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Correlations of Vitamin D Status with Osteocalcin, Parathyroid Hormone, and Minerals in Osteoporotic and Diabetic Patients, and Healthy Individuals

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Abstract

Background: Osteocalcin, parathyroid hormone, and ions such as calcium, magnesium, and phosphate are integral to the vitamin D hormone system and skeletal metabolism. The production or absorption of these biomarkers is considered to be affected by the body's vitamin D status.

Methods: The study population consisted of 165 female and 161 male adults (326 in total), including 99 osteoporotic, 53 type 1 diabetic, 51 type 2 diabetic, and 123 healthy individuals, all from northern Greece. We investigated the possible association of vitamin D status with osteocalcin and parathyroid hormone (PTH) measured by ELISA, as well as calcium, phosphate, and magnesium measured by enzymatic assays.

Results: The highest mean osteocalcin concentration was observed in osteoporotic subjects (40.33 pg/ml), followed by type 2 diabetics (26.37 pg/ml), healthy individuals (19.65 pg/ml), and type 1 diabetics (17.79 pg/ml). Parathyroid hormone was highest in osteoporotic (163.33 pg/ml), followed by type 1 diabetics (61.33 pg/ml), type 2 diabetics (59.61 pg/ml), and healthy individuals (17.65 pg/ml). Osteocalcin and parathyroid hormone concentrations showed inverse correlations ($p< 0.001$) with those of total and free calcidiol. The correlations of calcium and magnesium with total and free calcidiol were significant ($p< 0.05$), whereas phosphate concentration was not correlated with vitamin D metabolites. A strong inverse correlation ($R^2 = 0.489$) between calcium and parathyroid hormone was observed ($p< 0.001$). Osteocalcin showed a weak inverse correlation ($R^2 = 0.049$) with calcitriol ($p< 0.001$).

Conclusions: Osteocalcin, parathyroid hormone, calcium, and magnesium correlate with vitamin D status, whereas phosphate does not.

Keywords: Calcium, Magnesium, Osteocalcin, Parathyroid Hormone, Phosphates, Vitamin D.

Introduction

The osteocalcin gene (*BGLP*) is one of the first genes in which vitamin D-responsive elements (VDRE) were identified in 1990; i.e., it is subject to the basic regulation of calcitriol and its intracellular receptor (1). Osteoblasts produce osteocalcin and carboxylate it by a vitamin K-dependent process, which results in the accumulation of the carboxylated form (carboxylated Osteocalcin, cOC), that is incorporated into the osteoid. The measurement of osteocalcin is widely used as a marker of bone turnover (2). An

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undercarboxylated form of osteocalcin (undercarboxylated Osteocalcin, ucOC) plays an active role in glucose metabolism (3). Type 1 and 2 diabetics are at an increased risk of fractures and hyperglycemia, and insulin administration affects bone metabolism. Thus, osteocalcin is considered a potential link, with a hormonal and structural role, between osteoporosis and the two types of diabetes examined in this study (4).

Parathyroid hormone (PTH) regulates how the kidneys handle calcium, phosphate, sodium and hydrogen ions (5). It also regulates the hydroxylation of calcidiol to produce calcitriol (6).

It is known that intestinal calcium absorption depends on the adequacy of vitamin D (7). Absorption efficiency is reduced by more than 75% in experimental animals with vitamin D deficiency (8) as well as in patients undergoing hemodialysis who have low circulating calcitriol levels due to impaired renal function (9).

Although phosphate metabolism throughout the body is regulated mainly through the control of renal phosphate reabsorption, much research has also been conducted on the mechanisms of intestinal absorption (10). In humans, Davis et al. (11) showed that calcitriol in the jejunum increases phosphate absorption with chronic renal failure patients and presumably low serum calcitriol levels.

In both male and female type 2 diabetics, a significant increase in serum magnesium levels has been observed after vitamin D_3 supplementation, suggesting that vitamin D enhances the intestinal absorption of magnesium (12). Conversely, the enzymatic actions of hepatic 25-hydroxylase, renal 1 α-hydroxylase and 24-hydroxylase, essential for the conversion of vitamin D to calcidiol, then to calcitriol, and finally for their catabolism, as well as the vitamin D transport protein (DBP), all depend on magnesium (13,14). Magnesium deficiency results in reduced calcitriol levels and increased parathyroid response. Magnesium supplementation has been shown to significantly reduce resistance to vitamin D therapy in patients with rickets (15).

This study aimed to investigate whether and to what extent osteocalcin, PTH, and the aforementioned minerals are related to the body's vitamin D status through correlations with total and free calcidiol, as well as calcitriol in the r samples.

Materials and Methods

Study design

From March 15 to 31, 2018, each participant donated a fasting serum sample, which was stored in a deep freeze (-70 °C) until examined. None of the participants were receiving vitamin D supplementation, and all individuals aged≥ 60 years old were independent and living in the community. All the diabetic patients were receiving insulin treatment. Participants were excluded from the study if, during the last six months, they had used a medication known to affect calcium and vitamin D metabolism, except for antiresorptive therapies used by osteoporotic participants, according to the ELIOS guidelines issued in 2018 by the Hellenic Osteoporosis Foundation. Other exclusion criteria included diseases that affect calcium homeostasis, such as metabolic bone diseases other than uncomplicated osteoporosis, osteoporotic fractures, and liver or kidney diseases.

The concentrations of hormones and minerals in the samples were measured at the private diagnostic laboratory "Bioanalysis," which is ISO 9001:2008 certified by the Hellenic Accreditation System (ESYD).

Osteocalcin and PTH were measured using an Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany), via the chemiluminescence method. This assay detects undercarboxylated (ucOC) and carboxylated (cOC) osteocalcin together. Concentrations of calcium (CPC, colorimetric method), phosphate (UV, phosphomolybdate method), and magnesium (xylidyl blue, colorimetric method) ions were measured on a Hitachi 902 biochemical

analyzer (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, version 23 (IBM, Armonk, NY), following previously reported procedures (16,17). For correlations between mean concentrations, the parametric independent samples t-test was used. Where t-test assumptions were violated, the non-parametric Mann-Whitney U test was used instead. Statistical significance was determined at a probability level of $\alpha = 0.05$.

Ethics

Written informed consent was obtained from each participant. Approval of the protocol, after thorough review, was granted by the Committee of Ethics and Deontology in Research of the Democritus University of Thrace (protocol number: Θ24/Δ.Σ7/28.2.2081).

Results

Subjects

As previously reported in detail (16), the demographics and conditions of the study participants are shown in Table 1. There was no age or gender matching between groups.

Osteocalcin

The mean osteocalcin concentration was significantly higher in females across the entire sample, with a weak correlation. Mean osteocalcin concentrations did not differ significantly between sexes within any of the individual study groups (Fig. 1).

Table 1. Demographics and conditions of the study participants.

Subtotal	Male		Female		Age		Condition
	Count	%	Count	%	Mean	SD	
123	70	56.9	53	43.1	46.4	13.3	Healthy (No known condition)
99	24	24.2	75	75.8	56.3	11.0	Osteoporosis
53	27	50.9	26	49.1	36.7	8.8	Type 1 Diabetes
51	40	78.4	11	21.6	58.9	7.0	Type 2 Diabetes
326	**161**		**165**				**Total**

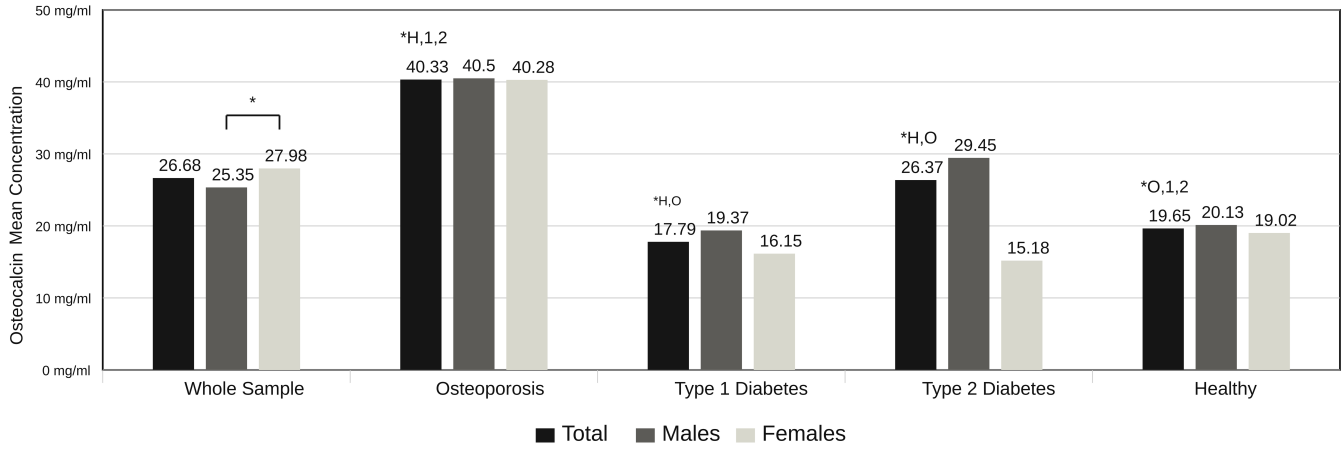

Fig. 1. Osteocalcin mean concentrations by gender in the study groups and the whole sample. Whole sample: 326 participants, 165 females and 161 males. Osteoporotic: 99 participants, 75 females and 24 males. Type 1 diabetics: 53 participants, 26 females and 27 males. Type 2 diabetics: 51 participants, 11 females and 40 males. Healthy: 123 participants, 53 females and 70 males. Osteocalcin was determined by chemiluminescence. Males had significantly lower mean concentrations than females in the whole sample ($p < 0.001$), with a weak correlation. Among study groups, osteoporotic had significantly higher mean concentrations than the other three groups ($p \leq 0.001$ in all cases) with very strong correlations, and the healthy had significantly lower mean concentrations than type 2 diabetics ($p = 0.002$) with a weak to moderate correlation, but higher than type 1 diabetics ($p \leq 0.001$), with a moderate correlation. Asterisks (*) indicate the statistically significant differences. H: Healthy, O: Osteoporosis, 1: Type 1 Diabetes, 2: Type 2 Diabetes.

The mean osteocalcin concentration in osteoporotic subjects was significantly higher than that in the other three study groups, with very strong correlations. The mean osteocalcin concentration was significantly higher in healthy controls than in type 1 diabetics, showing a moderate correlation. In type 2 diabetics, the concentration was lower, with a weak to moderate correlation. Mean concentrations did not differ significantly between type 1 and type 2 diabetics (Fig. 1).

For all constructed simple linear regression models, the model equations, p-values, and coefficients of determination (R^2) are summarized in Table 2. There was an inverse correlation between total calcidiol and osteocalcin concentrations ($p< 0.001$). The R^2 for this model was 0.171, indicating that 17.1% of the variation in osteocalcin concentration could be explained by total calcidiol concentration, reflecting a moderate correlation. The R^2 values for the other models presented in Table 2 were interpreted similarly.

There was a moderate inverse correlation between free calcidiol and osteocalcin concentrations ($p< 0.001$). Additionally, a weak inverse correlation was observed between calcitriol and osteocalcin concentrations ($p< 0.001$).

Table 2. Simple Linear Regression Models.

Correlations	Equations	p	R^2
Total calcidiol - osteocalcin	$\hat{Y} = 36.805 - 0.459x$	<0.001	0.171
Free calcidiol - osteocalcin	$\hat{Y} = 46.165 - 3.991x$	<0.001	0.204
Calcitriol - osteocalcin	$\hat{Y} = 32.537 - 0.169x$	<0.001	0.049
Total calcidiol - PTH	$\hat{Y} = 181.408 - 4.304x$	<0.001	**0.666**
Free calcidiol - PTH	$\hat{Y} = 229.400 - 29.615x$	<0.001	**0.498**
Total calcidiol - calcium	$\hat{Y} = 9.464 + 0.005x$	0.032	0.014
Free calcidiol - calcium	$\hat{Y} = 9.212 + 0.073x$	<0.001	0.046
PTH - calcium	$\hat{Y} = 15.643 - 0.043x$	<0.001	**0.489**
Total calcidiol - magnesium	$\hat{Y} = 1.784 + 0.006x$	<0.001	0.054
Free calcidiol and magnesium	$\hat{Y} = 1.766 + 0.031x$	0.005	0.024

R^2: Coefficient of determination. **Bold**: A strong or very strong correlation.

Parathyroid hormone

The mean PTH concentration was significantly lower in males across the entire sample, showing a moderate correlation (Fig. 2), the exact inverse of the pattern observed in the corresponding total calcidiol correlation (16).

The mean PTH concentration in osteoporotic individuals was significantly lower in males, with a very strong correlation, again the reverse of the results of total calcidiol (16). The concentration was not significantly different between genders in type 1 and type 2 diabetics, just as in the case of total calcidiol (16). However, it was significantly higher in healthy males, with a weak but not negligible correlation (Figure 2), whereas in total calcidiol there were no significant gender differences (16).

PTH levels were significantly elevated in osteoporotic individuals compared to the other three study groups, with strong correlations. Very strikingly, all the osteoporotic individuals in our study had hyperparathyroidism, probably secondary, as their total calcidiol status was very poor (16). The mean PTH concentration was significantly higher in type 1 and type 2 diabetics than in healthy controls, with strong correlations. The mean PTH concentration did not differ significantly between the two diabetic groups (Fig. 2). Total calcidiol and PTH were very strongly and inversely correlated ($p< 0.001$).

Our next model supported a strong inverse correlation between free calcidiol and PTH concentrations ($p< 0.001$).

Minerals

No significant differences in mean phosphate concentrations were found among the study groups (Fig. 3). However, significant differences in mean calcium concentrations were found among the groups in osteoporotic subjects, followed by healthy controls. The highest concentrations were found in the two diabetic groups, which did not differ significantly from each other.

Regarding magnesium, healthy controls had the highest mean concentration, followed by osteoporotic subjects and the two diabetic groups, which again showed no significant difference between them. All correlations were strong (Fig. 2).

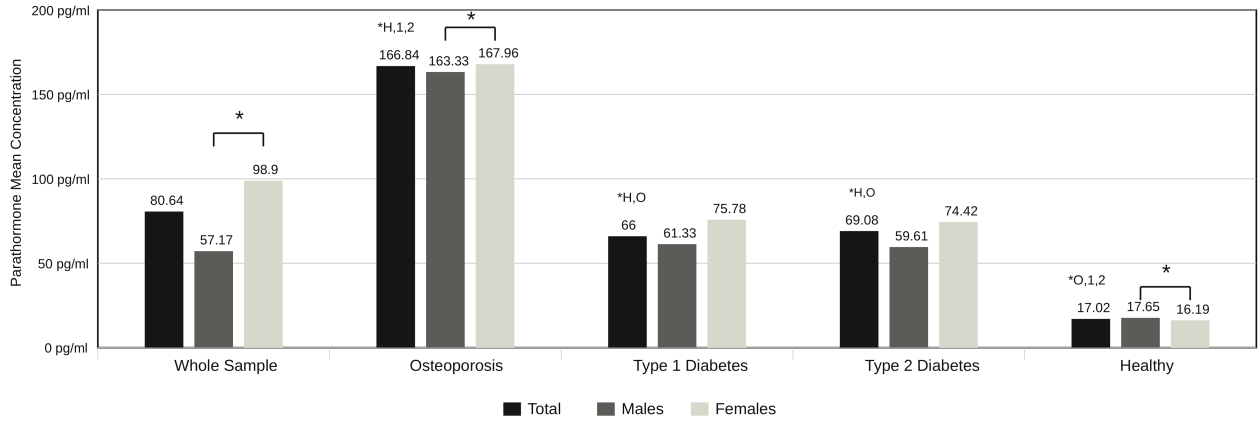

Fig. 2. Parathormone mean concentrations by gender in the study groups and the whole sample. Whole sample: 326 participants, 165 females and 161 males. Osteoporosis: 99 participants, 75 females and 24 males. Type 1 diabetes: 53 participants, 26 females and 27 males. Type 2 diabetes: 51 participants, 11 females and 40 males. Healthy: 123 participants, 53 females and 70 males. Parathormone was determined by chemiluminescence. Males had significantly lower mean concentrations than females in the whole sample ($p < 0.001$) with a moderate correlation, and in osteoporotic ($p \leq 0.001$) with a very strong correlation. Among study groups, osteoporotic had significantly higher mean concentrations than the other three groups ($p \leq 0.001$ in all cases) with very strong correlations, and the healthy had significantly lower mean concentrations than the two diabetic groups ($p \leq 0.001$ in both cases) with very strong correlations. Asterisks (*) indicate the statistically significant differences. H: Healthy, O: Osteoporosis, 1: Type 1 Diabetes, 2: Type 2 Diabetes.

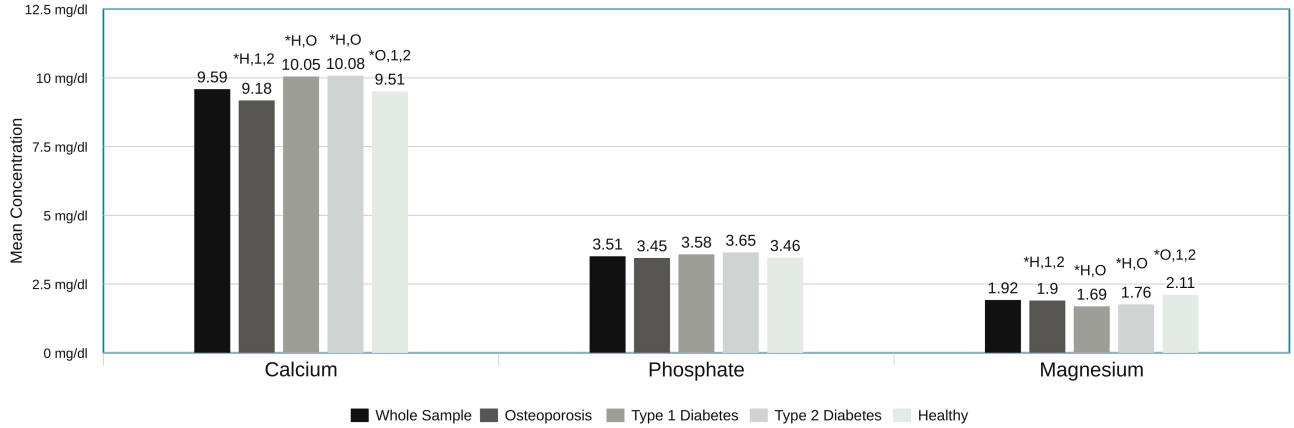

Fig. 3. Mean mineral concentrations in the study groups and the whole sample. Whole sample: 326 participants, 165 females and 161 males. Osteoporosis: 99 participants, 75 females and 24 males. Type 1 diabetes: 53 participants, 26 females and 27 males. Type 2 diabetes: 51 participants, 11 females and 40 males. Healthy: 123 participants, 53 females and 70 males. Calcium ions were determined by the CPC colourimetric method, Phosphate ions by the UV phosphomolybdate method, and Magnesium ions by the xylidyl blue colourimetric method. Calcium: Osteoporotic had a significantly lower mean concentration than all other study groups ($p \leq 0.001$ in all cases), with very strong correlations. The healthy group had a lower mean concentration than the two diabetic groups ($p \leq 0.001$ in both cases), with very strong correlations. Phosphate: No significant differences were found among the study groups. Magnesium: The healthy group had a higher mean concentration than all the other study groups ($p \leq 0.001$ in all cases), with very strong correlations. Osteoporotic had a significantly higher mean concentration than the two diabetic groups ($p \leq 0.001$ with type 1 diabetics, $p = 0.028$ with type 2 diabetics), also with very strong correlations.

Total calcidiol and calcium concentrations were very weakly correlated ($p = 0.032$). Free calcidiol and calcium concentrations were weakly correlated ($p< 0.001$). There was a strong inverse correlation between PTH and calcium concentrations ($p< 0.001$). Additionally, a weak correlation was observed between total calcidiol and magnesium concentrations ($p< 0.001$).

Finally, we observed a very weak correlation between free calcidiol and magnesium concentrations ($p = 0.005$).

For the remaining correlations, the construction of simple linear regression models was not feasible because all assumptions were violated, so we used the non-parametric Spearman's ρ test, since at least the monotonicity condition held. The data demonstrated that across the entire sample, there were no significant correlations between the concentrations of PTH and calcitriol ($p = 0.447$), calcitriol and calcium ($p = 0.387$), PTH and phosphate ($p = 0.387$), total calcidiol and phosphate ($p = 0.604$), free calcidiol and phosphate ($p = 0.747$), calcitriol and phosphate ($p = 0.403$), and calcitriol and magnesium ($p = 0.732$).

Discussion

Overall, the osteocalcin concentration showed a moderate inverse correlation with total and free calcidiol and a weak inverse correlation with calcitriol. The mean osteocalcin concentration was significantly higher in osteoporotic subjects and lower in patients with type 1 diabetes compared to the other study groups, and in the whole sample, it was significantly higher in females.

The weak effect size of this finding and the nonsignificant differences found in the individual study groups suggest that there are likely no significant differences in mean osteocalcin concentrations between genders.

The correlations of osteocalcin with total and free calcidiol are consistent with the findings of Lai et al. (18) and Demiaux et al. (19). Osteocalcin was the only variable that correlated with calcitriol in the present study (17). As this specific correlation has not been previously reported in the literature, it warrants further investigation.

The mean osteocalcin concentration in males in the present study (25.35 ng/ml) was higher than in males from Athens participating in a study published by Kassi et al. (18.32 ng/ml) (20), the only Greek study of vitamin D status to date that included osteocalcin measurements.

The significantly higher mean osteocalcin concentration in osteoporotic subjects was consistent with expectations, since in the previous literature osteocalcin was significantly higher in osteoporotic postmenopausal women compared to non-osteoporotic postmenopausal controls and has been suggested as a screening test for this group (21,22). Interestingly, in the present study, this conclusion is extended to men, who were not included in those previous studies.

The mean osteocalcin concentration in patients with type 1 diabetes was significantly and moderately lower compared with the healthy group. Osteocalcin regulates, among other things, glucose metabolism (3), rendering this an expected finding.

In patients with type 2 diabetes, this trend was reversed, as the mean osteocalcin concentration was significantly higher than in the healthy group, with a weak to moderate correlation. Hwang et al. reported that high levels of both cOC and ucOC correlate with increased glucose tolerance in type 2 diabetic subjects (23). It should be kept in mind that all diabetic patients were treated with insulin. In the study by Okazaki et al., serum osteocalcin levels in type 2 diabetics were low before and elevated after insulin treatment (2).

Overall, PTH showed strong correlations with total and free calcidiol, whereas there was no correlation with calcitriol. The mean PTH concentration was significantly higher in osteoporotic subjects and lower in the healthy controls, compared with the other study groups; furthermore, it was significantly higher in females than males.

The very strong inverse correlation of PTH with total calcidiol is consistent with previous literature (24). The same finding was observed in the study by Singhellakis et al., in males and females from Athens; however, in that case, the correlation was moderate (25). Most correlations of PTH concentration between genders and between study groups followed exactly the inverse pattern of total calcidiol (16), with similar strength of correlations, except for the healthy control group, as discussed below.

To our knowledge, the strong inverse correlation of PTH with free calcidiol has not been previously reported in the literature. However, this finding was expected, since both in present study (17) and previous literature (26), the concentrations of total and free calcidiol were strongly correlated.

The absence of correlation between PTH and calcitriol is an interesting finding, as it highlights why calcitriol is not considered a good and sensitive marker of the body's vitamin D status, as previously demonstrated. (17).

The significant difference in mean PTH concentration between genders in the healthy control group was unexpected, since such differences did not occur with total calcidiol, with which PTH generally exhibited the same, but inverted, pattern of correlations (16). However, the mean concentrations in both genders were within the normal range (8-76 pg/ml), suggesting that this finding probably has no clinical significance.

The mean PTH concentration in e males across the entire sample was more than twice as much as that measured by Kassi et al. in males from Athens (23.63 pg/ml) (20). However, the present study included a substantial proportion of patients with osteoporosis and diabetes who were assessed at the end of winter. In contrast, the study by Kassi et al. evaluated exclusively healthy males during autumn, a time point at which the mean total calcidiol concentration increases significantly. This seasonal variation has been demonstrated previously (16, 27), so lower PTH concentrations were expected in that study.

Overall, osteoporotic subjects had a significantly lower mean calcium concentration, and healthy subjects had a higher mean calcium concentration than the other study groups, with very strong correlations. Healthy controls and osteoporotic subjects had higher mean magnesium concentrations than diabetic patients, and no significant differences in mean phosphate concentrations were found between the study groups. Calcium had weak correlations with total and free calcidiol, a strong inverse correlation with PTH, and no correlation with calcitriol. Phosphate concentration was not correlated with any of the vitamin D metabolites. Magnesium had weak correlations with total and free calcidiol.

There are several reports in the literature of higher blood calcium concentrations in type 2 diabetic patients compared with healthy controls (28). Conversely, Rana et al. observed a significantly lower mean circulating calcium concentration in type 2 diabetic patients compared to healthy controls (29).

The effect of total calcidiol on calcium concentration could be considered negligible. However, total calcidiol levels have been previously correlated with blood calcium concentrations (30). On the other hand, Need et al. reported that calcium concentration does not correlate with total calcidiol concentration, except at concentrations <10 ng/ml (severe deficiency) (31).

The correlation between free calcidiol and calcium concentrations was again weak, but stronger than that between total calcidiol and calcium concentrations. This finding is consistent with the results reported by Schwartz et al. (32).

The absence of a correlation between calcitriol and calcium concentrations was expected, as Wishart et al. (33) reported that serum calcitriol concentration does not correlate with intestinal calcium uptake.

The correlation between PTH and calcium concentrations was consistent with expectations, as it has been reported in

previous studies (34,35). The strong effect size, unlike the weak correlation of total calcidiol, suggests that the minute-to-minute regulation of calcium concentration is primarily driven by PTH, rather than vitamin D (36).

The absence of significant differences in mean phosphate concentration between the study groups has not been previously reported in the literature, as this aspect has not been investigated for these specific health outcomes. The absence of correlations between phosphate and the various vitamin D metabolites was consistent with expectations, as the majority of the effects on renal phosphate excretion are mediated by FGF23 (37) and PTH (5), whereas most of its intestinal absorption occurs via a non-saturable component that is independent of calcitriol and the VDR (10,11).

Furthermore, PTH concentration was not correlated with phosphate. The complex interaction between vitamin D, PTH, and FGF23 (38) likely prevents such correlations from being easily detected.

Due to the synergistic effect of magnesium and vitamin D, it is expected that healthy controls would exhibit the highest levels of magnesium and total calcidiol. Notably, however, the mean concentrations across all study groups were within the normal range, and no participant presented with values outside this reference interval (1.5–2.5 mg/dl).

Osteoporotic subjects demonstrated a higher mean magnesium concentration, but a lower mean total calcidiol concentration compared with the two diabetic groups. It can be hypothesized that the higher mean magnesium concentration in osteoporotic versus diabetic patients is due to bone resorption, which releases skeletal magnesium content into circulation (39).

The present data demonstrated that circulating magnesium ion concentration correlated weakly with total calcidiol concentration.

However, a hierarchical multiple regression model showed that magnesium concentration was a confounder for total calcidiol concentration, a finding that has not been previously reported in related literature. The same could be assumed for the weak correlation found between magnesium and free calcidiol concentrations, for which no literature references from previous studies were identified.

Furthermore, no literature references were found regarding the absence of a correlation between magnesium and calcitriol.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Concept and design: C.A., and S.M.; Acquisition, analysis, and interpretation of data: C.A., S.M., and M.C.; Drafting of the manuscript: C.A., K.A.; Critical review and editing of the manuscript for important intellectual content: K.A., M.C., and S.M.; Supervision and project administration: T.L. All authors have read and agreed to the published version of the manuscript.

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