DR. EMMA BILLINGTON (Orcid ID : 0000-0002-2556-588X)

Article type : Original Paper

Serum Phosphate is Related to Adiposity in Healthy Adults

Emma O Billington MD[1,2], Greg D Gamble MSc[1], Sarah Bristow PhD[1], Ian R Reid MD[1]

[1]Bone & Joint Research Group, University of Auckland, Private Bag 92019, Auckland, 1142, New Zealand

[2]Division of Endocrinology & Metabolism, University of Calgary, 1820 Richmond Road SW, Calgary, Alberta, T2T 5C7, Canada

Keywords: phosphate, adiposity, body mass index, bone density, fibroblast growth factor 23

Corresponding author and person to whom reprint requests should be addressed:

Emma O Billington, MD

Richmond Road Diagnostic & Treatment Centre

1820 Richmond Road SW, Calgary AB, Canada, T2T 5C7

Email: emma.billington@ahs.ca

Telephone: 1-403-955-8328

Fax: 1-403-955-8249

Abstract

Background: Inorganic phosphate is a crucial component of cellular energy metabolism. We have identified an inverse relationship between serum phosphate concentration and fat mass in a cohort of healthy men. This study reports those data and determines whether this association is present in two female populations.

Methods: Cross-sectional data from three independent cohorts, consisting of healthy adult males (Male Cohort, n=323) and healthy postmenopausal women (Female Cohort 1, n=185; and Female Cohort 2, n=1471), are reported. Associations between serum phosphate and weight, body mass index (BMI), fat mass, and bone mineral density (BMD) were assessed. In a fourth cohort of postmenopausal women (FGF23 Cohort, n=20), associations between fibroblast growth factor 23 (FGF23), weight and BMI were assessed.

Results: Serum phosphate correlated inversely with weight, BMI and fat mass across all three cohorts ($r = -0.13$ to -0.31, p-value <0.0001 to 0.02). Associations were diminished after adjustment for PTH, but remained significant. In the FGF23 Cohort, FGF23 was positively correlated with weight ($r=0.60$, $p=0.007$) and BMI ($r=0.49$, $p=0.03$). Phosphate was inversely associated with BMD in Female Cohorts 1 & 2 ($r = -0.08$ to -0.29, p-value <0.0001 to 0.02). This relationship was attenuated, but remained significant at most sites, following adjustment for age, fat mass, renal function, and 25-hydroxyvitamin D.

Conclusions: Serum phosphate is inversely associated with measures of adiposity in both women and men, largely independently of PTH. FGF23 might mediate these associations. This relationship may be an unrecognized confounder in some of the correlates of serum phosphate already described.

Introduction

Inorganic phosphate is an essential component of many biological processes, and its serum levels are widely measured in clinical practice. Adequate phosphate concentrations are required for bone mineralization, and phosphate has a crucial role in energy metabolism, being required for generation of the primary cellular energy substrate, adenosine triphosphate (ATP). We have incidentally noticed an inverse correlation between serum phosphate levels and fat mass in a cohort of healthy men, and report that finding in this manuscript. In large databases, such associations can easily occur by chance, so we have also looked for similar associations between serum phosphate and measures of adiposity in two independent data sets, and used what other variables are available in these databases (e.g. parathyroid hormone [PTH]) to gain insight into the possible mediators of this relationship.

Although fibroblast growth factor 23 (FGF23) levels were not available in any of the three populations studied, this hormone is an important regulator of serum phosphate concentrations [1] and has been associated with fat mass in other cohorts [2, 3], suggesting that it may be a mediator of the relationships reported in this study. For this reason, we have also looked for associations between FGF23 and body mass index (BMI) in a further cohort in whom FGF23 levels were available.

Methods

Participants

This analysis uses baseline data from four independent cohorts of adults who participated in clinical trials at our center. The original trials have been described previously [4-7]. A brief description of each cohort is given below. Since the trial interventions took place after the collection of the data used in these analyses, the details of the trials are only relevant in that they determined the entry criteria to the cohorts. Sample size was dictated by the number of individuals from each cohort in whom the variables of interest had been assessed. All studies were approved by the local ethics committee, and informed consent was obtained from each participant at the time of study enrolment.

Male Cohort

This cohort (n=323) of community-dwelling men, aged 40 and older, were randomized to receive calcium supplements (600 or 1200 mg per day) or placebo for a two-year period [5]. Exclusion criteria included major active disease (including coronary heart disease, hypertension, diabetes mellitus, untreated thyroid disease, renal dysfunction, liver disease, malignancy, or known metabolic bone disease), current lipid-lowering therapy, 25-hydroxyvitamin D <25 nmol/L, and BMD Z-score <-2.0 at the spine or total hip.

Female Cohort 1

This cohort (n=185) of healthy postmenopausal women, age < 75 years, were randomized to receive hydrochlorothiazide 50 mg or placebo daily for a two-year period [4]. Exclusion criteria included disorders of calcium metabolism, previously treated osteoporosis, systemic illness (including renal, thyroid or hepatic dysfunction), glucocorticoid use, use of calcium supplements at a dose of >500mg per day, and use of hormone replacement therapy within 12 months.

Female Cohort 2

This cohort (n=1471) of healthy postmenopausal women, aged 55 or older, were randomized to receive either 1g elemental calcium (as citrate) or placebo daily for five years [6]. Women receiving therapy for osteoporosis or taking calcium supplements were ineligible. Additional exclusion criteria included major ongoing disease, malignancy, metabolic bone disease, 25-hydroxyvitamin D <25 nmol/L, and BMD Z-score of <-2.0 at the spine.

FGF23 Cohort

This cohort (n=20) of healthy women, at least 5 years postmenopausal, were randomized to receive either 1g elemental calcium (as carbonate) or to a placebo containing no calcium for three months [7]. Exclusion criteria included known cardiovascular disease, recent treatment with medications known to affect calcium concentrations or bone metabolism, and active systemic illness.

Measurements

In all cohorts, height was measured with a Harpenden stadiometer, and weight was determined using electronic scales. Dietary calcium intake was assessed with a validated food frequency questionnaire [8]. Baseline blood pressure was measured using either a Dynamap automatic monitor (Johnson & Johnson, Tampa, FL) (Male Cohort, Female Cohort 2) or a manual sphygmomanometer (Female Cohort 1), after a 5-10 minute rest period. In the Male Cohort and in Female Cohort 2, three blood pressure readings were taken three minutes apart, and results were averaged.

BMD and body composition were measured using dual-energy x-ray absorptiometry with a Lunar Prodigy (Male Cohort), Lunar DPX-L (Female Cohort 1), or Lunar Expert (Female Cohort 2) instruments (GE-Lunar, Madison, Wisconsin). BMD was assessed at the spine, hip and the total body.

Each participant provided a fasting serum sample at baseline, and concentrations of phosphate, total calcium, creatinine, glucose, and lipids were assessed. Glomerular filtration rate was estimated using the MDRD equation [9].

In the Male Cohort, 25-hydroxyvitamin D was measured using either a radioimmunoassay (DiaSorin, Stillwater, Minnesota) or a chemiluminescent assay (Nichols, San Juan Capistrano, California), both assays meeting the performance targets for the Vitamin D External Quality Assessment Scheme (DEQAS) [10]. Results using the Nichols assay were converted to DiaSorin results as previously described [11]. Serum PTH was measured in 151 participants using Roche autoanalyzers (Roche Diagnostics, Indianapolis, Indiana).

In Female Cohort 1, 25-hydroxyvitamin D levels were measured using the Incstar assay (Incstar Corporation, Stillwater, Minnesota). Serum PTH was measured using an Allegro assay (Nichols Institute, San Juan Capistrano, CA). Fasting insulin levels were measured using an in-house radioimmunoassay, with an intra-assay CV of 3.9%, and an inter-assay CV of 7.8%.

In Female Cohort 2, 25-hydroxyvitamin D levels were measured using a DiaSorin radioimmunoassay (DiaSorin, Stillwater, Minnesota). PTH levels were not determined in this cohort.

In the FGF23 Cohort, FGF23 was measured using an intact ELISA kit (Kainos Laboratories, Tokyo, Japan, CV 6%). Phosphate and PTH concentrations were assessed using a Cobas modular autoanalyzer (Roche Diagnostics).

Statistical analyses

In the three principal cohorts, visual inspection of frequency distribution histograms and Q-Q plots values were done to determine whether linear variables had parametric or non-parametric distributions. Associations between serum phosphate and other baseline parameters were assessed using Pearson's correlation for normally distributed data. Relationships between serum phosphate and measures of adiposity were further examined using linear regression analyses with phosphate as the dependent variable and fat mass as an independent variable, with stepwise inclusion of PTH, age and eGFR in the model. Relationships between serum phosphate and other cardiometabolic variables were also examined using linear regression analyses with inclusion of fat mass in the model. Associations between serum phosphate and BMD were similarly explored following adjustment for fat mass, in addition to the following established determinants of BMD: age, eGFR and 25-hydroxyvitamin D.

In the fourth (FGF23) cohort, results from the Shapiro-Wilk test for normality and the appearance of frequency distribution histograms and Q-Q plots were used to assess whether variables were normally distributed. Associations between FGF23, phosphate, PTH and measures of adiposity were determined using Pearson's correlation.

All data analysis was done with SAS v9.4 (SAS Institute, NC), and figures were created using Prism v6.0 (GraphPad Software Inc, 2013). The threshold for statistical significance was $p < 0.05$.

Where applicable, reporting of this study conforms to the recommendations laid out in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [12, 13].

Results

A total of 1979 individuals (1656 women, 323 men) from the three principal cohorts were studied. Their baseline characteristics are presented in Table 1.

Bivariate correlations of serum phosphate

Bivariate correlations of serum phosphate are shown in Table 2. All variables displayed in this table conformed to a normal distribution.Significant inverse correlations of serum phosphate with weight, BMI, and fat mass were observed in each of the cohorts ($-0.13 > r > -0.31$, $0.02 > p > 0.0001$). The correlations with fat mass are shown in Figure 1, and suggest that the relationships are robust and not influenced by outlying data points. Serum phosphate was also inversely related to diastolic blood pressure, lean mass and BMD in Female Cohorts 1 and 2, and to PTH, glucose and insulin in Female Cohort 1. PTH and insulin were not measured in Female Cohort 2.

Serum phosphate and adiposity

The inverse associations between serum phosphate and adiposity were further explored using multiple regression analysis to assess the possible role of PTH, which was measured in the Male Cohort and in Female Cohort 1. PTH was positively associated with BMI ($r=0.24$, $p=0.004$; $r=0.22$, $p=0.003$, in the respective cohorts) and fat mass ($r=0.23$, $p=0.005$; $r=0.28$, $p<0.0001$, respectively), and tended to be negatively related to serum phosphate ($r=-0.08$ $p=0.31$; $r=-0.24$, $p=0.001$, respectively). Multiple regression with phosphate as the dependent variable demonstrated that the relationship between phosphate and fat mass was independent of PTH, though PTH influenced phosphate in Female Cohort 1 (Table 3). When age and eGFR were added to the models, the results were little changed (Table 3).

Serum phosphate and cardiometabolic parameters

To better understand the other bivariate correlations of serum phosphate shown in Table 2, adjustments for fat mass were made. In the Male Cohort, the association between serum phosphate and serum glucose was no longer significant after adjusting for fat mass. In Female Cohort 1, the associations of serum phosphate with diastolic blood pressure and insulin were no longer significant after adjustment for fat mass (p=0.07 for diastolic blood pressure and 0.10 for glucose) but the relationship between phosphate and glucose remained significant (p=0.0005). In Female Cohort 2, the inverse association between serum phosphate and diastolic blood pressure remained statistically significant after adjusting for fat mass (p=0.01). Collectively, these findings suggest that fat mass is independently related to both phosphate and to these metabolic parameters, and that this co-dependence on fat mass mediates much of the apparent correlations between them.

Serum phosphate and skeletal parameters

Phosphate was negatively correlated with BMD at all sites in Female Cohorts 1 and 2, but not in the Male Cohort (Table 2). Because fat mass is a strong predictor of BMD, we adjusted these analyses for fat mass, in addition to age, eGFR, and 25-hydroxyvitamin D (Table 4). This was done using multivariable models in which phosphate was the dependent variable and the other parameters were predictor variables. Adjustment attenuated the relationships between phosphate and BMD, but they remained statistically significant at some skeletal sites (Table 4). Of the variables that were adjusted for, fat mass had the largest influence on the relationship between phosphate and BMD. The impact of adjustment was similar when BMI or weight were used in place of fat mass (data not shown).

Fibroblast growth factor 23 (FGF23) Cohort

The FGF23 Cohort consisted of 20 women, one of whom was excluded from analysis due to an outlying FGF23 level and subsequent diagnosis of primary hyperparathyroidism. For the remaining women (n=19), mean (SD) age was 67.3 (3.7) years, body weight 72.8 (11.7) kg, BMI 26.9 (4.1) kg/m^2, serum phosphate 1.14 (0.18) mmol/L, serum PTH 4.2 (1.0) pmol/L and FGF23 52.6 (12.4) pg/mL. FGF23 was significantly associated with both body weight (r=0.60, p=0.007) and BMI

(r=0.49, p=0.03) (Figure 2). In this cohort, there were trends towards an inverse correlation between BMI and phosphate (r=-0.39, p=0.11), and a positive correlation between BMI and PTH (r=0.35, p=0.14), although these did not reach statistical significance because of the much smaller size of this cohort.

Discussion

In the present study, we have demonstrated an inverse association between serum phosphate concentrations and measures of adiposity in three cohorts of healthy individuals, and identified that this relationship is independent of circulating PTH concentrations. The strong correlation between FGF23 and BMI observed in a smaller fourth cohort indicates that this hormone may be a mediator of the relationship between serum phosphate and fat mass. We also observed an inverse relationship between phosphate and BMD in two female cohorts, which is small in magnitude but appears to persist, to some extent, independently of fat mass.

Though the relationship between serum phosphate concentration and measures of adiposity is not widely appreciated, there are some corroborative data in the literature which have been largely overlooked since these were not the primary endpoints of those studies [14-18]. Lind and colleagues assessed 2183 50-year old males, selected from the general population, and observed inverse correlations between serum phosphate and both BMI (r=-0.24, p <0.0008) and waist-to-hip ratio (r=-0.33, P <0.0001). These relationships persisted after adjustment for age, sex and serum creatinine [15]. Haglin assessed 2265 adults (1272 women), most of whom were obese, and demonstrated an inverse correlation between serum phosphate and BMI in women (r= -0.18, p=0.0001) but not men (r=-0.06, p=0.07) [16]. Haap *et al* assessed 881 healthy adults (540 women) with a family history of type 2 diabetes, and found serum phosphate to be negatively correlated with BMI, an association that remained significant after adjustment for age and gender (r=-0.17, p <0.0001) [14]. In an analysis of 46,798 healthy Korean adults (16,568 women), Park and colleagues reported a negative correlation between phosphate and both BMI and waist circumference, although the relationship with BMI was no longer statistically significant after adjustment for age, sex, and serum calcium [17]. Individuals

with metabolic syndrome have been reported to have lower phosphate levels than controls ($p < 0.0001$) [19]. Our findings confirm an inverse relationship between serum phosphate and adiposity in cohorts of both men and women, spanning a large range of ages.

Because PTH is correlated with fat mass [20], and because PTH directly influences serum phosphate concentrations, consideration of PTH status is important when assessing the relationship between phosphate and fat mass. In this study, we have shown that variance in PTH accounts for only part of the association between serum phosphate and fat mass.

Fibroblast growth factor-23 (FGF23) is a second hormone that regulates serum phosphate. This was not assessed in our three primary cohorts, but we have demonstrated, using a smaller fourth cohort, that circulating FGF23 levels are related to adiposity. A number of pieces of evidence corroborate this finding. In the population-based Swedish MrOS cohort of 964 men, body weight was positively correlated with FGF23 after adjustment for age ($r=0.18$, $P<0.0001$) and after further adjustment for indices of mineral metabolism ($r=0.20$, $P<0.0001$) [21]. Similar relationships were seen with fat mass. In a community-based cohort of 946 70-year olds from Uppsala, correlations of FGF23 with body weight and fat mass were also found ($r=0.07$, $P<0.05$) [21]. In 2134 middle-aged men and women from the EPIC-Germany cohort, waist circumference and BMI increased across the quartiles of FGF23 [22]. In the Health ABC study, a similar significant relationship between quartiles of FGF23 and BMI was observed, mean BMI being 26.6 in FGF23 quartile 1 and 28.0 in quartile 4 [23]. Finally, in patients with primary hyperparathyroidism, FGF23 levels are related to BMI ($P<0.001$) [24]. Obesity may be causative in these relationships, since leptin directly stimulates FGF23 expression in primary rat osteoblasts[25], and administration of leptin to ob/ob mice almost doubles serum FGF-23 concentrations [26]. The causative role of obesity could also be inferred from the effects of weight loss on FGF23. This does not appear to have been

comprehensively assessed, but there is a report of 10 obese men who, after non-surgical weight loss of 20kg, showed a decline in FGF23 levels of about 20% [27]. PTH and FGF23 may act together to produce these effects since both are related to weight and both are phosphaturic. Their secretion may be inter-dependent, since FGF23 has been shown to promote proliferation of parathyroid cells [28], and reduction of PTH levels after parathyroidectomy reduces FGF23 levels by about 20% [24].

There are other mechanisms which could contribute to the lower serum phosphate levels of adiposity. Overweight individuals tend to have a diet that is high in carbohydrates but low in protein, and therefore are more likely to have inadequate phosphate intake [16]. Intracellular phosphate shifts are promoted by glucose entry into cells, which is facilitated by insulin release. Many overweight and obese individuals develop hyperinsulinemia, which might contribute to lower extracellular phosphate concentrations. In keeping with this hypothesis, we identified negative correlations between serum phosphate and glucose levels in the Male Cohort, and in Female Cohort 1, as well as an inverse relationship between serum phosphate and insulin in Female Cohort 1. As expected, these relationships were largely attenuated after adjustment for fat mass. Similarly, in their study of healthy Korean adults, Park *et al* identified an inverse association between serum phosphate and both fasting glucose ($r=-0.07$, $p<0.0001$) and fasting insulin ($r=-0.04$, $p<0.0001$), and these relationships were no longer present once analyses were adjusted for BMI [17].

Phosphate is an important component of bone mineral. Adequate local phosphate concentrations are required for bone mineralization, and very low phosphate levels are associated with osteomalacia [29]. We found inverse associations between serum phosphate and BMD in the two female cohorts that were assessed, but not in the cohort of males. Following adjustment for age, PTH, fat mass, renal function, and 25-hydroxyvitamin D, associations between phosphate and BMD were attenuated. However, most associations remained statistically significant following these adjustments, suggesting that the relationship between phosphate and BMD may also be mediated by other factors.

The cross-sectional design of this study precludes conclusions about the direction of causality and the mechanisms underlying the observed associations between serum phosphate and measures of adiposity and BMD. We were also limited in our ability to assess potential mediators of these relationships, as PTH and FGF23 levels were not available in all cohorts. Cross-sectional evaluation of large databases can lead to the identification of spurious associations, and assessment of cohorts recruited to satisfy specific inclusion and exclusion criteria can introduce selection bias. However, a major strength of the present study is its demonstration of consistent relationships between phosphate and measures of adiposity in three independent cohorts of men and women. This indicates that our findings are unlikely to be spurious and suggests that they are representative of the general population.

In conclusion, this study suggests that serum phosphate is associated with markers of adiposity, adding substantially to the existing evidence for this. The biological significance of this phosphate-adiposity relationship remains to be determined, but it is relevant to any other studies that consider associations of serum phosphate, and may well represent an unrecognized confounder in some of the relationships that have already been described. This study also raises the important question of the association between adiposity and FGF23 levels, which requires further exploration, but may explain the observed relationship between serum phosphate and fat mass. Finally, it provides another facet to the already complicated relationship between bone metabolism and glucose-fat homeostasis.

Author Contributions

EOB and IRR conceived of the study idea. EOB, GDG and SMB extracted and analyzed the data. EOB drafted the manuscript, and GDG, SMB and IRR contributed important intellectual content to revisions of the final article.

Funding

The original studies described in this paper were supported by the Health Research Council of New Zealand.

References

1 Liu S and Quarles LD. How fibroblast growth factor 23 works. J Am Soc Nephrol 2007;18:1637-47.

2 Mirza MA, Alsio J, Hammarstedt A, Erben RG, Michaelsson K, Tivesten A *et al.* Circulating fibroblast growth factor-23 is associated with fat mass and dyslipidemia in two independent cohorts of elderly individuals. Arterioscler Thromb Vasc Biol 2011;31:219-27.

3 di Giuseppe R, Kuhn T, Hirche F, Buijsse B, Dierkes J, Fritsche A *et al.* Potential Predictors of Plasma Fibroblast Growth Factor 23 Concentrations: Cross-Sectional Analysis in the EPIC-Germany Study. PLoS One 2015;10:e0133580.

4 Reid IR, Ames RW, Orr-Walker BJ, Clearwater JM, Horne AM, Evans MC *et al.* Hydrochlorothiazide reduces loss of cortical bone in normal postmenopausal women: a randomized controlled trial. Am J Med 2000;109:362-70.

5 Reid IR, Ames R, Mason B, Reid HE, Bacon CJ, Bolland MJ *et al.* Randomized controlled trial of calcium supplementation in healthy, nonosteoporotic, older men. Arch Intern Med 2008;168:2276-82.

6 Reid IR, Horne A, Mason B, Ames R, Bava U and Gamble GD. Effects of calcium supplementation on body weight and blood pressure in normal older women: a randomized controlled trial. J Clin Endocrinol Metab 2005;90:3824-9.

7 Bristow SM, Gamble GD, Pasch A, O'Neill WC, Stewart A, Horne AM *et al.* Acute and 3-month effects of calcium carbonate on the calcification propensity of serum and regulators of vascular calcification: secondary analysis of a randomized controlled trial. Osteoporos Int 2016;27:1209-16.

8 Angus RM, Sambrook PN, Pocock NA and Eisman JA. A simple method for assessing calcium intake in Caucasian women. Journal of the American Dietetic Association 1989;89:209-14.

9 Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW *et al.* Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766-72.

10 Carter GD, Carter CR, Gunter E, Jones J, Jones G, Makin HL *et al.* Measurement of Vitamin D metabolites: an international perspective on methodology and clinical interpretation. J Steroid Biochem Mol Biol 2004;89-90:467-71.

11 Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD *et al.* Determinants of vitamin D status in older men living in a subtropical climate. Osteoporos Int 2006;17:1742-8.

12 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC and Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. The Lancet;370:1453-7.

13 Simera I, Moher D, Hoey J, Schulz KF and Altman DG. A catalogue of reporting guidelines for health research. Eur J Clin Invest 2010;40:35-53.

14 Haap M, Heller E, Thamer C, Tschritter O, Stefan N and Fritsche A. Association of serum phosphate levels with glucose tolerance, insulin sensitivity and insulin secretion in non-diabetic subjects. Eur J Clin Nutr 2006;60:734-9.

15 Lind L, Jakobsson S, Lithell H, Wengle B and Ljunghall S. Relation of serum calcium concentration to metabolic risk factors for cardiovascular disease. Bmj 1988;297:960-3.

16 Haglin L, Lindblad A and Bygren LO. Hypophosphatemia in the metabolic syndrome. Gender differences in body weight and blood glucose. Eur J Clin Nutr 2001;55:493-8.
17 Park W, Kim BS, Lee JE, Huh JK, Kim BJ, Sung KC *et al.* Serum phosphate levels and the risk of cardiovascular disease and metabolic syndrome: a double-edged sword. Diabetes Res Clin Pract 2009;83:119-25.
18 Uza G, Pavel O, Uza D and Vlaicu R. Effect of propranolol on hypophosphatemia in overweight. International journal of obesity 1981;6:507-11.
19 Kalaitzidis R, Tsimihodimos V, Bairaktari E, Siamopoulos KC and Elisaf M. Disturbances of Phosphate Metabolism: Another Feature of Metabolic Syndrome. American Journal of Kidney Diseases 2005;45:851-8.
20 Bolland MJ, Grey AB, Ames RW, Horne AM, Gamble GD and Reid IR. Fat mass is an important predictor of parathyroid hormone levels in postmenopausal women. Bone 2006;38:317-21.
21 Mirza MAI, Alsio J, Hammarstedt A, Erben RG, Michaelsson K, Tivesten A *et al.* Circulating Fibroblast Growth Factor-23 Is Associated With Fat Mass and Dyslipidemia in Two Independent Cohorts of Elderly Individuals. Arteriosclerosis Thrombosis and Vascular Biology 2011;31:219-+.
22 Di Giuseppe R, Kühn T, Hirche F, Buijsse B, Dierkes J, Fritsche A *et al.* Potential predictors of plasma fibroblast growth factor 23 concentrations: Cross-sectional analysis in the epic-Germany study. PLoS ONE 2015;10.
23 Isakova T, Cai X, Lee J, Katz R, Cauley JA, Fried LF *et al.* Associations of FGF23 With Change in Bone Mineral Density and Fracture Risk in Older Individuals. Journal of Bone and Mineral Research 2015.
24 Nilsson IL, Norenstedt S, Granath F, Zedenius J, Pernow Y and Larsson TE. FGF23, metabolic risk factors, and blood pressure in patients with primary hyperparathyroidism undergoing parathyroid adenomectomy. Surgery (United States) 2016;159:211-7.
25 Saini RK, Kaneko I, Jurutka PW, Forster R, Hsieh A, Hsieh J-C *et al.* 1,25-Dihydroxyvitamin D3 Regulation of Fibroblast Growth Factor-23 Expression in Bone Cells: Evidence for Primary and Secondary Mechanisms Modulated by Leptin and Interleukin-6. Calcified Tissue International 2013;92:339-53.
26 Tsuji K, Maeda T, Kawane T, Matsunuma A and Horiuchi N. Leptin stimulates fibroblast growth factor-23 expression in bone and suppresses renal 1a,25-dihydroxyvitamin D3 synthesis in leptin-deficient mice. J. Bone Mineral Res. 2010;25:1711–23.
27 Fernández-Real JM, Puig J, Serrano M, Sabater M, Rubió A, Moreno-Navarrete JM *et al.* Iron and Obesity Status-Associated Insulin Resistance Influence Circulating Fibroblast-Growth Factor-23 Concentrations. PLoS ONE 2013;8.
28 Kazuki K, Ai T, Kenryo F, Keiichi K, Masayasu M, Ikuji H *et al.* FGF23 plays an important role in the pathogenesis of secondary hyperparathyroidism. Australian and New Zealand Bone & Mineral Society. http://www.anzbmsconference.com/downloads/2015-handbook/ANZBMS%20HANDBOOK%202015%20FINAL/3.%20ANZBMS_MEPSA_MBS ANZ_%20ABSTRACTS.pdf.; 2015.
29 Ruppe MD and Jan de Beur SM. Disorders of Phosphate Homeostasis. In: Rosen CJ, editor. Primer on Metabolic Bone Diseases and Disorders of Mineral Metabolism, 8th Edition. Washington, DC: American Society for Bone and Mineral Research; 2013.

Table 1: Baseline characteristics of three independent cohorts

	Male Cohort (n=323)	**Female Cohort 1** (n=185)	**Female Cohort 2** (n=1471)
Age (y)	56.5 (10.3)	62.8 (5.8)	74.2 (4.2)
Height (cm)	1.77 (0.07)	1.62 (0.05)	1.59 (0.06)
Weight (kg)	82.7 (12.1)	68.1 (11.4)	66.9 (11.3)
BMI (kg/m^2)	26.5 (3.3)	26.0 (4.2)	26.4 (4.2)
Dietary calcium intake (mg/d)	870 (450)	1000 (510)	860 (390)
Systolic BP (mmHg)	131 (13)	130 (14)	141 (23)
Diastolic BP (mmHg)	78 (8)	82 (9)	73 (11)
Phosphate (mmol/L)	1.0 (0.1)	1.2 (0.1)	1.2 (0.1)
Total calcium (mmol/L)	2.33 (0.09)	2.29 (0.08)	2.32 (0.09)
PTH (pmol/L)	4.06 (1.25)*	2.96 (1.24)	
eGFR ($mL/min/1.73m^2$)	73.3 (10.8)	66.1 (9.7)	57.3 (10.0)
25OH vitamin D (nmol/L)	92.5 (32.9)	55.0 (23.2)	53.5 (17.9)
Glucose (mmol/L)	5.04 (0.48)	5.03 (0.90)	5.11 (0.70)
Insulin		10.6 (10.7)	
Fat mass (kg)	19.4 (7.6)	27.8 (8.7)	27.1 (9.6)
Lean mass (kg)	59.4 (6.9)	36.7 (3.6)	36.2 (4.1)
Total body BMD (g/cm^2)	1.26 (0.09)	1.06 (0.08)	1.03 (0.09)
Lumbar spine BMD (g/cm^2) †	1.25 (0.16)	1.07 (0.16)	1.06 (0.18)
Femoral neck BMD (g/cm^2)	1.01 (0.14)	0.87 (0.13)	0.82 (0.12)
Total hip BMD (g/cm^2)	1.08 (0.14)		0.86 (0.13)

*151 men in this cohort had PTH levels measured
†Represents BMD at L2-L4 in Female Cohort 2, and L1-L4 in the other two cohorts
BMI = body mass index, BP = blood pressure, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate, BMD = bone mineral density

Table 2: Correlation coefficients between serum phosphate and other parameters in the three cohorts

	Male Cohort (n=323)		**Female Cohort 1** (n=185)		**Female Cohort 2** (n=1471)	
	r	***p-value***	***r***	***p-value***	***r***	***p-value***
Age (y)	0.06	0.28	0.08	0.26	**0.08**	**0.004**
Height (cm)	-0.07	0.24	0.03	0.70	-0.04	0.16
Weight (kg)	**-0.15**	**0.007**	**-0.29**	**<0.0001**	**-0.19**	**<0.0001**
BMI (kg/m^2)	**-0.13**	**0.02**	**-0.31**	**<0.0001**	**-0.19**	**<0.0001**
Dietary calcium intake (mg/d)	**0.16**	**0.004**	0.12	0.12	-0.02	0.47
Systolic BP (mmHg)	-0.06	0.25	-0.12	0.18	-0.04	0.10
Diastolic BP (mmHg)	-0.06	0.30	**-0.21**	**0.02**	**-0.09**	**0.001**
Total calcium (mmol/L)	-0.02	0.75	-0.10	0.22	0.03	0.33
PTH (pmol/L)*	-0.08	0.31	**-0.24**	**0.001**		
eGFR (mL/min/17.3m^2)	0.01	0.90	-0.05	0.48	0.00	0.95
25OH vitamin D (nmol/L)	0.03	0.61	0.11	0.24	0.00	0.94
Glucose (mmol/L)	**-0.13**	**0.02**	**-0.28**	**0.0002**	-0.03	0.26
Insulin			**-0.24**	**0.02**		
Fat mass (kg)	**-0.18**	**0.002**	**-0.31**	**<0.0001**	**-0.18**	**<0.0001**
Lean mass (kg)	-0.06	0.29	-0.11	0.13	**-0.10**	**<0.0001**
Total body BMD (g/cm^2)	-0.07	0.22	**-0.21**	**0.005**	**-0.12**	**<0.0001**
Lumbar spine BMD (g/cm^2)	-0.02	0.77	**-0.19**	**0.009**	**-0.09**	**0.0004**
Femoral neck BMD (g/cm^2)	0.02	0.78	**-0.29**	**<0.0001**	**-0.08**	**0.002**
Total hip BMD (g/cm^2)	0.00	0.95			**-0.13**	**<0.0001**

Significant associations are bolded.
*151 men in this cohort had PTH levels measured
BMI = body mass index, BP = blood pressure, PTH = parathyroid hormone, eGFR = estimated glomerular filtration rate, BMD = bone mineral density
r = Pearson's correlation coefficient

Table 3: Parathyroid hormone (PTH) and fat mass as predictors of serum phosphate in two cohorts before and after adjustment in a multivariable model*

	Male Cohort		**Female Cohort 1**	
	β-coefficient	***p-value***	***β-coefficient***	***p-value***
Unadjusted Model				
Fat Mass	-0.18	0.002	-0.31	<0.0001
Model adjusted for PTH				
Fat Mass	-0.21	0.01	-0.27	0.0004
PTH	-0.04	0.67	-0.16	0.03
Model adjusted for PTH, age, eGFR				
Fat Mass	-0.16	0.06	-0.27	0.0005
PTH	-0.05	0.53	-0.17	0.02
Age	0.17	0.06	0.04	0.54
eGFR	0.21	0.02	-0.07	0.31

*Based on a multivariable model with serum phosphate as the dependent variable and the other parameters as predictor variables.

Table 4: Bone mineral density (BMD) as a predictor of serum phosphate in three cohorts before and after adjustment in multivariable models*

BMD Site	**Male Cohort** (n=323)		**Female Cohort 1** (n=185)		**Female Cohort 2†** (n=1471)	
	β-coefficient	***p-value***	***β-coefficient***	***p-value***	***β-coefficient***	***p-value***
Unadjusted Model						
Total body	-0.07	0.22	**-0.21**	**0.005**	**-0.12**	**<0.0001**
Lumbar spine	-0.02	0.77	**-0.19**	**0.009**	**-0.09**	**0.0004**
Femoral neck	0.02	0.78	**-0.29**	**<0.0001**	**-0.08**	**0.002**
Total hip	0.00	0.95			**-0.13**	**<0.0001**
Adjusted Model						
Total body	0.01	0.93	-0.12	0.27	**-0.06**	**0.03**
Lumbar spine	0.04	0.59	**-0.23**	**0.02**	-0.05	0.09
Femoral neck	0.13	0.14	**-0.26**	**0.02**	-0.04	0.14
Total hip	0.10	0.26			**-0.07**	**0.007**

*Data are from a multivariate model in which serum phosphate is the dependent variable and BMD, parathyroid hormone (PTH), age, fat mass, estimated glomerular filtration rate (eGFR), and 25-hydroxyvitamin D are independent variables. Separate regression analyses were carried out for the four skeletal sites. PTH levels were not assessed in Female Cohort 2, so adjusted correlations reported for this cohort do not include adjustment for PTH.

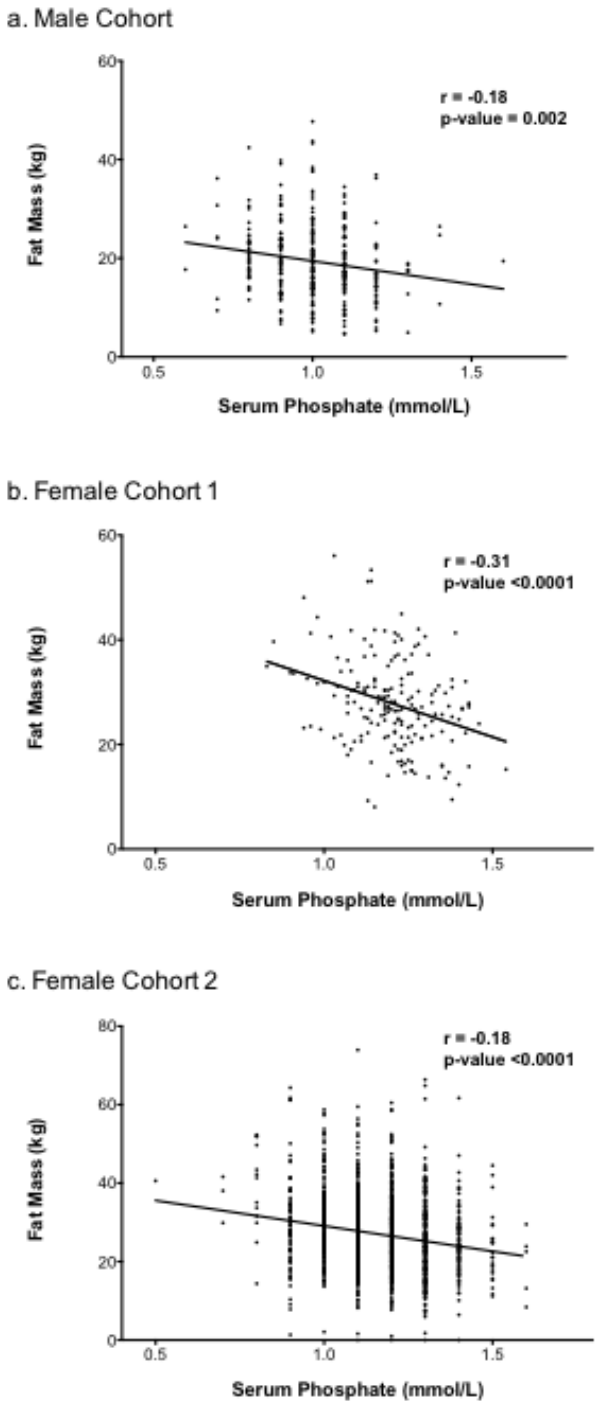

Figure 1: Relationship between serum phosphate and fat mass in three independent cohorts, (a) Male Cohort (n=323), (b) Female Cohort 1 (n=185), and (c) Female Cohort 2 (n=1471).

r = Pearson’s correlation coefficient

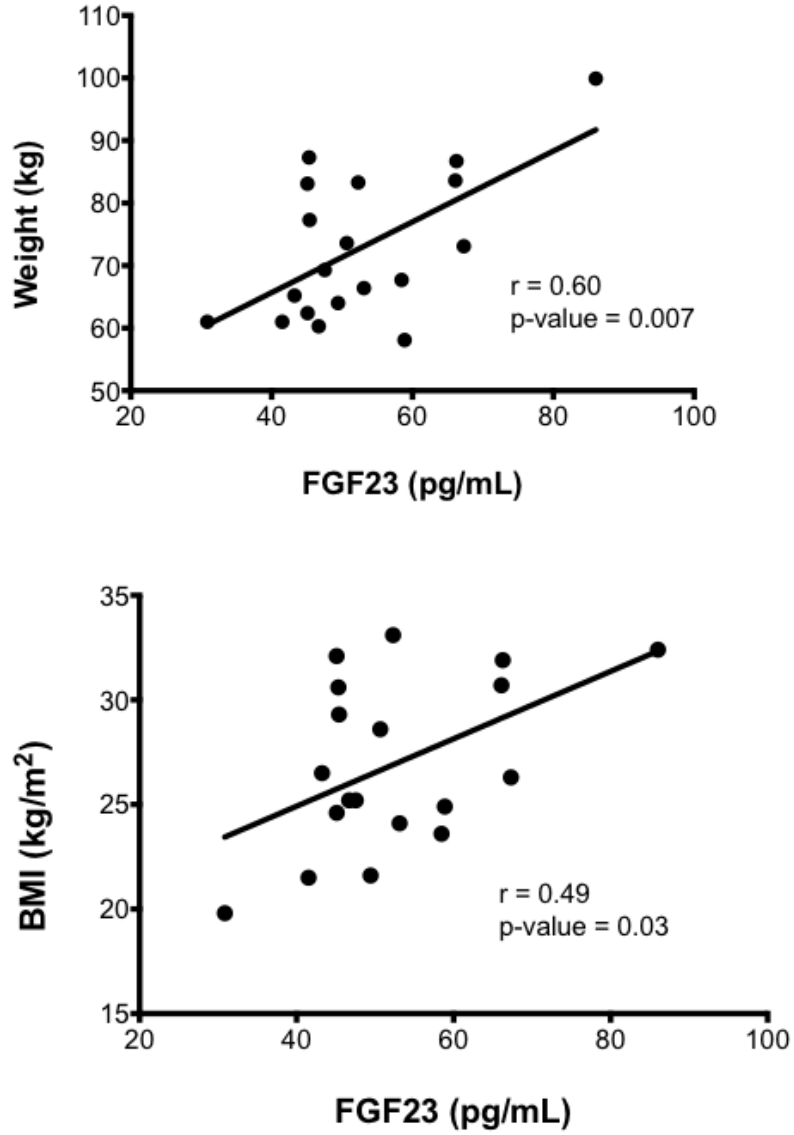

Figure 2: Relationship between fibroblast growth factor 23 (FGF23) and both weight (top) and body mass index (BMI, bottom) in a cohort of healthy postmenopausal women (n=19).

r = Pearson’s correlation coefficient