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ORIGINAL ARTICLE

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Vitamin D deficiency in the elderly in Athens, Greece

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Abstract Vitamin D deficiency characterized by low 25-hydroxyvitamin D [25(OH)D] levels has been found to be prevalent among the elderly in many regions of the world. To investigate the vitamin status in elderly community-living persons in Athens, we measured 25(OH)D and parathyroid hormone (PTH) in elderly persons and young blood donors during the winter and summer. The changes in these parameters in a subgroup of the elderly were studied longitudinally. The blood donors had mean 25(OH)D levels similar in winter and summer and twice as high in winter compared to the elderly. At the end of the winter, about 20% of the elderly had severe vitamin D deficiency, with 25(OH)D below 25 nmol/l, and only 6.5% could be judged as vitamin D sufficient with values above 80 nmol/l. The situation improved during summer, although 64.8% of the elderly continued to have levels below 80 nmol/l. Mean plasma PTH in the elderly in summer was not different from that of blood donors; however, it was doubled during the winter. Regression of PTH on 25(OH)D demonstrated that PTH starts to rise when 25(OH)D falls below approximately 80 nmol/l. We conclude that severe vitamin deficiency associated with secondary hyperparathyroidism is not uncommon in the elderly in Athens during the winter; it subsides during summer, although only one-third of the elderly population attain vitamin D sufficiency during summer. We found that a threshold value of 25(OH)D exists at approximately 80 nmol/l, below which secondary hyperparathyroidism ensues, as described previously.

Key words vitamin D deficiency · elderly · threshold value of 25(OH)D · secondary hyperparathyroidism

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Introduction

The criteria for the diagnosis of vitamin D deficiency based on serum 25-hydroxyvitamin D [25(OH)D] levels have been revised recently, as discussed by Heaney [1] and Holick [2]. Three forms or grades of bone disease associated with vitamin D deficiency are recognized [1,3]. Severe vitamin D deficiency characterized by serum 25(OH)D <20–25 nmol/l causes clinical osteomalacia. Levels of 25(OH)D between 20 and 80 nmol/l may be associated with secondary hyperparathyroidism, osteoporosis, and increased risk for hip fracture. Levels of serum 25(OH)D above 80 nmol/l are considered to be sufficient to protect from bone disease caused by vitamin deficiency [1] and result in maximal intestinal absorption of calcium [4]. An inverse relationship between parathyroid hormone (PTH) and 25(OH)D in the elderly has been reported by several authors [5–9]. The 80 nmol/l value of serum 25(OH)D has been proposed and adopted as a threshold level because it was demonstrated by Chapuy et al. [5] that when 25(OH)D falls below 78 nmol/l plasma PTH starts to rise and secondary hyperparathyroidism ensues. However, the existence of such a threshold has been disputed by Vieth et al. [10]. During recent years, using the criteria mentioned above, it has been reported that vitamin D deficiency is common among the elderly in many regions of the world [11,12].

In 2003, we measured serum 25(OH)D and PTH in independent community-living elderly residents of Athens, Greece (latitude 38° N) during the winter and summer. We found that during the winter about 20% of the elderly had serum 25(OH)D in the osteomalacia range (<25 nmol/l) and only about 7% had values above 80 nmol/l. The situation improved during the summer. The vitamin D deficiency we observed was associated with abnormally high PTH levels during the winter. Regression of PTH on 25(OH)D values using four different regression models confirmed the existence of a threshold level of 25(OH)D at approximately 80 nmol/l, as reported by Chapuy et al. [5].

Methods and materials

Subjects

The first group of the population of this study consisted of 231 independent community-living and apparently healthy women aged 60–89 years (median age, 71 years) and 48 men aged 64–86 years (median age, 72 years). A fasting blood sample was obtained at about 9 AM in late winter season (between February 15 and March 15; winter group). From 56 subjects (51 women and 5 men) randomly selected among the elderly persons who were studied in winter, a blood specimen was also obtained during the next summer (August 15 to September 15; summer group). These subjects were examined in the municipal recreation centers for the elderly of Athens during their regular daytime visits and after their informed consent was obtained. A blood sample was also obtained in our hospital from 44 blood donors (29 men and 15 women) aged 19–46 years (median, 34) during the winter, and from another group of 30 blood donors (25 men and 5 women) aged 25–48 years (median, 38) during the summer, after their informed consent. Exclusion criteria were any current treatment or treatment during the past 5 years for osteoporosis, as well as treatment with vitamin D supplements of any kind or drugs known to affect calcium metabolism. Patients with primary hyperparathyroidism were excluded, and only euthyroid individuals and those with serum creatinine not higher than 1.5 mg/dl were included in the study. The calcium intake of the elderly persons of this study was estimated to vary between 400 and 700 mg daily and that of the blood donors between 400 and 900 mg daily; this estimation was based on the consumption of diary products. The study was approved by the Scientific Committee of the Hospital.

Methods

Parathyroid hormone (PTH) was determined using the Nichols Advantage Bio-Intact PTH (1–84) two-site immunochemiluminometric assay. This assay detects only the bioactive PTH (1–84) molecule, excluding PTH fragments (7–84) or smaller, with a sensitivity of 0.16 pmol/l. 25(OH)D was measured with a liquid-phase radioimmunoassay (25-Hydroxyvitamin D RIA; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The sensitivity of this method was 3.0 nmol/l. The cross-reactivity in the assay of $25(OH)D_2$ was 75%, that of 24,25-dihydroxyvitamin D_3 100%, and that of cholecalciferol less than 0.3%. Serum calcium, phosphorus, proteins, and creatinine were measured by an autoanalyzer.

Statistical analysis

The mean and SD of various parameters of each group are presented in the text and mean and SEM in the figures. The statistical analysis, including the regression models, was performed using GraphPad Prism Software (San Diego, CA, USA).

Results

Serum 25(OH)D

The two groups of blood donors (summer and winter) did not have different 25(OH)D levels (mean ± SD), 80.3 ± 27.58 and 85.7 ± 44.64, respectively (Fig. 1). Serum 25(OH)D in the elderly during the winter was 41.6 ± 19.45 nmol/l for the women and 49.2 ± 22.56 for men. Because of the similarity of values, the data from women and men were combined in a single winter group (n = 279) with 25(OH)D 42.9 ± 20.18 (Fig. 1). The change of 25(OH)D between winter and summer was studied longitudinally in the same 56 randomly selected elderly individuals among the 279 who were examined in winter. Their mean 25(OH)D, from 41.3 ± 29.7 during the winter, increased to 89.8 ± 65.67 nmol/l during the summer ($P < 0.0001$, paired t test) (Fig. 2). The mean 25(OH)D in the elderly during summer was not different

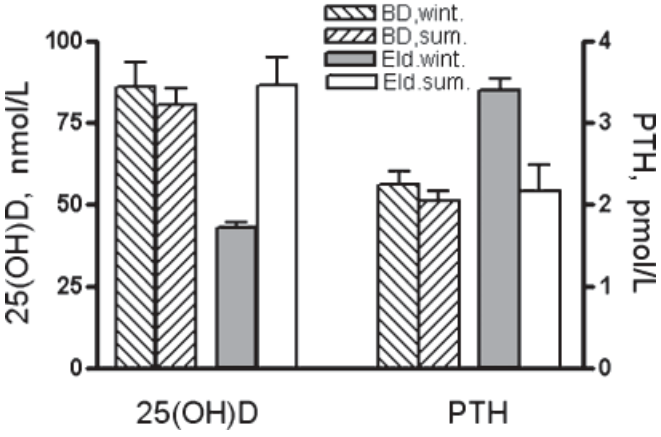

Fig. 1. Cross-sectional seasonal changes in 25-hydroxyvitamin D [*25(OH)D*] and parathyroid hormone (*PTH*). *BD, wint.*, blood donors in winter, n = 44; *BD, sum.*, blood donors in summer, n = 30; *Eld. wint.*, elderly in winter, n = 279; *Eld. sum.*, elderly in summer, n = 56. Note that the 56 elderly studied in summer are also included in the winter group. (Mean and SEM are shown)

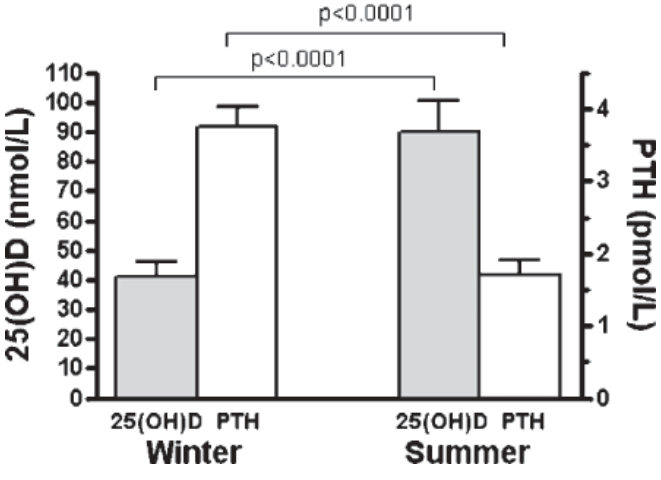

Fig. 2. Longitudinal changes of *25(OH)* and *PTH* in the same (n = 56) community-living elderly individuals between summer and winter. Mean and SEM are shown. Statistical analysis by paired t test

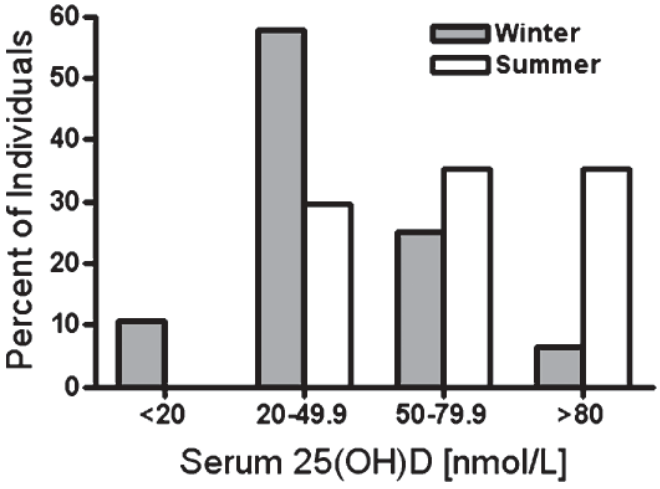

Fig. 3. Frequency distribution of elderly individuals in categories according to 25(OH)D levels in winter (n = 279) and summer (n = 56). Note that during summer only about one-third of the community-living elderly were vitamin D sufficient [25(OH)D >80 nmol/l]

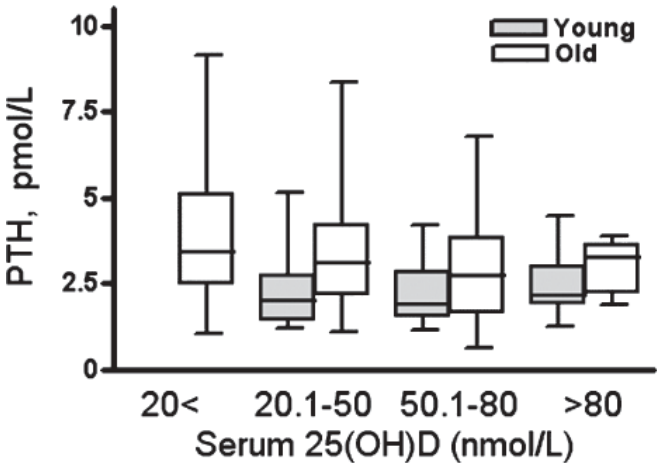

Fig. 4. Parathyroid hormone (*PTH*) in young (*shaded*) and old (*white*) subjects with similar 25(OH)D levels. The *boxes* extend from the 25th to the 75th percentile, the *horizontal lines* are at the median, and the *whiskers* extend to the smallest and to the largest value. In all categories of 25(OH)D, old persons had higher PTH values compared to the young, although the differences were not significant (Tukey–Kramer test). Among the older persons, those with 25(OH)D <20 nmol/l had significantly higher PTH compared to those with 25(OH)D >50 or >80 nmol/l ($P < 0.05$; Dunnett's multiple comparison test). Note that the winter as well the summer values are included for the 56 elderly subjects who were studied longitudinally

from the summer or winter level of blood donors (see Fig. 1). The frequency distribution of the elderly population of this study in categories according to 25(OH)D values is shown in Fig. 3. Thus, in the elderly group examined during the winter, only 6.5% had 25(OH)D values above 80 nmol/l, while about 20% had values below 25 nmol/l and 11% below 20 nmol/l. In the group of the elderly studied during summer, no persons had values less than 20 nmol/l, although only 35.2% had values above 80 nmol/l.

Plasma PTH

PTH was significantly higher (P = 0.02) in the group of blood donors examined in winter compared to those examined during summer (2.24 ± 0.92 vs. 2.04 ± 0.66) (see Fig. 1). Plasma PTH in the elderly during the winter was 3.4 ± 2.07 pmol/l (Fig. 1). In the 56 individuals who were studied longitudinally, mean plasma PTH was 3.7 ± 1.45 pmol/l during the winter and decreased to 1.7 ± 1.11 pmol/l during the summer ($P < 0.0001$, paired t test) (see Fig. 2). Mean plasma PTH in the elderly during the summer was not different from that of blood donors during summer or winter (Fig. 1). During the winter, 43.3% of the elderly had plasma PTH above 4.0 pmol/l (the upper limit for blood donors); during summer, 3.3% had PTH above this value.

To investigate if PTH levels were different between young (<48 years old, n = 74) and old (>60 years old, n = 335) subjects with similar 25(OH)D values as described by Vieth et al. [10], the subjects were classified in the following four categories according to their 25(OH)D values: <20, 25–49.9, 50–79.9, and >80 nmol/l (Fig. 4). In all these categories mean PTH was higher in the older individuals compared to the young, although not significantly (Tukey–Kramer test). Among the elderly, mean PTH was significantly higher in the category with 25(OH)D <20 nmol/l compared to the two categories with 25(OH)D above 50 nmol/l ($P < 0.05$; Dunnett's multiple comparison test).

Correlation between plasma PTH and serum 25(OH)D or serum creatinine

In the combined group of elderly persons examined during winter (n = 279) and summer (n = 56), the relationship between plasma PTH and serum 25(OH)D and creatinine was evaluated. There was a weak although significant negative Pearson correlation between PTH and 25(OH)D (r = –0.20, P = 0.002) and a positive correlation between PTH and serum creatinine (r = 0.18, P = 0.004). Multiple correlation of PTH simultaneously with serum 25(OH)D and creatinine showed a significant relation (r = 0.29, P = 0.001, R^2 = 0.083). Regression of the values of plasma PTH on serum 25(OH)D from all the groups of the study combined (n = 409) is shown in Fig. 5. The combination of elderly and young persons in one group for this analysis was necessary to include an adequate number of high 25(OH)D values. There was a significant negative correlation between PTH and 25(OH)D (r = –0.275, $P < 0.0001$). A Lowess curve fitted to the data showed a horizontal part for values of 25(OH)D higher than 80 nmol/l and a second part with an ascending slope for 25(OH)D values below 80 nmol/l, the deflection point of the curve being close to this critical value. An one-phase exponential decay best-fitting curve is also shown with the following best-fitting values: span = 4.411, plateau = 2.295, R^2 = 0.1733, but no Gaussian residuals. A polynomial third-order curve was also fitted to the data with R^2 = 0.172. Both the latter curves showed a similar pattern to the Lowess curve, i.e., a horizontal part for 25(OH)D values higher than 80 nmol/l and an ascending part with the deflection point approximately at this 25(OH)D value. This pattern

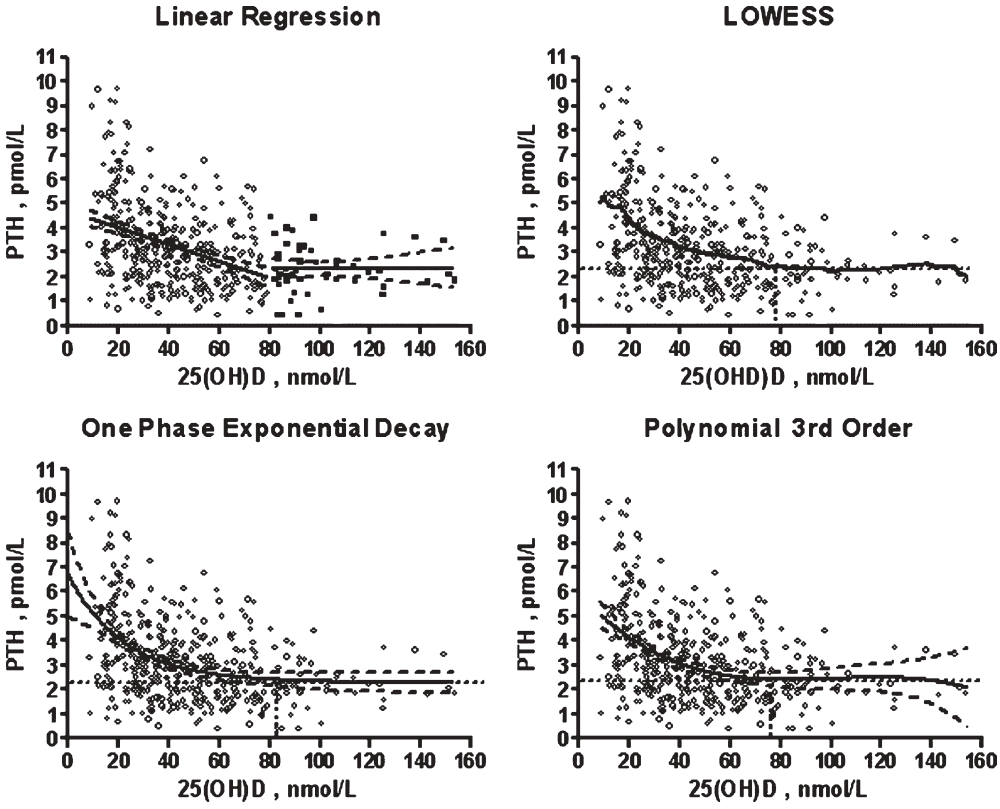

Fig. 5. The four regression models of PTH on 25(OH)D gave equivalent results. There is a horizontal part of each *line* for 25(OH)D values above approximately 80 nmol/l. For values of 25(OH)D below this critical level, PTH started to rise. The 95% CI are shown with the regression lines

shared by the three regression models described above could be obtained by fitting two linear regression lines separately on the values of 25(OH)D lower or higher than 80.0 nmol/l, the threshold value proposed by Chapuy et al. [5]. For the first line, the goodness of fit values were r^2 = 0.1299, $P = < 0.0001$, and runs test for linearity with $P = 0.13$. The second line was horizontal with P (runs test) = 0.56 (Fig. 5).

The relationship between PTH and 25(OH)D was also evaluated strictly in the group of the 56 elderly subjects who were studied longitudinally, including their winter and summer values. Thus, for the whole group (n = 112) the linear regression coefficient was $R^2 = 0.14$ ($P = 0.005$); if only patients with 25(OH)D values less than 80.0 nmol/l were considered (n = 84), a linear regression coefficient of $R^2 = 0.44$ was found [$P < 0.0001$; P value (runs test) = 0.07].

Serum calcium and phosphorus were not significantly different among the groups of this study (data not shown).

Discussion

In several epidemiological studies, it has been reported that vitamin deficiency, as defined by low serum 25(OH)D levels, is common in elderly populations in various regions of the world [11,12]. This vitamin deficiency in the elderly is frequently associated with secondary hyperparathyroidism and increased risk for hip fracture [13,14]. Oily fish is one of the very few foods that naturally contain vitamin D [2,15]. Fish consumption seems to be important in maintaining fairly adequate vitamin status in elderly Japanese during the winter [15]. Generally, the main source of vitamin D in humans is considered to be the skin, where vitamin D is produced during exposure to the UVB sunlight [2]. Thus, it was somehow unexpected that people in southern Europe tended to have lower 25(OH)D levels compared to the populations living in central or northern Europe and other parts of the world at higher northern latitudes [11,12]. In the present study, we found that in Athens (northern latitude 38°) and at the end of the winter about 20% of elderly independent and apparently healthy persons have 25(OH)D levels below 25 nmol/l and only 6.5% have levels above 80 nmol/l, which is considered to be the lower value compatible with vitamin D sufficiency [1,2,5]. The situation improves during the summer, when no persons had values in the osteomalacia range (<20 nmol/l) [1,2]. However, even at the end of the summer only about one-third of the elderly population could be judged as vitamin D sufficient with serum 25(OH)D higher than 80 nmol/l. These findings agree with the seasonal variation of 25(OH)D levels described previously by Lips et al. [16] and Bouillon et al. [17] in northern Europe and also by Rapuri et al. for residents of Omaha, Nebraska (latitude, 41° N) [18]. It should be noted that Athens is located above 37° latitude, and in areas north of this latitude very little of the UVB solar radiation reaches the surface of the earth during the months of November through February [2].

The low 25(OH)D levels in the elderly during the winter were associated with secondary hyperparathyroidism be-

cause the mean PTH during this period of time was twice as high compared to that during summer. Several investigators have found that PTH is negatively correlated with serum 25(OH)D in the elderly [5–9]. Chapuy et al. [5], by fitting a three-phase decay regression model to their data, found that PTH starts to rise for values of 25(OH)D below 78 nmol/l. Thus, a threshold value of serum 25(OH)D in the vicinity of 80 nmol/l was adopted to discriminate vitamin D insufficiency from adequacy [1,2,5]. However, the existence of a threshold value was challenged by Vieth et al., who by fitting a Lowess regression model to their data did not detect any clear-cut deflection point in the regression line [10]. After testing four different regression models in our data (including a Lowess model), we confirmed the finding of Chapuy et al. [5]. However, in the present study we found that the regression coefficient of plasma PTH on serum 25(OH)D was $R^2 = 0.04$ for the whole range of 25(OH)D values and $R^2 = 0.13$ for values of 25(OH)D lower than 80 nmol/l. Thus, only 4%–13% of the variation of PTH could be explained by the serum 25(OH)D. These findings indicate that plasma PTH values in the elderly may not be obligatorily high at any time when low 25(OH)D values are detected (see Fig. 5). A limitation of this regression analysis is the heterogeneity of the group analyzed, which included elderly as well as young individuals, especially in view of our finding that younger subjects tend to have lower PTH levels compared with elderly with similar 25(OH)D levels. However, we think that because the differences in PTH between young and elderly were not statistically significant (see Fig. 4), this regression analysis may be considered as valid. When the relationship between PTH and 25(OH)D was examined strictly in the elderly who were studied longitudinally, we found that 14% of the variation of PTH could be explained by 25(OH)D for the whole range of 25(OH)D values, whereas 44% of this variation could result from 25(OH)D for values of 25(OH)D below 80.0 nmol/l. The latter finding implies that a strong negative correlation between PTH and 25(OH)D is manifest, especially if the seasonal changes of 25(OH)D and PTH are examined in the same elderly subjects and within the vitamin D deficiency range of 25(OH)D levels.

We also found a positive correlation between PTH and serum creatinine in the elderly, as reported by Vieth et al. [10]. However, this relationship, albeit significant, was weak, and only 3% of the variation of PTH could be explained by changes in serum creatinine level. Our data agree with the the finding of Vieth et al. [10] that, for any 25(OH)D level, the elderly tend to have slightly higher (but not significantly) PTH levels compared to the younger individuals (see Fig. 4), and therefore other factors apart from low serum 25(OH)D, such as relatively higher serum creatinine or old age, may account partly for the relatively high PTH in the elderly [6,10].

We consider that the secondary hyperparathyroidism we found to be prevalent in the elderly in Athens during the winter may be ascribed mainly to the vitamin deficiency caused by reduced sun exposure and not to low calcium intake. Our data did not allow a correlation of the PTH levels with calcium intake. However, the fact that the high PTH of our elderly persons during the winter decreased significantly during summer indirectly implies that hyperparathyroidism during the winter was mainly the result of vitamin D deficiency because it is unlikely that the longitudinally studied elderly changed their calcium intake considerably within a few months.

In conclusion, we have found that low 25(OH)D levels, diagnostic of vitamin deficiency, and high serum PTH are not uncommon among independent community-living elderly persons in Athens, especially during the winter. Our findings are in agreement with the findings of other investigators that there is a threshold value of serum 25(OH)D (at about 80 nmol/l) below which plasma PTH starts to rise. There was a marked seasonal reciprocal change of PTH and 25(OH)D in the same elderly subjects, and within the range of vitamin D deficiency [25(OH)D <80 nmol/l] the two parameters were strongly negatively correlated. The contribution of serum creatinine and old age to relatively higher PTH levels in the elderly that we found seems to be small.

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