Bone Densities in Patients Receiving Isotretinoin for Cystic Acne

Sancy A. Leachman, MD, PhD; Karl L. Insogna, MD; Lee Katz, MD; Alice Ellison, RN; Leonard M. Milstone, MD

Background: Few studies have been done of bone densities in humans receiving retinoids, despite a substantial amount of literature concerning retinoid-induced osteoporosis in animals. We prospectively measured bone density and calcium metabolism in young men (aged 17-25 years) receiving oral isotretinoin for cystic acne and in a group of healthy volunteers (aged 19-26 years).

Observations: Compared with that in healthy control subjects, mean bone density was lower at all sites (spine, femoral neck, and Ward triangle) and was considerably more variable at the spine in young men with cystic acne even before treatment. Bone density at the Ward triangle decreased a mean of 4.4% ($P= .03$) after 6 months of isotretinoin use (1 mg/kg of body weight). Four patients showed decreased density of more than 9% at the Ward triangle. The difference between the mean change in bone density in the patient group and in the control group was significant at the Ward triangle ($P=.04$) but not at the other sites. Measurements of calcium metabolism did not change over time in either group.

Conclusions: A loss of bone density occurring in the absence of measurable alterations of calcium metabolism is likely to be a direct effect of retinoids on bone. Further study of retinoid-induced osteoporosis in humans and of bone density in patients with cystic acne is needed.
PARTICIPANTS AND METHODS

STUDY DESIGN

Bone densities and indices of calcium metabolism were measured during a 6-month period in men receiving isotretinoin (1 mg/kg of body weight) for acne and in an age-matched untreated control group.

PARTICIPANTS

Subjects in the treatment group were referred from the dermatology faculty practice of Yale University School of Medicine, New Haven, Conn, or from local dermatologists for the treatment of cystic acne. Control subjects were recruited from local universities. Subjects were limited to men between the ages of 17 and 26 years to minimize age-related decreases in bone density and to avoid intrinsic differences in the response of men and women and the confounding effects of estrogen-containing oral contraceptives used by many women who take retinoids orally. Other exclusion criteria included a history of acne fulminans, the prior use of isotretinoin, the prior or current use of corticosteroids or diuretics, and a personal or family history of metabolic bone disease. The Human Investigation Committee of the Yale University School of Medicine approved this study. All participants gave written informed consent and were instructed not to make notable changes in their diets or to take supplemental vitamin A.

BONE DENSITY MEASUREMENTS

Bone densitometry was performed using absorptiometry (Hologic 1000 QDR, Hologic, Inc, Waltham, Mass) following the manufacturer’s recommended protocols. The manufacturer uses the following measurements of precision for this machine: lumbar spine, 0.00806 g/cm²; femoral neck, 0.0104 g/cm²; and the Ward triangle, 0.0161 g/cm². To ensure that there were no technical anomalies in the raw data and that the density readouts were correct, all scans were reanalyzed independently, in a blinded manner, by a company scientist.

At all sites in the patient group than in the control group (Figure 1). Two subjects receiving isotretinoin for the treatment of acne had densities at the spine that were more than 2 SDs below the reference means supplied by the manufacturer of the measuring device. Initial mean bone densities at all sites were lower in the patient group than in the control group (Table). In both groups, however, the mean densities were well within the age- and sex-matched reference ranges.

During the 6 months of observation, bone density decreased at all sites in both groups, except for an increase at the lumbar spine in the group receiving isotretinoin (Figure 2). Patients receiving isotretinoin had a mean decrease in bone density at the Ward triangle of 4.4% (P = .03) and an increase at the lumbar spine of 1.1% (P = .02). The mean change of −4.4% at the Ward triangle in the patient group was significantly different (P = .04) from the −0.01% change at the Ward triangle in the control group (Table).

Four of the 18 men treated with isotretinoin had a mean decrease in bone density at the Ward triangle of more than 9%. These 4 had 4 of the 5 lowest spine densities at the start of treatment. No significant differences in the other measurements of bone density or calcium metabolism characterized this group of 4 compared with the control group or the rest of the isotretinoin-treated men.

Serum and urine measurements of calcium metabolism were obtained at baseline and at 6 months, and selected values are shown in the Table. Serum calcium, phosphorous, PTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D concentrations were within the reference range at both times for every patient. A mean increase in the 25-hydroxyvitamin D levels in the patient group and a mean decrease in the urinary nephrogenous cAMP response to the calcium load in the control group were noted. These changes were not associated with significant differences in values between the patient and control groups.

An oral calcium tolerance test was used to demonstrate that men receiving isotretinoin absorbed oral calcium and had an appropriate rise in serum calcium lev-

METABOLIC MEASUREMENTS

Circulating levels of 25-hydroxyvitamin D were measured by a previously described competitive protein-binding assay using rat plasma as the source of vitamin D–binding protein. Circulating levels of 1,25-dihydroxyvitamin D were determined by a competitive protein-binding assay using calf thymus receptor protein for 1,25-dihydroxyvitamin D. The serum immunoreactive parathyroid hormone (PTH) concentration was measured using an antiserum to the midregion of human PTH. Bovine PTH (amino acids 37-84) labeled with iodine I 125 was used as a tracer with standards from a human PTH adenoma extract, as previously described. Urinary and plasma cyclic adenosine monophosphate (cAMP) levels were measured by radioimmunoassay, and nephrogenous cAMP was calculated using the measured plasma and urine values and serum creatinine concentrations. The renal tubular maximum for the absorption of phosphorus was normalized to the glomerular filtration rate. The oral calcium tolerance test was performed as previously reported following an overnight fast after subjects had been receiving a low-calcium diet for 10 days. Briefly, baseline blood and urine specimens were collected between 7 and 9 AM. At 9 AM, oral calcium, 1 g, was given, and blood and urine specimens were again collected between noon and 1 PM after an equilibration period from 9 to 11 AM. Total serum and urinary calcium values were determined by atomic absorption spectrometry (model 2380; Perkin-Elmer Corp, Norwalk, Conn). Serum and urinary phosphorous levels were determined by a colorimetric method adapted for the automated centrifugal analyzer (Gemini; Electro-Nucleonics Inc, Silver Springs, Md). Serum creatinine, electrolyte, triglyceride, cholesterol, aspartate and alanine aminotransferase, alkaline phosphatase, and bilirubin concentrations were measured by standard automated methods.

DATA ANALYSIS

Each parameter was analyzed using paired t tests. Differences in changes between groups were assessed using t tests, adjusted for unequal variances where necessary.
els and an appropriate fall in nephrogenous cAMP concentrations (Table). The response to the calcium tolerance test after 6 months of isotretinoin treatment was the same as the response at baseline. The response in the patient group was not significantly different from that in the control group.

**COMMENT**

The decrease in bone density at the Ward triangle in our patients who received isotretinoin should not be surprising. Research published during the past 70 years has demonstrated that the profound effects of retinoids on bone growth and mineralization are dependent on the dose and duration of treatment. Normal bone growth and development require adequate levels of vitamin A.36 Animals fed excess vitamin A or synthetic retinoids have accelerated bone remodeling10,37 that leads to poor bone growth, radiolucency, loss of mineral content,38-40 and spontaneous fractures.18,41,42 Retinoids act directly on bone, causing demineralization of intact bones in vitro43,44 and the activation of isolated osteoblasts45 and osteoclasts.46 Retinoids act by binding to a family of nuclear receptors, and each receptor shows tissue-specific and developmentally regulated expression. In evaluating the risk-benefit ratios of retinoids that have therapeutic actions on skin, the finding47,48 that the retinoic acid receptor is predominantly expressed in skin, cartilage, and developing bone is likely to be important, although still cryptic.

Hypervitaminosis A causes bone demineralization and even fractures in humans, most of which have occurred in children.12,17 Yet, a number of considerations fostered the hope that these toxic effects of vitamin A on the skeleton might not be a problem in adults using synthetic retinoids such as isotretinoin. First, the synthetic retinoids available for clinical use were marketed because of their tissue-selective effects: compared with vitamin A, they have relatively strong effects on epithelial tissues and weak effects on bone.18 Second, it was hoped that skeletal toxic reactions might not be directly relevant to synthetic retinoid use in adult humans. Most of the experiments in animals have been done on growing rather than adult bones, and most of the toxic effects of vitamin A in humans have been observed in children.

![Figure 1. Bone mineral densities were measured during a 6-month period in control subjects and in patients receiving isotretinoin for acne. Each line represents 1 patient; the baseline measurement is at the left end of the line, and the 6-month measurement is at the right end.](image)

### Measurements of Bone Density and Calcium Metabolism*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group (n = 14)</th>
<th>Patient Group (n = 18)</th>
<th>Intergroup Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 6 mo % Change</td>
<td>Baseline 6 mo % Change</td>
<td>% Difference† P‡</td>
</tr>
<tr>
<td>Bone density, g/cm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>1.102 ± 0.062 1.101 ± 0.080</td>
<td>1.011 ± 0.120 1.022 ± 0.123</td>
<td>1.14 .02 1.20 .07</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>1.039 ± 0.119 1.021 ± 0.103</td>
<td>0.928 ± 0.116 0.915 ± 0.117</td>
<td>−1.33 .09 0.20 .85</td>
</tr>
<tr>
<td>Ward triangle</td>
<td>0.949 ± 0.123 0.948 ± 0.122</td>
<td>0.843 ± 0.127 0.805 ± 0.138</td>
<td>−4.43 .03 4.42 .04</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, mmol/L (mg/dL)</td>
<td>2.38 ± 0.08 (9.5 ± 0.3)</td>
<td>2.38 ± 0.08 (9.5 ± 0.3)</td>
<td></td>
</tr>
<tr>
<td>Phosphate, mmol/L (mg/dL)</td>
<td>0.98 ± 0.15 (3.9 ± 0.6)</td>
<td>0.98 ± 0.11 (3.9 ± 0.45)</td>
<td></td>
</tr>
<tr>
<td>Parathyroid hormone, (pg/dL)</td>
<td>112 ± 18 (157 ± 85)</td>
<td>137 ± 95 (138 ± 22)</td>
<td>22.4 .06 20.4 .20</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D, nmol/L</td>
<td>59.7 ± 7.7 (59.2 ± 7.7)</td>
<td>53.4 ± 11.7 (59.4 ± 16.0)</td>
<td>13.4 .02 6.4 .45</td>
</tr>
<tr>
<td>1,25-Dihydroxyvitamin D, pmol/L</td>
<td>115.2 ± 21.1 (121.7 ± 23.1)</td>
<td>104.8 ± 21.8 (116.7 ± 22.9)</td>
<td>18.2 .11 17.8 .22</td>
</tr>
<tr>
<td>Response to calcium load</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔSerum calcium, mmol/L (mg/dL)</td>
<td>0.12 ± 0.07 (0.50 ± 0.29)</td>
<td>0.09 ± 0.08 (0.35 ± 0.30)</td>
<td>1.3 .08 0.9 .58</td>
</tr>
<tr>
<td>ΔUrine nephrogenous cAMP, nmol/L §</td>
<td>−3.9 ± 1.2 (−3.5 ± 1.3)</td>
<td>−4.0 ± 1.5 (−4.5 ± 1.2)</td>
<td>−0.1 .97 4.8 .18</td>
</tr>
</tbody>
</table>

*Values are given as group mean ± SD. cAMP indicates cyclic adenosine monophosphate.
†Difference indicates mean change in patient group minus the mean change in the control group.
‡Adjusted for unequal variances.
§Glomerular filtrate calculated according to Broodus et al.33

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Although several reports have suggested that isotretinoin use is associated with radiolucency of long bones and abnormal findings on bone scans, 3 recent patient series published following the initiation of our study were reassuring in their assessment of isotretinoin use in adults. None found deleterious effects of isotretinoin use on measurements of bone density.

Why are the results and conclusions of the bone density measurements in those 3 series different from ours? Kocijancic prospectively measured bone densities at a single location, the lumbar spine, in males receiving isotretinoin for acne. He attributed a small increase in density of the spine to an age-expected change at that site. We, too, saw a small increase in density of the spine and would add that mild isotretinoin-induced calcification of the anterior longitudinal spinal ligament, which has been reported in patients receiving isotretinoin for acne, might contribute to that increase. DiGiovanna et al took single measurements of bone density in patients who had received retinoids long term for inherited disorders of keratinization and compared them with those of age- and sex-matched “normals” provided by the manufacturer of the densitometer. The use of etretinate, but not of isotretinoin, was associated with low bone density at the Ward triangle compared with the reference values. Reservations about that report have been raised because of the heterogeneity of the patient population in underlying diagnosis, age, sex, and ancillary treatments and because some patients had diseases that predisposed them to elevations in PTH levels and abnormalities in calcium metabolism. In addition, long-term therapy with retinoids would have predisposed those patients to widespread ligamentous calcifications that could confound the density measurements. Margolis et al prospectively studied patients who received isotretinoin for acne. In contrast to our study, they identified no effect of isotretinoin on bone density. The Margolis study differed from our study in several respects: it lacked a control group; it used a variable and, on average, lower dose of isotretinoin (0.89 mg/kg); the treatment course lasted 20 weeks instead of 28 weeks; both men and women (many of whom were using oral contraceptives) were enrolled; and the patients’ ages ranged from 19 to 35 years.

Our study, too, has flaws and raises questions. Larger samples might have added weight to the statistical differences and allowed a detection of changes in addition to the change at the Ward triangle. It is regrettable that we have no measures of bone turnover, such as osteocalcin or procollagen telopeptide levels. A randomized, prospective comparison of treated and untreated patients with cystic acne would have been better. We question, however, whether administering a mildly toxic medicine to healthy persons or withholding a uniquely effective treatment from young men with cystic acne is justified. Other impediments to prospective studies of retinoid effects on bones—such as the reluctance of insurers to pay for monitoring tests—have been discussed; perhaps our identification of a potentially serious toxic effect now provides adequate justification for such a study.

Retinoid dosage and individual susceptibility may each be determinants of toxicity. Case reports and experimental studies generally point to the importance of dosage in provoking more serious toxic effects, such as hypercalcemia. Our inability to detect measurable changes in PTH levels or in calcium and vitamin D metabolism is consonant with other studies of comparable doses and suggests that subtle, direct effects of isotretinoin on bone cause the loss of bone density at the Ward triangle without causing hypercalcemia.

The baseline findings of generally low bone densities and great variability of spine density in the patients with acne were unexpected and deserve further study. That patients with acne fulminans have lytic lesions in bone, but patients with cystic acne apparently do not, is well known. In our series, the 4 persons showing the greatest decrease in bone density at the Ward triangle had 4 of the 5 lowest baseline densities at the spine. That observation suggests that some persons may be at increased risk for retinoid-induced osteoporosis at the Ward triangle and that they might be identifiable by pretreatment measurements of spine density. Villablanca et al have suggested that individual susceptibility may be a determinant of retinoid-induced toxic effects: peak serum concentrations of retinoid did not correlate with the degree of hypercalcemia after high doses of isotretinoin were given for neuroblastoma.

The important question raised by our study is the clinical significance of a mean 4% decrease in bone density at the Ward triangle in young men. In a dietary study of Scandinavian women, a high intake of vitamin A was associated with osteoporosis—most notably at the Ward triangle—and with an increased risk for hip fracture. Is progressive demineralization likely in persons who need long-term retinoid therapy for chemoprevention or for chronic, serious skin disease such as psoriasis or ichthyosis? Is the isotretinoin-induced osteoporosis at the Ward triangle reversible after short courses of retinoids? Are some persons predisposed or sensitized to the demineralizing effect of retinoids, and how can those persons be identified?

As the clinical uses for retinoids expand, it is important to determine whether retinoid-induced loss of bone density is reversible, whether it is progressive with longer courses of treatment, and whether the effect applies to all treatment populations and to all retinoids having a thera-
peutic action on skin. If the loss of bone density leads to more serious medical sequelae, such as fractures, can preventive measures, such as dietary supplements of calcium, vitamin D, or vitamin E, prevent retinoid-induced demineralization without increasing the risk of ligamentous calcifications or decreasing efficacy? Further studies are needed so that this unique and potent class of drugs can be used with the highest possible margin of safety.

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REFERENCES


