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ORIGINAL COMMUNICATION

High prevalence of vitamin D insufficiency in healthy elderly people living at home in Argentina

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Objective: To evaluate the nutritional status of vitamin D in urban populations of healthy elderly people living at home, in different regions of Argentina.
Design: Cross-sectional study.
Subjects: In total, 386 ambulatory subjects over 65 y of age from seven cities (between latitude 26°S and 55°S) were asked to participate between the end of winter and the beginning of spring. Of these, 369 accepted, 30 were excluded because of medical history or abnormal biochemical determinations. Finally, 339 subjects (226 women and 113 men) ($X \pm$ s.d.) (71.3 $\pm$ 5.2 y) were included.
Results: Serum 25OHD levels were lowest in the *South* (latitude range: 41°S–55°S): 14.2 $\pm$ 5.6 ng/ml ($P<0.0001$ *vs* North and Mid regions); highest in the *North* (26°S–27°S): 20.7 $\pm$ 7.4 ng/ml ($P<0.03$ *vs* Mid, $P<0.0001$ *vs* South); and intermediate in the *Mid* region (33°S–34°S) 17.9 $\pm$ 8.2 ng/ml. Serum *mid-molecule PTH (mmPTH)* and 25OHD were inversely related: ($r=-0.24$, $P<0.001$). A cutoff level of 25OHD at which serum mmPTH levels began to increase was established at 27 ng/ml. A high prevalence (87–52%) of subjects with 25OHD levels in the deficiency-insufficiency range (25OHD levels $<$20 ng/ml) was detected.
Conclusion: This study shows that vitamin D deficiency/insufficiency in the elderly is a worldwide problem. Correction of this deficit would have a positive impact on bone health of elderly people.
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Introduction

Vitamin D insufficiency is common in elderly populations (particularly in institutionalized subjects) (Mc Kenna, 1992) and leads to secondary hyperparathyroidism, high bone turnover, bone loss and osteoporotic fractures (Brazier *et al*, 1995; Chapuy *et al*, 1996; Dawson-Hughes *et al*, 1997; Le Boff *et al*, 1999). Different categories have been proposed to classify vitamin D status: vitamin D deficiency that leads to osteomalacia, with the ensuing histomorphometric changes (Parfitt *et al*, 1982), vitamin D insufficiency that has an effect on calcium homeostasis, such as a diminution in calcium absorption and 1,25(OH)2D levels, leading to secondary hyperparathyroidism (Peacock *et al*, 1985), and vitamin D sufficiency with no effect on calcium homeostasis. The threshold of serum 25OHD that separates vitamin D

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sufficiency from insufficiency is generally defined by its biological effect, primarily by the increase in serum parathormone. A new category of desirable levels has been introduced, based on the 25OHD levels required to diminish hip fractures (Chapuy *et al*, 1992; Mc Kenna & Freaney, 1998). Reports in the literature have established serum 25OHD levels below which PTH begins to increase to be between 12 and 20 ng/ml (Bouillon *et al*, 1987; Gloth *et al*, 1995; Ooms *et al*, 1995; Mc Kenna & Freaney, 1998). More recently, higher levels of 25OHD have been proposed as limits of insufficiency: 25,31 or 44 ng/ml (Chapuy *et al*, 1997; Dawson-Hughes *et al*, 1997; Haden *et al*, 1999). However, different techniques were used to measure 25OHD: competitive protein binding assay (CPBA), radioimmunoassay and immunoradiometricassay, and this may have influenced the different values obtained.

Several studies have been performed in Argentina to determine 25OHD levels (Ladizesky *et al*, 1987; Oliveri *et al*, 1990, 1991, 1993a, 1993b, 1994; Plantalech *et al*, 1997; Fradinger & Zanchetta, 2001) and in Australia at similar latitude (Need *et al*, 1993, 2000), but none were designed to assess simultaneously the vitamin D status of elderly populations living in different regions of a country that covers 3700 km from north to south, between latitude 22°S and 55°S.

The aim of the present study was:

(1) To evaluate the nutritional status of vitamin D in urban populations of elderly people living at home, from different regions in Argentina.
(2) To study the influence of geographic and climatic characteristics.
(3) To investigate the threshold of insufficiency, evaluating the relation between mmPTH and 25OHD.

Subjects and methods

Subjects

In all, 386 ambulatory subjects over 65 y of age, from seven cities in Argentina (between latitude 26°S and 55°S) were asked to participate in the study, to be performed between the end of winter and the beginning of spring (15 August–15 October). The subjects were relatives or guardians of patients attending hospitals or subjects included in hospital preventive programs, in the selected cities. In all, 369 volunteers accepted to participate, but only 351 subjects (234 women and 117 men) were studied because they had no previous history of malignancy, osteoporotic fractures, renal, malabsorptive or metabolic bone diseases. None of the subjects was receiving vitamin D or any medication that could affect mineral metabolism. The results of biochemical determinations led to the exclusion of two women who presented hypercalcemia due to primary hyperparathyroidism, and of 10 subjects with high serum creatinine levels (above 1.4 mg/dl).

Table 1 Average latitude, temperature and sunshine hours (heliophany) in each region during the study period

Region	*Latitude (°South)*	*Temperature (°Celsius)*	*Heliophany (h/day)*
North	27	16.1	6.3
Mid	33	10.6	5.3
South	48	3.7	3.6

The data of the study population, which finally consisted of 339 subjects ($X \pm$ s.d.) (71.3 ± 5.2 y) (226 women,113 men), were analyzed. They were grouped according to location in three geographical regions: South (Cities: Bariloche: 41°S, $n=28$; Comodoro Rivadavia: 45°S, $n=28$ and Ushuaia: 55°S, $n=32$); Mid (cities: Buenos Aires 34°S, $n=161$ and Mendoza: 33°S, $n=32$) and North (cities: Corrientes: 27°S, $n=28$ and Tucumán: 26°S, $n=30$).

Methods

Dietary calcium intake was assessed by means of a food frequency questionnaire that enquired about weekly consumption of dairy products. The number of hours of sunlight per day for each city and the mean winter temperature were obtained from the National Meteorological Service, and were expressed as the mean of the 3 months during which the study was performed. The mean daily hours of sunlight and temperature of each city are shown in Table 1. All the patients gave informed consent prior to the onset of the study; the study protocol was approved by the Asociación Argentina de Osteología y Metabolismo Mineral. Fasting blood samples of all the patients were obtained. The serum samples were frozen and submitted to the central laboratory, where they were stored until processing.

The following serum biochemical determinations were performed: calcium (atomic absorptiometry spectrophotometry), creatinine (colorimetric), bone alkaline phosphatase (BAP) (wheat lectin precipitation), 25 hydroxyvitamin D (25OHD) (RIA-IDS) and mid-molecular parathormone (mmPTH) (RIA, employing the antiserum that recognizes intact hormone as well as mid-molecular and carboxyterminal fragments). All biochemical determinations were performed at the laboratory of the Hospital de Clínicas using methods described elsewhere (Ladizesky *et al*, 1987; Oliveri *et al*, 1990, 1991, 1993a; Zeni *et al*, 2001). The interassay and intra-assay CV of 25OHD (RIA-IDS) were 8.2% and 5.3%, respectively. Samples from all the cities were included in each assay to diminish interassay variations.

Statistical analysis was conducted using the SPSS statistical software package (SPSS, Inc., Chicago, IL, USA). Comparison of mean values between regions was evaluated by unpaired *t*-test. Comparisons among groups divided according to 25OHD level ranges were performed using the Kruskall–Wallis test corrected by Bonferroni (*post hoc* test) and comparison among means was performed using the Mann–

Table 2 Study population: age and serum biochemical determinations for each region

Region	*Age (years)*	*C intake (mg/day)*	*Calcium (mg/dl)*	*BAP (IU/l)*	*25OHD (ng/ml)*	*PTH (pg/ml)*	*Creatinine(mg/dl)*
Ref. value			8.9–10.4	31–95	15–45	20–100	0.7–1.3
North	70.0±4.9	554±304	9.6±0.4	62±15	20.7±7.4	38.1±14.4	0.8±0.2
Mid	72.0±5.5#	477±305#	9.5±0.3	60.8±19.5	17.9±8.2*	55.9±38.7##	0.8±0.2
South	70.6±4.9	589±313	9.5±0.4	59.2±16.8	14.2±5.6**	43.3±17.7	0.9±0.1

Data are mean ± s.d. C intake, calcium intake; BAP, bone alkaline phosphatase; 25OHD, 25 hydroxy vitamin D; PTH: parathormone. #$P<0.05$ vs North and South; ##$P<0.001$ vs North and South; *$P<0.03$ vs North; **$P<0.001$ vs North and Mid; unpaired *t*-test.

Table 3 Mean values of serum PTH according to 25OHD levels ranges

25OHD level range	*n*	*25OHD (ng/ml)*	*PTH (pg/ml)*	*n above normal (>100 pg/ml)*
Deficiency (<10 ng/ml)	35	7.8±1.2	83.3±68.6**(25–420)	6
Insufficiency (10–19 ng/ml)	194	14.0±2.8	47.9±23.2 *(20–170)	4
Moderate hypovitaminosis (20–29 ng/ml)	78	23.2±2.4	44.1±19.4 (19–100)	0
Mild hypovitaminosis/desirable (30–39 ng/ml/≥40 ng/ml)	32	34.5±5.6	36.6±11.7 (25–60)	0

Data are mean ± s.d. (range of values). 25OHD, 25 hydroxyvitamin D; PTH, parathormone. **$P<0.001$ vs the other three categories; *$P<0.01$ vs mild hypovitaminosis/desirable. Comparisons among groups divided by 25OHD levels ranges were performed using the Kruskall–Wallis test corrected by Bonferroni (*post hoc* test) and the comparison of these groups means was performed by the Mann–Whitney test.

Whitney test. Linear associations were analyzed by Rho–Pearson correlation coefficients and multivariate linear regression. A value of P below 0.05 ($P<0.05$) was considered to be significant. The relation between mmPTH and 25OHD was expressed according to the Box–Tidwell model.

Results

Serum levels of calcium ($X\pm$s.d. 9.5±0.3 mg/dl), bone alkaline phosphatase (61.0±18 UI/l) and creatinine (0.8±0.2 mg/dl) were within normal range, and no significant differences among regions were observed (Table 2).

As regards serum 25OHD levels, the South exhibited the lowest levels: 14.2±5.6 ng/ml ($P<0.0001$ *vs* North and Mid regions); the North had the highest levels of 25OHD: 20.7±7.4 ng/ml ($P<0.03$ *vs* Mid); and the Mid region was found to have intermediate levels, between South and North values: 17.9±8.2 ng/ml.

Vitamin D status was determined according to 25OHD levels, following Mc Kenna and Freaney's (1998) Classification and the percentage of subjects in each category was determined for each region: deficiency: <10 ng/ml (North: 2%, Mid: 11%, South 14%), insufficiency ≥10 to <20 ng/ml (North: 50%, Mid: 53%, South: 73%), hypovitaminosis ≥20 to <40 ng/ml (North: 46%, Mid: 35%, South: 13%), and desirable: equal to or higher than 40 ng/ml (North: 2%, Mid: 1%, South: 0%). Within the South region, Ushuaia, the southernmost city in Argentina (55°S), exhibited the highest deficiency prevalence (25%) and only 3% of the population exhibited 25OHD levels above 20 ng/ml. The levels of mmPTH were found to be increased in the groups with lowest levels of 25OHD. In total, 10 subjects with mmPTH values above the normal range (>100 pg/ml) were in the deficiency and insufficiency groups of 25OHD levels (Table 3). mmPTH levels correlated positively with age ($r=0.15$, $P<0.005$) and inversely with serum 25OHD levels ($r=-0.24$, $P<0.001$) (Figure 1). No correlation was found between mmPTH and creatinine. The relation between mmPTH and 25OHD levels was analyzed in the whole population (box–tidwell regression model). The 'cutoff level' of 25OHD at which serum mmPTH levels began to increase was established at 27 ng/ml (301 subjects had levels below 27 ng/ml). Analysis of data according to age (<75 and ≥75 y) evidenced that the oldest group had significantly higher levels of mmPTH (53.8±37.5 pg/ml) and lower 25OHD (16.3±6.1 ng/ml) than the youngest group (43.9±22.0 pg/ml and 18.9±9.2 ng/ml) ($P<0.003$).

No differences in serum calcium, BAP, or creatinine were found. The levels of 25OHD were found to correlate positively and strongly with heliophany ($r=0.86$, $P<0.014$) and average temperature ($r=0.89$, $P<0.007$), and negatively with latitude ($r=0.81$, $P<0.027$) (Figure 2) in all seven cities.

Analysis according to sex showed that women exhibited significantly higher levels of BAP than men (62.3±17.7UI/l *vs* 57.5±18.2 UI/l; $P<0.05$), whereas men

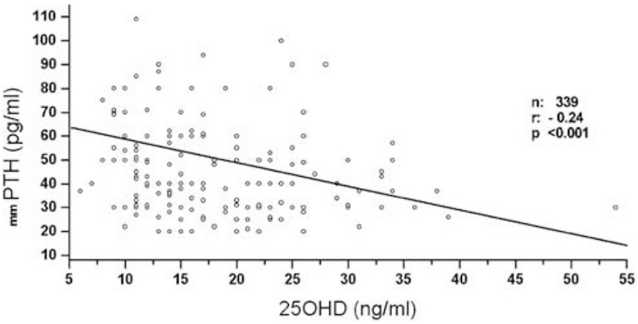

Figure 1 Correlation between mid-molecule parathormone (mmPTH) and 25 hydroxyvitamin D (25OHD) in the whole population.

showed significantly higher levels of 25OHD (18.7 ± 8.5 ng/ml *vs* 16.7 ± 7.2 ng/ml; $P<0.05$) and creatinine (0.9 ± 0.2 mg/dl *vs* 0.8 ± 0.2 mg/dl) than women.

The average calcium intake was found to be below adequate levels established for this age group, in all three regions (Standing Comittee on the Scientific Evaluation of Dietary reference Intakes, 1998). The Mid region exhibited the lowest calcium intake values (Table 2).

Discussion

To our knowledge, this is the first study to assess end-of-winter vitamin D status of elderly populations living in different regions of a large country which covers 3700 km from North to South. Inhabitants of seven cities located between latitude 26°S and 55°S were studied, the examinations were performed during the last month in Winter and the first month in Spring. Although climatic conditions differ, ethnicity—mostly Caucasian of European descent with negligible native Indian ancestry—diet and lifestyle are largely similar throughout the country.

Previous studies in Argentinean newborns, children and young adults performed at different times found the average range of 25OHD in the South region to be significantly lower than in the Mid and North (Ladizesky *et al*, 1987; Oliveri *et al*, 1990, 1991, 1993a, 1994; Plantalech *et al*, 1997), due to the lack of skin photoproduction of vitamin D during winter in the latter region (Ladizesky *et al*, 1995).

Differences in 25OHD levels have been found among different geographic areas (Mc Kenna, 1992). Values are usually higher in North America than in Europe, both in winter and summer (Mc Kenna, 1992), probably as a consequence of sunshine availability as well as the inclusion of vitamin D-enriched foods in the average diet. The levels of 25OHD found in the present study are in between the relatively high US and low European values. This is most likely due to the combined effect of adequate sunshine availability in most of Argentina on the one hand, and low intake of vitamin D supplemented foods on the other.

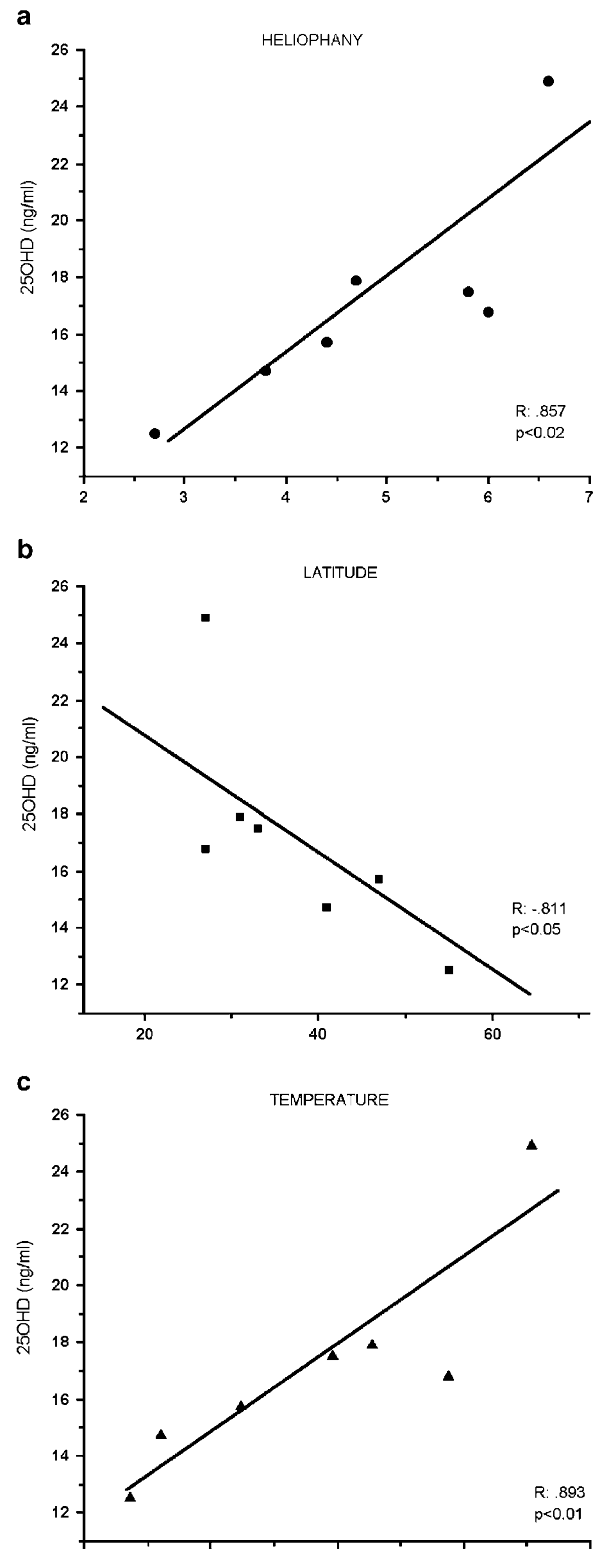

Figure 2 Correlations between average sunshine hours (heliophany), temperature (°Celsius), latitude (°South) and 25 hydroxy vitamin D (25OHD) levels for each city during the study period.

In view of the above, one of the most remarkable findings of the present study is the markedly high prevalence of deficient/insufficient levels of 25OHD (<20 ng/ml) in ambulatory elderly subjects, not only in the South (87% of the population), but also in the Mid (64%) and North (52%) regions.

A very high correlation (~0.8) was observed between the average 25OHD values obtained in each of the seven cities and the corresponding average sunlight hours, temperature and latitude. Although this is an expected finding (Chapuy *et al*, 1997; Holick, 2002), at least one study in Europe revealed higher levels of 25OHD in the Central and Northern areas than in the Mediterranean regions which are sunnier and have milder weather conditions (van der Wielen *et al*, 1995); this is probably due to differences in ethnicity, diet or lifestyle. Since the present study was performed in the same country, and consequently the study population was homogenous as regards ethnicity and living and dietary habits, climate can be considered the main determinant of vitamin D status.

In agreement with previous reports (Brazier *et al*, 1995; Chapuy *et al*, 1997; Haden *et al*, 1999; Melin *et al*, 1999), a significant but relatively weak correlation (*r*: −0.24) was found between serum 25OHD levels and serum PTH. On the other hand, there is a remarkable change in average mmPTH levels for each category of vitamin D status, from 37 pg/ml in the 'desirable' level to 83 pg/ml in the 'deficient' vitamin D status level (Table 3). In the present study the 'cutoff' 25OHD value, above which serum mmPTH levels remained stable and relatively low, was found to be 27 ng/ml. This value is close to 30 ng/ml, which is in the range of hypovitaminosis according to Mc Kenna and Freaney's (1998) definition of different vitamin D status. This 'cut-off' value is similar to that proposed by Haden *et al* (1999): 25 ng/ml, Melin *et al* (1999): 30 ng/ml and Chapuy *et al* (1997): 31 ng/ml, and is similar to the cutoff level associated with an increase in bone markers (Need *et al*, 2002). However, other authors have proposed lower and higher levels as cutoff values: 12–16 ng/ml (Ooms *et al*, 1995; Gloth *et al*, 1995; Thomas *et al*, 1998) and 44 ng/ml, respectively (Dawson-Hughes *et al*, 1997). This last value is very similar to the level proposed as being desirable to diminish fracture risk (Chapuy *et al*, 1992; Mc Kenna & Freaney, 1998).

Average serum mmPTH observed in the Mid-region was higher than that found in the South, in spite of the higher 25OHD level in the former compared to the latter region. A probable explanation for this is the significantly lower calcium dietary intake from dairy products (477±305 *vs* 588±313, $P<0.003$) and the slight but significant difference in age between the inhabitants included in the study (72.0 *vs* 70.6 y) ($P<0.05$). Thus, vitamin D status is an important but not exclusive determining factor of PTH secretion and the importance of an adequate calcium intake cannot be neglected in the light of this study, as has been suggested by other authors (Clemens *et al*, 1987; Chapuy *et al*, 1996).

Within our population, the 'very' elderly (above 75 y of age) presented more pronounced vitamin D insufficiency compared to the group aged 65–75 years, probably because their exposure to the sun and the capacity of their skin to synthesize vitamin D from its precursor are more limited. This observation emphasizes the fact that the very elderly are at greater risk of having increased PTH levels and, consequently, decreased bone mass (Brazier *et al*, 1995; Chapuy *et al*, 1996; Le Boff *et al*, 1999).

In agreement with previous reports (van der Wielen *et al*, 1995; Dawson-Hughes *et al*, 1997; Carnevale *et al*, 2001), the women in our study had significantly lower 25OHD levels and higher mmPTH and bone alkaline phosphatase compared to the men. Other studies have shown that bone resorption markers are also higher in women, and most likely this increased bone turnover status is in part responsible for the increased rate of bone fragility fractures found almost universally in women. This finding emphasizes the importance of correcting vitamin D deficiency/insufficiency in women, as it has proved to be efficacious in reducing the rate of bone fracture (Chapuy *et al*, 1992, 2002).

The present study has important implications regarding the proper care of our elderly population, since it shows a very high prevalence of vitamin D deficiency/insufficiency in all the studied communities. Although the sample is highly selected, the results emphasize that the problem of vitamin D deficiency is a worldwide problem in both hemispheres: in the South as previously reported in Australia, New Zeland and Argentina (Plantalech *et al*, 1997; Inderjeeth *et al*, 2000; Need *et al*, 2000) and in the North (US and Europe populations), where it has been extensively studied (Ooms *et al*, 1995; Thomas *et al*, 1998; Chapuy *et al*, 1996; Dawson-Hughes *et al*, 1997; van der Wielen *et al*, 1995; Le Boff *et al*, 1999).

Hip fractures—as well as other bone fragility fractures are unfortunately developing as one of the most significant problems for the health care of the elderly, both from the point of view of morbidity/mortality and financial costs. Correction of vitamin D deficit has proven to be effective to significantly reduce fracture rates. With the knowledge that vitamin D deficit is a worldwide problem for the elderly and that correction of the deficit will have an impact on their health care, the following question must be addressed. Should supplementation with vitamin D be recommended to the elderly population at large? Or should it be restricted to the groups at higher risk, such as women and the 'very elderly'? If such a policy is advised, the therapeutic regimen should also be defined, stating the recommended dose and periodicity to achieve the desired levels of 25OHD (above 'cutoff' mmPTH raising level), which will not necessarily be the customary 800 IU/per day. In fact, recent studies on young males with normal vitamin D status have shown that 1.000 IU of vitamin D per day produces a very small impact on 25OHD levels, while a dose of 5.000 IU per day is able to raise 25OHD levels without side effects (Heaney *et al*, 2002). Important data on the widespread occurrence of vitamin D deficit and on the beneficial effect of vitamin D supplementation on bone health have been reported, and evidence the need to examine the long-term recommended dose and use of vitamin D for the elderly.

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