

# Phosphate Metabolism in Intensive Care Patients with Acute Respiratory Failure

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## ABSTRACT

**Objective:** To determine the variables predicting the change of plasma phosphate over the first 24 hr period in intensive care in patients with acute respiratory failure

**Methods:** Fifty-seven patients were studied prospectively in a university teaching hospital intensive care unit (ICU). Thirty two patients were classified as having acute respiratory failure and a primary respiratory system diagnosis (group I), 10 were classified as having acute cardiogenic pulmonary oedema (group II) and 15 were general ICU patients (group III). Arterial blood specimens at intensive care unit admission ( $T_0$ ) and at 24 hr post-admission ( $T_{24}$ ) were assayed for multiple plasma biochemical parameters including phosphate ( $PO_4$ ) and red blood cell 2,3-diphosphoglycerate (2,3-DPG). Timed urine collections were used to determine 24 hr urine phosphate loss and renal phosphate threshold concentration (RTP). During the measurement period glucose-free fluids only were infused.

**Results:** Fifty seven patients had a mean ( $\pm$  SD) age of  $67 \pm 12$  years and Apache II score of  $22 \pm 6$ . The plasma  $PO_4$  at  $T_0$  was  $1.55 \pm 0.71$  mmol/L and showed a significant 24 hr decrease of 0.55 mmol/L ( $p < 0.0001$ ) at  $T_{24}$ . Hypophosphataemia at  $T_0$  was observed in 26% of patients. Red blood cell 2,3-DPG was not elevated at  $T_0$  ( $13.5 \pm 3.3$   $\mu$ mol/gHb) and showed a non-significant increment over 24 hr. Urine phosphate loss over the 24 hr period was  $21.8 \pm 14.0$  mmol with RTP being reduced below the lower reference range limit in groups I ( $0.65 \pm 0.29$  mmol/L) and II ( $0.57 \pm 0.29$  mmol/L). The naïve form of phosphate change ( $PO_4T_{24} - PO_4T_0$ ) was significantly related to initial plasma  $PO_4$  and was subject to regression to the mean, which was estimated to have inflated the relationship by 25%. The appropriate form of phosphate change was found to be log ratio  $T_{24}/T_0$  phosphate. Independent predictors of log ratio  $T_{24}/T_0$  phosphate were 24 hr change ( $T_{24}-T_0$ ) in both 2,3-DPG and arterial pH, RTP, prescription of aminophylline (categorical factor) and the interaction of aminophylline and RTP ( $R^2 = 0.65$ , ordinary least squares regression).

**Conclusions:** Twenty-four hour plasma phosphate decrement in intensive care unit patients was multifactorial and was attended by a lowered renal threshold phosphate concentration. (**Critical Care and Resuscitation 2002; 4: 93-103**)

**Key words:** phosphate metabolism, acute respiratory failure, 2,3-diphosphoglycerate, renal threshold phosphate concentration, multivariable regression, bootstrap, multiple imputation, model uncertainty, regression to the mean, logarithmic transformation

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Profound disturbances of inorganic phosphate metabolism have been observed during the course of respiratory illness associated with acute respiratory failure. In non-intensive care unit patients, phosphate metabolic changes (e.g. hypophosphataemia, low muscle phosphorus content with defective muscle metabolism and "inadequate" renal phosphorus handling) have been reported in the clinically stable state.<sup>1-5</sup> In the critically ill intensive care unit (ICU) patient, both admission hyperphosphataemia and acute hypophosphataemia associated with acute respiratory failure and mechanical ventilation have also been reported.<sup>6-9</sup> Cellular phosphate changes and renal phosphate handling have usually been inferred from either observed changes of blood inorganic phosphate and other blood chemistry variables (e.g. arterial blood gas parameters). However, phosphate metabolism in the acute phase of respiratory failure necessitating ICU admission and ventilation would not appear to have been systematically evaluated. A prospective study was undertaken to investigate phosphate metabolism in patients over the first 24 hr after admission to the ICU, by measuring the change ( $\Delta$ ) of plasma phosphate and red blood cell 2,3-diphosphoglycerate concentration (as an index of intracellular phosphate shift)<sup>10</sup> and the renal phosphate handling during the 24 hr period.<sup>4</sup>

## METHODS

The study was approved by the Queen Elizabeth Hospital Ethics of Research Committee.

### *Patients and patient definitions*

Patients were enrolled on admission to the ICU with diagnoses of:

- i) acute respiratory failure (Group I). Acute respiratory failure was defined as a syndrome comprising clinical signs (cyanosis, dyspnoea, impaired conscious state and signs of respiratory muscle exhaustion) with a  $\text{PaO}_2 < 60$  mm Hg on room air (or  $\text{PaO}_2/\text{FIO}_2 < 286$  on oxygen) and/or decompensated respiratory acidosis.<sup>11</sup> Patient diagnostic categories were: a) asthma and chronic obstructive pulmonary disease as defined by the 1987 American Thoracic Society statement<sup>12</sup> and, b) pneumonia, defined as a clinical syndrome of respiratory distress as evidenced by a combination of fever, leukocytosis, purulent sputum and chest X-ray opacity.<sup>13</sup>
- ii) cardiogenic acute pulmonary oedema (Group II).<sup>14,15</sup> Cardiogenic acute pulmonary oedema was defined as a syndrome of acute onset of dyspnoea with clinical findings consistent with pulmonary oedema (increased work of breathing, gallop rhythm, widespread auscultatory crepitations in the absence of any

history of respiratory infection) and appropriate radiological changes.<sup>16</sup>

- iii) general ICU patients (Group III), with primary diagnoses other than respiratory failure and/or cardiogenic pulmonary oedema.

Plasma phosphate reference range was 0.8 - 1.45 mmol/L. Hypophosphataemia was classified as mild (0.61 - 0.8 mmol/L), moderate (0.32 - 0.6 mmol/L) and severe ( $< 0.32$  mmol/L).<sup>17,18</sup> Hyperphosphataemia was defined as a plasma concentration exceeding 1.45 mmol/L.

### *Protocol*

Patients were observed over a 24 hr period.

1. All patients had continuous monitoring of heart rate, intravascular blood pressure via indwelling arterial catheters (Sirecust 404-1, Siemens, Erlangen, Germany) and pulse oximetry (Biox 3740, Ohmeda, Boulder, CO). Where indicated, mechanical ventilation was provided by Puritan-Bennett PB7200ae microprocessor controlled ventilators (Puritan Bennett Corp, Overland Park, KS).
2. Intravenous fluids as 0.45% or 0.9% saline only were used for the first 24 hr in the ICU, to avoid hypophosphataemia due to concomitant infusion of glucose-containing fluids.<sup>7</sup> No phosphate or magnesium-containing fluids were given during this period. Potassium (as potassium chloride) was supplemented in maintenance fluids to maintain plasma potassium  $\geq 4.0$  mmol/L.
3. At study entry (ICU admission  $T_0$ ) and at 24 hr post ICU admission ( $T_{24}$ ), arterial blood specimens were taken for measurements of:
  - i) plasma biochemical variables (measured on a Technicon DAX random access analyser, Bayer Australia Ltd, using standard chemical analyses). Calcium levels corrected for plasma albumin by 0.02 mmol/L per g of albumin (to a value of 40 g/L).<sup>19</sup>
  - ii) arterial blood gases (analysis was performed using the ABL3 Acid-Base Laboratory Radiometer, Copenhagen, Denmark).
  - iii) plasma lactate (measured by conversion to pyruvate with reduction of NAD to NADH).<sup>20</sup>
  - iv) intact parathyroid hormone (2-site chemi-luminometric immunoassay, Ciba Corning Magic Lite Intact PTH).
  - v) red blood cell 2,3-diphosphoglycerate (RBC 2,3-DPG); concentration was determined after the method described by Luzzato (reference range 9.4 - 16.9  $\mu\text{mol/g}$  of haemoglobin).<sup>21</sup>
4. At  $T_0$  a random urine sample was taken followed by a 24 hr urine collection. Both specimens were assay-

ed for concentrations of sodium, potassium, creatinine, calcium, magnesium and phosphate.

5. Between 1000 to 1200 hr on the day after admission, a two hour urine collection and arterial blood sample at mid-point were taken. The time from admission to the beginning of the two hour collection was recorded (time delay of collection for real threshold phosphate). Urine and plasma concentrations of creatinine and phosphate urine volume were measured.

- i) Clearance of creatinine and phosphate were calculated according to the formula:

Clearance (mL/min) =

$$\frac{\text{Urine conc (mmol/L)}}{\text{Plasma conc (mmol/L)}} \times \frac{\text{Urine vol (mL/2 hr)}}{120}$$

- ii) Renal threshold phosphate concentration (RTP) was calculated using the Walton and Bijvoet nomogram, which derives RTP from concomitant values of plasma phosphate and the ratio of phosphate to creatinine clearance.<sup>22</sup>
- iii) Fractional excretion of phosphate (FE<sub>PO4</sub>) was calculated according to the formula:<sup>23</sup>

$$\text{FE}_{\text{PO}_4} = [(U/P)_{\text{PO}_4} / (U/P)_{\text{Cr}}] \times 100$$

Where,

U and P = urine and plasma concentrations

PO<sub>4</sub> = phosphate (mmol/L)

Cr = creatinine (mmol/L)

6. An APACHE II score<sup>24</sup> was recorded over the first 24 hr. Patient details, pertinent to the study (e.g. diagnosis, age, sex, mechanical ventilation and prescription of diuretic, corticosteroids, aminophylline and sympathomimetic agents), were recorded separately.

#### Statistical analysis

Variables are reported as mean ± SD unless otherwise indicated. Interval data were analysed by t-test (differences (Δ) T<sub>24</sub> relative to T<sub>0</sub>) and categorical by Pearson χ<sup>2</sup> and Fisher exact tests, where appropriate. Variable relations were analysed by analysis of variance and ordinary least squares regression (OLS). Stata® statistical software was used.<sup>25</sup> Multivariable linear regression was used to establish quantitative relationships between key physiological predictor variables of Δ phosphate.

The appropriate analytic form for Δ phosphate was investigated by regression of candidate variables: a) *change* (T<sub>24</sub>-T<sub>0</sub> phosphate), b) *percentage change* [(T<sub>24</sub>-T<sub>0</sub> phosphate)/T<sub>0</sub> phosphate × 100], c) *ratio* (T<sub>24</sub>

phosphate/T<sub>0</sub> phosphate) and d) *log ratio* (log [T<sub>24</sub> phosphate/T<sub>0</sub> phosphate] = [log (T<sub>24</sub> phosphate) - log (T<sub>0</sub> phosphate)]) against both *initial phosphate* (T<sub>0</sub> phosphate) and *average phosphate* [(T<sub>0</sub> phosphate + T<sub>24</sub> phosphate)/2], as suggested by Harrell,<sup>26</sup> to find a dependent variable not related to initial values (T<sub>0</sub> phosphate). Normality was assessed by kernel density estimation,<sup>27</sup> and specific tests (Shapiro-Wilk). To establish the propriety of *change* vs *percentage change*, Kaiser's R was also calculated.<sup>28</sup>

The effect of regression to the mean on the β coefficient of the of the *change* vs *initial phosphate* relationship, was computed after Blomqvist's method:<sup>29,30</sup> β\* = [β<sup>#</sup> + (1-p)]/p, where β<sup>#</sup> is the regression slope of *change* on *initial phosphate*, as derived from the data-set and p = 1 - δ<sup>2</sup>/s<sup>2</sup>, where s<sup>2</sup> is the observed variance of measured initial (T<sub>0</sub>) values and δ<sup>2</sup> is an estimate from an "external" source of within-subject variance. Estimates of δ<sup>2</sup> were obtained from computerised records of patients admitted to the ICU; duplicate values of phosphate were obtained at admission from patients admitted from 1998-1999, where the difference between time of admission and time of duplicate observation was < 3 hr.

Predictor variables were defined by a backward selection process,<sup>31</sup> with Akaike information criterion,<sup>32</sup> corresponding to a nominal p value of 0.157 for stepwise selection.<sup>33</sup> Consistency of variable selection was investigated by using bootstrap (1000 samples) of the backward selection with p = 0.157 and recording the variables selected with a frequency > 50% in the OLS regression.<sup>33-35</sup> Multi-collinearity (variance inflation factors [VIF] < 10 and condition number < 15),<sup>36</sup> interactions (adjusted for multiple comparisons after Holm<sup>37</sup>) and predictor non-linearity were also investigated.<sup>38</sup> Where non-linearity was evident, covariate effect was modelled using fractional polynomials.<sup>39</sup> Specific attention was paid to the selection problems with highly correlated (r ≥ 0.5) variables:<sup>40</sup> a) arterial pH and PaCO<sub>2</sub>, b) fractional excretion of phosphate, 24 hr urine phosphate excretion and renal threshold phosphate. Models assessed were: OLS and generalised linear model (GLM) with the log link.<sup>41</sup> Models were compared using estimates of fit:<sup>41-43</sup> R<sup>2</sup>, adjusted R<sup>2</sup>, AIC and adjusted likelihood ratio index.

A set of key predictor variables in addition to the candidate variables for Δ phosphate were defined and any missing value pattern was identified.<sup>44</sup> The data set was tested for "missingness" being completely at random (MCAR; that is, the missing values were a random subsample of the entire data-set),<sup>45</sup> utilising the missing value module provided in Systat® statistical software.<sup>46</sup> To explore the impact of missing values on regression estimates (i) a complete data set of key

predictors was generated using the expectation-maximisation (EM) algorithm and (ii) a multiple imputation approach (k=10 data sets, using data-augmentation techniques) after Schafer<sup>47</sup> was adopted.

## RESULTS

Fifty seven patients, 32 males and 25 females with a mean ( $\pm$  SD) age of  $67 \pm 12$  years and Apache II score  $22 \pm 6$ , of whom 32 were ventilated, were enrolled into the study. In Group I, patient diagnoses (n = 32) were: exacerbation of chronic obstructive pulmonary disease

(COPD) with acute respiratory failure (n = 20), pneumonia (n = 10) and status asthmaticus (n = 2). Group II comprised 10 patients with acute pulmonary oedema; no patient had a diagnosis of myocardial infarct based upon 12 lead electrocardiogram and creatine kinase estimation. In Group III diagnoses were: postoperative abdominal aortic aneurysm (n = 3), gastrointestinal perforation (n = 3), septic shock (n = 4); post cardiac arrest (n = 3), overdosage (n = 1) and subarachnoid haemorrhage (n = 1). Details of the 3 diagnostic groups are given in Table 1.

**Table 1. Patient diagnostic groups**

	Group I	Group II	Group III	p
Number	32	10	15	
Age (years)	$67 \pm 11$	$67 \pm 12$	$68 \pm 16$	NS
APACHE II score	$20 \pm 5$	$24 \pm 3$	$24 \pm 9$	NS
Sex (M/F)	18/14	6/4	8/7	NS
Mechanical ventilation	18	6	11	NS
Diuretics	9	10	4	0.0001
Corticosteroids	23	1	3	0.0001
Aminophylline	26	3	1	0.0000
Sympathomimetics	4	4	5	0.0002

Values for age and Apache II score are given as mean  $\pm$  SD.

**Table 2. Initial (T<sub>0</sub>) values (mean  $\pm$  SD) for group variables**

Variable (normal reference range)	Group I	Group II	Group III
Phosphate (0.8 - 1.45 mmol/L)	$1.45 \pm 0.66$	$2.06 \pm 0.81$	$1.42 \pm 0.66$
RBC 2,3-DPG (9.4 - 16.9 $\mu$ mol/gHb)	$13.5 \pm .85$	$10.85 \pm 2.68$	$14.67 \pm 3.81$
pH (7.36 - 7.44)	$7.31 \pm 0.14$	$7.23 \pm 0.12$	$7.36 \pm 0.17$
PaCO <sub>2</sub> (35 - 45 mmHg)	$62 \pm 32^{\Delta}$	$47 \pm 10$	$37 \pm 11$
Bicarbonate (22 - 30 mmol/L)	$28 \pm 8^{\Delta}$	$19 \pm 4$	$22 \pm 4$
PaO <sub>2</sub> /FIO <sub>2</sub>	$227 \pm 116$	$143 \pm 76$	$256 \pm 150$
Lactate (0.5 - 2.0mmol/L)	$2.4 \pm 1.5^*$	$6.5 \pm 5.3^*$	$3.7 \pm 3.2$
Parathyroid hormone (1.0 - 7.0 pmol/L)	$5.7 \pm 4.4^{**}$	$20.7 \pm 19.4^{***}$	$4.2 \pm 2.0^{##}$
Glucose (3.0 - 5.0 mmol/L)	$11.4 \pm 5.1^*$	$20.2 \pm 11.7^{*#}$	$10.6 \pm 4.7^{\#}$
Calcium (2.10 - 2.60 mmol/L)	$2.31 \pm 0.16$	$2.24 \pm 0.14$	$2.39 \pm 0.21$
Creatinine (0.05 - 0.12 mmol/L)	$0.093 \pm 0.036^*$	$0.201 \pm 0.151^{*#}$	$0.115 \pm 0.054^{\#}$
Urine phosphate (5 - 80 mmol/L)	$21.3 \pm 21.4$	$14.6 \pm 6.5$	$14.7 \pm 10.2$
Urine sodium (0 - 300 mmol/L)	$71 \pm 41$	$85 \pm 32$	$66 \pm 46$
24 hr urine phosphate (16 - 48mmol)	$22.5 \pm 14.0$	$22.7 \pm 10.6$	$19.9 \pm 16.6$
24 hr urine sodium (0 - 300 mmol)	$130 \pm 103$	$218 \pm 183$	$117 \pm 129$
RTP (0.8 - 1.35 mmol/L)	$0.65 \pm 0.29$	$0.57 \pm 0.29$	$1.09 \pm 0.50^{\Delta\Delta}$
FE <sub>PO4</sub> (6 - 20%)	$27 \pm 22$	$35 \pm 23$	$19 \pm 13$

(\* or #, p < 0.01 and \*\* or ##, p < 0.001) indicate significant differences in pairwise comparisons (across groups), ( $\Delta$ , p < 0.01 and  $\Delta\Delta$ , p < 0.001) indicate single group difference with respect to two other groups, RBC 2,3-DPG, Red blood cell 2,3-diphosphoglycerate, FE<sub>PO4</sub>, fractional excretion of phosphate, RTP, renal threshold phosphate concentration.

**Table 3. Initial (T<sub>0</sub>) and 24 hour (T<sub>24</sub>) values (mean ± SD) for variables**

Variable ( normal reference range)	T <sub>0</sub>	T <sub>24</sub>	<i>p</i>
Phosphate (0.8 - 1.45 mmol/L)	1.55 ± 0.71	1.00 ± 3.0	< 0.0001
RBC 2,3-diphosphoglycerate (9.4 - 16.9 $\mu$ mol/gHb)	13.5 ± 3.3	14.1 ± 3.0	NS
pH (7.36 - 7.44)	7.31 ± 0.15	7.42 ± 0.07	< 0.0001
PaCO <sub>2</sub> (35 - 45 mmHg)	53 ± 7	44 ± 15	0.008
Bicarbonate (22 - 30 mmol/L)	25 ± 8	27 ± 7	< 0.0001
PaO <sub>2</sub> /FIO <sub>2</sub>	222 ± 122	220 ± 111	NS
Lactate (0.5 - 2.0 mmol/L)	3.4 ± 3.1	1.8 ± 1.0	< 0.001
Parathyroid hormone (1.0 - 7.0 pmol/L)	7.8 ± 10.1	7.3 ± 7.05	NS
Glucose (3.0 - 5.0 mmol/L)	12.7 ± 7.4	8.3 ± 2.3	< 0.0001
Calcium (2.10 - 2.60 mmol/L)	2.32 ± 0.18	2.31 ± 0.19	NS
Creatinine (0.05 - 0.12 mmol/L)	0.12 ± 0.08	0.108 ± 0.08	NS
Urine phosphate (15 -45 mmol/L)	18.3 ± 17.1		
Urine sodium (0 - 300 mmol/L)	73 ± 41		
24 hr urine phosphate (16 - 48 mmol)	21.8 ± 14.0		
24 hr urine phosphate/creatinine (1.8 - 2.7)	2.8 ± 1.7		
24 hr urine sodium (0 - 300 mmol)	142 ± 129		
RTP (0.8 - 1.35mmol/L)	0.76 ± 0.39		
FE <sub>PO4</sub> (6 - 20%)	26 ± 20		

RTP, renal threshold phosphate concentration, RBC = Red blood cell, FE<sub>PO4</sub>, fractional excretion of phosphate. Phosphate/creatinine measured in mmol, *P* refers to significance of t-test for T<sub>24</sub>-T<sub>0</sub>.

Groups were comparable for age, sex, ventilatory status and Apache II scores, but differed in frequency of prescription of the therapeutic agents parenteral diuretic, corticosteroids, aminophylline and sympathomimetics.

Initial (T<sub>0</sub>) values for variables in the three groups are shown in Table 2. At T<sub>0</sub>, 3 patients (one in each Group) had mild hypophosphataemia, 25 (17 in Group I and 8 in Group III) had normal levels and 29 (14 in Group I, 9 in Group II and 6 in Group III) were hyperphosphataemic. All groups showed a 24 hr decrement of plasma phosphate (non-significant in Group III).

Red blood cell 2,3-diphosphoglycerate concentrations showed 24 hr increments in Groups I and II, but a slight decrease in Group III (the changes were non-significant). Arterial blood gas variables showed initial acidemia in all groups and hypercapnia in Group I. All variables tended to normalise over the 24 hr observation period.

Variables for which intergroup differences existed were: plasma lactate (T<sub>0</sub>), parathyroid hormone (T<sub>0</sub> and T<sub>24</sub> - T<sub>0</sub> value) and glucose (T<sub>0</sub> and T<sub>24</sub> - T<sub>0</sub> value). Statistically significant differences (T<sub>24</sub> - T<sub>0</sub>) were found for the following variables: phosphate, pH, PaCO<sub>2</sub>, bicarbonate, lactate and glucose (Table 3). At T<sub>24</sub>, 15 patients (26%) had hypophosphataemia; 13 in Group I and one patient each in Groups II and III. Of these 15 patients, 9 had mild, 4 had moderate and 2, both Group I, had severe hypophosphataemia.

Twenty four hour urinary phosphate and sodium excretions showed no significant difference between groups, whether expressed as absolute amount (mmol/24 hr) or relative to urine volume and/or creatinine excretion per 24 hr. Two-way analysis of variance revealed no diagnostic-group/frusemide prescription effect with respect to 24 hr urine sodium excretion. Time delay of collection before assessment of renal threshold phosphate was 16.6 ± hr and no relationship was demonstrated between renal threshold phosphate and this time delay (*p* = 0.11; no evidence of non-linear relationship). Renal threshold phosphate concentration showed a reduction below reference range in Groups I and II (0.65 ± 0.29 and 0.57 ± 0.29, respectively). Values were significantly lower for both groups compared with Group III (1.09 ± 0.50, *p* = 0.001). Of the 15 hypophosphataemic patients at T<sub>24</sub>, 13 had renal threshold phosphate concentrations < 0.8 mmol/L, compared with 20 of 40 patients who were normo- or hyperphosphataemic (Fisher exact, *p* = 0.01).

Concerning the relations between "candidate"  $\Delta$  variables and initial and average phosphate: phosphate change was significantly related to both initial (phosphate change = 0.673 - 0.787 x T<sub>0</sub>PO<sub>4</sub>; R<sup>2</sup> = 0.72, *p* = 0.0001) and average (phosphate change = 0.522 - 0.836 x average phosphate; R<sup>2</sup> = 0.35, *p* = 0.0001) phosphate values. Kaiser's R, computed at 0.73, favoured percentage change as a predictor. Percentage change, ratio and

log-ratio demonstrated a significant relation to  $T_0$  phosphate, but no relation ( $p > 0.12$  for all) to average phosphate suggesting an independence from initial values for this variable. However, only log-ratio phosphate was normally distributed and was thus adopted as the appropriate form of  $\Delta$  phosphate.

The confounding effect of regression to the mean was quantified by the use of Blomqvist's adjustment of the regression relationship change vs  $T_0$  phosphate.<sup>29</sup> The initial relation was: phosphate change =  $0.673 - 0.787(T_0\text{PO}_4)$ ;  $p = 0.000$ ,  $R^2 = 0.72$ ,  $\text{RMSE} = 0.35$ .  $s^2$  ( $T_0$  variance) was 0.51 and  $\delta^2$  (within-subject "external variance") was 0.243, yielding a corrected  $\beta^*$  of -0.593, a 25% reduction in effect.

#### Predictive models

Using log ratio  $T_{24}/T_0$  phosphate as the form of  $\Delta$  phosphate (the dependent variable), the significant predictors initially identified by backward selection were  $\Delta$  2,3-diphosphoglycerate,  $\Delta$  pH and renal threshold phosphate concentration. No effect of time delay of collection before renal threshold measurement was evident ( $p = 0.58$ ). Further exploration, guided by theoretical considerations, identified a significant ( $p = 0.003$ ) interaction between the prescription of aminophylline and renal threshold phosphate concentration. No multi-collinearity was present. By bootstrap of the backward selection process, the variables selected (frequency  $> 50\%$ ) were  $\Delta$  2,3-diphosphoglycerate,  $\Delta$  pH, renal threshold phosphate concentration and aminophylline. This occurred in both the initial and complete (EM) data sets. Parameter estimates with standard errors (SE) and  $p$  values and indices of model fit for the models are seen in Table 4. Non-linearity of covariate effect was not demonstrated. Estimates of fit ( $R^2$ , adjusted  $R^2$  and adjusted likelihood ratio index,  $R^2_{\text{adj}}$ ) were comparable between OLS and GLM, but the lower AIC favoured the GLM model.

#### Missing data

The initial variables considered in the full data set were 19 in number and missing values occurred in 10 (all continuous variables). The frequency of missing values (per variable) ranging from 2 to 17%. The  $p$ -value for Little's MCAR test was 0.8, indicating that the missing data was in fact a random sub-sample of the dataset. Model parameters using multiple imputation are seen in Table 5; parameter estimates and standard errors were consistent with those of the original dataset. Because of missing values in the initial data set, observation number ( $n$ ) for the multivariable models was reduced to 46 compared with 57 for the complete (EM) and multiple imputation data sets. No difference existed between the set of means of (continuous) key predictor

variables of the initial data set and i) the complete EM data set (Hotelling's T-squared test) and ii) the  $k = 10$  multiply imputed data sets (t-test, data not shown). Data summaries are therefore reported from the initial data set.

#### DISCUSSION

Previous studies have suggested an association between respiratory illness and both hyper- and hypophosphataemia. Fisher *et al*,<sup>1</sup> in a retrospective study, reported a 13% incidence of admission hypophosphataemia in "acute respiratory illness", but no correlation between arterial blood gases and serum phosphate levels. In stable COPD patients, serum phosphate levels were reported to be significantly lower than controls ( $0.96 \pm 0.25$  vs  $1.17 \pm 0.11$  mmol/L, respectively). In hospitalised patients studied a mean of 14 days post admission, 23% had serum phosphate levels  $< 0.81$  mmol/L and 9%  $< 0.64$  mmol/L.<sup>2,9</sup> However, in critically ill COPD patients ( $n = 14$ ) with acute respiratory failure, Labaan *et al*,<sup>7</sup> found high-normal initial phosphate levels (1.21 mmol/L) in 9 patients and hyperphosphatemia in 5 patients. Both acute respiratory acidosis,<sup>7,48</sup> and metabolic acidosis<sup>49,50</sup> are associated with hyperphosphatemia. Our study confirmed this association with a 40% incidence of hyperphosphatemia in patients admitted with acute respiratory failure (Groups I & II). This was best demonstrated by contrasting Group II patients, where a mixed acidosis was present and 9 of 10 patients were hyperphosphataemic with Groups I & III combined, where 20 of 47 patients were hyperphosphataemic (Fisher exact test,  $p = 0.01$ ).

Decrease in plasma phosphate over the initial treatment period of acute respiratory failure has been previously noted, especially during mechanical ventilation<sup>5-7</sup> and was evident in the current study, being significant (compared with  $T_0$  values) in Groups I and II only. Delta pH was a significant independent variable in this study and mechanical ventilation (postulated as a "key" factor in previous studies),<sup>5-7</sup> is thus a surrogate for the induced change of  $\Delta$  pH (and  $\Delta$  PaCO<sub>2</sub>, with which  $\Delta$  pH was obviously highly correlated;  $\rho = -0.84$ ,  $p = 0.0001$ ).

The effects of other potentially important predictors must also be considered: i) pre-admission treatment variables (while these may have affected phosphate metabolism, there was no group difference in time-zero urine phosphate or sodium excretion, expressed absolutely, Table 2, or relative to creatinine excretion), ii) extracellular volume change (possible group differences were not reflected in any urinary excretion index nor in the effect of administered diuretics), iii) mechanical ventilation (the effect of different modes of ventilatory support was not explored), iv) gastrointestinal phosphate

**Table 4. Parameter and SE estimates with performance indices of multivariable models**

<i>Model</i> <i>Parameter (SE)</i>	<i>OLS</i>	<i>p</i>	<i>GLM: log link</i>	<i>p</i>	<i>GLM: eform</i>
del RBC 2,3-DPG	0.043 (0.024)	0.07	0.057 (0.03)	0.06	1.059 (0.032)
del pH	- 2.01 (0.344)	0.0001	- 2.715 (0.598)	0.0001	0.066 (0.04)
RTP	0.965 (0.30)	0.003	0.740 (0.364)	0.04	2.10 (0.763)
aminophylline	0.836 (0.261)	0.003	0.611 (0.351)	0.08	1.841 (0.646)
amin_rtp	- 0.99 (0.349)	0.007	- 0.771 (0.408)	0.05	0.462 (0.188)
R <sup>2</sup>	0.65		0.6		
R <sup>2</sup> adj	0.6		0.55		
AIC	32.2		25.4		
R <sup>2</sup> <sub>alri</sub>	0.52		0.5		

del = difference ( $T_{24} - T_0$ ), RBC 2,3-DPG = Red blood cell 2,3-diphosphoglycerate, RTP = renal threshold phosphate concentration, amin\_rtp = interaction between aminophylline and RTP, OLS = ordinary least squares, GLM = generalised linear model, eform = exponentiated form.

**Table 5. Parameter and SE estimates of multiple imputation data sets (k = 10)**

<i>Model</i> <i>Parameter (SE)</i>	<i>OLS</i>	<i>p</i>	<i>GLM: log link</i>	<i>p</i>
del RBC 2,3-DPG	0.047 (0.021)	0.03	0.052 (0.024)	0.03
del pH	- 2.048 (0.308)	0.0001	- 2.720 (0.483)	0.001
RTP	0.914 (0.266)	0.001	0.656 (0.296)	0.03
aminophylline	0.687 (0.229)	0.003	0.481 (0.286)	0.08
amin_rtp	- 0.847 (0.181)	0.004	- 0.65 (0.335)	0.05

del = difference ( $T_{24} - T_0$ ), RBC 2,3-DPG = Red blood cell 2,3-diphosphoglycerate, RTP = renal threshold phosphate concentration, amin\_rtp = interaction between aminophylline and RTP, OLS = ordinary least squares, GLM = generalised linear model.

losses (presumed small), were not measured, and v) the effect of endogenous stress hormones such as insulin and adrenaline (which were not quantified). Potentially dissimilar patients were also combined in Group I, but this was thought reasonable as there were no differences in the set of mean values between the patients with exacerbation of COPD and pneumonia (Hotelling's  $T^2 = 64.37$ ,  $p < 0.89$ ).

Red blood cell 2,3-diphosphoglycerate concentrations are decreased in metabolic acidosis,<sup>51</sup> normal or increased in uncompensated respiratory disease<sup>10</sup> and increased in respiratory alkalosis.<sup>52</sup> However, such concentration changes are time dependent and, in normal subjects at least, begin at  $> 4$  hr after initiation of extracellular pH change and are maximal between 24 to 48 hr.<sup>53</sup> Thus, where rapid changes of arterial pH occur, as in the treatment of acute respiratory failure, the so-called "characteristic" changes in red blood cell 2,3-diphosphoglycerate may not necessarily be found.

In the current study,  $T_0$  2,3-diphosphoglycerate for all patients ( $13.5 \pm 3.3$  umol/gHb) was not raised above the reference range nor significantly different from previously reported levels in critically ill ventilated

patients ( $14.5 \pm 1.1$  umol/gHb,<sup>54</sup>;  $p > 0.2$ ). There was no significant  $T_0$  nor  $\Delta$  2,3-diphosphoglycerate difference between the three diagnostic groups and the overall  $\Delta$  2,3-diphosphoglycerate was non-significant. Similarly, there was no treatment-effect of ventilation nor ventilation-diagnostic group interaction for  $\Delta$  2,3-diphosphoglycerate ( $p = 0.86$  and  $0.69$  respectively). Our results were thus in contrast to the study of Agusti *et al*,<sup>10</sup> who noted higher levels of 2,3-diphosphoglycerate at 24 hr post-admission in ventilated COPD patients compared with those not ventilated. In our study the  $T_{24}$  2,3-diphosphoglycerate difference in Group I, ventilated vs non-ventilated was non-significant at  $1.1 \pm 1.0$  (SE) umol/gHb ( $p = 0.31$ ), although there was no difference between the  $T_{24}$  2,3-diphosphoglycerate levels in ventilated patients between the Augusti study<sup>10</sup> and our study ( $16.1 \pm 3.3$  vs  $14.93 \pm 3.1$  umol/gHb,  $p > 0.3$ ). Such a contrast may indicate different treatment regimens and/or patient characteristics.

George *et al*,<sup>52</sup> suggested that under conditions of extreme (to tetany) hyperventilation a major portion of the inorganic phosphate lost from the serum may be accounted for as phosphate esters within the erythro-

cytes with 2,3-diphosphoglycerate representing approximately 50% of the total change in phosphate ester. Although the  $\Delta$  2,3-diphosphoglycerate was an independent predictor in the current series, no information was available regarding other red cell esters. Moreover, as the 24 hr  $\Delta$  pH was modest at  $0.10 \pm 0.14$ , a large  $\Delta$  2,3-diphosphoglycerate may not have been expected (and was not found) by virtue of arterial pH change alone. Thus, under the acute conditions of this study,  $\Delta$  2,3-diphosphoglycerate may have been a confounder or surrogate and not a direct index of intracellular phosphate shift, as, for example, a change in muscle phosphate concentration.<sup>2</sup>

Renal threshold phosphate concentration, which reflects the set-point of renal phosphate reabsorption as a function of both plasma phosphate concentration and glomerular filtration rate,<sup>55,56</sup> was depressed below the lower reference range limit in Groups I and II. This presumably reflected the prescription of threshold lowering agents in the therapy of acute respiratory failure (e.g. methylxanthine derivatives, corticosteroids, loop diuretics and sympathomimetics).<sup>2,9,57</sup> For those patients with renal threshold phosphate below the lower limit of normal ( $< 0.8$  mmol/L,  $n = 34$ ), there was a statistically significant difference between the threshold of those hypophosphataemic at  $T_{24}$  ( $n = 13$ ) compared with those who were not ( $n = 13$ ,  $0.43 \pm 0.05$  vs  $n = 21$ ,  $0.56 \pm 0.14$  mmol/L respectively; one-sided t-test,  $p = 0.006$ ). Renal threshold phosphate concentration values must also be interpreted with some caution, being nomogram derived and potentially unreliable when glomerular filtration rate is  $< 50$  mL/min. Five patients in the cohort had plasma creatinine levels ( $T_{24}$ )  $> 0.2$  mmol/L.

At  $T_0$  the urine phosphate concentration was  $18.3 \pm 17.1$  mmol/L (range 0.2 - 83 mmol/L) and the 24 hr excretion was  $21.8 \pm 14.0$  mmol (range 1.1 - 57.0 mmol/24 hr), which was in the lower range of our normal reference range of 16 - 48 mmol. These "low" 24 hr excretion values must be interpreted in the context of no phosphate supplementation, although in trauma cases, where in the unsupplemented condition, mean urine phosphate losses were  $29 \pm 16$  (SEM) mmol/24 hr, supplementation was reported not to produce a significant difference in phosphate excretion.<sup>58</sup> That there was no urinary phosphate loss above "normal" over the first 24 hr, in the presence of a decreasing plasma phosphate concentration and a low renal threshold phosphate concentration (0.76 mmol/L, 95% confidence interval 0.65 - 0.86), would suggest: i) pre-admission urinary phosphate loss and subsequent (inappropriate) renal phosphate excretion under the influence of phosphaturic agents and/or ii) body compartment redistribution of phosphate.<sup>6</sup>

A considerable literature has addressed the appropriate analytic form of "change". Initial analysis was not unreasonably directed at finding a measure of change that was independent of the initial ( $T_0$ ) phosphate value. Plots of candidate measures against both initial and average phosphate, as recommended by various authors,<sup>26,30</sup> were used to overcome the intrinsic mathematical relationship involved in analysing raw change variables; in particular, the simple difference ( $T_{24} - T_0$ ). In this study, the measure adopted as the dependent variable was the log ratio ( $\log T_{24}/T_0$  phosphate) which, being normally distributed, was considered the most appropriate dependent variable for linear regression. Hayes,<sup>30</sup> reviewing measures to mathematically adjust for the potential effects of regression to the mean, found Blomqvist's adjustment<sup>29</sup> the most robust. In the current study the "effect" of regression to the mean was to inflate by 25% the coefficient for the regression relation, phosphate change versus initial phosphate. An alternate analysis could have considered  $T_{24}$  phosphate as the dependent regression variable, with  $T_0$  phosphate as baseline, in an ANCOVA analysis.<sup>59</sup> Such a strategy would, however, have yielded the same analytic results as the use of phosphate change as the dependent variable.<sup>60</sup> Baseline corrections have a significant impact when the correlation, "baseline" vs "current value", is  $> 0.5$ .<sup>61</sup> In the current study, the correlation  $T_{24}:T_0$  phosphate was 0.26.

In the presence of missing values, multivariable analysis is usually accompanied by complete-case analysis. That is, only complete observations are considered across variables, resulting in a decrease in the total  $n$  and potential bias and/or loss of efficiency in estimation. Recent recommendations on the conduct of multivariable analysis<sup>62</sup> have been unusually silent on appropriate statistical procedures to deal with missing values. The Little test for MCAR was non-significant in this data set, suggesting that there would be minimal bias in the parameter estimates from conventional analysis and such was generally found (Tables 4 and 5). Thus effective reduction of observation number to  $n = 46$  in variable selection and model construction in the initial data set appeared to relatively robust to "missingness". That this may not be the case in other data sets is, of course, an empirical question.

The three variables which were demonstrated to predict log ratio phosphate were consistent from the physiological viewpoint. The full model (all predictors considered) had an  $R^2$  of 0.68, but an adjusted  $R^2$  of only 0.36, suggesting that a more parsimonious model was appropriate. The OLS model used a log-transformed dependent variable ( $\log$  ratio  $T_{24}/T_0$  phosphate); fitted values and parameter estimates were in



terms of the log response (the geometric mean). Natural log differences ( $\log [T_{24} \text{ phosphate}/T_0 \text{ phosphate}] = [\log(T_{24} \text{ phosphate}) - \log(T_0 \text{ phosphate})]$ ) correspond to fractional differences on the original scale; that is, the expected value will change by  $100\{\exp(\beta_j)-1\}\%$  for each 1-unit change in the independent variable  $x_j$  ( $\beta$  being the appropriate regression coefficient), regardless of whether  $x_j$  is continuous or dichotomous<sup>63</sup>. A particular advantage of the GLM log-link model is that it provides estimates of the (exponential) conditional mean function (ratio  $T_{24}/T_0$  phosphate). The GLM log-link logs the predictor ( $x\beta$ ), rather than the response, to linearise the relationship between response and predictors. That is, if we consider a transformation (say, logarithmic)  $g$ , then the expectation ( $E$ ) of classic linear model has the form:  $E\{g(Y_i)\} = a + x\beta$  whereas the GLM has the form:  $g\{E(Y)\} = a + x\beta$ .<sup>64</sup> Thus parameters are equal to the logs of arithmetic means and their ratios (the ratios being for either groups defined by discrete predictors, in this case "aminophylline, yes/no", or changes in response to a unit increase in a continuous predictor). The original arithmetic means and ratios are given by the exponential form (Table 4; GLM: eform). In this sense, the GLM log-link model provides more convenient and intuitive estimates than the traditional log-transformed OLS.

### Conclusions

Over the first 24 hr following admission to the ICU, critically ill patients with acute respiratory failure experienced a significant decline in plasma phosphate. At 24 hr, 26% of patients were hypophosphataemic, but this was severe in 4% only. The decline in phosphate was attended by a lowered renal threshold phosphate concentration, presumably multifactorial. Admission red blood cell concentrations of 2,3-diphosphoglycerate were not elevated beyond reference limits and the observed 24 hr increment was non-significant. Explanation of observed changes in phosphate levels over time reflected key underlying physiologic variables, albeit these were modified by certain treatment factors.

Modern statistical techniques, for example the bootstrap, data-augmentation and generalised linear models, are able to supplement traditional approaches to multivariable prediction. The effect of regression to the mean may be quantified, missing values are able to be managed appropriately, modelling of skewed variables may be more effectively accomplished and model uncertainty, in its various forms, may be quantified.

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