Protective Effect of an Aldose Reductase Inhibitor Against Bone Loss in Galactose-Fed Rats: Possible Involvement of the Polyol Pathway in Bone Metabolism

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Many patients with diabetes mellitus show a moderate reduction in bone mass. Our recent in vitro studies showed that sustained exposure of osteoblast-like MG-63 cells to high glucose by itself impairs their functions partly via the polyol pathway. To investigate the role of hyperglycemia in the etiology of diabetic osteopenia in vivo separately from insulin deficiency, we determined whether epalrestat, an aldose reductase (AR) inhibitor (ARI), lessens the abnormalities in calcium (Ca) metabolism in galactose-fed rats. Weight gain was impaired in the rats, which was not altered by epalrestat. Galactose feeding temporarily enhanced bone resorption as reflected by increased biochemical markers for bone resorption (urinary excretion of pyridinoline [PYR] and deoxypyridinoline [DPYR]) at 1 to 3 months, which were significantly decreased by epalrestat. Epalrestat also restored the positive correlation between a bone-formation marker (serum osteocalcin [OC]) and a bone-resorption marker (urinary DPYR excretion) at 6.5 months. Histomorphometric analysis of bone performed 6.5 months after galactose feeding showed that both the bone volume and osteoblast numbers in the tibia, which were significantly suppressed by galactose feeding, were partly restored to a significant extent by the simultaneous administration of epalrestat. In summary, epalrestat partially protected against the development of osteoblast dysfunction and reduced the temporary increase in biochemical markers for bone resorption induced by galactose feeding, with a resultant increase in bone volume, suggesting that the polyol pathway may be intimately involved in the development of abnormal bone metabolism in galactose-fed rats.

DIABETES MELLITUS is known as one of the major causes of secondary osteopenia.[1-5] The degree of osteopenia depends on the quality of diabetic control.[6,7] A greater loss of Ca into the urine caused by hyperglycemia and/or glycosuria followed by the development of secondary hyperparathyroidism is proposed as one of the major mechanisms of these metabolic abnormalities.[8-10] However, impaired bone formation due to a deficit of osteoblasts has also been recognized as the most important factor in the development of diabetic osteopenia.[11-17] We reported recently that sustained exposure to a high glucose concentration by itself is sufficient to impair the growth potential[18] and the responsiveness of osteoblast-like MG-63 cells to 1,25-dihydroxyvitamin D_3 (1, 25-$(OH)_2D_3$)[19] and parathyroid hormone (PTH),[20] and that poor glycemic control impairs the response of osteoblasts and osteoclasts to 1,25-$(OH)_2D_3$ in normoinsulinemic type 2 diabetic patients.[17] The mechanism was explained in part by a process involving aldose reductase (AR) as evidenced by the protective effect of an AR inhibitor (ARI), epalrestat.[21]

The importance of hyperglycemia in the development of diabetic complications has been demonstrated by studies using nondiabetic animals fed galactose.[22] Retinopathy[23,24] and neuropathy[25,26] morphologically indistinguishable from that found in diabetic rats develop in galactose-fed rats in the absence of several metabolic and pathophysiologic disorders, including insulin deficiency. The protective effect achieved by pharmacologic inhibition of AR against the development of these diabetic complications in galactose-fed animals clearly supports the importance of the polyol pathway.[26]

These findings prompted us to quantify the protective effects of an ARI, epalrestat, on the development of osteopenia in galactose-fed rats and particularly on the parameters of bone metabolism.

MATERIALS AND METHODS

Rats

Male 12-week-old Wistar rats (N = 40) purchased from Keari (Osaka, Japan) were caged individually with free access to food and water. They were maintained on a 12-hour light/dark cycle. They were divided into four groups of 10 rats each and maintained for 6.5 months according to the following protocol. Control rats (n = 10) were maintained on a regular MF diet (Oriental Yeast, Tokyo, Japan) containing 30% (wt/wt) cellulose (Asahi Kasei, Tokyo, Japan) and 20% (wt/wt) corn starch (Nihon Shokuhin Kako, Tokyo, Japan). Galactose-fed rats (n = 30) were fed a MF diet supplemented with 50% (wt/wt) galactose (Pfanstiehl Laboratories, Waukegan, IL). Galactose-fed rats were subdivided into three groups: an untreated group (galactose-fed rats, n = 10); a 0.1% (wt/wt) epalrestat (5-[(1Z, 2E)-2-methyl-3-phenylpropenylidene]-4-oxo-2-thioxo-3-thiazolidine acetic acid; Ono Pharmaceutical, Osaka, Japan)-treated group (n = 10); and a 0.3% (wt/wt) epalrestat-treated group (n = 10). Administration of epalrestat at a dose of 0.1% reportedly prevents the development of diabetic complications in galactose-fed rats and streptozotocin (STZ)-diabetic rats without any toxic effects.[27-29]

The rats were weighed biweekly and checked for general health and food intake. For monthly collection of 24-hour urine, the rats were placed in individual metabolic cages with free access to the same diet and water. After a 2-day acclimatization period, urine samples were collected for 24 hours in jars containing sodium azide and protease inhibitors. The rats were each fed one of the specified diets for 6.5 months during the study. After food deprivation overnight but with free access to water, the rats were bled by aortic puncture and killed under ether anesthesia. The blood was centrifuged and the serum was stored at −80°C for biochemical analysis. Tibiae were excised for bone histomorphometry.

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Biochemical Parameters

Serum calcium (Ca), phosphate (Pi), magnesium (Mg), creatinine (Cr), glucose, and albumin levels were measured by an autoanalyzer. The serum fructosamine level was measured by a conventional assay method. Serum PTH levels were measured using a rat PTH immunoradiometric assay kit (Nichols Institute, San Juan Capistrano, CA).[30] The serum osteocalcin (OC) level was measured with a commercial radioimmunoassay kit (Yamasa Shoyu, Chosi, Japan) using rat OC as a standard. Urinary Ca and Pi levels were measured by an autoanalyzer, and the urinary Cr level was measured by a method previously described.[31] Urinary albumin was assayed by conventional radioimmunoassay, and urinary pyridinoline (PYR) and deoxypyridinoline (DPYR) levels were measured by high-performance liquid chromatography as previously described.[32] Urinary excretion of Ca, PYR, and DPYR was corrected for urinary Cr excretion.

Histomorphometry of Right Proximal Tibia

Histomorphometric analysis of the right proximal tibia was performed as described previously.[33] Briefly, the proximal tibia metaphysis was fixed with 70% ethanol, dehydrated in graded concentrations of ethanol, defatted in acetone, and then embedded in methyl methacrylate. A frontal section of the proximal tibia metaphysis at 5-μm thickness was cut using a microtome (Supercut; Reichelt-Yung, Nussloch, Germany) and stained by Goldner's trichrome method. A digitizing image-analysis system (OsteoMeasure Version 2.2; OsteoMetric, Atlanta, GA) was used for static histomorphometric measurements of the secondary spongiosa of the proximal tibial metaphysis between 1 and 4 mm distal to the growth plate–epiphyseal junction. The measured parameters included total tissue area (TV), cancellous bone area (BV) and bone surface (BS), osteoid area and osteoid surface (OS), eroded surface (ES), and number of osteoblasts (N.Ob) and osteoclasts (N.Oc). From these values, BV/TV, OS/BS, N.Ob/BV, ES/BS, and N.Oc/BV were calculated.[34]

Statistical Analysis

Values are expressed as the mean ± SD unless otherwise indicated. Statistical analysis was performed by ANOVA with Fisher's paired least-significant difference (PLSD) test. A P level less than .05 was regarded as statistically significant.

RESULTS

Body Weight, Glycemia, and Food Consumption

Galactose-fed rats developed polyuria and polydipsia. The body weight of the control group increased significantly by 98% during the 6.5-month period. Administration of 0.3% epalrestat to control rats did not affect the weight gain or nutritional state (data not shown). Weight gain was significantly less in the galactose-fed groups, including those simultaneously administered epalrestat (Fig 1). Administration of epalrestat to galactose-fed rats did not affect weight gain during the experiment period. Serum glucose levels were significantly lower in galactose-fed rats versus controls. Serum fructosamine levels increased significantly by approximately 80% due to a persistent elevation in the serum level of galactose (Table 1). Epalrestat did not affect serum albumin, Cr, Ca, Pi, and Mg levels or urinary excretion of albumin in galactose-fed rats. Since bone metabolism is known to be affected by the nutritional state[35] and renal function,[36] these data appear to validate the protocol for evaluating the direct effect of ARI on bone metabolism in galactose-fed rats.

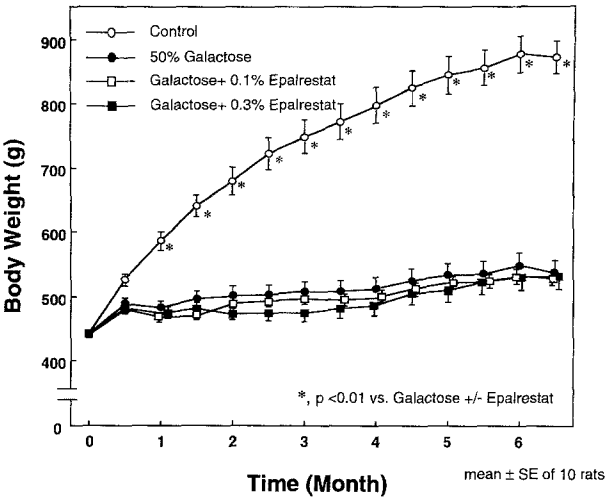


Fig 1. Changes in body weight every 2 weeks during the experiment. Wistar rats were aged 12 weeks at the beginning of the experiment (n = 10 per group). Weight gain was significantly impaired in galactose-fed rats by 1 month after initiation of galactose feeding. Statistical analysis was performed by ANOVA with Fisher's PLSD test.

Urinary Excretion of Ca, PYR, and DPYR

Figure 2 shows time-course changes in the urinary excretion of Ca, PYR, and DPYR during the experiment. Urinary DPYR showed an age-related increase in 12-week-old rats and declined thereafter. Urinary excretion of either Ca, PYR, or DPYR was significantly increased by 1 month after the initiation of galactose feeding, with a maximal increase of urinary PYR and DPYR excretion at 2 months. Epalrestat significantly reduced the galactose-induced increases in urinary excretion of Ca, PYR, and DPYR. The reduction appeared dose-dependent in terms of the urinary excretion of PYR and DPYR (Table 2).

Serum OC Level

The serum OC level determined 6.5 months after initiation of galactose feeding was 22.7 ± 9.59 ng/mL (mean ± SD) in control rats (n = 10) and 8.34 ± 3.33 ng/mL in galactose-fed rats (n = 10), suggesting that galactose feeding significantly suppressed serum OC levels. Administration of epalrestat to galactose-fed rats did not significantly affect serum OC levels (6.65 ± 5.46 ng/mL in 0.1% epalrestat-treated rats [n = 10] and 5.13 ± 4.58 ng/mL in 0.3% epalrestat-treated rats [n = 10]).

Restoration of a Positive Correlation Between Urinary DPYR Excretion and Serum OC

We next examined the coupling state between bone formation and resorption in a noninvasive way. Among the various biochemical markers, the relationship between the serum OC level and urinary DPYR excretion is the best biochemical index to estimate the coupling state of bone.[37] Urinary DPYR excretion was significantly correlated with the serum OC level in rats fed a normal diet, suggesting the existence of a coupling phenomenon between bone resorption and bone formation in normal rats (Fig 3A). However, in galactose-fed rats, the correlation between these two parameters disappeared (Fig 3B). Of interest, epalrestat seemed to improve osteoblast function in

Table 1. Serum Parameters at 6.5 Months After Initiation of Galactose Feeding

Parameter	Control (n = 10)	Galactose-Fed (n = 10)	Galactose-Fed + 0.1% Epalrestat (n = 10)	Galactose-Fed + 0.3% Epalrestat (n = 10)
Glucose (mg/dL)	180.5 ± 16.1	125.4 ± 29.4*	119.6 ± 13.2*	126.6 ± 12.0*
Fructosamine (μmol/L)	143.2 ± 11.8	250.9 ± 56.1*	273.5 ± 27.6*	277.4 ± 22.0*
Albumin (g/dL)	2.3 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.3
Cr (mg/dL)	0.55 ± 0.08	0.38 ± 0.06	0.41 ± 0.06	0.37 ± 0.05
PTH (pg/mL)	17.0 ± 5.9	16.3 ± 5.6	16.8 ± 3.9	18.7 ± 8.0
Ca (mEq/L)	5.1 ± 0.2	5.2 ± 0.1	5.3 ± 0.3	5.1 ± 0.2
P (mg/dL)	5.1 ± 0.9	5.9 ± 0.6	5.8 ± 0.3	6.0 ± 0.6
Mg (mg/dL)	2.5 ± 0.4	2.5 ± 0.3	2.5 ± 0.2	2.6 ± 0.3

NOTE. Data are the mean ± SD.
*$P < .05$ *v* control.

response to bone resorption, as evidenced by epalrestat's restoration of a positive correlation between these two parameters in galactose-fed rats (Fig 3C and D).

Bone Histomorphometric Analysis

Since it was suggested previously that epalrestat improves bone metabolism in galactose-fed rats both by restoring osteoblast function and by inhibiting bone resorption, we next examined the effect of epalrestat on bone histomorphometric parameters in the proximal tibia from rats maintained on each diet for 6.5 months. BV/TV declined by 60% in galactose-fed rats 6.5 months after initiation of the feeding (Fig 4). Epalrestat significantly attenuated this bone loss, although BV/TV was still less than the ratio in control rats. However, since the body weight, which is known to increase bone mineral content,[35] was significantly less in galactose-fed rats versus control rats, the reduction of body weight may explain why an ARI did not completely prevent the reduction in BV/TV caused by galactose feeding. A significant reduction in N.Ob/BV in galactose-fed rats was reversed by simultaneous administration of epalrestat. In contrast, epalrestat did not significantly increase N.Oc/BV in epalrestat-treated galactose-fed rats (Table 3).

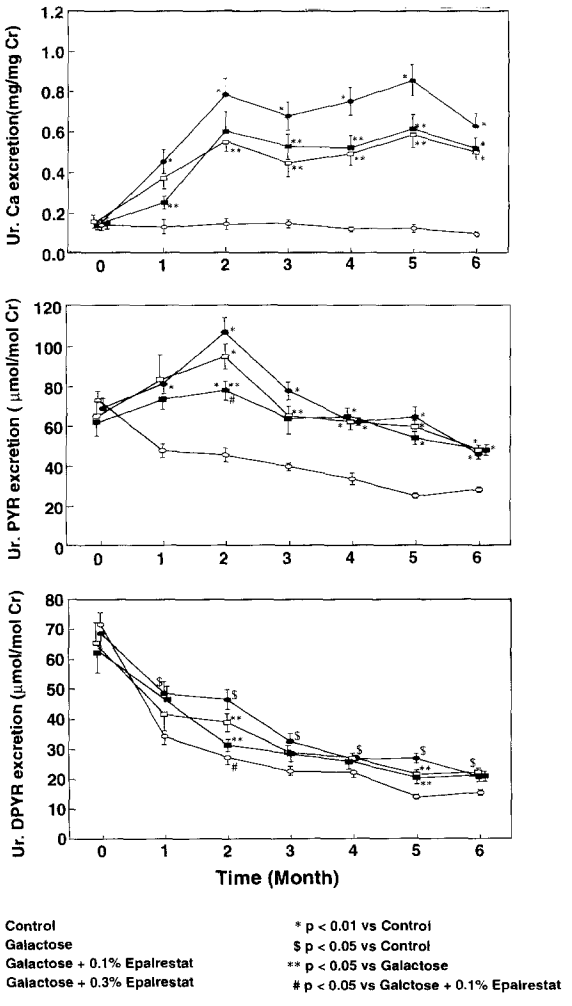


Fig 2. Changes in 24-hour urinary excretion of Ca, PYR, and DPYR during the experiment. Epalrestat significantly prevented galactose-induced increases in urinary excretion of Ca, PYR, and DPYR. Statistical analysis was performed by ANOVA with Fisher's PLSD test.

DISCUSSION

Galactosemia increases the tissue formation of polyol (galactitol) by an increase in the cellular uptake of galactose, as hyperglycemia causes intracellular accumulation of sorbitol, leading to the development of retinopathy[23,24] or neuropathy[25,26] that are morphologically indistinguishable from these conditions observed in diabetic rats. However, the process by which galactose enters the cells is independent of insulin, unlike the case for glucose. No less significant is the fact that galactose-fed animals do not show any abnormality in blood levels of insulin, free fatty acids, branched-chain amino acids, or fibrinogen or other disorders that are often associated with diabetes.[38] With these data as the background, the galactose-fed rat has thus served as a useful in vivo model for studying the role of the polyol pathway in the development of diabetic osteopenia.

The present study demonstrated that galactose impaired the osteoblast function to secrete OC in coordination with bone resorption, as reflected both by a significant suppression of serum OC levels in galactose-fed rats and by a disappearance of the close correlation of serum OC with urinary DPYR excretion. Furthermore, it was demonstrated that the impairment of osteoblast function was significantly ameliorated by epalrestat at a dose that reportedly protects against the development of diabetic complications.[27-29]

As in STZ-treated rats shortly after STZ treatment, the present study showed that the galactose-fed rat had a temporary increase of bone resorption 1 to 3 months after galactose feeding, as reflected by increased urinary excretion of Ca, PYR, and DPYR. As with hyperglycemia/hyperglycosuria in STZ-rats, galactosemia/galactosuria induced an increase of Ca loss

Table 2. Effect of Epalrestat on Biochemical Parameters of Bone Resorption

Parameter	Control (n = 10)	Galactose-Fed (n = 10)	Galactose-Fed + 0.1% Epalrestat (n = 10)	Galactose-Fed + 0.3% Epalrestat (n = 10)
Urinary Ca/Cr (mg/mg)	0.14 ± 0.08	0.78 ± 0.26*	0.55 ± 0.14*†	0.60 ± 0.34*
Urinary PYR/Cr (μmol/mol)	45.2 ± 10.7	106.4 ± 25.5*	94.4 ± 20.1*	77.3 ± 14.5*†‡
Urinary DPYR/Cr (μmol/mol)	27.2 ± 7.0	46.6 ± 10.0*	39.0 ± 9.3*†	31.4 ± 6.6†‡

(Mean ± SD).

*$P < .01$ *v* control.

†$P < .05$ *v* galactose-fed.

‡$P < .05$ *v* galactose-fed + 0.1% epalrestat.

into the urine followed by the development of secondary hyperparathyroidism in galactose-fed rats, which is assumed to be one of the major causes of a temporary stimulation of bone resorption. Of great interest, epalrestat significantly lessened the temporary increase in bone resorption induced by galactose feeding in vivo. The presence of AR enzyme is demonstrated in rat bone by immunocytochemistry.[39] No less important are the data that epalrestat did not affect serum glucose or fructosamine in galactose-fed rats. Neither the nutritional state (serum albumin and body weight) nor renal function (serum Cr and urinary excretion of albumin) could account for the beneficial effect of epalrestat, suggesting that epalrestat exerts a beneficial effect on bone metabolism in galactose-fed rats via AR inhibition. Supportive of this speculation is our recent finding that a sustained high-glucose condition enhances the formation of tartrate-resistant multinucleated giant cells in a long-term in vitro murine bone marrow culture system, and that this stimulation was not mimicked by an iso-osmolar control, ie, a high concentration of mannitol.[40]

Bone remodeling is a complex process that involves several cellular functions directed toward the coordinated resorption and formation of new bone. Bone formation is coordinated with bone resorption by coupling factors to maintain a mineralized bone matrix. The markers for bone resorption are the urinary excretion of Ca, PYR, and DPYR corrected for Cr excretion; among these, DPYR is the best marker, since it exists exclusively in bone tissue in significant amounts. Furthermore, it is best correlated with bone turnover measured by Ca kinetics[41] and bone histomorphometry.[42] OC, also called bone Gla-protein, is a small noncollagenous protein specific for bone tissue. Serum OC is known as a specific marker of bone formation even when formation and resorption are uncoupled.[43,44] Serum OC levels in galactose-fed rats were significantly suppressed, as they are in diabetic patients[17,45] and STZ-diabetic rats.[46] In contrast to its beneficial effect on high-glucose–exposed osteoblast-like MG-63 cells in vitro,[21] epalrestat did not significantly increase serum OC levels in galactose-fed rats in vivo. The finding that epalrestat did not increase serum OC in vivo seemed to result from lower bone turnover secondary to epalrestat-induced suppression of bone resorption 1 to 2 months after galactose feeding. To investigate this point, we examined the correlation between serum OC and urinary DPYR excretion, which is reportedly useful for assessing the coordination of bone formation and resorption noninvasively.[37]

The restoration by epalrestat of a positive correlation between serum OC and urinary DPYR excretion (Fig 3) clearly demonstrates that epalrestat improved osteoblast function at least in terms of OC secretion in coordination with bone resorption. This notion is supported by a histomorphological analysis showing that epalrestat partially prevented a galactose-induced reduction in the osteoblast number to a significant extent. These results suggested that epalrestat may partially restore osteoblast function by AR inhibition. Supportive of this

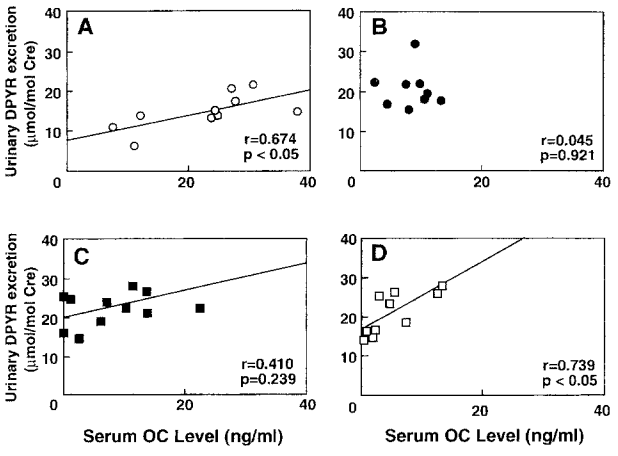
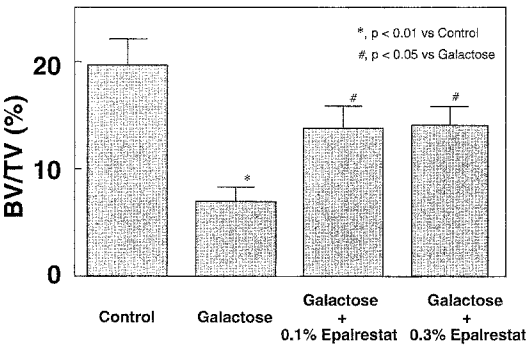


Fig 3. Correlation between a marker of bone formation (serum OC level) and a marker of bone resorption (urinary DPYR excretion) in control rats (A), rats fed 50% (wt/wt) galactose (B), rats fed 50% (wt/wt) galactose plus 0.1% (wt/wt) epalrestat (C), and rats fed 50% (wt/wt) galactose plus 0.3% (wt/wt) epalrestat (D). Epalrestat restored a positive correlation between the 2 markers in a dose-dependent manner in galactose-fed rats.



mean ± SE of 10 rats

Fig 4. Protective effect of epalrestat against the loss of cancellous bone volume (BV/TV) in galactose-fed rats. Histomorphometric analysis showed that cancellous bone volume in the proximal tibia declined 60% in galactose-fed rats 6.5 months after initiation of galactose feeding. The decline in bone volume was significantly lessened by epalrestat. Statistical analysis was performed by ANOVA with Fisher's PLSD test.

Table 3. Effect of Epalrestat on Bone Histomorphometry Parameters

Group	BV/TV (%)	OS/BS (%)	N.Ob/BV	ES/BS (%)	N.Oc/BV	Rm.S/BS (%)
Control	19.5 ± 8.39	7.89 ± 6.34	1.58 ± 0.42	0.46 ± 0.62	0.242 ± 0.090	8.35 ± 6.27
Galactose	6.84 ± 4.79*	7.18 ± 6.36	0.23 ± 0.48*	0.39 ± 0.66	0.055 ± 0.055	7.57 ± 6.04
Galactose + 0.1% epalrestat	13.6 ± 7.11†	7.39 ± 5.28	1.70 ± 1.46†	0.46 ± 0.01	0.304 ± 0.090	7.86 ± 5.65
Galactose + 0.3% epalrestat	12.3 ± 7.15	9.17 ± 8.28	1.57 ± 1.94†	0.45 ± 1.58	0.329 ± 0.231	9.23 ± 8.32

NOTE. Data are the mean ± SD of 10 rats.

Abbreviations: BV/TV, cancellous bone volume; OS/BS, osteoid surface per bone surface; N.Ob/BS, osteoblast number per bone volume; ES/BS, eroded surface per bone surface; N.Oc/BV, osteoclast number per bone volume; Rm.S/BS, remodeling surface per bone surface.

*$P < .05$ *v* control.

†$P < .05$ *v* galactose-fed.

notion, in addition to our reports, is the recent report that a reduction in the long-term change in the *z*-score for bone mineral density of the radius caused by suppression of bone turnover due to osteoblast dysfunction in type 2 diabetic patients is significantly negatively correlated with the severity of diabetic retinopathy,[14] in which the intracellular accumulation of sorbitol is known to play a major role. Since bone histomorphometric analysis was performed at 6.5 months after galactose feeding, and thus, the inhibitory effect of epalrestat on a galactose-induced temporary increase of bone resorption observed 1 to 3 months after galactose feeding was no longer significant at 6.5 months, the effect of epalrestat on the histological measures of resorption was not evident. As a result of its beneficial effect on impaired bone metabolism in galactose-fed rats, epalrestat partially prevented bone loss, particularly in trabecular bone, in galactose-fed rats (Fig 4). Although bone loss is usually evident in cortical bone but not in trabecular bone in patients with diabetes mellitus, galactose-fed rats showed a significant bone loss in trabecular bone. However, since bone loss is also evident in trabecular bone in STZ-diabetic rats,[39] the duration of diabetes seems more important to determine the location of bone loss rather than the nonspecific effect of galactose. It was previously reported that administration of an ARI, sorbinil, to STZ-diabetic rats for 3 months failed to improve the biochemical and bone histomorphometric abnormalities associated with diabetes.[39] In that study, the ARI tended to attenuate a glycosuria-induced increase of serum amino-terminal PTH 14 days after STZ injection. The method used in the study to measure the serum PTH level was less sensitive than that used in the present study. Furthermore, the duration of the previous experiment was not sufficient to permit the development of diabetic complications such as nerve and eye abnormalities.[47] These factors may explain the lack of a protective effect of an ARI against the development of osteopenia in STZ-diabetic rats. It is also possible that the cellular insults resulting from increased polyol formation and accumulation of glycation products are more severe with galactosemia than with hyperglycemia,[48] since AR has a nearly fourfold higher affinity for galactose than for glucose.[49] Furthermore, the relative insulin deficiency in STZ-diabetic rats might prevent the entry of glucose into bone cells, lessening the protective effect of an ARI. These speculations may explain, in part, the difference between the beneficial effect of an ARI in galactose-fed rats and its lack of apparent effect in STZ-diabetic rats.

In summary, the present study demonstrates that epalrestat prevented the development of osteoblast dysfunction and lessened the temporary increase in biochemical markers for bone resorption induced by galactose feeding, with a resultant increase in the volume of cancellous bone and the number of osteoblasts. Therefore, the present study clearly demonstrates that the polyol pathway is important for the development of galactose-induced abnormalities in bone metabolism. Therefore, it is suggested that diabetic osteopenia might be caused, in part, by bone cell injury through an accumulation of intracellular sorbitol, and thus, there is a possibility that ARIs might be clinically useful agents for the treatment of patients with diabetic osteopenia.

REFERENCES

1. Albright F, Reifenstein EC: Parathyroid Glands and Metabolic Bone Disease: Selected Studies. Baltimore, MD, Williams & Wilkins, 1948, pp 150-160
2. Levin ME, Boisseau VC, Avioli LV: Effect of diabetes mellitus on bone mass in juvenile and adult onset diabetes. N Engl J Med 294:241-244, 1976
3. Wu K, Schubeck KE, Frost HM: Haversian bone formation rates determined by a new method in human diabetes and osteoporosis. Calcif Tissue Res 6:204-219, 1970
4. Hough S, Avioli LV, Bergfeld MA, et al: Correction of abnormal bone and mineral metabolism in chronic streptozotocin-induced diabetes mellitus in the rat by insulin therapy. Endocrinology 108:2228-2234, 1981
5. Okuno Y, Nishizawa Y, Sekiya K, et al: Total and regional bone mineral content in patients with non–insulin dependent diabetes mellitus. J Nutr Sci Vitaminol 37:S43-S49, 1991 (suppl)
6. Imura H, Seino Y, Nakagawa S, et al: Diabetic osteopenia in Japanese—A geographic study. J Jpn Diabetes Soc 30:9929-9934, 1987
7. McNair P, Madsbad S, Christiansen C, et al: Bone loss in diabetes: Effects of metabolic state. Diabetologia 17:283-286, 1979
8. McNair P, Madsbad S, Christiansen C: Bone mineral loss in insulin treated diabetes mellitus; studies on pathogenesis. Acta Endocrinol (Copanh) 90:463-472, 1979
9. Gallagher JC, Melton LJ, Riggs BL: Examination of prevalence rates of possible risk factors in a population with a fracture of the proximal femur. Clin Orthop 153:158-165, 1980
10. Raskin P, Stevenson MR, Barilla DE, et al: The hypercalciuria of diabetes mellitus: Its amelioration with insulin. Clin Endocrinol (Oxf) 9:329-335, 1978
11. Silberberg R: The skeleton in diabetes mellitus; a review of the literature. Diabetes Res 3:329-338, 1986
12. Klein M, Frost HM: The numbers of bone resorption and formation in rib. Henry Ford Hosp Med Bull 12:527-536, 1964
13. Rico H, Hernandez ER, Cabranes JA, et al: Suggestion of a deficient osteoblastic function in diabetes mellitus. The possible cause of osteopenia in diabetics. Calcif Tissue Int 45:71-73, 1989

14. Krakauer JC, McKenna MJ, Buderer NF, et al: Bone loss and bone turnover in diabetes. Diabetes 44:775-782, 1995

15. Bouillon R, Bex M, Herck EV, et al: Influence of age, sex, and insulin on osteoblast function: Osteoblast dysfunction in diabetes mellitus. J Clin Endocrinol Metab 80:1194-1202, 1995

16. Verhaeghe J, Suiker AM, Van Bree R, et al: Increased clearance of 1,25-$(OH)_2D_3$ and tissue-specific responsiveness to 1,25-$(OH)_2D_3$ in diabetic rats. Am J Physiol 265:E215-E223, 1993

17. Inaba M, Nishizawa Y, Mita K, et al: Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-$(OH)_2D_3$ in patients with type 2 diabetes. Osteoporosis Int (in press)

18. Terada M, Inaba M, Yano Y, et al: Growth-inhibitory effect of a high glucose concentration on osteoblastic-like cells. Bone 22:17-23, 1998

19. Inaba M, Terada M, Koyama H, et al: Influence of high glucose on 1,25-dihydroxyvitamin D_3–induced effect on human osteoblast-like MG-63 cells. J Bone Miner Res 10:1050-1056, 1995

20. Yoshida O, Inaba M, Terada M, et al: Impaired response of human osteosarcoma (MG-63) cells to human parathyroid hormone induced by sustained exposure to high glucose. Miner Electrolyte Metab 21:2201-2204, 1995

21. Terada M, Inaba M, Yoshida O, et al: Diabetic osteopenia; impaired proliferation in human osteoblast-like cell, MG-63, by high glucose. J Bone Miner Res S351, 1994 (suppl 1, abstr)

22. Berry GT: The role of polyols in the pathophysiology of hypergalactosemia. Eur J Pediatr 154:S53-S64, 1995 (suppl 2)

23. Robison WG Jr, Nagata M, Laver N, et al: Diabetic-like retinopathy in rats prevented with an aldose reductase inhibitor. Exp Eye Res 50:355-366, 1990

24. Kern TS, Engerman RL: Galatose-induced retinal microangiopathy in rats. Invest Ophthalmol Vis Sci 36:490-496, 1995

25. Cameron NE, Cotter MA, Rebertson S, et al: Muscle and nerve dysfunction in rats with experimental galactosemia. J Exp Physiol 77:89-108, 1992

26. Mizisin AP, Powell HC: Schwann cell injury is attenuated by aldose reductase inhibition in galactose intoxication. J Neuropathol Exp Neurol 52:78-86, 1993

27. Shindo H, Tawata M, Aida K, et al: The role of cyclic adenosine 3′,5′-monophosphate and polyol metabolism in diabetic neuropathy. J Clin Endocrinol Metab 74:393-398, 1992

28. Suzuki E, Yasuda K, Miyazaki S, et al: ^{1}H-NMR analysis of nerve edema in the streptozotocin-induced diabetic rat. J Lab Clin Med 124:627-637, 1994

29. Itagaki I, Shimizu K, Kamanaka Y, et al: The effect of an aldose reductase inhibitor (Epalrestat) on diabetic nephropathy in rats. Diabetes Res Clin Pract 25:147-154, 1994

30. Finch JL, Rapp N, Martin KJ, et al: A new sensitive homologous radioimmunoassay for amino-terminal parathyroid hormone in the rat. J Bone Miner Res 7:229-233, 1992

31. Cook JGH: Creatinine assay in the presence of protein. Clin Chim Acta 32:485-486, 1971

32. Uebelhardt D, Duboeuf F, Meunier PJ, et al: Lateral dual photon absorptiometry: A new technique to measure the bone mineral density at the lumber spine. Bone Miner 8:87-96, 1990

33. Lane NE, Kimmel DB, Nilsson MHL, et al: Bone-selective analogs of human PTH(1-34) increase bone formation in an ovariectomized rat model. J Bone Miner Res 11:614-625, 1996

34. Parfitt AM, Drezner MK, Glorieux FH, et al: Bone histomorphometry: Standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Committee. J Bone Miner Res 2:595-610, 1987

35. Heaney RP: Nutrition and risk for osteoporosis, in Marcus R, Feldman D, Kelsey J (eds): Osteoporosis. San Diego, CA, Academic, 1996, pp 483-509

36. Imanishi Y, Koyama H, Inaba M, et al: Phosphorus intake regulates intestinal function and polyamine metabolism in uremia. Kidney Int 49:499-505, 1996

37. Nakayama K, Fukumoto S, Takeda S, et al: Differences in bone and vitamin D metabolism between primary hyperparathyroidism and malignancy-associated hypercalcemia. J Clin Endocrinol Metab 81:607-611, 1996

38. Engerman RL, Kern TS: Experimental galactosemia produces diabetic-like retinopathy. Diabetes 33:97-100, 1984

39. Glajchen N, Epstein S, Ismail F, et al: Bone mineral metabolism in experimental diabetes mellitus: Osteocalcin as a measure of bone remodeling. Endocrinology 123:290-295, 1988

40. Inaba M, Nishizawa Y, Shioi A, et al: Importance of high glucose condition in the development of diabetic osteopenia: Possible involvement of the polyol pathway. Osteoporos Int 7:S209-S212, 1997 (suppl 3)

41. Eastell R, Hampton L, Colwell A: Urinary collagen crosslinks are highly correlated with radioisotopic measurements of bone resorption, in Christiansen C, Overgaard K (eds): Proceedings of the Third International Symposium on Osteoporosis. Aalborg, Denmark, Osteopress, 1990, pp 469-470

42. Delmas PD, Schlemmer A, Gineyts E, et al: Urinary excretion of pyridinoline crosslinks correlates with bone turnover measured on iliac crest biopsy in patients with vertebral osteoporosis. J Bone Miner Res 6:639-644, 1991

43. Brown JP, Delmas PD, Malaval L, et al: Serum bone Gla-protein: A specific marker for bone formation in postmenopausal osteoporosis. Lancet 1:1091-1093, 1984

44. Delmas PD, Malaval L, Arlot M, et al: Serum bone gla-protein compared to bone histomorphometry in endocrine diseases. Bone 6:329-341, 1985

45. Palmeri E, Pedrazzoni M, Malaquinoo AM, et al: Osteocalcin levels in diabetes mellitus, in Christiansen C, Arnaud CD, Parfitt AM, et al (eds): Osteoporosis. Aalborg, Denmark, Aalborg Stiftsbogtrykkeri, 1984, pp 809-810

46. Ishida H, Seino Y, Taminato T, et al: Circulating levels and bone contents of bone γ-carboxyglutamic acid–containing protein are decreased in streptozotocin-induced diabetes: Possible marker for diabetic osteopenia. Diabetes 37:702-706, 1988

47. Tilton RG, Pugliese G, LaRose LS, et al: Discordant effects of the aldose reductase inhibitor, sorbinil, on vascular structure and function in chronically diabetic and galactosemic rats. J Diabetes Complications 5:230-237, 1991

48. Dvornik D, Millen J, Hicks D, et al: The pharmacokinetics of aldose reductase inhibitors, in Sakamoto N, Kinoshita JH, Kador PF, et al (eds): Polyol Pathway and Its Role in Diabetic Complications. The Netherlands, Amsterdam, Elsevier Science, 1988, pp 599-605

49. Sato S, Kador PF: NADPH-dependent reductases of the dog lens. Exp Eye Res 50:629-634, 1990