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Research Article

# Abnormal Mineral Metabolism in Diabetic Patients -Relevance to Micro and Macrovascular Disease and to Bone Metabolic Disorder

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## Abstract

**Background:** Diabetic patients present an increased fracture risk despite an increased bone mineral density; serum Parathyroid Hormone (PTH), phosphate and 25-hydroxivitamin D (25OHD) are now considered independent cardiovascular risk factors. We systematically evaluated mineral metabolism to assess these contrasting and emerging factors.

**Patients and Methods:** We studied 350 diabetic patients. Besides standard clinical care, serum calcium, phosphate, magnesium, PTH and 25OHD were measured. The Statistical Package for the Social Sciences Program (IBM SPSS 24th version) was used to analyze the data.

**Results:** Mineral metabolism was commonly abnormal (13%). Insulin and thiazide diuretics alter serum calcium levels. Most patients (85%) presented low 25OHD levels, inversely related to metabolic control and to PTH levels and 21% presented increased PTH levels. Serum PTH levels were higher in patients with retinopathy and in those with high blood pressure.

**Discussion:** Because of nephropathy, diuretic and insulin use, diabetic patients commonly present abnormal mineral metabolism. Almost all diabetic patients are deficient/insufficient regarding 25OHD levels in relation to metabolic control. Secondary hyperparathyroidism and low levels of 25OHD, a unique combination in diabetic patients may contribute to the increased bone fragility in diabetic patients and may be a risk factor for micro- and macrovascular disease.

**Keywords:** Brittle Bones; Low Vitamin D; Micro and Macrovascular Disease; Mineral Metabolism; Secondary Hyperparathyroidism

## Introduction

Diabetes Mellitus (DM) is one of the most common chronic diseases in the western world, with a prevalence between 8-10% in the general population, that is rapidly increasing [1]. Because of long term biochemical abnormalities and micro- and macrovascular disease, DM is a systemic disease with the widespread organ involvement [2,3]. Because of long term use of multiple medications - antidiabetic, anti-hypertensive, hypolipidemic, and anti-platelet drugs - secondary adverse effects are common [4]. An increased risk of bone fractures has been reported in diabetic patients - up to six-fold in Type 1 Diabetics (DM1) and two-fold in Type 2 Diabetics (DM2) - that does not seem to be accounted for by an increased risk of falls and everyday trauma due to hypoglycemia, reduced visual acuity and peripheral or autonomic neuropathy (5-6). Despite this, Bone Mineral Density (BMD) although reduced in DM1 has been consistently reported as increased in the much larger group of DM2 [7-9]. This apparent paradox suggests decreased bone quality in DM patients although the mechanisms remain largely speculative [10-14].

Minerals accumulate in bone (99%, 85% and around 50% of total body content for calcium, phosphate and magnesium respectively) and together they comprise more than 65% of the skeleton weight (15). Data regarding mineral metabolism is therefore relevant to understand bone quality, bone mineral density

and fracture risk in diabetic patients.

Abnormal mineral metabolism is common in the general population with an estimated prevalence of 1-3% for primary hyperparathyroidism, more than 50% for vitamin D insufficiency/deficiency and 9% for nephrolithiasis [16]. Hyper or hypocalcemia are also relatively common in the general population and may occur in 1% and 25% of hospital admissions respectively [17]. Disordered mineral metabolism may be even more common in DM patients; because of autonomic neuropathy, mineral intake, gastrointestinal absorption and excretion may be altered, while because of nephropathy mineral losses may be increased, and because of obesity and mobility limitations mineral distribution may be different [2,3,18-20]. Also, inhibitors of Sodium Glucose Linked Transporters (SGLT-2), insulin, thiazide and loop diuretics may alter the renal handling of water, sodium and minerals [4,21].

More recently, serum PTH is emerging as an independent risk factor for cardiovascular disease and all-cause mortality, although the relation is stronger with the former [22,23]. Since the increased risk is apparent in either primary or secondary hyperparathyroidism, Chronic Kidney Disease (CKD) cannot be the only explanation. In fact, the increased risk occurs within the normal range of serum PTH and persists after correction for the glomerular filtration rate. This effect is a major one with a one standard deviation increase in serum PTH being associated with a 30-40% increase in cardiovascular risk and in fact is equivalent to other well-established risk factors for cardiovascular disease like systolic blood pressure and total cholesterol. These data are further reinforced by intervention studies with calcimimetic drugs that reduce the cardiovascular risk [22]. A direct effect of PTH on vascular stiffening, remodeling, calcification and more general in the atherogenic process has been invoked, that includes the differentiation of vascular smooth muscle cells into chondrocytes with a osteoblast-like phenotype [22,24]. Curiously enough, the increased risk persists even when correcting for low serum 25OHD, while low serum 25OHD is also emerging as independent risk factor for cardiovascular disease [25].

We purposed to evaluate mineral metabolism in diabetic patients assisted at the out patient endocrine department. We wanted to assess how common abnormalities of mineral metabolism were in the real setting of diabetes care, to explore if these abnormalities could help explain the increased fracture risk despite an increased bone mineral density and to verify whether indexes of mineral metabolism were associated with micro- or macrovascular disease.

## Patients and Methods

We studied all DM assisted by one of us, at a tertiary public center. The database regarding those patients includes – gender and actual age, time since diagnosis, height and actual weight from which the body mass index was computed [weight(kg)/height(m)$^2$, BMI], and the last available glycated hemoglobin [$HbA_{1c}$]; presence or absence of retinopathy – no retinopathy, retinopathy without laser therapy and retinopathy with previous laser therapy (summed up when indicated as retinopathy yes or no), nephropathy - no nephropathy, microalbuminuria > 30 mg/24h and renal failure with serum creatinine > 1.5 mg/dL (summed up when indicated as nephropathy yes or no), and peripheral neuropathy - yes or no on clinical questioning, were also recorded as well as the presence or absence of High Blood Pressure (HBP) and dyslipidemia; drugs being used for diabetes mellitus, HBP, dyslipidemia and anti-platelet agents were recorded; a specific inquiry regarding use of Drugs or Supplements (DS) that included anti-osteoporotic drugs, calcium or vitamin D were also included.

Besides the usual care [26], serum calcium, phosphate, magnesium, Parathyroid Hormone (PTH) and 25-hidroxyvitamin D (25OHD) were obtained at the last appointment in a venous sample drawn in the morning. Analytical measurements were performed at the Chemical Pathology Department of the hospital using standardized methods; chemical colorimetric methods for serum calcium, phosphate and magnesium and Chemiluminescence Immunoassay (CLIA) or Radioimmunoassay (RIA) methods for serum parathyroid hormone - Immulite 2500 (Diagnostics Product Corporation, Los Angeles, California) - and 25-hydroxyvitamin D - 25-HydroxyvitaminD $^{125}$I RIA kit (Dia Sorin Inc., Stillwater). Intra- and inter-Assay variation coefficients were always below 10%. Units and reference values are as follows: Serum calcium - 8.6-10.2 mg/dL; serum phosphate - 2.4-5.1 mg/dL; serum magnesium - 1.3-2.7 mg/dL; serum parathyroid hormone (PTH) - 14-72 pg/mL; serum 25-hydroxyvitamin D (25OHD) - 30-80 ng/mL, with values < 20 ng/mL defining deficiency, values between 20-29 ng/mL insufficiency and values > 100 ng/mL toxicity.

Clinical and analytical data were entered on a Statistical Package for the Social Sciences (IBM SPSS 24$^{th}$ version) database and statistical analysis used the same program. Results are presented as the mean ± standard deviation or as the percent as appropriate. The normal distribution of continuous variables was assessed with the Kolmogorov-Smirnov test and non-normal distributed variables were log transformed prior to analysis; however, when no differences regarding the non-transformed variables were found, these are presented instead for the sake of simplicity. Comparison between variables used the chi-squared test or Anova with the Least Significant Difference (LSD) for pos hoc comparisons as appropriate and the relation between continuous variables used multiple regression analysis. The limit of significance is 0.05 [27,28].

## Results

### Patient Characteristics

Only patients with Type 1 Diabetes Mellitus (DM1) - 56 patients (16%) or patients with Type 2 Diabetes Mellitus treated

with insulin (DM2IT) - 164 patients (47%) or Type 2 Diabetes Mellitus treated with oral agents (DM2OT) - 130 patients (37%) were included in the analysis.

Clinical characteristics of the patients are summarized in Table 1. In short, patients were both sexes, old (DM2IT and DM2OT) or young (DM1) adults, with long standing disease and overweight (DM2IT and DM2OT). Metabolic control was less than desirable, and they presented chronic microvascular disease and macrovascular risk factors mainly in those with long standing Type 2 Diabetes (DM2IT). In both DM2IT and DM2OT groups more than 80% of the patients were using drugs to control blood pressure and more than 60% were using both hypolipidemic drugs and anti-platelet agents. Drugs to treat osteoporosis or containing calcium or vitamin D were only marginally used.

Differences between groups are presented although they were not used later in the analysis; instead individual variables were selected to explore mineral metabolism.

| | DM1 (56) | DM2IT (164) | DM2OT (130) | Differences between Groups |
|---|---|---|---|---|
| Sex (F/M) (%) | 51/49 | 44/56 | 40/60 | NS |
| Age (years) | $39\pm14^{a}$ | $67\pm11^{b}$ | $66\pm12^{b}$ | $F(2,348)=128.5$, $p<0.01$ |
| Years since diagnosis | $21\pm14^{a}$ | $19\pm11^{a}$ | $15\pm10^{b}$ | $F(2,348)=9.3$, $p<0.01$ |
| BMI ($kg/m^2$) | $24.3\pm3.9^{a}$ | $28.9\pm5.2^{b}$ | $28.8\pm5.1^{b}$ | $F(2,348)=16.7$, $p<0.01$ |
| HbA1c (%) | $8.5\pm1.7^{a}$ | $8.4\pm1.6^{a}$ | $7.3\pm1.3^{b}$ | $F(2,348)=28.1$,$p<0.01$ |
| Retinopathy (yes) (%) | $45^{a}$ | $56^{a}$ | $21^{b}$ | $\chi^2 = 36.6$, df=2, $p<0.01$ |
| Nephropathy (yes) (%) | $38^{a}$ | $56^{b}$ | $40^{a}$ | $\chi^2 = 14.2$, df=2, $p<0.01$ |
| Neuropathy (yes) (%) | $16^{a}$ | $38^{b}$ | $33^{b}$ | $\chi^2 = 18.6$, df=2, $p<0.01$ |
| HBP (yes) (%) | $25^{a}$ | $85^{b}$ | $78^{b}$ | $\chi^2 = 88.1$, df=2, $p<0.01$ |
| Dyslipidemia (yes) (%) | $27^{a}$ | $63^{b}$ | $62^{b}$ | $\chi^2 = 25.9$, df=2, $p<0.01$ |
| HBP drugs (yes) (%) | $35^{a}$ | $91^{b}$ | $81^{c}$ | $\chi^2 = 84.2$, df=2, $p<0.01$ |
| Hypolipidemic drugs (yes) (%) | $29^{a}$ | $62^{b}$ | $61^{b}$ | $\chi^2 = 20.4$, df=2, $p<0.01$ |
| Antiplatelet drugs (yes) (%) | $21^{a}$ | $68^{b}$ | $66^{b}$ | $\chi^2 = 49.3$, df=2, $p<0.01$ |
| DS (yes) (%) | 5 | 11 | 8 | NS |
| *Significant different groups are presented with different indexes $^{a,b,c}$ | | | | |

**Table 1:** Clinical Characteristics.

## Abnormal Mineral Metabolism

Results regarding common parameters of mineral metabolism are presented across the three defined groups (Table 2). Again, differences regarding groups although presented are not further used in the analysis.

| | DM1 (56) | DM2IT (164) | DM2OT (130) | Differences between groups |
|---|---|---|---|---|
| Calcium (mg/dl) | $9.5\pm0.5$ [8.1-10.5] | $9.5\pm0.4^{a}$ [8.3-10.5] | $9.7\pm0.5^{b}$ [8.1-12.0] | $F(2,348) = 3.212$, $p < 0.05$ |
| Phosphorus (mg/dl) | $3.6\pm0.8^{a}$ [1.9-6.4] | $3.5\pm0.6$ [1.8-5.8] | $3.4\pm0.6^{b}$ [1.4-5.2] | $F(2,348) = 3.248$, $p < 0.05$ |
| Magnesium (mg/dl) | $2.0\pm0.2^{a}$ [1.6-2.9] | $1.9\pm0.3^{b}$ [1.3-2.9] | $1.9\pm0.3^{b}$ [0.9-2.9] | $F(2,348) = 5.678$, $p < 0.01$ |

| | | | | |
|---|---|---|---|---|
| PTH (ng/dl) | 80±183 [11-1210] | 63±50 [9-363] | 54±44 [28-124] | NS |
| 25OHD (ng/dl) | 21±10 [4-44] | 22±14 [3-113] | 20±10 [4-54] | NS |
| *Range is indicated within square brackets. Significant different groups are presented with different indexes [a,b,c] | | | | |

**Table 2:** Parameters of Mineral Metabolism.

Regarding minerals, available as routine, results outside the reference range were mild and uncommon: 28 cases for calcium (8%); 14 cases for phosphate (4%) and 7 cases for magnesium (2%). Only four patients presented more than one abnormal result. Analysis of the individual patient records revealed the 45 patients (13% of the total) to present: primary hyperparathyroidism previously undiagnosed (3 patients), secondary hyperparathyroidism in patients with diabetic nephropathy (12 patients), isolated hypocalcemia (2 patients) and isolated hypophosphatemia (2 patients) secondary to serious gastrointestinal disease, hypocalcemia secondary to hypovitaminosis D (2 patients), hypercalcemia secondary to vitamin D toxicity (1 patient), hypercalcemia secondary to neoplastic disease (1 patient), hypercalcemia due to familial hypocalciuric hypercalcemia (1 patient), and 14 patients with isolated mild abnormalities secondary to diuretic use (loop diuretics or thiazide-like diuretics); in 7 patients no obvious reason for the very mild abnormalities of serum calcium, phosphate or magnesium could be found.

Decreased 25OHD levels were very common with 85% presenting deficient/insufficient vitamin D levels (< 30 ng/mL) and 55% presenting deficient vitamin D levels (< 20 ng/ml); only one patient presented increased 25OHD levels (> 100 ng/mL) suggesting vitamin D toxicity. As noted most of these patients presented normal serum values of calcium, phosphate and magnesium (see later).

Increased PTH values were less common (21% of the patients) with more than half of these presenting values above 100 pg/mL (13%). Low PTH values were only found in 4 patients and were borderline with normal serum calcium levels.

## Relation to Diabetes Characteristics

Mineral indexes were not significantly different across sex and were not significantly related to age. Time since diagnosis was significantly related to both serum calcium (parcial r=-2.043, p<0.05) and to serum PTH (partial r=2.036, p<0.05). Actual BMI was significantly related to serum calcium (r=0.105, p<0.05) the relation increasing when all other mineral parameters were simultaneously considered (partial r=0.268, p<0.01).

Metabolic control evaluated by the last HbA1c, was significantly related to serum calcium (r=-0.129, p<0.05) to serum phosphate (r=0.162, p<0.01) to serum PTH (r=0.171, p<0.01) and to 25OHD (r=-0.146, p<0.05); however, when all variables were simultaneously considered only vitamin D remained significant (partial r=-0.156, p<0.05) (Figure 1). Significance persisted after correcting for nephropathy.

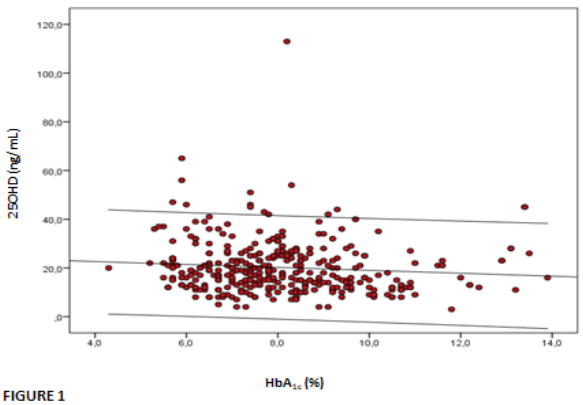


**Figure 1:** Linear regression of serum 25OHD vs HbA1c (r=-0.146, p<0.05).

Patients with retinopathy presented significantly higher levels of serum PTH compared to patients without retinopathy - 89±140 pg/mL vs. 52±40 pg/mL, t=3.039, df=348, p<0.01 - with no differences regarding other indexes of mineral metabolism. Significance remained after correction for nephropathy.

Differences regarding mineral metabolism indexes in patients with or without nephropathy are presented in Table 3.

| | No nephropathy (192) | Proteinuria (98) | Renal failure (60) | Differences between groups |
|---|---|---|---|---|
| Calcium (mg/dL) | 9.6±0.4[a] | 9.5±0.4[b] | 9.5±0.5[b] | F(2,348) = 3.8, p<0.05 |
| Phosphate (mg/dL) | 3.5±0.6[a] | 3.4±0.7[a] | 3.8±0.8[b] | F(2,348) =5.4, p<0.01 |
| Magnesium (mg/dL) | 1.9±0.2[a] | 1.9±0.3[a] | 2.1±0.3[b] | F(2,348) = 12.3,p<0.01 |
| PTH (pg/mL) | 47±29[a] | 58±45[a] | 151±211[b] | F(2,348) = 18.6, p<0.01 |
| 25OHD (ng/mL) | 24±14[a] | 20±9 | 17±6[b] | F(2,348) = 4.2, p<0.05 |
| [*]Significant different groups are presented with different indexes [a,b,c] | | | | |

**Table 3:** Mineral Metabolism Across Diabetic Nephropathy.

Magnesium levels were significantly lower in those patients with peripheral neuropathy - 1.9±0.3 mg/dL vs. 2.0±0.3 mg/dL, t=3.174, df=348, p<0.01 - with no differences regarding serum calcium, phosphate, PTH or 25OHD. Again, the differences persisted after correcting for nephropathy.

PTH levels were significantly higher in those patients with HBP - 70±103 pg/mL vs. 45±30 pg/mL, t=-1.965, df=348, p<0.05 – with no significant differences regarding serum calcium, phosphate, magnesium or 25OH; the significance was lost when nephropathy was taken into account; however, in a model that includes HBP, nephropathy and age, HBP is again a significant factor for serum PTH levels - r= 0.378, p<0.01, partial rs 0.155, 0.320 and -0.178, respectively, p<0.05 in every case. There were no significant differences regarding indexes of mineral metabolism in patients with or without dyslipidemia.

As noted in Table 2, type 2 diabetic patients using insulin (DM2IT) presented significantly lower calcium levels regarding type 2 diabetic patients not using insulin (DM2OT) - 9.5±0.4 mg/dL vs. 9.7±0.5 mg/dL, t=2.489, df=348, p <0.05 - with no other significant differences regarding the indexes of mineral metabolism; however, the difference was reduced to a trend (p<0.1) when nephropathy was taken into account. Patients using thiazide-like diuretics - most of them in fixed associations with angiotensin converting enzyme inhibitors or angiotensin receptor antagonists - presented significantly higher calcium levels – 9.7±0.5 mg/dL vs. 9.5±0.5 mg/dL, t=3.274, df=348, p<0.05 - in comparison with patients taking other HBP drugs or no drugs, the differences persisting after correction for nephropathy, with no other significant differences regarding the indexes of mineral metabolism. There were no significant differences regarding the same indexes between patients using statins compared to those not using them, nor in relation to patients using or not acetylsalicylic acid as an anti-platelet agent. The number of patients using drugs or supplements with calcium, vitamin D or anti-osteoporotic agents was too small and furthermore with contradictory effects on mineral metabolism to allow any meaningful comparisons.

## Relation Between Indexes of Mineral Metabolism

Neither calcium (KS z=1.510, p<0.05), nor phosphate (KS z=1.341, p<0.06) nor magnesium (KS z=1.806, p<0.05) were normal distributed; this non-normal distribution persisted even if abnormal values were excluded. Also, calcium, phosphate and magnesium were independent variables - there was only a small albeit significant direct relation between serum phosphate and serum magnesium (r=0.166, p<0.05).

Serum PTH (KS z=4.787, p<0.01) and 25OHD (KS z=2.009, p<0.01) were also not normally distributed. Serum PTH was inversely and significantly related to calcium (r=-0.187, p<0.01) and directly and significantly related to phosphate (r=0.338, p<0.01) and to magnesium (r=0.227, p<0.01); when the three variables were simultaneously considered (n=350, r=0.345, p<0.01) all three remained as significant variables (β = -27.752, 17.037 and 66.031, p< 0.01, 0.05 and 0.01, respectively for calcium, phosphate and magnesium as dependent variables of PTH). On the contrary 25OHD was not significantly related to serum calcium, to serum phosphate or to serum magnesium not even when all three variables were simultaneously considered or when PTH was also simultaneously entered in the regression analysis.

25OHD levels were inversely related to serum PTH (r=-0.157, p < 0.05) (Figure 2), suggesting that for levels of 25OHD less than 20 ng/dL, serum PTH progressively increases. However only 21% of those presenting deficient vitamin D levels (< 20 ng/mL) and only 16% of those with insufficient vitamin D levels (20-29 ng/mL) presented increased PTH levels. Therefore, most patients with decreased vitamin D values (< 30 ng/mL) presented normal PTH levels (81%). Only 2 patients with low 25OHD levels (< 30 ng/mL) (<1%) presented low serum calcium levels.

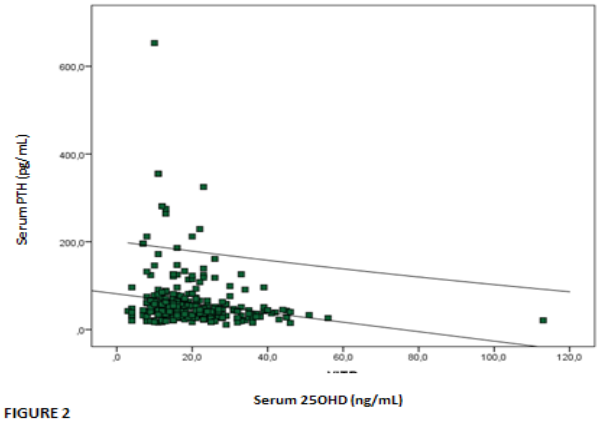


**Figure 2:** Linear regression of serum 25OH vs serum PTH (r=-0.157, p<0.05).

On the other hand, 74% of those patients presenting with increased PTH levels presented low levels of 25OHD (< 30 ng/mL) and nephropathy, with 18% presenting only low levels of 25OHD without nephropathy, 4% only nephropathy with normal 25OH levels and 4% both normal levels of 25OHD and normal renal function. In fact, only 35% of those patients with nephropathy presented high PTH levels. Ninety-one % of the patients with nephropathy presented low levels of 25OHD, compared with only 78% of those without nephropathy $\chi^2 = 9.49$, df= 1, p<0.01.

## Discussion

Abnormal mineral metabolism may be expected in DM patients, since this is a chronic systemic disease with widespread organ involvement and multiple drug use, and mineral metabolism may therefore be altered at several levels - intake, absorption, distribution and gastrointestinal and renal excretion [1-4].

Data regarding mineral metabolism in DM patients may help explain the apparent paradox of an increased fracture risk in both type 1 and type 2 diabetics, even when accounting for several confounding variables, despite an increased bone mineral density in type 2 DM patients [5-7]. This apparent paradox suggests decreased bone quality beyond bone mineral density [10-12].

Data regarding mineral metabolism may be important by itself since several indexes of mineral metabolism, including serum PTH, 25OHD and hyperphosphatemia are now emerging as powerful independent risk factors for cardiovascular disease and even all-cause mortality [22-25].

The sample of diabetic patients considered is large enough and representative of diabetic patients assisted in tertiary centers in the western world, to allow for meaningful conclusions; furthermore, any conclusions should apply to the real practice conditions of everyday life, since they are significant for the relatively small number of patients considered. Patients are middle aged or old adults, with long standing disease, metabolic control less than what would be desirable, and with evidence for microvascular disease and risk factors for macrovascular disease. Multiple drugs were simultaneously being used in these patients (average 13 “Pills” a day, data not shown).

Abnormal mineral metabolism is indeed common in diabetic patients - 45 patients or one in every 7-8 patients (13%) - even if abnormalities of calcium, phosphate and magnesium are generally mild, isolated and for the most part secondary to diabetic nephropathy - 12 patients - or to diuretic use - 14 patients. It should be noted that besides the recognized effects of thiazide and loop diuretics, the first one also evident in this sample, insulin use was associated with lower serum calcium levels in this sample. Other cases - 14 patients - can also be expected in the general population including undiagnosed cases of primary hyperparathyroidism, serious gastrointestinal disease, hypovitaminosis D, vitamin D toxicity, neoplasia and familial hypocalciuric hypercalcemia [15,17,29-32].

Much more common however are the low levels of 25OHD, that are indeed the “norm” in diabetic patients. These results agree well with other larger scale studies recently reported [33]. Interestingly enough in that study, an inverse relation of serum 25OHD levels with metabolic control was also reported, such as the one found in this sample [33]. That relation may explain the strikingly high prevalence of deficient/insufficient vitamin D found in diabetics. However, the relation may also be bidirectional since some studies have suggested that normal levels of vitamin D may protect against the development of type 2 diabetes mellitus as recently reviewed (34). Vitamin D receptors have been described in pancreatic β cells and vitamin D insufficiency/deficiency has been associated with impaired insulin secretion [34]. In fact, protective effects have been described for vitamin D supplementation on what relates do retinopathy, nephropathy and neuropathy [34].

The clinical relevance of this data is however controversial since as also observed in this sample almost all patients present normal calcium levels (>99%) and normal serum PTH levels (79%). Only 21% of those presenting deficient vitamin D levels and 16% of those with insufficient vitamin D levels present increased PTH levels.

As recommended, we measure total 25OHD (D2 and D3, bound and free) not the active form 1,25-dihydroxivitamin D, only one measurement was considered despite the well-known seasonal variability and the reference range is controversial, even if theoretically justified by maximal intestinal calcium absorption and lowest PTH levels [33-38]. Furthermore, no routine assay for the vitamin D binding protein is currently available. Even so it is remarkable that 9% of the patients present extremely low levels of 25OHD (< 10 ng/mL), generally believed to be associated with

rickets/osteomalacia [35-38].

Much remains to be known about this hormone with ubiquitary receptors distribution and widespread biologic effects [39]. Recent studies suggest, serum 25OHD levels to be an independent risk factor for cardio- and cerebrovascular disease and mortality at levels probably lower than those considered appropriate for normal bone health [25,34,35,39].

Almost a fourth of the sampled patients also present increased PTH levels, that for the most part seem to depend on the combined effect of decreased 25OHD and nephropathy. In fact, diabetic patients may unique combine these two factors, in a way similar to those with CKD (40,41). However, in diabetes mellitus low levels of 25OHD occur prior to the development of nephropathy in apparent relation to metabolic control. Besides in this report at least, nephropathy was also associated unexpectedly to low levels of 25OHD, not only the theoretically expected decrease of 1,25-dihydroxyvitamin D.

Contrariwise to the decreased 25OHD levels, the increased PTH may be indeed real, since PTH was significantly and independently related to serum calcium, phosphate and magnesium, and these variables were not normally distributed which suggests a certain degree of constraint.

It should be noted that increased PTH levels - in fact even within the normal range - are now emerging as a powerful independent risk factor for cardiovascular disease and all-cause mortality in the general population and in diabetic patients, generally associated with increased vascular stiffness [23-25]. Also in this study, higher PTH levels were found both in patients with HBP and in patients with retinopathy compared to patients without those conditions and the significance remained after correcting for nephropathy. PTH may therefore be a risk factor for both macro- and microvascular disease. How can the abnormal findings now reported help explain bone fragility in diabetic patients?

Since no major widespread changes of serum levels of calcium, phosphate or magnesium were found, a major role for hormones regulating mineral metabolism and bone turnover is suggested.

The relevance of the decreased 25OHD seems doubtful. Firstly, because the clinical relevance of low 25OHD is by itself unclear [34-38]; secondly because decreased 25OHD would be expected at least in line with classic cases of rickets and osteomalacia to result in mineralization defects of the expanding bone matrix that would result in decreased bone mineral density not increased bone mineral density and would result in more “soft” deforming bones [35-39].

The relevance of increased PTH seems more probable. First because the increased levels seem real, explained by both the nephropathy and the decreased 25OHD levels, and to be significantly related to serum calcium, phosphate and magnesium [15]. Secondly because on bone PTH initiates the cycle of reabsorption-formation that remodels bone, and increased PTH levels may lead to uncoupling of the process resulting in bone cysts and bone brown soft tumors that are the typical bone changes in primary hyperparathyroidism not osteoporosis per se [42-45]. This is also suggested by the therapeutic use of teriparatide in the treatment of serious osteoporosis and lack of effectiveness in promoting bone healing after a fracture [46,47].

BMD is generally extremely low in osteitis fibrosa cystica the classic bone disease of primary hyperparathyroidism [42-45]. Classic osteitis fibrosa cystica however is now a rare manifestation of primary hyperparathyroidism that more commonly is an incidental analytical finding in asymptomatic subjects [42-45]. In a recent study half of the patients with primary hyperparathyroidism presented normal BMD scores although decreased Trabecular Bone Score (TBS) [43]. Despite this a much-increased risk of bone fractures is found both at the vertebral and non-vertebral bone sites [46,47].

Bone disease in diabetic patients resembles in several ways bone disease of CKD, namely because it includes low levels of vitamin D (25OHD or 1,25-dyhydroxyvitamin D) and increased PTH, even if in diabetic patients low levels of 25OHD in relation to poor metabolic control may precede nephropathy for many years and nephropathy seems associated in diabetic patients with decreased levels of both 25OHD and 1, 25-dihydrovitamin D [41,48,49]. In both cases chronic renal disease also leads to increased levels of Fibroblast Growth Factor (FGF-23) induced by hyperphosphatemia, that may further reduce 1,25-OH-vitamin D, by inhibiting kidney 1-α-hydroxylase activity [50].

In End Stage Renal Disease (ESRD) and milder forms of CKD an increased fracture risk is found, but data regarding BMD is also contradictory; an apparent decreased BMD in CKD disappears after correcting for confounding factors in the National Health and Nutrition Examination Survey, and the Kidney Disease Improving Global Outcomes recently recommended against BMD testing in CKD stages 3-5, since it does not predict fracture risk as it does in the general population and does not predict the type or renal osteodystrophy [41,48,49]. In the Health, Aging, and Body Composition study including 2754 old (70-79 years) well functioning community-living subjects, BMD at the femoral neck and total hip were not significantly different in patients with or without CKD [49].

Tentatively, therefore the increased PTH levels may contribute both to the increased bone mineral density and to the increased porosity - more brittle bones found in diabetic patients.

Of course, factors related to diabetes may be thought to explain the distinctive bone disease of diabetes. Hyperglycemia per se, Advanced Glycosylation Products (AGE), oxidative

stress, insulin, IGF1, hormones of the adipose tissue, cytokines of the inflammatory cells of the adipose tissue, etc. All have been tentatively implicated, but none so far seems to explain the increased fracture risk despite an increased bone mineral density [10-12].

In brief we found that in the real conditions of diabetic care, abnormal mineral metabolism is common and should be considered mainly in relation to diabetic nephropathy, diuretic use and also insulin use. Apparent vitamin D deficiency or insufficiency is extremely common and is inversely related to metabolic control. Increased PTH levels although less common seem to depend on low 25OHD levels and nephropathy, significantly relate to serum calcium, phosphate and magnesium, may tentatively explain the increased bone mineral density and more porous brittle bone, and to be by itself a significant factor for micro- and macrovascular disease.

## Conclusion

Standardized clinical care of diabetic patients reveals common abnormalities of mineral metabolism (13%) mainly hyper- or hypocalcemia in relation to diabetic nephropathy, diuretic and insulin use that must be specifically addressed. Low levels of 25OHD are extremely common (85%) and although inversely related to metabolic control and even lower in patients with diabetic nephropathy are of uncertain relevance.

Increased PTH levels, although less common (21%) may be more relevant. Increased PTH levels are found in relation to diabetic nephropathy and low 25OHD levels a unique combination in diabetic patients, significantly relate to serum calcium, phosphate and magnesium, may explain the increased bone mineral density and more porous brittle bone found in diabetic patients and may be a significant factor not only for macro- but also microvascular disease. Evaluation of mineral metabolism must be added to the standardized care of diabetic patients.

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