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Vitamin D Binding Protein (DBP), Free Calcidiol, and Total Calcitriol in Adults from Northern Greece

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Abstract

Background: An ongoing debate has been raised on whether is better to use total or free calcidiol as a screening test in the population.

Methods: In winter and summer, free calcidiol, total calcitriol, and vitamin D binding protein (DBP) concentrations were determined by immunoenzymatic assays in 326 adults (161 males, 165 females). These included 99 osteoporotic patients, 53 type 1 and 51 type 2 diabetics, and 123 athletic healthy persons, all from northern Greece.

Results: In the whole sample, free calcidiol mean concentrations differed significantly ($p < 0.001$) between males (5.53 pg/ml) and females (4.68 pg/ml). Free calcidiol was significantly greater in the athletic healthy group (6.02 pg/ml) than in the three patient groups, and lowest in the osteoporosis group (3.69 pg/ml). Total calcitriol mean concentration did not differ significantly between genders in the whole sample ($p = 0.896$) or in the study groups, except for type 2 diabetics (males 38.33 pg/ml, females 54.52 pg/ml, $p = 0.001$). It was significantly less in the osteoporotics (34.61 pg/ml) than in the athletic healthy group (41.65 pg/ml, $p = 0.037$) and type 1 diabetics (43.73 pg/ml, $p = 0.030$), whereas it did not differ significantly between the other study groups. The DBP mean concentrations were not significantly different between genders in the whole sample and the study groups nor among the study groups ($p = 0.467$).

Conclusions: Comparisons with our previously reported results of total calcidiol suggest the measurement of free calcidiol offers nothing more than that, and total calcitriol is not a sensitive measure for assessing vitamin D status.

Keywords: Calcidiol, Calcitriol, Vitamin D, Vitamin D Binding Protein.

Introduction

Vitamin D_3 (cholecalciferol), photosynthesized in the epidermis or ingested and absorbed from the intestine, is metabolized in the liver where it is converted into 25-hydroxyvitamin D (calcidiol), the main vitamin D metabolite found in the bloodstream, by the action of various cytochromes that play the role of 25-hydroxylase (1). Further, in the kidneys and many extrarenal tissues, it is converted by the action of the 1a-hydroxylase, to 1,25 dihydroxyvitamin D (calcitriol), its biologically active metabolite, considered to be a secosteroid hormone (2).

Total serum calcidiol (25-hydroxyvitamin D) is considered a measure of vitamin D status. However, an ongoing debate has been raised as to whether total or free calcidiol levels are accurate and representative indicators of vitamin D status (3).

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The activity of 1a-hydroxylase is tightly regulated in the kidneys to produce calcitriol (1,25-hydroxyvitamin D), which directs the endocrine functions of vitamin D in the body. However, the extrarenal endocrine and paracrine mechanisms responsible for some of the actions of vitamin D change the picture (4). Thus, serum total calcitriol concentration is not considered a valid indicator of vitamin D action, since it probably does not reflect total calcitriol levels in extrarenal tissues and cells, nor the overall status of vitamin D in the body (3).

Vitamin D binding protein (DBP) transports the vast majority of the various vitamin D family metabolites into the bloodstream. Calcidiol binds to DBP with high affinity (5). A smaller, but significant, fraction of calcidiol in circulation is bound to albumin, but with much lower affinity than DBP, and this complex is called bioavailable calcidiol. Finally, a minimal amount of calcidiol, 0.03%, remains free, i.e., not bound to any protein (3). The same partitioning, bound to DBP, bound to albumin and free, also applies to calcitriol, although the relative percentages are slightly different due to the different affinity constants of calcitriol binding to the corresponding proteins (6).

The clinical utility of measuring free/bioavailable calcidiol has not yet been adequately established. The usefulness of this depends on the ability of free calcidiol to yield information that total calcidiol measurement does not already provide (3).

Our research, of which this study is a part, aims to assess vitamin D status in adults from northern Greece, by measuring free calcidiol and total calcitriol levels in late winter and late summer in athletic healthy individuals and patients with osteoporosis and types 1 and 2 diabetes. The present study also aims to investigate the association of DBP with vitamin D status and assess free calcidiol and total calcitriol and compare them with total calcidiol levels.

Materials and Methods

Subjects

326 people in total were recruited for this study. These included 161 males (49.4%) and 165 females (50.6%) with an average age of 49.8 ± 13.5 years and an age range of 20–77 years. Of these, 99 individuals, 24 males (24.2%) and 75 females (75.8%), 56.3 ± 11 years old were osteoporotic patients; 53 individuals, 27 males (50.9%) and 26 females (49.1%), 36.7 ± 8.8 years were type 1 diabetics (T1D) and 51 individuals, 40 males (78.4%) and 11 females (21.6%), 58.9 ± 7 years were type 2 diabetics (T2D). These participants formed the patient study groups. 123 more individuals, 70 males (56.9%) and 53 females (43.1%), 46.4 ± 13.3 years, were healthy and participated regularly in outdoor sports, forming the athletic healthy (AH) group. The participants, both in the whole sample and in study groups, were divided into young (20–39 years), middle-aged (40–59 years), and elderly (≥ 60 years) subgroups. Details of the study groups, including the age subgroups, have been previously published (7). The individuals of each group were not matched by gender and age.

All participants donated one serum sample each from 15 to 31 March 2018 (late winter), from 8 to 10 am, and the samples were stored frozen (-70 °C) until analysed. 106 of them (42.5% males, 57.5% females) donated a second serum sample from 20 to 30 September 2018 (late summer). Free calcidiol, total calcitriol, and DBP concentrations were measured in the second sample, and those 106 subjects were divided by osteoporosis (35 participants), diabetes (20 participants), and athletic healthy (51 participants). Demographic and other characteristics, as well as the exclusion criteria by study group, have been previously reported (7).

Further, biochemical markers in all samples were measured with automated methods in the "Bioanalysis" diagnostic laboratory, which is certified by the Hellenic Accreditation System (ESYD) with ISO 9001:2008.

Measurement of FSH, LH, and total estrogen concentrations, using a chemiluminescence method on the Elecsys immunoanalyser (Roche Diagnostics, Germany), confirmed that 16 of the 75 osteoporotic women were pre-, while the remaining 59 were post-, menopausal.

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Each participant completed a questionnaire including information such as gender, age, underlying diseases, diabetes type, medical history, and medications. In addition, osteoporotic patients reported their bone mass as previously measured.

At the Clinical Chemistry Laboratory, Faculty of Biomedical Sciences, IHU, we measured free calcidiol, total calcitriol, and DBP concentrations, using commercial immunoenzymatic kits: Free 25OH Vitamin D ELISA (Immuno-Biological Laboratories Inc., USA) for free calcidiol, 1,25$(OH)_2$ Vitamin D ELISA (DIAsource ImmunoAssays, Belgium) for total calcitriol, and Human Vitamin DBP Immunoassay Quantikine® ELISA (China R&D Systems Ltd., China) for DBP, according to the manufacturer's instructions. The ELISA for the determination of DBP was based on the use of polyclonal antibodies, so it is unbiased towards DBP isotypes (3).

Statistics were analyzed using IBM SPSS Statistics, version 23 (IBM, NY), as previously reported (7). As a threshold for statistical significance, we considered a probability level of $\alpha = 0.05$, in all cases.

Ethics: Each participant was thoroughly informed of all matters relating to their participation in this research and provided written informed consent. The Democritus University of Thrace's Committee of Ethics and Deontology in Research reviewed and approved the protocol by document number Θ24/Δ.Σ7/28.2.2081.

Results

Vitamin D metabolites

The results of total calcidiol measurements have been previously reported and discussed in detail (7).

First measurement (late winter)

Genders: The correlations of free calcidiol and total calcitriol mean concentrations by gender in the whole sample and the study groups in late winter are summarized in Figures 1 and 2, respectively. The correlations of the vitamin D metabolite concentrations in the study groups in late winter are also shown, using an asterisk for every statistically significant correlation found for the total of participants among each study group, also between males and females within each study group.

Regarding the differences in mean concentrations between genders in the whole sample, the results for free calcidiol (Fig. 1) were the same as those for total calcidiol, with the same effect size, whereas the mean concentrations of total calcitriol did not differ significantly (Fig. 2).

Concerning the study groups, the results of free and total calcidiol between genders in osteoporotics were similar, but the association of free calcidiol was much weaker. Total calcitriol showed no difference between genders in osteoporotics. In the three other study groups, the results of free calcidiol and total calcitriol between genders were the same as those of total calcidiol, since in all cases no significant differences were found here, with the sole exception of the calcitriol in T2D (Fig. 2).

Study Groups: The difference in mean free calcidiol concentration between osteoporotics and the AH group was consistent with that of total calcidiol, whereas between T1D and AH, the association was weaker in the case of free calcidiol (Fig. 1). On the contrary, between AH and T2D, the association was stronger in free calcidiol, versus total calcidiol. However, here we conducted Welch's ANOVA, which yields a single effect size result for all correlations, whereas in the case of total calcidiol, we conducted individual Mann-Whitney U analyses, with separate calculation of the effect size in each analysis. Between osteoporotics and the two diabetic groups, as well as between the latter two study groups, the free calcidiol results agreed with the corresponding total calcidiol analysis.

The results of total calcitriol were not consistent with those of either total or free calcidiol, since only osteoporotics had a significantly lower mean concentration than T1D and AH subjects, with weak correlations, and no other significant differences appeared (Fig. 2).

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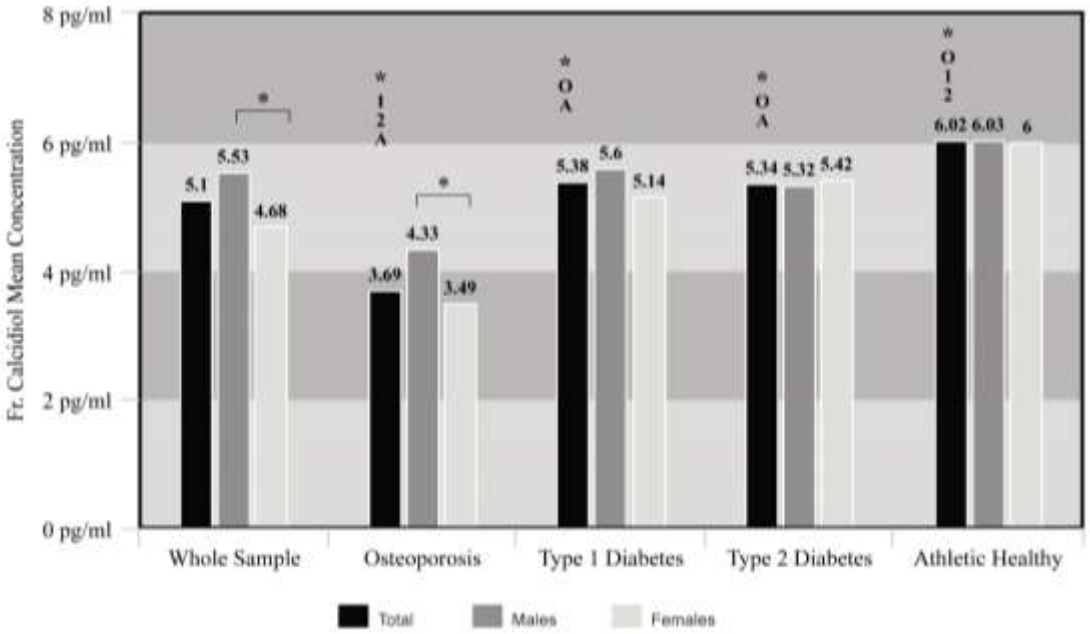

Fig. 1. Mean concentrations of free calcidiol in the whole sample and the study groups in late winter, in total and by gender. Whole sample: 326 individuals, 161 males and 165 females. Osteoporosis: 99 individuals, 24 males and 75 females. Type 1 diabetes: 53 individuals, 27 males and 26 females. Type 2 diabetes: 51 individuals, 40 males and 11 females. Athletic healthy: 123 individuals, 70 males and 53 females. Free calcidiol was measured by ELISA. Males had significantly greater mean concentration than females in whole sample ($p < 0.001$) and osteoporosis ($p = 0.030$), with mild correlations. In total, osteoporosis had a significantly lower mean concentration than type 1 & 2 diabetes, and athletic healthy ($p < 0.001$ in all cases), with very strong correlations. Type 1 & 2 diabetes had a significantly lower mean concentration than athletic healthy ($p = 0.017$, $p = 0.011$), with very strong correlations. *: A statistically significant difference. O: Osteoporosis, 1: Type 1 diabetes, 2: Type 2 diabetes, A: Athletic healthy.

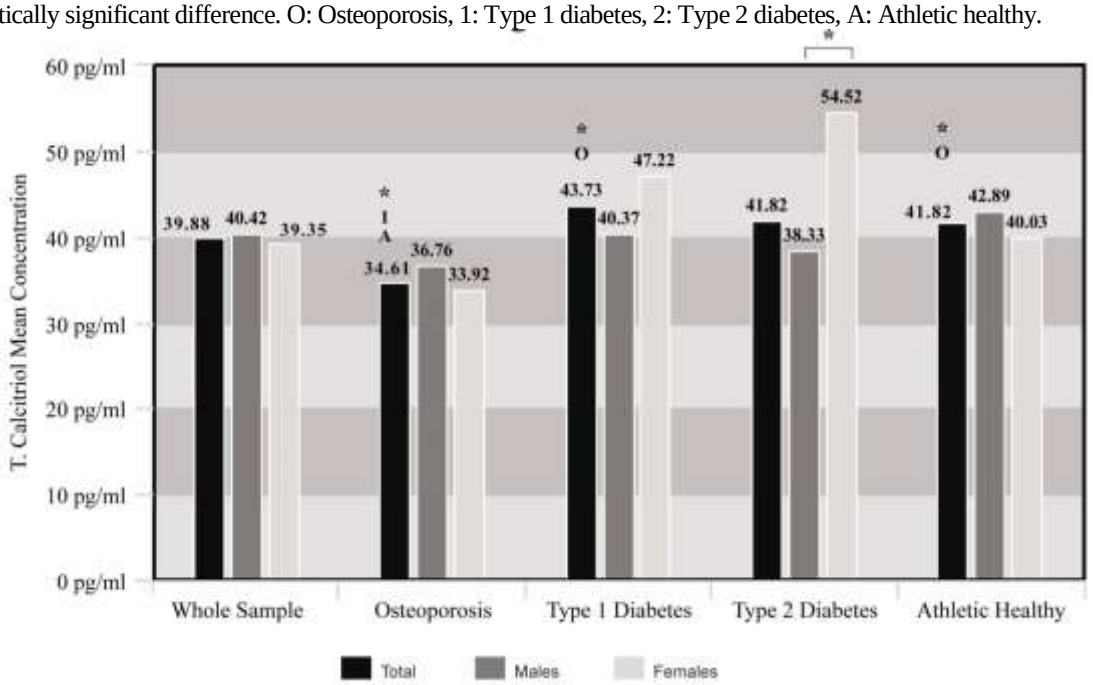

Fig. 2. Mean concentrations of total calcitriol in the whole sample and the study groups in late winter, in total and by gender. Whole sample: 326 individuals, 161 males and 165 females. Osteoporosis: 99 individuals, 24 males and 75 females. Type 1 diabetes: 53 individuals, 27 males and 26 females. Type 2 diabetes: 51 individuals, 40 males and 11 females. Athletic healthy: 123 individuals, 70 males and 53 females. Total calcitriol was measured by ELISA. In type 2 diabetes, females had a significantly greater mean concentration than males ($p < 0.001$) with a strong correlation. In total, osteoporotics had a significantly lower concentration than type 1 diabetics ($p = 0.030$) and athletic healthy ($p = 0.037$) with weak correlations. *: A statistically significant difference. O: Osteoporosis, 1: Type 1 diabetes, A: Athletic healthy.

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Age subgroups

Mean free calcidiol and total calcitriol concentrations, and correlations among age subgroups in the whole sample and study groups are summarized in Figures 3 and 4, respectively.

In the whole sample, the results of free calcidiol were not entirely consistent with those of total calcidiol (7), as the size effect was less, and no difference was seen between the middle-aged and elderly subgroups (Fig. 3).

In osteoporotics, the results of free calcidiol agreed with the corresponding correlations of total calcidiol, since no differences between the age subgroups appeared.

In T1D, the results of free calcidiol were similar to those of total calcidiol, but with weaker correlations.

The difference in mean free calcidiol concentration between the middle-aged and elderly subgroups was not significant in the T2D group (Fig. 3), whereas the corresponding analysis of total calcidiol showed a significant difference with a mild correlation between these two age subgroups (7).

In AH subjects, the free calcidiol results were not consistent with those of total calcidiol, since no significant differences between age subgroups were seen here.

The total calcitriol means concentrations did not differ between age subgroups in the whole sample and in the study groups, except for T2D, where the elderly had a significantly greater mean concentration than the middle-aged with a mild to strong correlation (Fig. 4).

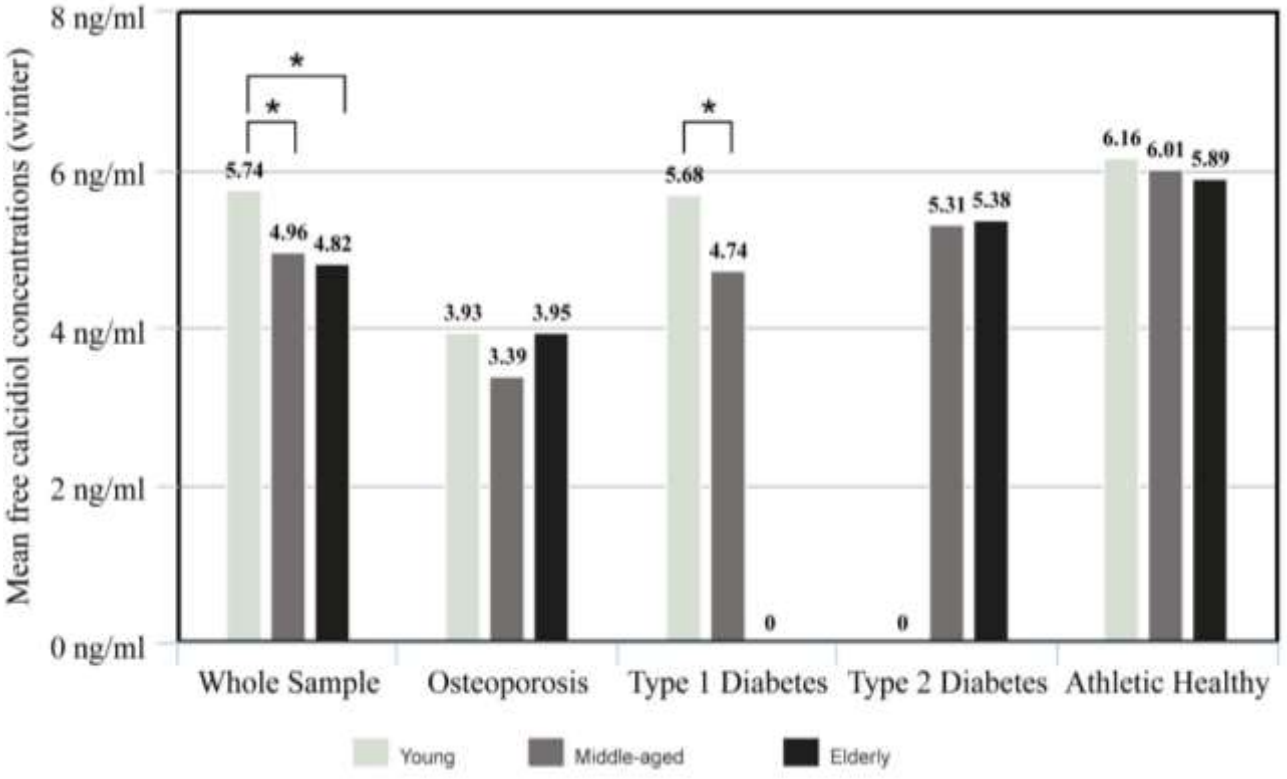

Fig. 3. Mean free calcidiol concentrations and the correlations among age subgroups in the whole sample and study groups. Whole sample: Young = 76, middle-aged = 156, elderly = 94. Osteoporosis: Young = 7, middle-aged = 46, elderly = 46. Type 1 diabetes: Young = 34, middle-aged = 18, elderly = 1 (mean concentration not applicable). Type 2 diabetes: Young = 0, middle-aged = 28, elderly = 23. Athletic healthy: Young = 35, middle-aged = 64, elderly = 24. Free calcidiol was measured by ELISA. In the whole sample, the young had a significantly greater mean concentration than the middle-aged ($p = 0.004$) and the elderly ($p < 0{,}001$) with mild correlations. In type 1 diabetes, the young had a significantly greater mean concentration than the middle-aged ($p = 0.004$) with a strong correlation *: A statistically significant difference.

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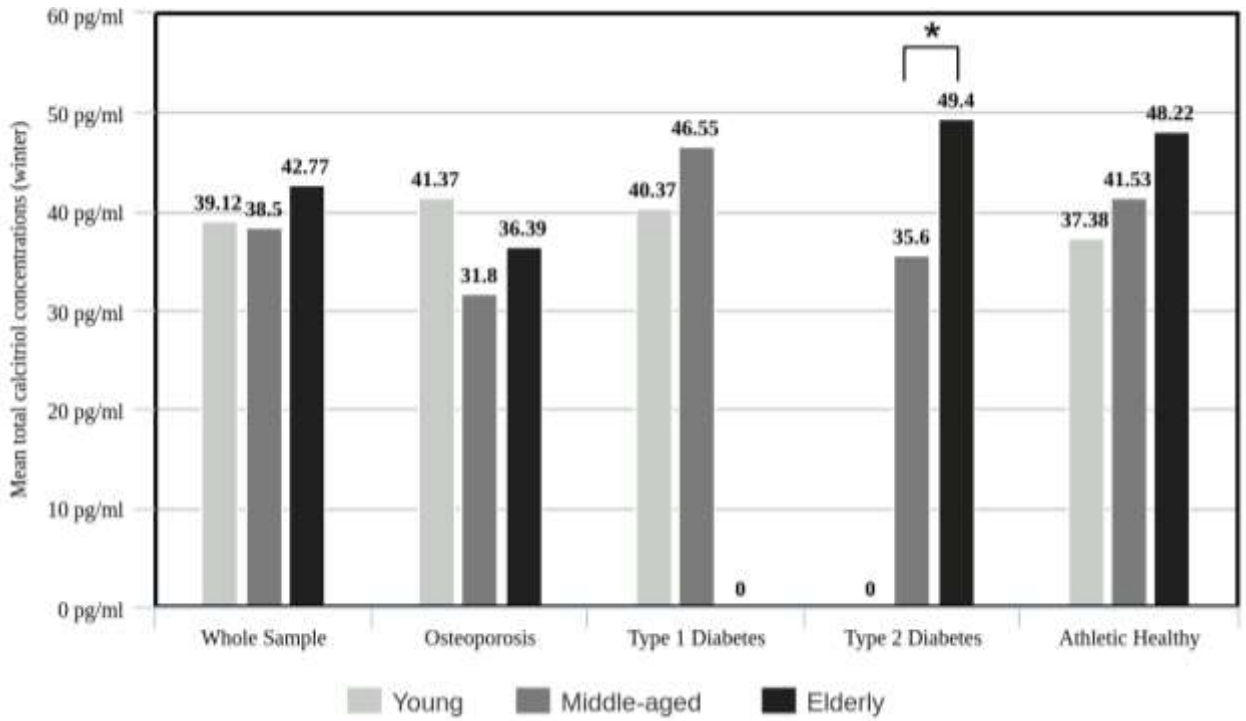

Fig. 4. Mean total calcitriol concentrations among age subgroups in the whole sample and study groups. Whole sample: Young = 76, middle-aged = 156, elderly = 94. Osteoporosis: Young = 7, middle-aged = 46, elderly = 46. Type 1 diabetes: Young = 34, middle-aged = 18, elderly = 1 (mean concentration not applicable). Type 2 diabetes: Young = 0, middle-aged = 28, elderly = 23. Athletic healthy: Young = 35, middle-aged = 64, elderly = 24. Total calcitriol was measured by ELISA. In type 2 diabetics, the elderly had a significantly greater mean concentration than the middle-aged ($p = 0.006$) with a mild to strong correlation. No other correlations were found.

Correlations between vitamin D metabolites: The simple linear regression model we constructed supported that total calcidiol concentration was very strongly correlated with, and to some extent predicts, free calcidiol concentration ($p < 0.001$). The regression equation was: $\hat{Y} = 2.738 + 0.99x$. The R^2 for this equation was 0.617, i.e., 61.7% of the variation in free calcidiol concentration could be explained by the total calcidiol concentration.

To investigate the correlation between total calcidiol and total calcitriol, we did not attempt to construct a simple linear regression model, since all assumptions were violated; therefore, we resorted to the non-parametric Spearman's ρ test, as the scatterplot showed that the monotonicity condition held. The two variables showed no statistically significant correlation ($p = 0.416$). The same was observed between free calcidiol and total calcitriol ($p = 0.956$).

Second measurement (late summer)

The correlations of free calcidiol and total calcitriol mean concentrations in late winter and summer in the 106 subjects with a second measurement are presented in Figure 5.

One unexpected finding was the significantly less free calcidiol concentration in osteoporotics in late summer than in late winter, with a strong correlation, while in diabetics and AH subjects, free calcidiol concentrations were significantly greater late summer than in late winter, with mild and strong correlations, respectively. The mean total calcitriol concentration in osteoporotics was significantly greater in summer than in winter, although the correlation was marginal ($p = 0.049$) and weak, and in diabetic patients it was also significantly greater but with a strong correlation. Noteworthy is that total calcitriol mean concentration in AH subjects was significantly less during the summer, with a strong correlation.

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Welch's ANOVA and Games-Howell Post Hoc tests were used to compare the mean concentrations of total calcitriol in late summer between the three study groups, as the condition of homogeneity of variance was violated. Differences were significant ($p < 0.001$). The mean total calcitriol concentration in late summer was significantly less in AH subjects than in osteoporotics ($p = 0.005$) and diabetics ($p < 0.001$), while it was not significantly different between diabetics and osteoporotics. The effect size parameter was $\eta^2_p = 0.281$, i.e., the correlations were strong.

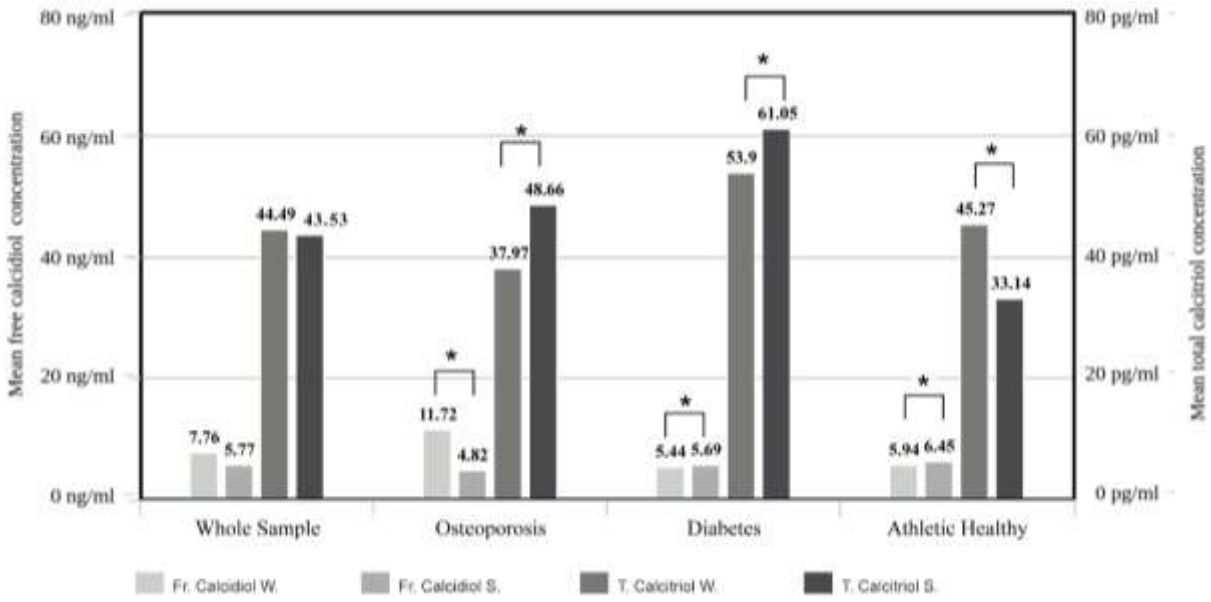

Fig. 5. Free calcidiol and total calcitriol mean concentrations and their correlations in late winter and late summer among the whole of subjects with a second measurement and the study groups. Whole sample: 106 individuals. Osteoporosis: 35 individuals. Diabetes: 20 individuals. Athletic healthy: 51 individuals. Free calcidiol and total calcitriol were measured by ELISA. In osteoporotics, free calcidiol concentration was significantly greater in the winter ($p < 0.001$) with a strong correlation, whereas total calcitriol concentration was significantly greater in the summer ($p = 0.049$) with a weak correlation. In diabetics, both free calcidiol and total calcitriol was significantly greater in summer ($p = 0.005$ with a mild correlation, and $p < 0.001$ with a strong correlation). In athletic healthy subjects, free calcidiol was significantly greater in summer ($p < 0.001$) with a strong correlation, whereas total calcitriol was significantly lower in summer ($p < 0.001$) with a strong correlation. *: A statistically significant difference. W: Winder. S: Summer.

DBP

1st measurement (late winter)

The DBP mean concentrations in the whole sample and study groups, in total and by gender in late winter are summarized in Figure 6. No significant differences were seen between genders or study groups.

Correlations of DBP with vitamin D metabolites: The simple linear regression models we constructed demonstrated that the correlation between DBP and total calcidiol concentrations was significant ($p = 0.007$). The regression equation was $\hat{Y} = 17.848 + 0.02x$. The R^2 for this equation was 0.022, i.e., only 2.2% of the variation in total calcidiol concentration could be explained by the DBP concentration, a very weak correlation.

The correlation between DBP and free calcidiol concentrations was significant ($p = 0.002$). The regression equation was $\hat{Y} = 4.237 + 0.03x$. The R^2 for this equation was 0.029, i.e., only 2.9% of the variation in total calcidiol concentration could be explained by DBP concentration, with the correlation being very weak. To investigate the correlation between DBP and total calcitriol, we used the non-parametric Spearman's ρ test for the same reasons as above. No statistically significant correlation was observed ($p = 0.055$).

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2nd measurement (late summer)

DBP mean concentrations and their correlations in late winter and late summer among the whole of subjects with a second measurement and the study groups are summarized in Figure 7. To assess the differences in DBP concentration in the total of subjects with second measurement and individual groups between winter and summer, the non-parametric Wilcoxon Signed Rank test was performed, as the conditions of parametric Mixed Design ANOVA and repeated-measures t-test were violated. To assess the effect size, the parameter r was calculated. Mean DBP concentrations differed significantly with strong correlations between winter and summer in both the whole sample and study groups.

Welch's ANOVA and Games-Howell Post Hoc tests were used to compare the mean late summer DBP concentrations between the three study groups. The mean DBP concentration was not significantly different between AH subjects and osteoporotics ($p = 0.628$); however, DBP concentrations in both were significantly less than in diabetics ($p = 0.001$). The effect size parameter was $\eta^2_p = 0.310$, i.e., the correlations were strong.

No significant differences between pre- and post-menopausal females were observed in any of the parameters tested in the study.

Discussion

Overall, we observed that although a strong correlation was seen between total and free calcidiol concentrations, consistent with the literature to date (8,9), the analyses of free calcidiol in no case revealed a correlation that the analyses of total calcidiol concentrations failed to reveal. On the contrary, at best, they gave the same results, but usually with lower effect size, while at worst they failed to reveal some correlations, some of them well documented in the literature, which the analyses of total calcidiol concentrations revealed, such as the deterioration of vitamin D status with increasing age (10,11). In the few cases where the effect size appeared stronger in free than in total calcidiol, this could be attributed to the different characteristics of the statistical methods used in each case. We conclude, therefore, that the measurement of free calcidiol offers nothing more than that of total calcidiol; on the contrary, the latter is more sensitive and robust.

Indeed, several studies failed to show any difference between the association of total and free calcidiol or showed a stronger association of total calcidiol with various health outcomes (12–17).

The decrease in mean free calcidiol concentration in osteoporotics in late summer is remarkable, since the increase in mean total calcidiol concentration in this group, in the subjects with a second measurement, was significant, with a strong correlation (7). This is presumably because the concentrations of both total and free calcidiol during the winter in osteoporotics were so low that when their levels were raised due to increased sun exposure, they began to produce calcitriol, enter homeostasis, and repair some musculoskeletal defects that had accumulated during the winter. Thus, free calcidiol was consumed continuously, so that in the second measurement significantly lower values were found. This hypothesis is supported by the results of total calcitriol in late summer, as indeed the mean concentration in osteoporotics was significantly greater than in AH subjects. In previous literature it has been reported that vitamin D supplementation in rachitic children increases serum total calcitriol to high levels, indicating strong replenishment to rectify the effects of vitamin D deficiency (18,19), similar to what was seen here.

Regarding total calcitriol, significant differences with a mild correlation were found between total calcitriol concentrations of middle-aged and elderly T2D, whereas the exact opposite was observed in the mean concentrations of total calcidiol (7), while the mean free calcidiol concentrations in T2Ds did not differ significantly between these age subgroups. This study was not designed to investigate in depth the physiological differences that are likely to underlie this finding and further research on this is required.

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According to our data, total calcidiol concentration did not correlate with or predict total calcitriol concentration, contrary to the study of Singhellakis et al., who found a mild correlation (20). However, Need et al. reported that total calcitriol concentration did not correlate with total calcidiol, except at total calcidiol concentrations of <10 ng/ml (21).

Free calcidiol and total calcitriol concentrations in the whole of the participants with a second measurement did not increase from late winter to late summer, contrary to what was observed for total calcidiol concentration. The strong drop in mean free calcidiol concentration in the osteoporotics, combined with the results of the other two groups, probably accounts for the overall, although not significant, decrease in free calcidiol during the summer in the whole of subjects with a second measurement.

About total calcitriol, this finding is probably because the regulation of calcitriol production, exerted at the level of the CYP27B1 and CYP24A1 genes, is so detailed and the control so tight (4,22) that an increase in total calcidiol does not automatically cause an increase in calcitriol production. Indeed, Li et al., in a fairly large population sample of 1,066 men with prostate cancer, found no increase in serum total calcitriol in summer (23). On the contrary, Singhellakis et al. observed in 625 healthy men and women a significant increase in total calcitriol concentration in late summer (20).

One of the most striking findings of the present study is the significant decrease in mean total calcitriol concentration in summer in the AH subjects with a second measurement, while the reverse result was found in osteoporotics and diabetics.

In the AH subjects, who have adequate total calcidiol levels throughout the year, the body presumably chooses to reduce total calcitriol levels, always within normal limits, to mitigate any risk of side effects from the highly metabolically active hormonal form of vitamin D. This is a very interesting finding, as is the above hypothesis, but further study is needed to confirm it.

In general, in most cases, total calcitriol concentration did not differ significantly between study groups, genders, and age subgroups, with few exceptions, discussed above. Therefore, our data confirmed that total calcitriol measurement is neither a sensitive nor accurate indicator of vitamin D status, and its measurement does not provide important information at this level.

The DBP mean concentrations in late winter did not significantly differ between genders. In previous studies (24,25), was been found that females have significantly greater DBP concentrations than males, something that was not verified in the present study. However, one of these works (24) studied only subjects with T1D and healthy controls.

The mean DBP concentration was significantly greater in summer than in winter with a strong correlation, both in all participants with a second measurement and in the respective study groups, something also mentioned in previous literature (26).

The mean DBP concentration in late summer was significantly greater in diabetics than in the other two study groups, whereas it was not significantly different between osteoporotics and AH subjects. Given that in winter the mean DBP concentrations did not differ significantly between study groups, it seems that the mean DBP concentration increases especially in diabetics in summer, something we have not found in previous literature.

Our data supported that the concentration of DBP correlates with and to some extent predicts the concentration of both total and free calcidiol in the circulation; however, the correlations were very weak, which could mean that these correlations were possibly type 2 errors (false positive). Nonetheless, when constructing a multiple linear regression model involving several variables from our study, which we have not yet presented, we confirmed the contribution of DBP concentration in determining the total calcidiol concentration. According to that model, a one-unit increase in DBP concentration, with all other variables held constant, would cause the

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total calcidiol concentration to increase by 0.133 units (data not shown), a contribution considerably stronger than the one we found with the simple linear regression model. Therefore, we conclude that the contribution of DBP to the partial determination of total calcidiol concentration is a valid finding. This contrasts with those of Lauridsen et al. (27) and Blanton et al. (24), who found no correlation between these two variables.

Regarding the weak correlation of DBP concentration with that of free calcidiol, we found no relevant reports in the previous literature, but it is nevertheless expected, since the concentrations of total and free calcidiol showed a very strong correlation to each other, as aforementioned.

The concentration of DBP does not correlate with the concentration of total calcitriol in the circulation. This contradicts the finding of Lauridsen et al., according to which DBP concentration is an independent predictor of total calcitriol concentration, even after correcting for various other factors such as smoking, sun exposure, supplementation, etc. (27). This could be because in that study the number of participants was significantly larger and more homogeneous in terms of gender (595 women) and in terms of age and underlying diseases (osteoporotic, immediately after menopause).

No significant difference was found between pre- and post-menopausal women in all the parameters analyzed in this study, in agreement with the results of Abdulmahdi Mokif et al (28).

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Conflict of interests

None.

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