

# The Calcium/Phosphorus Homeostasis in Chronic Kidney Disease: From Clinical Epidemiology to Pathophysiology



## A Homeostase Fosfo-Cálcica na Doença Renal Crónica: Da Epidemiologia Clínica à Fisiopatologia

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### ABSTRACT

**Introduction:** A simple data filtering process together with some basic concepts of control theory applied to electronically stored clinical data were used to identify some of the pathophysiological mechanisms underlying the perturbations of the calcium/phosphorus homeostasis in chronic kidney disease.

**Material and Methods:** Retrospective data (a set per patient of serum single value concentrations of creatinine, calcium, phosphorus, parathormone, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D) from 2507 patients with stable chronic kidney disease not on renal replacement therapy were studied. The variables were paired and subjected sequentially to a moving average and partitioned into frequency classes. The plots were interpreted using the concept of a feedback loop comprising two branches of opposite sign and of set point of the loop. The set point for each pair of variables is displaced in the course of the disease and this displacement indicates which of the two factors involved (the serum concentrations of calcium or parathormone, for example) is primarily affected.

**Results:** This analysis showed that in the course of the development of chronic kidney disease the relationships between the observed variables progressed following a monotonous, a biphasic or a triphasic pattern.

**Discussion:** As chronic kidney disease progresses, calcium/phosphorus metabolism regulation evolves through different phases. Later, there is a progressive loss of the parathyroid gland sensitivity to the control by the serum concentrations of calcium and phosphorus. The sensitivity to the inhibitory action of 1,25-dihydroxyvitamin D decreases monotonously but never releases the gland.

**Conclusion:** The clinical data analysis used permits to illustrate the underlying pathophysiological mechanisms.

**Keywords:** Calcium; Homeostasis; Hyperparathyroidism; Kidney Failure, Chronic; Phosphorus

### RESUMO

**Introdução:** O propósito do presente trabalho consistiu em descrever alguns dos mecanismos fisiopatológicos envolvidos nas perturbações do metabolismo fosfo-cálcico na doença renal crónica a partir de um processo simples de filtragem de dados e de alguns conceitos básicos de teoria de sistemas.

**Material e Métodos:** Foram estudados os valores (um conjunto por doente, obtido numa única colheita de sangue) de creatinina, cálcio, fósforo, paratormona, 25-hidroxitamina D e 1,25-dihidroxitamina D num grupo de 2507 doentes com doença renal crónica não sujeitos a substituição renal. As variáveis foram emparelhadas e tratadas, primeiro por um processo de média deslizante e depois por distribuição por classes de frequência. Os resultados obtidos foram interpretados à luz do conceito de anse de retro-controlo contendo dois ramos de sinais opostos cuja intersecção define o 'ponto operacional' do sistema. Este ponto desloca-se ao longo da evolução da doença e o sentido do deslocamento indica qual dos dois fatores (a calcémia ou a concentração de paratormona, por exemplo) está a afetar primariamente o sistema.

**Resultados:** Esta análise mostrou que a evolução das relações entre as variáveis observadas seguiu um padrão monótono, bifásico ou trifásico.

**Discussão:** À medida que a doença renal crónica progride ocorrem alterações na regulação do metabolismo fosfo-cálcico, passando por diferentes fases. Numa fase mais avançada há uma perda do controlo das glândulas paratiróides pela calcémia e pela fosfatémia. A sensibilidade à ação inibitória da 1,25-dihidroxitamina D diminui progressivamente mas nunca desaparece.

**Conclusão:** A metodologia utilizada na análise de uma base de dados clínicos permitiu ilustrar mecanismos fisiopatológicos subjacentes.

**Palavras-chave:** Cálcio; Fósforo; Hiperparatiróidismo; Homeostase; Insuficiência Renal Crónica

### INTRODUCTION

The conceptual basis of present day medical practice is largely grounded on the findings of molecular biology and on statistical guidelines (evidence based medicine) regularly produced by a large number of respected scientific and professional bodies, guidelines that the clinicians cannot ignore.

This evolution entailed a progressive loss of interest in the application of a pathophysiological approach in a clinical environment and the progressive disappearance of

the departments of physiology in European and American medical schools. Yet, confronted with a patient, only the knowledge - not necessarily profound - of the underlying disease mechanisms, will enable the definition of the reality boundaries of his/her condition, essential if one wants to avoid big mistakes.

Our aim in this paper is to illustrate how any clinician without a specialized training and using data bases now available in most medical institutions, can apply a

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quantitative approach based on simple concepts of control theory to unveil the physiopathology subjacent to clinical situations.

We chose as a case study the disturbances in the calcium (Ca) and phosphorus (P) metabolism that take place in chronic kidney disease (CKD).

## MATERIAL AND METHODS

### Study population

This study was conducted in the Nephrology departments of three general hospitals in the Lisbon district (Santa Cruz, São Bernardo and Fernando Fonseca). It allowed us to analyse data regarding the CKD population followed as outpatients, and was an expansion of a previously published study<sup>1</sup> which was continued through the period between the years 2008 and 2011.

The data were gathered retrospectively from electronic records that included demographic information on age, gender and race, pharmacy dispensations on medication with vitamin D (calcitriol or paricalcitol) and phosphorus binders (calcium carbonate, sevelamer carbonate and/or aluminium hydroxide), and laboratory evaluation of serum calcium ( $[Ca]_s$ ), phosphorus ( $[P]_s$ ), creatinine ( $[creatinine]_s$ ), parathyroid hormone ( $[PTH]_s$ ), 25-hydroxyvitamin D ( $[25(OH)D]_s$ ) and 1,25-dihydroxyvitaminD ( $[1,25(OH)_2D]_s$ ).

Exclusion criteria comprised patients: 1) with a unstable renal function during the previous 6 months defined as the variation of serum creatinine of more than 0.5 mg/dl; 2) on vitamin D therapy; 3) on dialysis or submitted to a renal transplant; 4) with glomerular filtrations rates less than 15 ml/min per 1.73 m<sup>2</sup> and above 160 ml/min per 1.73 m<sup>2</sup>; 5) with serum concentrations of intact PTH less than 10 pg/ml.

The present study was approved by the ethical committees of the hospitals where it took place.

### Laboratory evaluation

Fasting serum samples were collected during a routine follow-up outpatient visit. For each patient all variables studied were measured in the same blood sample.

Serum creatinine, calcium and phosphorus were measured using standardized autoanalyser techniques.

Serum intact parathyroid hormone concentration was determined by chemiluminescent immunometric assay with two systems: Immulite 2000 Intact PTH from Diagnostic Products Corporation, Los Angeles, Calif., USA and by Elecsys Systems and Modular Analytics E170 from Roche Diagnostic, GmbH, Mannheim, Germany.  $[25(OH)D]_s$  and  $[1,25(OH)_2D]_s$  were measured by radioimmunoassay either with Biosource Europe, SA, Nivelles, Belgium or Immunodiagnostic Systems, Ltd., United Kingdom.

The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease Study (MDRD) formula<sup>2</sup> as follows: eGFR (in milliliters per minute per 1.73 m<sup>2</sup>) = 186.3 X [serum creatinine]<sup>-1.154</sup> X age<sup>-0.203</sup> X [0.742 if a woman] X [1.21 if black]

Since data on race were available for only 51% of the patients, we decided not to include it in the estimation of GFR. This missing value had the effect of underestimating eGFR by 21% for the black race. However, in the available data, about 3% of our patients were black, so the overall effect was small.

### Principles of the analysis

#### Regulated and regulatory variables. The setpoint.

Serum ionic calcium participates, as an activator, in a large variety of very important metabolic processes such as bone formation and remodeling, coagulation, synaptic transmission, excitation/contraction and excitation/secretion, in general with an affinity for this ion below the micromolar range. Its survival value might explain why its concentration in serum is so tightly regulated.

Physiological regulation can be analyzed in terms of feedback loops. In the case of calcium homeostasis the most important operate through the control of PTH secretion which involve the state variables:  $[Ca]_s$ ,  $[P]_s$ ,  $[PTH]_s$ ,  $[1,25(OH)_2D]_s$  and fibroblast growth factor 23 (FGF23).<sup>3</sup>

Parathormone production is inhibited by  $[Ca]_s$ ,  $[1,25(OH)_2VD]_s$  and  $[FGF23]_s$  through the action on its parathyroid glands receptors, respectively CaR, VDR and FGFR coupled to klotho.<sup>4</sup>  $[P]_s$  stimulates PTH production through an extracellular putative receptor not yet identified.<sup>5</sup>

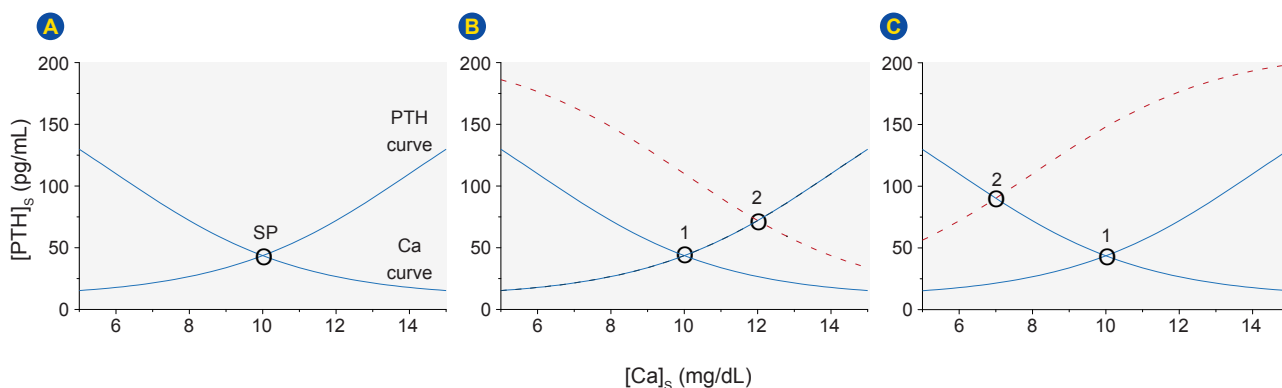


Figure 1 – Schematic representation of variations of  $[PTH]_s$  as a function of  $[Ca]_s$  and of  $[Ca]_s$  as a function of  $[PTH]_s$ . The intersection of both lines defines the set point of the system (A). The set point moves from position 1 to position 2 when  $[PTH]_s$  is the driving variable as in primary hyperparathyroidism (B) or in hypocalcemia, when  $[Ca]_s$  is the driving variable (C).

On the other hand, PTH leads to  $[P]_s$  decrease by promoting its kidney excretion and increases  $[Ca]_s$ ,  $[1,25(OH)_2VD]_s$  and  $[FGF23]_s$ , stimulating Ca bone and renal resorption,  $1,25(OH)_2VD$  renal production and FGF23 bone production.<sup>6</sup>

As an example, the Ca/PTH loop comprises two branches: one branch is the hypercalcemic action of PTH<sup>7</sup> and the other is the inhibitory action of Ca on the secretion of PTH.<sup>8</sup> Each of these actions can be represented by a line on a plot of  $[Ca]_s$  versus  $[PTH]_s$ . At any particular moment the concentrations of the two factors must satisfy both curves, defining the so called setpoint (SP) (Fig. 1A). The concept of SP imported from control system theory<sup>9</sup> was first introduced in medicine by Guyton in the analysis of the cardiac output.<sup>10</sup>

To any pathological situation entailing a sustained displacement of  $[Ca]_s$  from its normal value will correspond a displacement of the SP either along the line describing a primary change in  $[PTH]_s$ , (primary hyperparathyroidism, positive slope line in Fig. 1B) or along the negative slope line reflecting a primary change in  $[Ca]_s$  (Fig. 1C) or a combination of both. These figures should be seen as representing 'thought experiments', since in real situations one should expect the involvement of the other loops participating in the calcium homeostasis.  $[Ca]_s$  is regulated even in disease situations.<sup>1</sup> In any case the primary participation of Ca or PTH is revealed by the polarity of the slope of the Ca/PTH plot.

In a clinical environment this analysis cannot be applied to a single patient. We propose instead to use it in the characterization of the different phases of the evolution of the perturbations in Ca/P metabolism resulting from chronic renal failure. If we measure  $[Ca]_s$  and  $[PTH]_s$  in the same blood sample these two quantities will define the SP of the metabolic state of the patient. If we perform these measurements in a large number of patients it is reasonable to assume that the further away the SP is from its normal value the more advanced the disease and, in average, the 'older' it is. If so, the trajectory of the SP (following a positive or negative slope) will indicate if it is driven by primary alterations of  $[Ca]_s$  or of  $[PTH]_s$  (Fig. 1).

### Trend extraction from the raw data

The method used in processing our data was previously reported.<sup>1</sup> We showed above that the slope of the displacement of the SP provides an indication of which of two variables studied is driving the metabolic process. To analyze the relations between different pairs of variables measured in the same blood sample, tables have to be constructed. It is, then, important to address the question of how to extract the signal (the slope of their relationship) from the noise present in the raw data. There are two main sources of noise. Firstly, that due to the effects of medications the patient may be taking, of the diet or mood, pulsatility, variability in the laboratory assays, etc.. In sufficiently large populations it is hoped that this noise, unrelated to the signal, can be partially filtered by conventional statistical methods such as smoothing or moving average. The data is first sorted by the

independent variable ( $[PTH]_s$  in the example presented in Fig. 2). The first *n* values of the dependent variable (say, 5) are averaged and the 3<sup>rd</sup> value is replaced by this average. Then sample 2 to 6 are averaged and sample 4 is replaced by this average. The procedure is applied to the whole set of data. The random effects are likely to be distributed above and below the average and, at least in part, they will cancel out each other. Fig. 2, panel A, represents a plot of the raw data obtained from a population of 2507 patients. Panel B reports the same data subjected to a smoothing with a window of 11 consecutive samples while panel C shows the extracted noise obtained by subtracting B from A. There is a considerable reduction of the noise in B making now apparent the emergence of two noisy trends with opposite slopes. As expected, the noise displayed in C is distributed around an average of zero. The moving average technique is conventionally used to detect trends.<sup>11</sup>

Another source of noise is the variability to be expected of the setting point between individuals. This is one of the sources of dispersion when one tries to define 'normal' values for a variable. It can also be partially filtered out by partitioning the results into classes of frequency.

In this procedure the data sorted as above is binned and the population of each bin is replaced by its mean and standard error of the mean (SEM). Panel D of Fig. 2 reports the application of this procedure directly to the data of panel A and shows already the underlying trends of the evolution of the disease. In panel E we plot the data treated first by smoothing (panel B) and then by the binning procedure<sup>1</sup>.

When the raw data were plotted representing the relationship between  $[Ca]_s$  and  $[PTH]_s$  as shown in Fig. 2A no clear pattern of association between the two variables appeared. When the data were binned directly two trends emerged but with very large SEMs.

After sequentially smoothing and binning the data as described above it became possible to observe the relations between  $[Ca]_s$  and  $[PTH]_s$  (Fig.s 2E, 3F) and, similarly, for all the pairs of variables as represented in Fig. 3.

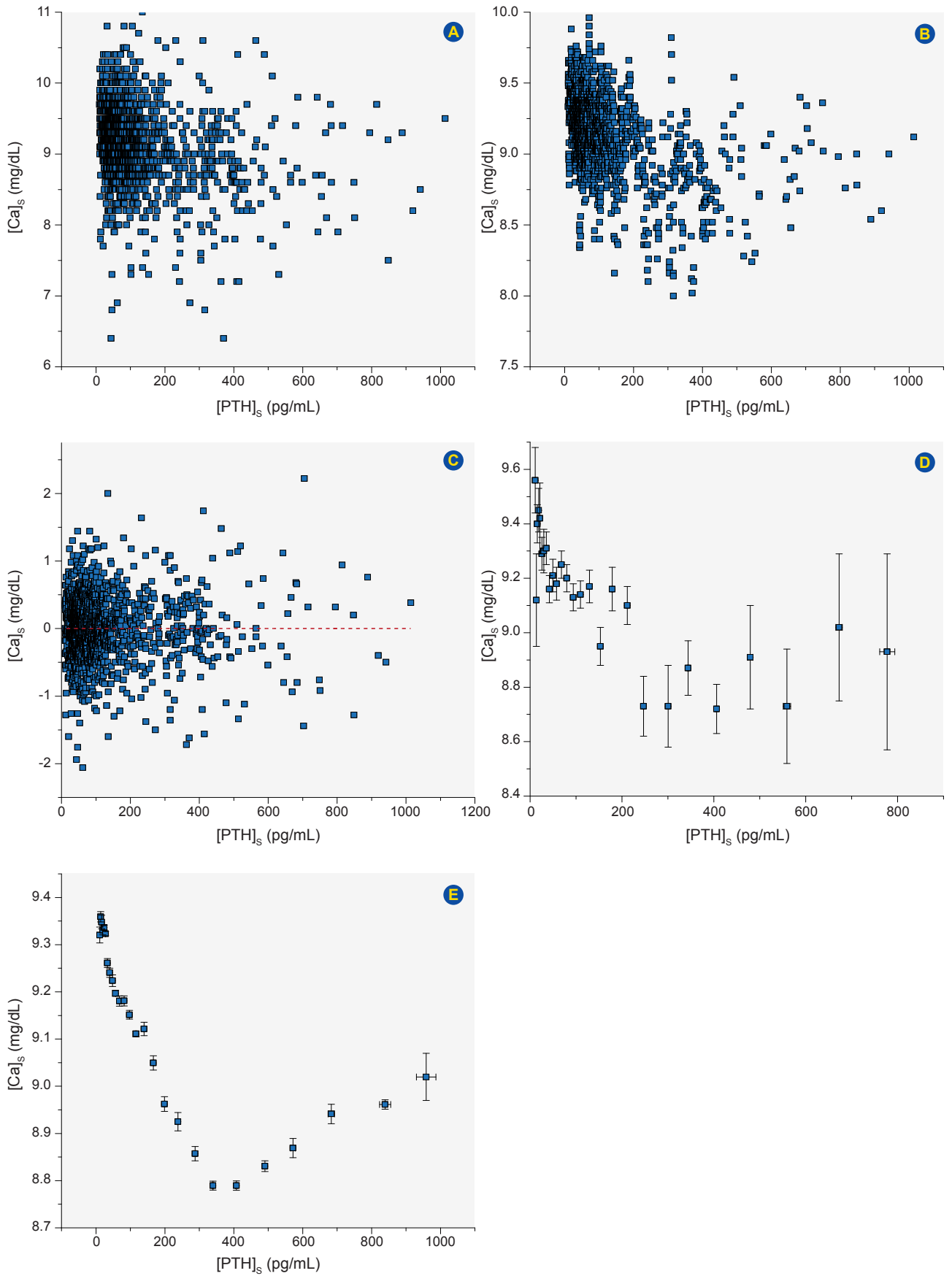
## RESULTS

### Participant characteristics

The demographic, clinical and laboratory characteristics of the population are described in Table 1. Patients were between 16 and 96 years old and had a mean eGFR of  $56.8 \pm 34.3$  ml/min per  $1.73m^2$ , varying from 15.03 to 159.4 ml/min per  $1.73 m^2$ . The primary renal diseases were: diabetes (11%), tubulointerstitial disease (7.3%), chronic glomerulonephritis (5.6%), hypertension (12.8%), cystic kidney disease (2.2%), miscellaneous (5.7%) and unknown or missing (55.4%).

### Functional relationships between the state variables

Some of the relationships between the state variables (eGFR,  $[Ca]_s$ ,  $[P]_s$ ,  $[PTH]_s$ ,  $[25(OH)D]_s$  and  $[1,25(OH)_2D]_s$ ) studied according to the methodology described above are represented in Fig. 3. It can be seen that some of the relations are monotonous but others are biphasic or even



**Figure 2** – The filtering of the data. **A:** Raw data. **B:** Data filtered by moving average. **C:** Noise filtered out by moving average. **D:** Data filtered by the binning procedure applied directly to the raw data. **E:** Data obtained by applying in succession the moving average and the binning procedure to the raw data.

triphasic. This diversity could not have been perceived by visual inspection of the raw data, as exemplified in Fig. 2, panel A, and the analysis could not have been easily carried out by the use of more conventional statistical methods. The physiologic meaning of some of the associations herein found will be discussed in detail further below.

**DISCUSSION**

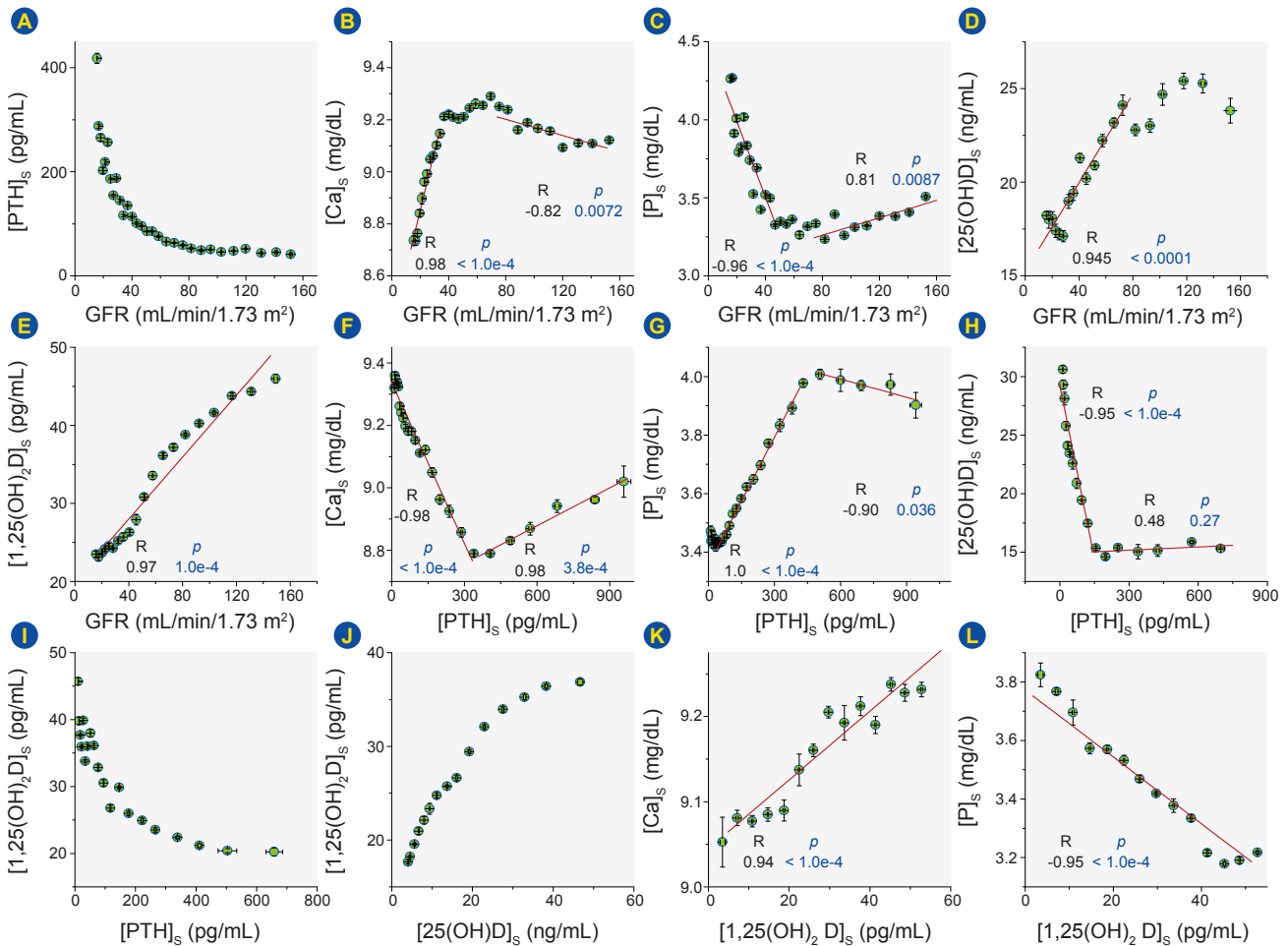
The serum concentration of calcium and the external balance of phosphorus are regulated through the operation of a number of feedback loops, which involve the bone, the parathyroid glands, the gut and the kidney<sup>13-16</sup> and are thus altered in the course of chronic kidney disease with the development of secondary hyperparathyroidism.<sup>19,20</sup> There is evidence that this is associated with low levels of [25(OH)D]<sub>S</sub>,<sup>21,22</sup> impairment in the renal production of [1,25(OH)<sub>2</sub>D]<sub>S</sub>,<sup>23-25</sup> serum calcium decrease<sup>26,27</sup> and phosphorus retention by the kidney.<sup>28,29</sup> More recently it was reported that FGF23, which is produced in the bone, increases early in CKD and may reach very high levels in the late stages of kidney disease.<sup>30-32</sup> It is known that FGF23 inhibits kidney 1-alfa hydroxylase, hence inhibiting [1,25(OH)<sub>2</sub>D]<sub>S</sub> production, and promotes kidney phosphorus excretion.<sup>33</sup> As CKD progresses, PTH continued demand is

associated with a parathyroid gland hyperplasia together with a loss of the calcium, vitamin D and FGF23/klotho receptors, rendering the parathyroid glands partially resistant to the inhibition by those factors.<sup>6</sup>

As expected, the plots of Fig. 3 indicate that the changes in trends in the course of CKD probably result from the interplay between the production of PTH and its controlling factors: [Ca]<sub>S</sub>, [P]<sub>S</sub>, [1,25(OH)<sub>2</sub>D]<sub>S</sub> and also [25(OH)D]<sub>S</sub>. In a population of CKD patients in different stages of the disease, one should expect the values of these state variables to be spread but, in average, to follow those relationships. Since these patients were studied in quasi steady state, the concentrations of PTH in serum are a direct reflection of its rate of secretion. As the measurements of [FGF23]<sub>S</sub> were unavailable in our study this hormone is a 'hidden variable'. We will discuss in more detail the most important relationships unveiled by our analysis.

**Dependence on GFR**

The reduction in eGFR is linearly correlated with a decrease in [1,25(OH)<sub>2</sub>D]<sub>S</sub> (Fig. 3E) ( $R = 0.97, p = 0.0001$ ), showing that its production is compromised from the early stage of the CKD as also reported by others.<sup>28,31,34</sup> This may be due both to the effect of kidney tubular mass reduction<sup>35</sup>,



**Figure 3** – Correlations between the observed variables. The number of samples for each of the variable pairs is given in each panel. The linear fitting and computation of correlation coefficients were performed with Origin version 6.1.

p: Probability; R: Coefficient of correlation.

and to the increase in serum levels of FGF23 through its inhibiting action on  $1,25(\text{OH})_2\text{D}$  production.<sup>33</sup>

A decrease in eGFR was accompanied by a monotonous, non-linear, increase in  $[\text{PTH}]_s$  (Fig. 3A). The increase in  $[\text{PTH}]_s$  together with the fall in  $[\text{Ca}]_s$  which accompanies the decline in eGFR are conventionally attributed to the fall in  $[1,25(\text{OH})_2\text{D}]_s$  and to the increase in  $[\text{P}]_s$  (Fig. 3A, 3B, 3C and 3E).<sup>36,37</sup>

As opposed to  $[1,25(\text{OH})_2\text{D}]_s$  and  $[\text{PTH}]_s$ , the curves describing the variations of  $[\text{Ca}]_s$  (Fig. 3B) and  $[\text{P}]_s$  with the eGFR (Fig. 3C) were biphasic, changing the polarity of the slopes at values of eGFR of approximately 50 ml/min per  $1.73 \text{ m}^2$ .

The correlation between the concentrations of  $[\text{Ca}]_s$  and eGFR (Fig. 3B) is positive ( $R = 0.98$ ;  $p < 0.001$ ) for values of this variable below 50 ml/min per  $1.73 \text{ m}^2$  and negative ( $R = -0.82$ ;  $p = 0.007$ ) above this value, while the correlation between  $[\text{P}]_s$  and eGFR (Fig. 3C) is negative ( $R = -0.96$ ;  $p < 0.001$ ) for values below 50 ml/min per  $1.73 \text{ m}^2$  and positive ( $R = 0.81$ ;  $p = 0.009$ ) above this value. The reasons for this behavior are far from clear. From the numerical data it is possible to show that the glomerular loads of both Ca and P fall linearly with eGFR regardless of the trends of their concentrations in the serum. This indicates that the variations with opposite slopes of the two ions cannot be explained by the changes in GFR which would affect both ions in the same direction.

The negative correlation between  $[\text{Ca}]_s$  and eGFR for values of this variable above 50 ml/min per  $1.73 \text{ m}^2$  (Fig. 3B) coincides with the observations of Evenepoel *et al*<sup>31</sup> and is, so far, a finding for which we have no explanation. It cannot be attributed to a change in  $[1,25(\text{OH})_2\text{D}]_s$  since, in the same range, this hormone is positively correlated with eGFR (Fig. 3E). The positive correlation between  $[\text{Ca}]_s$  and eGFR below 50 ml/min per  $1.73 \text{ m}^2$  occurs while there is a dramatic rise of  $[\text{PTH}]_s$  with the fall in eGFR. It probably results, among other factors, from a reduction in the intestinal uptake of that ion due to the positive correlation between eGFR and  $[1,25(\text{OH})_2\text{D}]_s$  (Fig.s 3E, 3K).

The positive correlation between eGFR and  $[\text{P}]_s$  (Fig. 3C) for values of eGFR above 50 ml/min per  $1.73 \text{ m}^2$ , is an observation previously reported by others<sup>30,36</sup> which cannot be explained by the simultaneous rise in  $[\text{PTH}]_s$ , but may be due to the early rise in  $[\text{FGF23}]_s$  together with the fall in  $[1,25(\text{OH})_2\text{D}]_s$ .<sup>37</sup> When eGFR falls below 50 ml/min per  $1.73 \text{ m}^2$  there is a progressive increase in  $[\text{P}]_s$  (Fig. 3C), as also reported by Isakova *et al*<sup>30</sup> probably because the fall in the glomerular load can no longer be compensated by an increased tubular excretion.<sup>1,38</sup>

The element  $[25(\text{OH})\text{D}]_s$  is independent of eGFR for values above 80 ml/min per  $1.73 \text{ m}^2$  (Fig. 3D) but below this threshold both variables decline in parallel, as also reported by Isakova *et al*.<sup>30</sup> This has been attributed to a poor nutritional state and to underexposure to the sun in chronic patients in combination with vitamin D metabolism disturbance, which includes an impaired endogenous synthesis of vitamin D in the skin and the compromise of

the uptake of  $25(\text{OH})\text{D}$  from the glomerular ultrafiltrate for its recycling to the circulation and maintenance of its serum levels.<sup>39</sup> Moreover, FGF23 promotes  $25(\text{OH})\text{D}$  catabolism by stimulation of the 24-hydroxylase.<sup>40</sup> The dependence of  $[1,25(\text{OH})_2\text{D}]_s$  on  $[25(\text{OH})\text{D}]_s$  (Fig. 3J) suggests that in CKD the synthesis of the active vitamin is substrate dependent.<sup>41</sup>

### Dependence on PTH

The panels in which  $[\text{PTH}]_s$  was used as independent variable (Fig. 3, panels F – G) reveal a biphasic or triphasic behaviour.  $[\text{Ca}]_s$  (Fig. 3F) is negatively correlated ( $R = -0.98$ ;  $p < 0.001$ ) with PTH for concentrations of this hormone below 360 pg/ml and positively correlated ( $R = 0.98$ ;  $p < 0.001$ ) above this value. This reflects the development of a lower sensitivity to factors controlling PTH production ( $[\text{Ca}]_s$ ,  $[1,25(\text{OH})_2\text{D}]_s$ ,  $[\text{FGF 23}]_s$ ) and eventually of tertiary hyperparathyroidism.<sup>42</sup>

It was noted that  $[\text{PTH}]_s$  and  $[\text{P}]_s$  show a triphasic relationship (Fig. 3G). For values of  $[\text{PTH}]_s$  below 630 pg/ml phosphorus drives the displacement of the set point. As the production of PTH becomes partially or totally autonomous, PTH promotes the excretion of phosphorus ( $R = -0.96$ ;  $p = 0.04$ ) in spite of the reduced tubular mass available to respond to this physiological mechanism, thus resulting in a decrease of  $[\text{P}]_s$ . For very low values of  $[\text{PTH}]_s$ , the  $[\text{PTH}]_s/[\text{P}]_s$  curve exhibits a unexplained negative slope. A possible explanation is that for values of eGFR above 80 ml/min per

Table 1 - Characteristics of the subject population

	All patients (n = 2507)
<b>Demographics</b>	
Age* (years)	64 ± 17 [16 - 96]
Gender (male) (%)	52.3
Race (white/black) (n = 1280)	97/3
<b>Renal Function</b>	
Serum creatinine* (mg/dL)	1.8 ± 0.89 [0.6 - 4.8]
eGFR* (mL/min per $1.73 \text{ m}^2$ )	56.8 ± 34.3 [15.03 - 159.4]
<b>CKD Staging**</b>	
Stage 1 (%)	18.4
Stage 2 (%)	16.9
Stage 3 (%)	40.6
Stage 4 (%)	24.1
<b>Phospho-calcium metabolism</b>	
Serum calcium* (mg/dL) (n = 2016)	9.1 ± 0.6 [6.4 - 11.7]
Serum phosphorus* (mg/dL) (n = 2078)	3.5 ± 0.7 [1,18 - 7.0]
Serum iPTH* (pg/mL) (n = 1576)	134.4 ± 155.8 [10.5 - 2220]
Serum $1,25(\text{OH})_2\text{D}$ * (pg/mL) (n = 629)	32.8 ± 15.7 [1.25 - 77.6]
Serum $25(\text{OH})\text{D}$ * (ng/mL) (n = 554)	20.1 ± 15.4 [2.5 - 98.5]
<b>Medication</b>	
Non-calcium phosphorus binder (%)	0.1
Calcium supplements (%)	3.1

n: Number of patients

\* The results are presented as means ± standard deviation and range (square brackets)

\*\* CKD Stages according to K/DOQI clinical practice guidelines for CKD<sup>12</sup>

1.73 m<sup>2</sup> (when [PTH]<sub>s</sub> values are the lowest), [P]<sub>s</sub> is driven by [PTH]<sub>s</sub>.<sup>43</sup> Alternatively or in addition, an early rise of FGF23 when [PTH]<sub>s</sub> has not increased yet (Fig. 3A) might produce the same effect.<sup>44</sup>

Another important determinant of [PTH]<sub>s</sub> is [25(OH)D]<sub>s</sub>, the decreased levels of which are common in CKD patients.<sup>45</sup> Fig. 3H shows a clear association between [25(OH)D]<sub>s</sub> and [PTH]<sub>s</sub> for values of this hormone below 150 pg/ml (R = -0.95; p < 0.001), which disappeared at higher values of [PTH]<sub>s</sub> probably because the parathyroid cells become much less sensitive. The stability of [25(OH)D]<sub>s</sub> with values of [PTH]<sub>s</sub> above 150 pg/mL (Fig. 3H) is difficult to explain since [1,25(OH)<sub>2</sub>D]<sub>s</sub> is correlated with [PTH]<sub>s</sub> over the range of values measured (Fig. 3I) and [25(OH)D]<sub>s</sub> and [1,25(OH)<sub>2</sub>D]<sub>s</sub> are almost linearly correlated (Fig. 3J). This stability may reflect a residual capacity of synthesis/absorption of vitamin D independent of the progression of the CKD.

It was clear that [PTH]<sub>s</sub> and [1,25(OH)<sub>2</sub>D]<sub>s</sub> are negatively associated, although with a progressively decreasing slope as [PTH]<sub>s</sub> increases (Fig. 3I). This indicates that the inhibitory branch of the corresponding loop dominates all the way and is explained by the decreasing number of receptors of 1,25(OH)<sub>2</sub>D in the parathyroid glands. The effect of the increase in [PTH]<sub>s</sub> in the later stages of the CKD is masked by the progressive loss of the kidney response.

#### Dependence on 1,25(OH)<sub>2</sub>D

Items [25(OH)D]<sub>s</sub> and [1,25(OH)<sub>2</sub>D]<sub>s</sub> were positively associated (Fig. 3J). This plot can be fitted by a Michaelis-Menten saturation curve (VM = 41.4 ± 1.2; K = 6.78 ± 0.63) suggesting that the production of [1,25(OH)<sub>2</sub>D]<sub>s</sub> is a function of the substrate [25(OH)D]<sub>s</sub> and is not saturated for levels of [25(OH)D]<sub>s</sub> up to 40 ng/ml. Also, [1,25(OH)<sub>2</sub>D]<sub>s</sub> is negatively correlated with [P]<sub>s</sub> (Fig. 3L) (R = 0.95; p < 0.001). This may be explained in part by the phosphorus inhibition of 1,25(OH)<sub>2</sub>D production either directly or indirectly through FGF23.<sup>46-49</sup> In studies evaluating CKD stages 1 to 3, it was found that [1,25(OH)<sub>2</sub>D]<sub>s</sub> was independently and positively associated with [25(OH)D]<sub>s</sub>, and negatively associated with

[FGF-23]<sub>s</sub> and [P]<sub>s</sub>.<sup>31,35</sup> The assumption of an early [FGF23]<sub>s</sub> increase is supported by the observation<sup>50</sup> that it occurs as eGFR decreases below 90 ml/min per 1.73 m<sup>2</sup> but the exact mechanisms of increased FGF23 in CKD remains to be determined.<sup>51,52</sup> As well, [1,25(OH)<sub>2</sub>D]<sub>s</sub> is positively and linearly correlated with [Ca]<sub>s</sub> (Fig. 3K) (R = 0.94; p < 0.001). Finally, [1,25(OH)<sub>2</sub>D]<sub>s</sub> (Fig. 3I) is non-linearly negatively correlated to [PTH]<sub>s</sub> throughout the whole range.

#### CONCLUSION

The application of simple methods of data processing (moving average and partitioning into frequency classes) to numerical clinical data of the nephrology departments from three general hospitals, allowed the clear cut identification of trends in the behaviour of Ca/P homeostasis in the evolution of CKD.

Using the concept of feedback loop together with our knowledge of the physiological mechanisms in which the observed state variables participate, it was possible to illustrate previously known mechanisms but also to unveil relations so far practically disregarded or even hitherto unknown and unexplained.

#### PROTECTION OF HUMANS AND ANIMALS

The authors declare that the present study was approved by the ethical committees of the hospitals where it took place and developed according to the Helsinki Declaration of the World Medical Association.

#### DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

#### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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