walking, cycling, stair climbing, organized sports, and recreational activity over the past 12 mo. The collected data were converted to physical activity energy expenditure (kilocalories per day) using validated formulas developed by Ainsworth et al. (41). Established clinical risk factors of fractures were assessed as part of the evaluation of the 10-y probability of fracture using the Fracture Risk Assessment (FRAX) tool (www.shef.ac.uk/FRAX/) (42). In addition, the use of calcium and vitamin D supplements and anti-osteoporotic drugs were recorded.

Statistical analysis

Variables are presented as means ± SDs, SEs, or percentages. Data distributions were checked for normality using the Shapiro-Francia W test and skewness/kurtosis tests. Whenever feasible, non-Gaussian variables, including covariates, were normalized using simple mathematical transformations. To account for the lack of normality of some of the variables included in the models, most of the statistical analyses were performed using nonparametric tests, and the Huber-White robust sandwich estimator of SEs was used for linear regression analyses. For the cross-sectional analysis, bone parameters at baseline were expressed

as sex-specific z scores calculated on the study population, and all analyses were performed in men and postmenopausal women separately. Comparisons of participants' characteristics and baseline values and changes in bone parameters according to the 3 DAL groups were performed using Kruskal-Wallis tests for continuous data and χ^2 tests for categorical variables. In case of a significant group effect, pairwise comparisons were performed using Mann-Whitney tests. The associations between bone traits and PRAL were assessed using multivariate linear regression models adjusted for potential confounders, which were selected on the basis of available literature and/or differences among DAL groups (at baseline or during the follow-up). We further explored whether the effects of DAL group were different according to baseline BMD. Cox proportional hazards models adjusted for age or FRAX with/without BMD were used to estimate the HRs with 95% CIs for incident fractures in both sexes separately. All analyses were performed using STATA software, version 14.0 (StataCorp LP).

Results

Subjects' characteristics

Baseline characteristics of the study population stratified by PRAL groups are presented in **Table 1**. The majority of the participants (59%) had an Alk-D, whereas 23% had a Neut-D and 18% an Acid-D. There were no age differences between PRAL groups, but the proportion of men was greater in the Acid-D group ($P < 0.0001$). Therefore, the results of all subsequent analyses on bone traits are presented in the total cohort, but also by sex. Compared with participants consuming an Alk-D and a Neut-D, those with an Acid-D had a higher BMI and ALM ($P < 0.001$ for both). Participants with an Acid-D also had a greater prevalence of obesity than participants consuming an Alk-D. Physical activity energy expenditure ($P = 0.097$) and smoking status ($P = 0.052$), albeit nonsignificant, tended to differ between PRAL groups, with those with a Neut-D reporting the lowest physical activity energy expenditure and the highest smoking prevalence. In accordance with the inclusion criteria of this cohort, the prevalence of diabetes was very low and not different between groups. The use of antiresorptive drugs did not differ at baseline but tended to be lower in the Acid-D group over the follow-up period ($P = 0.080$). The use of vitamin D/calcium supplements was lower in the Acid-D group at baseline and tended to be lower ($P = 0.067$) in this group during the follow-up as well. Based on these group differences, we added these 2 latter variables as potential confounders in the adjusted models looking at the associations between bone traits and DAL.

With regard to dietary intakes, participants in all 3 PRAL groups had adequate calcium and protein intakes according to dietary recommendations (calcium: 700–1300 mg/d; protein: 0.8–1.2 g kg body weight^{-1} d^{-1}) for this age group (43, 44). Participants consuming an Acid-D according to PRAL also had greater NEAP and higher total energy, dietary protein (in grams per day or grams per kilogram per day) and phosphorus intake, whereas dietary potassium and magnesium intakes were higher in those with an Alk-D. Calcium intake tended to differ between PRAL groups, although it did not reach statistical significance ($P = 0.056$).

Biological markers at baseline in relation to PRAL score

Vitamin D (calcifediol) concentrations were lower in subjects consuming an Acid-D compared with those consuming a Neut-D ($P = 0.035$) and an Alk-D ($P < 0.001$) (Table 1). In analyses stratified by sex, vitamin D concentrations were lower in women consuming an Acid-D compared with those consuming a Neut-D (Δ median: −20%; $P = 0.016$) or an Alk-D (Δ median: −19%; $P < 0.001$) (**Supplemental Figure 2**). Vitamin D concentrations were also lower in the Acid-D group compared with the Alk-D group when restricting the analysis to women not taking vitamin D supplements (Δ median: −14%; $P = 0.011$). The proportions of participants with vitamin D insufficiency (< 50 nmol/L) were 27%, 29%, and 35% in the Alk-D, Neut-D, and Acid-D groups, respectively ($P = 0.144$), while very few participants had vitamin D deficiency (<25 nmol/L; 2%, 4% and 2%, respectively; $P = 0.478$). When tested in multiple regression models, the negative association between vitamin D concentrations and PRAL in women was attenuated after adjustment for age, weight, height, physical activity, total energy intake, smoking, alcohol consumption, and use of vitamin D supplements (nonadjusted β: −0.012; $P < 0.001$; adjusted β: −0.005; $P = 0.066$). Renal function, PTH, IGF-1, β-CTX, or P1NP concentrations did not differ between groups of PRAL in women or men (P values ranging from 0.296 to 0.930) (Supplemental Figure 2). Similar results were obtained when excluding participants taking antiresorptive drugs, and when using NEAP (data not shown).

BMD, microstructure, and strength at baseline in relation to PRAL

The prevalence of osteoporosis, defined as at least one BMD T-score ≤ −2.5 SDs at the spine or hip, was not different between groups ($P = 0.419$) (Table 1). Overall, at baseline, aBMD, vBMD, bone strength, and microstructural bone parameters did not differ or were slightly better in subjects with an Acid-D compared with those with an Alk-D and a Neut-D. Specifically, women in the Acid-D group had a higher Tb.N (Δ z score: Acid-D vs Alk-D: +0.28 SD; $P = 0.010$; Acid-D vs Neut-D: +0.40 SD; $P = 0.001$) and lower Tb.Th (−0.28 SD, $P = 0.013$, and −0.25 SD, $P = 0.055$, respectively), Tb.Sp (−0.21 SD, $P = 0.026$, and −0.30 SD, $P = 0.003$, respectively), and Tb.Sp.SD (−0.19 SD, $P = 0.062$, and −0.31 SD, $P = 0.005$, respectively) at the tibia (**Figure 1**). Men in the Acid-D group had higher Tb.Th at the radius compared with men with an Alk-D (Δ z score: +0.37 SD; $P = 0.042$) (**Figure 2**). Compared with men with a Neut-D, men in the Acid-D group also had greater hip (Δ z score: +0.53 SD; $P = 0.008$) and radius (Δ z score: +0.46 SD; $P = 0.024$) aBMD and a greater radius bone strength (Δ z score for failure load: +0.47 SD; $P = 0.026$) in relation to a larger cross-sectional area (Δ z score: +0.42 SD; $P = 0.043$). In regression models adjusted for age, weight, height, physical activity, total energy intake, creatinine, vitamin D, smoking habits, alcohol consumption, and the use of calcium/vitamin D supplements and antiresorptive drugs, the differences in Tb parameters between women with an Acid-D and those with an Alk-D remained significant (e.g., adjusted β: tibia Tb.N, β = 0.24; 95% CI: 0.01, 0.46; $P = 0.037$) (**Supplemental Table 1**). In men, the observed differences between PRAL groups remained significant only for radius Tb.Th ($\beta = 0.38$; 95% CI: 0.01,

TABLE 1 Characteristics of participants by PRAL groups[1]

	Alk-D (PRAL <5) ($n = 506$)	Neut-D ($-5 \leq$ PRAL ≤ 5) ($n = 192$)	Acid-D (PRAL >5) ($n = 155$)	P[2]
PRAL, mEq/d	−20.5 ± 12.8	−0.3 ± 3.0[3]	15.7 ± 12.0[4,5]	<0.001*
NEAP, mEq/d	32.5 ± 8.3	47.7 ± 7.05[3]	62.3 ± 18.1[4,5]	<0.001*
Age, y	65.1 ± 1.4	65.1 ± 1.5	65.1 ± 1.4	0.935
Sex, F %	84	78	65[4,5]	<0.001*
Height, cm	165 ± 8	165 ± 8	167 ± 9[4]	0.026*
Weight, kg	67.4 ± 12.2	69.3 ± 14.2	74.5 ± 14.2[4,5]	<0.001*
BMI, kg/m^2	24.9 ± 4.0	25.4 ± 4.5	26.8 ± 4.4[4,5]	<0.001*
ALM/height, kg/m^2	6.64 ± 1.03	6.76 ± 1.08	7.26 ± 1.28[4,5]	<0.001*
Creatinine, mmol/L	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.493
GFR,[6] mL min^{-1} 1.73 m^{-2}	86.7 ± 10.4	86.9 ± 11.4	88.1 ± 9.8	0.648
25-Hydroxyvitamin D (calcifediol), nmol/L	69.6 ± 27.2	66.7 ± 26.7	61.0 ± 22.4[4,5]	0.035*
Lifestyle factors				
Energy intake, kcal/d	1927 ± 493	1939 ± 462	2143 ± 566[4,5]	<0.001*
Protein, g/d	72 ± 18	79 ± 18[3]	100 ± 34[4,5]	<0.001*
Protein, g kg body weight^{-1} d^{-1}	1.1 ± 0.32	1.18 ± 0.3[3]	1.39 ± 0.55[4,5]	<0.001*
Dietary calcium, mg/d	1173 ± 400	1087 ± 379	1191 ± 490	0.056
Dietary magnesium, mg/d	388 ± 120	326 ± 86[3]	335 ± 87[4]	<0.001*
Dietary phosphorus, mg/d	1215 ± 352	1196 ± 338	1396 ± 339[4,5]	<0.001*
Dietary potassium, mg/d	3682 ± 999	2953 ± 797[3]	2959 ± 74[4]	<0.001*
Physical activity, kcal/d	584 ± 314	543 ± 345	617 ± 381	0.097
Smoker, current, %	6	11	10	0.052
Alcohol consumption (≥30 g/d), %	8	9	13	0.219
Comorbidities and treatments, %				
Obesity[7]	10	15	21[4]	0.001*
Type 2 diabetes	3	4	4	0.818
Vitamin D insufficiency (<50 nmol/L)	27	29	35	0.144
Low ALM/height-squared[8]	13	11	5[4,5]	0.033*
Osteoporosis[9]	16	19	14	0.419
Prevalent low-trauma fractures	19	18	15	0.500
FRAX MOF without BMD	12.9 ± 6.7	12.9 ± 6.9	11.1 ± 5.9[4,5]	<0.001*
FRAX MOF with BMD	11.4 ± 6.2	11.4 ± 6.4	10.3 ± 5.9[4,5]	0.013*
Vitamin D–calcium supplements (baseline)	37	36	23[4,5]	0.003*
Vitamin D–calcium supplements (follow-up)	59	53	48	0.067
Antiresorptive treatment at baseline	7	6	5	0.792
Osteoporosis treatment at follow-up[10]	27	29	18	0.080

[1] Values are means ± SDs or percentages. Women, $n = 676$; men $n = 177$. *Significant, $P < 0.05$. Acid-D, acidic diet; Alk-D, alkaline diet; ALM, appendicular lean mass; BMD, bone mineral density; EWSOP2, European Working Group on Sarcopenia in Older People; GFR, glomerular filtration rate; NEAP, net endogenous acid production; Neut-D, neutral diet; PRAL, potential renal acid load; FRAX MOF, 10-y probability of a major osteoporotic fracture.

[2] Three-group comparisons by Kruskal-Wallis test.

[3] Significantly different at $P < 0.05$ using a Mann-Whitney test in participants consuming a Neut-D vs those consuming an Alk-D.

[4] Significantly different at $P < 0.05$ using a Mann-Whitney test in participants consuming an Acid-D vs those consuming an Alk-D.

[5] Significantly different at $P < 0.05$ using a Mann-Whitney test in participants consuming an Acid-D vs those consuming a Neut-D.

[6] Estimated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.

[7] Defined as a BMI > 30 kg/m^2.

[8] Defined according to the European Working Group on Sarcopenia in Older People (EWSOP2) criteria as a ALM/height^2 < 7 kg/m^2 in men and < 5.5 kg/m^2 in women (36).

[9] Defined as at least one BMD T-score ≤ − 2.5 SDs at the lumbar spine, total hip, or femoral neck.

[10] Biphosphonates, denosumab, or raloxifene.

0.76; $P = 0.047$) and aBMD ($\beta = 0.44$; 95% CI: 0.04, 0.85; $P = 0.034$).

Longitudinal changes of BMD and microstructure in relation to DAL

Changes in BMD, bone microstructure, and strength over the follow-up, in relation to PRAL, are reported for the total population in **Table 2** and by sex in **Figure 3**.

Bone loss at the distal radius (changes in Tt.BMD, $P < 0.001$) and spine (changes in spine aBMD, $P = 0.040$) were attenuated with increasing PRAL (continuous variable) in the total population. In particular, a higher PRAL was associated with a lower age-related increase in radius Tb.Ar ($P < 0.001$), and parallel lower age-related decreases in Ct.Th and Ct.Ar ($P = 0.001$ for both), reflective of attenuated endocortical bone resorption. After controlling for age, sex, weight, height, smoking habits, alcohol consumption, dietary energy intake, physical activity, calcifediol and creatinine concentrations at baseline,

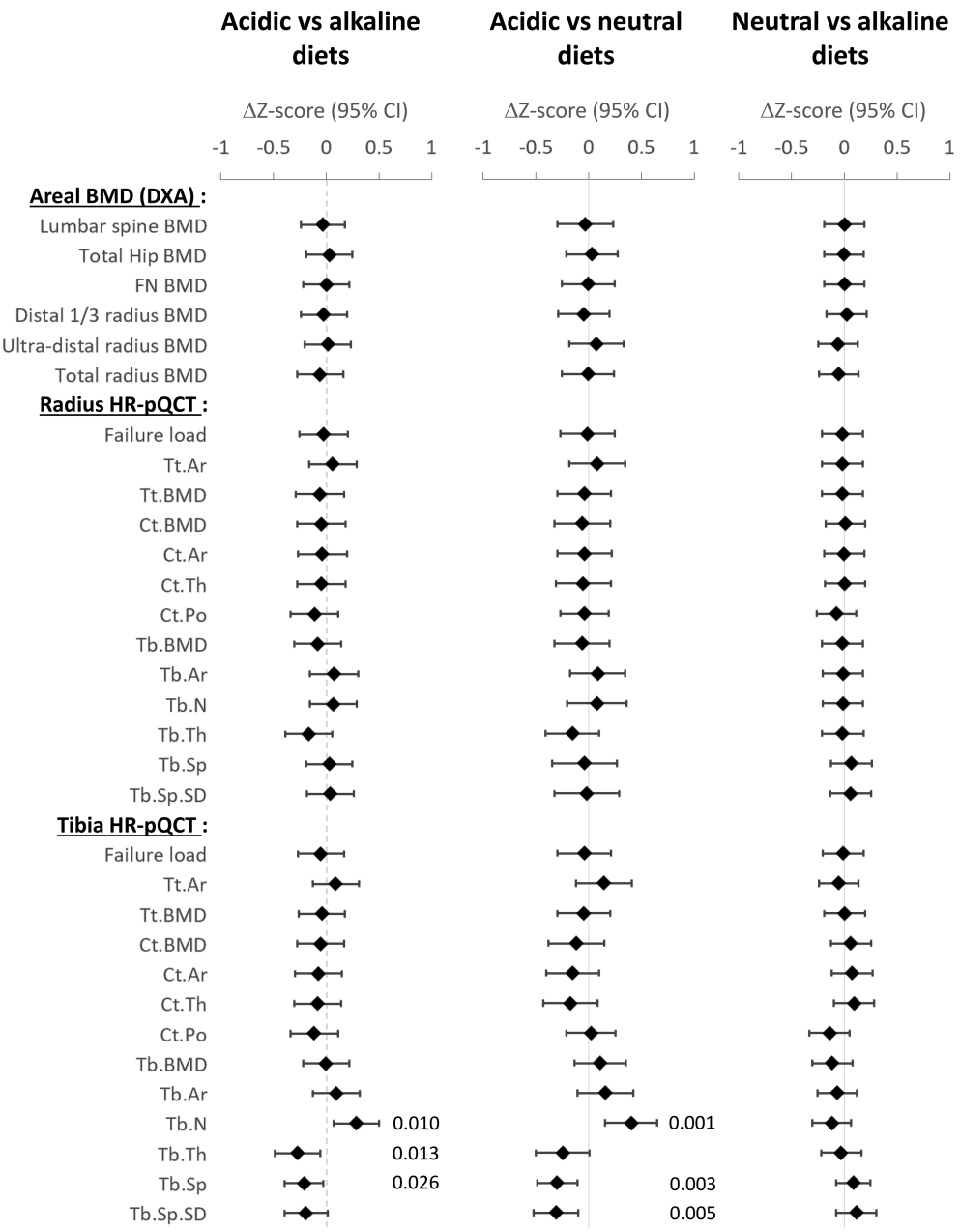

FIGURE 1 Differences in aBMD and bone microstructure and strength at baseline between PRAL groups in postmenopausal women ($n = 676$). Data are expressed as differences in sex-specific z scores calculated on the study population (Δ z score, 95% CI). Three-group comparisons were performed by Kruskal-Wallis test. In case of a significant group effect, pairwise comparisons were performed using Mann-Whitney tests. Significant P values are indicated in the graph. aBMD, areal bone mineral density; BMD, bone mineral density; Ct.Ar, cortical area; Ct.BMD, cortical volumetric BMD; Ct.Po, cortical porosity; Ct.Th, cortical thickness; FN, femoral neck; HR-pQCT, high-resolution peripheral quantitative computed tomography; PRAL, potential renal acid load; Tb.Ar, trabecular area; Tb.BMD, trabecular volumetric BMD; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Sp.SD, Tb separation SD; Tb.Th, trabecular thickness.

and use of calcium/vitamin D supplements and osteoporosis treatment at follow-up, almost all the observed associations persisted, with the exception of the associations of spine aBMD and radius Ct.BMD with PRAL, which were no longer significant (Table 2). There were no significant associations between changes in tibia bone traits or bone strength at the radius or tibia and PRAL. When repeating these analyses in subjects who did not receive antiresorptive therapies during the follow-up, the results remained largely unchanged (**Supplemental Table 2**).

We further looked at the associations between changes in bone traits and PRAL groups in women and men separately (Figure 3). Similar to the results for the whole population, women with an Acid-D experienced lower bone loss (Tt.BMD) at the distal radius compared with those with the Alk-D ($P = 0.002$) or Neut-D ($P < 0.001$). This finding was accompanied by Ct thinning (Ct.Ar and Ct.Th) and endocortical expansion (Tb.Ar) in women with an Acid-D. Women with the lowest BMD at baseline experienced greater bone loss at the distal radius over the follow-up compared

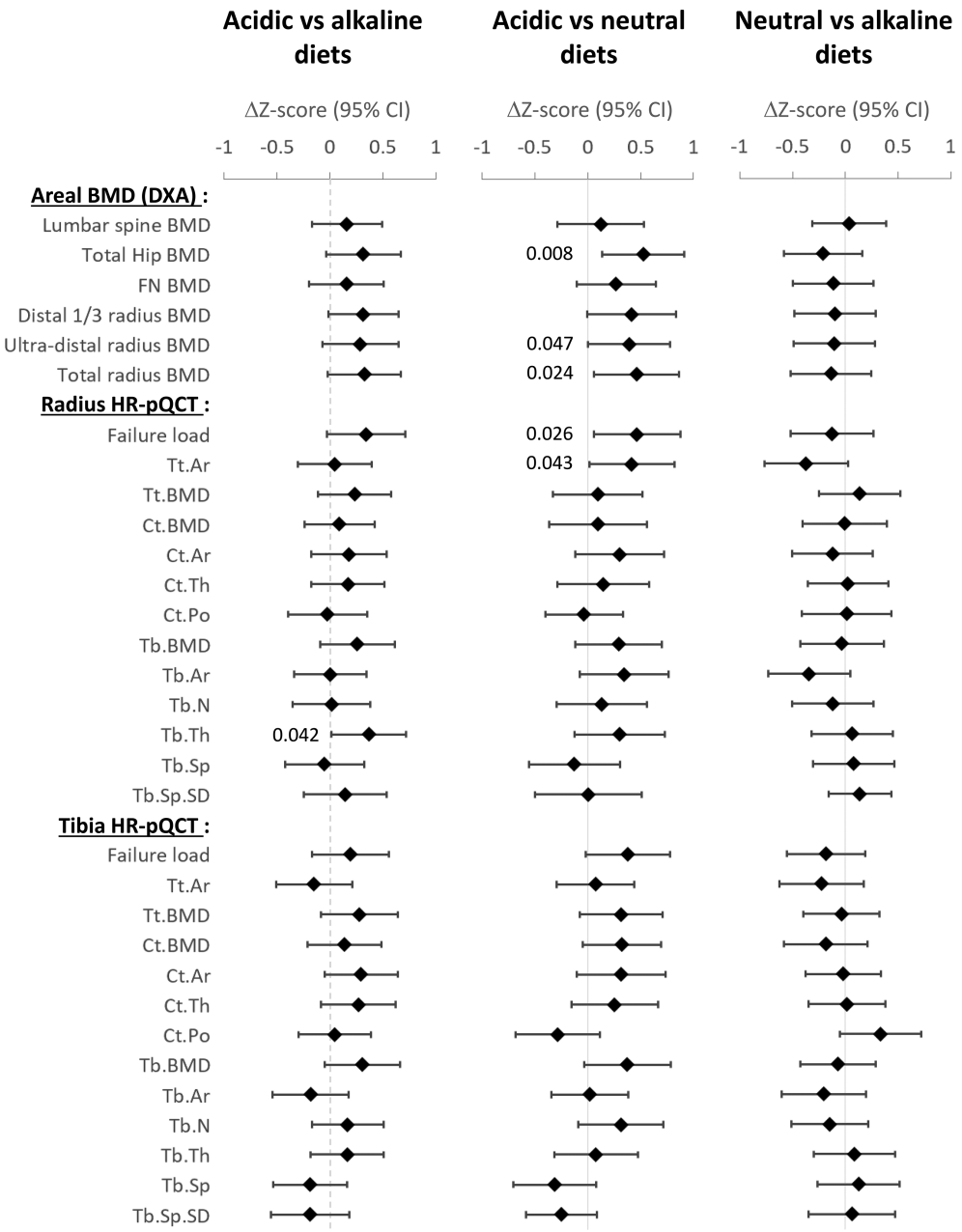

FIGURE 2 Differences (Δ *z* score, 95% CI) in BMD and bone microstructure and strength at baseline between PRAL groups in men ($n = 177$). Significant *P* values are indicated. Data are expressed as differences in sex-specific *z* scores calculated on the study population (Δ *z* score, 95% CI). Three-group comparisons were performed by Kruskal-Wallis test. In case of a significant group effect, pairwise comparisons were performed using Mann-Whitney tests. Significant *P* values are indicated in the graph. BMD, bone mineral density; Ct.Ar, cortical area; Ct.BMD, cortical volumetric BMD; Ct.Po, cortical porosity; Ct.Th, cortical thickness; FN, femoral neck; HR-pQCT, high-resolution peripheral quantitative computed tomography; PRAL, potential renal acid load; Tb.Ar, trabecular area; Tb.BMD, trabecular volumetric BMD; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Sp.SD, Tb separation SD; Tb.Th, trabecular thickness.

with women with higher baseline BMD ($P < 0.001$), but there was no interaction between baseline BMD and PRAL group on these BMD changes (**Supplemental Figure 3**). In men, bone loss was, as expected, less than in women, and there were no differences by PRAL group in changes of aBMD, vBMD, bone microstructure, or strength (all *P* values >0.05). Taken together, these observations suggest attenuated endocortical and Ct bone loss with higher PRAL in postmenopausal women (Figure 3I) but not in men.

To further evaluate whether some PRAL components predominantly drive the relation between the attenuation of bone loss and PRAL, we performed linear regression analysis models for changes in Tt.BMD, Ct.Th and Tb.Ar at the radius (dependent variables), and NEAP and the various PRAL components (dietary protein, calcium, phosphorus, potassium, and magnesium intakes) as independent variables (**Table 3**). When these individual nutrients were tested separately, only protein and phosphorus intakes were associated with attenuated

TABLE 2 Associations (linear regressions) between changes in aBMD and bone microstructure and strength (dependent variables, % change/y) and PRAL (continuous independent variable, per SD increase) in the total study population[1]

	Nonadjusted models		Adjusted models[2]	
	β (95% CI)	P	β (95% CI)	P
aBMD (DXA)				
Lumbar spine BMD ($n = 704$)	0.0012 (0.0001, 0.0024)	0.040*	0.0008 (−0.0004, 0.0020)	0.194
Total hip BMD ($n = 695$)	0.0005 (−0.0001, 0.0011)	0.108	0.0001 (−0.0005, 0.0007)	0.739
Total radius BMD ($n = 697$)	0.0004 (−0.0002, 0.0010)	0.239	− 0.0001 (−0.0007, 0.0004)	0.621
Radius (HR-pQCT) ($n = 644$)				
Tt.BMD	0.0015 (0.0007, 0.0023)	<0.001*	0.0009 (0.0002, 0.0017)	0.019*
Ct.BMD	0.0006 (0.0001, 0.0011)	0.013*	0.0004 (−0.0001, 0.0008)	0.135
Ct.Ar	0.0024 (0.0010, 0.0038)	0.001*	0.0017 (0.0002, 0.0031)	0.023*
Ct.Th	0.0026 (0.0011, 0.0041)	0.001*	0.0019 (0.0004, 0.0033)	0.013*
Tb.BMD	0.0007 (−0.000, 0.0014)	0.061	0.0002 (−0.0005, 0.0009)	0.520
Tb.Ar	− 0.0005 (−0.0008, −0.0002)	<0.001*	− 0.0004 (−0.0007, −0.0001)	0.007*
Tb.N	0.0002 (−0.0013, 0.0018)	0.777	− 0.0003 (−0.0019, 0.0012)	0.668
Tb.Th	0.0006 (−0.0008, 0.0021)	0.376	0.0008 (−0.0007, 0.0023)	0.303
Tb.Sp	− 0.0001 (−0.0018, 0.0016)	0.908	0.0006 (−0.0011, 0.0023)	0.489
Failure load	0.0394 (−0.0970, 0.1758)	0.571	− 0.0353 (−0.1730, 0.1023)	0.614
Tibia (HR-pQCT) ($n = 681$)				
Tt.BMD	− 0.0000 (−0.0006, 0.0006)	0.925	− 0.0003 (−0.0008, 0.0003)	0.365
Ct.BMD	0.0001 (−0.0003, 0.0006)	0.591	0.0000 (−0.0005, 0.0005)	0.944
Ct.Ar	0.0003 (−0.0010, 0.0015)	0.647	− 0.0000 (−0.0012, 0.0012)	0.991
Ct.Th	0.0002 (−0.0010, 0.0015)	0.698	− 0.0000 (−0.0012, 0.0012)	0.959
Tb.BMD	− 0.0002 (−0.0007, 0.0004)	0.524	− 0.0004 (−0.0010, 0.0002)	0.187
Tb.Ar	0.0000 (−0.0001, 0.0001)	0.991	0.0000 (−0.0001, 0.0001)	0.937
Tb.N	− 0.0002 (−0.0015, 0.0012)	0.798	− 0.0008 (−0.0021, 0.0006)	0.287
Tb.Th	− 0.0000 (−0.0013, 0.0013)	0.993	0.0004 (−0.0010, 0.0017)	0.577
Tb.Sp	0.0002 (−0.0012, 0.0015)	0.820	0.0008 (−0.0006, 0.0022)	0.244
Failure load	− 0.0406 (−0.1758, 0.0945)	0.555	− 0.0237 (−0.1719, 0.1245)	0.754

[1] Values are expressed per 1 SD increase in PRAL and 1 percentage-point annual change in dependent variables; $n = 708$ including 565 women and 143 men. *Significant, $P < 0.05$. aBMD, areal bone mineral density; BMD, bone mineral density; HR-pQCT, high-resolution peripheral quantitative computed tomography; Ct.Ar, cortical area; Ct.BMD, cortical volumetric BMD; Ct.Th, cortical thickness; PRAL, potential renal acid load; Tb.Ar, trabecular area; Tb.BMD, trabecular volumetric BMD; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Tt.BMD, total volumetric BMD.

[2] Adjusted for age, sex, weight, height, current smoking status, alcohol consumption, dietary energy intake, physical activity, 25-hydroxyvitamin D and creatinine concentrations, and use of osteoporosis treatment and calcium/vitamin D supplements during follow-up.

bone loss (for change in radius Tt.BMD—protein: $P = 0.001$; phosphorus: $P = 0.002$). These data suggest that the higher protein and phosphorus intakes observed in the group with the Acid-D are beneficial for bone and account for the attenuation of bone loss observed in participants with higher PRAL.

Prevalence and incidence of fractures in relation to DAL

The prevalence of low-trauma fractures at baseline was not different between the Alk-D, Neut-D, and Acid-D groups (women: 20%, 19%, and 16%, respectively; $P = 0.628$; men: 13%, 12%, and 13%, respectively; $P = 0.972$). Incident fracture rates were assessed in 830 participants over a follow-up of 5.9 ± 1.4 y (mean ± SD). Ninety-two participants (12%), including 87 women (14%) and 5 men (3%), experienced low-trauma fractures (Alk-D, 11%; Neut-D, 14%; and Acid-D, 13%; $P = 0.514$). In Cox regression models adjusted for age, the risk of low trauma fractures was not different between PRAL groups—women: HR (95% CI): Acid-D vs Alk-D, 1.17 (0.88, 1.56); $P = 0.268$; Acid-D vs Neut-D, 0.92 (0.48, 1.77); $P = 0.795$; men: HR (95% CI): Acid-D vs Alk-D, 0.88 (0.28, 2.79); $P = 0.826$; Acid-D vs Neut-D, 0.72 (0.04, 11.59), $P = 0.815$. Similar results were obtained in models adjusted for the probability of major osteoporotic fracture assessed by FRAX with or without aBMD. Restricting the analyses to participants who were not receiving osteoporotic treatment at follow-up did not modify these results.

Discussion

In this cross-sectional and longitudinal study performed in a homogenous cohort of 65-y-old healthy individuals, we observed null or favorable, but no adverse, associations between BMD and bone microstructure and strength at baseline and an Acid-D. Over the 6-y follow-up period, postmenopausal women with an Acid-D experienced slower rates of age-related bone loss at non–weight-bearing skeletal sites compared with women with an Alk-D and/or a Neut-D, as suggested by lower decreases in radius Tt vBMD and attenuated endocortical and Ct bone loss in those with an Acid-D. In men, despite some favorable bone characteristics observed cross-sectionally in the Acid-D group, patterns of changes in bone traits were not different between PRAL groups. Given that men represented 21% of the study population and experienced less rapid bone loss than postmenopausal women, any prospective association may have been less easily identifiable in males. In terms of hormonal control or biochemical markers, there were no differences between DAL groups in bone-related

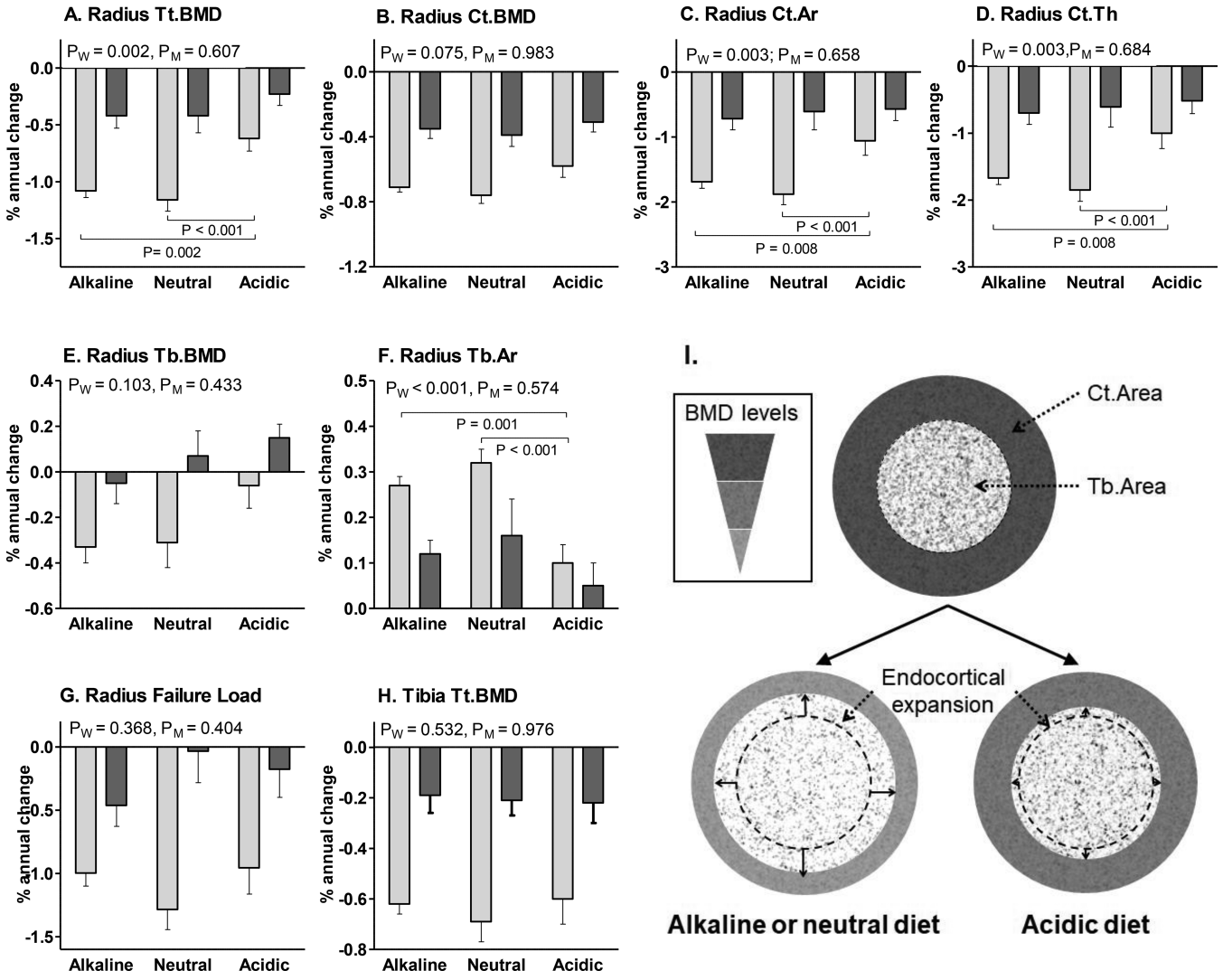

FIGURE 3 Annual changes (%) in radius Tt.BMD (A), Ct.BMD (B), Ct.Ar (C), Ct.Th (D), Tb.BMD (E), Tb.Ar (F), failure load (G), and tibia Tt.BMD (H) in postmenopausal women (radius, $n = 512$; tibia, $n = 542$; light gray) and men (radius, $n = 132$; tibia, $n = 139$; dark gray) according to PRAL groups. The columns represent means and the whiskers indicate SEs. The *P* values of the 3-group comparisons performed by Kruskal-Wallis tests are indicated for women (P_W) and men (P_M). In case of a significant group effect, significant *P* values of pairwise comparisons performed using Mann-Whitney tests are also indicated in the graph. The observed changes resulted in attenuation of endocortical and cortical bone loss in women with an acid diet compared with those with alkaline diet or a neutral diet, as illustrated in panel I. BMD, bone mineral density; Ct, cortical; Ct.Ar, cortical area; Ct.BMD, cortical volumetric BMD; Ct.Th, cortical thickness; PRAL, potential renal acid load; Tb, trabecular; Tb.Ar, trabecular area; Tb.BMD, trabecular volumetric BMD; Tt.BMD, total volumetric BMD.

hormones or bone turnover markers. In accordance with these findings, no associations were observed between DAL and prevalent or incident fractures in either sex. Collectively, our findings do not support negative associations of DAL with changes in bone parameters or fractures.

In line with our findings, several studies do not suggest negative DAL effects on bone (14, 20, 25–28, 30). In the Aberdeen Prospective Osteoporosis Screening Study, postmenopausal women in the highest quartile of NEAP and PRAL experienced lower aBMD loss at the femoral neck and lumbar spine compared with the women in the lowest quartile, but these differences did not persist after adjustments for confounders (29). Similarly, in a cohort of community-living women aged ≥60 y old, no significant associations were seen between PRAL and loss of Tt, Ct, or Tb vBMD (assessed by pQCT) over 6 y (30). Likewise, fracture risk does not appear to be influenced by PRAL/NEAP (20, 25, 28), with these results being independent of renal function (25). In contrast, a few (19–22), albeit not all (23, 25–27) cross-sectional studies have reported negative associations between DAL and aBMD. Nevertheless, the inconsistent patterns of these negative associations [i.e., in isolated subgroups of the total population such as premenopausal women (22, 29) or elderly women with fractures (19), at single bone sites (21), or by using one DAL measure (NEAP), but not another (PRAL) (24)], and the fact that these are not confirmed in longitudinal studies, raise questions about the significance of the "acid-ash hypothesis" in skeletal integrity.

Our findings that a higher DAL is linked to nondifferent or favorable, rather than adverse, bone outcomes are puzzling. Although proteins are major DAL contributors through the

TABLE 3 Associations between annual changes (%) in radius Tt.BMD, Ct.Th, and Tb.Ar (dependent variables) and NEAP and individual PRAL components (independent variables) in separate models in the total study population[1]

	Change in radius Tt.BMD		Change in radius Ct.Th		Change in radius Tb.Ar	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
NEAP	0.0013 (0.0004, 0.0023)	0.005*	0.0025 (0.0010, 0.0040)	0.001*	−0.0006 (−0.0009, −0.0002)	0.001*
Calcium	0.0008 (−0.0001, 0.0017)	0.067	0.0011 (−0.0005, 0.0027)	0.175	−0.0003 (−0.0006, 0.0000)	0.063
Protein	0.0014 (0.0006, 0.0023)	0.001*	0.0024 (0.0008, 0.0040)	0.003*	−0.0006 (−0.0009, −0.0002)	0.001*
Magnesium	0.0000 (−0.0008, 0.0009)	0.969	−0.0005 (−0.0019, 0.0009)	0.517	0.0000 (−0.0003, 0.0003)	0.854
Phosphorus	0.0012 (0.0004, 0.0020)	0.002*	0.0016 (0.0003, 0.0029)	0.019*	−0.0004 (−0.0007, −0.0002)	0.002*
Potassium	0.0000 (−0.0008, 0.0008)	0.983	−0.0002 (−0.0015, 0.0012)	0.814	−0.0000 (−0.0003, 0.0002)	0.748

[1] Values are expressed per 1 SD increase in independent variables and 1 percentage-point annual change in dependent variables; $n = 644$ including 512 women and 132 men. *Significant, $P < 0.05$. Ct.Th, cortical thickness; NEAP, net endogenous acid production; PRAL, potential renal acid load; Tb.Ar, trabecular area; Tt.BMD, total volumetric bone mineral density.

metabolism of some amino acids (e.g., methionine, cysteine) to sulfuric acid, they also have well-established anabolic effects on bone (12), which are not considered by the "acid-ash hypothesis". These include supply of amino acids as substrates of bone matrix, enhanced calcium absorption, increases in circulating IGF-1, and maintenance of muscle mass and strength (2, 12). In previous analyses in postmenopausal women from the same cohort, we demonstrated beneficial influences of animal and dairy protein intakes on bone Tb microstructure, which potentially drove the observed increases in bone strength (34). Likewise, we observed skeletal benefits in women who consumed fermented dairy products, which are good sources of protein, calcium, phosphorus, and other nutrients important for bone health (39, 45). Given that participants in the Acid-D group in this analysis had higher protein and phosphorus intakes compared with those with an Alk-D and/or a Neut-D, our results could at least partially reflect the beneficial skeletal effects of these nutrients, as suggested by reduced bone loss with higher protein and phosphorus intakes (Table 3). Alternatively, an Acid-D/Alk-D may be associated with additional dietary factors, which are not incorporated in the DAL algorithms but affect bone metabolism and could not be controlled for (46). For instance, a potentially high intake of dairy products in an Acid-D may be related to positive metabolic and bone effects, with these effects being attributed not only to the provision of proteins and phosphorus but also to other nutrients such as calcium, pre- or probiotics (39, 45).

Another explanation of our results may be that participants with an Acid-D differ from those with Alk-D and/or Neut-D in anthropometric and lifestyle characteristics with positive influences on bone health. For example, participants in the Acid-D group were heavier, had higher ALM, and tended to report higher energy expenditure due to physical activity, suggesting increased mechanical loading on their skeleton. Conversely, they reported fewer vitamin D/calcium supplements at baseline and were less likely to use osteoporosis treatment (trend) at follow-up. Nevertheless, when we controlled for height, weight, physical activity, but also other factors (age, smoking habits, alcohol consumption, dietary energy intake, 25-hydroxyvitamin D and creatinine concentrations, and use of vitamin D/calcium supplements and osteoporosis treatment at follow-up), the associations between changes in bone traits and DAL persisted, suggesting that the observed relations are independent of several confounders.

Our study has several strengths, including its prospective design, complete follow-up, and detailed assessment of bone outcomes and confounding factors. By repeating HR-pQCT scans at follow-up visits, we quantified age-related changes of densitometric, microarchitectural, and mechanical properties of Ct and Tb bone in the appendicular skeleton (distal radius and tibia). As such, we provide a longitudinal, noninvasive, in vivo, 3-dimensional characterization of bone properties, which is rarely available in large epidemiological studies. To estimate PRAL, we utilized a validated algorithm, previously shown to be closely correlated with renal net acid excretion in 24-h urine samples (5). The nutrients comprising PRAL were assessed by 3-d food records, a method of dietary assessment based on real-time data collection and potentially little reliance on memory, which may thus provide more accurate information on dietary intake than food frequency questionnaires or single 24-h recalls (47). In addition, blood samples used to assess biological markers of bone turnover or linked with bone metabolism were collected just at the end of the dietary assessment.

We should also acknowledge the limitations of our study. Our findings apply to a homogenous and relatively healthy cohort with normal renal function, limiting the extrapolation of the observed associations to more heterogeneous populations or older individuals with compromised renal acid excretory ability and poor buffering capacity as a result of lower muscle and/or bone mass (3). Participants in this study were well nourished with normal to high protein and calcium intakes and a relatively alkaline diet (mean PRAL: -9.4 ± 18.2 mEq/d); thus, these results may not be applicable to populations with higher PRAL values or insufficient calcium or protein dietary intakes. Given that the estimation of PRAL and subsequent diet group categorization were based on data collected at baseline, there is the possibility of misclassification bias. Other limitations of our investigation include the inherent limitation of the PRAL equation to disentangle the anabolic from the catabolic influences of some of its components (e.g., protein), the unavailability of acid-base biomarkers (e.g., urinary pH, ammonium, or citrate) to evaluate actual metabolic acidosis (48), and the possibility of residual confounding factors.

In conclusion, baseline BMD and bone microarchitecture and strength did not differ or were slightly better in men and postmenopausal women with an Acid-D than in those with Alk-D and Neut-D. Longitudinally, the consumption of an Acid-D was associated with attenuated age-related bone loss at

non–weight-bearing bone sites in women, and these associations were independent of main confounders. These findings may at least partially be explained by the higher protein and phosphorus intakes in participants with an Acid-D; these nutrients are major acid contributors in DAL equations but also have well-established benefits on skeletal health. Finally, there was no evidence that history or incidence of fractures was related to an Acid-D in either women or men. Collectively, our findings do not advocate unfavorable DAL-mediated effects on bone in a healthy, well-nourished, elderly population.

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