Glomerular filtration rate and serum phosphate: an inverse relationship diluted by age

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Abstract

Background. Available data indicate that serum phosphate increases only when glomerular filtration rate (GFR) falls into the low range (<60 mL/min \times 1.73 m\textsuperscript{2}). GFR and serum phosphate decrease with ageing. This population-based study investigated by age-controlled analyses the relationship of GFR with serum phosphate in adults with GFR above the low range.

Methods. Data were collected on age, sex, menstrual status, anthropometry, overnight urinary creatinine, dietary protein (overnight urinary urea), reported intake of milk/yogurt, serum creatinine, phosphate, calcium and total protein in 4034 adults (age 18–91 years) with GFR \geq 60 mL/min \times 1.73 m\textsuperscript{2} as assessed by estimated GFR (eGFR, simplified MDRD equation) and creatinine clearance (overnight urinary creatinine/serum creatinine).

Results. The relationship of eGFR with serum phosphate was positive in men and null in women in univariate analyses \((P = 0.001\) and 0.148\), negative in both sexes with age adjustment \((P < 0.001)\). Age-adjusted results did not depend on colinearity between age and eGFR because the relationship was inverse also replacing eGFR with creatinine clearance \((P < 0.001\) in both sexes). In univariate regression analysis done separately by gender and six age-strata (18–24, 25–34, 35–44, 45–54, 55–64 and \geq 65\), the line of serum phosphate over eGFR was constantly inverse \((\text{range of } P = 0.010/0.089)\) with the progressively lower y-axis intercept from young to older ages. The inverse relationship of eGFR or creatinine clearance with serum phosphate was significantly inverse also controlling for other variables \((P < 0.01)\).

Conclusions. GFR differences in the range \geq 60 mL/min \times 1.73 m\textsuperscript{2} are inversely and independently related to serum phosphate. The relationship is undetectable without age-controlled procedures because, for serum phosphate, the
effect of GFR differences above \( \geq 60 \text{ mL/min} \times 1.73 \text{ m}^2 \) is much smaller than the effect of age.

**Keywords:** age; GFR; Gubbio study; phosphate

### Introduction

Serum phosphate (P) is used to monitor P retention that is a consequence of low glomerular filtration rate (GFR) and a determinant of severe complications in the bone and in the cardiovascular system of patients affected with kidney failure [1]. A number of studies show that serum P increases only when there is a substantial reduction of glomerular filtration rate (GFR) but is stable when GFR ranges close to or above the so-called near normal range (GFR \( \geq 60 \text{ mL/min} \times 1.73 \text{ m}^2 \)) [2–20].

An influence of age on serum P has been known since 1969 [21–26]. Recent data showed that the relationship between age and serum P differs between men and women and reflects a relationship of age with renal tubular P reabsorption in both sexes [27]. In men, renal tubular P reabsorption and serum P are continuously and progressively lower with increasing age. In women, the curves of renal tubular P reabsorption and serum P over age have three different phases: progressively lower values during the fertile age, transiently higher values during the climacteric and progressively lower values in post-menopausal age [27]. As far as age and GFR are concerned, large studies consistently show that GFR is progressively lower with increasing age whatever is the estimate used to index GFR [28–30]. Thus, secondary to the effect of age per se, high values of serum P and GFR are common in younger ages whereas low values of serum P and low GFR are common in older ages. The possibility exists that the combination of these age-associated differences in serum P and GFR could play a confounding role and/or a dilution effect in the analysis of the relationship between GFR and serum P when GFR ranges \( > 60 \text{ mL/min} \times 1.73 \text{ m}^2 \). To investigate this possibility, the relationship between GFR and serum P was cross-sectionally investigated with control for age in a population-based sample of adults with indices of GFR \( \geq 60 \text{ mL/min} \times 1.73 \text{ m}^2 \).

### Methods

The Gubbio Study is an epidemiological investigation targeting the whole population resident within the medieval wall of the city of Gubbio (Italy) [31,32]. Activities were approved by the local institutional committee and included an informed consent by participants. Previous papers reported information on response rate, time of examinations, similarities between the Gubbio population and the Italian population [31,32]. As previously described [27,30], the second examination of the Gubbio Study included the collection of timed overnight urine under fed condition for analyses of renal tubular function; a brief medical examination with inclusion of measurements of blood pressure and anthropometrics and the administration of questionnaires on medical history and life styles.

Laboratory procedures and definition of variables

Creatinine and P in freshly collected serum and urine samples were measured by automated colorimetric methods (kinetic alkaline picrate and ammonium molybdate, respectively). The intra-assay error in daily blind duplicates was \(< 5\%\) for serum variables and \(< 10\%\) for urinary variables. GFR was assessed by two different indices: estimated GFR (eGFR) and creatinine clearance. The abbreviated equation of the Modification Diet in Renal Disease study [33] was used for eGFR calculation as reported [30,34]. Creatinine clearance was calculated as overnight urinary creatinine excretion rate/serum creatinine. Weight and height were used for calculation of body surface area (BSA = height\(m^2\) \( \times \) weight\(kg\)/1.73 m\(^2\)) and body mass index (BMI = weight\(kg\)/height\(m^2\)). Information on menstrual status was included in the analysis because sex hormones affect phosphorus homeostasis [35]. Cessation of menstrual cycles was defined as the lack of cycles in the last year. Dietary protein was estimated with the use of overnight urinary urea excretion after conversion of urea to nitrogen [36]. Overnight urea excretion was extrapolated to 24-h urea excretion on the basis of the evidence that urea excretion rate is similar and correlated between overnight urine and 24-h urine [37]. Information about average intake of milk or yogurt per week was used as an index of dairy products intake and expressed as cups/day. Serum total calcium was normalized per 7 g of serum protein to reduce the confounding of protein-bound calcium [30].

### Results

**Descriptive statistics**

The population sample participating in the second examination of the Gubbio Study included 4680 persons with age \( \geq 18 \) years. A total of 646 persons were excluded from this analysis because of missing data for some variables \((n = 13, 0.3\%\)) or creatinine clearance \(< 60 \text{ mL/min} \times 1.73 \text{ m}^2\) \((n = 606, 12.9\%\)) and creatinine clearance \(< 60 \text{ mL/min} \times 1.73 \text{ m}^2\) \((n = 27, 0.6\%\)). Thus, the cohort for the present analysis is made of 4034 persons (1939 men and 2095 women, age range 18–91 years) with complete data, with both eGFR and creatinine clearance \( \geq 60 \text{ mL/min} \times 1.73 \text{ m}^2\).

Table 1 reports descriptive statistics by sex. In both sexes, creatinine clearance was higher than eGFR \((P < 0.001\)) and correlated with eGFR \((R = +0.673 \text{ and } +0.690, P < 0.001\)). Measured serum total Ca correlated with the serum total protein in men and women \((R = +0.136 \text{ and } +0.162, P < 0.001\)). As expected, after the exclusion of persons...
with values <60 mL/min x 1.73 m², eGFR and creatinine clearance were slightly skewed (skewness >0.8, P < 0.05). Due to the eGFR skewness, the median was slightly lower than the mean (81.6 and 75.5 mL/min x 1.73 m², men and women, respectively; interquartile range = 73.2–91.2 and 68.1–85.6). Similarly for creatinine clearance, the median was slightly lower than the mean (102.5 and 101.8 mL/min x 1.73 m²; interquartile range = 91.6–116.5 and 90.9–115.1). Serum P was slightly skewed in men (skewness = +0.402, median = 3.3 mg/dL, interquartile range = 2.9–3.7) but not in women (skewness = +0.083, median = 3.5 mg/dL, interquartile range 3.1–3.8). The intake of milk/yogurt was the only highly skewed variable (skewness >1) and was logarithm (log) transformed to reduce the effects of non-normal distribution (log-transformed intake = 0 for persons with intake = 0). The median of milk/yogurt intake was 1.7 cups/week in men and 3.5 cups/week in women (inter-quartile range = 0.0–5.0 and 0.0–6.0, respectively).

Time variability in serum P was assessed by two measures repeated over a 6.3-month mean period in a subset of 88 men and 112 women (age range = 18–82). The two measures were similar (3.42 and 3.43 mg/dL, P = 0.878 by the paired t-test) and correlated (R = 0.719, P < 0.001). Time variability in serum creatinine and eGFR was similarly low as previously reported [30].

### Relationship of serum P over indices of GFR: analyses in the whole cohort

Figure 1 shows that, in univariate analyses, the relationship between eGFR and serum P was significantly positive in men and non-significant in women. The regression coefficient of serum P over eGFR was +0.0045 in men (P < 0.001, 95% CI = +0.0025/+0.0065) and −0.0014 in women (P = 0.148, 95% CI = −0.0032/+0.0005). In contrast, in age-controlled analyses, the relationship between eGFR and serum P was significantly negative in men and women (P < 0.001, 95% CI = −0.0055/−0.0035) and non-significant in men (P = 0.153, 95% CI = 0.0012/0.0000).
Relationship of serum P over indices of GFR: analyses by age stratum

The relationship between eGFR and serum P was investigated by univariate analyses in separate age strata to explain the contrast in the results between univariate analyses and age-adjusted analyses on the whole cohort. Persons with age ≥65 were combined in a single stratum to avoid the bias of strata with low sample size (age strata 75–84 and ≥85). The results of univariate analyses by age stratum are graphically shown in Figures 2 and 3 (men and women, respectively) and as coefficients of the regression lines in Table 2. The results indicated a consistently inverse relationship between eGFR and serum P. The relationship was weak (R ranging from −0.143 to −0.092) but significant or borderline significant in all age strata of both sexes. With increasing age, the regression line of serum P over eGFR had similar values of slope but progressive lower values of the y-axis intercept in both sexes. The reduction in the value of the y-axis intercept across age strata was present in both sexes: large and significant in men (95% CI of the y-axis intercept at age 18–24 did not overlap the 95% CI at age ≥65), weak and nonsignificant in women. The shift of the line towards the lower range of serum P from young to older ages reflected the fact that, independent of eGFR, serum P levels were significantly lower with increasing age as previously described [27].

In accordance with the similarity of the slopes in Table 2, there was not an interaction of age with the slope of the relationship between eGFR and serum P because the age × eGFR product was not associated with serum P in a multiple regression of serum P over eGFR and age (P of the age × eGFR product = 0.848 in men and 0.480 in women). Findings were similar when creatinine clearance instead of eGFR in age-controlled analyses in men (regression coefficients = −0.0036, P < 0.001; 95% CI = −0.0050/−0.0023) and women (−0.0036, P < 0.001; 95% CI = −0.0049/−0.0024).

Multivariate analyses on the independence of the relationship between indices of GFR and serum P

Table 3 shows the results of multivariate linear regression models where serum P was regressed as a dependent variable over GFR indices and other variables in men and women. The analyses were done using eGFR in model 1 and creatinine clearance in model 2. The reference interval of numerical variables was defined as an approximation of 1 SD of the independent variable in men and women. In both sexes there was a significantly inverse relationship between the GFR index (either eGFR or creatinine clearance) and serum P. As far as the other variables are concerned, the results were significant for age in both sexes and for body mass index and cessation of menstrual cycles only in women. In all models, the standardized regression coefficient (beta) was several times smaller for the GFR index (range of values = −0.115/−0.036) than for age (range of values = −0.402/−0.315).

Discussion

This cross-sectional study shows the novel finding of an inverse relationship between GFR indices and serum P in men and women selected from a population sample for having GFR indices above the threshold of 60 mL/min, hence in a range where previous studies reported the absence of relationship between GFR and serum P. The relationship between GFR indices and serum P appeared only in statistical procedures performed with control for age or focused in separate subgroups with a narrow age range because the effect of GFR on serum P was much smaller in comparison to the effect of age on serum P. Also, the study shows that the inverse relationship between GFR indices and serum P was independent not only of age but also of other variables including anthropometry, serum calcium, dietary indices and menopause in women.

A limitation of the study was the lack of information on modulators of P metabolism such as parathyroid hormone, phosphatonin and active vitamin D [38]. It is unlikely that these modulators might have a primary causal role in the inverse relationship between GFR and serum P. Previous papers unanimously show that the hormonal changes associated with low GFR include high levels of parathyroid hormone [1,2,4,5,7] in combination with high levels of phosphatonin [1,18] and low levels of active vitamin D [1,6,7,10,11,14]. The components of this combination, altogether and each one per se, are known to reduce serum P [38]. Thus, a reasonable inference is that the expected changes in the levels of parathyroid hormone, phosphatonin and active vitamin D secondary to GFR reduction should have counterbalanced and blunted the inverse relationship between GFR and serum P. In a similar way, the limited information on dietary factors should have played a minor role, if any. An inverse relationship between GFR and serum P could be explained by dietary factors only if a P-rich diet lowered kidney function, an effect not supported by any observation. Another possible limitation was the collection of data in a single ethnic group residing in one town. Several data support the idea that the Gubbio cohort is similar to other population samples at least looking at the prevalence of kidney dysfunction [30] and the relationship of this dysfunction with various endpoints [30,39]. However, further studies are needed to verify whether the present findings are generalizable to other populations.
Altogether, the present results support the interpretation that there is a continuous relationship of GFR with serum P, hence with P retention. This conclusion is in contrast with all previous papers on this point [2–19] and with the view, derived from those papers, that kidney dysfunction increases serum P only in the presence of a substantial decrease in GFR [1]. The use of a large set of population-based data and age-controlled statistical procedures are reasonable explanations for the contrast between the conclusions of the present paper and the conclusions of past papers. The majority of the past papers was in fact without control for age and had limited statistical power due to low
number of participants (n of individuals ranging from 15 to 256 in [2–18]). Three previous papers were based on large sets of data about serum P [19,20,39]. The results of the paper by Kestenbaum et al. [19] cannot be compared with the results of the present study because those authors analysed with control for age only the relationship between serum P and mortality, but not the relationship between renal function and serum P. Onufrań et al. reported data on age, eGFR and serum P in 13,340 examinees of the ARIC study with age 45–64 years and eGFR ≥ 45 mL/min [20]. The higher eGFR in the ARIC cohort reflected the lack of data in older persons because the prevalence of eGFR < 60 mL/min is known to increase steeply for ages ≥ 65 years (approximately up to 20% in the Gubbio cohort).
Table 2. Univariate regression coefficients (95% CI) of the relationship between eGFR and serum P separately analysed by age stratum in men and women

| Age   | Men | | | Women | | |
|-------|-----| | |       |   | |
|       | y-axis intercept (mg/dL) | Slope (mg/dL per mL/min × 1.73 m²) | y-axis intercept (mg/dL) | Slope (mg/dL per mL/min × 1.73 m²) |
| 18–24 | 4.42 (3.79/5.04) | −0.0067 (−0.0113/−0.0003) | 4.27 (3.85/4.69) | −0.0062 (−0.0109/−0.0015) |
| 25–34 | 3.96 (3.53/4.39) | −0.0049 (−0.0098/−0.0001) | 3.88 (3.50/4.25) | −0.0046 (−0.0090/−0.0002) |
| 35–44 | 3.99 (3.53/4.45) | −0.0070 (−0.0124/−0.0016) | 3.81 (3.43/4.20) | −0.0063 (−0.0111/−0.0015) |
| 45–54 | 3.66 (3.28/4.03) | −0.0060 (−0.0106/−0.0014) | 3.96 (3.56/4.36) | −0.0069 (−0.0120/−0.0017) |
| 55–64 | 3.40 (3.04/3.75) | −0.0038 (−0.0081/+0.0006) | 3.85 (3.50/4.21) | −0.0051 (−0.0099/−0.0003) |
| ≥65   | 3.35 (2.97/3.73) | −0.0045 (−0.0095/+0.0004) | 3.81 (3.44/4.18) | −0.0056 (−0.0108/−0.0004) |

Table 3. Multiple regression of serum P over indices of GFR and other variables: difference in serum P (mg/dL) associated with the reference interval of the independent variable (95% CI)

| Independent variable | Reference intervala | Men | | | Women | | |
|----------------------|---------------------|-----| | |       |   | |
|                      | Model 1: eGFR as the index of GFR | Model 2: creatinine clearance as the index of GFR | Model 1: eGFR as the index of GFR | Model 2: creatinine clearance as the index of GFR |
| eGFR                 | 15 mL/min × 1.73 m² | −0.051** (−0.085/−0.016) | Not included | −0.086** (−0.121/−0.052) | Not included |
| Creatinine clearance | 15 mL/min × 1.73 m² | −0.049** (−0.074/−0.024) | Not included | −0.061** (−0.083/−0.039) |
| Age                  | 15 years           | −0.222** (−0.250/−0.193) | −0.222** (−0.249/−0.195) | −0.178** (−0.220/−0.136) | −0.161** (−0.201/−0.120) |
| Serum normalized calcium | 0.6 mg/dL        | n.s. (−0.054/+0.006) | n.s. (−0.045/+0.016) | n.s. (−0.012/+0.038) | n.s. (−0.007/+0.043) |
| Body mass index      | 4 kg/m²           | n.s. (−0.033/+0.029) | n.s. (−0.021/+0.042) | −0.035* (−0.057/−0.013) | −0.029* (−0.052/−0.007) |
| Dietary protein      | 0.4 g protein/kg weight | n.s. (−0.021/+0.063) | n.s. (−0.021/+0.044) | n.s. (−0.023/+0.025) | n.s. (−0.008/+0.044) |
| Intake of milk/yogurt  | 0.4 log cups/day  | n.s. (−0.014/+0.036) | n.s. (−0.014/+0.036) | n.s. (−0.035/+0.010) | n.s. (−0.036/+0.009) |
| Cessation of menstrual cycles | Yes/no | Not included | Not included | +0.323** (+0.238/+0.408) | +0.301** (+0.216/+0.386) |

For numerical variables, the reference interval was defined as an approximation of one SD in men and women.

**P < 0.001, *P < 0.01, n.s., not significant (>|0.05).

[30]. The results differed between the ARIC cohort and the Gubbio cohort also in the relationships of age and eGFR with serum P. The relationship of age with serum P was direct and significant only among women of the ARIC cohort whereas it was significantly inverse in both sexes of the Gubbio cohort. Moreover, the age-adjusted relationship of eGFR with serum P was not significant in the ARIC cohort whereas it was significantly inverse in the Gubbio cohort. The contrast between the two sets of results was reasonably explained by differences in the age range of the two cohorts and in the availability of information on menstrual status. In fact, when the analysis was limited to ages 45–64, the relationship of age with serum P was direct also in women of the Gubbio cohort because serum P tends to increase from age 45 to age 64 among them [27]. The increase in serum P among middle-aged women, however, reflects the influence of climacteric on renal P reabsorption, not the effect of age [27,35]. Thus, the lack of information on menstrual status and the narrow range of age in the ARIC cohort biased the evaluation of the true effect of age on serum P. In turn, the biased assessment of the relationship between age and serum P necessarily precluded a correct age-adjustment in the analysis on the relationship between eGFR with serum P. Finally, the paper by Dhingra et al. [40] did not focus at all on the relationship between GFR and serum P.

The practical implications of the present results depend on the role of serum P in the pathogenesis of osteodystrophy and vascular calcifications typical of kidney failure. An investigation on these end-points was beyond the scopes of the study. Nevertheless, it is reasonable to hypothesize that even the mild differences in serum P found in the present study in association with low GFR might play some role in the pathogenetic sequence leading to skeletal and vascular alterations typical of kidney failure. In fact, differences in the so-called normal range of serum P are able to modulate certain functions of parathyroid cells and to activate calcifying vascular cells [41–43]. Thus, the present data raise the possibility that P-induced alterations in parathyroid glands, bone tissue and vascular wall could develop in kidney disease before than previously believed and secondarily to slight increases in serum P within its clinically normal range. A support to this possibility comes from the epidemiological evidence of a direct relationship between serum P and incident cardiovascular disease among persons...
with eGFR >60 mL/min [39]. If even slight increases in serum P might have detrimental effects, further investigations would be needed about P homeostasis in youth when serum P is high secondary not to kidney dysfunction but to the physiological interplay of P-saving hormones as growth hormone, estrogen, etc.

In conclusion, the results of this population-based cross-sectional study indicate that, in contrast with the present views, there is an inverse relationship between GFR and serum P also when GFR is above the low range. However, the effects of GFR differences on serum P in the range >60 mL/min are much smaller in comparison to the effects of differences in age. Thus, the relationship between GFR and serum is detectable only when analyses are focused on large samples of individuals with a narrow range of age or with the use of age-controlled statistical procedures able to reduce the confounding of age on serum P.

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Low-density lipoprotein clearance in patients with chronic renal failure

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Abstract

Background. Chronic renal failure increases the risk of atherosclerosis. The clearance of low-density lipoprotein (LDL), a major risk factor for atherosclerosis, has been reported as being disturbed in dialysis patients. We studied LDL metabolism in non-dialyzed patients with chronic kidney disease (CKD).

Methods. LDL clearance was studied with a radiotracer method in 57 CKD patients and 10 healthy controls.

Results. In the CKD patients, the fractional catabolic rate of LDL apo B (LDL FCR), an indicator of LDL clearance from plasma, ranged from 0.13 to 0.56 pools/day with a mean value of 0.34 pools/day being comparable to that of the control subjects. In the renal patients, LDL FCR correlated significantly with estimated glomerular filtration rate (eGFR) (r = 0.340, P = 0.010) and this association remained significant after the adjustment with age, body mass index, gender, presence of diabetes and LDL cholesterol concentration (P = 0.004). In CKD patients with eGFR <15 mL/min/1.73 m² the mean LDL FCR was significantly reduced when compared to that of CKD patients with eGFR >30 mL/min/1.73 m² (P = 0.005). LDL apo B production rate was not associated with renal function or different between renal patients and control subjects.

Conclusions. The clearance of LDL seems to be related to the severity of renal impairment, but a remarkable reduction in LDL catabolism can be observed only in patients with advanced renal failure.

Keywords: chronic renal failure; LDL cholesterol; lipoprotein metabolism

Introduction

Patients with chronic renal failure are at a substantially elevated risk of developing atherosclerosis. Approximately 40% of these patients have cardiovascular disease even before they reach end-stage renal failure requiring dialysis therapy [1]. The risk of cardiac mortality has been shown to be elevated more than 100-fold among dialysis patients aged 45 years or younger compared to the general population [2]. Even mild renal impairment has been associated with an increased risk for death, cardiovascular events and hospitalization in the general population [3], suggesting that cardiovascular disease begins to develop early in the course of chronic renal failure.