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Parathyroid and bone response of the diabetic patient to uremia

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Parathyroid and bone response of the diabetic patient to uremia. Biochemical and radiologic indices of bone disease were assessed in 26 insulin-dependent diabetic patients and 28 nondiabetic patients with endstage kidney disease. The two groups were comparable in age, sex, duration of renal failure, and length of time on dialysis. Diabetic patients showed significantly lower serum calcium and immunoreactive parathyroid hormone (iPTH) levels than nondiabetic patients. iPTH was not related to total serum calcium, but was positively correlated with serum phosphorous ($r = 0.37$, $P < 0.05$ and $r = 0.54$, $P < 0.005$, in nondiabetic and diabetic patients, respectively). iPTH correlated with alkaline phosphatase ($r = 0.59$, $P < 0.0009$) and calcitonin ($r = 0.51$, $P < 0.05$) only in nondiabetic patients. Osteitis fibrosa was noted radiologically in 30% of nondiabetic patients and in none of the diabetic patients ($P < 0.03$). Bone morphology in eight diabetic patients who underwent iliac bone biopsy was characterized by reduced trabecular and osteoid bone volume, no woven bone, and marked reduction in indices of bone formation and resorption. The small amount of bone and lack of osteomalacia are a unique feature of the diabetic patient with chronic renal disease. The long-term sequelae of low bone turnover and reduced circulating iPTH may present a special problem to the long term diabetic survivor on the current therapies of uremia.

Réponses parathyroïdienne et osseuse de malades diabétiques en urémie. Les indices biochimiques et radiologiques d'ostéopathie ont été déterminés chez 26 malades diabétiques insulino-dépendants et 28 malades nondiabétiques avec une néphropathie terminale. Les deux groupes étaient comparables en âge, sexe, durée de l'insuffisance rénale, et durée de la dialyse. Les malades diabétiques avaient un calcium sérique et une hormone parathyroïdienne immuno-réactive (iPTH) significativement plus faibles que les malades nondiabétiques. iPTH n'était pas reliée au calcium sérique total, mais était positivement corrélée avec le phosphore sérique ($r = 0{,}37$, $P < 0{,}05$ et $r = 0{,}54$, $P < 0{,}005$, chez les nondiabétiques et les diabétiques, respectivement). iPTH était corrélée à la phosphatase alcaline ($r = 0{,}59$, $P < 0{,}0009$) et à la calcitonine ($r = 0{,}51$, $P < 0{,}05$) uniquement chez les malades nondiabétiques. Une ostéïte fibreuse a été notée radiologiquement chez 30% des malades nondiabétiques et chez aucun des diabétiques ($P < 0{,}03$). La morphologie osseuse chez huit diabétiques qui avaient subi une biopsie de la crête iliaque était caractérisée par une réduction des volumes osseux trabéculaire et ostéoïde, l'absence d'os spongieux, et une réduction marquée des indices de formation et de résorption osseuses. La faible quantité d'os et l'absence d'ostéomalacie sont une caractéristique unique du malade diabétique atteint de néphropathie chronique. Les séquelles à long terme d'un renouvellement osseux faible et d'une diminution d'iPTH circulante pourraient constituer un problème spécial pour le diabétique survivant à long terme avec les traitements habituels de l'urémie.

Diabetes mellitus results in pathologic changes and functional derangements in many organs. Several abnormalities have been described in the skeleton of patients with insulin-dependent type I and noninsulin-dependent type II diabetes [1–7]. However, the consequences of these abnormalities are not clear [2, 8]. The development of diabetic nephropathy and renal failure adds another dimension to the bone pathology. We and other investigators have noted reduced secondary hyperparathyroidism in diabetic patients on hemodialysis and a lower incidence of aseptic necrosis following transplantation [9, 10]. The present study was undertaken to further characterize the mineral metabolism and to determine the relationship of PTH to static and dynamic indices of bone histology in the uremic diabetic patient. Several features of metabolic bone disease which appear to be unique to the diabetic patient with endstage renal disease prompt this report.

Methods

Fifty-four patients with renal failure referred for kidney transplantation were selected for this study. None of the patients had undergone parathyroidectomy or were on cimetidine or vitamin D therapy. Nondiabetic patients did not have overt carbohydrate intolerance and remained euglycemic on glucocorticoids after kidney transplantation. The diabetic patients had insulin-dependent diabetes for over 20 years and continued to require insulin in renal failure. Since diabetic nephropathy is characterized by a brief duration of renal failure [11], diabetic and nondiabetic patients were selected for this study with the following criteria: *(1)* the course of the renal failure documented to have been less than 5 years; *(2)* renal disease characterized by proteinuria and hypertension.

Serum immunoreactive parathyroid hormone (iPTH) was measured by the method of Arnaud, Tsao, and Littledike [12]. The assay uses an antiserum (GP-1M) with major specificity for the carboxyl terminal region of the PTH molecule, quantitating both intact species of circulating hormone and fragments. Calcitonin was measured in plasma by the method of Heath and Sizemore [13], and 25 hydroxyvitamin D [14], and 1,25 dihydroxyvitamin D [15] in serum by competitive protein binding assays following the isolation of the metabolite by chromatography. Total calcium and magnesium were measured by atomic absorption spectrometry, phosphorous by the standard Subbarow method, and albumin and alkaline phosphatase by the autoanalyzer.

Conventional screen-film radiography was performed on all subjects and included radiographs of the thoracic and lumbar

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spine, chest, abdomen, and pelvis. Radiographs of the hands were not obtained. The radiographs for each subject were reviewed in composite and without access to clinical data. Radiographic findings of osteopenia, osteosclerosis, and osteitis fibrosa were evaluated as follows:

- Osteopenia was determined radiographically by generalized radiolucency of the skeleton, accentuation of primary trabeculae, and biconcave or wedged deformities of the vertebral bodies.
- Osteosclerosis was determined radiographically by generalized increase in radiodensity, coarse thickening of trabeculae, and radiodense bands paralleling the endplate of the vertebral bodies.
- Osteitis fibrosa was defined radiographically by subperiosteal bone resorption at sites of musculotendinous insertions such as the coracoclavicular ligaments, or by subchondral bone resorption at articular sites such as the sacroiliac joints, symphysis, or acromioclavicular joints.

Transilial bone biopsies were performed after double tetracycline labelling at the time of renal transplantation in eight consecutive diabetic patients using the Bordier trephine, 8 mm in diameter. The specimens were taken from a standard area 2 cm below and posterior to the anterior iliac spine from the inner table of the pelvis. The core of bone was placed in Mellonigs fixative or 70% ethanol and sent without delay to the Department of Medicine, Omaha, Nebraska. After dehydration and defatting by successive changes of alcohol and acetone, the specimens were embedded in methyl methacrylate. Sections were cut 10-μ thick and left unstained for fluorescent microscopy and, 5-μ thick to stain with Goldner's light microscopy. Quantitative histomorphometry was done using an integrating eyepiece with alternating hemispherical lines intersecting at 36 points with a grid. An eyepiece micrometer was used for measurement of distances. Indices measured were: *(1)* trabecular bone volume as space occupied by mineralized and unmineralized trabecular bone tissue, as a fraction of whole bone tissue (including marros); *(2)* osteoid volume as the fraction of unmineralized bone matrix per unit of whole bone tissue including marrow; *(3)* fractional osteoid surface as the fraction of trabecular bone surface covered by osteoid; *(4)* bone formation rate as the volume of new bone formed per unit volume of pre-existing bone per year, derived from the linear appositional rate, bone volume, surface density and double-labeled surface. The equation is $F_v = S_v \cdot \bar{M} \cdot S_{fa}$ where S_v is mm^2 trabecular surface area per mm^3 bone tissue, $\bar{M}$ is new bone per year and S_{fa} is mm^2 double-labeled surface area/mm^2 trabecular surface area [16]; *(5)* fractional resorption surface is the fraction of trabecular bone surface occupied by Howships lacunae.

Yates' corrected χ^2 was used to compare frequency distributions between groups. Comparisons of biochemical and metabolic findings were made with Student's *t* test for independent samples. Pearson's r was computed in the correlational analyses of biochemical values within a group. All calculations were performed with the SPSS [17] or BMD-P [18] series of computer programs.

Results

Relevant clinical data are summarized in Table 1. Biochemical and metabolic data are shown in Table 2. Diabetic patients showed significantly lower serum calcium, albumin, and iPTH than the nondiabetic patients. There was no correlation between serum albumin and calcium in either group. Serum iPTH was not related to total serum calcium but was positively correlated with serum phosphorous ($r = 0.37$, $P < 0.05$ in nondiabetic patients and $r = 0.54$, $P < 0.005$ in diabetic patients). iPTH levels correlated with alkaline phosphatase ($r = 0.59$, $P < 0.0009$) and calcitonin ($r = 0.51$, $P < 0.05$) only in nondiabetic patients. Vitamin D metabolites were measured in the eight patients who had bone biopsies. Serum 25-hydroxyvitamin D values ranged from undetectable to 30 ng/ml (mean ± SD = 14 ± 10); four of eight patients had values within the range of nutritional deficiency or less than 10 ng/ml. 1,25 dihydroxyvitamin D values were 6 to 26 pg/ml (mean ± SD = 13 ± 6); the values were reduced in six of eight patients.

Table 1. Clinical data in 28 nondiabetic (NDM) and 26 diabetic (DM) patients

	NDM	DM
Male, *number*	20	19
Female, *number*	8	7
Age, *years*, mean ± SE	32.5 ± 2.1	35.9 ± 1.6
Time on dialysis, *months*, mean ± SE	12.3 ± 2.3	14.8 ± 2.0

Table 2. Biochemical and metabolic data in 28 nondiabetic (NDM) and 26 diabetic (DM) patients

	NDM		DM
Calcium, *mg/dl*[a]	9.95 ± 0.22	$P < 0.05$	9.38 ± 0.16
Phosphorous, *mg/dl*	4.69 ± 0.30		5.68 ± 0.43
Magnesium, *mg/dl*	3.08 ± 0.09		3.00 ± 0.09
Alkaline P, *IU/liter*	94 ± 17		96 ± 13
Albumin, *g/dl*	4.6 ± 0.11	$P < 0.002$	4.1 ± 0.1
iPTH, *μlEq/ml*	1733 ± 271	$P < 0.002$	734 ± 141
Calcitonin, *pg/ml*	303 ± 59		301 ± 31

[a] Values are reported as mean ± SEM.

Table 3. Radiologic axial skeletal survey in 28 nondiabetic (NDM) and 26 diabetic (DM) patients

	NDM		DM
Osteitis fibrosa, %	30	$P < 0.03$	0
Osteosclerosis, %	20	NS	0
Osteopenia, %	39	NS	41

The results of the radiologic survey of the axial skeleton in nondiabetic and diabetic patients are shown in Table 3. Specific findings of secondary hyperparathyroidism, for example, osteitis fibrosa, were observed only in nondiabetic patients. The relevant clinical and metabolic data from the eight patients who underwent transilial bone biopsy at the time of renal transplantation are shown on Table 4. The histologic appearance of osteoporosis, decreased cellularity, and absence of deposition of tetracycline was found in five of the eight patients. Only patient 8 showed increased osteoclast numbers, resorption surfaces, and excess osteoid consistent with the secondary hyperparathyroidism seen in renal osteodystrophy. However, this patient's bone volume was within the low normal range. The numerical data of bone histomorphometry are given in Table 5 [19] in order of increasing serum iPTH. The eight patients showed either reduced ($N = 5$) or low normal ($N = 3$)

Table 4. Clinical and biochemical data in eight uremic diabetic patients who underwent bone biopsy

Patient no.	Age *years*	Sex	Time on dialysis *months*	Total calcium	Magnesium	Phosphorous	iPTH *μlEq/ml*	Calcitonin *pg/ml*	25(OH)D *ng/ml*	$1,25(OH)_2D$ *pg/ml*
				mg/dl						
1	41	M	8	8.9	2.5	2.3	124	235	8	11
2	32	F	10	9.1	3.1	3.7	160	235	8	9
3	43	M	0	9.5	2.1	1.8	184	115	25	18
4	35	F	18	9.0	3.7	5.8	258	315	10	11
5	53	M	8	8.7	3.2	4.0	385	174	18	26
6	34	M	7	10.0	2.9	3.6	550	263	U	14
7	43	M	6	9.0	3.8	2.6	1150	405	30	6
8	33	F	13	7.9	1.0	5.0	2090	145	10	9

Abbreviation: U, undetected.

Table 5. Measurements of bone histomorphometry in eight uremic diabetic patients

		Diabetic patients (case number)[a]							
	Normal[b]	1	2	3	4	5	6	7	8
Trabecular bone volume (V_v) mm^3/mm^3, mean ± SD	0.21 ± 0.04	0.12	0.11	0.14	0.12	0.12	0.17	0.19	0.18
Osteoid volume (V_{os}) mm^3/mm^3, mean ± SEM	0.005 ± 0.003	0	0.009	0.013	0.009	0.010	0.003	0.002	0.027
Fractional osteoid surface (Sf), mm^3/mm^2, mean ± SD	0.123 ± 0.54	0	0.129	0.419	0.169	0.597	0.003	0.091	0.519
Osteoid seam width, μm, mean ± SD	6.9 ± 6	0	7	9	11	7	0	7	19
Bone formation rate, (F_v)[c] $mm^3/mm^3/yr$	0.15 − 0.25	0	0	0	0	0.085	0	0.005	0.697
Fractional resorption surface, (S_r)	0.063 ± 0.025	0.029	0.008	0.017	0.011	0.024	0.238	0.094	0.294
Aluminum stain[d]		+	0	0	++	+	ND	++	0

[a] The patients are numbered according to the degree of secondary hyperparathyroidism as assessed by the serum iPTH level.
[b] Values were obtained from Meunier and Corupron [19].
[c] Comparable normal data were not available; the normal range is estimated.
[d] Aluminum stain is reported qualitatively that is, 0, normal, + to +++, mild to severe, and ND, not done.

bone volume. Seven patients exhibited osteoid volume less than normal, three an increase in fractional osteoid surface, and two increased resorption surfaces. There was no relationship between any of the biochemical measurements and the morphologic indices except for a correlation between serum PTH and resorption surfaces ($r = 0.81$, $P < 0.05$).

The results of the staining of the biopsy sections for aluminum generously provided by Dr. Don Sherrard are found in Table 5 and show no relationship to the other indices of bone histomorphometry.

Discussion

This study demonstrates several unique features in the manifestations of secondary hyperparathyroidism in uremic diabetic patients. We confirmed our earlier observations of lower circulating iPTH in diabetic patients with endstage renal disease than nondiabetic patients with comparable diseases and duration of dialysis. The antiserum of the assay which we used in this study was of a different specificity for PTH than the antiserum used in our earlier report [9] and does not detect abnormal differences in diabetic patients without renal disease [20]. Three other immunoassays revealed lower values for the hormone in the serum of diabetic patients with renal failure [10], during pregnancy [21], and in nonuremic insulin-treated diabetic patients [22] than control subjects. Measurements of the endogenous biologically active PTH through stimulation of adenylate cyclase in an in vitro bioassay [23] in the sera of eight diabetic patients were similar to those of eight nondiabetic patients (Dr. R. A. Nissenson, personal communication). The assay we used detects primarily the carboxyl terminal end of the molecule, including nonbiologically active fragments and, characteristically, shows increases from 10 to 200 times the upper limit of normal in patients with chronic renal failure. Differences in the metabolism of PTH between diabetic and nondiabetic patients cannot be eliminated and need further study.

The reduced secondary hyperparathyroidism appears to be unrelated to the course of diabetic nephropathy and length of time on dialysis. Diabetic endstage renal disease is characterized by a relatively brief duration of renal failure associated with proteinuria and hypertension [11]. Nondiabetic uremic

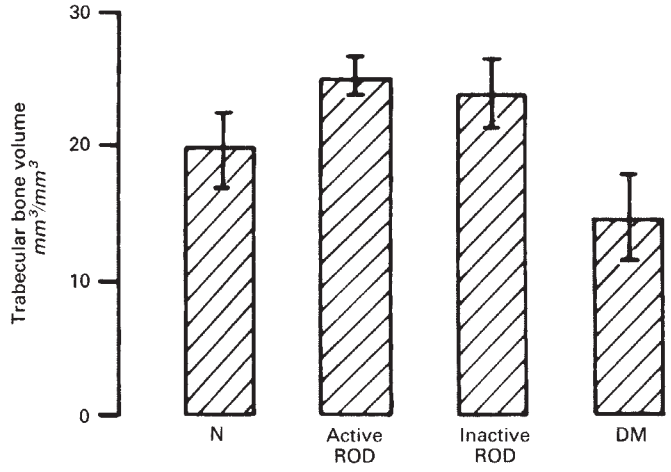

Fig. 1. *Trabecular bone volume* (mm^3/mm^3 ± SD) *in nonuremic control subjects* (N) *and in renal osteodystrophy* (ROD) *in nondiabetic patients (active and inactive) and diabetic patients* (DM). Data on N were obtained from Meunier and Corupron [19] and on ROD from Frost et al [29], respectively.

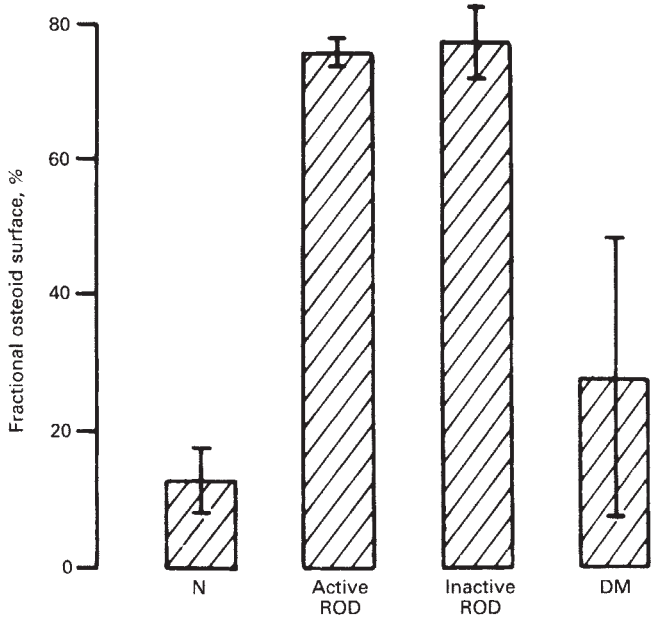

Fig. 2. *Fractional osteoid surface* (% ± SD) *in nonuremic control subjects* (N) *and in renal osteodystrophy* (ROD) *in nondiabetic patients (active and inactive) and diabetic patients* (DM). Data on N were obtained from Meunier and Corupron [19] and on ROD from Frost et al [19], respectively.

control subjects for the biochemical indices were selected in this study if their renal disease and clinical course were similar to diabetic nephropathy. The two groups were closely matched for age, sex, and duration of dialysis. Several biochemical differences were noted between diabetic and nondiabetic uremic patients. The serum calcium of diabetic patients was significantly lower than nondiabetic patients. The absence of a relationship between albumin and calcium in the serum of diabetic patients suggests that the lower total serum calcium in the diabetic patient is related to one or more factors regulating serum calcium. These include retention of phosphate [24], resistance of the skeleton to circulating PTH [25], nutritional deficiency [26], or failure of renal hydroxylation of vitamin D [27]. Neither 25-hydroxyvitamin D nor 1,25 dihydroxyvitamin D are lower than normal in nonuremic diabetic patients [20]. The patients who had biopsies showed marked variation in their nutritional status of vitamin D which did not appear to be related to any biochemical or histomorphometric measurement, and the 1,25-dihydroxy vitamin D values in this group were all measurable and similar to values reported in patients with endstage renal failure.

Despite the difference in secondary hyperparathyroidism between diabetic and non-diabetic patients, serum alkaline phosphatase was similar; however, alkaline phosphatase correlated positively with PTH only in the nondiabetic patients. The activity of this enzyme, as reported by other investigators, may well reflect the severity of renal osteodystrophy in high bone turnover states which tend to be more responsive to treatment [28, 29]. The role of calcitonin in bone disease is not clear [30–32]. Modest increases in serum calcitonin levels were observed in both diabetic and nondiabetic patients with chronic renal failure and do not account for differences in bone morphology between the two groups.

Radiologic assessment of the axial skeleton did not reveal findings of osteitis fibrosa in diabetic patients, in contrast to 30% of the nondiabetic patients. Osteosclerosis, a marker of severe secondary hyperparathyroidism, was similarly observed only in nondiabetic patients. These data and our previous findings of decreased uptake in bone scans of uremic diabetic patients indicate that diabetic patients with renal failure have reduced manifestations of secondary hyperparathyroidism [9]

The main feature of the histology of bone in the diabetic patients with chronic renal failure is the reduced or low normal volume of bone associated with reduced indices of both bone formation and resorption (Table 5). Only one patient whose serum iPTH concentration was 50 times the upper limit of normal showed the more typical bone histology of secondary hyperparathyroidism in endstage renal failure, for example, increased osteoclastic resorption and an excess of unmineralized osteoid. A less typical aspect of this patient's bone histology was the small amount of bone tissue. Although the bond morphology in endstage renal failure is variable, most patients show three types of osteodystrophy with the features of osteomalacia or osteitis fibrosa predominating or mixed which can be separated according to the dynamic measurements of bone turnover and iPTH concentrations [33]. In another series of patients in whom both the static and dynamic indices of bone histology in dialysis patients are recorded, one-third of the patients were reported to show 'inactive' renal osteodystrophy, a variant of the osteomalacic form [29], characterized by normal or increased bone volume, a mineralization defect, increased osteoid volume, a low rate of bone turnover, and normal or low concentrations of PTH. The diabetic patients we studied differ from these patients, as illustrated in Figures 1 and 2, in the low volume of bone and the smaller amount of osteoid. The paucity of osteoid tissue suggests that the osteopenia in diabetic bone is related to a decrease in matrix synthesis. We do not, however, have serial measurements of bone mass of diabetic patients on dialysis to document bone loss, and techniques for direct quantitation of resorption rates are not available. The static measures of resorption surface are

unexpectedly low and not correlated with the rates of bone formation in our study group. This reflects the limitations of current methodology but also suggests uncoupling of the process by which the integrity of the adult skeleton is maintained; for example, activation of new remodeling sites initiated by osteoclastic resorption followed by synthesis of matrix and mineralization at the same site [34]. One explanation for our findings is an uncoupling of this process associated with increased bone loss of previously normal volume of bone, as reported by DeLeeuw, Mulkens, and Vertommen in juvenile diabetic patients [35]. Another possibility, consistent with an earlier study of diabetic bone [7], is that bone volume was low in our patients prior to renal failure and that the reduction in bone turnover persists with the progression of renal failure.

Whether or not the changes in the bone of diabetic patients with chronic renal failure can be attributed to the underlying abnormality in glucose metabolism is uncertain. Evidence of aluminum toxicity which has been incriminated in the etiology of the inactive osteomalacic form of renal osteodystrophy [36, 37] was present in only two of six patients. Osteomalacia associated with bone pain and hypercalcemia after vitamin D is also reported to follow the surgery in 7% of patients on maintenance dialysis treated by parathyroidectomy [38]. In both these situations, however, there is abundant osteoid tissue which is not calcified. This has led investigators to attribute the hyperosteoidosis to a defect in mineralization rather than matrix synthesis. The abnormality in the remodeling of diabetic bone from the early studies of Frost and colleagues [7] reveals reduced rates of bone formation and resorption. He and his coworkers found the ratio of resorption spaces to the number of osteoid seams to be higher in diabetic rib lamelar bone than in nondiabetic bone, suggesting that diabetic bone was more slowly remodeled. There is considerable evidence that the skeleton of the diabetic patient contains less mineral than that of nondiabetic patients [1–5, 39]. However, osteomalacia, osteoporosis, or increased fragility of diabetic bone is not well-documented [2, 8]. These observations favor the view that the bone of diabetic patients has a reduced rate of metabolism which influences the 'response' to chronic renal failure. That the defect in bone cells in diabetic patients is common to the cells of the parathyroid, resulting in reduced secretion of the hormone, is entirely speculative. Since most of our patients were insulin-dependent juvenile onset diabetic patients, treated with insulin for many years, deficiency of this hormone is an unlikely explanation for our findings.

The clinical relevance of these findings is complex. How do patients with diabetes mellitus and pre-existing osteopenia fare with the progression of renal failure? There may be short-term benefits from low bone turnover and reduction in the concentration of circulating PTH in the uremic diabetic patient. They exhibit few, if any, manifestations of secondary hyperparathyroidism and have a reduced risk of aseptic necrosis following kidney transplantation [9]. In contrast to these short-term benefits, the long-term consequences of the bone disease we have described is disconcerting. The low bone volume and low bone turnover in a disease which promotes increased resorption of bone may lead to skeletal collapse (patient 4 in Tables 4 and 5 refused dialysis and died 3 months after returning to hemodialysis following a failed kidney transplant partly because of the complications of multiple fractures). The results of our study and reports of bone disease following parathyroidectomy [38] mandate against parathyroidectomy in uremic diabetic patients. As diabetic patients are maintained on dialysis or with kidney transplantation, the impact of uremia on the diabetic skeleton will be better understood. In the interim serial monitoring of bone mass with bone densitometry or bone biopsy could be useful in the management of the individual diabetic patient with endstage renal disease. Additional studies will be needed to indicate which of the current therapies of uremia is least detrimental to the skeleton of the diabetic patient.

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