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POSTTRANSPLANTATION BONE DISEASE

The effect of immunosuppressive drugs on
bone: clinical and experimental studies

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To the memory of my parents,

To my brothers and sisters,

To my wife Amiera and my kids Saga, Dania, and Wahbi.

ABSTRACT

Osteoporosis and pathologic fractures are frequent complications in patients with end-stage liver or kidney diseases. Although successful transplantation eliminates some factors related to poor organ function, an increased incidence of pathologic fractures was reported in the early post-transplant period in spite of normalization of the allograft function. The aims of this thesis were to study the effect of organ transplantation on bone density and bone mineral metabolism as well as to evaluate the effect of immunosuppressive drugs on bone mineral metabolism.

Twenty-five patients with end-stage liver disease and candidates for liver transplantation were measured for bone mineral density (BMD) before transplantation and measurements were repeated in 9 patients at 3 months, 6 months, and 12 months after liver transplantation (Paper I). We found that an early and accentuated bone loss occurs during the first 6 months following transplantation, predominantly in sites enriched with trabecular bone and less in cortical bone. This bone reduction seems to mainly be the result of increased bone resorption, possibly related to the immunosuppressive therapy.

One hundred and twenty-eight patients with different types of hyperparathyroidism (HPT) were included for evaluation of bone mineral density before and after parathyroidectomy (Paper II). Bone mineral density (BMD) measurements were performed in all patients once before parathyroidectomy and measurements were repeated longitudinally for 3 years. Regardless of the etiology, a large proportion of HPT patients had reduced bone density. In patients with primary symptomatic HPT and patients with HPT associated with hemodialysis, bone density increases after parathyroidectomy to an extent that largely restores their preoperative bone loss. In contrast, parathyroidectomy does not appear to improve BMD in patients with HPT associated with renal transplantation, possibly because of the adverse effects of immunosuppressive agents in these patients.

The effect of the immunosuppressant drugs cyclosporine A (CsA), Tacrolimus (FK506), and prednisolone on bone mass in the rat was studied in two experiments using densitometry of femur bone and bone morphometry of decalcified tibial bone (Paper III, IV). Bone density and histology revealed that both CsA and FK506 affect bone by different mechanisms, possibly growth retardation, and that a high dosage of CsA or FK506 alters both cortical and trabecular bone in rats. Prednisolone reduced bone turnover without affecting bone mass, and the combined therapy of both CsA and prednisolone induced a low bone turnover osteopenia.

To evaluate the association between statin use and BMD in our patient population, hospital records for all adult patients who had received a kidney transplant between 1973 and 1998 were reviewed and 59 patients were identified as receiving statins. Bone density measurements were performed in 32 renal transplant patients taking statins as well as in age- and sex- matched controls. Statins users had a higher bone density in the lumbar spine compared to their controls.

In conclusion, patients undergoing organ transplantation are at high risk to develop osteoporosis and pathologic fractures due to the accentuated bone loss, which occurs during the first posttransplant year. The immunosuppressive therapy could have a contribution in the early decrease of bone mass in patients following transplantation as well as in abolishing the anabolic effect of parathyroidectomy on bone mass in patients with HPT associated with renal transplantation. Experimental administration of CsA and FK506 to rats in doses comparable to those used in human subjects provided direct evidence that both drugs have adverse effects on bone. Moreover, CsA seems to augment the effect of prednisolone on bone when both drugs were given together. Preliminary data of the effect of statins on bone showed a modest increment of trabecular bone density (lumbar spine) in statin users compared to controls. However, large randomized controlled studies are needed to accurately determine if statins have a salutary effect on bone mass and bone mineral metabolism.

LIST OF PUBLICATIONS

I.   **Abdelhadi M**, Eriksson SAV, Ljusk Eriksson S, Ericzon B-G, & Nordenström J
     Bone mineral status in end-stage liver disease and the effect of liver transplantation.
     Scand J Gastroenterol 30: 1210- 1215, 1995.

II. **Abdelhadi M** and Nordenström J.
     Bone mineral recovery after parathyroidectomy in patients with primary and renal hyperparathyroidism.
     J Clin Endocrinol Metab 83: 3845-3851, 1998

III. **Abdelhadi M**, Ericzon B-G, Hultenby K, Sjöden G, Reinholt Finn P, and Nordenström J.
     Structural skeletal impairment induced by immunosuppressive therapy in rats: Cyclosporine A versus Tacrolimus (FK506).
     Transpl Int 15: 180-187, 2002

IV. **Abdelhadi M**, Ericzon B-G, and Nordenström J.
     Effects of Cyclosporine and prednisolone and their combination on bone mass and bone mineral metabolism in rat.
     (Manuscript)

V   **Abdelhadi M**, Fehrman-Ekholm I, and Nordenström J.
     The effect of statins on bone mineral density in renal transplant patients
     (Manuscript)

CONTENTS

LIST OF ABBREVIATIONS

ARF	Activation, resorption, and formation sequence
BMC	Bone mineral content
BMD	Bone mineral density
BMU	Basic multicellular (remodeling) units
CT	Calcitonin
CsA	Cyclosporine A
DPA	Dual photon absorptiometry
DXA	Dual energy X-ray absorptiometry
HMG-CoA	Hydroxy-methylglutaryl-coenzyme A
HPT	Hyperparathyroidism
IRMA	Immunoradiometric assay
PTH	Parathyroid hormone
SPA	Single photon absorptiometry
TBVv	Trabecular bone volume density
WHO	World Health Organization

1 INTRODUCTION

1.1 Bone composition and structure

The skeleton is not only an adaptable and well-articulated frame but also a dynamic mineral reserve bank in which the body stores its calcium and phosphate in a metabolically stable and structurally useful manner. Approximately 99% of total body calcium (1.1 to 1.5 kg) and 75% to 80% of total phosphorus (500 to 700 g) are stored in the skeleton in the form of hydroxyapatite. Water makes up about 20% of the wet weight of bone tissue, and the dry weight consists of about 70% inorganic (mineral) material and 30% organic (bone cells and organic matrix) (Netter, 1987). The organic matrix is composed of about 95% collagen (predominantly type 1) and the remainder is a complex of noncollagenous proteins such as osteocalcin, osteonectin, sialoproteins, proteoglycans, and bone morphogenic protein. Four types of cells can be distinguished in bone: lining cells (flat cells along the surface of bone), osteoblasts (which are involved in bone formation), osteoclasts (large cells which are involved in bone resorption), and ostocytes (located within the bone).

All of the bone tissue making up the skeleton exists in either one of two general structural categories, compact bone (cortical bone) or trabecular bone (cancellous bone). Compact bone is the dense outer shell of the skeleton and trabecular bone is the system of plates, rods, arches, and struts, which is encased within the shell. About 80% of the mass of the skeleton is compact bone, and the rest is trabecular bone (Recker, 1994).
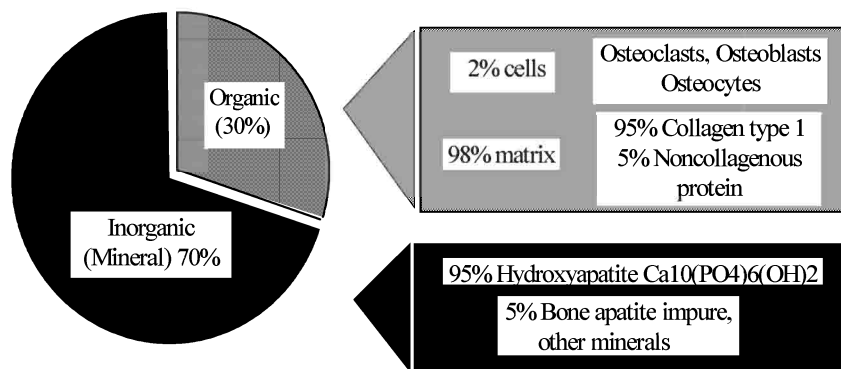
Figure I: Bone composition

1.2 Calcium homeostasis

In a normal adult the amount of calcium in the extracellular fluids and soft tissues does not exceed 10 g with approximately 0.3 g present in the plasma and 9.7 in the extracellular fluids (Albright, 1979). Calcium is found in the serum in form of three fractions (ionized calcium 50%, protein-bound calcium 40% and calcium complexes to citrate and phosphate ions 10%). Some 90% of the protein-bound calcium is bound to

albumin and the remainder to globulin. The plasma calcium is maintained at a remarkably constant concentration by a complex homeostatic mechanism (Mundy, 1990). Parathyroid hormone (PTH) and 1,25(OH)$_2$D regulate the transport of calcium between the extracellular fluid and bone, kidney, and gut. The major effect of PTH on the kidney is to increase the distal tubular reabsorption of calcium, increase fractional excretion of phosphate, and stimulate the renal synthesis of 1,25 (OH)$_2$D. PTH and 1,25 (OH)$_2$D promote net release of calcium and phosphate from bone. 1,25 (OH)$_2$D directly stimulates intestinal absorption of calcium and phosphate (Brown, 1994) (Fig. II).
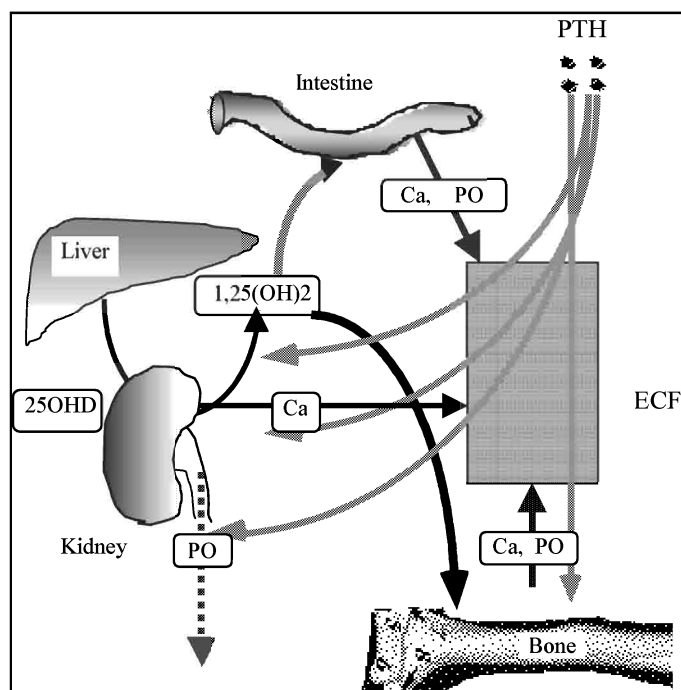

Figure II: Role of PTH and 1,25(OH)$_2$D in calcium homeostasis

1.3 Bone remodeling

Bone remodeling is a dynamic process of the adult skeleton after closure of the epiphyseal growth plates, maintained through the continuous process of destruction and renewal of bone tissue that takes place throughout life. Bone remodeling is achieved by the activity of basic multicellular (remodeling) units (BMUs) located on free bone surfaces and along the vascular channels (Albright and Skinner, 1979). The sequence of events at the remodeling site is the activation-resorption-formation (ARF) sequence (Baron, 1996). Initiation of the remodeling cycle begins with activation of precursor (osteoprogenitor) cells in a localized area of bone. A team of osteoclasts is produced first, resorbing bone for about 1 month. After resorption subsides, the reversal phase lasts about 1 week, when bone-forming cell (osteoblasts) are attracted and which during a 3-month period infill the erosion cavity with new bone. Once the cycle has

been completed cellular activity returns to its resting state, leaving the remaining osteoblasts as inactive lining cells. The action of osteoclasts and osteoblasts are tightly coupled (Albright, 1979; Kanis, 1991a). Bone remodeling is regulated by systemic hormones and by local factors (Canalis, 1996b). (Fig. III)

1.4 Bone remodeling and physiological bone loss

At any time, approximately 15-20% of the bone surface is involved in resorption, formation, or mineralization, and the remainder is quiescent (Kanis, 1991b). The end result of a bone-remodeling event implies a net deficit of bone. Although trabecular bone comprises only 20-25% of total skeletal mass in healthy individuals, the surface of trabecular bone is greater than that of cortical bone (Kanis, 1991b). Because of the high surface-to-volume ratio of trabecular bone tissue, the metabolic activity of trabecular bone is nearly eight times that of cortical bone. This may help to explain why disorders of skeletal homeostasis (metabolic bone disease) have a greater effect on trabecular bone than on cortical bone.

Two factors determine the level of bone mass at any age: peak bone mass achieved at puberty, duration and rate of bone loss thereafter. As a feature of normal physiological aging the rate of bone loss in the lumbar spine is accounted for -0.5 to -0.8% per year in men and between (-0.8 to -1.0%) per year in women (-2% to -3% per year during menopause) (Vaananen, 1991).

1.5 Systemic hormones and local factors regulating bone remodeling

1.5.1 Parathyroid hormone (PTH)

Parathyroid hormone is a polypeptide with a molecular weight of approximately 9500 Daltons. PTH does not act directly on the cytoplasm of its target cells in kidney and bone. Instead, it binds to specific receptors on the surface of target cells; this binding triggering the release of second messengers that mediate the multiple distant effects of PTH. (Kronenberg, 1994). PTH affects bone turnover by increasing rates of bone resorption and new bone formation. This stimulatory action on osteoclast and bone resorption is only thought to be mediated through stimulation of osteoblasts, since osteoclasts lack PTH receptors (Dempster et al., 1993). PTH also influences osteoblasts in terms of both function and number. Reported dual effects of PTH in bone formation, anabolic and catabolic are both time-and dose-dependent. Intermittent exposure to PTH increases and stimulates bone formation, whereas continuous exposure to PTH decreases bone formation (Dempster et al., 1993; Marcus, 1994; Reeve, 1996). The anabolic action of PTH is thought to be mediated through increased synthesis or release of local factors such as insulin-like growth factor (IGF-I) and possibly transforming growth factor-β (Dempster et al., 1993).

1.5.2 Vitamin D

Vitamin D or cholecalceferol is biologically inactive, since that synthesized in the skin as well as that ingested in food must undergo two successive hydroxylations in the liver and kidney to become the biologically active 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) (Mundy, 1990). The major biological function of vitamin D is to maintain sufficient concentrations of calcium and phosphate in the plasma and extracellular fluids. It achieves this function through the action of its metabolites on the target organs, the intestine and bone, which contain a specific nuclear vitamin D receptor (VDR) for

1,25(OH)$_2$D (Holick, 1996). In the intestine, vitamin D facilitates the absorption of calcium and phosphate. Although the absorption of calcium and phosphate occurs along the entire length of the small intestine, most calcium absorption principally occurs in the duodenum, and that of phosphate in the jejunum and ileum.
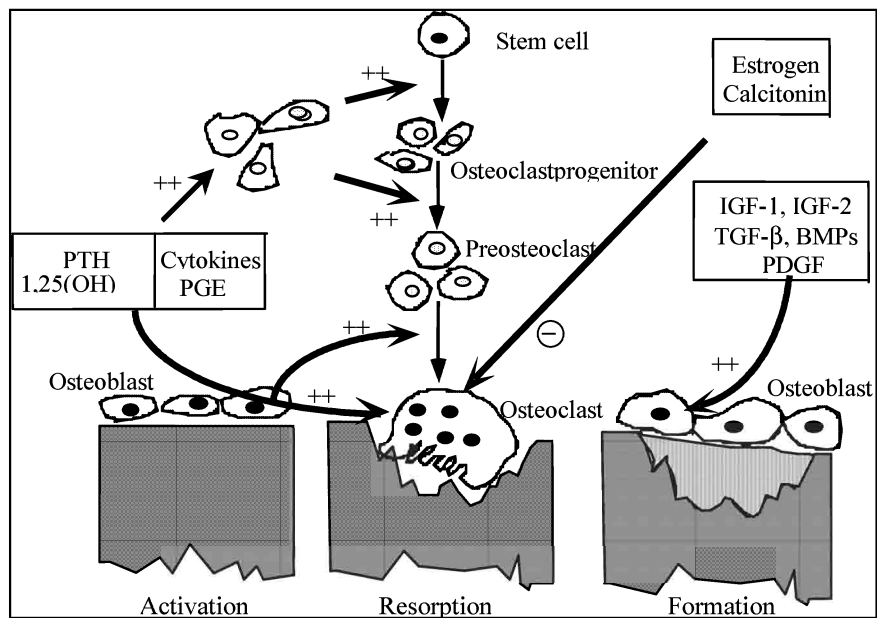

Figure III: Systemic hormones and local factors in bone remodeling

1.5.3   Calcitonin

Calcitonin (CT), a 32-amino-acid peptide, is primarily produced by thyroidal C-cells. Its major physiological effect is to inhibit osteoclastic bone resorption. In spite of intensive studies, it is not clear whether it also enhances bone formation or alters cell number (Rasmussen and Bordier, 1974). Although there are some data suggesting a stimulatory effect of CT on bone formation, long-term anabolic CT-actions may be preferentially mediated by osteoclast inhibition., Even transient osteoclast inhibition might possibly alter remodeling cycles on bone surfaces in a predominant and prolonged formative phase (Skjodt and Russell, 1992)

1.5.4   Skeletal Growth Factors

A number of local growth factors are secreted by bone cells, mainly by osteoblasts, with either autocrine or paracrine action on cell proliferation and differentiation. Some of these factors have important influences on the regulation of bone formation and bone resorption. These growth factors include insulin-like growth factors (IGF-I and -II), transforming growth factor-β (TGF-β), platelet-derived growth factor AA (PDGF), and several bone morphogenetic factors (BMFs). Skeletal cells also synthesize and respond to cytokines such as interleukins 1 and 6 and selected colony-stimulating factors which

have known primary effects on immune and hematological cells (Canalis, 1996b; Robey et al., 1992).

1.6 Osteoporosis

Osteoporosis has been defined by consensus at conferences as " a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (WHO, 1993). The final clinical outcome of the osteoporotic process is a fracture, which can occur spontaneously or following minimal trauma. Osteoporosis has become increasingly recognized as a major health problem. In Sweden, the total annual cost for the care of hip fractures alone recently estimated to be 2.7 milliard SEK (Läkemedelsverket, 2001).

Osteoporosis is generally categorized as primary or secondary, based on the absence or presence of associated medical diseases, surgical procedures, or medications known to be associated with accelerated bone loss. Low bone density has been used to predict risk of fractures as well as to diagnose osteoporosis. This led the World Health Organization (WHO) working group to propose that osteoporosis should be diagnosed when bone mineral density is 2.5 SDs or more below the mean for healthy young adult women at the spine, hip, or wrist (Kanis et al., 1994).

1.7 Posttransplantation osteoporosis

Organ transplantation is used nowadays for a substantial number of patients with chronic and life-threatening heart, kidney, liver, and bone marrow diseases. The introduction of potent immunosuppressive agents such as Cyclosporine (CsA) and Tacrolimus (FK506) has contributed tremendously to overall improvements in long-term graft survival and many transplant patients are expected to have a good quality of life and a long life expectancy.

Successful transplantation eliminates some factors related to the pre-transplant period that have had a negative influence on bone status. However, a high incidence of pathological fractures was reported following organ transplantation (Epstein, 1996; Porayko et al., 1991; Shane et al., 1993). Vertebral fractures occur in 4% to 65% of patients after liver transplantation, the lowest range being in patients with chronic active hepatitis and the highest in patients with PBC (Eastell et al., 1991; McDonald et al., 1991; Porayko et al., 1991).

The frequency of prevalent fractures reported in other series of organ transplant recipients ranged from 17% to 37% in lung transplant patients (Ferrari et al., 1996; Shane et al., 1993) and about 18% to 50% of patients after heart transplantation (Rich et al., 1992; Shane et al., 1993). Severe osteopenia has also been reported at both the lumbar spine and femoral neck within the initial 2 posttransplant years in renal transplant patients. However, bone mass was shown to stabilize thereafter and only moderate and age-related bone loss was apparent (Grotz et al., 1995).

1.8 Posttransplant factors affecting bone mineral metabolism

1.8.1 Glucocorticoids (GCs)

Glucocorticoid use has long been recognized as a risk factor for bone loss and increased incidence of pathological fractures in humans. About 30% to 50% of patients exposed to glucocorticoids develop fractures (Lukert and Raisz, 1990). The incidence and severity are directly related to the dose and the duration of therapy: Trabecular bones are more severely affected than cortical bones.

Glucocorticoid-induced bone loss is primarily due to a decrease in bone formation and an increase in bone resorption. Glucocorticoids directly suppress osteoblast function, impairing collagen synthesis and indirectly through a decrease in production of certain local growth factors, in particular, insulin-like growth factor 1 (IGF-I) and transforming growth factor ß (TGF ß). Glucocorticoids indirectly increase bone resorption through enhanced secretion or activity of PTH, in part as the result of a decrease in intestinal calcium absorption and increase in renal calcium excretion, and in part due to the direct effect of glucocorticoids on osteoblasts by increasing the expression of PTH receptors in these cells. (Canalis, 1996a)

1.8.2 Cyclosporine (CsA) and Tacrolimus (FK506)

CsA and FK506 are potent immunosuppressive agents that exert their effects through inhibition of IL-2 release as well as of T lymphocyte activation (Epstein, 1996). In experimental studies, CsA has been shown to inhibit bone resorption mediated by parathyroid hormone (PTH), prostaglandin E_2 (BGE_2) and 1,25 dihydroxyvitamin D in fetal rat limb bone cultures (Stewart and Stern, 1989), while *in vivo* studies using CsA indicated increased bone turnover and the development of severe osteopenia (Movsowitz et al., 1988; Movsowitz et al., 1990). Similar results of high turnover osteopenia have been reported in rats treated with FK506 (Cvetkovic et al., 1994; Katz et al., 1991).

The mechanisms involved in the alteration of bone mineral metabolism following immunosuppressant therapy with CsA or FK506 are still largely unknown. Hypogonadism is thought to make a minor contribution since testosterone replacement does not prevent CsA-induced osteopenia (Bowman et al., 1997). It has been suggested that T lymphocytes play an essential role in the bone-depleting effects of CsA (Buchinsky et al., 1995). However, this contrasts with the observation that T lymphocyte-deficient (nude) rats have apparently normal skeleton. Moreover, the protective effect of CsA in adjuvant-induced arthritis suggesting that T cell activation, which is readily blocked by CsA, does not seems to be involved in the development of inflammation-dependent osteopenia (del Pozo et al., 1990).

1.9 Quantitative bone mass measurement

Considerable progress has been made in the development of non-invasive techniques for quantitative assessment of bone mineral density. These methods calculate bone mass on the basis of tissue absorption of photons derived from either a radionuclide or an X-ray tube.

The measurement of bone mineral density (BMD) is important in quantifying the development and degree of osteoporosis. A reduced BMD predisposes the patients to fractures. The most frequent and important of the related fractures are those of the proximal femur, vertebrae, and distal forearm, but fractures also commonly occur at many other locations.

The approximate contribution of the cortical and trabecular components of bone at the five scanning sites is as follow: Distal radius, 75% cortical and 25% trabecular bone; Mid-radius, > 95% cortical bone; Lumbar spine >66% trabecular bone, Intertrochanteric region of the femur, 50% cortical and 50% trabecular bone; and cervical region of the femur, 75% cortical and 25% trabecular bone (Riggs et al., 1981)

There are four basic techniques currently used for non-invasive assessment of the skeleton: Single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT).

Single photon absorptiometry (SPA)
Cameron and Sorenson originally introduced photon absorptiometry in 1963 (Cameron and Sorensen, 1963). The difference in photon absorption between bone and soft tissue allows calculation of the total bone mineral content (BMC) in the scan path, which is inversely related to the transmission count rate measured. The major radionuclide source is I^{125} (28 keV). SPA is relatively fast, inexpensive, has a low associated radiation dose, and is accurate and precise. It is only adapted for appendicular bone measurement.

Dual photon absorptiometry (DPA)
DPA is a modification of the SPA using a radioisotope that emits photons at two energy levels. The addition of a dual source allows the measurement of sites that cannot be surrounded with constant soft tissue thickness across the scan bath, such as the lumbar spine and the hip regions. The isotope source is $Gadolinium^{153}$ with radiation peaks at 44 keV and 100 keV (Mazess et al., 1974).
The common disadvantages of DPA are: Time consuming (20-30 min. for one site), older Gd sources give too high density values, and difficulties in defining precise region of interest in spinal area in elderly patients.

Dual energy X-ray absorptiometry (DXA)
DXA is developed as a modification in DPA using an X-ray source in place of an isotope. The photon energies commonly used for DXA scanning are 38 keV and 70 keV. These low energy photons enable detection of very small changes in the two-compartment system (bone mineral and soft tissue). The advent of DXA provides: Better spatial resolution, better precision, much lower scanning time, improvement in automation of analysis procedures, and reduced exposure to radiation.

Quantitative computed tomography (QCT)
QCT can determine in three dimensions the true volumetric density of trabecular or cortical bone at any skeletal site. While QCT of the peripheral skeleton is performed on dedicated equipment, vertebral QCT can readily performed on commercial body CT

scanners with the use of specially designed calibration phantoms. More recent implementations of the technique have reduced the intially high radiation dose and improved the precision by employing commercially available automated scanning and analysis software. However, the radiation dose is still at least 30-fold hifher than that of DXA (Wahner and Fogelman, 1995). Therefore, CT seems less useful in comparison to DXA in terms of precision, accuracy, radiation exposure, quality assurance, and day-to-day logistical ease of operation, particularly in a clinical practice setting.

2 AIMS OF THE STUDY

To evaluate bone mass values at different skeletal sites in patients with end-stage liver disease, and to examine the effect of liver transplantation on bone mineral metabolism.

To evaluate and compare the impact of parathyroidectomy on bone mineral density in patients with primary symptomatic HPT, HPT associated with hemodialysis, and HPT associated with renal transplantation.

To investigate and compare the effect of Cyclosporine (CsA) and Tacrolimus (FK506) on bone mass and bone mineral metabolism in rats.

To study the effect of combined immunosuppressive drugs on bone mass and bone mineral metabolism in the rat.

To characterize the degree of skeletal bone mass changes after renal transplantation in our patient population and to evaluate the effect of HMG CoA reductase inhibitors (statins) on bone mineral density.

3 MATERIALS AND METHODS

3.1 Clinical studies

3.1.1 Subjects

Paper 1: Twenty-five patients with end-stage liver disease who were candidates for liver transplantation were measured for bone mineral density of the lumbar spine, proximal femur, and distal radius once before transplantation. Twelve patients underwent liver transplantation, two patients died a few weeks after transplantation and one patient refused further investigation. The remaining nine patients (six males, three females) were followed-up for more than one year (Table I).

Paper II: One hundred and twenty-eight patients with different types of hyperparathyroidism (HPT) were included for evaluation of bone mineral density before and after parathyroidectomy. Bone mineral density (BMD) measurements of the total body, lumbar spine, proximal femur and distal radius were performed in all patients once before parathyroidectomy and measurements were repeated longitudinally for 3 years (Table I).

Table I: Demographic and clinical characteristics of the study patient groups (Paper I, II)

	Paper I	Paper II			
	End-stage liver disease	Primary HPT symptomatic	asymptomatic	Renal HPT Hemodialysis	renal transplant
No. of patients	25	54	24	20	30
Age (years)	52±9	56 ± 2	69 ± 2	56 ± 3	50 ± 2
Weight (kg)	66±12	71 ± 2	72 ± 3	66 ± 3	72 ± 2
Sex (M / F)	15 / 10	13 / 41	3 / 21	6 / 14	17 / 13
Management	Ltx.	Ptx.	Follow-up	Ptx.	Ptx.
No. of patients	9 (12)	52	24	19	15
Follow-up	12 months	3 years	3 years	3 years	3 years

Ltx. Liver transplantation; Ptx Parathyroidectomy

Paper V: Hospital records of two hundred and forty adult patients who had received a kidney transplant at the Dept. of Transplantation Surgery between 1973-1998 were reviewed. Fifty-nine patients were identified as receiving HMG CoA reductase inhibitors (statins). Patients were excluded from enrolment in the study if they had: chronic rejection and received maintenance dialysis; recent treatment with drugs known to affect bone metabolism such as biphosphonates, oestrogen, progesterone, or calcitonin. After inclusion of patients according to exclusion criteria, one hundred and fifty-six patients (49 statin users, 107 nonusers) were eligible and invited to participate in the study. Sixty-three patients (32 statin users, 31 nonusers) agreed to participate and performed bone density measurements and blood sampling. A major number of statin users received a simvastatin preparation (14 patients; 44%) or fluvastatin (13 patients; 40%). Nonusers were renal transplants without lipid-lowering drugs and served as controls.

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3.1.2   Methods
Bone density measurements

Dual Photon Absorptiometry
Bone mineral density (BMD; g/cm^2) of the lumbar spine (L$_2$-L$_4$) and proximal femur (*paper I*) was measured by dual photon absorptiometry using a commercial instrument (DP3 Lunar Radiation, Madison, WI, USA). This instrument uses the ^{153}Gd isotope, with radiation peaks at 44 and 100 keV. In all measurements the scanning speed was 2.5 mm/s. The proximal femur analysis program provides data for 3 different regions of interest; the femoral neck, Ward's triangle (the area with the lowest bone mineral), and the greater trochanteric. The coefficient of variation (reproducibility) for dual photon absorptiometry is 1.1% at our center (Eriksson et al 1989).

Single Photon Absortiometry
A single photon absorptiometer (SP2) with ^{125}I isotope (12), from the same vendor was used for measurements of bone mineral content (BMC; g/cm) in the distal and proximal radius (*paper I*). BMC of the distal radius was measured in an area starting at a point of junction between the radius and ulna and extending 12 mm in the proximal direction. BMC of the proximal radius was measured in the area starting 8 cm from the tip of the ulnar process and extending 9 mm in the proximal direction.

Dual Energy X-ray Absorptiometry
Dual energy X-ray absorptiometry (DXA) was used for the assessment of BMD using a Lunar DPX-L absorptiometer (Lunar Radiation Corporation, Madison, Wisconsin. U.S.A.). In each patient, separate scans of the whole body, the lumbar spine L$_2$ - L$_4$, the proximal femur (femoral neck) and distal radius (*paper III, V*) were made and analyzed using the manufacturer's version 1.3 software. The in vivo measuring precision was assessed by three scans in each of eight volunteers. The coefficient of variation (CV) for the different sites were: total body 0.6%, femoral neck 2.0%, lumbar spine (L$_2$-L$_4$) 1.0%, and distal radius 1.0%. The precision (CV) based on repeated measurements with the Lunar Spine Phantom was 0.4%.

Blood and urine analysis
Blood and urine samples were collected from patients on the same day and intervals of bone density measurements for determination of bone mineral metabolism analysis.
Serum concentrations of calcium, phosphate and magnesium (*paper I, II*), as well as liver function tests: alkaline phosphatase [ALP], aspartate aminotransferase [AST], bilirubin and albumin (paper I), were conducted following the standard procedures of the hospital. Intact PTH (*paper I, II, V*) was measured using the Allegro Immunoradiometric Assay (Nichols Institute, CA, USA). The serum osteocalcin concentration (*paper I, V*) was measured by a radioimmunoassay (OSTK-PR, Cis Bio International, Gif-sur-Yvette, Cedex, France).

Reference controls
Reference data for bone mineral density (*paper I*) was obtained from 25 normal subjects, matched for age, sex and weight to our patients. These participants were part of a larger group of healthy controls who were recruited from the hospital staff. None

had any condition or was receiving any medication known to influence bone metabolism

The reference data for bone mineral density (*paper II, V*) consisted of the data for age- and sex-matched controls obtained from the DXA manufacturer's reference population. Although this reference population consists of healthy North American individuals, healthy Swedish controls have been shown to have bone mineral densities that are similar to those of healthy Americans (Karlsson et al., 1993).

3.2 Experimental Studies

3.2.1 Animals

Paper III: Male Sprague-Dawley rats (Forty rats for each experiment), each weighing approx. 350 g, were randomly allocated to five groups of 8 rats. Each of the five groups was given one of the following: FK506 1.5 mg/kg, FK506 3 mg/kg, CsA 15 mg/kg, CsA 30 mg/kg or distilled water (control group) for 28 days by daily oral gavage. All animals were housed under similar conditions at 21^{0}C in a 12-h light/12-h dark cycle and maintained on a standard rat diet of 0.98% calcium, 0.75% phosphorus, and 1500 IU/kg vitamin D3, and tap water *ad libitium*.

Paper IV: Fifty, eleven-week-old male Sprague-Dawley rats, weighing approximately. 450 g, were randomly divided into five groups and received Cyclosporine 15 mg/kg alone or combined with prednisolone 3mg/kg daily by gavage (p.o). The other groups received either prednisolone 3 mg/kg or distilled water 1 ml/kg daily by gavage (p.o) for 30 days according to the following protocol:

Group 1: (n=8). baseline control were sacrificed at the start of the experiment; Group 2: (n = 10) Control (distilled water); Group 3: (n = 10) 15-mg/kg Cyclosporine neoral; Group 4: (n = 10) 3-mg/kg Prednisolone; Group 5: (n = 10) 15-mg/kg Cyclosporine neoral and 3-mg/kg b.w Prednisolone.

Rats were weighed and bled on days 0, 15, and 30 under ketamine anesthesia (0.1 mg/100 g i.m.). Blood samples were centrifuged and the sera stored at -70°C until assayed.

All rats received 2 mg/100g tetracycline hydrochloride by s.c. injection on days 16 and 15, and Calcein 1.0 mg/100 g on days 27 and 28 for histomorphometric determination of dynamic parameters of bone remodeling.

3.2.2 Methods

Densitometric analysis of rat femurs (paper III, IV)

The bone mineral content (BMC) and bone mineral density (BMD) of the intact left femur of each rat was measured using a commercially available standard dual-energy X-ray densitometer (DXA, Lunar Corporation, Madison, WI, USA). Bone scan and analysis were performed using the manufacturer's software version 1.3 without additional modification for small-animal measurements. The scan resolution (pixel size) was 0.3 x 0.6 mm, with a collimator diameter of 0.84 mm. The scan width was 40 mm and the length 45 mm. The scanning time per femur was 8-9 minutes. The coefficient of variation (CV) for measurements of BMC and BMD of the femur was respectively 2.6% and 1.2% (mean values) based on repeated measurements of 6 femurs with 10 scans each.

Bone ash weight (*paper III*)
After DXA measurements, each femur was dissected free of soft tissue and the bones were ashed in a muffle oven at 700^0C for 24 hours, and the ash weight per bone was determined.

Histomorphometry (*paper III*)
One tibia from each animal was demineralized in a 4% EDTA solution, pH 7.4, for 4 weeks. Following demineralization, specimens were briefly rinsed in distilled water, dehydrated in ethanol followed by acetone, and embedded in LX-112 (Ladd, Vermont, USA). Semi-thin sections (approx. 0.8 μm) were cut with a diamond knife and stained with toluidine blue and the sections were examined by light microscopy. Micrographs were made at final magnification of 110 and 275 X. Morphometric analyses were performed on the printed copies by point counting using a square lattice (with 2 or 4 cm between test lines) or a semiautomatic interactive image analyzer (Videoplan, Zeiss, Oberkochen, Germany).
   One overview micrograph (magnification 110 x) covering the epiphyseal growth plate and the proximal metaphysis of one tibia in each animal, one micrograph (magnification 275 x) covering the epiphyseal growth plate and 2-3 micrographs (magnification 275 x) covering the methaphysis were analyzed and the following parameters were measured:
   1. Volume density (Vv) of trabecular bone: The relative volume of bone trabeculae in the proximal metaphysis. The compartment of interest was defined as the region between the distal end of the growth plate and 1.5 mm into the metaphysis proper. The measurements were made by point counting according to stereological principles.
   2. The height of the growth plate and the volume density (Vv) of the hypertrophic zone. Using higher power micrographs of the epiphyseal growth plate, the former was made by three random ruler measurements on one micrograph per animal; the latter by point counting.
   3. Estimation of osteoclast volume density was performed by measuring the relative area of all osteoclast profiles on the micrographs of the metaphysis using the image analyzer (Videoplan). The measurements were performed in duplicate and the mean value calculated for each animal. Final results are given as mean values for all animals in the different groups.

Femur bone Length (*paper IV*)
Each femur was dissected free of soft tissue and the length was measured from the proximal end of the caput femoris to the distal end of the medial femoral condyles using a sliding caliper.

Blood collection and analysis
Blood was collected at the time of sacrifice and serum was frozen and stored at -70^0C until assayed. Serum calcium, phosphate, creatinine, urea, and alkaline phosphates were determined by a photometric procedure using an Automatic Multichannel Analyzer (Hitachi 917/ BM-regents; Hitachi Ltd, Naka, Japan).
Serum PTH (*paper III, IV*) was measured by immunoradiometric assay (IRMA) using a commercially available kit specific for the rat (Nichols Institute, Diagnostics). This assay uses two different goat antibodies to the N-terminal region (1-34) of the rat

molecule purified by affinity chromatography. Intra- and interassay coefficients of variation were 4.5% and 6.7 %, respectively. Serum IGF-1 was determined using a rat IGF-1 RIA kit (Diagnostic Systems Laboratories, Texas, USA). Serum osteocalcin was measured by a two-site immunoradiometric assay (IRMA) using a commercially available rat- specific kit (Immutopics, San Clemente, CA, USA) (*paper IV*).

3.3 Ethical considerations
The study protocol for (*paper I, II, V*) involving human subjects was approved by the hospital's Ethics Committee and the informed consent of each patient was obtained.
Experimental procedures for (*paper III, IV*) were reviewed and approved by the Regional Animal Research Ethics Committee.

3.4 Statistical analysis
Student's paired t-test was used to compare preoperative bone mass and biochemical values with those obtained after surgery (*paper I, II*). The unpaired t-test and ANOVA were used to compare differences between the various groups of patients (*paper II, V*). Linear regression was used to correlate bone mass at different sites with other variables. The Z-score (*paper II, V*) was calculated for each bone density measurement from the mean for the relevant control group (Z-score = patient's value - group mean ÷ group SD).
The non-parametric Mann-Whitney U test and ANOVA were used to test differences between normal controls and treated animals (*paper III, IV*). Linear regression was used to correlate body weight and BMC with other variables (*paper III*).

4 RESULTS AND COMMENTS

4.1   **Bone mass in liver disease and effect of liver transplantation (*I*)**

Aims: The aim of this study was to determine bone mass at different skeletal sites in patients with end-stage liver disease and to determine the effect of liver transplantation on bone mineralization.

Results: Bone mineral content (BMC) and bone mineral density (BMD) measured at different skeletal sites in 25 patients with end-stage liver disease was not significantly different from that in matched normal controls. In nine patients followed up after transplantation, an accelerated decrease in bone mass was detected during the first 3 to 6 months after transplantation at the distal radius (-10.2±9.6%), lumbar spine (-8.2±7.0%) and Ward's triangle of the femur (−11.8±12.9%). At the end of the first transplant year some regain in bone mass was detected in the majority of patients.

Table II. Bone mass changes as percentages of the initial values in 9 patients after liver transplantation. Means ± SD

	Before transplantation	After transplantation			
		1 month	3 months	6 months	12 months
BMC	**%**	**%**	**%**	**%**	**%**
Distal radius	100	101.5±11.4	95.3±8.7	89.6±10.1**	91.8±14.0*
Proximal radius	100	98.4±3.0	100.3±4.9	100.2±4.5	100.0±5.9
BMD					
Lumbar spine	100	96.2±4.8	91.8±7.0**	95.1±8.3*	94.5±8.7*
Femoral neck	100	97.5±6.1	95.1±8.9	91.6±12.9*	94.5±17.3
Ward's triangle	100	97.3±6.9	93.7±9.4	88.1±12.9**	91.2±18.7*
Trochanteric	100	97.3±2.0	96.3±5.7	94.6±10.9	97.9±15.4

* p < 0.05   ** p < 0.01 as compared to baseline values.

Comments: Some previously reported studies of bone mass in liver patients have used histomorphometry or single photon absorptiometry, thus restricting analysis to one or two skeletal regions (Long et al., 1978; Stellon et al., 1987). Our finding of normal bone mass at different skeletal sites in liver patients are not in agreement with the severe form of bone loss reported in several other studies (Floreani et al., 1991; Hodgson et al., 1985; Jorge-Hernandez et al., 1988; Matloff et al., 1982). The reason for the observed discrepancy between different studies regarding bone mass changes in chronic liver disease is not known but methodological differences used for evaluating bone mineral status and patient diagnosis may have contributed.

Although successful transplantation eliminates some factors related to poor liver function that have a negative influence on bone status, an accentuated decrease in bone

mass in the first 6 months following liver transplantation and a net regain at the end of first transplant year has been noted (Eastell et al., 1991; McDonald et al., 1991)

In our prospective study of liver transplant recipients followed for one year, one patient (1/9; 11%) developed multiple fractures in the pelvis, hip and ribs after the 6th posttransplant month. Two other patients had bone disease symptoms (hip and back pain) after transplantation without evidence of fracture or osteonecrosis. In previous studies vertebral fractures have been reported in 4% to 65% of patients after liver transplantation. The lowest range was in patients with chronic active hepatitis and the highest in patients with PBC (Eastell et al., 1991; McDonald et al., 1991; Porayko et al., 1991). In a recent cross-sectional study by Thaillandier *et al* (Taillandier et al., 1999), osteoarticular complications occurred in 90 patients (10%) among the 920 surviving patients after liver transplantation. The most common was collapse of vertebral bodies. About 90% of fractures occurred during the first 12 months and 88% of fractures occurred in patients with cirrhosis.

4.2 Bone mass in HPT and the effect of parathyroidectomy (*II*).

Aims: To study the longitudinal effects of parathyroidectomy in patients with primary symptomatic HPT, HPT associated with chronic renal failure, and HPT following successful kidney transplantation as well as study of the effect of conservative (non-operative) management in a group of patients with asymptomatic (mild) primary HPT

Results: Bone density values in 128 patients with different types of HPT were lower than that of age and sex-matched reference groups. A greater reduction of bone mineral density was observed at cortical bone sites (distal radius) than in predominantly trabecular bone (lumbar spine).
After parathyroidectomy, a modest increase (1 to 8%) in BMDs values at all sites was detected in patients with primary symptomatic HPT and HPT associated with a renal transplant. The largest increase in bone mass was observed in patients with HPT associated with hemodialysis, in whom the improvement amounted to 7 - 23%.

Comments: This study demonstrates that skeletal osteopenia is present in a major proportion of patients with HPT and confirms the findings of previous studies of HPT patients using bone densitometry or histomorphometry (Copley et al., 1993; Lindergard et al., 1977; Richardson et al., 1986; Silverberg et al., 1995; Silverberg et al., 1996; Silverberg et al., 1989). Furthermore, the magnitude of bone loss in distal radius was greater than that observed in the lumbar spine. This difference could result from the selective action of PTH on cortical bone loss and preservation of trabecular bone (Christiansen et al., 1992; Dempster et al., 1993; Parisien et al., 1995).

The effect of parathyroidectomy on bone mineral density in patients with HPT associated with hemodialysis has not been as well characterized as in patients with primary HPT, and there are studies reporting either favorable (Abugassa et al., 1990; Copley et al., 1993; Lindergard et al., 1977) or disadvantageous (Felsenfeld et al., 1982) effects.

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This prospective study demonstrated that in patients with primary hyperparathyroidism, BMD improvement after surgery was modest but persisted over the study period (Abugassa et al., 1990; Horiuchi et al., 2002; Nakaoka et al., 2000; Silverberg et al., 1995; Silverberg et al., 1999). The most marked increases in BMD after parathyroidectomy were evident in patients with HPT associated with hemodialysis. In these patients bone density increased by 7 to 23%, which nearly normalized their preoperative reduced bone density (Abugassa et al., 1990; Chou et al., 2001).
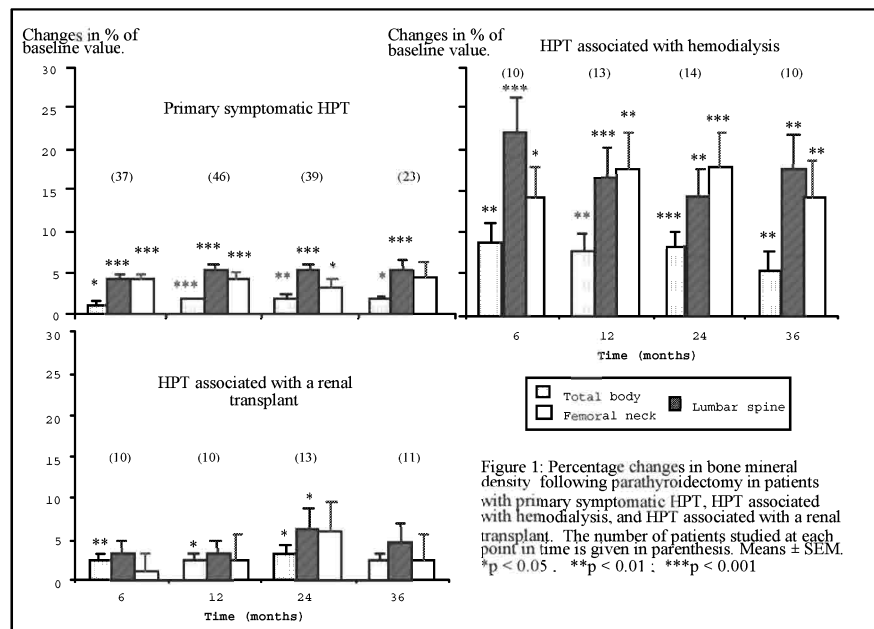
Figure 1: Percentage changes in bone mineral density following parathyroidectomy in patients with primary symptomatic HPT, HPT associated with hemodialysis, and HPT associated with a renal transplant. The number of patients studied at each point in time is given in parenthesis. Means ± SEM. $*p < 0.05$. $**p < 0.01$; $***p < 0.001$

Relatively few studies have addressed bone mass changes in patients with hyperparathyroidism associated with a renal transplant. While successful renal transplantation usually corrects the mineral metabolism disturbances that lead to renal osteodystrophy, persistent HPT plays a major threat to bone mass in these patients (Heaf et al., 2000; Kokado et al., 2000; Setterberg et al., 1996). Despite a marked decrease in the bone density of patients with HPT associated with renal transplant, bone mass increased only marginally after parathyroidectomy. It is most likely that the immunosuppressive therapy which is given after renal transplantation has potential side-effects on bone metabolism and may impair bone recovery (Aroldi et al., 1997; Epstein, 1996; Kokado et al., 2000)

Our findings emphasize the beneficial skeletal effect of parathyroidectomy in patients with symptomatic primary HPT and in those with secondary HPT due to renal failure. In contrast, parathyroidectomy does not appear to improve BMD in patients with renal transplant associated with hyperparathyroidism, possibly because of the adverse skeletal effects of immunosuppressive agents in these patients. These findings suggest that it may be preferable to perform parathyroidectomy before rather than after renal transplantation.

4.3 Effects of CsA and Tacrolimus (FK506) on bone (*III*)

Aims: To examine and compare the effect of the immunosuppressants cyclosporine and FK506 on rat bone using bone densitometry and histomorphometry.

Results: Densitometric analysis of the whole femur demonstrated a significant reduction in BMC (-11% to -14%) in rats treated with the low or high dose of FK506, and high dose CsA compared to normal controls. Conversely, decalcified sections revealed a lower volume density of trabecular bone of the tibia metaphysis in both CsA-treated groups (-39.1%, and -44.9%), in the high-dose FK506 group (-20.9%), and unchanged in low-dose FK506 treated group.

Comments: In previous experimental studies, the effect of CsA on bone mineral metabolism has given contradictory results. *In vitro* CsA has been shown to inhibit bone resorption mediated by parathyroid hormone, prostaglandin E and 1,25 dihydroxyvitamin D in fetal rat limb bone cultures (Stewart et al 1986). While some *in vivo* studies using CsA indicate regression of bone loss in adjuvant arthritic rats (del Pozo et al., 1990) and decreased bone resorption with increased bone formation (Orcel et al., 1989), other studies reported increased bone turnover and the development of severe osteopenia (Movsowitz et al., 1988; Movsowitz et al., 1990). However, our approach to measuring the effect of these drugs by bone densitometry and histomorphology provides several advantages: First, each methodology reflects bone changes in a particular type of bone, e.g. densitometry of the whole femur bone reflects mainly changes in bone at the organ level and histology of the proximal metaphysis reflects changes in bone at the tissue level. Secondly, metabolic bone disorders are heterogeneous diseases and changes at a specific site or type of bone may not reflect changes occurring in other sites or bones.
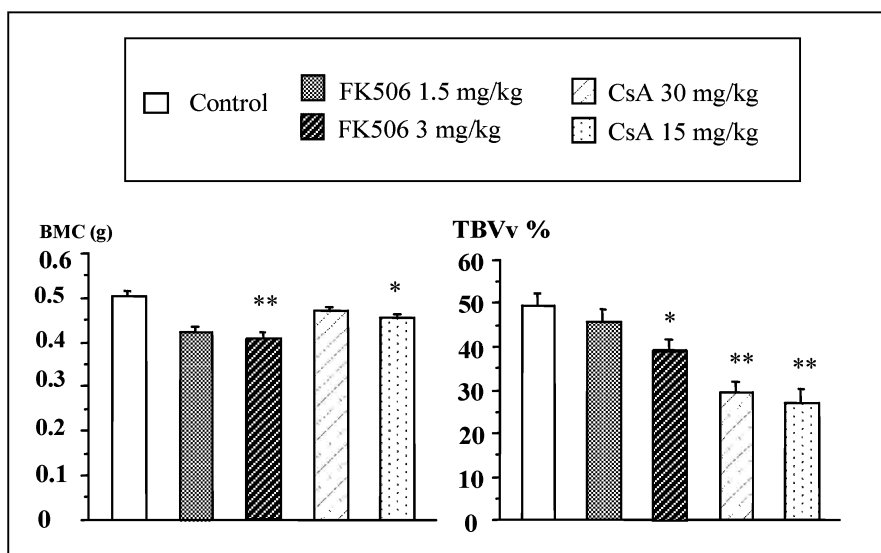

Figure 3: BMC and TBV in treated and control rats

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The present experimental study demonstrated that FK506 as well as CsA reduced bone mass, the effect being most pronounced by FK506 on cortical bone of the femur (organ level), and by CsA on trabecular bone of the tibia (tissue level). The low rate of body weight gain and bone changes in treated rats suggest a possible growth inhibitory effect of these drugs. This concord with the findings of (Bennett 1991) who demonstrated that CsA administered to rats in doses higher than 5 mg/kg exerted a growth-inhibiting effect.

One interesting finding in our study was that the growth plates of the rats treated with high dose CsA exhibited expanded hypertrophic zones, to some extent mimicking rickets. Thus our observation could raise the question of whether CsA exerts a direct toxic action on chondrocytes interfering with the local mineral metabolism. Furthermore, it seems likely that CsA also disturbs local skeletal metabolism at the epiphyseal/metaphyseal border, as observed with the markedly lower trabecular bone volume density of the primary spongiosa along with increased volume density of osteoclasts in rats treated with CsA than in controls. These findings concord with recent studies showing that CsA in doses between 7.5 mg/kg and 15 mg/kg induces a high bone turnover osteopenia of the proximal tibial metaphysis (Mann et al., 1996; Romero et al., 1995).

4.4 Effect of combined CsA and prednisolone on bone (*IV*)

Aims: To investigate and compare the effect of CsA, prednisolone and their combination on bone mass and bone mineral metabolism in rats.

Results: Rats given CsA alone or in combination with prednisolone exhibited significant lower values of bone mineral density (BMD) at the whole and distal metaphysis of the femur compared to normal control rats. BMD values in rats treated with prednisolone were not significantly different from those obtained in normal controls. Osteocalcin (a bone formation marker) was increased in CsA treated rats and decreased in both prednisolone and CsA+ prednisolone treated groups. (-6.3, p< 0.001; -5.0, p< 0.05.

Comments: The present study was designed to address the following consideration: Firstly, the independent effects of cyclosporine A (CsA) and prednisolone on bone mass following organ transplantation have been difficult to characterize in clinical studies since transplant patients receive combined therapy of CsA together with a corticosteroid. Secondly, previous studies of the effect of corticosteroids on rat bone metabolism were based on either parenteral administration of steroids by s.c. injection or by adding drugs to food pellets. Since the parenteral formulation of CsA or corticosteroids differs from the oral formulation and the side-effect are reported to be more severe with the parenteral formulation, in our experiment we aimed to study the effects of CsA and prednisolone on bone when animals were given an oral formulation. In a clinical setting, oral formulations are almost exclusively used.

In contrast to the well-known side effect of glucocorticoids on bone mass in humans, no detrimental effect on bone mass was observed in rats. Nevertheless, there are some

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common effects of prednisolone in humans and rats including decreases in serum osteocalcin, a marker of osteoblastic function, which suggests a slowing of bone formation.

Conversely, CsA-treated rats displayed a lower bone mass in the femur (whole femur and distal metaphysis) accompanied by biochemical indices of increased bone turnover as evidenced by significantly increased osteocalcin levels. It has been repeatedly documented that CsA induces high bone turnover osteopenia predominantly in trabecular bone and that cortical bone is less affected by therapeutic or even supratherapeutic dosages (10-30 mg/kg).

Interestingly, when both CsA and prednisolone were given to rats, a lower bone mass of the femur was observed together with a biphasic pattern in biochemical parameters such that the values at the end of the experiment were similar to those of rats treated with prednisolone alone.

4.5 Effect of statin on bone mass in renal transplant (*V*)

Aims: To evaluate the effect of statin on bone by comparison of bone mass values in patients with renal transplants taking statins and those not taking statins.
Results: BMD z-score of the lumbar spine was higher in statin users compared to nonusers (p< 0.05), and patients receiving fluvastatin had better bone mass than those taking simvastatin in comparison to nonusers (p< 0.05). No significant differences between statin users and nonusers were recorded in other skeletal sites such as femoral neck or distal radius.

Comments: Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (statins) are the most widely prescribed class of drugs for lowering cholesterol and reducing heart attacks and provide an important and effective approach to the treatment of hyperlipidemia and arteriosclerosis (Crouch, 2001; Hunninghake, 1998; Vaughan et al., 2000)

The recent finding that statins have positive effects on bone formation was discovered by Mundy *et al* (Mundy et al., 1999) while searching for agents that enhance osteoblastic differentiation and bone formation. More than 30,000 compounds from a natural products collection were screened and statin was identified as the only natural product in this collection with ability to stimulate bone morphogenetic protein-2 (BMP-2) promoter in an osteoblastic cell line. This effect on bone formation was further confirmed by adding statins to bone cultures or when given systemically in rats.

The observation that statins may have anabolic effects on bone has received much attention by investigators to explore whether there is supporting evidence of statin effects on the human skeleton.

Our previous studies (Abdelhadi and Nordenstrom, 1998; Setterberg et al., 1996) as well as studies performed by others (Grotz et al., 1995; Heaf et al., 2000; Kokado et al., 2000; Parker et al., 1999) have demonstrated that a reduced bone mass is a constant

finding in renal transplant patients, but the present cross-sectional study showed a significant higher bone mineral density of the lumbar spine in renal transplant patients taking statins compared to those who did not take statin (nonusers). Our findings are consistent with the report of Chung *et al* (Chung et al., 2000) in which patients with type 2 diabetics had mean bone mineral density at the femoral neck increased significantly during 15 months of statin therapy. In addition, other cross-sectional or epidemiological studies have indicated that statins increase bone mineral density and protect against fractures (Chan et al., 2000; Meier et al., 2000; Pasco et al., 2002; Wang et al., 2000). However, some clinical and experimental studies have determined no reduction in risk of bone fractures among statin users (Reid et al., 2001; Sirola et al., 2002), and conversely a decreased bone density and lower bone formation in rats treated with statins (Maritz et al., 2001).

5 SUMMARY AND CONCLUSIONS

Bone densitometry is essential to confirm the diagnosis of osteoporosis in patients where there is some question, and to establish degree of osteopenia or the risk of fracture in all patients. The introduction of DXA has enabled more precise measurements of BMD in humans as well as in small animals.

Patients with end-stage liver diseases were not found to have significant decrease in bone mass. After liver transplantation an early and accentuated bone loss was observed during the first 6 months following transplantation, predominantly of trabecular bone and less cortical bone. This bone reduction seems to have mainly been the result of increased bone resorption, possibly related to the immunosuppressive therapy. At 12 months some regain of the postoperative bone loss was observed although full recovery had not occurred at this time point.

In patients with primary or renal HPT a large proportion of patients had a reduced bone density. In patients with primary symptomatic HPT and patients with HPT associated with hemodialysis bone density increased after parathyroidectomy to an extent that largely restores their preoperative bone loss. However, no anabolic effect of parathyroidectomy on bone mass was observed in patients with HPT associated with a renal transplant, probably because of their immunosuppressive therapy.

In experimental animals, bone densitometry and histology indicated that the immunosuppressive drugs CsA and FK506 may affect bone by different mechanisms, possibly growth retardation, and that a high dosage of CsA or FK506 alters both cortical and trabecular bone in rats. The toxic effect of high dose-CsA showed histological changes of the growth plate resembling rickets. In addition, the combination of prednisolone (which markedly inhibits osteoblasts function) and CsA (which stimulates bone turnover) act in opposition, resulting in a normal or low bone turnover associated with osteopenia.

In a cross-sectional study, renal transplant patients taking statins had higher bone mineral density of the lumbar spine than those not taking statins. Conceivably, fluvastatin may have a more favorable effect on bone mass than simvastatin. However, prospective randomised controlled studies are needed to accurately determine if statins have a salutary effect on bone mass and bone mineral metabolism in renal transplant patients.

6 ACKNOWLEDGEMENTS

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