REVIEW ARTICLE

J. Sci. Soc. Thailand, 9 (1983) 205 - 222

CALCIUM HOMEOSTASIS AND BONE METABOLISM IN DIABETES MELLITUS

NATEETIP KRISHNAMRA

Faculty of Science, Department of Physiology, Mahidol University, Rama VI Rd., Bangkok 10400, Thailand

(Recieved 16 August 1983)

Abstact

Diabetes mellitus is associated with bone mineral loss and increased urinary excretion of calcium and phosphate, while the reports on total plasma calcium concentrations of ionized calcium and plasma protein are inconsistent. Many hypotheses have been put forward to explain the underlying mechanism for such changes. Osteopaenia may be a consequence of defective renal tubular reabsorption of calcium which would lead to a secondary increase in PTH secretion. On the other hand, the absence of insulin may have a direct effect on bone or osteopaenia may have resulted from inherited defects in diabetic bone. Phosphate depletion secondary to urinary loss also contributes to bone defect. Since the iPTH concentration in diabetic subjects are normal, it is likely that a primary defect in diabetic bone is responsible for diabetic osteopaenia.

The studies of calcium metabolism in experimentally induced diabetes provide useful information. However, a distinction must be made between short and long term adaptation when considering changes in elements of calcium homeostasis.

Introduction

Bone Mineral Loss in Diabetes Mellitus

Although a large amount of information has accumulated regarding the manifestation and complications of diabetes mellitus, relatively little information is currently available regarding the effects of this disorder on the integrity and metabolism of bone. In 1949,

Albright and Reifenstein[1] reported the occurrence of osteoporosis in patients with long standing and poorly controlled diabetes. The coexistence of diabetes and osteoporosis was re-emphasized by Berney in 1952[2]. Later, Menezel *et al.*[3] reported a 22 percent incidence of proximal hip fractures in diabetics. In the past, the diagnosis of osteopaenia could be made only when more than 30 percent of the mineral content of bone had been lost[4,5]. Nowadays, more advanced techniques make it possible to detect the loss of mineral contents at an early stage and since then the decreased bone mineral content has been recognized as a general feature of insulin treated diabetes mellitus[6-11]. A reduction in skeletal density has been demonstrated in both juvenile[9] and maturity-onset[8] diabetes mellitus. However, some other studies have failed to detect osteopaenia in diabetics[12-17]. In any case, the weight of recent evidence favours some reduction of bone mass below age and sex matched normal in many diabetics. The earliest effect of diabetic environment on bone is seen in the increased rate of foetal anomalies involving skeletal system of foetuses of diabetic mothers[18]. Moreover, Kelin and Frost[19] have observed that although the size of the bone osteon is normal in diabetes, the rate of osteous formation is decreased to approximately 36% of normal so that it requires two to eight times longer to form an average osteon in diabetics than in nondiabetic persons. The reduction in bone mass develops during the first 3 to 5 years of clinical diabetes and becomes most severe in patients with diabetes onset before 21 years of age[11]. The degree of osteopenia certainly depends on the quality of diabetic control[21], but the way in which defective glucose homeostasis interferes with calcium metabolism remains to be established.

Possible Causes of Decreased Bone Mineral in Diabetes Mellitus

One possible explanation for low bone mineral content in diabetics is that hyperglycaemia and/or glycosuria decreases the tubular reabsorption of calcium[21,22] leading to secondary hyperparathyroidism and loss of bone mineral. Alternatively, changes in metabolism induced by the lack of insulin may act directly on bone, causing bone loss with a secondary rise in the urinary excretion of calcium and phosphate and a suppression of parathyroid hormone secretion[23].

Previous studies have demonstrated that intravenous glucose increased urinary calcium excretion in normal subjects[21,22]. In parallel to this, McNair *et al.* [23] found a weak but positive correlation between blood glucose levels and urinary excretion of both calcium and phosphate in a cross-sectional study of 215 insulin-dependent diabetic patients. The tubular reabsorption of calcium and phosphate must be decreased in the presence of hyperglycaemia since the filtered load of the two qualities were unchanged. Through which mechanism abnormal glucose homeostasis caused bone mineral loss in diabetes was evaluated in their study by measuring serum levels of calcium and immunoreactive parathyroid hormone (iPTH). If the decreased tubular reabsorption calcium and phosphate seen with raising blood glucose levels was the primary pathogenetic factor, theoretically the calcium loss would lead to low serum levels of calcium and secondary increase in serum iPTH. McNair *et al*[23] found a minute decrease in serum calcium of 1 mg/l and a low PTH values, therefore

they concluded that the low serum iPTH levels in diabetic patients may indicate a direct action of the abnormal glucose homeostasis on bone, probably mediated through increased renal excretion of bone mineral. But recent work by Hoskin[24] showed a decreased calcium uptake by slices of kidney cortex from alloxan-diabetic rat. This decrease could be prevented by early institution of insulin therapy even though the dose of insulin was not sufficient to maintain normoglycaemia suggesting that lack of insulin may directly impair the tubular absorption of calcium.

As PTH and 1,25-dihydroxycholecalciferol ($1,25(OH)_2$-D_3) are the hormones principally concerned with calcium homeostasis, changes in their concentation in the diabetic state could provide evidence for either of the alternative hypotheses. Studies of iPTH concentration in diabetics have not shown any deviation from control values[22,25,26]. Studies of adult diabetics also show normal levels of vitamin D metabolites in these patients[25,26]. In diabetic children, however, Gertner et al.[26] and Frazier et al[27] have reported slightly reduced $1,25(OH)D_3$ levels compared with levels in age matched healthy subjects. From these data, the authors concluded that a primary defect in bone formation induced by the diabetic state was most likely to be responsible for diabetic osteopaenia. Studies on mammalian bone *in vitro* indicate that insulin may stimulate bone formation rather than resorption[22] but high doses of insulin (1-4 U/ml) have been shown to enhance bone resorption in chick[28,29]

Another explanation for the decreased bone mass seen in diabetes is suggested by the work of Goldstein *et al*[30] who demonstrated a decreased proliferative capacity of the diabetic fibroblast in tissue culture and suggested an early senescence of all cells as basic to the diabetic problem. This degeneration, when applied to bone, would also lead to early osteopaenia. On the other hand, Levin[8] suggested that osteopaenia reflects the underlying disease since it occurred early and was not related to severity as evidenced by the need for insulin, to duration or to treatment with insulin or diet alone. The effect of insulin was blunted in diabetes because of the inherited defects in diabetic bone[16, 18, 19].

In insulin dependent diabetic patients, functional glomerular changes are known to occur from the early stages of the disease. Fasting urinary excretion of inorganic phosphate is increased and the maximal capacity of renal tubular reabsorption of phosphate per litre of filtrate (Tm_{PO_4}/GFR) is significantly decreased[31-34]. Therefore it is possible that phosphate depletion secondary to urinary losses in poorly controlled diabetics might make an important contribution to such a primary defect of bone formation, since both negative calcium balance[35] and increased bone resorption[36] have been reported as consequences of phosphate depletion. While hypophosphataemia is known to occur during acute diabetic ketoacidosis and its treatment, much less is known about phosphate metabolism during long term treatment of diabetes. In the normal state, the renal threshold for phosphate reabsorption is the chief determinant of plasma phosphate. However, the commonly observed fall in plasma phosphate seen acutely when higher doses of insulin are administered occur too rapidly to be due to

changes in renal phosphate handling[37], and therefore must be due to shifts of phosphate from extra to intracellular compartments. It has been reported that a significant relationship exists between glucose and phosphate uptake by tissues, both *in vivo*[38-40] and *in vitro*[41-43]. These findings have been intepreted as the phosphate molecule following the glucose molecule as it moves from the extracellular into the intercellular space under the influence of insulin[42-46]. In contrast, patients chronically receiving insulin infused at a basal rate of 13-16 mU/kg/h showed a rise in plasma phosphate as Tm_{PO_4}/GFR rose[26]. This was due in part to the abolition of glycosuria, as glucose is known to compete with phosphate for proximal tubular reabsorption. The abnormalities in renal handing of phosphate were closely related to the blood glucose concentration and to the rate of tubular reabsorption of glucose[34]. The higher the fasting blood glucose, and therefore the reabsorption rate of glucose, the lower the Tm_{PO_4}/GFR. The inhibitory effect of glucose in linked to glucose reabsorption *per se* and not to a nonspecific osmotic intraluminal effect[47,48]. However, the increase in Tm_{PO_4}/GFR could also have been due to a direct insulin effect on the renal tubule, as experimental studies have shown that administration of insulin may have an antiphosphaturic effect even under conditions in which plasma glucose in held constant[49].

Serum Calcium Concentration in Diabetes Mellitus

Diabetes mellitus is associated with bone mineral loss and increased urinary excretion of calcium and phosphate [8,22,23,26,49-51]. The changes related to indices of poor metabolic control, but the underlying mechanism remains unknown[25]. Fogh-Andersen and his group [52,53] demonstrated lowered serum ionized calcium in diabetic patients. This agrees with Jasinski *et al*[54] who reported a decrease in ionized calcium of 0.18 and 0.38 mmol/l in 11 uncomplicated and 17 complicated cases of adult onset diabetes. Jasinski explained this decrease by increased calcium binding to the plasma proteins, but that observation was not reproduced in the studies of Fogh-Andersen[52] which showed identical mean values for protein-bound calcium. Fogh-Andersen and his group found that the lower ionized calcium of the diabetics was associated with increased calcium binding to calcium-complexing small anions. The calcium complexed with small anions is the least well examined calcium fractions, as direct determination is difficult. Nevertheless, they may possibly have physiological importance as suggested by Toffaletti and Bowers[55]. Heath and coworkers[25] have found that regardless of plasma glucose, glucosuria, ketouria, age or treatment status, 7 untreated adult diabetic subjects had no abnormalities of several elements of calcium homeostasis. In a recent study, increased ionized calcium was found in 3 diabetic patients together with normal to low protein bound calcium[56]. No explanation was offered for the discrepancies. Could the change in acid-base status in diabetics cause changes in serum ionized calcium? Acute metabolic acidosis is known to be associated with increments in serum ionized calcium levels[57-60]. Chronic metabolic acidosis is also known to result in hypercalciuria[61-64] and during chronic metabolic acidosis, PTH does not exert its normal anticalciuric effect[65]. To the author's knowledge, there is no information in the literature concerning the influence of metabolic acidosis developed in uncontrolled diabetes on serum ionized calcium levels.

Inspite of some reports of lowered ionized calcium, the mean serum PTH concentration of the diabetics was low to normal [23,52,66]. It is still difficult to explain why the secretion of PTH was not increased to correct the decrease in ionized calcium. It has been suggested that the hypomagnesaemia observed in diabetics[67 68] may have contributed to the apparent absence of response of the parathyroid gland[69-71]

Calcium Homeostasis and Bone Metabolism in Experimentally Induced Diabetes Mellitus

Diabetes could be chemically induced in experimental animals by alloxan or streptozotocin which specifically destroy the cells of the islets of Langerhans. Because of metabolic stresses, survival of the animal with uncontrolled diabetes depends on adaptations in intestinal absorption. Experimental diabetes produces initial loss of body weight followed by reduced rate of growth indicated by lower body weight in the first few days after injection of the diabetogenic agent. In contrast with decreased body weight, total intestinal and mucosal dry weight later exceed these of control. In comparison with normal rats, diabetic rats show enhanced transport activity for nutrients such as carbohydrate[72] and amino acids[73].

Bone morphology has been studied in long term streptozotocin diabetic rats in order to examine the nature of the effect of diabetes mellitus upon bone mineralization. Ten months duration of diabetes in rats caused significant decrease in the cortical thickness and density of femoral shaft bone[74]. However, there was no evidence of osteomalacia in diabetic bone in term of the accumulation of osteoid. Schedl and his group have revealed possible pathogenetic mechanisms by a series of important studies of alloxan and streptozotocin-induced diabetes mellitus in rats[75-78]. Short term (5-12 days) diabetes in the rat is characterized by impaired duodenal and colonic calcium absorption although the calcium absorption by illium and caecum are normal[79,80]. This defect has been ascribed to low levels of circulating $1,25(OH)_2D_3$[51,77,82,83] due to impaired synthesis[84] which in turn leads to a decrease in duodenal calcium binding protein[76,85]. Decreased calcium absorption in the rat with short-term diabetes results in a negative calcium balance[79] and is attended by a decrease in serum calcium[77,86] an elevation in circulating PTH[75,86] and a diminished calcitonin (CT) response[86]. Hypocalcaemia in these short-term diabetic rats is however limited to the fasting state because fed diabetic animals maintain normal to slightly elevated serum calcium levels[75]. It has been suggested that calcium malabsorption was apparently compensated by mobilization of bone calcium through secondary hyperparathyroidism. It is however difficult to explain the rapid development after overnight fasting of hypocalcaemia and decreased serum ionized calcium in the diabetic rat[79], especially since animals have increased blood levels of PTH[75,87] and suppressed CT secretion[84,86]. Treatment with insulin results in normal ization of plasma 1, $25(OH)_2D_3$ levels and correction of intestinal calcium malabsorption[77,82,83,88]. It is not known whether insulin acts directly on the renal 1,α-hydroxylase but it apparently has permissive effect on PTH stimulations of $1,25(OH)_2D_3$ production in cultured kidney cells[89]. However, insulin is probably not an absolute requirement in 1, α hydroxylation since diabetic rats when fed a low calcium diet can significantly increase serum $1,25(OH)_2$-D_3 concentration compared with diabetic rats fed a normal calcium diet[90].

In contrast to these observations, chronically diabetic rats maintain normal to significantly elevated fasting serum calcium levels despite severe hypercalciuria and a pronounced decrease in bone turnover without any evidence of osteitis fibrosa[91]. A distinction must be made between short term and long term adaptation when considering changes in calcium absorption and mineral metabolism in the adaptive response to insulin deficiency. Although the diabetic rats are in negative calcium balance 2 weeks after the induction of diabetes, these animals are in positive balance 4 weeks later[83].

In fact the accumulated data clearly demonstrated enhanced absorption of calcium in long standing experimental diabetes despite low level of $1,25(OH)_2D_3$ and hypercorticosterone, which normally would have resulted in impaire dintestinal calcium absorption[73,92,94] Experimental insulin deficiency is not only associated wiht markedly increased food intake[83], but also characterrized by several compensatory intestinal adaptions that enable the animal to survive the stresses of uncontrolled diabetes. These include intestinal hypertrophy, increased mucosal cell proliferation, and stimulation of several membrane transport systems[72,95,97]. Further investigation is required before the finding in rats can be related to reports of an insulin reversible increase in intestinal calcium absorption in insulin deficient humans[98].

In contrast to short-term diabetic animals, the chronic insulin deficient rat was charcaterized by markedly decreased circulating PTH and urinary cyclic AMP excretion[83]. A primary alteration of parathyroid function in uncontrolled diabetes cannot be excluded but seems less likely because the diabetic rat manifests an appropriate parathyroid response to diminished calcium absorption in the acute stage of experimental diabetes[75,86]. Insulin replacement normalized the intestinal absorption of calcium and corrected the decrease in circulating PTH. Low levels of circulating $1,25(OH)_2D_3$ was noted in chronically diabetic rats[83] despite normal levels of circulation 25-hydroxyvitamin D.

Effects of Insulin on Calcium Homeostasis

There are conflicting reports on the effect of insulin on plasma calcium concentration in rats. In 1977, Yamaguchi and Yamamoto[99] reported that the administration of insulin significantly decreased the serum calcium concentration in intact and thyroparathyroidectomized (TPTX) rats. Even at dose level of 10 mU/100 g body weight which did not produce the reduction of serum glucose level, insulin by intraperitoneal route caused a significant decrease in the serum calcium concentration. On the other hand, a rise in serum calcium provokes a temporary release of insulin[100,101]. Since the enhancement of serum calcium concentration after the administration of calcium caused an increase in serum glucose level[102], it was suggested that the hypocalcaemic effect of insulin may play an important role in order to prevent the elevation of serum glucose level in rats.

In contrast, hypoglycaemic doses of approximately 1 U/100 g body weight corrected the hypocalcaemia of TPTX rats but had no effect in intact animals[28] and higher doses

(12 U/100 g body weight) of commercial but not highly purified insulin provoked hypercalcaemia in intact rats. Moreover, a peptide extracted from the pig pancreas, devoid of insulin immunoreactivity has been shown to elevate plasma calcium in the rat [103].

In our study of the effect of insulin on the plasma levels of glucose, calcium and inorganic phosphate (Figures 1-3) a hypoglycaemic dose of 0.5 U/100 g body weight administered intramuscularly did not have any effect on the plasma calcium concentration in either fed or fasted intact rats but induced hypercalcaemia within 2 h in fed short-term (2 day) diabetic rats (Fig.4), suggesting that insulin may have an acute and direct action on the absorption of calcium. However, it was surprising that the effect may be observed in 2 h. Interestingly, we found that the same dose markedly increased the levels of plasma calcium (Fig.5) in fasted and fed TPTX, diabetic rats confirming that the action of insulin at least in this condition, was independent of PTH. The insulin-induced hypercalcaemia in TPTX and not in intact rats suggested a possibility of the role of an antihypercalcaemic hormone, calcitonin in intact diabetic rat. This point is being investigated.

Since birds are known to be very sensitive to PTH and have resistance to the hypoglycemic effects of insulin, the effect of commercial and highly purified insulin on plasma calcium concentration was studied in 10 day old chicks 60 min after the administration of the hormone [104]. Both types of insulin provoked a dose related elevation of plasma calcium. Hypercalcaemia was even observed with 0.05 U insulin a dose that did not modify the plasma phosphate concentration.

Administration of insulin to aves provokes marked stimulation of the autonomic nervous system and secretion of catecholamines, which apparently is independent of the degree of hypoglycaemia [105]. Moreover, it was reported that catecholamines induce PTH secretion in mammals, an effect that is blocked by β-blocking agents [106], however, propranolol did not modify the hypercalcaemic effect of insulin [104], suggesting that insulin-induced β adrenergic stimulation [105] was not responsible for the hypercalcaemic effect of insulin and also that the possibility of a catecholamine-mediated stimulation of PTH secretion [106] by insulin was therefore unlikely. Body and his group [107] sought to determine to what extent the change observed during insulin-induced hypoglycaemia might be attributable to epinephrine. In contrast to the study in bird, they found that during infusion of physiological doses of epinephrine in human, serum ionized calcium, iPTH and iCT concentrations were unaltered but the serum inorgonic phosphate was significanly decreased. Therefore, it can be concluded that catecholamines do not mediate the insulin-induced change in calcium metabolism.

Conclusions

The pathophophysiological lesions responsible for alteration in skeletal density in diabetes mellitus have not been delineated. However there are many possible mechanisms of explaining the disturbance of bone mineral metabolism in diabetes mellitus, for instance.

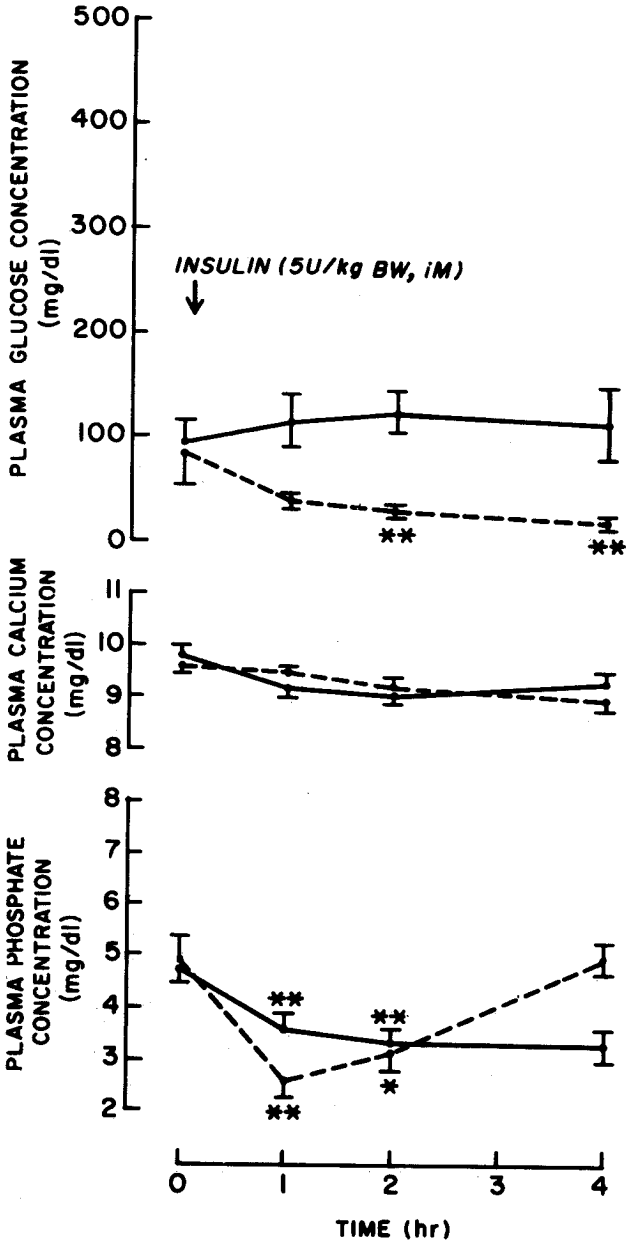

Figure 1. The plasma concentrations (mg/dl) of glucose, calcium and phosphate in 17 hr fasted, intact rat after normal saline (—, n = 9) or insulin (5 U/kg BW, im) (----, n = 13) administration. The vertical bars indicate S E M. Asterisks denote significant difference from time zero value *P< 0.05, ** P< 0.01.

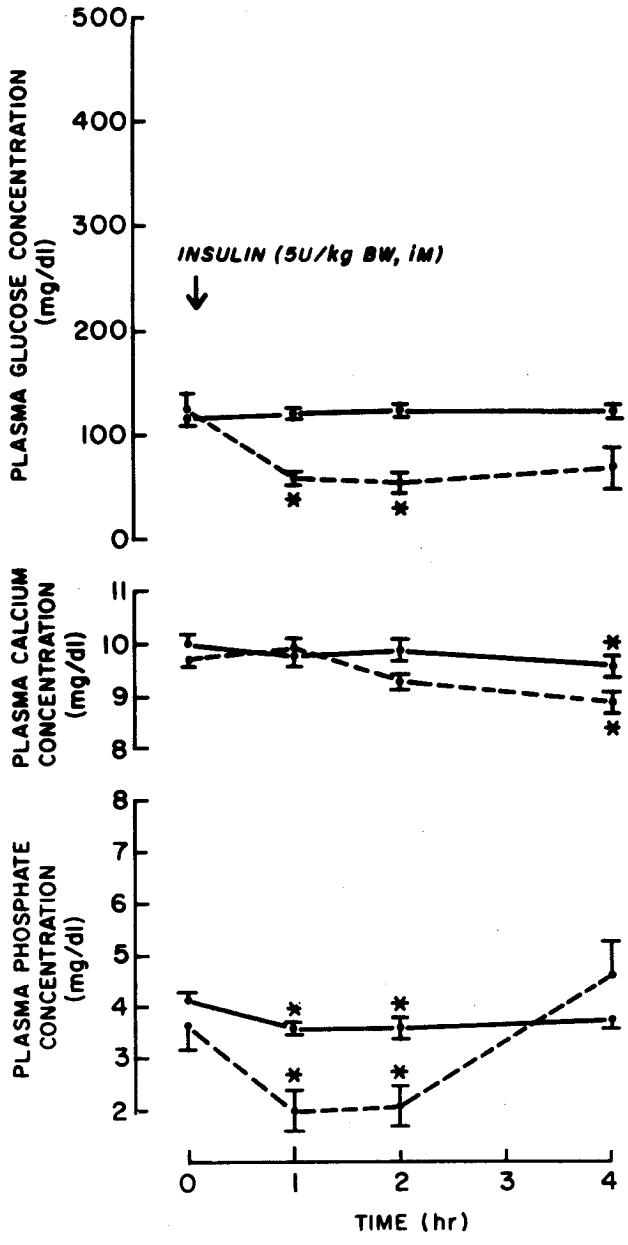

Figure 2: The plasma concentration (mg/dl) of glucose, calcinm and phosphate in fed intact rat after normal saline (____,n = 8) or insulin (5 U/kg BW, im) (----, n = 13) administration. The vertical bars indicate SEM. *denotes $P<0.05$ when compared with time zero value.

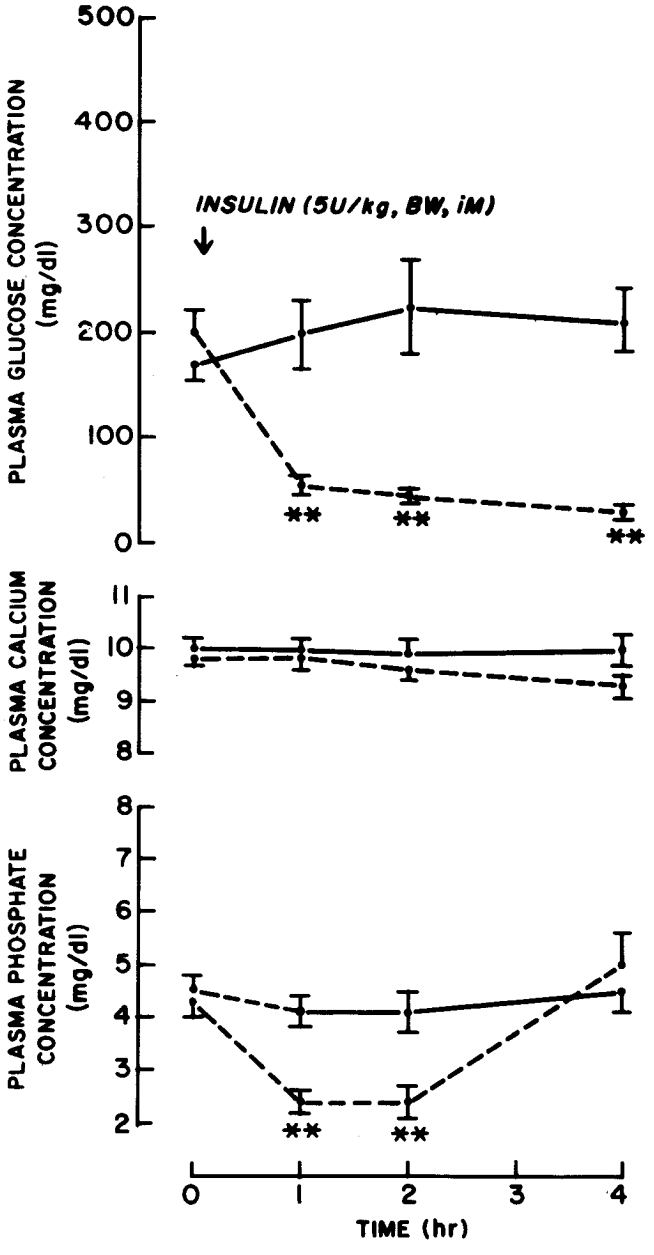

Figure 3: The plasma concentrations (mg/dl) of glucose, calcium and phosphate in 17 hr fasted alloxan-induced diabetic rat after normal saline (——, n = 9) or insulin (5 U/kg BW, im) (- - - -, n = 11) administration. The vertical bars indicate SEM. **denotes $P<0.01$ when compared with time zero value.

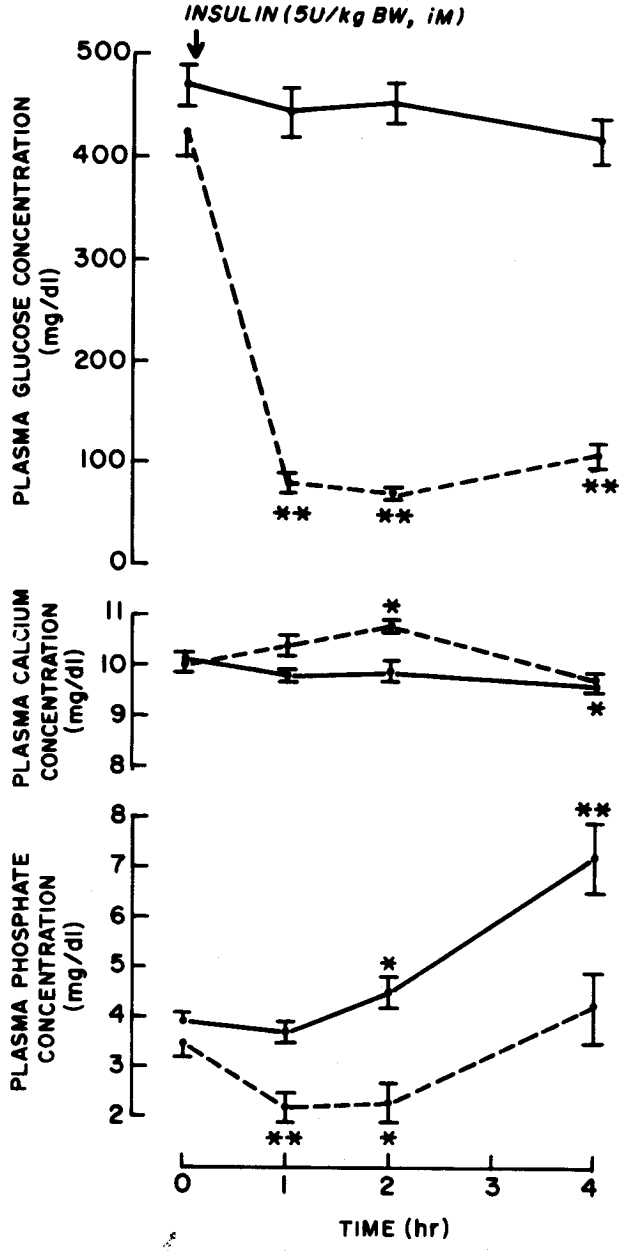

Figure 4: The plasma concentrations (mg/dl) of glucose, calcium and phosphate in fed alloxan-induced diabetic rat after normal saline (——, n = 9) or insulin (5 U/kg BW, im) (- - - -, n = 11) administration. The vertical bars indicate SEM.* denotes $P<0.05$, **$P<0.01$, when compared with time zero value

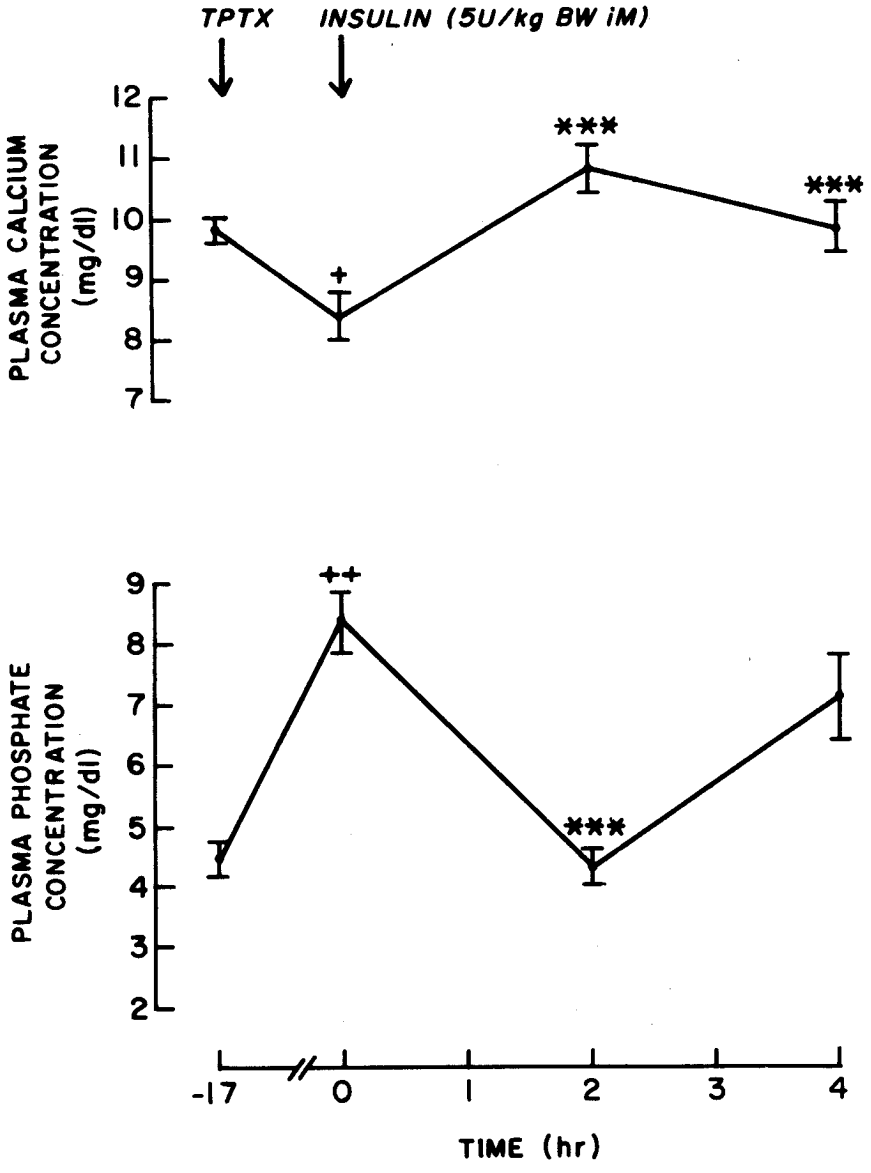

Figure 5: The plasma concentrations (mg/dl) of calcium, and phosphate in 17 hr fasted, thyroparathyroidectomized (TPTX) alloxan-induces diabetic rat after insulin (5 U/kg BW, im) (n = 8) administration. The vertical bars indicate SEM. Comparisons were made between the values at 0 hr and -17 hr, ** denotes $P<0.01$ and *** denotes $P<0.001$. Comparisons were also made between the values at 2 and 4 hrs with that at 0 hr, ***denotes $P<0.001$.

1. hypercalciuria due to osmotic diuresis leading to secondary hyperparathyroidism.
2. direct effect of the lack of insulin on bone
3. hypophosphataemia as a result of a decrease in Tm_{PO_4}/GFR.

The studies of experimentally induced diabetes mellitus in animals have suggested disturbances of calcium homeostasis eg. impaired vitamin D metabolism and calcium absorption as the cause of decrease in bone density. This coupled with increased urinary calcium loss from osmotic diuresis could result in secondary hyperparathyroidism to preserve plasma calcium levels at the expense of bone. However, studies in humans have shown that adult diabetic subjects have little or no abnormalities of calcium homeostasis, therefore the likely explanation for the observed diabetic osteopaenia is a defect in bone metabolism which may be completely or partially corrected by insulin treatment.

References

1. Albright, F. and Reifenstein, E.C.(1952) *Parathyroid glands and metabolic bone disease:Selected studies.* Williams and Wilkins Company, Baltimore, p.156.
2. Berney P.W.(1952) Osteoporosis and diabetes mellitus. *J.Iowa Med.Soc* **42.** 10-12
3. Menezel, J., Makin, M. and Robin, G. (1972) Prevalence of diabetes mellitus in Jerusalem: its association with presenile osteoporosis. *Isr.J.Med. Sci.* **8,** 918-919.
4. Ardran, G.M. (1951) Bone destruction not demonstrable by radiography. *Br.J.Radiol.* **24,** 107-109.
5. Smith, D.M., Johnston, Jr., C.C. and Yu, P.L.(1972) In vivo measurement of bone mass: its use in demineralized states such as osteoporosis. *J.Am.Med.Assoc.* **219,** 325-329
6. Jurist, T.M (1970) In vivo determination of the elastic response of bone. II ulnar resonant frequency in osteoporotic, diabetic and normal subjects, *Phys.Med.Biol* **15,**427-434.
7. Forgaç, S., Halmos, T. and Salamon, F. (1972) Bone change in diabetes mellitus *Isr. J. Med. Sci* **8,** 782-787.
8. Levin, M.E., Boisseau, V.C. and Avioli, L.V. (1976) Effects of diabetes mellitus on bone mass in juvenile and adult onset diabetes. *N. Eng. J.Med.* **294,** 241-245.
9. Rosenbloom, A.L., Lezotte, D.C. Weber, F.T., Gudat, J., Heller, D.R., Weber, M.L., Klein, S. and Kennedy, B.B. (1977) Diminution of bone mass in childhood diabetes, *Diabetes* **26,**1052-1056.
10. Santiago, J.V., McAlister , W.H., Ratzan, S.K., Bussman, Y., Haymond M.W., Schackelford, G. and Weldon, V.V. (1977) Decreased cortical thickness and osteopenia in children with diabetes mellitus. *J. Clin. Endorinol. Metab.* **45,** 845-853.
11. McNair, P., Madabad, S., Christiansen, C., Faber, O.K., Transbol, I. and Binder, C.(1978) Osteopenia in insulintreateddiabetes mellitus, its relation to age at onset, sex and duration of disease. *Diabetologia* **15,** 87-90.
12. Frost, H.M. (1964) Papers of the orthopaedic research laboratory: lamellar bone physiology in diabetes mellitus. *Henry Ford Hosp Med . Bull.* **12,** 495-501.
13. Klein, M.D., Frost, H.M. and Sedlin, E.(1964) A pilot study of lamellar bone physiology in diabetes mellitus. *Henry Ford Hosp. Med. Bull* **12,**55-59
14. Meema, H.E. and Meema, S. (1967) The relationship of diabetes mellitus and body weight to osteoporosis in elderly females. *Can. Med. Assoc. J.* **96,** 132-139.

15. Meema, H.E. and Reid, D.B.W. (1969) The relationship between skin and cortical bone thickness in old age with special reference to osteoporosis and diabetes mellitus: a roentgenographic study. *J.Gerontol* **24**, 28-35.
16. Wu, K., Schubeck, K.E., Frost, H.M. and Villanueva, A. (1970) Haversian bone formation rates determined by a new method in a mastadon and in human diabetes mellitus and osteoporosis. *Calcif. Tissue Res.* **6**, 204-219.
17. Deleeuw, I. and Abs, R. (1977) Bone mass and bone density in maturity type diabetes measured by the $^{125}_{I}$ photon absorption technique. *Diabetes* **26**, 1130.
18. Pedersen, J.(1967) The pregnant diabetics and her new born. Problem and management. Williams and Wilkins Company, Baltimore, p.60-107.
19. Kelin, M. and Frost, H.M.(1964) The numbers of bone resorption and formation foci in rib. *Henry Ford Hosp.Med.Bull.* **12**, 527-536.
20. McNair, P.,Madsbad, S., Christiansen,C.,Christensen, M.S.,Faber,O.K.,Binder,C.,Tranbl, I.(1979) Bone loss in diabetes : effects of metabolic state. *Diabetologia* **17**, 283-286.
21. Lemann, J.Jr., Lennon, E.J.,Piering, W.R.,Prien,E.L.Jr. and Ricinati, E.S.(1970) Evidence that glucose ingestion inhibits net renal tubular reabsorption of calcium and magnesium in man. *J.Lab.Clin.Med.* **75**,578-585.
22. Raskin, P.,Stevenson, M.R.M.,Barilla, D.E., and Pak, C.Y.C.(1978) The hypercalciuria of diabetes mellitus: Its amelioration with insulin. *Clin.Endocrinol.* **9**.329-335.
23. McNair, P., Madsbad, S., Christensen, M.S., Christensen, C.,Faber, O.K., Binder, C. and Transbol, I. (1979) Bone mineral loss in insulin-treated diabetes mellitus: Studies on pathogenesis. *Acta Endocrinol.* **90**,463--472.
24. Hoskin, B. (1983) Insulin prevention of impaired renal calcium transport in alloxan diabetic rats. *Life Sciences* **31**, 377-383.
25. Heath, H., Lambert, P.W., Service, F.J. and Arnaud, S.B. (1979) Calcium homeostasis in diabetes mellitus. *J.Clin. Endocrinol. Metab.* **49**, 462-466.
26. Gertner, J.M., Tramborlane, W.V., Horst, R.L., Sherwin, R.S., Felig, P. and Genel, M. (1980) Mineral metabolism in diabetes mellitus. Changes accompanying treatment with a potable subcutaneous insulin infusion system. *J.Clin. Endocrinol. Metab.* **50**, 862-866.
27. Frazier, T.E., McGee, B.R., Santiago, J.W., White, N., Bryce, G., Mallon, J. and Avioli, L. (1979) Reduction in 1,25 $(OH)_2$ vitamin D levels in diabetic children. *Diabetes* **28**, 368-373.
28. Puche, R.C., Romano, M.C., Locatto, M.E. and Ferretti, J.L. (1973) The effect of insulin on bone resorption. *Calcif. Tissue Res.* **12**, 8-13.
29. Guthman, H.M. III., Palmieri, G.M.A., Hinton, A.B. and Guthnam, A.L. (1982) The mechanism of insulin--induced hypercalcemia in the chick. *Endocrinology,* **110**, 571-574.
30. Goldstein, S., Littlefield, J.W. and Soeldner, J.S. (1969) Diabetes mellitus and aging diminished plating efficiency of cultured human fibroblast. *Proc. Natl. Acad. Sci. USA.* **64**, 155-160.
31. Astrug, A.K. (1966) Studies on the clearance and tubular reabsorption of phosphates in diabetes and some of its complications. *Diabetologia,* **2**, 198-201.
32. Ditzel, J., Brochner-Mortensen, J., Hansted, C. and Rodbro, R. (1978) Changes in the renal handling of phosphate in juvenille diabetes. *Acta Endocrinol Suppl (Copenh)* **88**, 24, (Abstract).
33. Raskin, P. and Pak, C.Y.C. (1981) The effect of chronic insulin therapy on phosphate metabolism in diabetes mellitus. *Diabetologia* **21**, 50-53.
34. Ditzel, J., Brochner-Mortensen, J. and Kawahara, R. (1982) Dysfunction of tubular phosphate reabsorption related to glomerular filtration and blood glucose control in diabetic children. *Diabetologia* **23**, 406-410.
35. Domniquez, J.H., Gray, R.W. and Lehmann, J. (1976) Dietary phosphate deprivation in women and men: effects on mineral and acid balance, parathyroid hormone and metabolite of 25 (OH) vitamin D. *J.-Clin. Endocrinol. Metab.* **43**, 1050-1058.

36. Baylink, D., Wegedal. J. and Stanffer, M. (1971) Formation, mineralization and resorption of bone in hypophosphatemic rats. *J.Clin. Invest.* **50,** 2519-2527.
37. Riley, M.S., Schade, D.S. and Eaton, R.P. (1979) Effects of insulin infusion on plasma phosphate in diabetic patients. *Metabolism* **29,** 191-198.
38. Pollack, H., Millet, R.F., Essex, H.E., Mann, F.C. and Bollman, J.L. (1934) Serum phosphate changes induced by injections of glucose into dogs under various conditions. *Am. J. Physiol.* **110,** 117-122.
39. Levine, R., Loube, P.D. and Weisberg, H.F. (1949) Nature of action of insulin on level of serum inorganic phosphate. *Am. J. Physiol.* **159,** 107-110.
40. Hargenfeldt, L. and Wahlberg, F. (1971) Blood inorganic phosphate, pyruvate and lactate during an intravenous glucose tolerance test in ischemic cardiovascular disease. *Acta Med. Scand.* **189,** 207-211.
41. Bollinger , A.and Hartman, F.W. (1925) Observations on blood phosphate as related to carbohydrate metabolism. *J.Biol. Chem.* **64,** 91-109
42. Kipnis, D.M. (1959) Regulation of glucose uptake by muscle:functional significance of permeability and phosphorylating activity. *Ann. N.Y. Acad.Sci.* **82,** 355-365.
43. Kaji, H. and Park, C.R. (1961) Stimulation of phosphate uptake by insulin and its relation to sugar transport in the perfused rat heart. *Fed. Proc.* **20,** 190-196.
44. Forsham, P.H. and Thorn, G.W. (1950) Changes in organic serum phosphorus during the intravenous glucose tolerance test as an adjunct to the diagnosis of early diabetes mellitus. *Proc. Am. Diabetes Assoc.* **9.** 101-122.
45. Randle, P.J. and Smith, G.H. (1958) Regulation of glucose uptake by muscle 2. The effect of insulin, an aerobiosis and cell poisons on the penetration of isolated rat diaphragm by sugar. *Biochem. J.* **70,** 501-508.
46. Zampa, G.A. Attula, F., Braechetti, O., Geminiani, G.O., Gorgatti, E. and O'Diffeddi, M.J. (1967) Studies on peripheral glucose metabolism using the experimental human forearm preparation. *Diabetologia* **3,** 35-46.
47. Fox, M., Thur, S., Rosenburg, L. and Segal, S.(1964) Impaired renal tubular function induced by sugar infusion in man *J. Clin. Endocrinol, Metabl.* **24,** 1318-1327.
48. Corman, B.C., Touvay, C., Poujiol, P. and deRouffinac, C. (1978) Glucose mediated inhibition of phosphate reabsorption in rat kidney. *Am. J. Physiol.* **235,** F430-439.
49. DeFronzo, R.H., Cooke, C.R., Andres, R., Faloona, G.R. and Davis, P.J. (1975) The effect of insulin on renal handling of Na, K, Ca and PO_4 in man. *J. Clin. Invest.* **55,** 845-855.
50 DeLeeuw, I., Vertommen, J. and Abs, R. (1976) The mineral content of the trabecullar bone of diabetic subjects. *Diabetologia 12,* 386-392.
51. DeLeeuw, I., Vertommen, J. and Abs, R. (1978) The magnesium content of trabecular bone in diabetic subjects. *Biomedicine* **29,** 16-17
52. Fogh-Andersen, N., McNair, P., Moller-Petersen, J. and Madsbad, S. (1982) Serum calcium fraction in diabetes mellitus. *Clin. Chem.* **28,** 2073-2076.
53. Fogh-Andersen, N., McNair, P., Moller-Petersen, J. and Madsbad, S. (1983) Lowered serum ionized calcium in insulin treated diabetic subjects. *Scand. J. Clin. Lab. Invest.* **43,** suppl. 165, 93-97.
54. Jasinski, K., Adamski, A. and Smars, C. (1968) Plasma calcium fractions in diabetes mellitus. Pol. Med. J. **7,** 1033-1040.
55. Toffaletti, J. and Bowers G.N. (1979) The possible physiological importance of calcium complexes: An opinion, *Clin. Chem. Acta.* **97.** 101-105.
56. Duncan. P.H. Wills, M.R., Smith, B.J. and Savory, J. (1982) Clinical studies of protein-bound calcium in various diseases. *Clin. Chem.* **28,** 672-675.
57. Kaplan, E.L., Hill, B.J., Locke, S., Toth, D.N. and Perkin, G.W. (1971) Metabolic acidosis and parathyroid hormone secretion in sheep, *J. Lab. Clin. Med.* **78,** 819-827.
58. Beck, N. and Webster, S.K. (1976) Effects of acute metabolic acidosis on parathyroid hormone action and calcium mobilization. *Am. J. Physiol.* **230,** 127-131.
59. Mahnensmith, R., Thiele, S.O., Cooke, C.R., Broadus, A. and DeFronzo, R.A.(1979) Effect of acute metobolic acidemia on renal electrolyte transport in man. *Metab. Clin. Exp.* **28,** 831-842.

60. Oster, J.R., Perez, G.O., Canterbury, J.M., Alpert, H.C. and Vaamonde, C.A. (1982) Plasma parathyroid hormone and divalent cation responce to induction of acute metabolic acidosis. *Can. J. Physiol. Pharmacol.* **60**, 1505-1513.
61. Lemann, J.Jr., Litzow, J.R. and Lennon, E.J. (1967) Studies of the mechanism by which metabolic acidosis augments urinary calcium excretion in man. *J.Clin. Invest.* **46**, 1318-1328.
62. Stacy, B.C. and Wilson, B.W. (1970) Acidosis and gypercalciuria: Renal mechanism affecting Ca, Mg and Na excretion in the sheep. *J. Physiol.* **210**, 549-564.
63. Coe, F.L., Firpo, J.J., Hollandsworth, D.L., Canterbury, S.L. and Reiss, J.M. (1975) Effect of acute and chronic metabolic acidosis on serum immunoreactive parathyroid hormone in man. *Kidney Int.* **8**, 262-273.
64. Ral, S., Wong, N.L.M. and Dirks, J.H. (1979) Effect of metabolic acidosis and alkalosis on sodium and calcium transport in the dog kidney. *Kidney Int.* **15**, 520-533.
65. Batlle, D., Itsarayoungyuen, K., Hays, S., Arruda, J.A.L. and Kurtzman, N.A. (1982) Parathyroid hormone is not anticalciuric during chronic metabolic acidosis. *Kidney Int.* **22**, 264-271.
66. McNair, P., Christensen, M.S., Madsbad, S., Christiansen, C. and Transbol, I. (1981) Hypoparathyroidism in diabetes mellitus. *Acta Endocrinol.* **96**, 81-86.
67. McNair, P., Christiansen, C., Madsbad, S., Lauritxen, E., Faber, O.K., Binder, C., and Transbol, I. (1978) Hypomagnesemia, a risk factor in diabetic retinopathy. *Diabetes* **27**, 1075-1077.
68. Levin, G.E., Mather, H.M. and Pilkington, T.R.E. (1981) Tissue magnesium status in diabetes mellitus. *Diabetologis* **21**, 131-134.
69. Chase, L.R. and Slatopolsky, E. (1974) Secretion and metabolic efficacy of parathyroid hormone in patients with severe hypomagnesaemia. *J. Clin. Endocrinol.* **38**, 363-371.
70. Anast, C.S., Winnacker, J.L., Forte, L.R. and Burns, T.W. (1976) Impaired release of parathyroid hormone in magnesium deficiency. *J. Clin. Endocrinol. Metab.* **42**, 707-717.
71. Rude, R.K., Oldham, S.B. and Single, R.F. (1976) Functional hypoparathyroidism and parathyroid endorgan resistance in human magnesium deficiency, *Clin. Endocrinol,* **5**, 209-224.
72. Schedl, H.P. and Wilson, H.D.(1971) Effects of diabetes on intestinal growth and hexose transport in the rat. *Am. J. Physiol.* **220**, 1739-1745.
73. Lal, D. and Schedl, H.P. (1974) Intestinal adaptation in diabetes: amino acid absorption. *Am. J. Physiol,* **227(4)**, 827-831.
74. Brown, D.M. and Jowsey, J.(1977) Osteoporosis in diabetes mellitus rats, *Diabetes* **26**, 370 A.
75. Schneider, L.E., Hargis, G.K., Schedl, H.P. and Williams, G.A. (1974) Parathyroid function in the alloxan diabetic rat. *Endocrinology* **95**, 749-752.
76. Schneider, L.E., Wilson, H.D. and Schedl, H.P. (1974) Effects of alloxan diabetes on duodenal calcium-binding protein in the rat. *Am. J. Physiol,* **227**, 832-838.
77. Schneider, L.E., Schedl, H.P., McCain, T. and Haussler, M.R. (1977) Experimental diabetes reduces circulating 1,25 dihydroxyvitamin D in the rat. *Science* **196**, 1452-1454.
78. Schneider, L.E., Wilson, H.D. and Schedl, H.P. (1977) Effects of diabetes and vitamin D depletion on duodenal and ilial calcium transport in the rat. *Acta Diabetol* **14**, 18-24.
79. Schneider, L.E. and Schedl, H.P. (1972) Diabetes and intestional calcium absorption in the rat. *Am. J. Physiol.* **223-1323.**
Physiol. **223**, 1319-1323.
80. Petith, M.M. and Schedl, H.P. (1978) Effects of diabetes on cecal and colonic calcium transport in diabetic rat. *Am. J. Physiol.* **235**, E 699-702.
81. Schneider, L.E., Omdahl, J. and Schedl, H.P. (1976) Effects of vitamin D and its metabolites on calcium transport in the diabetic rat. *Endorinology* **99**, 793-799.

82. Horst, R.L., Spoede, S.L. and Littledike, E.T. (1979) Vitamin D metabolism in the diabetic rat. In Vitamin D, Basic Research and Its Clinical Application, Nowman, A.W. et al eds., Walter de Gruyter and Co., New York p. 1003.

83. Hough, F.S., Russell, J.E., J.E., Teitellbaum, S.L. and Avioli, L.V. (1982) Calcium homeostasis in chronic steptozotocin-induced diabetes mellitus in the rat. *Am. J. Physiol.* **242.** E451-456.

84. Wongsurawat, N., Armbrecht, H.J., Zenser, T.V., Davis, B.B., Thomas, M.L. and Forte, L.R. (1983) 1, 25-dihydroxyvitamin D_3 and 24, 25- dihydroxyvitamin D_3 production byisolated renal slices is modulated by diabetes and insulin in the rat. *Diabetes* **32(4),** 302-306.

85. Schneider, L., Wilson, H.D. and Schedl, H.P. (1973) Intestinal calcium binding protein in the diabetic rat. *Nature* **245,** 327-330.

86. Schedl, H.P., Heath III., H. and Wenger, J. (1968) Serum calcitonin and parathyroid in experimental diabetes: effects of insulin treatment. *Endocrinology* **103,** 1368-1373.

87. Weber, C.J., Hardy, M.A., Williams, G.A., Lerner. R.L. and Reemtsma, K. (1976) Pancreatic islet transplantation and hyperparathyroidism in diabetic rats *Surg. Forum* **27,** 317-323.

88. Schnedier, L.E., Omdahl, J.L. and Schedl, H.P. (1977) Insulin treatment of diabetic rats: effects on ducodenal calcium absorption. *Endocrinology* **100,** 67-73.

89. Henry, H.L, (1981) Insulin permits parathyroid hormone stimulation of 1, 25-dihydroxy vitamin D_3 production in cultured kidney cells. *Endocrinology* **108,** 733-735.

90. Wilson, H.D., Horst, R.L, and Schedl, H.P. (1982) Calcium intake regulates 1, 25-dihydroxy vitamin D_3 formation in the diabetic rat. *Diabetes* **31,** 401-405.

91. Hough, F.S., Teitelbaum, S.L., Russell, J., Bergfeld, M., Slatopolsky, E. and Avioli, L.V.(1980) Normal lization of deranged mineral homeostasis and skeletal morphology in the chronically diabetic rat by insulin therapy (Abstract) *Clin.Res.* **28,** 261A.

92. Harrison, J.G. and Harrison, H.C.(1960) Transfer of ^{45}Ca across intestinal wall in vitro in relation to action of vitamin D and cortisol. *Am. J. Physiol.* **199,** 265-271.

93. Kimberg, D.V., Baerg, R.D., Gershon, E. and Grandusius, R.T. (1971) Effect of cortisone treatment on the active transport of calcium by the small intestine. *J Clin. Invest.* **50,** 1309-1321.

94. Myrtle, J.F. and Norman, A.W. (1971) Vitamin D: a cholecaliferol metabolite highly active in promoting intestinal calcium transport. *Science,* **171,** 79-82.

95. Caspary, W.F.(1973) Effect of insulin and experimental diabetes mellitus on the digestive-absorptive function of the small intestine. *Digestion* **9,** 248-263.

96. Walker, B.E.and Schedl, H.P.(1979) Small intestinal calcium absorption in the rat with experimental diabetes. *Proc. Soc. Exp. Biol. Med.* **161,** 149-152.

97. Spencer, E.M., Khalil, M.and Tobiassen, B. (1980) Experimental diabetes in the rat causes an insulin reversible decrease in renal 25-hydroxy vitamin D_3-1-$\overset{\alpha}{\wedge}$-hydroxylase activity. *Endocrinology,* **107,** 300-305.

98. Monnier, L.C., Colette, C., Aguirre, L., Sany, C. and Mirouze, J.(1978) Intestinal and renal handling of calcium in human diabetes mellitus: influence of acute oral glucose loading and diabetic control. *Eur. J. Clin.Invest.* **8,** 225-231.

99. Yamaguchi, M. and Yamamoto, T.(1977) Effect of insulin on serum calcium concentration in rats. *Chem. Pharm. Bull.* **25(10),** 2767-2769.

100. Grodsky, G.M. and Benneft. L.L. (1966) Cation requirements for insulin secretion in the isolated perfused pancreas. *Diabetes* **15**, 910-913.

101. Davis, G., Somers, G. and Malaisse, W.J.(1974) Stimulation of insulin release by calcium. *Biochem. Biophy. Res.Commun.* **67**, 525-529.

102. Lundquist, I., Fanska, R and Grodsky, G.M. (1976) Interaction of calcium and glucose on glucagon secretion. *Endocrinology* **99**, 1304-1312.

103. Taylor, J., Tager, H.S. and Kaplan, E.L.(1976) A hypercalcaemic factor in commercially prepared insulin. *J.Surg.Res* **20**, 361-365.

104. Kaplan, E.L., Tager, H.S. and Logocki, R.(1977) A calcium elevating peptide from pig pancreas, *Clin. Res.* **25**. 622 (Abstract).

105. Palmieri, G.M.A., Hawrylko, J, and Hinton, A. (1979) Hypercalcemic effect of insulin in the chick. *Endocri nology* **104(6)**, 1774-1777.

106. Pittman, R.P. and Hazelwood, R.L. (1973) Catecholamine response of chickens to exogenous insulin and tolbutamide. *Comp. Biochem. Physiol. (A)* **45**, 141-146.

107. Fischer, J.A., Blum, J.W. and Binsawanger, U.(1973) Acute PTH response to epinephrine in vivo. *J.Clin Invest.* **52**, 2434-2441.

108. Body, J., Cryer, P.E., Offord, K.P.and Heath III, H.(1983) Epinephrine is a hypophosphatemic hormone in man. Physiological effects of circulating epinephrine or plasma calcium, magnesium, phospho rus, parathyroid hormone and calcitonin. *J. Clin. Invest.* **71**, 572-578.

บทคัดย่อ

ความผิดปกติของสภาวะแคลเซี่ยมในโรคเบาหวานนั้นอาจสังเกตได้เช่น ความผิดปกติของกระดูก (osteopaenia) มีการขับถ่ายแคลเซี่ยมและฟอสเฟทออกทางปัสสาวะมากกว่าปกติ ส่วนระดับของแคลเซี่ยมและโปรตีนในเลือดนั้นมีรายงานซึ่งต่างกันไป อย่างไรก็ตาม มีกลุ่มนักวิจัยหลายกลุ่มพยายามอธิบายถึงสาเหตุของความผิดปกติของกระดูก เช่นอาจเกิดจากความผิดปกติของระบบดูดกลับของแคลเซี่ยมในท่อไต ซึ่งจะยังผลให้มีการหลั่งพาราไทรอยด์ฮอรโมนเพิ่มขึ้น ซึ่งมีผลกระตุ้นการสลายกระดูก หรือสาเหตุอาจเนื่องมาจากผลโดยตรงของโรคเบาหวานต่อกระดูก หรือความผิดปกติของกระดูกสืบเนื่องมาจากสาเหตุทางกรรมพันธุ์ นอกจากนั้นการเสียฟอสเฟทไปมากในปัสสาวะในโรคเบาหวานก็เป็นสาเหตุหนึ่งได้ อย่างไรก็ตามรายงานส่วนมากดูจะชี้ว่าสาเหตุของ osteopaenia เนื่องจากผลโดยตรงของการขาดอินซูลินต่อกระดูก

การทดลองในสัตว์ทดลองเป็นประโยชน์มากในการศึกษาเกี่ยวกับสภาวะแคลเซี่ยมในโรคเบาหวาน แต่ในการสรุปผลนั้นพึงสังวรณ์ว่าการตอบสนองของสภาวะแคลเซี่ยมต่อการขาดอินซูลินนั้นแตกต่างกันไปแล้วแต่จะศึกษาในโรคเบาหวานแบบเฉียบพลันหรือเรื้อรัง