Osteoporos Int (1997) 7 (Suppl. 3):S209–S212

Osteoporosis International

Importance of Sustained High Glucose Condition in the Development of Diabetic Osteopenia: Possible Involvement of the Polyol Pathway

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Diabetes mellitus is associated with various disorders of calcium (Ca) metabolism, such as impairment of Ca absorption [1,2] and loss of Ca from bone [3], which can be followed by the development of diabetic osteopenia [4–8]. The degree of osteopenia certainly depends on the quality of diabetic control [9,10]. Regarding the pathogenetic mechanism of diabetic osteopenia, greater loss of Ca into urine as a result of hyperglycemia and/or glycosuria has been proposed as a major cause of bone loss [11–13] by inducing secondary hyperparathyroidism. However, it should be emphasized that, in diabetics, bone formation does not increase sufficiently to compensate for an increase in bone resorption [14]. Therefore, impaired bone formation due to a deficiency of osteoblasts has recently been proposed as the most important factor [15,16]. This hypothesis is supportd by data indicating a decrease in serum levels of osteocalcin (OC) in diabetic patients [17,18], since OC is specifically produced by osteoblasts and is thus a clinically useful marker for osteoblast function [19]. It is not known whether a decrease in serum OC levels in diabetics derives from the loss of insulin stimulation of osteoblasts [14,20] or from defective cell function following long exposure to high glucose levels.

This review focuses on the effect of sustained high glucose condition on bone and mineral metabolism in the diabetic state, and in particular on the involvement of the polyol pathway.

Effect of Sustained High Glucose Condition on Bone Cell Function In Vitro

Effect of High Glucose on the Proliferation of Human Osteoblast-Like MG-63 Cells In Vitro

Sustained 7-day exposure to high glucose significantly inhibited growth of human osteoblast-like MG-63 cells in a dose-dependent manner up to 49.5 mM, as compared with cells maintained under normal glucose (5.5 mM) or a high mannitol condition (iso-osmolar control). It is of interest that the inhibitory effect of high glucose was partially reversed by the simultaneous addition of the aldose reductase inhibitors (ARI), epalrestat. High glucose also attenuated the insulin-like growth factor I (IGF-I)-induced stimulation of cell growth. Again the effect of glucose was not mimicked by mannitol, strongly suggesting a specific effect of glucose [21].

These observations suggested that high glucose per se may significantly impair the proliferation of osteoblasts in either the basal or IGF-I-stimulated condition, and that the inhibitory effect of high glucose may be in part explained by an intracellular accumulation of sorbitol.

Effect of High Glucose on the Responsiveness of MG-63 Cells to Parathyroid Hormone (PTH) and 1,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$)

Human PTH (1–34) induced a prompt rise in intracellular cAMP and cytosolic Ca^{2+} in MG-63 cells in a time- and dose-dependent manner. Sustained 7-day exposure to high glucose significantly impaired cellular responsiveness to human PTH (1–34) in either response as compared with cells maintained under normal glucose [22].

1,25-$(OH)_2D_3$, an active form of vitamin D_3, stimulated OC secretion from MG-63 cells in a time- and dose-dependent manner. Sustained 7-day exposure to high glucose significantly impaired 1,25-$(OH)_2D_3$-induced OC secretion from the cells in a concentration-dependent manner as compared with cells maintained under normal glucose. High glucose attenuated the 1,25-$(OH)_2D_3$-induced increase in the stationary level of OC mRNA. Furthermore, high glucose significantly decreased the 1,25-$(OH)_2D_3$ receptor content without any changes in the equilibrium constant for 1,25-$(OH)_2D_3$ [23]. The impairment of the response of MG-63 cells to either human PTH (1–34) or 1,25-$(OH)_2D_3$ was not mimicked by treatment with high mannitol, suggesting a specific effect of glucose.

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These observations suggest that high glucose per se may significantly impair the responsiveness of osteoblasts to two major hormones that activate osteoblasts, and thus may be one of major factors causing osteoblast dysfunction in diabetes mellitus.

Effect of High Glucose on the Generation of Multinucleated Osteoclast-Like Cells In Vitro

To determine whether high glucose may modulate the differentiation of osteoclasts from hematopoietic stem cells, the effect of high glucose on in vitro osteoclastogenesis was examined using the co-culture of murine bone marrow cells with a stromal cell line (ST2) in the presence of 1,25-$(OH)_2D_3$ and dexamethasone, as previously described [24]. High glucose significantly enhanced the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells. Again, high mannitol, an iso-osmolar control, did not show any effect, indicating the specific effect of glucose. These data suggest that high glucose per se may enhance the formation of osteoclasts in diabetic states.

Effect of a High Galactase Diet on Bone and Mineral Metabolism in Rats In Vivo and its Partial Reversal by the Simultaneous Administration of ARI

The importance of hyperglycemia in the development of diabetic complications has been demonstrated by studies of non-diabetic animals fed galactose [25]. Retinopathy [26,27] and neuropathy [28,29] that are morphologically indistinguishable from those seen in diabetic rats develop in galactose-fed rats in the absence of several metabolic and pathophysiological disorders that are characteristic of diabetes. Pharmacological inhibition of aldose reductase reportedly inhibits a variety of anatomical and pathophysiological abnormalities in galactose-fed rats [25]. Therefore, the effect of epalrestat, an ARI, on the abnormalities of bone metabolism in rats fed galactose was examined to investigate the role of hyperglycemia in the etiology of diabetic osteopenia in vivo, separately from insulin deficiency.

Weight gain was impaired in such rats, which was not altered by epalrestat. Galactose temporarily stimulated bone resorption at 1–2 months after the initiation of galactose feeding, as reflected by increased urinary excretion of pyridinoline (PYR) and deoxypyridinoline (DPYR). These increases were significantly lessened by epalrestat in a dose-dependent manner. Whereas a positive correlation between a bone formation marker (serum OC) and a bone resorption marker (urinary DPYR excretion) disappeared in galactose-fed rats, simultaneous administration of epalrestat restored the positive correlation between the two parameters.

Histomorphometric analysis of bone performed 6.5 months after galactose feeding showed that both cancellous bone volume and the number of osteoblasts in tibia, which were significantly suppressed by galactose feeding, were reversed to a significant extent by epalrestat.

These data together suggest that epalrestat may prevent the development of osteoblast dysfunction and attenuate the temporary increase in bone resorption induced by galactose feeding, with a resultant increase in bone volume. It was therefore hypothesized that an intracellular accumulation of galactitol, resulting from a sustained elevation of the serum galactose level, may be an important factor in the development of abnormal bone and mineral metabolism in galactose-fed rats. This suggests, in turn, that an intracellular accumulation of sorbitol resulting from sustained high glucose exposure is an important factor for the development of abnormal bone and mineral metabolism in the diabetic state.

Influence of Glycemic Control on Osteoblast Function in Male NIDDM Patients Without Overt Diabetic Complications or Insulin Deficiency

To further investigate the importance of a sustained high glucose condition on the perturbation of osteoblast function in man, the influence of glycemic control on biochemical markers for osteoblast function was examined in both the basal and 1,25-$(OH)_2D_3$-stimulated state. The non-insulin-dependent diabetes mellitus (NIDDM) patients enrolled were all male, below 60 years of age and without any overt complications, to avoid the influence of age, menstrual cycle, sex hormone level and diabetic complications. The other exclusion criteria were renal disease that may cause secondary hyperparathyroidism, endocrine disorder, liver disease, malnutrition, or any other disease or medication that could influence bone and mineral metabolism. None of the patients was insulin-deficient as reflected by normal or elevated urinary excretion of C-peptide immunoreactivity. During the stimulation of 9 NIDDM patients with oral administration of 1,25-$(OH)_2D_3$ at 2.0 μg/day for 6 consecutive days once at 2200 hours, the skeletal response was assessed by monitoring bone alkaline phosphatase (BALP), OC and carboxyterminal propeptide of human type 1 procollagen in serum drawn at 0800 hours after an overnight fast and 24-h urinary excretion of PYR and DPYR at days 1, 2, 4 and 7. As parameters of glycemic control, mean level of fasting blood sugar (mFBS) and HbA_{1c} were used. In the basal state, serum BALP tended to correlate negatively with HbA_{1c}, although not significantly. 1,25-$(OH)_2D_3$ administration increased the serum 1,25-$(OH)_2D$ level significantly by 2 days, followed by a significant reduction in serum intact PTH, indicating the relevance of the dose of 1,25-$(OH)_2D_3$ for osteoblast stimulation. A maximal incremental response of serum OC upon 1,25-$(OH)_2D_3$ administration was negatively correlated with both mFBS and HbA_{1c}. Furthermore, the magnitude of 1,25-$(OH)_2D_3$-induced bone resorption (maximal increase in

urinary DPYR excretion) and its ratio (maximal increase in urinary DPYR excretion/basal urinary DPYR excretion) were both significantly correlated in a negative manner with mFBS, although basal urinary excretion of DPYR per se was not. These data together suggest that sustained high glucose condition attenuated, possibly in a concentration-dependent manner, 1,25-$(OH)_2D_3$-induced stimulation of osteoblasts either to secrete OC or to transmit a signal to osteoclasts to resorb bone in NIDDM patients even without overt diabetic complications or insulin deficiency. Therefore, it was hypothesized that a sustained high glucose condition may be a major factor contributing to osteoblast dysfunction in NIDDM patients.

Discussion

We demonstrated that high glucose per se impaired the function of osteoblast-like MG-63 cells in vitro and that high glucose significantly enhanced the formation of TRAP-positive multinucleated cells in an in vitro osteoclastogenesis system using the co-culture of murine bone marrow cells with ST2 stromal cells. Epalrestat, an ARI, attenuated a temporary increase in bone resorption and restored osteoblast function in galactose-fed rats in vivo, and attenuated the inhibitory effect of high glucose on MG-63 cell growth in vitro. Histomorphometric analysis showed that the significant reduction in cancellous bone volume and the number of osteoblasts in tibia brought about by galactose feeding could be reversed to a significant extent by the simultaneous administration of epalrestat.

It was therefore suggested that glucose may have a harmful effect on bone and mineral metabolism through an accumulation of intracellular sorbitol. Furthermore, we provided evidence that, even in those without overt diabetic complications, osteoblast function was significantly impaired in NIDDM patients possibly due to sustained exposure to high glucose. Firstly, serum OC was indeed lower in NIDDM patients when compared with age- and sex-matched controls. Secondly, although not significant (probably due to the small number of subjects), serum BALP tended to correlate negatively with serum HbA_{1c}. Lastly, the incremental responses of urinary DPYR excretion and of serum OC during 1,25-$(OH)_2D_3$ stimulation correlated negatively with both these markers of glycemic control. These data together clearly demonstrate that sustained exposure to high glucose may be a major factor in explaining osteoblast function in diabetic patients.

A previous report demonstrated that BALP, a marker of bone formation, was a significant predictor of age-related reduction in BMD *Z*-score [30]. Reduced bone turnover resulting from osteoblast hypofunction, as reflected by a lower BALP value, is supposed to retard age-related bone loss. Although a reduction in bone turnover may attenuate age-related bone loss and thus result in a higher BMD *Z*-score [31], it may increase bone fragility, independently of BMD, by promoting the accumulation of fatigue microdamage. The repair of microdamage by bone remodelling normally keeps pace with its production, but if repair is incomplete or delayed for too long, fatigue damage will accumulate [32]. This factor may contribute to the risk of overt fracture [33] or to the occurrence of brittle bone [34] in the diabetic state.

In summary, it is suggested that a sustained high glucose condition per se certainly impairs osteoblast function and that a harmful effect of high glucose may at least in part be explained by an accumulation of intracellular sorbitol.

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