

Measurement of Markers of Osteoclast and Osteoblast Activity in Patients with Acute and Chronic Diabetic Charcot Neuroarthropathy

A. Gough^{1*}, H. Abraha², F. Li², T.S. Purewal¹, A.V.M. Foster¹, P.J. Watkins¹, C. Moniz², M.E. Edmonds¹

¹Diabetic Department, ²Department of Clinical Biochemistry, King's College Hospital, Bessemer Road, London, UK

Excess osteoclast activity is believed to be responsible for the early bone changes associated with Charcot neuroarthropathy in diabetes mellitus. Markers of osteoclast and osteoblast activity were measured in four groups of patients: 16 with an acute Charcot foot, 16 with a chronic Charcot foot, 10 diabetic controls, and 10 non-diabetic controls. Serum carboxyterminal telopeptide of type 1 collagen (1CTP), a marker of osteoclastic bone resorption, was significantly raised in the dorsal venous arch of the acute Charcot foot, $6.1 \pm 1.5 \mu\text{g l}^{-1}$ (mean \pm SD) compared with the chronic Charcot foot 4.1 ± 1.4 , diabetic controls 3.3 ± 1.4 , and non-diabetic controls 2.8 ± 1.4 , $p < 0.0001$. This local increase in 1CTP was also reflected systemically in a study subgroup of 6 patients with acute Charcot neuroarthropathy, in whom peripheral antecubital vein 1CTP was 9.2 ± 2.6 compared with 9.0 ± 3.1 in the foot. In 6 chronic Charcot neuroarthropathy patients, foot (3.8 ± 1.3) and systemic (4.0 ± 1.5) 1CTP values were similar. Serum procollagen carboxyterminal propeptide (P1CP), an indicator of osteoblastic bone formation, was not significantly different between the feet of patients with acute Charcot neuroarthropathy $112 \pm 1.5 \mu\text{g l}^{-1}$, patients with chronic Charcot neuroarthropathy $109 \pm 1.5 \mu\text{g l}^{-1}$, diabetic controls $93.5 \pm 2.3 \mu\text{g l}^{-1}$, and non-diabetic controls $90.1 \pm 1.5 \mu\text{g l}^{-1}$. These results suggest that the acute Charcot foot demonstrates excess osteoclastic activity without concomitant increase in osteoblastic function. This may be important in its pathogenesis. © 1997 by John Wiley & Sons, Ltd.

Diabet. Med. 14: 527–531

No of Figures: 0. No of Tables: 3. No of Refs: 44

KEY WORDS Charcot neuroarthropathy Osteoclast Osteoblast Carboxyterminal telopeptide of type 1 collagen (1CTP) Procollagen Carboxyterminal propeptide (P1CP)

Received 18 September 1996; revised 7 March 1997; accepted 9 March 1997

Introduction

The development and progression of Charcot neuroarthropathy can be a devastating consequence of diabetes. Bone deformity associated with extreme cases may lead ultimately to amputation. Recent evidence suggests that early features may be found in 3% of people with diabetes.^{1,2} The pathogenesis however remains unclear. One proposed mechanism^{3–5} is that repeated trauma in an insensitive joint leads to intracapsular effusions, ligamentous laxity, and joint instability, with resultant bone and articular surface damage. This almost certainly occurs in association with neurovascular abnormalities,⁶ where neuropathy, including autonomic dysfunction, produces an exaggerated vasodilatory response, with increased blood flow⁷ and arterio-venous shunting,

leading eventually to osteoclastic bone resorption.^{8,9}

Type 1 collagen makes up more than 90% of bone matrix. The pyridinoline cross-linked carboxy-terminal telopeptide domain of type 1 collagen (1CTP) is released during collagen degradation.¹⁰ Serum concentrations correlate with osteoclast activity and bone resorption rates measured either histomorphometrically or by calcium kinetic studies.^{11–13} The carboxy-terminal propeptide of type 1 collagen (P1CP)^{14,15} is released by specific endoprotease cleavage from the precursor molecule procollagen, which is synthesized by osteoblasts. P1CP is a validated marker of bone formation and osteoblast function.^{11,13,16,17}

To investigate if excessive osteoclast activity is a feature of early Charcot neuroarthropathy, we measured foot venous levels of 1CTP and P1CP in Charcot patients with acute and chronic disease. Local (foot) and systemic concentrations of both markers were assayed simultaneously in a study subgroup, to evaluate any potential

* Correspondence to: Dr Andrew Gough, Diabetes Centre, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham B9 5SS, UK.

role for 1CTP and P1CP for monitoring disease activity and the effects of treatment.

Patients and Methods

We studied four groups, patients with an acute Charcot foot ($n=16$), those with chronic disease ($n=16$), controls with diabetes ($n=10$), and non-diabetic controls ($n=10$). Clinical characteristics are summarized in Table 1.

Charcot patients were recruited from the Diabetic Foot Clinic, controls with diabetes from the General Diabetic Clinic, and control subjects without diabetes from staff at the same hospital. Individuals with a coexisting illness or on medication likely to affect bone/calcium metabolism were excluded. Insulin-dependent (Type 1) diabetes mellitus (IDDM) was defined as onset at less than 30 years of age and insulin treatment within 1 year of diagnosis.

Criteria for acute Charcot neuroarthropathy were: typical clinical features of a swollen, erythematous foot; $> 2^\circ\text{C}$ temperature difference between affected and non-affected foot, measured using a Mikron® infra-red thermometer (Mikron Instrument Company Inc., Wyckoff, New Jersey, USA) (under normal circumstances foot temperatures should not differ by more than 2°C ¹⁸); and increased bone isotope uptake on three phase ^{99m}technetium methylenediphosphonate scintigraphy.¹⁹ Osteomyelitis, if suspected, was excluded as far as possible with gallium-67 or indium-111 labelled white cell studies^{20,21} or magnetic resonance imaging (MRI).^{22,23} Patients with osteomyelitis were not included.

Diagnosis of chronic Charcot neuroarthropathy was based on the presence of characteristic bone destruction, fragmentation, and disorganized joint architecture on plain radiographs of the foot, that had remained unchanged over 6 months. This was associated with no foot temperature difference.

Blood for 1CTP and P1CP assays was collected with venous stasis and determined using radioimmunoassay (RIA) kits (Orion Diagnostica, Finland). Local samples were drawn from the dorsal vein of the foot and systemic

samples from the antecubital vein. Normal ranges were 1CTP $1.7\text{--}5.0\ \mu\text{g l}^{-1}$, P1CP $< 150\ \mu\text{g l}^{-1}$. Blood was kept for measurement of serum creatinine (analysed on a SMAC Analyser, Technicon Ltd, Basingstoke, UK; normal $< 120\ \mu\text{mol l}^{-1}$), alkaline phosphatase (normal range $30\text{--}120\ \text{IU l}^{-1}$), and glycated haemoglobin (HbA_{1c}) by HPLC (Primus CLC 330TM), non-diabetic range $4\text{--}6\%$. Dipstick urinalysis was performed using Albustix® (Bayer Diagnostics, Basingstoke, UK) on random samples; proteinuria was deemed present if one + or greater was detected on three occasions.

The study was approved by the local Ethics Review Committee.

Statistical Analysis

Data not normally distributed were logarithmically transformed prior to analysis. Statistical comparisons between the groups were performed by One-Way ANOVA for multiple comparisons, and are expressed as the mean \pm standard deviation (SD) or the geometric mean if log-transformed. Simultaneous values from foot and arm were compared with paired *t*-tests. Fisher's Exact test was used for categorical variables and correlations performed using Pearson's linear correlation coefficient on log-transformed data. Results were considered significant with $p < 0.05$.

Results

Baseline clinical characteristics are shown in Table 1 and were comparable in all four groups. There were more females among the chronic patients, although this was not significant, chronic vs acute ($p=0.223$), diabetic controls ($p=0.387$), non-diabetic controls ($p=0.387$). Importantly, the number of post-menopausal women was similar in all groups ($n=3, 4, 2, 2$ for acute, chronic, diabetic, and non-diabetic, respectively). Median (range) duration for acute Charcot neuroarthropathy was 32 (15–252) days compared to 730 (328–1825) days for chronic disease.

Table 1. Clinical details of patients and controls

	Non-diabetic controls ($n=10$)	Diabetic controls ($n=10$)	Acute Charcot neuroarthropathy ($n=16$)	Chronic Charcot neuroarthropathy ($n=16$)
Age (yr)	53.7 ± 12.1	55.2 ± 12.4	52.6 ± 9.8	54.8 ± 12.1
Sex	7M/3F	7M/3F	12M/4F	9M/7F
IDDM	–	3	6	4
Insulin treated	–	6	10	8
Duration diabetes (yr)	–	19.1 ± 9.9	18.2 ± 11.6	18.3 ± 11.6
HbA _{1c}	–	9.4 ± 1.9	9.0 ± 1.9	9.6 ± 2.3
BMI* (kg m^{-2})	27.4 ± 6.3	27.0 ± 5.3	28.7 ± 7.2	29.7 ± 6.1
Creatinine ($\mu\text{mol l}^{-1}$)	78.2 ± 27.0	83.7 ± 24.9	80.9 ± 32.0	76.0 ± 34.3
% with proteinuria	–	40	37.5	43.7

Results as mean \pm SD.

*Body mass index: weight in kg divided by height in m^2 .

No significant difference between any group.

Table 2. Localized foot levels of osteoclast (1CTP) and osteoblast (P1CP) markers and alkaline phosphatase in Charcot patients and controls

	Non-diabetic controls (<i>n</i> = 10)	Diabetic controls (<i>n</i> = 10)	Acute Charcot neuroarthropathy (<i>n</i> = 16)	Chronic Charcot neuroarthropathy (<i>n</i> = 16)	<i>p</i> value
1CTP ($\mu\text{g l}^{-1}$)	2.8 \pm 1.4	3.3 \pm 1.4	6.1 \pm 1.5	4.1 \pm 1.4	<0.0001
P1CP ($\mu\text{g l}^{-1}$)	90.1 \pm 1.5	93.5 \pm 2.3	112 \pm 1.5	109 \pm 1.5	0.767
Alkaline phosphatase (IU l ⁻¹)	50.8 \pm 1.2	66.7 \pm 1.5	78.7 \pm 1.4	92.5 \pm 1.4	0.032

Results as geometric mean \pm SD.

1CTP differed significantly between the four groups, $p < 0.0001$, Table 2. The acute patients had significantly higher values than the chronic, $p = 0.013$. P1CP levels showed no significant differences amongst the groups studied, $p = 0.767$, Table 2. Alkaline phosphatase also differed between groups, $p = 0.032$, although there was no difference between groups with acute and chronic Charcot disease.

In the study subgroup of 12 patients in whom simultaneous samples were taken from the foot and antecubital vein (Table 3), there was significant correlation between local (foot) and systemic levels of both 1CTP and P1CP, in both acute and chronic patients; 1CTP: acute ($r = 0.986$, $p < 0.001$), chronic ($r = 0.945$, $p < 0.001$) and P1CP: acute ($r = 0.889$, $p < 0.001$), chronic ($r = 0.878$, $p < 0.001$).

No correlation between 1CTP and P1CP was found for any group studied. Similarly no significant relationship was found between either bone marker and age, BMI, HbA_{1c}, presence of proteinuria, creatinine, and duration of diabetes in any patient group. P1CP levels did correlate with alkaline phosphatase activity in acute ($r = 0.565$, $p < 0.05$) and chronic ($r = 0.633$, $p < 0.02$) patients but not in controls with diabetes ($r = 0.192$, $p > 0.5$) or those without ($r = 0.231$, $p > 0.5$).

Discussion

In this study, measurement of 1CTP, a marker of collagen degradation, supports the idea that excessive osteoclast activity and bone resorption is a feature of the early

stages of Charcot neuroarthropathy. Serial assays of 1CTP may provide a useful means of monitoring disease activity, especially as systemically measured values seem to correspond to localized levels within the foot. Certainly in other disease states with significant bone pathology, such as post-menopausal osteoporosis,²⁴ hyperthyroidism,^{8,24} primary hyperparathyroidism,²⁵ multiple myeloma,²⁶ and prostatic carcinoma²⁷ the levels of 1CTP correlate with clinical condition and prognosis. In Paget's disease, levels have been used to follow the effect of treatment with bisphosphonates.²⁸

It is possible that elevated 1CTP is a consequence of an acute phase reaction. However, in conditions with marked inflammatory reactions such as active rheumatoid arthritis, 1CTP shows strongest correlations to the presence of destructive joint disease²⁹ and only weaker associations with other inflammatory markers of disease activity such as erythrocyte sedimentation rate (ESR) and C-reactive protein (C-RP), suggesting it reflects bone and tissue destruction, more than the acute phase response.

As 1CTP is excreted by the kidneys,¹⁰ circulating 1CTP levels may be raised once glomerular filtration rate (GFR) is reduced to less than two-thirds of normal.¹⁰ Therefore care is needed in interpreting results in the presence of renal impairment, which itself may cause elevation via renal osteodystrophy.³⁰ No patient in this study had abnormal renal function as measured by serum creatinine, although patients with proteinuria were included. Proteinuria itself, certainly of nephrotic proportions, in the presence of normal renal function does not seem to be significantly associated with bone disease^{31,32} and

Table 3. Localized (foot) and systemic 1CTP and P1CP values in acute and chronic Charcot patients

	Acute Charcot neuroarthropathy (<i>n</i> = 6)			Chronic Charcot neuroarthropathy (<i>n</i> = 6)		
	Foot	Systemic	<i>p</i> value	Foot	Systemic	<i>p</i> value
1CTP ($\mu\text{g l}^{-1}$)	9.0 \pm 3.1	9.2 \pm 2.6	> 0.5	3.8 \pm 1.3	4.0 \pm 1.5	> 0.5
P1CP ($\mu\text{g l}^{-1}$)	94 \pm 16	91 \pm 18	> 0.5	97 \pm 15	92 \pm 18	> 0.5

Results as mean \pm SD.

therefore should not affect 1CTP levels. However, one study has shown raised urinary hydroxyproline excretion in diabetic patients with microalbuminuria, reflecting changes in type 1 collagen metabolism similar to 1CTP.³³ Interestingly, excretion rates were not increased in frank proteinuria (albumin excretion rate, AER, $> 150 \mu\text{g min}^{-1}$).³³ Other studies however have failed to demonstrate a relationship between increased hydroxyproline excretion and microalbuminuria or established proteinuria.³⁴ We found no statistical correlation between the presence of proteinuria and 1CTP levels. However it is likely that using Albustix® urinalysis the majority of our patients who were positive had an AER $> 150 \mu\text{g min}^{-1}$ and therefore our results with 1CTP only confirm previous observations of no relationship between albuminuria and excess collagen metabolism.³⁴ Any potential effects on serum 1CTP levels from hyperfiltration associated with undetected early renal disease or which may be present in non-insulin-dependent diabetes^{35,36} remain unknown. P1CP is degraded by binding to mannose receptors within the reticuloendothelial system of the liver.^{37,38} All patients participating in this study had normal hepatic function. P1CP is less affected by renal insufficiency³⁹ and is not filtered by the glomerulus⁴⁰ and indeed was not related to proteinuria in our study.

Alkaline phosphatase (AP) activity, a bone formation marker, although within the normal range in all groups, was significantly higher in the Charcot patients compared to controls, whereas P1CP levels were similar. It is believed however that AP may be related mainly to the calcification process and may not reflect bone matrix or collagen synthesis.⁴¹ Disease-specific discrepancies between AP and P1CP have been noted in other metabolic bone conditions and may reflect differences in the production rates of the various formative markers at the level of individual osteoblasts in the differing pathologies.¹⁷

Clinically it is often difficult to distinguish between Charcot neuroarthropathy and osteomyelitis. It is unlikely that these markers will be helpful in this respect, as increased 1CTP is seen in an experimental model of canine osteomyelitis.⁴² Similarly, urinary excretion of hydroxypyridinium crosslinks, which also reflects type 1 collagen degradation, was equally elevated in both osteomyelitis and Charcot neuroarthropathy in a small pilot study.⁴³ However, once the diagnosis is established, 1CTP may prove beneficial in monitoring disease activity and in assessing the effects of therapeutic intervention with agents such as the bisphosphonates.⁴⁴ Practically, 1CTP and P1CP offer advantages over other biochemical markers, such as osteocalcin (an osteoblast marker), in that they are stable in sera following repeated thawing and freezing of specimens or if stored at room temperature for up to 15 days.¹⁴ Unlike other measures of bone turnover, such as urinary hydroxyproline, the contribution from non-skeletal sources³⁸ and diet¹⁶ is negligible.

We noted with interest that foot and systemic levels

of 1CTP were raised in acute Charcot neuroarthropathy patients. These patients had no clinical evidence of generalized bone disease to account for the systemic rise, and we therefore conclude that the foot and systemic levels are both related to the Charcot foot. This may be of practical importance in that routine arm venepuncture may be sufficient to follow disease activity.

In conclusion our data with 1CTP and P1CP suggest that the acute Charcot foot demonstrates excess bone matrix resorption as a result of increased osteoclastic activity, without concomitant increase in osteoblast synthetic function. Therefore bone resorption and formation which are normally 'coupled', to maintain the integrity of the skeleton, have become 'uncoupled'. This may be important in the pathogenesis of diabetic Charcot neuroarthropathy.

References

1. Cavanagh PR, Young MJ, Adams JE, Vickers KL, Boulton AJM. Bony abnormalities in the feet of neuropathic diabetic patients (oral presentation). In: Bakker K, ed. *The Diabetic Foot: First International Symposium and Workshop*. Amsterdam: Excerpta Medica, 1991: 5.
2. Cofield RH, Morrison MJ, Beabout JW. Diabetic neuroarthropathy in the foot: patient characteristics and patterns of radiographic change. *Foot Ankle* 1983; **4**: 15–22.
3. Bruckner FE, Howell A. Neuropathic joints. *Semin Arthritis Rheum* 1972; **2**: 47–49.
4. Johnson JTH. Neuropathic fractures and joint injuries. Pathogenesis and rationale of prevention and treatment. *J Bone Joint Surg* 1967; **49A**: 1–30.
5. Slowman-Kovacs SD, Braunstein EM, Brandt KD. Rapidly progressive Charcot arthropathy following minor joint trauma in patients with diabetic neuropathy. *Arthritis Rheum* 1990; **33**: 412–417.
6. Brower AC, Allman RM. Pathogenesis of the neurotrophic joint: neurotraumatic vs neurovascular. *Radiology* 1981; **139**: 349–354.
7. Edmonds ME, Clarke MB, Newton S, Barrett J, Watkins PJ. Increased uptake of bone radiopharmaceutical in diabetic neuropathy. *Q J Med* 1985; **57**: 843–855.
8. Johnson LC, Williams LE, Blard JH. Cervical spine subluxations and massive osteolysis in the upper extremities in rheumatoid arthritis. *Arthritis Rheum* 1966; **9**: 348.
9. Brooks AP. The neuropathic foot in diabetes. Part II: Charcot's neuroarthropathy. *Diabetic Med* 1986; **3**: 116–118.
10. Risteli J, Elomaa I, Niemi S, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type 1 collagen: a new serum marker of bone collagen degradation. *Clin Chem* 1993; **39**: 635–640.
11. Eriksen EF, Charles P, Melsen F, Mosekilde L, Risteli L, Risteli J. Serum markers of type 1 collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. *J Bone Miner Res* 1993; **8**: 127–132.
12. Eriksen EF, Charles P, Mosekilde L, Risteli L, Risteli J. Cross-linked carboxy-terminal telopeptide of type 1 collagen in serum: a new bone resorption marker. *J Bone Miner Res* 1991; **6** (suppl 1): S243.
13. Charles P, Mosekilde L, Risteli L, Risteli J, Eriksen EF. Assessment of bone remodelling using biochemical

- indicators of type 1 collagen synthesis and degradation: relation to calcium kinetics. *Bone Miner* 1994; **24**: 81–94.
14. Melkko J, Niemi S, Risteli L, Risteli J. Radioimmunoassay of the carboxy-terminal propeptide of human type 1 procollagen. *Clin Chem* 1990; **36**: 1328–1332.
 15. Pedersen BJ, Bonde M. Purification of human procollagen type 1 carboxy-terminal propeptide cleaved as *in vivo* from procollagen and used to calibrate a radioimmunoassay of the propeptide. *Clin Chem* 1994; **40**: 811–816.
 16. Robins SP. Biochemical markers for assessing skeletal growth. *Eur J Clin Nutr* 1994; **28** (Suppl 1): S199–S209.
 17. Charles P, Hasling C, Risteli L, Risteli J, Mosekilde L, Eriksen EF. Assessment of bone formation by biochemical markers in metabolic bone disease: separation between osteoblastic activity at the cell and tissue level. *Calcif Tissue Int* 1992; **51**: 406–411.
 18. Sanders LJ, Murray-Leisure K. Infections of the diabetic foot. In: Abramson C, McCarthy, eds. *Infectious Diseases of the Lower Extremity*. Baltimore: Williams and Wilkins, 1991: 193–211.
 19. Eymott MJ, Alavi A, Dalinka MK, Kyle GC. Bone scintigraphy in diabetic osteoarthropathy. *Radiology* 1981; **140**: 455–475.
 20. Hetherington VJ. Technetium and combined gallium and technetium scans in the neuropathic foot. *J Amer Podiat Assoc* 1982; **72**: 458–463.
 21. Seabold JE, Flickinger FW, Kao SC, Gleason TJ, Kahn D, Nepola JV, Marsh JL. Indium-111 leucocyte/technetium-99m-MDP bone and magnetic resonance imaging; difficulty in diagnosing osteomyelitis in patients with neuropathic osteoarthropathy. *J Nucl Med* 1990; **31**: 549–556.
 22. Yuh WTC, Corson JD, Baraniewski HM, Rezai K, Shamma AR, Kathol MH, et al. Osteomyelitis of the foot in diabetic patients. Evaluation with plain films, 99m Tc-MDP bone scintigraphy and MR imaging. *AJR* 1989; **152**: 795–800.
 23. Beltran J, Scott Campanini D, Knight C, McCalla M. The diabetic foot: magnetic resonance imaging evaluation. *Skel Radiol* 1990; **19**: 37–41.
 24. Ebeling PR, Peterson JM, Riggs BL. Utility of type 1 procollagen propeptide assays for assessing abnormalities in metabolic bone diseases. *J Bone Miner Res* 1992; **7**: 1243–1250.
 25. De la Piedra C, Diaz-Martin MA, Diaz-Diego EM, Lopez-Gavilanes E, Gonzalez-Parra E, Caramelo C, et al. Serum concentrations of carboxy-terminal cross-linked telopeptide of type 1 collagen (1CTP), serum tartrate resistant acid phosphatase, and serum levels of intact parathyroid hormone in parathyroid hyperfunction. *Scand J Clin Lab Invest* 1994; **54**: 11–15.
 26. Elomaa I, Virkkunen P, Risteli L, Risteli J. Serum concentrations of the cross-linked carboxy-terminal telopeptide of type 1 collagen (1CTP) is a useful prognostic indicator in multiple myeloma. *Br J Cancer* 1992; **66**: 337–341.
 27. Kylmala T, Tammela TL, Risteli L, Risteli J, Kontturi M, Elomaa I. Type 1 collagen degradation product (1CTP) gives information about the nature of bone metastases and has prognostic value in prostate cancer. *Br J Cancer* 1995; **71**: 1061–1064.
 28. Filipponi P, Pedetti M, Beghe F, Giovagnini B, Miam M, Cristallini S. Effects of two different bisphosphonates on Paget's disease of bone: 1CTP assessed. *Bone* 1994; **15**: 261–267.
 29. Hakala M, Risteli L, Manelius J, Nieminen P, Risteli J. Increase type 1 collagen degradation correlates with disease severity in rheumatoid arthritis. *Ann Rheum Dis* 1993; **52**: 866–869.
 30. Mazzaferro S, Pasquali M, Ballanti P, Bonucci E, Costantini S, Chicca S, De-Meo S, et al. Diagnostic value of serum peptides of collagen synthesis and degradation in dialysis renal osteodystrophy. *Nephrol Dial Transplant* 1995; **10**: 52–58.
 31. Tessitore N, Bonucci E, D'Angelo A, Lund B, Valvo E, Lupo A, Loschiavo C, et al. Bone histology and calcium metabolism in patients with nephrotic syndrome and normal or reduced renal function. *Nephron* 1984; **37**: 153–159.
 32. Korkor A, Schwartz J, Bergfield M, Teitelbaum S, Avioli L, Klahr S, Slatopolsky E. Absence of metabolic bone disease in adult patients with nephrotic syndrome and normal renal function. *J Clin Endocrinol Metab* 1983; **56**: 496–500.
 33. Selby PL, Shearing PA, Marshall SM. Hydroxyproline excretion is increased in diabetes mellitus and related to the presence of microalbuminuria. *Diabetic Med* 1995; **12**: 240–243.
 34. Olmos JM, Perez-Castrillon JL, Garcia MT, Garrido JC, Amado JA, Gonzalez-Macias. Bone densitometry and biochemical bone remodelling markers in type 1 diabetes mellitus. *Bone Miner* 1994; **26**: 1–8.
 35. Silveiro SP, Friedman R, Gross JL. Glomerular hyperfiltration in NIDDM without overt proteinuria. *Diabetes Care* 1993; **16**: 115–119.
 36. Vora JP, Doblen J, Dean JD, Thomas D, Williams JD, Owens DR, Peters JR. Renal haemodynamics in newly presenting non-insulin-dependent diabetes mellitus. *Kidney Int* 1992; **41**: 829–835.
 37. Smedsrod B, Melkko J, Risteli L, Risteli J. Circulating C-terminal propeptide of type 1 procollagen is cleared mainly via the mannose receptor in liver endothelial cells. *Biochem J* 1990; **271**: 345–350.
 38. Jensen LT, Olesen HP, Risteli J, Lorenzen I. External thoracic duct-venous shunt in conscious pigs for long-term studies of connective tissue metabolites in lymph. *Lab Anim Sci* 1990; **40**: 620–624.
 39. Hamdy NA, Risteli J, Risteli L, Harris S, Beneton MN, Brown CB, Kanis JA. Serum type 1 procollagen peptide: a non-invasive index of bone formation in patients on haemodialysis? *Nephrol Dial Transplant* 1994; **9**: 511–516.
 40. Fleischmajer R, Timpl R, Tuderman L, Raisher L, Wiestner M, Perlsh JS, et al. Ultrastructural identification of extension aminopeptides of type I and type III procollagens in human skin. *Proc Natl Acad Sci USA* 1981; **78**: 7360–7364.
 41. Robey PG, Fisher LW, Young MF, Termine JD. The biochemistry of bone. In: Riggs BL, Melton III LJ, eds. *Osteoporosis*. New York: Raven Press, 1988: 95–109.
 42. Philipov JP, Pascalev MD, Aminkov BY, Grosev CD. Changes in serum carboxy-terminal telopeptide of type 1 collagen in an experimental model of canine osteomyelitis. *Calcif Tissue Int* 1995; **57**: 152–154.
 43. Edelson GW, Jensen JL, Kaczynski R. Comparison of urinary hydroxypyridinium crosslinks in diabetics with Charcot foot disease versus osteomyelitis (Abstract). *Diabetes* 1996; **45** (suppl 2): 108A.
 44. Selby PL, Young MJ, Boulton AJM. Bisphosphonates — a new treatment for diabetic Charcot neuroarthropathy? *Diabetic Med* 1994; **11**: 28–31.