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The relative influence of serum ionized calcium and 25-hydroxyvitamin D in regulating PTH secretion in healthy subjects

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ABSTRACT

Background: While the inverse relationship between serum ionized calcium (Ca^{2+}) and PTH is well-established, the relationship between 25(OH)D and PTH showed conflicting results. The study aimed to evaluate the relative contributions of age, sex, serum Ca^{2+}, ionized magnesium (Mg^{2+}), 25(OH)D and $1,25(OH)_2D$ in regulating PTH secretion in healthy subjects.

Methods: This is a secondary analysis of an observational study performed from March 2014 to July 2015 carried out in 2259 blood donors (1652 men and 607 women, age range 18–68 years). Subjects with parathyroid disorders and taking drugs that affect mineral metabolism were excluded.

Results: Significant correlations [between Ca^{2+} and PTH ($r = -0.223$, $p < 0.001$), 25(OH)D and PTH ($r = -0.178$, $p < 0.001$) and between PTH and age ($r = 0.322$, $p < 0.001$)] were found. As a preliminary step to multivariate analysis, a regression tree analysis was performed using PTH as response variable and age, Ca^{2+}, Mg^{2+}, 25(OH)D, $1,25(OH)_2D$ and sex as explanatory variables to determine the effect of each covariate on the response variable. For subjects < 38 years, 25(OH)D was the most important parameter in regulating PTH. For subjects ≥38 both 25(OH)D and Ca^{2+} levels regulated PTH secretion. Subjects with 25(OH)D < 13 ng/mL had average higher PTH; in this group only, subjects with $Ca^{2+} \geq 1.30$ mmol/L had average lower PTH compared to subjects with $Ca^{2+} < 1.30$. The multivariate analysis showed that all variables had a significant effect ($p < 0.001$) on PTH. Anova Type III errors c indicated that 25(OH)D accounted for 32.1% of the total variance in PTH, Ca^{2+} accounted for 18% of the total variance, BMI for 14.3%, and $1,25(OH)_2D$ for 11.1%. The remaining percentage was attributable to age and sex. This was confirmed by the regression tree approach, where 25(OH)D and Ca^{2+} accounted for the largest variation in the average levels of PTH.

Discussion: Under stable conditions 25(OH)D plays a significant role in regulating PTH secretion. Under conditions of relative vitamin D sufficiency, Ca^{2+} also plays an important role.

1. Introduction

Parathyroid hormone (PTH) plays a pivotal role in regulating calcium homeostasis. It is a single-chain peptide composed of 84 amino acids, with a molecular weight of 9.3 Kilo-Daltons and a rapid elimination time. PTH is secreted by the parathyroid glands in response to varying concentrations of serum calcium [1]; however, changes in the concentrations of other electrolytes or hormones, such as serum levels of magnesium [2], phosphate [3], 25-hydroxyvitamin D [25(OH)D] [4,5], 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] [6] and fibroblast growth factor 23 [7], may also modulate PTH secretion.

Several investigations have demonstrated the relative importance of

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each single factor that influences PTH secretion. Besides the well-established inverse relationship between serum ionized calcium and PTH, some recent studies examined the relationship between 25(OH)D and PTH, but results have been conflicting in terms of the strength of the correlations [8,9] and the putative value at which PTH starts to increase [10–12].

Most studies, both in vitro and in vivo, that attempted to understand the regulation of PTH secretion investigated only one referent, such as total calcium and PTH [13], 25(OH)D and PTH [14] and $1,25(OH)_2D$ and PTH [15]. Many of these studies included a relatively small number of subjects or patients at tertiary centers for metabolic bone diseases, which introduce inherent biases that may be significant. Further, it is well known that PTH secretion is not regulated by total serum calcium but by its ionized (Ca^{2+}) fraction, which represents the metabolically active portion (the same is true for magnesium). Ionized calcium can be deduced indirectly from total serum calcium values after adjusting for serum albumin; however, this method is imprecise. Accordingly, most practitioners believe that quantitative determination of extracellular ionized serum calcium represents the gold standard, especially when differentiating primary hyperparathyroid patients from normal subjects [16].

This study aimed to evaluate the relative importance of ionized calcium, ionized magnesium, 25(OH)D and $1,25(OH)_2D$ in the regulation of PTH secretion in a large cohort of > 2000 healthy volunteers.

2. Materials and methods

This study is a secondary analysis of an ongoing project aimed at evaluating the prevalence of normocalcemic primary hyperparathyroidism (NPHPT) in otherwise healthy subjects. Enrollment for the NPHPT investigation began in March 2014 and ended in July 2015 with 2355 blood donors at the Policlinico Umberto I Hospital, Sapienza University of Rome (latitude 41°54′39″24 N).

We selected blood donor volunteers as a target population because, by definition, they are supposedly normal subjects; before donation they were checked for possible biochemical abnormalities, such as elevated serum transaminases, hyperglycemia, clotting factors abnormalities, etc.

Of 2355 enrollees, we excluded 96 subjects: 31 patients with NPHPT (8 of whom had renal hypercalciuria) who are the focus of another paper; 30 patients with primary hyperparathyroidism; 26 patients who were considered as outliers based upon their serum ionized calcium and parathyroid hormone values (see statistical analysis section), 7 patients with idiopathic hypoparathyroidism, and 2 subjects for technical concerns (i.e., inadequate blood samples, storage problems).

The 2259 subjects in our analysis included 1652 men and 607 women aged 18–68 years (mean age 40.3 ± 11.85). All agreed to participate in this investigation and signed informed consent. The unbalanced number between males and females reflects the primary NPHPT investigation requirement for consecutive enrollment. Subjects were given a self-administered questionnaire to document lifestyle habits, previous disease or drug intake that could interfere with calcium and phosphorus metabolism. The first 159 subjects included in the study completed the questionnaire twice, wherein the second interview was conducted by one of the authors (FF). Concordance between answers to both questionnaires was 99.9%; therefore, only the self-administered questionnaire was collated for the remaining individuals. No subjects were excluded for medical conditions, aside from a few who were initially eliminated from the study because they declared to have taken drugs that affect mineral metabolism (i.e., thiazides). Individuals taking supplemental calcium and/or vitamin D were not excluded.

Upon inclusion, nurses at the blood donation center accurately measured weight to the nearest 0.1 kg using a calibrated bathroom scale with subjects wearing light clothing and no shoes; height was measured by stadiometer to the nearest 0.001 m for body mass index (BMI) calculation.

Briefly, blood samples were taken between 8.00 and 10.00 a.m. for measurement of serum ionized calcium (Ca^{2+}), ionized magnesium (Mg^{2+}), 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and parathyroid hormone in the fasting state. Except for Ca^{2+} and Mg^{2+} whose levels were measured immediately after collection (within 2 h of sampling, while room temperature was kept at °4C), aliquoted blood samples were stored at −80 °C and assayed at a later time in batch. Serum Ca^{2+} and Mg^{2+} determinations were carried out using an ion-selective electrode with the fully automated biochemical analyzer NOVA 8 (Nova Biomedical, Waltham, MA) [17]; PTH and 25(OH)D were measured by chemiluminescence-immunoassay (CLIA) with the fully automated LIAISON® analyzer, while $1,25(OH)_2D$ was measured on the LIAISON XL®. Due to technical limitations imposed by use of the LIAISON® instrument, the 25(OH)D and PTH assays were performed on a weekly basis while $1,25(OH)_2D$ values were measured during three long-lasting batch sessions. The PTH assay employed (1–84 PTH, DiaSorin USA, Stillwater, MN, USA) has 100% specificity to PTH 1–84 with no cross-reactivity to the 7–84 PTH fragment. Intra- and inter-assay coefficients of variation were 4.1% and 5.2%, respectively. The determinations of serum 25(OH)D were performed with a competitive one-step backfill chemiluminescence assay (Vitamin D TOTAL Assay, DiaSorin USA, Stillwater, MN, USA) having a measurement range of 4–150 ng/mL and functional sensitivity ≤ 4.0 ng/mL; intra- and inter-assay precision were 8.9% and 12.8%, respectively, with reported 100% detection of both 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3. The quantitative determinations of serum $1,25(OH)_2D$ were performed with an automated chemiluminescent immunoassay (1,25 Dihydroxyvitamin D, DiaSorin USA, Stillwater, MN, USA) with a measurement range of 5–200 pg/mL and functional sensitivity ≤ 5.0 pg/mL. The LIAISON XL® 1,25 dihydroxyvitamin D assay is a modified 3-step sandwich assay that uses a unique (recombinant VDR LBD fused with a proprietary chaperone) protein for capture of the $1,25(OH)_2D$ molecule and a murine monoclonal antibody which specifically recognizes the conformation acquired upon binding of the $1,25(OH)_2D$ molecule to the LBD. The 1,25 dihydroxyvitamin D assay employed has 100% specificity to $1,25(OH)_2D_3$ and $1,25(OH)_2D_2$ without cross-reactivity to other forms of vitamin D. The intra- and inter-assay coefficients of variation were 2.1% and 4.9%, respectively.

The investigation was approved by the Institutional Review Board of Department Internal Medicine and Medical Disciplines and then approved by the Ethical Committee of “Sapienza” Rome University. The research was carried out complying with the World Medical Associations Declaration of Helsinki (Ethical Principles for Medical Research involving Human Subjects).

2.1. Statistical analysis

Data are presented as mean ± SD. Normality of the continuous covariates was visually assessed; for variables with severely skewed distribution, log-transformed values were considered if they resulted in more symmetric normal distributions. Single quantitative variables were inspected using boxplots. Patients displaying values outside the range $Q1 - 2 * IQR$; $Q3 + 2 * IQR$ (Q1 = first quartile, Q3 = third quartile, IQR = inter quartile range) for any of the considered variables were further inspected for outliers. Where possible, outliers were substituted with correct values, otherwise they were eliminated from the study.

The correlations between variables were analyzed with the Spearman rank correlation test. As a preliminary mining tool, a regression tree approach was used to assess the set of variables relevant for the determination of PTH levels. Regression trees are a powerful data mining technique which uses a cluster weighted approach to model fitting. They operate by partitioning the variable space into disjoint regions which are characterized for minimizing the within-group variance of the response variable. The splitting is achieved by selecting at each iteration the variable that attains the largest decrease

of the within variance. Tree growth stops when the decrease of the within variance becomes negligible or when the number of elements in a node falls below a predefined threshold. Regression trees are a non-parametric technique that imposes minimal assumptions on the data, can handle outliers and missing values, and is therefore effective for large datasets with several independent variables. They are also most effective for feature selection when there are a large number of variables, subsets of which may be more influential in explaining the variability of the response variable.

After the selection of the most influential covariates via regression tree, the relation between log-PTH and age, Mg^{2+}, Ca^{2+}, 25(OH)D, $1,25(OH)_2D$, BMI and sex was numerically assessed using a linear model considering the interaction effect of all covariates with sex:

$$\text{Log PTH} \sim (\text{age} + Mg^{2+} + Ca^{2+} + 25(OH)D + 1,25(OH)_2D + \text{BMI}) * \text{sex}$$

A stepwise procedure was used to select the best model using Bayesian Information Criterion (BIC). Relevance of the effect of each remaining covariate on log-PTH was assessed using Anova Type-III error. The results were considered significant when a probability < 0.05 was obtained.

Statistical elaborations were conducted with the statistical package R v. 3.0.2.

3. Results

Table 1 shows the main anthropometric and biochemical parameters of the 2259 healthy subjects. Tables 2 and 3 recast the same anthropometric and biochemical parameters in men and women, respectively.

For the study population, a significant inverse correlation was observed between serum ionized calcium and PTH values ($r = -0.223$, $p < 0.001$) (Fig. 1). This correlation was slightly stronger for men (-0.231) than for women (-0.186), although the difference was not statistically significant ($p = 0.323$). A negative correlation was also found between serum 25(OH)D and PTH values ($r = -0.178$, $p < 0.001$) (Fig. 2) with different, although not significant, values for sex (-0.161 for men vs -0.232 for women; $p = 0.121$). A significant direct correlation between serum PTH and age ($r = 0.322$, $p < 0.001$) was found with sizes for men ($r = 0.349$) and women ($r = 0.225$) that were significantly different ($p = 0.0044$) (Fig. 3). A significant direct correlation was also found between serum $1,25(OH)_2D$ and PTH values ($r = 0.055$, $p = 0.0089$) although it was only significant for males ($r = 0.082$, $p < 0.001$). There was a significant positive correlation between serum ionized magnesium and PTH values ($r = 0.0469$, $p = 0.026$) that was lost within men and women, respectively, suggesting the presence of spurious correlations. Finally, a positive correlation was found between BMI and serum PTH values ($r = 0.232$, $p < 0.001$) (Fig. 4) with a slight difference between men (0.208) and women (0.219).

As a preliminary step to the multivariate analysis, a regression tree analysis was performed using PTH as response variable and age, Ca^{2+}, Mg^{2+}, 25(OH)D, $1,25(OH)_2D$ and sex as explanatory variables to determine the effect of each covariate on the response variable. Since regression trees are non-parametric techniques, no log transformation was necessary for PTH values. According to the results obtained, there is a difference in the effect of the covariates on PTH level for patients below or at/above age 38 years; this was confirmed by the simple regression analysis depicted in Fig. 5. For younger patients, 25(OH)D values were the most important parameter in regulating the levels of PTH; indeed, subjects with 25(OH)D levels below 14 ng/mL had an average value of PTH equal to 23 pg/mL, while subjects with 25(OH)D levels > 14 ng/mL had an average value of PTH equal to 20. For patients aged 38 years or older, both 25(OH)D and Ca^{2+} levels were effective in regulating the secretion of PTH. However, 25(OH)D values

Table 1
Anthropometric and biochemical parameters of the study population (n = 2259).

Variable	Mean	Standard deviation	Min	Max	Normal range
Age (years)	40.3	11.85	18	68	–
Weight (kg)	76.1	13.3	45	135	–
Height (m)	1.75	0.08	1.5	2.05	–
BMI (kg/m^2)	24.9	3.4	14.6	46.5	18.5–25
Ca^{2+} (mmol/L)	1.29	0.04	1.14	1.37	1.17–1.33
Mg^{2+} (mmol/L)	0.53	0.05	0.33	1.56	0.45–0.6
PTH (pg/mL)	23.7	8.6	6.3	63.1	6.5–36.6
25(OH)D (ng/mL)	20.2	8.9	4	89.60	> 30
$1,25(OH)_2D$ (pg/mL)	50.7	13.3	5.7	109	19.9–79.3

Table 2
Anthropometric and biochemical parameters in male subjects (n = 1652).

Variable	Mean	Standard deviation	Min	Max	Normal range
Age (years)	40.3	11.9	18	68	–
Weight (kg)	76.1	13.3	50	135	–
Height (m)	1.75	0.08	1.58	2.05	–
BMI (kg/m^2)	24.9	3.4	14.6	41.2	18.5–25
Ca^{2+} (mmol/L)	1.29	0.04	1.14	1.37	1.17–1.33
Mg^{2+} (mmol/L)	0.53	0.05	0.33	1.56	0.45–0.6
PTH (pg/mL)	23.6	8.6	6.4	63.1	6.5–36.6
25(OH)D (ng/mL)	20.2	8.9	4	68.2	> 30
$1,25(OH)_2D$ (pg/mL)	50.7	13.3	5.7	98.5	19.9–79.3

Table 3
Anthropometric and biochemical parameters in female subjects (n = 607).

Variable	Mean	Standard deviation	Min	Max	Normal range
Age (years)	40.3	11.85	18	64	–
Weight (kg)	76.1	13.3	45	116	–
Height (m)	1.75	0.08	1.46	1.86	–
Body mass index (kg/m^2)	24.9	3.4	17.4	46.5	18.5–25
Ca^{2+} (mmol/L)	1.29	0.04	1.18	1.37	1.17–1.33
Mg^{2+} (mmol/L)	0.53	0.05	0.40	0.68	0.45–0.6
PTH (pg/mL)	23.7	8.6	6.3	53.6	6.5–36.6
25(OH)D (ng/mL)	20.2	8.9	4	89.6	> 30
$1,25(OH)_2D$ (pg/mL)	50.7	13.3	8.9	109	19.9–79.3

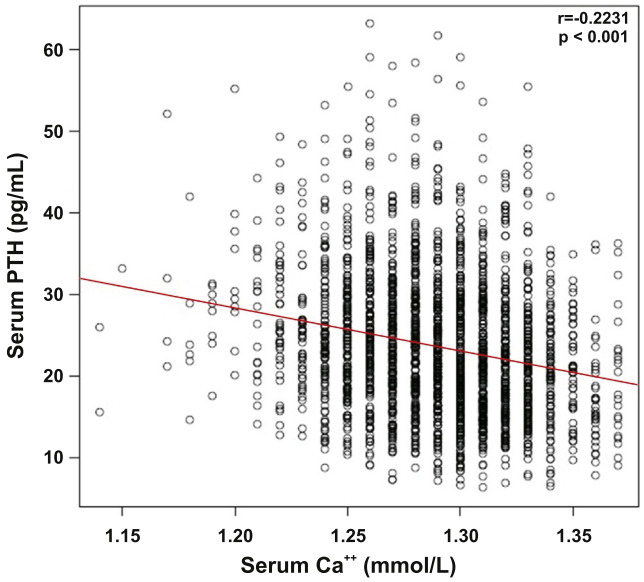

Fig. 1. The inverse correlation between serum parathyroid hormone (PTH) and ionized calcium (Ca^{++}) values, in the population as a whole (2259 subjects).

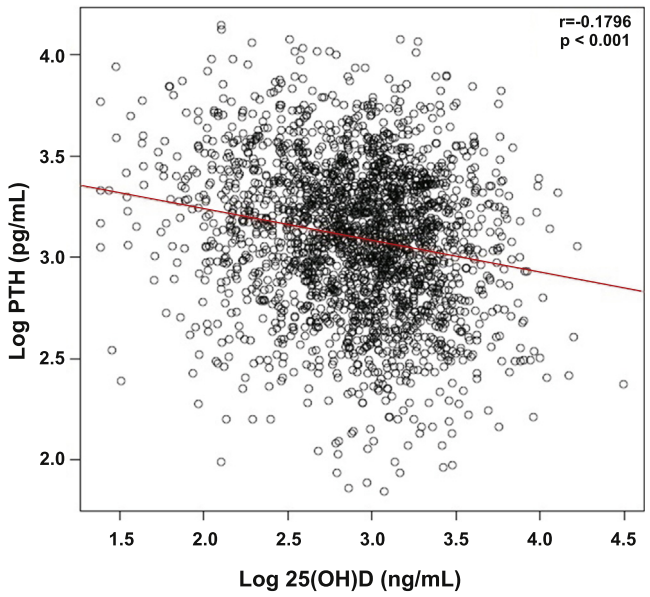

Fig. 2. The inverse correlation between parathyroid hormone (PTH) and 25(OH)D serum values, in the population as a whole (2259 subjects).

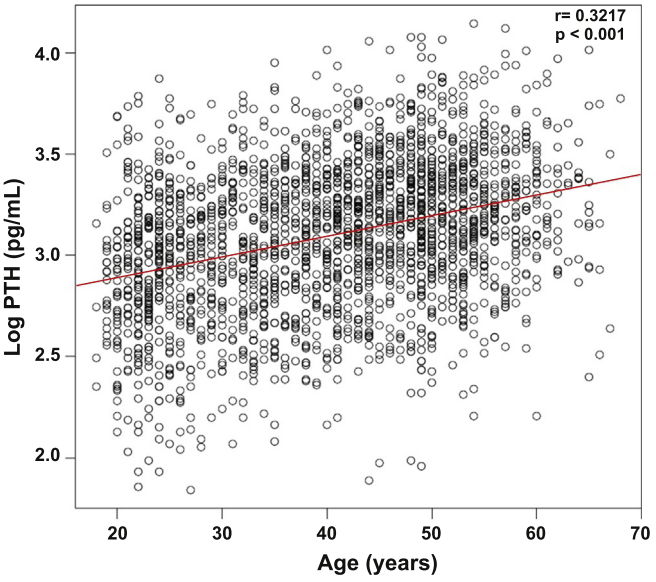

Fig. 3. The direct correlation between parathyroid hormone (PTH) and age, in the population as a whole (2259 subjects).

had the strongest effect since patients with levels of 25(OH)D below 13 ng/mL showed an average value of PTH equal to 29 pg/mL while the others showed an average value of PTH equal to 25 pg/mL. The latter group can be further partitioned considering the levels of Ca^{2+} which are effective in explaining the variations of PTH only in this subset of patients. Indeed, patients with $Ca^{2+} \geq 1.30$ mmol/L had lower average PTH value (23 pg/mL) than those with $Ca^{2+} \leq 1.30$ mmol/L (average PTH value of 26 pg/mL).

To detail the effect of each covariate on the levels of PTH, we performed a multivariate analysis via a linear model to quantitatively assess the most important parameters influencing PTH serum levels; results are reported in Table 4. After stepwise selection, all variables retained in the model had a significant effect ($p < 0.001$) on the log transformed value of PTH. Except for age, there was no interaction between all covariates and sex, suggesting that they did not determine a differentiated effect for males and females. With respect to age, on

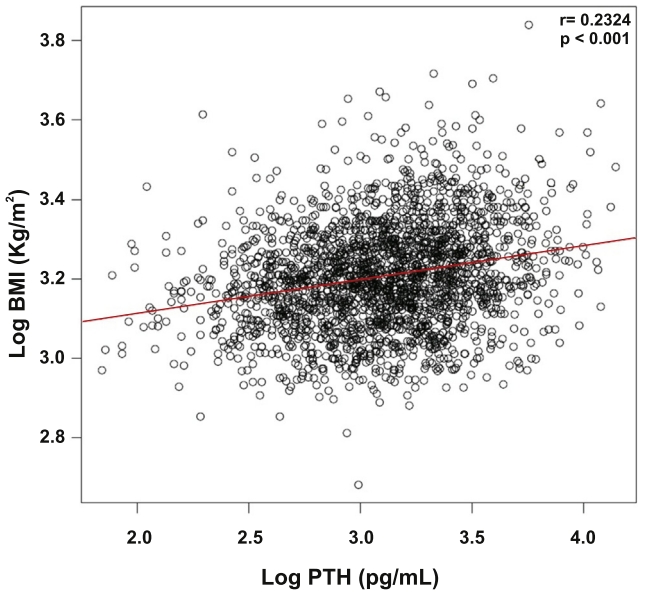

Fig. 4. The direct correlation between body mass index (BMI) and parathyroid hormone (PTH), in the population as a whole (2259 subjects).

average, the effect for female patients was positive, i.e., the levels of log PTH increase with age (an increase of 0.005 of log PTH for each year, corresponding to a percentage increase of 0.5% for PTH). Male subjects showed an almost doubled effect of age on log PTH (increment of 0.00965 of log PTH for each year, corresponding to a percentage increment of around 0.97% for PTH). Fig. 6 shows a divergent effect of the lines regressing average values of PTH in males and females over age; notably, the intersection point is around the time of menopause in females. Controlling for all the other covariates, males show, on average, levels of log PTH that are lower than those of females (-0.17463, $p < 0.001$) although the effect of age on PTH is stronger for men. Therefore, even if men tend to show lower levels of PTH at younger ages, they rapidly increase their levels of PTH with time and show higher levels of PTH on average after the age of 40.

Finally, Anova Type III errors for the selected model were employed to assess the percentage of variability of log PTH due to each covariate in the model when entered last to the regression. This accounts for the amount of variability owing to that particular covariate since it is the part of the variation of the response variable that is not explained by the other covariates. Considering the total variance explained by the covariates in the model without the intercept, 25(OH)D accounted for 32.1% of the total variance in PTH explained by the covariates when entered last in the model, Ca^{2+} accounted for 18% of the total variance, BMI for 14.3%, and 1,25$(OH)_2$D for 11.1%. The remaining percentage was attributable to age and sex. This was confirmed by the regression tree approach, where 25(OH)D and Ca^{2+} accounted for the largest variation in the average levels of PTH.

4. Discussion

This investigation showed that, in a hierarchical order under stable non-acute conditions, 25-hydroxyvitamin D has a significant role in regulating parathyroid hormone secretion compared to other variables examined, including serum ionized calcium. Importantly, this finding remained consistent across three statistical approaches. The influence of 25(OH)D may relate not only to the peripheral effect of 25(OH)D on intestinal calcium absorption [18,19] but also to a direct effect on PTH secretion at a central level. Indeed, a number of studies, including previously published pivotal findings [4], have consistently shown an inverse association between PTH and 25(OH)D [20]. This is probably related to the expression by parathyroid cells of megalin (a D binding

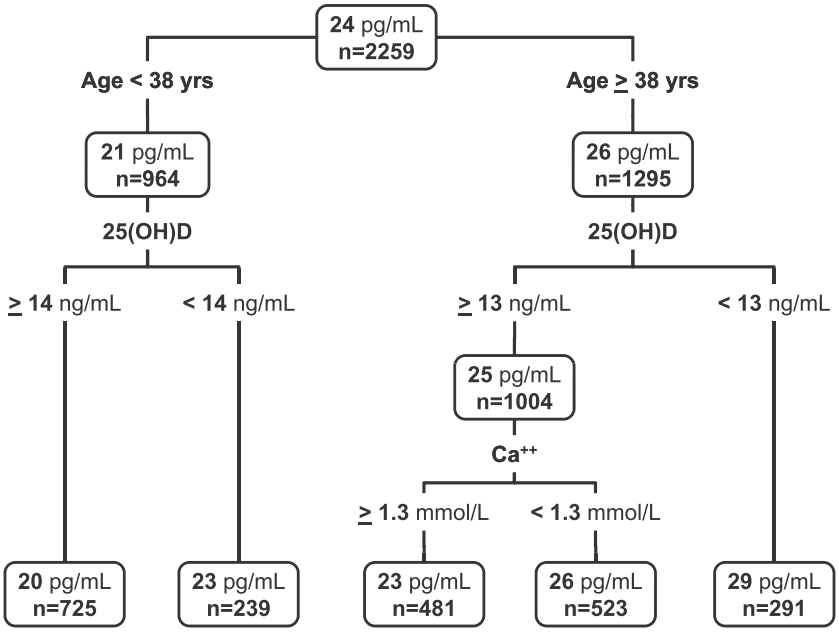

Fig. 5. The correlation between parathyroid hormone (PTH) and age for males (black line), females (red line) and the entire population (blue dotted line). Balls indicate average PTH values for males and females. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Estimates of the parameter for the multivariate model after stepwise selection using Bayesian Information Criterion.

Variable	Estimate	*p*-Value
Intercept	4.43583	< 0.001
Age (years)	0.00505	< 0.001
Serum ionized calcium (mmol/L)	−1.49130	< 0.001
Serum25(OH)D (ng/mL)	−0.00795	< 0.001
Serum1,25$(OH)_2$D (pg/mL)	0.00310	< 0.001
Body mass index (kg/m^2)	0.01482	< 0.001
Sex = M	−0.17463	< 0.001
Age ∗ sex = M	0.00465	< 0.001

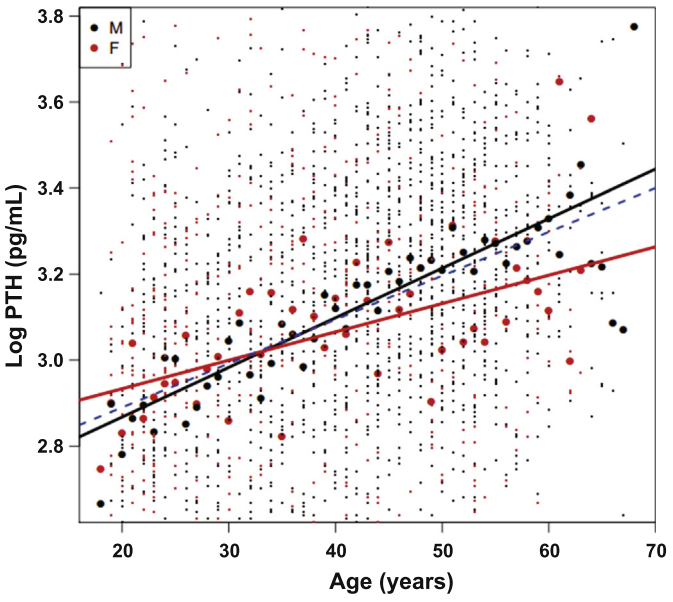

Fig. 6. Regression tree fitted on the data, built through a process of binary recursive partitioning, i.e. an iterative process of splitting the data into partitions, and then splitting it up further on each of the branches, until terminal nodes are reached (see text for better explanation).

protein receptor) that enables internalization of the substrate which is then converted to active 1,25$(OH)_2$D by the enzyme 1-alpha hydroxylase, CYP27B1 [21,22]. We do not want to disregard the importance of serum ionized calcium and the critical role of the calcium sensing receptor (CaSR) in regulating PTH secretion; in our study this was clearly established in subjects older than 38 years with 25(OH)D values above 13 ng/mL. Furthermore, under acute perturbations of calcium homeostasis, such as those encountered in acute hypo- or hypercalcemia, the most important driving factor regulating PTH secretion is represented by sensing of this ion through the CaSR expressed by the parathyroid glands [23,24].

Both experimental and human studies support these findings. Silver and co-workers [25] showed that the administration of 1,25$(OH)_2$D [the final product of intracrine 25(OHD) conversion] markedly decreased transcription of the PTH gene without concomitant changes in serum calcium concentrations. When studied “in vivo”, the suppressive action of vitamin D on preproPTH mRNA was dominant over that of high Ca^{2+} [25,26]. “In vitro” studies have shown that stimulation of vitamin D receptor with both doxercalciferol and calcidiol directly suppresses parathyroid hormone gene expression. These studies constitute the basis for utilization of vitamin D analogues in the setting of uremic secondary hyperparathyroidism [27–29] to decrease parathyroid hormone secretion with little or no change in serum calcium concentrations.

Two previous studies are of particular importance in this context. First, a pharmacokinetic investigation of oral and intramuscular administration of 300,000 IU of cholecalciferol disclosed that variations in PTH serum levels are independent of concomitant non-significant changes in serum Ca^{2+}. The general linear model demonstrated that, among all variables considered, 25(OH)D plays a significant role in influencing PTH serum levels both in the short (3 days) and long term (30 and 60 days) [30]. The second study examined administration of 600,000 IU and found similar results, the only exception being a 0.2 mg/dL increment of serum calcium at the third day of observation [17]; increases in serum 25(OH)D levels were inversely related to changes of circulating parathyroid hormone levels. Both studies, have been confirmed by subsequent data from other investigators [31] that unequivocally demonstrate the prominent role of 25(OH)D compared to serum calcium in regulating parathyroid hormone secretion, at least in the previous clinical conditions.

Another important finding from this study is linked to the behavior

of PTH serum levels with aging. Several studies have shown an increase in PTH with advancing age [13,32,33]. This has been attributed to many reasons including decreased intestinal calcium absorption, secondary to decreased $1,25(OH)_2D$ production [34] and/or theoretically increased resistance to target organs. To the best of our knowledge, this study is the first carried out in > 2000 normal subjects that separately analyzed changes of serum PTH with aging in both sexes. Interestingly, we found a divergent behavior between males and females so that at about age 40 years the lines begin to diverge. This could be related to the peri- and menopausal period where bone resorption increases following estrogen deficiency, with efflux of calcium from bone thus temporarily limiting the increase of parathyroid hormone in females [35]. An important corollary of our investigation is represented by the fact that normal values of serum PTH should be age and sex specific.

Concerning other correlations, parathyroid hormone levels' relation to BMI was probably mediated by 25(OH)D levels. Studies have reported that sequestration of calcidiol in adipose tissue underlies this association [36]; however, other factors such as high leptin levels and interleukin 6 produced by fat tissue, which have an inhibitory effect on 25(OH)D synthesis, may also play a role [37].

The direct correlation between $1,25(OH)_2D$ and serum PTH levels may be explained by the conversion of 25(OH)D to $1,25(OH)_2D$ as a function of circulating PTH. This is similar to what is generally expected to occur in primary hyperparathyroidism [38–40] or following the administration of parathyroid hormone for therapeutic purposes [41,42]. The absence of correlations when considering only females may be ascribed to the well-known effect of estrogen deficiency on $1,25(OH)_2D$ production [43]. We do not have an obvious explanation for the correlation between serum ionized magnesium and PTH; however, the absence of any correlation when considering the effect of sex suggests a spurious or chance finding and thus further studies are needed in this area.

Major strengths of this study are represented by: 1) the large number of normal subjects evaluated; 2) use of an accurate method to measure circulating PTH levels; 3) the measurement of serum ionized calcium, the metabolically active form; and 4) the simultaneous evaluation of several parameters known to influence parathyroid hormone secretion.

The major limitation was not including glomerular filtration rate as an estimate of kidney function. However, the subjects studied were blood donor volunteers, periodically checked for major health problems, including kidney function; therefore, it is unlikely that they would have had diseases to significantly damage the kidney. Furthermore, it is conceivable that adding other factors that are known to also contribute to parathyroid hormone secretion, such as for example fibroblast growth factor 23, could modify the results obtained in our model.

In conclusion, we propose that under clinically stable conditions not challenged by acute perturbation of calcium shifts or derangements, vitamin D plays a significant role in regulating parathyroid hormone secretion. Under clinical conditions of relative vitamin D sufficiency, serum ionized calcium also plays an important role.

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