THE PATHOGENESIS AND TREATMENT of GLUCOCORTICOID-INDUCED OSTEOPOROSIS

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The work described in this thesis has resulted in the following publications:

1. Reid IR, France JT, Pybus J, Ibbertson HK. Low plasma testosterone levels in glucocorticoid-treated male asthmatics. Br Med J 1985; 291:574.

2. Reid IR, Chapman GE, Fraser TRC, Davies AD, Surus AS, Meyer J, Huq NL, Ibbertson HK. Low serum osteocalcin levels in glucocorticoid-treated asthmatics. J Clin Endocrinol Metab 1986; 60: 379-383.

3. Reid IR, Katz J, Ibbertson HK, Gray DH. The effects of hydrocortisone, parathyroid hormone and the bisphosphonate APD on bone resorption in neonatal mouse calvaria. Calcif Tissue Int 1986; 38: 38-43.

4. Reid IR, Ibbertson HK. Calcium supplements in the prevention of steroid-induced osteoporosis. Am J Clin Nutr 1986; 44: 287-290.

5. Reid IR, Schooler BA, Hannon S, Ibbertson HK. Biochemical sequelae of an oral calcium load : a comparison of four proprietary supplements. Aust NZ J Med 1986; 16: 193-7.

6. Reid IR. Serum testosterone levels during chronic glucocorticoid therapy. Ann Int Med 1987: 106: 639 (letter).

7. Reid IR, Ibbertson HK. Evidence for decreased tubular reabsorption of calcium in glucocorticoid-treated asthmatics. Hormone Res 1987; 27: 200-204.

8. Reid IR, Ibbertson HK. Corticosteroids and osteoporosis. Aust NZ J Med 1987; 17: 611-612 (letter).

9. Reid IR, King AR, Stewart AW, Alexander CJ, Ibbertson HK. Prevention of steroid-induced osteoporosis. In: Christiansen C et al (eds). Osteoporosis 1987, Kobenhavn, Osteopress pp 1016-1020.

10. Reid IR, King AR, Alexander CJ, Ibbertson HK. Prevention of steroid-induced osteoporosis with (3-amino-1-hydroxypropylidene)-1,1-bisphosphonate (APD). Lancet, 1988; i:143-146.

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CHAPTER 1 : REVIEW OF THE LITERATURE

1.1 Introduction

The original description of endogenous glucocorticoid excess by Cushing included reference to the "increased tendency to fracture" in these patients (Cushing,1932). The introduction of cortisone as a therapeutic agent in the following decade was followed by case reports of vertebral fractures (Curtess et al 1954), indicating that hypercortisolism of whatever cause is deleterious to the skeleton. Since that time, many workers have documented that steroid-treated patients have decreased bone mass, whether assessed by histomorphometry of bone biopsies (Dempster et al, 1983), the prevalence of fractures (Adinoff and Hollister, 1983) metacarpal cortical thickness (Greenberger et al, 1982) or more sophisticated techniques (Hahn and Hahn, 1976; Hahn et al, 1979; Rickers et al,1982, Dykman et al, 1984; Schaadt and Bohr, 1984; Reid DM et al, 1986;). Prospective studies of bone mass during steroid treatment are fewer in number but confirm that these drugs do cause progressive bone loss. Deding et al (1977) found a 2.5% decrease in distal forearm bone mineral content during the first 12 weeks of prednisone treatment for haematological or connective tissue diseases. In a similar study, Rickers et al (1984) found that this rate of loss fell to 0.6% in the second 12 weeks of therapy. No untreated control group was studied so the contribution of the underlying disease to the negative calcium balance is not known. Comparable studies in asthmatic patients have found dose-related losses of peripheral trabecular bone of between 1 and 7% per annum

(Ruegsegger et al, 1983). Factors determining the rate of bone loss in a particular patient remain to be determined. Some studies have found that it is related to steroid dose (Chesney et al, 1978; Hahn, 1978; Ruegsegger et al, 1983), that younger patients are more at risk (Ruegsseger et al, 1983) and that the underlying disease determines susceptibility (Mueller, 1976; Reid DM et al, 1986). Evidence is conflicting on each of these points, in particular with regard to the deleterious effects of sub-replacement doses of glucocorticoids. Two studies in patients with rheumatoid arthritis found that mean bone mass was less than control in those receiving low doses of glucocorticoids (Nagant de Deuxchaisnes et al, 1984; Sambrook et al,1986) though the difference was not statistically significant in Sambrook's study.. It thus appears unlikely that there is a threshold below which glucocorticoid therapy is without some effect on bone metabolism.

Glucocorticoid-induced bone loss is more marked in trabecular than cortical bone (Hahn et al, 1974; Schaadt and Bohr, 1984). It is therefore the vertebrae, ribs and pubic bones which fracture most frequently (Maldague et al, 1984). Vertebral bone loss caused by steroids is characterised by the radiographic appearence of "hollow vertebrae" in which vertical and horizontal trabeculae are equally diminished (Maldague et al, 1984). In contrast, in involutional osteoporosis there is preservation and possibly thickening of vertical trabeculae.

1.2 Pathogenesis

Glucocorticoid drugs affect calcium and bone metabolism at many

sites. Bone biopsies from patients receiving glucocorticoids show increased bone resorption and decreased bone formation (Jowsey and Riggs, 1970; Meunier et al, 1984). There is thus an uncoupling of these usually tightly linked processes. This is the basis of the rapid bone loss which glucocorticoid drugs can produce. The extent to which these changes in osteoblast and osteoclast function are the direct effect of glucocorticoids will now be considered, along with other possible mechanisms by which steroids might modulate bone cell function .

1.2.1 Osteoblasts: Bone cells have cytoplasmic glucocorticoid receptors (Feldman et al, 1975) and therefore, the direct action of steroid hormones on bone is possible. Rodent osteoblasts grown in culture show inhibition of protein and collagen synthesis (Peck et al, 1967; Wong, 1979; Guenther et al, 1984), cell growth (Chen and Feldman, 1971) and RNA synthesis (Peck et al, 1967; Peck et al, 1969) when exposed to glucocorticoids.Human osteoblast cells show similar responses during long-term incubation with supraphysiological steroid concentrations but low glucocorticoid levels and short incubation periods stimulate cell growth and collagen synthesis (Gallagher et al, 1984).

In bone organ culture the situation is more complex. Cortisol ($3x10^{-8}$- $3x10^{-6}$M) stimulates [^{3}H]-proline incorporation into both collagenase-digestible and other proteins when present for periods of up to 48 hours. At 96 hours however, [^{3}H]-proline incorporation is inhibited along with synthesis of DNA and RNA (Dietrich et al, 1979). Histological examination confirms that osteoblast and fibroblast numbers are decreased (Dietrich et al, 1979). Hahn et al (1984) have also found that a number of

indices of osteoblast activity are increased by cortisol exposure for 6-24 hours. Both this initial stimulation and the subsequent depression of osteoblast activity requires the presence of the periosteum (Canalis,1984) and it seems likely that glucocorticoids not only directly affect osteoblast function but also inhibit the proliferation of the periosteal cells from which osteoblasts are derived (Chyun, 1984). A recent study of isolated chick periostea contradicts this hypothesis by demonstrating sustained stimulation of cell proliferation by dexamethasone (Tenenbaum and Heersche,1985). The reason for this discrepancy is not clear.

Animal (Sheagren et al,1976; Tam et al, 1981; Korkor et al,1983; Jowell et al,1987) and human (Jowsey and Riggs,1970; Hahn et al,1979; Meunier et al 1984) studies confirm the in vitro evidence of impaired bone formation following steroid treatment. Histomorphometry of biopsies taken from steroid-treated subjects shows that osteoid surfaces are increased but that osteoid thickness is diminished as is the calcification rate (Meunier et al, 1984). Dempster et al (1983) have found that the diminished bone formation rate is attributable to a shortened life-span of the active osteoblastic cells in each basic multicellular unit. As in the in vitro studies, the glucocorticoid effect on bone formation may be biphasic with respect to time. In support of this, Gennari et al (1984b) have found increases in alkaline phosphatase, non-dialysable urinary hydroxyproline excretion and bone mineralisation rate after 30 days treatment with prednisone.

1.2.2 Osteoclasts: In cell culture, mouse osteoclast-like cells show a large decrease in hyaluronate synthesis at physiological

glucocorticoid levels but protein synthesis is unaffected (Wong,1979). In cultures of cat bone marrow, hydrocortisone reduces the activity, survival and generation of osteoclasts (Suda et al,1983). In contrast, Teitelbaum et al (1981) have shown glucocorticoid-enhancement of bone resorption by rat peritoneal macrophages, putative osteoclast precursors. This appears to be mediated by increased binding of these cells to bone, brought about by alterations in cell-surface oligosaccharides (Bar-Shavit et al, 1984). Bone organ culture studies are in general agreement with those of cell cultures; i.e. basal and PTH-stimulated bone resorption is inhibited by glucocorticoids (Stern,1969; Raisz et al 1972; Caputo et al,1976). Most animal studies fit with this view also and demonstrate a reduction in indices of bone resorption (Korkor et al, 1983; Lindgren et al, 1983; Jowell et al, 1987). These studies have generally used much higher doses of steroid per unit body weight than those prescribed to human subjects and one study using only moderately supraphysiological steriod doses has indeed shown increased bone resorption (Jee et al, 1972).

In human histomorphometic studies, there is a consistent finding of increased bone resorption in steroid-treated subjects (Jowsey & Riggs,1970; Hahn et al,1979; Meunier et al,1984). After one month of steroid treatment, urine hydroxyproline excretion (an index of bone resorption) is significantly elevated (Gennari et al,1984b). In a recent study of patients on glucocorticoids long-term, however, this difference was not found Need et al, 1986). It might be speculated that this is because the mean bone mass of these patients is significantly reduced and that a normal urine hydroxyproline excretion represents a higher

than normal fractional bone resorption rate. Thus expression of hydroxyproline exceretion per unit bone mass [as is done with histological indices and as has been suggested by Hyldstrup et al 1984] would resolve this discrepancy.

The differences between the clinical and laboratory studies have been explained as representing either inter-species differences in the cellular response to glucocorticoids or as being due to the presence of indirect steroid effects on bone in vivo, such as a stimulation of PTH secretion (vide infra). As suggested above, differences in steroid dose may also be important.

1.2.3 Intestinal calcium and phosphate absorption: One of the few effects of glucocorticoids on calcium metabolism about which there is general agreement is that they cause a decrease in intestinal calcium absorption. This has been documented in animals (Lindgren and DeLuca, 1983) and man, whether studied by calcium balance (Nordin et al, 1981) or radio-calcium absorption techniques (Klein et al,1977; Hahn et al, 1979; Hahn et al, 1981; Gennari et al, 1984b). This change is apparent within two weeks of the initiation of steroid treatment (Hahn et al, 1981) and its extent is dependent upon the steroid administered and the dose used (Gennari et al,1983). Dietary phosphate absorption is mediated by a similar but probably distinct mechanism to calcium (Gennari et al,1984a) and is also impaired by glucocorticoids (Gennari et al, 1983). The mechanism of the reduction in calcium and phosphate absorption is unknown. Whereas vitamin D and its metabolites were initially thought to be implicated, this is no longer generally accepted (vide infra). Investigations in chicks have shown that cortisol reduces levels of the soluble intestinal calcium-binding protein in intestinal mucosal cells

and that the levels of this protein correlate closely with intestinal calcium absorption. (Feher and Wasserman,1979). This suggests that there is a direct glucocorticoid action on the intestinal cell.

1.2.4 Urinary calcium and phosphate excretion: Glucocorticoid receptors have been identified in the human kidney thus making a direct effect of these drugs on renal calcium handling possible (Fuller and Funder,1976). Laake pointed out in 1960 that tubular reabsorption of calcium was decreased in some patients taking steroids, with a consequent rise in urine calcium loss. Since that time, an increase in 24 hour urine calcium excretion during the first month of glucocorticoid therapy has been documented by a number of workers (Wajchenberg et al,1969; Hahn et al,1980; Cannigia et al,1981; Gennari et al,1984b). In patients taking steroids long-term, 24 hour urine calcium excretion may be normal (Hahn et al,1979) but fasting urine calcium loss remains elevated (Suzuki et al,1983).In contrast, the infusion of cortisol in normal volunteers, over a 3 hour period does not affect calcium excretion (Lemann et al, 1970).

Various interpretations have been placed upon this data. The absence of an acute effect of glucocorticoids on urine calcium has been interpreted as ruling out a direct action on the renal tubule. Likewise, the increased urine calcium during the first weeks of glucocorticoid treatment is accompanied by increased urine hydroxyproline excretion (Cannigia et al,1981; Gennari et al,1984b) and could thus be attributed to increased bone lysis alone. However, the higher level of fasting calcium excretion in patients taking glucocorticoids long-term (Suzuki et al,1983) in whom hydroxyproline is not elevated, implies that

there is an alteration in renal calcium handling.

The effects of steroid treatment on renal phosphate handling have been less fully assessed. In the first few weeks of steroid therapy, urinary phosphate excretion increases (Wajchenberg et al, 1969; Cannigia, et al,1981; Gennari et al,1984b) and the tubular resorption of phosphate declines (Gennari et al,1984b). These changes could be secondary to the consistent rise in PTH levels accompanying the introduction of steroids. In those on chronic steroid therapy, however, urinary phosphate levels are not significantly elevated above control (Suzuki et al,1983). In vitro, sodium-dependent phosphate transport by brush-border membranes from the proximal renal tubules of rats is diminished by pretreatment of the animals with dexamethasone (Boross et al,1986).

1.2.5 Vitamin D: The effects of glucocorticoids on vitamin D metabolism have been widely studied but with apparently inconsistent results. Prospective studies in patients or normal subjects beginning steroid therapy have shown no changes in either 25(OH)D or 24,25(OH)D but significant increases in $1,25(OH)_2D$ after 2-15 days of treatment (Hahn et al,1981; Braun et al,1982; Gennari et al,1983). A fall in intestinal calcium absorption occurs simultaneously which cannot, therefore, be attributed to altered vitamin D metabolism. There is a strong correlation between the increases in $1,25(OH)_2D$ and PTH which follow the introduction of steroids ($r=0.87$), (Gennari et al,1983) and no increase in $1,25(OH)_2D$ occurs in hypoparathyroid patients (Braun et al,1982). These results indicate that glucocorticoids impair intestinal calcium absorption by a mechanism not involving changes in vitamin D metabolism and that there is a secondary

elevation of $1,25(OH)_2D$ mediated by PTH.

The rise in $1,25(OH)_2D$ appears to be transient and a fall back towards pre-treatment values is apparent within the first week of therapy (Braun et al,1982). This is confirmed by longer term human studies which show no significant change in production, metabolic clearance or plasma levels of $1,25(OH)_2D$ (Seeman et al,1980; Rickers et al, 1982). In the severe hypercortisolism of endogenous Cushing's syndrome the elevation of $1,25(OH)_2D$ may persist, however (Findling et al,1982).

One clinical study is in apparent conflict with those already cited and suggests that $1,25(OH)_2D$ levels are actually reduced by glucocorticoid treatment (Chesney et al,1978). These findings, however, were in children with proteinuric renal disease and are probably accounted for by loss of vitamin D metabolites and their binding protein in the urine.

Animal studies have provided conflicting results, suggesting that $1,25(OH)_2D$ levels are either elevated (Colette et al, 1986) or depressed (Carre et al, 1974). In vitro studies of renal 1α-hydroxylase activity are also contradictory, indicating either inhibition (Henry,1986) or no change (Kano and Jones,1984; Chen et al,1986) after glucocorticoid exposure.

Studies of 25(OH)D levels during long-term steroid use suggest that there may be some reduction (Klein et al,1977; Seeman et al,1979) though this is not a universal finding (Hahn et al,1979; Findling et al,1982). Changes in levels of D-binding protein do not seem to contribute to this change (Braun et al,1982) and there is evidence that the half-life of vitamin D is shortened

during steroid therapy with increased conversion of the vitamin to a biologically inactive metabolite (Avioli et al,1968). These changes may exacerbate calcium malabsorption but are not fundamental to its development, for the reasons set-out above.

Glucocorticoids may also affect calcium metabolism by modulating the sensitivity of cells to $1,25(OH)_2D$. Receptors for $1,25(OH)_2D$ in dog intestinal cells are increased following prednisone administration but no associated change in calcium-binding protein is found (Korkor et al,1985). In rat bone cells, glucocorticoids increase $1,25(OH)_2D$ receptor number (Chen et al,1983; Manolagos et al,1984; Chen et al,1986) and tissue responsiveness to this hormone (Chen et al,1986). In cells of mouse origin however, both the same (Wong et al,1986) and conflicting (Chen et al,1982) results have been found. Whether such changes occur in human cells is unknown.

1.2.6 Calcitonin: The changes in calcitonin levels associated with glucocorticoid use have received much less scrutiny than those of the other two major calcium-regulating hormones. Lo Cascio et al (1984) found a reduction in mean plasma calcitonin levels to less than half baseline values in seven patients studied during the first eight months of steroid treatment. However, four of these subjects were cortisol-deficient initially and were thus receiving physiological steroid replacement rather than pharmacological therapy. A further two had other illnesses which affect calcium metabolism (sarcoidosis and nephrotic syndrome, respectively). The relevance of these findings to patients with normal calcium metabolism is, therefore, quite unclear and the matter requires further study.

1.2.7 Parathyroid Hormone: The infusion of cortisol into normal human subjects results in an increase in serum PTH level within 15 minutes and a maximum level of 160% of the basal is reached after one hour (Fucik et al,1975). A similar increase has been found during the first few weeks of steroid therapy by some (Gennari et al,1983; Gennari et al,1984b) but not all (Hahn et al, 1981) investigators. Studies of patients chronically exposed to high glucocorticoid levels have repeatedly found a 50-100% increase in PTH levels in comparison with controls (Fucik et al,1975; Hahn et al,1979; Findling et al,1982; Suzuki et al,1983) though this finding is not universal (Seeman et al,1980). The increase in serum PTH is usually attributed to a tendency to hypocalcaenia produced by both diminished enteral calcium absorption and increased urinary calcium excretion. While these factors may operate in the long-term they cannot account for the acute increase in PTH levels that Fucik observed folowing cortisol administration to fasting subjects. In this study no fall in serum calcium was detectable and other studies (Lemann et al,1970) have shown that there is no change in urine calcium excretion during this period after glucocorticoid administration either. This implies that glucocorticoids have a direct action on the parathyroid gland. In vitro studies support this conclusion. Exposure of cultured rat parathyroid glands to cortisol (10^{-8}-10^{-6} M) results in a dose-related stimulation of PTH release which reaches six times control at 48 hours (Au,1976).

As with $1,25(OH)_2D$, the PTH sensitivity of cells may also be modified by glucocorticoids. There is evidence from in vitro studies that glucocorticoids increase the cAMP response to PTH in bone cells (Chen and Feldman,1979; Hahn and Halstead,1979; Korkor

et al,1983; Rodan et al,1984). The mechanism of this action is unclear. Rodan et al (1984) have suggested that it results from promotion of receptor-adenylate cyclase coupling whereas others have attributed it to increased activity of the adenylate cyclase catalytic unit (Chen and Feldman,1979; Rizzoli and Fleisch, 1984). There is also evidence for glucocorticoid inhibition of phosphodiesterase activity (Chen and Feldman,1978 and 1979). In late passage cultures of the clonal osteogenic sarcoma cell line, ROS 17/2.8, PTH-induced cAMP production is not followed by activation of cAMP-dependent protein kinase unless the cells have been treated with glucocorticoid (Zajac et al,1986) suggesting that steroids can also modulate later sections of this pathway.

The actions of PTH on bone cells are also mediated by changes in the intracellular calcium concentrations (Reid IR et al,1987). Recently, it has been demonstrated that the calmodulin activity in the bones of steroid-treated rabbits is increased, suggesting that PTH actions on this messenger system might also be potentiated (Lehman et al,1984). Other bioresponses to PTH also show steroid modulation. The effects of PTH on citrate decarboxylation in osteoblast-like cells, hyaluronate synthesis in osteoclast-like cells (Wong,1979) and prostaglandin production in rat calvariae (Simmons and Raisz, 1984)are all potentiated by glucocorticoids.

From this evidence it seems probable that hyperparathyroidism does accompany both acute and chronic glucocorticoid treatment. This is likely to have a significant effect upon bone metabolism in these patients and may contribute to increased bone resorption and the development of osteopenia.

1.2.8 Other locally-produced and systemic factors: Prostaglandins are synthesised in bone tissue (Sandberg et al, 1982) and they promote bone resorption in organ culture systems (Katz et al 1981 and 1983) though they have the opposite effect on isolated osteoclasts (Chambers et al,1985). The effect of prostaglandins on bone formation is biphasic with respect to dose, there being a stimulatory effect on cell replication and collagen synthesis at low concentrations with a reversal of these changes at higher doses (Raisz,1984). PGE_2 has been used in most studies though PGI_2 appears to have similar effects. Their synthesis is blocked by cortisol (Sandberg et al,1982; Chyun and Raisz, 1983) suggesting that this may be a mechanism by which cortisol modulates bone cell function. In addition, epidermal growth factor, fibroblast growth factor and platelet-derived growth factor promote bone resorption by prostaglandin-dependent mechanisms (Raisz and Kream,1983). Glucocorticoids might, therefore, be expected to block actions of these factors also.

Bone metabolism is also regulated by factors released from lymphocytes (e.g osteoclast activating factors) (Raisz and Kream,1983) and monocytes (the interleukins) (Beresford et al,1984). Since glucocorticoid drugs modulate the numbers of these cells in the circulation, an alteration in the concentration of their products is to be expected in patients taking steroids.

Growth hormone treatment of embryonic rat bone in organ culture causes an increase in alkaline phosphatase levels and stimulates the local production of insulin-like growth factor 1 (IGF-1)

(Strake,1984). The addition of IGF-1 itself to this system increases DNA and protein synthesis (Canalis,1980), cortisol inhibiting the former effect but potentiating the latter. In man, growth hormone stimulates intestinal calcium absorption, (Reid and Ibbertson, unpublished data; Chipman et al, 1980) and bone turnover (Aloia et al, 1987). It has been suggested that steroids decrease circulating levels of growth hormone (Hartog et al, 1964)) and they would therefore be expected to blunt its effects on bone cell metabolism and intestinal calcium absorption.

Sex hormones are potent regulators of bone metabolism and hypogonadism in either sex is associated with the development of osteoporosis (Nordin et al, 1981). Glucocorticoid administration results in an acute fall in plasma testosterone levels in men (Kirschner et al, 1965; Doerr and Pirke, 1976; Schaison et al, 1978) apparently as a result of a direct action on the testis (Schaison et al, 1978). Studies in rats suggest that the mechanism of this effect is a decrease in the number of gonadotropin binding sites on the testicular interstitial cells (Saez et al, 1977). The effect of chronic administration of steroids on serum testosterone levels in the male has not been assessed, hitherto.

In post-menopausal women, the adrenal gland is the principal source of sex hormones and levels of both androgens and oestrogens are substantially reduced by glucocorticoids (Crilly et al, 1 979). These changes are likely to accelerate the rate of bone loss in steroid-treated postmenopausal women.

1.3 Treatment

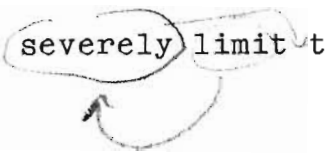
In contrast to the large number of published studies investigating the pathogenesis of glucocorticoid-induced osteoporosis, there is a dearth of information relating to its prevention and treatment. Possible therapeutic strategies can be divised from a consideration of the sites at which glucocorticoids exert their deleterious effects on bone. Thus, inhibition of bone formation might be expected to be reversed by fluoride, increased osteolysis by anti-resorptive agents such as the bisphosphonates and calcitonin, impaired intestinal calcium absorption by calcium and/or vitamin D supplements and hypercalciuria by thiazide diurectics. The evidence regarding the efficacy of each of these therapeutic options will be examined and other approaches to the problem discussed.

1.3.1 Fluoride: It was first suggested that fluroride might be an effective therapy for osteoporosis because subjects exposed to high environmental fluoride levels were found to have increased radiographic bone density. (reviewed by Nagant de Deuxchaisnes,1983). Subsequent studies of fluoride use in the management of postmenopausal and senile osteoporosis have demonstrated increases in trabecular bone volume of the iliac crest and bone mineral content of the distal radius and lumbar vertebrae. Furthermore, the Mayo Clinic group have documented that the vertebral fracture rate in patients with postmenopausal osteoporosis is substantially decreased following fluoride treatment (Riggs et al,1982). The increase in bone mass produced by fluoride appears to result from an increase in the bone formation rate (Jowsey et al, 1972).

The only study of fluoride in the prevention of steroid osteoporosis is that of Rickers et al (1982) in which patients beginning steroid treatment were randomly allocated to either receive vitamin D, sodium fluoride and calcium or to act as controls. During the first 6 months, both groups showed a 2.5% decline in bone mineral content measured in the distal forearm. The authors concluded that fluoride was without benefit in the early prevention of steroid-induced bone loss. Unfortunately, this study did not measure bone density in the spine which is the region most affected by both glucocorticoids and fluoride. In another study, patients with osteoporosis of various aetiologies including steroid therapy, were treated with fluoride (Briancon and Meunier,1981). A beneficial effect was observed in the group as a whole but separate data for the steroid-treated sub-group was not given. These workers have recently presented further data from a subsequent study in which the trabecular bone volume of steroid-treated patients increased following fluoride administration (Meunier et al, 1987). While these results appear promising, the role of fluoride treatment in steroid osteoporosis still requires further study.

1.3.2 Calcitonin: While there has been increasing interest in the use of calcitonin in the treatment of postmenopausal osteoporosis (Gruber et al,1984; Aloia et al,1985; Mazzuoli et al 1986) it has received scant attention in steroid osteoporosis. The acute osteolytic response to glucocorticoid administration in the rabbit can be prevented by calcitonin (Thompson et al,1972) and a single case report (Palmieri et al, 1974) has documented reduced hydroxyproline excretion in a steroid-treated patient. Ringe et al (1987) recently demonstrated a beneficial effect of

calcitonin on bone mass in a prospective study of six months duration in steroid-treated subjects. However, its expense, side-effects and the necessity for parenteral administration continue to severely limit the use of calcitonin.



1.3.3 Bisphosphonates: Like calcitonin, bisphosphonates are potent inhibitors of bone resorption. They have been found to increase bone mass in normal rats (Reitsma et al,1983) and pigs (de Vernejoul et al,1987) and to inhibit the bone loss accompanying castration (Wink et al,1985) or glucocorticoid treatment (Jee et al,1981) of animals. Preliminary reports have suggested that APD increases the bone mineral content of patients receiving the drug for the treatment of Paget's disease (Nagant de Deuxchaisnes et al, 1983) and in those with osteoporosis (Huaux et al,1985). Bisphosphonates are also of value in disuse (Schneider et al,1981) and juvenile (Hoekman et al,1985) osteoporosis. They have yet to be assessed in patients with steroid-induced osteoporosis.

1.3.4 Calcium and vitamin D: Because glucocorticoids cause a reduction in calcium absorption, many authors have recommended calcium supplementation of the diet in order to restore calcium balance. Formal assessment of this strategy is virtually non-existent, with only one trial in the literature (Nilsen et al,1978). This study was carried out in elderly patients with rheumatoid arthritis, half of whom received 6 g/day of microcrystalline hydroxyapatite, the remainder acting as controls. There was a slight reduction in the rate of radial (but not ulnar) bone loss in the treated group (4.8%/year)in

comparison with the control subjects (5.3%/year, $p < 0.05$). Much greater energy has been directed towards correcting the malabsorption of calcium with vitamin D or its metabolites. In rats, vitamin D (Tam et al, 1981), 1α-hydroxyvitamin D_2, 1α-hydroxyvitamin D_3 (Sjoden et al,1984) and $1,25(OH)_2D_3$ (Lindgren and DeLuca,1983; Lindgren et al,1983) diminish the bone loss caused by glucocorticoids. 1α-hydroxyvitamin D_2 appears to cause less stimulation of bone resorption than the D_3 metabolite and thus may be preferable (Sjoden et al,1984). In contrast, $1,25(OH)_2D$ appears to accelerate prednisone-induced bone loss in rabbits (Lindgren et al,1984) implying that these effects are species-specific, and thus of questionable relevance to clinical osteoporosis.

Hahn and co-workers have published a series of human studies assessing the use of vitamin D and its metabolites. They found that vitamin D 50,000u three times per week plus calcium 500 mg/day induced a 2.0 ± 0.8% increase in the bone mineral content of the radial shaft and a 8.4 ± 3.5% increase in metaphyseal bone mineral content over a 12 month period (Hahn and Hahn, 1976). When 25(OH)D (40 mg/day) was used (Hahn et al, 1979) there was a 46% increase in intestinal radio-calcium absorption and serum parathyroid hormone levels declined by 54%. This was associated with a fall in the number of osteoclasts seen in bone biopsies. Forearm bone mineral content increased over one year by 2.1±0.4% and 13.2±5.1% at the diaphyseal and metaphyseal sites respectively. Control subjects showed a non-significant decline in bone mineral content.

In contrast, the same investigators failed to find any benefit

from treating patients taking steroids with $1,25(OH)_2D$ (0.4 μg/day) (Dykman et al, 1984). Over an 18 month period, there was a 7% increase in metaphyseal bone mineral content in these patients but the control group receiving 500 mg of elemental calcium daily alone showed a similar improvement. Two other groups have found a beneficial effect of 1 α-hydroxyvitamin D on the bone mass of steroid-treated patients (Sorensen et al, 1977; Braun et al, 1983) but these studies were very short-term. The role of vitamin D and its metabolites in this condition is thus uncertain. When considering its use, its toxicity must also be borne in mind (Schwartzman and Franck, 1987).

1.3.5 Thiazides: The capacity of thiazides to increase renal tubular calcium reabsorption has led to the suggestion that they may be beneficial in steroid osteoporosis. It has been shown that bone density is increased in patients receiving thiazides for treatment of hypertension (Wasnich et al, 1983) and bendrofluazide causes a slowing of postmenopausal bone loss (Christiansen et al, 1980). Suzuki et al (1983) have shown that trichlormethiazide not only reduces the elevated urinary calcium excretion of steroid-treated patients but that nephrogenous cAMP and serum PTH levels are also suppressed. These changes would be expected to result in decreased bone resorption but the long-term effect of thiazide therapy on the bone mass of steroid-treated subjects remains to be assessed. Condon et al (1978) have reported balance studies in 3 steroid-treated subjects given bendrofluazide and vitamin D. Calcium balance tended to improve on the combined therapy but the relative contributions of the two drugs to this are not known. There is, thus, evidence suggesting a beneficial effect of thiazides in steroid-treated subjects but further studies of bone density in these patients are required.

1.3.6 Other therapies: The most obvious way of preventing steroid osteoporosis is to remove the glucocorticoid excess. A study in patients with Cushing's syndrome, has shown that correction of hypercortisolism is accompanied by a dramatic renewal of osteoblastic activity and consequent increase in trabecular bone volume (Bressot et al, 1979). Measurement of total body calcium following cure of Cushing's syndrome did not however, show such a dramatic improvement (Williams et al, 1986), possibly because this technique reflects predominantly cortical bone mass. A more realistic alternative to the withdrawal of steroid drugs is to administer them on alternate days only. This approach results in less marked inhibition of intestinal calcium absorption in human subjects (Klein et al, 1977) and in rabbits it lessens the growth retardation and degree of osteoporosis. (Sheagren et al, 1976). A prospective study in children, however, has shown that osteoporosis still develops when steroid drugs are taken on alternate days (Chesney et al, 1978). Gluck et al (1981) measured forearm bone mass in patients taking alternate day steroid treatment and in matched patients on daily therapy. The groups showed comparable degrees of bone loss. A longitudinal study of tibial trabecular bone mass in asthmatics receiving steroids on alternate days has confirmed that bone loss does occur with this regimen (Ruegsegger et al, 1983). There is thus little support for the belief that alternate day treatment has a less deleterious effect on bone mass, though the data remain scanty.

Another approach to prevention is provided by the development of deflazacort, a glucocorticoid which appears to have a reduced

effect on calcium metabolism relative to its anti-inflammatory potency. Deflazacort, an oxazoline derivative of prednisone, causes smaller increases in urine calcium and hydroxyproline excretion (Hahn et al, 1980; Caniggia et al, 1977) than does prednisone. It may also impair intestinal calcium absorption to a lesser degree (Gennari et al, 1983; Caniggia et al, 1977) though Hahn et al (1980) were unable to confirm this. A prospective 7 month study has shown that the reduction in trabecular bone volume during deflazacort therapy is approximately one third of that accompanying comparable doses of prednisone (Lo Cascio et al, 1984). Bone-sparing properties of deflazacort have also been suggested by a recent prospective, controlled trial in which patients with rheumatoid arthritis were studied over a 15 month period (Nagant de Deuxchaisnes et al, 1986). There was no change in lumbar bone mineral content in those receiving deflazacort, whereas this index declined by 4.35% ($p < 0.05$) in patients taking prednisone. The crucial question about which uncertainity continues, is the relative glucocorticoid potency of deflazacort. The results found to date would be consistent with its having a significantly lower milligram potency than prednisone and consequently being used in non-equivalent doses in the comparative studies. The clinical data with respect to anti-inflammatory efficacy do not really support this explanation but the imprecision of measuring clinical response to glucocorticoids is such that it cannot be ruled out at the present time.

It has been shown that progesterone can compete with glucocorticoids for occupancy of the glucocorticoid receptor in bone cells (Feldman et al, 1975). Progesterone therefore, might

be expected to block glucocorticoid effects. There is a case report (Roof et al, 1983) of 17-hydroxyprogesterone treatment resulting in a doubling of vertebral mineral density over one year and a recent prospective study appears to confirm this beneficial effect (Grecu et al, 1987).

Anabolic steroids have been used for some years in glucocorticoid osteoporosis, though only recently has any controlled data documenting their efficacy been published (Need, 1987). Likewise, oestrogen replacement therapy in steroid-treated postmenopausal women and regular exercise would both be expected to help maintain bone mass, but their value in steroid-treated patients has not been specifically studied.

In conclusion, no fully effective and safe therapeutic or prophylactic regimen for steroid osteoporosis has been established, though some of the options discussed appear promising. At present, the clinician must base a patient's treatment on the available, inadequate data and await the results of further clinical studies.

CHAPTER 2: THE EFFECTS OF HYDROCORTISONE, PARATHYROID HORMONE AND THE BISPHOSPHONATE, APD ON BONE RESORPTION IN NEONATAL MOUSE CALVARIA

2.1 Introduction:

Histological studies in patients taking glucocorticoid drugs show osteopenia, diminished osteoblast activity and increased bone resorption (see chapter 1). In bone tissue culture, glucocorticoids produce the same effect on indices of bone formation as is seen in vivo but bone resorption is usually found to be suppressed. There are a number of possible explanations for this discrepancy. Laboratory animals show an inter-species variation in the responses of bone cells to glucocorticoids(Chen et al, 1982, 1983a and 1983b) so the effects seen in cultured fetal rat bones may not necessarily be applicable to man. Secondly, glucocorticoid therapy in man results in secondary hyperparathyroidism (section 1.2.7) which may outweigh any direct suppression of osteoclast function by glucocorticoids. Finally, osteoclast sensitivity to parathyroid hormone (PTH) appears to be increased in the presence of glucocorticoids (section 1.2.7).

In order to assess, in vitro, the significance of these changes in PTH concentration and sensitivity, it would be of interest to compare bone resorption in the presence of physiological PTH and hydrocortisone levels with that resulting from the levels of these hormones found in steroid-treated subjects. The effects of such combined changes in PTH and glucocorticoid levels upon in vitro bone metabolism have not been studied previously.

Since increased bone resorption is one of the hallmarks of glucocorticoid-induced osteoporosis in man, inhibitors of this process might be expected to lessen the rate of steroid-induced bone loss. (3-Amino-1-hydroxypropylidene)-1,1-bisphosphonate (APD) is a potent inhibitor of osteolysis (Reitsma et al, 1980) of proven efficacy in conditions in which bone resorption is pathologically increased, such as Paget's disease (Frijlink et al, 1979) and the hypercalcaemia of malignancy (Sleeboom et al, 1983). Its effects in the proposed model have also been assessed.

2.2 Method:

2.2.1 Organ culture: The culture procedure used was developed by Dr June Katz and has been described by her, elsewhere (Katz et al, 1981). Hemi-calvariae were dissected from 6 day old Charles River CD-1 mice and incubated on steel mesh grids in 5ml Gibco 199 medium containing human serum albumin (1 g/l), pencillin (200,000 U/l), streptomycin (100mg/l) and ascorbic acid (5.7×10^{-4} M). After 24 hours pre-culture, the bones were transferred to the treatment media containing hydrocortisone, PTH or APD and incubation continued for a further 48 hours. The cultures were maintained in an atmosphere of 5% CO_2, 20% O_2, 75% N_2, and regassed daily.

Hydrocortisone was dissolved in absolute alcohol, PTH in 0.01M acetic acid and APD in distilled water. Equivalent quantities of these diluents were added to the control cultures and the final concentrations of ethanol and acetic acid were 0.036% and 3.3×10^{-6}M, respectively.

In some experiments, pairs of hemi-calvariae were used as treatment and control explants and in others all bones were pooled and the treatment and control groups randomly selected.

2.2.2 Measurement of ^{45}Ca Release: The mice were injected subcutaneously with 5uCi $^{45}CaCl_2$ at 2 days of age. At the conclusion of each experiment, ^{45}Ca content of the media and bones was estimated and ^{45}Ca release into the medium expressed as a percentage of the total (Wilson et al, 1981). To determine non-cell-mediated ^{45}Ca release, 2-4 hemi-calvariae were killed by thrice freezing and thawing prior to incubation. The mean percent ^{45}Ca release from these bones was subtracted from that of the live bones to yield the percent active resorption which was then expressed as a ratio to active ^{45}Ca release in control calvariae.

2.2.3 Measurement of hydroxyproline synthesis: Hydroxyproline synthesis was measured by incubating calvariae in media containing [5-^{3}H] proline (1uCi/ml), for 48 hours. When ^{45}Ca release was being measured simultaneously, media were sampled for ^{45}Ca and the bones were decalcified in 2ml of 20mM EGTA (in 0.05M Tris-HCl, pH 7.5) for 24 hours at 37°C in order to determine ^{45}Ca retained in the bone (Katz et al, 1983). The decalcified tissue matrix was hydrolysed in 6 M HCl with the addition of [2-^{14}C] hydroxyproline to determine procedural losses. After evaporation of the acid, the hydrolysate residues were adjusted to pH 8.2, and the labelled proline and hydroxyproline were separated, after oxidation by chloramine T, by 5 extractions into toluene. The aqueous phase was then heated and the pyrrole product of the ^{3}H-hydroxyproline extracted into toluene and counted.

2.2.4 Measurement of protein and DNA synthesis: Unlabelled calvariae were precultured for 24 hours and then incubated in the experimental media for a further 44 hours. At that time, 1uCi L-[U-^{14}C] leucine and 5uCi[methyl-^{3}H] thymidine were added and incubation continued for a further 4 hours. The bones were removed from the media, homogenized in 0.5ml 5% trichloracetic acid (TCA), filtered (Whatman GF/C paper) and the precipitate washed (25ml 5% TCA, 4°C). An equal volume of 10% TCA was added to the medium from each dish and then centrifuged (IEC centrifuge, model HN, 3/4 speed, 10min). The precipitate was washed three times with 5ml 5% TCA. Radioactivity in the precipitate was determined and values for the bones and media summed.

2.2.5 Materials: Medium 199 was obtained from Grand Island Biological Company, New York; human serum albumin from Commonwealth Serum Laboratories, Melbourne; hydrocortisone from the Sigma Chemical Company, St Louis and radiochemicals from the Radiochemical Company, Amersham. Bovine PTH was kindly donated by Dr P M Barling and APD by Professor O L M Bijvoet. All other reagents and solvents were Analar grade.

All isotopes were measured by liquid scintillation counting.

Results are expressed as mean ± SEM and comparisons made using Student's 't' test, for paired or unpaired data, or analysis of variance, as appropriate.

2.3 Results

Hydrocortisone 10^{-8}M caused no significant change in ^{45}Ca release from calvariae but at both 10^{-7}M and 10^{-6}M, osteolysis was significantly increased (fig 1). PTH caused a dose-related rise in bone resorption at concentrations between 0.3 and 0.9 ug/l (fig 2).

When calvariae were incubated in the presence of approximately physiological concentrations of PTH and hydrocortisone (0.3ug/l and 10^{-8}M, respectively), neither ^{45}Ca release nor ^{3}H-proline incorporation were significantly different from those in control cultures (fig 3 and table). Doubling the PTH concentration from 0.3 to 0.6 ug/l, in the presence of hydrocortisone 10^{-8}M, produced a 10% increase in mean bone resorption (non-significant) and increasing hydrocortisone from 10^{-8}M to 10^{-7}M, in the presence of PTH 0.3ug/l, augmented ^{45}Ca release by 25% ($p<0.02$). When both PTH and hydrocortisone were present in the higher concentrations (0.6ug/l and 10^{-7}M, respectively) ^{45}Ca release increased by 39% above that resulting from the lower levels of both hormones ($p< 0.005$). In contrast, the incorporation of ^{3}H-proline and ^{14}C-leucine was diminished (table 1).

The effect of APD on bone resorption was studied in control explants without the addition of hydrocortisone or PTH (fig 4). A marked, dose-related inhibition was found at concentrations greater than 10^{-5}M. APD was then added to bones cultured in the presence of PTH 0.6ug/l and hydrocortisone 10^{-7}M (fig 5). The increased bone resorption previously noted in the presence of the higher PTH and cortisol levels (fig 3) was confirmed and completely suppressed by APD 10^{-5}M.

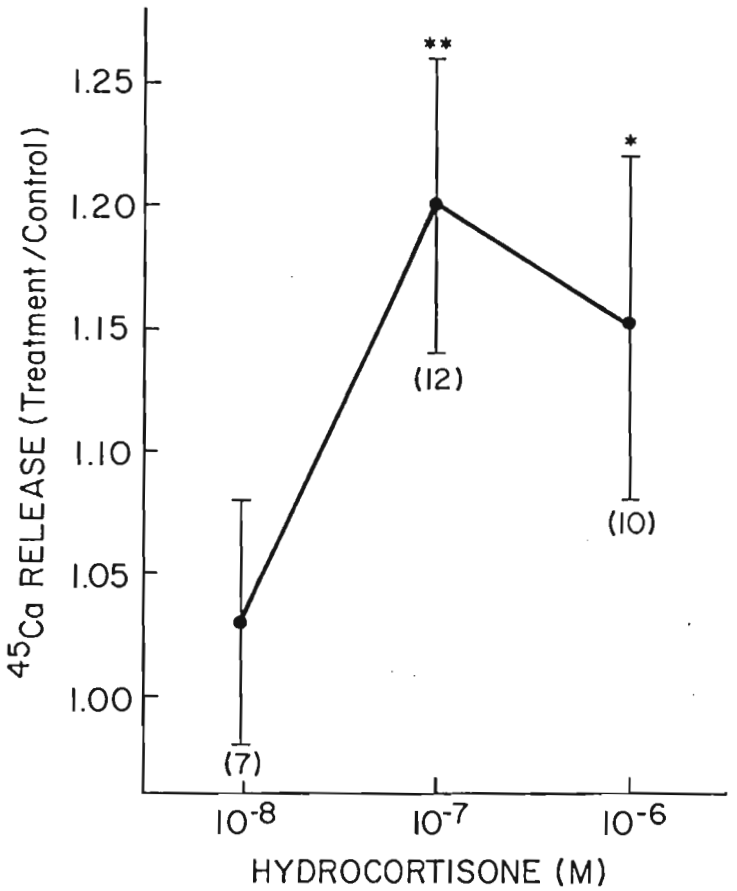


Figure 1: Effect of hydrocortisone on ^{45}Ca release from mouse calvariae. The data are mean ± SEM and have been pooled from 3 experiments. The number of treatment hemi-calvariae in each experiment is indicated in brackets. In the 10^{-8}M and 10^{-6}M experiments treatment and control bones were paired. In the 10^{-7}M experiment, 7 unpaired control bones were used. Significant differences from control are shown: *, $p<0.05$, **, $p<0.005$.

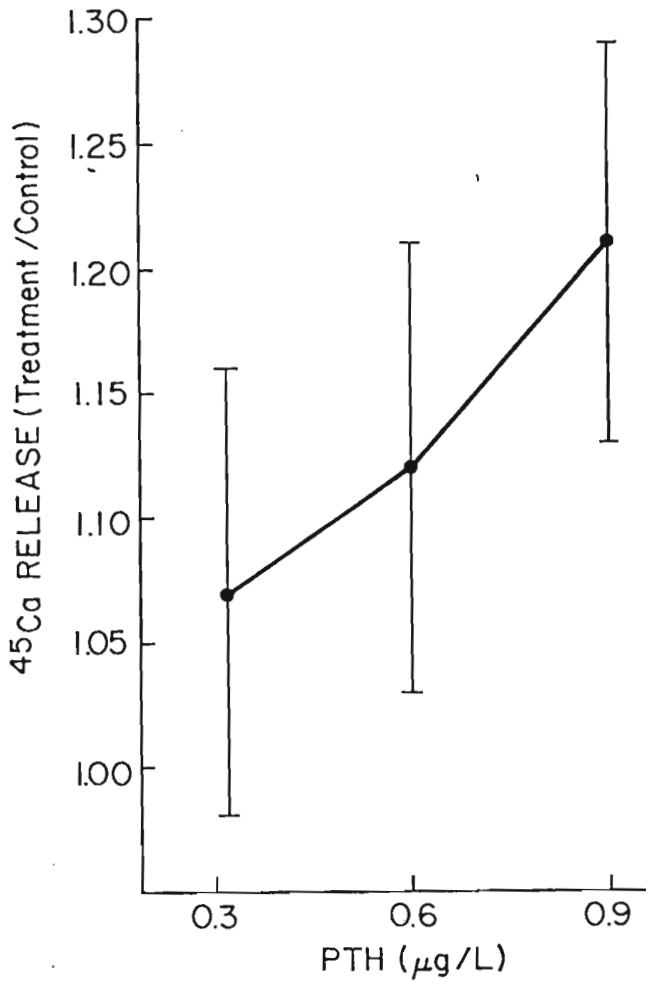


Fig 2: Effect of PTH on ^{45}Ca release. n=6 in each group. Data are mean ± SEM. ^{45}Ca release at 0.9μg/l PTH is significantly greater than control (Tukey's multiple comparison procedure) and there is a significant linear trend ($p<0.005$).

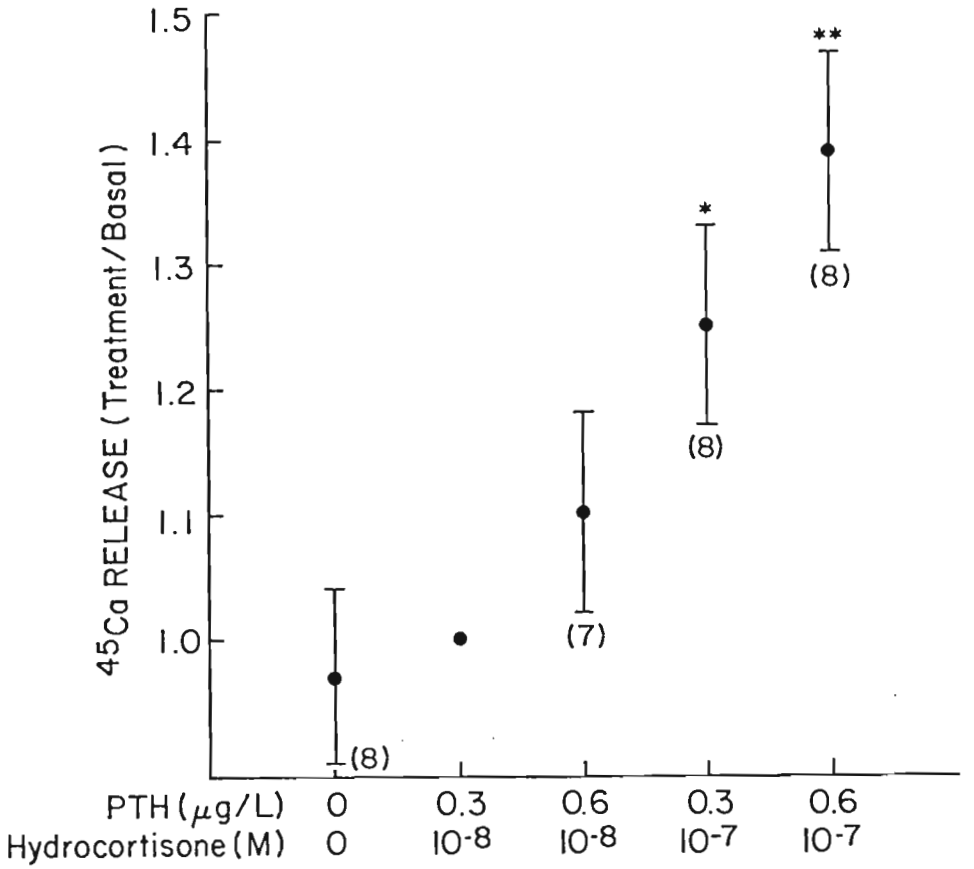


Fig 3: ^{45}Ca release in the presence of various concentrations of PTH and hydrocortisone as indicated on the abscissa. Isotope release was measured in paired bones, one being treated with the hormone levels indicated and the other with PTH 0.3μg/l and hydrocortisone 10^{-8}M (referred to as "basal" levels). Results are calculated as a ratio of treatment/basal and expressed as mean ± SEM. The data have been pooled from 3 experiments. Significant differences from basal values are shown: *, $p<0.02$; **, $p<0.005$.

TABLE 1: Effect of hydrocortisone, PTH and APD upon protein and DNA synthesis. Leucine and thymidine data were derived from one experiment.

PTH (μg/l)	Treatment H/c (M)	APD (M)	^{3}H-proline incorporation into hydroxproline (nCi)	^{14}C-leucine incorporation into protein (nCi)	^{3}H-thymidine incorporation into DNA (nCi)
-	-	-	88 ± 4 (6)	3.9 ± 0.2 (5)	8.1 ± 2.1 (5)
0.3	10^{-8}	-	87 ± 4 (6)	4.6 ± 0.1[b] (4)	15.0 ± 1.3[b] (4)
0.6	10^{-7}	-	64 ± 3[a] (5)	3.6 ± 0.2[a] (5)	11.2 ± 1.2 (5)
0.6	10^{-7}	3×10^{-6}		3.6 ± 0.2 (5)	17.3 ± 1.2[c] (5)
0.6	10^{-7}	10^{-5}	72 ± 2 (6)	3.5 ± 0.3 (5)	19.1 ± 2.1[d] (5)
0.6	10^{-7}	3×10^{-5}	67 ± 3 (5)		
0.6	10^{-7}	10^{-4}		4.1 ± 0.7 (4)	13.2 ± 0.5 (4)

Data are mean ± SEM. n indicated in parentheses.

a, significantly different from PTH 0.3 μg/l-H/c 10^{-8}M, $p<0.005$;

b, significantly different from control, $p<0.05$;

c, significantly different from PTH 0.6 μg/l-H/c 10^{-7}M, $p<0.01$;

d, significantly different from PTH 0.6 μg/l-H/c 10^{-7}M, $p<0.02$;

H/c = hydrocortisone

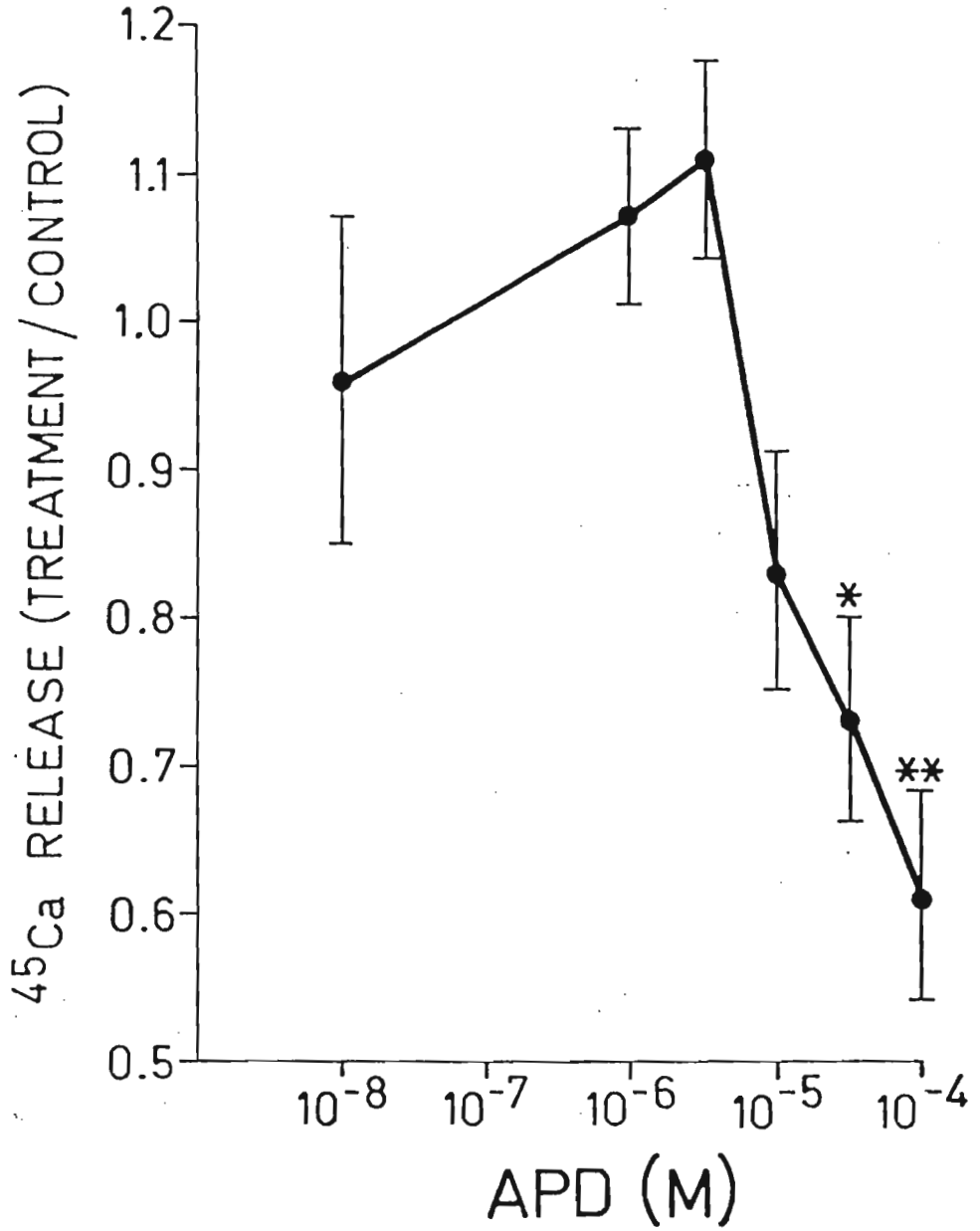


Figure 4: Effect of APD on ^{45}Ca release in unstimulated mouse calvariae. Results expressed as mean ± SEM. n=5 in each group. Significant differences from control are shown: *, $p<0.005$; **, $p<0.001$.

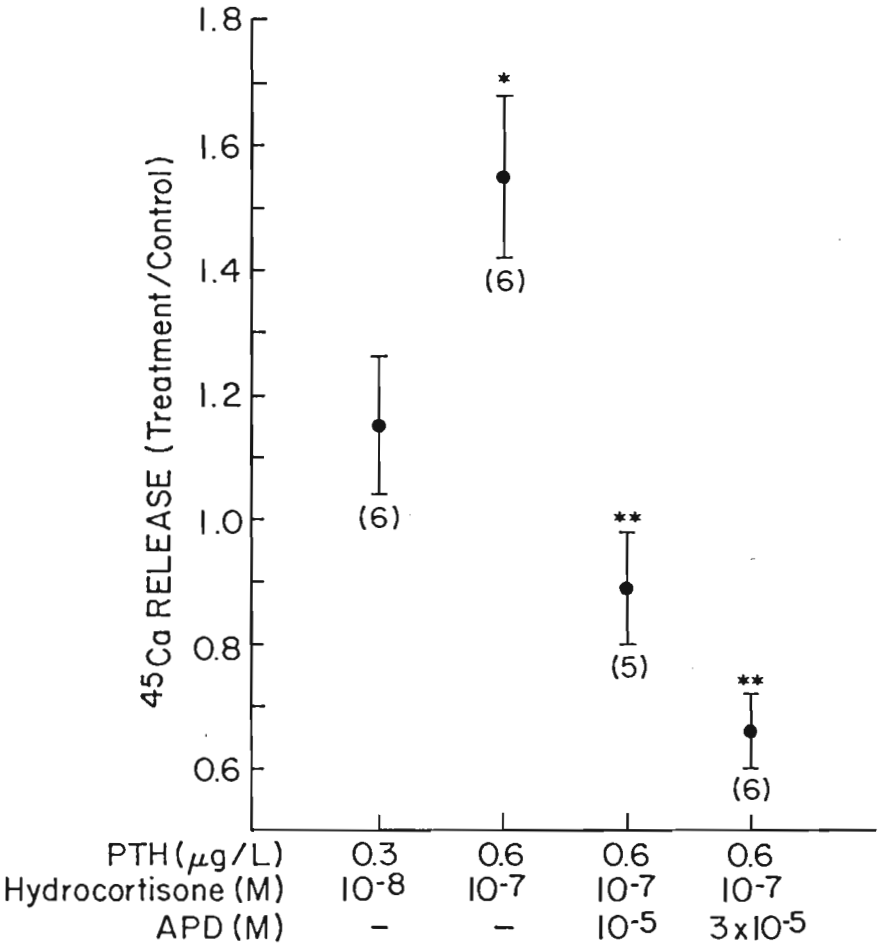


Fig 5: Effect of APD on ^{45}Ca release in the presence of PTH and hydrocortisone. The concentrations of PTH, hydrocortisone and APD in each treatment group are indicated on the abscissa. Results expressed as mean ± SEM. *, significantly different from PTH 0.3μg/l+hydrocortisone 10^{-8}M, $p < 0.01$; **, significantly different from PTH 0.6μg/l+hydrocortisone 10^{-7}M, $p < 0.001$.

Further suppression below control values occurred with APD 3 x 10^{-5}M. APD produced no change in ^{3}H- proline incorporation (table 1) and actually stimulated thymidine incorporation into DNA. The incorporation of leucine was unaffected by APD.

2.4 Discussion

The present studies using neonatal mouse calvariae have demonstrated two significant differences from results obtained previously in fetal rat bone culture systems. Firstly, supraphysiological levels of hydrocortisone result in increased calcium mobilisation from bone and, secondly, this effect is additive to the stimulation of bone resorption caused by parathyroid hormone. In contrast, fetal rat bone resorption rates are reduced by glucocorticoids, both in the basal state (Raisz et al, 1972; Caputo et al, 1976) and following PTH stimulation (Stern, 1969). There are differences between the models studied which may account for this divergence of results, in particular the species of origin of the bones and the use of neonatal rather than fetal bones. There is considerable evidence of inter-species differences in the response to glucocorticoids at both the cellular and organism levels. Thus, it is difficult to demonstrate glucocorticoid-induced osteoporosis in rats unless their calcium intake is restricted (Jee et al, 1972), in marked contrast to the situation in humans. Glucocorticoid-treated rats (Lindgren et al, 1983) and dogs (Korkor et al, 1983) do not usually show the increase in osteoclast numbers that has repeatedly been observed in humans, though this may be partly accounted for by the higher steroid doses given to the animals (Jee et al, 1970). There are even differences between mice and rats in their responses to glucocorticoids. The administration of cortisone to pregnant rats for example, produces osteopetrotic changes in the offspring whereas similar treatment of mice results in osteoporosis (Ornoy, 1971; Kaduri & Ornoy, 1974). At the cellular level, Chen et al (1982 & 1983) have found divergent effects of glucocorticoids on 1,25-dihydroxyvitamin D_3 receptor number and on cell growth in rat and mouse osteoblast-like cells. There is less known about differences in

bone cell function between the fetus and neonate. However, glucocorticoids inhibit the differentiation of osteoclasts from their progenitor cells (Suda et al, 1983; Teitelbaum et al, 1984) and it is possible that differences in the number of mature osteoclasts between fetal and neonatal bone may affect the response to glucocorticoids. Finally, physiological PTH concentrations have been used in the present studies whereas levels 100 to 1000 times greater have been studied in the past (Stern, 1969; Raisz et al, 1972; Caputo et al, 1976). This factor may contribute to differences in the interaction of PTH and hydrocortisone.

The finding of a stimulatory effect of hydrocortisone upon bone resorption is not unique. It has previously been described by Teitelbaum et al (28), using a devitalised bone particle/peritoneal macrophage model and by Glowacki (1983), who measured the rate of resorption of bone particles in subcutaneous pouches in rats. This phenomenon, however, has not been demonstrated previously in a bone organ culture system. The mechanism of the stimulation of bone resorption in either the present study or those referred to is uncertain but may be related to enhancement of resorbing cell attachment to bone. This appears to be mediated by glucocorticoid-induced changes in cell surface glycoproteins (Bar - Shavit et al, 1984).

The hormone levels studied in these experiments were similar to those which exist in patients receiving glucocorticoids. These subjects often take doses of steroids which are tenfold greater than physiological replacement levels and they have been found to have iPTH levels twice those of control subjects (Hahn et al, 1979; Suzuki et al, 1983). Therefore, the effects of these increments in PTH and hydrocortisone

concentrations were assessed, both individually and together, in this study. The resultant data suggest that the increases in both hydrocortisone and PTH may contribute to the increased osteolysis of steroid-treated subjects.

The inhibition of bone resorption by APD described previously (Shinoda et al, 1983) is reproduced in this study. In addition, APD is shown to overcome the stimulation of bone resorption caused by hydrocortisone and PTH without inhibition of other indices of cellular function. Since increased osteolysis contributes to the development of osteoporosis in patients taking glucocorticoids, these results provide support for studies of APD in the treatment of glucocorticoid-induced osteoporosis. The use of APD in vivo, however, might be expected to result in a transient fall in serum calcium and so a further rise in PTH. Its effects would also be modified by coupling of osteoblast and osteoclast function which may lead to a fall in bone formation. For these reasons the therapeutic value of APD in glucocorticoid osteoporosis remains to be determined.

CHAPTER 3 : HORMONAL AND METABOLIC INDICES IN GLUCOCORTICOID-TREATED SUBJECTS : INTRODUCTION AND METHODS

3.1 Introduction

The literature review carried out in Chapter 1 of this thesis indicated that a number of studies have been undertaken in which levels of calcitropic hormones and other indices of calcium metabolism have been compared between patients treated with glucocorticoids and control subjects. There is general agreement from these reports that steroid use is associated with inhibition of intestinal calcium absorption and increased circulating PTH levels. It is not the intention of this thesis to reproduce that work, but rather to study those areas in which consensus has not been reached (e.g. levels of vitamin D metabolites, renal tubular reabsorption of calcium), to study the effect of glucocorticoids on newly developed indices of calcium metabolism (e.g. serum osteocalcin) and to explore areas peripheral to but impinging on calcium and bone (e.g. androgens and growth hormone). Because of the diversity of these areas, each will be considered in a separate chapter. The method of patient selection, however, was the same throughout and will be described at this point.

3.2 Patients

Two groups of patients were studied; those receiving long-term steroid therapy and those who had received no steroid treatment in the previous three months. The largest single group of patients to which we had access was those with asthma or chronic obstructive airways disease and it was decided to restrict the

study population to this group to eliminate effects of diverse underlying diseases and their treatments. Patients known to have other diseases likely to affect calcium metabolism, including liver or renal disease, were excluded. Patients taking medications which might affect calcium metabolism, other than beta-adrenergic agonists, theophylline preparations and thiazide diuretics, were also not studied. Subjects receiving thiazides, however, were excluded from the studies of urine calcium excretion (Chapter 5).

Steroid treated subjects were referred to us by physicians in the Auckland area in response to a letter outlining our proposed study. The non-steroid-treated control group was recruited from the patient records of the Greenlane Hospital Asthma Clinic. Subjects were matched for age, sex and menopausal status. All patients were European. Descriptive data for the subjects studied is given in Table 2. Each matched pair is identified with a number which will be referred to in subsequent chapters. The two groups were comparable in terms of age, weight and serum creatinine concentrations. In spite of screening of the patients' medical records before sample collection, two patients with slight elevations of serum creatinine (see pairs 12 and 14, Table 2) were studied. The inclusion or exclusion of these two subjects does not affect any of the findings reported herein. Accordingly, their data has been included throughout, except in the consideration of renal calcium handling.

The steroid-treated patient in pair 4 is noteworthy in that she had been receiving prednisone 160 mg/day for over one year and yet had virtually no clinical evidence of Cushing's syndrome. This implies that she was resistant to prednisone's effects in

Table 2 : Descriptive data for glucocorticoid-treated and control subjects

		Control			Glucocorticoid-treated				
Pair No.	Sex	Age	Weight(kg)	Serum Creatinine (mmol/L)	Age	Weight(kg)	Serum Creat-inine (mmol/L)	Glucocorticoid Dose (mg/day)**	Duration glucocorticoid treatment (years)
1	M	36	73.1	0.10	38	79.2	0.10	45	14
2	M	38	70.8	0.09	41	86.8	0.10	12.5	12
3	F	42	54.5	0.08	38	61.6	0.12	20	10
4	F	20	63.6	0.07	19	68.0	0.07	160	4
5	M	52	89	0.08	49	66.5	0.11	4	5
6	F	16	60	0.05	17	73.8	0.07	10	1
7	M	60 +	74.3	0.10	60	63.8	0.08	4.5	18
8	M	57	89.5	0.09	58	63.5	0.10	15	16
9	F*	55	59.7	0.09	55	49.0	0.08	10	3
10	M	21	72.5	0.09	24	68.1	0.11	15	2
11	F	22	84.3	0.07	22	62.0	0.07	15	7
12	M	49	76.1	0.15	51	105.0	0.08	10	20
13	M	63	62.5	0.08	64	69.0	0.10	15	1.3
14	M	64	63.0	0.08	64	62.8	0.14	20	7
15	M	77	60.9	0.12	74	86.8	0.09	15	7
16	M	56 +	80.0	0.10	54	57.5	0.10	16	2
17	M	72	88.4	0.10	73	69.4	0.09	20	3
18	M	72	83.1	0.10	72	67.0	0.09	10	3.5
19	F*	49	80.5	0.08	51	80.4	0.10	10	3
20	M	54	91.6	0.10	54	78.4	0.10	15	4
Mean±Sem		49±3	75±3	0.091±0.005	49±3	71±3	0.095±0.004	22.1±7.5 (n=20) 14.8±2.0 (n=19++)	7.1±1.3

++ Excluding patient number 4 whose prednisone dose was 160 mg/day

** Mean daily dose in prednisone equivalents during the 4 weeks prior to blood sampling

\+ Taking thiazide diuretic

* Both members of pair postmenopausal

some way, possibly because of reduced conversion of the ingested drug to prednisolone in the liver or because of accelerated metabolism of the active species. Whatever the mechanism of this resistance, her data are atypical in many respects and will be commented on subsequently.

Informed consent was obtained from all patients studied and the study protocols were approved by the Auckland Hospital Ethical Committee.

All blood and urine samples were obtained after an overnight fast. Patients emptied their bladders on rising, and then took only water until the sampling was completed about 2 hours later. All subjects were outpatients at the time of study and their asthma was clinically stable.

3.3 Assay Methods

Serum ionized calcium was measured using an ICA1 calcium electrode (Radiometer, Copenhagen). Zinc was measured by atomic absorption spectrometry following protein precipitation using the method of Sunderman and Roszel (1967). Other serum and urine electrolytes, creatinine, urea and albumin were assayed using Technicon autoanalysers (Tarrytown, New York). Total urine hydroxyproline was estimated spectrophotometrically using a modification of the method of Zender (1972) and serum alkaline phosphatase was also measured by spectrophotometry using a p-nitrophenylphosphate substrate.

Serum 25(OH)D was measured by a competitive protein-binding assay, following extraction into ethanol and chromatogrpahy on a

Sephadex LH-20 column. Serum samples for $1,25(OH)_2D$ measurement were initially purified by solvent extraction followed by HPLC, using the method of Eisman et al (1976). Their Sephadex column separation step was omitted and a back-extraction using 0.1M phosphate buffer added (D Lissner, personal communication). $1,25(OH)_2D$ was then estimated by a radioreceptor assay using intestinal cytosol receptor protein from normal 6 week old chickens (Dokoh et al, 1981).

The osteocalcin (OC) radioimmunoassay used was developed by Dr George Chapman and associates and has been described in detail elsewhere (Reid I R et al 1986). It uses antisera raised in rabbits against ovine OC and an ovine OC standard. Serial dilutions of human serum samples with high OC values gave assay displacement curves parallel to that of the ovine OC standard. There was 100% cross-reaction with ovine OC peptide 15-49, which has an identical sequence to that of the corresponding region of the human OC sequence (Poser et al, 1980; Mende et al, 1984).

Sex hormone and gonadotropin assays were carried out in the Hormone Assay Laboratory at National Women's Hospital. Highly specific antisera for the steroid radioimmunoassays (RIAs) were raised in Chinchilla rabbits by multiple subcutaneous injections of steroid - BSA conjugates obtained from Steraloids, Wilton, New Hampshire, USA. Radioactive tracers were purchased from Amersham International, Amersham, England. Testosterone was measured in diethyl ether extracts of the plasma samples by RIA using an antiserum to 4-androst-11α, 17β-diol-3-one, 11 hemisuccinate : BSA; free and bound fractions were separated by addition of a suspension of dextran coated charcoal. Sex hormone binding globulin (SHBG) capacity was determined by the method of Rudd et

al (1974). Plasma free testosterone concentration was derived from the total testosterone concentration and the SHBG capacity using the calculation of Wiest et al (1978). DHEAS was determined by a direct RIA of DHEA following hydrolysis of the plasma samples with Triton X-100 solubilised steroid sulphatase prepared from placental microsomes (McNaught and France, 1980). An antiserum to 5-androsten-3β-ol-7,17-dione 7-CMO: BSA was used in the assay and separation of free and bound fractions was achieved with dextran coated charcoal. LH and FSH were measured by Amersham Amerlex RIA kit.

Insulin-like growth factor 1 (IGF-1) assay was measured by RIA following extraction with acid-ethanol (Gluckman et al, 1983).

Table 3 sets out the normal ranges for each of the assays used.

Table 3 : Adult Normal Ranges for Serum and Urine Biochemistry

Serum	
Total calcium (Ca_t)	2.15 - 2.60 mmol/L
Ionised calcium (Cai)	1.18 - 1.28 mmol/L
Osteocalcin (OC) or bone gla-protein (BGP)	4 - 20 µg/L
Alkaline phosphatase (Alk phos)	30-95 units/L
25-hydroxyvitamin D [25(OH)D]	18 - 56 µg/L
1,25 dihdroxyvitamin D [$1,25(OH)_2D$]	20 - 59 ng/L
Total testosterone*	13 - 40 nmol/L+
Sex hormone binding globulin capacity (SHBC)+	40 - 60 nmol/L+
Free testosterone+	380 - 1200 pmol/L+
Luteinising hormone (LH)+	2 - 12 (IU/L)+
Follicle stimulating hormone (FSH)+	1 - 9 (IU/L)+
Creatinine	0.05 - 0.12 mmol/L
Zinc*	12 - 20 µmol/L
Urine	
Calcium/creatinine (uCa/Cr)	0.06 - 0.40 mol/mol
Ca_E	<0.03 mmol/L
TmCa	1.98 - 2.71 mmol/L
Hydroxyproline/creatinine (uHP/Cr)	< 30 mmol/mol
Sodium/creatinine (uNa/Cr)	1 - 16 mol/mol

* Measured in plasma

\+ Normal range for adult males.

CHAPTER 4 : SERUM OSTEOCALCIN AND RELATED INDICES

4.1 Introduction

Osteocalcin (OC, also known as bone Gla-protein) is a 49-amino acid protein, probably synthesised in osteoblasts (Nishimoto and Price, 1980; Gallop et al, 1980) which constitutes 20% of the non-collagenous protein of bone (Gallop et al, 1980). Its function is unknown, but, by virtue of its γ-carboxyglutamic acid residues, it has a high affinity for hydroxyapatite and is thus closely associated with bone mineral. OC is detectable in serum and its level correlates with histomorphometric indices of bone formation (Brown et al, 1984). Circulating OC is freshly synthesised and not derived from resorption of existing bone (Price et al, 1981). For these reasons, it has been used as a biochemical marker of osteoblast function and its level studied in patients with various metabolic bone diseases. Glucocorticoids have significant effects on bone metabolism and their prolonged use results in osteoporosis. Their effect upon serum OC levels had not been previously described.

The production of OC is stimulated by $1,25(OH)_2D$ in rat osteosarcoma cells (Price and Baukol, 1980), rats (Price and Baukol, 1981) and patients with inherited rickets (Gundberg et al, 1983) or osteoporosis (Zerwekh et al, 1985). Glucocorticoids influence the number of $1,25(OH)_2D$ receptors on osteoblast-like cells in vitro (Chen et al, 1982 and 1983) and increase the production of OC by osteoblasts in response to $ol,25(OH)_2D$ (Chen et al, 1986). Since the acute effect of $1,25(OH)_2D$ administration upon OC had not been studied in either

normal or glucocorticoid-treated man, we measured OC levels in these two groups before and after the administration of $1,25(OH)_2D$.

4.2 Subjects and Procedures

Fasting morning blood samples were obtained from the patients described in Table 2. Data were incomplete in one subject from pair 15, so only 19 steroid-treated patients and their matched controls were considered. No patient was receiving coumarin anti-coagulants.

The response of serum OC to $1,25(OH)_2D$ administration was determined in 6 normal subjects (3 men, 3 women; mean age, 30 ± 14 (SD) years and 6 other patients with chronic glucocorticoid excess. The latter group consisted of 4 women with Cushing's syndrome (pituitary-dependent in 3 and secondary to metastatic small cell carcinoma of the lung in one) and two asthmatic patients (one man, one woman) receiving prednisone 20 mg per day. The mean (±SD) age of this group was 47 ± 14 years. $1,25(OH)_2D$ was administered orally at 1300h on days 1-4 (1.5 ug on day 1 and 1 ug daily thereafter) and blood was drawn for OC measurement at 0900h on days 1 and 5. In some subjects, additional blood samples were taken at 0900h on days 2-4.

4.3 Results

In the glucocorticoid-treated asthmatic patients, serum OC levels were approximately 50% less than those of matched asthmatic

control subjects ($p < 0.001$, paired "t" test, Table 4). The other indices of bone turnover, serum alkaline phosphatase and urine hydroxyproline, were not different between the groups, however. The correlations between these three indices are shown in Table 5, and were generally higher in the control group.

Because OC production is regulated by $1,25(OH)_2D$, vitamin D metabolites were also measured. Again no significant difference between the groups was present. However, there was a significant correlation between serum OC and $1,25(OH)_2D$ in the glucocorticoid group but not in the control group (Figure 6). Data from a further 20 glucocorticoid-treated patients were added to allow more detailed analysis. These patients were receiving glucocorticoids for treatment of asthma (n=6), connective tissue diseases (n=8), ulcerative colitis (n=1) and cranial arteritis (n=5). The serum creatinine levels were normal in all and their mean levels of $1,25(OH)_2D$ and OC were 36 ± 3 (SEM) ng/L and 3.6 ± 0.4 mg/L, respectively. The correlation between serum OC and $1,25(OH)_2D$ was again present ($r=0.57$, $p < 0.0002$). Multiple regression analysis of OC against $1,25(OH)_2D$, 25(OH)D, alkaline phosphatase, urine hydroxyproline and glucocorticoid dose, indicated that OC correlated positively with $1,25(OH)_2D$ ($p < 0.02$), inversely with glucocorticoid dose ($p < 0.05$), and was independent of the other variables. The relationship between OC, $1,25(OH)_2D$ and dose is described by the equation:

$$OC = 4.1 + 0.05\ [1,25(OH)_2D] - 0.09\ \text{dose}$$
$$(R^2 = 0.37,\ p < 0.005)$$

Table 4 : Serum osteocalcin levels and related indices in glucocorticoid-treated and control subjects

	Control					Glucocorticoid-treated				
Pair No	OC (mg/L)	Alk phos (u/L)	uHP/Cr (mmol/mol)	25(OH)D (µg/L)	1,25(OH)$_2$D (ng/L)	OC (mg/L)	Alk Phos (u/L)	uHP/Cr (mmol/mol)	25(OH)D (µg/L)	1,25(OH)$_2$D (ng/L)
1	9.6	70	18	30	53	2.5	65	20	26	28
2	12.7	25	19	22	46	4.0	105	26	26	56
3	4.1	35	7	30	24	3.1	50	12	24	23
4	8.5	40	27	16	47	14.3	85	37	31	72
5	6.0	80	13	29	35	7.0	65	10	27	47
6	13.1	90	46	37	43	10.6	80	34	17	90
7	10.2	45	12	24	36	2.2	70	16	28	42
8	7.9	75	19	24	54	2.6	40	16	22	21
9	7.9	60	11	14	49	2.7	70	24	32	24
10	30.2	120	32	21	56	9.1	80	19	25	71
11	4.0	45	10	29	40	3.7	60	21	13	78
12	10.4	70	13	53	33	4.4	80	14	28	35
13	6.8	90	23	18	62	4.4	80	11	15	59
14	12.0	80	15	29	26	1.9	60	8	15	30
16	9.0	100	21	23	34	1.8	85	41	18	44
17	5.8	70	12	8	24	0.9	70	19	11	18
18	6.7	60	16	28	42	4.4	80	25	4	30
19	8.6	70	16	19	60	5.2	85	20	34	32
20	20.4	125	39	22	48	5.7	55	15	25	42
	10.2±1.4	71±6	19±2	25±2	43±3	4.8±0.8*	72±4	20±2	22±2	44±5

* Significantly different from control, $p < 0.001$

Abbreviations defined in Table 3

TABLE 5 : Correlation between biochemical indices of bone turnover

		AP	uHP/Cr
OC	Control	0.62**	0.64**
	Steroid	0.32	0.38
AP	Control	-	0.61*
	Steroid	-	0.54*

Data are correlation coefficients calculated for the steroid-treated and control groups separately. $n = 19$ in each group.

*$p<0.05$ **$p<0.01$

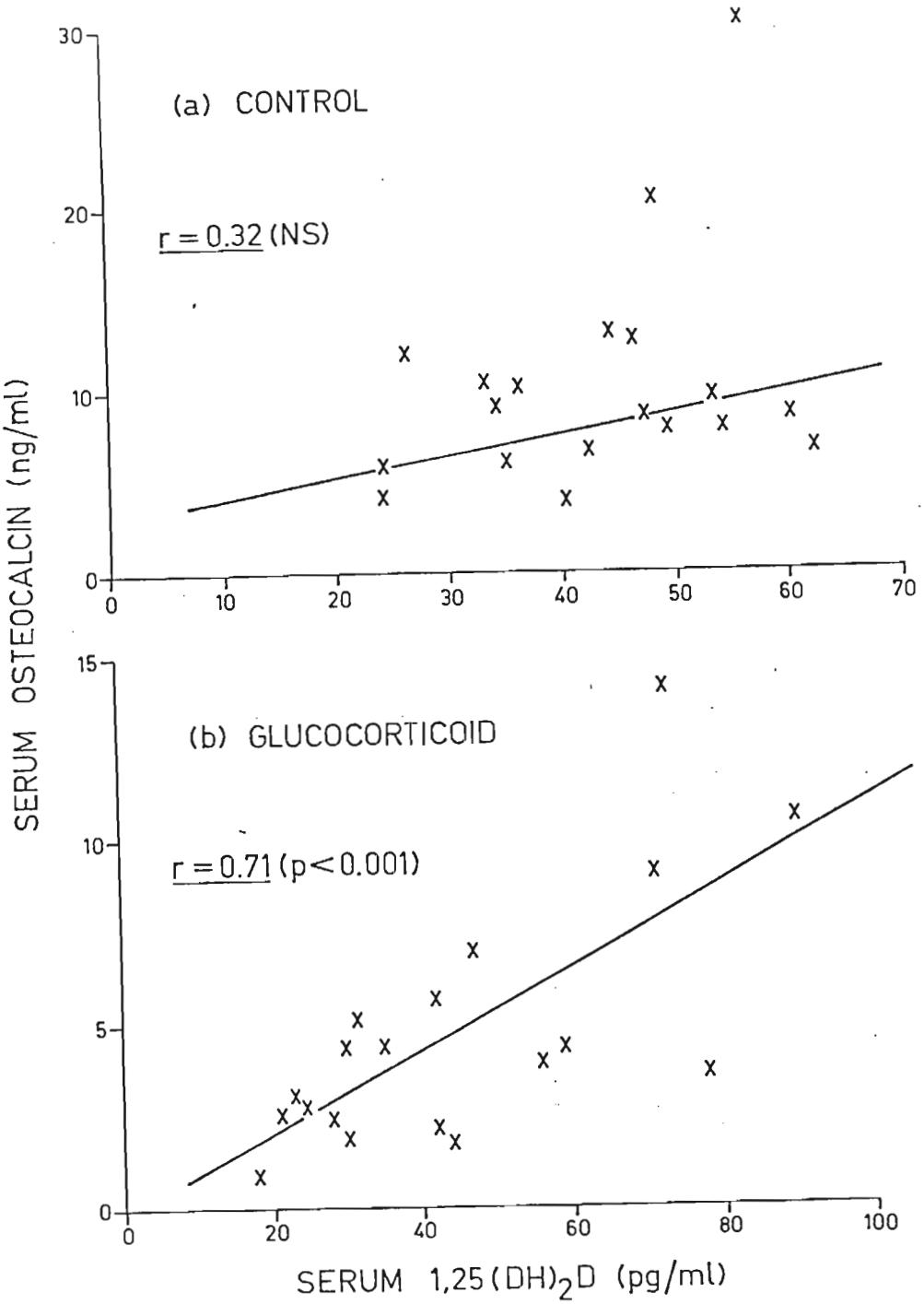


Figure 6: Regression lines for serum osteocalcin on serum 1,25-$(OH)_2D$ in asthmatic patients: (a), not receiving glucocorticoids, and (b), receiving long-term prednisone therapy.

Patient 4 (from Table 2) was excluded from this analysis because her very high prednisone dose was found to have a disproportionate influence on the results.

Following $1,25(OH)_2D$ administration, all normal subjects had to rise in OC levels by the third day (Figure 7). In half, OC levels continued to rise to day 5, but in the remainder the peak was on days 2 or 3. The mean increase was significant on day 2 ($p < 0.05$) and day 3 ($p < 0.02$). A similar percentage increase occurred in the patients with glucocorticoid excess, though the absolute increments were considerably smaller. In only one of these patients did OC levels peak during the 4 days of $1,25(OH)_2D$ therapy. The increase above basal levels was statistically significant on day 5 ($p = 0.025$).

4.4 Discussion

We have found substantially reduced serum OC levels in patients receiving chronic glucocorticoid therapy and this effect is dose-related. Values in the control asthmatic patients are similar to those in the normal subjects, indicating that asthma and its treatment have no significant influence. Histological studies of bone in glucocorticoid-treated patients have shown a reduction in indices of bone formation. Since serum OC correlates with these indices the low OC levels probably reflect the glucocorticoid-induced reduction in bone formation. This finding provides further validation for the use of OC measurements as an index of osteoblast function and suggests they may be useful in assessing the degree of impairment of bone formation in individual corticosteroid-treated patients.

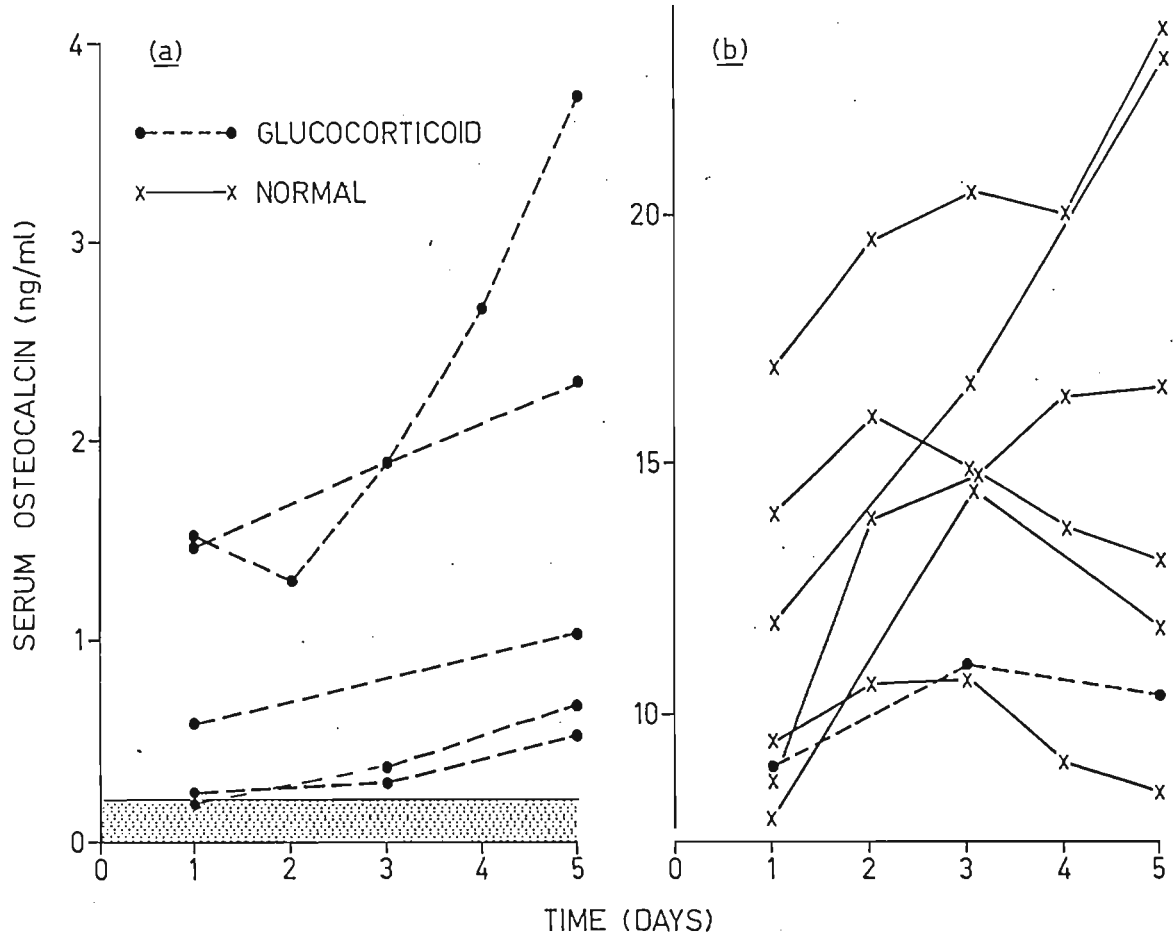


Figure 7: Serum osteocalcin levels before and during 1,25-$(OH)_2D$ administration (4.5µg total in 4 daily doses) in normal subjects and patients with chronic glucocorticoid excess.

(a) Subjects with basal osteocalcin levels <2ng/ml. The horizontal line indicates the limit of detection of the assay.

(b) Subjects with basal osteocalcin levels >2ng/ml.

Despite the low OC, there is no reduction in serum alkaline phosphatase, suggesting a differential effect of glucocorticoids on these two indices of osteoblast activity. However, bone contributes only one half of circulating alkaline phosphatase activity so a depression in this fraction might be overwhelmed by random fluctuation in the non-bone component. Similarly, urine hydroxyproline was not different between the groups. This is consistent with published data from other studies and is apparently at odds with histological evidence of increased bone resorption in steroid-treated subjects, as discussed in Section 1.2.2.

The significant correlation between serum OC and $1,25(OH)_2D$ in the glucocorticoid-treated patients is unexpected since there is no such relationship in normal subjects, either in this or other studies (Epstein et al, 1984). These findings could be explained by postulating that factors other than $1,25(OH)_2D$ have a predominant influence on OC production in normal subjects and that the action of some of these factors is blocked by high glucocorticoid levels. The relative importance of $1,25(OH)_2D$ in determining OC production would thus be increased in steroid-treated subjects. Regulators of osteoblast function that are affected by glucocorticoids include prostaglandins (Chyun and Raisz 1984) and the interleukins (Beresford et al, 1984).

The administration of $1,25(OH)_2D$ increases OC production in normal subjects. This stimulatory effect has also been demonstrated in patients with inherited rickets and more recently in post-menopausal osteoporosis (Zerwekh et al, 1985). In the

latter study, OC levels were elevated after a two week course of $1,25(OH)_2D$. This contrasts with the present findings in normal young adults, in about half of whom serum OC levels increased but then fell towards baseline after only 4 days treatment. Whether this difference is a function of age, $1,25(OH)_2D$ dose or the presence of osteoporosis is not known.

In patients with glucocorticoid excess, basal OC levels and the absolute increases following $1,25(OH)_2D$ were considerably reduced. The percentage increase, however, was similar to that of the normal subjects and possibly more sustained. The samples with low OC values were measured in a more sensitive non-equilibrium RIA and the changes were greater than can be attributed to the imprecision of the measurement. The consistency of the response among the patients and the significant increase in mean levels indicate that the increase is a real phenomenon. Thus, in the presence of glucocorticoid excess, OC production is responsive to $1,25(OH)_2D$ and, when the low basal levels of osteoblast function are taken into account, the proportionate responses were similar to those in normal subjects.

The reduction in basal levels of OC in glucocorticoid-treated patients does not result from a reduction in serum levels of $25(OH)D$ or $1,25(OH)_2D$, since there was no significant difference in these values between steroid-treated and untreated patients. These findings are consistent with a number of other recent studies (see Section 1.2.5). Furthermore, the correlation between basal $1,25(OH)_2D$ and OC levels, and the normal proportional OC response to administered $1,25(OH)_2D$ in the

presence of glucocorticoid excess indicate that these drugs do not interfere with the action of $1,25(OH)_2D$ on osteoblasts. It seems likely that glucocorticoids reduce OC production either by a direct action on osteoblasts or by inhibition of stimulators other than $1,25(OH)_2D$.

4.5 Postscript

Since the publication of the data dealt with in this chapter, several other groups have confirmed and amplified its findings. Lukert et al (1986) have shown that serum OC concentrations are reduced in glucocorticoid-treated patients and Godschalk and Downs (1986) have confirmed this prospectively in normal subjects. They have also shown that a fall in OC is apparent within 24 hours of starting steroid treatment, maximal between 48 and 96 hours, rapidly reversible and dose-related. Using rats, Jowell et al (1987) have demonstrated a similar time-course and dose-dependence and that the glucocorticoid effect is antagonised by the administration of $1,25(OH)_2D$.

CHAPTER 5 : URINARY CALCIUM EXCRETION IN GLUCOCORTICOID-TREATED ASTHMATIC PATIENTS

5.1 Introduction

Increased urinary calcium loss has been observed during both acute and chronic glucocorticoid treatment, as discussed in Section 1.2.4. Recently, Suzuki et al (1983) have demonstrated a significant positive correlation (r=0.63) between fasting urine calcium and nephrogenous cAMP in steroid-treated patients, suggesting that the increased urine calcium loss may be a major contributor to the development of hyperparathyroidism in these patients. Some authors contend that the hypercalciuria merely reflects the increased rate of bone resorption (Hahn et al, 1980; Bringhurst and Potts, 1979) but others believe that it results from a direct glucocorticoid effect on the kidney (Suzuki et al, 1983). In order to clarify this issue, we have measured various indices of urinary calcium excretion and calculated Nordin's notional tubular maximum for calcium reabsorption (TmCa) in the two groups of asthmatic patients.

5.2 Subjects and Methods

The subjects studied were those described in Table 2. Pairs 7 and 16 were excluded because one member of each was taking a thiazide diuretic and pairs 12 and 14 because of slight elevations of serum creatinine levels. Pair 13 was also excluded because of incomplete data.

Fasting blood and urine samples were collected, as described in

Section 3.3. Urine calcium excretion was expressed as a molar ratio to creatinine (Ca/Cr) and as Ca_E, the urinary calcium excretion per litre of glomerular filtrate. This is equal to the product of the urinary calcium/creatinine ratio and the serum creatinine concentration. TmCa was derived using the method of Marshall (1976) :

$$TmCa=(Ca_i-Ca_E)/[1-0.08\ \log_e(Ca_i/Ca_E)]$$

where Ca_i is the serum ionized calcium concentration.

Comparisons were made using Student's two-tailed "t" tests for paired data.

5.3 Results

Results for the individual patients are shown in Table 6 and the means±SEM derived from these are shown in Table 7. Serum total and ionized calcium levels did not differ between the groups. The mean fasting urinary Ca/Cr in the glucocorticoid-treated patients was more than twice that found in the control subjects ($p < 0.005$). A similar difference was present when calcium excretion was expressed per litre of glomerular filtrate ($p < 0.005$). The mean calculated TmCa values in the glucocorticoid group were significantly less than those of the control subjects ($p < 0.005$).

A more conventional way of studying tubular reabsorption of calcium is to plot Ca_E against serum calcium concentration and relate an individual's position on this graph to the normal

Table 6 : Urinary calcium excretion and related indices in glucocorticoid-treated and control subjects

	Control						Glucocorticoid-treated					
Pair No	Ca_t (mmol/L)	Ca_i (mmol/L)	uCa/Cr (molar)	Ca_E (mmol/LGF)	TmCa (mmol/L)	uNa/Cr (molar)	Ca_t (mmol/L)	Ca_i (mmol/L)	uCa/Cr (molar)	Ca_E (mmol/LGF)	TmCa (mmol/L)	uNa/Cr (molar)
1	2.44	1.24	0.17	0.017	1.86	13.5	2.58	1.30	0.24	0.026	1.86	21.6
2	2.58	1.25	0.51	0.046	1.64	19.3	2.32	1.23	0.55	0.043	1.54	26.5
3	2.38	1.23	0.12	0.010	2.00	7.6	2.33	1.20	0.79	0.094	1.31	14.2
4	2.40	1.20	0.21	0.015	1.83	14.9	2.39	1.20	0.13	0.009	1.95	1.4
5	2.51	1.29	0.29	0.023	1.86	12.6	2.46	1.23	0.59	0.065	1.58	9.8
6	2.49	1.28	0.31	0.016	1.96	27.7	2.63	1.33	0.32	0.023	1.97	11.1
8	2.37	1.26	0.18	0.016	1.91	8.1	2.38	1.23	0.89	0.089	1.60	19.1
9	2.47	1.27	0.51	0.046	1.67	9.6	2.57	1.30	1.40	0.111	1.31	17.4
10	2.60	1.27	0.20	0.018	1.90	11.4	2.53	1.26	0.30	0.033	1.60	10.3
11	2.44	1.28	0.17	0.012	2.03	11.6	2.46	1.26	0.59	0.042	1.52	12.3
15	2.44	1.26	0.13	0.016	1.92	3.5	2.43	1.24	0.48	0.043	1.67	25.0
17	2.36	1.21	0.17	0.017	1.81	15.1	2.42	1.27	1.13	0.101	1.37	32.0
18	2.54	1.30	0.27	0.027	1.85	23.8	2.44	1.24	0.36	0.032	1.71	18.6
19	2.39	1.24	0.20	0.016	1.87	10.5	2.44	1.27	0.28	0.028	1.83	11.2
20	2.50	1.29	0.35	0.035	1.76	18.1	2.46	1.24	0.29	0.029	1.67	17.9

For clarity, means ± SEM are shown separately in table 7

Table 7 : Urinary calcium excretion and related indices in glucocorticoid - treated and control subjects: mean data.

	Control	Glucocorticoid	p
Ca_t (mM)	2.46±0.02	2.47±0.02	NS
Ca_i (mM)	1.26±0.01	1.25±0.01	NS
uCa/Cr (molar ratio)	0.25±0.03	0.56±0.09	< 0.005
Ca_E(mmol/LGF)	0.022±0.003	0.051±0.008	< 0.005
TmCa (mM)	1.86± 0.03	1.63±0.06	< 0.005
uNa/Cr(molar ratio)	13.8±1.6	16.6±2.0	NS
Serum creatinine (mM)	0.087±0.004	0.092±0.004	NS

All samples were taken in the fasting state.

Data are mean ± SEM, n = 15.

range. This has been done in Figure 8, where the mean normal relationship determinied by Nordin (1976) is also shown (solid line). When Ca_E was related to total serum calcium concentration, it was above the mean normal value in 9/15 glucocorticoid-treated patients and below that value in 12/15 control subjects ($p < 0.05$, chi-square test). Urinary sodium excretion did not differ between the two groups (Table 7) and thus does not account for the observed differences in calcium loss.

The relationship between TmCa and prednisone dose is shown in Figure 9. The correlation coefficient between dose and TmCa for all 15 patients is +0.48 but this is heavily weighted by patients 1 and 4. Following their omission, the correlation coefficient becomes -0.44 ($0.1 < p < 0.2$). The regression line for these 13 patients is shown in the figure.

5.4 Discussion

These data confirm that fasting calcium excretion is increased in subjects treated with glucocorticoids. Since this increase is still found when calcium loss is expressed per litre of glomerular filtrate, it cannot be attributed to increased glomerular filtration rates in the steroid-treated subjects. Furthermore, there is no difference in serum ionized calcium concentrations between the glucocorticoid and control groups, indicating that the filtered loads of calcium per litre of glomerular filtrate are similar. It can thus be deduced that the increased calcium excretion must result from altered tubular

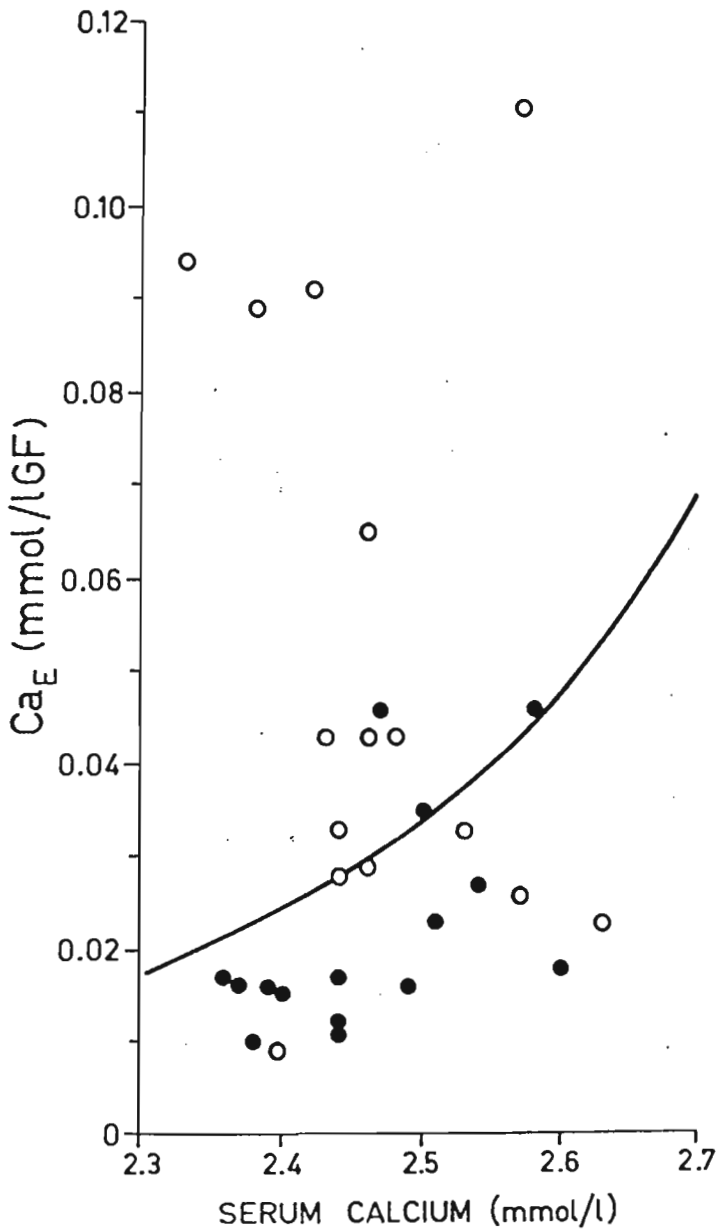


Figure 8: Fasting urinary calcium excretion per litre of glomerular filtrate as a function of serum calcium in glucocorticoid-treated asthmatics (open circles) and asthmatic control subjects (closed circles) matched for age, sex and menopausal status. The solid line represents the mean normal relationship as determined by Nordin (1976). The two groups are significantly different ($p<0.05$, chi-square test).

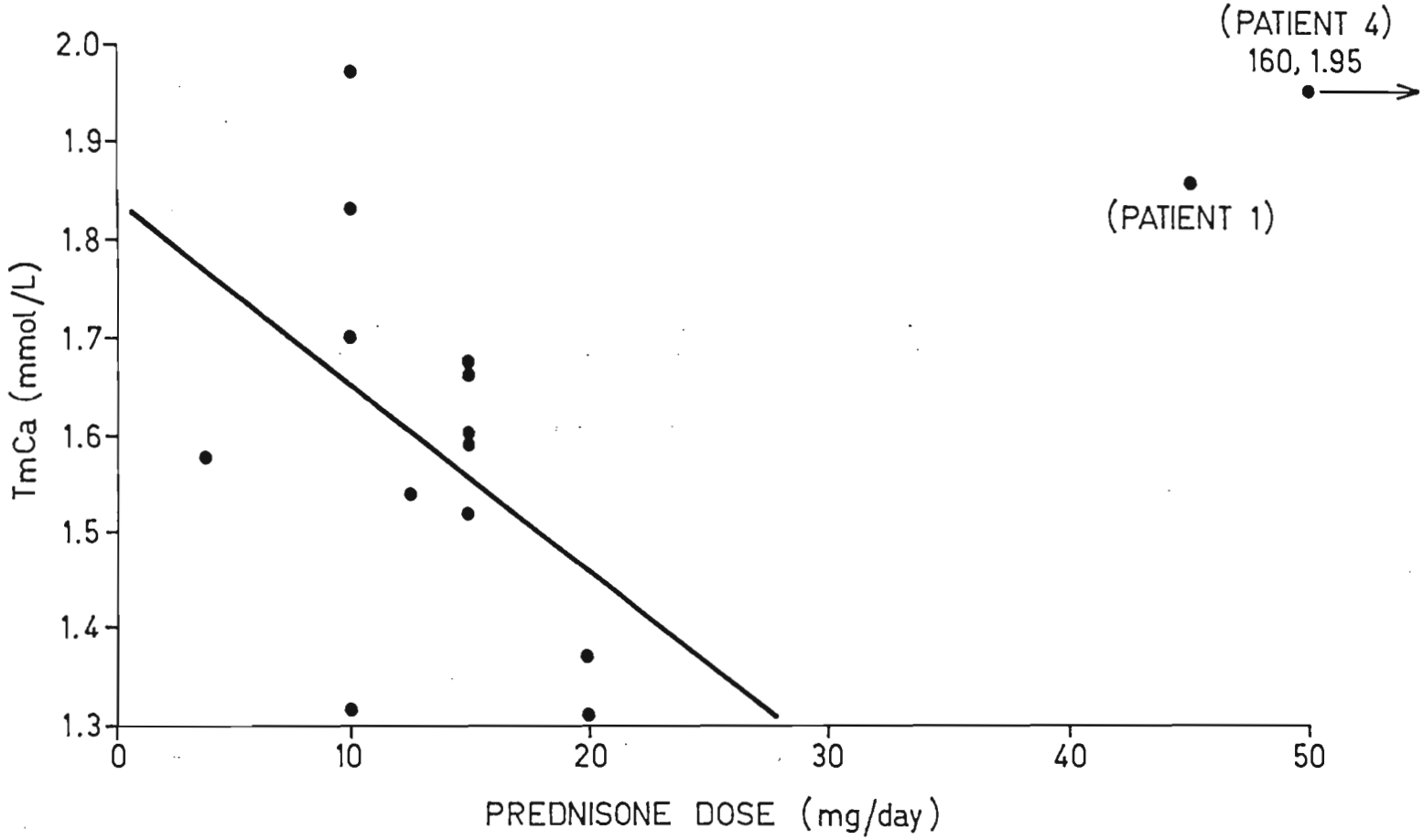


Figure 9: Relationship between TmCa and prednisone dose in glucocorticoid-treated asthmatics. The regression line was calculated following the exclusion of patients 1 and 4. The correlation for the remaining 13 patients is -0.44 ($0.1<p<0.2$).

handling of calcium via a sodium-independent mechanism (since sodium excretion was the same in the two groups). Marshall's notional TmCa provides a convenient numerical index of tubular reabsorption of calcium and comparison of values obtained in the two groups confirms a lower renal tubular calcium reabsorption in the patients receiving glucocorticoids. The same conclusion is reached if tubular resorption of calcium is inferred graphically, as has been done in Figure 8. The data in this figure also suggest that the tubular reabsorption of calcium is higher in the control asthmatics studied than in the normal subjects from whom Nordin derived the mean normal line. This small difference may reflect differences in measurement techniques between laboratories or it may be an effect of asthma or its treatment. Theophylline is unlikley to be responsible, since its acute effect is to increase urine calcium loss (Colin et al, 1984). However, adrenergic agonists raise PTH levels (Kukreja et al, 1975) and thus may increase the tubular reabsorption of calcium.

These data do not clearly establish a dose-response relationship between glucocorticoids and TmCa, but are suggestive of one. As has been mentioned earlier, patient 4 appears to be resistant to the effects of oral steroids as judged by both clinical and biochemical parameters, so her deviation from the regression line is not surprising. There is no ready explanation for the normal TmCa of patient 1, however, though laboratory error cannot be excluded. Even after the exclusion of these 2 patients, the correlation coefficient does not reach conventional levels of statistical significance, so the matter cannot be finally resolved on the basis of this data.

The present findings are apparently at variance with those of Lemann et al (1970), who failed to detect any change in urine calcium loss within three hours of cortisol injection. In their studies, however, there was also a glucocorticoid-induced reduction in sodium excretion and probably an increase in PTH levels, judging from the similar studies of Fucik et al (1975). Both these changes may have obscured any effect of cortisol on urine calcium. Sodium excretion was the same in both groups in the present study and so is unlikely to play a role in the altered handling of calcium by the kidney. It is also very unlikely that the impairment of calcium reabsorption found in the present studies is mediated by PTH, since PTH has been shown to be elevated in states of glucocorticoid excess and so would tend to increase calcium reabsorption rather than diminish it. The impairment of calcium reabsorption may represent a direct action of glucocorticoids on tubular calcium transport, similar to that thought to occur in the gut. This hypothesis is supported by the finding that vitamin D-dependent calcium binding proteins are present in both the duodenum and kidney and that, in the mouse at least, these appear to be biochemically and immunologically identical (Delorme et al, 1983). In the chick, cortisol reduces both intestinal calcium excretion and the soluble fraction of intestinal calcium binding protein (Feher and Wasserman, 1979). The effects of glucocorticoids on the renal protein do not appear to have been studied.

Whatever mechanism may be operating at the tubular level, this decrease in renal tubular calcium reabsorption is likely to contribute to the development of negative calcium balance, and, thus, osteoporosis. It is possible that the extent to which

tubular calcium reabsorption is inhibited in a given individual is one factor determining the likelihood of that person developing significant osteoporosis.

CHAPTER 6 : PITUITARY - GONADAL FUNCTION IN GLUCOCORTICOID-TREATED MALE ASTHMATIC PATIENTS

6.1 Introduction

The catabolic effects of glucocorticoids on bone are accompanied by similar damage to soft tissues, such as muscle and skin. These changes resemble those occurring in hypogonadal males in whom osteoporosis and muscle wasting are also prominent. The possibility that hypogonadism contributes to the catabolic effects of glucocorticoid excess is further suggested by the finding of a reduction in plasma testosterone levels in males with Cushing's syndrome (Smals et al, 1977; Luton et al, 1977) and in normal men following the acute administration of glucocorticoids (see Section 1.2.8). Further evidence of glucocorticoid interference with testicular function comes from the observation of arrested spermatogenesis in testicular biopsies taken after 30 days of prednisone therapy to normal volunteers (Mancini et al, 1966). The effect of long-term administration of glucocorticoids on the pituitary-gonadal axis of men had not been studied previously and is addressed in this chapter. In the light of these results, blood samples were subsequently collected for plasma zinc measurement also, as will be discussed subsequently.

6.2 Patients & Methods

The male patients listed in table 2 were studied, with the exception of pairs 5,7 and 8 in whom data were incomplete. The mean prednisone dose (±SEM) was 17.6 ± 2.9mg/day and the mean duration of this therapy, 6.9 ± 1.8 years. The mean ages of the glucocorticoid and control groups were 55.4 years (range 24-74)

and 54.7 years (range 21-77), respectively. All subjects were studied in the outpatient clinic and were free of acute symptoms of asthma.

Fasting blood samples were drawn and hormone assays carried out as described in section 3.3. Blood for zinc measurement was drawn, with minimal venostasis, into plastic tubes containing zinc-free heparin. Comparisons have been made using Student's"t" test for paired data.

6.3 Results

Individual values for the assayed hormones are shown in table 8 and the derived means ± SEM in table 9. Mean plasma total testosterone in the steroid-treated group, was approximately 60% of that in the control subjects. There was no difference in levels of sex hormone binding globulin between the groups and, accordingly, the calculated free testosterone concentration was also significantly reduced in the steroid-treated patients.

Multiple regression analysis of free testosterone concentration against prednisone dose and age indicated no significant relationship with either variable. However, data from patient 1 again heavily weighted these conclusions (see figure 10) and after his exclusion from the analysis, there was a significant correlation between free testosterone and prednisone dose ($r=-0.72$, $p < 0.02$). Serum testosterone was also measured in 2 asthmatic men (ages 49 and 60 years) receiving sub-replacement doses of prednisone (4 and 4.5 mg/day respectively). These are included in figure 10 to provide a wider range of prednisone doses for analysis. The correlation coefficient in this combined

TABLE 8 : Plasma testosterone and related indices in glucocorticoid-treated and control male asthmatics

	Control							Glucorticoid-treated						
Pair No.	T (nmol/l)	SHBG (nmol/l)	Free T (pmol/l)	LH (IU/l)	FSH (IU/l)	DHEAS (µmol/l)	Zinc (µmol/l)	T (nmol/l)	SHBG (nmol/l)	Free T (pmol/l)	LH (IU/l)	FSH (IU/l)	DHEAS (µmol/l)	Zinc (µmol/l)
1	26	39	952	5.0	3.2	10	14	10	45	297	4.7	5.4	0.3	14
2	24	57	728	8.6	4.6	2.7	11	15	51	425	4.6	3.8	1.1	13
10	22	51	684	8.5	4.8	9.6	12	10	56	251	29	9.7	2.2	11
12	21	62	569	8.1	5.4	0.8	13	14	61	376	12.2	13.1	0.4	10
13	16	72	375	8.4	6.0	0.5	11	18	63	464	9.5	4.7	1.5	10
14	23	50	738	4.8	7.1	0.8	12	8.7	52	234	11.5	12.5	0.4	11
15	17	58	478	18	14.1	0.4	10	9.7	53	262	28	13.1	0.2	12
16	14	49	418	8.3	6.8	3.8	-	16	53	456	8.5	7.2	1.1	-
17	16	62	434	6.2	7.5	3.2	12	3.5	62	83	39	16.0	0.2	9
18	20	59	560	6.9	6.2	2.6	12	23	56	682	7.7	11.4	1.4	9
20	14	45	419	7.7	5.6	1.0	12	7.0	39	211	28	11.7	0.5	13

For clarity, means ± SEM are shown separately in table 9

TABLE 9: Plasma testosterone and related indices in glucocorticoid-treated and control male asthmatics : mean data.

	Control	Glucocorticoid	p
Total testosterone (nmol/l)	19.4 ± 1.2	12.2 ± 1.7	< 0.01
SHBG (nmol/l)	54.9 ± 2.7	53.6 ± 2.2	NS
Calculated free testosterone (pmol/l)	578 ± 54	340 ± 49	< 0.01
FSH (mIU/ml)	6.5 ± 0.9	9.9 ± 1.2	< 0.02
LH (mIU/ml)	8.2 ± 1.1	16.5 ± 3.6	< 0.05
DHEAS (umol/l)	3.2 ± 1.1	0.9 ± 0.2	< 0.05
Zinc * (umol/l)	11.6 ± 0.3	11.1 ± 0.5	NS

* Measured in 10 pairs of subjects only.

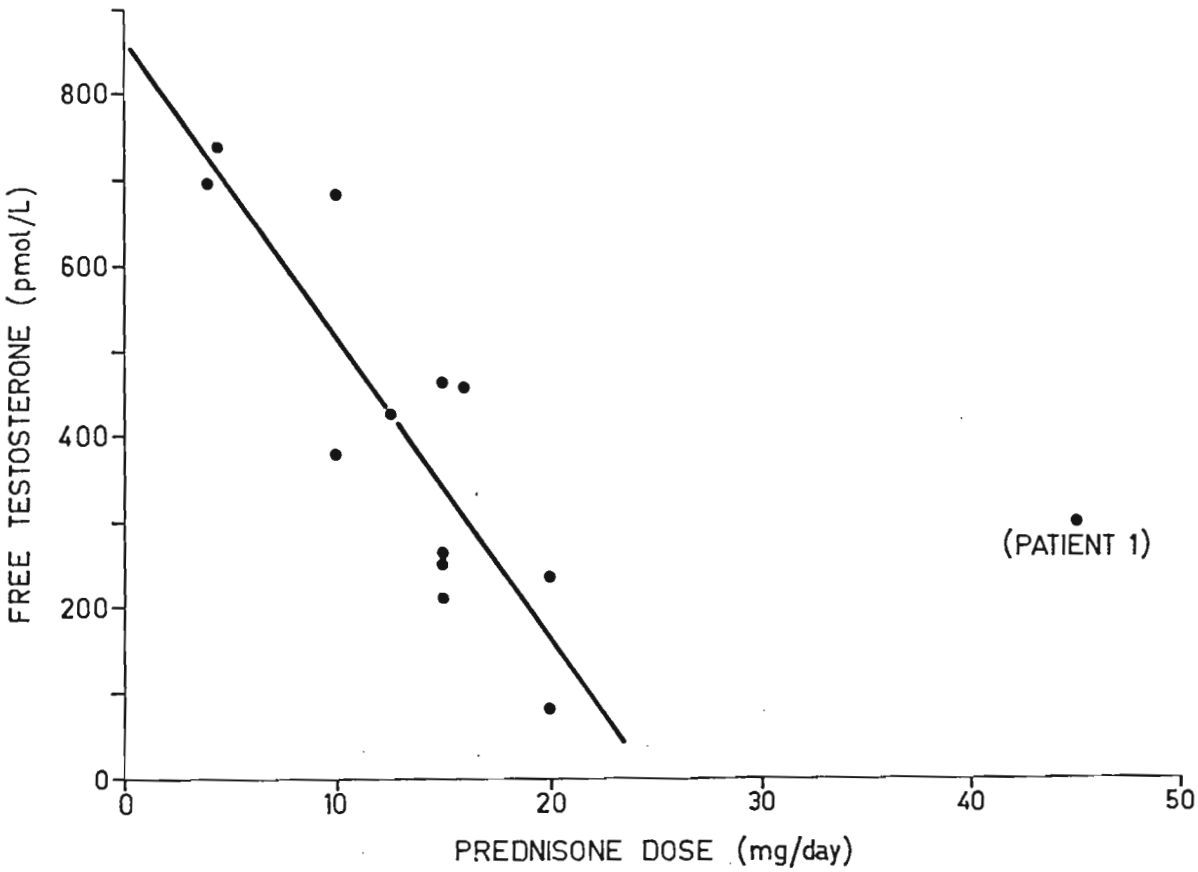


Figure 10: Relationship between plasma free testosterone concentration and prednisone dose in 13 asthmatic men. The regression line was calculated after the exclusion of data from patient one. $r=0.86$, $p<0.001$.

patient group, excluding patient 1, is -0.86 ($p < 0.001$).

The results of gonadotropin measurements showed the opposite trend. Mean FSH levels were 50% higher in those on steroids and LH levels approximately doubled (table 9). The relationship between LH and free testosterone is shown graphically in figure 11. There was a negative correlation between free testosterone and LH in the steroid-treated patients ($r=-0.73$, $p=0.01$). Of the 7 steroid-treated patients with subnormal free testosterone levels, 6 had elevated LH levels. Patient 1 had a low testosterone level and normal LH but was taking a much higher dose of prednisone (45mg/day) than the others. Inspection of table 8 indicates similar appropriate elevations of FSH in those with low free testosterone levels, though multiple regression analysis of free testosterone against FSH and LH, showed only testosterone and LH to be significantly related.

Plasma levels of the adrenal androgen DHEAS were also suppressed in those taking steroids (tables 8 & 9) but zinc concentrations were the same in both groups.

6.4 Discussion

These data show that long-term administration of prednisone to male asthmatics is associated with decreased plasma testosterone levels. This phenomenon is dose-related with clearly subnormal levels of free testosterone found in those receiving 15 mg of prednisone daily, or more. The patient receiving the highest dose of prednisone in this group, however, had an only modestly subnormal plasma testosterone concentration. The same patient also lay outside the general dose-response relationship for

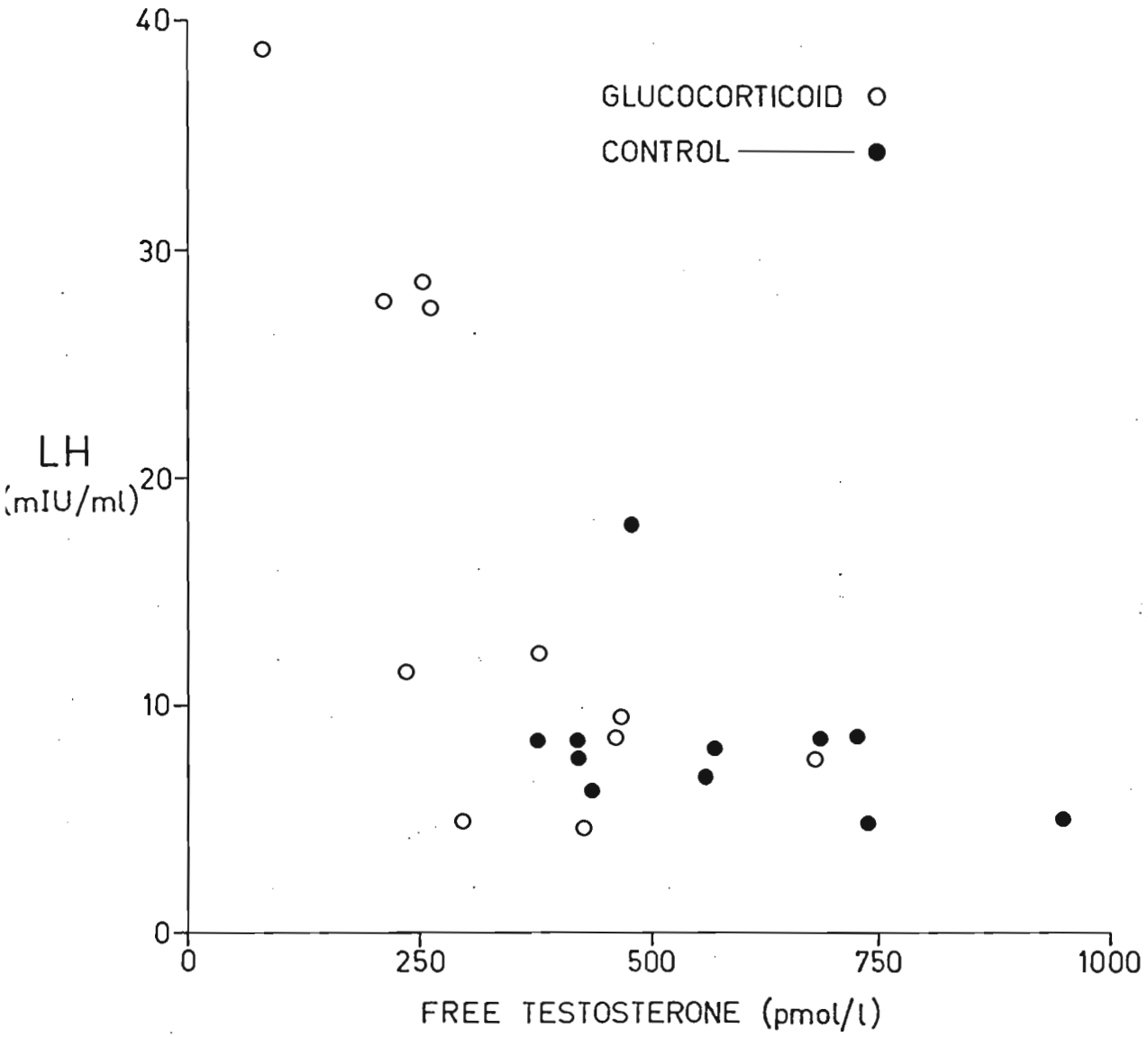


Figure 11: Relationship between plasma free testosterone and plasma LH in 11 glucocorticoid-treated asthmatic men and age-matched asthmatic men not receiving glucocorticoids. The correlation coefficient between these two variables in the glucocorticoid-treated patients is -0.73 ($p=0.01$).

tubular calcium reabsorption (see chapter 5) and either subnormal activation of prednisone to prednisolone in the liver or supranormal metabolism of the active steroid might account for these aberrant results.

A possible confounding factor in this study would be any difference in the severity of asthma between the two groups. While the glucocorticoid group may have had more frequent and severe asthmatic attacks in the past, all subjects were free of significant asthmatic symptoms at the time of study and so an effect of their underlying illness on gonadal function is unlikely.

The gonadotropin data suggest that pituitary function is normal and the response to diminished circulating levels of testosterone, appropriate. This is indicated by the elevation of mean levels of both LH and FSH in patients taking glucocorticoids and the strong negative correlation between LH and free testosterone in this group. Were the site of glucocorticoid action the pituitary, a positive correlation between LH and testosterone would be expected.

The depression of DHEAS production by steroid treatment is unlikely to account for these findings. While some DHEAS is converted to testosterone in the testis, this normally accounts for only 10-20% of total testosterone production (Mauvais-Jarvis and Baulieu,1965). Thus the 40% reduction in testosterone levels observed in these patients cannot be ascribed to the fall DHEAS. This conclusion is supported by the fact that acute ACTH infusion in men also reduces plasma testosterone (Irvine et al, 1974; Schaison et al, 1978) whereas it would be expected to elevate

DHEAS levels (Vaitukaitis et al, 1969). In post-menopausal women, in contrast, loss of adrenal androgen production may be of great pathophysiological significance (Crilly et al, 1979; Need, 1987).

These considerations lead to the conclusion that glucocorticoid-induced hypogonadism is caused by an effect on the testis. One possible mechanism of this effect is zinc deficiency. A significant reduction in serum zinc levels has been documented in steroid-treated asthmatics in comparison with asthmatic patients not receiving these drugs (Ellul-Micallef et al, 1976). Zinc deficiency in other contexts has been associated with testicular hypofunction. Dietary zinc deficiency in Iran has been found to be associated with delayed sexual maturation and this is reversible when zinc supplements are taken (Halsted et al, 1972). Patients on haemodialysis for renal failure have reduced plasma zinc and testosterone, low sperm counts and elevated levels of LH and FSH. Zinc supplementation changes all this indices towards normal (Mahajan et al, 1982). Experimentally induced zinc deficiency in normal subjects reproduces these findings, which are again reversible (Abbasi et al, 1980). The present data, however, do not support the original finding of zinc deficiency in steroid-treated subjects. The reason for this is inapparent. Steroid-treated and control subjects were matched for age, sex and race (all European) in this study and their intake of other medications was comparable. None of this data is provided in the original publication of Ellul-Micallef's group. It is concluded that zinc deficiency does not underlie the testicular dysfunction of the patients described herein.

Saez et al (1977) have studied the effect of dexamethasone and ACTH treatment on gonadal function in rats. Both agents reduced plasma testosterone and testicular synthesis of DNA and protein even when hCG was administered simultaneously. They related these changes to their finding of reduced numbers of hCG-binding sites in the Leydig cells of steroid-treated rats, conferring a relative resistance to gonadotropin action. These results have been confirmed (Bambino and Hsueh, 1981) and are consistent with the findings of the present study.

There is also data, however, suggesting that glucocorticoids can directly inhibit LH release from the pituitary. Thus, corticotropin releasing factor (Rivier and Vale, 1984), ACTH (Mann et al, 1982; Rivier and Vale, 1984) and cortisol (Ringstrom and Schwartz, 1985) all diminish either basal or GnRH-stimulated LH levels in rats, and prednisolone reduces stimulated LH levels in women (Sakakura et al, 1975). Cushing's disease is associated with reduced LH reserve in both men and women (Bocuzzi et al, 1975; Luton et al, 1977). It thus appears that glucocorticoids affect the pituitary-gonadal axis at at least two levels, though in the majority of patients described in the present study, the gonadal effect predominated.

Review of the data describing the acute effect of glucocorticoids on pituitary-gonadal function in men, suggests that the gonadal defect is usually dominant in this context also. Cortisol (Doerr and Pirke, 1976), dexamethassone (Schaison et al, 1978) and ACTH (Schaison et al, 1978; Irvine et al, 1974) all reduce plasma testosterone with either no change (Schaison, Irvine) or an

increase (Doerr) in gonadotropins. Furthermore, administration of dexamethasone to normal men for 3 days depresses the testosterone response to hCG (Schaison et al, 1978).

In the male, testosterone is thought to promote bone formation, inhibit bone resorption and so lead to positive calcium balance (Nordin et al, 1981). Hypogonadism in men is associated with progressive bone loss and the occurrence of fractures (Francis et al, 1986). Therefore, the major reduction in plasma testosterone seen in these patients is likely to contribute significantly to the development of osteoporosis and also to the soft tissue atrophy associated with glucocorticoid use. Since established glucocorticoid-induced osteoporosis is such a difficult clinical problem, consideration should be given to the routine measurement of testosterone levels in all males on long-term steroid therapy and the institution of appropriate replacement therapy.

6.5 Postscript

Since the publication of the work described in this chapter, MacAdams et al (1986) have confirmed that serum testosterone levels are reduced in steroid-treated subjects with chronic pulmonary disease. An inverse relationship between plasma testosterone concentration and glucocorticoid dose was also found by these workers. Basal gonadotropin levels, however, did not differ between the steroid-treated and control groups and the responses to GnRH were the same in both groups. These gonadotropin data conflict with those of the present study, though the discussion above has pointed out that glucocorticoids can also impair LH release. It is not clear why the principal site of glucocorticoid action on the pituitary-gonadal axis

should differ between these two studies. The principal difference between our study and that of MacAdams is the age of the subjects studied. Their patients were aged 67 ± 4 years (mean ± SD) whereas ours ranged from 24-74 years, with a mean of 55 years. Since elderly males demonstrate proportionately smaller increases in gonadotropin levels in response to a decrease in testosterone, possibly because of higher estrogen levels inhibiting gonadotropin secretion (Goldstein,1979), this age difference may account for their failure to observe increased gonadotropin levels.

CHAPTER 7 : SERUM INSULIN-LIKE GROWTH FACTOR 1 LEVELS IN GLUCORTICOIDS-TREATED ASTHMATIC SUBJECTS

7.1 Introduction

Both growth hormone and the circulating mediator of some of its actions, insulin-like growth factor 1 (IGF-1), have anabolic effects on bone cells (see Section 1.2.8). It is well established that glucocorticoids retard linear bone growth in children (Blodgett et al, 1956) and it has been suggested that this effect might be mediated by glucocorticoid suppression of growth hormone secretion. Were this the case, reduced levels of growth hormone or IGF-1 might contribute to the development of osteoporosis in steroid-treated patients.

Data describing the effects of glucocorticoid excess on circulating growth hormone levels are conflicting. In children, basal and stimulated growth hormone levels are apparently unaffected by steroid treatment (Morris et al, 1968; Sturge et al, 1970; Vazques et al, 1972) though this is not the case when long-acting steroid preparations are used (Stempfel et al, 1968). Studies in adults, on the other hand, suggest that growth hormone levels are reduced. In normal subjects given prednisone for 8-10 days, integrated circulating growth hormone levels and growth hormone production rates are diminished (Thompson et al, 1972). Arginine-induced growth hormone release is also reduced in these circumstances (Takeda et al, 1974). In patients receiving glucocorticoid therapy, growth hormone response to hypoglycaemia is probably blunted (Hartog et al, 1964; Frantz and Rabkin, 1964) as it is in patients with Cushing's disease (Hartog et al, 1964;

James et al, 1968; Strauch et al, 1969; Demura et al, 1972). In the latter group of patients, it is probable that the impaired growth hormone response is at least in part secondary to glucocorticoid excess, since it is reversed in some patients following pituitary irradiation (Demura et al, 1972). It does not seem to result from a direct steroid effect on the pituitary, however, since dexamethasone-treated rats have a supra-normal rise in growth hormone levels following sitmulation with growth hormone-releasing factor (Wehrenberg et al, 1983). Furthermore the growth-inhibiting actions of glucocorticoids are not entirely mediated by diminished growth hormone release, since steroids also inhibit growth in hypopituitary subjects (humans and rats) receiving growth hormone therapy (Soyka and Crawford, 1965).

The growth-promoting effects of growth hormone are thought to be mediated by the somatomedins, in particular IGF-1 (also known as somatamedin-C) (Daughaday et al, 1972). IGF-1 is a 70 amino acid peptide which has significant homology with proinsulin. Like that molecule, it contains two disulphide bridges joining opposite ends of the peptide to produce an approximately circular secondary structure (Rinderknecht and Humbel, 1978; Klapper et al, 1983). Unique among peptide hormones, it circulates bound to a 145 kilodalton carrier protein (Zapf et al, 1975; Hintz and Liu, 1977) and its basal levels are both easily measurable and relatively stable. The latter two characteristics make it a much more convenient measure of growth hormone status than assays of the pituitary hormone itself. For these reasons, we have measured IFG-1 levels in serum from the steroid-treated and control asthmatic patients under study.

7.2 Patients and Methods

The patients studied are those shown in Table 2 and their preparation and the timing of blood sampling is as described previously. Pairs 13 and 19 were excluded because of missing data. The IGF-1 assay is described in Section 3.3.

7.3 Results

Figure 12 shows graphically individual serum IGF-1 levels in the two groups of patients. The matched pairs are indicated with solid lines. The mean (± SEM) IGF-1 concentration in the control group was 65.4 ± 9.5 mg/L and in those taking steroids it was 84.2 ± 16.4 mg/L. Comparison using Student's "t" test for paired data indicated that there was no significant difference between the groups.

It is clear from Figure 12 that 4 patients have values which are clearly higher than those of the other patients studied. Both members of pair 6 were teenagers and their high levels may reflect the pubertal peak in IGF-1. There is no reason apparent for the higher values found in the steroid-treated members of pairs 16 and 20.

Others have observed a decline in IGF-1 with age, from puberty onwards (Johanson and Blizzard, 1981). Figure 13 shows the relationship between age and IGF-1 levels in these two groups of patients. In the control subjects, there was a highly significant inverse relationship ($r = -0.79$, $p < 0.001$). In those receiving steroids the correlation was not significant ($r = -0.44$,

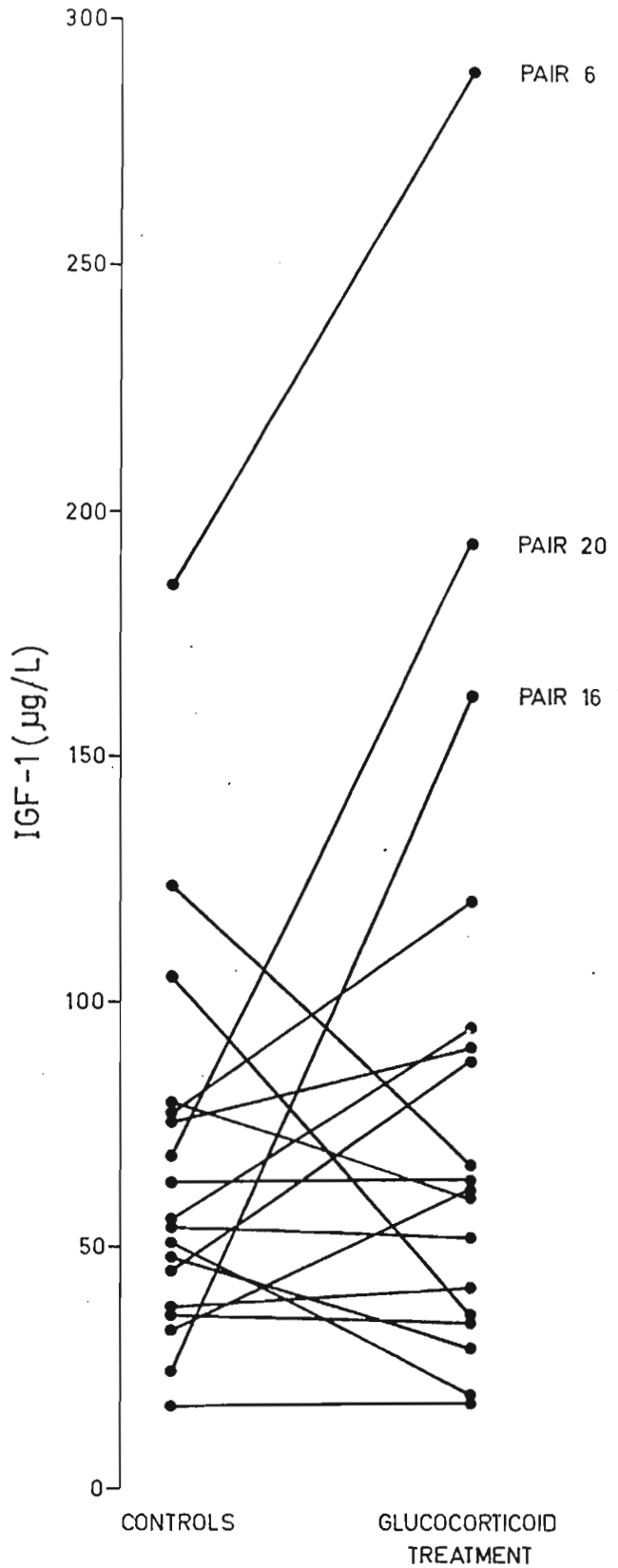


Figure 12: Individual values of insulin-like growth factor 1 in matched pairs of asthmatic subjects either treated or not treated (control) with oral glucocorticoids. The pair numbers indicated refer to table 2. There was no significant difference between the groups.

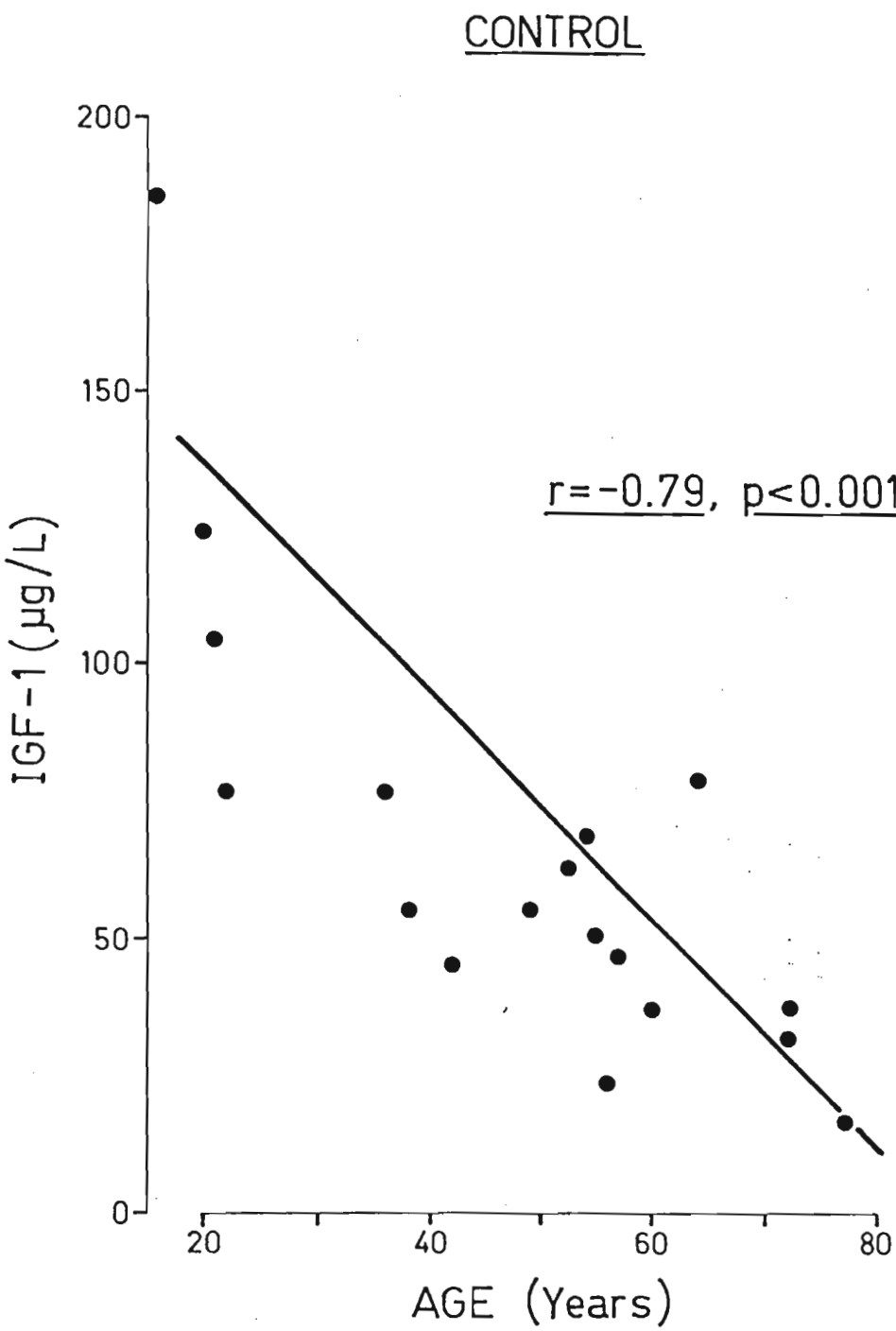


Figure 13a Relationship between serum IGF-1 and age in asthmatic subjects not treated with glucocorticoids.

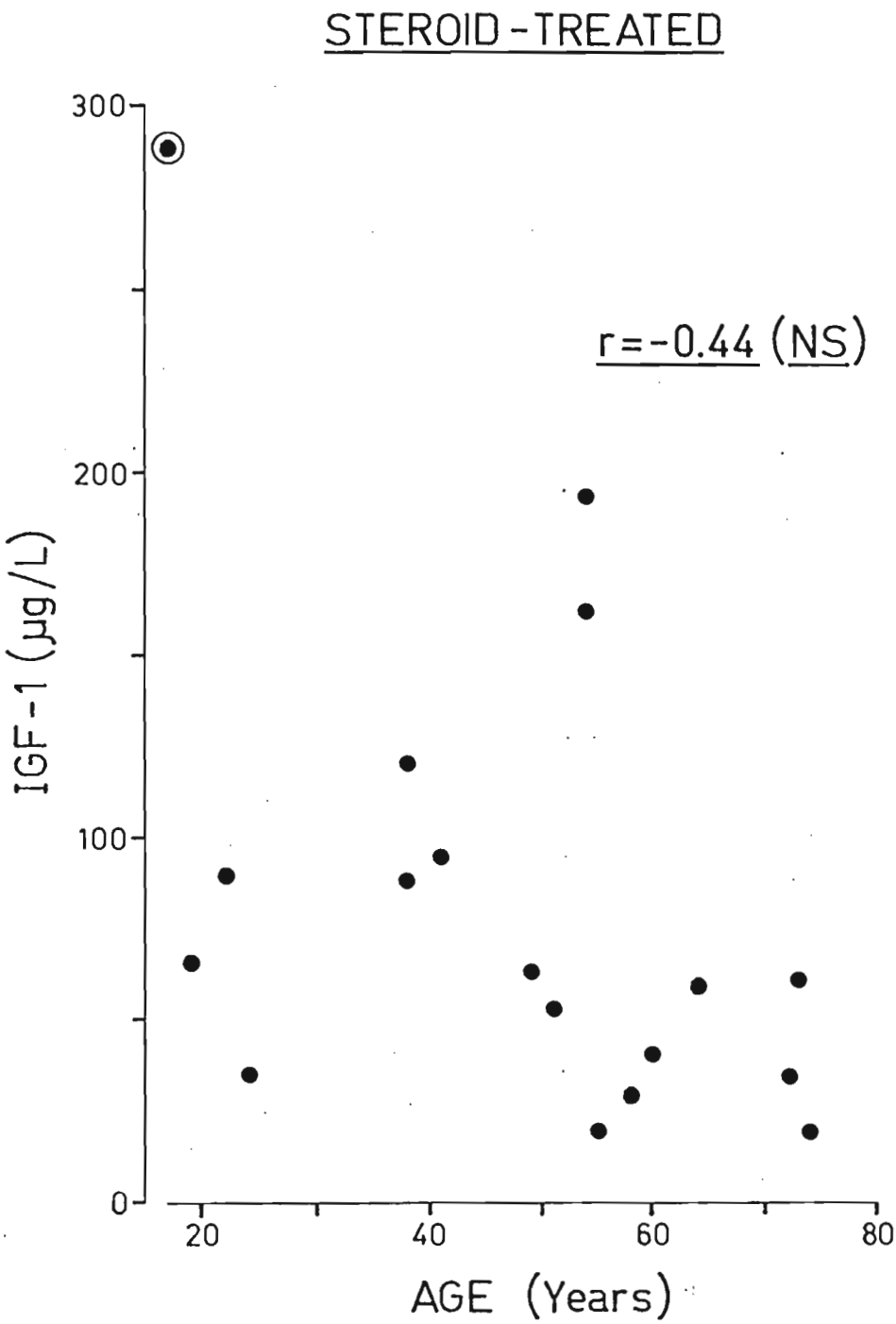


Figure 13b. Relationship between serum IGF-1 and age in glucocorticoid-treated asthmatic subjects.

$0.05 < p < 0.1$) and was disproportionately influenced by the data of patient 6, whose omission reduced the correlation coefficient to -0.21.

The relationships between circulating IGF-1 and biochemical indices of bone turnover were also studied. The relevant correlation coefficients are shown in Table 10. The values are not randomly distributed, since all are positive. However, only urine hydroxyproline/creatinine correlated significantly with serum IGF-1 and this was most marked in the subjects not taking steroids (fig 14). How real the apparent difference between the steroid-treated and control group is, is unclear, since omission of the highest IGF-1 value from the control group reducesthat correlation coefficient to 0.47 ($p = 0.05$).

7.4 Discussion

The data presented here do not suggest that glucocorticoid treatment causes any change in circulating levels of IGF-1. This implies that growth hormone levels are comparable in the two groups. IGF-1 levels are influenced by nutritional status but this is unlikely to have obscured any difference between the groups since all patients were taking a normal home diet and the two groups were of comparable mean weight (Table 2).

These findings are in agreement with observations of normal levels of immuno-reactive somatomedins in patients with Cushing's syndrome (Thoren et al, 1981; Gourmelen et al, 1982) and the failure of a single 60mg dose of prednisone to suppress IGF-1 (Unterman and Phillips, 1985). In contrast, studies using bioassays of somatomedin have found an acute fall in bioactivity

TABLE 10 : Correlations between serum IGF-1 and indices of bone turnover in steroid-treated and control asthmatic subjects.

	Control	Steroid-treated	Total Group
Serum osteocalcin	0.36	0.27	0.14
Serum alkaline phosphatase	0.20	0.11	0.13
Urine hydroxyproline/ creatinine	0.72 **	0.41	0.51 *
n	18	18	36

* $p < 0.005$; ** $p < 0.001$

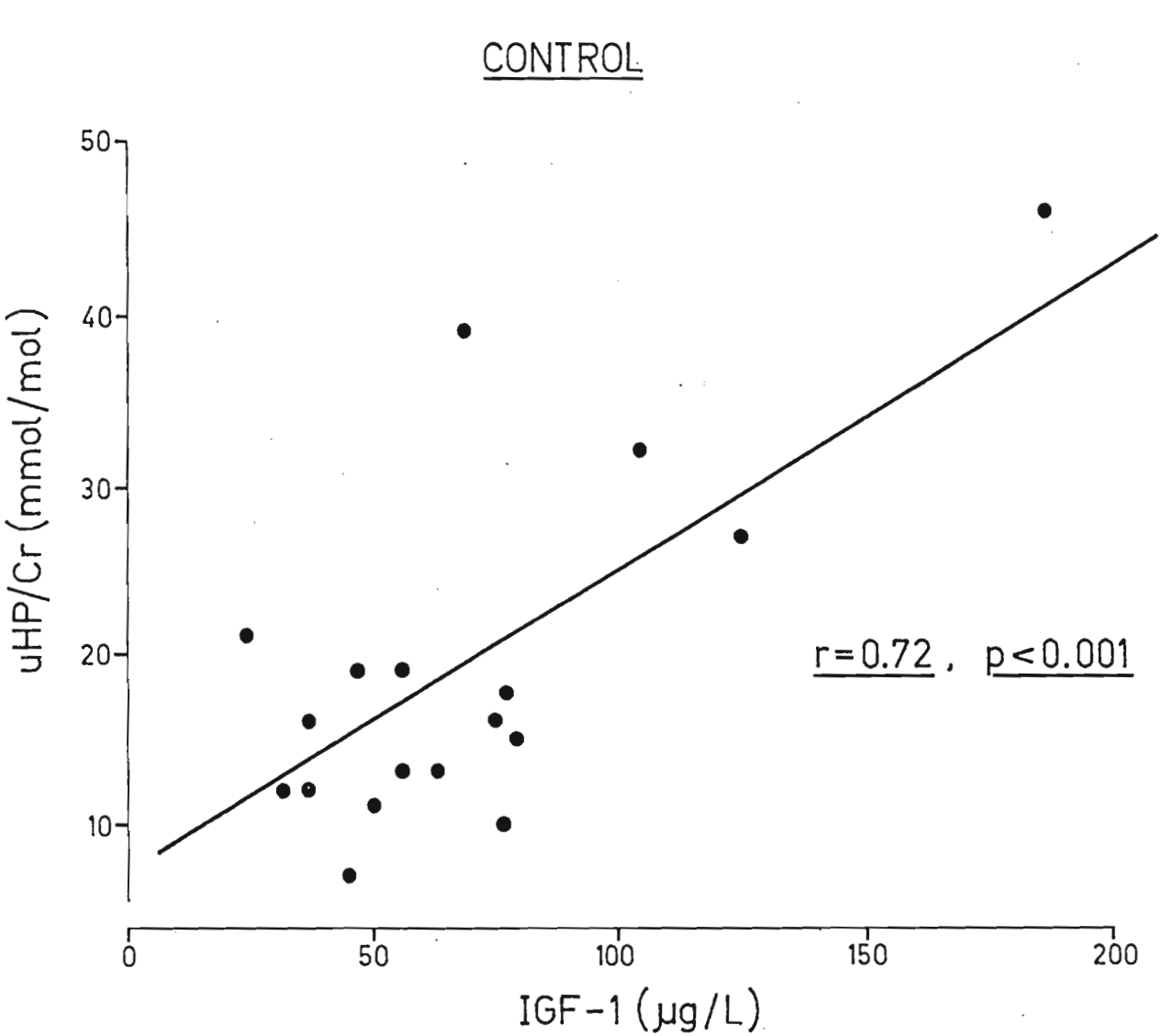


Figure 14a Relationship between urinary hydroxyproline/ creatinine ratio and serum IGF-1 in asthmatic subjects not treated with glucocorticoids.

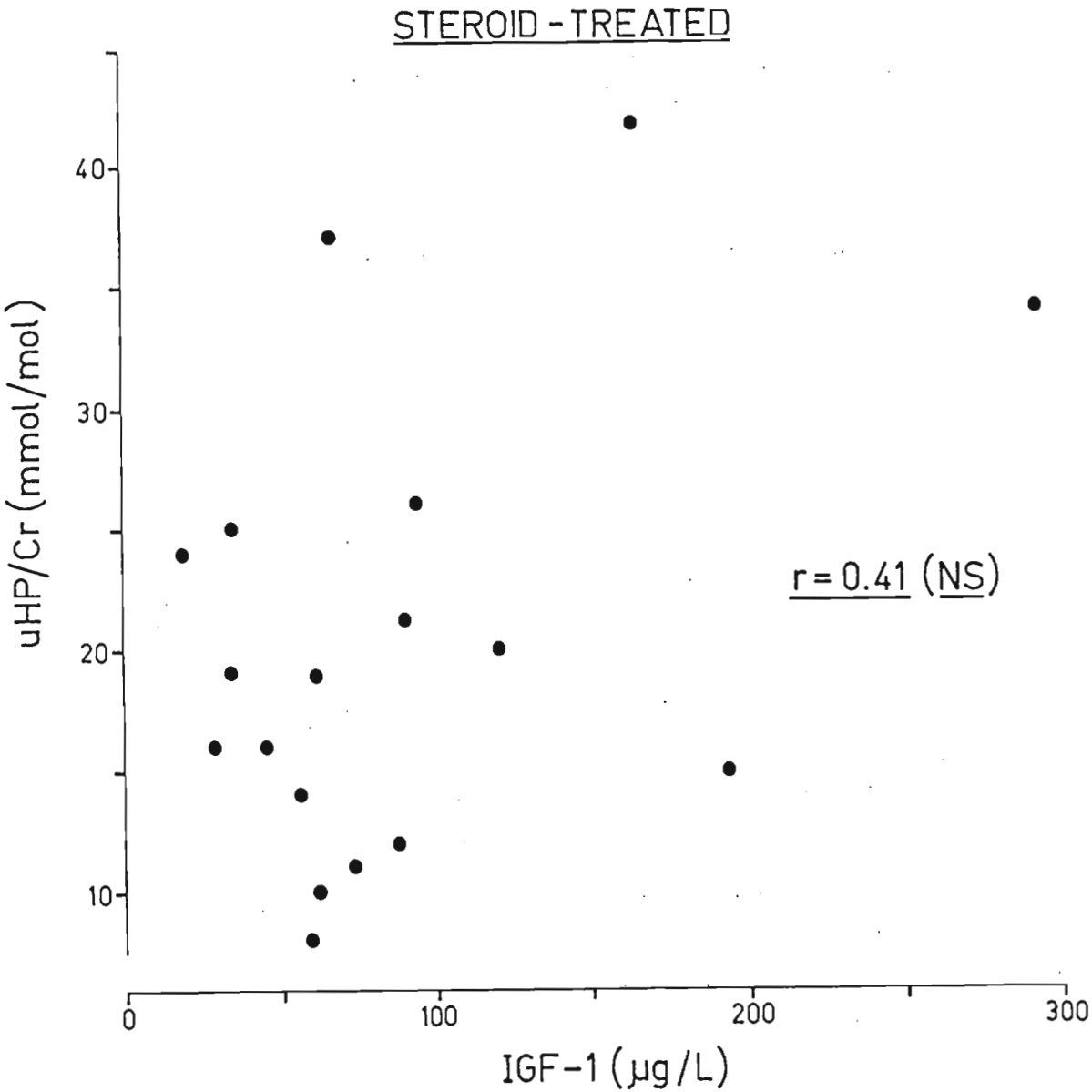


Figure 14b Relationship between urinary hydroxyproline/ creatinine ratio and serum IGF-1 in glucocorticoid-treated asthmatic subjects.

following glucocorticoid administration (Unterman and Phillips, 1985; Elders et al, 1975) and a sustained inhibition in patients receiving daily steroid therapy (Green et al, 1978). This discrepancy appears to be due to the presence of inhibitors of somatomedin activity (Phillips et al, 1979) which can be chromatographically separated from the somatomedins themselves. Unterman and Phillips (1985) have used these techniques to demonstrate that the activity of isolated somatomedins does not change following steroid administration, but that an increase in inhibitor activity does occur.

The control subjects in the present study manifest the expected fall in IGF-1 with age, whereas this is less marked in those taking prednisone. The reason for this difference is not apparent though it does suggest that glucocorticoids do interfere with the regulation of IGF-1, even if mean levels are unchanged. The positive correlation between IGF-1 and urinary hydroxyproline implies that this hormone might have a role in the regulation of bone turnover in vivo. The fact that the correlation is higher in the control group may simply be due to weighting of the statistic by a single outlying value (see above) or it may imply that in patients taking steroids, the role of IGF-1 in the regulation of bone turnover is diminished. The direct or indirect stimulation of osteolysis by steroids (see Section 1.2.2) might account for such a diminution.

Other data suggests a role for growth hormone in the regulation of skeletal homeostasis. Heaney's group have shown that growth hormone stimulates skeletal turnover in the dog (Heaney et al, 1972; Harris et al, 1972), and this has now been demonstrated in children (Delmas et al, 1986) and adults (Peacock et al, 1987).

In spite of this, Bennet et al (1984) found no relationship between IGF-1 levels and either serum alkaline phosphatase or urine hydroxyproline in normal and osteoporotic women. Their subjects were older than those in the present study and the majority were postmenopausal. These differences may account for apparently discrepant results.

In conclusion these data indicate that the deleterious effects of glucocorticoids on bone are not mediated by changes in the level of immunoreactive IGF-1 but that this hormone may have a significant role in the regulation of normal bone turnover. The latter finding and the increasing availability of human growth hormone suggest that it may have a place in the clinical manipulation of bone turnover (Aloia et al, 1987).

CHAPTER 8 : CALCIUM SUPPLEMENTS IN THE PREVENTION OF GLUCOCORTICOID - INDUCED OSTEOPOROSIS

8.1 Introduction

The preceding chapters have considered the mechanisms by which glucocorticoids produce a progressive reduction in bone mass. The present and subsequent sections will address some of the ways in which this process might be slowed or reversed.

One of the most firmly established effects of glucocorticoids on calcium metabolism is an inhibition of intestinal calcium absorption (see section 1.2.3). This, in turn, may be a factor contributing to the development of hyperparathyroidism (section 1.2.7). This postulated sequence of effects has led to the use of vitamin D and its metabolites to promote intestinal calcium absorption and thus suppress parathyroid secretion and bone resorption. Both vitamin D (Hahn & Hahn, 1976) and 25(OH)D (Hahn et al, 1979) appear to be effective in this regard and their use is associated with an increase in bone mass. The more active metabolite, $1,25(OH)_2D$ also increases intestinal calcium absorption but does not appear to exert a beneficial effect on bone mass (Dykman et al, 1984). This may be because of its direct stimulation of bone resorption, though it might be expected that the other vitamin D metabolites would also have this deleterious effect. The direct bone-resorbing effect of the vitamin D metabolites and these inconsistencies among the published studies cast doubt upon the usefulness of the vitamin D metabolites in steroid osteoporosis, as does their propensity to cause

significant hypercalcaemia and hypercalciuria (Schwartzman and Franck, 1987). Net calcium absorption can also be increased without the use of vitamin D, however, by increasing the dietary intake of calcium (Wilkinson, 1976). Adequate doses of calcium supplements might therefore be expected to produce similar beneficial effects upon calcium metabolism to those seen with vitamin D and thus lead to a decrease in bone resorption. They have additional advantages in safety and cost but, in spite of this, their role in the prevention of steroid osteoporosis has not been widely studied. This may in part stem from the belief that patients with relative malabsorption of calcium do not benefit from dietary supplements of this ion (Need, 1987). This investigation assesses the ability of a daily calcium supplement to suppress urine hydroxyproline, an index of bone resorption, in glucocorticoid-treated patients.

8.2 Patients

Thirteen patients (6 men, 7 women) receiving chronic prednisone therapy gave their informed consent to participation in the study. Relevant descriptive data are given in Table 11. Nine were receiving steroids for treatment of asthma, one each for cranial arteritis, dermatomyositis, polymyositis, and dermatitis. These underlying conditions were in remission throughout the study period and no patient suffered from other conditions or was taking other drugs known to affect calcium metabolism. The patients' median age was 60 years (range 18-84) and the median duration of steroid therapy was 3 years (range 0.2-35). The median prednisone dose was 15 mg per day (range 6-160) and it

Table 11 : Descriptive data for trial subjects

Age (yr)	Sex	Underlying Disease	Duration of Steroid Therapy (yrs)	Prednisone Dose (mg/day)
74	F	Asthma	3	10
82	F	Cranial arteritis	0.5	15
71	F	Dermatomyositis	0.5	8.5
54	M	Asthma	4	15
18	F	Asthma	4	160
40	F	Polymyositis	0.2	14
25	M	Asthma	2	16
73	M	Asthma	3.5	10
74	M	Asthma	7	15.5
60	M	Asthma	18	6
68	F	Dermatitis	35	15
37	F	Asthma	1.5	25
52	M	Asthma	3	10

remained stable throughout the study period.

8.3 Methods

Three, fasting, double-voided, one hour urine samples were collected on consecutive mornings and a single blood sample obtained from each subject at both the beginning of the study and after two months taking a nightly supplement containing 1g of elemental calcium (Calcium-Sandoz). Hydroxyproline, calcium and creatinine were measured in urine and ionised calcium, phosphate, alkaline phosphatase and osteocalcin in serum, using the techniques described in section 3.3. Urinary hydroxyproline and calcium were expressed as molar ratios to creatinine (HP/Cr and Ca/Cr, respectively) and the mean of the three values calculated. Results are given as mean ± SEM and have been analyzed using Student's "t" test for paired data.

8.4 Results

Figure 15 demonstrates the effect of calcium supplementation on urine hydroxyproline excretion. There was a 20% reduction in the mean HP/Cr from 27.1 ± 2.5 to 21.8 ± 2.4 mmol/mol during the 2 month period of calcium therapy ($p < 0.001$). The decrease was observed in all but one patient (patient number 5) whose urine hydroxyproline subsequently fell by 50% after a further four months of calcium supplementation. In the remaining 12 patients, the extent of the suppression of HP/Cr was related to the basal level, those with the highest basal values showing the greatest fall (Figure 16). During the same period, there was an increase

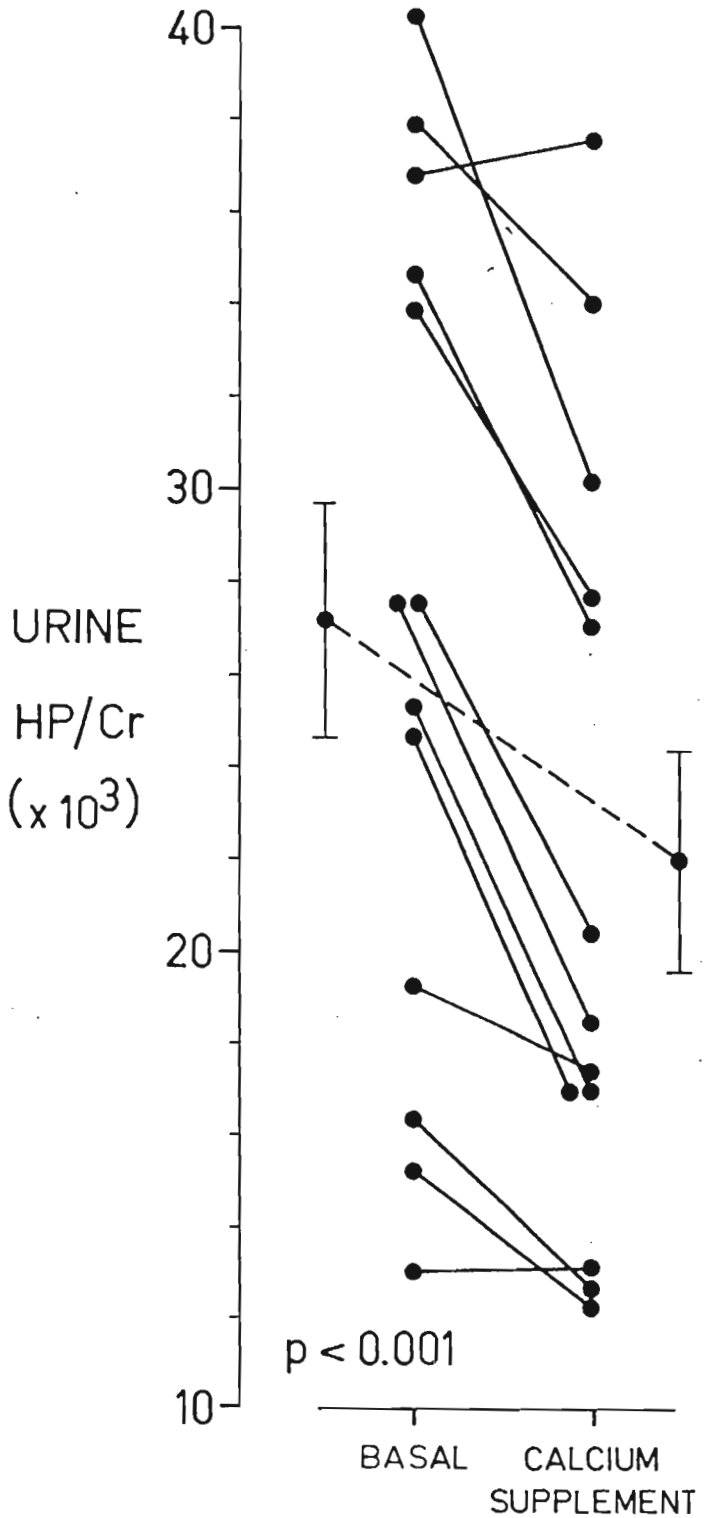


Figure 15 Fasting urinary hydroxyproline/creatinine ratios in glucocorticoid-treated asthmatic patients before and after 2 months therapy with calcium (1 g per day). Mean (± SEM) values are indicated by the broken line.

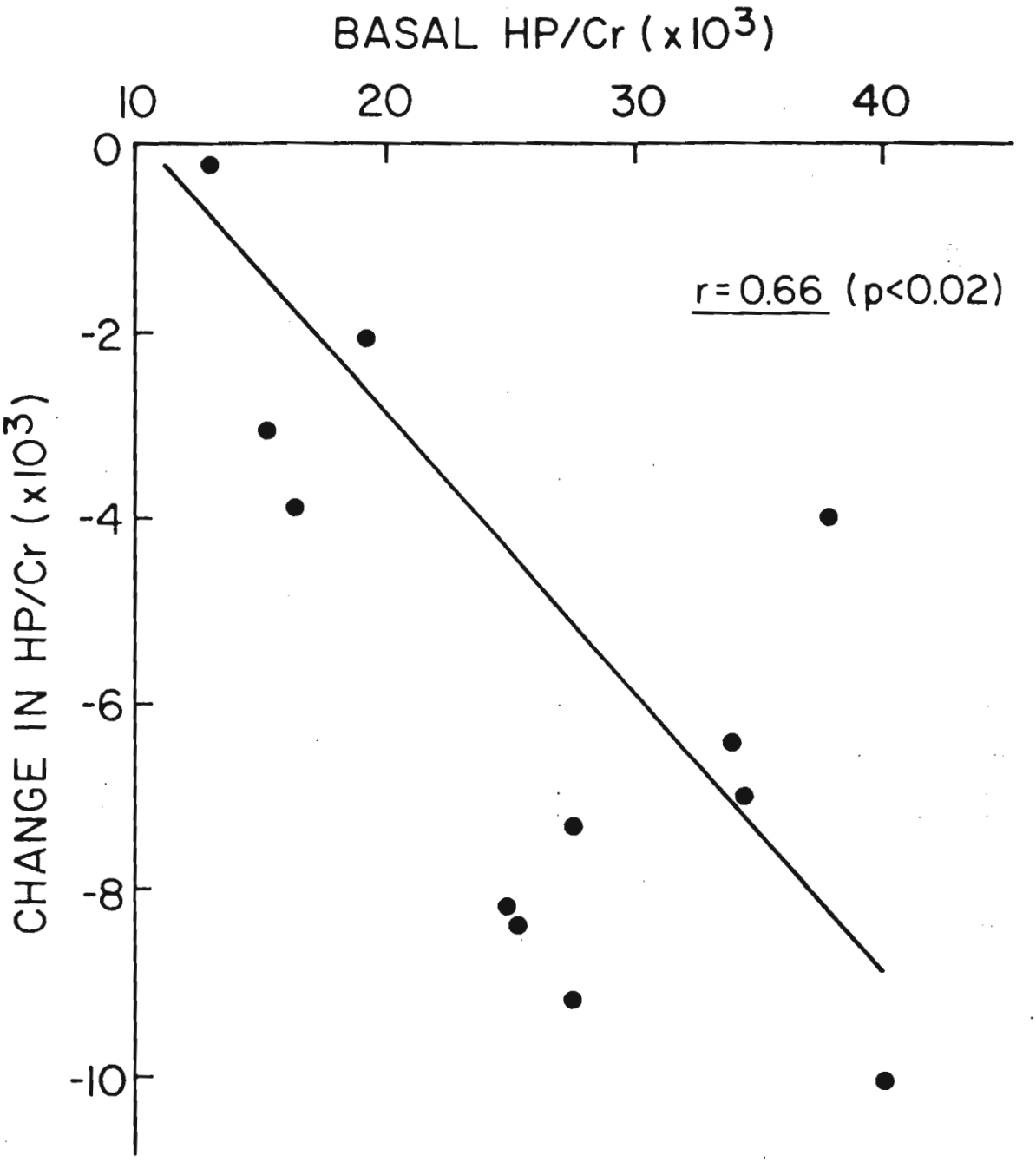


Figure 16 Regression of the changes in HP/Cr during calcium supplementation on basal values in the 12 subjects who showed a response.

in Ca/Cr ($p < 0.05$), but no significant change in the other measured indices (Table 12)

8.5 Discussion

These results demonstrate that calcium supplementation consistently suppresses urinary hydroxyproline excretion in glucocorticoid-treated patients. Excretion of this imino acid reflects collagen breakdown in bone and soft tissue but, as there is no reason to suspect that soft tissue catabolism changed during the study period, the findings probably indicate a reduction in bone resorption. The consistency of this reduction among the patients and the absence of alterations in clinical status or prednisone dosage during the study suggest that the decreased osteolysis can be attributed to the calcium supplementation. The probable mechanism of this effect is a suppression of PTH secretion (see Chapter 9), though increased calcitonin levels may also be contributory (Austin et al, 1979).

Hahn et al (1979) have shown that the administration of 25-hydroxyvitamin D to steroid-treated subjects also reduces bone resorption and that this is accompanied by an increase in bone mass. If this vitamin D metabolite is assumed to act by increasing net intestinal calcium absorption and thus suppressing PTH, then the present data imply that calcium supplementation alone can achieve a similar effect on bone metabolism and an associated increase in bone mass is to be expected. Similarly, in postmenopausal osteoporosis calcium supplements have been shown to reduce hydroxyproline excretion (Horowitz et al, 1984), and their long-term use is associated with increased bone mass

Table 12 : Effect of calcium supplementation on indices of calcium metabolism in 13 glucocorticoid-treated subjects

	Basal	Calcium-Treated
Ca/Cr (molar ratio)	0.42 ± 0.05 (0.06 - 0.30)	0.74 ± 0.14*
Ionized calcium (mmol/l)	1.24 ± 0.01 (1.18 - 1.28)	1.26 ± 0.01
Phosphate (mmol/l)	1.12 ± 0.05 (0.9 - 1.6)	1.09 ± 0.05
Alk. phosphatase (u/l)	66 ± 3 (30 - 95)	60 ± 5
Osteocalcin (µg/l)	5.2 ± 1.4 (4 - 20)	5.9 ± 0.3

All samples were collected in the fasting state and indices other than Ca/Cr were measured in serum. Ca/Cr = urinary calcium/creatinine ratio. Data are mean ± SEM. Normal ranges are shown in parentheses.

* = significantly different from basal value, $p < 0.05$.

All other values were not significantly changed from baseline.

(Recker et al, 1977; Nordin et al, 1978) and a decreased fracture rate (Riggs et al, 1982). Studies of the effect of simple calcium supplementation upon the bone mass of glucocorticoid-treated subjects have not been carried out, although microcrystalline hydroxyapatite has been shown to reduce the rate of loss of height and radial bone density in such patients (Nilsen et al, 1978). This is consistent with the results of the present study.

The extent of the HP/Cr suppression is a function of the basal level, suggesting that those patients most likely to benefit from calcium supplements can be selected on the basis of their initial measurements. The slope of this regression line (-0.23) also indicates that suppression of bone resorption is only partial, even when allowance is made for the soft tissue contribution to HP/Cr (probably equivalent to a value of 10×10^{-3}). It is possible that higher doses or more frequent administration of calcium might produce more complete arrest of osteolysis.

Associated with the fall in HP/Cr was a 76% increase in Ca/Cr. This large change is probably caused by the proximity of the time of urine sampling to that of calcium administration. Of more importance, is the absence of any change in the two measured indices of osteoblast function, alkaline phosphatase and osteocalcin. This implies that bone formation has not significantly declined despite the reduction of bone resorption. Thus, an increase in bone mass is to be expected. Absence of the normally tight coupling between bone formation and resorption has been noted previously in steriod-treated subjects (see Chapter 1) and may account for these findings. It is also possible that a

decline in bone formation may have occurred more than 2 months after the introduction of calcium supplementation.

While these data are preliminary and do not directly measure changes in bone mass, they do suggest that a safe and inexpensive form of prophylaxis against glucocorticoid-induced osteoporosis is available. The dose of calcium used and its timing require further study as do its effects on bone density. Until such information is available, the use of calcium supplements in steroid-treated subjects would appear to have a reasonable basis.

CHAPTER 9 : THE ACUTE BIOCHEMICAL EFFECTS OF FOUR PROPRIETARY CALCIUM PREPARATIONS

9.1 Introduction

The data described in the previous chapter indicate that oral calcium supplementation is able to suppress bone resorption in steroid-treated subjects. For the clinician, this raises the question of which calcium supplement should be used, an issue which is also relevant to the management of patients with osteoporosis of other causes. There is very little data comparing the absorption of the various calcium salts or the effect of different formulations of these salts (e.g. tablet versus solution). The bioavailability of calcium in proprietary calcium preparations has been almost totally neglected.

A further factor that might affect efficacy, is the other constituents of a supplement, in particular the sodium content. The renal handling of sodium and calcium are tightly linked and urine sodium loss (reflecting intake) correlates with that of both calcium and hydroxyproline (Goulding, 1981). Furthermore, an increase in dietary sodium is accompanied by increased bone resorption as measured by hydroxyproline excretion (Goulding and Lim, 1983). The sizeable sodium content of some preparations makes this a relevant issue.

This study assesses the amount of calcium absorbed from single doses of four proprietary calcium preparations in common use in various parts of the world. In addition, it addresses the

mechanism of action of supplements on bone turnover and assesses their palatability, which is important since long-term compliance is a pre-requisite for the success of any prophylactic or therapeutic regimen in osteoporosis.

9.2 Methods

9.2.1 Procedure and Subjects: The calcium absorption from each preparation was compared using a modification of the oral calcium tolerance test of Broadus et al (1978). A fasting blood and 2 hour urine sample were collected (period 1) before ingestion of the test preparation in a dose containing 1g of elemental calcium. This was followed immediately by a standard light breakfast of peaches and cereal (calcium content 14mg). Two consecutive 2 hour urine collections (periods 2 and 3) were then obtained and blood samples taken 1 and 3 hours after the oral calcium dose. Ten normal subjects (8 female, 2 male) aged 20 to 35 years, took part in the trial. Each subject took the four calcium preparations shown in Table 13 in a random order with an interval of at least one week between tests. The procedure was repeated on a fifth occasion without the calcium supplement (control).

9.2.2 Assays: Serum and urine sodium and creatinine and urine calcium were measured in all samples. Urine hydroxyproline, serum ionised calcium (Ca_i) and serum parathyroid hormone (sPTH) (in-house, c-terminal specific, immunoradiometric assay) (Addison et al, 1971; Evans, 1979) were assayed in periods 1 and 3 only. The palatability of each preparation was rated by the subjects on

TABLE 13 COMPOSITION OF CALCIUM PREPARATIONS

	Composition	Formulation	Sodium Content (mmol/g calcium)
SPAR-CAL®	- Calcium carbonate, citric acid, potassium bicarbonate	Effervescent tablet	<1.4
CALCIUM-SANDOZ	- Calcium lactate-gluconate, calcium carbonate, sodium bicarbonate, citric acid	Effervescent tablet	17.7
OS-CAL®	- Calcium carbonate (from oyster shell)	Film coated tablet	0.4
OSSOPAN®	- Micro-crystalline hydroxy-apatite (from bovine bone)	Powder	1.6

a 5 point scale (1 = unpleasant, 3 = neutral, 5 = pleasant).

9.2.3 Calculations and Statistics: Urine sodium and calcium were expressed as molar ratios to creatinine (uNa/Cr, uCa/Cr) and urine hydroxyproline as millimoles per mole of creatinine (uHP/Cr). The change (Δ) in each parameter between periods 1 and 3 was calculated.

The total calcium absorbed at 4 hours was assumed to equal the sum of the rise in extracellular fluid (ECF) calcium at this time plus the cummulative urinary calcium excretion in excess of basal levels. The rise in ECF calcium is in turn comprised of intravascular (i.e. plasma) calcium and that of the interstitial fluid. The rise in interstitial fluid calcium concentration is equal to ΔCa_i since this ion diffuses freely across capillary walls. In plasma, Ca_i is only 47% of the total calcium concentration because of binding of the ion to albumin (Hays, 1980), so the rise in total calcium concentration in this compartment is $\Delta Ca_i/0.47$.

Since interstitial fluid volume and plasma volume are 20% and 4.5% of body weight, respectively (Hays, 1980), the increase in ECF calcium content can be calculated to be :

$$\Delta Ca_i . BW . 0.296$$

where BW is the body weight in kg. Similarly, the urine calcium excretion in periods 2 and 3 in excess of the projected basal level is :

$$uCa_{2+3} - [u(Ca/Cr)_1 \cdot uCr_{2+3}]$$

where uCa and uCr are the urine calcium and creatinine excretions respectively and the subscripts refer to the collection periods. The total absorbed calcium was derived by summing these two expressions thus:

Total absorbed calcium =

$$[\Delta Ca_i \cdot BW. \ 0.296] + uCa_{2+3} - [u(Ca/Cr)_1 . uCr_{2+3}]$$

The results were analysed using analysis of variance and t-tests for paired data with Bonferroni's inequality for multiple t-tests. Relationships between variables were examined by regression analysis. Palatability scores were compared using the Sign test.

9.3 Results

9.3.1 Calcium absorption (Table 14): There was a significant rise in serum ionised calcium following each calcium preparation and in 20 of the 40 individual tests it exceeded the upper limit of normal (1.28 mmol/L). The mean increase was the same following Spar-Cal and Calcium-Sandoz, but was smaller after Os-Cal ($p < 0.02$) and Ossopan ($p < 0.005$). There was also a significant rise in urinary calcium excretion and this gave the same ranking of the preparations, Ossopan being significantly inferior to the others ($p < 0.002$). The mean total calcium

TABLE 14: INDICES OF CALCIUM ABSORPTION

	SPAR-CAL®(SC)	CALCIUM SANDOZ®(SA)	OS-CAL®(OC)	OSSOPAN®(OP)	CONTROL
Δ Ca_i(mmol/l)	0.08±0.02*†	0.08±0.01*†	0.05±0.01*	0.03±0.01*	0 ±0.01
Δ uCa/Cr (molar ratio)	0.38±0.06*	0.34±0.05*	0.25±0.05*	0.12±0.03*‡	-0.03±0.01
Total absorbed calcium @ 4 hours (mmol)	2.01±0.32*	1.87±0.25*	1.22±0.29*	0.75±0.14*	-
Δ PTH (µg/l)	-0.03±0.02	-0.06±0.01*	-0.06±0.02*	-0.05±0.01*	-0.01±0.01
Δ HP/Cr (molar ratio x 10^3)	-2.4 ±1.0**	-2.5 ±2.1	-3.5 ±2.2	-	-0.8 ±1.8

Data shown are means ± SEM. See "Methods" for abbreviations.
Each preparation was given in a dose containing 1g elemental calcium.

* = Significantly different from zero ($p<0.01$)

† = Greater than OP ($p<0.002$) and OC ($p<0.02$)

‡ = Less than SC, SA or OC ($p<0.002$)

** = Significantly different from zero ($p<0.05$).

absorbed from the preparations during 4 hours ranged from 0.85mmols to 2.01mmols (3-8% of the administered dose) and was again significantly greater for Calcium Sandoz and Spar-Cal than for Os-Cal and Ossopan (Fig 17).

9.3.2 PTH and hydroxyproline: There were no significant differences between the four preparations in the changes in sPTH and urine hydroxyproline (Table 14). For this reason, the four results from each subject were averaged (Fig 18). Following the calcium load, there was a reduction in mean sPTH from 0.16±0.01 to 0.10±0.02 ug/l ($p < 0.001$) and a decline in urine hydroxyproline/creatinine ratio from 20±1 to 17±1 ($p < 0.02$). Since Ossopan contains hydroxyproline, the data from this preparation were excluded from the latter analysis.

9.3.3 Urine sodium excretion: The urine sodium excretion rose significantly following each preparation except Ossopan (Fig 19) but this rise did not reflect the sodium content of the individual calcium supplements (compare Fig 19 and Table 13). Regression analysis of the pooled data showed that the rise in sodium excretion significantly correlated with the increases in both calcium excretion ($r=0.63$, $p<0.001$) and serum ionised calcium ($r=0.50$, $p<0.001$) and also with the interaction of these two variables ($r=0.63$, $p<0.001$). The relationship between Na/Cr and Ca/Cr is described by the equation:

$$\Delta Na/Cr = 0.10 + 18 \ Ca/Cr.$$

9.3.4. Control Studies: None of the indices measured showed a

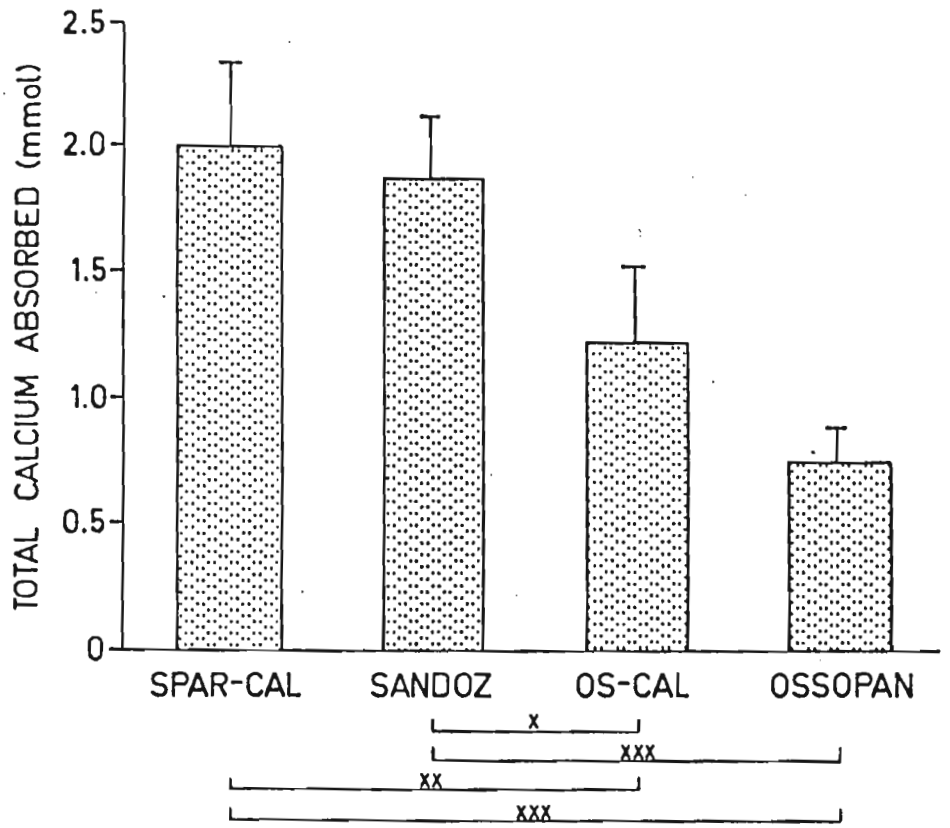


Figure 17: Total calcium, absorbed (mean ± SEM) 4 hours after the ingestion of each of 4 calcium preparations by 10 healthy, young subjects. Significant differences between preparations are indicated: X, $p<0.05$; XX, $p<0.02$, XXX, $p<0.005$ (1mmol calcium = 40mg).

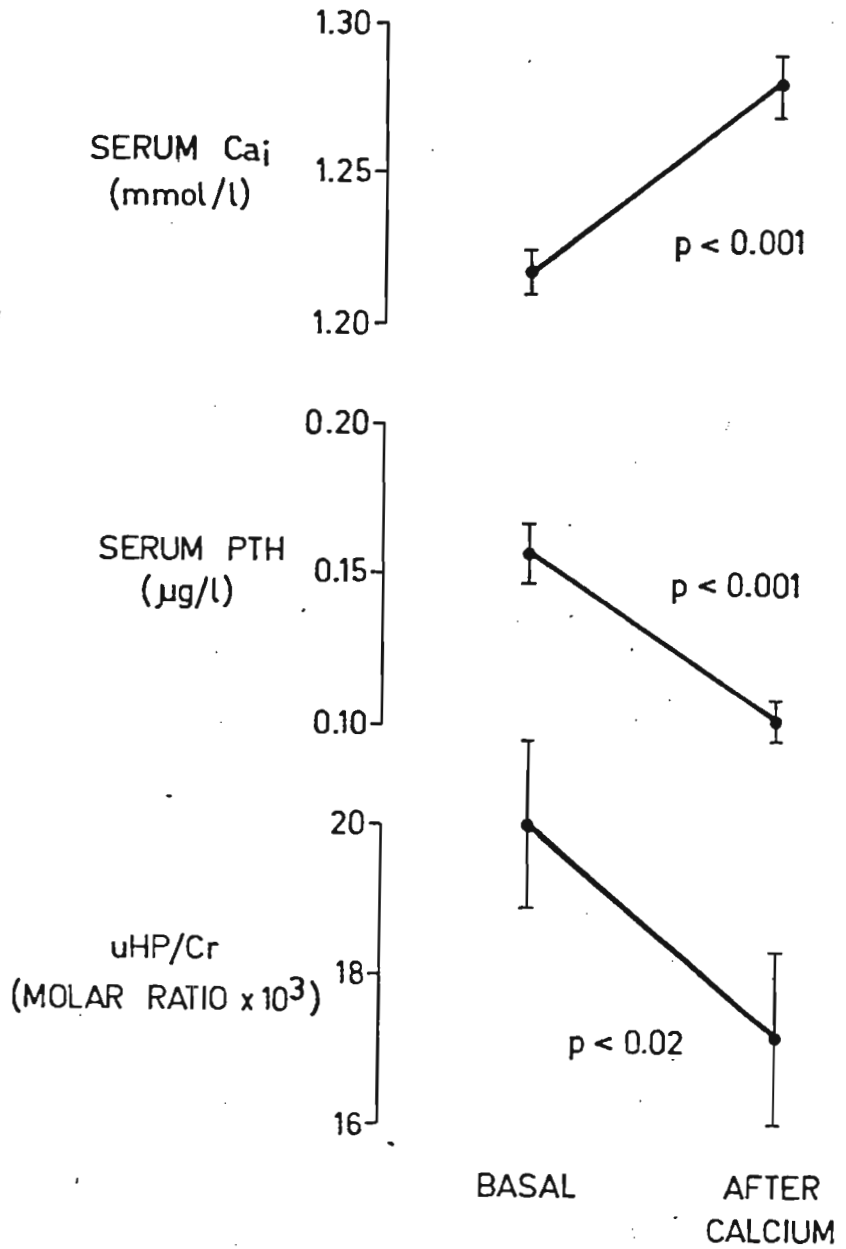


Figure 18: Serum ionised calcium, serum parathyroid hormone and urinary hydroxyproline/creatinine ratio in the fasting state and 2-4 hours after ingestion of 1g of calcium in 10 healthy, young subjects. Mean values for each subject were derived by pooling data for the 4 different calcium supplements, excluding Ossopan from the uHP/Cr data (see text). The mean ± SEM of these values are shown above.

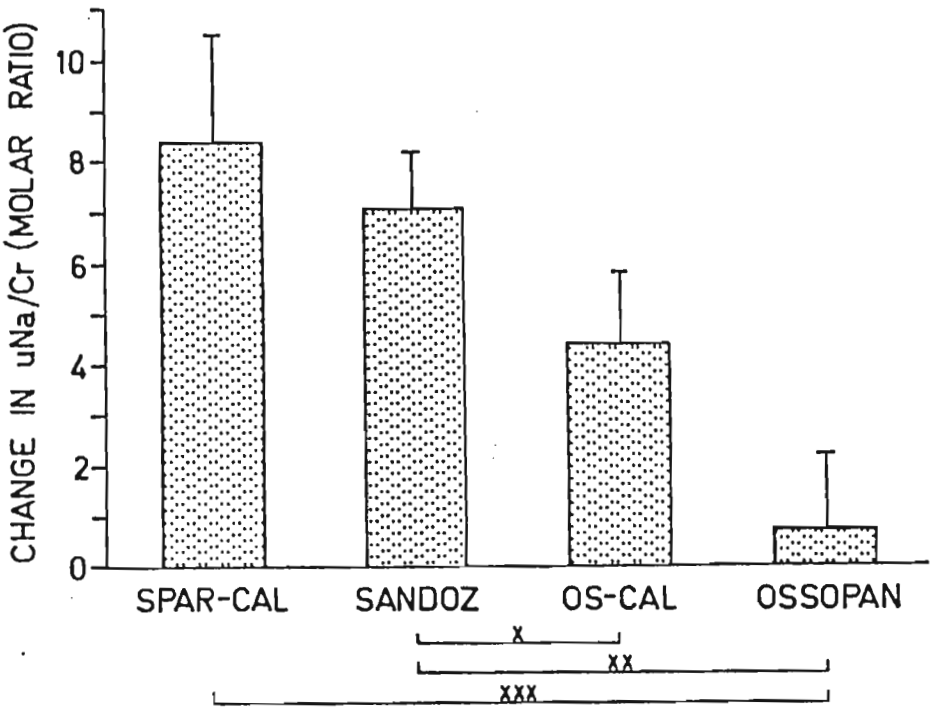


Figure 19: Change in urinary sodium excretion (mean ± SEM) expressed as a molar ratio to creatinine, following each of 4 calcium preparations. Significant differences between preparations are indicated: X, $p<0.02$; XX, $p<0.005$; XXX $p<0.001$. In the control studies the mean change in uNa/Cr was - 1.0±1.3.

significant deviation from zero in the control studies.

9.3.5. Palatability: The mean palatability scores (± SEM) were as follows: Spar-Cal, 3.9±0.3; Calcium-Sandoz, 3.9±0.3; Os-Cal, 3.3±0.3; Ossopan, 1.4±0.2. Using the Sign test, the scores for Ossopan were found to be significantly lower than those for each of the other preparations ($p < 0.01$).

9.4. Discussion

Previous studies have shown that the intestinal absorption of calcium can be assessed by measurement of the rise in serum and urine calcium within 4 hours of an oral calcium load (Broadus et al, 1978). This is so because the principal site of absorption is the upper small intestine (Avioli, 1982; Roth and Werner, 1983; Krejs et al, 1983) and the passage of a light meal through this region is completed 2-3 hours after ingestion (Lagerlof et al, 1976). Thus, absorption of a calcium load is 95% complete within 2.5 hours of an oral dose (Birge et al, 1969).

We have chosen to combine the changes in serum and urine calcium in order to estimate the total calcium absorbed over 4 hours. There is a small error in this calculation as Ca_i was measured 3, rather than 4, hours after the dose. This is unlikely to influence these comparisons, however, since radiocalcium tracer studies using a comparable dose of calcium carrier, show a plateau in serum tracer levels during this period (Marshall, 1976). The calculation also takes no account of the possibility of net calcium deposition in bone during the four hour study and

may therefore slightly underestimate true absorption. Since bone mass is stable in young adults, the fraction of absorbed calcium resulting in a net increase in skeletal mass is likely to be negligible.

The four calcium preparations are similarly ranked (Spar-Cal and Calcium-Sandoz > Os-Cal > Ossopan) whether assessed by ΔCa_i, ΔuCa/Cr or the total absorbed calcium. There are no significant differences between Spar-Cal and Calcium-Sandoz but the other preparations are significantly different in one or more of the indices of calcium absorption. This order possibly reflects both the solubility and formulation of each preparation. Spar-Cal and Calcium-Sandoz are already in solution when ingested. Os-Cal is a large, film-coated tablet which requires gastric acid to become soluble, while Ossopan requires gastric acid and probably enzymatic degradation also, to release its calcium. Previously published data have suggested that, in patients with normal gastric acid production, different calcium salts are equally well absorbed (Ivanovich et al, 1967; Patton and Sutton, 1952; Berstad et al, 1976), though the physical form in which the salt is administered (e.g. tablet versus powder) may affect bioavailability (Watkins et al, 1962). Since absorption is most rapid in the upper small intestine, it is time-limited and any significant delay in the release of calcium will reduce the fraction absorbed. Though some calcium absorption does occur in the ileum, its low rate and the fact that more than 90% of each preparation is still unabsorbed when it reaches this site, rule out the possibility of the poorly soluble preparations achieving a compensatorily higher distal absorption. Isotopic studies of

calcium absorption have confirmed the importance of the duodenal absorption rate in determining total absorption (Roth and Werner, 1983).

The similar degree of suppression of PTH levels and hydroxyproline excretion by the four preparations is, at first, surprising. Both these indices, however, are subject to greater measurement imprecision than calcium concentrations and this may contribute to the apparent uniformity of effect. It is also possible that PTH secretion is completely suppressed by even a small increase in ECF ionised calcium. Thus, the acute changes in PTH and hydroxyproline would be similar despite differences in the amount of calcium absorbed. The duration of suppression, however, might be expected to be more prolonged following those preparations with a higher bioavailability. The present data do not permit us to test this hypothesis.

A previous study (Windsor et al, 1973) using a radiocalcium tracer, suggested that calcium absorption from Ossopan was superior to that from calcium gluconate, but the data could equally well indicate a lower yield of soluble calcium carrier from the Ossopan. The advantages claimed for Ossopan in some clinical studies (Durance et al, 1973; Dent and Davies, 1980; Epstein et al, 1982) could result from its other mineral and organic constituents rather than from a higher calcium availability.

The acute changes following these oral calcium loads provide information on the mechanism of action and possible side-effects

of calcium supplements. Three hours after the load, the serum ionised calcium had risen, in some instances to above the normal range. This was accompanied by a 40% fall in sPTH and a fall in urine hydroxyproline, indicating a reduction in bone resorption. Such an acute change had not been documented previously, though reduced hydroproxyline excretion after 8 days of calcium supplementation has been reported (Horowitz et al, 1984). This suggests that bone resorption is immediately responsive to variations in dietary calcium intake. The mechanism of this responsiveness is unknown. The acute fall in sPTH may be important but a rise in calcitonin secretion in response to the increased serum calcium may also be involved.

An unexpected finding of this study was the marked rise in sodium excretion following oral calcium. The regression analysis suggests that this is related to the change in urine calcium excretion and that each millimole increase in calcium is accompanied by 18 millimoles of additional sodium loss. The same relationship has been described following calcium infusion (Levitt et al, 1958) and attributed to the similar permeability of the proximal renal tubule to the two ions and to the action of solvent drag when either is reabsorbed (Parfitt and Kleerekoper, 1980). This phenomenon may also be responsible for the rise in aldosterone reported to follow calcium loading (Raymond et al, 1984). It probably contributes to the volume depletion seen in patients with severe hypercalcaemia and could cause sodium depletion, particularly in elderly patients receiving high-dose calcium.

The palabability of Ossopan was very much less than that of the other preparations. This could lead to poor compliance and reduced efficacy, since calcium supplements must be taken regularly over a period of years to be effective.

These short term studies have demonstrated immediate metabolic effects from calcium ingestion and have also shown significant differences in net calcium absorption between available proprietary preparations. Further studies are needed to assess whether these differences are sustained during long-term administration.

9.5 Postscript

Since this study was performed there have been a number of studies published assessing the availability of calcium from dietary supplements. These have generally shown similar fractional absorptions from salts of different solubilities in normal subjects (Recker, 1985; Goddard et al, 1986; Sheikh et al, 1987) though some have found superior absorption from more soluble salts (Nicar and Pak, 1985; Marchandise et al, 1987). (In Nicar's study, calcium carbonate and citrate were compared using urine calcium excretion as the index of absorption. The higher urine calcium loss following citrate ingestion may be contributed to by the effects of a citrate load on renal calcium handling, for example, by the formation of calcium-citrate complexes in the urine). In the absence of normal gastric acid production, however, absorption of carbonate in the fasting state appears to be reduced (Recker, 1985). This is unlikely to be

relevant to the poorer performance of Os-Cal in the present study since all the subjects involved were young and healthy and thus unlikely to have achlorhydria. These studies generally support the conclusions of the present study; that the calcium salt used is less important than the form of its administration, which will determine the fraction in solution as the supplement passes through the upper small bowel.

CHAPTER 10 : PREVENTION OF STEROID-INDUCED OSTEOPOROSIS WITH (3-AMINO-1-HYDROXYPROPYLIDENE)-1,1-BISPHOSPHONATE (APD)

10.1 Introduction

The two preceding chapters have addressed calcium supplementation as a means of suppressing PTH hypersecretion and thus inhibiting that element of bone resorption which is PTH-dependent. The advent of the second generation bisphosphonates provides a much more potent method of inhibiting bone resorption and has the advantage of acting directly at the bone/osteoclast level and thus inhibiting osteolysis of whatever cause. Since there is clear histological evidence of increased osteolysis in patients receiving glucocorticoids (Section 1.2.2), bisphosphonates would be expected to have a beneficial effect on the bone mass of these subjects. This hypothesis is tested in the present study using the potent, second generation bisphosphonate, APD, in a prospective, placebo-controlled study over 12 months.

10.2 Patients

Adult patients receiving long-term supraphysiological doses of glucocorticoids were considered for admission to the study. Patients with disorders of bone and mineral metabolism other than osteoporosis, those receiving drugs (other than glucocortocoids) which affect mineral metabolism and those with renal impairment or liver disease were excluded. Patients meeting these criteria who gave informed consent were grouped according to sex, menopausal status, age and duration of steroid therapy and randomly assigned to receive either APD 150mg/day, in the form of a single enteric-coated tablet, or placebo. This dose of APD was

chosen since it is adequate to block bone resorption in postmenopausal subjects (O.L.M. Bijvoet, personal cummunication). All subjects also received a daily supplement of 1g of elemental calcium (calcium lactate-gluconate, Sandoz). This was begun at least two weeks before their baseline biochemical evaluation.

Twenty patients were allocated to each treatment group (Tables 15a and 15b). In the control group, one patient (number 34) withdrew for personal reasons. In the APD group, one patient (number 18) was found to have osteomalacia at her initial bone biopsy and was therefore withdrawn, and one patient withdrew for personal reasons (number 10). Two patients (numbers 9 and 11) randomised to receive APD died as a result of exacerbations of their underlying chronic obstructive airways disease. Thus 19 patients receiving placebo and 16 patients receiving APD completed 12 months of study. Descriptive data for these patients at the time of entry to the study and their average steroid intake during the study period (calculated from drug diaries) are summarised in Table 16. There were no significant differences between the two groups.

10.3 Methods

Lumbar vertebral mineral density was assessed at 0 and 12 months by quantitive computed tomography using a Technicare 2020 scanner and the method of Cann and Genant (1985). In brief, a lateral computed radiograph of the lumbar spine was obtained and used to permit precise positioning of the scanner. The scan from which measurements were made was then performed through the mid-plane of the third lumber vertebra, the scan thickness being 10mm. Scans were performed at 120 kVp. Bone mineral density was

Table 15a : Patient data at entry to study - APD group

Patient No.	Sex	Age(yr)	Weight(kg)	Duration of glucocorticoid treatment (yr)	Average glucocorticoid dose*	25(OH)D (µg/L)	Underlying disease
1	M	38	81	14	26.4	27	Asthma
2	M	30	58	4	6.8	34	Asthma
3	M	51	80	13	11.4	23	Asthma
4	M	64	70	7	19.7	37	CORD
5	M	56	77	9	15.0	22	Bechet's disease
6	M	65	69	1	16.2	13	CORD
7	F‡	55	51	5	11.0	17	Asthma
8	F	30	58	2	11.2	9	MCTD
9+	M	58					CORD
10+	M	36					Rheumatoid arthritis
11+	M	54					Asthma
12	M	73	69	3	12.9	9	CORD
13	F‡	55	54	3	12.2	29	Asthma
14	F	17	74	1	16.4	21	Asthma
15	F‡	66	63	0.5	6.9	36	Cranial arteritis
16	F	21	57	7	15.2	7	SLE
17	M	21	61	4	20.0	17	SLE
18+	F	48					Ulcertative colitis
19	F	45	54	0.1	15.9		Churg-Strauss granulomatosis
20	F	22	67	7	23.7	15	Asthma

* In mg prednisone equivalent/day. Calculated from drug diaries kept during study.

\+ Did not complete study.

‡ Postmenopausal.

Abbreviations: CORD, chronic obstructive respiratory disease.
SLE, systemic lupus erythematosis
MCTD, mixed connective tissue disease.

Table 15b: Patient data at entry to study - placebo group

Patient No.	Sex	Age(yr)	Weight(kg)	Duration glucocorticoid treatment	Average glucocorticoid dose*	25(OH)D (µg/L)	Underlying disease
21	F	44	52	0.8	10	29	SLE
22	M	51	105	20	10.8	30	CORD
23	M	70	80	0.5	9.9	29	Cranial arteritis
24	M	41	87	12	22.0	34	Asthma
25	F	34	45	4	8.6	38	Ulcerative colitis
26	F	38	62	10	22.9	20	Asthma
27	F	47	63	22	20	20	Asthma
28	F‡	58	99	3	10.3	14	Cranial arteritis
20	M	60	64	18	5.6	-	Asthma
30	M	74	87	7	14.1	10	CORD
31	M	49	67	5	4.4	43	Asthma
32	F‡	51	58	1	18.3	17	Cranial arteritis
33	F	40	78	0.1	11.5	9	Polymyositis
34+	F	19					Asthma
35	M	54	78	4	29.3	25	Asthma
36	M	25	68	2	13	25	Asthma
37	F‡	51	80	3	7.5	23	Asthma
38	M	56	74	6	8.2	33	Asthma
39	M	73	67	4	10.2	4	CORD
40	M	77	59	2	3.2	28	Cranial arteritis

* In mg prednisone equivalent/day. Calculated from drug diaries kept during study.

\+ Did not complete study.

‡ Postmenopausal.

Abbreviations: CORD, chronic obstructive respiratory disease
SLE, systemic lupus erythematosis

Table 16 : Patient data and bone mass at entry to study

	Placebo	APD
n	19	16
Male : premen F : postmen F	11:5:3	8:5:3
Age (years)	52 ± 3	48 ± 5
Weight (kg)	72 ± 4	65 ± 3
Duration glucocorticoid treatment (years)	6.5 ± 1.6	5.0 ± 1.1
Average glucocorticoid dose* (mg prednisone equivalents/day)	12.6 ± 1.6	15.1 ± 1.4
Serum 25-hydroxyvitamin D (µg/L)	23.9 ± 2.5	20.5 ± 2.5
Vertebral mineral density (mg/cm^3)	62.3 ± 9.4	64.2 ± 10.4
Metacarpal cortical index (CA/TA)	0.765 ± 0.023	0.747 ± 0.015

Data are mean ± SEM. There are no significant differences between the groups.

* Average during trial period.

Premen F = premenopausal female; postmen F = postmenopausal female.

calculated from the mean CT number of the vertebral body and the CT numbers of the calibration solutions within a phantom which was scanned simultaneously. No patients had fractures of scanned vertebrae at the time of either their baseline or follow-up scans. The coefficient of variation of this technique is 3.3% (duplicate measurements made on the same day after repositioning of the patients, N = 13).

Metacarpal cortical area (expressed as a ratio to total cross-sectional metacarpal area) was measured in the second, third and fourth metacarpals of the non-dominant hand using the method of Horsman and Simpson (1975). Posterior-anterior contact radiographs were produced, all films being taken by the same radiographer using a standard protocol. Measurements were made on all three films from a single patient at the conclusion of the study. On the baseline radiograph, the longitudinal axis was drawn and the bone's mid-point determined and marked. The measurement sites on subsequent films were identified by superimposition of the later films on the base-line radiograph and were also marked. Measurements of total and medullary width were made using Vernier calipers and recorded to the nearest 0.05mm. All measurements were made in duplicate and the mean of these values used. Making the assumption that the metacarpals were approximately circular at their mid-points, cortical area was calculated as the difference between the cross-sectional area of the whole bone and that of the medullary cavity. Results were expressed as the mean of the ratio of cortical area/total area for the three bones.

In the latter part of the study, bone mineral content of the non-dominant, distal radius and ulna was measured using a Novo GT 35

osteodensitometer with an ^{125}I source. The subject's arm was placed in a water bath and the most distal point at which the separation of the radius and ulna exceeded 8mm was selected automatically by the instrument. Six scans at intervals of 4mm in a proximal direction were carried out and the average bone mineral content recorded. The mid-point of the scanned area is, on average, 3.6 cm proximal to the styloid process of the radius (Nielsen et al, 1980) and 20% of the bone of this region is trabecular (Jensen et al, 1982). Measurements made using this technique correlate well with total body calcium (Christiansen and Rodbro, 1975).

Trans-iliac bone biopsies were performed at entry to the study and at 12 months. Biopsies were taken using a Jamshidi needle, fixed in neutral buffered formalin, embedded undecalcified in methylmethacrylate and cut into 5μm sections with a Jung Model-K Sledge microtome. Histomorphometric measurements were made with a Zeiss Integrating Eyepiece II or a linear micrometer, by Dr Alan King. The sections were stained with Goldner's trichrome and examined at 156 x magnification.

Biochemical measurements were made using standard techniques, as described in Section 3.3

All measurements were made with the operator blinded to the patient's treatment group. Data were analysed by two-tailed "t" tests unless otherwise indicated and are presented as mean±SEM.

10.4 Results

10.4.1 Biochemistry: The serial biochemical data for the two

groups of patients are shown in Figure 20. A repeated measures analysis of variance design was used to assess the changes over time for these variables. Depending on the particular measure different comparisons between time intervals were used; for all variables each time was compared to the next time while for serum alkaline phosphatase and urine hydroxyproline each time was also compared with the mean of all the subsequent times. All analyses were done through the use of PROC GLM in the statistical package, SAS.

There was a fall in urine hydroxyproline excretion during the first six weeks of APD treatment and this remained significantly below the baseline level throughout the remainder of the study period ($p < 0.05$). No change occurred in the control group. A fall in alkaline phosphatase also occurred but this was more gradual and the nadir was not reached until 12 weeks of treatment. Values subsequently remained below the baseline level ($p < 0.0001$). In the control group, alkaline phosphatase activity also fell significantly below the initial level ($p < 0.05$) but this was much less dramatic than in those receiving APD. The other index of osteoblast function that was assessed, bone gla-protein, showed a more complex pattern of response. In the APD group, the only significant change between consecutive measurements was a fall between 6 and 12 weeks ($p < 0.01$). In contrast, there were significant increases in the control group at 6 ($p < 0.002$) and 9 ($p < 0.02$) months and a fall between 9 and 12 months ($p < 0.05$). The responses of the two groups were significantly different ($p < 0.01$).

Serum ionised calcium fell in the first 6 weeks of APD therapy ($P < 0.02$) but was not significantly different from baseline

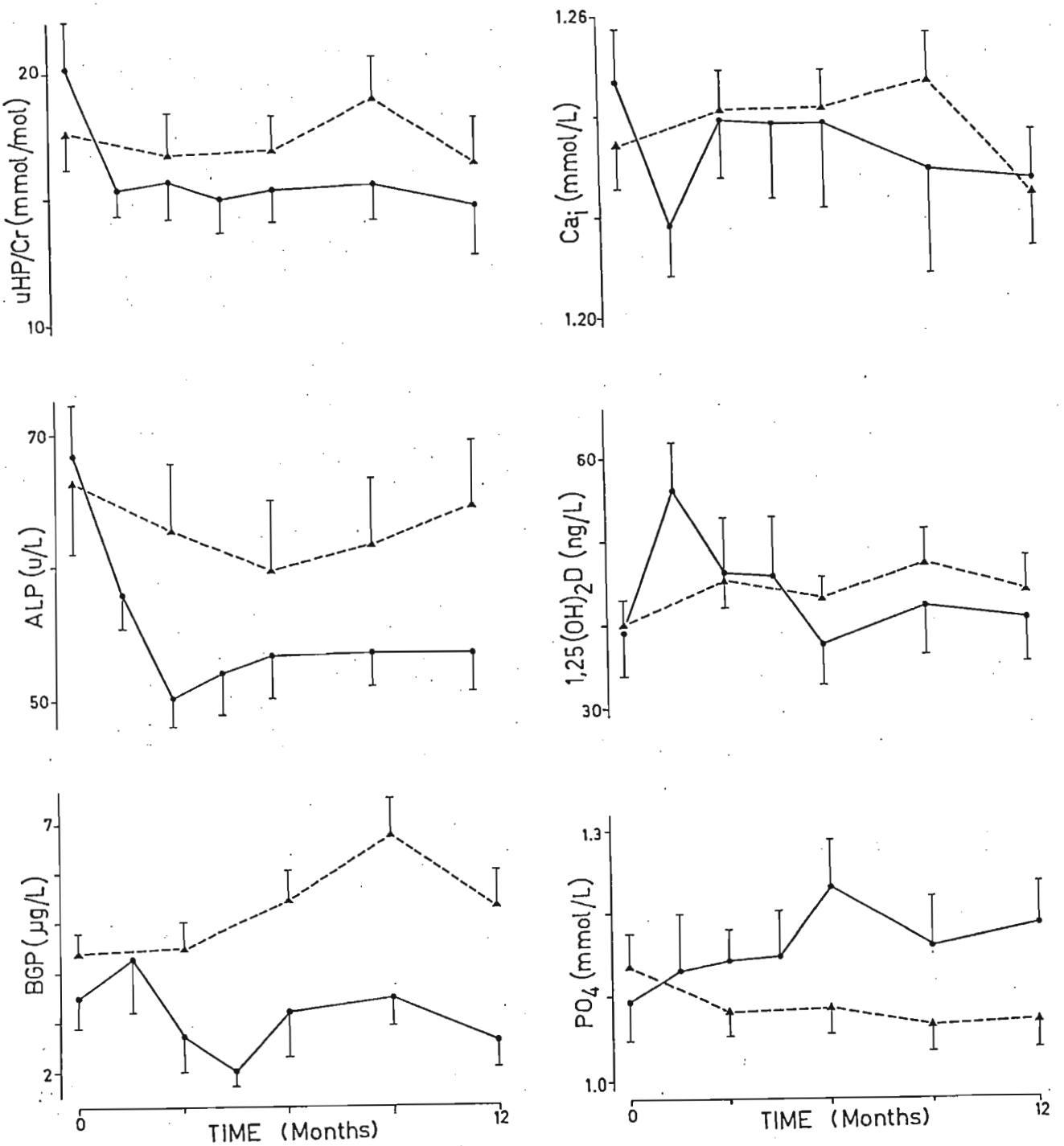


Figure 20: Biochemical indices in glucocorticoid-treated patients receiving APD plus calcium (circles) or placebo plus calcium (triangles). Calcium supplements (1g/day) were begun at least 2 weeks before the initial biochemical assessment.

subsequently. In the placebo group, there was no change until the last 3 months of the study when ionised calcium also fell ($p < 0.05$). Serum $1,25(OH)_2D$ rose during the first 6 weeks in the APD group ($p < 0.001$) but was not different from baseline subsequently. It did not change significantly in the control group. Mean serum phosphate levels progressively diverged in the two groups, but this was not statistically significant.

10.4.2 Bone histomorphometry: Table 17 shows the histomorphometric indices of bone formation and resorption. There were no significant differences between the groups initially. All indices declined significantly after 12 months treatment with APD. In the placebo group, there was a small but significant increase in the thickness of osteoid surfaces but no change in the other indices.

10.4.3. Bone mass: Individual patient data for metacarpal indices are shown in Figures 21a and b. Radiographs from patients 5, 20 and 35 were missing and results for these individuals are therefore not shown.

Individual data for vertebral mineral density appear in Figures 22a and b. Baseline scans in patients 22, 28 and 30 were lost because of a computer error and patients 7 and 17 failed to attend for one or both scans.

Group data expressed as mean change from baseline values for both metacarpal index and vertebral mineral density are shown in Figure 23. The mean metacarpal cortical area of the APD-treated patients rose by 1.2% ($p < 0.06$) in the first 6 months but did not change further between 6 and 12 months (Figure 23). In contrast,

Table 17 : Bone histomorphometry at entry to study and at 12 months

	Placebo		APD		APD vs Placebo
	Baseline	12 months	Baseline	12 months	at 12 months++
Relative trabecular osteoid volume (%)	2.95±0.55	2.79±0.58	3.65±0.45	1.49±0.49***	$p < 0.02$
Relative trabecular osteoid surfaces (%)	28.9±4.0	21.6±3.5	33.6±3.4	16.9±2.9***	NS
Thickness index of osteoid surfaces	9.6±1.0	13.1±1.3*	11.1±0.9	7.8±1.2**	$p < 0.005$
Osteoclast number (/mm^2)	0.12±0.09	0.23±0.09	0.23±0.05	0.09±0.02**	$p < 0.01$
Resorption surfaces+(%)	0.36±0.06	0.57±0.19	0.63±0.15	0.27±0.09**	$p < 0.01$

Significant changes from baseline are shown : *$p < 0.05$, **$p < 0.02$, ***$p < 0.001$.

\+ Percentage of trabecular surface with resorption bays containing osteoclasts. Other indices are as defined by Meunier et al.

++ Significance of between-group comparisons of changes from initial values. NS = not significant.

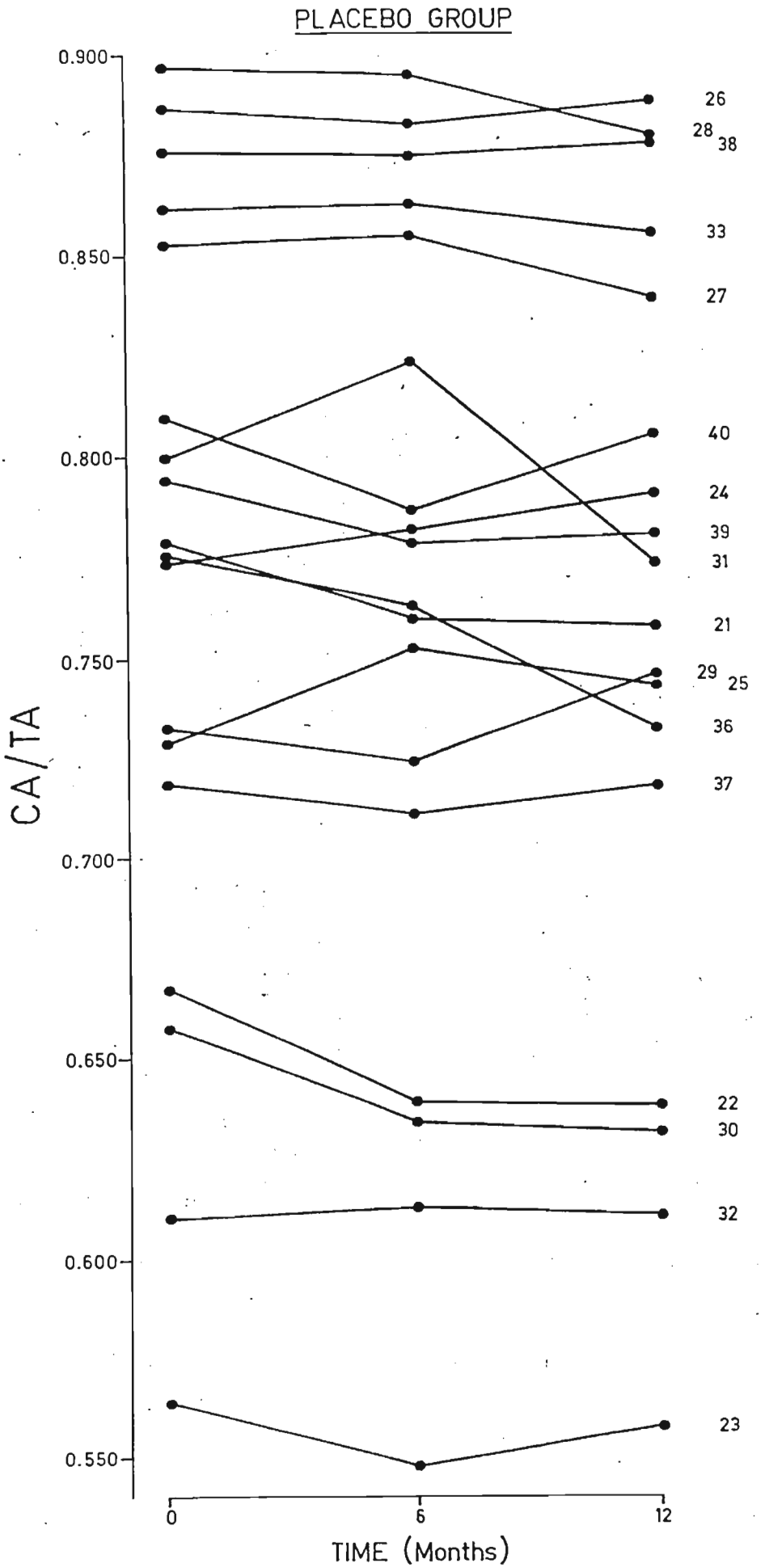


Figure 21a: Measurements of metacarpal cortical area (as a ratio to total area) during the 12 month trial period in patients receiving placebo plus calcium (1g/day). Patients' code numbers are shown to the right of their 12 month values and correspond with those given in Table 15.

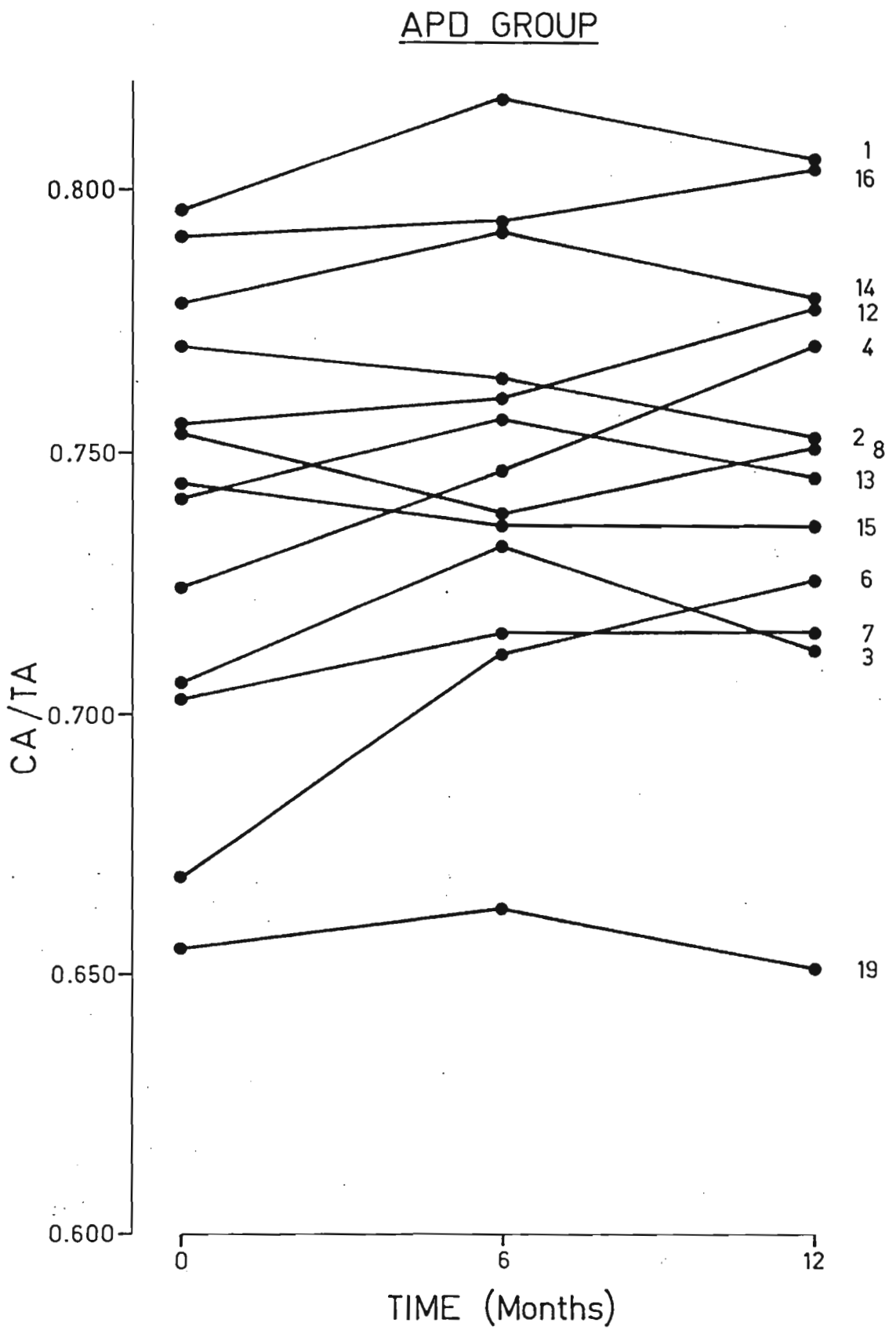


Figure 21b: Measurements of metacarpal cortical area in APD-treated patients. The format is as described for Figure 21a.

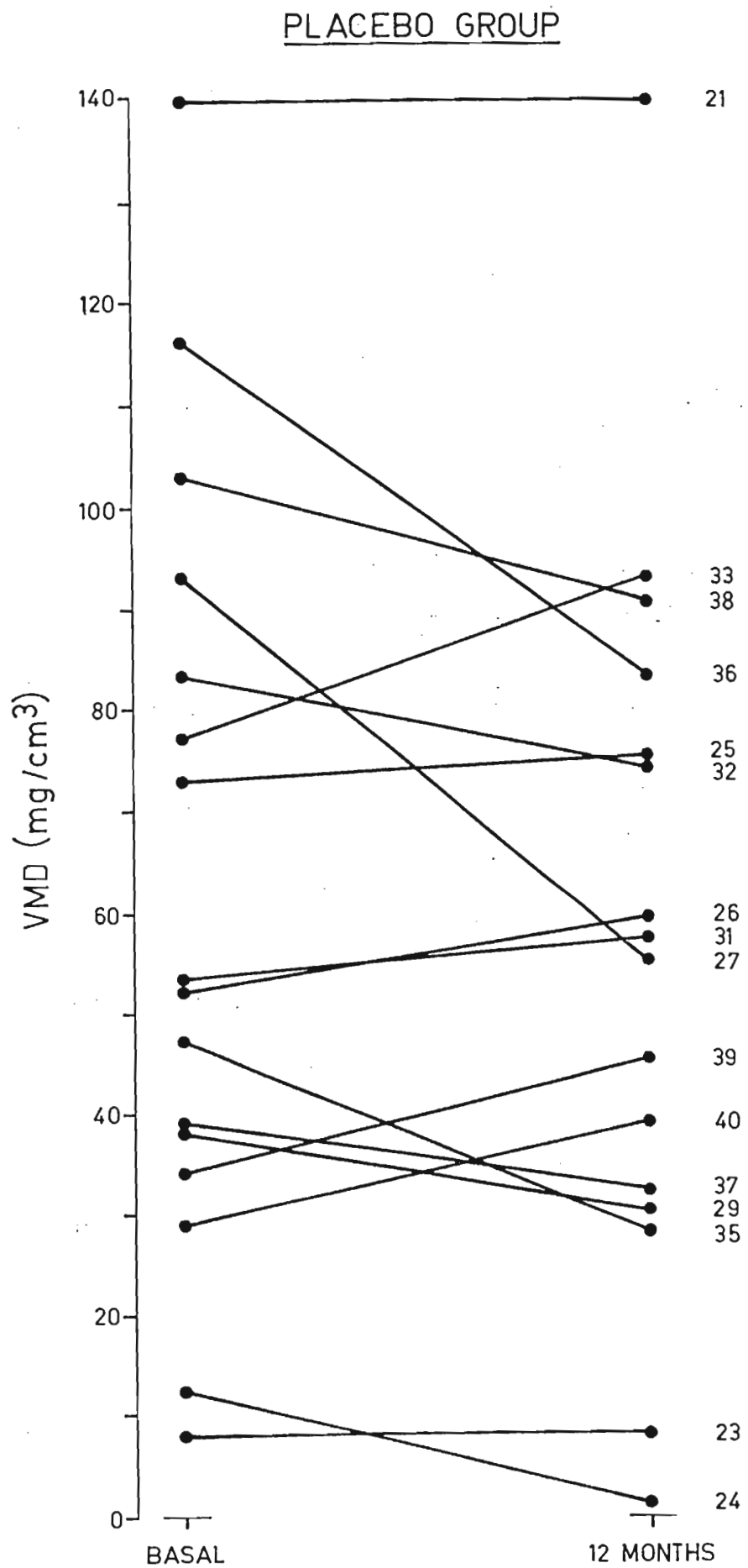


Figure 22a: Measurements of vertebral mineral density by computed tomography over the 12 month trial period in patients receiving placebo plus calcium (1g/day). Format as in Figure 21a.

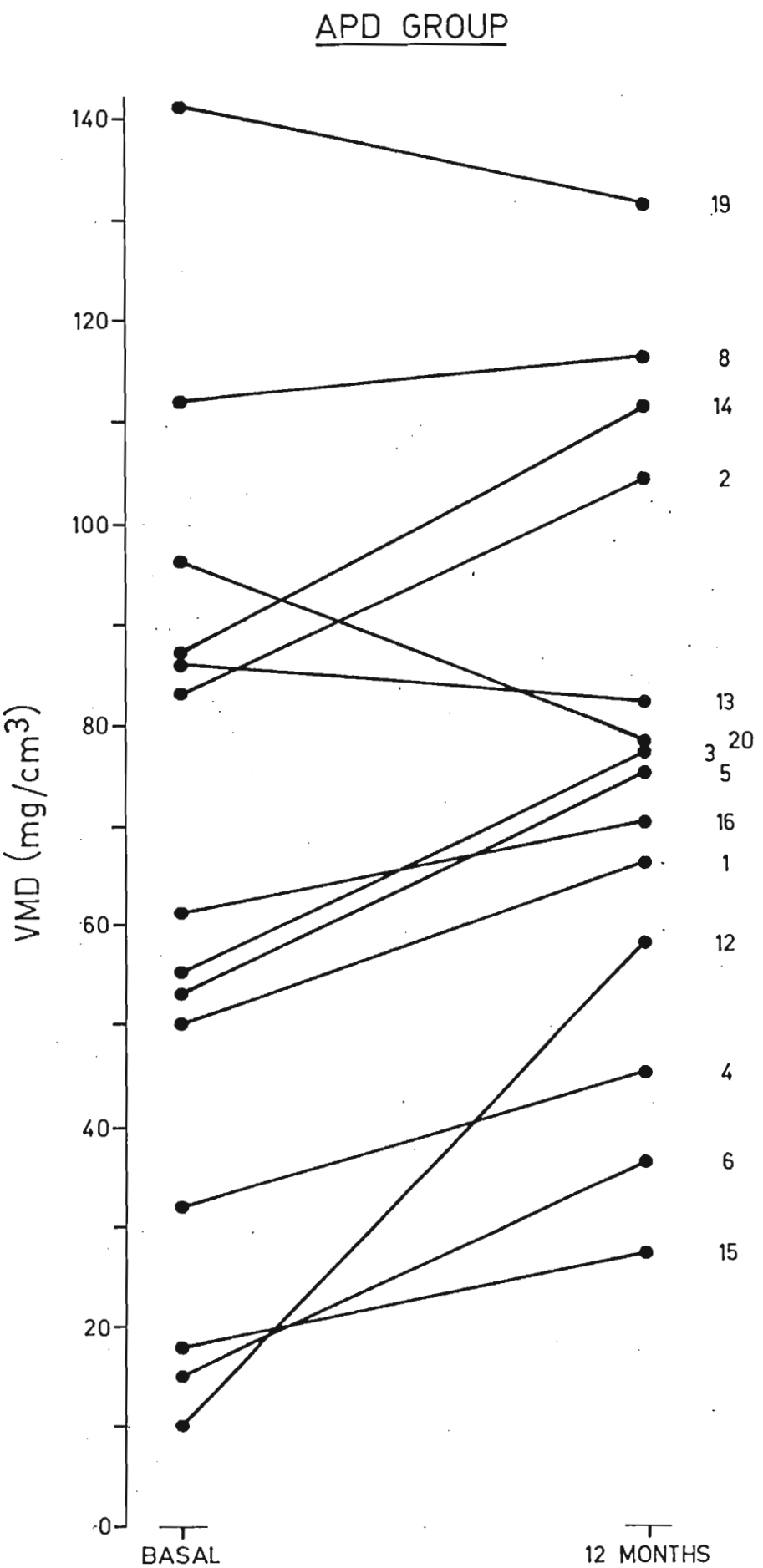


Figure 22b: Measurements of vertebral mineral density in APD-treated patients. The format is as in Figure 21a.

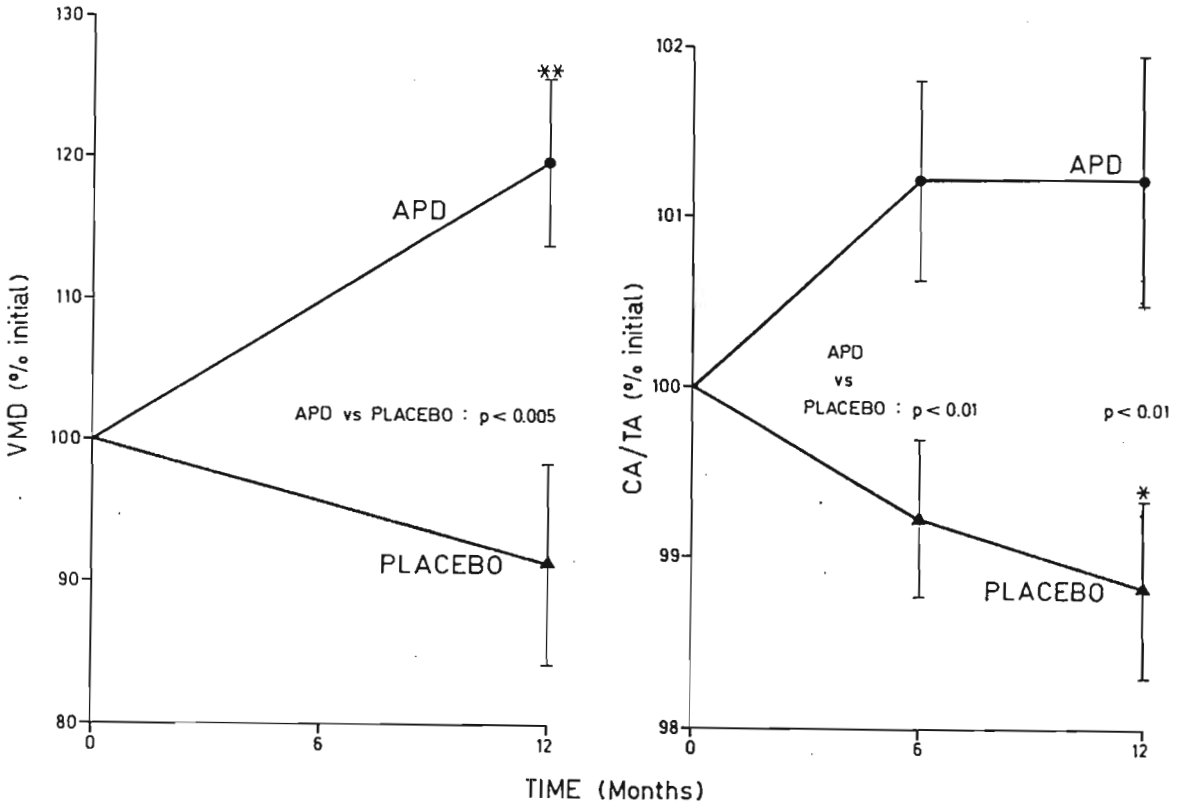


Figure 23: Changes in vertebral mineral density (VMD) and metacarpal cortical area (expressed as a ratio to total area, CA/TA) in glucocorticoid-treated patients receiving either oral APD, 150 mg/day or placebo. All subjects also took a daily supplement of 1g of elemental calcium. These figures represent the measurements derived from the data in Figures 21 and 22. Significant changes from baseline are shown : * $p<0.05$, ** $p<0.02$.

there was a progressive fall in metacarpal cortical area in the control group reaching 1.2% at 1 year ($p < 0.05$). The two groups were significantly different at both time points ($p < 0.01$). Vertebral mineral density increased by 19.6% during the 12 month period in the group receiving APD ($p < 0.02$), but decreased by 8.8% in the placebo group (non-significant). The mean changes in the two groups were significantly different ($p < 0.005$).

Forearm bone mineral content measurements were not available at the beginning of the trial but were made at 3 and 12 months. Individual patient data are shown in Figure 24. During this period, bone mineral content did not change significantly in patients receiving APD (-1.1±0.8%) but declined in the control group (-2.6±0.6%, $p < 0.001$). The mean changes in the two groups were not significantly different.

10.4.4. Side-effects: Six patients experienced nausea following ingestion of APD at some time during the study. In three, this resolved when the tablets were taken with food, in one, the nausea persisted but was not severe enough to cause withdrawal from the study and in two, nausea first developed between 10 and 12 months and no attempt was made to alter the relation of dosage to meals. Two other patients receiving APD were endoscopically diagnosed as having duodenitis and gastric ulceration, respectively. The latter patient was also taking indomethacin, aspirin and significant quantities of alcohol.

In the placebo group, two patients complained of epigastric discomfort during the study. One was found to have an erosive gastritis and the other declined endoscopy. Peripheral blood cell counts, liver function tests and serum levels of creatinine

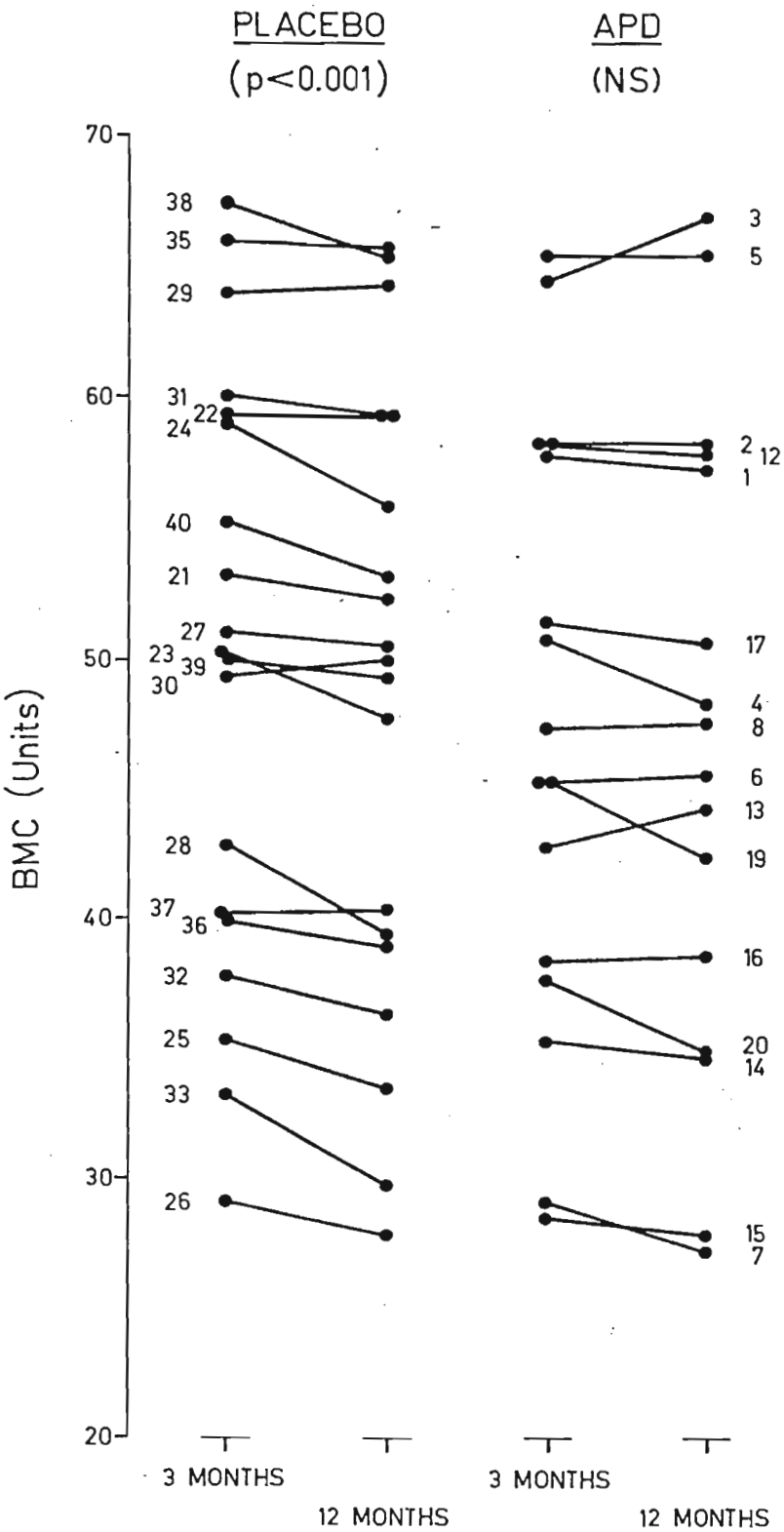


Figure 24: Individual measurements of distal forearm bone mineral content (in arbituary units) in both the placebo and APD-treated groups. The numbers in each margin are patient numbers as in Table 15 and Figures 21 and 22. There was a significant decline in BMC in the placebo group and no significant change in those receiving APD.

and urea did not change during the study period in either group.

10.5 Discussion

10.5.1 Biochemistry: The biochemical changes observed are those to be expected from the use of a potent anti-resorptive agent. There was an early and sustained inhibition of hydroxyproline excretion consistent with the fall in the histomorphometric indices of bone resorption. The two biochemical indices of bone formation, alkaline phosphatase and bone gla-protein, declined more gradually than did hydroxyproline. This is probably because of coupling of formation to resorption, though Marie et al (1985) have suggested that APD has a direct inhibitory effect on bone formation. The decline in alkaline phosphatase levels in the control group may be a late effect of the calcium supplementation that was started just before the beginning of the study period. It suggests that the absence of change in alkaline phosphatase commented on in Section 8.5 is attributable to the short duration of that study.

The changes in bone gla-protein are more complex than those of alkaline phosphatase. In the APD group, there is actually an upward trend at 6 weeks in contrast to the fall in alkaline phosphatase. It is probable that this reflects the abrupt increase in $1,25(OH)_2D$ levels that occurred at this time. We and others have clearly shown that $1,25(OH)_2D$ increases circulating bone gla-protein levels in both normal subjects and those receiving glucocorticoids (see Chapter 4). Once $1,25(OH)_2D$ concentrations have returned to baseline, the fall in bone turnover caused by APD becomes the dominant effect and bone gla-protein levels decline. A similar dissociation of alkaline

phosphatase and bone gla-protein levels has been observed in patients receiving APD for treatment of Paget's disease (Papapoulos et al, 1987). Both placebo and APD groups show a peak in bone gla-protein levels in the second six months, the reason for which is not apparent. Average prednisone dose was constant throughout the study period so this is not the cause. It might represent some "drifting" of the assay's standards, though the staff performing the assay do not think this to be likely.

The temporal dissociation between the decline in bone resorption and bone formation probably accounts for the transient fall in serum ionised calcium during the first 6 weeks of APD treatment. In turn, the rise in $1,25(OH)_2D$ is probably a response to this, being mediated by the parathyroid gland. Unfortunately, PTH data are unavailable from this study but the stimulatory effect of hypocalcaemia on PTH secretion and the role of PTH in regulating the renal hydroxylation of 25(OH)D are firmly established. A similar sequence of biochemical responses to APD has recently been described in patients receiving the drug for the treatment of Paget's disease (Papapoulos, 1986).

Serum phosphate levels appear to diverge in the two groups throughout the study period, though this does not quite reach statistical significance. The downward trend in the placebo group could be attributed to the phosphate-binding effect of the calcium supplements they were receiving. The opposite trend in the APD group is more puzzling. It contrasts with the hypophosphataemic effect of APD in short-term human studies (Adami et al, 1982) but is consistent with the finding that intestinal phosphate absorption rises in rats receiving APD (Reitsma et al, 1983). This has been attributed to the

complexing of dietary calcium by APD with a resultant increase in availability of dietary phosphate. The first generation bisphosphonate, etidronate, also increases serum phosphate concentrations in man, apparently as a result of increased renal tubular phosphate reabsorption (Walton et al, 1975; Recker et al, 1973). Unfortunately, urine phosphate excretion was not assessed in the present study so it is not possible to determine whether this mechanism is operative with APD also.

The upward trend in serum phosphate is unlikely to be related to the phosphate content of the APD itself, both because bisphosphonates are thought to be very stable in vivo (Fleish, 1981) and because the quantity of phosphate in the absorbed APD is trivial in comparison with the normal dietary intake. Thus, only 1-2% of the bisphosphonate is absorbed (Recker and Saville, 1973; Reitsma et al, 1983) giving a net daily absorption of 3mg, with a phosphorus content of less than 1mg. This is insignificant in comparison with the normal dietary intake of phosphorus of approximately 1.5g.

10.5.2 Bone histomorphometry: As mentioned above, the histomorphometric and biochemical indices of both bone formation and resorption showed consistent decreases, confirming the tight coupling that exists between these two phenomena. Importantly, there was no evidence of osteomalacia in those receiving APD consistent with other evidence that this drug has a wide margin of safety between doses which inhibit resorption and those that inhibit mineralisation (Reitsma et al, 1980; Shinoda et al, 1983). This is in contrast to etidnonate which sometimes causes osteomalacia in treated patients (Krane, 1982).

The very low rate of bone turnover following APD treatment raises the possibility of impaired micro-fracture repair (Frost, 1985) with the possible consequence of patients having more frequent fractures in spite of having a higher bone mass. It is not possible to dismiss this possibility on the basis of the data presented here, though it is worth noting that oestrogen, the only agent proven to decrease fracture incidence in postmenopausal women, appears to function primarily as an inhibitor of bone resorption. Long-term studies with fracture rate as an end-point are required to ensure that the demonstrated improvement in bone mass does translate into a lower fracture incidence. It is possible that lower dosage of APD or its cyclical use with stimulators of bone formation might overcome this problem.

10.5.3 Side-effects: The only side-effects observed were in the gastro-intestinal system and they were predominantly minor, as observed in previous studies using oral APD (Fraser et al, 1984; Scurr and Cutting, 1984). The significance of the endoscopic abnormalities is unclear, because persistent dyspepsia was equally common in both groups and because patients receiving glucocorticoids are already at risk of peptic ulceration. Such side-effects might limit the use of oral APD in the future though no patient was withdrawn from the trial on this account.

10.5.4. Bone mass - individual data: (Figs 21,22,24). The individual data for metacarpal indices appears somewhat confusing at first sight because of the wide range of basal values and the relatively small percentage changes during the study period.

Patients 40 and 31 show marked inconsistencies in consecutive readings, suggesting some possible measurement error in the 6 month value in each case. Failure to precisely position the hand for each radiograph could account for such discrepant results.

There is a greater within-group uniformity in the individual vertebral mineral density data. Only three patients in the placebo group (33, 39, 30) show a significant rise in this index during the study period and in two (33, 40) this may be attributable to reductions in their average prednisone dose (from 15 to 10 mg/day and from 5.5 to 1.5 mg/day, respectively). Similarly, patients in the APD group almost all gained bone mass, the clearest exception to this being patient 20. This patient also lost significant forearm bone mass and showed no suppression of the biochemical indices of bone turnover. This suggests that she was either not adequately absorbing the APD or not taking it. The forearm bone mineral content results are also quite consistent within each group, there being a fall between 3 and 12 months in the placebo group and no real change in those on APD.

The very low values for vertebral mineral density in some of the patients deserve comment. This is probably a reflection of at least two separate phenomena. Firstly, the predilection of glucocorticoids to cause trabecular osteopenia, as discussed in Section 1.1, and secondly, the increased fat mass associated with glucocorticoid use. While there is no data describing the vertebral fat content in steroid excess, it is likely to be increased in parallel with the general increase in adiposity found in these patients. This will lead to an artefactual depression of vertebral mineral density because of the low radiological density of fat. The extent to which this occurs

could be assessed by comparing single and dual energy CT scans. Such an artefact would not affect the conclusions of this study, however, since we are primarily concerned with changes in bone mass and both the pre- and post- APD values will be equally influenced by fat artefact.

10.5.5 Bone mass-group data: (Fig. 23) The reduction in mean bone mass in the control group confirms prospectively what is already well known from retrospective studies; that glucocorticoid therapy leads to progressive bone loss. Consistent with previous cross-sectional data (Hahn et al, 1974), the rate of bone loss is much greater in trabecular than in cortical bone and intermediate at sites of mixed histology (i.e. the distal forearm). In spite of the effect of calcium supplementation on bone resorption demonstrated in Chapter 8, this therapy alone is clearly not adequate to arrest bone loss completely in steroid-treated subjects.

At all three sites of bone assessment, APD-treated patients fared better than those receivng calcium and placebo. The measurements of metacarpal cortical area indicate that APD treatment led to an early increase in bone mass which was maintained subsequently. There were similar changes in vertebral mineral density, though the time-course of the APD-induced increase is not defined. Stabilisation of bone mass in those already established on APD therapy is confirmed by the forearm bone mineral content measurements at 3 and 12 months. This pattern of an early increase in bone mass followed by a subsequent plateau is consistent with the observed effects of APD on calcium balance in both animals (Reitsma et al, 1980) and man (Frijlink et al, 1979). It reflects the primary inhibitory action of APD on

osteoclast-mediated bone resorption with a delay of some weeks before local coupling mechanisms result in a reduction in bone formation. At the histological level, the increase probably represents a "filling in" of existing osteoclast resorption bays and a failure to intiate further areas of resorption. This sequence is probably common to the action of all anti-resorptive agents (Parfitt, 1980). Following this period of re-equilibration, the steroid-induced excess of bone resorption over formation is no longer present and bone mass is thus maintained.

The extent of the increase in vertebral mineral density in those receiving APD is at first surprising. However, other authors have observed comparable increases in bone mass after treatment of patients with steroid osteoporosis. Hahn and Hahn (1976) observed an 8.4% increase in distal forearm bone mineral content following treatment with vitamin D and calcium and the same group found a 16% increase when 25-hydroxyvitamin D and calcium were used (Hahn et al, 1979). Vertebral bone mass was not measured in either of these studies but substantially greater changes would be expected at this site because of its higher content of trabecular bone in comparison with the forearm. This lability of bone mass in steroid-treated subjects probably reflects the increased extent of bone resorption, and thus the larger number of osteoclast resorption bays which can be filled in when an anti-resorptive regimen is introduced.

This is the first report describing the use of APD in the treatment of steroid osteoporosis. Its results are in accord with those from other animal and human studies. Thus, bisphosphonates have been found to increase bone mass in normal rats (Reitsma et al, 1983) and pigs (de Vernejoul et al, 1987)

and to inhibit the bone loss accompanying castration (Wink et al, 1985) or glucocorticoid treatment (Jee et al, 1981) of animals. Preliminary reports have suggested that APD increases bone mineral content in patients receiving the drug for treatment of Paget's disease (Nagant de Deuxchaines et al, 1983) and in those with osteoporosis (Huaux et al, 1985). Bisphosphonates are also of value in disuse (Schneider and McDonald, 1981) and juvenile (Hoekman et al, 1985) osteoporosis. The present study differs from those cited, however, in that it is prospective and includes a randomly allocated control group. It thus provides the best evidence to date for a positive effect of bisphosphonates on bone mass. While this study includes only steroid-treated patients, it is possible that its findings may have relevance to other types of osteoporosis associated with increased bone resorption.

CHAPTER 11 : CONCLUSIONS

In the introduction to this study, the pathogenesis and treatment of steroid osteoposoris were discussed. It was concluded that glucocorticoids have a direct inhibitory action on bone formation and that, in man at least, bone resorption is stimulated. This has generally been regarded as resulting from hyperparathyroidism but at least one study has suggested a direct stimulatory effect of glucocorticoids on osteolysis. The results of the present studies provide support for the existence of such a mechanism. They also confirm the inhibition of osteoblast function, both in-vitro (where leucine and proline incorporation into calavariae were found to be diminished in the presence of high cortisol levels) and in vivo, using serum bone gla-protein levels as an index of osteoblast function. These studies have provided further insight into the pathogenesis of steroid osteoporosis by confirming that steroid ingestion is associated with hypercalciuria and suggesting that this is mediated by a direct renal effect of the glucocortocoid. Of potentially great clinicial significance, it has been demonstrated that glucocortocoids produce hypogonadism in male patients; thus an easily detectable and correctable contributing factor to steriod osteoporosis has been identified. On the other hand, the present studies tend to discount any direct effect of glucocorticoids on serum levels of insulin-like growth factor -1 and support the conclusion of other workers that any effect of cortisol is on somatomedin action rather than production.

Two general therapeutic strategies have been studied. Firstly, it has been confirmed that calcium supplements can indeed inhibit bone resorption in steroid treated subjects and the mechanism and time course of this effect have been explored in normal individuals. Furthermore, the in vivo availability of calcium from a number of proprietary preparations has been assessed and it is concluded that absorption is probably better when calcium is ingested in solution or suspension rather than in the solid phase. Finally, a new strategy in the treatment of steroid osteoporosis has been evaluated, in the form of the second generation bisphosphonate, APD. When given in a dose of 150 mg per day, this drug is able to significantly depress bone turnover below levels achieved with calcium supplementation alone. This results in beneficial effects on bone mass at both metacarpal and vertebral sites in comparison with patients receiving calcium supplementation alone. These results require confirmation over longer study periods but provide exciting possibilities for the future management of both this and other osteopenic states.

From the results of these studies, a number of further questions arise. Can the demonstrated changes in tubular reabsorption of calcium and in serum levels of bone gla-protein be used to assess patients' risk of developing significant osteopenia whilst on steroids? What degree of benefit will result from the use of testosterone replacement in males taking steroids? Does the suppression of bone resorption by calcium supplements result in a significant beneficial effect on bone mass and is the dose of 1g per day of calcium optimal? Finally, what are the risks and benefits of the use of APD over a period of years in patients at

risk of glucocorticoid-induced or post-menopausal osteoporosis, what is the optimal dose of APD and is it most effective when given continuously, intermittently or cyclically with stimulators of bone formation? It is beyond the scope of the present study to address these questions but it is hoped that that answers to them will be forthcoming in the next few years.

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