Fracture is additionally attributed to hyperhomocysteinemia in men and premenopausal women with type 2 diabetes

Jianbo Li1,2*, Hongman Zhang1, Lingfei Yan1, Min Xie1, Jiawei Chen1
1Department of Endocrinology and Metabolism, The First Affiliated Hospital of Nanjing Medical University, Nanjing, and 2Department of Endocrinology and Metabolism, The Affiliated Jiangsu Shenze Hospital of Nanjing Medical University, Suzhou, China

Keywords
Fracture, Homocysteine, Men and premenopausal women with type 2 diabetes

*Correspondence
Jianbo Li Tel: 0086-25-83718836 Fax: 0086-25-83716602 E-mail address: ljbjlx18@aliyun.com

J Diabetes Invest 2014; 5: 236–241
doi: 10.1111/jdi.12149

ABSTRACT

Aims/Introduction: Data on hyperhomocysteinemia in relation to fractures in diabetes are limited. We aimed to explore the relationship between plasma total homocysteine concentrations and fractures in men and premenopausal women with type 2 diabetes.

Materials and Methods: Diabetic and control participants (n = 292) were enrolled in a cross-sectional hospital-based study. Bone mineral density and fractures were documented by dual energy X-ray absorptiometry and X-ray film, respectively. Plasma total homocysteine concentrations were measured using fluorescence polarization immunoassay. Risk factors for low bone mineral density or fractures and determinants of homocysteine were obtained from blood samples and the interviewer questionnaire.

Results: Plasma total homocysteine levels were higher in diabetic participants with fractures than without (8.6 [2.1] μmol/L vs 10.3 [3.0] μmol/L, P = 0.000). Diabetic participants with fractures had similar bone mineral densities as control participants. The association of homocysteine with the fracture was independent of possible risk factors for fractures (e.g., age, duration of diabetes, glycated hemoglobin, body mass index, thiazolidinediones and retinopathy) and determinants of homocysteine concentration (e.g., age, sex, serum folate and vitamin B12, renal status and biguanide use; odds ratio 1.41, 95% confidence interval 1.05–2.03, P = 0.020). Furthermore, per increase of 5.0 μmol/L plasma homocysteine was related to the fracture, after controlling for per unit increase of other factors (odds ratio 1.42, 95% confidence interval 1.12–1.78, P = 0.013).

Conclusions: Plasma total homocysteine concentration is independently associated with occurrence of fractures in men and premenopausal women with type 2 diabetes. Future prospective studies are warranted to clarify the relationship.

INTRODUCTION

Type 2 diabetes has become a major public health problem1. Type 2 diabetes is a potential cause of compromised bone mechanical properties, and might increase a risk for a fracture2. Once a fracture has occurred, healing is delayed. However, the underlying pathogenesis of fractures in diabetes has not been well defined. This is important to the predication and intervention of fracture among disorders associated with diabetes.

Homocysteine is associated with decreases on bone blood flow and biomechanical properties3. Hyperhomocysteinemia results in increasing production of oxidation products, homocysteine thiolactone and homocysteine mixed disulphides, which can damage the endothelium by excessive sulphation of connective tissues4,5. As such, fractures might increase. Furthermore, hyperhomocysteinemia is more common in patients with type 2 diabetes6,7. Therefore, the hypothesis that homocysteine might act as a factor, and even as a predictor of diabetes-associated bone strength and fractures, deserves consideration.

There are some determinants of plasma homocysteine levels8. Homocysteine concentration is closely related to folate or renal status in the elderly9. Homocysteine metabolism is dependent on vitamin B12 and folate concentration, or on betaine in men10. In addition, other factors, such as lifestyles (e.g., smoking,
alcohol and coffee intake) and drugs, can affect plasma homocysteine levels. Recently, bone mineral density and fracture risk have been studied in diabetes. However, a meticulous study on homocysteine in association with fracture risks in Chinese men and premenopausal women with type 2 diabetes is limited. For this reason, we explored the relationship.

**MATERIALS AND METHODS**

**Diabetes Status and Participant Selection**

Diabetes status was biochemically confirmed in patients according to the World health Organization diagnostic criteria for the classification of diabetes. Chinese men and premenopausal women with regular menstruation (aged 20 years to premenopause) with type 2 diabetes registered consecutively as outpatients from The First Affiliated Hospital of Nanjing Medical University, Nanjing, China (tertiary care hospital) between May 2010 and July 2011 were routinely examined for bone density and screened for recently occurring fractures (within 4–6 weeks) with a negative history of prior fractures. Healthy participants from the hospital health check-up center were examined for their bone density. The participants were mainly residents from seven districts of the local city (Nanjing), and divided into three groups with some variables matched among: the healthy control, diabetes with fractures and without. They all signed informed consent, and the study was approved by the hospital and university scientific and ethics committee. Patients with severe renal dysfunction (creatinine >147 mmol/L), severe liver disease (e.g., aspartate aminotransferase or alanine aminotransferase >3 times the normal level), heart failure New York Heart Association class III or IV, and any other conditions (hypercortisolism, hyperparathyroidism, hypogonadism, hyperthyroidism, etc.) or drugs (glucocorticoids, sex steroids, warfarin, bisphosphonates, etc.) related to affecting bone mineral density or fractures were excluded. We also eliminated injury-associated fractures. All prior fractures were excluded through questionnaire and X-ray tests or magnetic resonance imaging if necessary. The selected patients were documented on their previous regimen of hypoglycemic agents or insulin during the present study: 83% were on metformin (biguanide), 45% were on thiazolidinediones, 77% were on sulfonylureas, 30% were on insulin and 89% were on more than one of these agents. In addition, they continued the use of antihypertensive and lipid regulating agents if necessary.

**Bone Assessment**

Bone mineral density of all participants was measured using dual energy X-ray absorptiometry (QDR Discovery, Hologic, Inc., Bedford, MA, USA) by the same qualified examiner who was blinded to other data of the present study. Participant positioning and scan analysis procedures were standardized for all scans with coefficient variation <0.01, bone mineral density (g/cm²) at lumbar spine (L1 to L4) and the femoral neck were collected and analyzed. Fracture status was assessed at the site of the lumbar spine and hip using X-ray films. In all participants, conventional thoracic and spinal radiographs, and hip radiographs in lateral and anteroposterior projections were obtained. In situations where X-ray alone was insufficient, magnetic resonance imaging was carried out. A fracture was diagnosed if 20% or more reduction in the site of the bone tested was observed by two investigators who were blinded to each other’s readings.

**Clinical Feature Measurement**

Bodyweight was measured on the same scales in light clothing and no shoes before breakfast, and upright height was measured on the same wall-mounted stadiometer. Individual body mass index (BMI) was then calculated as weight (kg)/height (m)². Smoking status, total calcium intake, alcohol intake, history of myocardial infarction, stroke and parental history of hip fractures were documented through questionnaire. Physical activities were classified on the basis of frequency, and duration of mild, moderate and strenuous activities in the prior weeks. Kilocalories of energy expended was calculated (metabolic equivalent [MET] score = kcal h/week/kg). Retinopathy and neuropathy were screened using stereoscopic retinal photographs or electromyography, respectively.

**Blood Homocysteine and Other Biochemical Parameter Measurement**

Fasting plasma homocysteine concentration was measured using a commercially available fluorescence polarization immunoassay (AXSYM; Abbott Diagnostics, Abbott Park, IL, USA). This was repeated twice in all participants. Fasting plasma glucose and serum creatinine were measured using an automatic analyzer (AU5400; Olympus, Tokyo, Japan). Fasting plasma insulin, serum osteocalcin and serum testosterone for men were measured using chemiluminescence immunoassay (ROCHE E170; Roche, Basel, Switzerland). Glycated hemoglobin (HbA1c) was measured using high-performance liquid chromatogram (Bio-Rad D10; Bio-Rad, Berkeley, CA, USA). Serum C-reactive protein (CRP) was assessed using nephelometry (Siemens BNII; Siemens, Munich, Germany). Serum folate and vitamin B₁₂ were determined using automated test assays (Access; Beckman, Brea, CA, USA). Urinary albumin concentration was measured using immunonephelometry (DCA 2000; Bayer, Leverkusen, Germany). Urinary creatinine concentration was measured using an alkaline picrate method. Individual urinary albumin to creatinine ratio was then calculated as albumin (mg)/creatinine (g).

**Statistical Analysis**

We used SPSS version 13 for Windows software (SPSS Inc., Chicago, IL, USA) for statistical analysis. Data were expressed as median with 25th and 75th quartiles for skewed data or mean (standard deviation) for normally distributed data. Non-normally distributed variables were log-transformed before analysis. The multiple comparisons among groups were assessed using ANOVA for variables with homogeneous variances or non-parametric test.
if non-homogeneous. Percentages were compared using the \( \chi^2 \)-test. The potential variables were first assessed in univariate analyses. The variables were then analyzed using logistic regression analysis. Plasma homocysteine was later added to subsequent models controlled for possible risk factors for bone mineral density or fractures and the known determinants of homocysteine. Finally, an analysis was made of per unit increase plasma homocysteine as an independent variable for fractures using multivariate logistic regression models. All odds ratios (OR) were given with their 95% confidence intervals (CI). The fit of each model was tested, and the Nagelkerke \( R^2 \) approximation was compared. The data were cross-sectional observations. \( P < 0.05 \) was considered statistically significant.

All procedures were carried out according to the 19th revision of the Declaration of Helsinki.

RESULTS

The study was completed by 292 patients (101 men aged 44–65 years, 191 premenopausal women aged 41–53 years), including 124 healthy control, 115 diabetic patients without fractures and 53 diabetic patients with fractures: 23 men and 26 women with vertebral fractures, four men and five women with femoral neck fractures, and two men and two women with a mixture of the two fractures. In a univariate analysis for variables (Table 1), type 2 diabetes with fractures, type 2 diabetes without fractures and healthy controls were compared. Fractures were identified in the participants with higher levels of HbA1c and longer duration of diabetes, lower fasting insulin levels, and higher incidence of diabetic retinopathy and higher serum CRP levels, as compared with the non-fracture participants. There were no differences in variables among the three groups of patients; that is, bone mineral density, distribution of sex, active smokers, age, serum osteocalcin, body mass index, total calcium intake, alcohol intake, energy expended from physical activity (MET), serum folate, serum \( B_12 \), urinary albumin to creatinine ratio and serum creatinine (insignificant \( P \)-values not shown in Table 1). Both diabetic groups did not have obviously different percentages of thiazolidenediones use. Diabetic participants tended to have increased levels of homocysteine, in contrast to healthy control participants. In addition, plasma homocysteine levels were higher in diabetic participants with fractures than without. Furthermore,

Table 1 | General variables in healthy control participants and type 2 diabetic participants with fractures and without

| Variables | HC \((n = 124)\) | \(P\)-value* | DM \((n = 115)\) | \(P\)-value† | DM + F \((n = 53)\) | \(P\)-value‡ |
|-----------|----------------|-------------|----------------|-------------|----------------|-------------|
| Age (years) | 53.7 (43.9, 58.2) | NS | 54.6 (45.7, 60.3) | NS | 55.3 (46.2, 63.5) | NS |
| Testosterone in men (nmol/L) | 13.5 (6.2) | NS | 13.6 (8.2) | NS | 12.8 (6.7) | NS |
| Female/male (%) | 65.7 | NS | 64.5 | NS | 66.7 | NS |
| Fasting glucose (mmol/L) | 5.5 (0.3) | 0.000 | 7.4 (1.3) | 0.000 | 8.2 (2.9) | 0.037 |
| HbA1c (%) | 5.3 (0.5) | 0.000 | 6.6 (1.7) | 0.002 | 7.4 (2.4) | 0.017 |
| Duration of diabetes (years) | – | – | 7.1 (3.6, 8.6) | – | 8.5 (5.7, 9.2) | 0.001 |
| Active smoker (%) | 5.9 | NS | 6.0 | NS | 5.7 | NS |
| Fasting insulin (mIU/L) | 19.7 (2.4) | NS | 20.3 (2.6) | NS | 18.7 (2.5) | 0.042 |
| Body mass index (kg/m²) | 21.3 (2.0) | NS | 20.5 (2.5) | NS | 22.7 (2.8) | NS |
| Total calcium intake (mg/day) | 825.57 (574.8) | NS | 830.6 (589.2) | NS | 820.7 (447.2) | NS |
| Alcohol intake, 1 drink per day (%) | 8.2 | NS | 8.0 | NS | 8.1 | NS |
| MET | 21.5 (18.7) | NS | 22.6 (20.3) | NS | 20.6 (19.0) | NS |
| History of myocardial infarction (%) | – | – | 3.0 | – | 3.4 | NS |
| History of stroke (%) | – | – | 2.4 | – | 2.6 | NS |
| Presence of retinopathy (%) | – | – | 5.2 | – | 6.0 | 0.045 |
| Presence of neuropathy (%) | – | – | 3.8 | – | 4.1 | NS |
| Parental history of fracture (%) | – | – | 15 | – | 17 | NS |
| Thiazolidenediones use (%) | – | – | 42.5 (4.9) | – | 48.1 (5.2) | NS |
| Osteocalcin (µg/L) | 33.6 (5.2) | NS | 32.3 (4.0) | NS | 35.2 (4.8) | NS |
| C-reactive protein (mg/L) | 6.5 (0.6) | 0.029 | 6.69 (2.0) | 0.03 | 7.2 (2.3) | 0.038 |
| Plasma homocysteine (µmol/L) | 8.0 (2.0) | 0.012 | 8.6 (2.1) | 0.03 | 10.3 (3.0) | 0.000 |
| Serum folate (nmol/L) | 23.9 (5.7) | NS | 27.4 (5.6) | NS | 25.7 (7.5) | NS |
| Serum \( B_12 \) (µmol/L) | 391 (173) | NS | 3378 (159) | NS | 383 (147) | NS |
| Urinary ACR (mg/g) | 25.4 (35) | NS | 260 (33) | NS | 282.3 (30) | NS |
| Serum creatinine (µmol/L) | 99.7 (7.73) | NS | 105.2 (11.3) | NS | 103.5 (9.6) | NS |
| Metformin use (%) | – | – | 80 | – | 86 | NS |

All values are mean (standard deviation) for normally distributed data and median (interquartile range) for skewed data; active smoker and sex data are prevalence (%). Healthy control (HC): without diabetes (DM) and bone fracture (DM + F). *HC vs (DM + F); †DM vs (HC); ‡DM + F vs DM. ACR, albumin to creatinine ratio; HbA1c, glycated hemoglobin; MET, energy expended from physical activity; NS, not significant (\( P > 0.05 \)).
a 1-µmol/L increase in plasma homocysteine concentration appeared to be associated with increasing the occurrence of fractures by 1.2–2.5%. The two groups of diabetic patients had similar variables that could potentially influence the homocysteine concentration; for example, urinary ACR, serum creatinine level, serum folate and vitamin B₁₂ concentration, as well as the percentage of metformin use. No sex-related differences in mean homocysteine concentrations were observed. In the univariate model, the three groups had similar bone mineral density of the lumbar spine (L1-4) and femoral neck with well-matched sex-related age (Table 2, an extension of Table 1) among others (Table 1).

In a multivariate model (not shown) of analysis for these factors and clinical variables, in the absence of homocysteine, duration of diabetes was significantly associated with fractures (OR 1.26, 95% CI 1.05–1.37; P = 0.018), and the relationship of HbA1c to fractures was also significant (OR 1.18, 1.08–1.32; P = 0.023). Interestingly, fasting glucose and insulin levels, the presence of retinopathy, and CRP levels, which were discernable between diabetic participants with fractures and without in the univariate analysis (Table 1), were not closely related to fractures in this model.

In further sequential multivariate models, homocysteine levels were assessed as continuous variables. Initially in a model (Table 3), plasma homocysteine was shown to be associated with fractures (OR 1.50, 95% CI 1.14–2.5%). The two groups of diabetic patients had similar variables that could potentially influence the homocysteine concentration; for example, urinary ACR, serum creatinine level, serum folate and vitamin B₁₂ concentration, as well as the percentage of metformin use. No sex-related differences in mean homocysteine concentrations were observed. In the univariate model, the three groups had similar bone mineral density of the lumbar spine (L1-4) and femoral neck with well-matched sex-related age (Table 2, an extension of Table 1) among others (Table 1).

Femoral neck BMD (g/cm²) Table 2: Bone mineral density in healthy control participants and type 2 diabetic participants with fractures and without

| Variables          | HC (n = 124) | P-value* | DM (n = 115) | P-value† | DM + F (n = 53) | P-value‡ |
|--------------------|-------------|----------|--------------|----------|----------------|----------|
| Male age (years)   | 56.8 (48.6, 61.2) | NS       | 58.4 (47.2, 62.7) | NS       | 57.3 (50.8, 59.1) | NS       |
| Female age (years) | 48.2 (42.5, 49.7) | NS       | 50.2 (43.8, 51.5) | NS       | 49.8 (44.6, 52.2) | NS       |
| L1                 | 1.038 (0.201) | NS       | 1.033 (0.220) | NS       | 0.982 (0.211) | NS       |
| L2                 | 1.041 (0.230) | NS       | 1.032 (0.210) | NS       | 1.015 (0.206) | NS       |
| L3                 | 1.037 (0.209) | NS       | 1.031 (0.209) | NS       | 0.976 (0.204) | NS       |
| L4                 | 1.036 (0.201) | NS       | 1.035 (0.204) | NS       | 0.981 (0.209) | NS       |
| Femoral neck BMD (g/cm²) | 0.732 (0.110) | NS       | 0.674 (0.109) | NS       | 0.743 (0.115) | NS       |

All values are odds ratio (OR) and 95% confidential interval (95% CI). Model Nagelkerke R² = 0.27. *Homocysteine adjusted for risk factors for fracture and determinants of homocysteine. ACR, albumin to creatinine ratio; BMD, bone mineral density; CI, confidence interval; CRP, C-reactive protein; HbA1c, glycated hemoglobin.

**DISCUSSION**

We found that diabetic patients had elevated plasma homocysteine levels, and diabetic patients with fractures had a further increased level of homocysteine. The relationship between elevated plasma homocysteine levels and fractures was independent of other possible risk factors for fractures, including bone mineral density, and determinants of higher homocysteine concentration in men and premenopausal women with type 2 diabetes. In addition, a unit increment of plasma homocysteine appeared to be linked to a fracture, and every 5.0-µmol/L increase in plasma homocysteine was obviously associated with the
occurrence of the fracture independent of other variables. Interest-
ing, among the fractures, most were vertebral fractures for both sexes. This is in contrast to one previous study on postmen-
opausal women without diabetes, in which high homocysteine
levels (as a result of poor renal function) were associated with an
increased risk of hip fractures. Furthermore, our participants
with fractures had a mean homocysteine level below 11 μmol/L,
and a mean plasma homocysteine concentration of 10.3 μmol/L,
which could directly or indirectly damage bone. Some authors
found that hyperhomocysteinemia was associated with serum
osteocalcin levels in postmenopausal women. However, the
link was not evident in our participants (men and premeno-
pausal women with type 2 diabetes). This shows that hyperhom-
ocysteinemia did not greatly affect the activity of osteoblasts on
the aspect of mechanism of hyperhomocysteinemia-related frac-
ture occurring in our patients. In fact, we did not find obvious
bone mass loss in the fracture participants. Compromised bio-
mechanical properties might be a main contributor to fracture.
Apart from homocysteine, the present study showed that HbA1C
duration of diabetes and duration of diabetes were independently associated with fractures, respectively, and the duration appeared to be more important, with comparable BMI among the participants. In addition to microangiopathy and increased inflammation, long-standing diabetes results in elevated concentration of advanced glycation end-products in the bone, thus affecting bone
strength. Long term tight glycemic control has a proven benefi-
cial effect on diabetic chronic complications, and this benefi-
cial effect might be extended to diabetes-related fracture. In contrast to some observations that certain antidiabetic agents result in secondary fractures, we did not find the occurrence of fractures to be increased by thiazolidinediones use (data not shown).

The present findings were observed after potential variables were considered; for example, bone mineral density, sex, active
smokers, age, body mass index, total calcium intake, alcohol
intake, energy expended from physical activities (MET) and a
family history. However, genes that might be associated with
the development of fractures and insulin-like growth factor 1 levels
that are thought to be decreased in diabetes, and are also associ-
ated with bone mineral density, were not included in this inves-
tigation. The relationship between diabetes and fractures
might be a more complex issue. In addition, bone mineral
density assessments with dual energy X-ray absorptiometry were
not carried out at other sites of the body for our participants,
such as humeri, ribs, hands and feet. Furthermore, genetic deter-
minants of homocysteine levels (a novel polymorphic site in
MTHFR [G1793A]) could influence the homocysteine levels
were not evaluated. Finally, the study sample was relatively
small, and these data might be influenced by the patient selec-
tion. Future prospective studies are warranted on a larger scale,
which include more potentially confounding factors. This could clarify the homocysteine–fractures relationship observed in the present cross-sectional study, thus providing a potential value of guiding management of fractures in type 2 diabetes.

The present study shows that the plasma homocysteine level is related to and is possibly a useful indicator of fractures in men and premenopausal women with type 2 diabetes. If prospective studies could further define an increase of plasma homocysteine as a causal factor of fractures in population with type 2 diabetes, this would be a timely aid in the prevention and treatment of this disorder.

ACKNOWLEDGMENTS

We thank the National Natural Science Foundation (81070655) of China and Jiangsu Provincial Natural Science Foundation (BK2009441) & PAPD of China for supporting this project. This work would not have been possible without the funding. There is no conflict of interest.

REFERENCES

1. Yang W, Lu J, Weng J, et al. Prevalence of diabetes among men and women in China. N Engl J Med 2010; 362: 1090–1101.
2. Hofbauer LC, Brueck CC, Singh SK, et al. Osteoporosis in patients with diabetes mellitus. J Bone Miner Res 2007; 22: 1317–1328.
3. Tyagi N, Vacek TP, Fleming JT, et al. Hyperhomocysteinemia decreases bone blood flow. Vasc Health Risk Manag 2011; 7: 31–35.
4. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-oxidized hydrogen peroxide generation from homocysteine. Clin Invest 1986; 77: 1370–1376.
5. Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelial-derived relaxing factor and related oxides of nitrogen. Clin Invest 1993; 91: 308–318.
6. Abdella NA, Mojiminiyi OA, Akanji AO, et al. Associations of plasma homocysteine concentration in subjects with type 2 diabetes mellitus. Acta Diabetol 2002; 39: 183–190.
7. Yang G, Lu J, Pan C. The impact of plasma homocysteine level on development of retinopathy in type 2 diabetes mellitus. Zhonghua Nei Ke Za Zhi 2002; 41: 34–38.
8. Jacques PF, Bostom AG, Wilson PW, et al. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001; 73: 613–621.
9. Chico A, Perez A, Cordoba A, et al. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? Diabetologia 1998; 41: 684–693.
10. Mudd SH, Pool JR. Labile methyl balance for normal humans on various dietary regimens. Metabolism 1975; 24: 721–733.
11. Ganji V, Kafai MR. Demographic, lifestyle, and health characteristics and serum B vitamin status are determinants of plasma total homocysteine concentration in the post-folic acid fortification period, 1999-2004. J Nutr 2009; 139: 345–352.
12. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporos Int 2007; 18: 427–444.
13. Shan PF, Wu XP, Zhang H, et al. Age-related bone mineral density, osteoporosis rate and risk of vertebral fracture in mainland Chinese women with type 2 diabetes mellitus. J Endocrinol Invest 2011; 34: 190–196.
14. WHO. World Health Organization: Diabetes Mellitus: Report of a WHO Study Group. World Health Org., Geneva, 2006.
15. Leboff MS, Narweker R, LaCroix A, et al. Homocysteine levels and risk of hip fracture in postmenopausal women. J Clin Endocrinol Metab 2009; 94: 1207–1213.
16. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004; 50: 1482–1483.
17. Hao L, Ma J, Zhu J, et al. Vitamin B-12 deficiency is prevalent in 35- to 64-year-old Chinese adults. J Nutr 2007; 137: 1278–1285.
18. Viégas M, Costa C, Lopes A, et al. Prevalence of bone mineral density and vertebral fractures in postmenopausal women with type 2 diabetes mellitus and their relationship with duration of the disease and chronic complications. J Diabetes Complications 2011; 25: 216–221.
19. Kuyumcu ME, Yesil Y, Oztürk ZA, et al. The association between homocysteine (hcy) and serum natural antioxidants in elderly bone mineral densitometry (BMD). Arch Gerontol Geriatr 2012; 55: 739–743.
20. Kim DJ, Park BL, Koh JM, et al. Methionine synthase reductase polymorphisms are associated with serum osteocalcin levels in postmenopausal women. Exp Mol Med 2006; 38: 519–524.
21. Holman RR, Paul SK, Bethel MA, et al. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008; 359: 1577–1589.
22. Hong XM, Hsu YH, Henry TD, et al. Association of the Methyleneetetrahydrofolate reductase C677T polymorphism and fracture risk in Chinese postmenopausal women. Bone 2007; 40: 737–742.
23. Li JB, Wang CY, Chen JW, et al. Expression of liver IGF-1 Gene and its serum IGF-1 Level in rats with diabetes. World J Gastroenterol 2004; 10: 255–259.
24. Raisz LG. Pathogenesis of bone mineral density: concepts, conflicts, and prospects. J Clin Invest 2005; 115: 3318–3325.
25. Mao R, Fan Y, Chen F, et al. Genetic polymorphism of MTHFR G1793A in Chinese populations. Eur J Epidemiol 2008; 23: 363–368.
26. Kurra S, Siris E. Diabetes and bone health: the relationship between diabetes and osteoporosis-associated fractures. Diabetes Metab Res Rev 2011; 27: 430–435.