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Type 2 Diabetes Is Associated with Increased Bone Mineral Density in Mexican-American Women

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Background. Our purpose was to determine whether type 2 diabetes is associated with altered bone mineral density (BMD) and whether fasting serum insulin levels are correlated with BMD.

Methods. In a population-based family study of Mexican-Americans, we obtained measurements of BMD, diabetes status (by 2-h oral glucose tolerance test), obesity, and serum insulin concentrations in 600 subjects from 34 families. Analyses were stratified by sex and conditioned on the pedigree structure to account for residual correlations among related individuals.

Results. Women with diabetes had significantly higher BMD at hip than women without diabetes ($p = 0.03$) even after adjustment for age, body mass index (BMI), and menopause status. BMD at spine was also higher in diabetic women than in nondiabetic women, although the association was no longer statistically significant after adjustment for BMI. Diabetes was not associated with BMD in men. In nondiabetic men and women, insulin levels were significantly correlated with BMD after adjustment for age and other lifestyle covariates, but correlations were diminished and were no longer statistically significant after further adjustment for body mass index.

Conclusions. These results suggest that Mexican-American women with type 2 diabetes have higher BMD compared to their nondiabetic counterparts, with the association independent of obesity at hip, although not at spine or forearm. Increased BMD was also correlated with serum insulin levels, although this association was not independent of obesity. Longitudinal studies may be required to better define the mechanisms underlying the observed association between BMD and diabetes.

Key Words: Osteoporosis, Diabetes, Mexican-Americans, Insulin.

Introduction

The relationship between diabetes and osteoporosis is complex. Compared to nondiabetic controls, individuals with type 1 diabetes are at increased risk of fracture (1). This relationship is likely due at least in part to a corresponding decrease in bone mineral density (BMD) (2–8). In contrast, the relationship between type 2 diabetes and osteoporosis is less clear. In several (9,10) but not all (11–17) studies, fracture risk is observed to be lower in subjects with type 2 diabetes than in nondiabetic controls. In at least some studies, BMD is reported to be higher in individuals with type 2 diabetes than in those without (10,18–20).

Potential mechanisms that might lead to an increase in BMD in individuals with type 2 diabetes are not clear.

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Obesity is associated with both type 2 diabetes and BMD, but in at least some studies the association observed between type 2 diabetes and BMD remained even after accounting for differences in body mass index (BMI) between diabetic and nondiabetic subjects. Some investigators have hypothesized that hyperinsulinemia may contribute to higher BMD, and indeed a positive correlation between serum insulin levels and BMD has been reported in several studies (19,20). But this association is also complicated by the presence of obesity, because obesity-associated insulin resistance leads to compensatory hyperinsulinemia (21).

These prior observations provided the motivation for us to examine the relationship between type 2 diabetes and BMD in a large population-based sample of Mexican-Americans in whom we obtained measurements of BMD, diabetes status, and a large set of other cardiovascular risk factors including serum insulin levels. We hypothesized first that the association observed in certain prior studies between diabetes and BMD would also be apparent in our population-based sample of Mexican-Americans. Second, we speculated that long-term metabolic consequences of diabetes would accelerate bone loss and, therefore, that BMD would be inversely correlated with duration of diabetes. Finally, we hypothesized that long-standing hyperinsulinemia may play a role in increased BMD observed in diabetes, and that support for this hypothesis could be obtained by detecting a correlation among nondiabetic subjects between serum insulin concentrations and BMD.

Methods

To test the previously mentioned hypotheses, we studied subjects from the San Antonio Family Osteoporosis Study (SAFOS). The SAFOS was initiated in 1997 with the goal of identifying genetic and environmental determinants of bone mineral density in large Mexican-American families. Families recruited into the SAFOS were concurrently enrolled in a related study, the San Antonio Family Heart Study (SAFHS), initiated in 1991. Probands for these families were originally identified from a low-income neighborhood in San Antonio, TX, USA using a house-to-house recruitment procedure. Eligibility criteria for study probands were that they be 40–60 years of age and have large families in the San Antonio area. All first-, second-, and third-degree relatives of probands and probands' spouses were invited to participate; the invitation was extended regardless of the probands' (or relatives') medical history. Between 1991 and 1996, a total of 1,431 individuals from 41 large families were recruited into the SAFHS. Details of the sampling and recruitment procedures have been previously described (22).

In 1997, a subset of the 34 largest SAFHS families was invited to participate in SAFOS. Recruitment into SAFOS was held in conjunction with a 4- to 5-year follow-up examination of SAFHS families. Subjects participating in the follow-up phase of SAFHS received a medical examination in our clinic on the morning following a 12-h fast. Fasting blood samples were collected for determination of serum glucose and insulin levels. Serum was separated from clotted blood by centrifugation and then stored at −80°C until assayed. Diabetes was diagnosed using plasma glucose criteria of the World Health Organization (WHO) (23) and/or by self-reported use of antidiabetic medications. Those indicating that a physician had never previously told them that they had diabetes but who met WHO glucose criteria were considered to have newly diagnosed diabetes. Serum concentrations of insulin were measured by commercial radioimmunoassay (Diagnostic Products, Los Angeles, CA, USA). The coefficient of variation between duplicate aliquots measured in a single laboratory run was 6.5% for fasting insulin. Height and weight were measured with participants wearing no shoes. Body mass index (BMI) was calculated as weight (in kilograms [kg]) divided by height (in square meters [m^2]). All procedures were approved by the Institutional Review Board at the University of Texas Health Science Center at San Antonio (1997).

Bone mineral content was measured at spine (L1–L4), hip (intertrochanter), and forearm (ultradistal radius) using dual energy x-ray absorptiometer (DXA) (Hologic 1500W, Hologic, Inc., Bedford, MA, USA). Areal bone mineral density (BMD, g/cm^2) was determined by dividing bone mineral content (BMC, g) by projected area of region scanned (cm^2). Coefficients of variation for repeat *in vivo* measurements of spine (L2–L4), femoral neck, and femoral trochanter were 1.3, 3.9, and 2.0%, respectively. Variability in repeat measurements of phantoms is much lower.

A questionnaire was administered to obtain information on subject's medical history, medication use, dietary habits, physical activity patterns, and smoking and alcohol consumption behaviors. Calcium intake was assessed by a 104-item food frequency questionnaire (22). Diuretic use, including loop diuretics, thiazide diuretics, hydrochlorothiazide, osmotic diuretics, and other diuretics was coded as a binary variable based on the medication questionnaire.

Physical activity was assessed using a modified version of the Stanford 7-day physical activity recall instrument (24,25). Subjects reported the weekly number of hours they slept and engaged in moderately strenuous, heavy, and very heavy physical activities. Examples of activities corresponding to each category were provided to assist subject responses. Light physical activity was defined as the difference between total possible hours of weekly activity (i.e., 7 days × 24 h/day = 168 h) and number of hours accounted for by sleep and moderate, heavy, and very heavy activity. Each category of physical activity was scored in metabolic equivalents (or METS; one MET = energy expenditure of 1 kg of body weight per h) and expressed on a per day basis.

Because type 2 diabetes did not occur in any individuals <30 years of age, we restricted our analyses to individuals aged 30 years and older. Of 653 SAFOS participants aged 30 years or older, diabetes status could not be determined in

48 subjects due to a missing or incomplete oral glucose tolerance test. Bone mineral density measurements were not usable in an additional five subjects, leaving 600 individuals for whom both BMD and diabetes status were assessed.

Statistical analyses were conducted for men and women separately. Variance component models were used to partition variation in BMD into effects due to diabetes status and other individual-specific covariates including age, sex, and behavioral factors. We further conditioned these analyses on pedigree structure to allow for residual familial correlations in BMD between related individuals. Diabetes status was parameterized as a 0/1 dummy variable that allowed estimation of mean BMD in subjects with and without diabetes. Model parameters were estimated using maximum likelihood methods. Significance testing was performed by comparing the likelihood of the pedigree data under competing models using the SOLAR software package (San Antonio, TX, USA) (26). Specifically, we compared the likelihood of data under a full model in which effect of diabetes status was estimated to that of a nested model in which effect of diabetes status was constrained to be zero. Model likelihoods were compared by likelihood ratio test; twice the difference in logarithms of the model likelihoods is distributed as a chi-square distribution with degrees of freedom equal to the difference in parameters between the two models.

Among diabetic subjects, we tested in analogous fashion whether BMD differed between subjects with newly and previously diagnosed diabetes while adjusting for effects of current age and other covariates. Similarly, we tested whether BMD was higher among those using medications vs. diabetic subjects not under treatment with pharmacologic agents.

We next considered whether serum insulin levels were correlated with BMD. We rank-ordered men and women separately according to fasting insulin values and then compared mean BMD between those in the highest and lowest quartiles of the insulin distribution. In computing mean BMD levels in each quartile, we adjusted for age and other covariates using the variance component approach by creating dummy variables corresponding to highest or lowest insulin quartile. We tested whether insulin levels were significantly correlated with mean BMD by regressing insulin (the independent variable) against BMD (the dependent variable). In addition, we simultaneously estimated effects of age and other covariates (diuretics, smoking, alcohol consumption, physical activity, calcium intake, and in women, estrogen use and menopause status) on BMD by including them in the model as covariates. As carried out previously, significance testing was conducted using the likelihood ratio test by comparing the likelihood of a nested model (value of regression coefficient constrained to be zero) to that of a full model (value of regression coefficient estimated). Fasting insulin levels were transformed by their natural logarithms prior to these analyses to reduce skewness of the distribution.

Results

The study sample included 217 men and 383 women aged 30–96 years. Characteristics of these individuals are shown in Table 1. There were 55 men with diabetes and 162 without and 98 women with diabetes and 285 without. On average, diabetic men were 6.0 years older than nondiabetic men (54.3 vs. 48.3 years, $p = 0.005$) and diabetic women were 6.4 years older than their nondiabetic counterparts (54.8 vs. 47.4 years, $p < 0.001$). As expected, subjects with diabetes had higher body mass index than those without ($p = 0.006$

Table 1. Characteristics of study participants >30 years of age according to sex and diabetes status. The San Antonio Family Osteoporosis Study

	Men			Women		
Characteristic	Nondiabetic	Diabetic	*p*	Nondiabetic	Diabetic	*p*
n	162	55		285	98	
Age (years)	48.3 ± 13.7	54.3 ± 13.3	0.005	47.4 ± 12.6	54.8 ± 12.5	<0.001
Body mass index, kg/m^2	29.9 ± 6.3	33.0 ± 6.2	0.006	31.0 ± 6.6	35.2 ± 8.3	<0.001
Diuretic use, % (current)	0	12.2	<0.001	3.2	6.9	ns
Menopause status, %						
Hysterectomy	–	–		12.1	8.7	ns
Pre-menopausal	–	–		55.7	32.6	
Peri-menopausal	–	–		1.8	4.3	
Post-menopausal	–	–		30.4	54.3	
Estrogen use, % (current)	–	–		16.8	18.4	ns
Smoking, % (current)	29.0	31.5	ns	16.3	7.1	0.025
Alcohol use, % (current)	59.9	30.9	<0.001	30.6	9.2	<0.001
Physical activity (METS)	277 ± 60	263 ± 48	ns	254 ± 42	240 ± 19	ns
Calcium intake (mg/day)	948 ± 535	910 ± 563	ns	878 ± 401	852 ± 359	ns

ns = not significant.

and $p < 0.001$ in men and women, respectively). There was little difference in reported physical activity levels or calcium intake between diabetic and nondiabetic individuals. Men were more likely to smoke and to drink alcohol than women, although diabetic individuals were less likely than nondiabetic individuals to engage in these behaviors, with the exception that frequency of smoking differed little between diabetic and nondiabetic men. Diabetic women (who were slightly older) were more likely than nondiabetic women to have transitioned into menopause. The proportion of women reporting current use of estrogen did not differ significantly between those with and without diabetes.

Table 2 summarizes associations between diabetes and BMD at the hip, lumbar spine, and forearm in men and women separately. Three models are shown: in model 1, mean levels of BMD are adjusted for age and age squared; in model 2, means are adjusted for age, for an additional set of lifestyle, and, for women, reproductive history-related variables; and in model 3, means are adjusted for all model 2 variables as well as for body mass index. In men, there was no evidence for an association between diabetes and BMD at any site, and the lack of association persisted after adjusting for potential confounders. In contrast, BMD tended to be higher in diabetic women than in nondiabetic women, with this association achieving statistical significance at the hip ($p < 0.001$) and spine ($p = 0.003$) following adjustment for age, age squared, and various lifestyle and reproductive factors. The association between diabetes and BMD remained statistically significant even after further adjustment for body mass index at the hip ($p = 0.037$) but not at the spine ($p = 0.11$). At the hip, presence of diabetes was associated with 3.7% increase in BMD in women even after accounting for differences in body mass index and other covariates between diabetic and nondiabetic women.

Among diabetic subjects, we next examined whether BMD was correlated with duration of diabetes. First, we compared mean BMD, adjusted for age, age squared, and other covariates, between newly and previously diagnosed diabetic subjects. There were 15 men with newly diagnosed diabetes and 40 men with previously diagnosed diabetes; comparable numbers for women were 21 newly and 77 previously diagnosed cases of diabetes. There were no significant differences in BMD between those with newly diagnosed and previously diagnosed diabetes at any of the three sites in either men or women ($p > 0.10$ for all comparisons; data not shown). We further estimated the correlation between BMD and duration of diabetes in men and women and again observed no significant differences in either sex ($p > 0.10$ at all three sites; data not shown). Additional analyses revealed neither fasting glucose levels nor medication type (oral agents or insulin) as significantly associated with BMD.

We subsequently examined the relationship between BMD and serum insulin levels. We initially restricted analysis to nondiabetic subjects because insulin secretory capacity may decline in diabetic subjects as a secondary consequence of hyperglycemia. Table 3 shows mean BMD among nondiabetic individuals in the highest and lowest quartiles of insulin distribution and also beta coefficients describing the association between insulin levels and BMD adjusted for selected covariates. In both men ($n = 162$) and women ($n = 283$), insulin levels were significantly and positively correlated with BMD at the hip and forearm when adjusting for age, age squared, and various lifestyle and reproductive history variables (models 1 and 2, all p values <0.01). In women, insulin levels were also significantly correlated with BMD at the spine. However, when further adjustment was made in the analysis for BMI (model 3),

Table 2. Bone mineral density (g/cm^2) at the hip, spine, and forearm according to diabetes status in men and women. The San Antonio Family Osteoporosis Study

	Men			Women		
Site	Nondiabetic ($n = 162$)	Diabetic ($n = 55$)	% increase associated with diabetes	Nondiabetic ($n = 285$)	Diabetic ($n = 98$)	% increase associated with diabetes
Hip (intertrochanter)						
Model 1	1.309 ± 0.021	1.330 ± 0.032	1.60	1.207 ± 0.016	1.307 ± 0.024	8.28[c]
Model 2	1.309 ± 0.026	1.357 ± 0.034	3.67	1.211 ± 0.018	1.310 ± 0.024	8.18[c]
Model 3	1.299 ± 0.023	1.311 ± 0.031	0.92	1.217 ± 0.016	1.262 ± 0.022	3.70[a]
Spine (L1–L4)						
Model 1	1.063 ± 0.020	1.057 ± 0.030	−0.56	1.011 ± 0.014	1.071 ± 0.019	5.93[b]
Model 2	1.055 ± 0.024	1.050 ± 0.032	−0.47	1.012 ± 0.015	1.070 ± 0.019	5.73[b]
Model 3	1.048 ± 0.023	1.018 ± 0.031	−2.86	1.014 ± 0.014	1.043 ± 0.019	2.78
Forearm (ultradistal radius)						
Model 1	0.547 ± 0.008	0.535 ± 0.013	−2.19	0.463 ± 0.006	0.477 ± 0.008	3.02
Model 2	0.545 ± 0.010	0.542 ± 0.014	−0.55	0.463 ± 0.006	0.478 ± 0.008	3.24
Model 3	0.544 ± 0.010	0.532 ± 0.014	−2.20	0.464 ± 0.006	0.468 ± 0.008	0.86

Model 1 adjusted for age, age^2; model 2 = adjusted for model 1 covariates + diuretics, smoking, alcohol, physical activity, calcium intake, estrogen use, and menopause status; model 3 = adjusted for model 2 covariates + body mass index. [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

Table 3. Mean bone mineral density (g/cm^2) at the hip, spine, and forearm in nondiabetic men and women in the highest and lowest quartiles of the insulin distribution. The San Antonio Family Osteoporosis Study

	Men				Women			
	Mean BMD among those in:				Mean BMD among those in:			
Site	Highest insulin quartile (n = 40)	Lowest insulin quartile (n = 41)	β*	p	Highest insulin quartile (n = 70)	Lowest insulin quartile (n = 71)	β	p
Hip (intertrochanter)								
Model 1	1.368	1.220	0.101	<0.001	1.258	1.185	0.082	<0.001
Model 2	1.390	1.237	0.103	<0.001	1.215	1.136	0.080	<0.001
Model 3	1.313	1.278	0.017	0.58	1.174	1.168	0.034	0.11
Spine (L1–L4)								
Model 1	1.068	1.032	0.036	0.13	1.042	1.001	0.041	0.01
Model 2	1.066	1.030	0.034	0.15	0.984	0.933	0.044	0.007
Model 3	1.020	1.034	−0.056	0.07	0.968	0.947	0.022	0.23
Forearm (ultradistal radius)								
Model 1	0.568	0.520	0.036	<0.001	0.476	0.450	0.020	0.005
Model 2	0.575	0.526	0.038	<0.001	0.459	0.431	0.021	0.003
Model 3	0.560	0.537	0.021	0.09	0.447	0.443	0.001	0.89

Model 1 adjusted for age, age^2; model 2 = adjusted for model 1 covariates + diuretics, smoking, alcohol, physical activity, calcium intake, and, in women only, estrogen use and menopause status; model 3 = adjusted for model 2 covariates = body mass index. Means are for subjects in whom exposure is absent (i.e., nonsmokers, nondrinkers, those not on diuretics, and [in women] those not taking estrogens or having undergone menopause), or at mean level of continuously distributed covariate (age, physical activity level, calcium intake, and BMI). β* = beta coefficient describing change in BMD associated with a 1-unit increase in insulin level (measured on natural logarithm scale).

the strength of the correlation decreased substantially, and in every case the correlation was no longer statistically significant. These analyses were then repeated after including diabetic subjects not taking medications (26 men and 34 women) and the results were virtually unchanged. These results indicated that the correlation between insulin levels and BMD was largely dependent on obesity.

Discussion

Although there have been conflicting studies of the relationship between type 2 diabetes and elevated BMD, the majority have concluded that individuals with type 2 diabetes have equal (7,16,27–29), if not higher (10,18,30–33), BMD than nondiabetic control subjects. Results from our study in Mexican-Americans also support this overall conclusion, although we observed BMD to be significantly associated with diabetes in women only. The relationship of diabetes to bone health is complex and is probably age-dependent. Krakauer and colleagues have proposed that diabetes may interfere with normal accumulation of bone mass at younger ages, but may retard age-related bone loss at older ages (34). Mean age of our study population was 49.4 years, while mean age of diabetes onset was 46.9 years.

Perhaps the largest study to have examined the association between diabetes and BMD was the Rotterdam Study. In this study, diabetes was observed to be associated with a 3% increase in hip and spine BMD in both men and women (10). However, unlike our study, the Rotterdam Study focused on an elderly population (mean age 68 years) and used a single nonfasting glucose value taken 2 h after glucose load for diagnosis of diabetes rather than the standard fasting oral 2-h glucose tolerance test, as used in our study. In contrast to the Rotterdam Study, several previous studies, including ours, reported BMD to be more strongly associated with type 2 diabetes and BMD in women than in men. For example, in their study of a community-based population of older adults, Barrett-Connor and Holbrook reported BMD to be higher in diabetic than nondiabetic women at the spine, hip, and forearm, although at no site were there significant differences between diabetic and nondiabetic men (9). Furthermore, the association between diabetes and BMD observed in women could not be explained by differences in age, obesity, smoking, alcohol, exercise, or medication use. However, this study included only 41 men and 39 women with diabetes. El Miedany and colleagues also reported higher BMD in 40 postmenopausal women with diabetes compared to 40 nondiabetic control women, with no corresponding difference between diabetic and nondiabetic men (30). Although intriguing, these two studies included relatively small numbers of men, and it is possible that failure to detect associations between diabetes and BMD in men may be attributable to low power. Our study also included a relatively small number of men with diabetes—only 55, approximately one half the number of diabetic women.

Mechanisms that might account for an association between diabetes and BMD are not clear. Type 1 diabetes appears to be associated with increased bone turnover and lowered BMD, possibly arising from actions of inflammatory processes and/or growth factor deficiencies (35). These processes may interfere with acquisition of peak bone mass.

However, the role of hyperglycemia per se on bone turnover may be negligible. In our subjects with type 2 diabetes, we observed no correlation between BMD and either duration of diabetes or level of hyperglycemia. If longstanding diabetes does lead to decreased BMD, it is possible that the correlation is sufficiently small that it could not be detected in our sample.

The possibility that hyperinsulinemia could mediate in part an association between type 2 diabetes and elevated BMD has received considerable attention. The idea is attractive insofar as subjects with type 1 diabetes lack insulin and generally have reduced bone mass, while individuals with type 2 diabetes usually have an excess of insulin, at least in the early stages of the disease, and have been shown to have increased bone mass. Physiologically, insulin plays an important role in maintenance of normal bone formation (36). It promoted cell proliferation in bone cells (37,38) and may also affect bone metabolism indirectly because of its structural homology to IGF-1, allowing it to bind to either insulin receptor or insulin-like growth factor (IGF) receptor, both of which are expressed on osteoblasts (39). Both IGF-I and IGF-II are potent bone-stimulating growth factors (40,41) and have been shown to decrease collagen degradation and increase collagen synthesis in cultures of intact calvariae (42). Serum insulin levels have been correlated with BMD in several epidemiologic studies (19,43,44). Although diminished, the correlation between insulin and BMD in some of these studies remained after additional adjustment for obesity (19).

The relationship between insulin resistance and BMD may also be mediated through sex hormones due to their effects on bone metabolism. For example, estrogen replacement therapy slows the rate of bone loss (45) and also elevated levels of IGF-1 (46). Insulin resistance, hyperinsulinemia, and diabetes are associated with decreased levels of sex hormone-binding globulin (47,48), which in turn lead to higher free estrogen and testosterone levels and increased BMD. Post-menopausal Hispanic women with type 2 diabetes have also been shown to have higher levels of free testosterone and androstenedione (49). Prospectively, androgen levels have been shown to predict incident type 2 diabetes and change in visceral fat (50,51); therefore, it is possible that the relationship between type 2 diabetes and increased BMD is mediated through increased androgen levels. This may also explain some of the sex differences observed in the relationship between type 2 diabetes and BMD.

The relationship among obesity, diabetes, and BMD is potentially complex. On the one hand, obesity could be associated with elevated BMD strictly by the mechanical force of increased load to the skeleton. However, it has also been suggested that the effect of obesity on BMD may extend beyond this mechanical aspect and have more to do with the biological effects of fat mass tissue on BMD (52). For example, increased fat mass could be the initiating factor in stimulation of osteoblast activity and bone loss retardation by setting off a chain of events that includes hyperinsulinemia and its ensuing effects on sex hormones.

The majority of previous studies that evaluated the relationship between diabetes and osteoporosis were conducted in Caucasian populations. The relationship between diabetes and osteoporosis risk may have particular relevance for Mexican-Americans, in whom the prevalence of diabetes is especially high (25.5% in subjects aged 30 years and older in our study). Mexican-American women appear to be at lower risk than non-Hispanic white women for hip fracture (53–56), which may be accounted for in part by their having a relatively higher BMD (57). However, it is not yet clear whether these two observations are in any way related. It may be relevant that the high prevalence of diabetes in Mexican-Americans is also accompanied by a high frequency of insulin resistance with concomitant hyperinsulinemia (58). Consequently, we were able to evaluate the relationship of BMD with insulin levels in study participants across a wide range of insulin values. Our results confirm an association between hyperinsulinemia and elevated BMD, although this association was not independent of BMI. Either changes in BMI mediate an effect of insulin on BMD, or insulin may merely be a marker for some other osteogenic factor. Finally, an important limitation of our study design was that we measured BMD at only a single point in time, and an individual's BMD at any point in time will be a function of factors influencing his or her acquisition of peak bone mass occurring in early adulthood as well as factors influencing bone turnover. We also did not perform x-rays of measured sites to identify other processes affecting bone density. A more thorough evaluation of the effects of insulin and/or diabetes on bone may require longitudinal examination of the effects of these variables on change in BMD.

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References

1. Kelsey JL, Browner WS, Seeley DG, Nevitt MC, Cummings SR. Risk factors for fractures of the distal forearm and proximal humerus. The Study of Osteoporotic Fractures Research Group. Am J Epidemiol 1992;135:477–489.
2. Mathiassen B, Nielsen S, Ditzel J, Rodbro P. Long-term bone loss in insulin-dependent diabetes mellitus. J Intern Med 1990;227:325–327.
3. Mathiassen B, Nielsen S, Johansen JS, Hartwell D, Ditzel J, Rodbro P, Christiansen C. Long-term bone loss in insulin-dependent diabetic patients with microvascular complications. J Diabet Complications 1990;4:145–149.
4. Compston JE, Smith EM, Matthews C, Schofield P. Whole body composition and regional bone mass in women with insulin-dependent diabetes mellitus. Clin Endocrinol (Oxf) 1994;41:289–293.

5. Kayath MJ, Dib SA, Vieiaa JG. Prevalence and magnitude of osteopenia associated with insulin-dependent diabetes mellitus. J Diabet Complications 1994;8:97–104.
6. Forst T, Pfutzner A, Kann P, Schehler B, Lobmann R, Schafer H, Andreas J, Bockisch A, Beyer J. Peripheral osteopenia in adult patients with insulin-dependent diabetes mellitus. Diabet Med 1995;12:874–879.
7. Tuominen JT, Impivaara O, Puukka P, Ronnemaa T. Bone mineral density in patients with type 1 and type 2 diabetes. Diabetes Care 1999; 22:1196–1200.
8. Muñoz-Torres M, Jodar E, Escobar-Jiménez F, López-Ibarra PJ, Luna JD. Bone mineral density measured by dual X-ray absorptiometry in Spanish patients with insulin-dependent diabetes mellitus. Calcif Tissue Int 1996;58:316–319.
9. Barrett-Connor E, Holbrook TL. Sex differences in osteoporosis in older adults with non-insulin-dependent diabetes mellitus. JAMA 1992; 268:3333–3337.
10. Van Daele PL, Stolk RP, Burger H, Algra D, Grobbee DE, Hofman A, Birkenhager JC, Pols HA. Bone density in non-insulin-dependent diabetes mellitus. The Rotterdam Study. Ann Intern Med 1995;122: 409–414.
11. Isaia G, Bodrato L, Carlevatto V, Mussetta M, Salamano G, Molinatti GM. Osteoporosis in type II diabetes. Acta Diabetol Lat 1987;24: 305–310.
12. Forsen L, Meyer HE, Midthjell K, Edna TH. Diabetes mellitus and the incidence of hip fracture: results from the Nord-Trondelag Health Survey. Diabetologia 1999;42:920–925.
13. Heath H III, Melton LJ III, Chu CP. Diabetes mellitus and risk of skeletal fracture. N Engl J Med 1980;303:567–570.
14. Meyer HE, Tverdal A, Falch JA. Risk factors for hip fracture in middle-aged Norwegian women and men. Am J Epidemiol 1993;137:1203–1211.
15. Kwon DJ, Kim JH, Chung KW, Kim JH, Lee JW, Kim SP, Lee HY. Bone mineral density of the spine using dual energy X-ray absorptiometry in patients with non-insulin-dependent diabetes mellitus. J Obstet Gynaecol Res 1996;22:157–162.
16. Wakasugi M, Wakao R, Tawata M, Gan N, Koizumi K, Onaya T. Bone mineral density measured by dual energy x-ray absorptiometry in patients with non-insulin-dependent diabetes mellitus. Bone 1993;14:29–33.
17. Sosa M, Domínguez M, Navarro MC, Segarra MC, Hernández D, de Pablos P, Betancor P. Bone mineral metabolism is normal in non-insulin-dependent diabetes mellitus. J Diabetes Complications 1996;10:201–205.
18. Rishaug U, Birkeland KI, Falch JA, Vaaler S. Bone mass in non-insulin-dependent diabetes mellitus. Scand J Clin Lab Invest 1995;55:257–262.
19. Stolk RP, Van Daele PL, Pols HA, Burger H, Hofman A, Birkenhager JC, Lamberts SW, Grobbee DE. Hyperinsulinemia and bone mineral density in an elderly population: the Rotterdam Study. Bone 1996;18:545–549.
20. Barrett-Connor E, Kritz-Silverstein D. Does hyperinsulinemia preserve bone? Diabetes Care 1996;19:1388–1392.
21. Bogardus C, Lillioja S, Mott D, Reaven GR, Kashiwagi A, Foley JE. Relationship between obesity and maximal insulin-stimulated glucose uptake *in vivo* and *in vitro* in Pima Indians. J Clin Invest 1984;73:800–805.
22. Mitchell BD, Kammerer CM, Blangero J, Mahaney MC, Rainwater DL, Dyke B, Hixson JE, Henkel RD, Sharp RM, Comuzzie AG, VandeBerg JL, Stern MP, MacCluer JW. Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. Circulation 1996;94:2159–2170.
23. World Health Organization Expert Committee Second Report on Diabetes Mellitus 1980.
24. Blair SN, Haskell WL, Ho P, Paffenbarger RS Jr, Vranizan KM, Farquhar JW, Wood PD. Assessment of habitual physical activity by a seven-day recall in a community survey and controlled experiments. Am J Epidemiol 1985;122:794–804.
25. Sallis JF, Haskell WL, Wood PD, Fortmann SP, Rogers T, Blair SN, Paffenbarger RF Jr. Physical activity assessment methodology in the Five-City Project. Am J Epidemiol 1985;121:91–106.
26. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198–1211.
27. Giacca A, Fassina A, Caviezel F, Cattaneo AG, Caldirola G, Pozza G. Bone mineral density in diabetes mellitus. Bone 1988;9:29–36.
28. Weinstock RS, Goland RS, Shane E, Clemens TL, Lindsay R, Bilezikian JP. Bone mineral density in women with type II diabetes mellitus. J Bone Miner Res 1989;4:97–101.
29. Hampson G, Evans C, Petitt RJ, Evans WD, Woodhead SJ, Peters JR, Ralston SH. Bone mineral density, collagen type 1 alpha 1 genotypes and bone turnover in premenopausal women with diabetes mellitus. Diabetologia 1998;41:1314–1320.
30. El Miedany YM, El Gaafary S, El Baddini MA. Osteoporosis in older adults with non-insulin-dependent diabetes mellitus: is it sex related? Clin Exp Rheumatol 1999;17:561–567.
31. Meema HE, Meema S. The relationship of diabetes mellitus and body weight to osteoporosis in elderly females. Can Med Assoc J 1967;96: 132–139.
32. De Leeuw I, Abs R. Bone mass and bone density in maturity-type diabetics measured by the 125I photon-absorption technique. Diabetes 1977;26:1130–1135.
33. Johnston CC Jr, Hui SL, Longcope C. Bone mass and sex steroid concentrations in postmenopausal Caucasian diabetics. Metabolism 1985; 34:544–550.
34. Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM. Bone loss and bone turnover in diabetes. Diabetes 1995;44: 775–782.
35. Ziegler R. Diabetes mellitus and bone metabolism. Horm Metab Res 1992;26(Suppl):90–94.
36. Thomas DM, Ng KW, Best JD. Insulin and bone: a clinical and scientific review. Endocrinol Metabol 1997;4:5–17.
37. Hickman J, McElduff A. Insulin promotes growth of the cultured rat osteosarcoma cell line UMR-106-01: an osteoblast-like cell. Endocrinology 1989;124:701–706.
38. Heinze E, Vetter U, Voigt KH. Insulin stimulates skeletal growth *in vivo* and *in vitro* comparison with growth hormone in rats. Diabetologia 1989;32:198–202.
39. Pun KK, Lau P, Ho PW. The characterization, regulation, and function of insulin receptors on osteoblast-like clonal osteosarcoma cell line. J Bone Miner Res 1989;4:853–862.
40. Ernst M, Froesch ER. Osteoblastlike cells in a serum-free methylcellulose medium form colonies: effects of insulin and insulinlike growth factor I. Calcif Tissue Int 1987;40:27–34.
41. Verhaeghe J, Suiker AM, Visser WJ, Van Herck E, Van Bree R, Bouillon R. The effects of systemic insulin, insulin-like growth factor-I and growth hormone on bone growth and turnover in spontaneously diabetic BB rats. J Endocrinol 1992;134:485–492.
42. McCarthy TL, Centrella M, Canalis E. Regulatory effects of insulin-like growth factors I and II on bone collagen synthesis in rat calvarial cultures. Endocrinology 1989;124:301–309.
43. Haffner SM, Bauer RL. The association of obesity and glucose and insulin concentrations with bone density in premenopausal and postmenopausal women. Metabolism 1993;42:735–738.
44. Reid IR, Evans MC, Cooper GJ, Ames RW, Stapleton J. Circulating insulin levels are related to bone density in normal postmenopausal women. Am J Physiol 1993;265:E655–E659.
45. Doren M, Samsioe G. Prevention of postmenopausal osteoporosis with oestrogen replacement therapy and associated compounds: update on clinical trials since 1995. Hum Reprod Update 2000;6:419–426.
46. Dawson-Hughes B, Stern D, Goldman J, Reichlin S. Regulation of growth hormone and somatomedin-C secretion in postmenopausal women: effect of physiological estrogen replacement. J Clin Endocrinol Metab 1986;63:424–432.
47. Khaw KT, Barrett-Connor E. Fasting plasma glucose levels and endogenous androgens in non-diabetic postmenopausal women. Clin Sci (Colch) 1991;80:199–203.

48. Nestler JE. Sex hormone-binding globulin: a marker for hyperinsulinemia and/or insulin resistance? J Clin Endocrinol Metab 1993;76:273–274.
49. Phillips GB, Tuck CH, Jing TY, Boden-Albala B, Lin IF, Dahodwala N, Sacco RL. Association of hyperandrogenemia and hyperestrogenemia with type 2 diabetes in Hispanic postmenopausal women. Diabetes Care 2000;23:74–79.
50. Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB. Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men- prospective results from the Massachusetts Male Aging Study. Diabetes Care 2000;23:490–494.
51. Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY. Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. Int J Obes Relat Metab Disord 2000;24:485–491.
52. Reid IR, Plank LD, Evans MC. Fat mass is an important determinant of whole body bone density in premenopausal women but not in men. J Clin Endocrinol Metab 1992;75:779–782.
53. Silverman SL, Madison RE. Decreased incidence of hip fracture in Hispanics, Asians, and blacks. California Hospital Discharge Data. Am J Public Health 1988;78:1482–1483.
54. Lauderdale DS, Jacobsen SJ, Furner SE, Levy PS, Brody JA, Goldberg J. Hip fracture incidence among elderly Hispanics. Am J Public Health 1998;88:1245–1247.
55. Bauer RL. Ethnic differences in hip fracture: a reduced incidence in Mexican Americans. Am J Epidemiol 1988;127:145–149.
56. Bauer RL, Deyo RA. Low risk of vertebral fracture in Mexican American women. Arch Intern Med 1987;147:1437–1439.
57. Looker AC, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP, Johnston CC Jr, Lindsay RL. Proximal femur bone mineral levels of US adults. Osteoporos Int 1995;5:389–409.
58. Haffner SM, Stern MP, Hazuda HP, Pugh JA, Patterson JK. Hyperinsulinemia in a population at high risk for non-insulin-dependent diabetes mellitus. N Engl J Med 1986;315:220–224.