THE ABSORPTION OF CALCIUM AND ITS INCORPORATION INTO BONE DURING CORTICOSTEROID THERAPY.

Allan Geoffrey Need

Department of Medicine

University of Adelaide

Adelaide

and

Division of Clinical Chemistry

Institute of Medical and Veterinary Science

Frome Road

Adelaide

South Australia

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DECLARATION

I declare that the work described herein contains no material that has been accepted for the award of any degree or diploma in any university and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference is made in the text.

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ABSTRACT

Previous studies indicate that corticosteroid osteoporosis is unique in that it is marked by both an increase in bone resorption and a decrease in the bone formation rate. The increased bone resorption has been demonstrated in this series by an increase in urinary hydroxyproline excretion which has been shown to be associated with decreased radiocalcium absorption and increased urinary calcium excretion. An index (radiocalcium absorption - calcium excretion) discriminated between osteoporotic (OP) and non-osteoporotic (N) corticosteroid-treated cases better than any parameter alone.

When radiocalcium absorption was regressed on the serum 1,25-dihydroxycalciferol (1,25D) level it was found that the slope was normal for the N patients (0.0050 ± 0.0010) but significantly flatter for the OP patients (0.0024 ± 0.0008; $p<0.001$). This indicates a decreased efficiency in the gut response to 1,25D in the OP patients.

Treatment of the calcium malabsorption with 1,25D caused the hourly fractional radiocalcium absorption to rise from 0.37 ± 0.04 to 0.64 ± 0.07 of the dose per hour ($p<0.01$) and, when combined with a calcium supplement, a fall in urinary hydroxyproline excretion to normal.

The radiokinetic bone formation rate was found to be decreased in patients on corticosteroids, when they were compared with postmenopausal osteoporotic women. Treatment with nandrolone decanoate increased the bone formation rate

from 45±17 to 134±37 mg Ca/d ($p<0.025$) in those given nandrolone alone.

The effects of nandrolone on the forearm bone mineral density, measured by photon absorptiometry, were investigated in a cross-over trial. There was a significant (time-weighted) gain in bone density on nandrolone (+1.6±0.6 mg/ml/month;$p<0.05$) and a significant (time-weighted) loss off the drug (-1.3±0.3 mg/ml/month;$p<0.01$). The difference between these 2 rates was highly significant ($p<0.001$).

The results confirm that corticosteroid osteoporosis is associated with a)decreased intestinal calcium absorption and b)increased urinary calcium excretion and a new index, combining both parameters, has been developed which discriminates well between osteoporotic cases and those with normal spines ($p<0.0001$). This may be useful for predicting the risk of the disease in any individual patient on corticosteroid therapy. The biochemical response to calcitriol and calcium therapy suggests that this is a useful combination for treatment and the response of bone mineral density to nandrolone suggests that further improvement may be gained from anabolic steroid therapy which appears to correct the abnormality in bone formation.

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I am truly indebted to all those patients who, in the presence of serious illness, volunteered for investigations aimed at increasing knowledge of corticosteroid osteoporosis, hoping that the future for others on corticosteroids may be less uncomfortable.

This work, I trust, demonstrates that the further study of glucocorticoid drugs and their side effects will be of help to those receiving them and, furthermore, suggests some new approaches which may be used in the study of more common forms of osteoporosis.

CHAPTER ONE

REVIEW OF THE LITERATURE

Clinical features of steroid osteoporosis.

Harvey Cushing, when first describing the syndrome of endogenous hypercortisolism (Cushing,1932), noted a "marked thoracic kyphosis" in 6 out of 12 patients and an "increased tendency to fracture". These skeletal effects are now recognized as serious side effects of treatment with cortisone and its analogues. Indeed, shortly after the first report of the remarkable clinical efficacy of cortisone (Hench et al,1949) descriptions appeared of fractures through bone which had become osteoporotic during such therapy (Boland and Headley,1950;Demartini et al,1952;Curtiss et al,1954). The vertebrae and ribs are most often affected (Reifenstein,1956) and fractures may appear within weeks of beginning therapy. As well as causing pain and restricting physical activity, these fractures can lead to a worsening of respiratory function, in asthmatic patients, by splinting of the chest wall (by rib fractures) and by reducing vital capacity (with vertebral fractures causing a reduction of trunk height and increasing dorsal kyphosis).

Prevalence in Cushing's syndrome.

The prevalence of osteoporosis in Cushing's syndrome is difficult to assess because there is no generally accepted definition of osteoporosis. An early review (Eisenhardt and Thompson,1939) noted "osteoporosis" to be present, on standard x-ray films, in 53 out of 61 cases of Cushing's syndrome. Sprague et al (1956) estimated that at least 40% of such patients became osteoporotic, Reifenstein (1958) estimated the prevalence at 91%, Soffer et al (1961) at 50% and Urbanic and George (1981) estimated it at 48%, with women being affected more often than men (Reifenstein,1956). These estimates were made by subjective judgement about vertebral translucency in standard radiographs.

A more objective estimate of the prevalence of osteoporosis in patients with Cushing's syndrome may be obtained by analysing the proportion of such patients with fractures. Sprague et al (1956) reported that 20 out of 100 such patients (20%) had fractured ribs. Howland et al (1958) reported that 29 out of 69 cases (42%) had vertebral fractures . Forty six (67%) of these patients had rib fractures and 6 (9%) had pelvic fractures. Soffer et al (1961) reported that 20 out of 52 patients (38%) had pathological fractures and these were mainly in the spine.

Prevalence during Corticosteroid therapy.

The prevalence of osteoporosis during steroid therapy is also difficult to assess, especially as the diseases for which corticosteroids are prescribed (rheumatoid arthritis and renal disease, for example) are commonly associated with

osteoporosis themselves. McConkey et al (1962) reported that steroid therapy for rheumatoid arthritis did not increase the prevalence of osteoporosis, as judged by spine radiographs, but 5 out of 61 steroid treated patients (8%) in their series had vertebral fractures and none of those not on steroids. Saville and Karmosh (1967) found a detrimental effect of steroids on the spine, but only in patients over 50 years old. The prevalence of osteoporosis in asthmatic patients on steroids has been reported to be nearly twice that of those not on steroids (Arnoldsson,1958) but Mueller (1976), using forearm photon densitometry, claimed that corticosteroid therapy caused no bone loss in asthmatic patients.

On analysing the prevalence of fractures in patients on long term corticosteroid therapy (table1.1), a more consistent picture emerges. Steinbrocker et al (1951) found that 2 out of 128 patients (2%) on long term corticosteroid therapy had fractures (1 vertebral and 1 humeral), Boland (1951) found 3 out of 76 (4%), Burrage et al (1955) found 5 out of 30 patients (17%) to have fractures, Arnoldsson (1958) found 3 vertebral and 2 pelvic fractures in 142 patients (4%), Kendall (1960) found fractures in 5 out of 70 patients (7%), McConkey et al (1962) found 5 out of 61 corticosteroid treated patients (8%) had fractures and none of 36 non corticosteroid-treated rheumatoid patients while Saville and Kharmosh (1967) found 7 out of 42 corticosteroid treated (17%) and 9 out of 52 non corticosteroid treated (17%) had fractures. Adinoff and Hollister (1983) found fractures in 14 out of 128 corticosteroid treated (11%) and none of 58 non corticosteroid-treated patients with asthma.

AUTHORS	DATE	PREVALENCE OF FRACTURES
Steinbrocker et al	1951	2/28 (2%)
Boland	1951	3/76 (4%)
Burrage et al	1955	5/30 (17%)
Arnoldsson	1958	5/142 (4%)
Kendall	1960	5/70 (7%)
McConkey et al	1962	5/61 (8%)
Saville and Karmosh	1967	7/42 (17%)
Adinoff and Hollister	1983	14/128 (11%)

Table 1.1.
Prevalence of fractures of all types reported in retrospective surveys of corticosteroid-treated patients.

These surveys, being retrospective, can be considered as minimum estimates of the prevalence rates, especially in the earlier studies where awareness of fractures may not have been as widespread as it was later. In a prospective study Adinoff and Hollister (1983) found that fractures developed in 8 out of 19 corticosteroid-treated patients (40%) and 0 out of 11 non corticosteroid-treated patients with similar degrees of severity of asthma. Thus the incidence of corticosteroid-induced fractures may be higher than generally supposed. Finally, Luder (1954) and Chesney et al (1978) have reported the occurrence of osteoporosis in children on steroid therapy.

It is clear that osteoporosis is a common complication of corticosteroid therapy but it probably occurs less frequently in these patients than in those with Cushing's syndrome. Postmenopausal women (Saville and

Karmosh,1967;Arnoldsson,1958;McConkey et al,1962),the elderly and immobilized (Reifenstein,1958) and children (Luder,1954;Chesney,1978) appear to be most at risk.

Objective assessments of bone demineralization

No useful series of bone mass assessments in patients with Cushing's syndrome is known to the author. Measurements of bone mass during corticosteroid therapy, however, have been reported in 13 studies. Saville and Karmosh (1967) measured the cortical thickness of the radial shaft in patients with rheumatoid arthritis and found it significantly reduced in patients who had been treated with corticosteroids when compared with similar patients who had not received corticosteroids. Gallagher et al (1973) found that the metacarpal cortical area to total area ratio was below the mean for age in 16 patients on corticosteroids, with 13 of the 16 measurements being below the normal mean and 3 being more than 3 standard deviations below the mean. Similar decreases in per cent bone volume were found on examining the trabecular bone histology in these patients.

Mueller (1976), using photon densitometry, found no decrease in forearm bone mineral content in 114 asthmatic patients whether or not they had been treated with corticosteroids. These findings are at variance with those of Hahn (1978) who found that radial metaphyseal bone mineral content decreased by 11.6±2.8% in 16 patients after 1-3 years and by 39.3±2.0% in 33 patients after 3 years glucocorticoid therapy in patients with rheumatoid arthritis. Diaphyseal bone mineral content decreased by 6.3±3.1% at 1-3 years and

17.4±2.1% after 3 years in the same patients. As trabecular bone makes up 75% of metaphyseal bone and only 10% of diaphyseal bone (Schlenker and von Segg&n,1976), the results suggest that trabecular bone is lost preferentially during corticosteroid therapy. Similar findings were reported by Chesney et al (1978) where 25 children treated with corticosteroids for renal disease had a mean reduction in forearm bone mineral mass of 16.7±1.2% ($p<0.001$) when compared with 768 controls).

In 1979 Bressot et al reported a decreased per cent bone volume in histological sections from 51 corticosteroid treated female patients (10.6±0.04%) compared with that in 109 controls (16.0±0.03%,$p<0.05$). Gluck et al (1981) showed that 10 patients on alternate day corticosteroids had decreased cortical bone mass in the forearm (95% of normal) as did 15 patients on daily therapy (90% of normal). The decreases in trabecular bone mass in the forearm (87% and 86% of normal respectively) were greater. Nordin et al (1981) reported histological findings in 32 corticosteroid treated patients where the per cent bone volume was 11.4±0.80%, compared with 17.6±0.59% in controls ($p<0.001$).

Reid et al (1982) found the total body calcium content, by neutron activation analysis, to be decreased by 11.5% ($p<0.01$) in males and 15.5% ($p<0.001$) in females with rheumatoid arthritis on corticosteroid therapy while similar patients not on corticosteroid therapy had decreases of 5.3% ($p<0.05$) and 6.8% ($p<0.01$) respectively. Greenberger et al (1982) found the combined cortical thickness in metacarpals to be at or below the mean for age in 21 corticosteroid

treated individuals ($p<0.001$), with 19% having values more than 2 standard deviations below the mean. Rickers et al (1982) showed that the forearm bone mineral content, measured by photon absorptiometry, could decrease by 2.5% in 6 months after the initiation of corticosteroid therapy in patients with haematological disorders. Finally, Adinoff and Hollister (1983) found the forearm bone mineral content to be decreased by a mean of 1.4 standard deviation units in 128 corticosteroid-treated asthmatic patients when compared with 98 normal controls ($p<0.01$). This loss was thought to be greater in the trabecular than the cortical bone because of a greater fall in distal than proximal radial density, there being a greater proportion of trabecular bone distally (Schlenker and von Seggen,1976). Asthmatic patients with similar degrees of respiratory dysfunction, but who received either intermittent corticosteroid therapy, or none at all, had no significant decrease in bone mineral content. Finally, Rickers et al (1984) reported similar findings in 31 patients treated with prednisolone for 24 weeks where a 2.5% loss of proximal forearm bone mineral and a 3.5% loss of distal (more trabecular) forearm bone mineral were noted.

Thus it can be said that long term continuous corticosteroid therapy does lead to a significant loss of bone, and that intermittent therapy may be less hazardous in this respect. Bone mineral content has been related to bone strength (Dalen et al,1976;Carter et al,1976), and thus loss of bone is almost certainly the cause of the large number of fractures which occur during steroid therapy. The mechanisms by which corticosteroids cause this bone loss will be

examined in this thesis.

Effects of corticosteroids on calcium metabolism

Several authors have shown that the adrenal glands have effects opposite to those of the parathyroid glands. On the one hand, adrenalectomy leads to a rise in plasma calcium concentration (Loeb,1932;Sprague et al,1953;Myers,1962). Such a rise has also been reported with the onset of hypoadrenalism in the presence of hypoparathyroidism (Forbes,1956). On the other hand, glucocorticoids tend to lower the plasma calcium level (Stoerk et al,1963) and are used in the treatment of hypercalcaemia (Anderson et al,1954;Connor et al,1956;Henneman et al,1956;Morgan et al,1956;Verner et al,1958;Slater et al,1959;Mundy and Martin,1982), although it must be admitted they are not usually effective in cases of primary hyperparathyroidism (Dent,1956).

Parathyroid hyperplasia has been reported in Cushing's syndrome in man (Wajchenberg,1965) and following glucocorticoid therapy in animals (Williams et al,1974). However the observation of Forbes, already mentioned, and that of Segerstrom and Ohrn (1972) that cortisone further lowered the plasma calcium in parathyroidectomized rats show that parathyroid hormone and corticosteroids can act independently. Corticosteroids do not consistently lower the plasma calcium in the normal intact animal(Fox et al,1978) and the evidence supports the hypothesis that parathyroid hormone secretion increases to maintain the plasma calcium during corticosteroid therapy. This normally overcomes any

tendency for corticosteroids to produce hypocalcaemia.

Aetiology of steroid osteoporosis.

Reifenstein (1956) believed that glucocorticoid therapy produced an imbalance between catabolic effects (cortisol-like) and anabolic effects (androgen-like) and that the resultant decrease in formation of bone protein matrix led to osteoporosis. He suggested that anabolic steroid therapy would reverse the bone loss during corticosteroid therapy. Studies since that time, as already alluded to, have suggested, however, that steroid osteoporosis is associated with abnormalities of calcium metabolism leading to increased bone resorption (Bunim et al,1958;Baylink,1983). Furthermore, correction of these abnormalities with calcium and vitamin D may reverse the bone loss suffered by patients taking glucocorticoids (Hahn et al,1979).

It is obvious that osteoporosis may be caused by either decreased bone formation, increased bone resorption or both, and therefore it is necessary to consider both factors in a study of steroid osteoporosis.

The bone formation rate.

The skeleton is continually remodelling, with an annual turnover up to 25% in adults (Parfitt,1980). Bone resorption is a function of osteoclasts, which are stimulated to resorb bone by parathyroid hormone. Bone formation, on the other hand, is a function of osteoblasts which are responsible for production of a protein matrix (osteoid) and the subsequent mineralization of it. The factors controlling bone formation

are poorly understood but there is evidence for a defect in this function in corticosteroid treated patients, as outlined below.

Histomorphometry of bone biopsy samples from corticosteroid treated patients, in addition to showing a decrease in the amount of bone contained within the biopsy, shows a decrease in thickness of the osteoid seam (Jowsey and Riggs,1970;Bressot et al,1979;Hahn et al,1979). Decreased osteoblast numbers have been reported by Kendall (1960) and decreased bone forming surfaces by Riggs et al (1966). In two studies where calcification fronts were labelled in vivo, with two courses of oral tetracycline fourteen days apart, the bone appositional rate, i.e. the rate of progression of the calcification front, was also found to be decreased(Klein et al,1965;Bressot et al,1979).

Eisenberg and Gordan (1961), using stable strontium, have demonstrated decreased bone turnover during steroid therapy. The bone formation rate can also be measured radio-isotopically by following the rate at which ^{47}Ca leaves the plasma and subtracting the amount leaving the body in the urine and faeces (Burkinshaw et al,1969). This method suggests decreased incorporation of calcium into bone during steroid therapy (Gallagher et al (1973;Crilly et al,1978a).

Hydrocortisone has been shown to reduce osteoblastic activity in rib biopsies (Frost and Villaneuva,1961) and to inhibit protein synthesis by and uridine incorporation into isolated bone cells (Peck et al,1967). Corticosteroids have also been shown to inhibit growth of osteoblastic cells in vitro (Chen et al,1979) and to inhibit type I collagen

synthesis in cultured rat calvaria (Canalis,1983). Thus osteoblastic collagen synthesis, osteoid formation and the bone formation rate are all decreased during steroid therapy.

Increased bone resorption rate.

There are several reports of increased bone resorption during steroid therapy (Riggs et al,1966;Wajchenberg et al,1969;Jowsey and Riggs,1970;Lukert et al,1973;Gallagher et al,1973;Crilly et al,1978;Bressot et al,1979). The cause of this increase is not established but the findings of both decreased intestinal calcium absorption (Harrison and Harrison,1960;Bhandarkar et al,1961;Sallis and Holdsworth,1962;Wajchenberg et al,1969;Winter et al,1969;Kimberg et al,1971;Cannigia and Gennari,1973;Klein et al,1977;Crilly et al,1978;Adams et al,1981;Shultz,1982) and increased urinary calcium loss (Irwin et al,1954;Pechet et al,1959;Laake,1960;Cannigia and Gennari,1973;Adams et al,1981;Suzuki et al,1983) suggest that bone calcium may be mobilized to maintain the plasma calcium concentration when insufficient calcium is absorbed from the gut to balance renal losses. Such a model has been described in calcium-deprived rats given a large sodium intake to increase urinary calcium excretion (Goulding,1980).

An alternative explanation for these findings in corticosteroid-induced osteoporosis would be that increased bone resorption leads to decreased intestinal calcium absorption and increased urinary calcium excretion because of increased flow of calcium into the extracellular fluid. Cortisol has an inhibitory effect on bone resorption in

vitro, however (Nisbet and Nordin,1969;Stern,1969;Raisz et al,1972a), although this effect can be overcome with sufficient parathyroid hormone (Raisz,1972a).

If the calcium malabsorption and increased urinary calcium in corticosteroid osteoporosis result from an increase in bone resorption then the calcium released should suppress parathyroid hormone release. On the other hand, if increased bone resorption is the result of decreased intestinal calcium absorption and increased urinary calcium excretion, then serum parathyroid hormone levels should be increased.

Parathyroid hormone secretion during corticosteroid therapy.

The increased bone resorption found in steroid osteoporosis is mediated by osteoclasts in the same way that it is in hyperparathyroidism. Also, glucocorticoids do not cause bone loss in parathyroidectomized animals (Jee et al,1970). Serum parathyroid hormone levels have been reported to be increased during steroid therapy (Fucik et al,1975;Lukert et al,1976;Hahn and Hahn,1976;Suzuki et al,1973) and to fall after treatment of Cushing's syndrome (Findling et al,1982) or during treatment of steroid osteoporosis (Suzuki et al,1983). For these reasons and because of the tendency of corticosteroids to decrease bone resorption in vitro (Nisbet and Nordin,1969;Wong et al,1979;Canalis,1983;Korkor et al,1983) it seems more likely that calcium malabsorption and increased urinary calcium are causes of the increased bone resorption than vice versa.

25 hydroxycalciferol (25D).

This compound is a useful index of the body stores of vitamin D as it is the major circulating metabolite. Normal levels vary widely within populations and throughout the world, depending on the amount of sunlight exposure of each individual. Overt bone disease has been found only with levels well below those obtained in normal people but the possibility that variations within the normal range could have an effect on calcium metabolism has not been ruled out.

Avioli et al(1968) claimed that prednisolone caused an increase in the rate of disappearance of 25D from the plasma, with an increased appearance rate of a more polar metabolite. This change was postulated to cause a decrease in plasma 25D and a subsequent fall in calcium absorption. Klein et al (1977) produced evidence which tended to support this hypothesis, showing that patients on corticosteroid therapy had lower 25D levels than controls (42 nmol/l versus 64 nmol/l,$p<0.001$) and that radiocalcium absorption was positively related to the 25D level ($r=0.58$,$p<0.01$). Bressot et al (1979) again reported that 25D levels were lower in corticosteroid treated patients than in controls (34 ± 3 versus 74 ± 2 nmol/l,$p<0.05$). Similar results were reported by Seeman et al (1980) and Slovik et al (1980), with the differences between the groups being just significant and rather small.

However, Hahn et al (1977) found normal 25D levels in 21 patients on long term corticosteroid therapy and found that the level correlated with the dietary intake of vitamin D. The same group did report later (Hahn et al,1979) that administration of supraphysiological doses of 25D could

correct the calcium malabsorption found in corticosteroid treated patients, the radiocalcium absorption increasing by 46% in 15 patients ($p<0.001$). Similar results with vitamin D therapy have also been reported by Cannigia and Gennari (1972). Hahn et al (1981) gave prednisolone to 12 normal subjects for 14 days and reported no change in 25D although there was a 31% decrease in radiocalcium absorption ($p<0.001$).

Rickers et al (1982) observed no significant change in 25D levels after 6 month's prednisolone therapy in 15 patients but Findling et al (1982) found normal 25D levels in 7 patients with Cushing's syndrome and the levels did not change after successful treatment of the condition. Similarly Gennari et al (1983) showed that corticosteroids given for 15 days could cause a decrease in radiocalcium absorption and a rise in serum parathyroid hormone levels with no change in serum 25D levels. Yamada et al (1984) could find 25D levels decreased only in the group of corticosteroid treated patients on the highest doses.

Given that 25D levels are, by and large, a reflection of sunlight exposure it is entirely possible that the lower levels found in corticosteroid treated patients by some authors are a result of severe illness confining the patient indoors. Although corticosteroids may hasten the conversion of 25D to inactive metabolites as do anticonvulsants (Hahn et al,1972;Tollman et al,1973), reductions of 25D, within the normal range, as reported here have not been shown to cause any major change in calcium metabolism.

1,25 dihydroxycalciferol (1,25D).

This vitamin D metabolite is produced by hydroxylation of 25D within the renal tubule, under the influence of parathyroid hormone. It is the most potent vitamin D metabolite known, being approximately one thousand times more active than 25D. Its production is under negative feedback control and changes in this hormone have a great influence on calcium metabolism. 1,25D stimulates intestinal calcium absorption (Avioli,1972) and causes increased bone resorption in vitro (Raisz et al,1972b). These 2 effects will tend to lead to an increase in the plasma calcium.

There is a significant correlation between serum levels of this hormone and radiocalcium absorption in man (Morris et al,1985) and a dose-related response of radiocalcium absorption when 1,25D is given orally (Need et al,1984).

Serum levels of 1,25D are not altered acutely during steroid therapy or on treatment of Cushing's syndrome (Crilly et al,1981b;Seeman et al,1980;Findling et al,1982), although there is evidence they may fall after longer periods of corticosteroid treatment (Yamada et al,1984). It has been reported that osteoporotic patients on corticosteroid therapy have lower levels of 1,25D than similarly treated patients who are not osteoporotic (Nordin et al,1984). The failure to sustain normal 1,25D levels may be one factor predisposing to osteoporosis, although a primary defect in the gut response to 1,25D is probably more important (Kimberg et al,1971;Klein et al,1977;Crilly et al,1978;Adams et al,1981;Shultz,1982).

Other contributing factors.

Children (Luder,1954;Chesney et al,1978) and postmenopausal females (Reifenstein,1958;Arnoldsson,1958;McConkey et al,1962) are particularly prone to develop steroid osteoporosis. It may be that total androgen deficiency caused by adrenal suppression is the reason, as both these patient groups depend on their adrenal glands as the sole source of androgens. Older men , however, are not immune (Saville and Karmosh,1967).

Although spinal fractures have been reported to be more common in postmenopausal than in premenopausal corticosteroid-treated women, similar reductions in metacarpal cortical index have been reported for each group (Crilly et al (1982). Measurements of cortical bone, however, may not be appropriate for the study of steroid osteoporosis which primarily affects trabecular bone.

The degree of bone loss has been related to the duration of therapy (Hahn et al,1974) but crush fractures may occur in the first weeks of therapy (Boland and Headley,1950;Steinbrocker et al,1951;Demartini et al,1952;Luder,1954). Crilly et al(1984) found that metacarpal bone mass was a function of duration of therapy and dose of steroid combined. Deding et al(1977) reported a negative correlation between total body calcium and dose of corticosteroid.

Alternate day therapy

Sheagren et al (1979) have reported that alternate day steroid therapy in young rabbits does not cause osteoporosis

as does daily therapy, but Chesney et al 1978) showed that alternate day steroid therapy in children was still associated with significant osteoporosis. Gluck et al (1981) found similar degrees of osteoporosis in 28 patients on daily steroids and 25 patients on alternate day steroids and Ruegsegger et al (1983) followed 20 patients on alternate day prednisone therapy for for one year, finding a substantial average annual loss (3.5%) in trabecular bone of the tibia.

Summary

The reports discussed above indicate that fractures are commonly found in corticosteroid treated patients and that they are associated primarily with reduced amounts of trabecular bone. There is decreased calcium absorption and increased urinary calcium excretion during such therapy and a consequent rise in parathyroid hormone levels which maintains normal plasma calcium levels at the expense of the skeleton. The relative importance of intestinal calcium malabsorption and increased urinary calcium loss have not been examined but correction of either will lower the parathyroid hormone level and probably reduce the bone loss.

There is decreased new bone formation during corticosteroid therapy and this is a direct effect, which is demonstrable in vitro. Effective treatment for this abnormality is yet to be found, although Reifenstein suggested forty years ago that anabolic steroid therapy would be useful.

Aims of this study

1. To assess the separate effects of calcium malabsorption

and increased urinary calcium loss on bone resorption during corticosteroid therapy and to assess the effects of treatment of calcium malabsorption in these patients.

2. To confirm that the bone formation rate is decreased during corticosteroid therapy and to assess whether an anabolic steroid, nandrolone decanoate, can correct this abnormality.

CHAPTER TWO

THE EFFECTS OF CALCIUM MALABSORPTION AND INCREASED URINARY CALCIUM EXCRETION ON BONE RESORPTION DURING CORTICOSTEROID THERAPY.

It has been claimed that both decreased intestinal calcium absorption (Kimberg et al,1969;Krawitt,1972;Crilly et al,1978) and increased renal excretion of calcium (Adams et al,1981;Suzuki et al,1983) contribute to the increased bone resorption of corticosteroid-induced osteoporosis but the effects of the former two variables on bone resorption have not been studied simultaneously in a group of corticosteroid-treated patients.

To study the effects of corticosteroid therapy on calcium metabolism it is important to use suitable controls, as several of the variables measured are age and sex dependent. The plasma calcium level rises at the menopause and hence the urinary calcium excretion rises also. Postmenopausal women are also known to be more susceptible to corticosteroid-induced osteoporosis than premenopausal women. For these reasons, only postmenopausal women have been selected for this study.

Measurements of radiocalcium absorption, plasma calcium and fasting urinary calcium, sodium and hydroxyproline

excretion were made in postmenopausal patients on prednisolone therapy and compared with the results found in a group of closely age-matched normal postmenopausal women. Among the corticosteroid-treated patients, those with osteoporotic spines were compared with those with normal spines. Skinfold thickness on the dorsum of the hands was used as a measure of the corticosteroid effect on collagen synthesis (Peck et al,1967), because there is little subcutaneous fat in this site.

METHODS

Subjects gave their informed consent for the procedures performed in this study which was approved by the Research Review Committee of the Royal Adelaide Hospital.

The series comprises 30 postmenopausal women aged from 48 to 80 years treated with prednisolone in doses ranging from 5 to 30 mg/d and 30 closely age-matched women referred for investigation for osteoporosis but found to have normal spine radiographs and no history of peripheral fracture. The conditions for which prednisolone were prescribed are listed in table 2.1.

	"Normal" spines	Osteoporotic spines
Obstructive Airways Disease	7	7
Vasculitis	4	3
Rheumatoid Arthritis	2	2
Haemolytic Disease	1	2
Eczema	-	1
Systemic Lupus	-	1
TOTAL	14	16

TABLE 2.1
Primary diagnoses of 30 corticosteroid-treated postmenopausal patients.

Corticosteroid-treated patients were classified as osteoporotic if spinal radiographs showed one or more crush fractures (loss of both anterior and posterior vertebral height) or a wedge fracture (loss of anterior vertebral height only) together with a history of a peripheral fracture since the menopause (Nordin et al,1975). Those whose spines showed no deformity or a wedge fracture in the absence of peripheral fracture were classified as normal. Single vertebral wedge fractures are often found in normal individuals and are, presumably, the result of previous trauma.

PROCEDURES

A standard protocol was followed. Skinfold thickness was

measured with Harpenden calipers at 3 sites across the back of each hand and the mean value calculated. The patients fasted overnight, voided on waking, and then drank 250 ml of distilled water. Urine was collected between 0800 and 1000 hours, approximately, and blood at 0900 hours. After collection of the urine, the patients were given an oral dose of 5 mcCi of ^{45}Ca in 20 mg of calcium carrier (as the chloride) in 250 ml of distilled water, and blood was collected exactly one hour later.

Plasma radioactivity was measured in a Packard 3375 liquid scintillation counter. Plasma calcium, creatinine and albumin were measured by a sequential multiple analyser with computer (Technicon SMAC II). Urinary sodium was measured by ion-selective electrode on the Beckman Astra. Urinary calcium was measured by a centrifugal analyser (Cobas Bio-Roche) by the method of Connerty and Briggs (1966) modified by monitoring the reaction before and after adding EDTA. Urinary hydroxyproline was measured by the method of Bergman and Loxley (1970).

CALCULATIONS

Plasma calcium was corrected to what it would have been at an albumin concentration of 44 g/l using a coefficient of 0.015 mmol of calcium per gm of albumin (obtained from regression of plasma calcium on plasma albumin in 467 normal postmenopausal women - Professor B.E.C. Nordin, personal communication).

Fasting urinary calcium, sodium and hydroxyproline excretions are expressed as the molar ratios to creatinine

concentration (Ca/Cr, Na/Cr and OHPr/Cr) which represent the excretion rates relative to lean body mass and avoid bladder emptying errors. The Ca/Cr, corrected for urinary sodium, [Ca/Cr(Na corr)] was also calculated, using a coefficient of 0.01 mmol of calcium per mmol of sodium (Goulding,1981;Need et al,1985b). The tubular maxima for calcium and phosphate reabsorption (TmCa,TmP) were calculated as described elsewhere(Marshall et al,1976;Need et al,1985b). The TmCa, corrected for sodium excretion [TmCa(Na corr)] was also calculated as previously described (Need et al,1985b).

The plasma radioactivity (fraction of ^{45}Ca dose per litre of plasma) was multiplied by 15% of body weight to yield the fraction of the dose circulating at 1 hour and the hourly fractional absorption (α)(Marshall and Nordin,1981) was read off a calibration curve (Hartley et al,1982).

Because calcium absorption has a positive effect on calcium balance and urinary calcium a negative effect, the difference between these two opposing variables provides a summation of their effects. We have therefore subtracted the Ca/Cr from the hourly fractional absorption of radiocalcium to provide a single index of the effect of these two variables on calcium balance.

STATISTICAL ANALYSIS

Differences between means for the normal controls and corticosteroid-treated patients were tested for statistical significance by Student's t test for paired data and the corticosteroid-treated patients with osteoporotic spines were compared with those with "normal" spines by Student's t test

for unpaired data.

RESULTS

Comparison of corticosteroid-treated patients with age-matched controls.

The mean values of the measured variables in the patients are compared with those in the normal postmenopausal women in tables 2.2 and 2.3. The corticosteroid-treated patients as a whole had lower plasma albumin ($p<0.001$), Na/Cr ($p<0.05$) and skinfold thickness ($p<0.01$) than the normal controls but there was no significant difference between them in height or body weight. OHPr/Cr, Ca/Cr and calcium absorption were not significantly different in the corticosteroid-treated group when compared with the controls.

However, when the urinary calcium was corrected for sodium, it was significantly higher in the patients than in the controls ($p<0.05$), and similarly when TmCa was corrected for sodium excretion it was lower in the patients than in the controls, although this difference was not significant. The plasma phosphate concentration and TmP were lower in the corticosteroid-treated patients than in the controls, but the differences were not statistically significant. The mean α - Ca/Cr index was lower in the patients than the controls, but this also did not quite reach significance.

Variable	NORMAL CONTROLS	p	CORTICOSTEROID-TREATED PATIENTS All	"Normal" Spines	p	Osteoporotic Spines
Number	30		30	14		16
Age (yr)	62(1)	ns	63(1)	62(1)	ns	63(2)
Years since menopause	14(2)	ns	16(2)	16(3)	ns	16(2)
Current dose of prednisolone(mg/d)	nil		13(1)	14(1)	ns	12(1)
Duration of therapy (yr)	nil		6(1)	6(1)	ns	6(1)
Weight (Kg)	63(2)	ns	64(2)	69(2)	< 0.05	60(2)
Height (cm)	159(1)	ns	158(1)	162(1)	< 0.01	155(1)
Skinfold thickness (mm)	2.0(0.1)	< .01	1.7(0.1)	1.7(0.1)*	ns	1.7 (0.1)*

Significance of difference from controls
*p<0.05

TABLE 2.2

Comparison of clinical variables (±SE) in normal postmenopausal subjects and corticosteroid treated women with "normal" and osteoporotic spines.

Variable	NORMAL CONTROLS	p	CORTICOSTEROID-TREATED PATIENTS All	"Normal" Spines	p	Osteoporotic Spines
Number	30		30	14		16
Plasma albumin (g/l)	44(0.5)	<.001	41(0.7)	42(0.7)*	ns	41(0.7)†
⁴⁵Ca absorption (fx/hr)	0.74(0.04)	ns	0.64(0.06)	0.85(0.05)	< .001	0.45(0.05)‡
Plasma corrected calcium(mmol/l)	2.40(0.01)	ns	2.43(0.01)	2.41(0.01)	ns	2.45(0.02)*
Plasma phosphate (mmol/l)	1.05(0.02)	ns	1.02(0.02)	1.01(0.02)	ns	1.03(0.02)
Plasma creatinine(mmol/l)	0.08(0.003)	ns	0.08(0.003)	0.09(0.003)	<.05	0.07(0.003)
Urinary Ca/Cr	0.26(0.03)	ns	0.34(0.04)	0.25(0.03)	<.05	0.43(0.05)
Urinary Na/Cr	14.5 (1.3)	<.05	10.0(1.8)	9.2 (1.0)	ns	10.8 (1.2)
Urinary Ca/Cr (Na corr)	0.12(0.03)	<.05	0.24(0.04)	0.16(0.03)	<0.05	0.32(0.04)†
TmCa (mmol/lGF)	2.03(0.03)	ns	2.03(0.04)	2.06(0.03)	ns	2.00(0.04)
TmCa (Na corr)(mmol/lGF)	2.30(0.03)	ns	2.22(0.04)	2.25(0.04)	ns	2.20(0.04)
TmP(mmol/lGF)	1.14(0.04)	ns	1.10(0.05)	1.10(0.05)	ns	1.11(0.05)
Urinary OHPr/Cr	0.020(0.001)	ns	0.023(0.002)	0.016(0.001)	<0.001	0.029(0.002)‡
α-Ca/Cr index	0.48(0.05)	ns	0.29 (0.09)	0.62 (0.07)	<0.0001	0.01 (0.10)‡

Significance of difference from controls

*p<0.05 †p<0.01 ‡p<0.001

TABLE 2.3

Comparison of measured variables (mean±SE) in normal postmenopausal subjects and long-term corticosteroid treated patients with 'normal' and osteoporotic spines.

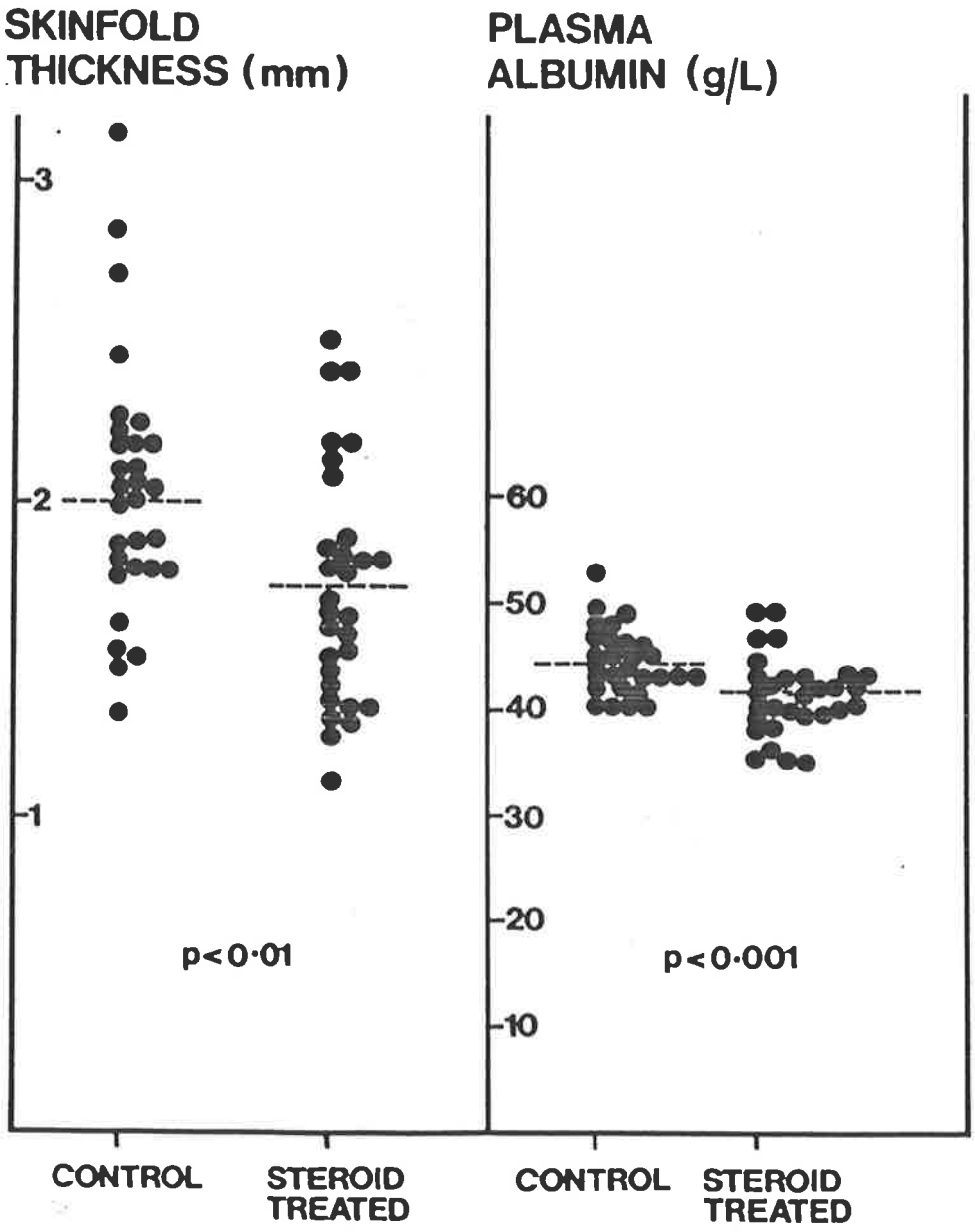

Figure 2.1.

Comparison of skinfold thickness, on the dorsum of the hands, and plasma albumin values between 30 postmenopausal women on prednisolone therapy and 30 closely age-matched controls.

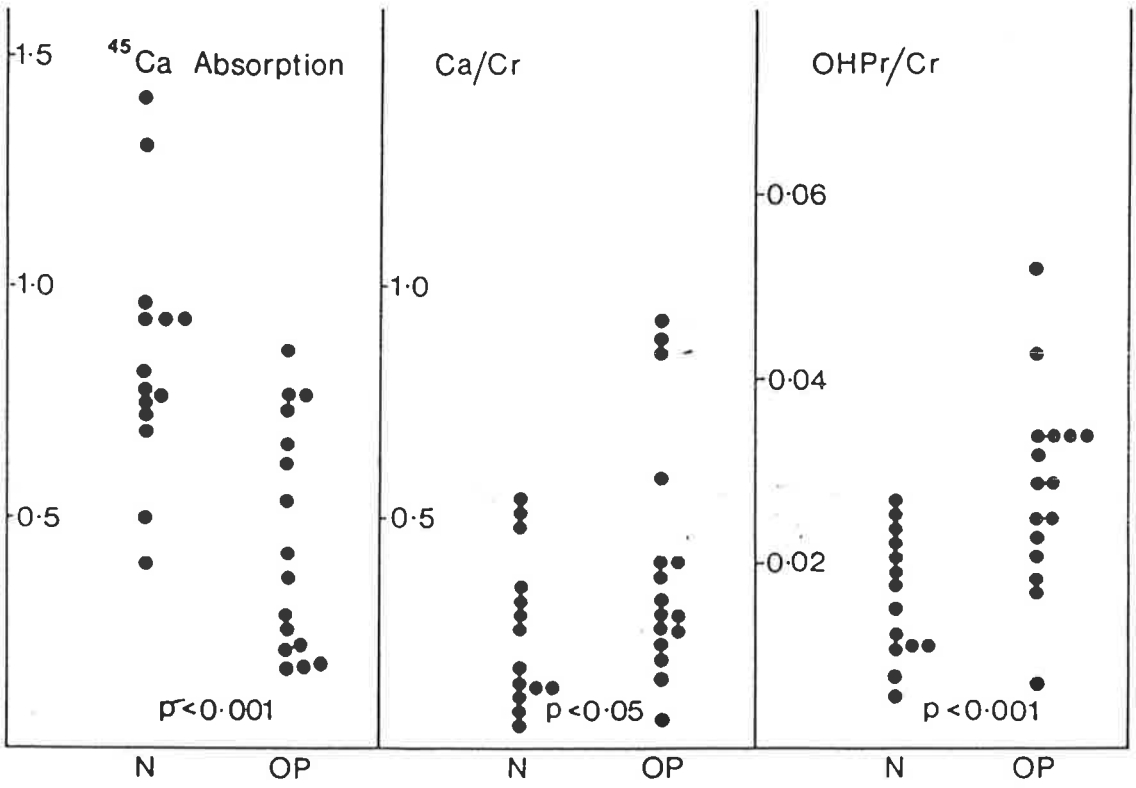

Figure 2.2.

Comparison of hourly fractional radiocalcium absorption and fasting urinary calcium/creatinine and hydroxyproline/creatinine ratios in 30 postmenopausal women on prednisolone therapy.

N = normal spine, OP = osteoporotic spine.

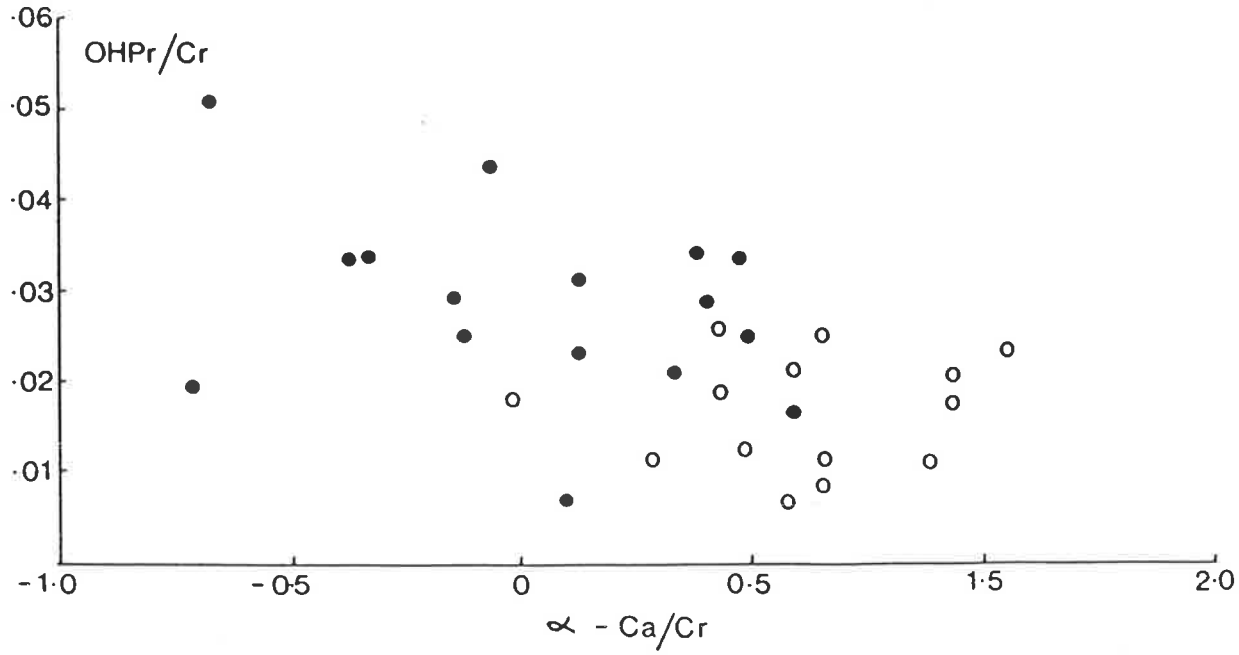

Figure 2.3.

Regression of fasting urinary hydroxyproline/creatinine ratio on the difference between hourly fractional radiocalcium absorption (∝) and fasting urinary calcium/creatinine ratio in 30 postmenopausal women on prednisolone therapy.

● = osteoporotic spine.

O = normal spine.

Comparison of "normal" and osteoporotic corticosteroid-treated cases.

The measured variables in the normal and osteoporotic groups are compared in table 2.2. The osteoporotic cases were not significantly older (there was a one year difference in mean age only) but they were significantly shorter ($p<0.01$) and lighter ($p<0.05$). There was [illegible] no significant difference in skinfold thickness between the two groups. Calcium absorption and plasma creatinine were significantly lower in the osteoporotic than the normal group ($p<0.001$ and <0.05 respectively) and Ca/Cr and OHPr/Cr were higher ($p<0.05$ and <0.001 respectively)(figure 2.1). Ca/Cr, when corrected for the urinary sodium, was also significantly higher ($p<0.05$) in the osteoporotic than the normal spine group. Plasma calcium was higher and TmCa was lower in the osteoporotic than the normal group but this was not statistically significant and The α - Ca/Cr index was highly significantly lower in the osteoporotic group than in the group with normal spines ($p<0.0001$).

When OHPr/Cr was regressed on the α - Ca/Cr index in the whole corticosteroid-treated group, the correlation coefficient was -0.50 ($p<0.01$) and the equation was OHPr/Cr = 0.027 - 0.012x(α - Ca/Cr)(figure 2.3).

DISCUSSION

The significant differences between the normal postmenopausal women and the corticosteroid-treated patients as a whole are in respect of skinfold thickness, plasma

albumin, fasting urinary sodium and the sodium-corrected fasting urinary calcium.

The thinner skin of the corticosteroid-treated cases presumably reflects the decreased collagen synthesis associated with corticosteroid therapy (Sheppard and Meema,1966;Peck et al,1967;Uitto et al,1972;Dykes and Marks,1977;Hall,1977;Canalis,1983). Their lower plasma albumin may simply be a result of the associated diseases for which they were being treated. The lower fasting urinary sodium may be fortuitous but is important because it obscures the real difference in fasting urinary calcium between the two groups. The uncorrected urinary calcium is higher in the corticosteroid-treated patients than in the controls but the significance of this difference only emerges when the urinary calcium is corrected for sodium excretion on the basis of the well documented relationship between urinary calcium and sodium (Goulding,1981;Need et al,1985b). Despite the difference in Ca/Cr between the controls and the corticosteroid-treated patients, the mean TmCa is not different between these groups because the plasma calcium is slightly higher in the corticosteroid-treated cases than in the controls. However, after correction for sodium excretion, there is no significant difference in mean TmCa between the corticosteroid-treated group and the controls. We therefore conclude that, in postmenopausal women at least, corticosteroid therapy may increase urinary calcium partly by reducing tubular reabsorption and partly through a slightly raised plasma calcium. The non-significant decrease in TmP in the corticosteroid-treated group may be a result of increased

parathyroid hormone secretion (Anderson and Foster,1959), although it remains possible that this is a direct effect of corticosteroids as it has been reported to occur in parathyroidectomised rats (Laron et al,1957). There is also an increase in urinary hydroxyproline in the corticosteroid-treated group which, though not statistically significant, possibly represents a real increase in bone resorption (Kirivikko et al,1970;Nordin et al,1976), the importance of which is enhanced by the strong probability that bone formation rate is reduced in these cases (Jowsey and Riggs,1970;Bressot et al,1979). In view of the well known rise in urinary hydroxyproline and bone resorption at the menopause (Crilly et al,1979), it is tempting to suggest that suppression of adrenal androgens by corticosteroid therapy, and the resultant further reduction in serum oestrogens (Crilly et al,1978a) also contributes to the increased bone resorption in postmenopausal women on corticosteroids.

However, comparison of the normal and osteoporotic subsets in the corticosteroid-treated group shows that this apparent increase in bone resorption is entirely confined to the osteoporotic cases (see below).

The significant differences between the "normal" and osteoporotic corticosteroid-treated groups are in respect of weight, height, radiocalcium absorption and plasma creatinine (all of which are lower in the osteoporotics) and fasting urinary calcium and hydroxyproline (which are higher in the osteoporotics). We have no explanation to offer for the difference in weight between these two groups but presume that the difference in height is due to vertebral compression

in the osteoporotic cases (Nordin et al,1978). The cause of the calcium malabsorption is not clear. It is unlikely that it reflects primarily a deficiency of 1,25D because serum levels of this hormone are generally normal in corticosteroid patients(Crilly et al,1981a;Seeman et al,1980;Findling et al,1982). Animal studies suggest either a post-receptor abnormality(Shultz et al,1982) or a decrease in 1,25D receptors(Hirst and Feldman,1982). Yamada et al (1985) have also reported, in abstract form, that 1,25D levels may fall with time in long term corticosteroid-treated patients. Whatever the cause may be, it is probable that the calcium malabsorption in the osteoporotic group is a significant factor in the pathogenesis of the osteoporosis, just as it is in the pathogenesis of other forms of osteoporosis. This interpretation is strongly supported by the effect of calcitriol therapy on urinary hydroxyproline excretion which the author and others have reported in postmenopausal osteoporosis (Need et al,1985a;Haas et al,1979) and which our group has observed in corticosteroid-induced osteoporosis (see chapter 4).

However, it is clear the high bone resorption in the osteoporotic group is not merely a result of calcium malabsorption. The increase in fasting urinary hydroxyproline also appears to be a response to their increased urinary calcium excretion. This increased urinary calcium loss is probably, as already indicated, mainly due to reduced tubular reabsorption of calcium which, like the calcium malabsorption, is likely to be a direct effect of corticosteroids on calcium transport (Windhager et al,1981).

Nevertheless it is true that there is also a very small increase in plasma calcium in the osteoporotic group and it is not possible at the present stage to establish which of these factors is responsible for the urinary calcium loss.

There is also a lower plasma creatinine in those corticosteroid-treated patients with osteoporotic spines than in those with normal spines and this is interpreted as a result of corticosteroid-induced increase in the glomerular filtration rate(Baylis and Brenner,1978).

In the author's analysis, duration of therapy did not differ between the cases with and without osteoporosis. A finite time must clearly elapse, however, before osteoporosis can develop. Trabecular bone loss can occur during the first 6 months of therapy(Rickers et al,1982), but the patients in the series reported here had been treated for a mean period of 6 years. Forearm bone mass has been reported to decrease further after the first three years of therapy (Hahn,1978) but this measurement includes cortical bone which must have a slower response than trabecular bone to any resorptive process because of its smaller surface area/volume ratio. Crilly et al(1983) reported that the loss of metacarpal cortical bone was related to the product of the dose and duration of corticosteroid treatment.

It is concluded that corticosteroid therapy tends to reduce intestinal absorption and renal tubular reabsorption of calcium and that this combination of effects leads to an increase in bone resorption to maintain the plasma calcium. This is reflected in an increase in the urinary hydroxyproline. This probably explains why the difference

between α and Ca/Cr yields an empirical index which discriminates more between osteoporotic and non-osteoporotic patients on corticosteroid therapy than either variable alone. The α - Ca/Cr index could probably be used to estimate the risk of osteoporosis in patients on corticosteroids and is likely to be more useful than the urinary hydroxyproline, although highly correlated with it. Thus the author agrees with Baylink (1983) that the combination of low calcium absorption and high calcium excretion produced by corticosteroid therapy is a major risk factor for osteoporosis but does not agree that absorption is so difficult to measure as to be unsuitable for clinical use.

If the above concepts are valid, the appropriate treatment in patients shown to be at risk of corticosteroid osteoporosis would be to increase calcium absorption or reduce urinary calcium. Calcitriol therapy can be used to increase calcium absorption, if it is low,(Klein et al,1977) and a low sodium diet, with or without a thiazide diuretic, can reduce urinary calcium (Suzuki et al,1983). A combination of vitamin D and thiazide therapy, however, has the potential to produce hypercalcaemia (Condon et al,1978) and it must be emphasized that this type of therapy could only be expected to reduce bone resorption; it will do nothing to increase bone formation.

The malabsorption of calcium in corticosteroid-treated patients is studied further in the next chapter.

CHAPTER THREE

SERUM 1,25-DIHYDROXYCALCIFEROL LEVELS AND THE CONTROL OF CALCIUM ABSORPTION DURING CORTICOSTEROID THERAPY.

The absorption of dietary calcium has both active and passive (diffusion) components, with the former being under the control of 1,25D (Morris et al,1985). After injection of 1 alpha-hydroxycalciferol in rats, the active metabolite, 1,25D localizes mainly in the upper small bowel (Walling et al,1974) and this is the site of the maximum rate of calcium absorption in man (Wensel et al,1969) and other species (Kimberg et al,1961;Avioli,1972).

Although a reduction in net absorbed calcium could be a result of decreased active absorption, decreased passive absorption or increased endogenous calcium excretion it is the early, active phase of calcium absorption, as described in chapter 2, which can be impaired during corticosteroid therapy and this impairment is related to the presence of osteoporosis. This defect also probably leads to impaired reabsorption of digestive juice calcium, which is normally 85% absorbed(Avioli,1972), thus producing a further tendency to negative calcium balance.

The malabsorption of calcium during corticosteroid therapy has been attributed to an impaired response to 1,25D at the

gut mucosal level (Kimberg et al,1971;Shultz et al,1982;Hirst and Feldman,1982). However, another study(Yamada et al,1984) suggests that, with the passage of time, blood levels of 1,25D decrease in patients maintained on corticosteroids, and it has also been reported that those corticosteroid-treated patients who have osteoporosis have lower levels of 1,25D than those with normal spines (Nordin et al,1984).

In this study the relations between radiocalcium absorption and serum 1,25D levels have been examined in corticosteroid-treated patients with and without osteoporosis to further investigate the cause of the calcium malabsorption in the former group.

METHODS

This study examined 39 patients (mean age 58±3 years) on long term prednisolone therapy with mean duration of therapy 7±1 years and mean current dose of steroid 17±3 mg/d. There were 12 males and 27 females. None of the patients was receiving therapy for osteoporosis or had evidence of renal disease or osteomalacia. Patients were classified as osteoporotic if they had more than one wedged vertebra or one or more crushed vertebrae.

Calcium absorption was measured as described in chapter 2, using ^{45}Ca and a small carrier dose of calcium (20mg), to measure the early (active) transport of calcium into the blood stream.

1,25D was measured, after separation from other vitamin D

metabolites using HPLC, by radioimmunoassay (Taylor et al,1980).

Differences between groups were tested with Student's unpaired t test. Radiocalcium absorption was regressed on age, current dose of prednisolone, duration of corticosteroid therapy and 1,25D level for the whole group and separately for those patients with osteoporotic (OP) spines and "normal" (N) spines. Differences between regression coefficients (slopes) were tested for significance by analysis of variance (Snedecor and Cochran,1980).

RESULTS

The osteoporotic and normal spine groups are compared in table 3.1.

Group	n	age (yr)	alpha (fx/hr)	1,25D (pmol/l)	dose (mg/d)	duration (yr)
All	39	58(3)	0.67(0.06)	81(8)	17(3)	7(1)
N	22	53(4)	0.86(0.21)	89(10)	20(5)	7(2)
p		<0.05	<0.001	ns	ns	ns
OP	17	64(2)	0.44(0.08)	72(14)	13(3)	6(2)

TABLE 3.1
Comparison of patients with osteoporotic (OP) and "normal" (N) spines on long-term corticosteroid therapy. Alpha = hourly fractional radiocalcium absorption, dose = dose of prednisolone at time of study, duration = period during which patient has been receiving prednisolone. Results expressed as mean(±)SE.

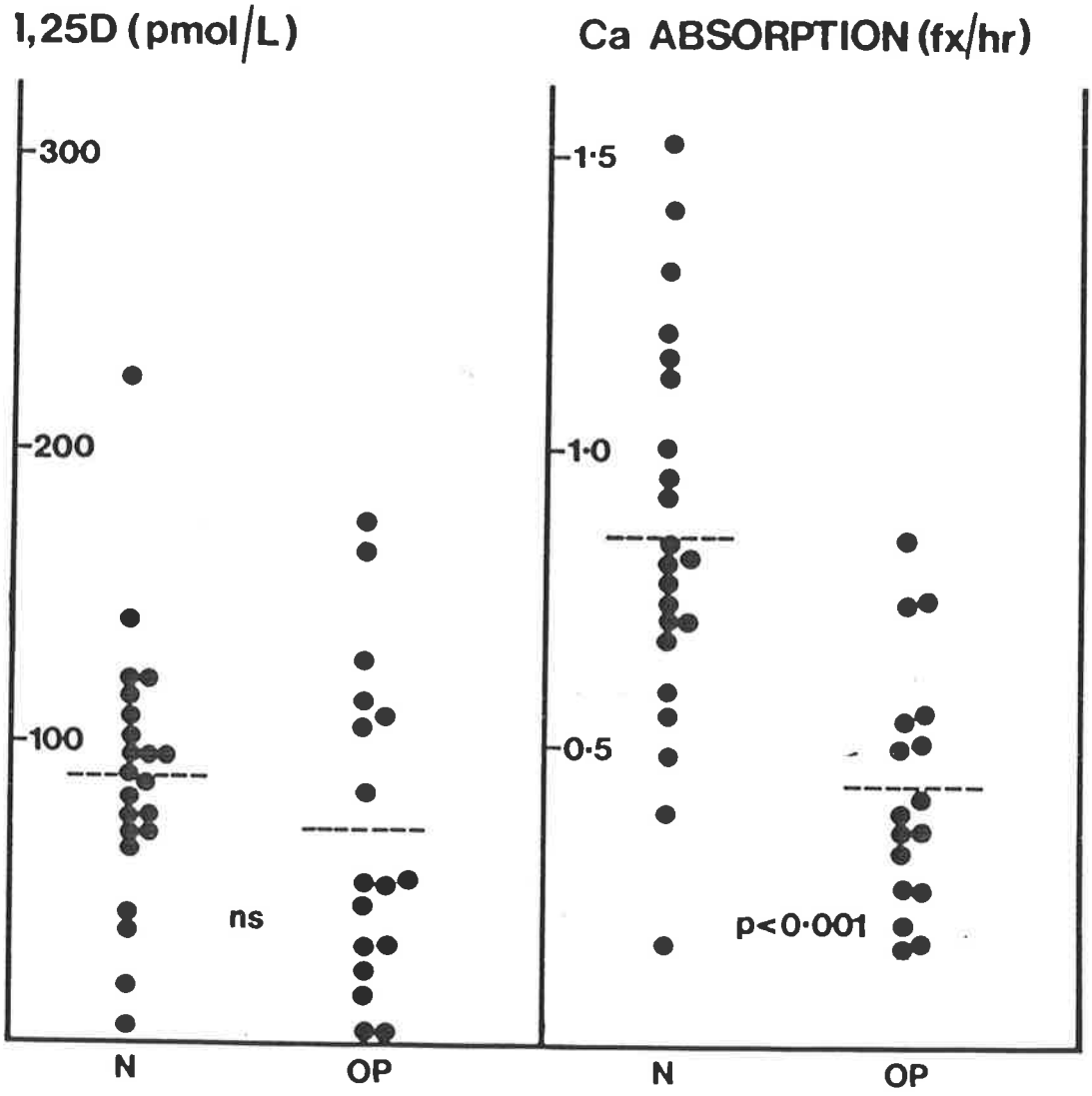

Figure 3.1.

Comparison of serum 1,25-dihydroxycalciferol levels and hourly fractional radiocalcium absorption in prednisolone treated patients with normal (N) and osteoporotic (OP) spines.

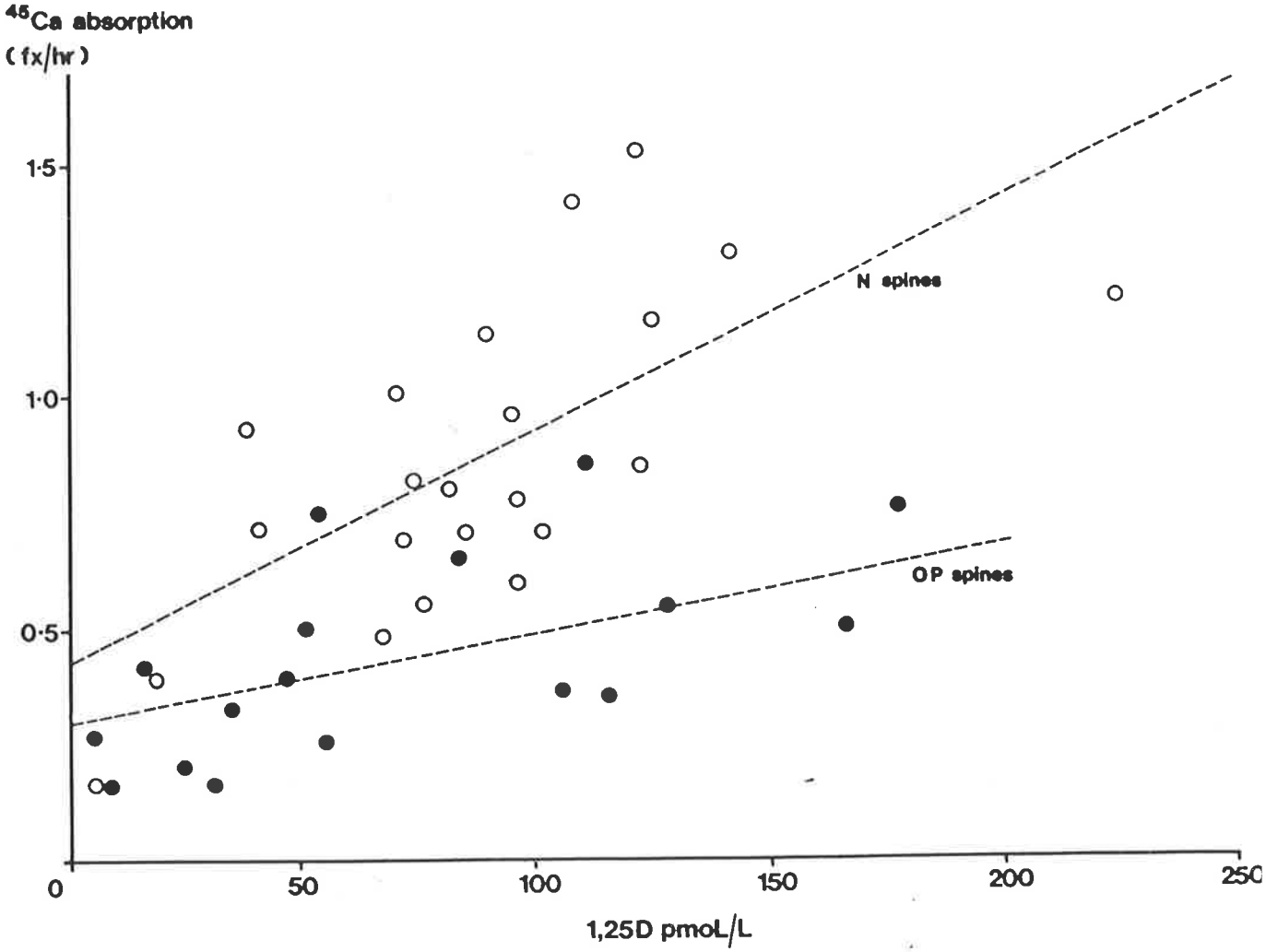

Figure 3.2.

Relation between hourly fractional radiocalcium absorption and serum 1,25-dihydroxycalciferol levels in 39 patients on long term prednisolone therapy. ● = osteoporotic spine, O = normal spine.

For normal spines $y = 0.0050x + 0.42$

($r = 0.67; p<0.001$)

For osteoporotic spines $y = 0.0024x + 0.27$

($r = 0.61; p<0.05$)

The OP patients were older than the N group by 11 years ($p < 0.05$). 1,25D levels (normal range 50-140 pmol/l), did not differ significantly between the two groups and the difference, using the regression coefficient of radiocalcium absorption on 1,25D for the whole group, would be expected to account for a difference in fractional radiocalcium absorption of only 0.05 per hour. There was no relationship between radiocalcium absorption and age, current dose of prednisolone or duration of corticosteroid treatment whether the OP and N groups were tested separately or together.

The fractional radiocalcium absorption (normal range 0.30 - 1.30 per hour) was at the lower end of the normal range in the OP group (0.44 ± 0.08), but was in the middle of the normal range (0.88 ± 0.21) in the N group. The difference between the groups was highly significant ($p<0.001$).

The regression of radiocalcium absorption on 1,25D is shown in figure 3.2. For the N group the regression coefficient is 0.0050 ± 0.0010 and for the OP group it is very significantly lower (0.0024 ± 0.0008, $p<0.001$). The correlation coefficients for the two subgroups are significant ($p<0.001$ and <0.05 respectively) and the correlation coefficient for all 39 cases combined is 0.59 ($p<0.001$).

DISCUSSION

A predictable finding in this study is the significantly decreased radiocalcium absorption in the OP cases. However, although calcium absorption is related to 1,25D in the group

as a whole, 1,25D is not decreased in the OP group and does not differ significantly between the groups. This suggests that the malabsorption of calcium is not a result of low 1,25D levels but is the result of a deficient response to 1,25D at the gut level.

If the slope of radiocalcium absorption on 1,25D can be used as an indicator of gut sensitivity to 1,25D, then the N group demonstrated similar sensitivity to that of normal postmenopausal women (slope 0.0042±0.0009) (Morris et al,1985). The response of radiocalcium absorption to endogenous 1,25D in the OP group, however, seems deficient, and similar results have been reported for postmenopausal women with osteoporosis (slope 0.0029±0.0008) (Morris et al,1985). Similarly, a decreased response of intestinal calcium transport to 1,25D has been reported in rats treated with prednisolone (Carre et al,1974).

The findings reported here support those of Seeman et al (1980), Crilly et al (1981b), Findling et al (1982) and Dykman et al (1984) who found no decrease in 1,25D in corticosteroid-treated patients. Again, similar findings have been reported in rats (Favus et al,1973). Animal studies suggest either a post receptor abnormality (Shultz et al,1982) or a decrease in 1,25D receptors to explain the calcium malabsorption. The report of Yamada et al (1984), in abstract form, that 1,25D levels may fall with time in corticosteroid-treated patients needs further evaluation and the data reported here would not support such a model.

The reports of an acute rise in 1,25D levels on initiation of corticosteroid therapy (Crilly et al,1981b;Hahn et

al,1981;Braun et al,1982;Yamada et al,1984) are further evidence for a decreased gut response to 1,25D. The suggested model for the observed events is that decreased calcium absorption leads to a compensatory rise in PTH secretion which stimulates 1,25D production until calcium absorption is restored or bone resorption increases to compensate for the fall in intestinal calcium.

It is surprising, then, that in spite of continuing secondary hyperparathyroidism during corticosteroid therapy (Fucik et al,1975;Lukert et al ,1976;Hahn and Hahn,1976;Suzuki et al,1983), the 1,25D levels fall to normal instead of remaining elevated. This is at variance with the 1,25D levels found in primary hyperparathyroidism (Haussler et al,1976) and suggests that with long term corticosteroid therapy there is a decrease in the ability to produce 1,25D in appropriate quantities. This aspect needs further investigation, for example by testing the renal 1-alpha hydroxylase "reserve" with parathyroid hormone in corticosteroid-treated patients.

CHAPTER FOUR

CORTICOSTEROID-INDUCED OSTEOPOROSIS: CORRECTION OF CALCIUM MALABSORPTION WITH CALCITRIOL.

Calcitriol (1,25D), in a dose of 0.40 mcg/d, has been reported to increase calcium absorption in patients undergoing corticosteroid therapy (Klein et al,1977). A smaller dose, of 0.25 mcg/d, stimulates calcium absorption in postmenopausal women with osteoporosis and calcium malabsorption and also appears to decrease bone resorption as a direct consequence (Need et al,1985a). The effects of calcitriol on bone resorption have been studied histomorphometrically in corticosteroid-treated patients (Dykman et al,1984) but the effect on urinary hydroxyproline was not described. There is always the risk that the beneficial effect of calcitriol on calcium absorption may be nullified by its bone-resorbing action (Raisz et al,1972b), and this has possibly limited its use.

In the study reported here, patients on prednisolone therapy who had calcium malabsorption, defined by a simple radiocalcium absorption test, were given a daily oral dose of calcitriol. Because the object of the treatment with calcitriol was to correct the increased bone resorption resulting from the high parathyroid hormone levels found in

corticosteroid osteoporosis, a calcium supplement was added to the calcitriol therapy. The effects on radiocalcium absorption and urinary hydroxyproline were analysed.

METHODS

Subjects gave their informed consent for this study which was approved by the Research Review Committee of the Royal Adelaide Hospital.

The series comprises 8 patients, aged from 41 to 72 years treated with prednisolone in a mean dose of 27±14 mg/d. There were 5 females and 3 males. Calcium malabsorption was defined, as previously, as an hourly fractional absorption of ^{45}Ca of less than 0.5 (Need et al,1985a). All patients had spinal osteoporosis as defined by the presence of one or more vertebral crush fractures or one or more vertebral wedge fractures together with a history of of a peripheral fracture since the menopause. The conditions for which prednisolone was prescribed are listed in table 4.1.

PROCEDURES

A standard protocol was followed. The patients were fasted overnight, voided on waking, and then drank 250 ml of distilled water. Urine was collected between 0800 and 1000 hours, approximately, and blood at 0900 hours. After collection of the urine, the patients were given an oral dose of 5 mcCi of ^{45}Ca in 20 mg of calcium carrier (as the chloride) in 250 ml of distilled water, and blood was collected exactly one hour later.

Five patients were then treated with calcitriol

(Rocaltrol-Roche) in a dose of 0.50 mcg/d and 3 patients with 0.25 mcg/d. All patients were given a 1g calcium supplement (Sandocal-Sandoz) at night. After a variable period on this therapy, ranging from 2 to 6 months, the tests were repeated.

Plasma radioactivity was measured in a Packard 3375 liquid scintillation counter. Plasma calcium, albumin, creatinine and phosphate were measured by a sequential multiple analyser with computer (Technicon SMAC II). Urinary calcium was measured by a centrifugal analyser (Cobas Bio-Roche) by the method of Connerty and Briggs (1966) modified by monitoring the reaction before and after adding EDTA. Urinary hydroxyproline was measured by the method of Bergman and Loxley (1970).

CALCULATIONS

Plasma calcium was corrected to that predicted for an albumin concentration of 44 g/l using a coefficient of 0.015 mmol of calcium per gram of albumin (obtained from regression of plasma calcium on plasma albumin in 467 normal subjects - BEC Nordin, personal communication).

Fasting urinary calcium, phosphate and hydroxyproline excretions are expressed as the molar ratios to creatinine concentration (Ca/Cr, P/Cr and OHPr/Cr) which represent the excretion rates relative to lean body mass and avoid bladder emptying errors. The tubular maxima for calcium and phosphate reabsorption (TmCa, TmP) were calculated as described elsewhere (Marshall et al,1976).

The plasma radioactivity (fraction of ^{45}Ca dose per litre of plasma) was multiplied by 15% of body weight to yield the

fraction of the dose circulating at 1 hour and the hourly fractional absorption (α) (Marshall et al,1981) was read off a calibration curve (Hartley et al,1982).

STATISTICAL ANALYSIS

Results before and after therapy are expressed as the mean $\pm$ standard error and were analyzed for statistical significance using Student's t test for paired data.

RESULTS

Eight patients were assessed before and after calcitriol and calcium therapy but only 7 had sequential calcium absorption measurements. The individual results and patient details are shown in tables 4.1, 4.2 and 4.3.

Plasma calcium and phosphate levels were normal before therapy but several patients had increased fasting urinary OHPr/Cr, Ca/Cr and plasma ALP activity (tables 4.1, 4.2 and 4.3). In 1 patient the plasma calcium rose above the normal range on 0.50 mcg/d of calcitriol with calcium, but it returned to normal when the dose of calcitriol was reduced to 0.25 mcg/d and in 1 patient on 0.25 mcg/d with calcium (case 8) the plasma calcium was increased at 4 months and normal again at 5 months after treatment began.

In 2 patients, studied before and after 2 to 6 months on 0.5 mcg daily of calcitriol, mean α rose from 0.27 to 0.61 per hour and in 5 patients on 0.25 mcg it rose from 0.42 to 0.66. For all 7 patients with baseline and treated absorption values, mean rose from 0.37 ± 0.04 to 0.64 ± 0.07 ($p<0.01$).
In all 8 patients given calcitriol and calcium, mean OHPr/Cr

fell from 0.025±0.003 to 0.013±0.001 ($p<0.01$) and ALP from 128±16 to 77±16 ($p<0.01$) in the same time period (table 4.2). The fall in OHPr/Cr was proportional to the initial value (figure 4.1) and the slope of the regression was -0.91. The fall in ALP tended to be proportional to the initial value but the correlation coefficient (-0.32) was not significant.

There were no significant changes in plasma calcium or phosphate or in urine calcium and phosphate excretion.

Case	Age	Sex	Primary disease	^{45}Ca absorption (fraction/hour)		Therapy		Duration of therapy (months)
				Before	After	1,25D mcg/d	Ca g/d	
1.	72	F	pulmonary fibrosis	0.17	0.61	0.50	1	2
2.	59	F	pulmonary fibrosis	0.37	0.60	0.50	1	6
3.	45	M	pemphigus	0.17	-	0.50	1	2
4.	54	F	lupus	0.42	0.56	0.25	1	2
5.	70	F	pulmonary fibrosis	0.50	0.65	0.25	1	2
6.	65	F	rheumatoid arthritis	0.49	0.87	0.25	1	2
7.	41	M	chronic lymphatic leukaemia	0.33	0.37	0.25	1	2
8.	71	M	pulmonary fibrosis	0.34	0.85	0.25	1	4

TABLE 4.1

Patient details and response of radiocalcium absorption to calcitriol in 7 patients on prednisolone therapy.

	Plasma Calcium		Plasma Phosphate		ALP	
Normal range	2.20-2.55 mmol/l		0.75-1.35 mmol/l		30-110 l/l	
	BASE	TRT	BASE	TRT	BASE	TRT
Case						
1.	2.42	2.58	1.06	1.12	90	48
2.	2.49	2.40	0.93	1.30	98	81
3.	2.50	2.40	1.03	0.68	70	65
4.	2.55	2.51	1.26	1.10	128	69
5.	2.34	2.39	0.91	0.98	136	48
6.	2.39	2.35	1.19	1.08	80	69
7.	2.43	2.37	1.08	1.09	208	184
8.	2.51	2.58	1.02	0.99	128	55
Mean	2.45	2.45	1.06	1.04	117	77
SE	0.02	0.03	0.04	0.06	16	16
p	ns		ns		<0.01	

TABLE 4.2
Plasma biochemical variables at baseline (BASE) and during treatment with calcitriol and calcium (TRT) in 8 patients on prednisolone therapy.

	Ca/Cr		P/Cr		TmCa		TmP		OHPr/Cr	
Normal range	0.00-0.32		0.8-3.0		1.8-2.6 mmol/lGF		0.6-1.4 mmol/lGF		<0.017	
	BASE	TRT	BASE	TRT	BASE	TRT	BASE	TRT	BASE	TRT
Case										
1.	0.88	0.69	2.8	2.0	1.7	1.9	0.9	1.2	0.018	0.013
2.	0.51	0.79	2.0	3.9	1.9	1.7	1.0	1.1	0.025	0.014
3.	0.66	0.67	4.0	2.1	1.9	1.6	0.9	0.5	0.035	0.015
4.	0.57	0.19	2.2	2.3	2.0	2.2	1.5	1.1	0.029	0.012
5.	0.65	0.22	1.6	1.6	1.6	2.0	0.9	1.1	0.007	0.009
6.	0.53	0.55	1.1	2.5	1.9	1.8	1.6	1.1	0.018	0.017
7.	0.34	0.23	1.1	2.5	1.9	1.9	1.3	1.0	0.033	0.013
8.	0.19	0.25	0.7	1.2	2.2	2.1	1.3	1.1	0.032	0.012
Mean	0.54	0.45	1.9	2.3	1.9	1.9	1.2	1.0	0.025	0.013
SE	0.07	0.09	0.4	0.3	0.1	0.1	0.1	0.1	0.003	0.001
p	ns		ns		ns		ns		<0.01	

TABLE 4.3
Urinary variables at baseline (BASE) and during calcitriol and calcium treatment (TRT) in 8 patients on prednisolone.

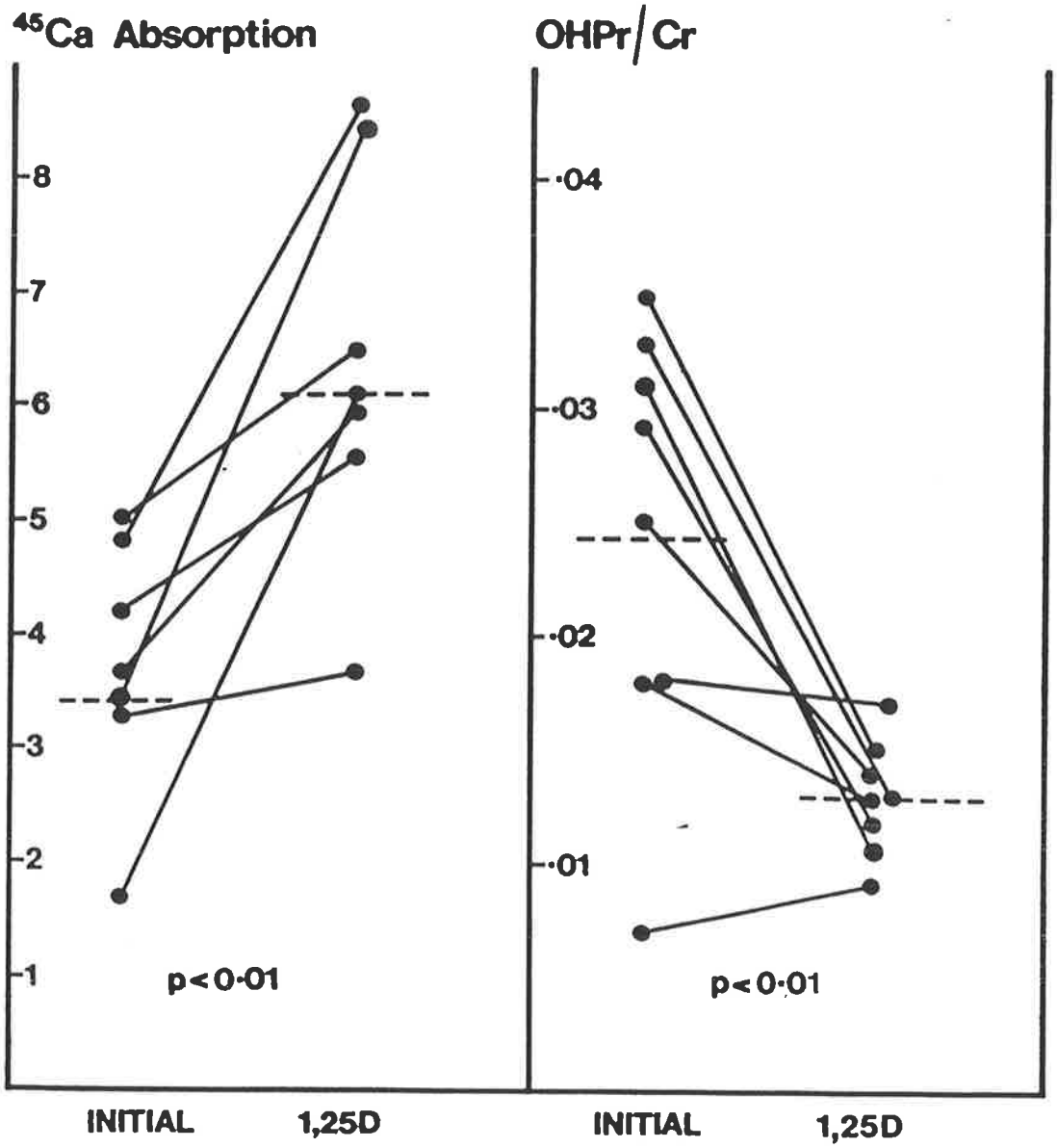

Figure 4.1.

Effect of calcitriol and calcium therapy on hourly fractional radiocalcium absorption and fasting urinary molar hydroxyproline/creatinine ratio in corticosteroid-treated patients.

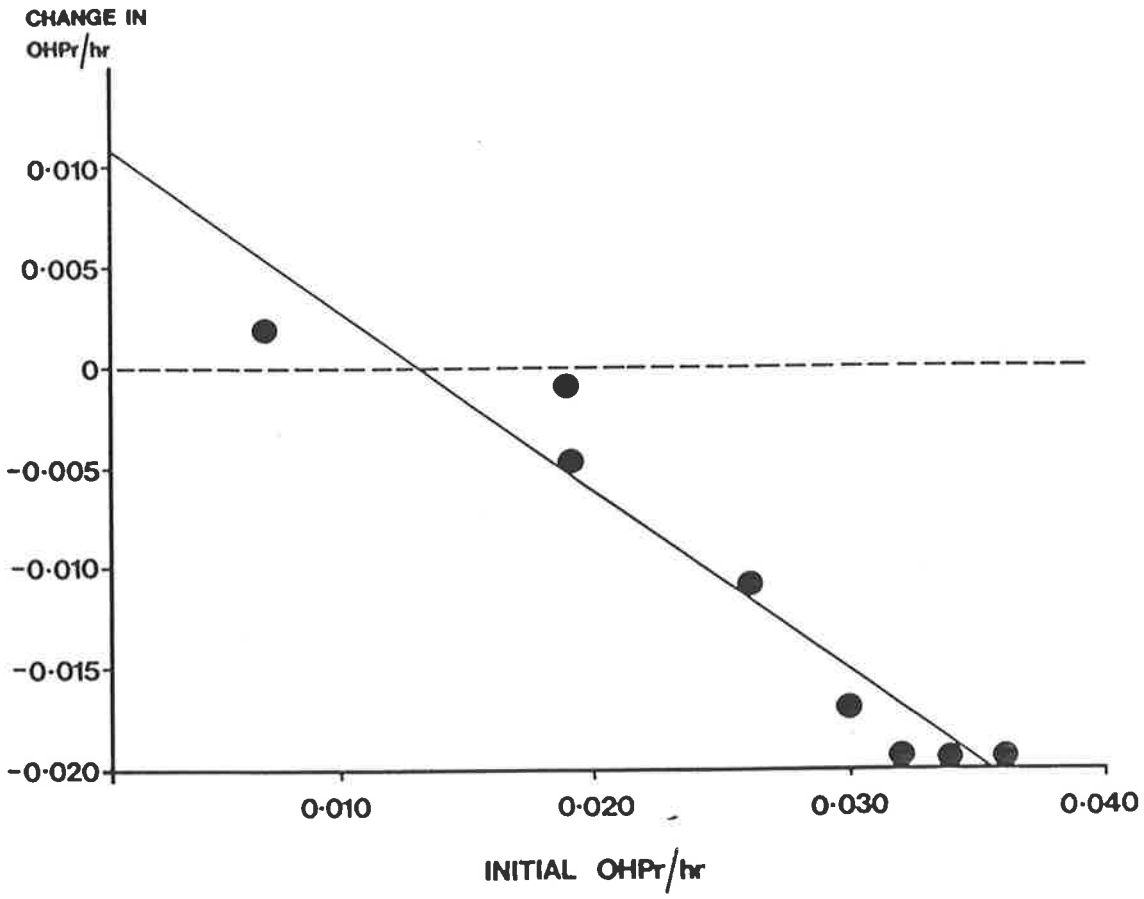

Figure 4.2.

Regression of change in fasting urinary hydroxyproline/creatinine ratio (OHPr/Cr) on initial OHPr/Cr in 8 corticosteroid-treated patients given calcitriol and calcium therapy for 2 to 6 months.

$y = 0.011 - 0.91x$ ($r = 0.97; p<0.001$)

DISCUSSION

The most significant change in the measured variables after 2 to 6 month's calcitriol therapy is in radiocalcium absorption, with an increase of 60% ($p<0.01$) of the initial value. Similar increases in calcium absorption have been reported previously with similar doses of calcitriol in corticosteroid-treated patients (Klein et al,1977), and this response has also been shown by our group in postmenopausal osteoporotic patients with calcium malabsorption (Need et al,1985a).

Although calcium absorption was not restored to the normal arithmetical mean value of 0.81 (Nordin et al,1985), there was a significant fall in OHPr/Cr, towards normal, indicating a fall in bone resorption, which implies a decrease in parathyroid hormone secretion. It may not be necessary to restore calcium absorption fully to normal to ensure adequate calcium availability if a 1g calcium supplement is given.

The regression of fall in OHPr/Cr against the initial value (coefficient -0.91 ± 0.10) indicates partial suppression of OHPr/Cr towards the non bone component of 0.10 (Nordin et al,1976). A slope of -1.0 would indicate complete suppression of bone turnover. The intercept of initial OHPr/Cr on the y axis (the point at which no change is expected) is 0.012 which is similar to that found after treatment of postmenopausal osteoporotic women not on corticosteroids (Need et al,1985a).

The decrease in bone resorption is, however, accompanied by

a decrease in ALP which is probably due to a fall in bone formation rate as occurs with oestrogen therapy (Nordin and Peacock,1983). This effect will tend to offset the beneficial effect of decreased bone resorption but a decrease in bone turnover is likely to lead to a lower rate of bone loss in the long term.

The response of α to what are probably physiological doses of calcitriol (Gray et al,1978) could indicate either calcitriol deficiency or a primary malabsorption of calcium at the gut level. However the ready response of bone resorption to the therapy suggests that calcium malabsorption is a primary cause of the increased bone resorption.

It is likely that the changes with treatment follow the sequence of increased calcium absorption leading to decreased PTH activity, and subsequently, in bone resorption , and that decreased bone formation follows as a consequence. This interpretation is in keeping with histomorphometric findings from a study of 12 corticosteroid treated patients given calcitriol and calcium therapy for 18 months (Dykman et al,1984). There was a significant fall in the number of osteoclasts per mm^2 in the trabecular bone and similar falls in osteoblastic osteoid surface, relative osteoid volume and total osteoid surface. Only longitudinal bone density measurements can establish whether the long term effect of calcitriol and calcium on calcium balance is favourable, and the initial data (Dykman et al,1984) suggest they produce no significant change in forearm bone mineral density. It is likely that the suppression of the bone formation rate which follows suppression of bone resorption nullifies much of the

benefit gained by this therapy. The problem of decreased bone formation during corticosteroid therapy is studied in the next chapter.

CHAPTER FIVE

EFFECTS OF AN ANABOLIC STEROID, NANDROLONE DECANOATE, ON BONE FORMATION RATE DURING CORTICOSTEROID THERAPY.

INTRODUCTION

Over 40 years ago Albright (1941) suggested that postmenopausal osteoporosis was a result of decreased bone formation due to insufficient adrenal androgen production in later life. He showed that androgens produced an anabolic effect which was opposite to the catabolic effects produced by cortisol-like steroids (glucocorticoids) and claimed that the production rate of protein bone matrix was the important determinant of bone mass. Albright (1941) produced positive nitrogen and calcium balances in postmenopausal osteoporotic women given testosterone.

The side effects of testosterone therapy in postmenopausal women proved unacceptable, however, and, when it was demonstrated that calcium deprivation could cause osteoporosis in animals and that postmenopausal osteoporosis was associated with increased bone resorption, interest in androgen therapy waned. Most workers have concentrated on treating osteoporosis with agents that reduce bone resorption such as oestrogens, calcium, vitamin D and its metabolites ,calcitonin, diphosphonates or progestagens (Lindsay et

al,1978;Nordin et al,1980;Erlik et al,1981;Horsman et al,1983;Gruber et al,1984;Lindsay et al,1984).

Interest in androgen therapy has continued for other reasons , and a series of compounds have been developed which have the anabolic effects of testosterone with much less effect on the secondary sexual characteristics (Novakowski,1961). These compounds are evaluated by their ability, in rat experiments, to selectively increase the mass of the levator ani muscle with little increase in prostatic mass.

There have been several reports on the use of these agents in osteoporosis in the last 15 years. Oxandrolone has been reported to have similar histological effects to oestrogen on the bone of postmenopausal osteoporotic women (Riggs et al,1972), with a decrease in bone resorbing surfaces being followed, after 2 to 4 months, by a decrease in bone forming surfaces. No effects on the bone mass were reported in that study but the author's conclusions were that the maximal benefit that could be derived from the therapy would be "arrest or slowing of the bone loss". Wilson and Griffin (1980) later stated that anabolic steroids had no role to play in the treatment of osteoporosis.

Other reports have been more positive, with Chesnut et al (1977), Chesnut et al (1983) and Dequeker and Geusens (1985) reporting increases in bone mass with anabolic hormone therapy, using neutron activation analysis and forearm photon absorptiometry.

This study was designed to confirm the decrease in bone formation rate caused by corticosteroids and to investigate

the possible role of an anabolic steroid, nandrolone decanoate, in correcting this abnormality.

METHODS

Patients gave their informed consent for this study, which was approved by the Research Review Committee of the Royal Adelaide Hospital. The series comprises 18 patients on long term prednisolone therapy (13 females and 5 males) and 38 postmenopausal women with varying degrees of spinal osteoporosis as controls. None of the patients had biochemical or clinical evidence of renal failure, osteomalacia or Paget's disease. The steroid-treated patients were aged 60±2 years and the osteoporotic patients 67±1 years.

BONE FORMATION RATE.

The bone formation rate was measured using the expanding pool model (Burkinshaw et al,1969) as follows:

The patients were admitted to hospital. After intravenous injection of 10 mcCi of ^{47}Ca, the body content of isotope was measured in a whole body monitor. Blood samples were taken 5 minutely for 30 minutes, then at 60 minutes, 120 minutes, 300 minutes, 700 minutes and at 1500 minutes and then daily up to 1 week, whereupon the whole body count was repeated.

The log of the specific activity of ^{47}Ca fell linearly when plotted against the log of the elapsed time (a power function) for the first 16 hours, which is consistent with

diffusion of the isotope into an ever expanding pool. The reciprocal of the specific activity of ^{47}Ca at any instant is thus a measure of the size of the pool, and extrapolation of the line allows calculation of the theoretical pool size at 1 week. The specific activity of ^{47}Ca deviates from this straight line after 16 hours and this deviation is attributed to both calcium loss from the body in faeces and urine and to loss into a permanent site in the bone (mineralization).

The amount of isotope in the exchangeable pool after 1 week can be calculated from the pool size which is the reciprocal of the theoretical (extrapolated) specific activity at 1 week if no calcium had left the pool (S^*_t) (figure 5.1) and from the measured specific activity at 1 week (S'_t). The amount in the pool is S'_t/S^*_t.

The mineralization rate is = Retention of ^{47}Ca - S'_t/S^*_t per week, or the amount retained in the body minus the amount in the exchangeable pool.

On discharge the corticosteroid-treated patients were commenced on either a course of nandrolone decanoate, 50 mg intramuscularly every 2 weeks or the same dose of nandrolone together with calcium 1g/d and calcitriol 250 ng/d orally.

The bone formation rate was measured again after treatment for 3 months.

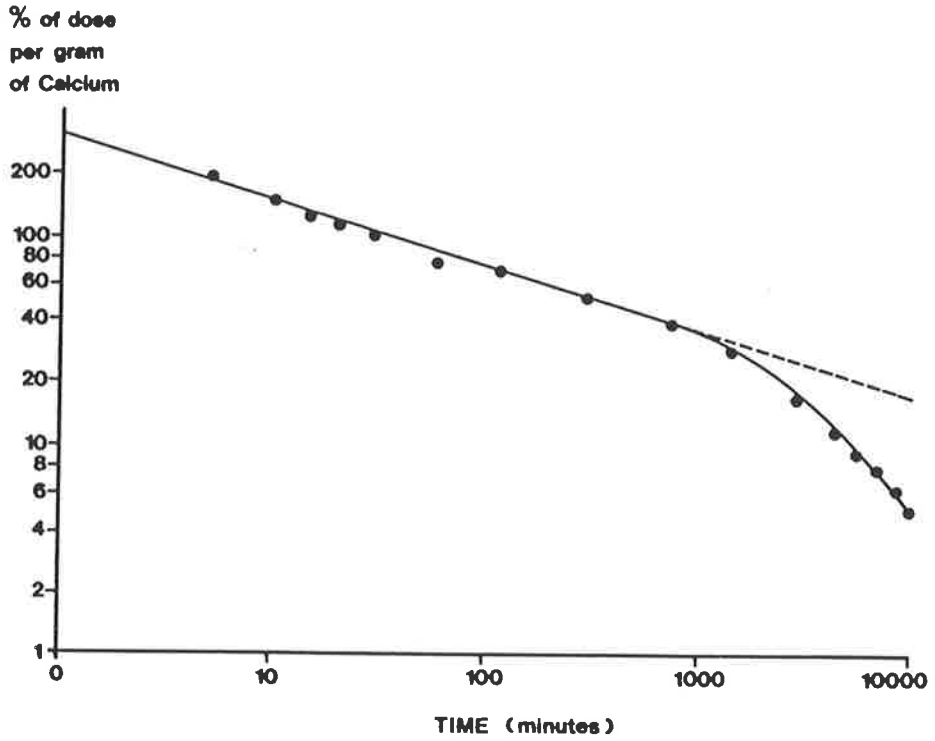

Figure 5.1.

Plot of log plasma specific avtivity of ^{48}Ca against log time in a patient given 10 mcCi of ^{47}Ca intravenously.

RESULTS

The radio-isotopic bone formation rate in 18 patients (13 female and 5 male) on prednisolone therapy was 65.5±15.6 mg of calcium per day whereas in 38 postmenopausal women with osteoporosis it was 138.0±16.7 mg/d./d ($p<0.01$)(table 5.1). There was no significant difference in bone formation rate between male and female patients on prednisolone.

Fourteen patients on prednisolone therapy (5 male and 9 female) were treated with nandrolone decanoate 50 mg intramuscularly every 2 weeks for 3 months and the bone formation rate was then remeasured. There was

no significant change in bone formation rate (from 50.5±13.6 to 98.5±27.6 mg/d)(table 5.2).

There was no significant change in the bone formation rate in those patients who had received calcium and calcitriol as well as nandrolone decanoate (from 60.6±25.0 to 34.4±22.4 mg/d). When the group given nandrolone decanoate alone was examined separately, it was found that there was a significant rise in the bone formation rate from 45.0±17.1 to 134.1±36.8 mg/d ($p<0.02$) (figure 5.3).

SIDE EFFECTS

No patient reported any increase in facial hair or hoarseness of the voice during the 3 month's course of nandrolone therapy.

	n	BONE FORMATION RATE (mg/d)	SE
CORTICOSTEROID-TREATED			
Male	5	30.0	13.4
Female	13	79.1	19.9
All	18	65.5	15.6
p		<0.01	
POSTMENOPAUSAL OSTEOPOROTIC WOMEN	38	138.0	16.7

TABLE 5.1
Bone formation rate in prednisolone-treated patients compared with postmenopausal osteoporotic women.

		BONE FORMATION RATE mg/d		
	n	Before	p	After
All patients	14	50.5(13.6)	ns	98.5(28.0)
Nandrolone, calcium and calcitriol.	5 (3F,2M)	60.5(25.0)	ns	34.4(22.4)
Nandrolone alone	9 (6F,3M)	45.0(17.1)	<0.025	134.1(36.8)

TABLE 5.2
Change in bone formation rate (±SE) in corticosteroid-treated patients given nandrolone decanoate or nandrolone decanoate plus calcium and calcitriol for 3 months.

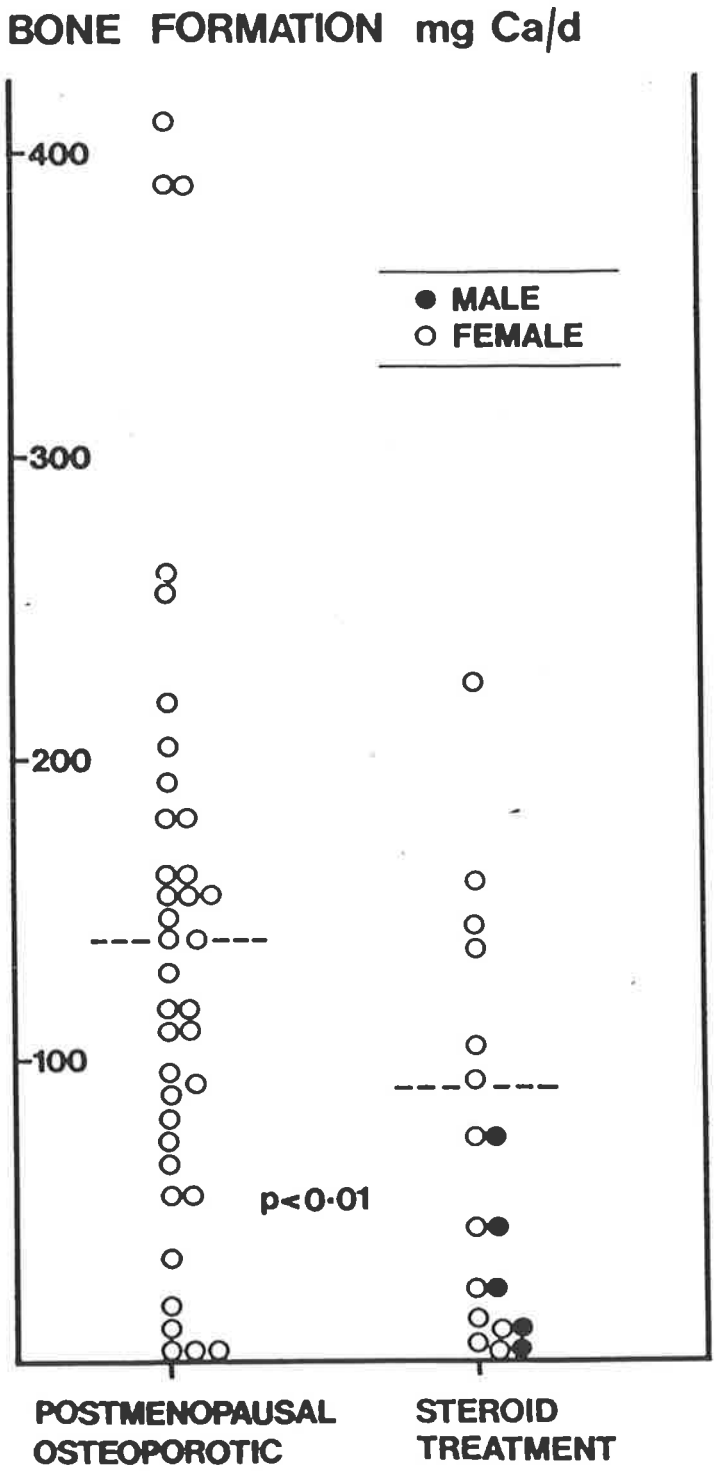

Figure 5.2.

Comparison of radiokinetically measured bone formation rate in postmenopausal osteoporotic women and steroid-treated cases.

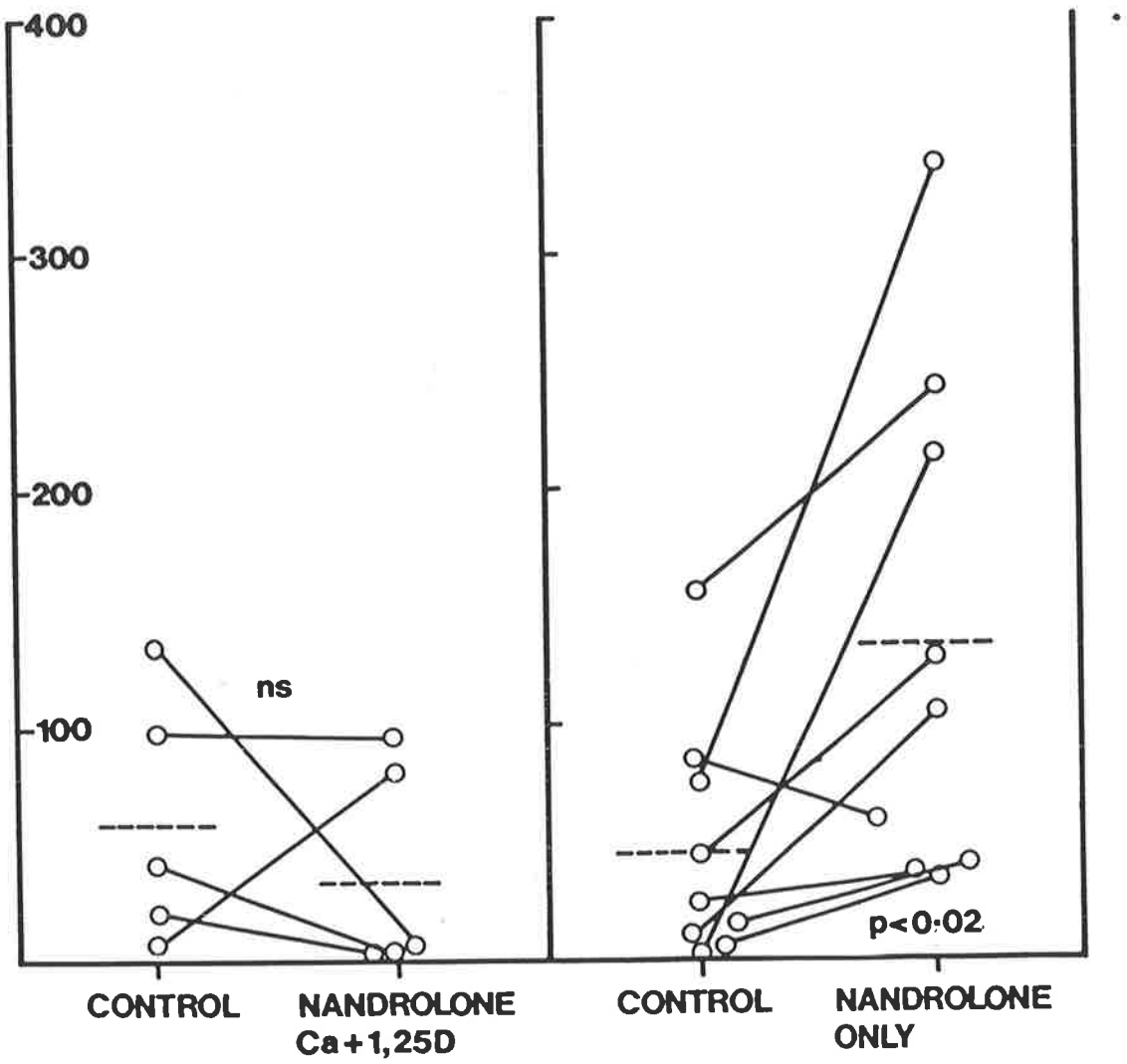

Figure 5.3.

Effects of treatment with nandrolone decanoate combined with calcium and calcitriol and nandrolone alone on radiokinetically measured bone formation rate in prednisolone-treated patients.

DISCUSSION

Some of the postmenopausal osteoporotic patients were chosen for this measurement because of a suspected low bone formation rate, having osteoporosis with a normal bone resorption rate (measured by the urinary excretion of hydroxyproline). There are no normal controls in this series, however, and it is impossible to say whether the bone formation rate of 138 mg/d in the postmenopausal osteoporosis group is really low or whether it just reflects differences in technique from earlier studies which give a normal mean of 250 mg/d (Burkinshaw et al,1971). Whatever the case for the postmenopausal osteoporotic women, the corticosteroid-treated patients had an even lower bone formation rate, in keeping with the histomorphometric findings of a low bone formation rate in patients treated with corticosteroids (Jowsey and Riggs,1970;Bressot et al,1979). The magnitude of the decrease in ^{47}Ca bone formation rate is similar to that found in the histomorphometric appositional rate by Bressot et al (1979) which was 0.34 ± 0.25 micron/d for 50 corticosteroid-treated patients and 0.72 ± 0.12 micron/d for normal controls, $p<0.001$).

After nandrolone decanoate therapy there is almost a doubling of the bone formation rate, indicating that this therapy may be of use in treating corticosteroid osteoporosis, especially if the increased bone resorption can be controlled. The fall in bone formation rate in those patients given calcium and calcitriol as well as nandrolone is probably a consequence of the fall in bone resorption

caused by these agents as detailed above. Whenever increased bone resorption is corrected, whether with oestrogen (Nordin and Peacock,1983) or with calcium and calcitriol (Need et al,1985a) there is a subsequent fall in plasma alkaline phosphatase activity, which almost certainly indicates a fall in bone formation. Indeed, this is probably the factor which limits the usefulness of agents designed to decrease bone resorption.

It is not known whether the beneficial effect of nandrolone decanoate therapy on bone formation in corticosteroid-treated patients will lead to an increase in the bone mass, but the following study was designed to test such an hypothesis.

CHAPTER SIX

EFFECTS OF NANDROLONE DECANOATE ON FOREARM BONE MINERAL DENSITY IN CORTICOSTEROID-INDUCED OSTEOPOROSIS.

Corticosteroid-induced osteoporosis is a consequence of both reduced bone formation and increased bone resorption, but treatment up to now has been largely focussed on reducing bone resorption with agents such as calcium and vitamin D (Hahn et al,1976), thiazide diuretics (Suzuki et al,1974) and calcium and calcitriol (Dykman et al,1984). Perhaps this is because changes in bone resorption have been emphasised in postmenopausal osteoporosis and they can be monitored effectively by measurement of the urinary excretion of hydroxyproline. Changes in bone formation are usually measured by sequential bone biopsy or radiokinetic analyses which are more difficult to perform on large numbers of patients. Fluoride therapy has been suggested as the most efficient way of increasing trabecular bone formation during corticosteroid therapy (Baylink,1983) and vigorous exercise has also been suggested to be useful. The former therapy is less likely to increase appendicular bone (Baylink,1983), can cause intestinal upset and fascial pain and the bone formed is histologically abnormal. The latter therapy is impractical for most corticosteroid treated patients.

Albright considered that virtually all osteoporosis was due to decreased bone formation, and that it would respond to testosterone therapy (Albright,1941) but the virilizing effects of this hormone made it unsuitable for use in postmenopausal women, who make up the bulk of the osteoporotic population.

Anabolic steroids, designed to have the anabolic effects of testosterone without the virilizing effects, have been subsequently developed. These drugs are generally believed to have little beneficial effect in osteoporosis (Wilson and Griffin,1980), but some positive reports have appeared (Chesnut et al,1977;Chesnut et al,1983;Dequeker and Geusens,1985). This report describes the change in forearm mineral density, using photon absorptiometry, in a group of patients with steroid-induced osteoporosis who were observed both on and off nandrolone decanoate therapy.

METHODS

Ten patients on long term prednisolone therapy, and with varying degrees of osteoporosis, were included in this trial. The mean dose of prednisolone was 11±1.7 mg/d. Patient details and diseases for which prednisolone were prescribed are given in table 6.1.

Seven of the patients (with normal urinary hydroxyproline excretion) received no other treatment than the anabolic steroid. Calcitriol and calcium was given to one patient with calcium malabsorption and calcium to 2 patients with

increased bone resorption, to control increased bone resorption (detected by increased urinary hydroxyproline excretion). In 6 of the patients the first period of observation was on nandrolone decanoate and the second (control) period off this therapy. In 4 patients nandrolone was given after an appropriate control period of observation. The only change in therapy between each period of observation was the removal or addition of nandrolone decanoate at a dose of 50 mg every 2 weeks by intramuscular injection. The details of other treatments received are given in table 6.1.

The bone mineral density of the distal right forearm was measured on a Molsgaard Bone Mineral Analyser by photon absorptiometry, using a ^{125}I source. The arm was immersed in water and 6 rectilinear scans performed, in automated fashion, beginning at a fixed distance from the wrist. The hand gripped a fixed post, with the metacarpo-phalangeal joints opposite a mark on the post to standardize supination at the wrist. The first scan was programmed to begin at a site where the gap between radius and ulna reached 8 mm and then each successive scan was performed 4 mm proximal to the previous one. After 6 scans the attached computer calculated the integral of the absorption across both radius and ulna for each scan, giving the water a value of zero, and then calculated the mean for all scans. The instrument was calibrated against a human radius and ulna, which were subsequently ashed, so as to give a direct result for forearm mineral content (FMC) in mg/cm. The coefficient of variation of duplicate measurements on 20 individuals on different days was 0.5%. The FMC was divided by the cross sectional area of

the radius and ulna (Horsman and Leach,1974) to give the forearm mineral density (FMD) in mg/ml. In sequential studies the FMC was divided by the original bone area to yield sequential values of FMD.

Initial and final FMD were compared using Student's t test for paired data. Rates of change were compared in 2 ways using Student's t test for paired and unpaired data. In the first (paired) analysis the mean rates on and off nandrolone decanoate were simply compared. However this analysis does not take account of the differences in periods of observation between cases. On the assumption that longer periods of observation should carry more weight, the time-weighted mean rates of change were calculated by entering each rate in the sum as many times as there were months of observation.

RESULTS

Details of the responses to nandrolone are given in Table 6.2. In the group as a whole, the mean FMD deceased from 357±22 mg/ml to 344±21 mg/ml ($p<0.01$) during the control periods. The FMD fell at a comparable rate whether the control period was after nandrolone decanoate therapy (-1.49±0.56 mg/ml/month) or before nandrolone decanoate therapy (-1.47±0.62 mg/ml/month). In the group as a whole there was a significant increase in FMD from 346±23 mg/ml to 357±23 mg/ml ($p<0.025$) on nandrolone decanoate.

The unweighted mean rate of change in FMD in the control

periods was -1.48 mg/ml/month ($p<0.025$). The unweighted mean rate of change on nandrolone decanoate therapy was +1.47 mg/ml/month ($p<0.05$). The difference between the unweighted mean rates of change was definitely significant for the whole group ($p<0.01$).

The time-weighted mean rate of change off nandrolone decanoate was significantly negative for the group as a whole ($p<0.01$) and the weighted rate of change was significantly positive on nandrolone decanoate ($p<0.025$) but the difference between the weighted rates of change during the control and nandrolone periods was highly significant ($p<0.001$)(figure 3).

The treatment was well tolerated. There were no side effects in the men but 1 woman developed an increase in facial hair after 17 months on the drug and 1 woman reported an unwanted increase in libido.

Case	Age	Sex	Primary Disease	Initial FMD	Other Treatment	Dose of Prednisolone (mg/d)
1.	58	F	Asthma	308	Nil	15
2.	72	M	Pacemaker Failure	366	Nil	15
3.	54	M	Asthma	442	Nil	15
4.	60	F	Asthma	433	Nil	15
5.	72	F	Asthma	347	Ca 1g/d	Intermittent
6.	63	F	Asthma	216	1,25D;0.25ug Ca 1g/d	20
7.	48	F	Asthma	424	Nil	10
8.	66	F	Asthma	265	Nil	10
9.	66	F	Colitis	380	Nil	Intermittent
10.	58	F	Poly-neuritis	327	Ca 1g/d	10

TABLE 6.1
Details of 10 patients in crossover trial of the effects on forearm bone density of nandrolone decanoate 50 mg intramuscularly every 2 weeks.

	PRE NANDROLONE		ON NANDROLONE		POST NANDROLONE	
Case	Time (months)	Rate (/mth)	Time (months)	Rate (/mth)	Time (months)	Rate (/mth)
1.	–	–	17	+0.12	11	-1.36
2.	14	-1.86	4	+3.25	–	–
3.	9	-2.33	16	+1.50	–	–
4.	–	–	3	+2.67	19	-0.42
5.	–	–	3	-3.33	15	-0.40
6.	–	–	11	+2.27	9	-2.00
7.	–	–	6	+1.00	3	-4.33
8.	–	–	4	+1.75	3	-0.33
9.	9	-2.67	7	+2.00	–	–
10.	7	-0.14	6	+3.50	–	–

TABLE 6.2
Observation periods and rates of change of forearm bone density in 10 patients in crossover trial of nandrolone decanoate therapy.

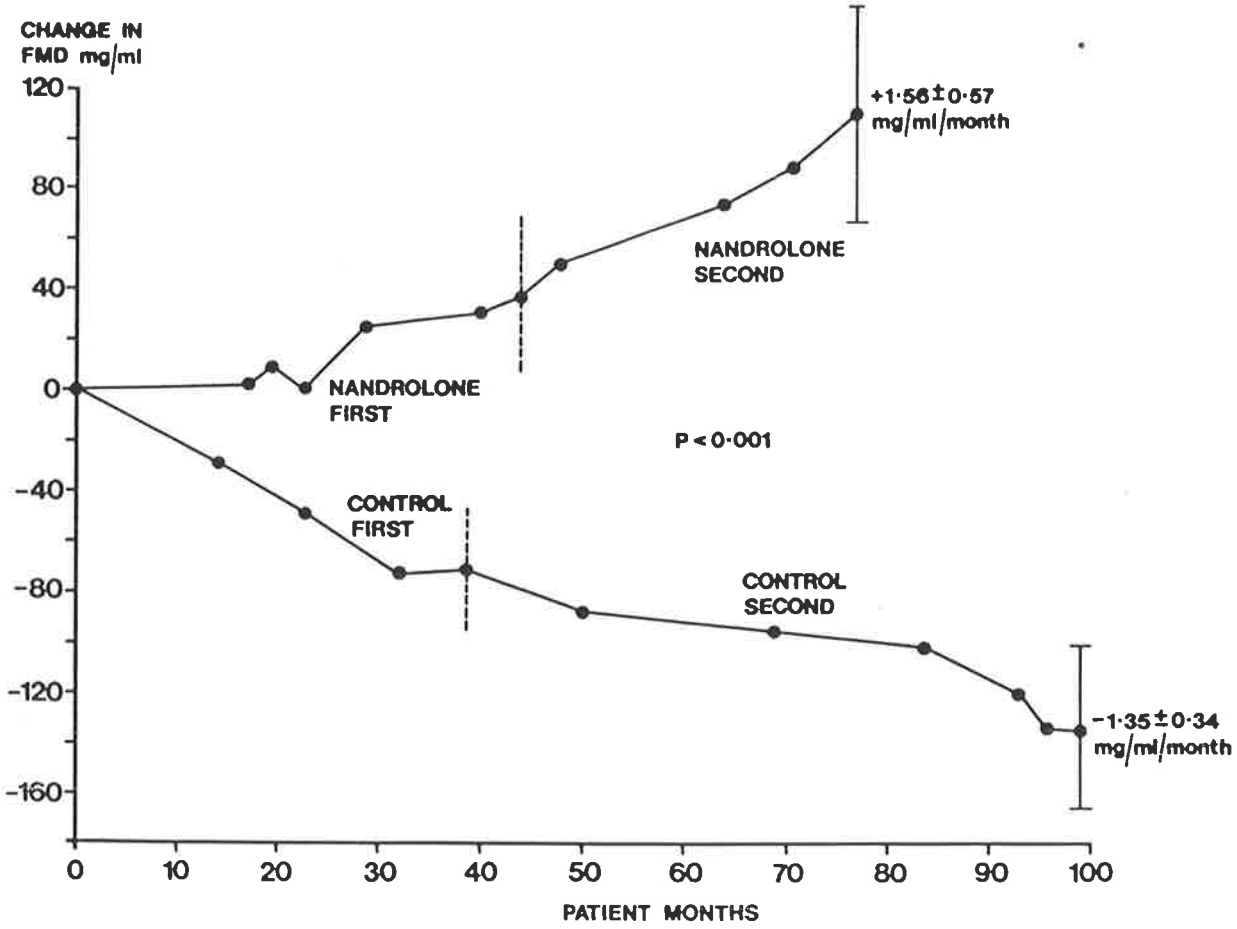

Figure 6.1.

Comparison of changes in forearm mineral density (FMD) on and off nandrolone decanoate therapy , 50mg intramuscularly every 2 weeks, in 10 corticosteroid-treated patients. Each point represents one patient.

DISCUSSION

The treatment of corticosteroid-induced osteoporosis remains unsatisfactory at the moment because most patients present with severe bone mineral loss and most therapies, at best, merely arrest the process of bone resorption. The striking finding in this study is a significant increase in bone mass during the treatment period.

Nandrolone has been used for over 20 years to treat osteoporosis, but there is a surprising lack of data on its effectiveness in this condition. Previous authors have found no evidence of biochemical (Bijlsma et al,1982) or radiological (Vose et al,1978) change during anabolic steroid therapy, but other studies, using neutron activation analysis (Chesnut et al,1977;Chesnut et al,1983) and photonabsorptiometry (Dequeker et al,1985) suggest that these agents may increase bone mass.

Although the increase in bone mass in our patients may eventually be limited by an increasing absolute resorption rate (if bone mass is determined by the ratio of formation to fractional resorption (Nordin et al,1981)) any increase in bone mass is remarkable in itself. Most therapies for osteoporosis have been designed to limit further loss of bone by decreasing bone resorption and thus tend to cause a subsequent fall in the bone formation rate which prevents any increase in the bone mass (Nordin et al,1981).

Previous studies showing increased total body calcium (by neutron activation analysis) (Chesnut et al,1977;Chesnut et al,1983) have used a method with much less precision than the

one described here and patient dropout and differences between patients and controls have hampered interpretation of the results. The site of increased calcium deposition was also not determined. This study is the first to use patients as their own controls and this has become possible because the greater precision of photon absorptiometry allows detection of significant changes over a relatively short time.

The rate of fall in FMD during the control periods overall was 16 mg/ml per annum. This is rather faster than the 6 mg/ml per annum loss that might be expected in untreated normal subjects in this age group (Nordin BEC - personal communication) and indicates how bone mass may fall rapidly on corticosteroid therapy in spite of control of bone resorption. This, of course, is because of the decreased bone formation rate in these cases. The author's results are compatible with this concept, i.e. that continuing bone loss after normalization of urinary hydroxyproline excretion implies impaired bone formation. The results are also compatible with the low androgen levels in osteoporosis which is reported elsewhere (Nordin et al,1985).

Bone mineral content has been related to the structural strength of the bone (Cater and Hayes,1976;Dalen et al,1976) so that it is anticipated that the patients treated with nandrolone will sustain less fractures in the long term than those patients treated with anti-resorptive treatment alone. Studies designed to confirm this are now indicated, but will require large numbers of patients to be followed for longer periods of time.

Anabolic steroid therapy can only increase the bone mass if sufficient calcium is available to mineralize the new bone formed and so in this study nandrolone was only given to patients with normal calcium absorption, or where malabsorption of calcium had been corrected with calcitriol. However, anabolic steroids could possibly be beneficial even when calcium absorption is impaired, if they reduce the urine calcium, as has been reported (Chesnut et al,1983). This has not been specifically examined by the author or anyone else. The results reported here suggest the need for further studies of the factors governing bone formation, and ways of identifying cases in which this is grossly impaired.

CONCLUSIONS

One of the commonest side effects of corticosteroid therapy is osteoporosis, frequently heralded by the onset of vertebral and rib fractures. These fractures affect 10% or more of corticosteroid-treated patients, with postmenopausal women being especially at risk. There is a significant decrease in bone density during such therapy, whether the assessment is made by histomorphometry, metacarpal morphometry, photon absorptiometry or neutron activation analysis. Trabecular bone mass appears to be affected more than cortical bone mass.

Corticosteroid therapy can have marked effects on calcium metabolism but there is little effect on the plasma calcium level itself in the intact animal. However, adrenalectomy can lead to a rise in the plasma calcium level and corticosteroids can be used to treat hypercalcaemia. Parathyroid hyperplasia has been reported in Cushing's syndrome in man and also after corticosteroid treatment in animals, and the evidence suggests that parathyroid secretion rises to maintain the plasma calcium level during corticosteroid therapy. This normally overcomes any tendency for corticosteroids to cause hypocalcaemia.

BONE RESORPTION

Bone resorption is increased in corticosteroid osteoporosis (Riggs et al,1966;Crilly et al,1978;Bressot et al,1979) and

this appears to be the result of both decreased intestinal absorption of calcium (Kimberg et al,1971;Crilly et al,1978) and increased urinary calcium excretion (Adams et al,1981;Suzuki et al,1983). Such a model has been described in calcium-deprived rats given a large sodium intake to increase urinary calcium excretion (Goulding,1980).

Serum parathyroid hormone levels are increased during corticosteroid therapy in man, further supporting the calcium deprivation hypothesis (Fucik et al,1975;Lukert et al,1976;Hahn and Hahn,1976;Suzuki et al,1983). In this connection it is worth noting that glucocorticoids actually decrease bone resorption in vitro, but this effect can be overcome with sufficient parathyroid hormone (Raisz et al,1972a).

Serum levels of 25 hydroxyvitamin D, the major circulating vitamin D metabolite, are either normal or slightly reduced in corticosteroid osteoporosis and it is unlikely that this has any effect on calcium metabolism.

The author has analysed the effects of calcium malabsorption and increased urinary calcium excretion in 30 postmenopausal corticosteroid treated women. The hourly fractional ^{45}Ca absorption (α) was lower (0.45 ± 0.05 vs 0.85 ± 0.05, $p<0.001$) and fasting urinary calcium/creatinine (Ca/Cr) higher (0.43 ± 0.05 vs 0.25 ± 0.03, $p<0.05$) in the osteoporotic patients (n = 16) than in those with normal spines (n = 14). Because calcium absorption has a positive effect on calcium balance and urinary calcium a negative effect, the difference between these 2 variables (α - Ca/Cr) was calculated for each patient. The result was very

significantly lower in the osteoporotic patients (0.02±0.08) than in those with normal spines (0.60±0.06, $p<0.0001$). This index may prove to be a useful predictor of the risk of osteoporosis developing in any patient on corticosteroid therapy.

The calcium malabsorption in corticosteroid – treated patients has been further investigated by simultaneous measurement of calcium absorption and its major regulating hormone, 1,25-dihydroxyvitamin D (1,25D). Levels of 1,25D were similar (and normal) in the OP and N patients in spite of a very significant difference in radiocalcium absorption ($p<0.001$).

Regression of radiocalcium absorption on 1,25D levels shows that the slope for OP patients (0.0024±0.0008) differs from that for N patients (0.0050±0.0010) very significantly ($p<0.001$). Because the slope for the N patients corresponds with that for normal postmenopausal women (Morris et al,1985), the data indicate that the OP patients have a deficient intestinal calcium absorption response to 1,25D. Results of further studies to define the site and nature of this defective response, and to determine the behaviour of the 1,25D receptors and relevant post receptor events are anticipated.

Treatment of the calcium malabsorbing patients with oral 1,25D and a calcium supplement resulted in a significant fall in the fasting urinary hydroxyproline, consistent with a decrease in bone resorption. This ready response is taken as further evidence that the malabsorption of calcium is an important cause of the increased bone resorption in

corticosteroid osteoporosis.

BONE FORMATION RATE

Reifenstein (1956) believed that corticosteroid osteoporosis was due to an imbalance between catabolic (cortisol-like) and anabolic (androgen-like) effects and that, as a result, there was a decrease in the formation of the protein bone matrix. The bone formation rate, measured by histomorphometric methods (Jowsey and Riggs,1970;Bressot et al,1979;Hahn et al,1979) or radiokinetic methods (Gallagher et al,1973) does appear to be reduced in corticosteroid osteoporosis and in vitro studies support this hypothesis (Peck et al,1967;Chen et al,1979;Canalis,1983).

The data reported here, derived from a radiokinetic measurement using the expanding pool model (Burkinshaw,1969), confirm that the bone formation rate is decreased during corticosteroid therapy. The mineralization rate was 66±16 mg of calcium per day in 18 corticosteroid treated patients and 138±17 mg Ca per day in 38 postmenopausal osteoporotic women not on corticosteroids ($p<0.01$).

In 9 patients treated for 3 months with nandrolone decanoate 50 mg intramuscularly every 2 weeks, there was an increase in the bone formation rate from 45±17 mg/d to 134±37 mg/d ($p<0.02$), indicating that the abnormality could be responsive to anabolic agents. This rekindled interest in Reifenstein's 30 year old impressions and raised the question whether some of the decrease in bone formation might be caused by the suppression of adrenal androgen production which accompanies corticosteroid therapy. Low adrenal

androgens have been found in osteoporotic patients not on corticosteroids by our group previously (Nordin et al,1985).

Further experiments were performed to see if the increase in bone formation rate after nandrolone therapy could have a beneficial effect on the bone mineral mass. For this purpose sequential measurements of forearm mineral density were performed, in 10 corticosteroid treated patients, on the Molsgaard Bone Mineral Analyser. This instrument measures the absorption of ^{125}I gamma rays on 6 scans across the forearm with an overall imprecision of 0.5% CV. In a cross-over study patients were observed during a course of nandrolone or during an adjacent control period.

During nandrolone therapy the patients gained bone mineral at a mean rate of 1.6 ± 0.6 mg/ml/month ($p<0.05$). During the control period there was a mean loss of 1.3 ± 0.3 mg/ml/month ($p<0.01$). The difference between the 2 observation periods was highly significant ($p<0.001$).

Methods of identifying patients with a decreased bone formation rate now need to be further developed as they may be useful in selecting patients for anabolic hormone therapy.

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