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ORIGINAL ARTICLE

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

Jianbo Li[1,2]*, Hongman Zhang[1], Lingfei Yan[1], Min Xie[1], Jiawei Chen[1]

[1]Department of Endocrinology and Metabolism, The First Affiliated Hospital of Nanjing Medical University, Nanjing, and [2]Department of Endocrinology and Metabolism, The Affiliated Jiangsu Shenze Hospital of Nanjing Medical University, Suzhou, China

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 B_{12}, 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 problem[1]. Type 2 diabetes is a potential cause of compromised bone mechanical properties, and might increase a risk for a fracture[2]. 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 properties[3]. Hyperhomocysteinemia results

in increasing production of oxidation products, homocysteine thiolactone and homocysteine mixed disulphides, which can damage the endothelium by excessive sulphation of connective tissues[4,5]. As such, fractures might increase. Furthermore, hyperhomocysteinemia is more common in patients with type 2 diabetes[6,7]. 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 levels[8]. Homocysteine concentration is closely related to folate or renal status in the elderly[9]. Homocysteine metabolism is dependent on vitamin B_{12} and folate concentration, or on betaine in men[10]. In addition, other factors, such as lifestyls (e.g., smoking,

alcohol and coffee intake) and drugs, can affect plasma homocysteine levels[8,11].

Recently, bone mineral density and fracture risk have been studied in diabetes[12,13]. 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[14]. Chinese men and premenopausal women with regular menstruation (aged 20 years to premenopause) with type 2 diabetes registered consecutively as outpatients from the clinic or inpatients from the ward of 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 (glucocorticods, 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 thiazolidenediones, 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/cm^2) 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 B$_{12}$ 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 χ^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/m^2)	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	–	3.1	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)	6.53 (0.6)	0.029	6.69 (2.0)	NS	7.2 (2.5)	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} (pmol/L)	391 (173)	NS	3378 (159)	NS	383 (147)	NS
Urinary ACR (mg/g)	25.4 (3.5)	NS	26.0 (3.3)	NS	28.2 (3.0)	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 25%, 75%) 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 B_{12} 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 lumber 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 1.14–2.12; $P = 0.016$) after controlling for other risk factors including bone mineral density and thiazolidenediones use for fractures and known determinants of homocysteine concentrations. The relationship remained significant (OR 1.41, 95% CI 1.05–2.03; $P = 0.020$) when biguanide prescription was added to the same model. Furthermore, increases in plasma homocysteine when categorized in increments of 5.0 μmol/L model (Table 4) were significantly associated with the occurrence of fractures (OR 1.42, 95% CI 1.12–1.78; $P = 0.013$), after adjusting for per 5-year increase of duration of diabetes, per 1% increase of HbA1c, per 10-nmol/L increase of serum folate, per 100-pmol/L increase of serum B_{12}, per 10-μmol/L increase of serum creatinine and per 5-mg/g increase of urinary ACR.

Table 3 | Odds ratios (95% confidence intervals) associated with risk factors for fractures in multivariable logistic regression

Variables	OR (95% CI)	P-value
Age (years)	0.94 (0.83–1.06)	0.613
Duration of diabetes (years)	1.10 (1.02–1.21)	0.027
HbA1c (%)	1.11 (1.03–1.24)	0.047
Fasting insulin (mIU/L)	0.76 (0.44–1.08)	0.732
Body mass index (kg/m^2)	0.78 (0.35–1.04)	1.034
Lumber spine BMD (g/cm^2)	0.75 (0.31–1.13)	0.715
Femoral neck BMD (g/cm^2)	0.86 (0.55–1.12)	0.480
Parental history of fracture (%)	0.81 (0.65–1.07)	0.567
Presence of retinopathy (%)	1.02 (0.83–1.10)	0.122
Thiazolidenediones use (%)	0.89 (0.73–1.04)	0.478
Male sex	0.43 (0.10–1.15)	1.175
Active smoker (%)	0.65 (0.39–1.01)	0.730
Alcohol intake (%)	0.82 (0.43–1.08)	0.862
CRP (mg/L)	0.97 (0.73–1.15)	0.186
Serum folate (nmol/L)	0.95 (0.84–1.16)	0.399
Serum B_{12} (pmol/L)	0.81 (0.42–1.07)	0.873
Urinary ACR (mg/g)	0.92 (0.77–1.13)	0.525
Serum creatinine (μmol/L)	0.78 (0.30–1.16)	1.042
Homocysteine (μmol/L)*	1.50 (1.14–2.12)	0.016

All values are odds ratio (OR) and 95% confidential interval (95% CI). Model Nagelkerke R^2: 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

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
Lumber spine BMD (g/cm^2)						
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/cm^2)	0.732 (0.110)	NS	0.674 (0.109)	NS	0.743 (0.115)	NS

Values are the mean (standard deviation) for normally distributed data. Healthy control (HC): without diabetes (DM) and bone fracture (DM + F); *HC vs (DM + F); †DM vs (HC); ‡(DM + F) vs DM. BMD. Bone mineral density; NS, not significant ($P > 0.05$).

Table 4 | Odds ratios (95% confidence interval) associated with per 5-mmol/L increase of plasma homocysteine in relation to fracture

Independent variables	OR (95% CI)	P-value
Homocysteine (per 5 mmol/L increase)	1.42 (1.12 –1.78)	0.013
Duration of diabetes (per 5-year increase)	2.17 (1.11 –2.95)	0.025
HbA1c (per 1% increase)	1.21 (1.03 –1.52)	0.038
Urinary ACR (per 5-mg/g increase)	0.67 (0.25 –1.01)	0.826
Serum creatinine (per 10-µmol/L increase)	0.85 (0.54 –1.05)	0.641
Serum folate (per 10-nmol/L increase)	0.82 (0.65 –1.03)	0.657
Serum B_{12} (per 100-pmol/L increase)	0.98 (0.81 –1.08)	0.968

All values are the odds ratios (OR) and 95.0% confidence intervals (CI). Model Nagelkerke R^2: 0.26. Homocysteine was adjusted for duration of diabetes, glycated hemoglobin (HbA1c), urinary albumin to creatinine ratio (ACR), serum creatinine, serum folate and vitamin B_{12}.

occurrence of the fracture independent of other variables. Interestingly, among the fractures, most were vertebral fractures for both sexes. This is in contrast to one previous study on postmenopausal women without diabetes, in which high homocysteine levels (as a result of poor renal function) were associated with an increased risk of hip fractures[15]. 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 was a potential threshold demarcating fractures from non-fracture in type 2 diabetes. The participant selection (e.g., diabetic or not, pre- or postmenopausal, and young adult or older) and ethnicity difference (Asians or Westerners) could contribute to these differences in results among studies. A larger prospective study is required to confirm the present findings.

A decrease to subnormal levels of previously normal serum vitamin B_{12} levels might occur, possibly because of interference with B_{12} absorption from the B_{12}-intrinsic factor complex by metformin[16]. We added metformin use (83% participants) as an additional confounding factor to the multivariable analysis. Nevertheless, metformin did not show its significant effect on the serum B_{12} of patients with or without fractures from our observation. The concentration of serum folic acid, another potential factor influencing homocysteine concentration, was not found to be different among the patients. The nutrient level appears to be affected by dietary preference, as shown by some differences in its serum concentration between the southerners and the northerners of China[17]. In addition, homocysteine concentrations were observed to be similar between the two sexes. This disagrees with one report in normal young adults[11]. Sex hormones that were normal in both sexes of the present study cannot explain this discrepancy. Consistently, as reported previously[6], we observed that diabetic conditions influence plasma homocysteine concentration to some extent.

In our fracture patients with increased homocysteine levels, there was a slightly elevated percentage of retinopathy, a chronic diabetic complication, pathologically due to ischemia or hypoxia within the retina, suggesting that in addition to retinopathy-related fall risks[18], homocysteine-related compromised microcir-

culation might, at least in part, be involved in fractures. This is supported by one experimental study that found that homocysteine decreases bone blood flow and biomechanical properties[3]. Homocysteine might also be related to inflammation participating in fractures, as shown by our finding that CRP increased in the diabetic patients with fractures with hyperhomocysteinemia. From our observation, unlike homocysteine, CRP was not an independent risk factor for fracture, and appeared to be a reflector of homocysteine-related inflammation. Homocysteine decreases the level of natural anti-oxidants[19], and thus increases inflammation and CRP level. In addition, hyperhomocysteinemia results in increasing production of oxidation products, homocysteine thiolactone and homocysteine mixed disulphides[4,5], which could directly or indirectly damage bone. Some authors found that hyperhomocysteinemia was associated with serum osteocalcin levels in postmenopausal women[20]. However, the link was not evident in our participants (men and premenopausal women with type 2 diabetes). This shows that hyperhomocysteinemia did not greatly affect the activity of osteoblasts on the aspect of mechanism of hyperhomocysteinemia-related fracture occurring in our patients. In fact, we did not find obvious bone mass loss in the fracture participants. Compromised biomechanical properties might be a main contributor to fracture. Apart from homocysteine, the present study showed that HbA1c 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[12]. Long term tight glycemic control has a proven beneficial effect on diabetic chronic complications[21], and this beneficial 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 thiazolidenediones 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 associated with bone mineral density, were not included in this investigation[22–25]. The relationship between diabetes and fractures might be a more complex issue[26]. 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 determinants of homocysteine levels (a novel polymorphic site in MTHFR [G1793A] could influence the homocysteine levels[25]) were not evaluated. Finally, the study sample was relatively small, and these data might be influenced by the patient selection. 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

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