THE EFFECTS OF TOPICAL CALCIPOTRIOL ON SYSTEMIC CALCIUM HOMEOSTASIS IN PATIENTS WITH PSORIASIS

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by

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ABSTRACT

Calcipotriol is a new and effective topical treatment for chronic plaque psoriasis vulgaris. It is an analogue of calcitriol (1,25 dihydroxyvitamin D - the active metabolite of vitamin D), and so has the potential to affect systemic calcium metabolism even when used topically. Animal studies indicate that parenteral calcipotriol has a weaker effect on systemic calcium homeostasis than calcitriol. Extensive clinical trials using relatively small amounts of calcipotriol (50μg/g) ointment (on average 30-40g/wk) detected no effect on serum calcium *in vivo* provided that the recommended maximum weekly dose of 100g was not exceeded. Cases of hypercalcaemia from calcipotriol ointment have been reported, both after excessive use and in relation to manufacturers recommendations.

Short wave ultraviolet light (UVB) is commonly used to treat psoriasis in combination with topical agents such as dithranol and tar. The use of calcipotriol in combination with UVB is becoming common practice. UVB initiates synthesis of vitamin D in the skin and therefore might enhance the calciotropic effects of calcipotriol when used in combination.

The aims of this study were to detect any alteration of calcium homeostasis in patients treated with topical calcipotriol, to identify the mechanism(s) of any detected effects and to determine whether the addition of UVB would enhance those effects.

In summary, we have confirmed that topical calcipotriol does have an effect on systemic calcium homeostasis. Intestinal absorption of calcium, and probably phosphate, is increased, when large doses are applied (up to 360g of the 50μg/g ointment per week). Serum calcium and phosphate rise while serum PTH and 1,25 dihydroxyvitamin D_3 levels fall. Urinary excretion of calcium and phosphate are increased. The suppressive effects on PTH and endogenous 1,25 dihydroxyvitamin D_3 levels may be due to direct inhibition by calcipotriol as well as indirect effects of rising serum calcium and phosphate.

At the upper limit of the recommended dose (100g/wk), calcipotriol has a small but measurable effect on systemic calcium homeostasis as manifested by a small rise in 24h urine calcium and serum ionized calcium. These changes are probably not of clinical significance. The addition of short wave ultraviolet light has no additive effect on systemic calcium homeostasis at recommended doses.

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ETHICAL APPROVAL

Ethical approval was obtained for all of the studies reported herein from the Leicestershire Ethics Committee.

ABBREVIATIONS

1,25vitD = $1\alpha,25(OH)_2D_3$ = $1\alpha,25$dihydroxyvitamin D_3 = Calcitriol		
ALP	=	Alkaline phosphatase
Ca_E	=	Calcium excretion index
GMCSF	=	Granulocyte Macrophage Colony Stimulating Factor
GFR	=	Glomerular Filtration Rate
HLA-DR	=	Class II human leukocyte antigen
HPLC	=	High performance liquid chromatography
IFNα	=	Interferon Alpha
IFNγ	=	Interferon Gamma
IL-1	=	Interleukin 1
IL-2	=	Interleukin 2
IL-4	=	Interleukin 4
IL-6	=	Interleukin 6
IL-8	=	Interleukin 8
MED	=	Minimal erythema dose
mRNA	=	Messenger ribonucleic acid
PASI	=	Psoriasis Area and Severity Index
PTH	=	Parathyroid hormone
PUVA	=	Psoralen plus UVA (photochemotherapy for psoriasis)
SER	=	Standard error of the mean
Tm_{PO4}/GFR	=	Renal threshold phosphate concentration
UVA	=	Long wave ultraviolet light (320-400nm)
UVB	=	Short wave ultraviolet light (290-320nm)
Vitamin D_2	=	Calciferol

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CHAPTER 1 INTRODUCTION

1.1 Vitamin D

1.1.1 Synthesis and metabolism

1.1.1.1 *Synthesis of vitamin D in the skin*

The skin is the principal source of vitamin D in man. UVB photolyses 7-dehydrocholesterol in the skin to previtamin D_3. Vitamin D_3 (cholecalciferol) is formed from previtamin D_3, over a period of a few hours,[1] by a time- and temperature-dependent isomerization process.[2] Further exposure to UVB results in the formation of biologically inert products; previtamin D_3 is photoisomerized to lumisterol and tachysterol[3] and vitamin D_3 is photodegraded to 5,6-trans-vitamin D_3 and suprasterols 1 and 2 (Fig. 1).[4] Melanin, by absorbing UV radiation, may also partly limit formation of previtamin D_3.[5] Thus, sunlight regulates the production of previtamin D_3 and limits the systemic availability of vitamin D_3.

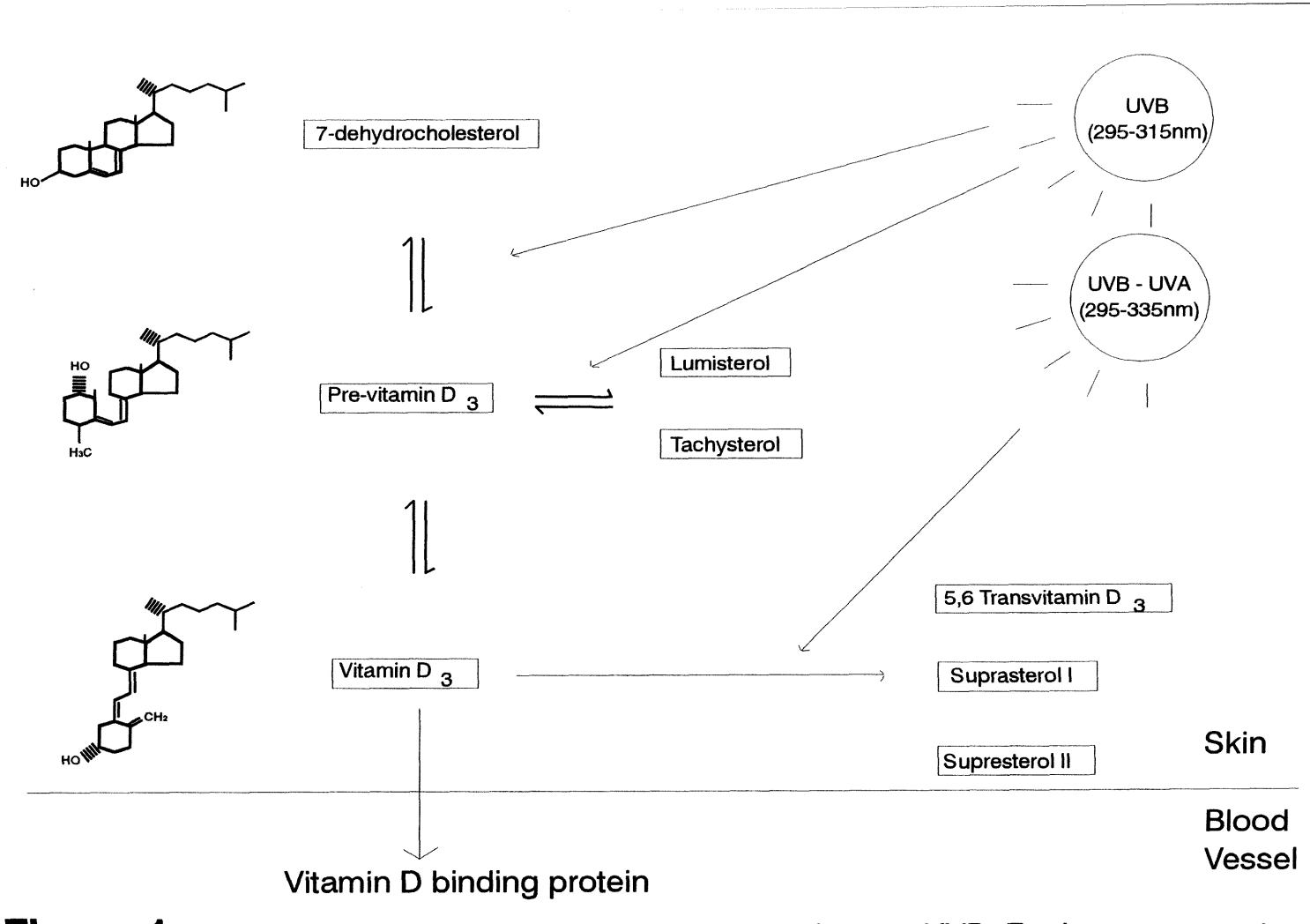


Figure 1. Synthesis of vitamin D_3 in the skin is dependent on UVB. Further exposure to UVB and UVA leads to photodegradation of vitamin D_3 to biologically inert products.

1.1.1.2 *Synthesis and metabolism of calcitriol*

Although the skin is the more important source of vitamin D in man, dietary intake of both vitamin D_2 and vitamin D_3 also contribute to vitamin D status. This dietary intake may be critical when exposure to sunlight is lacking. Vitamin D_2 and D_3 are inactive and must be hydroxylated to form the active metabolite, calcitriol (1,25 dihydroxyvitamin D). Vitamin D binds to serum vitamin D binding protein (Fig. 2) and, together with dietary vitamin D_2 and D_3 (incorporated into chylomicrons), is transported to the liver where it undergoes 25-hydroxylation to 25-hydroxyvitamin D (a cytochrome P-450 dependent reaction). Plasma concentrations of 25-hydroxyvitamin D reflect vitamin D input from endogenous and exogenous sources.

25-hydroxyvitamin D is then carried to the proximal tubule of the kidney where it undergoes 1α-hydroxylation to calcitriol (1,25 dihydroxyvitamin D - the active metabolite of vitamin D). 1α-hydroxylation is delicately controlled by a negative feedback system[6] which involves parathyroid hormone, serum calcium (indirectly) and serum phosphate, and also serum levels of calcitriol itself (Fig. 2). There is little regulation of hepatic 25 hydroxylation.[7]

Calcitriol is metabolized to 1,24,25 trihydroxyvitamin D by 24R-hydroxylase. This enzyme also converts 25-hydroxyvitamin D_3 to 24,25 dihydroxyvitamin D (Fig. 2). These two metabolites are less active than calcitriol and are themselves metabolized by other side chain hydroxylases, ultimately resulting in the production of calcitroic acid which is water soluble and inactive. In general when 1α-hydroxylase is suppressed, 24R-hydroxylase is stimulated thus increasing inactivation of calcitriol and 25-hydroxyvitamin D.

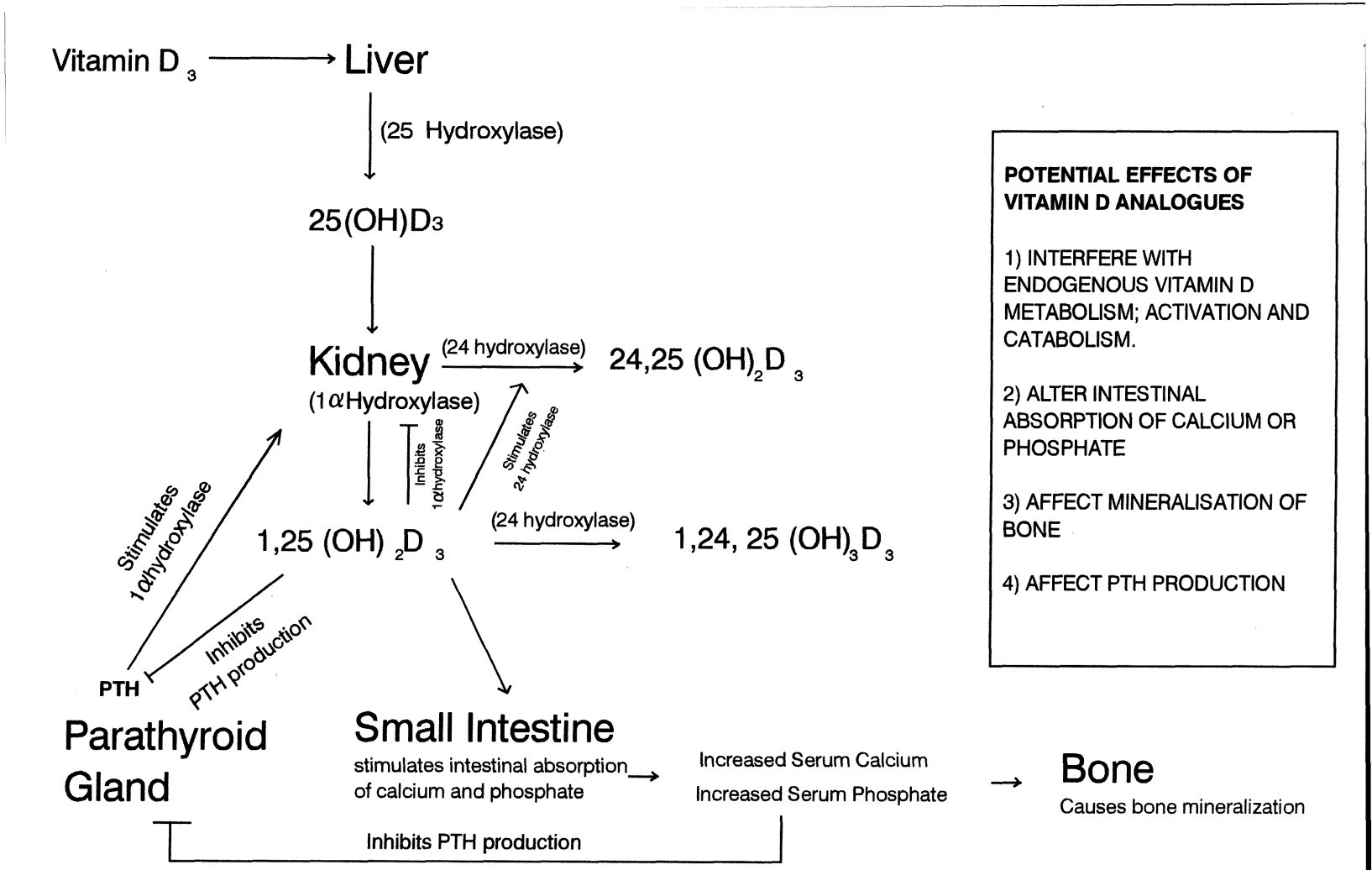


Figure 2. Activation of vitamin D_3 to 1,25 dihydroxyvitamin D_3 (calcitriol) is delicately controlled by a negative feedback system.

Extra-renal production of calcitriol has also been demonstrated *in vitro* in several tissue types, including the placenta,[8] bone,[9] malignant melanoma[10] and keratinocytes.[11] Under normal circumstances, extrarenal production of calcitriol contributes little to systemic calcium homeostasis. However, in certain pathological conditions, such as sarcoidosis,[12] extra-renal production can cause significant disturbance of calcium homeostasis.

1.1.2 Mode of action

Calcitriol exerts its effects through the vitamin D receptor which is a nuclear/cytosol protein of approximately 50kDa. The vitamin D receptor has a wide tissue distribution having been located in intestinal epithelial cells, parathyroid chief cells, lymphocytes, keratinocytes and many other tissues.[13] It has also been identified in malignant tumour cell lines.[14] The vitamin D receptor bears structural homology to the superfamily of steroid hormone receptors, which includes the glucocorticoid, oestrogen, progesterone, androgen, thyroid and retinoic acid receptors. These receptors each have a domain which binds to the appropriate steroid, a domain which interacts with other members of the nuclear receptor superfamily and a DNA binding domain. Calcitriol binds to the vitamin D receptor and the resulting complex then forms a dimer with a retinoid receptor, the retinoid X receptor (RXR).[15] Interaction of this dimer with the vitamin D response element induces gene transcription and protein synthesis. Vitamin D response elements are present in the promoter region of target genes with which calcitriol interacts. Some of the proteins known to be produced as a result include the calcium binding proteins, calbindin D_{9K} and calbindin D_{28K},[16] and the extracellular bone matrix protein, osteocalcin.[17] The calcium binding proteins are involved in transmembrane transport of calcium in the intestine. Osteocalcin binds calcium in the bone matrix.

Recently, evidence has also emerged for effects of calcitriol which are independent of the vitamin D receptor. Rapid vitamin-D-dependent absorption of calcium from the intestine has been demonstrated *in vitro*.[18] Rapid increases in intracellular calcium in keratinocytes and osteoblasts have also been detected.[19,20] The significance of these alternative pathways *in vivo* is uncertain.

1.1.3 Effects on systemic calcium homeostasis

Amongst the most important actions of calcitriol which are known to be of physiological significance in man are those on the intestine, bone, parathyroid gland and kidney.

Calcitriol increases intestinal calcium absorption by stimulating the production of calcium binding proteins.[21] Intestinal phosphate absorption is also increased.[22]

Calcitriol has a dual effect on bone. Bone mineralization is increased, indirectly, through increased availability of calcium and phosphorus. It also interacts, via its receptor, with the osteoblast lineage of cells to produce a variety of proteins. Paradoxically, calcitriol also initiates increased reabsorption of bone by stimulating osteoblasts to produce resorption factors that stimulate osteoclastic bone resorption.[23] It also influences haemopoetic\macrophage precursors to differentiate towards osteoclasts.

Calcitriol inhibits parathyroid hormone (PTH) production.[24] PTH increases serum calcium levels by stimulating resorption of bone and inhibiting urinary excretion of calcium. PTH synthesis is stimulated by falling serum calcium levels. Synthesis is inhibited serum calcium levels rise[25] and also when serum calcitriol levels rise (Fig. 2).

In the kidney, calcitriol suppresses activation of vitamin D by inhibiting 1α hydroxylase and stimulating 24 hydroxylase.

As outlined above, and illustrated in figure 2, calcitriol synthesis is delicately regulated by intricate links and feedback mechanisms. In the situation of hypercalcaemia, there is compensation for moderate changes in serum calcium, but this may result in precipitation of calcium in urine and formation of renal calculi. In more severe toxicity, once the standard homeostatic mechanisms are overcome, ectopic calcification and demineralization of bone may occur with potentially serious effects such as pancreatitis and renal failure.[2] Deficiency of vitamin D, on the other hand, may lead to osteomalacia.

1.1.4 Effects on cell proliferation and differentiation

In addition to a pivotal role in systemic calcium homeostasis, calcitriol has immunosuppressive and anti-proliferative effects. *In vitro* enhancement of maturation of monocytes/macrophages[26] has been demonstrated. Calcitriol inhibits cell proliferation and promotes differentiation in mouse[27] and human keratinocyte cultures.[28,29] Cornified envelope formation and transcription of transglutaminase are enhanced by calcitriol.[27,29,30,31,40,41] Human keratinocytes grown on dermis stripped of its epidermis are able to reconstruct a morphologically normal stratified and keratinizing epidermis[32] and, in air liquid interface culture, calcitriol increases the number of stratum corneum layers and reduces water permeation.[33]

Calcitriol also inhibits proliferation and promotes differentiation of many benign and malignant cell lines including skin fibroblasts,[27,34] breast cancer cells,[13] and leukaemia cells.[35,36] Several vitamin D analogues[37,38,39] including calcipotriol[40,41] also demonstrate these actions. These properties have prompted the investigation of the use of calcitriol and its analogues in the treatment of haematological malignancies[42] and also in breast cancer.[43,44] Inhibition of proliferation and enhanced differentiation of keratinocytes is one possible explanation for the effect of calcitriol in psoriasis.

1.1.5 Modulation of inflammation

Lymphocytes

Calcitriol and its analogues are also potent modulators of inflammation. Calcitriol inhibits interleukin-1 (IL-1) induced T-cell proliferation and the production of cytokines such as IL-2 and IL-6 by these cells.[45,46,47,48] and inhibits accumulation of mRNA for IL-2, IFNγ and GMCSF. CD8+ activity is promoted and generation of cytotoxic and natural killer cells is inhibited.[49]

Macrophages

Calcitriol increases cytotoxicity of macrophages.[50] Interferon alpha (IFNα), IFNγ and calcitriol have synergistic effects on the proliferation of monocytes-macrophages (U-937 leukaemia cell line). IFNα increases the expression of the vitamin D receptor.[51] Interaction between IFNγ and calcitriol is less clear. Calcitriol has been shown both to enhance[52] and to antagonize[53,54] IFNγ induction of class II HLA antigens in tumour cell lines. In human peripheral blood monocytes, calcitriol has been shown to down-regulate HLA-DR expression.[55] Calcitriol promotes increased expression of CD14 but decreased expression of CD23.

Myeloid cells

Calcitriol inhibits platelet-activating factor receptor gene expression in HL-60 cells, thus interfering with a potent lipid mediator of inflammation[56] Calcitriol inhibits proliferation and enhances differentiation of the HL-60 line.[57]

Polymorphs

The release of arachidonic acid by polymorphonuclear leukocytes is inhibited by calcitriol,[58] which also inhibits migration of these cells.[59]

Putative mechanisms of effect in psoriasis

The effects calcitriol and its analogues on keratinocyte proliferation and differentiation as well as their immunosuppressive and anti-inflammatory actions may explain their efficacy in the treatment of psoriasis. As outlined below, while keratinocyte hyperproliferation and de-differentiation are hallmarks of the disease, the importance of the lymphocytic infiltrate in psoriasis has been realized over the past 15 years. Thus these agents may have a dual effect in psoriasis therapy.

1.2 Psoriasis

1.2.1 *Pathophysiology*

Psoriasis is a common skin disorder affecting approximately 2% of the population, presenting most commonly in the teens although it may develop at any age. It is characterized by erythematous scaly plaques of variable size which are found most commonly on the elbows and knees but any skin site may be affected. Histopathologically the disease is characterized by hyperproliferation of the epidermis, producing characteristic papillomatosis, accumulation of inflammatory cells particularly T lymphocytes, monocytes and neutrophils and elongation and increased tortuosity of the dermal papillary blood vessels. The pathogenesis of psoriasis is incompletely understood. There appears to be a genetic predisposition but the mode of inheritance is uncertain. Both autosomal dominant[60] and multifactorial inheritance[61] have been described. Thirty-six percent of psoriatics have a first degree relative with psoriasis and twin studies reveal a 72% concordance between monozygotic twins.[62] Psoriasis is also associated with the presence of HLA A1, B13, B17, B37, DR7 and HLA-Cw6. Factors other than inheritance also come into play. Streptococcal infections are a well recognized trigger factor and it has been proposed that this is due to a streptococcal superantigen with structural homology to keratins in the epidermis.[63] The presence of such a superantigen could lead to autoimmunity and the development of chronic disease. In support of this is the finding of restricted T cell clones in chronic plaque and guttate psoriasis.[64]

The fact that there is an early accumulation of predominantly memory T cells in the dermis of evolving psoriatic plaques lends support to an autoimmune basis as does the association between psoriasis and certain major histocompatibility antigens. The evidence in favour of psoriasis being a T cell mediated disease is strengthened by the fact that cyclosporin, a selective inhibitor of T lymphocyte function, is a highly effective treatment for psoriasis.

The hallmark of psoriasis is hyperproliferation of keratinocytes[65] which leads to a thickened epidermis and a raised scaly plaque. There is shortening of the epidermal germinative cell cycle, an increase in the number of cells in the proliferative pool and marked shortening of the epidermal turnover time in psoriatic lesions.[66] As well as being hyperproliferative, keratinocyte differentiation is also abnormal. Cytokeratin expression is altered and involucrin and membrane-bound transglutaminase appear prematurely.[67] While, in the past, these changes were thought to be of primary importance in the pathophysiology of psoriasis, it is now felt that the dermal T cell inflammatory infiltrate drives the keratinocyte proliferation and de-differentiation via cytokines such as IFN-γ.

1.2.2 *Treatment*

Treatments for psoriasis can be divided into topical and systemic. Topical treatments include emollients, tar, salicylic acid, dithranol, vitamin D and corticosteroids. These may be used alone in mild to moderate disease and are commonly used in combination with systemic therapy in more severe disease. The efficacy of some topical therapies, particularly corticosteroids and emollients, may be helped by occlusion with polythene or similar dressings. Occlusion increases absorption into and through the epidermis by increasing temperature and hydration of the skin.[68] Occlusion also appears to have an intrinsic therapeutic effect of its own in psoriasis, the mechanism of which is unknown.[69]

Systemic therapy for psoriasis includes the use of phototherapy (UVB and PUVA), antimetabolites such as methotrexate, hydroxyurea and thioguanine, the retinoids acitretin, etretinate and isotretinoin, and the immunosupressants azathioprine and cyclosporin. The mode of action of many of these treatments is now considered to be either anti-proliferative; inhibiting keratinocyte turnover, or immunosuppressive; inhibiting the dermal lymphocyte infiltrate which drives the inflammatory process, or a combination of both. Calcitriol, the active metabolite of vitamin D_3, being both a potent immunosuppressive and anti-proliferative agent, would appear to be an ideal treatment for psoriasis.

1.3 Vitamin D and psoriasis

1.3.1 *Vitamin D and its metabolites*

Vitamin D, in some form, has been used to treat a variety of skin disorders including psoriasis from the early part of this century.[70] It was claimed to be effective for the treatment of pemphigus vulgaris,[71] scleroderma,[72] tuberculosis,[73] and dermatitis.[74] Several open studies were published in the 1930's and 1940's demonstrating the efficacy of vitamin D in the treatment of psoriasis.[67,75,76] However, interest in vitamin D waned in the 50's with the advent of topical steroids, dithranol and oral methotrexate.

The serendipitous observation of Morimoto *et al*[77] that 1α hydroxycholecalciferol (1-alpha), given to a patient to treat osteoporosis, improved coexistent psoriasis, reawakened interest in vitamin D. The authors subsequently studied a total of 52 patients with psoriasis and reported responses to oral 1-alpha-hydroxyvitamin D_3 (1μg daily), oral calcitriol (0.5μg daily) and topical calcitriol at concentrations ranging from 0.1μg/g to 0.5μg/g.[78,79] Two small open studies[80,81] also demonstrated a beneficial effect of oral and topical calcitriol and placebo-controlled studies[82,83] confirmed these findings.

Hollick and coworkers[84] first investigated oral calcitriol and demonstrated a significant improvement in psoriasis but at some cost with regard to toxicity. Up to 2μg of calcitriol was sufficient to induce a 26% remission rate and a mean 60% improvement in chronic plaque psoriasis but resulted in transient hypercalciuria in 40% of patients. By giving the oral calcitriol at night, toxicity was reduced. Hollick *et al.*[84] then investigated topical calcitriol. They found that calcitriol 15μg/g ointment was an effective treatment for chronic plaque psoriasis and detected no effect on systemic calcium homeostasis in patients who used up to 60μg/d.

The majority of other studies also found that topical calcitriol was safe. However Langner *et al.*[83,85] found that patients using larger amounts of calcitriol to treat areas greater than 600cm^2 developed hypercalciuria and some of the patients even developed asymptomatic hypercalcaemia. Recently Sipps *et al*[86] investigated the use of a mean of 50g of topical calcitriol (3μg/g) per week in 9 patients over a period of 6 weeks. They found no effect on serum calcium, PTH, calcitriol, osteocalcin, alkaline phosphatase, 24h urine calcium or gastrointestinal absorption of calcium as estimated by the stable strontium absorption test.

Concerns about possible toxicity of calcitriol prompted a search for naturally occurring or synthetic analogues of calcitriol with similar (or greater) efficacy but without the calciotropic effects. 1α,24dihydroxycholecalciferol (tacalcitol, TV-02) and 1α,24-ene-25 cyclopropyl cholecalciferol (calcipotriol) are the first agents to be developed and licensed for the treatment of psoriasis (see below). A number of other analogues are in various stages of production and one in particular - 1(S), 3(R)-dihydroxy-20(R)-4^1-hydroxy-41^1-ethyl-1^1-hexyloxy-9, 10-secopregna 5 (Z), 1O(1g)-triene also known as KH 1060 - appears to be a very promising agent although clinical studies have not yet been carried out.

1.3.2 *1α,24-dihydroxycholecalciferol (Tacalcitol)*

This synthetic analogue of vitamin D has been used in a number of small studies to treat patients with psoriasis. It does not require activation and therefore bypasses initial control mechanisms. Metabolism and receptor-binding capacity have yet to be determined. Although it is said to be less toxic than 1α-hydroxycholecalciferol, Mortensen *et al* [87] have recently found that the effects on systemic calcium homeostasis when applied topically to female Lewis rats, were similar to calcitriol. In fairly limited open clinical trials, no toxicity has yet been demonstrated. Kato *et al* [88] evaluated 11 patients using tacalcitol at concentrations of between 1 and 4 μg/g in petrolatum. Occlusion was used initially and the response was good in all patients. In 8 patients the ointment applied twice daily without occlusion and 5 of those patients improved. No adverse effect on serum calcium or phosphate was noted. Two further open Japanese studies[89,90] have found tacalcitol to be effective and safe, although the numbers of patients were again small. More recently, Gerritsen *et al*[91] performed a double blind placebo controlled, right/left, within patient study with tacalcitol 4μg/g ointment. There was a significant clinical improvement with tacalcitol which was greater than placebo and this was reflected in significant reduction histologically in inflammation and keratinocyte proliferation. There were no alterations in serum calcium or phosphate.

1.4 Calcipotriol

1.4.1 *Introduction*

Calcipotriol (known as calcipotriene in the USA) was first synthesised by Calverley[92] in 1987, while Leo laboratories were searching for a suitable alternative to calcitriol with less effect on systemic calcium homeostasis. It is a synthetic analogue of vitamin D characterized by a terminal cyclopropyl ring and a 24- rather than a 25-hydroxyl group (Figure 3). The mode of action of calcipotriol appears to be identical to that of 1,25 dihydroxyvitamin D_3. It combines with the vitamin D receptor in the same manner as calcitriol (outlined above) and binds as avidly as calcitriol *in vitro*.[93] It has also been shown *in vitro*, to be equipotent to calcitriol in many respects. It is a potent inhibitor of proliferation and promoter of differentiation of U937 lymphoma cell.[94,40] Calcipotriol has also been shown to promote differentiation and inhibit proliferation of mouse and human keratinocytes.[41,95] Calcipotriol has been found to inhibit osteoblast-like cell proliferation,[96] increase osteocalcin and alkaline phosphatase activity,[97] and stimulate osteoclast-like cell formation in human bone marrow cultures.[98] These *in vitro* similarities have been confirmed *in vivo* in the treatment of psoriasis. Holland *et al*[99] demonstrated a reduction in expression of cytokeratin K16, which is a marker of hyperproliferation, in psoriasis, following treatment with calcipotriol. Cytokeratin K2, a marker of differentiation, increased to a level higher than normal. These changes may, however, be secondary to alterations in the dermal inflammatory infiltrate as De Jong *et al*,[100] found that the first change in psoriatic plaques treated with calcipotriol was a marked reduction

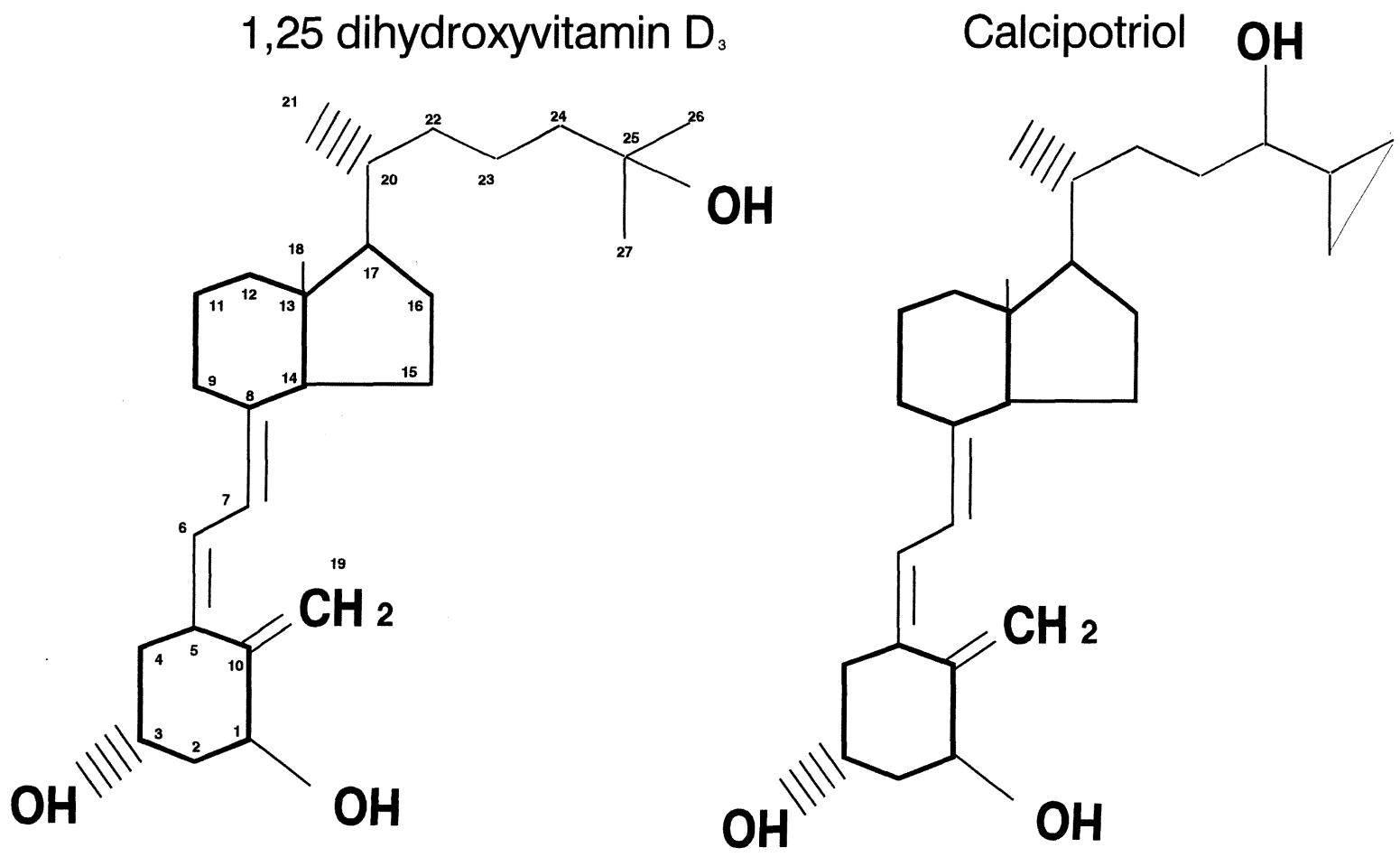


Figure 3. The vitamin D analogue calcipotriol differs from 1,25 dihydroxyvitamin D_3 (calcitriol) by the presence of a terminal cyclopropyl ring and a 22 rather than a 25 hydroxyl group.

in intensity of the polymorph infiltrate in the epidermis. This was followed by a reduction in epidermal proliferation and subsequent alterations in cytokeratin expression.

Calcipotriol has also been shown to reduce expression of IL6 and IL8 in psoriatic plaques.[101,102] These two cytokines are thought to be important in the pathophysiology of psoriasis.[103,104]

While calcipotriol and calcitriol have similar potencies in most respects there are two areas where they appear to differ. Firstly, in animal studies, calcipotriol has been shown to have a much weaker effect on systemic calcium homeostasis. It is this function which makes calcipotriol such an attractive agent for the treatment of psoriasis. In rats calcipotriol is metabolized much more rapidly than calcitriol. Consequently, it has a much weaker effect on systemic calcium homeostasis, in these animals. Oral, intravenous and intraperitoneal injection of calcipotriol has a 100- to 200-fold weaker effect on systemic calcium homeostasis than calcitriol.[40] Similar findings have been reported when calcipotriol was administered topically to female Lewis rats.[87] Bouillon *et al,*[105] found that calcipotriol did not bind as avidly to serum vitamin D binding protein as calcitriol. This has been proposed as a probable explanation for the more rapid metabolism of calcipotriol. However a subsequent study involving a number of vitamin D analogues failed to demonstrate a relationship between T_2 and affinity for serum vitamin D binding protein.[106] Two principal metabolites of calcipotriol, MC 1080 and MC 1046, have been identified. Calcipotriol is metabolized to these two inactive products by rat and human hepatocytes *in vitro* by oxidation at the 24 position.[107,108]

The second area in which calcipotriol appears to differ from calcitriol is in its effects on receptor-independent pathways. Norman *et al*,[109] found that calcipotriol had a much weaker effect on acute gastrointestinal calcium absorption *in vitro*.[*1] The significance of this finding is uncertain.

Recently, Naveh-Many *et al.*[110] reported a differential suppressive effect of calcipotriol on PTH mRNA production *in vitro* by bovine parathyroid cells. They found that calcipotriol was less effective by a factor of approximately 10 as compared to both 1,25 dihydroxyvitamin D_3 and oxacalcitriol. The clinical significance of this finding is uncertain.

[1] Calcipotriol is referred to as BT in this paper.

1.4.2 *Absorption and metabolism of topical calcipotriol in man*

There have been two studies investigating the metabolism of calcipotriol. In the first, carried out in healthy human volunteers, approximately 1% of radio-labelled topical calcipotriol was absorbed and all of the calcipotriol was excreted within 48hrs, as judged by radioactivity levels.[111] In the second, 3 groups were investigated; healthy human volunteers, psoriatics after a single dose and psoriatics after treatment for 2 weeks.[112] Between 2.6 and 12% of the administered dose was absorbed. The results of these studies must be interpreted with caution as direct measurement of calcipotriol levels was not performed. Radiolabelled calcipotriol (^{3}H-calcipotriol) was used and urine, faeces and serum measured at frequent intervals for 21 days for radioactivity and tritiated water. A sensitive assay of calcipotriol has recently been developed and it should now be possible to study the metabolism of calcipotriol *in vivo* in patients using it topically.[113]

1.4.3 *Formulation and guidelines for use*

Calcipotriol is poorly absorbed orally and has therefore been developed as a topical treatment for psoriasis. Until very recently, calcipotriol was available only as a 50μg/g ointment in 30g and 100g tubes. It is now also available as a 50μg/g cream and scalp application. The 100g tube has been replaced by a 120g tube and a new 60g tube is also available. Most of the literature relates to use of calcipotriol (50μg/g) ointment. It is applied twice daily and the manufacturers recommend that no more than 100g be used per week. The studies described in chapters 2-4 relate to the 50μg/g ointment as the other preparations were not available when this work was carried out.

Topical calcipotriol is recommended for the treatment of mild to moderate chronic plaque psoriasis affecting up to 40% body surface area. Initially, its use was restricted to a period of 6 weeks but that restriction has now been removed. The maximum recommended dose is 100g of ointment or cream per week.

1.4.4 *Clinical efficacy in psoriasis*

Initial pilot studies, carried out by Kragballe *et al*,[114,115] confirmed the efficacy of topical calcipotriol in the treatment of chronic plaque psoriasis and suggested that the optimal concentration was 50μg/g. In the first, Kragballe *et al* studied 27 patients in double-blind fashion. Calcipotriol cream, at concentrations of 10, 33 and 100μg/g, applied over a period of 6 weeks, was significantly better than placebo. In the second study, 25, 50 and 100uμ/g were compared to placebo in a double-blind right-left within-patient placebo-controlled study involving 50 patients. 50μg/g was found to be the optimum concentration. Several subsequent large multicentre studies have confirmed the efficacy of topical calcipotriol in the treatment of chronic plaque psoriasis over periods of 6-8 weeks. Kragballe *et al*[116] compared calcipotriol in another double-blind right-left within-patient study with betamethasone and found calcipotriol to be superior in 347 patients. Cunliffe *et al*[117] subsequently found calcipotriol to be equivalent to betamethasone in a randomized double-blind parallel-group study involving 409 patients. Berth-Jones *et al*[118] found calcipotriol to be superior to dithranol in a randomized parallel-group study involving 478 patients. These studies confirmed the short term benefits of calcipotriol in the treatment of psoriasis. A number of studies have also examined long term use although not in a controlled fashion. Kragballe *et al* reported sustained benefit in 15 patients over periods of up to 6 months.[119] Ramsay *et al* also found sustained beneficial effects over periods of up to 1 year in 161 patients.[120]

Calcipotriol has been used as adjunctive therapy in the treatment of more severe psoriasis. In combination with UVB, it has been shown to be no more effective than UVB alone.[121] However the number of patients studied was small (20). One subsequent, as yet unpublished study involving 101 patients demonstrated enhanced efficacy of UVB plus calcipotriol over calcipotriol alone (personal communication Leo laboratories). This does not answer the question of whether the addition of calcipotriol to UVB has any benefit over UVB alone.

In a randomized double-blind parallel group study involving 107 patients, calcipotriol was found to enhance the effect of PUVA and reduce the total cumulative dose of UVA given.[122] The use of calcipotriol in combination with cyclosporin has also proved beneficial. In a randomized double-blind parallel group study involving 69 patients, the use of calcipotriol was shown to enhance the efficacy of cyclosporin such that very low doses could be used to clear extensive psoriasis.[123]

1.4.5 *Adverse effects of calcipotriol (excluding effects on systemic calcium homeostasis)*

Lesional and perilesional irritation are the principal adverse effects of calcipotriol and occur in up to 20% of patients.[124] Facial (4%)[118] and flexural irritation are also a problem. Facial dermatitis may develop even in patients who are not applying calcipotriol directly to the face,[115] presumably due to inadvertent transfer from the hands after application to other body sites. Contact allergic dermatitis has been reported rarely[125] and recently photosensitive dermatitis has also been reported[126] although this is probably very rare.

1.4.6 *Adverse effects of calcipotriol on systemic calcium homeostasis*

It is important to consider the evidence for calcipotriol toxicity in licensed and unlicensed usage separately. Calcipotriol is licensed for use in mild to moderately severe chronic plaque psoriasis provided that no more than 100g of the 50μg/g ointment are used per week. Calcipotriol is not licensed for use in severe or unstable psoriasis or in other unrelated disorders.

1.4.6.1 LICENSED USAGE

Mild/moderate chronic plaque psoriasis (40-50g of calcipotriol 50μg/g ointment per week)

Published safety data are available on approximately 1000 patients with mild to moderate chronic plaque psoriasis requiring less than 100g of calcipotriol (50μg/g) ointment per week. In these studies, mostly multicentre, no short term (6-8 weeks) effect on serum calcium could be demonstrated.[115-8] More sensitive parameters of calcium homeostasis were only measured in a small number of patients in 2 studies. No short term effects on serum alkaline phosphatase, osteocalcin, PTH, 25-vitD, 1,25-vitD, 24h urine calcium, urine calcium/creatinine ratio or tubular reabsorption of phosphate and calcium could be demonstrated in patients using on average 25g and 40g of the ointment per week.[127,128] Long term toxicity has also been examined. No effect on serum calcium was demonstrated in 15 patients using calcipotriol for 6 months[119] and, in a separate study, in 161 patients using calcipotriol for one year.[120] There has been no investigation of the effect of long term calcipotriol on more sensitive parameters of calcium homeostasis.

Moderately extensive chronic plaque psoriasis (requiring approximately 100g per week)

There is one report describing 2 patients, using 70-80g of calcipotriol 50μg/g ointment, who developed hypercalcaemia.[129] However, in one case, the level was measured 2 weeks after stopping calcipotriol and was only just above the normal limit. In the other, hypercalcaemia developed after prolonged use of calcipotriol (11 weeks). It is possible that these patients represent a small subgroup of patients particularly susceptible to calcipotriol toxicity.

Gumowski-Sunek *et al,*[130] studied 10 patients using a mean of approximately 90g of calcipotriol ointment per week and found no significant alteration in a wide variety of parameters of systemic calcium homeostasis including an oral calcium tolerance test.

1.4.6.2 UNLICENSED USAGE

Extensive psoriasis (requiring greater than 100g per week)

There is one reported case[111] and three unpublished reports (Personal communication Leo Laboratories Ltd) of hypercalcaemia in patients treating extensive chronic plaque psoriasis with excessive amounts of calcipotriol (200-700g per week). There is also a report of a patient who developed hypercalciuria, without any change in serum calcium, while using 150g per week.[131]

Unstable or pustular psoriasis

There have been 3 reports of hypercalcaemia in patients with unstable psoriasis, 2 published,[132,133] and one unpublished (Personal communication, Leo Laboratories Ltd.). In 2 of these patients, less than 100g per week were used. It is likely that excessive absorption of calcipotriol through inflamed skin was the cause of toxicity in these patients.

Non-psoriatic patients

Because of enhancement of cell differentiation, there has been considerable interest in the effects of vitamin D and its analogues on cancer cells. Bower *et al*[134] studied the effects of topical calcipotriol in patients with metastatic breast cancer. Of nineteen patients who used 1g of calcipotriol 100μg/g ointment under occlusion, 2 developed hypercalcaemia after 2 and 8 days of therapy. One patient required rehydration and a biphosphonate as well as withdrawal of calcipotriol. Whether this was a genuine effect of such a small dose of calcipotriol and how much of the hypercalcaemia was related to bony metastases are difficult questions to answer. Patients with bony metastases may possibly be hypersensitive to vitamin D and/or its analogues.

There has also been some interest in the treatment of congenital ichthyosis with topical calcipotriol.[135] One patient treated with 80-100g/d of calcipotriol (50μg/g) ointment for 1 week developed vomiting and weight loss and a serum calcium 3.55mmol/L.[136] Levels returned to normal 8 days after withdrawal of calcipotriol.

1.5 Phototherapy for psoriasis

Phototherapy has been in use for the treatment of psoriasis since the early part of this century.[137] The mechanism of action is uncertain, although immunosuppressive activity has been demonstrated.[138]

Two types of phototherapy are used to treat psoriasis; short wave ultraviolet light (UVB) and a combination of long-wave ultraviolet light and 8-methoxypsoralen (PUVA).

The use of UVB is of relevance to this study as this is the wavelength which stimulates synthesis of cholecalciferol in the skin (Figure 2). UVB may be of benefit in combination with calcipotriol (personal communication Leo laboratories), and this combination is likely to become common practice in dermatology units.

UVB is most commonly produced by mercury vapour lamps with alkaline earth phosphor coatings to convert the wavelength to broad band UVB (290 - 320). The Phillips TL12 lamp, which is probably the most common lamp in use, also emits small amounts of UVA and UVC. More recently, a narrow-band UVB lamp has been developed (Phillips; TL-01) which has a peak emission at 311nm with most of the emission (83%) occurring between 309 and 313nm. This has been shown to be highly effective in the treatment of psoriasis.[139]

Ultraviolet light is administered either by an overhead set of lamps with the patient lying on a bed (usually broad-band UVB), analogous to the modern sun-bed, or a cabinet with lamps on 4 sides in which the patient stands. Patients are initially tested with different doses of UVB on selected areas on the back and asked to return to the clinic 24hrs later. The minimum dose which causes erythema is noted (minimal erythema dose; MED) and the patient is then given whole body treatment with 70% of the MED at the initial visit. The dose of UVB administered is increased at each visit by 20 - 40% depending on the patient's sensitivity. If erythema develops, the dose is not increased and a dose may be omitted depending on the severity of the burn. Treatment is usually carried out three times a week until there has been 90% clearance of psoriasis or failure to respond. The usual treatment period is 6-8 weeks although longer periods are sometimes necessary.

UVB is usually combined with topical therapy. The initial regime described by Goeckerman involved tar.[140] Dithranol is also commonly used.[141] The use of calcipotriol in combination has been reported[121] and no adverse effect on serum calcium has been found. More detailed assessment of systemic calcium homeostasis in such patients has not been carried out.

1.6 An overview of systemic calcium homeostasis and its assessment.

1.6.1 *Serum*

1.6.1.1 *Serum calcium*

Calcium is essential for the function of many cells, particularly skeletal and cardiac muscle and nervous tissue. Maintenance of serum calcium within normal limits is central to systemic calcium homeostasis. Calcium exists in extracellular fluid in three states. Approximately 40% is bound to plasma proteins particularly albumin; approximately 10% freely diffuses through the capillary membrane but is complexed with other anions such as citrate and phosphate; the remaining 50% is free ionized calcium. Ionized calcium is the physiologically active fraction and variations levels, if sustained, may lead to serious complications. Hypocalcaemia can lead to tetany and death. Hypercalcaemia may lead to dehydration, coma and death. Serum calcium is maintained by a combination of bone mineralization and resorption, renal excretion and intestinal absorption. Serum ionized calcium is also dependent on pH. As pH increases, for example in hyperventilation, binding of calcium to albumen is increased, this leads to a reduced serum ionized calcium and may result in symptomatic hypercalcaemia in the face of normal serum total calcium.

Ninety-nine percent of body calcium is in bone where there is a readily exchangeable reservoir and a much larger pool of stable calcium that is only slowly exchangeable. This is under the control of vitamin D, PTH and calcitonin as well as being directly affected by serum concentrations of calcium and phosphate. In normal healthy adults, about 500mmol of ionized calcium per day moves into and out of the readily exchangeable pool in bone while only 7.5mmol/day is interchanged between plasma and the stable pool.

A large amount of calcium is filtered daily in the kidneys but 98-99% is reabsorbed initially in the proximal tubules (60%) and later in the distal tubule (20-25%) and the loop of Henle (10%). Distal tubular reabsorption is under the control of PTH.

Intestinal absorption is principally under the influence of 1,25 dihydroxyvitamin D_3 although some passive absorption also takes place.

In common clinical practice, total serum calcium is used as an index of calcium status. As the concentration of total calcium, in serum, is principally affected by albumin concentration, it is usually adjusted for serum albumin concentration. Normal serum albumin level is taken to be 40g/L and therfore serum total adjusted calcium is calculated for an alteration from this norm using the formula:

Adjusted Total Serum Calcium = Total Serum Calcium - [*(Serum Albumin - 40)* x *0.025*]

This value is known as the serum total adjusted calcium and is an indirect indicator of ionized calcium.[142] It is more accurate to measure serum total ionized calcium directly but this is a more difficult assay to perform. The patient must be resting and the sample taken without application of a tourniquet. The sample must then be analyzed immediately under anaerobic conditions. The resulting value is also adjusted for pH.

1.6.1.2 *Serum phosphate*

As with calcium, most body phosphorus is contained in bone (85-90%) and exchange between plasma and bone is controlled simultaneously with calcium. 85-90% of phosphorus filtered in the kidney is actively reabsorbed in the proximal tubule. This process is powerfully inhibited by parathyroid hormone. Phosphate is absorbed from the intestine partially under the control of 1,25 dihydroxyvitamin D.

Eighty-five percent of serum inorganic phosphate is in the free ionized state, approximately 85% of this being $HPO_4^{-\,-}$ and $NaHPO_4^-$, and 15% as $H_2PO_4^-$. 15% of inorganic phosphate in serum is protein bound. In common clinical practice, serum total inorganic phosphate is measured. Although serum phosphate is important for a wide range of structural and metabolic functions such as bone mineralization; is a component of phospholipids, for cell membranes; and as a component of ATP for energy transfer, it is not as finely regulated as serum calcium.

Serum phosphate is a useful parameter for the assessment of calcium homeostasis when used in conjunction with serum calcium. In many disorders of calcium homeostasis, serum phosphate changes reciprocally with serum calcium. The reverse is true of disorders of parathyroid function where a fall in serum phosphate in the presence of a rise in serum calcium, for example, usually indicates primary hyperparathyroidism.

1.6.1.3 *Serum alkaline phosphatase*

Serum alkaline phosphatase activity reflects a group of isoenzymes derived principally from liver and bone. It is also released into serum from the intestine, in approximately 20% of the population. In pregnancy, significant amounts are released from the placenta to be detectable in serum. Although not specific, serum total alkaline phosphatase is measured, in common clinical practice, to screen for diseases of bone and liver. It is possible to measure the individual isoenzyme derived from bone, bone specific alkaline phosphatase, which is derived mainly from osteoblasts. Osteoblasts are important in the growth and remodelling of bone. The exact function of alkaline phosphatase is uncertain. It may serve to hydrolyze pyrophosphate which is a potent inhibitor of mineralization. Serum alkaline phosphatase is normally elevated in children and adolescents. In adults, serum alkaline phosphatase is elevated in bone disorders resulting from vitamin D deficiency, Paget's disease of bone and primary or secondary malignancy involving bone.

1.6.1.4 *Serum Vitamin D*

25-hydroxyvitamin D is the most readily available measure of vitamin D. This is a reasonably accurate reflection of supply of vitamin D. Measurement of serum 25 hydroxyvitamin D_3 is of use in the diagnosis of privational vitamin D deficiency, for example in Asian or elderly patients. It is also useful in assessing adequacy of replacement therapy. It may be misleading in the diagnosis of osteomalacia.[143]

Measurement 1,25 dihydroxyvitamin D_3 should be a more accurate indication of vitamin D activity. However serum 1,25 dihydroxyvitamin D_3 is much more difficult to measure and reports from the literature have given conflicting results. For example, high normal, normal and low levels of 1,25 dihydroxyvitamin D_3 have all been recorded in chronic renal failure.[144,145] Poor analytical performance at the lower end of the reference range may have been responsible for some of the variability. A number of assays are available for the measurement of 1,25 dihydroxyvitamin D_3. The standard method relies on high performance liquid chromatography (HPLC) and is laborious and time consuming. A new assay using a monoclonal antibody and immunoseparation is now available (IDS immunodiagnostics Ltd.) which should overcome some of these difficulties. Serum 1,25 dihydroxyvitamin D_3 is useful in more complicated causes of disordered calcium homeostasis, for example sarcoidosis and vitamin D resistant hypophosphataemic rickets.

1.6.1.5 *Serum PTH*

Parathyroid hormone is an important regulator of systemic calcium homeostasis. It inhibits tubular reabsorption of phosphate and stimulates reabsorption of calcium in the kidney. It also stimulates 1α hydroxylase which is the ultimate enzyme involved in the activation of vitamin D. PTH production is in turn inhibited by 1,25 dihydroxyvitamin D_3 and secretion is inhibited by serum ionized calcium. PTH is responsible for the mobilization of calcium from bone. It exerts this effect by activation of adenyl cyclase which results in increased osteoclastic activity and triggering the formation of more osteoclasts. PTH undergoes diurnal variation with peak serum levels at 4am and trough levels at 9am. Until recently, direct measurement of PTH was carried out by radioimmunoassay which was not clinically helpful. Immunoreactive PTH includes several fragments of PTH and most of the immunological activity measured is not the active hormone. Secondly, different antibodies recognize different parts of the PTH peptide, which causes particular problems in renal failure where C-terminal fragments rise to abnormally high levels. Monoclonal antibodies which measure intact PTH are now available and are much more clinically useful.

1.6.1.6 *Serum Osteocalcin*

Osteocalcin is a protein synthesized by osteoblasts and released into circulation. The concentration in serum is elevated in states of high osteoblast activity and decreased in states of diminished bone synthesis.

1.6.1.7 *Serum C-terminal propeptide of collagen type I*

Procollagen is the precursor of collagen which is cleaved to form collagen. The C-terminal propeptide of procollagen is cleaved and released in to circulation where it is readily measurable in serum. C-terminal propeptide of collagen type I is synthesized by osteoblasts, type I collagen being the main constituent of the organic matrix of bone. Like osteocalcin, it is an indicator of osteoblastic activity.

1.6.2

1.6.2.1 *Urine Biochemistry*

Urinary calcium output is dependent on many factors which regulate systemic calcium homeostasis. Changes in urinary calcium excretion are often an early indicator of changes in systemic calcium homeostasis. A fasting calcium/creatinine ratio has also been used as a measure of bone reabsorption. Urinary phosphate is largely determined by dietary intake.

1.6.2.2 *Urine excretion indices*

A number of indices may be calculated from the serum and urine calcium, phosphate and creatinine concentrations.

Tubular excretion of calcium (Ca_E) may be calculated using the method of Peacock and Nordin,[146] by the formula: $Ca_E = (U_{Ca} \times P_{Cr}) / U_{Cr}$. Where U_{Ca} is the urine concentration of calcium, P_{Cr} is the serum concentration of creatinine and U_{Cr} is the urine concentration of creatinine.

Renal threshold phosphate concentration (Tm_{PO4}/GFR) may be calculated using the method described by Walter and Bijvoet.[147] This method involves the use of urine phosphate concentration (U_{PO4}), urine creatinine concentration (U_{Cr}), serum phosphate concentration ($[PO_4]$) and serum creatinine concentration ([Cr]) and the formula:

$$\frac{C_{PO4}}{C_{Cr}} = \frac{U_{PO4} \times [Cr]}{U_{Cr} \times [PO_4]}$$

A nomogram is then used to calculate the renal threshold phosphate concentration. This gives a more accurate indication of renal handling of phosphate than other measurements such as phosphate clearance, phosphate/creatinine ratio, phosphate excretion index or functional reabsorption of phosphate in that it distinguishes between tubular reabsorption of phosphate and glomerular filtration rate and the net inflow of phosphate into the extracellular space from bone gut and soft tissues.

1.6.2.3 *Urine hydroxyproline*

Hydroxyproline, glycine and proline make up most of the collagen molecule. Hydroxyproline is found in no other mammalian protein with the exception of elastin where it is found only in small amounts. Total urinary excretion of hydroxyproline therefore is a good indicator of collagen turnover and, as most of the turnover of collagen in normal circumstances occurs in bone, it is a reflection of bone turnover of collagen.[148]

1.6.2.4 *Urine concentration of deoxypyidinoline cross-links*

Pyridinium crosslinks are products of a unique series of reactions during the maturation of collagen fibrils that lead to the formation of pyridinoline and deoxypyridinoline. Bone collagen contains both pyridinoline and deoxypyridinoline and release of these components from bone undergoing resorption constitutes the main source of both crosslinks in urine.[149] Measurement of both types of cross-links or individual cross-link measurements give an accurate of collagen turnover in bone. Deoxypyridinoline has a slightly more restricted tissue distribution than pyridinoline which is also found in cartillage and tendon. Excretion of crosslinks is greatly increased in conditions of increased bone turnover such as Paget's disease and primary hyperparathyroidism.

1.6.3 *Intestinal absorption*

Intestinal absorption of calcium is under the direct control of 1,25 dihydroxyvitamin D_3. Intestinal absorption is stimulated both acutely by non-genomic mechanisms (transcalctachia) and over a period of hours by genomic mechanisms. The majority of calcium absorption takes place in the duodenum and jejunum. Assessment of intestinal absorption is difficult and unreliable. Two established methods are available; radioisotope studies using ^{45}Ca and the stable strontium absorption test.[150] Both of these tests are relatively insensitive and there is considerable inter- and intra-patient variability.[151,152,153] Of the two tests, the strontium absorption test has the advantages of being cheaper, easier to administer and safer for the patient in that no radioactive isotopes are used.

1.7 Plan of investigation

1.7.1 SUMMARY

1) There has been little detailed investigation of systemic calcium homeostasis in patients applying topical calcipotriol.

2) There have been no dose ranging studies.

3) There have been no detailed long term studies.

4) It is uncertain how the current maximum recommended dose (100g per week) was arrived at. There appears to be no information to support it.

5) Toxic effects on systemic calcium homeostasis have been reported in psoriatic patients even within the recommended guidelines.

6) Now that calcipotriol is in world-wide use and regular use in general practice in this country, it is essential that its effects on systemic calcium homeostasis are quantified.

7) Long term use of calcipotriol must be assessed in detail now that the 6 week restriction has been lifted.

8) It is also imperative that the mechanism of those effects is elucidated. If calcipotriol were to have a differential effect *in vivo* on any aspect of systemic calcium homeostasis, it could have serious consequences and could result in a state of relative vitamin D deficiency or toxicity. As outlined above, there is some *in vitro* evidence that calcipotriol has a weaker effect than calcitriol on intestinal absorption. Even small imbalances in systemic calcium homeostasis could well be important now that calcipotriol is being used on a long term basis.

1.7.2 AIMS OF THIS PROJECT

1) To confirm that topical calcipotriol has an effect on systemic calcium homeostasis when used topically to treat patients with psoriasis.

2) To elucidate the mechanisms of that effect.

3) To determine whether topical calcipotriol has any effect on systemic calcium homeostasis when used within the manufacturers guidelines.

4) To determine whether the use of recommended doses of calcipotriol and short-wave ultraviolet light (narrow-band UVB; TL-01) in combination has a measurable effect on systemic calcium homeostasis.

CHAPTER 2 METHODS

2.1 Patients

2.1.1 *Groups*

A series of studies were carried out. These studies can be divided into four groups.

1) High doses of calcipotriol were applied to patients with extensive chronic plaque psoriasis to confirm that topical calcipotriol could affect systemic calcium homeostasis and to elucidate the mechanisms of that effect.

2) Patients with mild to moderately extensive disease applied doses up the recommended maximum (100g per week) to determine whether there was any effect on systemic calcium homeostasis at those doses.

3) The addition of UVB was assessed in patients with moderately extensive disease applying the maximum recommended dose of calcipotriol (100g/wk).

4) Finally, patients with pustular psoriasis were given as much calcipotriol as necessary to treat their disease.

2.1.2 *Inclusion and exclusion criteria*

Male and female patients over the age of 18 years were recruited. Females who were pregnant or breast-feeding were excluded. Patients with renal impairment and those with a history of renal calculi were excluded. Patients with abnormal calcium homeostasis and patients receiving vitamin D, calcium supplements or thiazide diuretics were also excluded. Patients receiving systemic therapy or UV radiotherapy were excluded except in the final study when the effects of UVB were being specifically assessed.

2.1.3 *In-patients*

Two of the studies involved the administration of large doses of topical calcipotriol which were potentially toxic. These patients were, therefore, admitted to hospital for the duration of the study. While in hospital, patients were kept mobile in order to prevent hypercalciuria due to immobility. They were not allowed outside in order to exclude any exogenous effect on vitamin D synthesis from natural sunlight. An exception was made in patients with unstable psoriasis, described in the final study, who were kept on bed rest as is standard practice.

2.1.4 *Out-patients*

The remaining studies were carried out in the outpatient department. Patients attended fasting on the morning of each visit.

2.1.5 *Application of ointment*

Calcipotriol 50μg/g ointment, as the commercially available product, was used in all studies. It was applied twice daily except in one study where the effect of a single dose was specifically studied. Patients were carefully instructed, at the beginning of the study, as to how to apply the ointment and how much to apply per day and per week. They were asked not to apply any other topical preparations or take any systemic anti-psoriatic medication throughout the study.

2.1.6 *Sample collection*

Whole blood and spot urine samples were taken in the morning with the patients in a fasting state. Blood samples were centrifuged immediately with a Capricorn 'Bench Top' centrifuge (model No CEP 289, Capricorn Laboratory Equipment, Christchurch Rd, Ringwood, Hants, BH24 3BB) at 4000 rpm (1800 x g) for 15 minutes. Serum was drawn off and frozen immediately, initially in liquid nitrogen and later in a -70^{0}C freezer. Blood was also taken using a heparinized syringe and transported immediately to the laboratory for assessment of ionized calcium. Whole blood samples for peak PTH levels were taken at night, at 4am. Samples were centrifuged and the serum was frozen in similar manner to the morning samples. Twenty-four hour urine samples were collected the day before each visit. Patients were instructed carefully at the beginning of the study, how to collect these samples. Instructions were repeated regularly throughout the study to ensure compliance. The initial morning sample on day 1 was discarded. Patients then collected all urine passed throughout the remainder of that day and the first sample on day 2 to complete the 24 hour sample.

2.2 Assessment of psoriasis severity

Severity of psoriasis was assessed using the psoriasis area and severity index (PASI).[154] This involves scoring 4 defined areas (head and neck, upper limbs, trunk, lower limbs) for erythema, induration and scale on a 5-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). Area affected is scored on a 7 point scale (0 = 0%, 1 = >0<10%, 2 = ≥10%<30%, 3 = ≥30%<50%, 4 = ≥50%<70%, 5 = ≥70%<90%, 6 = ≥90%<100%). In each area the sum of the scores for erythema, scale and induration are multiplied by the area score and then multiplied by 0.1 for the head and neck, 0.2 for the upper limbs, 0.3 for the trunk and 0.4 for the lower limbs. The PASI equals the sum of the scores for each of the 4 areas. The calculation is described by the formula:

$$PASI = [A \times 0.1 \times (E+I+S)_{Head/neck}] + [A \times 0.2 \times (E+I+S)_{U\ limbs}] + [A \times 0.3 \times (E+I+S)_{Trunk}] + [A \times 0.4 \times (E+I+S)_{L\ limbs}]$$

Where E = Erythema score (0-4)

I = Induration score (0-4)

S = Scale score (0-4)

2.3 Studies

2.3.1 Pilot Study

The purpose of this study was to determine whether calcipotriol, applied topically in high doses, would affect systemic calcium homeostasis.

Patients were admitted to hospital for a period of 3 weeks. They were kept mobile but not allowed to expose themselves to sunlight during their stay. Patients applied white soft paraffin for 2 days to enable baseline measurements to be taken. This was followed by calcipotriol (50μg/g) ointment for 2 weeks and by 2% crude coal tar for a final week, which acted as a biochemical washout period. Two hundred grams of the calcipotriol ointment were applied over the first week and, if there were no clinically significant effects on systemic calcium homeostasis, 300g were applied over the second week. In the initial stages, this regime was chosen because the safety of large doses of calcipotriol was uncertain. Serum total adjusted calcium was measured at baseline and three times a week throughout the study. 24h urine calcium was measured at baseline and twice weekly during the study. Serum PTH was measured at 4am (peak) and at 9am (trough) at baseline, at the end of the treatment period (2wks) and at the end of the washout period (3wks). Serum 1,25 dihydroxyvitamin D_3 levels and serum calcipotriol levels were measured at baseline, at the end of weeks 2 and 3.

Given that an effect on systemic calcium homeostasis was demonstrated in the pilot study, two questions would then need to be answered. Firstly, what was the mechanism of this effect and secondly, would there be any significant effects at recommended doses of calcipotriol.

Two types of study were designed. In the first, variably high doses of calcipotriol were administered in hospital and detailed assessment of systemic calcium homeostasis was made with particular reference to intestinal absorption of calcium and bone metabolism. In the second, outpatients were treated with recommended doses of calcipotriol under various circumstances to detect any alteration of systemic calcium homeostasis.

2.3.2 High Dose Study

The purpose of this study was to elucidate the mechanism(s) of the effect of calcipotriol on systemic calcium homeostasis. The principal areas of interest were intestinal absorption of calcium and bone metabolism.

Patients were started on a fixed calcium intake diet of 1000mg per day, three weeks prior to admission and were maintained on that diet while in hospital. The patients were assessed by a dietitian and the diet explained in detail. They were given a sheet (Appendix 1) explaining what the diet involved.

When admitted to hospital, they were kept mobile but not allowed to expose themselves to sunlight during their stay. Patients applied white soft paraffin for 2 days to enable baseline measurements to be taken. This was followed by calcipotriol (50μg/g) ointment for 2 weeks. The amount of calcipotriol applied was in proportion to the extent of psoriasis up to a maximum of 360g per week. The initial intention had been to use the following nomogram: 15-20% of skin surface area affected - 150g/wk, 20-25% - 200g/wk, 25-30% - 250g/wk, 30-35% - 300g/wk, >35% - 350g/wk. However as calcipotriol ointment comes pre-packed in 30g tubes, it was more practical to use units of 30g. The amounts applied were as follows; 15-20% of skin surface area affected - 150g/wk, 20-25% - 210g/wk, 25-30% - 240g/wk, 30-35% - 300g/wk, >35% - 360g/wk.

Patients' height and weight were recorded on admission. For the purposes of analysis, the dose of calcipotriol was then calculated in grams of 50μg/g ointment per kilogram body weight per week and changes in systemic calcium homeostasis were plotted against this figure.

Intestinal absorption of calcium was assessed using the strontium absorption test, as described below. This test was carried out at the beginning of the study and after 2 weeks. The effects of calcipotriol on bone metabolism were assessed by measuring serum osteocalcin, serum C-terminal propeptide of type I collagen, serum bone specific alkaline phosphatase, and fasting (am) urine hydroxyproline and deoxypyridinoline cross-links at baseline and after 2 weeks.

As in the pilot study, serum total adjusted calcium was measured at baseline and three times a week throughout the study, 24h urine calcium at baseline and twice weekly during the study and serum 4am (peak) and 9am (trough) PTH, serum 1,25 dihydroxyvitamin D_3 levels, and calcipotriol levels at baseline and after 2 weeks' treatment. In addition measurements were made of serum phosphate, serum creatinine, serum urea and serum total alkaline phosphatase at baseline and three times a week during the study, 24h urine phosphate and creatinine at baseline and twice weekly during the study and serum ionized calcium, fasting (am) urine calcium, creatinine, and phosphate at baseline and at the end of the treatment period (2wks)

Urine excretion indices for phosphate and calcium and glomerular filtration rate (GFR) were calculated on the basis of the 24h and fasting am urine levels.

2.3.3 Recommended doses

A total of five studies were carried out at recommended doses. Three of these involved in-depth assessment of systemic calcium homeostasis after fixed doses of calcipotriol within the manufacturer's guidelines.

2.3.3.1 *100g of calcipotriol (50μg/g) ointment per week - maximum recommended dose*

In the first of these three, patients were instructed to apply exactly 100g of calcipotriol (50μg/g) ointment per week for 4 weeks. Serum total adjusted calcium, serum phosphate, serum alkaline phosphatase, serum 9am PTH and 24h urine calcium were measured at baseline, 2 and 4 weeks. Serum osteocalcin, c-terminal propeptide of type I collagen and serum bone specific alkaline phosphatase were measured in selected patients at baseline and after 2 weeks.

2.3.3.2 *90g of calcipotriol (50ug/g) ointment per week*

In the second, patients were randomly assigned, in double-blind fashion, to apply exactly 90g per week of either calcitriol (3μg/g) ointment or calcipotriol (50μg/g) ointment over 8 weeks. Serum total adjusted calcium, serum ionized calcium, serum phosphate, serum alkaline phosphatase, serum osteocalcin, serum 25 hydroxyvitamin D_3, serum 9am PTH and 24h urine calcium, phosphate, creatinine and hydroxyproline were measured at baseline, 1, 2, 4, 6 and 8 weeks. This study was carried out in parallel with a multicentre study designed to compare the efficacy and tolerability of calcipotriol (50μg/g) and calcitriol (3μg/g) ointments. The study was sponsored by Duphar Laboratories Ltd.

2.3.3.3 *A single 6g dose of calcipotriol (50ug/g) ointment*

In the third study, patients were randomly assigned to apply either a single 6g dose of calcipotriol (50μg/g) ointment or placebo (white soft paraffin) on 2 separate occasions, 3 weeks apart. Patients attended fasting and had baseline samples taken for serum total adjusted calcium, serum ionized calcium, serum phosphate, serum alkaline phosphatase, serum 9am PTH and spot urine calcium, phosphate and creatinine. These parameters were repeated at 1, 2, 4, 6, 8 and 9 hours after application of the ointment under controlled dietary conditions. Having attended fasting, the patients were given a fixed breakfast and fixed lunch at each visit. They also consumed one pint of milk, half with breakfast and half at 1100am.

The two remaining studies involved the assessment of patients using small amounts of calcipotriol (50μg/g) ointment, which would be the norm for most patients in clinical practice.

2.3.3.4 *Occlusion plus calcipotriol*

In this study, patients applied calcipotriol (50μg/g) ointment twice daily while occluding the limbs with polythene film at night (a commonly employed method of enhancing efficacy of topical preparations in the treatment of psoriasis) for a period of 8 weeks. The patients were randomized, in single blind fashion, to one of two groups. Group A applied calcipotriol to all affected areas twice daily and at night they wrapped the limbs on one side with polythene film. The patients in group B applied calcipotriol twice daily to the limbs on one side and applied placebo (white soft paraffin) to the opposite side. Both sides were occluded at night with polythene film. The study was conducted in single blind fashion, sealed envelopes being handed to the patients at the beginning of the study with instructions of which the investigator was unaware.

Serum total adjusted calcium, serum phosphate and 24h urine calcium and phosphate were measured at baseline and every 2 weeks thereafter. As individual limbs were being assessed for psoriasis severity, an adapted PASI was used where the scores for individual limbs were considered separately and not added together.

2.3.3.5 *Long term calcipotriol (1yr)*

In the final study, patients applied calcipotriol (50μg/g) ointment twice daily over a period of one year. Serum total adjusted calcium and 24h urine calcium were measured at baseline and monthly thereafter.

2.3.4 Calcipotriol plus short wave ultraviolet light (UVB)

Male and female patients, between the ages of 18 and 75yrs, with moderately extensive chronic plaque psoriasis were asked to take part in the study. Patients with renal impairment, a history of renal calculi, taking thiazide diuretics and pregnant or lactating females were excluded from the study. Patients receiving other systemic anti-psoriatic therapy and patients with a history of photosensitivity were also excluded.

Patients were assigned to one of three treatment groups; 100g of calcipotriol (50μg/g) ointment per week, UVB alone, or UVB plus 100g of calcipotriol (50μg/g) ointment per week. The groups were matched for age and sex. UVB therapy was given 3 times per week in incremental doses as described by Green *et al.*[155] In brief, all patients were tested on selected areas of the back to determine the minimum dose of UVB which resulted in erythema (Minimal Erythema Dose - MED). The patients were given 70% of the MED at the first treatment and the dose was increased by 40% thereafter unless they developed erythema. In cases of mild erythema, the dose was increased by 20%. In cases of moderate erythema, the dose was left unchanged. In cases of marked erythema, treatment was withheld and restarted when erythema had settled at 70% of the previous dose. All patients received narrow-band UVB in a Dixwell EMLY UVA + UVB cabinet (Dixwell, Zone Industrielle du Pontet, 69360 St Symphorien d'Ozon, Lyon Sud, France) using Phillips TL-01 tubes. Serum total adjusted calcium, serum ionized calcium, serum phosphate, serum alkaline phosphatase, serum 9am PTH and 24h urine calcium were measured at baseline and at 2-weekly intervals over a six week period.

2.3.5 Unstable pustular psoriasis

Patients admitted to hospital for treatment of severe unstable psoriasis were assessed for suitability for treatment with topical calcipotriol. The standard treatment for these patients in the initial period is bed rest and emollients. If that proved unsuccessful, and if there were no contraindications, a trial of treatment with topical calcipotriol was begun. Because of the nature of this condition, a strict study protocol was not devised for these patients and monitoring for toxicity from calcipotriol consisted of measurement of serum total adjusted calcium. Calcipotriol was applied in amounts sufficient to cover the area of skin affected.

2.4 Brief outline of methods used to determine the mechanism of action of calcipotriol on systemic calcium homeostasis

2.4.1 *Assessment of the effects on intestinal absorption of calcium*

The effect on the intestine was measured using the stable non-isotopic strontium absorption test. This test was chosen in preference to radioisotope studies because it is safer and easier to perform. It has been shown to be as effective as radioisotope studies in the assessment of intestinal absorption of calcium.[150,156] The induction of transcaltachia[18] (acute gastro-intestinal absorption of calcium) was studied by examining the acute effects of a single topical dose of calcipotriol on serum and urine over a nine hour period.

2.4.2 *Assessment of the effects on parathyroid hormone secretion and function*

The effect of calcipotriol on parathyroid hormone levels was studied by measuring peak (4am) and trough (9am) serum levels of the intact hormone. Tm_{PO4}/GFR was also measured as a further indicator of parathyroid hormone bioactivity.

2.4.3 *Assessment of the effects on bone*

Because we were uncertain what the effects on bone might be and because different bone markers show different patterns of alteration in given disease states, we chose a broad battery of markers of bone formation (serum bone specific alkaline phosphatase, osteocalcin, and c-terminal propeptide of type I collagen) and bone resorption (Urine hydroxyproline and deoxypyridinoline X-links).

2.4.4 *Assessment of any effects on renal function*

Any direct/indirect effect on the kidney was determined using measurements of serum and urine calcium and creatinine and the appropriate nomogram (see page 50).[146,147]

2.5 Analytical methods

2.5.1 Carried out by hospital laboratory

The following parameters were measured routinely by the hospital laboratory initially using the SMAC II Technicon Analyzer (1991-1993) and subsequently on the DAX Technicon Analyzer (1993-1995).

Serum

Urea, creatinine, total calcium, phosphate, albumin, total alkaline phosphatase. Serum total adjusted calcium was calculated using the formula:

Adjusted total serum calcium = Serum calcium + (40 - serum albumin)/40

Serum 25 hydroxyvitamin D_3 levels were measured by radioimmunoassay (INCSTAR 25-OH-D assay, INCSTAR Corporation, Stillwater, Minnesota, USA).

Urine

24 hour calcium, 24 hour Phosphate, 24 hour creatinine. Fasting (am) urine calcium, phosphate, and creatinine.

Normal Ranges:

Serum urea	2.5 - 6.5 mmol/L
Serum creatinine	60 - 120 μmol/L
Serum total calcium	2.1 - 2.6 mmol/L
Serum total adjusted calcium	2.1 - 2.6 mmol/L
Serum phosphate	0.8 - 1.4 mmol/L
Serum alkaline phosphatase	40 - 130 IU/L
24h urine calcium	2.5 - 7.5 mmol/24h
24h urine phosphate	15 - 50mmol/24h
Serum 25 hydroxyvitamin D_3	
March	19.6 - 33.8ng/ml
June	16.0 - 38.6ng/ml
September	27.2 - 45.4ng/ml
December	10.7 - 26.5ng/ml

2.5.2 Additional measurements

2.5.2.1 *Serum ionized calcium*

Principle

This was measured using a Ciba Corning Ca 634 ISE Ca++/pH analyzer (Ciba Corning Diagnostics Ltd, Halstead, Essex, UK). The calcium ion selective electrode consists of a neutral carrier based calcium sensor immobilized in PVC. The sensor is in contact with the sample on one side and with the electrode fill solution on the other. Electrical connection is via Ag/AgCl wire. The pH selective electrode consists of a glass capillary which is selective to hydrogen ions. The sample passes through the capillary with the outer surface in contact with the electrode fill solution. Electrical connection is via Ag/AgCl wire. The ionized calcium was measured at the actual and corrected (7.40) pH.

Method

Samples were taken in the rested state in the morning, without a cuff, ensuring that the patient was free of anxiety, pain and not hyperventilating. Heparinized syringes were used and the samples were taken immediately to the laboratory for analysis.

Normal Range: 1.0 - 1.25

2.5.2.2 *Bone specific Alkaline Phosphatase*

Total serum alkaline phosphatase was measured using standard laboratory methodology on the SMAC II/DAX Technicon analyzer.

Bone specific alkaline phosphatase was measured using the Alkphase-B kit (Metra Biosystems Inc. Mountain View, California, CA 94043, USA).

Principle

Alkaline phosphatase is measured by enzyme linked immunosorbant assay (ELISA). Alkphase-B is an immunoassay in a microtitre plate format utilizing a monoclonal anti-bone specific alkaline phosphatase antibody coated on the plate to capture bone specific alkaline phosphatase in the sample. The enzyme activity of the captured bone specific alkaline phosphatase is detected with a pNPP substrate.

Method

Serum samples, stored at -70°C, were thawed out and brought to room temperature. 125µL of assay buffer was added to each well. 25µL of each sample and 25µL of standards (0, 2, 20, 50, 80, 140 U/L) and high and low controls were added to individual wells and incubated for 3 hours at room temperature. Wells were washed 4 times in 1X wash buffer. 150µL of substrate solution was then added to each well and incubated at room temperature for 30 minutes. Finally, 100µL of 1N NaOH was added and the optical density was read at 405nm using a plate reader.

Normal Range: F - 11.6 - 30.6 U/L

M - 15.0 - 41.3 U/L

2.5.2.3 *Serum 1,25 dihydroxyvitamin D_3 levels*

1,25 dihydroxyvitamin D_3 levels in serum were measured using the gamma-B 1,25-dihydroxyvitamin D_3 kit (IDS immunodiagnostic systems Ltd., Boldon, Tyne and Wear, NE35 9PD, UK).

Principle

The method involves the purification of 1,25 dihydroxyvitamin D_3 in serum by immunoextraction followed by quantitation by radioimmunoassay (RIA). Samples are delipidated and dihydroxyvitamin D_3 extracted from potential cross-reactants by incubation for 3 hours with a highly specific solid phase monoclonal anti-D antibody. The immunoextraction gel is then washed and purified 1,25 vit D eluted into glass tubes. Reconstituted eluates and calibrators are incubated overnight with a highly specific 1,25 vitD sheep anti-1,25dihydroxyvitamin D_3 antibody. ^{125}I-1,25 dihydroxyvitamin D_3 is added and incubated for 2 hours. Separation of bound from free is achieved by a short incubation with Sac-Cel© followed by centrifugation, decantation and counting. Bound radioactivity is inversely proportional to the concentration of dihydroxyvitamin D_3.

Method

500µL of each sample was added to individual tubes. 50µL of delipidation reagent was added to each tube. Tubes were centrifuged at 2000 x g for 30 minutes. 100µL of delipidated sample was added to each immunocapsule which contains monoclonal antibody to 1,25 dihydroxyvitamin D_3. Capsules were rotated end over end for 3 hours and then centrifuged for 1 minute at 500-750 x g to remove the sample. Each immunocapsule was washed twice. Elution reagent was added and then centrifuged for 1 minute at 500-750 x g to collect the eluate. This step was repeated twice. Eluate was then evaporated in a water bath for 30 minutes. 100µL of eluate was decanted into individual tubes. 200µL of primary antibody was added to each tube and incubated overnight. 200µL of ^{125}I-1,25 dihydroxyvitamin D_3 is added to each tube and incubated for 2 hours.

100µL of Sac-Cel© was added to each tube and incubated for 30 minutes. Samples were then washed and counted in a gamma counter.

Normal Range: 48 - 110pmol/L

2.5.2.4 *Serum Parathyroid hormone*

The intact serum parathyroid hormone was measured initially by the SAS laboratory (University College Hospital, Cardiff, Wales) using the Ciba Corning Magic light Method (Ciba Corning Diagnostics Ltd., Halstead, Essex, UK). After the initial pilot study, all PTH assays were carried out using the Gamma-BCT Intact PTH assay (IDS immunodiagnostic systems Ltd., Boldon, Tyne and Wear, NE35 9PD, UK).

Principle

An N-terminal specific monoclonal antibody (anti-PTH 1-34) is coated onto the inner surface of a polystyrene tube (the solid-phase or capture antibody). Samples are incubated in antibody coated tubes for 4 hours at 2^0C. Tubes are then decanted and washed to remove unbound sample. They are then incubated at 4^0C overnight with affinity purified ^{125}I goat anti-PTH (39-84), decanted and washed twice before counting. The concentration of PTH in the patient's sample is calculated from the concentration of bound reactivity. The use of N-terminal capture antibody with a split incubation format avoids C-terminal fragment interference.

Method

200μL of each of each sample, standards and controls were added to appropriately labeled tubes. The samples were incubated for 4 hours at 2^0C. 2mL of diluted wash solution was added to all tubes, which were then decanted and drained briefly on a pad of absorbent paper. 200μL of ^{125}I-anti-PTH was then added to each tube and two additional tubes set aside as total counts. The tubes were covered and incubated overnight. The tubes were decanted and drained thoroughly on absorbent paper. 2mL of diluted wash solution was added and the tubes were left to stand for 5 minutes. This step was repeated, the tubes inverted and drained. Counting was carried out with a gamma counter. All samples were measured in duplicate.

Normal Range:

18 - 60yrs	0.8 - 5.2pmol/L	61 - 88yrs	1.5 - 6.4pmol/L

2.5.2.5 *Serum Osteocalcin*

This was measured by enzyme-linked immunosorbant assay (ELISA) using the NOVOCALCIN kit (Metra Biosystemc Inc. Mountain View, California, CA 94043, USA).

Principle

The assay is a competitive, enzyme-linked immunosorbant assay. It uses osteocalcin coated strips, a monoclonal anti-osteocalcin antibody and an anti-mouse alkaline phosphatase conjugate to quantify osteocalcin in serum. The osteocalcin in the sample competes with the osteocalcin on the strip well for the monoclonal antibody. A second antibody-enzyme conjugate (anti-mouse IgG Alkaline phosphatase conjugate) is then added to the strip well to bind to the anti-osteocalcin monoclonal antibody. A substrate, para-nitrophenyl phosphate (pNPP), is added to produce a yellow colour which is read at 405nm.

Serum samples, stored at -70°C, were thawed out and brought to room temperature. 25µL of each sample was added to individual wells. 25µL of Standards (0, 2, 4, 8, 16, 32ng/mL) and high and low controls were added to individual wells. 125µL of anti-osteocalcin antibody was added to each well and incubated for 2 hours at room temperature. Wells were washed 3 times in 1X wash buffer. 150µL of enzyme conjugate was added to each well, incubated at room temperature for 60 minutes and then washed as before. 150µL of working substrate solution was then added to each well and incubated at room temperature for 30 minutes. Finally, 50µL of 3N NaOH was added and the optical density was read at 405nm using a plate reader.

Normal Range:	Females	3.7 - 10.0 ng/ml
	Males	3.4 - 9.1 ng/ml

2.5.2.6 *Serum C-terminal propeptide of type I collagen assay*

Serum C-terminal propeptide of type I collagen (C1CP) levels were measured by ELISA using the PROCOLLAGEN-C kit (Metra Biosystemc Inc. Mountain View, California, CA 94043, USA).

Principle

This assay is a sandwich immunoassay in a microtitre plate format which uses a strip-well module coated with a monoclonal anti-C1CP antibody, a rabbit anti-C1CP anti-serum, a goat anti-rabbit alkaline phosphatase conjugate, and a pNPP substrate to quantify C1CP in human serum.

Method

Serum samples, stored at -70°C, were thawed out, brought to room temperature and diluted 1:12. 100µL of diluted samples, standards (0, 1, 2, 5, 20, 80 ng/mL) and high and low controls were added to individual wells and incubated at room temperature for 2 hours.. 100µL of rabbit anti-C1CP antibody were added to each well and incubated for 45 minutes at room temperature. Wells were washed 3 times in 1X wash buffer. 100µL of enzyme conjugate was added to each well, incubated at room temperature for 45 minutes and then washed as before. 100µL of substrate solution was then added to each well and incubated at room temperature for 30 minutes. Finally, 50µL of 3N NaOH was added and the optical density was read at 405nm using a plate reader.

Normal Range:	Females	69 - 147 ng/ml
	Males	76 - 163 ng/ml

2.5.2.7 *Tubular maximum reabsorption of phosphate and calcium (Tm_{PO4}/GFR, Tm_{Ca}) and Calcium excretion index (Ca_E).*

Tm_{PO4}/GFR was measured using the nomogram derived by Walton and Bijvoet[147] and described above. Ca_E was measured using the method described by Peacock and Nordin[146] as described above (see page 50)

2.5.2.8 *Hydroxyproline*

Urine hydroxyproline concentration was measured using the Hypronosticon[7] kit (Organon Teknika B.V., Boxtel, Holland).

Principle

Hydroxyproline occurs in the urine mainly in a peptide-bound form. It is bound to a strong acid cation exchange resin. The resin is washed with distilled water to eliminate interference. The residual peptides are then hydrolyzed by heating to 100^0C for 16 hours. Hydroxyproline is eluted from the resin and oxidized to a pyrrole derivative which is coloured with an Ehrlich's reagent and quantitatively determined photometrically.

Method

One resin tablet was added to each tube. 0.5ml of each sample was added to the corresponding tube. 1ml of standard solution was then added to each tube and distilled water added up to the 10ml mark. Tubes were shaken vigorously and then centrifuged at 1500 x g, after which the supernatant was decanted. Tubes were then incubated overnight for 16 hours at 102^0C. 1 drop of 1% phenolphthalein in ethanol was added to each tube followed by 2N NaOH until the indicator changed. Tubes were centrifuged for 5 minutes at 1500 x g, filled with distilled water to 2.5ml and centrifuged again. The supernatant was pipetted from the hydrolysis tubes and 1 drop of 2N HCL added. 1ml of isopropanol followed by 0.5ml of oxidant in buffer was then added. After standing for 4 minutes, the colour reagent was added and absorbance measured at 560nm with a spectrophotometer.

Normal Range: 22 - 65yrs 25 - 80mg hydroxyproline/24h/m^2

2.5.2.9 *Urine concentration of deoxypyidinoline cross-links*

Urine levels of deoxypyridinoline were measured using the PYRILINKS-D kit (Metra Biosystemc Inc. Mountain View, California, CA 94043, USA).

Principle

The Pyrilinks-D assay is a competitive enzyme immunoassay in a microtitre plate format utilizing a monoclonal anti-deoxypyridinoline antibody coated on the plate to capture deoxypyridinoline. Deoxypyridinoline in the sample competes with conjugated deoxypyridinoline-alkaline phosphatase for the anti-body and the reaction is detected with a paranitrophenol (pNPP) substrate.

Method

First morning urine samples, stored at -70°C, were thawed out and brought to room temperature. Samples, standards (0, 3, 10, 30, 100, 300 nM) and high and low controls were diluted 1:10 and 50:1 added to individual wells. 100µL of cold enzyme conjugate were added to each well, incubated at room temperature for 2 hours minutes and then washed 3 times in 1X wash buffer. 150µL of substrate solution was then added to each well and incubated at room temperature for 60 minutes. Finally, 100µL of 1N NaOH was added and the optical density was read at 405nm using a plate reader.

Normal Range:	Females	2.5 - 6.5 nmol deoxypyridinoline/mmol creatinine
	Males	2.5 - 5.5 nmol deoxypyridinoline/mmol creatinine

2.5.2.10 *Non-isotopic Strontium absorption test*

The modified method described by Milsom S *et al*[150] was used to measure intestinal calcium absorption.

Principle

Serum strontium was measured on a Hitachi Z-8200 graphite furnace analyzer. Samples were diluted to between 1:10 and 1:50 in 0.1M HNO_3. The result was compared to a standard curve of aqueous strontium solution.

Method

Non-radioactive stable strontium chloride (2.5mmol) was given with a standard breakfast which consisted of 100g of peaches, 50ml of peach juice, 2 slices of white bread, 10g of butter, 10g of honey (48mg/1.2mmol of elemental calcium), followed later by 250ml of orange juice and 2 plain cracker biscuits. 10ml of venous blood was taken before ingestion of the strontium and after 1, 2, and 4 hours. Blood was spun as outlined above and serum was pipetted off for assay of serum strontium concentration.

2.5.2.11 *Analysis of calcipotriol and 1,25-dihydroxyvitamin D_3 in serum (Pilot study)*

These assays were carried out simultaneously on selected patients by Dr. LW LeVan from LUNAR Corporation, Madison, WI, USA.

Principle

The serum was analyzed for calcipotriol and for 1,25-dihydroxyvitamin D_3 using a specific high-performance liquid chromatography (HPLC)/radioreceptor assay, which is summarized as follows.

Method

To each plasma sample (0.75ml) was added 50µl of ethanol containing radiolabeled internal standard (approximately 1300 cpm (^{3}H)-1,25-$(OH)_2D_3$). After mixing, each sample was extracted by adding 1.0 ml of acetonitrile and mixing thoroughly followed by centrifugation. The supernatant was mixed with 1.0mL of 0.4M phosphate buffer (pH 10.5), centrifuged, and applied to a Bond-Elut© solid-phase extraction cartridge. The Bond-Elut© cartridge was eluted sequentially as follows: water, 2ml; methanol:water (70:30), 5ml; hexane:dichloromethane (9:1), 5mL; hexane:isopropanol (99:1), 3mL; and hexane:isopropanol (95:5), 5mL. The latter fraction, containing calcipotriol and 1,25-$(OH)_2D_3$, was collected, concentrated, and applied to a HPLC system consisting of a Zorbax©-SIL column (4.6mm x 25cm) eluted with 4.2% isopropanol in hexane:dichloromethane (60:40) at 2.0mL/minute. Fractions corresponding to the elution times observed for calcipotriol and 1,25-$(OH)_2D_3$ standards were collected and evaporated to dryness, then reconstituted in absolute ethanol (50 to 150µL).

Aliquots of the reconstituted HPLC fractions containing the compounds of interest were quantified using a non-equilibrium competitive radioreceptor assay based on displacement of (^{3}H)-1,25-$(OH)_2D_3$) from the bovine thymus 1,25-dihydroxyvitamin D receptor. This assay has been described previously by Reinhardt *et al.*[157] Concentrations of calcipotriol and 1,25-$(OH)_2D_3$ were determined individually using separate standard curves (1 to 25pg/tube) prepared from each of the respective compounds. Results were calculated using the SPLINE curve fit (RIASMART©) software package, Packard Instrument Company). A known portion of the 1,25-$(OH)_2D_3$ fraction from each sample was taken for liquid scintillation counting to determine the method recovery of the radiolabeled internal standard. After allowance for sample volume (0.75mL) and method recovery (74%), the standard curves correspond to assay working ranges of approximately 2 to 45 pg/mL.

2.6 Statistical analysis

In all studies, the (two tailed) paired t test was used to compare the baseline and end of study values for biochemistry. The (two tailed) Wilcoxon signed rank test was used to compare baseline and end of study values of PASI. In the occlusion and UVB studies, the Mann Whitney U test was used to compare the PASI in each group.

In the high dose study, ordinary least squares analysis was used to correlate changes in systemic calcium homeostasis with dose of calcipotriol applied.

CHAPTER 3 RESULTS

3.1 Performance and quality control of the assays

3.1.1 SMAC/DAX technicon analyzer

Internal quality control samples of high and low values for each biochemical parameter were measured with each run of a given assay. External quality control samples were also assessed on a regular basis. All samples were within acceptable limits. The results of the assays are presented separately for each study below.

3.1.2 Serum 25 hydroxyvitamin D_3

Internal quality control samples of high and low values for each biochemical parameter were measured with each run of a given assay. External quality control samples were also assessed on a regular basis. All samples were within acceptable limits. The results of the assays are presented separately for each study below.

3.1.3 Ionized calcium

All samples were measured in duplicate to ensure precision of the assay. Coefficient of variation over the period of the study was 1%. Accuracy of the machine was assessed by the use of internal control samples which were measured in the morning before starting a series. The quality control samples were either Hi, Med or Lo, indicating levels for pH and ionized calcium at the upper, middle or lower range of expected sample values. The ranges for acceptable limits for each of these is given below as well as the individual measurements throughout the study.

Acceptable limits for quality control samples:

Lo	Ca++ (0.69-0.83)	pH (7.11-7.17)
Med	Ca++ (1.12-1.26)	pH (7.31-7.37)
Hi	Ca++ (1.64-1.78)	pH (7.68-7.72)

Date	Lo	pH	Med	pH	Hi	pH
18/8/94	0.64	7.16	1.08	7.35		
22/2/94	0.75	7.2	1.2	7.38		
28/2/94	0.69	7.18	1.17	7.38		
7/3/94	0.69	7.13	1.17	7.34		
14/3/94	0.7	7.18	1.19	7.34		
21/3/94	0.7	7.18	1.17	7.34		
23/3/94	0.7	7.0	1.28	7.31		
28/3/94	0.7	7.18	1.17	7.34		
6/4/94	0.71	7.2				
18/4/94	0.75	7.19				
25/4/94					1.7	7.72
3/5/94	0.67	7.16				
7/5/94					1.69	7.71
16/5/94	0.71	7.18				
23/5/94					1.66	7.7
13/6/94	0.76	7.19				
27/6/94					1.71	7.71
4/7/94			1.1	7.37		
11/7/94	0.72	7.18				
18/7/94			1.16	7.38		
25/7/94					1.7	7.71
11/8/94			1.21	7.38		
23/8/94	0.76	7.17				
24/8/94	0.72	7.15				
12/9/94	0.74	7.17				
19/9/94					1.67	7.72
3/10/94	0.78	7.16				
14/10/94					1.64	7.7
17/10/94			1.19	7.34		
10/11/94	0.72	7.19				
22/11/94					1.68	7.7
30/11/94	0.76	7.22				
29/12/94	0.78	7.18				
3/1/95					1.58	7.71
9/1/95	0.74	7.18				
16/1/95					1.67	7.71
18/1/95	0.72	7.18			1.14	7.35
23/1/95	0.75	7.18				
30/1/95					1.66	7.7
23/2/95	0.69	7.19				
28/2/95	0.75	7.16				
4/3/95	0.73	7.19				
13/3/95					1.7	7.7
27/3/95	0.75	7.18	1.19	7.35		
3/4/95	0.78	7.17				

Date	Lo	pH	Med	pH	Hi	pH
13/4/95	0.74	7.21				
19/4/95	0.74	7.18				
1/5/95	0.71	7.18				
15/5/95	0.75	7.16	1.15	7.34	1.62	7.71
22/5/95	0.75	7.17	1.2	7.32	1.69	7.71
31/5/95	0.78	7.18				
6/6/95					1.67	7.72
14/6/95	0.7	7.18				
10/7/95					1.7	7.71
20/7/95	0.73	7.18				
31/7/95			1.22	7.33		
7/8/95					1.62	7.7
1/9/95	0.78	7.11				
4/9/95	0.79	7.15				
13/9/95					1.74	7.69
1/10/95	0.78	7.11	1.18	7.32	1.7	7.65
6/11/95	0.83	7.11				

Stability of samples stored in a fridge at 4^0C

An assessment of the stability of ionized calcium when samples were stored in a fridge at 4^0C was also carried out. This was done because, although the samples were brought to the machine immediately, occasionally the machine malfunctioned or had to be calibrated. In that event, samples were stored in the fridge until the machine was functional up to a maximum of half an hour. The samples were found to be stable for up to 45mins (table 1).

Results

The results of the assay are presented separately for each study below.

3.1.4 Bone specific alkaline phosphatase

The assay ran well, without problems. The within run coefficient of variation is between 3.9% and 5.8%. Internal quality control Hi and Lo samples were measured at the beginning of the assay. The results were within the acceptable limits and are presented below. The acceptable ranges have been mislaid.

Hi 70 U/L

Lo 14 U/L

Results

The results of the assay are presented separately for each study below.

Table 1

	Base	5 mins	10 mins	25 mins	45 mins	60 mins
Patient 1	1.2	1.18				1.11
Patient 2	1.16	1.16				1.11
Patient 3	1.28	1.36	1.34	1.31	1.26	1.26
Patient 4	1.14	1.17				1.14
Patient 5	1.22	1.23		1.2	1.18	1.20
Patient 6	1.16	1.16	1.15	1.15	1.15	1.15
Patient 7	1.23	1.25				1.12
Patient 8	1.24	1.26			1.19	1.19

Serum adjusted ionized calcium (mmol/L) on selected samples repeated over a period of up to one hour. Samples stored in fridge at 4°C. After 60 minutes, 2 of 8 samples were unstable.

3.1.5 Serum 1,25 dihydroxyvitamin D_3

The assay ran well without problems. The within run coefficient of variation is between 5% and 8%. Internal quality control (Hi and Lo) samples were measured at the beginning of the assay. The results were within the acceptable limits and are presented below.

Hi 122 pmol/L (acceptable range: 113 - 174 pmol/L)

Lo 43 pmol/L (acceptable range: 38 - 63 pmol/L)

Results

The results of the assay are presented separately for each study below.

3.1.6 Serum Parathyroid hormone level

The assay ran well without problems. Within run coefficient of variation is between 2.5% and 6%. Internal quality control (Hi and Lo) samples were measured at the beginning of the run. The results were within the acceptable limits and are presented below.

Hi 19.0 pmol/L (acceptable range: 16 - 21 pmol/L)

Lo 4.0 pmol/L (acceptable range: 2.9 - 4.8 pmol/L)

Results

The results are presented for each individual study below.

3.1.7 Serum osteocalcin

The assay ran well. Within run coefficient of variation is between 4.8% and 10%. Internal (Hi and Lo) quality control samples were measured before and after the assay. The results were within the acceptable limits and are presented below. The acceptable limits have been mislaid.

Hi	21 ng/ml	17 ng/ml
Lo	6 ng/ml	4 ng/ml

Results

The results of the assay are presented for each individual study below.

3.1.8 Serum C-terminal propeptide of type I collagen

The assay ran well, without problems. The within run coefficient of variation is between 5.5% and 6.8%. Internal quality control (Hi and Lo) samples were measured at the beginning and end of the assay, were within the acceptable limits and are presented below. The acceptable ranges have been mislaid.

Hi	341 ng/ml	275 ng/ml
Lo	55 ng/ml	55 ng/ml

Results

The results are presented for each individual study below.

3.1.9 Urine hydroxyproline

The assay ran well. Internal quality control samples (Hi and Lo) were measured at the beginning of the assay and were within the acceptable limits. The results are presented below.

Hi 79μmol/L (acceptable range: 71 - 107μmol/L)

Lo 13μmol/L (acceptable range: 13 - 19μmol/L)

Results

The results of the assay are presented for each individual study below.

3.1.10 Urine deoxypyridinoline cross-links

The assay performed satisfactorily. The within run coefficient of variation is between 3.6% and 9.5%. Internal quality control samples (Hi and Lo) were measured at the beginning of the assay and were within the acceptable limits. The results are presented below. The acceptable ranges have been mislaid.

Hi 92 nmol/L

Lo 16 nmol/L

Results

The results of the assay are presented for each individual study below.

3.1.11 Non-isotopic strontium absorption test

The assay performed satisfactorily. Full precision and recovery experiments were performed. Coefficient of variation over the working range is between 5 and 8%. Duplicate samples from 5 patients were measured by Dr JP Day, Dept of Chemistry, University of Manchester, for method validation (table 4).

3.2 SECTION I Pilot Study

3.2.1 Results

Fourteen patients, 4 male and 10 female, were recruited. Mean age was 48 years with a range of 21 to 79 years. The regime was very effective therapeutically (Figure 4). Mean PASI at baseline was 18.9 (range 7.2 to 53.7) and fell to 6.7 (1.2 to 32.9) after 2 weeks' treatment with calcipotriol. Mean PASI fell somewhat further to 6.2 (1.2 to 24.8) after a final week of treatment with crude coal tar (Table 2).

Mean serum calcium rose from 2.26mmol/L to 2.35mmol/L after 2 weeks ($P<0.001$), and fell in week 3 to 2.29mmol/L (Fig. 5). Mean 24h urine calcium rose from 4.49mmol/24h to 6.74mmol/24h after 2 weeks ($P<0.0001$) and fell in week 3 to 5.1mmol/24h (Fig. 6). Serum 4am(peak) and 9am(trough) PTH levels were measured in seven patients (Fig. 7). Mean 4am PTH fell from 5.1pmol/L to 2.5pmol/L and mean 9am PTH fell from 4.1pmol/L to 2.0pmol/L after 2 weeks ($P<0.01$). Mean 4am PTH rose to 3.75pmol/L and mean 9am PTH rose to 3.2pmol/L in week 3. Mean serum 1,25 dihydroxyvitamin D_3 levels were measured by HPLC in four patients (Fig. 8) in whom they fell from 23.2 to 10.8pg/ml after 2 wks ($P<0.05$) rising in week 3 to 22.1pg/ml. Serum calcipotriol levels were measured in the same four patients (Fig. 9). Mean serum calcipotriol concentration rose from undetectable levels to a mean of 11.3pg/ml falling in week 3 to 4.05pg/ml. In one patient, treatment with calcipotriol had inadvertently been started prior to baseline measurements being taken and so calcipotriol was just detectable in serum.

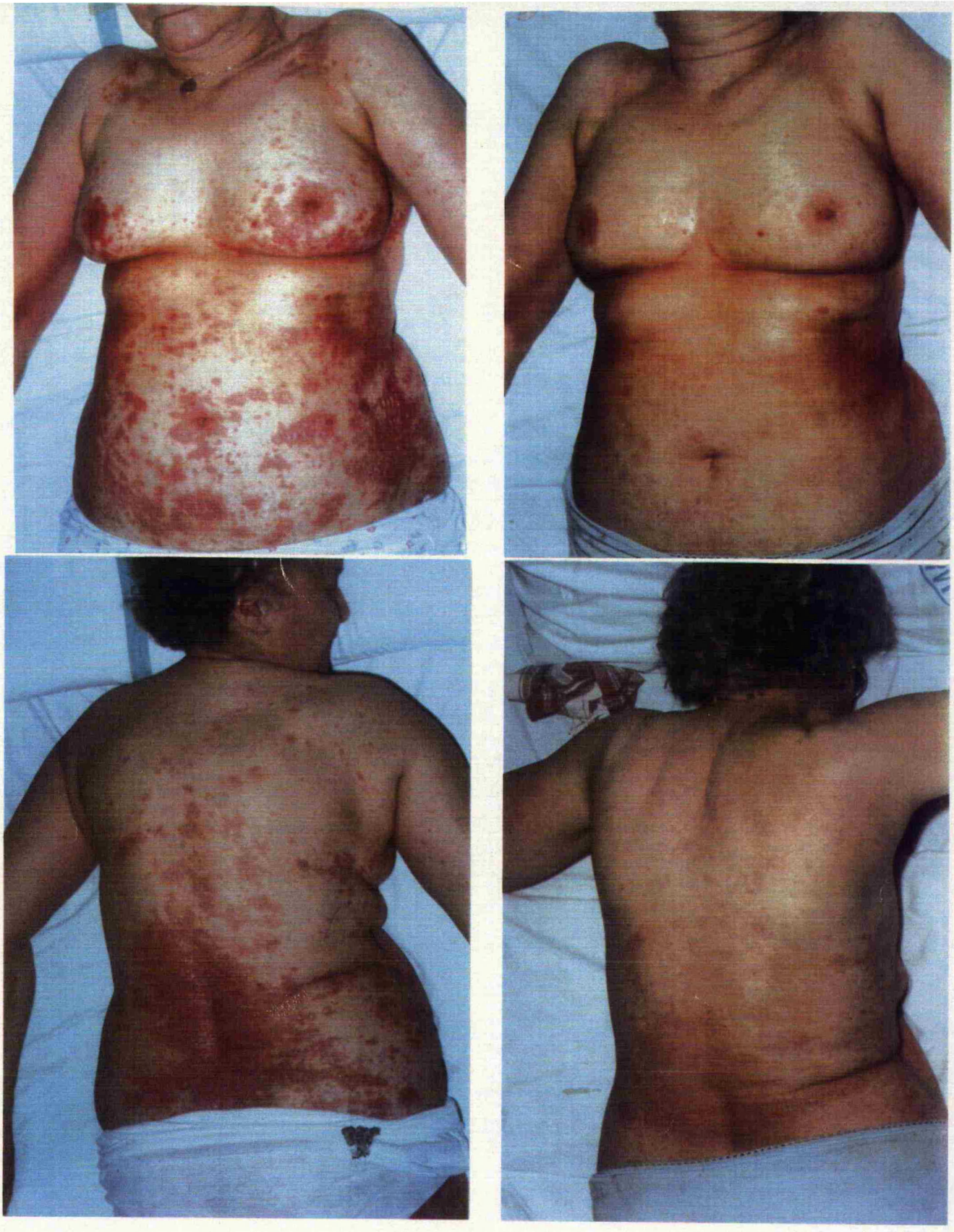


Figure 4. Patient with extensive chronic plaque psoriasis before and after treatment with high dose topical calcipotriol (pilot study).

Table 2

	Base	Wk2	Wk3
Patient 1	18.9	4.6	3.2
Patient 2	14.7	2.2	4.9
Patient 3	7.2	2.8	2.2
Patient 4	32.4	12.1	11.8
Patient 5	22.4	3.4	1.7
Patient 6	12.9	5.3	4.9
Patient 7	22.2	5.9	8.2
Patient 8	9.6	1.2	1.2
Patient 9	8.2	2.2	2.8
Patient 10	11.6	6.7	6.7
Patient 11	53.7	32.9	24.8
Patient 12	28	6.1	*
Patient 13	13.4	5.6	5.6
Patient 14	9	2.5	2.2
Mean	19	6.7	6.2
SEM	2.9	1.2	1.2

Individual PASI in pilot high dose study before treatment (Base) after 2 weeks treatment with calcipotriol (Wk2) and one week after withdrawal while treating psoriasis with tar (Wk3). *Result not available.

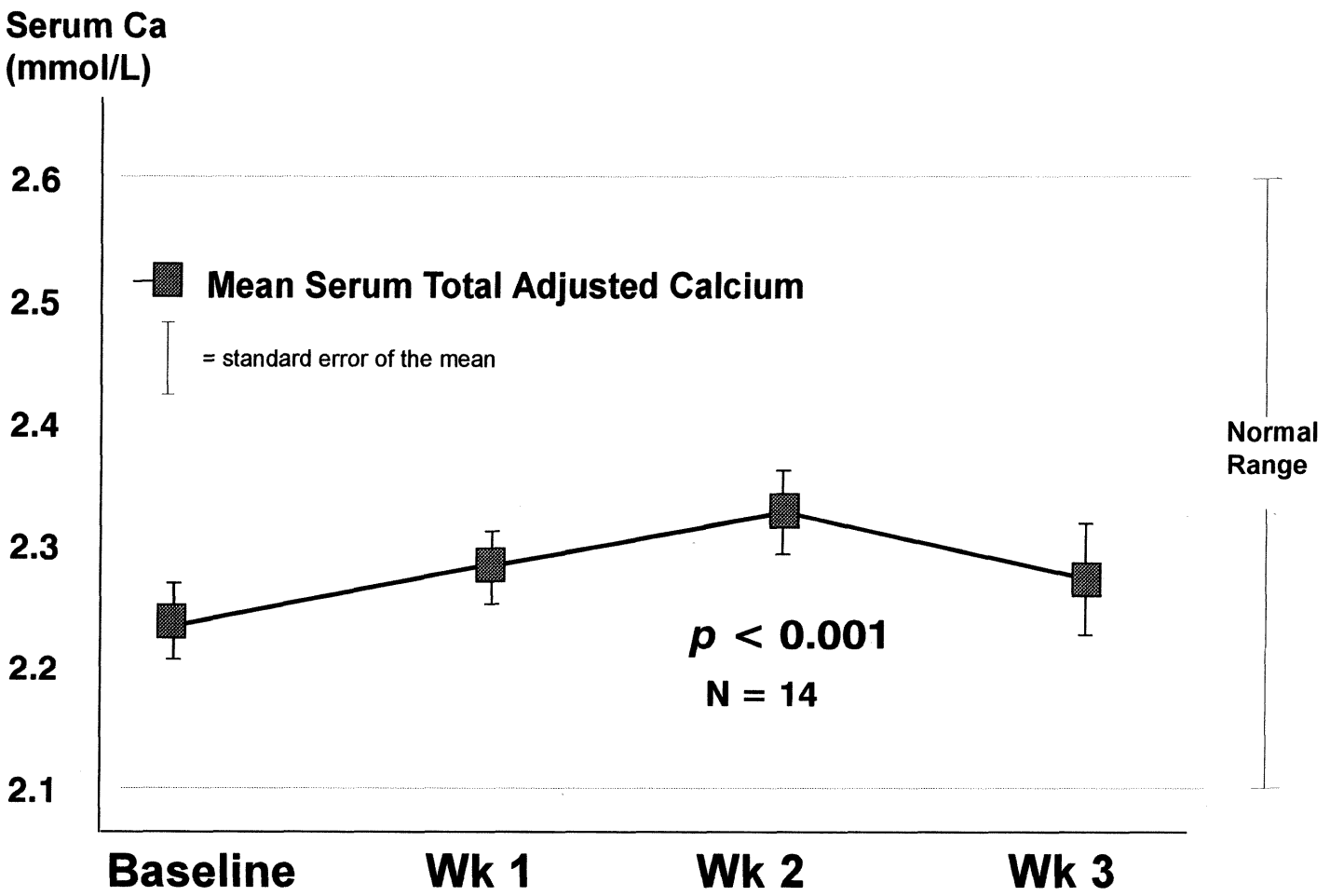


Figure 5. Mean and SEM serum total adjusted calcium at baseline, after treatment with 200g of calcipotriol (50μg/g) ointment over 1 week (Wk1), after treatment with 300g over a second week (Wk2) and 1 week after withdrawal of treatment (Wk3).

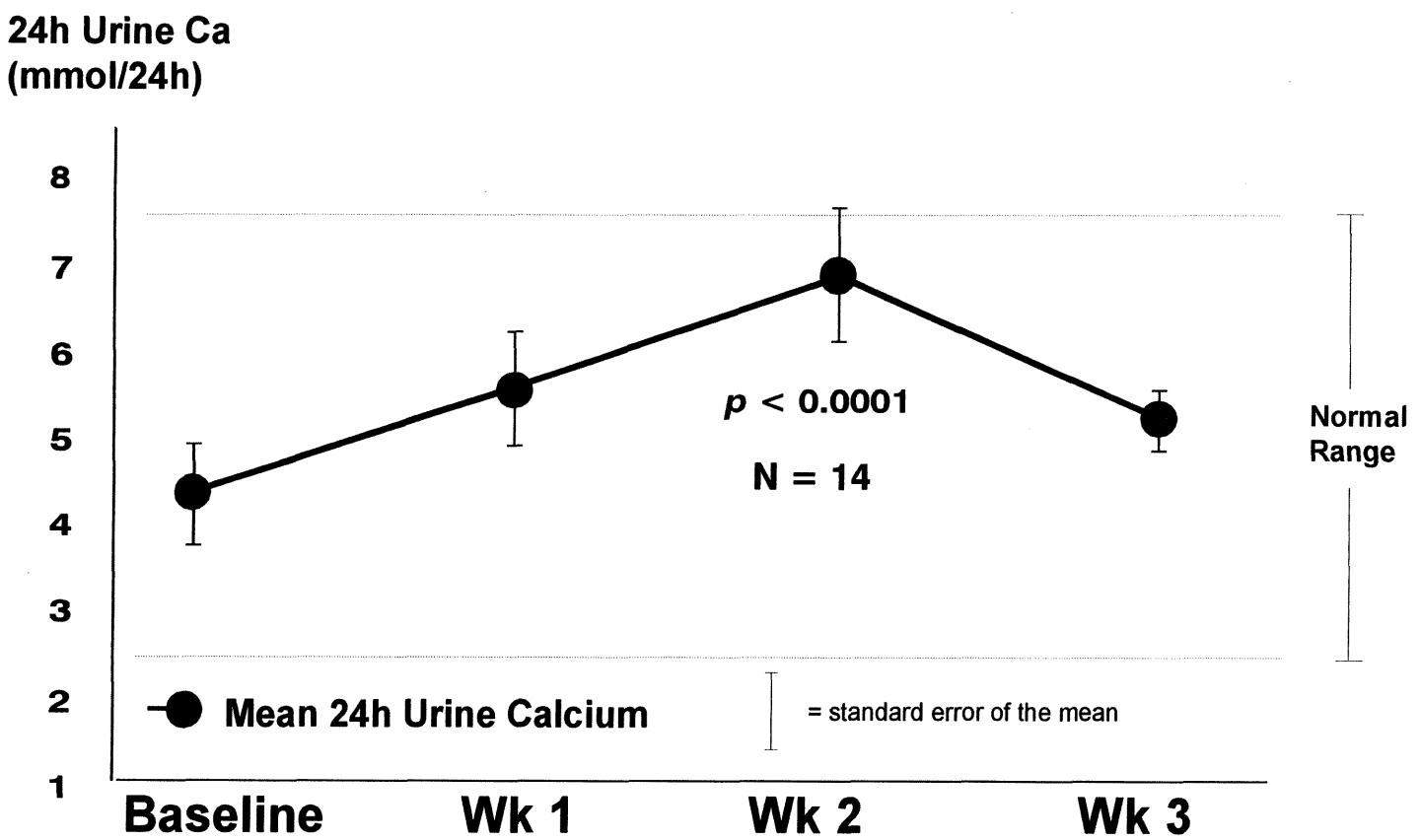


Figure 6. Mean and SEM 24 hour urine calcium at baseline, after treatment with 200g of calcipotriol (50μg/g) ointment over 1 week (Wk1), after treatment with 300g over a second week (Wk2) and 1 week after withdrawal of treatment (Wk3).

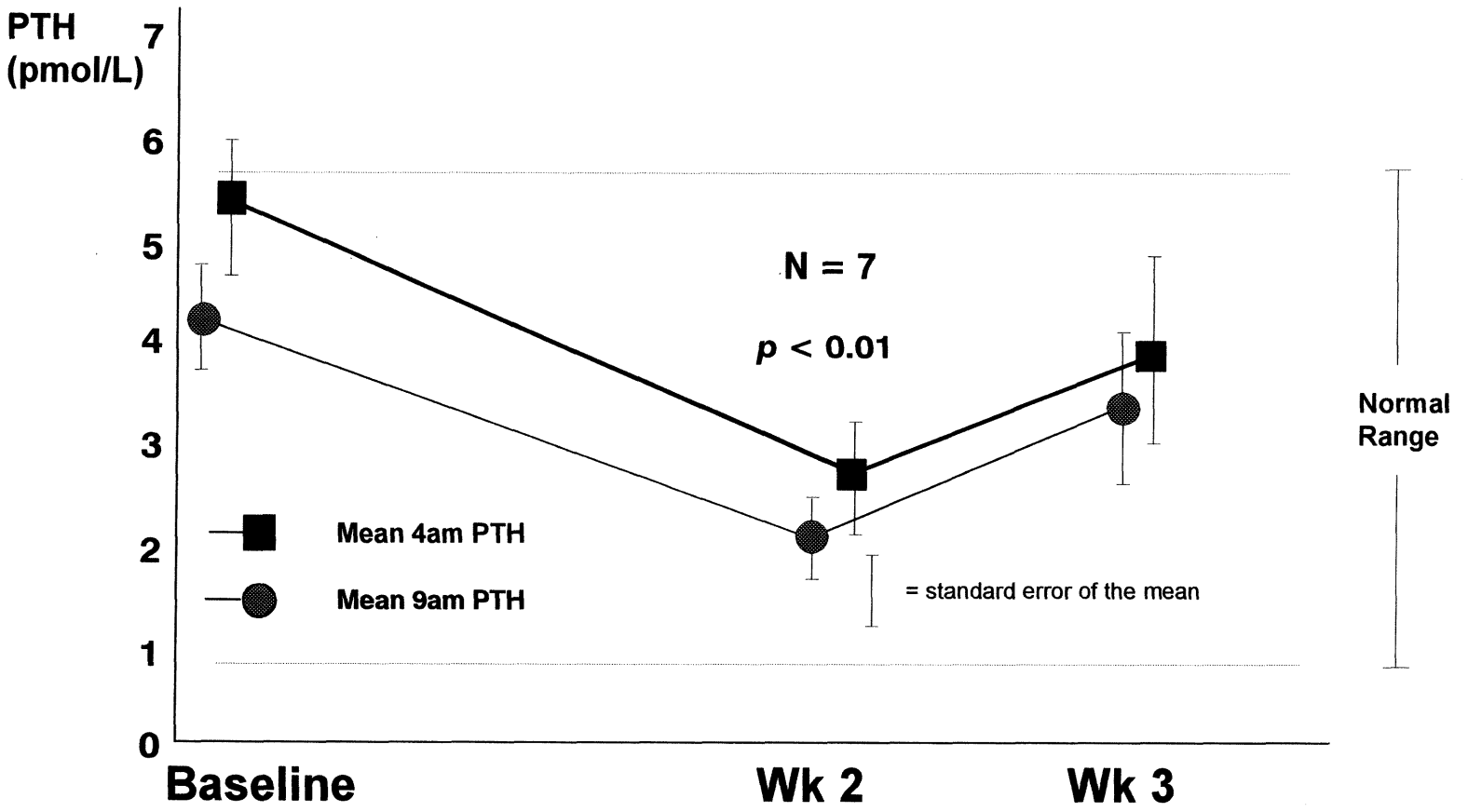


Figure 7. Mean and SEM serum 4am and 9am parathyroid hormone (PTH) levels measured in 7 of the 14 patients at baseline, after treatment with 200g of calcipotriol (50μg/g) ointment over 1 week followed by 300g over a second week (Wk2) and 1 week after withdrawal of treatment (Wk3).

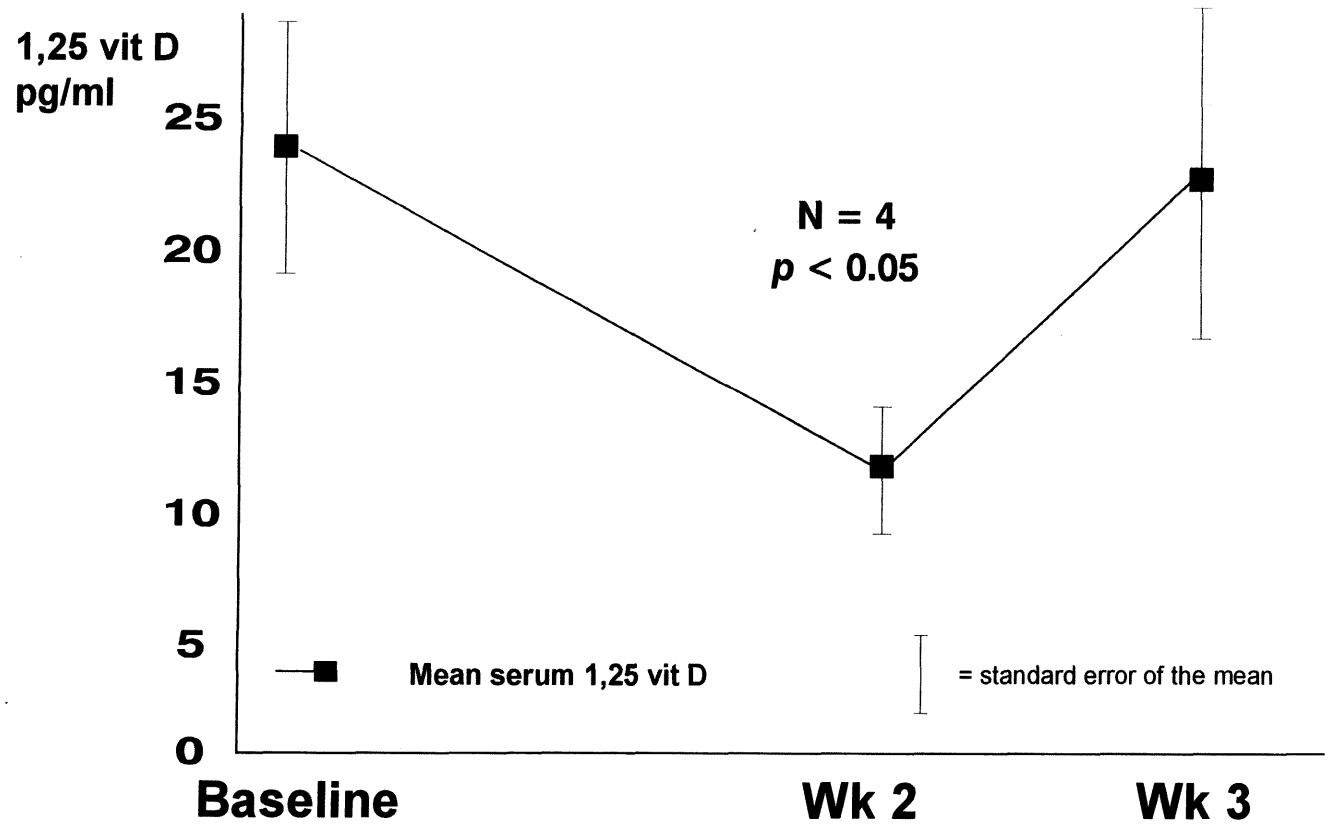


Figure 8. Mean and SEM serum 1,25 dihydroxyvitamin D_3 (1,25vitD) levels measured by Dr LeVan in 4 of the 14 patients at baseline, after treatment with 200g of calcipotriol (50μg/g) ointment over 1 week followed by 300g over a second week (Wk2) and 1 week after withdrawal of treatment (Wk3).

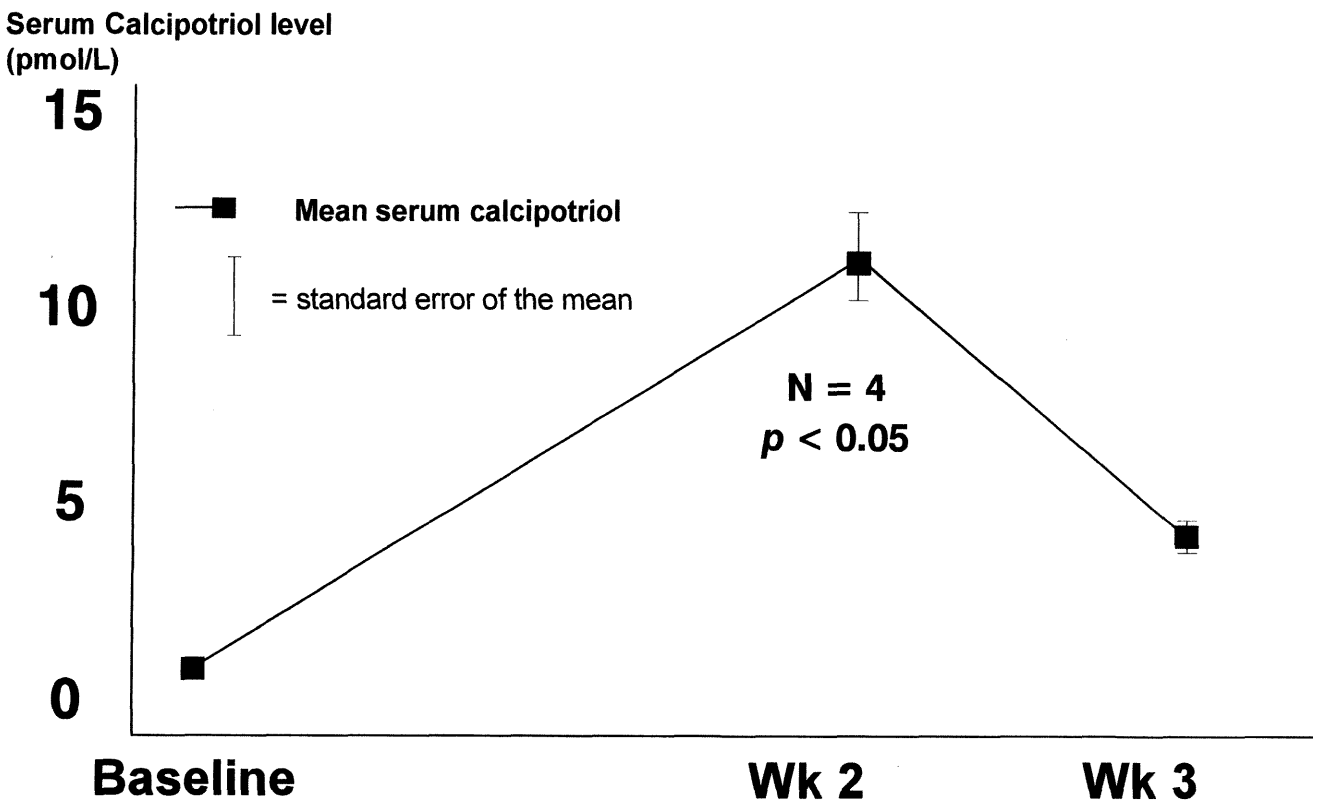


Figure 9. Mean and SEM serum calcipotriol levels measured in 4 of the 14 patients at baseline, after treatment with 200g of calcipotriol (50 g/g) ointment over 1 week followed by 300g over a second week (Wk2) and 1 week after withdrawal of treatment (Wk3). Calcipotriol was just detectable at baseline in one patient who had inadvertantly received calcipotriol before the baseline blood sample was taken.

3.2.2 Discussion

The results of the pilot study revealed that topical calcipotriol could affect systemic calcium homeostasis, if used in high doses. Serum total calcium and 24h urine calcium rose significantly while serum PTH and 1,25 dihydroxyvitamin D_3 fell during treatment with calcipotriol. The most likely explanation for these changes was a rise in intestinal absorption of calcium leading to a rise in serum calcium with subsequent inhibition of PTH production. The fall in PTH levels would then reduce activation of 25 dihydroxyvitamin D_3 to 1,25 dihydroxyvitamin D_3. Both the rise in serum calcium and the fall in serum PTH would result in a rise in 24h urine calcium. Other possible explanations for the findings included increased release of calcium from bone and a direct effect on PTH production by calcipotriol. Parallel effects on serum phosphate might also be important. Vitamin D enhances the intestinal absorption of phosphate and, therefore, calcipotriol would be expected to have a similar effect. A rise in serum phosphate would have inhibitory effects on 1 alpha hydroxylase thus inhibiting activation of vitamin D_3. One or more of these mechanisms could give rise to the changes observed. The effects appeared to be dose dependent in that serum and urine calcium rose initially on 200g per week and rose further on 300g per week. The effects were readily reversible returning towards normal 1 week after withdrawal of calcipotriol.

However, the parameters measured had not returned to normal in all patients and calcipotriol was still detectable in serum 1 week after stopping treatment. This was surprising given the rapid metabolism of calcipotriol in animals and suggested either a saturation effect of the enzymes metabolising calcipotriol or that calcipotriol was being stored perhaps in body fat and later released as serum levels began to fall.

Thus calcipotriol, at high doses could cause hypercalciuria and a significant rise in serum calcium. Apart from the concern about possible hypercalcaemia, hypercalciuria could lead to the development of renal calculi particularly with long term use of calcipotriol. The possibility of an adverse effect on bone mineralization had also to be considered.

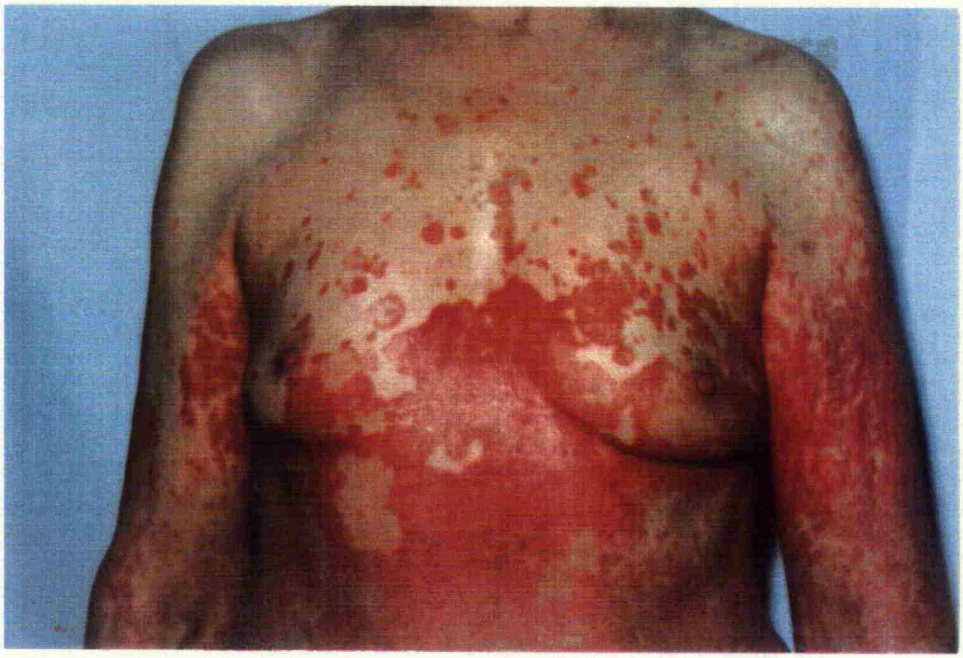
Two possible confounding factors arose following this study. Change in diet and immobilisation in hospital could, at least in part, explain some of the effects on systemic calcium homeostasis. It was felt that these factors were unlikely to have greatly contributed to the alterations observed as the patients were actively encouraged to mobilize while in hospital and the diet in hospital is not high in calcium (approx. 800mg/d). Furthermore, the rapid return of systemic calcium homeostasis towards normal after withdrawal of calcipotriol indicated a true effect of the drug. Nonetheless, it was felt that mobility and diet would have to be carefully controlled in further in-patient studies.

3.3 SECTION III High Dose Study

3.3.1 Results

16 patients, 9 male and 7 female were recruited. Mean age of the patients was 44 years with a range from 18 to 83 years. This regime was also highly effective therapeutically (Figures 10a and 10b). Mean PASI at baseline was 25.0 (range 13.3 to 41.3) and this fell to 7.9 (3.3 to 17.4) after 2 weeks' treatment with calcipotriol (Table 3).

Assessment of intestinal absorption revealed a dose dependent increase in strontium absorption at the end of the study. Although there was no significant alteration in strontium levels in the group as a whole at the end of the study, change in strontium levels correlated directly ($P < 0.05$; $r = 0.7$) with dose of calcipotriol in grams of calcipotriol (50μg/g) ointment per kg body weight per week (Fig 11, Table 4). Five of the patients developed hypercalcaemia (Fig 12, Table 5) and in that group, there was a significant rise in strontium levels (from 2305 to 3501μg/L, $P = 0.015$). There was considerable variation both within patients and between different patients which raised questions about the reliability of the test. The full list of results is tabulated in Table 4. Samples from 5 patients were assessed by Dr J Day, Manchester University for the purposes of quality control.



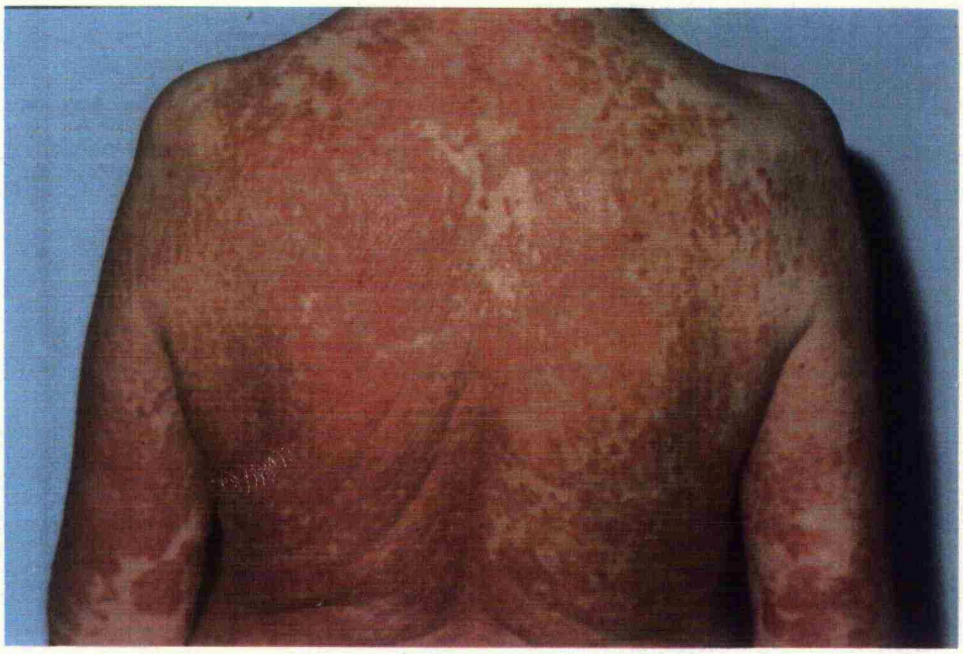


Figure 10a. Patient with extensive chronic plaque psoriasis before treatment with high dose topical calcipotriol.

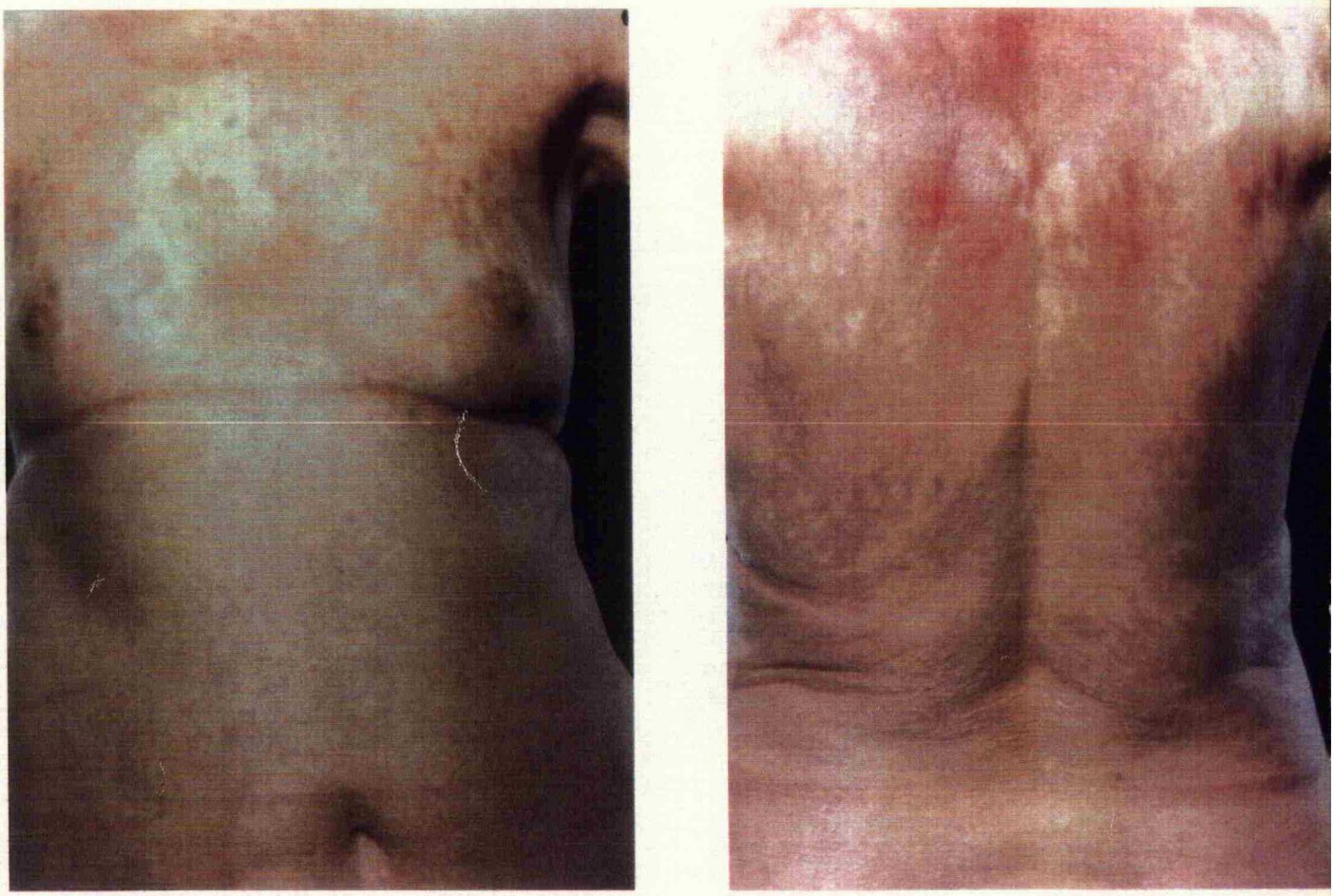


Figure 10b. Patient with extensive chronic plaque psoriasis after treatment with high dose topical calcipotriol.

Table 3

	Base	End
Patient 1	27	9.9
Patient 2	33.3	6.2
Patient 3	*	6
Patient 4	30.6	9.1
Patient 5	19.2	12.2
Patient 6	36.6	8.7
Patient 7	41.3	17.4
Patient 8	29.6	6.6
Patient 9	22.2	*
Patient 10	21.6	3.7
Patient 11	27	8.7
Patient 12	16.8	5.4
Patient 13	13.8	7.5
Patient 14	*	7.5
Patient 15	15.6	3.3
Patient 16	15	6
Mean	25	7.9
SEM	2.3	0.9

Individual PASI in second high dose study before treatment (Base) and after 2 weeks' treatment with high dose calcipotriol (Wk2). *Result not available.

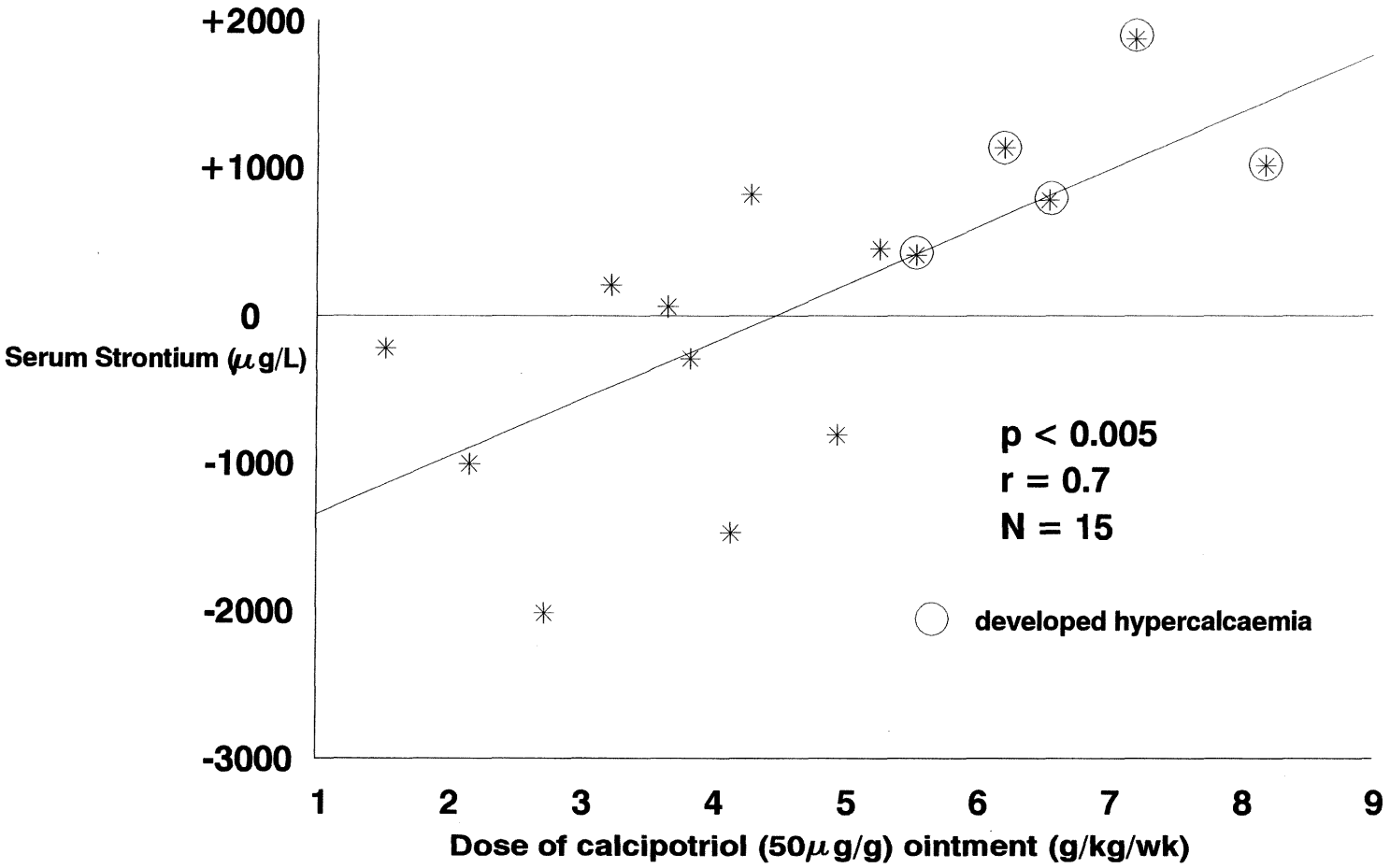


Figure 11. Change in serum strontium (4hrs after ingestion of 2.5mmol of stable strontium chloride) from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 4

	Leicester				Manchester			
	Pre Rx		Post Rx		Pre Rx		Post Rx	
	Base	4hrs	Base	4hrs	Base	4hrs	Base	4hrs
Patient 1	24	1635	45	1840	11	2399	13	2653
Patient 2	37	824	31	1833	*	*	*	*
Patient 3	0	1005	0	1415	*	*	*	*
Patient 4	*	*	*	*	*	*	*	*
Patient 5	0	705	0	414	*	*	*	*
Patient 6	0	2280	0	808	*	*	*	*
Patient 7	36	2780	272	3555	10	3719	169	5190
Patient 8	0	2403	0	3534	*	*	*	*
Patient 9	0	3214	41	5084	*	*	*	*
Patient 10	31	2021	76	1210	*	*	*	*
Patient 11	43	698	37	1510	13.2	791	23	2119
Patient 12	19	968	26	1029	*	*	*	*
Patient 13	10	1500	10	1950	10	2291	10	2776
Patient 14	0	2952	0	935	*	*	*	*
Patient 15	46	2945	100	1936	41	4961	86	2952
Patioent 16	28	1099	39	881	*	*	*	*
Mean	18	1802	45.1	1862	17	2832	60.2	3138
SEM	4.6	234	17.9	328	6.02	706	30.5	532

Serum strontium (μg/L) before (Base) and 4hrs after (4hrs) ingestion of 2.5mmol of stable strontium before (Pre Rx) and after (Post Rx) treatment with high dose topical calcipotriol. Results from Manchester on 5 patients for quality control.

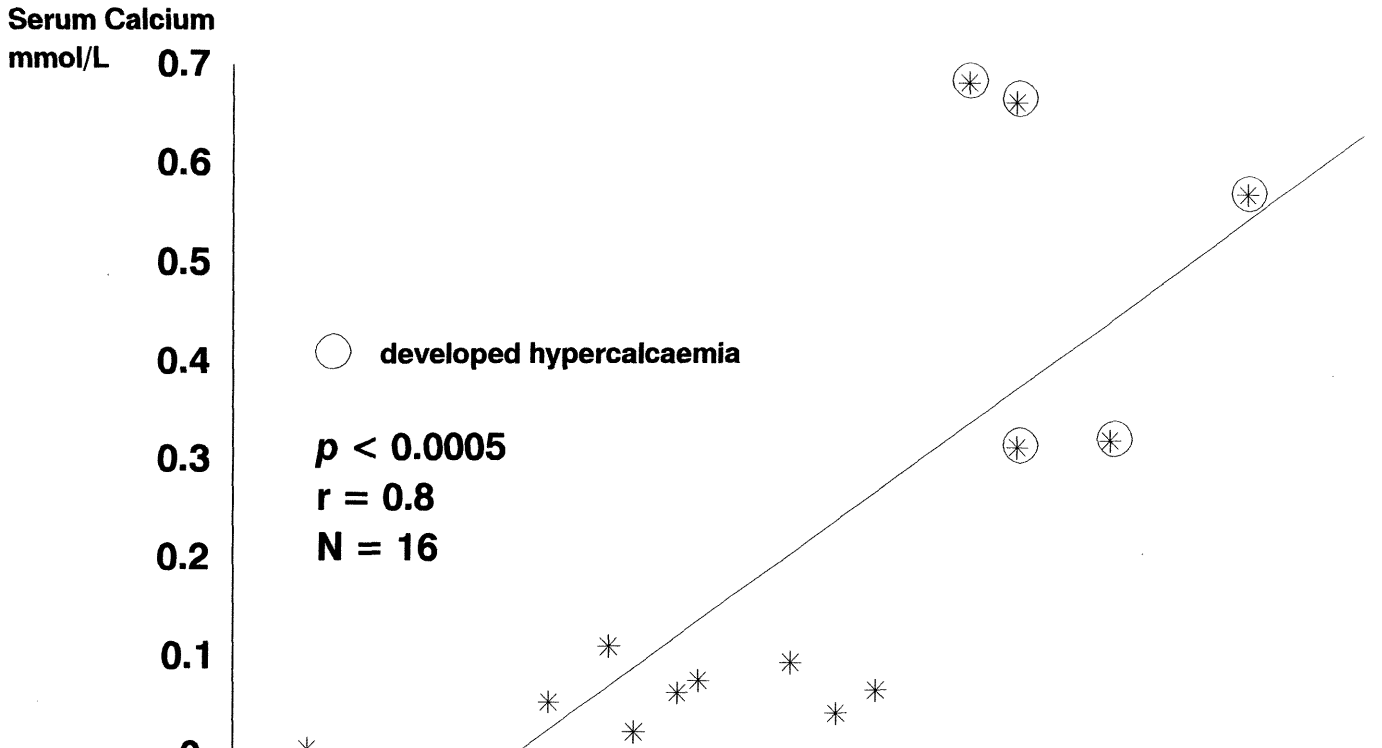


Figure 12. Change in serum total adjusted calcium from baseline to end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 5

Sca	Base	Base	Wk1	Wk1	Wk1	Wk2	Wk2	Wk2	Ica	Base	End
Patient 1	2.3	2.3	2.4	2.4	2.5	2.4	2.4	2.34		1.3	1.31
Patient 2	2.3	2.1	2.4	2.5	2.5	2.8	2.8	*		1.11	1.5
Patient 3	2.5	2.4	2.4	2.5	*	2.5	2.5	2.52		1.22	1.31
Patient 4	2.3	2.2	2.4	2.6	2.9	*	*	*		1.14	1.2
Patient 5	2.4	*	2.3	2.3	2.3	2.4	2.4	2.41		1.35	1.57
Patient 6	2.3	*	2.2	2.2	2.3	2.4	2.3	2.29		1.21	1.21
Patient 7	2.4	2.4	2.5	2.6	2.8	2.7	*	*		1.21	1.38
Patient 8	2.3	*	2.5	*	2.9	*	*	*		1.22	1.49
Patient 9	2.4	2.3	2.5	2.5	2.5	2.7	2.6	2.69		1.32	1.42
Patient 10	2.4	*	2.3	2.3	2.5	2.5	2.5	2.48		1.22	1.39
Patient 11	2.3	2.3	2.3	2.4	2.4	2.4	2.4	2.32		1.25	1.3
Patient 12	2.2	*	2.4	2.5	2.3	2.3	2.3	2.37		1.27	1.29
Patient 13	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.24		1.24	1.27
Patient 14	2.4	*	2.5	2.4	2.4	2.4	2.3	2.33		1.27	1.27
Patient 15	2.2	2.4	2.2	*	2.3	2.4	2.2	2.05		1.2	1.21
Patient 16	2.2	*	2.4	2.2	2.3	2.2	2.3	2.27		*	1.24
Mean	2.3	2.3	2.4	2.4	2.5	2.4	2.4	2.36		1.2	1.33
SEM	0.02	0.02	0.03	0.03	0.06	0.04	0.04	0.04		0.02	0.03

Serum total adjusted calcium (Sca) in mmol/L before treatment (Base) and during treatment (Wk1 and Wk2). Serum ionized calcium (Ica) in mmol/L before treatment (Base) and at the end of treatment (End) in patients applying up to 360g of ointment per week. *Result not available. Patients in italics were Asian.

Assessment of bone turnover revealed no significant alteration at the end of the study. Although there was a significant downward trend (Fig 13, Table 6) in serum total alkaline phosphatase (from 88.8 to 79.4; $P < 0.05$), there was no significant change in any of the specific parameters of bone turnover which were measured. Serum bone specific alkaline phosphatase, serum P1CP, serum osteocalcin, urine hydroxyproline and urine concentration of deoxypyridinoline cross-links remained unchanged (Tables 7,8). The reduction in serum total alkaline phosphatase may be explained by a reduction in hepatic alkaline phosphatase reflecting the fact that psoriatics are known to have a higher intake of alcohol than the general population. A period of abstention from alcohol while in hospital could result in a fall in hepatic alkaline phosphatase.

Other parameters of systemic calcium homeostasis were affected as in the pilot study. Mean serum calcium rose from 2.30 to 2.47mmol/L, five patients becoming hypercalcaemic (Fig. 12, Table 5). Four of these (patients 2, 4, 7 and 8)were withdrawn before they had received a full 2 weeks of treatment. Mean serum ionized calcium rose from 1.23 to 1.34mmol/L (Fig. 14, Table 5), mean 24h urine calcium from 4.29 to 6.45mmol/24h (Fig. 15, Table 9), 5 patients becoming hypercalciuric. Mean serum phosphate rose from 1.17 to 1.46mmol/L (Fig. 16, Table 10), 11 patients becoming hyperphosphataemic, and mean fasting urine calcium rose from 1.89 to 2.91mmol/L (Table 11). Mean 4am and 9am PTH fell from 3.7pmol/L and 3.2pmol/L to 1.3pmol/L and 1.6pmol/L, 6 patients becoming biochemically hypoparathyroid (Fig. 17, Table 12).

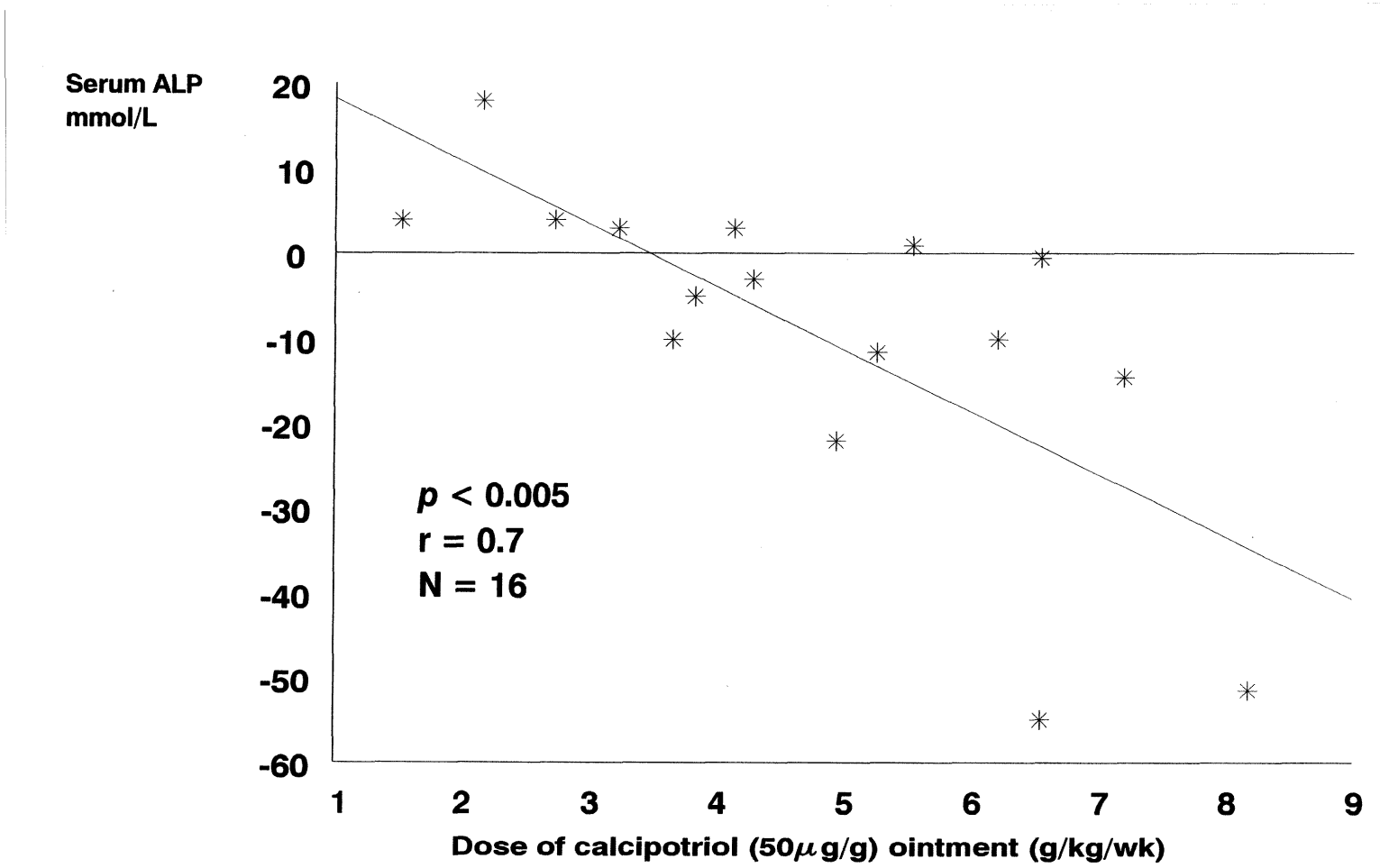


Figure 13. Change in serum total alkaline phosphatase from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 6

	Base	Base	Wk1	Wk1	Wk1	Wk2	Wk2	Wk2
Patient 1	61	49	55	55	54	52	63	58
Patient 2	200	147	154	138	112	120	122	*
Patient 3	121	*	120	110	115	113	114	122
Patient 4	61	58	55	57	59	*	*	*
Patient 5	84	*	82	91	82	81	83	79
Patient 6	77	*	80	80	80	78	80	80
Patient 7	92	*	90	87	82	37	*	*
Patient 8	70	*	70	*	60	*	*	*
Patient 9	57	58	61	59	56	59	52	43
Patient 10	121	*	113	132	120	122	112	99
Patient 11	69	69	68	62	63	66	66	66
Patient 12	91	*	95	87	94	92	87	81
Patient 13	61	64	52	63	62	55	53	51
Patient 14	106	*	106	103	106	105	108	110
Patient 15	106	110	122	*	115	130	132	126
Patient 16	73	*	78	82	80	77	83	77
Mean	91	79	88	86	84	85	89	82.7
SEM	9.3	15	7.3	7.2	5.9	7.9	7.4	8.11

Serum total alkaline phosphatase in patients applying high dose topical calcipotriol before (Base) and during (Wk1, Wk2) treatment. *Result not available.

Table 7

	P1CP		Osteocalcin		BS-ALP		Hyprol	
	Base	End	Base	End	Base	End	Base	End
Patient 1	110	88	13	15	16	13	*	*
Patient 2	198	154	5	13	22	16	*	*
Patient 3	88	55	17	20	22	23	186	83.6
Patient 4	88	132	13	10	12	12	237.6	*
Patient 5	99	77	6	10	26	34	129.6	36.3
Patient 6	77	66	13	10	18	15	182.6	89.6
Patient 7	110	88	18	23	14	16	*	*
Patient 8	99	66	7	13	9	12	*	*
Patient 9	121	143	19	38	18	13	348.8	324.8
Patient 10	66	99	9	6	21	16	374.9	86.8
Patient 11	143	143	5	7	17	19	*	*
Patient 12	143	198	13	17	37	26	305.9	*
Patient 13	550	583	14	14	16	16	*	*
Patient 14	77	88	10	14	20	21	53	94.4
Patient 15	*	88	17	6	21	20	37.8	158.1
Patient 16	66	*	8	17	18	18	33.8	*
Mean	136	138	12	15	19	18	189	125
SEM	31	33	1.2	2	1.6	1.5	40.2	31.7

Serum procollagen C peptide (P1CP; ng/ml), serum osteocalcin (ng/ml), serun bone specific alkaline phosphatase (U/L) and fasting (am) urine hydroxyproline (Hyprol; µmol/L) in patients applying high dose topical calcipotriol before (Base) and after (End) treatment. Several of the urine samples were lost or not analyzed for all parameters. *Result not available.

Table 8

	X-Links (Base)	X-Links (End)	Base/Creat	End/Creat
Patient 1	41	36	5.9	7.3
Patient 2	*	*	*	*
Patient 3	110	28	8.9	7.3
Patient 4	*	*	*	*
Patient 5	35	25	7.5	7.6
Patient 6	90	25	5.4	4.5
Patient 7	*	*	*	*
Patient 8	*	*	*	*
Patient 9	74	120	6.8	10.3
Patient 10	*	*	*	*
Patient 11	*	*	*	*
Patient 12	*	*	*	*
Patient 13	*	220	*	12.4
Patient 14	40	24	7.5	4.1
Patient 15	17	58	6.3	6.2
Patient 16	20	19	7.7	6.3
Mean	53.4	61.7	7.0	7.3
SEM	11.3	22.4	0.4	0.9

Fasting (am) urine deoxypyridinoline cross links (X-Links; nmol/L) and deoxypyridinoline/creatinine ratio (nmol/mmol) before treatment (Base) and after 2 weeks' treatment with calcipotriol (End). Several of the morning urine samples were lost or not analysed for cross-links. * Result not available

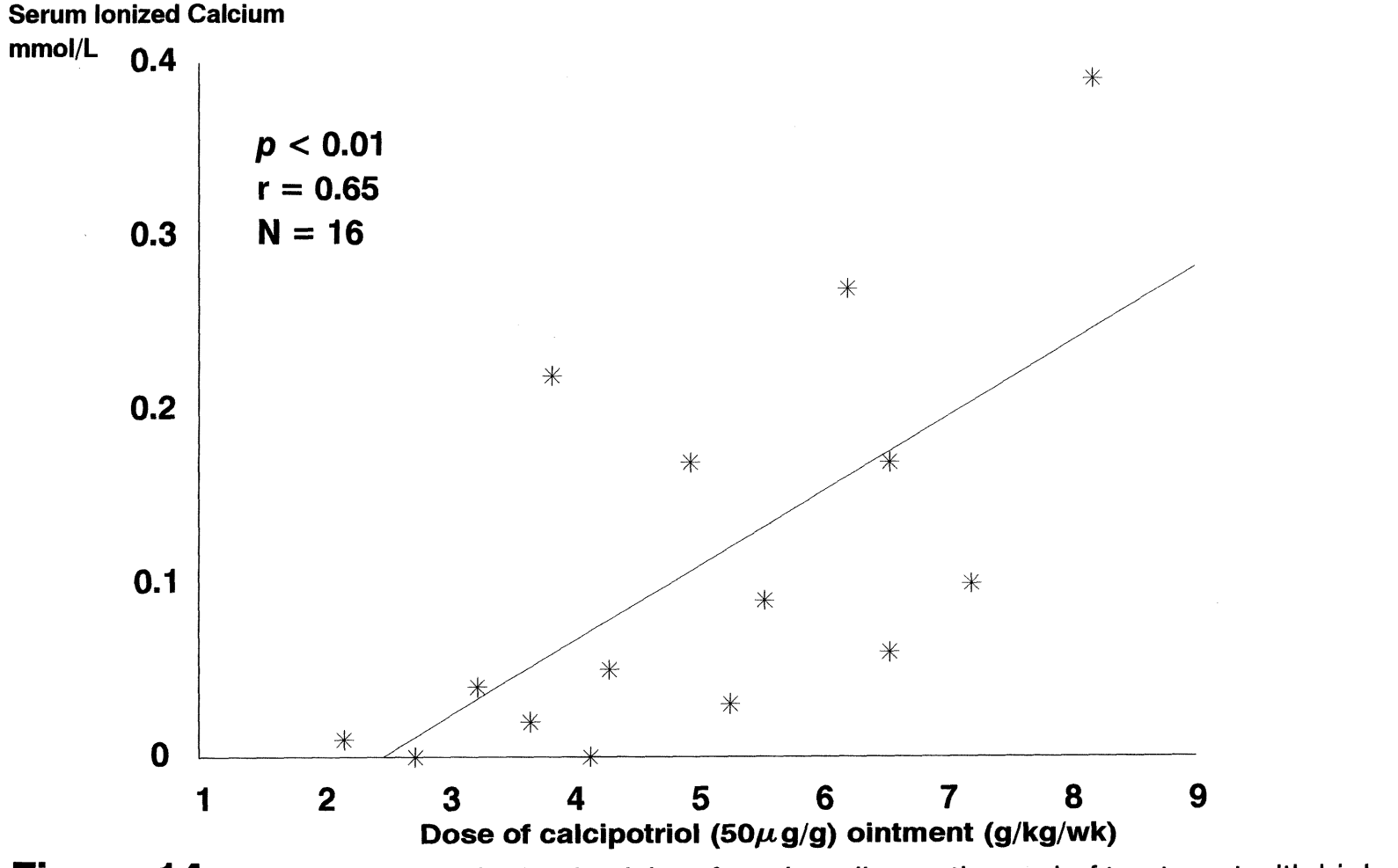


Figure 14. Change in serum ionized calcium from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

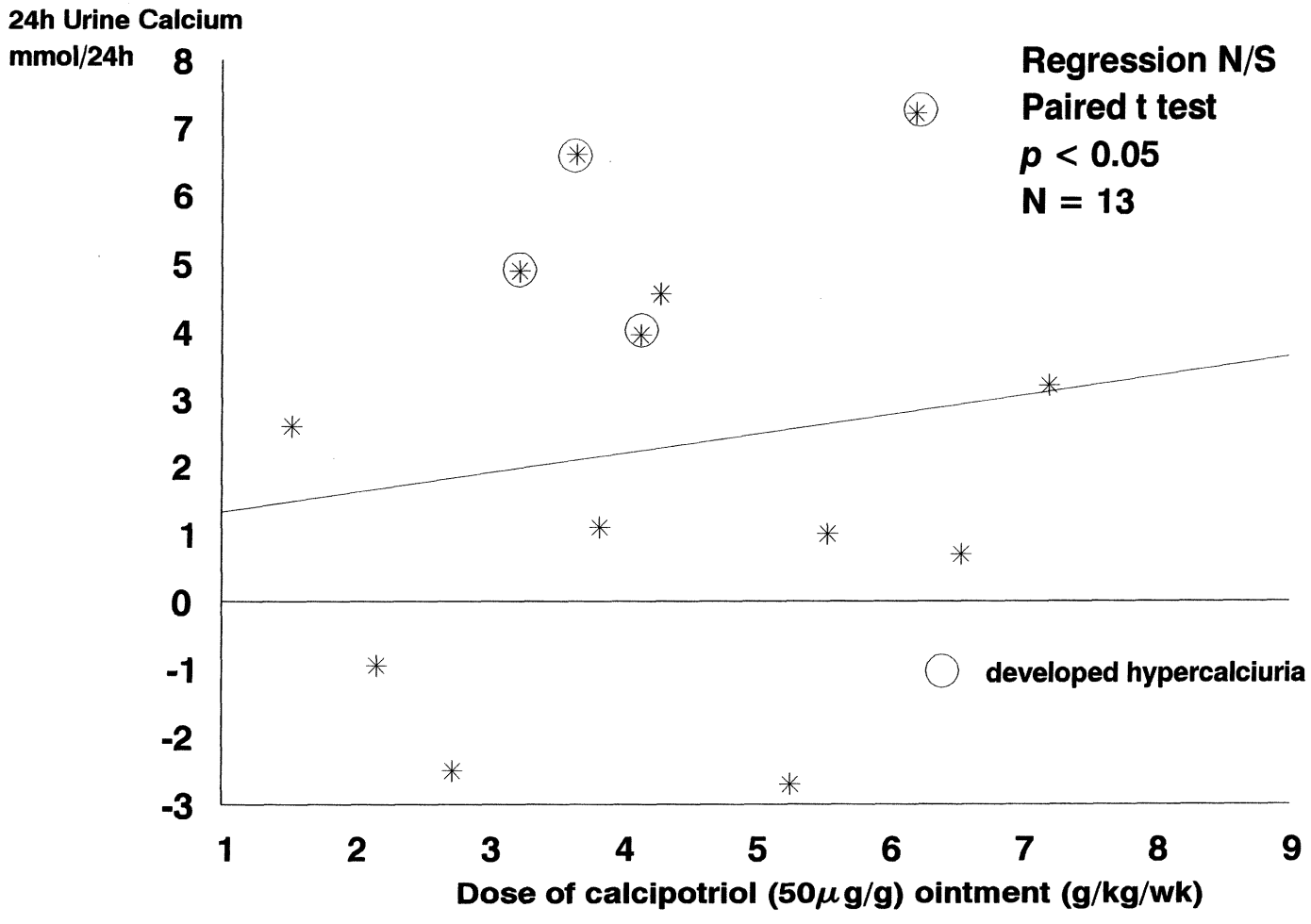


Figure 15. Change in 24h urine calcium from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 9

	Base	Base	Wk1	Wk1	Wk2	Wk2
Patient 1	3.2	*	9.4	12.7	7.26	8.9
Patient 2	*	*	11.6	*	*	11.2
Patient 3	.6	*	1.3	2.3	1.9	1.3
Patient 4	4.6	2.2	*	*	*	*
Patient 5	1.9	*	3.1	1.5	3	3
Patient 6	3.4	*	6.8	10.7	3.6	11.1
Patient 7	2.9	*	3.6	*	*	*
Patient 8	3.1	4.9	10.2	10.5	11.2	*
Patient 9	3.4	*	*	4.8	*	6.6
Patient 10	*	*	1.4	*	1.6	*
Patient 11	2	*	4.1	5.1	7.3	5.8
Patient 12	5.9	6.9	14.5	8.5	7.5	13
Patient 13	9	*	3.2	6.1	5.2	7.4
Patient 14	14	*	8.9	18.2	12.9	10.1
Patient 15	5.3	3	*	2.6	3.2	*
Patient 16	1.7	*	3.9	4.3	*	*
Mean	4.4	4.3	6.3	7.3	5.9	7.84
SEM	0.9	1	1.2	1.4	1.1	1.2

24h Urine calcium (mmol/24h) before treatment (Base) and during treatment (Wk1 and Wk2) in patients applying up to 360g of ointment per week. Several samples were incomplete collections and were therefore unsuitable for analysis of 24h urine calcium. *Result not available.

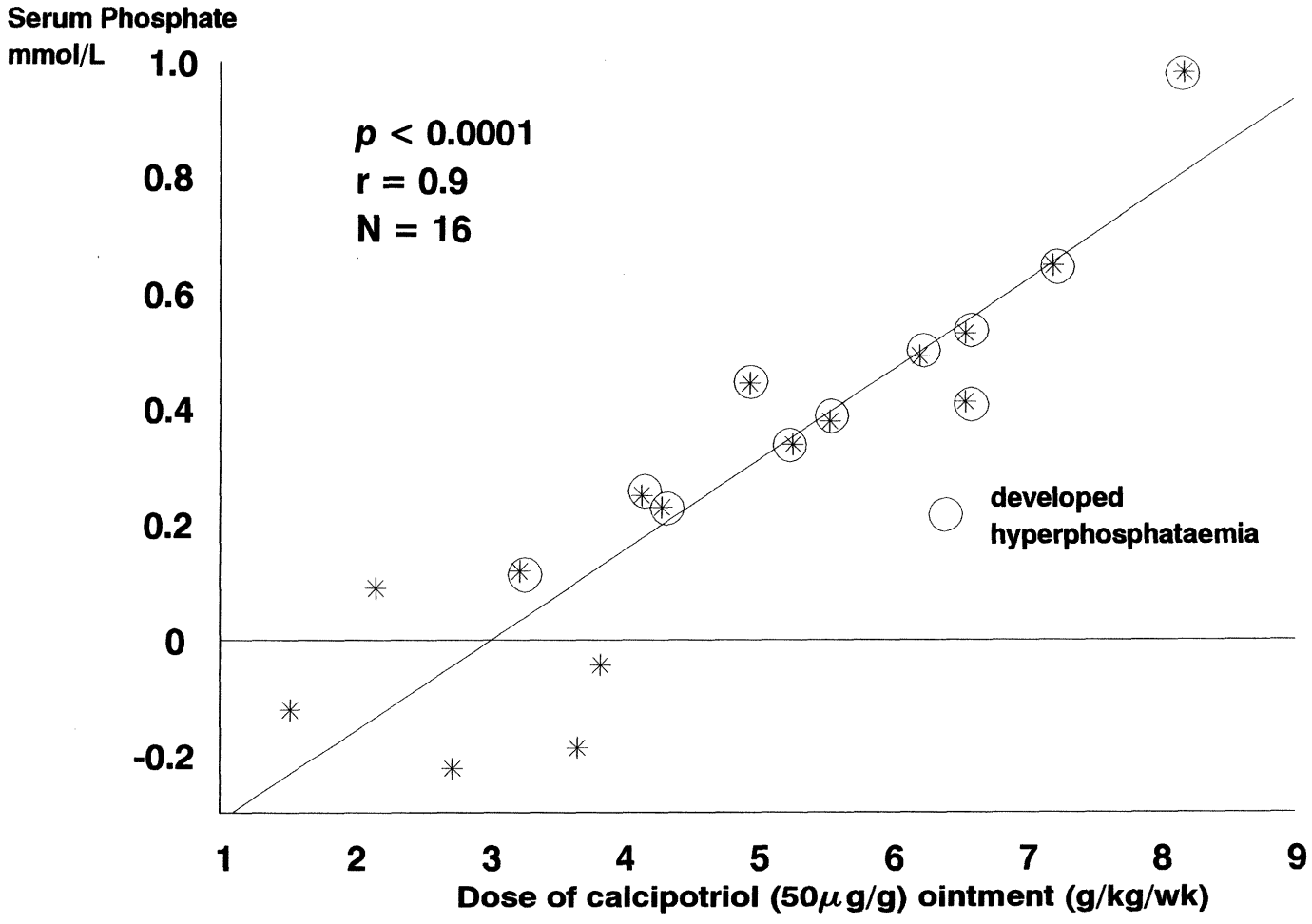


Figure 16. Change in serum phosphate from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 10

	Base	Base	Wk1	Wk1	Wk1	Wk2	Wk2	Wk2
Patient 1	1.3	1.2	1.1	1.5	1.4	1.4	1.3	1.5
Patient 2	0.9	0.9	1.2	1.4	*	2	1.75	*
Patient 3	1.3	1.1	1.3	1.5	*	1.4	1.72	1.51
Patient 4	1.1	0.9	1.2	1.6	1.4	*	*	*
Patient 5	1.3	*	1.1	1.1	1	1.3	1.17	1.26
Patient 6	1	*	1.5	1.1	0.9	1.1	1.31	1.47
Patient 7	1.1	*	1.1	1.2	1.2	1.6	*	*
Patient 8	1.4	*	1.6	*	1.9	*	*	*
Patient 9	1.4	1.3	1.6	1.9	1.7	1.7	2.09	2.13
Patient 10	0.9	*	0.9	1.3	1.3	1.5	1.44	1.23
Patient 11	1.1	1.6	1.1	1.4	1.1	1.6	1.41	1.72
Patient 12	1.5	*	1.5	1.4	1.3	1.3	1.4	1.19
Patient 13	1	0.9	1.3	1.3	1.5	1.5	1.07	1.29
Patient 14	1.3	*	1.3	1	1.4	1.1	0.99	1.2
Patient 15	0.7	1.1	0.9	0.8	0.8	*	0.87	1.05
Patient 16	1.3	*	1.2	1.2	1.1	1	1.12	1.24
Mean	1.2	1.1	1.3	1.3	1.3	1.4	1.36	1.40
SEM	0.1	0.1	0.1	0.1	0.1	0.1	0.09	0.09

Serum Phosphate (mmol/L) in patients applying high dose topical calcipotriol before (Base) and during (Wk1, Wk2) treatment. Patients applied up to 360g of ointment per week for a maximum of 2 weeks. Patients 2, 4, 7 and 8 stopped treatment early due to the development of hypercalcaemia. *Result not available

Table 11

Fasting (am) Urine	Calcium		Creatinine		Phosphate		Calcium Ex Index		Ca/Crt Ratio	
	Base	End	Base	End	Base	End	Base	End	Base	End
Patient 1	1.29	3.36	6.9	4.9	4.8	16.22	.19	.69	16.2	62.4
Patient 2	.5	5.5	4.7	*	4.5	14.3	.11	*	6.9	*
Patient 3	.77	1.5	12.4	3.84	3.71	20	.06	.39	7.1	44.1
Patient 4	*	*	7.2	*	*	*	*	*	*	*
Patient 5	1.24	1.21	4.69	3.3	16.6	9.51	.26	.37	22.5	30.8
Patient 6	1.15	3.31	16.6	5.6	16.9	9.13	.07	.59	6.4	56.7
Patient 7	2.4	4	47.6	18.2	28.8	4.9	.05	.22	4.1	17.6
Patient 8	2.27	3.3	*	4	7.15	7.3	*	.83	*	69.3
Patient 9	3.89	5.51	10.9	11.6	9.07	31.5	.36	.48	26.4	46.6
Patient 10	2.98	2.89	16.3	6.2	5.63	2.53	.18	.47	*	48.9
Patient 11	*	*	*	*	*	*	*	*	*	*
Patient 12	4.05	*	13.3	*	24	*	.3	*	25.9	*
Patient 13	6.17	6.41	12.9	17.7	21	45.9	.48	.36	35.2	26.4
Patient 14	2.43	2.63	5.3	5.9	8.1	18	.46	.45	40.3	41.9
Patient 15	.42	.6	2.7	9.3	7.37	19.45	.16	.06	14.2	5.8
Patient 16	.3	.6	2.6	3	3.6	5.1	.12	.2	14.3	22.2
Mean	2.1	3.1	12	7.8	11	15.7	0.2	0.4	18	39.4
SEM	0.5	0.5	3	1.5	2.2	3.36	0.03	0.03	3.4	5.5

Fasting (am) urine calcium (mmol/L), creatinine (μmol/L), phosphate (mmol/L), calcium excretion index (μmol/L), and calcium:creatine index (mmol/μmol) in patients applying high dose topical calcipotriol before (Base) and after (End) treatment. Patients applied up to 360g of ointment per week for a maximum of 2 weeks. Patient 11 was unable to provide fasting am urine samples. *Result not available.

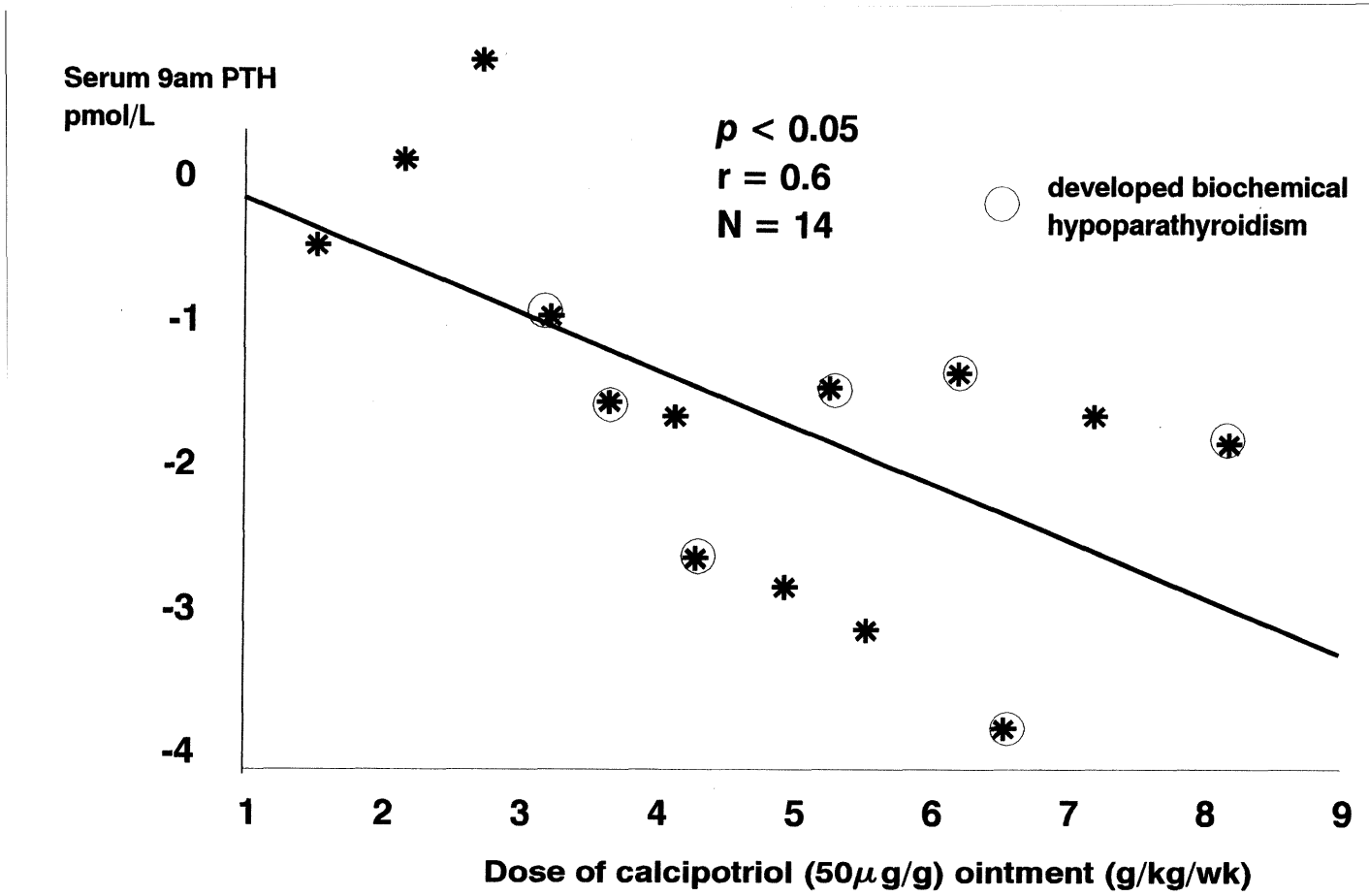


Figure 17. Change in serum 9am parathyroid hormone (PTH) levels from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 12

PTH	Base 4am	Base 9am	End 4am	End 9am	Tm_{PO4}/ GFR	Base	End
Patient 1	1.9	1.5	0.5	0.5		1.5	1.22
Patient 2	4.8	2.4	0.5	0.5		*	*
Patient 3	7.5	5.3	2.7	2.1		1.46	1.86
Patient 4	3.6	3.8	*	*		*	*
Patient 5	2.2	1.6	*	*		1.04	1.02
Patient 6	4	2.7	0.9	1		1.06	1.42
Patient 7	4.5	4.7	0.9	0.8		1.26	1.42
Patient 8	2	1.9	0.5	0.5		*	*
Patient 9	3.6	2.8	1.1	1.1		1.58	2
Patient 10	8.5	7	*	4.1		1.15	1.6
Patient 11	2.3	3.5	0.8	0.8		*	*
Patient 12	2.5	2.4	0.8	0.8		*	*
Patient 13	1.1	2.2	*	0.7		0.85	1.12
Patient 14	2.7	2.5	2.2	3.3		1.32	0.9
Patient 15	4.4	4.2	4.7	4.3		0.62	0.86
Patient 16	3.8	2	0.5	1.5		1.11	1.08
Mean	3.7	3.2	1.3	1.57		1.18	1.32
SEM	0.5	0.4	0.4	0.36		0.09	0.11

Serum PTH (pmol/L) andTm_{PO4}/GFR in patients applying high dose topical calcipotriol before treatment (Base) and after treatment (End). Patients applied up to 360g of ointment per week for a maximum of 2 weeks. *Result not available.

The changes in these parameters, with the exception of 24h urine calcium, correlated with dose of calcipotriol applied.

Urine indices of renal function were also affected. Mean calcium:creatinine ratio (Fig. 18, Table 11) rose from 216 to 356mmol/μmol and mean calcium excretion index rose from 18.7 to 29.5 (Table 11). Mean Tm_{PO4}/GFR rose from 1.17 to 1.31 (Fig 19, Table 12).

There was also rise in mean urine phosphate excretion - mean 24h urine phosphate rose from 13.5 to 20.2mmol/24h (Fig 20, Table13); and mean fasting urine phosphate (Table 11) rose from 11.5 to 15.7mmol/L. However, this was inconsistent and did not correlate well with dose of calcipotriol applied.

There was some concern about a rise in mean serum creatinine (from 88.6 to 93.1μmol/L), which was dose dependent (Fig 21, Table 14). However, glomerular filtration as judged by creatinine clearance was not altered (Fig 22, Table 14).

This study revealed one other interesting finding. 1,25 dihydroxyvitamin D_3 levels, as measured by the gamma-B 1,25-dihydroxyvitamin D kit (IDS immunodiagnostic systems Ltd., Boldon, Tyne and Wear, NE35 9PD, UK), rose markedly (Fig 23, Table 15) from a mean of 55.8 to 368.2pmol/L (10 of 11 patients above the normal range). This conflicted with the findings of our initial pilot study and it became apparent that calcipotriol in serum was interfering with the assay.

We therefore sent duplicate samples to Dr LeVan for further assessment. His HPLC method confirmed that 1,25 dihydroxyvitamin D_3 levels had actually fallen while calcipotriol levels had risen (Fig 23, Table 15). Mean serum 1,25 dihydroxyvitamin D_3 levels fell from 30.7 (SER=3.1) to 12.0pg/ml (2.0) while calcipotriol levels rose from undetectable or barely detectable in 4 patients who had received calcipotriol prior to baseline samples being taken to a mean of 25.7pg/ml (3.5).

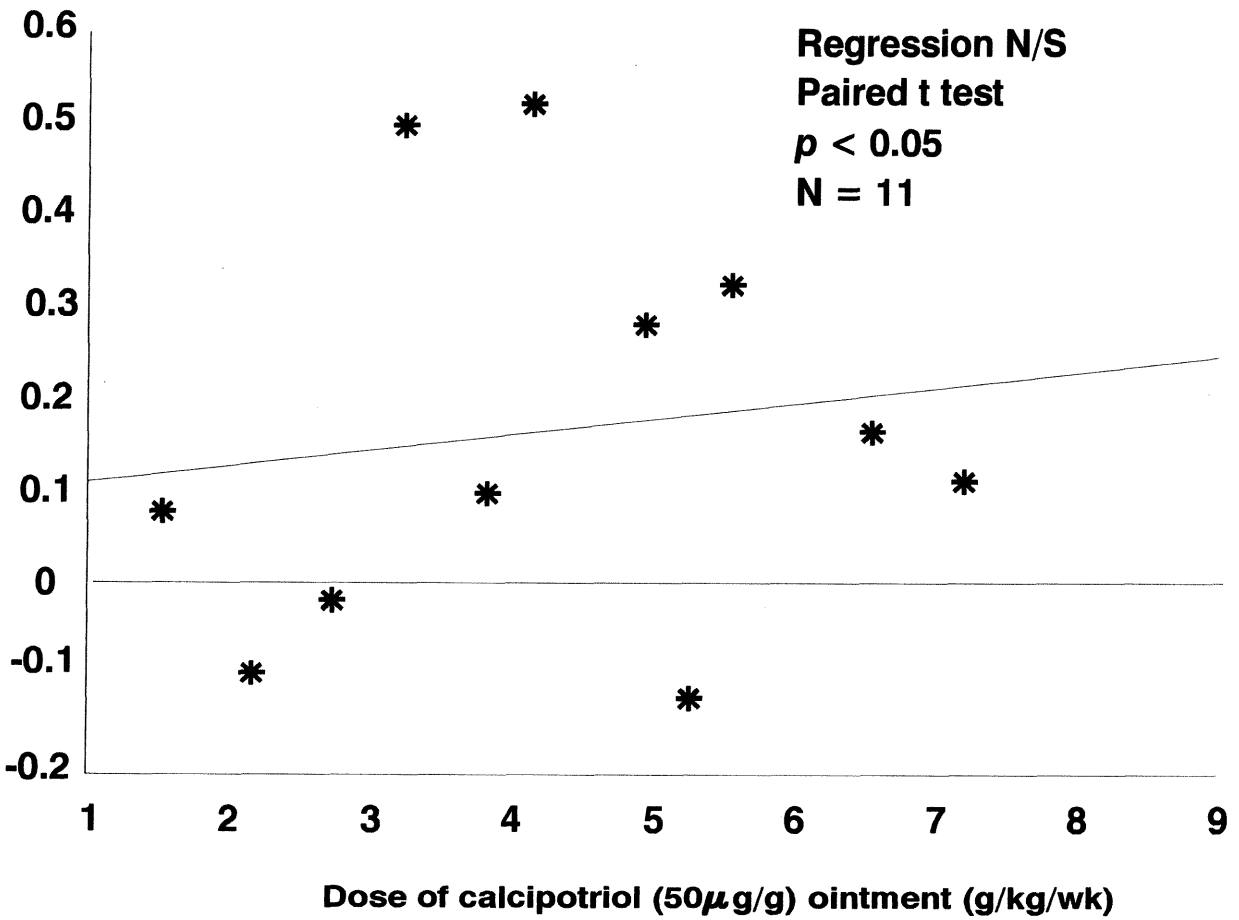


Figure 18. Change in fasting (a.m.) urine calcium/creatinine ratio from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

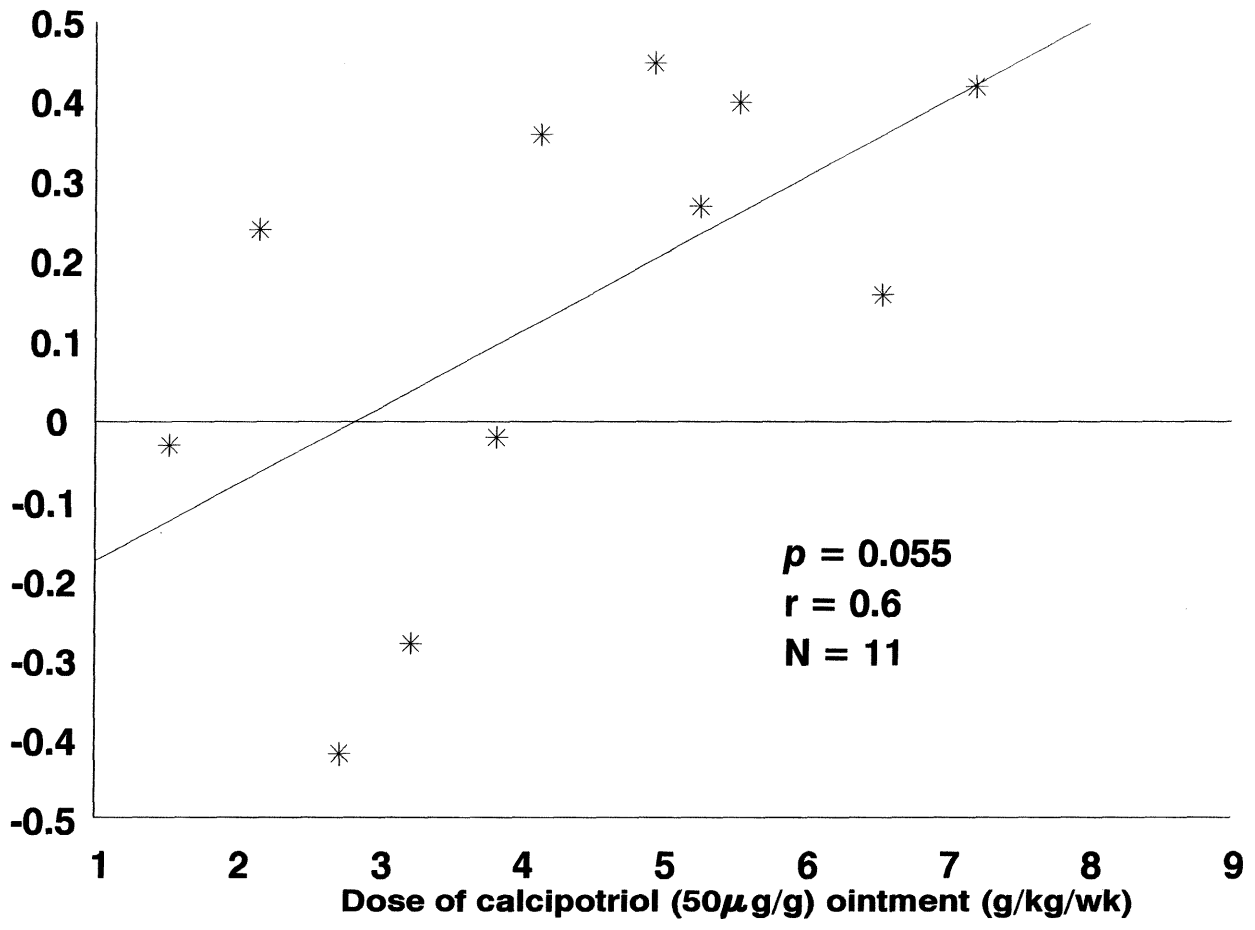


Figure 19. Change in Tm_{PO_4}/GFR from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

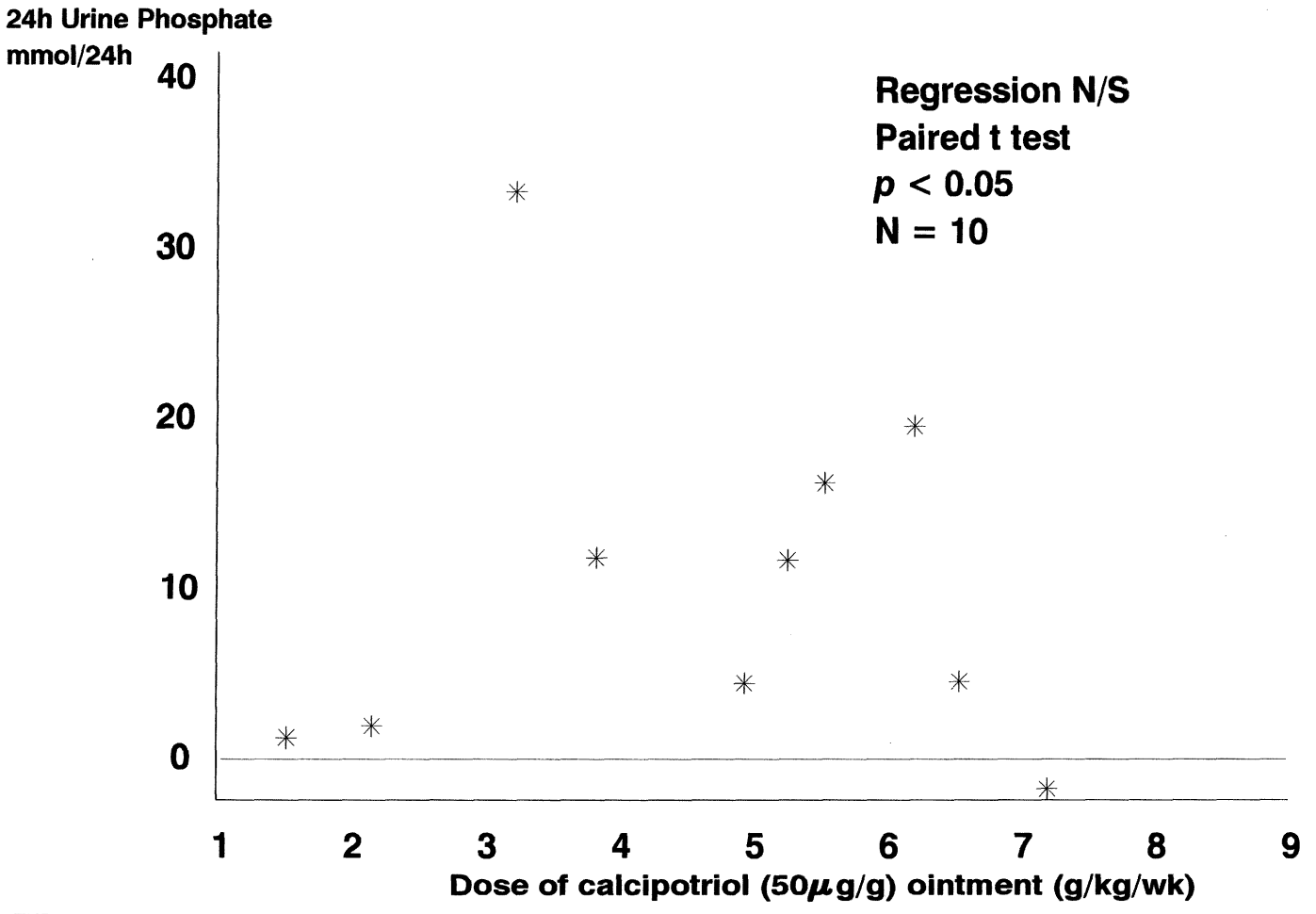


Figure 20. Change in 24h urine phosphate levels from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 13

	Base	Base	Wk1	Wk1	Wk2	Wk2
Patient 1	5.9	*	40	39.9	36.5	38.8
Patient 2	*	*	22.3	*	28.2	*
Patient 3	*	*	11	26.5	42.1	9.27
Patient 4	*	*	*	*	*	*
Patient 5	10.2	*	24.7	16.3	18.2	22.8
Patient 6	*	*	*	*	*	*
Patient 7	7.29	*	10.3	*	*	*
Patient 8	8.3	7.7	17.1	16.8	26	*
Patient 9	18.8	*	*	15.4	*	15.5
Patient 10	*	*	1	*	3.9	*
Patient 11	*	*	*	*	*	*
Patient 12	*	*	*	*	*	*
Patient 13	16.4	*	13.8	25.5	27	26.1
Patient 14	*	*	*	*	*	*
Patient 15	15.4	11	*	14	*	*
Patient 16	39.5	16.7	39.9	27.8	*	*
Mean	15	12	20	23	26	22.5
SEM	2.7	2.6	4.7	3.2	4.7	5.0

24h Urine phosphate (mmol/24h) in patients applying high dose topical calcipotriol before (Base) and during (Wk1, Wk2) treatment. Patients applied up to 360g of ointment per week for a maximum of 2 weeks. Urine phosphate concentration was not measured in many patients as regularly as 24h urine calcium. *Result not available.

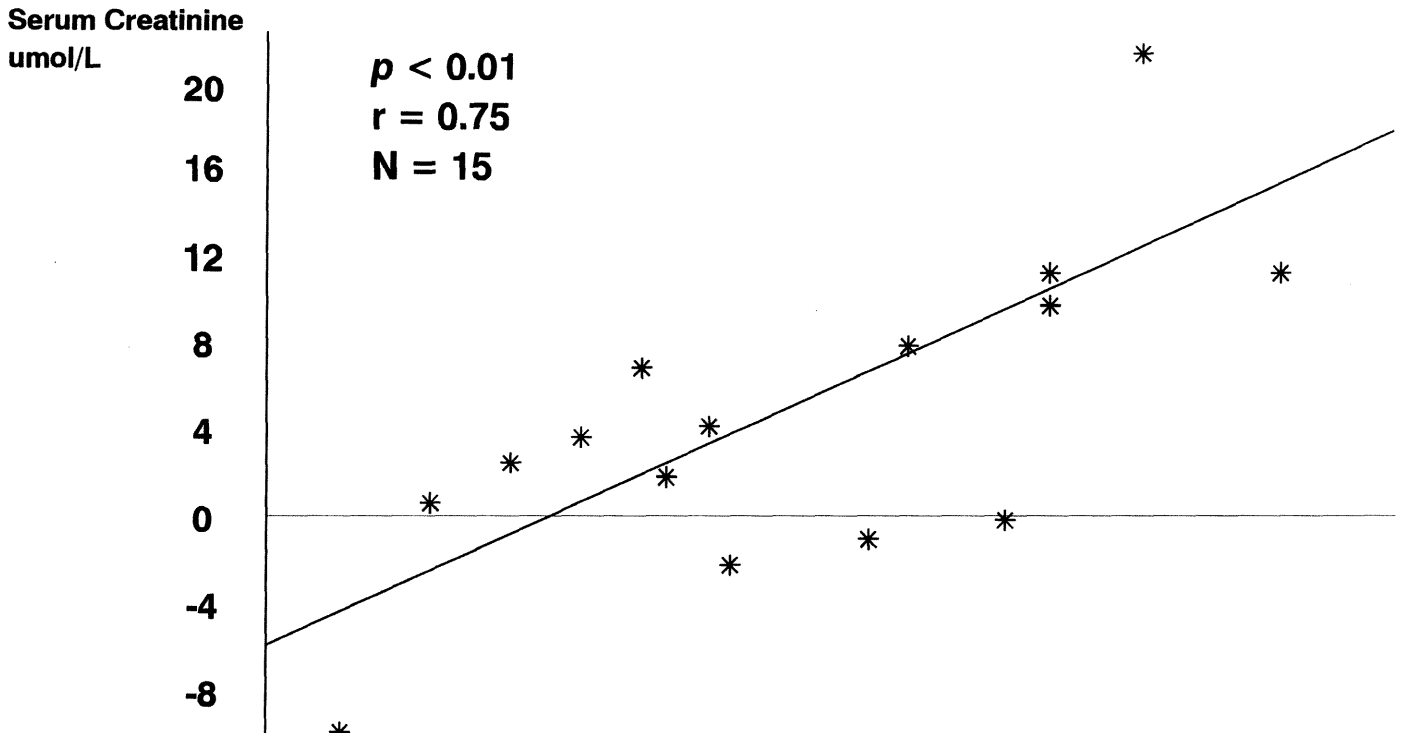


Figure 21. Change in serum creatinine from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 14

Creatinine	Base	Base	Wk1	Wk1	Wk1	Wk2	Wk2	Wk2	GFR Base	GFR End
Patient 1	91	82	93	87	95	89	90	91	104	90
Patient 2	72	58	72	69	74	74	78	*	*	66
Patient 3	116	112	94	114	*	114	138	113	38.8	25.9
Patient 4	83	78	72	98	100	*	*	*	50.8	*
Patient 5	85	*	92	88	93	90	86	84	45.3	69.1
Patient 6	92	*	113	104	98	105	87	96	105	124
Patient 7	81	*	97	99	80	*	*	*	44.7	43.5
Patient 8	84	*	*	*	*	84	*	*	46.8	84.9
Patient 9	72	76	83	89	97	97	90	98	82.4	137
Patient 10	*	*	105	*	*	*	106	105	*	35.2
Patient 11	107	103	97	100	93	105	98	105	59.5	*
Patient 12	85	*	104	97	105	96	92	87	*	*
Patient 13	77	70	75	71	77	72	72	73	145	139
Patient 14	88	*	89	87	85	87	90	94	*	*
Patient 15	91	91	*	*	*	*	93	90	62.7	*
Patient 16	124	*	109	125	124	112	119	111	90.2	79.4
Mean	90	84	93	94	93	94	95	96	72.8	81.3
SEM	3.9	6.2	3.7	4.5	4.1	4.1	4.8	3.2	9.8	12.4

Serum creatinine (μmol/L) and GFR (ml/min) in patients applying high dose topical calcipotriol before (Base), during (Wk1, Wk2) and after (End) treatment. Patients applied up to 360g of ointment per week for a maximum of 2 weeks. *Result not available.

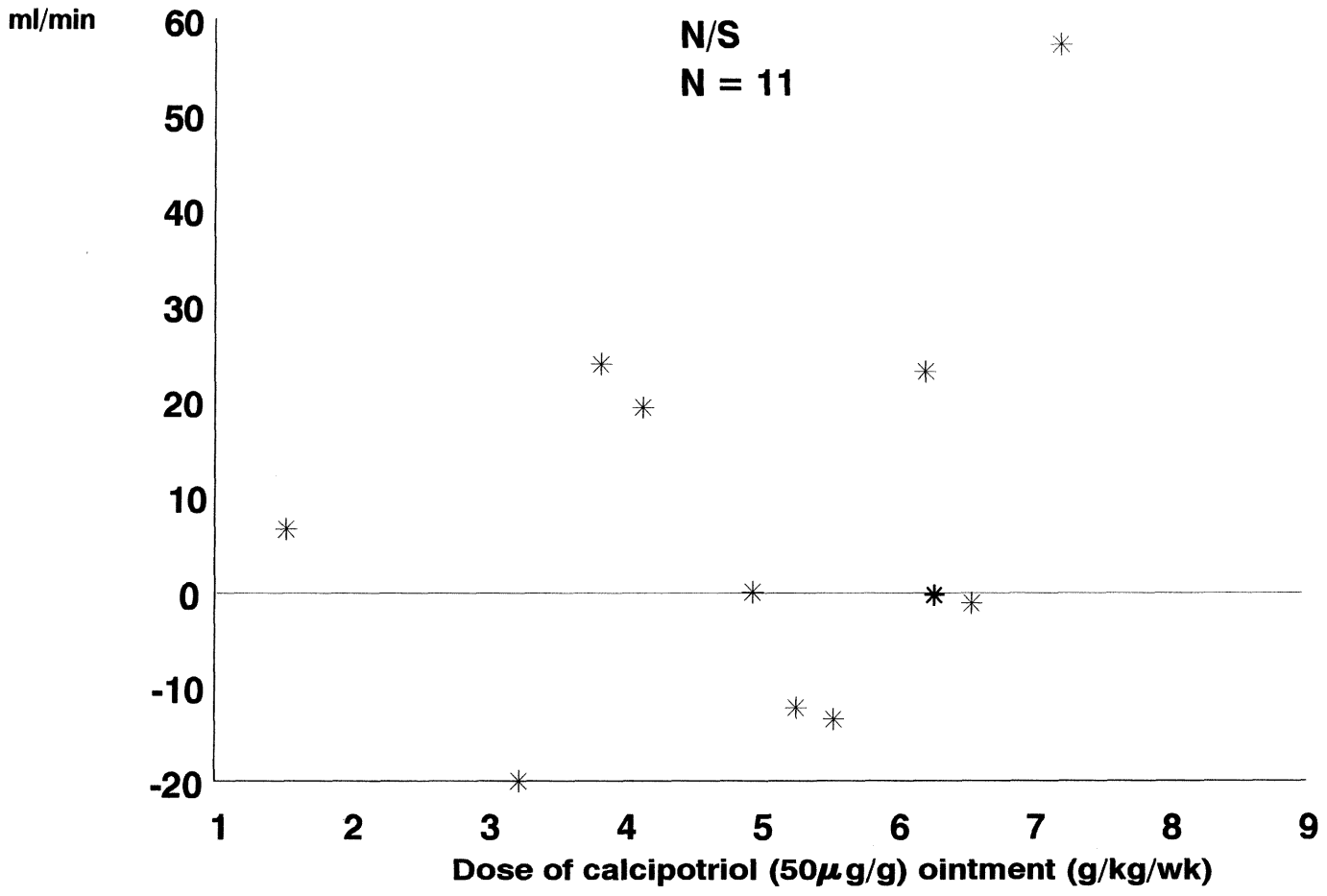


Figure 22. Change in GFR from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

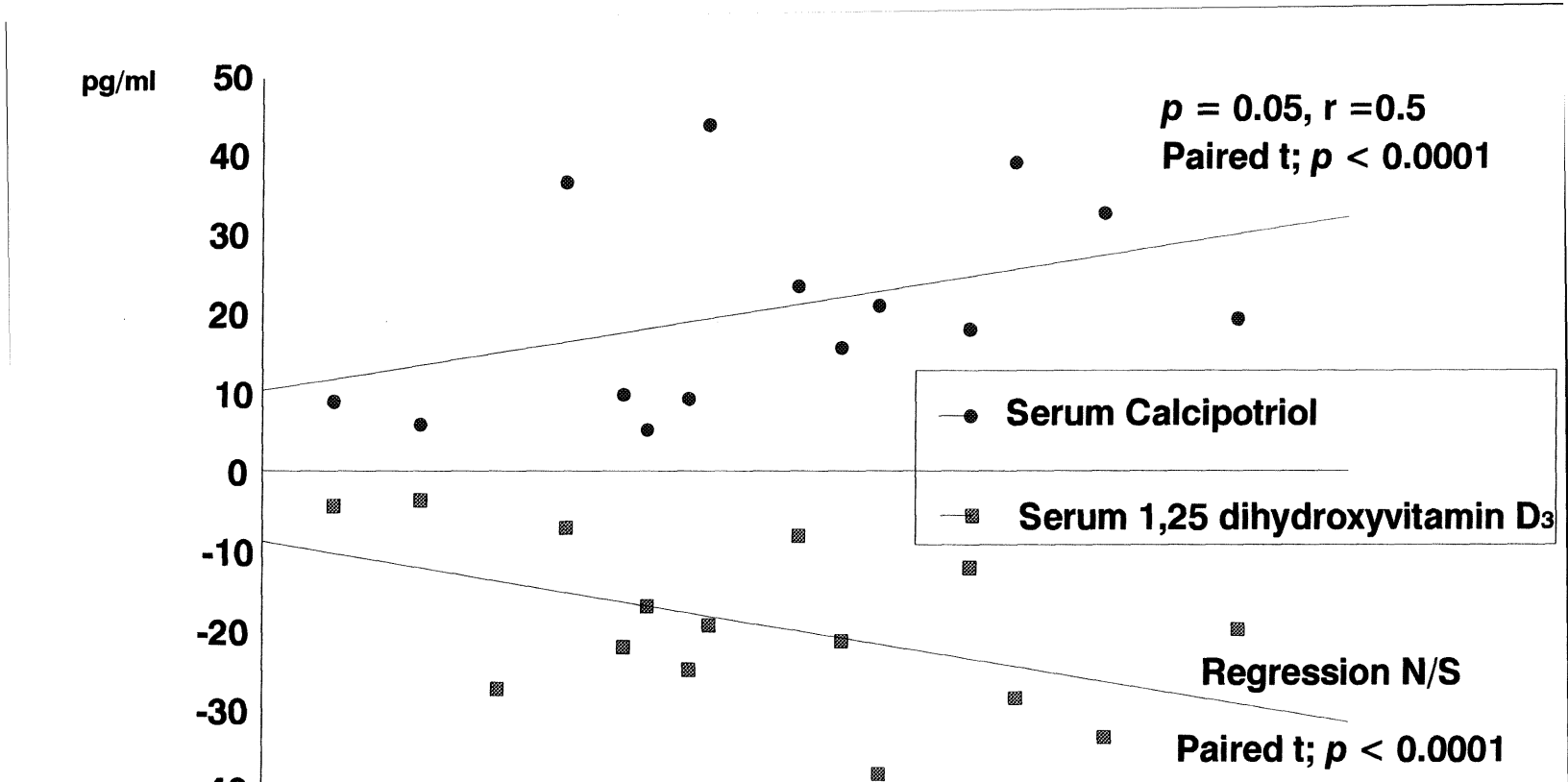


Figure 23. Change in serum calcipotriol and 1,25 dihydroxyvitamin D_3 levels (measured by Dr LeVan) from baseline to the end of treatment with high dose topical calcipotriol plotted against dose of calcipotriol applied per kg body weight.

Table 15

	25 vitD		1,25 vitD		Calcipotriol		1,25 vitD (HPLC)	
	Base	End	Base	End	Base	End	Base	End
Patient 1	19	17	37	500	0	39.3	17.3	10.6
Patient 2	13	7.2	52	*	0	22.3	27.7	8.3
Patient 3	8.2	6.9	46	187	5.8	27.1	44.4	6.7
Patient 4	*	*	*	*	*	*	*	*
Patient 5	11	11	51	287	0	8.6	29	12.5
Patient 6	19	10	*	*	0	13.6	30.7	6.1
Patient 7	13	13	51	500	0	42	37.7	9.6
Patient 8	7.7	10	24	395	4.7	23.1	19.1	7.4
Patient 9	7.5	12	51	500	4.7	37.7	39.1	6
Patient 10	5	5	134	*	14.4	38.2	22	14.3
Patient 11	17	16	46	500	0	47.4	26.5	7.6
Patient 12	21	16	64	500	5.1	15.3	28.5	6.8
Patient 13	7.9	9.6	62	*	0	19.4	42	21.1
Patient 14	25	10	53	300	0	*	57.3	30.3
Patient 15	11	17	53	87	0	9.6	29.8	26.5
Patient 16	22	16	57	294	6.8	16.1	10.1	6.1
Mean	14	12	56	368	2.8	26	31	12
SEM	6.4	1	6.6	42	1.1	3.5	3.1	2.0

Serum 25 hydroxyvitamin D (25 vitD; ng/ml), serum 1,25 dihydroxyvitamin D by radio-immunoassay (1,25 vitD; pmol/L), serum calcipotriol (pg/mg) and 1,25 dihydroxyvitamin D by high performance liquid chromatography (1,25 vit D; pg/mg) in patients applying high dose topical calcipotriol before (Base) and after (End) treatment. Patients applied up to 360g of ointment per week for a maximum of 2 weeks. Calcipotriol was detectable at baseline in a number of patients who had inadvertently started treatment with the ointment before the samples were taken. Calcipotriol interfered with the RIA assay giving a falsely high values for 1,25 dihydroxyvitamin D_3. *Result not available.

3.3.2 Discussion

In this study we confirmed that calcipotriol in large amounts increases serum total adjusted calcium and 24h urine calcium, suppresses PTH and 1,25 dihydroxyvitamin D_3. We also demonstrated a rise in serum ionized calcium, serum phosphate and urine phosphate. No effect on bone metabolism was demonstrated although the study period was very short and the numbers of patients studied small so that an effect on bone turnover cannot be excluded. Also, many of the patients did not have urine deoxypyridinoline cross-links measured which is felt, by many, to be the most sensitive index of bone turnover.

We have also demonstrated increased intestinal absorption of calcium in those patients receiving the highest doses of calcipotriol, particularly those who developed hypercalcaemia. That the strontium absorption test did not detect increased absorption in those applying lower doses is probably a reflection of the insensitivity of the test. There was marked inter and intra-individual variation in strontium levels after oral administration of a fixed dose under carefully controlled conditions. The change in levels from baseline to the end of the study varied from a two-fold increase to a 50% reduction. Most studies using this method in the past to estimate intestinal calcium absorption have relied on single measurements in each patient. Reynolds *et al*[158] found marked variation in levels on different days in the same individuals and concluded that it was a very insensitive test. Unfortunately, there is no evidence that any other methods of evaluating intestinal calcium absorption are better that the strontium absorption test.

Renal indices measured in these patients confirmed the dominant effect on PTH secretion, with calcium excretion index, calcium creatinine ratio and Tm_{PO4}/GFR all rising.

There were a number of other interesting findings in this study. Firstly, the changes in systemic calcium homeostasis correlated very closely with dose of calcipotriol applied per unit body weight. This is not surprising, but it has not previously been documented. This finding may explain the development of hypercalcaemia in patients using recommended amounts of calcipotriol while some studies using maximum or almost maximum recommended doses failed to demonstrate any effect whatsoever. Smaller patients are more likely to develop side effects from calcipotriol at lower doses.

The second interesting finding was the fact that calcipotriol interfered with the 1,25 dihydroxyvitamin D_3 assay kit. On reflection, this is not surprising, given that it is an antibody based test and that calcipotriol binds to the vitamin D antibody as avidly as 1,25 dihydroxyvitamin D_3 *in vitro*. This has important potential implications for patients using topical calcipotriol. Measurement of 1,25 dihydroxyvitamin D_3 using this type of assay is not reliable in such patients and HPLC measurement is necessary.

Finally, it is noteworthy that 3 of the patients who developed hypercalcaemia were Asian patients. Two out of four non-Asian patients applying greater than 5g per kg per week developed hypercalcaemia, whereas all three Asian patients applying those doses developed hypercalcaemia. The Asian patients developed hypercalcaemia earlier than the non-Asian, all developing hypercalcaemia within the first week (Table 5, Asian patients in italics). The Asian patients recorded higher serum total adjusted calcium levels than the two non-Asians who developed hypercalcaemia despite the fact that the two non-Asians were applying larger doses of calcipotriol. This may be of relevance, in that one of the two patients who was reported to have developed hypercalcaemia while using recommended amounts of calcipotriol, was Asian. However, the number of patients studied in this project was very small and these findings may well be a chance occurrence. Furthermore, we can suggest no explanation as to why these patients should be particularly susceptible to the toxic effects of calcipotriol.

3.4 SECTION III Recommended doses

3.4.1 Results

3.4.1.1 *100g of calcipotriol (50μg/g) ointment per week*

21 patients, 10 male and 11 female, were recruited. Mean age of the patients was 44yrs with a range from 19 to 74yrs. Mean PASI at baseline was 15.8 (range 38.5 to 7.2) and fell to 5.8 (11.7 to 0.9) after 4 weeks' therapy (Table 16).

Mean 24hr urine calcium rose significantly (Fig 24) from 4.71mmol/24h to 5.07mmol/24h ($P<0.05$). Six patients developed hypercalciuria. Almost all had abnormal baseline urine calcium levels and only one patient with a normal value at baseline developed an abnormally high level during treatment - 8.5mmol/24h. This level was not sustained and the patient's urine calcium returned to normal while still applying calcipotriol. Mean serum phosphate also rose (Fig 25, Table 17) from 0.97mmol/L to 1.12mmol/L ($P<0.005$). There was no significant change in serum total adjusted calcium (Fig. 24), serum 9am PTH, serum osteocalcin, c-terminal propeptide of type I collagen and serum bone specific alkaline phosphatase (Tables 17,18).

Table 16

	Base	Wk2	Wk4
Patient 1	*	*	*
Patient 2	*	*	*
Patient 3	*	*	*
Patient 4	35	*	5.4
Patient 5	*	*	*
Patient 6	24	*	8.8
Patient 7	18	*	9.7
Patient 8	12	7.7	5.2
Patient 9	9.6	*	5.4
Patient 10	14	13	3.8
Patient 11	19	8.8	6
Patient 12	8.4	*	8.2
Patient 13	12	7.6	5.2
Patient 14	7.8	2.6	1.3
Patient 15	9.9	2.8	3.6
Patient 16	7.2	4.9	4.6
Patient 17	11	7.4	10.5
Patient 18	39	27	11.7
Patient 19	14	6	2
Patient 20	11	2.8	0.9
Patient 21	*	*	*
Mean	16	8.2	5.8
SEM	2.3	2	0.8

Individual PASI in patients applying 100g of ointment per week before treatment (Base) after 2 and 4 weeks of treatment (Wk2 and Wk4). *Result not available.

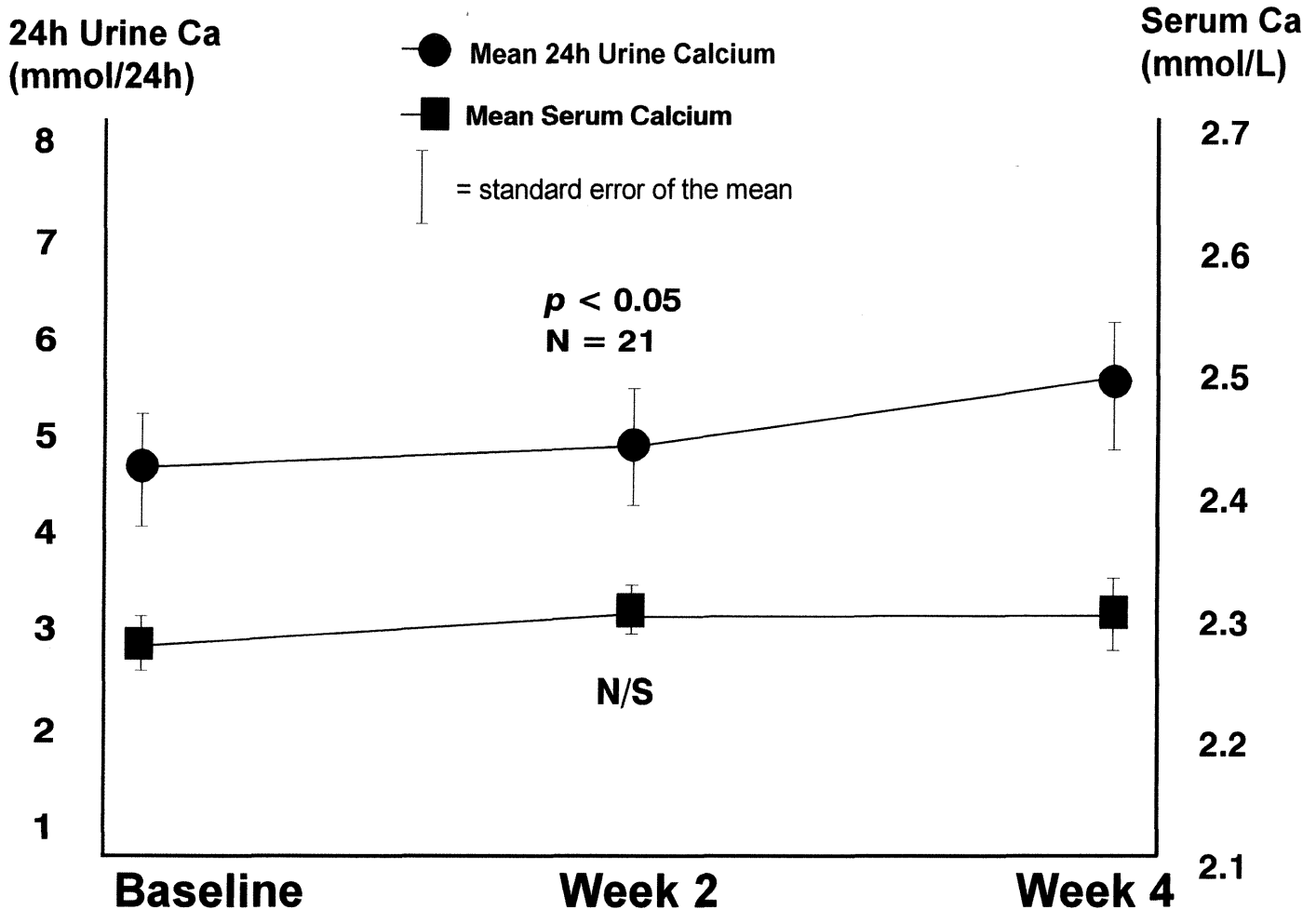


Figure 24. Mean serum total adjusted calcium and 24h urine calcium in patients applying exactly 100g of calcipotriol (50 μg/g) ointment per week over a 4 week period.

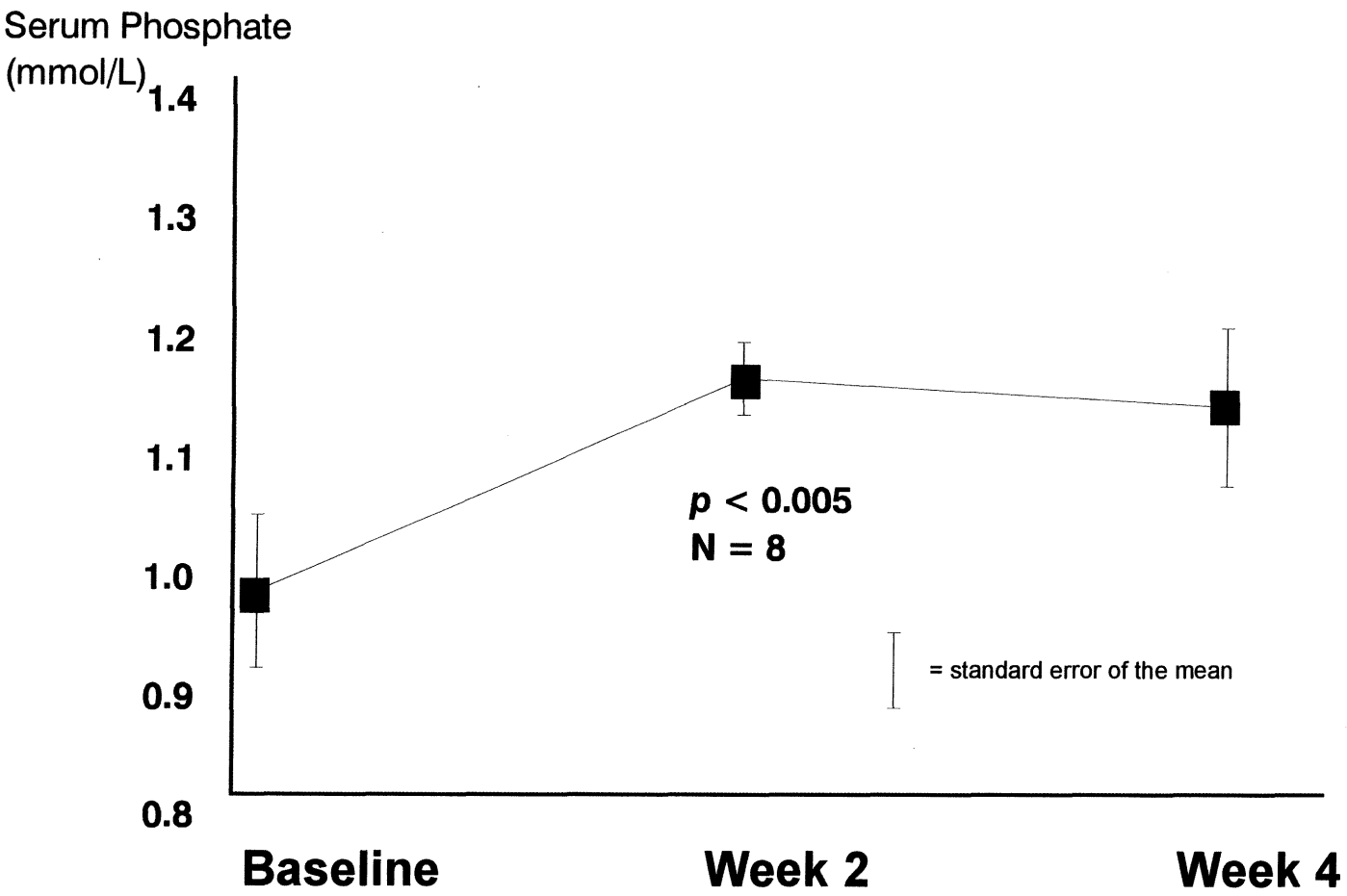


Figure 25. Mean serum phosphate in 8 patients applying exactly 100g of calcipotriol (50 μg/g) ointment per week over a 4 week period.

Table 17

	Serum PTH			Serum PO_4			Serum ALP		
	Base	Wk2	Wk4	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	2	0.9	2.5	0.58	*	0.65	114	*	104
Patient 2	5.9	7.7	6.3	*	1.12	1.29	*	88	102
Patient 3	4.5	2.8	3.8	1.07	1.31	1.4	64	65	67
Patient 4	2.3	1.7	3	0.89	1.23	1.1	101	77	82
Patient 5	3.2	3	3.9	1.11	1.19	1.17	68	62	75
Patient 6	3	2.9	2.9	*	1.08	1.06	*	89	99
Patient 7	3	2.2	3.1	1.03	1.19	1.21	149	183	162
Patient 8	2.4	2.8	3.1	0.99	1	1.06	66	63	62
Patient 9	6.7	8.6	6	0.93	1.08	1.01	59	60	62
Patient 10	2.6	3.1	*	1.17	1.15	1.31	46	47	48
Mean	3.7	3.5	3.9	0.9	1.2	1.41	88.7	76.6	81.9
SEM	0.5	0.8	0.4	0.1	0.1	0.3	12	15.8	12.8

Serum PTH (pmol/L), serum phosphate (mmol/L) and serum alkaline phosphatase (IU/L) before treatment (Base) and during treatment (Wk2 and Wk4) in patients applying 100g ointment per week.*Result not available

Table 18

Serum	P1CP		Osteocalcin		BS-ALP	
	Base	End	Base	End	Base	End
Patient 1	66	55	6	65	10	10
Patient 2	132	154	7	14	10	16
Patient 3	77	110	17	13	18	19
Patient 4	55	77	7	7	12	11
Patient 5	143	88	13	11	16	17
Patient 6	66	66	29	21	24	23
Patient 7	143	88	6	6	11	11
Patient 8	66	77	5	6	10	15
Patient 9	77	99	12	13	17	16
Mean	91.7	90	11.3	17.3	14.2	15.3
SEM	12.2	9.7	2.6	6.2	1.6	1.4

Serum Procollagen peptide (P1CP;ng/ml), Osteocalcin (ng/ml) and Bone specific alkaline phosphatase (BS-ALP;U/L) before and after 4 weeks' treatment in patients applying 100g ointment per week.
*Result not available

3.4.1.2 *90g of calcipotriol (50μg/g) versus 90g of calcitriol (3μg/g) ointment per week*

Twenty-four patients, 14 female and 10 male, were recruited. Mean age was 42 years with a range of 18 to 80 years. 12 were assigned to the calcipotriol group, 12 to the calcitriol group. Four patients in the calcipotriol group failed to complete the study. One of these was withdrawn after 6 weeks because of lack of efficacy and one, after 4 weeks, because of the possibility of pregnancy. The other 2 failed to attend after 2, and 4 week's treatment. Four patients in the calcitriol group failed to complete the study. Two were withdrawn after 6 weeks because of lack of efficacy, one patient went abroad on holiday after 1 week of treatment and one patient failed after 1 week of treatment. Baseline data were available on all patients, data from at least 4 visits on 21 patients and 'on-treatment' data on all but one patient.

There was no significant difference in the mean PASI (Table 19,20) of the two groups at baseline; calcipotriol group - 14.9 (range 3.7 to 38.2), calcitriol group - 13 (7.6 to 19.2). Mean PASI in patients applying calcitriol fell by 32% from 13 to 8.8 ($P = 0.013$) and in patients applying calcipotriol by 68% from 14.9 to 4.7 ($P < 0.005$). The reduction was significantly greater in the calcipotriol-treated group ($P = 0.014$).

There was a small but significant increase in serum ionized calcium (Fig 26) in the calcipotriol-treated group (from 1.21mmol/L to 1.25mmol/L, $P = 0.01$) but no significant changes in any of the other parameters of calcium homeostasis measured. There were no significant changes in any of the parameters measured in the calcitriol-treated group .

Table 19

Calcipotriol	Base	Wk1	Wk2	Wk4	Wk6	Wk8
Patient 1	11	2.3	3.1	0	0	0
Patient 2	3.7	5.2	2.8	1.7	1.3	*
Patient 3	15	7.4	3.2	1.8	1.6	0.9
Patient 4	11	7.8	4.7	4.4	3.7	3.7
Patient 5	38	16	6.2	8	*	*
Patient 6	19	9.8	7.6	9.6	*	7.3
Patient 7	12	4.6	3.8	*	1.6	1.4
Patient 8	6.2	3.8	4.2	*	*	*
Patient 9	16	8.1	3.2	3	0.5	2.2
Patient 10	16	*	6.4	3.8	2.7	1.7
Patient 11	16	14	13	15	16	*
Patient 12	14	10	9	9	*	*
Mean	15	8.1	5.6	5.6	3.5	2.5
SEM	2.5	1.3	0.9	1.5	1.9	0.9

Individual PASI in patients applying 90g of calcipotriol ointment per week for up to 8 weeks. *Result not available.

Table 20

1,25 vitD						
Patient 1	11	7	12	6.7	4.5	6.5
Patient 2	19	15	3.1	6.5	7.4	7.3
Patient 3	16	13	*	9.2	11	9.2
Patient 4	12	5.2	5.8	7.6	4.8	6.3
Patient 5	7.6	4.6	4.2	5.4	8.4	6.2
Patient 6	7.8	2	2.4	2	2.4	*
Patient 7	15	14	11	*	5	8
Patient 8	15	14	9	*	14	18.8
Patient 9	14	11	*	9.2	9.8	*
Patient 10	15	14	*	*	*	*
Patient 11	11	9.8	7.5	5.9	7.4	7.8
Patient 12	*	*	*	*	*	*
Mean	13	9.9	6.9	6.6	7.5	8.8
SEM	1.1	1.4	1.3	0.8	1.1	1.5

Individual PASI in patients applying 90g of 1,25 dihydroxyvitamin D_3 ointment. *Result not available

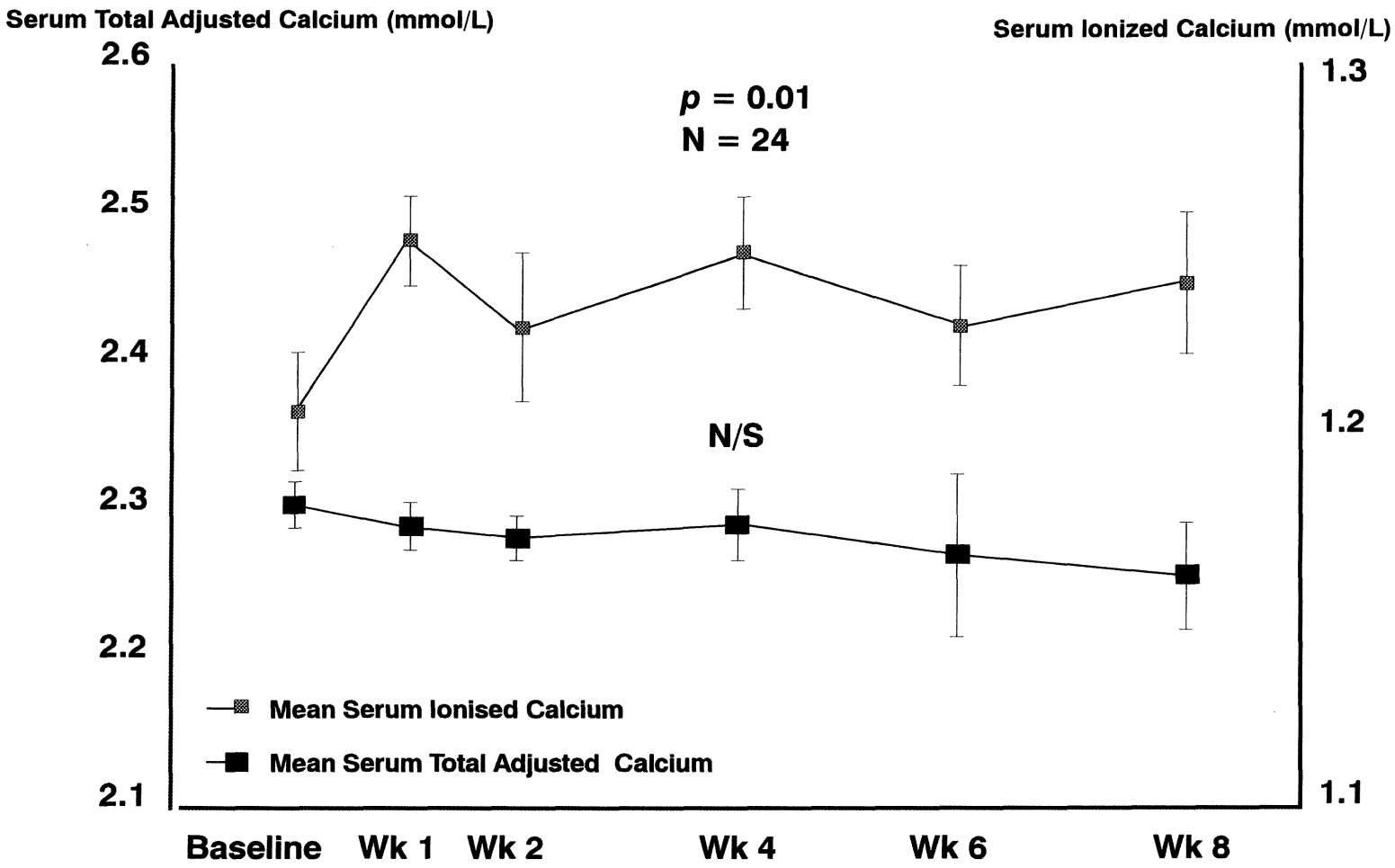


Figure 26. Mean serum total adjusted calcium and ionized calcium in patients applying exactly 90g of calcipotriol (50 μg/g) ointment per week over a 4 week period.

3.4.1.3 *A single 6g dose of calcipotriol (50μg/g) ointment compared to placebo*

10 patients, 3 male and 7 female, were recruited. Mean age was 48yrs, range 23 to 70yrs. There was no significant difference between the biochemical profiles over a nine hour period in each patient when calcipotriol was applied as compared to placebo (Figs 27-9).

3.4.1.4 *Calcipotriol (50μg/g) ointment plus occlusion*

48 patients, 22 male and 26 female, were recruited. Mean age was 43yrs, range 18 to 70yrs. Patients, on average, used 30-40g of the ointment per week. Seven patients failed to complete the study; 2 were withdrawn because of irritant reactions, 2 because of lack of efficacy and 3 were lost to follow-up. Data were available for analysis on 41 patients who completed the study. 19 were assigned to group A (calcipotriol Vs calcipotriol plus occlusion) and 22 to group B (calcipotriol plus occlusion Vs placebo plus occlusion). In group A, occlusion plus calcipotriol (mean severity score fell from 7.9 to 3.0, Table 21) was significantly more effective than calcipotriol alone (mean score fell from 7.6 to 4.6; Table 22), $P < 0.005$. In group B, occlusion plus white soft paraffin (Table 23) had no significant effect on severity of psoriasis while the response to calcipotriol (Table 24) plus occlusion was similar to group A (7.8 to 2.2, $P < 0.005$).

There was no significant change in mean serum total adjusted calcium, phosphate, alkaline phosphatase or mean 24h urine calcium and phosphate (Fig 30).

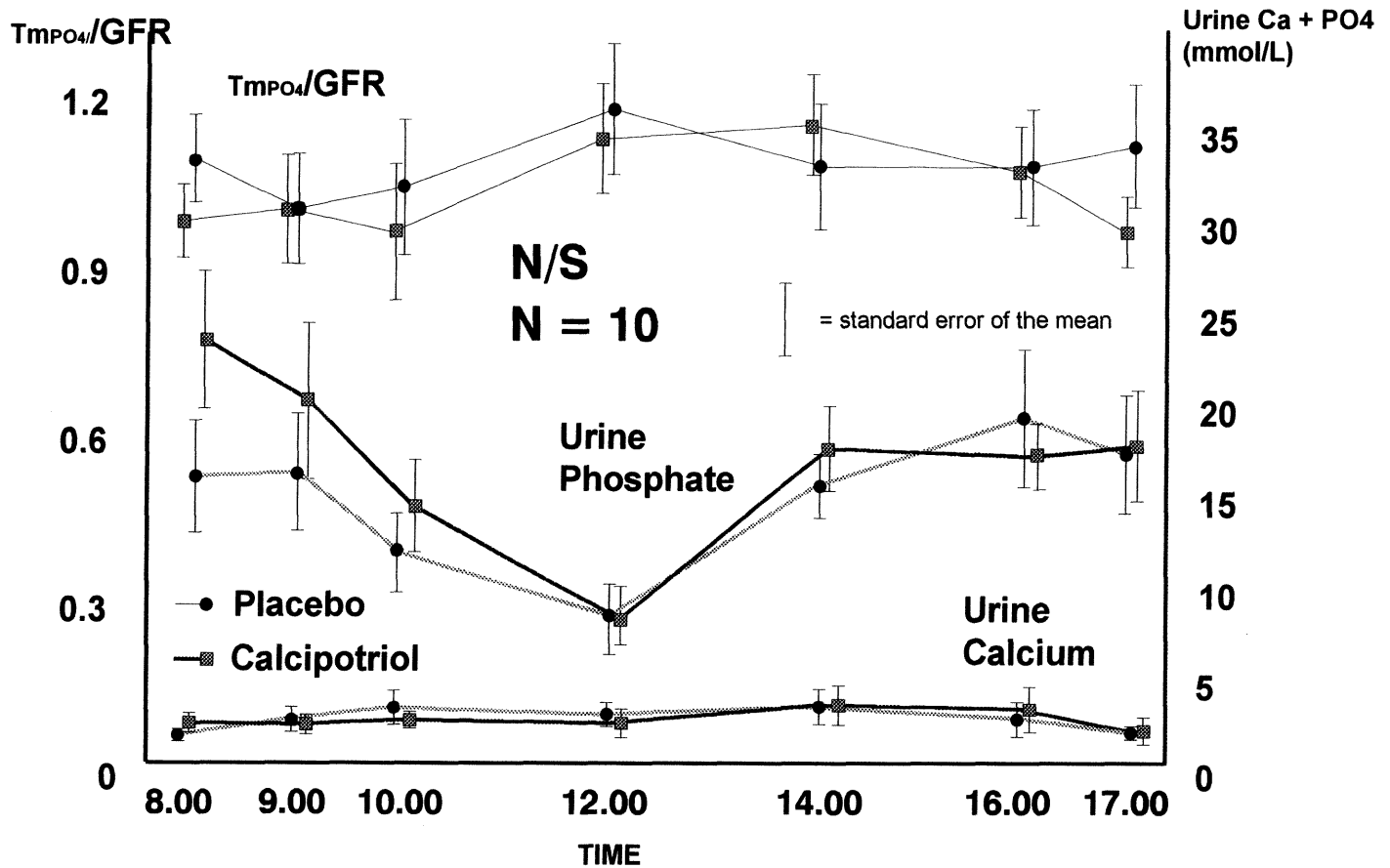


Figure 27. Mean and SEM spot urine calcium and phosphate and Tm_{PO4}/GFR before treatment and over nine hours after application of 6g of calcipotriol (50 μg/g) ointment or placebo.

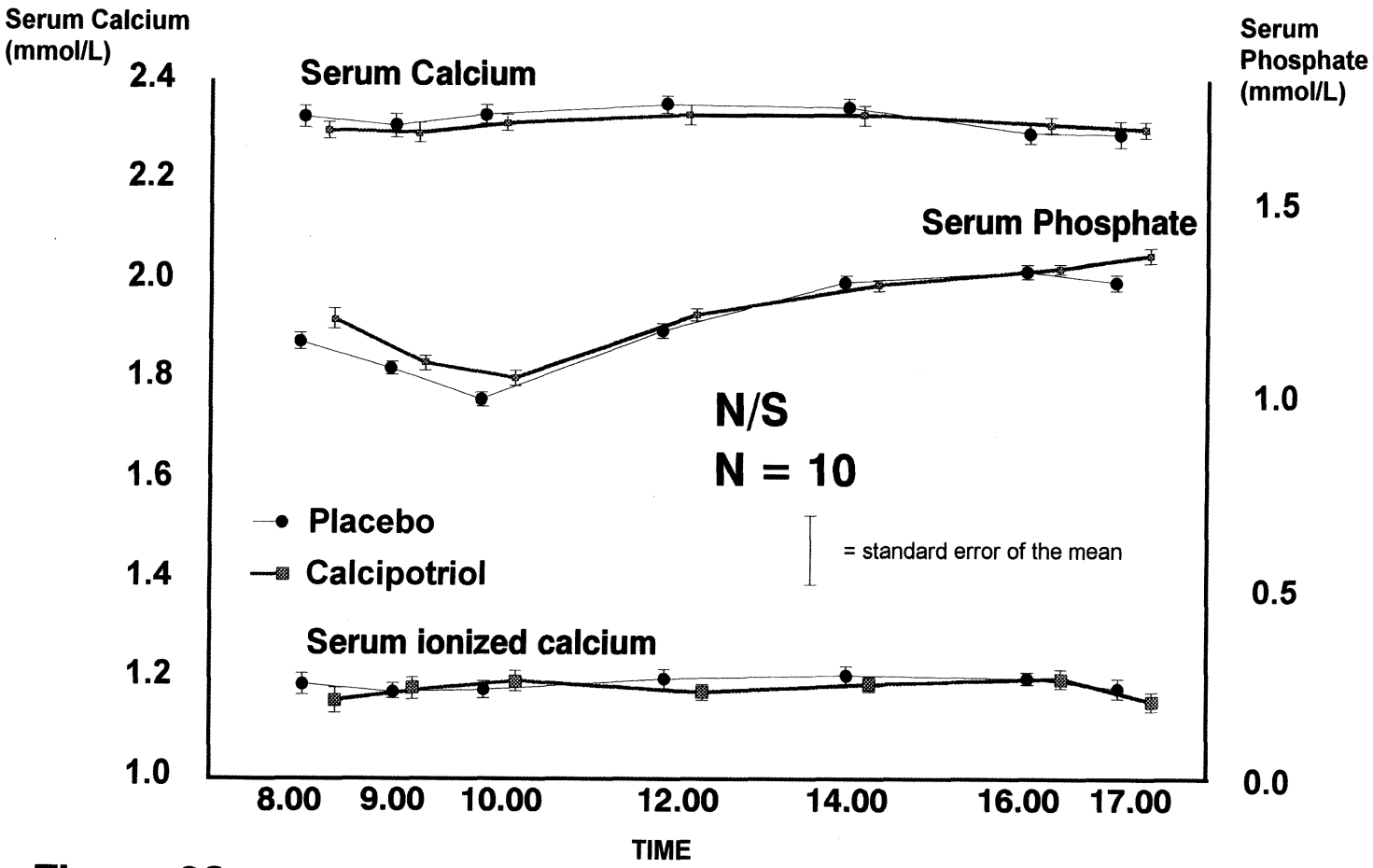


Figure 28. Mean and SEM serum total and ionized calcium and serum phosphate before treatment and over nine hours after application of 6g of calcipotriol (50μg/g) ointment or placebo.

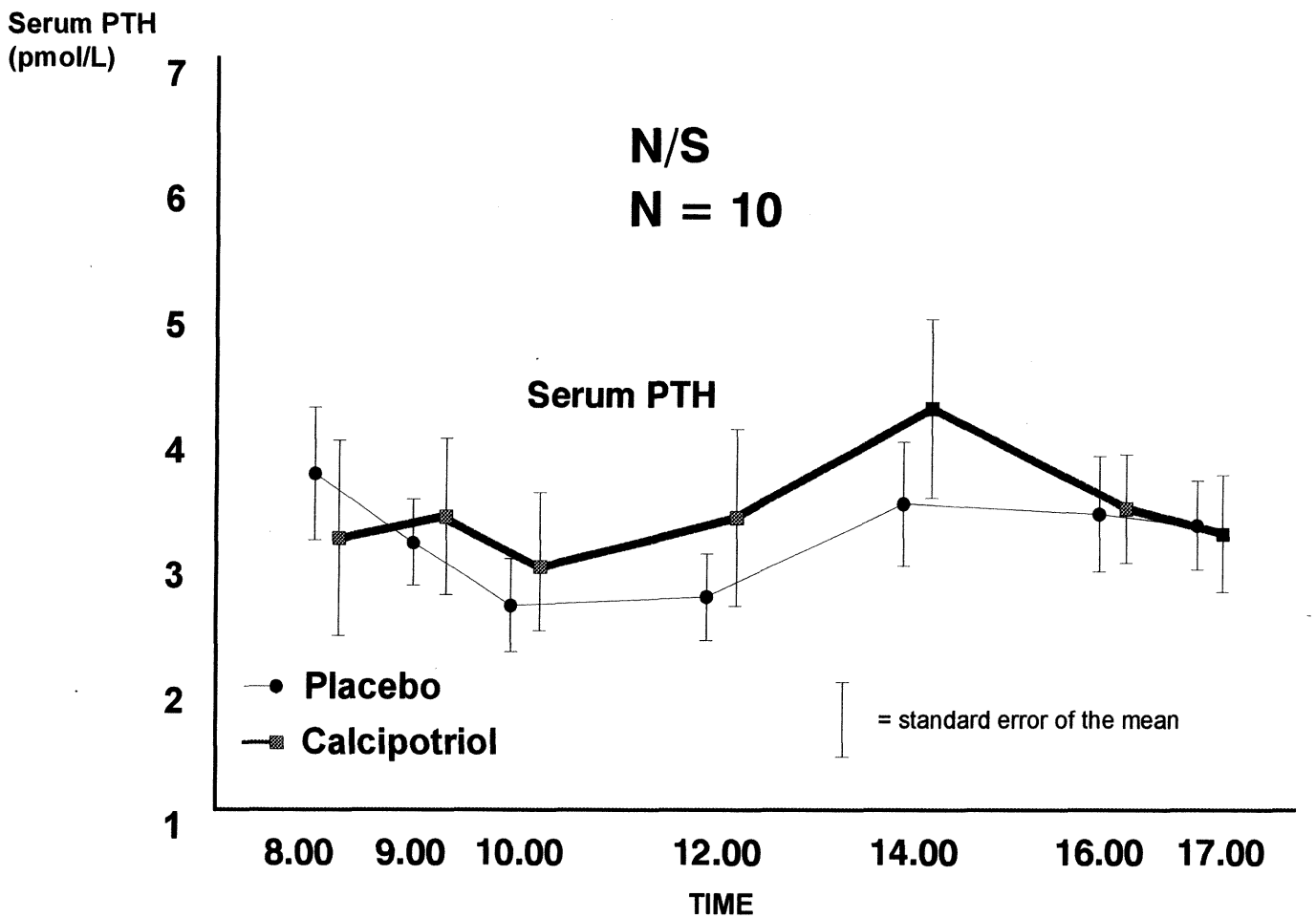


Figure 29. Mean and SEM serum parathyroid hormone (PTH) levels before treatment and after application of 6g of calcipotriol (50 μg/g) ointment over a nine hour period.

Table 21

	Base	Wk2	Wk4	Wk6	Wk8
Patient 1	5	1	4	1	2
Patient 2	8	8	8	8	6
Patient 3	9	*	6	*	5
Patient 4	8	4	3	6	3
Patient 5	7	3	2	2	2
Patient 6	8	6	5	5	6
Patient 7	11	6	4	2	1
Patient 8	6	6	5	4	8
Patient 9	9	9	9	8	8
Patient 10	8	7	6	3	3
Patient 11	7	4	6	5	3
Patient 12	7	3	3	2	*
Patient 13	9	3	3	2	2
Patient 14	9	4	2	1	1
Patient 15	8	5	3	2	0
Patient 16	9	3	5	1	0
Patient 17	7	4	3	1	1
Patient 18	8	2	2	1	1
Patient 19	7	4	4	3	3
Mean	7.9	4.6	4.4	3.2	3.1
SEM	0.3	0.5	0.5	0.5	0.6

Severity scores for the limb(s) assigned to be treated with calcipotriol plus occlusion in group A.
*Result not available.

Table 22

	Base	Wk2	Wk4	Wk6	Wk8
Patient 1	3	1	3	2	2
Patient 2	8	6	6	8	8
Patient 3	9	*	7	*	6
Patient 4	8	6	4	7	4
Patient 5	6	4	2	2	3
Patient 6	8	6	6	8	7
Patient 7	11	5	5	2	1
Patient 8	6	6	5	5	6
Patient 9	9	9	9	9	8
Patient 10	6	6	5	4	3
Patient 11	7	6	3	5	5
Patient 12	7	3	2	2	*
Patient 13	9	7	8	7	6
Patient 14	9	6	6	6	7
Patient 15	8	7	4	6	3
Patient 16	9	7	4	3	4
Patient 17	7	6	3	3	1
Patient 18	7	8	7	7	6
Patient 19	7	5	2	3	3
Mean	7.6	5.8	4.8	4.9	4.6
SEM	0.4	0.4	0.5	0.5	0.5

Severity scores for the limb(s) assigned to be treated with calcipotriol alone in group A. *Result not available.

Table 23

	Base	Wk2	Wk4	Wk6	Wk8
Patient 1	3	3	3	6	7
Patient 2	8	6	4	3	3
Patient 3	9	7	8	7	3
Patient 4	9	7	9	10	10
Patient 5	5	5	5	6	*
Patient 6	9	9	7	6	6
Patient 7	8	11	7	5	5
Patient 8	6	8	7	8	6
Patient 9	6	7	7	8	8
Patient 10	8	8	6	7	6
Patient 11	8	8	8	8	8
Patient 12	8	8	8	8	8
Patient 13	8	7	8	*	*
Patient 14	10	7	8	8	10
Patient 15	9	9	6	7	8
Patient 16	9	*	*	12	*
Patient 17	7	6	6	6	6
Patient 18	6	6	6	6	6
Patient 19	8	8	8	*	*
Patient 20	6	6	5	*	*
Patient 21	6	6	6	6	6
Patient 22	7	6	5	5	6
Mean	7.4	7	6.5	6.9	6.6
SEM	0.4	0.4	0.3	0.4	0.4

Severity scores for the limb(s) assigned to be treated with placebo plus occlusion in group B.
*Result not available.

Table 24

	Base	Wk2	Wk4	Wk6	Wk8
Patient 1	3	2	2	1	1
Patient 2	9	4	3	3	2
Patient 3	9	6	6	6	3
Patient 4	9	4	4	3	2
Patient 5	5	2	3	4	*
Patient 6	9	9	6	4	3
Patient 7	8	11	4	4	1
Patient 8	9	5	5	5	4
Patient 9	6	2	1	0	0
Patient 10	8	7	6	5	1
Patient 11	8	3	3	3	1
Patient 12	8	9	4	3	3
Patient 13	8	4	4	*	*
Patient 14	10	4	4	4	5
Patient 15	9	9	3	2	1
Patient 16	9	*	*	12	*
Patient 17	7	7	6	6	4
Patient 18	6	6	4	1	1
Patient 19	9	2	3	*	*
Patient 20	7	3	4	*	*
Patient 21	6	7	5	4	5
Patient 22	9	3	1	1	0
Mean	7.8	5.2	3.9	3.7	2.2
SEM	0.4	0.6	0.3	0.6	0.3

Severity scores for the limb(s) assigned to be treated with calcipotriol plus occlusion in group B.
*Result not available.

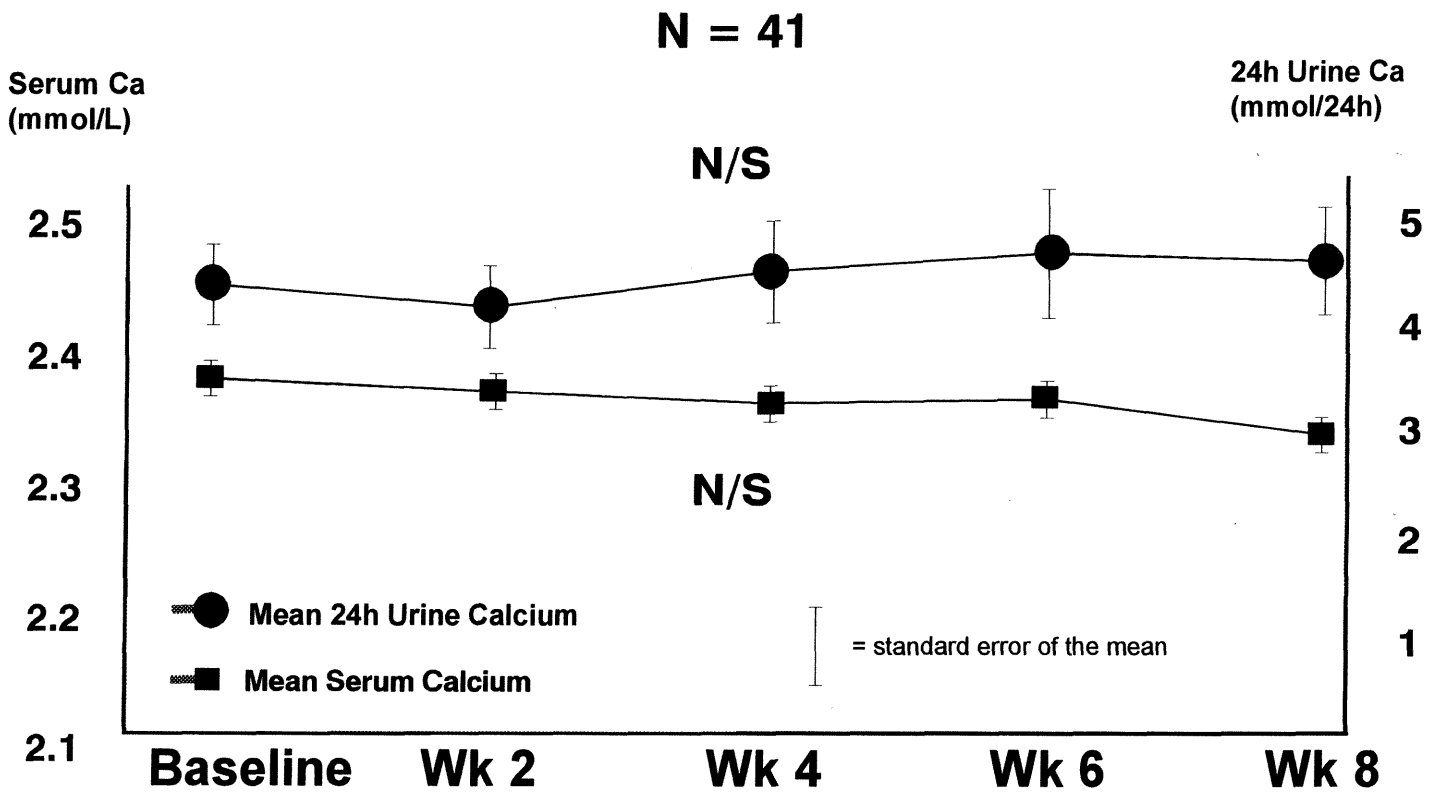


Figure 30. Mean and SEM serum total adjusted calcium and 24h urine calcium at baseline and during treatment with calcipotrio plus occlusion.

3.4.1.5 *Long term use of calcipotriol (50μg/g) ointment*

Twenty patients, 7 male and 13 female, were recruited. Mean age was 43 yrs, range from 37 to 50yrs. Mean PASI fell from 6.48 (range 0.9 to 10.8) to 3.35 (0 to 5.4). All 20 patients completed 12 months' treatment with calcipotriol. Patients used on average 30-40g per week with a range of 20-76g. There was no significant change in serum total adjusted calcium or 24h urine calcium throughout the study period (Fig 31).

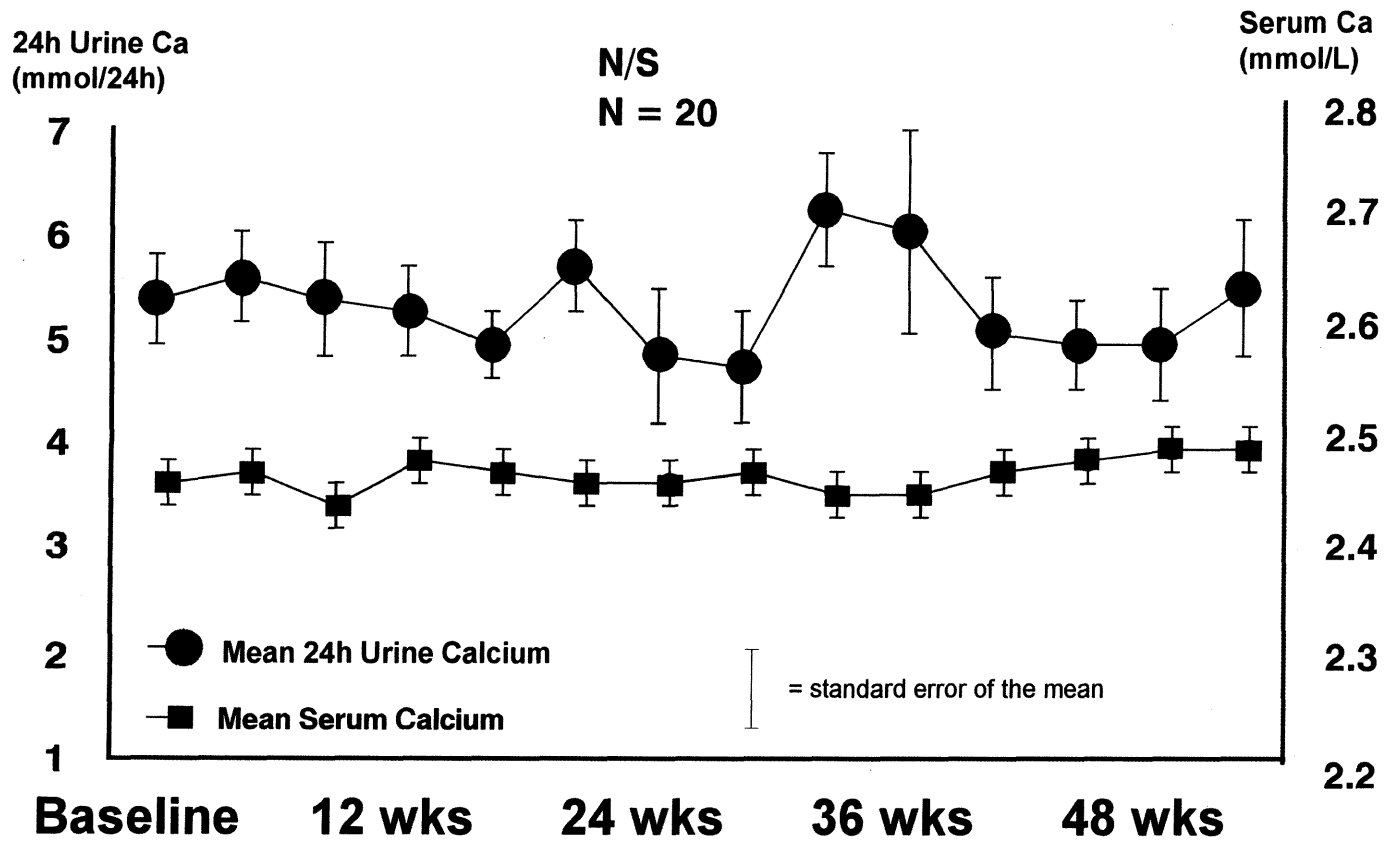


Figure 31. Mean and SEM serum total adjusted calcium and 24h urine calcium in patients applying 30-40g of calcipotriol ointment over a period of 12 months.

3.4.2 Discussion

These studies demonstrate that conventional doses of calcipotriol have no measurable effect on systemic calcium homeostasis. However, at doses approaching the maximum recommended (100g/wk), there is a small but significant effect. Serum phosphate, serum ionized calcium and 24h urine calcium appear to be more sensitive indices of that effect.

There appears to be no acute effect on gastro-intestinal absorption of calcium at these doses. This is in keeping with the *in vitro* work of Norman *et al.*[109] who found that calcipotriol had a much weaker effect on acute gastrointestinal absorption of calcium than calcitriol.

The clinical significance of these findings is uncertain but probably minor. The only possible significant consequence would be an increased risk of renal calculi due to a rise in urine calcium. The rise in urine calcium, however, was small and in most cases hypercalciuria did not develop. The risk of renal calculi rises exponentially with rise in urine calcium (Figure 32) but the curve is fairly flat until a level of 10mmol/24h is reached.[159] The mean rise in urine calcium in patients using the maximum recommended amount of calcipotriol was only 7.5% therefore the increased risk of renal calculi on average will be small.

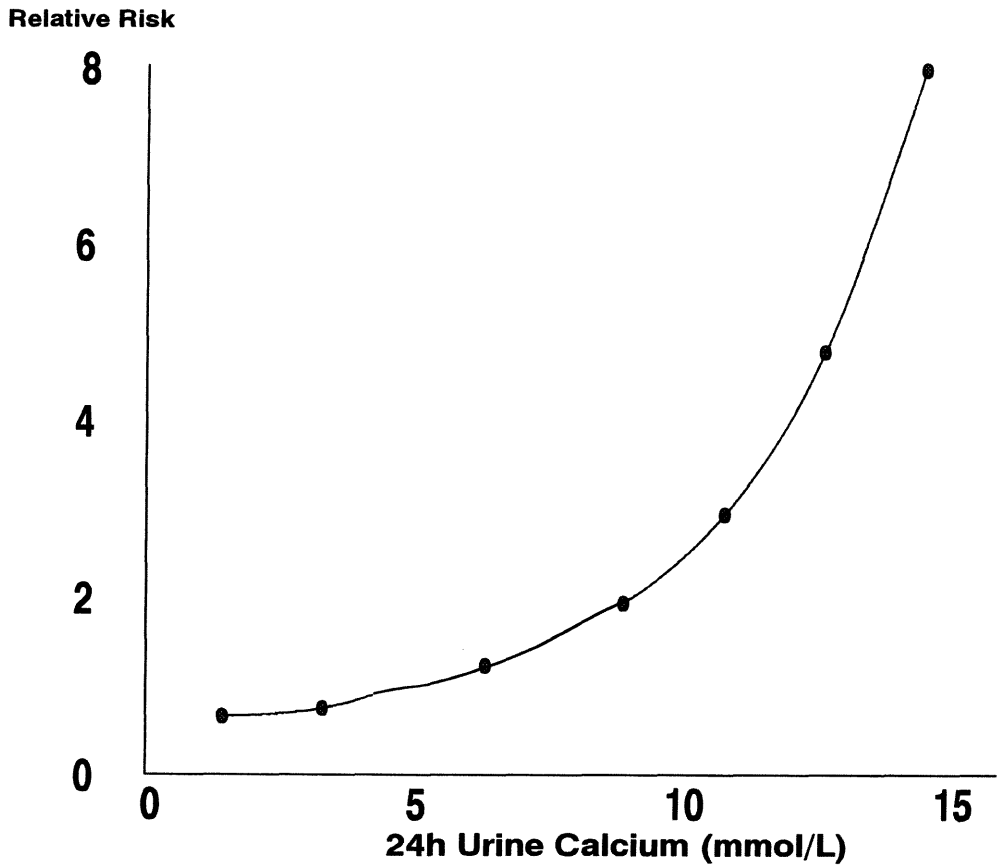


Figure 32. Risk of developing renal calculi rises exponentially with 24h urine calcium level. (Adapted from Nordin et al[159])

The only exception to this would be in people who already have a high urine calcium at baseline, because the risks of renal calculi rise exponentially above levels of 10mmol/24h. It would be wise to measure urine calcium at baseline in any patient with moderately extensive psoriasis and monitor those patients who have a high or high-normal urine calcium.

From a clinical efficacy point of view, it is surprising that occlusion alone had no effect on psoriasis in our study over an eight period. Given that there is extensive literature indicating a therapeutic effect of occlusion alone[69] some effect would have been expected. One possoble explanation is that our study involved the use of occlusion overnight only and polythene film rather than one of the more adherent occlusive dressings which have been previously studied.

3.5 SECTION IV Calcipotriol plus short wave ultraviolet light (UVB)

3.5.1 Results

All three treatments were effective. Mean PASI fell in the UVB group from 12.6 to 7.6 ($P < 0.005$). Mean PASI fell from 11.7 to 6.3 in the calcipotriol group ($P < 0.01$) and from 14 to 3.8 ($P < 0.005$) in the combined therapy group (Table 25). The combination of UVB and calcipotriol was slightly more effective than either agent alone although the difference was not statistically significant.

Mean serum phosphate rose ($P < 0.05$) in the group receiving therapy with UVB plus calciptriol from 0.92 (SER=.05) to 1.22(.09)mmol/L. However, the baseline serum phosphate in this group was significantly lower than either of the other groups and there was no difference between any of the groups in terms of mean serum phosphate at the end of the study (Figure 33). There was a small rise in serum phosphate in both of the other groups which fell short of statistical significance. There was no significant change in any of the other parameters of systemic calcium homeostasis measured in any of the groups (Tables 26-31).

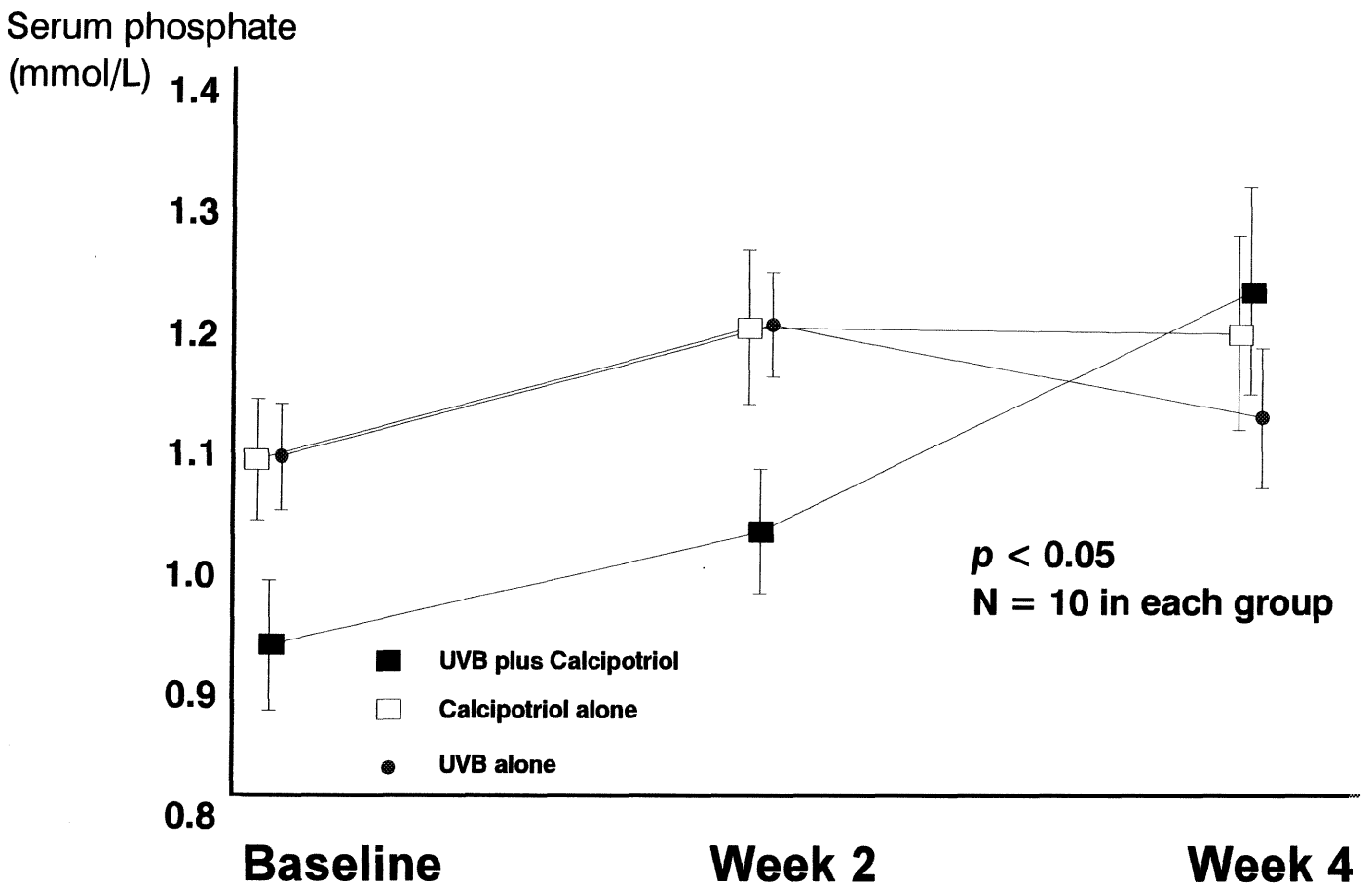


Figure 33. Mean and SEM serum phosphate in patients treated with UVB plus 100g of calcipotriol ointment, 100g of calcipotriol ointment alone or UVB alone.

Table 25

	UVB + Calcipotriol			Calcipotriol			UVB		
	Base	Wk2	Wk4	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	12	6.4	1.8	*	*	13.2	6.4	*	3.8
Patient 2	30	21	*	11.4	8.4	7	9	9	5.5
Patient 3	16	5.7	1.3	11.9	4.2	3.7	29.4	26.6	31.5
Patient 4	22	15	8.3	9.8	4.4	3	16.2	9.4	6.4
Patient 5	12	9.9	6.1	10.8	6.6	13.8	10	6.9	3
Patient 6	5.2	2.8	1.8	7.1	2.9	3.8	9.1	6.4	1.8
Patient 7	6	*	*	12.2	3.8	1.6	16.2	14.4	9.8
Patient 8	16	2.7	6	12.2	5	4.1	11.4	10.7	8.9
Patient 9	13	8.2	2.7	19	10.8	6.1	*	6.7	3.2
Patient 10	2.6	3.9	2.1	11.1	6.6	6.6	5.8	3.2	2
Mean	14	8.4	3.8	11.7	5.9	6.3	12.6	10.4	7.6
SEM	2.6	1.9	0.8	1.0	0.8	1.3	2.3	2.2	2.8

Individual PASI in patients being treated with UVB plus 100g of calcipotriol per week, 100g of calcipotriol ointment alone and UVB alone. *Result not available.

Table 26

	Serum Calcium			Ionized calcium			Serum phosphate		
	Base	Wk2	Wk4	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	2.18	2.21	2.26	1.31	1.26	1.28	1.13	1.32	1.25
Patient 2	2.18	2.28	*	1.26	1.31	*	.72	1.01	*
Patient 3	2.25	2.27	2.21	1.19	1.25	1.27	.88	1.21	1.35
Patient 4	2.33	2.33	2.29	1.27	1.3	1.26	.98	.8	1.04
Patient 5	2.41	2.3	2.31	1.26	1.25	1.26	.94	.9	.94
Patient 6	1.85	2.01	2.14	1.11	1	1.21	.74	1.23	1.66
Patient 7	*	*	*	1.18	*	*	*	*	*
Patient 8	2.07	2.11	2.26	1.23	1.31	1.32	.94	.99	1.32
Patient 9	2.33	2.38	2.31	1.39	1.41	1.36	.8	.96	.95
Patient 10	2.1	2.2	2.08	1.24	1.26	*	1.19	1.05	1.23
Mean	2.2	2.2	2.2	1.2	1.3	1.3	0.9	1.1	1.22
SEM	0.1	0.03	0.03	0.03	0.03	0.02	0.1	0.1	0.08

Serum total adjusted calcium (mmol/L), serum ionized calcium (mmol/L) and serum inorganic phosphate (mmol/L) in patients receiving UVB plus 100g of calcipotriol ointment per week.
*Result not available.

Table 27

	PTH			24h Urine calcium		
	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	1.9	1.1	2.6	4.2	4.3	5.3
Patient 2	3.2	2.1	*	1.6	*	*
Patient 3	1.5	1.6	2.1	2.4	6.9	13.5
Patient 4	4.7	4.3	3.9	.6	1.4	.8
Patient 5	5	3.9	4.3	3.5	5.15	1.5
Patient 6	26	8.5	5.6	.6	.5	.3
Patient 7	*	*	*	*	*	*
Patient 8	4	3.4	.7	11.1	3.69	3.4
Patient 9	2	2	2.6	2.2	3.1	5.3
Patient 10	1.2	1.9	*	2.6	3.9	2.1
Mean	5.5	3.2	3.11	3.2	3.62	4.03
SEM	2.60	0.76	0.61	1.13	0.71	1.51

Serum parathyroid hormone (pmol/L) and 24h urine calcium (mmol/24h) in patients receiving UVB plus 100g of calcipotriol ointment per week. *Result not available.

Table 28

	Serum calcium			Ionized calcium			Serum phosphate		
	Base	Wk2	Wk4	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	2.15	2.27	2.18	1.25	1.25	1.26	.9	1	.98
Patient 2	2.22	2.17	*	1.25	*	*	.9	1.23	*
Patient 3	2.24	2.23	2.19	1.29	1.32	*	.97	1.3	1.07
Patient 4	2.08	2.23	2.14	1.26	*	*	1.13	1.02	1.32
Patient 5	2.21	2.21	*	1.3	1.29	1.28	1.13	1.41	*
Patient 6	2.25	2.18	2.27	1.32	1.29	1.22	1.34	1.49	1.61
Patient 7	2.19	2.28	2.05	1.28	*	1.29	1.09	.98	.9
Patient 8	2.29	2.33	2.24	1.27	1.26	1.21	1.24	1.24	1.25
Patient 9	2.25	2.17	2.3	1.2	1.19	1.29	1	.99	1.06
Patient 10	2.33	2.29	2.34	1.17	1.24	1.28	1.36	1.05	1.27
Mean	2.2	2.2	2.2	1.3	1.3	1.3	1.1	1.2	1.18
SEM	0.02	0.02	0.03	0.02	0.01	0.01	0.06	0.06	0.76

Serum total adjusted calcium (mmol/L), serum ionized calcium (mmol/L) and serum inorganic phosphate (mmol/L) in patients applying 100g of calcipotriol ointment per week. *Result not available.

Table 29

	PTH			24h Urine calcium		
	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	6.1	5.9	6.3	3.3	2.5	3.4
Patient 2	.7	1.4	*	5.4	2.7	2.5
Patient 3	1.1	2.2	1.4	5.3	8.7	7.3
Patient 4	3	1.5	2.2	9.7	6.9	8.5
Patient 5	1.6	2.4	1.1	1.8	2.1	.9
Patient 6	*	2.1	.8	6.2	5.9	5.6
Patient 7	2.2	1.6	2.2	3.7	4.4	6.5
Patient 8	3.5	1.3	1.8	1.14	2.9	1.2
Patient 9	3.8	3.7	2.9	2.1	2.7	3.2
Patient 10	2.7	1.8	.5	1.5	3.4	3.1
Mean	2.7	2.4	2.1	4	4.2	4.22
SEM	0.5	0.5	0.6	0.9	0.7	0.87

Serum parathyroid hormone (pmol/L) and 24h urine calcium (mmol/24h) in patients applying 100g of calcipotriol ointment per week. *Result not available.

Table 30

	Serum Calcium			Ionized calcium			Serum phosphate		
	Base	Wk2	Wk4	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	2.27	2.33	2.22	*	*	1.44	.99	1.24	1.19
Patient 2	2.13	2.3	2.28	1.35	1.29	1.3	1.01	1.18	.97
Patient 3	2.26	2.24	2.21	1.27	1.28	1.25	1.16	1.35	.9
Patient 4	2.18	2.12	2.22	1.23	1.26	1.29	1.3	1.34	1.43
Patient 5	2.15	2.14	2.06	1.21	1.26	1.25	1.09	1.16	1.26
Patient 6	2.17	2.28	2.27	1.18	1.27	1.34	1.04	1.28	1.15
Patient 7	2.2	2.09	2.21	1.23	1.29	1.24	.86	1	1.17
Patient 8	2.28	2.21	2.14	1.27	1.28	1.27	.94	1.16	.9
Patient 9	2.24	2.26	2.26	1.25	1.29	1.24	1.26	1.2	1.13
Patient 10	2.27	2.22	2.27	1.23	1.27	*	1.15	1	1.06
Mean	2.2	2.2	2.2	1.2	1.3	1.3	1.1	1.2	1.11
SEM	0.02	0.02	0.02	0.01	.004	0.01	0.04	0.04	0.05

Serum total adjusted calcium (mmol/L), serum ionized calcium (mmol/L) and serum inorganic phosphate (mmol/L) in patients receiving UVB alone. *Result not available.

Table 31

	PTH			24h Urine calcium		
	Base	Wk2	Wk4	Base	Wk2	Wk4
Patient 1	1.7	1.8	1.8	5.1	3.1	3.6
Patient 2	1.1	1.6	*	3.6	1.9	6.3
Patient 3	1.9	1	1.9	*	3.1	*
Patient 4	6.4	3.8	4	1	1.1	1.3
Patient 5	1.4	2.6	0.7	3.8	5	9.5
Patient 6	2.3	2.2	1.7	6.3	8.1	8.5
Patient 7	3.1	1.6	1.5	3.3	3.8	6.8
Patient 8	2	3	3	5.9	8.4	5.1
Patient 9	1.4	*	1.6	5.5	8	5.1
Patient 10	3.6	1.3	2.5	6.7	7.4	6.5
Mean	2.5	2.1	2.1	4.6	5	5.86
SEM	0.5	0.3	0.3	0.6	0.9	0.82

Serum parathyroid hormone (pmol/L) and 24h urine calcium (mmol/24h) in patients receiving UVB alone. *Result not available.

3.5.2 Discussion

This study detected no clinically significant additive or synergistic effect on systemic calcium homeostasis when the combination of calcipotriol and UVB were used to treat chronic plaque psoriasis. The small effect on serum phosphate suggests that UVB plus calcipotriol may have a slightly greater effect than either agent alone but this is unlikely to be of clinical importance. It is perhaps surprising that there was no demonstrable effect on serum ionized calcium or 24h urine calcium. This may be due to the smaller number of patients in each limb as compared to our earlier studies looking at 90 and 100g per week.

In summary, although both calcipotriol and UVB are effective treatments for psoriasis, the combination of the two has not been found in this study to be superior to either agent alone. The combination of UVB plus calcipotriol not appear to be associated with clinically significant adverse effects on systemic calcium homeostasis.

3.6 SECTION V Unstable pustular psoriasis

3.6.1 Results

3 patients with generalised pustular psoriasis were treated with topical calcipotriol.

Case 1

A 68-year-old woman was admitted with pustular psoriasis covering 45% of her body. Calcipotriol ointment was applied twice daily and she improved rapidly, pustulation disappearing within 24 hours. She was treated in hospital for 3 weeks at a dose of 100g of calcipotriol (50ug/g) per week and then discharged on the same treatment. There was no change in serum calcium during that time period.

Case 2

A 76-year-old woman with long-standing unstable psoriasis who had 2 previous admissions with severe pustular psoriasis but been controlled with acitretin 25mg daily for the previous 2 years, was admitted with sudden deterioration. Ninety percent of her skin was covered with pustules. On admission, there was biochemical evidence of both renal and hepatic impairment and we were reluctant to institute systemic therapy. She was treated initially with bed rest emollients, and intravenous fluids. However, after 3 days she had deteriorated further and treatment with topical calcipotriol was started. Her psoriasis improved rapidly, pustulation settling within 24hrs.

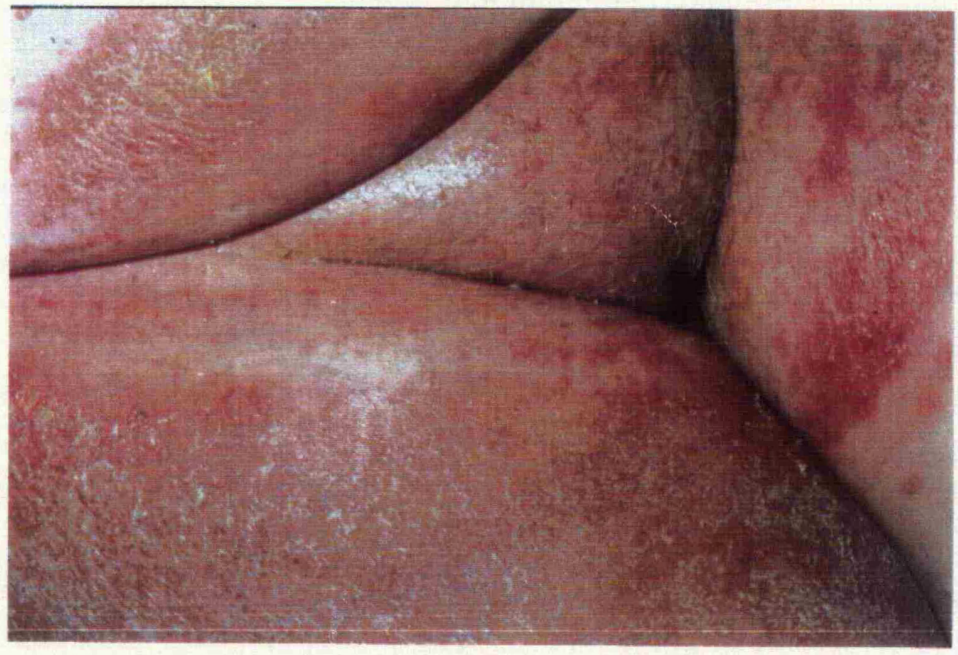
Approximately 300g of calcipotriol (50ug/g) ointment were used over the following 10 days. As she was then stable, treatment with etretinate was reinstituted. There was no significant change in serum calcium during the period of treatment with calcipotriol.

Case 3

An 81-year-old woman was admitted with flexural pustular psoriasis involving approximately 30% of the body. She was treated with topical calcipotriol and rapidly settled (Fig. 35). 100g of calcipotriol (50ug/g) ointment were used over one week and treatment was then stopped. 1 week later she relapsed, but responded again to calcipotriol. There was no change in serum calcium during the period of treatment with calcipotriol.

3.6.2 Discussion

Topical calcipotriol appears to be a very effective treatment for unstable pustular psoriasis. No side effects were detected in the patients we treated, however, because of the age and general condition of the patients it was not possible to carry out detailed assessment of systemic calcium homeostasis in these cases. Hypercalcaemia did not develop in our patients despite the use of up to 210g of calcipotriol (50ug/g) ointment per week. However, as outlined above, there have been reports of hypercalcaemia in similar patients using as little as 90g per week. Careful assessment of such patients is necessary if calcipotriol is to be used.



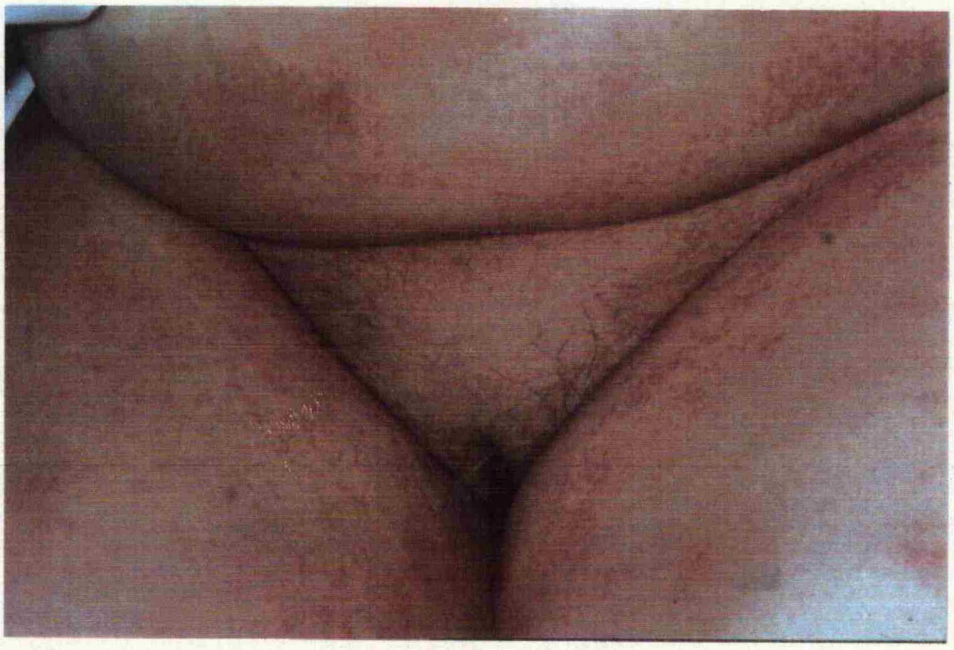


Figure 35. Patient with pustular psoriasis before and after treatment with topical calcipotriol.

CHAPTER 4 CONCLUSIONS

We have demonstrated that, despite calcipotriol's apparent reduced toxicity in animal studies, it does affect systemic calcium homeostasis when used topically to treat patients with chronic plaque psoriasis. The effect is detectable even at recommended doses.

4.1 Mechanisms of the effects of topical calcipotriol on systemic calcium homeostasis in patients with psoriasis

These results indicate that calcipotriol has similar effects on systemic calcium homeostasis to 1,25 dihydroxyvitamin D_3. When used topically, in high doses, it is absorbed percutaneously and may be detected in serum using the method described by LeVan.[160] Calcipotriol enhances gastrointestinal absorption of calcium and probably also phosphate. Serum total calcium and phosphate and serum ionized calcium both rise as a result. Serum levels of parathyroid hormone are depressed due to the rise in serum calcium and possibly a direct inhibitory effect of calcipotriol. Urine excretion of calcium and phosphate are both increased. Urinary calcium excretion is increased directly due to higher serum concentrations and also indirectly as a result of lower PTH levels. Serum 1,25 dihydroxyvitamin D_3 levels are suppressed due to inhibition of 1α hydroxylase. 1α hydroxylase is inhibited by reduced serum PTH, possibly directly by calcipotriol itself and also by rising serum phosphate levels.

There is no evidence of mobilization of calcium, or phosphate, from bone. However, the effects on bone markers were only studied at higher doses for very short periods. An effect of prolonged use cannot therefore be excluded.

Calcipotriol may differ in one respect from 1,25 vit D_3. We could detect no acute effect of calcipotriol on intestinal absorption of calcium at doses where chronic (8 weeks) usage did result in a rise in serum ionized calcium. This is in keeping with the findings of Norman *et al.*[109] who noted reduced induction of transcaltachia by calcipotriol *in vitro* as compared to 1,25 vit D_3. It is unlikely that this is of clinical significance as continued application of calcipotriol does result in an increase in serum calcium levels even at recommended doses.

4.2 Effects of recommended doses

Recommended doses of calcipotriol do appear to have a measurable effect on systemic calcium homeostasis which is small and only detectable when the maximum recommended dose is approached. At doses of 90g of the 50μg/g ointment per week, serum ionized calcium rose slightly but there were no changes in any of the other parameters measured. At 100g per week a rise in serum phosphate and 24h urine calcium excretion was detectable. The rise in urine calcium excretion has the potential for serious adverse effects in that it could result in or predispose to renal calculi, in susceptible individuals. However the mean rise in 24h urine calcium was only 0.36mmol/24h. Furthermore the risk of renal calculi relative to the general population only rises appreciably when the 24h urine calcium is greater than 10mmol/24h.[159] Although a number of patients using 100g per week did have levels this high, they had abnormal values prior to starting the study and only one patient with a normal value at baseline developed an abnormally high level during treatment - 8.5mmol/24h. This level was not sustained and the patient's urine calcium returned to normal while still applying calcipotriol.

Most patients using calcipotriol only use 30 - 40g per week and this amount appears to be safe. The addition of occlusion with polythene film does not appear to adversely affect systemic calcium homeostasis with these standard doses of calcipotriol. Long term use of standard amounts of calcipotriol also appears to be safe in this regard.

4.3 Effects of calcipotriol plus UVB

No clinically significant enhancement of the effect of topical calcipotriol on systemic calcium homeostasis has been demonstrated when it is used in combination with UVB. We have not investigated the use of high dose topical calcipotriol plus UVB, although the additive effect of UVB is likely to be minimal.

4.4 Effects of calcipotriol in unstable psoriasis

In our limited experience with the use of topical calcipotriol in unstable pustular psoriasis, the use of up to 210g per week did not cause hypercalcaemia over short periods. However, the experience of others[132,133] would indicate that hypercalcaemia may occur in these patients even with recommended doses. More detailed assessment of such patients would probably reveal some alteration in systemic calcium homeostasis, however that was not deemed possible in our particular patients.

4.5 Implications of these findings for the treatment of psoriatic patients with topical calcipotriol

These results indicate that topical calciptriol, even at recommended doses, may affect systemic calcium homeostasis.

At high doses, there is considerable risk of toxicity. The dose per kg body weight is the important measurement to take into consideration. Five of nine patients receiving 360g of 50ug/g ointment per week developed hypercalcaemia. Five out of five patients applying greater than 5.5g per kg of the ointment per week developed hypercalcaemia.

The significance of hypercalcaemia in 2 of 4 Asian patients is uncertain. Given the small numbers, it may be a chance occurrence. This is an issue which probably merits further assessment with greater numbers of patients.

Our high dose studies have not revealed any effect of calcipotriol which differs from 1,25 dihydroxyvitamin D_3 and therefore, it is unlikely that a situation of relative vitamin D deficiency would arise from long term use of this agent. The possible differential effect on acute intestinal calcium absorption which is supported by the results of our single dose study, is probably not of clinical significance as intestinal absorption does appear to be increased by long term use of calcipotriol. We cannot exclude a differential effect on bone at high doses over prolonged periods. This may also merit further investigation.

Patients with unstable pustular psoriasis are probably also at risk of toxicity from calcipotriol. Although we found in our three patients that it was effective and safe at doses as high as 210g per week, there are reports in the literature of hypercalcaemia even with doses less than 100g per week.

The principal concern for patients using recommended doses is the development of renal calculi after long-term use. The effect on urine calcium is only detectable when the maximum recommended dose of 100g of the 50ug/g ointment is approached and in most patients the change is so small as to be inconsequential. However, patients with pre-existing hypercalciuria are an exception in that as a small rise in urine calcium in these patients will increase the risk of developing renal calculi.

For most patients with mild to moderate chronic plaque psoriasis, who use in the region of 30 - 40g of calcipotriol 50μg/g ointment per week, there is no evidence of any toxic effect on systemic calcium homeostasis. Both long-term use and the addition of occlusion with polythene film at night to selected areas appears to be safe in these patients.

Finally, calcipotriol interferes with antibody-based kits used to measure 1,25dihydroxyvitamin D_3. If it is necessary to determine the level in patients applying calcipotriol, either the drug should be stopped for 2-3 weeks, or HPLC should be used to ensure accuracy of the results.

Our findings are in stark contrast to those of previous toxicity studies, where no effect of calcipotriol was demonstrated.[114-122] We feel that there are two principle reasons for this discrepancy. Firstly, ours were the first studies designed to investigate fixed doses of the drug. All previous studies had investigated patients using a wide variety of doses and any effect of those using higher doses would have been diluted by the rest of the group. Secondly, there have been very few published studies[128-30] which investigated doses approaching the manufacturers guidelines and none which investigated patients using potentially toxic doses.

We are aware of one, as yet unpublished study, [161] which assessed the use of higher doses of calcipotriol in some detail. This has been presented in poster form but has not been subjected to peer review, as far as we are aware.

Our findings are in keeping with the growing number of case reports of toxicity at higher doses[117, 131-6] and the occasional report at recommended doses. Given that hypercalcaemia can develop at doses of 210g of ointment per week, it would be very surprising if no effect could be detected whatsoever at 100g or 90g per week. We feel that our findings are more consistent with post marketing surveillance reports than previous toxicity studies.

We feel that our findings also have implications for future assessment of topical agents prior to licensing. Topical drugs should be assessed, as any oral formulation would, at fixed doses to accurately determine the maximum recommended dose and detect any significant side effects.

4.6 Monitoring for toxicity

We have found serum phosphate, 24 hour urine calcium and serum ionized calcium to be more sensitive indicators of calcipotriol toxicity. Serum ionized calcium is not widely available and must be taken under controlled conditions and assayed immediately. It is unlikely to be useful for monitoring outside specialist units.

24 hour urine calcium is a relatively simple assay to carry out although it does require the full co-operation of the patient. This assay also has important clinical implications, in that a high normal value at baseline is a contra-indication to treatment with calcipotriol because of the risk of developing renal calculi.

Serum phosphate is also a relatively simple assay to carry out and could be used as a screening test although in itself it is probably not clinically relevant.

4.7 Summary

Topical calcipotriol is a safe and effective treatment for mild to moderate chronic plaque psoriasis. In patients with more extensive psoriasis who use amounts approaching the maximum recommended dose of calcipotriol, serum total adjusted calcium, serum phosphate and 24 hour urine calcium should be measured regularly. Serum ionized calcium, if available, can also be used in the early detection of toxicity.

In patients with extensive chronic plaque psoriasis, high dose calcipotriol is an effective treatment but should only be administered in hospital with the patients being carefully monitored. Doses greater than 5.5g of the 50μg/g ointment per kg body weight week should not be used as this is likely to cause hypercalcaemia.

Although high dose calcipotriol is also effective in the treatment of patients with unstable psoriasis, they are at particular risk because of increased absorption of calcipotriol and should be closely monitored.

The combination of UVB and calcipotriol does not appear to confer any additional toxicity risk although the clinical benefit of this combination remains to be proven.

APPENDIX 1

DIETARY GUIDELINES FOR STABILIZING DIET

As part of this present trial we will be providing you with a fixed calcium intake in hospital. To allow the body to adjust, for 3 weeks prior to hospital admission, it would be helpful if you followed these dietary guidelines:

We would like you to maintain your calcium intake at approximately 1000g per day. This will meet all the body's requirements.

The main sources of calcium in the diet are milk, yogurt, cheese and white bread.

To help keep your intake constant you need to eat daily:

5 calcium exchanges (in any combination)

One calcium exchange (200mg) is equal to:

1/3 pint of milk

OR 1 carton of yogurt

OR 150g of ice-cream

OR 200g of milk pudding

OR 100g of milk chocolate

Preferably you should eat wholemeal bread, or no more than 4 slices of white bread a day

If your calcium intake is normally very low you will be provided with calcium supplements

Otherwise no calcium supplements should be taken

Large amounts of salt in the diet can cause you to excrete calcium. It is therefore advisable to avoid adding salt to your food at the table and avoid very salty foods

The list below will help you to do this

AVOID:

- tinned/processed meats, eg bacon, salami
- cheese (unsalted cream cheese and cottage cheese are allowed)
- salted nuts, crisps, savoury snacks
- stock cubes, gravy powders, granules

  (gravy browning can be used as an alternative)
- tinned and packet soup
- Oxo, Bovril, Marmite

A salt substitute may be used, eg Ruthamal or Seloral

PRESENTATIONS/POSTERS

1. Immediate and long term effects of topical calcipotriol on calcium homeostasis during treatment of psoriasis. Berth-Jones J, Bourke JF, Elouzi H, Iqbal SJ, Hutchinson PE. presented at the BAD, July 1992.
2. 5 years experience with vitamin D and its analogues in the treatment of psoriasis. Berth-Jones J, Bourke JF, Hutchinson PE. Poster at the AAD, Dec 1992 (SILVER MEDAL AWARD).
3. An assessment of the effects of topical calcipotriol on systemic calcium metabolism. Bourke JF, Berth-Jones J, Hutchinson PE. Poster at the EADV, Sept 1993.
4. Calcipotriol in children. Presented at the BSPD annual meeting, Leicester Oct 1993.
5. Occlusion enhances the efficacy of topical calcipotriene in the treatment of chronic plaque psoriasis. Bourke JF,Berth-Jones J,Iqbal SJ,Hutchinson PE.Poster at the AAD, Dec 1993.
6. The effects of topical calcipotriol on systemic calcium homeostasis. Bourke JF, Berth-Jones J, Wong M, Holland S, Iqbal SJ, Hutchinson PE. Poster at Ninth workshop on Vitamin D, Orlando, USA 1994.
7. Seven years of clinical experience with topical calcipotriene in psoriasis. Berth-Jones J, Bourke J, Hutchinson PE. Poster at the ninth workshop on vitamin D. Orlando 1994.
8. High dose calcipotriol consistently suppresses serum parathyroid hormone levels. Bourke JF, Berth-Jones J, Iqbal SJ, Hutchinson PE. Presented at BAD, London 1994.

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10. The effects of up to 100g of topical calcipotriol (50μg/g) on systemic calcium homeostasis. Bourke JF, Berth-Jones J, Wong M, Elouzi H, Iqbal SJ, Hutchinson PE. Presented at Vitamin D: Actions and applications in Dermatology. Aarhus 1995.

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2. High dose topical calcipotriol for extensive psoriasis vulgaris. Bourke J F, Berth-Jones J, Iqbal S J, Hutchinson P E. Br J Dermatol 1993; 129: 74-6.

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4. Occlusion enhances the efficacy of topical calcipotriol in the treatment of chronic plaque psoriasis vulgaris. Bourke J F, Berth-Jones J, Hutchinson P E. Clin Exp Dermatol 1993; 18: 504-6.

5. High dose topical calcipotriol consistently suppresses serum parathyroid hormone levels. Bourke JF, Berth-Jones J, Iqbal SJ, Hutchinson PE. Clinical Endocrinology 1994; 41: 295-7.

6. Vitamin D analogues in psoriasis - effects on systemic calcium homeostasis. (Review) Bourke JF, Iqbal SJ, Hutchinson PE. Br J Derm 1996; 135: 347-54.

7. A Randomized Double-Blind Comparison of the Effects on Systemic calcium Homeostasis of Topical Calcitriol (3μg/g) And Calcipotriol (50μg/g) in the Treatment of Chronic Plaque Psoriasis Vulgaris.
Bourke JF, Iqbal SJ, Hutchinson PE. Acta Dermatovenereol *In Press.*

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1. **Observations on the effects of high dose topical calcipotriol on systemic calcium homeostasis in patients with extensive chronic plaque psoriasis.** Bourke JF, Mumford R, Iqbal SJ, Hutchinson PE. *Submitted to the Journal of Investigative Dermatology.*

2. **A comparison of the effects of ultraviolet light and calcipotriol on systemic calcium homeostasis in patients with chronic plaque psoriasis.** Bourke JF, Iqbal SJ, Hutchinson PE. *Submitted to Clinical and Experimental Dermatology.*

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1. **Hypercalcaemia with topical calcipotriol.** Bourke JF, Berth-Jones J, Hutchinson PE. Br Med J 1993; 306: 1344-5.

2. **Possible interference with calcipotriol in the new gamma-B-125 dihydroxyvitamin D IDS assay.** Iqbal SJ, Whittaker P, Bourke JF, Mumford R, Hutchinson PE, LeVan LW. Clinical Chemistry *In Press.*

3. **A single topical dose of 6g of calcipotriol (50ug/g) ointment has no acute effect on systemic calcium homeostasis.** Bourke JF, Berth-Jones J, Elouzi H, Wong M, Hutchinson PE, Iqbal SJ. Clin Exp Dermatol *In Press.*

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1. **Immediate and long term effects of topical calcipotriol on calcium homeostasis during treatment of psoriasis.** Berth-Jones J, Bourke J F, Elouzi H, Iqbal S J, Hutchinson P E. Br J Dermatol 1992; 127(suppl 40): 17-8.

2. **An assessment of the effects of topical calcipotriol on systemic calcium metabolism.** Bourke JF, Berth-Jones J, Iqbal SJ, Hutchinson PE. Journal of the European Academy of Dermatovenereology book of abstracts(JemecGBE,ed.) Kandrup. Copenhagen 1993; 380.

3. **High dose topical calcipotriol suppresses serum parathyroid hormone levels.** Bourke JF, Berth-Jones J, Iqbal SJ, Hutchinson PE. Br J Dermatol 1994; 131 (suppl 44): 17.

4. **The effects of topical calcipotriol on systemic calcium homeostasis.** Bourke JF, Berth-Jones J, Wong M, Holland S, Iqbal SJ, Hutchinson PE. Proceedings of the ninth workshop on vitamin D (Norman AW, Bouillon R,Thomasset M, eds.) de Gruyter. New York; 1995: 587-8.

5. **Seven years clinical experience with topical calcipotriene in psoriasis.** Berth-Jones J, Bourke J, Hutchinson PE. Proceedings of the ninth workshop on vitamin D.(Norman AW, Bouillon R,Thomasset M, eds.) de Gruyter. New York; 1995: 586-7.

6. **A double blind comparison of calcitriol (3ug/g) and calcipotriol (50ug/g) in the treatment of chronic plaque psoriasis.** Bourke JF, Iqbal SJ, Hutchinson PE. Br J Dermatol 1995; 133(suppl 45): 17.

7. **The effects of up to 100g of topical calcipotriol (50μg/g) on systemic calcium homeostasis.** Bourke JF, Berth-Jones J, Wong M, Elouzi H, Iqbal SJ, Hutchinson PE. Presented at Vitamin D: Actions and applications in Dermatology. J Invest Dermatol *in Press.*

8. **The effects of high dose topical calcipotriol (50μg/g) on systemic calcium homeostasis.** Bourke JF, Mumford R, Holland S, Vasanji K, Farmer N, Iqbal SJ, LeVan LW, Hutchinson PE. Poster at Vitamin D: Actions and applications in Dermatology. J Invest Dermatol *in Press.*

9. **The effects of UVB plus calcipotriol on systemic calcium homeostasis in patients with chronic plaque psoriasis.** JF Bourke, SJ Iqbal, PE Hutchinson. Br J Dermatol 1996; (suppl 47): 31.

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11. **High dose calcipotriol suppresses 1,25dihydroxyvitamin D_3 and interferes with measurement of serum 1,25dihdyroxyvitamin D_3 levels in patients with chronic plaque psoriasis.** JF Bourke, P Whittaker, R Mumford, SJ Iqbal, PE Hutchinson. Br J Dermatol 1996; 134: 583.

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