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Serum Levels of Vitamin D Metabolites in Isotretinoin-treated Acne Patients

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Serum levels of vitamin D metabolites were determined in 11 patients treated for cystic acne with a four-month course of isotretinoin (Roaccutane©). The levels were measured before treatment and after two months of medication. We found a significant fall in the level of 1,25-dihydroxyvitamin D ($p < 0.01$) and a significant increase in the molar ratio of 24, 25-dihydroxyvitamin D to 25-hydroxvitamin D ($p < 0.05$). No significant changes were found for the vitamin D metabolites 25-hydroxyvitamin D or 24,25-dihydroxy-vitamin D, for serum calcium, phosphorus, alkaline phosphatase or parathyroid hormone. Our data indicate early changes in the metabolism of vitamin D in patients on retinoid treatment. ***Key words: 1,25 dihydroxyvitamin D; Retinoid; Skeletal metabolism.***

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Both isotretinoin and etretinate have been reported to cause skeletal side-effects comparable to those of hypervitaminosis A and mimicking diffuse idiopathic skeletal hyperostosis (DISH) (1–6). Most commonly observed are hyperostosis and ossification of ligaments, osteoporosis, periosteal thickening, reduced cortical thickness and premature epiphyseal closure (6–8). The skeletal changes are identical for etretinate and isotretinoin, but seem to appear earlier with isotretinoin (3). The skeletal effects have mainly been noted during long-term therapy. However, minor changes have also appeared during low-dose short-term therapy for cystic acne with isotretinoin (9). The biochemical mechanisms by which the retinoids influence skeletal tissue have not been fully explained. One suggested mechanism is that the retinoids have a direct effect on the bone substance (10). Another possible mechanism is that retinoids may interfere with calcium-regulating hormones such as vitamin D and PTH (11, 12). It has been shown in rats that retinoids cause reduced serum levels of the active vitamin D metabolite 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$) (12, 13). The present study was designed to ascertain whether short-term treatment with isotretinoin in acne causes changes in the vitamin D and calcium metabolism in humans.

MATERIAL AND METHODS

We examined 11 patients (9 men and 2 women, mean age 24.2; range 15 to 46) treated for cystic acne with a four-month course of isotretinoin (Roaccutane©). The dosage given was 0.5–1 mg/kg/day. The patients had no known skeletal, endocrine, renal or gastrointestinal disorders. They were not using anticonvulsant or glucocorticoid medication and had not been treated with tetracyclines during the two months prior to the isotretinoin treatment. Blood samples were taken before treatment started and again after two months of medication. The samples were taken throughout most of the year: the first patient entered the programme in March 1990, the last one in December 1990. The following analyses were performed: full blood count, serum albumin, serum creatinine, alkaline phosphatase, transaminases, serum cholesterol, triglyceride, serum calcium, phosphorus and serum parathyroid hormone levels (PTH). The PTH analyses were performed by a radioimmunoassay measuring the intact-chain hormone (IRMA, Allegro, Nichols institute).

Vitamin D measurements

Separate serum samples for vitamin D analysis were immediately centrifuged and frozen at −20°C. The vitamin D metabolites 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$), 24,25-dihydroxyvitamin D ($24,25\text{-}(OH)_2D$) and 25-hydroxyvitamin D (25-OHD) were measured in 1,5 ml serum. The serum samples were extracted by diethylether, and the vitamin D metabolites separated and purified in open silicic acid columns and HPLC before measurements in competitive protein binding assays were carried out, as described earlier (14, 15). Since the serum concentrations of $24,25\text{-}(OH)_2D$ are linearly correlated to its precursor, 25-OHD, the $24,25\text{-}(OH)_2D$ values are also expressed as the molar ratio to the corresponding 25-OHD concentration (16).

Statistics

Statistical analyses were performed using matched-pair Student's *t*-test. The level of significance selected was 5% ($p < 0.05$).

RESULTS

The mean serum levels of the vitamin D metabolites are shown in Table I. A significant fall in the concentration of 1,25-

Table I. *Mean serum levels (± SD) of vitamin D metabolites measured before and two months after start of isotretinoin treatment*

	$1,25\text{-}(OH)_2D$ pmol/l $n = 11$	25-OHD nmol/l $n = 11$	$24,25\text{-}(OH)_2D$ nmol/l $n = 11$	$24,25\text{-}(OH)_2D \times 100$ 25-OHD $n = 11$
Before treatment	88.4±20.4	75.5±32.2	4.0±2.8	5.1±1.9
Two months after start of treatment	75.0±18.5*	72.6±34.4	4.7±2.5	6.8±3.0†

* Significantly different from pretreatment values, $p < 0.01$.
† Significantly different from pretreatment values, $p < 0.05$.

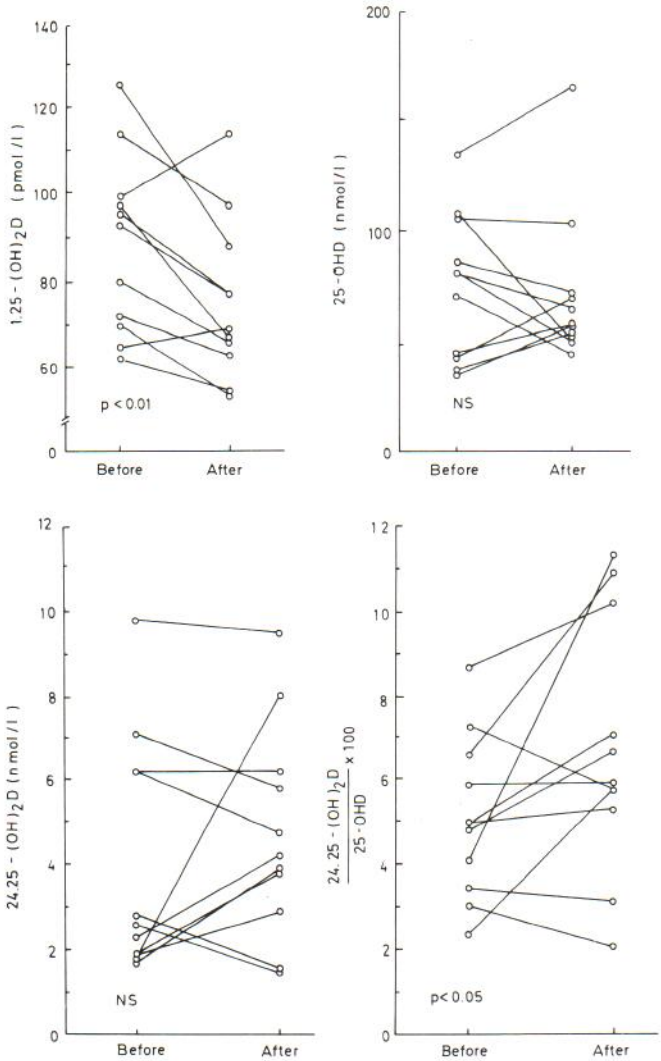

Fig. 1. Individual serum levels of 1,25-$(OH)_2D$, 25-OHD, 24,25-$(OH)_2D$ and the ratio 24,25-$(OH)_2D$/25-OHD before and two months after start of treatment with isotretinoin.

$(OH)_2D$ was observed after two months of treatment ($p < 0.01$). Individual serum levels of 1,25-$(OH)_2D$ are shown in Fig. 1. Nine out of 11 patients showed a notable fall in 1,25-$(OH)_2D$ serum concentration. We also found a significant increase in the relative levels of 24,25-$(OH)_2D$, expressed as the molar ratio of 24,25-$(OH)_2D$ to 25-OHD ($p < 0.05$), as shown in Fig. 1. No significant changes were observed in the serum concentrations of 25-OHD or 24,25-$(OH)_2D$, nor did we find significant changes of serum calcium, serum calcium corrected for albumin concentration, phosphorus, alkaline phosphatase or parathyroid hormone. Except for slight elevations in liver enzymes and serum triglycerides, no other laboratory changes were noted.

DISCUSSION

To the best of our knowledge this is the first study to show a reduction in serum levels of the active vitamin D metabolite 1,25-$(OH)_2D$ during retinoid treatment in humans. However, our findings are in line with results reported from animal models (12, 13). The observed reduction of 1,25-$(OH)_2D$ might be explained as a direct suppressive effect of the retinoids on the 1-alpha-hydroxylation in the kidneys (12). Trechsel & Fleisch have shown that retinol directly reduces the in vivo synthesis of 1,25-$(OH)_2D$ in rats (17). Another possible mechanism is that the retinoids have a direct effect on bone substance as a result of enhanced osteoclastic bone resorption (10) inducing secondary changes of the calcium and vitamin D metabolism. If stimulated osteoclastic bone resorption is the primary effect and the observed reduction of 1,25-$(OH)_2D$ just a secondary effect, one would expect to find elevated levels of serum calcium and reduced levels of PTH. Such changes in serum calcium and PTH were reported by Frankel et al. (12) studying the effects of hypervitaminosis A in rats. In our study, however, no changes were observed in either PTH or serum calcium. These findings might support the hypothesis that the retinoids have a direct effect on 1-alpha hydroxylation. It is possible, however, that the retinoids have both an effect on bone substance through the stimulation of osteoclastic bone resorption, and a suppressive effect on 1,25-$(OH)_2D$ synthesis in the kidneys.

In animals (18) as well as in humans (16) it has previously been shown that the synthesis of 1,25-$(OH)_2D$ and 24,25-$(OH)_2D$ is normally regulated in a reciprocal fashion. This would explain our observation of an increase in the molar ratio of 24,25-$(OH)_2D$ to 25-OHD as the serum levels of 1,25-$(OH)_2D$ fall.

Our investigations show early changes in vitamin D metabolism in patients on retinoid therapy. Such changes may have clinical relevance even for patients on a short-term low-dose regimen. However, our findings are based on a limited number of patients and more extensive studies are required to clarify the relationship between retinoids and vitamin D. The impact of changes in vitamin D metabolism on skeletal metabolism during retinoid therapy also remains to be further clarified.

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