Alterations of bone and mineral metabolism in diabetes mellitus

Part I. An overview

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Summary

A critical review of the literature leads to the conclusion that alterations of bone and mineral metabolism occur both in diabetic patients and in animals with experimentally induced insulin deficiency syndromes. The coexistence of juvenile insulin-dependent diabetes mellitus (type I) and radiological evidence of decreased bone mass (osteopenia) appears to be firmly established. Available data support the view that these patients have an increased propensity to skeletal fracture. Adult-onset, non-insulin-dependent diabetic populations, more heterogeneous as regards the type of diabetes, the therapy and the presence of complications or coexistent disease, are characterised by subpopulations with either a decreased, a normal or an increased bone mass. The pathogenesis of diabetic osteopenia is multifactorial. Data obtained from studies employing appropriate animal models of chronic insulin deficiency indicate that various metabolic and hormonal abnormalities may be involved.

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Despite the wealth of information available concerning the variety of systemic complications known to attend the chronic diabetic state, little is known about the effect of this disease on the metabolism of minerals and the integrity of bone. In recent years it has, however, become apparent that involvement of the skeletal system must be regarded as yet another complication of diabetes, both in man and in animals with experimentally induced insulin deficiency syndromes.

The earliest effect of the diabetic environment on bone is seen in the increased prevalence of skeletal malformations in the fetuses of diabetic mothers. Hypoplasia or deformities of the extremities, dislocation of the hips and agenesis of the sacrum or lumbar spine occur 3 - 5 times as frequently among these infants as among non-diabetic controls.[1] Both genetic and environmental metabolic factors are thought to contribute to the development of these skeletal abnormalities. The second category of bony abnormalities known to occur in those with diabetes results from the continuing trauma following diabetic neuropathy. This diabetic osteopathy is characterised by focal osteolysis, bone fragmentation, sclerosis, peri-osteal new bone formation and Charcot's neurogenic arthropathy, and is usually evident in the small bones of the foot and less frequently involves the knees, upper extremities or vertebrae.[2] It is, however, the role of diabetes as the cause of a metabolic bone disease resulting in a generalised decrease in bone mass (osteopenia) that has attracted much attention recently.

It is not surprising that alterations of bone mass in diabetic populations have been virtually ignored in the past, since other complications of the disease are usually more manifest clinically. Moreover, conventional radiographic evaluation of bone mass is most insensitive, since 30 - 50% of the skeletal mass must be lost before skeletal rarefaction can be detected by routine radiography.[3] Utilising more sensitive detection devices, altered bone mass in diabetic patients is documented with increasing frequency. However, the prevalence and significance of these alterations, their pathogenesis, natural evolution and response to therapy remain ill defined.

Alterations of bone mass in diabetic populations

As early as 1927 Morrison and Bogan[4] documented decreased skeletal mass and bone development in children with diabetes of long standing. In 1934 several cases of diabetes associated with vertebral crush fractures were reported from the Joslin clinic.[5] Albright and Reifenstein[6] confirmed these findings and Hernberg,[7] evaluating postmortem specimens of bone, reported in 1952 that osteoporosis was much more severe in young adults with diabetes. Subsequently, Berney[8] and others[9-13] re-emphasised the coexistence of diabetes and radiological evidence of decreased bone mass. In 1970 Jurist,[14] employing resonant frequency analysis, reported that elderly diabetic females had decreased skeletal strength compared with age-matched controls, and in 1972 von Neumann[15] documented diabetes in 23% of patients with vertebral crush fractures. Similar observations were reported in a large epidemiological study from Israel.[16]

In contrast, Meema and Meema[17] concluded that 'involutional osteoporosis' was *less* prevalent among 63 elderly (average age 78 years), institutionalised women with maturity-onset diabetes. Diabetic subjects in this heterogeneous study group had significantly higher body weights than an age-matched, non-diabetic control population. Similar findings were documented by a Hungarian survey of elderly diabetic patients.[18]

These conflicting findings were all based on the routine radiographic diagnosis of 'osteoporosis', which can be made only after advanced loss of bone mineral. Moreover, heterogeneous diabetic populations were usually studied. The development, by Cameron *et al.*[19] of an accurate method of determining bone mass *in vivo* by the photon absorption technique, greatly facilitated the evaluation of demineralising disorders. The evaluation of bone mass employing radiogrammetry[20] and total body neutron activation analysis[21] lent further accuracy and precision to the diagnosis of osteopenia.

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Applying photon absorptiometry techniques to 57 insulin-requiring diabetic patients, aged 50 years or less, Ringe *et al.*[22] noted that 31% of the females and 48% of the males (the male population was older) had a pathologically reduced bone mineral content. A positive correlation was also documented between osteopenia and the duration of diabetes, retinopathy and polyneuropathy. Using photon absorption densitometry Levin *et al.*[23] drew similar conclusions from a study of 35 insulin-treated juvenile-onset diabetics. A significant reduction in forearm trabecular mass was noted in 54% of patients (all under 31 years of age), with cortical bone loss greater in patients under 21 years of age. Bone loss was also detected in more than half of 101 non-insulin-dependent diabetics. Bone loss and the duration of diabetes did not correlate. The greatest decrease in bone mass was observed in the patients receiving oral agents and the decrease was already present in subjects with diabetes of less than 5 years' duration. The average loss of bone mass was greater in women (15%) than in men (8%). Eight of the adult-onset diabetic patients, however, demonstrated an increased bone mass. Densitometric measurements of forearm bone mass by De Leeuw and Abs[24] in 138 non-insulin-dependent diabetic subjects clearly revealed the existence of two diabetic populations: approximately 20% of patients had more than 10% bone loss; and a larger group, characterised by higher body weights, had an increased bone mass.

Until 1977 the majority of studies were limited primarily to adult diabetic populations. In 1977 Santiago *et al.*[25] noted that 25% of 107 insulin-dependent diabetic children (aged 4 - 18 years) had metacarpal cortical thickness values below the 5% limit for normal children. The bone loss was more common in boys and was unrelated to the duration of the diabetes. Rosenbloom *et al.*,[26] quantitating forearm bone mass by photon absorptiometry in 196 insulin-dependent diabetics aged 7 - 21 years, reported subnormal values in 48% of white females, 29% of white males and 19% of black diabetics. No significant correlation was noted between bone mass and insulin dose or physical activity. The bone loss was more severe in those individuals with the shortest duration of diabetes and significantly less severe after 5 years of treatment. Using similar techniques McNair *et al.*[27] recently reported their findings in 215 insulin-treated diabetics. As a group, the patients (aged 7 - 70 years) demonstrated a bone mineral deficit of 9,8% compared with sex- and age-matched controls. A comparison between patients who had developed diabetes before the age of 20 years and after 25 years, revealed a bone loss of 14% and 7% respectively, with no sex differences noted. These authors observed that the initiation of osteopenia coincided with the onset of clinical diabetes, and that the bone mass appeared to stabilise after 3 - 5 years — conclusions consistent with the earlier observations of Levin *et al.*,[23] Rosenbloom *et al.*,[26] and Santiago *et al.*[25]

Clinical significance of bone mass changes in diabetes

The pathogenesis of skeletal fracture is complex and undoubtedly multifactorial. While the amount of bone present is an important determinant, other factors such as the quality of bone, residual muscle strength and neuromuscular co-ordination are also involved in a subject's predisposition to fracture; changes in bone distribution, remodelling and turnover may also contribute significantly.[28] A reduction in bone turnover increases mean skeletal age. Furthermore, since the repair of naturally occurring microfractures depends on the normal remodelling process, a marked reduction in bone turnover would necessarily impair the repair mechanism. This relationship between repair and remodelling may account, in part, for the increased propensity to fracture associated with certain states of low bone turnover, such as that encountered in glucocorticoid excess syndromes.[29]

With regard to the relationship between the documented bone rarefaction in diabetic subjects and their predisposition to fractures, estimates vary between a two- to sixfold increased prevalence of hip and/or femoral neck fractures when compared with age-matched controls.[8-11,16] Moreover, fracture healing has been shown to be delayed in diabetic subjects and is presumed to result from the associated small-vessel disease, neuropathy and/or protein deficiency.[30] These reports of an increased propensity to fracture in diabetic subjects should be contrasted with a recent report by Heath *et al.*[31] who failed to reveal an increased fracture rate in a randomly selected, predominantly adult-onset diabetic population. This is not too surprising if we acknowledge previous reports that a given adult diabetic population comprises a mixture of patients, approximately 20% with decreased, but also many with increased bone mass. Heath *et al.*'s results may simply indicate that the 80% of diabetics who were not osteopenic had a disproportionately lower number of fractures.

Pathogenesis of diabetic osteopenia

In as complex a metabolic derangement as diabetes, the genesis of the documented decrease in bone mass could relate to alterations in various organ systems. The diabetic state could conceivably alter the integrity of bone directly. On the other hand, its adverse influence on the skeleton could also be mediated indirectly through primary alterations of mineral balance, vitamin D homeostasis or circulating levels of parathyroid hormone (PTH). Fig. 1 depicts those derangements known to attend the diabetic state which have to be considered potentially harmful to skeletal homeostasis.

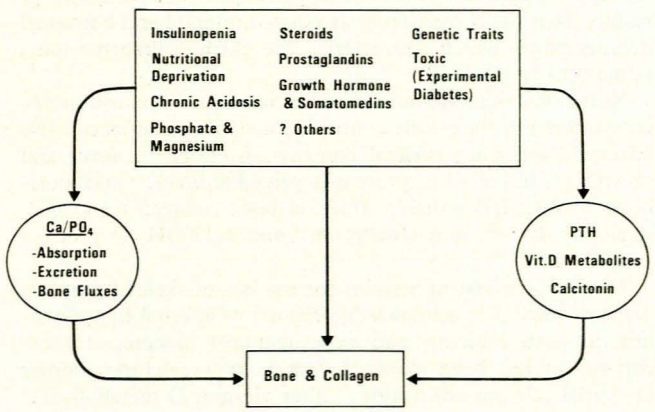

Fig. 1. Pathophysiological mechanisms considered to be involved in the development of diabetic osteopenia.

Insulin deficiency may well prove to be a key factor in the development of diabetic osteopenia. Insulin added to *in vitro* bone culture systems stimulates amino acid uptake[32] and enhances bone collagen synthesis.[33] Moreover, insulin has been shown to stimulate bone resorption *in vitro*,[34] to enhance the synthesis of chondroitin sulphate in costal cartilage slices[35] and to increase mesenchymal cell proliferation and endochondral bone growth.[36] Two models of experimental diabetes have generally been employed to study the influence of insulin deficiency on bone and mineral metabolism *in vivo*. The rat with short-term (5 - 12-day) insulinopenia is characterised by impaired intestinal absorption of calcium, which has been ascribed to low levels of circulating 1,25 dihydroxyvitamin D

($1,25(OH)_2D$) and duodenal calcium-binding protein.[37,38] Decreased calcium absorption in the rat with short-term diabetes results in a negative calcium balance, and is attended by a decrease in serum calcium, an elevation in circulating immunoreactive PTH and a diminished calcitonin (CT) response.[37-39] Apparent compensation for calcium malabsorption by the mobilisation of bone calcium through secondary hyperparathyroidism has thus been suggested. These abnormalities have been implicated in the genesis of diabetic osteopenia.

Utilising an experimental rat model of chronic (7-week) streptozotocin-induced diabetes, we[40-46] and others[47] were unable to document any evidence of osteitis fibrosa or of osteomalacia. Instead these chronically diabetic animals were characterised by a pronounced decrease in bone turnover; findings reminiscent of those in human diabetic patients reported by Landeros and Frost,[48] who noted decreased quantities of newly formed bone matrix and delayed osteon closure rates. Moreover, in contrast with the rat with short-term diabetes, intestinal absorption of calcium was found to be markedly enhanced in chronically insulin-deficient animals, resulting in a positive calcium balance despite severe hypercalciuria, and an appropriate suppression of both plasma PTH and urinary 3′5′-cyclic adenosine monophosphate (cAMP), and a marked elevation of circulating CT and $24,25(OH)_2D$.[40-44] Intestinal hyperabsorption of calcium in these animals was attended by a striking increase in the activity of an insulin-sensitive, small-intestinal alkaline phosphatase iso-enzyme[42] and occurred despite a paucity of circulating $1,25(OH)_2D$ and hypercorticosteronism, known to impair calcium absorption.[41]

Insulin therapy corrected intestinal hyperabsorption of calcium, hyperphosphatasaemia, renal calcium wasting, and decreased plasma PTH and increased plasma CT in the chronically diabetic rat.[40-42] Moreover, insulin treatment has been shown to normalise all histometric and biochemical parameters of bone turnover and growth.[40] Whether this response to therapy reflects a direct insulin effect on skeletal tissue or results from the correction of associated mineral and hormonal derangements which characterise the chronic diabetic state, remains to be resolved.

Nutritional deprivation and keto-acidosis, still too commonly encountered in the poorly controlled juvenile diabetic, can also adversely influence skeletal homeostasis. Bone turnover and growth are impaired in protein-deprived animals,[49] and metabolic acidosis has a direct effect on bone collagen turnover,[50] renal tubular calcium absorption[51] and $1,25(OH)_2D$ production.[52]

Theories derived to account for the loss of skeletal tissue in diabetes must also acknowledge reports of adrenal hyperfunction in both human[53] and experimental[54] diabetes. Hypercortisolism has been shown to cause hypercalciuria, impair intestinal calcium absorption,[55] alter vitamin D metabolism,[56] and directly suppress osteoblastic[57] and osteoclastic[58] activities.

It has been suggested that phosphorus and magnesium depletion, secondary to urinary losses of these minerals in poorly controlled diabetes, may contribute to the development of diabetic bone disease.[59] Phosphorus metabolism plays an integral role in bone mineralisation and also influences bone matrix synthesis,[60] intestinal calcium absorption[52] and renal vitamin D metabolism.[61] The direct effect of magnesium on bone is less well defined; however, a decreased secretion and/or synthesis of PTH,[62] and a blunted skeletal responsiveness to PTH[63] are well documented in magnesium deficiency.

Recent reports of increased plasma prostaglandins,[64] potent stimulators of bone resorption, and reduced levels of circulating somatomedins[65] in diabetes, also have to be acknowledged as potential aetiological factors in the genesis of diabetic bone disease. Finally, cognisance should be taken of the fact that skeletal abnormalities in diabetes may merely reflect the basic underlying disease and need not necessarily bear any relation to the metabolic and hormonal derangements known to attend this disorder. *In vitro* studies on the replicative lifespan of fibroblast cultures suggest that the diabetic state is associated with cellular characteristics of ageing.[66] Similar phenomena may involve other cells, including bone cells.

Conclusions

Alterations of mineral metabolism and bone morphology in diabetes mellitus have been amply documented. Apparently conflicting data can often be resolved by closer inspection of diabetic patient populations and differences in animal models of this disease. Critical analysis of available data allows us to arrive at the following conclusions:

1. A reduced bone mass in patients with diabetes has been documented by employing various techniques including conventional radiography,[4-12,16] radiogrammetry of cortical width,[25] photon absorption densitometry,[22-24,26,27] resonant frequency of the ulna,[14] and total body neutron activation analysis.

2. The coexistence of juvenile insulin-dependent (type I) diabetes and osteopenia appears to be firmly established.

3. Adult-onset non-insulin-dependent (type II) diabetic populations, more heterogeneous as regards type of diabetes, the therapy and the presence of complications or coexistent disease, are characterised by subpopulations with either decreased, normal or increased bone mass; the latter appears to be most prevalent among obese, elderly female subjects.[17,24]

4. The clinical significance of these alterations of bone mass in diabetic patients remains somewhat controversial. While many studies[8-10,15,16,67] have clearly implicated diabetes as a common and important aetiological factor in the development of skeletal fractures, others[31] have failed to reveal an increased fracture rate among diabetic populations as a whole. This is not surprising if we acknowledge previous reports[23,24] that a given adult diabetic population comprises a mixture of patients some with a decreased, but also many with an increased bone mass. Well-planned, prospective, longitudinal studies of clearly defined diabetic subpopulations are, in the view of the author, the only way to resolve this important issue.

5. The pathogenesis of diabetic osteopenia is unknown; theoretical consideration of potential aetiological factors indicates that various metabolic, hormonal and/or genetic influences may be involved. Data obtained from studies employing appropriate animal models of chronic insulin deficiency have shown that diabetic osteopenia represents a form of low-turnover osteoporosis, the genesis of which not only involves a direct insulin effect on skeletal tissue, but also relates to alterations in mineral, PTH, adrenocortical and vitamin D metabolism.

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REFERENCES

1. Pedersen J. *The Pregnant Diabetic and Her Newborn: Problems and Management.* Baltimore: Williams & Wilkins, 1967: 60-69.
2. Levin ME, Boisseau VC, Avioli LV. Effects of diabetes mellitus on bone mass in juvenile and adult onset diabetes. *N Engl J Med* 1976; **294:** 241-245.
3. Wray JB, Surgarman ED, Schneider AJ. Bone composition in senile osteoporosis. *JAMA* 1963; **182:** 118-124.
4. Morrison LB, Bogan IK. Bone development in diabetic children: a roentgen study. *Am J Med Sci* 1927; **174:** 313-319.
5. Root HF, White P, Marble A. Abnormalities of calcium deposition in diabetes mellitus. *Arch Intern Med* 1934; **53:** 46-52.
6. Albright F, Reifenstein EC, eds. *Parathyroid Glands and Metabolic Bone Disease: Selected Studies.* Baltimore: Williams & Wilkins, 1948: 145-204.
7. Hernberg CA. Skelettveränderungen bei Diabetes mellitus der Erwachsenen. *Acta Med Scand* 1952; **143:** 1-14.

8. Berney PW. Osteoporosis and diabetes mellitus. *J Iowa State Med Soc* 1952; **42:** 10-12.
9. Heuck F, Schmidt E. Zur Osteoporose bei Diabetes mellitus. *Verh Dtsch Ges Inn Med* 1956; **62:** 464-467.
10. Alffram PA. An epidemiologic study of cervical and trochanteric fractures of the femur in an urban population: analysis of 1664 cases with special reference to etiologic factors. *Acta Orthop Scand* 1964; **65:** 75-77.
11. Kuhlencordt H, Wieners H, Gocke H. Skelettuntersuchungen bei Diabetikern bis zum 45 Lebensjahr. *Dtsch Med Wochenschr* 1966; **91:** 1913-1916.
12. Fischer E, Hausser D. Kompaktadicke von Rippen und Schlüsselbein: Einfluss demineralisierender Erkrankungen. *Med Klin* 1970; **65:** 1212-1216.
13. Morgano G. Osteopatie diabetiche ed osteopatie senili. *G Gerontol* 1970; **18:** 379-384.
14. Jurist JM. *In vivo* determination of the elastic response of bone: II. Ulnar resonant frequency in osteoporotic, diabetic and normal subjects. *Phys Med Biol* 1970; **15:** 427-434.
15. Von Neumann HW, Arnold WD, Hein W. Das Vorkommen eines Diabetes mellitus bei 201 Patienten mit Schenkelhalsfrakturen und Wirbelkörperkompressionfrakturen. *Zentralbl Chir* 1972; **97:** 831-836.
16. Menczel J, Makin M, Robin G, Jaye I, Naor E. Prevalence of diabetes mellitus in Jerusalem: its association with presenile osteoporosis. *Israel J Med Sci* 1972; **8:** 918-919.
17. Meema HE, Meema S. The relationship of diabetes mellitus and body weight to osteoporosis in elderly females. *Can Med Assoc J* 1967; **96:** 132-139.
18. Forgacs S, Rosinger A, Vertes L. Diabetes mellitus and osteoporosis. *Endokrinologie* 1976; **67:** 343-350.
19. Cameron JR, Mazess RB, Sorenson JA. Precision and accuracy of bone mineral determination by direct photon absorptiometry. *Invest Radiol* 1968; **3:** 141-150.
20. Garn SM, Poznanski A, Larson K. Metacarpal lengths, cortical diameters and areas from the 10-state nutrition survey. In: Jaworski ZFG, ed. *Proceedings of the First Workshop on Bone Morphometry*. Ottawa: University of Ottawa Press, 1973: 367-391.
21. Cohn SH, Shukla KK, Ellis KJ. A multivariate predictor for total body calcium in man based on activation analysis. *Int J Nucl Med Biol* 1974; **1:** 131-134.
22. Ringe JD, Kuhlencordt F, Kruse HP. Bone mineral determinations on long-term diabetics. *AJR* 1976; **126:** 1300-1301.
23. Levin ME, Boisseau VC, Avioli LV. Effects of diabetes mellitus on bone mass in juvenile and adult onset diabetes. *N Engl J Med* 1976; **294:** 241-245.
24. De Leeuw I, Abs R. Bone mass and bone density in maturity-type diabetes measured by the ^{125}I photon absorption technique. *Diabetes* 1977; **26:** 1130-1135.
25. Santiago JV, McAllister WH, Ratzak SK *et al.* Decreased cortical thickness and osteopenia in children with diabetes mellitus. *J Clin Endocrinol Metab* 1977; **45:** 845-847.
26. Rosenbloom AL, Lezotte DC, Weber FT *et al.* Diminution of bone mass in childhood diabetes. *Diabetes* 1977; **26:** 1052-1055.
27. McNair P, Madsbad S, Christensen MS, Faber OK, Binder C, Transbøl I. Bone mineral loss in insulin-treated diabetes mellitus: studies on pathogenesis. *Acta Endocrinol* 1979; **90:** 463-469.
28. Christiansen C, Arnaud CD, Nordin BEC, Parfitt AM, Peck WA, Riggs BL, eds. Proceedings of the Copenhagen International Symposium on Osteoporosis. June 3 - 8, 1984.
29. Hough S, Teitelbaum SL, Bergfeld MA, Avioli LV. Isolated skeletal involvement in Cushing's syndrome: response to therapy. *J Clin Endocrinol Metab* 1981; **52:** 1033-1038.
30. Cozen L. Does diabetes delay fracture healing? *Clin Orthop* 1972; **82:** 134-136.
31. Heath H, Melton LJ, Chu CP. Diabetes mellitus and risk of skeletal fracture. *N Engl J Med* 1980; **303:** 567-570.
32. Hahn TJ, Downing SJ, Phang JM. Insulin effect on amino acid transport in bone: dependence on protein synthesis and Na^+. *Am J Physiol* 1971; **220:** 1717-1723.
33. Wettenhall REH, Schwartz PL, Bornstein J. Actions of insulin and growth hormone on collagen and chondroitin sulfate synthesis in bone organ cultures. *Diabetes* 1969; **18:** 280-285.
34. Puche RC, Romano MC, Locatto ME, Ferretti JL. The effects of insulin on bone resorption. *Calcif Tissue Res* 1973; **12:** 8-15.
35. Peck WA, Messinger K. Nucleoside and ribonucleic acid metabolism in isolated bone cells: effects of insulin and cortisol *in vitro*. *J Biol Chem* 1970; **245:** 2722-2729.
36. Weiss RE, Reddi H. Influence of experimental diabetes and insulin on matrix-induced cartilage and bone differentiation. *Am J Physiol* 1980; **238:** E200-E207.
37. Schneider LE, Schedl HP. Diabetes and intestinal calcium absorption in the rat. *Am J Physiol* 1972; **223:** 1319-1323.
38. Schneider LE, Nowosielski LM, Schedl HP. Insulin treatment of diabetic rats: effects on duodenal calcium absorption. *Endocrinology* 1977; **100:** 67-73.
39. Schedl HP, Heath H, Wenger J. Serum calcitonin and parathyroid hormone in experimental diabetes: effects of insulin treatment. *Endocrinology* 1978; **103:** 1368-1373.
40. Hough S, Avioli LV, Bergfeld MA, Fallon MD, Slatopolsky E, Teitelbaum SL. Correction of abnormal bone and mineral metabolism in chronic streptozotocin-induced diabetes mellitus in the rat by insulin therapy. *Endocrinology* 1981; **108:** 2228-2233.
41. Hough S, Russell JE, Teitelbaum SL, Avioli LV. Calcium homeostasis in chronic streptozotocin-induced diabetes mellitus in the rat. *Am J Physiol* 1982; **242:** E451-E456.
42. Hough S, Avioli LV, Teitelbaum SL, Fallon MD. Alkaline phosphatase activity in chronic streptozotocin-induced insulin deficiency in the rat: effect of insulin replacement. *Metabolism* 1981; **30:** 1190-1194.
43. Hough S, Slatopolsky E, Avioli LV. Hormonal alterations in experimental diabetes: role of a primary disturbance in calcium homeostasis. *Calcif Tissue Int* 1983; **35:** 615-619.
44. Hough S, Fausto A, Sonn Y, Dong Jo OK, Birge SJ, Avioli LV. Vitamin D metabolism in the chronic streptozotocin-induced diabetic rat. *Endocrinology* 1983; **113:** 790-796.
45. Hough S, Russell JE, Teitelbaum SL, Avioli LV. Regulation of epiphyseal cartilage metabolism and morphology in the chronic diabetic rat. *Calcif Tissue Int* 1983; **35:** 115-121.
46. Shires R, Teitelbaum SL, Bergfeld MA, Fallon MD, Slatopolsky E, Avioli LV. Effect of streptozotocin-induced chronic diabetes mellitus on bone and mineral homeostasis in rats. *J Lab Clin Med* 1981; **97:** 231-235.
47. Hammer WS, Soni NN, Fraleigh CM. Quantitative study of bone activity in the diabetic rat mandible: triple fluorochrome study. *Oral Surg* 1973; **35:** 718-729.
48. Landeros O, Frost HF. Radial rate of osteon closure measured by means of tetracycline labelling. *Henry Ford Hosp Med Bull* 1964; **12:** 499-505.
49. LeRoith D, Pimstone BL. Bone metabolism and composition in the protein deprived rat. *Clin Sci* 1973; **44:** 305-319.
50. Finlayson GR, Smith JG, Moore MJ. Effects of chronic acidosis on connective tissue. *JAMA* 1964; **187:** 659-662.
51. Lemann J, Litzow JR, Lennon EJ. Studies on the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. *J Clin Invest* 1967; **46:** 1318-1328.
52. Lee SW, Russell JE, Avioli LV. 25-Hydroxycholecalciferol to 1,25-dihydroxycholecalciferol: conversion impaired by systemic metabolic acidosis. *Science* 1977; **195:** 994-996.
53. Stancàková A, Merstenová E, Vajó J, Valcová M. The excretion of free cortisol, cortisone, cortisol sulfate and cortisone sulfate in peripheral vascular disease, diabetes mellitus and hyperthyroidism. *Horm Metab Res* 1978; **10:** 539-544.
54. De Nicola AF, Fridman O, Del Castillo EJ, Voglia VG. Abnormal regulation of adrenal function in rats with streptozotocin diabetes. *Horm Metab Res* 1977; **9:** 469-473.
55. Kimberg DV, Baerg RD, Gershon E, Grandusius RT. Effect of cortisone treatment on the active transport of calcium by the small intestine. *J Clin Invest* 1971; **50:** 1309-1321.
56. Chesney RW, Mazess RB, Hamstra AJ, DeLuca HF, O'Reagan S. Reduction of serum 1,25-dihydroxyvitamin D_3 in children receiving glucocorticoids. *Lancet* 1978; **ii:** 1123-1125.
57. Frost HM, Villanueva AR. Human osteoblastic activity: III. Effect of cortisone on lamellar osteoblastic activity. *Henry Ford Hosp Med Bull* 1961; **9:** 97-102.
58. Raisz LG, Trummel CL, Wener JA, Summons H. Effect of glucocorticoids on bone resorption in tissue culture. *Endocrinology* 1972; **90:** 961-967.
59. Gertner JM, Tamborlane WV, Horst RL, Sherwin RS, Felig P, Genel M. Mineral metabolism in diabetes mellitus: changes accompanying treatment with a portable subcutaneous insulin infusion system. *J Clin Endocrinol Metab* 1980; **50:** 862-866.
60. Rader JI, Baylink DJ, Hughes MR, Safilian EF, Haussler MR. Calcium and phosphorus deficiency in rats: effects on PTH and 1,25 dihydroxyvitamin D_3. *Am J Physiol* 1979; **236:** E118-E122.
61. Raisz LG, Mundy GR, Dietrich JW, Canalis EM. Hormonal regulation of mineral metabolism. In: McCann SM, ed. *Endocrine Physiology*, vol. 1. Baltimore: University Park Press, 1977: 199-240.
62. Rude RK, Oldham SB, Sharp CF, Singer FR. Parathyroid hormone secretion in magnesium deficiency. *J Clin Endocrinol Metab* 1978; **47:** 800-806.
63. Nagant de Deuxchaisnes C, Krane SM. Hypoparathyroidism. In: Avioli LV, Krane SM, eds. *Metabolic Bone Disease*, vol. 2. New York: Academic Press, 1978: 260-265.
64. Chase HP, Williams RL, Dupont J. Increased prostaglandin synthesis in childhood diabetes mellitus. *J Pediatr* 1979; **94:** 185-189.
65. Phillips LS, Orawski AT. Nutrition and somatomedin: III. Diabetic control, somatomedin and growth in rats. *Diabetes* 1977; **26:** 864-869.
66. Rowe DW, Starman BJ, Fujimoto WY, Williams RH. Abnormalities in proliferations and protein synthesis in skin fibroblast cultures from patients with diabetes mellitus. *Diabetes* 1977; **26:** 284-290.
67. Harrell RM, Drezner MK. Abnormal vitamin D metabolism in diabetes: contrasting defects in type I and type II models. (Abstract). 7th Annual Scientific Meeting of the American Society of Bone and Mineral Research, Washington DC, 15-18 June 1985.
68. Nordin BEC, Crilly RG, Smith DA. Osteoporosis. In: Nordin BEC, ed. *Metabolic Bone and Stone Disease*. New York: Churchill Livingstone, 1984: 1-70.