Calcif Tissue Int (1988) 43:199–201

Calcified Tissue International

Low Circulating Vitamin D in Obesity

Yair Liel, Enoch Ulmer, Judith Shary, Bruce W. Hollis, and Norman H. Bell

Veterans Administration Medical Center and Departments of Medicine, Pediatrics, and Pharmacology, Medical University of South Carolina, Charleston, South Carolina, USA

Summary. Previous studies demonstrated decreases in serum 25-hydroxyvitamin D in obese subjects. Studies were carried out to determine whether serum vitamin D is low in obesity. The results indicate that serum vitamin D is significantly lower in obese than in nonobese individuals and may contribute to lower serum 25-hydroxyvitamin D in obesity.

Key words: Obesity, vitamin D, 25-hydroxyvitamin D.

We previously reported that obesity is associated with alterations in the vitamin D-endocrine system in white individuals. There were increases in circulating immunoreactive parathyroid hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), and urinary cyclic adenosine 3′5′-monophosphate (cyclic AMP), and decreases in serum 25-hydroxyvitamin D (25OHD) and urinary calcium in obese as compared with nonobese age-matched men and women [1]. In the present study, we present evidence that serum vitamin D is reduced in white obese subjects.

Methods

Thirteen white obese subjects (5 men and 8 women) and 13 white nonobese subjects (7 men and 6 women), ranging in age from 20 to 35 years, were studied. The obese subjects were otherwise normal and in good health. All of them were 30% or more above their ideal body weight as determined from the tables of the Metropolitan Life Insurance Company, New York. None of the subjects, obese or nonobese, had an outdoor occupation. All of them were hospitalized at the General Clinical Research Center of the Medical University of South Carolina. Blood samples were collected after overnight fast for measurement of serum calcium, phosphorus, vitamin D, 25OHD, and $1,25(OH)_2D$. The studies were performed between the months of December and May.

Serum calcium [2] and phosphorus [3] were measured by automated colorimetric methods. Serum ionized calcium was measured with a solid-state electrode. Serum vitamin D was measured after deproteination of serum with an equal volume of methanol and extraction of serum two times with two volumes of hexane. ^3H-vitamin D_3, approximately 2,000 cpm, was added initially to each sample to estimate recovery. Vitamin D was separated from its metabolites by chromatography with cartridges of Bond Elut silica (Analytichem International, Harbor City, CA) developed in methylene chloride:isopropanol (99.8:0.2) [4]. Subsequently, the samples were submitted to high performance liquid chromatography with two solvent systems. The first was hexane:isopropanol (99.5:0.5), on a column of μPorasil (Waters Associates, Milford, MA) [5]. The second system was reverse-phase and was acetonitrile:methylene chloride (80:20) on a column of Vydac-ODS bonded 5μ silica, 300 Angstrom pore size, 25 × 0.4 mm (The Separation Group, Hesperia, CA) [6]. By employing the second system it was possible to separate vitamin D_3 from vitamin D_2. Recovery of ^3H-vitamin D_3 averaged 45.0 ± 1.9% (mean ± SE, N = 29). The concentrations of vitamin D_2 and D_3 were calculated from their UV absorption peaks at 265 nm. Results were combined and are expressed as ng/ml. Duplicate determinations were performed. Serum 25OHD was measured in duplicate at two concentrations by a competitive protein-binding assay with vitamin D-deficient rat serum [7] after extraction with acetonitrile, addition of phosphate buffer, chromatography on C-18 Sep-Pak, and elution with acetonitrile [8]. 25OHD was separated from other vitamin D metabolites before the binding assay by chromatography on silica Sep-Pak and elution with hexane:isopropanol (94:6). Serum $1,25(OH)_2D$ was measured by the method of Reinhardt et al. [8]. Weekly exposure to sunlight was estimated in a number of the subjects [9].

Statistical analyses were performed with Student's nonpaired *t* test. Correlation coefficient was performed with standard methods.

Address reprint requests to Dr. Norman H. Bell, Veterans Administration Medical Center, 109 Bee Street, Charleston, South Carolina 29403, USA.

Results

The results are summarized in Table 1. Serum total and ionized calcium were not different in the obese and nonobese subjects. Serum inorganic phosphate, serum vitamin D, and serum 25OHD were significantly lower and serum $1,25(OH)_2D$ was significantly higher in the obese compared with the nonobese subjects. Whereas none of the nonobese subjects had a serum vitamin D below 8 ng/ml, all of the obese men and women had serum values of 2.2 ng/ml or less and 8 of them had values that were undetectable (less than 1 ng/ml (Fig. 1). Vitamin D_3 accounted for all of the vitamin D present in the circulation of 9 of the nonobese individuals and in all of the obese ones. In the 4 other nonobese individuals, vitamin D_2 accounted for 22 ± 5% of the total serum vitamin D and averaged 2.2 ± 0.4 ng/ml. In all subjects, there was a significant negative correlation between the concentration of circulating vitamin D and percent ideal body weight ($r = 0.880$, $P < 0.01$) and a significant positive correlation between serum vitamin D and serum 25OHD ($r = 0.471$, $P < 0.02$). Exposure to sunlight, estimated in 8 nonobese and 8 obese subjects, was not different in the two groups (12.0 ± 1 vs. 12.8 ± 2 hours/week, N.S.).

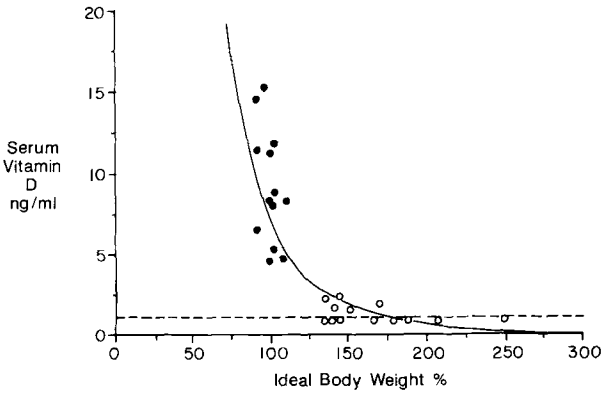

Fig. 1. Relationship between serum vitamin D and percent ideal body weight in obese and nonobese subjects. The hatched line is the lower limit of detection. The regression curve is plotted from the equation $Y = 1.953e + 7X^{-3.2257}$ where Y is the concentration in serum of vitamin D and X is the percent ideal body weight. Normals (•), obese subjects (○).

Discussion

Previous findings indicated that serum 25OHD is significantly lower [1, 10–12] and serum immunoreactive parathyroid hormone is significantly higher in obese compared with nonobese individuals [1, 13] and that these changes are reversed by weight loss [10, 13]. As already noted, we observed similar findings as well as increases in serum $1,25(OH)_2D$ and urinary cyclic AMP and decreases in urinary calcium in obese men and women and suggested that the changes resulted from skeletal resistance to parathyroid hormone [1]. The present results showing low values for serum vitamin D provide evidence for deficiency of vitamin D as an alternative or additional cause. This possibility is strengthened by our recent demonstration that decreases in urinary calcium and increases in serum $1,25(OH)_2D$ and urinary cyclic AMP is obese subjects are reversed by the administration of $25OHD_3$ [14]. Also, histomorphometric analysis of biopsies from the iliac crest revealed evidence of secondary hyperparathyroidism in 2 of 24 obese subjects and one of them showed changes suggesting mild osteomalacia [12]. Thus, vitamin D deficiency in obese individuals may not only be responsible for alterations of the vitamin D-endocrine system but, on occasion, may produce histologic changes of osteomalacia in the skeleton.

It should be noted that our values for serum vitamin D in normals are in the same range as those found in normal subjects during the summer months and that our values in obese subjects are in the same range as those found in normal subjects during the winter months in Boston [15]. We found that serum 25OHD increased appropriately in response to ultraviolet radiation in obese individuals (Y. Liel and N. H. Bell, unpublished observations).

Table 1. Serum values in nonobese and obese subjects

Group	Serum calcium mg/dl	Serum Ca^{2+} mg/dl	Serum phosphate mg/dl	Serum vitamin D ng/ml	Serum 25OHD ng/ml	Serum $1,25(OH)_2D$ pg/ml
Nonobese (13)	8.9 ± 0.1	4.95 ± 0.05	4.6 ± 0.2	9.1 ± 1.0	16 ± 2	27 ± 4
Obese (13)	8.9 ± 0.1	4.82 ± 0.04	3.9 ± 0.2	1.3 ± 0.1[a]	11 ± 1	37 ± 2
P value	NS	NS	<0.05	<0.001	<0.05	<0.01

[a] Values that were undetectable were calculated as 1 ng/ml

These studies provide evidence that low serum vitamin D values in obese subjects do not result from impaired dermal production. The extent to which changes in season influence vitamin D and mineral metabolism in obese subjects warrants further investigation.

The mechanism for low circulating vitamin D in obese subjects is not known. Increased metabolic clearance and enhanced uptake of vitamin D by adipose tissue are possible causes. Studies in rats and in human tissues indicate that labeled vitamin D is taken up and stored by adipose tissue [16, 17]. It is evident that additional studies are required to determine the means by which the metabolism of vitamin D is altered by obesity.

Acknowledgments. This study was supported by Grants HD 22542, HD 00781, AR 36066, and M01 RR 01070 (General Clinical Research Center) from the U.S. Public Health Service and by the Veterans Administration.

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Received November 27, 1987, and in revised form February 2, 1988.