

Bone mineral density and associated changes after kidney transplantation

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"In the final analysis very little is known about anything, and much that seems true today turns out to be only partly true tomorrow..."

Albright F, Reifenstein EC Jr. The Parathyroid Glands and Metabolic Bone Disease. Baltimore, Williams & Wilkins, 1948.

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List of papers

I. Szilveszter Dolgos, Anders Hartmann, Stine Bønsnes, Thor Ueland, Gunhild Aker Isaksen, Kristin Godang, Per Pfeffer and Jens Bollerslev. Determinants of bone mass in end-stage renal failure patients at the time of kidney transplantation.

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II. Szilveszter Dolgos, Anders Hartmann, Trond Jenssen, Gunhild Aker Isaksen, Per Pfeffer and Jens Bollerslev. Determinants of short-term changes in body composition following renal transplantation.

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III. Szilveszter Dolgos, Anders Hartmann, Stine Bønsnes, Gunhild Aker Isaksen, Kristin Godang, Thor Ueland, Per Pfeffer and Jens Bollerslev. Early changes in bone mass, biochemical bone markers and fibroblast growth factor 23 after renal transplantation.

The Scandinavian Journal of Clinical & Laboratory Investigation 2009; 69: 161–167.

IV. Szilveszter Dolgos, Anders Hartmann, Gunnhild Aker Isaksen, Svein Simonsen, Øystein Bjørtuft, Kirsten M Boberg and Jens Bollerslev. Osteoporosis is a prevalent finding in patients with solid organ failure awaiting transplantation – a population based study.

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Abbreviations

ABD	Adynamic bone disease
ALP	Alkaline phosphatase
BIA	Bioimpedance analysis
BMD	Bone mineral density
BMI	Body mass index
BSAP	Bone-specific alkaline phosphatase
CaSRs	Calcium sensing receptors
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease - Mineral and bone disorder
CRP	C-reactive protein
CsA	Cyclosporine A
CT	Computed tomography
CTX	C-terminal cross-linked telopeptide
DNA	Desoxyribonucleic acid
DPD	Deoxypyridinoline
DXA	Dual-energy x-ray absorptiometry
ESRD	End-stage renal disease
FGF-23	Fibroblast growth factor 23
GFR	Glomerular filtration rate
ICTP	Procollagen type I cross-linked carboxy-terminal telopeptide
K/DOQI	Kidney Disease Outcomes Quality Initiative
MMF	Mycophenolate mofetil
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NTX	N-terminal cross-linked telopeptide
Pi	Inorganic plasma phosphate
PTH	Parathyroid hormone
PICP	Procollagen type 1 amino-terminal extension peptide
PINP	Procollagen type 1 carboxy-terminal extension peptide
PYD	Pyridinoline
QCT	Quantitative computed tomography

RNA	Ribonucleic acid
RRT	Renal replacement therapy
RTx	Renal transplantation
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
SD	Standard deviation
SHPT	Secondary hyperparathyroidism
TRAP	Tartrate-resistant acid phosphatase
VDR	Vitamin D receptor
WHO	World Health Organization
WHR	Waist-hip ratio
1,25(OH) ₂ D ₃	1,25-dihydroxyvitamin D (calcitriol)
25-OHD	25-hydroxyvitamin D

Summary

Solid organ transplantation (SOT) is the best treatment of choice for patients with advanced organ failure. While patient and graft survival are obviously still the primary goals, increasing efforts have been directed towards long-term complications such as bone disease and alteration of body composition. The aims of our studies were to measure bone mineral density (BMD) and identify risk factors of low BMD in patients with chronic renal failure at the time of renal transplantation (RTx) (n=133), to quantify the early changes in body composition and identify risk factors of these changes following RTx (n=102), and to describe the magnitude of early post-transplant bone loss with corresponding changes in biochemical bone markers (n=44). In addition, BMD was compared in candidates for lung-, liver-, kidney- or heart transplantation (n=291). BMD and body composition measurements were performed by dual-energy x-ray absorptiometry at the single transplant centre in Norway. In body composition, we found a marked increase in body fat mass with a significant decrease in fat-free mass, without any significant changes in total body weight early after RTx. Low bone mass was present at all measured skeletal sites already at the time of RTx, and significant bone loss was observed as early as 10-12 weeks post-transplant. Serum osteocalcin and telopeptid in combination with parathyroid hormone were predictors of BMD changes in different skeletal parts; therefore seem to be reasonable choices for routine assessment of bone metabolism in RTx patients. Although, osteoporosis was a prevalent finding in all four SOT groups, lung failure patients consistently had the lowest Z-scores, followed by advanced liver-, kidney- and heart disease patients. Our findings raise awareness of bone disease before and early after transplantation and emphasise that screening and, if necessary, treatment should be initiated timely.

1. General introduction

1.1. Chronic kidney disease

The incidence of chronic kidney disease (CKD) and end-stage renal disease (ESRD) has been increasing steadily for decades. The CKD epidemic is a worldwide public health problem, associated with premature death, increased morbidity and, last but not least, enormous costs related to renal replacement therapy (RRT). The rise in ESRD globally is largely due to the increasing prevalence of diabetes mellitus and nephrosclerosis, the two major causes of CKD [1, 2]. An increasing number of older patients, particularly those over 65 years of age, is also a contributing factor.

CKD is usually associated with a progressive loss of renal function over several years or even decades. When patients with CKD progress to Stage 5 renal failure (glomerular filtration rate [GFR] below 15ml/min/1.73m²) [3] they usually suffer from uraemic symptoms; at this stage the possibility of starting RRT should have already been discussed. There are two options for RRT: chronic dialysis therapy (peritoneal dialysis or haemodialysis) or renal transplantation (RTx). The present work relates primarily to kidney transplant patients.

1.2. Organ transplantation

Many solid organs can be transplanted including the heart, kidneys, liver, lungs, pancreas, and intestine. The kidney, however, is the most commonly transplanted organ worldwide.

Renal transplantation is considered to be the best RRT option for patients with advanced CKD without comorbidities that may contraindicate elective surgery or long-term immunosuppressive treatment. Chronic dialysis is an alternative treatment for patients with ESRD not eligible for RTx.

Kidney transplantation has advanced remarkably over the past 50 years owing to a better understanding of the immunobiological mechanisms responsible for allograft rejection, the development of new immunosuppressive drugs, and improvements in surgical techniques. The introduction of cyclosporine A (CsA) in the early 1980s was responsible for a dramatic improvement in the short-term outcomes of renal transplant recipients, improved graft survival and a reduction in acute rejection rates during the

first year post-transplant. Furthermore, CsA played an important role in the development of non-renal solid organ transplantation [4].

Although transplantation improves survival and quality of life for most patients with CKD Stage 5, there is a shortage of allograft organs. The gap between the supply and demand of deceased allografts has led to growing waiting lists and waiting times. The lack of donor kidneys has led to a widening of the selection criteria for deceased donors, and the expansion of the living donor pool [5]. Overall, graft and patient survival rates are higher when living donor kidney grafts are used and the time on dialysis has been short, or even better if dialysis has not been initiated.

1.2.1. Kidney transplantation outcome

Overcoming organ rejection and prolonging the recipient's life for as long as possible have been major targets since the dawn of transplantation. With the availability of new immunosuppressive agents (e.g. calcineurin inhibitors, antiproliferative drugs plus mono- and polyclonal antibodies) and other potent drugs, acute allograft rejections can generally be prevented. Although acute rejection is now not as common, evidence suggests that a single episode of acute rejection may have an impact on long-term graft survival, at least if the increase in creatinine is not completely reversed [6]. While calcineurin inhibitors currently form the cornerstone of modern immunosuppressive treatment protocols, their nephrotoxic effect probably contributes to chronic allograft dysfunction that can lead to end-stage renal failure after non-renal organ transplantation [4].

Most post-transplant mortality occurs in the early postoperative period, and principally affects older transplant candidates (>65 years) with comorbid factors including diabetes and coronary heart disease [7, 8]. However, even the oldest patients eligible for transplantation gain survival benefit from kidney transplantation, compared to patients who remain on dialysis while being on the waiting list for transplantation [9].

Improvements in short-term outcomes have been associated with increased long-term survival for transplant patients. This has allowed physicians to focus on long-term post-transplant complications and patient quality of life. Areas of particular interest are chronic allograft injury, malignancies (in particular lymphoproliferative disease, skin cancer, renal cell cancer arising from the native kidney), infections (polyomavirus, cytomegalovirus, hepatitis B and C virus, human immunodeficiency virus),

cardiovascular complications (hypertension, vascular calcification, left ventricular hypertrophy), obesity and bone abnormalities.

1.3. Bone-kidney axis

1.3.1. Mineral homeostasis

A strong connection exists between mineral homeostasis, bone metabolism and kidney function. The kidneys play an essential role in regulating the net excretion of calcium and phosphate, and maintaining total body balance of both these minerals. Calcium and phosphate are absorbed from the intestine and can interchange between extracellular fluid and bone according to the homeostatic and metabolic requirements. The key hormonal factors coordinating this process are parathyroid hormone (PTH), calcitriol (1,25-dihydroxyvitamin D) and phosphatonins like fibroblast growth factor 23 (FGF-23). The normal regulation of mineral homeostasis is illustrated in Figure 1 (adapted from Saliba et al [10]).

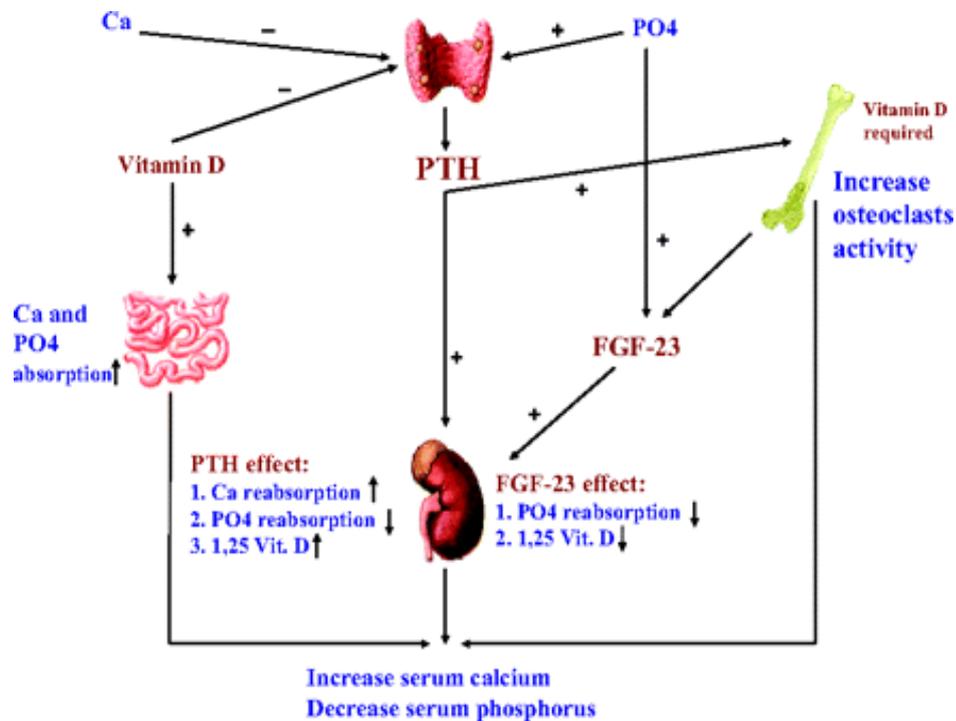


Figure 1. Normal calcium and phosphorus homeostasis (Saliba W et al. J Am Board Fam Med. 2009; 22:574-581. Reprinted with permission.)

1.3.1.1. Calcium

More than 99% of total body calcium is found in bone, predominantly in the form of hydroxyapatite crystals that play an essential role in skeletal integrity. During the daily bone remodelling process only a limited amount of skeletal calcium is exchanged between bone and extracellular fluid. Levels of intracellular and extracellular calcium (normal range: 2.1-2.5 mmol/l) are tightly controlled and play a key role for a variety of physiological processes. The most important component of total calcium is the ionized fraction (normal range: 1.1-1.3 mmol/l) that interacts directly with the calcium-sensing receptors (CaSRs) located in the parathyroid cells. Very small changes in ionized calcium concentration detected by CaSRs can modulate PTH secretion accordingly [11].

1.3.1.2. Phosphate

Phosphate is necessary for matrix mineralization and most of it (85%) is present in bone, with only 0.1% located in the extracellular space. Phosphate is present in cell membranes (phospholipids), and nucleic acid (RNA, DNA), and participates in energy metabolism of all biological systems. The maintenance of phosphate homeostasis, therefore, is of crucial biological importance. About 90% of inorganic plasma phosphate (Pi) is ultra-filterable, emphasizing the role of the kidney in phosphate excretion. Normal plasma concentration is maintained between 0.7-1.5 mmol/l. Until recently, phosphate homeostasis had been thought to be regulated by the same factors involved in maintaining calcium homeostasis i.e. PTH, calcitonin and vitamin D (1,25(OH)₂D₃). Recently, however, another hormone, FGF-23, has been identified that principally regulates phosphate homeostasis and bone formation [12].

1.3.2. Hormonal factors of mineral homeostasis

1.3.2.1. Parathyroid hormone

Biologically active PTH (1-84) is synthesized in parathyroid cells and released timely to maintain serum calcium concentrations within a tight physiologic range. When the serum calcium concentration drops, CaSRs become activated in the parathyroid cells and facilitate PTH release. PTH enhances the active reabsorption of calcium in the distal tubules and increases the absorption of calcium from the bowel by stimulating the synthesis of 1,25(OH)₂D₃. Furthermore, PTH indirectly affects bone degradation by stimulating the binding of the receptor activator of nuclear factor kappa-B ligand

(RANKL) to RANK, resulting increased osteoclastogenesis followed by bone resorption. PTH undergoes metabolic degradation within minutes. Intact PTH (iPTH; 1-84) and a fragment with an intact N-terminus, PTH (1-34), have the greatest biological activity. In the general population, the average PTH level is 1.1-6.9 pmol/l. As PTH is eliminated via glomerular filtration and tubular degradation, these fragments can accumulate in kidney failure.

1.3.2.2. Calcitonin

Calcitonin is peptide hormone secreted by specialized cells called C-cells in the thyroid gland. An increase in serum calcium stimulates calcitonin secretion. It decreases bone resorption by inhibiting osteoclasts through direct action on the osteoclastic calcitonin receptors.

1.3.2.3. Fibroblast growth factor 23 – “a new player in the field”

FGF-23 is a circulating phosphaturic hormone that plays an important role in phosphate homeostasis by regulating renal phosphate excretion via inhibition of Na-Pi-2a co-transport in the kidney. It is principally produced by osteocytes in response to increased serum phosphate. FGF-23 receptors are located in proximal tubular cells. Interaction between these receptors and a transmembrane protein called Klotho leads to the inhibition of phosphorus reabsorption and to impaired $1,25(\text{OH})_2\text{D}_3$ production via the inhibition of $1\text{-}\alpha$ -hydroxylase [13-15]. Recently, a vitamin D response element site was identified in the FGF-23 promoter region and it appears that $1,25(\text{OH})_2\text{D}_3$ stimulates FGF-23 activity in osteocytes. Therefore, FGF-23 can be considered as a counter-regulatory hormone for $1,25(\text{OH})_2\text{D}_3$ that maintains phosphate balance [16]. Its role is summarized in Figure 2. FGF-23 levels increase as renal function declines in response to phosphate retention. Depending on the assay used, the normal range of FGF-23 is approximately 10 to 108 RU/ml.

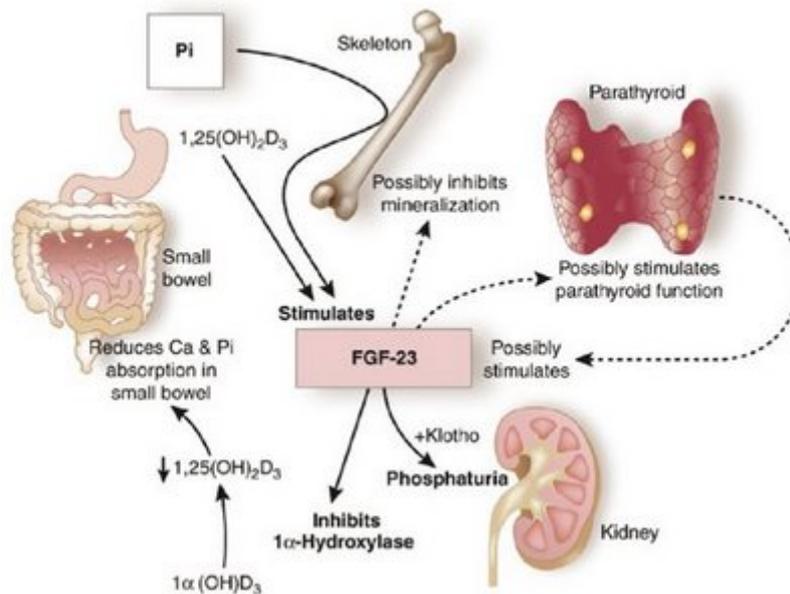


Figure 2. The regulation and action of FGF-23 (Emmett M. et al Kidney Int. 2008; 73:3-5. Reprinted with permission.)

1.3.2.4. Vitamin D

Precursors for active vitamin D hormones derive from dietary intake (plant sources, ergocalciferol - vitamin D₂, and from animal sources cholecalciferol - vitamin D₃) or from 7-dehydrocholesterol in the skin during sun exposure. Two important hydroxylation steps are necessary in the metabolism of vitamin D in order for its hormonal active forms to be produced. The first step is relatively fast and unregulated, and requires 25-hydroxylase enzymes in the liver to produce 25-hydroxyvitamin D (25-OHD). The biological half-life of serum 25-OHD is approximately 2-3 weeks, whereas the half-life of the active hormone, 1,25(OH)₂D₃ is only 4-6 hours. Measuring plasma 25-OHD level represents the best index of nutrition vitamin D intake. Further hydroxylation occurs by renal 1- α -hydroxylase located in cells of the proximal tubule, and results in the formation of the active hormone, calcitriol.

Calcitriol enters the target cell and binds to the vitamin D receptor (VDR). The liganded VDR then translocates to the nucleus where it interacts with the target DNAs and regulates gene transcription. Calcitriol is the major regulator of active intestinal calcium absorption and has a major effect on the differentiation of osteoclasts via the calcitriol-induced binding of RANKL to the RANK receptor [17, 18]. The renal synthesis of calcitriol is regulated by calcium, phosphorus, FGF-23 and PTH. Low levels of calcium

or phosphorus and increasing PTH level promote calcitriol synthesis. Furthermore, as described above, FGF-23 inhibits the activity of 1- α -hydroxylase [16].

The normal serum 1,25(OH)₂D₃ concentration in healthy individuals is 20-60 pg/ml. This decreases progressively as kidney function declines. According to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines [19], patients with CKD should undergo biochemical screening to detect vitamin D deficiency and receive timely treatment if needed. Plasma 25-OHD levels between 16 and 30 ng/ml qualify as vitamin D insufficiency, and values ranging from 5 to 15 ng/ml indicate vitamin D deficiency. These conditions can be treated with ergocalciferol, which should not be mistaken for the active vitamin D hormone (calcitriol) that is used to treat secondary hyperparathyroidism. Both inadequate vitamin D (25-OHD) and impaired calcitriol (1,25(OH)₂D₃) production can be observed in patients with CKD.

1.3.3. Bone physiology

1.3.3.1. Bone structure

Bone is a complex, highly organized and specialized connective tissue. Its organic matrix is composed of collagen fibers, which provide flexibility. The hard matrix of calcium salts (hydroxyapatite) deposited around the collagen fibers makes the bone rigid. There are two major types of bone in the human skeleton: cortical and trabecular. Nearly 80% of the total skeletal mass is cortical bone providing structural strength, while the rest is trabecular. The proportion of trabecular and cortical bone varies at different skeletal regions. In the vertebrae and the ultradistal forearm, trabecular bone makes up approximately 70% of the bone. In contrast, the proximal third of the radius consists entirely of cortical bone and the femur neck approximately 75%. The outer surface of bone is covered by periosteum with the endosteum lining the internal surface. There are two major categories of bone cell: osteoclast and osteoblast.

1.3.3.2. Bone remodelling

Bones are constantly undergoing remodelling via bone resorption by osteoclasts and bone formation by osteoblasts. Trabecular bone, with its large surface, is the predominant site of bone remodelling. Several hormones and local factors are involved in the control of this process. In normal adults, bone resorption and bone formation are well balanced. Imbalance between the two processes – i.e. osteoclast activity exceeding

osteoblast activity – leads to decreased bone mass and an increased risk of osteoporotic bone fracture.

1.3.4. Bone disease

1.3.4.1. Mineral and bone disorders in chronic kidney disease (CKD-MBD)

As CKD progresses, various bone disorders may develop. The classical term “renal osteodystrophy” has recently been replaced by “CKD-Mineral and bone disorder (CKD-MBD)” and is characterized by three components: laboratory abnormalities (of calcium or phosphorus or PTH or vitamin D), bone disease, and vascular calcification [20]. The classification of renal bone diseases is based on histological findings. High and low turnover bone diseases have been categorized based on over-stimulated or over-suppressed PTH, respectively.

- High-turnover bone diseases, characterized by increased bone remodelling, include *osteitis fibrosa* caused by secondary hyperparathyroidism (SHPT) and *mixed disorders*. The vast majority of CKD patients have some degree of SHPT [10, 20, 21]. The pathophysiology of SHPT is illustrated in Figure 3.

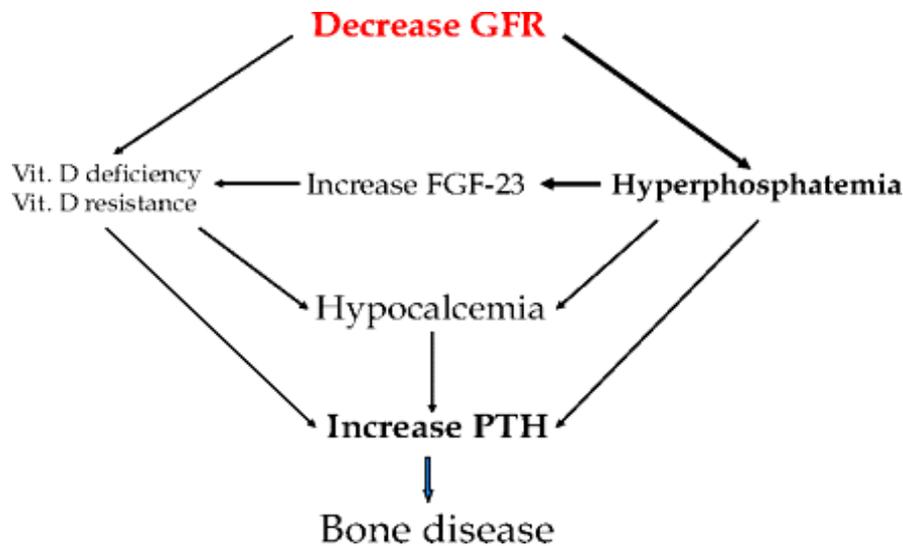


Figure 3. Calcium and phosphorus metabolism in renal failure (Saliba W et al. J Am Board Fam Med. 2009; 22:574-581. Reprinted with permission.)

- Low-turnover bone disease includes *osteomalacia* and *adynamic bone disorder* (ABD). Osteomalacia develops because of inappropriate bone mineralization. This was mainly caused by aluminum-based medical interventions which have now generally been abandoned so osteomalacia has become a rare condition. ABD is characterized by a low number of osteoblasts with decreased bone formation caused by inappropriate suppression of PTH [22].

1.3.4.2. Post-transplant bone disease

1.3.4.2.1. Bone mineral density after solid organ transplantation

Almost all patients have reduced bone mineral density (BMD), predominantly in trabecular bone, at the time of RTx and after transplantation [23-25]. Following renal transplantation, the functioning kidney allograft in most cases has a GFR of 30-60 ml/min, equivalent to CKD Stage 3. In kidney transplant recipients, BMD decreases by 4 to 9% in the lumbar spine and 5 to 8% in the femoral neck during the first post-transplant years. At the same time, 17-49% of patients have osteoporosis in the lumbar spine, 11-56% in the femoral neck and 22-52% in the radius [26-29]. The majority of post-transplant bone loss occurs within the first 6 months after RTx, mainly due to high dose glucocorticoid exposure. Consequently, bone disorders continue to be an important clinical problem after renal transplantation [30]. Low BMD and post-transplant osteoporosis is common and poses a significant problem among liver, lung, and heart transplant recipients during the first years post-transplant [28, 29, 31-36].

1.3.4.2.2. Fractures after solid organ transplantation

The main clinical implication of low BMD is the increased risk of osteoporotic bone fracture. Post-transplant fractures have a significant impact on patients' rehabilitation, quality of life, morbidity and related healthcare costs. After kidney transplantation, patients are up to four times more likely to have a bone fracture than the general population [24, 37]. The risk is even greater for patients previously treated with corticosteroids, and those who are older, post-menopausal or diabetic at the time of transplantation. The fractures can affect the appendicular skeletal sites such as the hip (prevalence 10-50%), and the axial sites such as vertebrae (prevalence 3-10%) [38].

After other solid organ transplantation, the fracture rate ranges from 18% - 40% during the first year after transplantation, with the majority of fractures affecting the vertebrae [28, 39, 40].

1.3.4.2.3. Mechanisms of transplantation-associated bone loss

Role of underlying disease: At the time of transplantation, candidates with end-stage organ failure have often lived with their primary disease (e.g. coronary heart disease, obstructive lung disease, liver cirrhosis or nephrosclerosis) for an extended period of time, with a deleterious effect on bone.

In chronic renal failure patients, bone disease frequently manifests when the GFR is below 60 ml/min. The pathogenesis of renal osteodystrophy is complex. Beyond the classical risk factors of bone loss, several specific mechanisms contribute to the poor bone condition in ESRD patients including vitamin D deficiency, hypocalcemia, hyperphosphatemia, metabolic acidosis, and still in some patients an overload of aluminum [41].

Advanced liver disease itself is associated with osteoporosis due to the essential role of the liver in 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D synthesis. Furthermore, chronic cholestatic liver disease is related to lipid malabsorption, which may reduce the enterohepatic circulation of vitamin D metabolites [42].

Many patients with obstructive pulmonary disease are long-time heavy smokers and have been receiving systemic corticosteroid therapy for an extended period. Besides obstructive lung disease, cystic fibrosis is another common indication for lung transplantation. In patients with cystic fibrosis reduces sex hormone secretion and calcium and vitamin D absorption can lead to low BMD [28].

Heart failure patients are predisposed to having a low BMD owing to a negative calcium balance. This is the result of prolonged physical inactivity, cardiac cachexia with poor nutrition, the use of loop diuretics, and because many patients have smoked [43].

Immunosuppressive treatment: Immunosuppressive regimens are needed for all transplanted patients to avoid organ rejection. The traditional regimen since 1983 consisted of CyA, azathioprine and glucocorticoids. However, during the past decade, mycophenolate mofetil (MMF) has replaced azathioprine. Furthermore, tacrolimus (instead of CsA) and mammalian target of rapamycin (mTOR) inhibitors like

everolimus and sirolimus are also in clinical use in combination with one or two of the other drugs. Mono- and polyconal antibodies for induction or rejection therapy may be added to this immunosuppressive regimen. No data is available on the effects of antibodies on bone. The degree of bone loss is directly related to the dose and duration of glucocorticoid exposure [44, 45]. Glucocorticoids hamper osteoblast proliferation and maturation therefore lead to the reduction of proteins essential for bone formation. Furthermore, glucocorticoids directly enhance osteoclast activity resulting in increased bone resorption. Prednisolone 10 mg daily is enough to cause bone loss during the first 6 months after the start of treatment. Even a long-standing, 7.5 mg prednisolone daily dose may lead to bone loss [46]. The effects of other immunosuppressive drugs on bone are not well studied and are summarized in Table 1. For calcineurin inhibitors data from various in vitro and in vivo studies are controversial. In rat model, the administration of CsA resulted in bone loss, especially of the trabecular bone but the significance of bone injury caused by CsA remains uncertain in patients. In human, CsA and tacrolimus may contribute to post-transplant bone loss by decreasing osteoprotegerin mRNA expression and increasing RANKL gene expression in osteoblasts [47-49]. The effect of other drugs such as sirolimus, MMF and azathioprine has been poorly investigated but they appear to have little or no effect on bone [50].

Table 1. Effect of immunosuppressive treatment on bone

Immunosuppressive agent	Effect
Glucocorticoids	
Systemic effects	Reduce intestinal calcium absorption Increase urinary calcium excretion Decrease the effect of vitamin D Increase parathyroid hormone Decrease adrenal and gonadal steroid synthesis
Local effect on bone	Decrease osteoblastic bone formation Increase osteoclasts and bone resorption
Calcineurin inhibitors	
Cyclosporine A (CsA)	Resorption rate higher than bone formation
Tacrolimus (FK506)	Resorption higher than formation (probably less severe than CsA)
Azathioprine	No effect on bone
Mycophenolate mofetil	No effect on bone
mTOR inhibitor	Hypophosphataemic osteomalacia by increasing hyperphosphaturia Inhibits longitudinal bone growth

Other renal transplant-related factors: Persistent hyperparathyroidism and mineral imbalance after transplantation, low post-transplant GFR, persistent hypogonadism, amyloidosis, diabetes mellitus, patient immobility, aluminum toxicity, and pre-existing high or low-turnover uremic osteodystrophy should also be considered as post-transplant risk factors of bone loss [51] and treated appropriately to prevent further bone loss.

1.3.5. Diagnosis of metabolic bone disease

1.3.5.1. Bone biopsy

The most accurate diagnostic test for determining the type of renal osteodystrophy is transiliac bone biopsy with double tetracycline labeling and bone histomorphometric analysis [19]. Bone biopsy is the gold standard, but as it is an invasive and painful procedure, it is infrequently used in routine clinical practice. It also requires technical expertise and a well-trained histomorphometrist to analyze the samples. At present, bone biopsy analysis by dynamic histomorphometry is not performed in Norway.

1.3.5.2. Measurement of bone mineral density

1.3.5.2.1. Dual-energy x-ray absorptiometry

Dual-energy x-ray absorptiometry (DXA) is a widely used, non-invasive method measuring bone mineral content (g/cm^2) and identifying osteopenia/osteoporosis. The technique implies a short scanning time (about 10 minutes), low radiation exposure (approximately $< 1\mu\text{Sv}$), low precision error independent of the operator (0.5-2%), and acceptable costs. The preferred skeletal sites for BMD measurement using DXA are the lumbar spine, total femur, radius, and whole body. The results of BMD measurements can be expressed as absolute values (g/cm^2), or as T-score and Z-score:

T-score: the number of standard deviations above or below the mean reference value for a healthy 30 year old adult of the same sex as the patient.

Z-score: the number of standard deviations above or below the mean reference value for the patient's age and sex.

Patients can be classified according to the World Health Organization (WHO) definition of osteoporosis (Table 2) [52]. However, it is important to note that white, post-menopausal population was used to define the WHO criteria. This has limited applicability to patients treated with immunosuppressants, or more heterogeneous patient populations (e.g. males, mixed ethnicity).

Table 2. World Health Organization definition of osteoporosis

Classification	Definition	Fracture Risk
Normal	BMD no more than 1 SD below the young adult mean	Very low
Osteopenia	BMD is 1 to 2.5 SD below the young adult mean (T score -1 to -2.5)	4x
Osteoporosis	BMD >2.5 SD below the young adult mean (T score >-2.5)	8x
Severe osteoporosis	BMD >2.5 SD below the young adult mean plus history of one or more fragility fractures	20x

BMD, bone mineral density
SD, standard deviation

Bone loss is more prevalent in trabecular bone, however, cortical bone, characterized by high bone mineral content, can hide small changes in trabecular bone during the DXA assessment [53]. As DXA does not provide specific information on bone turnover and the method is unable to differentiate between trabecular and cortical bone, the BMD results should be interpreted together with clinical assessment, bone biomarkers and bone histology if it is possible.

BMD is also the best method for assessment of overall body composition (see paragraph 1.4.5.9.).

1.3.5.2.2. Quantitative computed tomography

Another non-invasive procedure is quantitative computed tomography (QCT) that we did not use in our study. It measures BMD accurately and provides a three-dimensional image with separate assessment of cortical and trabecular bone. This technique measures the volumetric size (mg/ml) of the target bone. [54]. The main disadvantage of QCT is the high radiation exposure and a high cost.

1.3.5.3. Biochemical markers of bone metabolism

While bone histology is still the gold standard for the accurate assessment of bone turnover, the search for reliable biochemical bone markers has been ongoing for several years. With the development of new, more sensitive and specific assays, our ability to determine bone turnover by biochemical markers has improved considerably. Though measurement of iPTH has been still widely used and it provides basis for the assessment of bone turnover, serum iPTH levels alone are insufficient to clearly distinguish adynamic or normal bone from hyperparathyroid bone disease. Therefore the specificity of PTH as an indicator of bone turnover has been questioned [55]. According to Qi et al., serum iPTH levels between 65 and 450 pg/ml could not predict the degree of bone turnover in dialyzed patients, and bone biopsy was proposed to these patients [56].

A number of biochemical markers are available clinically to assess bone metabolism in patients with chronic renal failure. Biochemical markers respond within days or weeks after initiation of anti-resorptive therapy. Biochemical markers of bone resorption respond considerably faster than the markers of bone formation. All biomarkers have limitations and their clinical applicability remains to be established. Panels of simultaneously obtained bone formation and bone resorption markers need to be analyzed for an appropriate evaluation of bone disease in routine clinical practice. The most appropriate application for these biomarkers is to monitor trends over time.

1.3.5.3.1. Markers of bone formation

Osteocalcin: Osteocalcin is a major non-collagenous protein of the bone matrix produced by osteoblasts. Osteocalcin is released into the bloodstream during bone matrix synthesis. At the 'local' bone level, osteocalcin plays an important role in bone mineralization and may also induce bone resorption by stimulating adhesion and chemotaxis of osteoclasts [57]. At the 'systemic' level, recent data have shown that osteocalcin regulates pancreatic β cell proliferation and adipocyte gene expression, increasing insulin secretion and adiponectin synthesis, respectively [58, 59]. Its physiological role in bone energy regulation is shown in Figure 4 [59-61]. Osteocalcin has a short half-life in the blood and its serum concentration is determined mainly by renal clearance. In patients with CKD, serum osteocalcin increases as renal function declines.

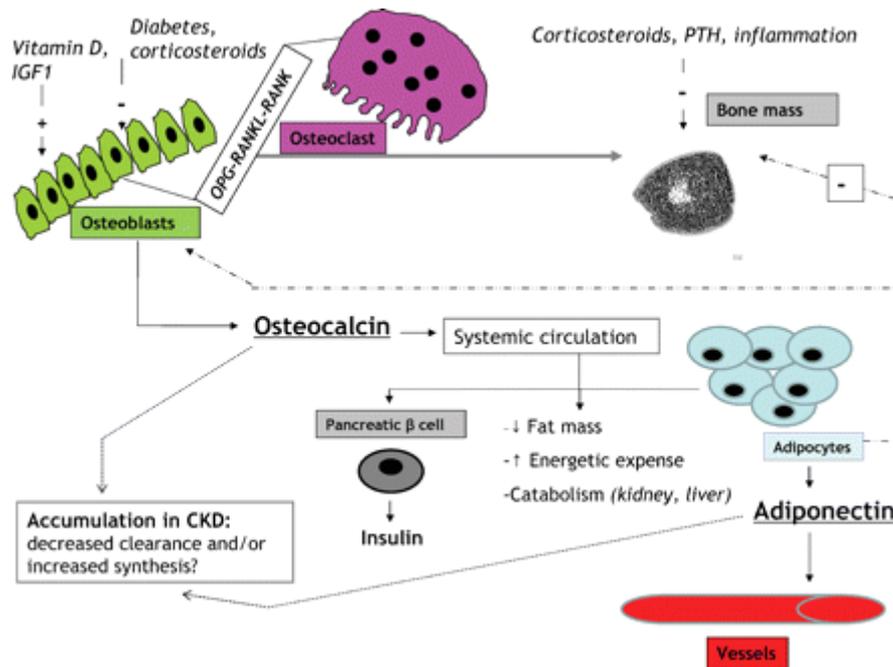


Figure 4. Hypothetical relationships between osteocalcin and adiponectin (Bacchetta J. et al., *Nephrol Dial Transplant* 2009; 24: 3120-3125. Reprinted with permission.)

Other markers of bone formation not measured in our study:

- *Bone-specific alkaline phosphatase*: Alkaline phosphatase (ALP) is a cell-membrane-associated enzyme expressed mainly by the liver and bone in adults. In bone, ALP is derived from osteoblasts and it influences bone mineralization. In the absence of liver failure, ALP is a useful indicator of osteoblast activity [62]. Furthermore, bone-specific alkaline phosphatase (BSAP) is the fraction of total alkaline phosphatase that is specific to the osteoblast. The serum level of BSAP is not influenced by renal failure. There is evidence that BSAP correlates well with iPTH levels and histomorphometric indices of SHPT in CKD patients [63, 64].
- *Propeptides of collagen type I*: Type I collagen is synthesized by osteoblasts from type I procollagen precursor proteins (Figure 5). These precursors have large extension domains at both ends. When type I collagen is synthesized, these propeptides, procollagen type 1 carboxy-terminal extension peptids (PINP) and procollagen type 1 amino-terminal extension peptids (PICP) respectively, are

enzymatically removed and released into the circulation. Higher levels of PINP and PINP in the plasma may indicate increased bone formation. PINP and PICP are degraded in the liver [65-67].

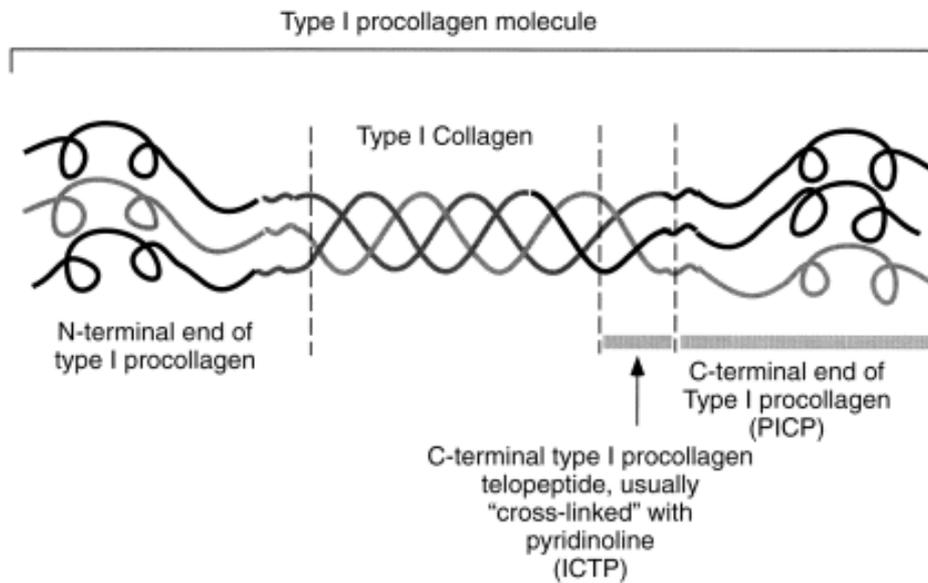


Figure 5. Schematic representation of a type I procollagen molecule (Ureña P. et al., *Kidney Int* 1999; 55:2141-2156. Reprinted with permission.)

1.3.5.3.2. Markers of bone resorption

Collagen breakdown products: The organic matrix of bone consists of type I collagens, which are held together by pyridinoline and deoxypyridinoline cross-links and provide mechanical force to the bone tissue. When collagen degrades, these cross-links and other fragments are released into the circulation, and excreted in the urine. The breakdown of type I collagen is mediated by acid proteases derived from osteoclasts. Telopeptides are small amino acid sequences originating from the nonhelical ends of collagen molecules as a result of enzymatic degradation. Fragments released by this process include N-, and C-terminal cross-linked telopeptides (NTX, CTX), procollagen type I cross-linked carboxy-terminal telopeptide (ICTP) and pyridinoline (PYD) and deoxypyridinoline (DPD) [68, 69]. We studied only the role of CTX in bone turnover.

Tartrate-resistant acid phosphatase (TRAP): Tartrate-resistant acid phosphatase is produced by osteoclast during bone resorption, and the TRAP 5b isoform has recently been identified as the osteoclast-specific portion of TRAP [70, 71]. During bone

degradation, TRAP5b is released into the circulation and is cleared mainly by the liver. The TRAP 5a isoform is a marker of inflammatory conditions and is synthesized by macrophages. We have not measured its level in our study.

1.3.6. Prevention of and most common treatments for transplantation-associated bone loss

As the most rapid bone loss occurs shortly after transplantation, it is important to introduce prophylaxis and treatment for post-transplant bone disease as soon as possible.

1.3.6.1. K/DOQI - General recommendations [19]

- a. Use the lowest possible steroid dose
- b. Stop smoking
- c. Initiate exercise
- d. Treat persistent hyperparathyroidism
 - Optimal management of calcium
 - Optimal management of vitamin D
 - Possible role of calcimimetics
 - Parathyroidectomy
- e. Avoid loop diuretics if possible
- f. Substitute for insufficient gonadal or thyroid function

1.3.6.2. Calcium and vitamin D supplements

Vitamin D metabolism is disrupted before and after kidney transplantation. It is recommended that transplant recipients with good kidney function and normal serum calcium level receive calcium (1000 to 1500 mg/day) and vitamin D in the form of cholecalciferol or ergocalciferol (400 to 800 IU/day) supplements in order to moderate post-transplant bone loss [72]. Small doses (0.25-0.5 µg/day) of active vitamin D derivatives – alfacalcidol, doxercalciferol or calcitriol – have also had a positive effect on BMD at the lumbar spine and femoral neck in kidney transplant patients [73, 74].

1.3.6.3. Bisphosphonates

Bisphosphonates ameliorate osteopenia or osteoporosis after transplantation through inhibition of osteoclasts [27, 74]. They can be administered either orally (alendronate, clodronate, etidronate, risedronate) or intravenously (ibandronate, pamidronate, zoledronic acid). They all have a long half-life and generally they are well-tolerated. It has been shown that long-term use of pamidronate and zoledronic acid is associated with renal failure and nephritic proteinuria. The risk of renal failure is directly related to the drug infusion time and dosage. The higher dose with shorter infusion time of bisphosphonate has higher nephrotoxic effect [75]. Ibandronate may be less problematic in this respect. In practice, however, nephrotoxicity from these drugs when used at recommended doses appears to be uncommon. Another area of concern relates to the skeleton. Use of bisphosphonates reduce bone turnover and might induce adynamic bone disease in patients with decreased kidney function [76].

1.3.6.4 .Calcitonin

Calcitonin treatment (salmon calcitonin 200 IU/day intranasally) has been shown to result in significant improvements in BMD (assessed by DXA) at the lumbar site after kidney transplantation [77] and prevent corticosteroid-associated bone loss [72]. Its effect has been small compared with other interventions as discussed above and it is also a very expensive treatment. Thus calcitonin has only gained limited importance in the treatment of post-transplant osteoporosis.

1.3.6.5. Hormone replacement therapy

Only a few small studies have assessed the effects of combined hormone replacement therapy – testosterone or selective oestrogen receptor modulators, or anabolic steroids – on bone loss. Oestrogen replacement therapy has been shown to inhibit bone loss in oestrogen-deficient women [78] and increase BMD in liver transplant candidates [79]. However, the benefit of oestrogen replacement therapy has been questioned by the negative cardiovascular effects and therefore cannot be generally recommended. Testosterone replacement therapy increases BMD in hypogonadal men [80], but data involving transplant patients are lacking.

In summary, according to a systematic review of “Interventions for preventing bone disease in kidney transplant recipients” in the Cochrane database: “treatment with bisphosphonates, vitamin D sterol or calcitonin has a favourable effect on BMD at any time following kidney transplantation. The optimal agent, route of administration, and duration of treatment are not known [74]”. However, none of these data were able to show an advantage of these individual treatments on bone fracture, so the effect on clinical outcomes is still unproven.

1.4. Nutritional considerations in kidney transplant patients

1.4.1. Body composition

The total body is composed of lean mass (water, protein, minerals) and fat mass. Standard body weight scales provide a measure of total weight, but don't determine the lean-to-fat ratio of that weight. Each fraction has an experimentally measurable constant density and many methods are available of assessing body composition in the clinical field. Fat primarily consists of triglyceride, while the fat-free fraction includes water, proteins and minerals (muscles, bones, ligaments, tendons, and internal organs) [81-83].

1.4.2. Malnutrition

Malnutrition is common in chronic renal failure patients receiving maintenance dialysis, and this condition is strongly associated with increased morbidity and mortality [84, 85]. Overall, dialysis patients are in a state of semi-starvation; the causes of malnutrition are multifactorial. Uremia caused by suppressed appetite plays an important role in reducing protein and energy intake. Furthermore, increased age and comorbidities, inflammation, inadequate dialysis, gastroparesis, metabolic acidosis, increased leptin level, and other uremia-related endocrine abnormalities i.e. insulin resistance, can lead to increased protein catabolism and renal anorexia [86]. Regardless of the mechanism, to survive this state of semi-starvation consequently requires adequate energy stores. Obesity, therefore, can be protective in dialysis patients. This is in contrast to the general population where obesity is an established risk factor of cardiovascular diseases. Underweight rather than overweight is a risk factor for premature death in dialysis patients [87, 88].

1.4.3. Obesity

There is a worldwide obesity epidemic [89]; consequently transplant services have to deal with obese patients. Obesity is characterized by altered metabolic function such as insulin resistance, dyslipidemia, hyperhomocysteinemia with increasing total body fat mass. A certain level of obesity can be protective in patients treated with chronic dialysis. The obvious negative health consequences of obesity, however, are important factors in deciding on the suitability for kidney transplantation. The deleterious metabolic effects of obesity, particularly of visceral obesity, frequently lead to severe general and transplant-related complications as discussed below. Obese patients also have increased risk for cardiovascular disease, arterial hypertension, hyperlipidemia, diabetes mellitus and other comorbidities [90-92].

1.4.4. Altered body composition after renal transplantation

With restoration of renal function and reversal of uremia following kidney transplantation, the appetite generally improves leading to weight gain and changes in body composition [93]. Other factors that impact on body composition following transplantation include the use of immunosuppressive agents, predominantly steroids, the presence of concomitant disease and changes in physical activity [94]. Obesity with fat mass accumulation increases the risk of delayed onset graft function and wound complications after kidney transplantation [95]. Furthermore, obese patients have more comorbidity associated with premature death with functioning allograft [96, 97].

In some cases, however, increase in body weight after transplantation may reflect normalization from a uremic malnourished state [94]. In general, body weight increases after renal transplantation regrettably mainly because of fat mass increases in parallel with decreases in lean body mass, principally in muscle [98, 99].

1.4.5. Methods of body composition assessment

Objective assessment of nutritional status is important because self-reported measures can frequently be misleading. Different methods are available to evaluate body composition in the clinical setting. Body composition assessment can range from the simple measurement of body weight as a single compartment analysis to the complexity of a six-compartment model [100]. The classic two-compartment model of body composition analysis involves assessing fat and fat-free compartments by measuring

body density. The three compartment model also includes assessment of total body water, usually using the dilution method. This model, however, does not improve the accuracy of body composition assessment. The four-compartment model is efficient in clinical use since it adds a separate protein and mineral measurement. The latest and most sophisticated model is the six-compartment model. It is a direct elemental analysis of the body, based on the principle of neutron activation analysis. It measures total body water, protein based on total body nitrogen, bone mineral content calculated from total body calcium, total body sodium (soft tissue), potassium and chloride in the body.

In our study, body mass index was used for nutritional assessment of the study population and DXA for evaluating body composition after renal transplantation.

The most common methods include the following.

1.4.5.1. Body mass index (BMI)

The most commonly used assessment index of obesity is BMI [89]. It is important to understand that BMI is not a measure, but rather a calculated index (body weight(kg)/height(m)²). Overweight is defined as a BMI between 25 and 29.9 kg/m², and obesity as a BMI greater than 29.9 kg/m². Based on self-reported or clinically assessed height and weight, this is an accepted, inexpensive measure to identify overweight or obese patients. BMI, however, fails to differentiate between lean body mass and fat mass, so it can be misleading in patients with high muscle mass [101].

1.4.5.2. Dual-energy x-ray absorptiometry

DXA has been recommended by the Kidney Disease Outcomes Quality Initiative (K-DOQI) as a reference method for assessment of body composition [102]. Although DXA uses uniform hydration status of lean body mass (LBM) at 0.732 liter/kg LBM during the assessment process [103], body composition analysis also appears adequate for patients undergoing kidney transplantation [104]. DXA is a widely used method for body composition assessment. It divides the body into three compartments; fat mass, lean mass, and bone mineral content, which attenuate the X-ray energy in a tissue-specific manner. The coefficient of variation for total body fat using DXA ranges from 1% to 3%.

1.4.5.3. Other methods not used in our studies

1.4.5.3.1. Waist circumference; Waist-hip ratio (WHR)

This is probably the most applied method for assessing abdominal fat distribution. The International Diabetes Federation has recently defined central obesity with increased waist circumference as the essential component for metabolic syndrome [105]. The waist circumference cut-off point for central obesity was defined as ≥ 94 cm and ≥ 80 cm in white men and women, respectively [106]. Furthermore, WHR is calculated by dividing the waist circumference with hip circumference. The suggested cut-off points for WHR are > 0.90 for females and > 0.85 for males.

1.4.5.3.2. Skinfold thickness

As more than 50% of body fat is located under the skin, measuring subcutaneous fat thickness in the triceps or other part of body can be used to calculate total body fat. The total error in body fat estimates using skinfold thickness ranges from 3% to 11%, and is influenced by sex, race and age [107, 108].

1.4.5.3.3. Near-infrared measurement

This technique is based on light absorption and reflection of organic tissues using near infrared light emission. Body fat absorbs light waves whereas lean body mass reflects them. The principle site of infrared measurement is the biceps. This device primarily assesses the subcutaneous tissue and a prediction equation estimates total body fat and calculates fat-free mass. This method is a radiation free, fast, simple bedside indirect technique and can be repeated frequently [109]. However, the reliability and accuracy of this method is lower than skinfold technique.

1.4.5.3.4. Underwater weighing

The procedure requires that the patient is completely submerged in a large tank of water, sitting on a special scale and asked to exhale all the air from his lungs. While the body displacement volume and the subject's underwater weight combined with the subject's laboratory weight are measured special calculation is used to determine body density and fat mass. The largest source of error in underwater weighing is the determination of residual volume (the amount of air remaining in the lungs following

maximal expiration). The total error for body fat mass is approximately 3-4% of body weight [81].

1.4.5.3.5. Dilution method

This is an accurate method for measuring total body water using an isotope labeled substance (e.g. deuterium oxide [D^2O]) which is injected intravenously and equilibrates with the total body water over hours. After the distribution and final equilibration of the tracer in plasma, the total body water compartment could be calculated as the ratio of dilution of D^2O . However, this technique is cumbersome, expensive and cannot be performed repeatedly [83].

1.4.5.3.6. Computed tomography and Magnetic resonance imaging

Computed tomography (CT) uses X-rays to identify adipose-, muscle-, skin-, visceral- and bone tissue. The CT images can be used to separate the subcutaneous and visceral component of total adipose tissue. It has excellent accuracy (<1% error) and precision (<1%) [110]. The major disadvantage is the high radiation dose and the relatively high cost.

Magnetic resonance imaging (MRI) is a useful technique for assessing body composition since the hydrogen densities of adipose and lean tissues are different. The relaxation time of protons is much shorter in fat than in water [111].

1.4.5.3.7. Bioimpedance analysis (BIA)

Bioimpedance analysis measures impedance of the body by applying a weak electric current. BIA measures the ability of tissues to conduct an electric current. The electricity flow through the tissue depends on the amount of body water. Body fat and bone have relatively poor conductive properties. This technique is inexpensive, rapid and non-hazardous, but less accurate. Overall accuracy of the various methods is 3 - 10%, due to differences in machines, methods, equations, variables, and the choice of reference method [112].

2. Aims of the study

The aims were to assess bone mineral density and body composition in solid organ transplant recipients, particularly in kidney transplant candidates.

Aims of the different papers included in this thesis:

2.1. Paper I

- To measure bone mineral density in kidney allograft patients and to identify predictors of bone loss and cumulative fracture rate at the time of renal transplantation.

2.2. Paper II

- To quantify the early changes in body composition after renal transplantation and identify predictors of these changes in a large number of generally well-nourished patients.

2.3. Paper III

- To describe the magnitude and distribution of early bone loss following renal transplantation and to evaluate the association between biochemical bone markers and alterations in bone mass.

2.4. Paper IV

- To compare bone mineral density among patients with different types of end-stage organ failure awaiting transplantation.

3. Patients and methods

This section highlights the most relevant aspects of the study populations and methods. Detailed descriptions of the studies have been published in the original papers.

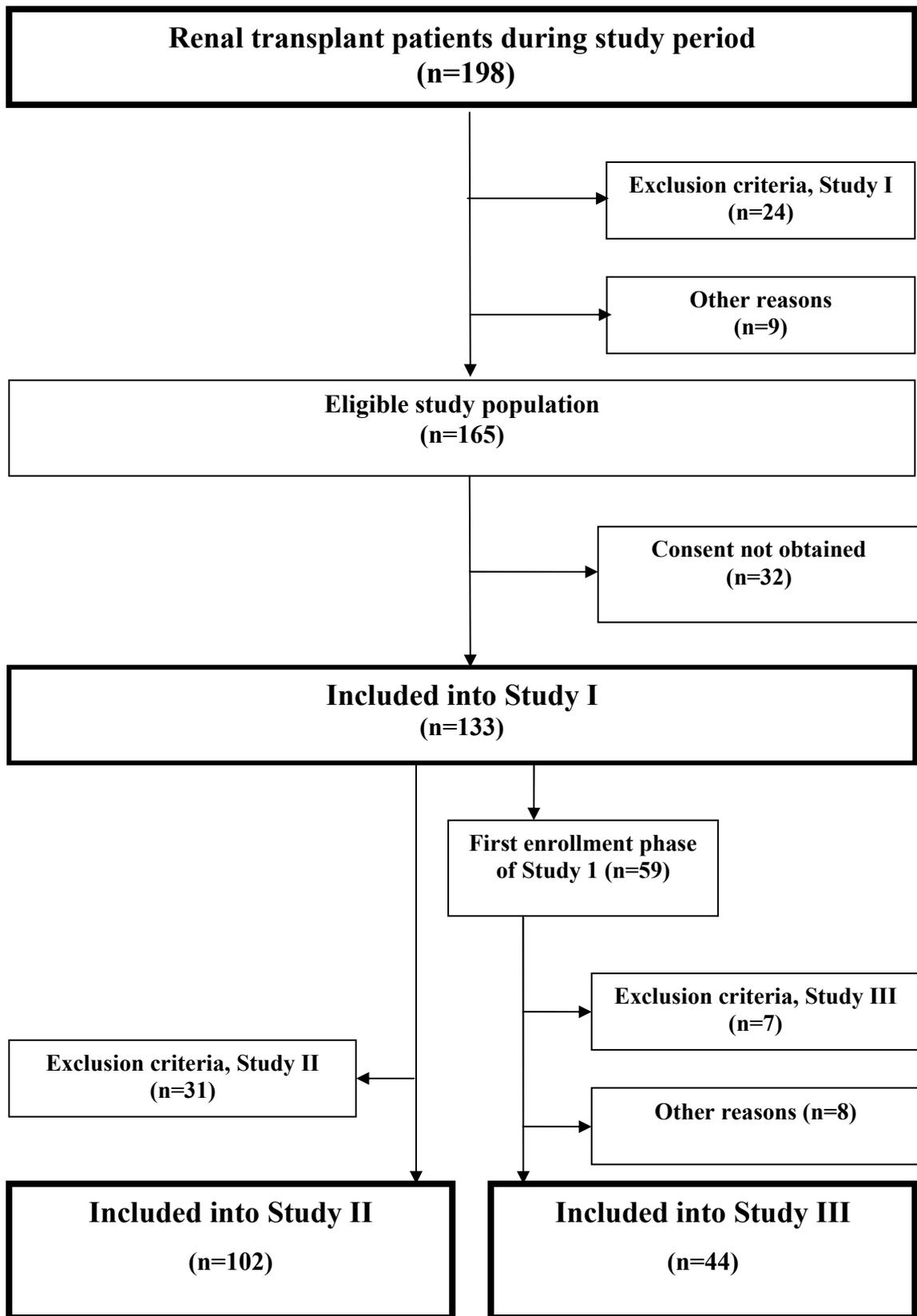
3.1. Papers I, II and III

3.1.1. Study populations

A total of 198 patients receiving a kidney allograft between February and early December 2006 at the Rikshospitalet University Hospital, Oslo comprised the patient cohort that was considered for enrollment in the studies. The disposition of patients for the three different studies is shown in Figure 6. Patients underwent BMD and body composition assessment performed by DXA, and blood samples were collected for biochemical analyses. A questionnaire was used to obtain relevant information on past medical history, pre-transplant fractures occurring after 18 years of age and concomitant medical treatment. Data quality was assured by subsequent interviews by one of the investigators. Patients were measured on the same day as their scheduled outpatient visit at the Section of Nephrology and at the Section of Endocrinology. In Study I (Paper I), of the 198 patients enrolled, 33 were not eligible (24 fulfilled the exclusion criteria being minors or had severe medical comorbidity and 9 were not measured due to other reasons e.g. referred to other hospital. Of the remaining 165 patients, 133 (81%) gave consent and were included.

Patients included in the first study (main cohort) were also considered for participation in Study II (Paper II). However, 31 patients were excluded due to previous transplantation or conditions which may influence body composition and the hydration status. The patients were generally non-vegetarians and no specific diet was recommended. In this second study, all patients (n=102) were out of dialysis treatment and the subjects were clinically stable without signs of overhydration. Patients emptied their bladder before the body composition measurements. The first 59 patients from the main cohort were also invited to participate in Study III (Paper III). Seven patients were excluded due to the exclusion criteria in this study (previous transplantation, conditions or treatment that may influence bone resorption). Two patients were lost during the study due to early graft loss (n=1) and death (n=1) and six patients for non-compliance. In total, 44 renal allograft recipients were enrolled in this study.

Figure 6: Patients' disposition in Papers I, II and III



Exclusion criteria:

Study I – less than 18 years of age, severe medical complication

Study II – previous transplantation, conditions which may influence body composition and hydration status

Study III – previous transplantation, conditions or treatment which may influence bone resorption

3.1.2. Immunosuppressive therapy

For every patient, immunosuppressive therapy consisted of initial high-dose glucocorticoids and an interleukin-2 blocker (basiliximab) for induction. Maintenance therapy consisted of prednisolone, MMF and either a calcineurin inhibitor (CyA or tacrolimus) or in a few cases (n=33) an mTOR inhibitor (sirolimus or everolimus). Two thirds of patients received calcineurin inhibitor-based immunosuppressive therapy. Basiliximab was administered intraoperatively and redosed on the 5th postoperative day. Methylprednisolone was administered on the day of RTx and the following day (580 mg and 80 mg, respectively). In the absence of acute rejection, prednisolone was tapered to 20 mg/day by day 9 after the transplantation and reduced during the next 8 to 12 weeks to a maintenance dose of 10 mg per day. Rejection episodes were diagnosed initially by an increase in serum creatinine of more than 20% without any other reasons (e.g. dehydration, urinary tract infection or post-renal obstruction). In cases of uncertain clinical condition with rising serum creatinine or recurrent (second/third) rejection episodes, ultrasound-guided core-needle biopsies were performed for histological diagnosis. Standard treatment of rejections was intravenous methylprednisolone bolus (usually 1375 mg within 5 days), and increased oral prednisolone dose (30 mg/day).

3.1.3. Measurement of bone density

Bone mineral density of the lumbar spine (LS), total femur (TF) and total body (TB) was measured between 5 to 10 days after transplantation using DXA (LUNAR Corp, Madison, WI, USA). The participants were positioned according to standard techniques. All analyses were controlled and read by trained operators to ensure that the interpretation of the skeletal anatomy (particularly the lumbar spine) was correct, and to identify any artifacts in the scans. The values were evaluated by the enCORE 2006 software (General Electrics Healthcare, V10.10, Madison, WI, USA) and were expressed as absolute BMD values in g/cm², and as T-score and Z-score. A quality assurance test of the mechanical operation was performed daily and the calibration of the scanner using a spine phantom was controlled weekly.

3.1.4. Measurement of body composition

Total body and segmental body composition (upper limbs, lower limbs and trunk) were measured by DXA using enCORE 2006 software. This calculated bone mineral content, fat-free mass, fat mass, and provided estimates of percent body fat applying a three-compartment model. Fat-free mass was calculated as the sum of lean tissue and bone mineral content. The baseline measurements were performed on average 7 days (range 5-10 days) after RTx and the follow-up measurements 10 weeks later (range 64-81 days).

3.1.5. Biochemistry

Fasting blood samples were collected at the Section of Nephrology as a part of the routine control and were measured using standard automatic analyser techniques at the Medical Laboratory for Biochemistry, Rikshospitalet, Oslo.

Ten weeks after RTx for each patient, on the day when the blood sample was collected, renal function was assessed via plasma clearance of ⁵¹Cr-labeled EDTA and the result was adjusted to 1.73 m² body surface.

The standard process of intact PTH analysis at our clinic uses electrochemiluminescence immunoassay (ECLIA, Elecsys PTH kit; Roche Diagnostics, Mannheim, Germany) which eliminates interference from inactive PTH fragments, and thereby offers improved sensitivity and specificity. Inter-assay variation is 7% in the lower and upper normal range, and 5% in the high concentration range.

Beyond the routine blood sample analyses, serum samples were taken after an overnight fast and frozen at - 80°C for later, biochemical assessment of bone turnover in a single batch analysis.

- Intact human osteocalcin 1-49 was analysed using iodine-125 immunoradiometric assay (IRMA); (N-tact® Osteo SP Osteocalcin IRMA kit made by DiaSorin, Stillwater, Minnesota, USA). The intra- and inter-assay coefficients of variation were both < 10 % as given by the manufacturer.
- The C-terminal cross-linked telopeptides (CTX-1) levels were measured using enzyme-linked immunosorbent assay (ELISA); (CrossLaps®, Nordic Bioscience Diagnostics A/S, Herlev, Denmark). The intra- and inter-assay coefficients of variation were both <10% as given by the manufacturer.

- The intact FGF-23 levels were determined using a two-site, sandwich ELISA system (ALPCO Diagnostics, Salem, USA) that detects epitopes within the amino-terminal and the carboxyl-terminal portions of FGF-23. The inter-assay variation in the normal and elevated concentration ranges was <6% as given by the manufacturer.
- Assessment of 25-hydroxyvitamin D (25-OHD) was performed using iodine-125 radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, USA). The intra- and inter-assay coefficients of variation of this assay were both <11% as given by the manufacturer.

3.1.6. Statistics

Normality was evaluated by the Kolmogorov-Smirnov test. Differences between and within groups were analysed by paired and unpaired samples T-test.

Crude associations between BMD and potential bone loss predictors were tested using bivariate correlations. Variables with p values less than 0.2 in this analysis were entered into a stepwise multiple linear regression model. R^2 is the determination coefficient that serves as a measure of the goodness-of-fit of the model; and the adjusted determination coefficient represents the proportion of variation of the dependent variable explained by the multivariate regression model. The same statistical methods were used to examine the relationship between changes in body composition from baseline until follow-up and potential predictors as well as between changes in BMD and baseline biochemical markers.

To examine the relationships between cumulative fracture rate and potential predictors, we used contingency tables (chi-square) *á priori* (dichotomizing continuous variables), followed by forward and backward stepwise logistic regression on variables with $p < 0.2$. In all studies the statistical program package SPSS was applied, and a two-sided p value < 0.05 was considered statistically significant.

3.2. Paper IV

3.2.1. Patient population

This single centre study assessed BMD in a large cohort of patients with the four most common types of end-stage organ failure (lung, liver, heart and kidney). Patients included in this study were registered on waiting list and all received subsequently a transplant at our centre. In total, 291 adult first-time transplant patients with end-stage organ failure were included in the study between August 2003 and December 2006. Of these, 210 were waiting for either lung (n=60), liver (n=84) or heart (n=66) transplantation, and were transplanted within one year after being measured for BMD. At our centre, BMD measurement has been a routine examination for all lung and liver failure candidates in the assessment process for transplantation. Measurement of BMD for heart failure patients awaiting transplantation was performed within a research study conducted at our clinic using no specific exclusion criteria.

Furthermore, a random selection of a comparable number of consecutive kidney allograft recipients (n=81) were included in the study. They were all from the main cohort described in Paper I. In our daily practice kidney patients are measured for BMD at the time of transplantation.

The study population represented 82% of lung-, 72% of liver-, and 86% of heart transplant candidates who were enrolled on waiting list and were transplanted in Norway in this time period. Similarly the kidney patients studied comprised 88% of kidney transplants in the same time period.

3.2.2. Bone mineral density

BMD was measured in the lumbar spine, total femur and two parts of the radius using the same DXA machine as previously described. All measurements were performed by the same two investigators using the same scanner.

3.2.3. Statistics

Differences between the four groups were determined by analyses of variance (ANOVA) using the Bonferroni method to adjust the p values. Multivariate logistic regression analyses were used in the assessment of different potential predictors for osteoporosis as outcome variables (osteoporotic versus normal).

3.3. Ethical considerations

All four studies included in this thesis were descriptive observations, meaning there was no interference with the standard treatment by introducing new drugs or new methods. Patients participating in these studies underwent routine examinations in relation to transplantation at the Rikshospitalet University Hospital, Oslo, Norway.

Precise measurement of BMD using DXA was used to observe the status of bone mass in these patients and to evaluate the risk of developing osteoporosis and fracture. Furthermore, the same DXA machine was able to analyse the body composition during the course of the BMD evaluation without increasing the dose of radiation during the measurement.

Taking blood samples is a part of standard routine practice at the outpatients' clinic at the Section of Nephrology during the follow up of renal transplant patients. Those patients who agreed to participate in the study were asked to give an extra sample of blood at the same time as their routine blood test.

Since most of the examinations performed in these studies are part of the routine evaluation, no ethical aspects of these studies were considered questionable. These studies and the information process used were also approved by the Norwegian Data Inspectorate to ensure enforcement of the Personal Data Act in Norway.

4. Results

4.1. Paper I

At the time of renal transplantation, DXA measurements revealed that Z-scores were significantly lower ($p < 0.05$), in lumbar spine, femur neck and whole body, in end-stage renal failure patients, compared with the reference population. Osteopenia was present in more than a third of the total group and osteoporosis (in varying parts of the skeleton) in 11-15%. The cumulative fracture rate was 29% (95% CI 21 to 37).

In univariate analysis, there was a strong negative correlation between total body BMD and age, former transplantation, time on haemodialysis, female sex, iPTH and post-menopausal status. BMI, physical activity and bisphosphonate treatment showed positive correlation with bone mass. The multivariate linear regression analysis revealed a significant relationship between total body BMD and age, former transplantation, female sex, iPTH, time on haemodialysis, BMI, and physical activity. The model explained 56% of the dependent variables. When the analysis was repeated for lumbar spine and the femur region, we found similar results.

Physical inactivity, BMI and presence of osteopenia were found to be independent determinants of cumulative fracture rate using logistic regression analysis.

4.2. Paper II

The overall patient population showed a numerical but non-significant weight loss of 0.9 kg [95% CI 0.3 to 2.2, $p = 0.106$] (78.1 kg to 77.2 kg) at follow-up. However, significant changes in body composition were found during the same period. There was a significant increase in total body fat mass (1.3 kg) accompanied by a significant reduction in fat-free mass (2.5 kg). Furthermore, regional body composition analyses showed significant changes in all measured compartments, principally fat mass increase in the abdominal region. In a separate analysis of men and women, changes in total body composition were consistent with the results for the total group.

When the total population was divided into three groups according to baseline body fat mass, the body composition analyses revealed a highly significant increase in total body fat mass (from 12.9 kg to 15.3 kg, $p < 0.001$) in the low-tertile group during follow-up. Meanwhile, a non-significant difference was observed in body fat mass (from 30.9 kg to 31.4 kg, $p = 0.360$) in the high-tertile group.

There was no noteworthy difference in the body composition of patients receiving or not receiving CsA-based immunosuppressive therapy, or between pre-emptive versus dialysis-treated patients.

All relevant variables associated with changes in body fat mass in the bivariate analysis ($p < 0.2$) were entered in a multivariate regression model. In the total group, increasing age, low-tertile body fat mass, prednisolone dose, longer time on dialysis, and lower C-reactive protein (CRP) level were independent predictors of increasing fat mass. Of the variations in body fat mass, 37% was explained by this model.

The same analyses were repeated to determine predictors for decrease in fat-free mass. The only independent predictor found for change in fat-free mass was cumulative prednisolone dose ($R^2=0.24$, $\beta=0.33$, $p=0.004$). Similar results were observed in separate analyses for men and women.

4.3. Paper III

Loss of bone mass occurred rapidly following renal transplantation, and BMD significantly declined in all examined compartments during the observation period. There was no significant difference in the BMD in patients with ($n=20$) or without ($n=24$) rejection episodes.

After transplantation, with the restoration of kidney function (median GFR=52 ml/min/1.73m²), osteocalcin and CTX-1 levels increased unexpectedly. FGF-23 levels significantly decreased after transplantation as did also phosphate and iPTH, but iPTH remained in the supra-physiological level. In addition, there was a significant increase in calcium, with no significant changes in 25-OHD levels. A significant association between serum osteocalcin and CTX-1 was seen at baseline ($r=0.25$, $p=0.001$) and at follow-up ($r=0.40$, $p<0.001$), indicating synchronisation of bone remodelling.

In the univariate analysis, iPTH correlated significantly with changes in lumbar spine BMD ($r=0.38$, $p=0.014$).

In the multivariate model, baseline osteocalcin was independently associated with bone loss in the total body ($p=0.049$), and CTX-1 in total femur ($p<0.001$). Baseline iPTH was significantly, though not consistently, associated with changes in the different bone compartments. Serum 25-vitamin D and FGF-23 did not predict BMD at any of the anatomical sites evaluated. Adding age, sex, serum urea level and cumulative prednisolone dose to the multivariate model did not affect the results.

4.4. Paper IV

In this middle-aged cohort including the four most common types of advanced organ failure groups (lung, liver, heart and kidney), a significant difference was observed in the proportion of males/females in the different transplant candidates. In the groups with liver, kidney, and heart failure, the majority of patients were male. Patients waiting for lung transplantation were mostly female and had a significantly lower ($p<0.001$) BMI, compared to the other groups.

Although low bone mass was found in patients with all four types of end-stage organ failure, lung failure patients consistently had the lowest Z-scores, followed by advanced liver, kidney and heart disease patients. Observations in males and females were similar to that seen in the total population. In the total group, significant differences were found in the Z-scores of the four organ failure groups at all skeletal sites measured, using one-way ANOVA analysis. Overall, the prevalence of osteoporosis was high and affected more than two thirds of patients awaiting lung transplantation. Of the patients with lung failure, 60% had chronic obstructive pulmonary disease (COPD). Significantly lower Z-scores in the lumbar spine and ultradistal radius regions were found in the COPD group, compared to non-COPD lung patients.

In the logistic regression model, age and BMI came out as risk factors for osteoporosis at all measured skeletal sites. However, there were no gender-specific differences in BMD, except in the distal radius region where men had a lower risk. Lung patients had a higher risk of developing osteoporosis in the lumbar spine [OR 2.56, 95% CI 1.02 to 6.46, $p=0.046$] and in the total femur [OR 3.81, 95% CI 1.20 to 12.01, $p=0.023$] than other transplant candidates.

5. Discussion

5.1. Transplant-related bone disease (Paper I, III, IV)

The issue of skeletal consequences following solid organ transplantation has recently got particular attention. Physicians have generally given more concern to graft rejection episodes, graft failure and other early or major complications. However, with better control of rejections and improved long-term patient as well as graft survival, bone status of solid organ transplant patients has become more important. Knowledge of the pathophysiology and of the prevention of bone loss is limited and little evidence exists based on randomised, intervention studies.

In young, healthy adults bone formation and resorption are in a state of balance, indicating synchronized bone remodelling. In kidney failure patients disturbances in mineral and bone metabolism become manifest as CKD progresses, resulting in complicated CKD-mineral and bone disorders (CKD-MBDs) [20]. Patients tend to undergo transplantation with pre-existing CKD-MBDs, therefore, bone disease is common in kidney allograft recipients, and progressive bone loss develops early after transplantation and persists for many years [26]. A “wait and see what happens” policy is inappropriate in these patients.

The aetiology of transplant-related bone disease is multifactorial, and the exact pathophysiological mechanism has not been fully characterised. Indeed, some studies are controversial [113]. Generally, rapid bone loss that occurs early after solid organ transplantation results from accelerated bone resorption owing to long-term skeletal insults from impaired bone formation. Nevertheless, bone status assessment early after transplantation is particularly important since transplant patients experience rapid post-transplant bone loss leading to a high risk of bone fracture [24]. If osteoporosis is identified (changes in BMD using DXA), initiation of early therapy to prevent subsequent bone loss is crucial.

The major challenge of this work was to describe the bone condition and the magnitude of early post-transplant bone loss, in addition to measuring corresponding changes in biochemical bone markers after renal transplantation. Furthermore, the research was performed for a broad evaluation of the BMD among different organ transplant candidates. In Norway, all transplantations are performed at a single centre, Rikshospitalet University Hospital, Oslo.

Our studies (Paper I, III) convincingly demonstrate that low bone mass is already present at all measured skeletal sites at the time of RTx, and significant bone loss can be observed as early as 10-12 weeks after renal transplantation. Similar findings were observed by Lindberg et al. [51] and Julian et al. [26] emphasizing that after renal transplantation, despite restoration of the excretory metabolic and hormonal abnormalities of chronic renal failure, osteoporosis remains a frequent and serious complication.

A major strength of the first study is that a large number of consecutive, and more than 80% of the eligible renal transplant recipients in the given time period were included. Our findings, therefore, are likely to be representative of ESRD patients who are eligible for renal transplantation in Norway. Dual-energy x-ray measurement was performed at the time of kidney transplantation to assess the skeletal complications in this ESRD population. In our study, Z-scores in all measured skeletal sites were lower, compared with the reference population.

5.1.1. Risk factors

Consistent with several previous studies we found that one of the major general risk factors for low BMD was ageing [114,115]. Older renal failure patients had a higher absolute bone loss, with a potentially higher age-dependent bone loss than the physiological decline. This finding can be explained by changes in hormonal status, nutrition and physical activity that parallel ageing. Female gender was also an independent risk factor. This was negatively associated with BMD, suggesting that the female skeleton is more vulnerable to transplant-related hormonal changes than that of the male [115]. The results showed that high BMI had a protective effect on bone mass owing to the higher level of oestrogens converted from androgens in adipose tissue [116]. Regular weight-bearing, physical activity also had a beneficial effect on bone mass, while prolonged bed rest had a powerful negative impact on skeletal health [117]. The anabolic effects of exercise on bone are related to changes in the homeostasis of various hormones, such as insulin-like growth factor-1 and sexual hormones, which are modified by exercise and may influence bone remodelling. Furthermore, it has recently been shown that exercise modifies calcium homeostasis and calciotropic hormones [118]. In addition to improving bone density, weight-bearing exercise reduces the risk of fractures by improving muscle strength and balance, thus helping to prevent falls.

One of the uremia-related factors associated with low BMD was former transplantation. This can be explained by the long-term immunosuppressive treatment (principally exposure to glucocorticoids), and possibly role of chronic inflammation related to chronic allograft rejection. Chronic inflammation may activate the RANKL/osteoprotegerin system which might accelerate osteoclastogenesis [17, 18]. Another risk factor for low bone mass was secondary hyperparathyroidism with a high level of circulating PTH. As described by Saliba et al., secondary hyperparathyroidism is associated with high bone turnover activity and is a leading cause of renal osteodystrophy [10]. High parathyroid hormone levels before kidney transplantation may induce significant bone loss within the first few months post-transplantation. In contrast, in two studies involving approximately the same number of transplant recipients as in our study, but late after RTx, time on haemodialysis had no significant effect on BMD [114, 115]. However, we found that the longer patients had received chronic dialysis the greater the likelihood for low bone mass. Such a reduction in BMD associated with time on dialysis has also been observed in other studies [119, 120].

5.1.2. Bone fractures

Fractures are the main clinical manifestation of osteoporosis. The cumulative fracture rate was high in our first study (29%). The detailed information on the level of trauma associated with each fracture were not available, thus we were not able to determine the exact proportion of low-energy fragility fractures. Data were collected only for symptomatic fractures; this rate may be a minimum estimate as certain types of fracture are not recognized by the health care system. Significant risk factors for high fracture rate were physical inactivity and presence of osteopenia. Cumulative corticosteroid dosage was not associated with increased fracture risk, a finding also reported by Vautour et al. [121]. This lack of association between glucocorticoid treatment and bone fracture is not easy to interpret since steroids play a major role in the loss of BMD after transplantation.

5.1.3. Dual-energy x-ray absorptiometry

Although, DXA is an important and widely used non-invasive diagnostic tool for determining bone density, it does not provide an accurate assessment of fracture risk in CKD [122], and its role in the evaluation of fracture risk in transplant patients is not

well established [123]. This is because a combination of bone density (measured easily by DXA) and bone quality (usually unmeasured) determines bone strength, which in turn influences fracture risks. Bone quality is determined by the micro-architecture of bone, bone turnover, mineralization, collagen properties, and accumulation of microdamage, which is more difficult to measure [124]. As bone quality contributes to bone strength, it is not surprising that there is an ongoing debate whether DXA measurement can predict fracture risk in CKD patients.

Despite some uncertainty of the value of BMD measurements in the evaluation of bone status in kidney transplant recipients, the KDIGO guidelines for post-transplant bone disease recommend measurement of BMD in the first 3 months after kidney transplantation if there are risk factors for osteoporosis [113].

5.1.4. Biochemical markers

The gold standard for assessing bone quality is the transiliac bone biopsy, which, as it is invasive, is infrequently used. There has, therefore, been great interest in developing non-invasive techniques that can provide a more accurate assessment of bone status than DXA. Another non-invasive way of assessing metabolic bone disease in patients with chronic kidney disease is the use of biochemical markers of bone turnover. Bone is constantly being renewed; bone markers are products derived from the bone remodelling process. Since different markers reflect different phases of the bone remodelling process, we used a rather comprehensive panel of bone turnover markers in Paper III to study the mechanisms of early post-transplant bone loss.

It is generally considered that bone remodelling is desynchronized in patients with CKD and that this leads to bone abnormalities before and shortly after transplantation [125]. Interestingly, we found a significant positive correlation between osteocalcin (marker of bone formation) and CTX-1 (marker of bone resorption), suggesting ongoing coupled bone turnover during the early post-transplant period. Our findings can be explained by the relatively large number of patients with pre-emptive RTx (29%), and the overall short length on dialysis (on average only one year). We did not find significant correlation between osteocalcin and CTX-1 in the subgroup of patients with acute rejection treated with high dose steroids suggesting a deleterious effect of glucocorticoids on bone [44, 45].

In uremia, reduced renal clearance leads to elevated osteocalcin and CTX-1 levels. The effectiveness of dialysis to remove osteocalcin and CTX-1 remain unknown. However, osteocalcin levels have been considered a useful marker to distinguish low from high turnover bone disease in patients on chronic maintenance dialysis [126]. In our study, despite impaired kidney function at baseline, circulating levels of osteocalcin and CTX-1 (which are cleared by the kidney) were in the lower normal range indicating low bone turnover at study inclusion. At follow-up after tapering of steroids, serum levels of both these markers were significantly raised demonstrating increased bone turnover, which partially explains the rapid deterioration of bone mass. Because these biochemical markers of bone turnover were elevated in the majority of patients, we hypothesized that increased rates of bone turnover contribute to early post-transplant bone loss.

A multivariate analysis was used to determine whether bone turnover biochemical markers of bone turnover are predictors of ongoing bone loss. We found that baseline osteocalcin and CTX-1 levels were independent predictors of early bone loss in total body and total femur region, respectively. The higher the level at baseline, the higher the risk of bone loss shortly after RTx. Similar findings were observed by Cruz et al. in 62 patients one year after renal transplantation [127].

The processes responsible for elevated bone resorption in this patient population remain unclear. However, pre-transplant secondary hyperparathyroidism has been implicated in early post-transplant bone loss [116]. In our study, we did not find a consistent association between iPTH and bone loss. This may be because excess PTH generally has catabolic effects on cortical bone and a relative preservation effect on trabecular bone. This has been shown consistently in several previous studies [128-130].

Considering the rapid bone loss and the relatively high prevalence of hypophosphatemia in kidney transplant patients, it seemed challenging to examine the potential role of the recently recognized FGF-23 in post-transplant bone diseases. FGF-23 has been identified as a regulator of the serum phosphorus and vitamin D, both of which are essential for bone health. In our study, not surprisingly, as kidney function recovered there was a rapid decrease in serum phosphate and FGF-23. We did not find an association between FGF-23 and other bone markers or BMD measurements, suggesting that this hormone does not directly affect bone metabolism early after RTx. Recent evidence suggests that FGF-23 is a counter-regulatory hormone for

1,25(OH)₂D₃ and maintains phosphate balance during vitamin D-mediated suppression of PTH [16].

More than 50% of the patients received vitamin D supplementation, primarily the vitamin D pro-hormone alfacalcidol (1 α -hydroxyvitamin D₃), prior to RTx. Alfacalcidol only requires hepatic C₂₅-hydroxylation to become the biologically active metabolite responsible for the effects of vitamin D on bone health and for the regulation of parathyroid function. The metabolic activation of vitamin D in liver is minimally regulated and vitamin D is mostly stored as 25-OHD. The level of 25-OHD, therefore, is a good measure of vitamin D nutrition in CKD patients. In our study, vitamin D deficiency was common with elevated iPTH levels. This emphasizes the importance of vitamin D supplementation (ergocalciferol or cholecalciferol) as an important step to halt abnormalities of bone and mineral metabolism in CKD. The KDIGO guidelines suggest that if plasma 25-OHD levels are normalized (>30ng/ml) and PTH levels remain elevated in CKD patients, active D sterol (calcitriol, alfacalcidol, or doxercalciferol) should be introduced [113].

5.1.5. Non-renal (lung, liver and heart) transplantation and osteoporosis

In Paper IV we assessed and compared bone status in a large number of lung, liver, heart and kidney transplant recipients. This study was not designed to identify independent predictors of bone loss but to better examine the issue of bone loss in solid organ transplant candidates in general. All subjects included in this study were registered on waiting list and received a transplant within a year after DXA measurement. One of the major strengths of our single centre study is that all BMD measurements were performed by the same two investigators using the same instrument. To the best of our knowledge, such study is the first to be conducted in this clinical setting.

Pre-transplant bone disease was common in all four transplant groups, despite the wide variety of primary diseases. Patients with end-stage lung disease, however, had the most pronounced bone loss and highest osteoporosis rate prior to transplantation, followed by liver, kidney, and heart patients. Many factors contribute to the pathogenesis of bone disease prior to transplantation, but our findings emphasized universal organ-specific adverse mechanisms of bone loss. As well as the general risk factors for bone loss (age, immobilization, low body weight, nutritional deficiency), pre-transplant bone

homeostasis is influenced by the diseased organ itself (i.e. lung, liver, kidney or heart failure) [131]. Furthermore, patients are treated with drugs (steroids, loop diuretics, heparin) which can promote negative calcium balance and bone loss.

The major finding of our study was that osteoporosis is extremely common in patients awaiting lung transplantation. A similarly high prevalence of osteoporosis, independent of the underlying lung disease, has also been reported by Tschopp et al. [132]. Shane et al. studied 70 patients awaiting transplant for end-stage lung disease and found glucocorticoid-treated patients with COPD were most severely affected [133]. We also found that COPD patients (most were long-time smokers) treated with steroids had decreased Z-scores, predominantly in the metabolically-active trabecular bone, compared to non-COPD lung patients. We found that, similar to patients awaiting lung transplantation, only a minority of patients with liver and cardiac failure had normal bone density [28, 29]. In general, more severe primary disease is associated with more severe bone loss prior to transplantation.

Interestingly, lung and liver transplant candidates had significantly lower Z-scores, compared to kidney transplant patients. We found it somewhat surprising that kidney failure patients had higher BMD than both lung and liver patients since CKD per se has a deleterious effect on bone. This may at least in part be explained by a high percentage of pre-emptive kidney transplantation and patients with a short time on dialysis. Moreover, metabolic bone disease may be recognised earlier and managed better in ESRD patients owing to a greater awareness of renal osteodystrophy among physicians treating renal patients. Although BMD measurements in kidney patients were performed a few days after RTx, the bone loss seen is probably an indication of the bone status in patients with ESRD rather than the effects of the RTx.

5.2. Alteration of body composition in kidney transplant patients (Paper II)

In this short-term, prospective study, we measured the body composition of more than one hundred, mainly well-nourished, first-time kidney allograft recipients. Measurement of DXA, the gold-standard non-invasive technique, revealed highly significant changes in body composition in the overall patient group after a short period post-transplant. There was a marked increase in body fat mass with a significant decrease in fat-free mass, without any significant changes in total body weight. Patients showed an early increase in total body fat with a central accumulation of fat mass. These alterations in

body composition could not be observed by simple body weight measurement and body mass index calculation, which emphasizes the importance of body composition analysis. In agreement with previous reports, we found that advancing age was an independent predictor of weight gain [134-135]. This can be explained by age-associated decreases in basal metabolic rate and physical activity. In sedentary people with declining energy needs not being matched with an appropriate reduction in energy intake, body fat content increases. Early after RTx most patients are inactive, and high steroid doses are used to avoid allograft rejection. Corticosteroids reduce lipolysis, which has an important effect on body composition. In our study the cumulative prednisolone dose was an independent predictor of fat mass distribution, mainly abdominal fat accumulation resembling Cushingoid features. Similar results were reported by van den Ham et al. in a study investigating the effect of early steroid withdrawal on body composition alterations after renal transplantation [94].

The duration of dialysis before transplantation was another significant predictor of body fat changes, suggesting that uremic toxicity and/or the haemodialysis procedure itself (bio-incompatibility of treatment, quality of the dialysate, loss of proteins, metabolic acidosis, psychological factors like depression, and socio-economic factors such as loneliness, poverty etc.) contribute to loss of muscle mass and body fat accumulation [136]. Ishimura et al. demonstrated similar effects of haemodialysis on body composition alterations in more than one hundred patients with ESRD [137]. Furthermore, we found that patients with poorer initial nutritional status, such as low-tertile fat mass, were more likely to have increased body fat mass. This can be explained by a brisk recovery with an increased appetite and calorie intake early after RTx, compared with their previous semi-starvation/malnourished uremic state prior to the transplant.

In our study, CRP, which is a marker of chronic systemic inflammation, was associated with body wasting. Chronic inflammation can lead to hypercatabolism with malnutrition and a decrease in fat mass [136, 138].

Lean body mass is the key marker of nutritional enhancement [139]. Although decreased lean body mass results mainly from loss of skeletal muscle mass after renal transplantation, the state of hydration does affect the DXA estimations of lean mass. In our study, measurement of baseline body composition was performed on average one week after RTx when the functioning kidney allograft had restored normal total body

water. We found that the cumulative prednisolone dose was the only independent predictor of decreased fat-free mass. High dose glucocorticoids early after RTx induce protein catabolism leading to negative nitrogen balance and muscle wasting [99].

Subgroup analyses by gender were performed to identify potential differences in body composition in response to different sex-hormone levels. We found that women had a higher body fat percentage than men owing to gender-specific patterns of fat distribution. There were significant changes in segmental body composition in both genders, except for changes in femoro-gluteal fat in females. Femoral fat is relatively insensitive to lipolytic stimuli and may play a protective role and cause less damage in organs such as the liver, pancreas and skeletal muscles by uptaking circulating free fatty acids [140].

6. Conclusions

6.1. Paper I

Significantly reduced bone mass and high cumulative fracture rate was found in this nationwide study involving a representative sample of ESRD patients who were eligible for renal transplantation in Norway. Beyond the well-known, generally accepted risk factors for reduced BMD, uremia-related independent factors were identified. For cumulative fracture rate, inactivity was the strongest predictor, followed by presence of osteopenia. Therefore, after normalization of the transplanted patients' physical performance, regular exercise should be strongly advised.

6.2. Paper II

Recovery from uremia with nutritional decline shortly after RTx can lead to significant changes in body composition despite no change in total body weight. Significant increase in fat mass along with reduction of fat-free mass was observed. The clinical consequence of these early changes remains to be explored in ongoing prospective studies. However, early nutrition intervention guided by qualified dietitians could be useful to avoid excessive weight gain early after transplantation.

6.3. Paper III

A significant reduction in BMD was observed due to increased bone turnover with inappropriate bone formation shortly after RTx. Serum osteocalcin and telopeptid in combination with iPTH seems to be a reasonable choice for routine assessment of bone metabolism. FGF-23 was not associated with either bone loss or any of the traditional bone markers. The exact role of FGF-23 on transplant-related bone loss needs further investigation.

6.4. Paper IV

Patients with end-stage lung-, heart-, liver- or kidney disease who are candidates for organ transplantation are all at risk for osteoporosis, most pronounced in lung patients. The finding suggests that increased awareness of bone disease before transplantation is warranted in all solid organ transplant groups.

7. Future perspectives

In our observational studies we have showed that reduced bone mass and osteoporosis is a frequent finding prior to solid organ transplantation and further accelerated bone loss can be observed in the first 3 months after kidney transplantation. However, reviews and meta-analysis of literature relating to this topic does not allow evidence-based pharmacological interventions to reduce bone loss and bone fractures in potential solid organ transplant recipients beyond what is known in the general population. Randomized controlled trials assessing effects of interventional drugs on fractures in this patient group are needed.

By today bisphosphonates may be the most promising agent.

Accordingly we have initiated an ongoing prospective, randomized, double blind, placebo controlled clinical study (Ibandronate Versus Placebo in the Prevention of Bone Loss After Renal Transplantation. Protocol no.: SMR-1471) including 130 patients to investigate whether bone loss and bone fracture may be better prevented with the addition of bisphosphonates to active vitamin D₃ compared to active vitamin D₃ alone.

If this study demonstrates a significant additive effect of ibandronate and appears safe in kidney transplant patients, a large-scale study with fracture end-points may be launched.

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Appendices

Paper I

Erratum for Paper II

Paper II

Paper III

Paper IV

Erratum for Paper II

In the Material and methods section of paper II there is a somewhat deceptive description of the study population. The correct description is given below:

Corrected version

Between February and December 2006, 198 patients received a kidney allograft at the transplant centre. Of these, 133 adult patients without severe comorbidity gave written consent for their medical data being used. Based on the exclusion criteria (previous transplantation, conditions which may influence body composition and the hydration status of the body), 31 patients were excluded. (Conditions included thyroid disease, limb amputation, and supportive haemodialysis due to delayed graft function at the time of measurements.) In total, 102 first time kidney allograft recipients were included in the study.

Original version

Between February and December 2006, 198 patients received a kidney allograft at our centre. Sixty-two patients were excluded from the study. The exclusion criteria for participation in this study were the following: age less than 18 years, limb amputation, known thyroid disease, repeat transplants and patients needed supportive haemodialysis due to delayed graft function at the time of measurements. Hundred and two first-time kidney allograft recipients of the remaining 136 (75%) agreed to participate, gave written informed consent for the data being used and were included in the study.