Changes in Sex Hormones and Calcium Regulating Hormones with Reference to Bone Mass Associated with Aging

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Synopsis

Measurement of bone mineral content (BMC), intestinal ⁴⁷Ca absorption, and calcium regulating hormones and sex steroids in serum were performed on 32 healthy aged subjects and 26 control young subjects. In BMC, there was a progressive fall after age 40, with the rate of decrease being greater in women than in men. A significant correlation was observed between BMC and testosterone in the men and between estrogens and BMC in the women, suggesting the possible importance of testosterone in men and estrogens in women in maintaining bone mass. Plasma PTH showed no change with age. However, the reserve capacity of the parathyroid was significantly reduced in the aged women. Serum levels of ionized calcium were low in aged subjects, indicating a possible alteration with age in the feedback control between ionized calcium levels and parathyroid hormone secretion. C-cell function was also decreased with age. Plasma 1,25-(OH)²D and ⁴⁷Ca absorption tended to decrease with age. Age-related bone loss could be a reflection of the interaction of these hormonal imbalance occurring with age.

It is widely known that there is normally a progressive loss of bone mass and an increase of the incidence of osteoporosis with age, especially in postmenopausal women. This bone loss is characterized by a chronic negative imbalance in bone formation and resorption in both cancellous and cortical bone. A number of factors are thought to be responsible for the bone loss.

We measured bone mass in aged people in relation to changes in calcium regulating hormones and sex hormones and investigated the role of these hormones in bone loss.

Materials and Methods

The bone mineral content and plasma parathyroid hormone, calcitonin, 1,25-(OH)²D and sex hormone levels were measured in 32 healthy aged subjects (17 men and 15 women), exhibiting normal laboratory findings, including hematological examination, liver function test, kidney function test and plasma protein analysis, ranging in age from 60 to 85 years and who were all leading ordinary lives. Complaints of a slight to moderate low back pain were present in 6 of the 17 men (35%), and in 7 of the 15 women (47%). Studies were also performed on 26 healthy younger subjects (15 men: 15-42 years old, 11 women: 17-40 years old) as controls. Bone mineral contents in the radius were measured by photon absorptiometry as previously reported (Yamamoto, 1976). Serum calcium, phosphorus and alkaline phosphatase levels were estimated by an automated analyzer (SMA 12/60). Ionized calcium was measured by a calcium ion analyzer (Orion Biomedical, Model SS-20).
Plasma levels of progesterone, pregnenolone, dehydroepiandrosterone, testosterone, dihydrotestosterone, androstenedione, and estradiol were determined by radioimmunoassay involving purification of steroid on celite columns as reported previously (Brenner, 1973, Guerrero, 1976). The sex steroids and their antisera were kindly donated by Dr. Egon Diczfalusy (Karolinska Institute, Sweden).

Plasma levels of 1,25-(OH)2D were measured by a competitive binding assay using rachitic chick intestinal cystosol as reported (Dokoh, 1979). Radioimmunoassay of calcitonin was performed using synthetic human calcitonin M and its goat antibody as reported previously (Morita, 1975).

**Calcium infusion test**

Calcium infusion test was carried out as follows: Calcium gluconate (15 mg of calcium per kg of body weight) was given intravenously in 500 ml of normal saline over a four-hour period. Venous blood was obtained at 0, 1, 2, 3 and 4 hr during the calcium infusion. All plasma samples were analyzed for calcium, ionized calcium and calcitonin.

**Radioimmunoassay of PTH with synthetic human (1-34) PTH**

A synthetic preparation of human (1-34) PTH, kindly donated by Dr. W. Rittel (Ciba-Geigy Ltd., Basle, Switzerland) was used for both radioiodination and standard. Antiserum was developed in a goat by repeated subcutaneous injections of a conjugate of synthetic human (1-34) PTH and complete Freund’s adjuvant. Iodination with 125I was carried out by the chloramine-T method to a specific activity of 100-200 µCi/µg. The labeled hormone was purified by a sequential gel filtration on Sephadex G25 (1×15 cm) and G75 (1×15 cm), and stored at -20°C. Serial dilutions of synthetic human (1-34) PTH containing 0 to 5,000 pg were made in 100 µl of assay buffer (10% hypoparathyroid serum, 0.05 M phosphate buffer, pH 7.5). To these (1-34) PTH standards or to 100 µl of test serum were added 100 µl of diluted antiserum (at final dilution of 1: 50,000) and 100 or 200 µl of the assay buffer. To equalize the protein concentrations in all standards and samples, 100 µl of hypoparathyroid serum was added to the standards tubes. Tubes containing the labeled hormone without antiserum served as controls with each standard curve.

The assays were preincubated at 4°C for 3 days without the labeled hormone. After addition of the tracer, the reaction mixture was incubated for an additional 2 days before separation. Phase separation was performed by the method of absorption with dextran-coated charcoal. The reaction tubes were equilibrated at 4°C for 1 hr after addition of 500 µl of dextran-coated charcoal suspension. Tubes were then centrifugated for 20 min at 3,000 rpm, separated by decantation, and counted in an automated counter for a period sufficient to yield a counting error of less than 1%. The assay was sensitive to about 80 pg/ml. The interassay coefficient of variation in sequential 10 assays was 11.0% for a pooled hyperparathyroid serum, assayed to be 4.2 ng/ml. The intrassay coefficient of variation of the pooled hyperparathyroid serum in 20 consecutive assays was 8.5%.

**EDTA infusion test**

Fifty mg per kg body weight of disodium ethylenediaminetetraacetate (EDTA) were given over a period of 60 min intravenously in 500 ml of normal saline containing 15 to 20 ml of 20% lidocaine. Venous blood sampling was done without tourniquet at 0, 1/2, 1 and 2 hr after the initiation of EDTA infusion with heparin in the collection tubes. All plasma samples were analyzed for calcium, ionized calcium and immunoreactive PTH.

**47Ca absorption test**

Intestinal calcium absorption was estimated by external counting with a whole body counter (Curtis, 1967). Examinees were placed on a constant diet of calcium and phosphorus (Ca 500 mg, P 1,000 mg per day) 1 week before the initiation of the test. On the first day, following an overnight fast, 10 nCi of 47Ca in 1 ml of saline was injected intravenously. Then the whole body count rate (A1) was measured by a low-level whole body counter. The whole body counting was made in both supine and prone position for 5 min, and the count rate was corrected for body background and radioactive decay. The coefficient of variation between duplicate determination of whole body activity was found to be 0.6%. Five days after the intravenous dose, the whole body count rate was again measured (A2). The five day fractional residual radioactivity in the body was given as (A2/A1).

On the 6th day, after overnight fasting, 500 nCi of 47Ca mixed with 100 mg of calcium as calcium gluconate in 100 ml of water, was given by mouth. The whole body count rate was determined immediately after the dose (B1) and again 5 days after the oral dose (B2). The five day fractional residual activity from the oral dose was given as (B2/B1), since the intravenous dose has little influence on the measurement of whole body activity from the 50 times greater oral dose. Then the absorbed fraction of 47Ca was expressed as (B2/A2)/(A2/A1).
Results

In serum calcium and alkaline phosphatase levels, no significant differences were observed between the control young subjects and the aged subjects in both sexes. However, ionized calcium showed lower values in about half of the aged subjects (Fig. 1). Serum inorganic phosphorus levels were distributed within the control range in a majority of the aged women, while the levels were lower in the aged men than those of the controls (Fig. 2). Fig. 3 shows changes in bone mineral content with age in the radial bone in 205 normal subjects, measured by photon absorptiometry. Bone mineral content increased rapidly in both sexes up to adolescence and thereafter there was little change until the age of 40. After the age of 40, it decreased slightly in men. In women, however, there was a significant and abrupt decline in bone mineral content, so that by age 70 to 80, bone mineral content was only 50% of that seen at age of 40.

![Fig. 1. Distribution of plasma ionized calcium levels in the aged subjects. Shaded areas represent the control range.](image1)

![Fig. 2. Distribution of serum inorganic phosphorus levels in the aged subjects. Shaded areas represent the control range.](image2)
Fig. 3. Age distribution of bone mineral content in the radius measured by photon absorptiometry.

- ●: man, ○: woman

### Plasma Levels for Sex Steroids and Gonadotropins in Aged Subjects

<table>
<thead>
<tr>
<th></th>
<th>Male aged subjects</th>
<th>Male controls</th>
<th>Female aged subjects</th>
<th>Female controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>testosterone (ng/ml)</td>
<td>4.35 ± 1.91</td>
<td>5.57 ± 1.34</td>
<td>0.13 ± 0.06</td>
<td>0.32 ± 0.19</td>
</tr>
<tr>
<td>androstenedione (ng/ml)</td>
<td>0.98 ± 0.39</td>
<td>1.18 ± 0.40</td>
<td>0.83 ± 0.43</td>
<td>1.66 ± 1.00</td>
</tr>
<tr>
<td>dehydroepiandrosterone (ng/ml)</td>
<td>1.76 ± 1.37</td>
<td>4.90 ± 2.28</td>
<td>2.35 ± 1.24</td>
<td>5.14 ± 3.40</td>
</tr>
<tr>
<td>pregnenolone (ng/ml)</td>
<td>0.58 ± 0.27</td>
<td>1.10 ± 0.35</td>
<td>0.54 ± 0.22</td>
<td>1.14 ± 0.64</td>
</tr>
<tr>
<td>estrone (pg/ml)</td>
<td>N.D.–34.4</td>
<td>N.D.–35.6</td>
<td>N.D.–21.2</td>
<td>54.0 ± 23.1</td>
</tr>
<tr>
<td>estradiol (pg/ml)</td>
<td>N.D.–117</td>
<td>N.D.–66.9</td>
<td>N.D.</td>
<td>66.7 ± 43.0</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>28.7 ± 23.9</td>
<td>N.D.–32.4</td>
<td>72.0 ± 22.1</td>
<td>20.3 ± 13.7</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>27.6 ± 21.8</td>
<td>5.0 ± 2.7</td>
<td>58.3 ± 26.9</td>
<td>11.8 ± 10.0</td>
</tr>
</tbody>
</table>

N.D.: not detectable
compared to 70% in men. The mean and standard deviation of plasma sex steroid and gonadotropin levels were listed in Table 1. In men, testosterone and androstenedione did not fall significantly with age, while dehydroepiandrosterone and pregnenolone were markedly decreased.

As regards to bone mass, among other steroids only testosterone levels showed a significant correlation \((r=0.60)\) with the bone mineral content. In women, estrone and estradiol levels were markedly reduced along with the loss of bone mass in the aged group.

Fig. 4 illustrates the relation between plasma testosterone and estradiol levels in relation to the bone mineral content in the aged subjects. In the aged men, most of the instances sustaining relatively high plasma testosterone levels also showed relatively high bone mineral content irrelevant to the estradiol levels. Four individuals with low testosterone (less than 2.4 ng/ml), however, showed a marked decrease in bone mass (less than 1.0 g/cm²). In the aged women, plasma estradiol levels were strikingly low with concomitant decrease in bone mass in all individuals.

As for the basal plasma PTH levels assayed with N specific antiserum, no significant differences were noted related to sex and age (Fig. 5). Then EDTA was infused and immunoreactive PTH and ionized calcium were measured. We employ-
ed a ratio of the increments of plasma PTH, $\Delta$-PTH, to the decrements of ionised calcium, $\Delta$Ca++, as an index to express the reactivity of the parathyroid glands. As shown on Fig. 6, many of the aged men, showed a fairly good response. In the aged women, however, all instances showed a very poor reactivity of the parathyroid gland. As regards plasma calcitonin levels, relatively low values were seen in the aged group of both sexes compared to the control young subjects (Fig. 7). Reactivity of C-cells in the thyroid in response to calcium infusion, also expressed as a ratio of the increments of plasma calcitonin to
those of plasma ionized calcium, tended to be low in the aged group compared to the controls (Fig. 8). The plasma levels of 1, 25-(OH)₂D, measured by a competitive binding assay, were distributed within the control range in a majority of the aged subjects (Fig. 9). It is noteworthy, however, that very low values were observed in some of the aged subjects. Intestinal absorption rate of ⁴⁷Ca tended to be low in the aged group compared to the control young subjects (Fig. 10).

**Discussion**

Although aging is not the direct result of deficiency states resulting from age-related hypofunction of the endocrine glands, aging does produce a variety of effects on hormone production, secretion and action. The age-related bone loss is thought to be a reflection of the multifactorial interaction of altered endocrine physiology. The significant correlation between the bone mineral content and the plasma testosterone, irrellevant to the estrogen levels, may lead to a speculation that testosterone might be an important determinant in maintaining bone mass in men. It is also speculated that estrogen might be important in women, possibly through the protective effects on bone (Orimo, 1972) and the possible effects on the conversion of 25-(OH)D₃ to an active form 1, 25-(OH)₂D₃ in the kidney (Tanaka, 1976).

In contrast to the sex steroids, age-related alteration is subtle in the calcium regulating hormones. Measured with N terminal specific antiserum, immunoreactive

![Graph](image-url)

**Fig. 9.** Plasma 1,25-(OH)₂D levels. Broken line represents the detection limit of 5 pg/ml.

**Fig. 10.** Intestinal ⁴⁷Ca absorption rate measured with a whole body counter.
PTH did not show significant differences between the aged and the control subjects. The reserve capacity of the parathyroid gland, however, was decreased in the aged women, indicating occult hypofunction of the parathyroid. It is noteworthy that the serum ionized calcium levels were distributed low in the may aged subjects. The low ionized calcium levels accompanied with normal PTH levels indicates that the feedback control between the blood concentration of ionized calcium and the PTH secretion from the parathyroid, which is strictly regulated in younger subjects, might be altered in senescence. C-cell function was reduced with age, both in basal secretion of calcitonin and in the reserve capacity. Plasma 1, 25-(OH)₂D levels and ⁴⁶Ca absorption rate tended to be low in the aged subjects comparing to the control young subjects. If the conversion of 25-(OH)D₃ to 1, 25-(OH)₂D₃ decreases with age, the result could be a decrease in rate of mineral turnover in bone. The possible importance of 1, 25-(OH)₂D₃ in aged subjects remains to be established by further study. Aging and calcium metabolism is characterized by a chronic negative imbalance in bone. A number of factors, such as genetics, diet, physical activity and sex hormones are generally considered contributory to the bone loss associated with age. In addition to these, hormonal imbalance occurring with age and its complex interaction appears to be the most important factor, and it would became more important to define these changes in order to devise both therapeutic and preventive measures for the bone loss associated with age.

References