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Serum Phosphate, BMI, and Body Composition of Middle-Aged and Older Adults: A Cross-Sectional Association Analysis and Bidirectional Mendelian Randomization Study

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ABSTRACT

Background: Observational studies have reported associations between serum phosphate and BMI in specific clinical settings, but the nature of this relation in the general population is unclear.

Objectives: The aim of this study was twofold: to investigate the association between serum phosphate and BMI and body composition, as well as to explore evidence of causality through a bidirectional one-sample Mendelian randomization (MR) in the population-based Rotterdam Study (RS).

Methods: Observational associations between phosphate (mg/dL) and BMI, lean mass, and fat percentage (fat%), estimated by DXA, were analyzed using multivariable regression models in 9202 participants aged 45–100 y from 3 RS cohorts. The role of serum leptin was examined in a subgroup of 1089 participants. For MR analyses, allele scores with 6 single-nucleotide polymorphisms (SNPs) for phosphate and 905 SNPs for BMI were constructed in 7983 participants.

Results: Phosphate was inversely associated with BMI in the total population (β: –0.89; 95% CI: –1.17, –0.62), and stronger in women (β: –1.92; 95% CI: –2.20, –1.65) than in men (β: –0.37; 95% CI: –0.68, –0.06) (P-interaction < 0.05). Adjustment for leptin did not change results in men. In women, adjustment for leptin attenuated the association, but it was not abolished (β: –0.94; 95% CI: –1.45, –0.42). Phosphate was inversely associated with fat%, but not with lean mass, in both sexes. MR analyses suggested a causal effect of BMI on serum phosphate (β: –0.01; 95% CI: –0.02, 0.00) but not vice versa.

Conclusions: Serum phosphate was inversely associated with BMI and fat% in a population-based study of middle-aged and older adults, with a stronger effect in women than in men. Adjusting for leptin attenuated this relation in women only. MR results suggest a causal effect of BMI on phosphate but not vice versa. An underlying sex dimorphism in phosphate homeostasis should be further explored. *J Nutr* 2022;152:276–285.

Keywords: phosphate, BMI, Mendelian randomization, fat mass, lean mass, population-based cohort

Introduction

Phosphate is a widely distributed mineral ion in the body that plays an important role as an essential component of cell signaling, energy metabolism, and nucleic acid synthesis (1). Most phosphate (85%) is present within bone tissue in hydroxyapatite crystals, whereas 15% is found in the intracellular compartment and only 1% circulates freely in the extracellular fluids (2).

Inverse associations between serum phosphate and BMI (in kg/m^2) but also between serum phosphate and waist-to-hip ratio (WHR), waist circumference, and fat mass have been described in specific populations such as in participants with nonmorbid obesity, hypertension, and metabolic syndrome (3–6). Only a few studies have been performed at the population level. The largest study to date included 46,798 South Korean adults older than 20 y without previous comorbidity, and the authors reported a negative correlation of serum phosphate with waist circumference and BMI. After adjustment for age, sex, and calcium concentrations, the association of serum phosphate with waist circumference remained robust, but the association between serum phosphate and BMI did not remain significant (7).

Several hypotheses have been proposed to explain the association between serum phosphate and BMI and body composition (8). Phosphate concentrations are regulated predominantly by parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) (9). In theory, the relation between phosphate and

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BMI and adiposity can be explained by either serum phosphate or its regulators {PTH, FGF23, or 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] or dietary phosphate} influencing adiposity or by adiposity influencing phosphate homeostasis. Billington et al. (8) reported an inverse association between phosphate and fat mass in 1676 postmenopausal women and 323 community-dwelling men without active disease. This association remained significant after adjusting for age, PTH, and estimated glomerular filtration rate (eGFR) (8). Leptin, synthesized by adipocytes and strongly associated with adiposity, has been shown to function as a FGF23 secretagogue in mice and could therefore influence phosphate (10, 11). A small case-control study in 20 women undergoing bariatric surgery showed higher leptin and FGF23 concentrations in cases compared with controls, but there was no difference in phosphate concentrations between the groups (10).

Recent studies have shown that phosphate is associated with all-cause mortality, cardiovascular mortality, and mortality from chronic obstructive pulmonary disease in men and progression of chronic kidney disease (CKD), among other adverse outcomes (12, 13). Moreover, conditions of low serum phosphate concentrations (hypophosphatemia) are characterized by defects at multiple levels other than bone, such as in glucose metabolism and muscle tissue (1, 5, 6, 14–16). A possible phosphate–adiposity relation may play a role in these associations, and if the relation between phosphate and mortality and morbidity can be explained by BMI, this may have consequences for health.

Due to lack of consistency and high heterogeneity of the previous findings on the association between phosphate and measures of adiposity, we aimed to investigate if serum phosphate was associated with BMI in a population-based setting with Caucasian elderly individuals with normal variation of both phosphate and BMI, as well as investigate sex differences, frequently reported for phosphate and several health-related outcomes (13, 17). Furthermore, we aimed to explore which body compartment drives this association and the role of potential confounders and regulators of phosphate homeostasis. For the purposes of testing causality and improving the inference of our results, Mendelian randomization (MR) analysis was applied. MR mimics a randomized controlled trial by using natural genetic variation, which makes it less susceptible to confounding (18). Importantly, MR analysis is considered unaffected by reverse causation (19). To this end, we performed a bidirectional MR analysis using genetic variants for BMI and for phosphate as instrumental variables (IVs).

Participants and Methods

Study population

We performed this cross-sectional observational study and one-sample bidirectional MR study in the Rotterdam Study (RS). RS is a population-based study of men and women aged 40 y or older and recruited in the district of Ommoord, Rotterdam (20). It is now composed of 4 cohorts named RS-I, RS-II, RS-III, and RS-IV (initiated in 1989, 2000, 2006, and 2016; total n ~18,000 participants). Participants have been followed through several visits since recruitment. Rationale and design have been described previously (20). The RS was approved by the Medical Ethics Committee of Erasmus MC and by the Ministry of Health, Welfare, and Sport of the Netherlands. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians. For the current study, BMI, WHR, and serum phosphate concentrations were assessed in the third visit of RS-I (RS-I-3, henceforth referred to as RS-I) and in the baseline visits of RS-II and RS-III. These visits are similar in design and data collections. Measures of body composition were assessed at the fifth visit of RS-I, the third visit of RS-II, and at baseline in RS-III (**Supplemental Figure 1**). A total of 3582 participants from RS-I, 2362 from RS-II, and 3258 from RS-III with complete information on phosphate, BMI, and covariates were included to study the observational association between serum phosphate and BMI. The total population of 9202 participants had a mean age of 64.9 y (range: 45–100 y), 56.5% was female, and mean BMI was 27.3. Genotype data for MR analysis were available for 3228, 1955, and 2800 participants from RS-I, RS-II, and RS-III, respectively. The total sample sizes for the analyses with WHR, fat mass, and lean mass modeled as the outcome are depicted in **Figure 1**.

Clinical outcomes

Fasting blood samples were collected at the research center in which serum phosphate concentrations were determined. The amount of phosphorus determined in blood corresponds to the inorganic fraction, or phosphate, present mostly under the forms of HPO_4^{2-} and $H_2PO_4^-$ with a 4:1 ratio at a physiological pH (2). The method for phosphate determination is based on the formation of ammonium phosphomolybdate; this compound is measured photometrically and directly proportional to phosphate concentration.

BMI was estimated from weight and height obtained in the standing position without shoes. Waist circumference was measured with a tape measure halfway between the ribcage and the pelvic bone. Hip circumference was measured at the maximal circumference of the hips. WHR was calculated from these measurements. Body composition variables, namely, fat mass (kg) and lean mass (kg), were determined from total body scans performed with iDXA equipment (GE Lunar) (20). Fat percentage (fat%) was estimated as fat mass (kg)/body weight (kg) × 100. Lean mass index (LMI) was estimated as lean mass (kg)/height $(cm)^2$ × 100.

The generation and management of GWAS genotype data for the Rotterdam Study (RS I, RS II, RS III) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The GWAS data sets are supported by the Netherlands Organisation of Scientific Research NWO Investments (No. 175.010.2005.011, 911-03-012), the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), and the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) Netherlands Consortium for Healthy Aging (NCHA), project No. 050-060-810.
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Supplemental Figures 1–4 and Supplemental Tables 1–13 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/.
Data can be obtained upon request. Requests should be directed towards the management team of the Rotterdam Study (datamanagement.ergo@erasmusmc.nl), which has a protocol for approving data requests. Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository.
Address correspondence to MCZ (e-mail: m.c.zillikens@erasmusmc.nl).
Abbreviations used: CKD, chronic kidney disease; CUE, continuously updating estimator; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; GRS, genetic risk score; GWAS, genome-wide associated study; IV, instrumental variable; LMI, lean mass index; MR, Mendelian randomization; PTH, parathyroid hormone; RS, Rotterdam Study; SNP, single-nucleotide polymorphism; WHR, waist-to-hip ratio; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; 2sls, 2-stage least squares.

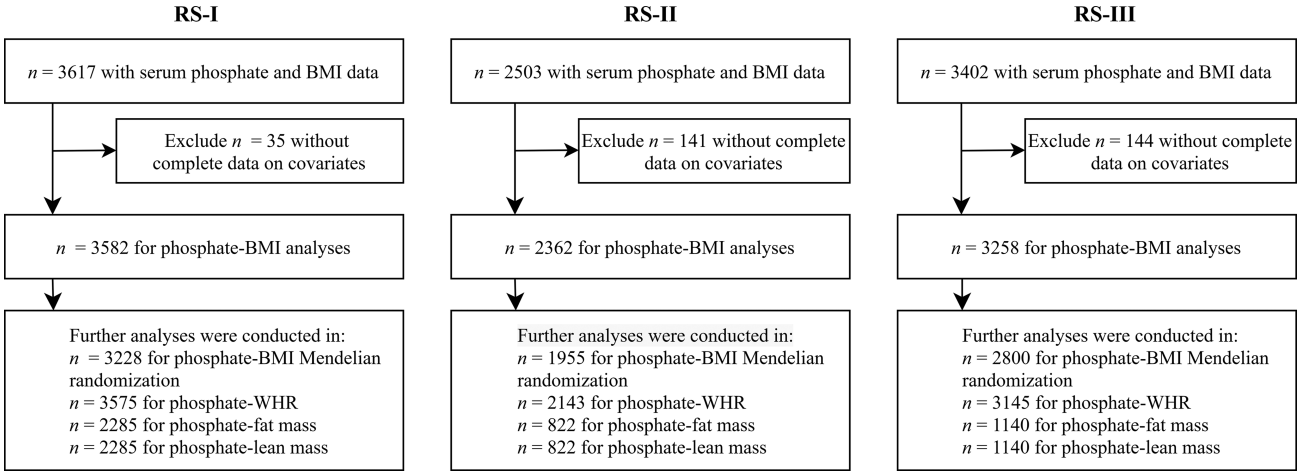

FIGURE 1 Participant flowchart summarizing sample sizes for the different analyses. MR, Mendelian randomization analyses; RS, Rotterdam Study; WHR, waist-to-hip ratio.

Confounder variables

Serum total calcium concentrations (mg/dL) were measured through a colorimetric o-cresolphthalein complexone method (Roche). Concentrations of serum 25-hydroxyvitamin D (25(OH)D) (nmol/L) were determined through an electrochemiluminescence immunoassay (Roche). Due to seasonal variability in sunlight exposure, 25(OH)D concentrations were adjusted for season and year of blood sampling, applying a cosinor regression method; from these models, population means were obtained and individual values were adjusted (21, 22). Serum creatinine concentrations were determined through an enzymatic colorimetric assay based on the formation of sarcosine. The Chronic Kidney Disease Epidemiology Collaboration equation was applied to calculate the eGFR (23). Serum 17β-estradiol and testosterone concentrations were determined by coat-a-count RIA (Siemens Diagnostics). Due to limited amount of plasma per participant, not all hormone concentrations could be determined in all participants.

Serum leptin concentrations were determined during the third visit of RS-I, in a random subset of participants selected as part of a separate case-cohort study (n = 489 men and n = 694 women). Leptin was quantified using a multiplex immunoassay on a custom-designed human multianalyte profile (Rules-Based Medicine) in a fasting blood sample. Smoking status and level of education were assessed during home interviews. Smokers were categorized as current smokers, ever smokers, or never smokers. Level of education was categorized as primary, low, intermediate, or high.

Genotyping

Participants were genotyped in the following platforms: Illumina HumanHap550 BeadChip, Illumina 550 duo, or Illumina 610 and 660 quad single-nucleotide polymorphism (SNP) arrays. Variants were filtered (24, 25) on call rate <95%, minor allele frequency <0.01, and Hardy–Weinberg equilibrium $P < 1.0 \times 10^{-6}$ and subsequently imputed to the Haplotype Reference consortium panel, release 1.1 (26). KING software (27) was applied to identify highly related participants (second degree or closer) through the estimation of kinship coefficients for each pair of individuals both between and within the cohorts. A kinship coefficient of 0.0884 was applied as a cutoff for second-degree relatedness.

Mendelian randomization

Mendelian randomization uses genetic instruments as instrumental variables to estimate the causal effect of a risk factor on an outcome. To this end, we constructed 2 genetic risk scores (GRSs) to instrument BMI by adding up the BMI-related SNPs reported in the genome-wide association study (GWAS) by Yengo et al. (28) in 2018 (29). This meta-analysis of GWAS on BMI identified 941 GWAS significant independent SNPs from a conditional and joint multiple-SNP analysis (COJO), including 655 SNPs from primary analysis, which explain 6.0% of the variance in BMI (28). Genotypes for all 941 SNPs were available in our study population, and we constructed 2 BMI GRSs: 1 including the 655 SNPs and 1 including all 941 SNPs. SNPs with an imputation quality score >0.8 were included for analysis (30). Furthermore, allele frequencies for palindromic SNPs were checked to decrease the possibility of strand coding errors. Palindromic SNPs with a minor allele frequency of >0.42 were discarded.

Currently, 2 GWAS on serum phosphate have been published: an European GWAS by Kestenbaum et al. (31) and a Japanese GWAS by Kanai et al. (32). We constructed a GRS for serum phosphate using the 13 phosphate-related SNPs that were reported in these GWAS. In the European GWAS, the RS was part of the discovery sample, which could result in bias from winner's curse (33). For this reason, GWAS summary statistics for serum phosphate were obtained from the UK Biobank, and the 13 SNPs were checked for GWAS significance using Neale Lab UK Biobank summary statistics (34, 35). Only the SNPs that were also GWAS significant in the UK Biobank (i.e., $P < 1.0 \times 10^{-8}$) were considered for inclusion in the phosphate GRS. Imputation quality and palindromic SNPs were checked as described above.

The European GWAS on serum phosphate by Kestenbaum et al. (31) and the GWAS on BMI by Yengo et al. (28) both included the RS. Deriving weights from the data under analysis can result in severe bias (29). Therefore, we performed the analyses with unweighted genetic risk scores.

Statistical analysis

Differences between men and women were compared using independent t test for continuous variables and χ^2 test for categorical variables. The cross-sectional associations between phosphate concentrations (in mg/dL) with BMI and measures of body composition were examined through multivariate linear regression models with BMI, WHR, fat mass, lean mass, and fat% modeled as the dependent variables and serum phosphate concentrations as the independent variable. Analyses with BMI modeled as the dependent variable were performed in each of the 3 different cohorts separately and were meta-analyzed by applying a random-effects model with Comprehensive Meta Analysis Version 3 (36). Analyses with WHR, fat mass, lean mass, and fat% modeled as the dependent variables were performed in RS-III, the cohort with simultaneous measurements of laboratory data and DXA. We explored potential sex differences in the association between serum phosphate and BMI by including interaction terms of phosphate with sex in age-adjusted models and performed sex-stratified analyses if there was evidence of a different association between serum phosphate and BMI across sexes (P-interaction < 0.10). The distribution of continuous variables was examined using frequency distribution histograms and Q-Q plots.

Basic analyses were age adjusted. All analyses were further adjusted for education level, smoking, eGFR, and for concentrations of total calcium, 25(OH)D, 17β-estradiol, and testosterone. These confounders were selected based on previously reported associations with phosphate and/or with the outcomes. BMI analyses in RS-I were further adjusted for leptin concentrations, which were available in ~30% of participants from RS-I.

Because fat mass and lean mass are related to one another, we tested the correlation between fat mass and lean mass using Spearman correlation coefficients. To avoid treating highly intercorrelated variables as independent ones, analyses with fat mass and lean mass modeled as outcomes were adjusted for LMI and fat%, respectively (37, 38). Because increased obesity is related to increased visceral obesity, BMI might be a confounder in the association between WHR and serum phosphate (39). Therefore, analyses for WHR were further adjusted for BMI.

We performed several sensitivity analyses. We restricted analysis to participants without CKD [defined as eGFR <60 mL/(min·1.73 m^2) (23), n = 8125]. Early stages of CKD are associated with hyperphosphaturia when there is still an adequate renal response to FGF23 (40). Furthermore, the measurements of serum phosphate and fat and lean mass were simultaneous in RS-III but not in RS-I and RS-II. We therefore proceeded to test the correlation for BMI and WHR measurements at the 2 different time points, and we performed the body composition analysis in RS-I and RS-II as a sensitivity analysis and meta-analyzed the results with those from RS-III. All analyses were performed with IBM SPSS software, version 21 (SPSS), Stata version 15 (StataCorp), and R version 3.6.1 (R foundation for Statistical Computing).

Mendelian randomization analysis

One-sample bidirectional MR was performed in participants with data on BMI, serum phosphate, covariates, and individual-level genotype data. First, we tested the 3 assumptions of MR (41). To correct for multiple testing of the independence assumption, a Bonferroni correction was applied resulting in a corrected P value < 0.006 (0.05/9) [testing age, sex, education level, smoking, total calcium, 25(OH)D, eGFR, testosterone, and 17β-estradiol]. Next, MR analyses were performed through 2-stage least squares (2sls) regressions (42) using Stata, with the GRS as the instrumental variable. Analyses were adjusted for age, sex, and the first 10 principal components to control for population stratification. β, P value, and F statistics were considered.

To account for potential overestimation of results due to family relatedness, 2sls MR analyses were repeated after randomly excluding first- and second-degree relatives, estimated using KING software. Because previous studies have shown an inverse relation of BMI with vitamin D deficiency, an observation that has been confirmed through MR, we performed an additional 25(OH)D-adjusted MR analysis by including the same BMI GRS in the models as a covariate but weighting each SNP for its effect on 25(OH)D in the study sample (43, 44).

MR–Egger, the weighted median estimator, and an adapted lasso regression were applied to investigate potential pleiotropy (45). Horizontal pleiotropy would violate the exclusion-restriction condition in MR (18). MR–Egger is able to assess directional pleiotropy (41, 45) The adaptive lasso regression provides a consistent estimate while allowing <50% of the instruments to be invalid (46). The weighted median approach assumes that genetic instruments representing >50% of the weights are valid IVs (42, 47). The adjusted continuously updating estimator (CUE) was applied to account for the presence of many weak instruments (48).

Results

The general characteristics of the study population, stratified by sex, are depicted in Table 1. More than 90% of the study population displayed serum phosphate concentrations within the normal range [2.5–4.5 mg/dL (49)]. On average, women had higher concentrations of serum phosphate, total calcium, and 17β-estradiol than men. Women tended to have higher BMI and higher prevalence of obesity. Leptin concentrations were higher in women than in men. Men generally had higher testosterone concentrations, higher levels of education, and higher values of WHR. Also, smoking was more prevalent in men than in women.

Phosphate and BMI

Cross-sectional sex-combined linear regression analyses showed a significant association between serum phosphate and BMI (β: –1.44; 95% CI: –1.62, –1.25; P < 0.001) (**Supplemental Table 1**). Further analyses were performed sex-stratified due to evidence of an interaction between phosphate and BMI across sexes (P-interaction < 0.001). Linear regression analyses (Table 2 and **Supplemental Table 2**) showed a significant inverse association between serum phosphate concentrations and BMI in men and a more pronounced inverse relation in women after adjustment for age, education level, smoking, total calcium, 25(OH)D, eGFR, 17β-estradiol, and testosterone.

Further adjustments of this analysis for leptin concentrations, available in ~30% of participants from RS-I (Table 3), did not affect results in men. In contrast, leptin adjustment attenuated but did not abolish the relation between serum phosphate concentrations and BMI in women.

Phosphate and body composition

There was a positive correlation between fat and lean mass in men and women (men: ρ: 0.248; P < 0.001; women: ρ: 0.369; P < 0.001). Furthermore, there was a positive correlation between BMI and WHR in men and women (men: ρ: 0.616; P < 0.001; women: ρ: 0.460; P < 0.001). To avoid treating highly intercorrelated variables as independent ones, analyses with fat mass modeled as the outcome were adjusted for LMI and lean mass was adjusted for fat%. Analyses with WHR modeled as the outcome were adjusted for BMI.

Table 4 displays the associations between serum phosphate and measures of body composition in RS-III, the cohort with simultaneous measurements of laboratory data and DXA. Serum phosphate concentrations were inversely associated with fat mass in women but not in men. Fat%, a measure of total adiposity, was found to be inversely associated with serum phosphate concentrations in both sexes. Serum phosphate concentrations were not significantly associated with lean mass in both sexes. WHR, a measurement of central adiposity, was not found to be associated with serum phosphate in men but women showed a significant inverse relation.

Sensitivity analyses

When repeating analyses in participants without CKD, we found a borderline significant inverse relation between BMI and serum phosphate concentrations in men and a significant inverse relation in women (**Supplemental Table 3**).

BMI and WHR measurements from both visits in RS-I and RS-II proved to be positively correlated in both sexes (BMI men RS-I: ρ: 0.897, RS-II: ρ: 0.898; P < 0.001; BMI women RS-I: ρ: 0.910, RS-II: ρ: 0.918; P < 0.001; WHR men RS-I: ρ: 0.632, RS-II: ρ: 0.780; P < 0.001; WHR women RS-I: ρ: 0.462, women RS-II: ρ: 0.800; P < 0.001). We performed the body composition analysis in RS-I and RS-II as a sensitivity analysis and meta-analyzed the results with those from RS-III (**Supplemental Tables 4–7**). Serum phosphate was inversely associated with fat mass in both sexes. Serum phosphate was also significantly associated with fat% in both sexes. Serum phosphate was inversely associated with lean mass in women but not in men. Serum phosphate was inversely associated

TABLE 1 Participant characteristics for men and women aged 45–100 y from RS-I, RS-II, and RS-III of the Rotterdam Study, stratified by sex[1]

	RS-I ($n = 3582$)			RS-II ($n = 2362$)			RS-III ($n = 3258$)		
Characteristic	Men	Women	*P* value[2]	Men	Women	*P* value[2]	Men	Women	*P* value[2]
n	1517	2065		1063	1299		1424	1834	
Age, y	71.8 (6.7)	72.5 (7.1)	0.005	64.5 (7.5)	65.0 (8.1)	0.13	57.0 (6.6)	57.2 (7.0)	0.28
BMI, kg/m^2	26.3 (3.2)	27.3 (4.4)	<0.001	26.9 (3.4)	27.4 (4.5)	0.001	27.9 (4.0)	27.6 (5.0)	0.11
Phosphate, mg/dL	3.15 (0.45)	3.62 (0.43)	<0.001	3.09 (0.44)	3.54 (0.44)	<0.001	3.22 (0.48)	3.66 (0.47)	<0.001
Normal phosphate,[3] *n* (%)	1412 (93)	1996 (97)	<0.001	967 (91)	1266 (98)	<0.001	1330 (93)	1743 (95)	0.045
Calcium, mg/dL	9.65 (0.38)	9.80 (0.41)	<0.001	9.57 (0.35)	9.69 (0.35)	<0.001	9.81 (0.41)	9.87 (0.44)	<0.001
25(OH)D, nmol/L	61.5 (25.5)	48.0 (22.6)	<0.001	65.3 (27.8)	58.9 (27.4)	<0.001	60.5 (27.0)	60.1 (26.9)	0.67
eGFR, mL/(min·1.73 m^2)	72.2 (14.6)	71.0 (13.8)	0.012	80.1 (15.1)	79.0 (15.0)	0.07	88.1 (14.6)	87.6 (14.2)	0.34
Renal impairment,[4] *n* (%)	285 (19)	414 (20)	0.35	107 (10)	146 (11)	0.36	48 (3)	77 (4)	0.22
Current smoking, *n* (%)	343 (23)	301 (15)	<0.001	272 (26)	272 (21)	0.008	434 (31)	436 (24)	<0.001
Education, *n* (%)									<0.001
Primary education	174 (12)	412 (20)	<0.001	69 (7)	131 (10)	<0.001	123 (9)	219 (12)	
Low/intermediate	489 (32)	1024 (50)		297 (28)	772 (59)		345 (24)	810 (44)	
Intermediate	575 (38)	527 (26)		415 (49)	271 (21)		481 (34)	408 (22)	
High/university	279 (18)	102 (5)		282 (27)	125 (10)		475 (33)	397 (22)	
Testosterone, nmol/L	17.5 (6.1)	1.1 (0.9)	<0.001	16.3 (5.8)	0.9 (0.9)	<0.001	17.5 (5.9)	0.9 (0.5)	<0.001
17β-estradiol, pmol/L	91.4 (36.0)	33.2 (35.6)	<0.001	130 (40.6)	72.9 (63.4)	<0.001	98.3 (36.7)	121 (289)	0.001
WHR	0.98 (0.07)	0.89 (0.10)	<0.001	0.97 (0.07)	0.86 (0.08)	<0.001	0.93 (0.07)	0.83 (0.07)	<0.001
Leptin,[5] ng/mL	5.53 (4.77)	17.7 (12.9)	<0.001	ND	ND	ND	ND	ND	ND
Fat mass,[6] kg	23.3 (7.4)	28.4 (8.7)	<0.001	23.7 (7.5)	29.4 (9.4)	<0.001	24.6 (9.0)	29.7 (11.2)	<0.001
Lean mass,[6] kg	55.5 (5.9)	40.4 (4.6)	<0.001	57.3 (6.0)	41.0 (4.6)	<0.001	59.5 (6.1)	41.7 (6.0)	<0.001
Fat percent[6]	27.8 (6.0)	38.8 (6.4)	<0.001	27.6 (5.9)	39.2 (6.5)	<0.001	27.3 (6.7)	39.1 (7.9)	<0.001

[1] Continuous values are displayed as mean (SD); categorical variables are displayed in absolute counts (%). eGFR, estimated glomerular filtration rate; ND, not determined; RS, Rotterdam Study; WHR, waist-to-hip ratio; 25(OH)D, 25-hydroxyvitamin D.

[2] Differences between men and women were compared using an independent *t* test for continuous variables and χ^2 test for categorical variables.

[3] Normal phosphate was defined as a serum phosphate within the normal range [2.5–4.5 mg/dL (49)].

[4] Renal impairment was defined as eGFR <60 mL/(min·1.73 m^2) (23).

[5] Serum leptin concentrations were available in a random sample of 471 men and 618 women from RS-I.

[6] Body composition parameters were available from the fourth and second follow-up visit of RS-I and RS-II, respectively, and the baseline visit of RS-III.

TABLE 2 Association between serum phosphate concentrations and BMI in men and women aged 45–100 y from the Rotterdam Study[1]

	Men			Women		
Model	*n*	β (95% CI)	*P* value	*n*	β (95% CI)	*P* value
Model 1[2]	4004	−0.33 (−0.62, −0.05)	0.022	5198	−2.22 (−2.50, −1.95)	<0.001
Model 2[3]	4004	−0.37 (−0.68, −0.06)	0.019	5198	−1.92 (−2.20, −1.65)	<0.001

[1] β and 95% CIs were estimated from linear regression models and represent the change in BMI per increase in 1 mg/dL of phosphate. Analyses were performed in each of the 3 cohorts of the Rotterdam Study separately, and estimates were meta-analyzed using a random-effects meta-analysis model. eGFR, estimated glomerular filtration rate; 25(OH)D, 25-hydroxyvitamin D.
[2] Model 1: adjusted for age.
[3] Model 2: adjusted for age, smoking, education level, calcium and 25(OH)D, eGFR, testosterone, and 17β-estradiol.

with WHR only in women. In men, serum phosphate was inversely associated with WHR, but after adjusting for BMI and confounders, this association was no longer significant.

Genetic instruments

For the BMI GRS, 30 of 941 SNPs were discarded due to an imputation quality score <0.8, and 6 SNPs were discarded as they were palindromic with an allele frequency close to 0.42. The remaining 905 SNPs from the COJO analysis and the 634 SNPs from the primary GWAS analysis were used to construct an unweighted GRS for BMI.

Concerning the first MR assumption, the 905 and 634 BMI GRSs were significantly associated with BMI, with *F* statistics >79 in all cohorts (**Supplemental Table 8**). Neither score was associated with serum phosphate. Concerning the second MR assumption, there was a significant inverse association between both the 905 and 634 BMI GRSs with 25(OH)D concentrations (**Supplemental Figures 2** and **3**).

For the phosphate GRS, 13 phosphate-associated SNPs from a European and Japanese GWAS were considered. GWAS significance was checked in the UK Biobank. Six of 13 SNPs were independently associated with phosphate or were in high linkage disequilibrium (r^2 >0.8) with an independently associated SNP in the UK Biobank. These 6 SNPs were used to construct a GRS for phosphate. The phosphate GRS was associated with serum phosphate concentrations, with *F* statistics >10 in all cohorts (**Supplemental Table 9**). The score was not associated with BMI. The phosphate GRS was not significantly associated with potential confounders (**Supplemental Figure 4**).

MR

The 2sls regression of BMI, instrumented by the 905 SNP GRSs, on serum phosphate as outcome showed a significant causal effect of genetically determined BMI on serum phosphate (**Supplemental Table 10**). The adapted lasso regression did not show evidence for invalid instruments. MR–Egger did not show evidence for pleiotropy (intercept, $P = 0.317$). The weighted median estimator and the adapted CUE returned similar estimates (**Figure 2**). Because the BMI GRS was associated with serum 25(OH)D, we included adjustment for genetically determined 25(OH)D by the same SNPs to assess the likely direct [25(OH)D-independent] effects of BMI on serum phosphate. In this 25(OH)D-adjusted model, estimates were similar to the unadjusted model (data not shown). 2sls regression with the 905 SNP GRSs was repeated after exclusion of first- and second-degree relatives and showed a significant causal effect of genetically determined BMI on serum phosphate in this group (**Supplemental Table 11**). On the other hand, 2sls regression with the 634 SNP GRSs on serum phosphate showed similar estimates, but it did not reach significance (**Supplemental Table 12**).

The 2sls regression of serum phosphate, instrumented by the 6 SNP GRSs, on BMI as outcome showed no evidence of a causal effect of genetically determined phosphate on BMI (**Supplemental Table 13**).

Discussion

Our analyses in 3 cohorts of a population-based study of Caucasian elderly individuals consistently showed that serum phosphate concentrations were inversely associated with BMI in both sexes, and this association was not influenced by education level, smoking, total calcium, 25(OH)D, eGFR, and gonadal steroids. Associations were significantly stronger in women compared with men, and in a subset analysis, we found leptin adjustments to attenuate but not abolish the significant results. Furthermore, phosphate was also associated with fat mass in women but not in men. Phosphate proved to be significantly associated with fat% in both sexes. With bidirectional MR analysis, we found that BMI lowers phosphate (1-unit higher BMI lowered phosphate by 0.01 mg/dL), but phosphate does not seem to affect BMI. Although the effect estimates from MR

TABLE 3 Association between serum phosphate concentrations and BMI in men and women aged 61–100 y with serum leptin measurements from RS-I[1]

	Men			Women		
RS-I	*n*	β (95% CI)	*P* value	*n*	β (95% CI)	*P* value
Model 1[2]	471	−0.91 (−1.53, −0.28)	0.005	618	−2.65 (−3.40, −1.89)	<0.001
Model 2[3]	471	−1.14 (−1.77, −0.51)	<0.001	618	−2.33 (−3.08, −1.58)	<0.001
Model 3[4]	471	−1.13 (−1.67, −0.59)	<0.001	618	−0.94 (−1.45, −0.42)	<0.001

[1] β and 95% CIs were estimated from linear regression models and represent the change in BMI per increase in 1 mg/dL of phosphate. eGFR, estimated glomerular filtration rate; RS, Rotterdam Study; 25(OH)D, 25-hydroxyvitamin D.
[2] Model 1: adjusted for age.
[3] Model 2: adjusted for age, smoking, education level, calcium and 25(OH)D concentrations, eGFR, testosterone, and 17β-estradiol.
[4] Model 3: adjusted for age, smoking, education level, calcium and 25(OH)D concentrations, eGFR, testosterone, 17β-estradiol, and leptin.

TABLE 4 Association between serum phosphate concentrations and measures of body composition in men and women aged 45–88 y from RS-III with measures of body composition[1]

	Men			Women		
Characteristic	*n*	β (95% CI)	*P* value	*n*	β (95% CI)	*P* value
Fat mass						
Model 1[2]	469	−1.93 (−3.79, −0.07)	0.042	671	−4.43 (−6.26, −2.59)	<0.001
Model 2[3]	469	−1.29 (−3.06, 0.48)	0.15	671	−4.11 (−5.97, −2.24)	<0.001
Model 3[4]	469	−1.34 (−3.08, 0.40)	0.13	671	−3.25 (−4.81, −1.69)	<0.001
Fat percentage						
Model 1[2]	469	−1.66 (−3.02, −0.30)	0.017	671	−2.34 (−3.63, −1.05)	<0.001
Model 2[3]	469	−1.45 (−2.76, −0.14)	0.031	671	−2.23 (−3.53, −0.93)	0.001
Model 3[4]	469	−1.44 (−2.75, −0.13)	0.032	671	−1.83 (−3.04, −0.62)	0.003
Lean mass						
Model 1[2]	469	−0.34 (−1.56, 0.87)	0.58	671	−1.02 (−2.00, −0.04)	0.042
Model 2[3]	469	0.08 (−1.18, 1.34)	0.92	671	−0.85 (−1.87, 0.16)	0.10
Model 3[4]	469	0.07 (−1.20, 1.33)	0.92	671	−0.28 (−1.25, 0.69)	0.57
WHR						
Model 1[2]	1370	−0.01 (−0.014, 0.002)	0.17	1775	−0.02 (−0.02, −0.01)	<0.001
Model 2[3]	1370	−0.01 (−0.02, 0.00)	0.045	1775	−0.02 (−0.03, −0.01)	<0.001
Model 3[4]	1370	−0.004 (−0.01, 0.003)	0.22	1775	−0.01 (−0.02, −0.002)	0.011

[1] β and 95% CIs were estimated from linear regression models and represent the change in outcome variable per increase in 1 mg/dL of phosphate. eGFR, estimated glomerular filtration rate; LMI, lean mass index; RS, Rotterdam Study; WHR, waist-to-hip ratio; 25(OH)D, 25-hydroxyvitamin D.
[2] Model 1: adjusted for age.
[3] Model 2: adjusted for age, smoking, education level, calcium and 25(OH)D concentrations, eGFR, testosterone, and 17β-estradiol.
[4] Model 3: adjusted for age, smoking, education level, calcium and 25(OH)D concentrations, eGFR, testosterone, 17β-estradiol, and body composition. WHR was adjusted for BMI, lean mass for fat percentage, and fat mass and fat percentage for LMI.

analyses should not be interpreted literally, they do provide us more insight in phosphate homeostasis (19, 50). This study adds to the existing knowledge of phosphate homeostasis. Recent studies have shown that phosphate is associated with several health-related outcomes (12, 13). Our findings imply that the phosphate–adiposity relation should be taken into account when considering associations of serum phosphate, and if the relation between phosphate and mortality and morbidity can be explained by BMI, this may have health consequences.

A key assumption of MR analysis is that the genetic instrument must influence the outcome only through the exposure and not through other pathways ("horizontal pleiotropy") (18). We performed several sensitivity analyses to test potential pleiotropic effects of the SNPs in the BMI GRS and to assess the credibility of our MR results (42). These analyses (including adapted lasso regression, MR–Egger, weighted median approach, and adjusted CUE) all returned similar estimates.

Our data suggest that BMI lowers phosphate. Several theories may explain this effect. A recent MR study showed that a higher BMI leads to a lower 25(OH)D concentration (43). The active form of vitamin D, 1,25$(OH)_2$D, is synthesized from 25(OH)D and increases phosphate absorption from the intestine. A decrease in 25(OH)D may therefore decrease phosphate absorption from the intestine. However, it must be added that, in contrast to its role on calcium homeostasis, 1,25$(OH)_2$D is likely to influence phosphate homeostasis in considerable magnitude only at the extremes of its concentration (51). We estimated the effect of genetically predicted BMI on phosphate, controlling for the genetically determined vitamin D by the same SNPs (44). This resulted in similar estimates with borderline significance.

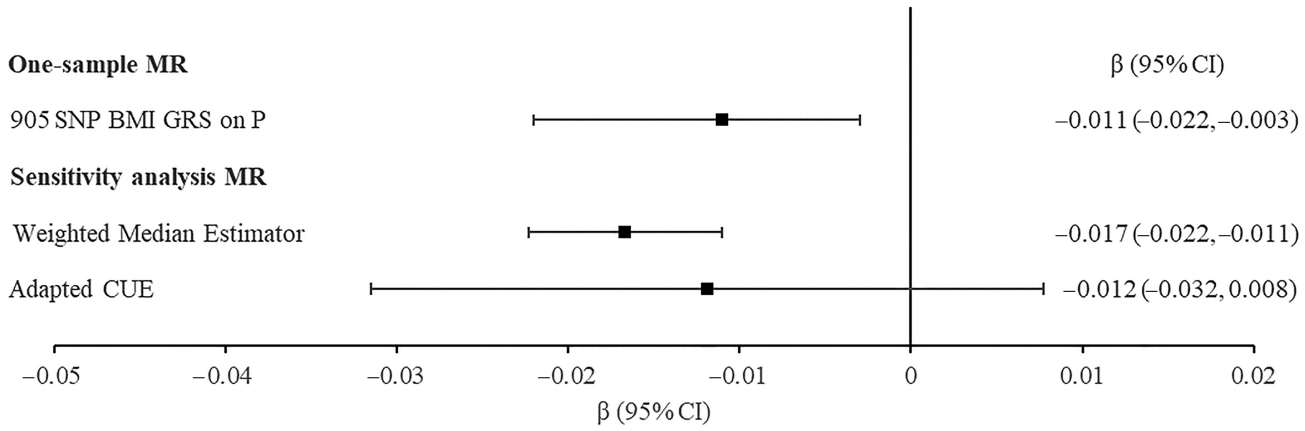

FIGURE 2 Mendelian randomization results for BMI and serum phosphate in men and women aged 45–100 y from the Rotterdam Study. CUE: continuously updated estimator; GRS, genetic risk score; MR, Mendelian randomization; P, phosphate; SNP, single-nucleotide polymorphism.

A positive relation between BMI, measures of central adiposity, and FGF23 concentrations has recently been described in several studies (52, 53). Hu et al. (54) found a positive association of serum FGF23 with abdominal obesity in 597 obese and nonobese men. In 591 postmenopausal women, both BMI and abdominal obesity were independently associated with serum intact FGF23, but there was no such association in premenopausal women ($n = 411$) (54). FGF23 is the most potent phosphaturic agent discovered so far. Holecki et al. (55) reported a positive association between phosphate and intact and cleaved FGF23 but found no association between BMI and measures of intact and cleaved FGF23 in 3115 elderly Polish male and female participants (9).

We observed a >50% attenuation of the effect estimate in women after adjusting for leptin. Leptin derives from white adipose tissue, and its concentrations reflect with high accuracy the amount of fat mass (11). Consistently, leptin-deficient mice display significantly higher concentrations of phosphate, calcium, and 1,25-dihydroxyvitamin D than wild-type mice (56). Interestingly, leptin has been recently described as a stronger predictor of FGF23 concentrations in women than $1,25(OH)_2D$ concentrations (10). Furthermore, the existence of leptin receptors at the kidney level (proximal straight tubules, loop of Henle, distal tubules, and collecting ducts) leaves room for a potential additional effect of leptin as a direct phosphaturic agent (11, 57). Thus, a phosphaturic effect of leptin through FGF23 and potentially also directly might partly explain the inverse association observed between BMI and phosphate concentrations, as reflected in the attenuation of the association after leptin adjustment in women. Previous studies have shown that leptin adjustments attenuate but not abolish the positive relation between FGF23 and body weight, BMI, and fat mass in both sexes (52). Collectively, these data support the concept that the relation between adipocytes and mineral metabolism is not fully mediated through leptin. On the other hand, leptin adjustment did not modify the association between BMI and phosphate in men, suggesting a sex dimorphism in the relation between leptin and phosphate concentrations. The potential role of "leptin resistance," in which leptin action is limited in obese states, on this sex dimorphism remains to be elucidated (58, 59). Further research will be needed to clarify our observations and to uncover the mechanisms underlying the sex dimorphism in this association.

We also considered the role of gonadal steroids as phosphate regulators as this has recently been reported. 17β-Estradiol treatment has been shown to induce phosphaturia in rats, but also in women, through a PTH-independent mechanism (60–62). In addition, testosterone concentrations were shown to exert an important role in regulating phosphate concentrations, even with a similar magnitude as PTH (63). We tested if 17β-estradiol or testosterone concentrations were playing a role as potential confounders of the observed associations. However, the adjustments for gonadal steroids did not change the association of phosphate with BMI in either sex.

Furthermore, we assessed the potential role for renal impairment, as obesity is associated with CKD progression and early stages of CKD are associated with hyperphosphaturia when there is still an adequate renal response to FGF23 (40, 64). We did not find that our results were confounded by CKD, as excluding participants with eGFR <60 mL/(min·1.73 m^2) yielded very similar results to those obtained from the entire study population.

We observed that phosphate concentrations were related to total adiposity, as reflected by fat%. This relation was found in both sexes but again stronger in women. The association between phosphate and WHR was mainly explained by BMI. This finding is in contrast with a previous report that suggested that phosphate was associated with fat distribution rather than with obesity itself (3).

This study has several limitations. The population is composed of European Caucasians, precluding inference to other populations or ethnic groups. We had no availability of serum FGF23, $1,25(OH)_2D$, or PTH concentrations. PTH decreases phosphate by increasing renal phosphate excretion. Furthermore, it has been shown that PTH is associated with BMI in obese participants and with fat mass in healthy postmenopausal women. Therefore, PTH could partly explain the association between phosphate and BMI (8–10, 65). Body composition was not measured in the same visit as the serum phosphate concentrations in the total research population, only in a subset, but results were mostly similar. Last, the F statistic for the MR analyses with instrumented phosphate was <10, which makes these analyses prone to weak instrument bias. Still, we found that the direction of the effect of the MR analyses with instrumented phosphate is opposite from the phenotypic analysis and from the MR analyses with instrumented BMI. Our study has several strengths, though. We were able to test and replicate findings in 3 large population-based cohorts, displaying normal variation of phosphate and BMI and therefore showing that this association is not restricted to subsets. Due to the sample size, sex-stratified analyses were feasible, and this highlighted the significant sex differences in our findings. Moreover, potentially important confounders could be investigated in this study. In addition, leptin measurements were available in a subset of the population, making it possible to further explore the potential mechanisms underlying the observed associations. An important strength of our study is the availability of genotype data, which allowed us to undertake a step forward in causal inference through the implementation of MR.

In summary, we found an inverse association between serum phosphate concentrations and BMI and fat% in Caucasian elderly individuals, with a significantly stronger effect in women compared with men. Bidirectional MR analysis indicated that BMI lowers phosphate and not the other way around. We found that serum leptin explained part of the association between phosphate and BMI in women, suggesting that fat mass is a regulator of phosphate homeostasis through production of leptin. Further research is needed to increase power and replicate our findings, especially regarding the role of leptin, and to elucidate the reasons underlying the observed sex differences. Our findings imply that the phosphate–adiposity relation should be taken in account when considering associations of serum phosphate.

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essential databases; AB and NC-O: performed the statistical analysis; AB, NC-O, and MCZ: wrote the paper and have primary responsibility for final content; and all authors: read and approved the final manuscript.

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