Dietary Calcium and Serum 25OHD Protect Chinese Women from Type 2 Diabetes

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Summary The Chinese dietary structure is different from Western and Mediterranean diets. This study aimed to assess the association of dietary calcium, serum 25-hydroxyvitamin D (25OHD), and other macronutrients with type 2 diabetes (T2D) in Chinese patients. This case-control study enrolled 605 patients (males, 337; females, 268) with T2D and 724 healthy subjects (males, 405; females, 319) at Sir Run Run Shaw Hospital from February to April 2014. Dietary calcium, total energy, fat to energy ratio, glucose administration, 2-h postprandial blood glucose, serum total cholesterol and high density lipoprotein (HDL)-cholesterol, and serum 25OHD level were assessed. Logistic regression was used to assess the associations of various parameters with T2D. Total energy, fat, and the fat-to-energy ratio were significantly higher in healthy male and female controls than in T2D patients (p<0.05). In addition, significant differences were obtained between the T2D and control groups in glucose management, 2-h postprandial blood glucose, serum total cholesterol, and HDL-cholesterol (all p<0.05). Interestingly, statistically significant inverse associations of dietary calcium and total energy intake with the risk of T2D were obtained in women: binary logistic regression analysis showed diet calcium and serum 25OHD were protecting factors against T2D (OR = 0.40, p = 0.034; OR = 0.50, p = 0.019). Dietary calcium and serum 25OHD may independently protect Chinese women from T2D. These findings highlight the importance of vitamin D and calcium in daily diet or supplement in the early period of T2D, meanwhile indicators associated with bone metabolism should be assessed in clinical nutrition. It is possible that dietary education and guidance should be implemented based clinical bone metabolism data.

Key Words T2D, dietary calcium, serum 25OHD, Chinese female

Type 2 diabetes (T2D) is a chronic metabolic disease with high blood glucose levels (1), which results from insulin deficiency and/or insulin resistance (2). T2D or metabolic disorder increased fracture risk (3), interfered with bone formation (4), and impaired fracture healing (5). Meta-analysis shown a consistent pattern of increased risk of fracture in the patients of T2D in studies conducted in the USA and Europe (6). Bone metabolism and blood glucose metabolism are considered to be closely related (7). Dietary factors play an important role in the development of diabetes (8, 9), providing additional means for prevention.

Circulating 25-hydroxyvitamin D (25OHD) is considered a valid biomarker for the vitamin D nutritional status (10). Markedly reduced 25OHD levels (vitamin D deficiency/insufficiency) were found in the youths with diabetes (11). In addition, higher serum 25OHD level were associated with markedly decreased risk of diabetes in Australians (12). Vitamin D deficiency was shown to be inversely correlated to insulin resistance regardless of the glucose tolerance status (13–18). These findings indicated that the relation between vitamin D and diabetess deserved further attention.

The notion that dietary calcium deficiency is associated with an increased risk of T2D, while diabetics typically have a high bone mineral density (BMD), remains controversial. In experimental studies, calcium intake improved pancreatic beta cell function and peripheral insulin sensitivity (19–21). In humans, related evidence was mainly derived from cross-sectional studies (22). Two cohort studies found a moderate but not statistically significant association between dietary calcium intake and the risk of diabetes after adjustment for other dietary factors (23, 24); however, the association with supplemental intake of calcium was shown to be statistically significant (25). A high intake of dairy foods, a major dietary source of calcium, was also associated with reduced T2D incidence (19, 26, 27). In addition, a large sample study demonstrated that dairy products and calcium intake was associated with a lower 9-y incidence of metabolic syndrome and impaired fasting glycemia or T2D (28). However, in a large-scale randomized, controlled trial conducted in American
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women (22), calcium plus vitamin D3 supplementation did not reduce the risk of diabetes over 7 y of follow-up. In agreement, higher dietary calcium appeared not to be associated with reduced risk of diabetes in Australian individuals (12). Given the scarcity and inconsistency of epidemiological data, it is unclear whether dietary calcium intake was helpful to decrease the incidence of bone metabolism diseases among T2D.

The Chinese dietary structure is different from Western and Mediterranean diets. Indeed, the traditional Chinese dietary structure prioritizes grains and vegetables, which are deficient in minerals and calcium (29). It was shown that the body fat content of Chinese patients with T2D is low compared with Caucasian counterparts (30). Reports assessing the association of calcium intake and T2D incidence in the Chinese population are scarce (31).

We therefore in this cross-sectional study aimed to assess the association of dietary calcium, serum 25OHD, and other macronutrients with type 2 diabetes (T2D) in Chinese patients.

MATERIALS AND METHODS

Participants and physical examinations. This was a case-control study conducted at Sir Run Run Shaw Hospital, Hangzhou, assessing T2D patients and healthy subjects aged 18 to 65 y. Data were collected between February and April 2014.

Inclusion criteria were (a) age ≤65 y and (b) diagnosis of T2D according to the World Health Organization (32) criteria (33). Exclusion criteria were as follows: body mass index (BMI) <19 kg/m²; heavy cigarette smoking (>20 cigarettes/d) (22); heavy alcohol consumption (>5 beer equivalent drinks per day) (24); use of drugs interfering with bone and calcium metabolism, including sex steroids, vitamin D metabolites, calcitriol, bisphosphonates, thyroid hormone, thiazolidinediones, heparin, warfarin, vitamin K, thiazides, and anticonvulsants; diseases that affect bone and calcium metabolism, including gastrointestinal diseases, Cush- ing syndrome, hypogonadism, primary or secondary hyperparathyroidism, cirrhosis, malignancy, other nutrition and renal insufficiencies; and bedriddenness. In total, 605 patients were included based on the above eligibility criteria.

Meanwhile, 724 age and gender-matched healthy individuals, undergoing a routine annual medical checkup at the physical examination center of Sir Run Run Shaw Hospital during the same period, were enrolled; 405 men and 319 women were volunteered into the control group.

All participants underwent standardized medical examinations including measurements of height, weight and waist circumference. Current smoking (yes/no) and physical activity (yes/no) were defined according to self-reports.

This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved by the Ethics Committee of Zhejiang Province (Approval ID: 20160121-2), and reviewed and accepted by all participants, who provided written informed consent prior to enrollment.

Lab measurements. At baseline, a clinical examination, including serum 25OHD, lipid profile, fasting plasma glucose (FPG), and 2-h 75-g OGTT, was administered at the initial visit. Prior to each further visit, participants were asked to fast for 12 h and refrain from drinking alcohol as well as vigorous activity for 24 h; they were also instructed not to smoke on the day of the visit. WHO criteria were used to determine the glucose tolerance status. Individuals currently taking oral hypoglycemic medications, with FPG≥7.0 mmol/L or OGTT≥11.1 mmol/L were considered T2D patients. Serum 25OHD (25-hydroxyvitamin D kit, Roche Diagnostics) concentrations were determined by the electrochemiluminescence method on a Roche Cobas 6000 analyzer. Fasting blood glucose (blood glucose kit, Beijing Leadman) was measured by the oxygen electrode method on a BeckmanDXI800 system, TC (total cholesterol kit, Beijing Leadman Biochemistry; Siemens ADVIA 2400 automatic biochemical analyzer), TG (triglyceride kit, Beijing Leadman Biochemistry; Siemens ADVIA 2400 automatic biochemical analyzer), LDL (low-density lipoprotein kit, Sekisui Medical, Japan; Siemens ADVIA 2400 automatic biochomic alanalyzer), and HDL (high-density lipoprotein kit, Randox, UK; Siemens ADVIA 2400 automatic biochemical alanalyzer) were assessed by enzymatic methods. HbA1c (D-10 glycosylated hemoglobin kit, Bio-Rad; Bio-Rad DiasTAT glycosylated hemoglobin analyzer) was measured by high performance liquid chromatography (HPLC).

Questionnaire surveys. Data were collected using 24-h dietary recall for 3 consecutive days, and condiment weighing as well as recording the daily consumption of food and condiments, including restaurant meals. Daily intake values of energy and macronutrients such as proteins, fats, and carbohydrates, were calculated according to “China Food Composition” (31). The formula for dietary nutrient intake was as follows:

$$Z_i = \sum_{j=1}^{n} \frac{x_i \times e_i \times c_i}{100 \times 100}$$

$Z_i$ is the intake of various nutrients per meal from food per person per day (g/d); $x_i$ is daily food consumption of the i kind (g); $e_i$ is the edible part of the j kind (refers to the content of edible part with per 100 g food); and $c_i$ is the food nutrient content of the j kind (g/100 g).

In addition, physical parameters, such as height and weight, were recorded, as well as non-dietary information, including marital status, work intensity, and education level.

Statistical analysis. Data were analyzed with the SPSS17.0 software (SPSS Inc., Chicago, IL). Quantitative data were assessed for normality by the Kolmogorov-Smirnov test. Non-normally distributed data were evaluated by the Mann-Whitney U test. To compare patient and healthy groups, the independent samples t-test and Mann-Whitney U test were used for continuous and categorical variables, respectively. Variables that significantly correlated with FPG and oral glucose tolerance
Table 1. Clinical data in T2D cases and controls by sex.

| Clinical features           | Males                          | p value | Females                          | p value |
|----------------------------|--------------------------------|---------|----------------------------------|---------|
| Age (y)                    | 53.16±13.65                    | 0.31    | 55.69±12.53                      | 0.46    |
| BMI (kg/m²)                | 26.37±4.10                     | 0.11    | 25.19±4.14                       | 0.19    |
| Education (≥High-school,%) | 15.28                          | 0.693   | 11.74                            | 0.643   |
| Rural (%)                  | 64.65                          | 0.052   | 68.69                            | 0.067   |
| Family history of T2D (%)  | 29.08                          | 0.04    | 37.31                            | 0.001   |
| Current smoker (%)         | 13.56                          | 0.641   | 0.079                            | 0.972   |
| Current drinker (%)        | 10.98                          | 0.870   | 0.94                             | 0.125   |
| Alcohol consumption (g/d)  | 30.38±56.72                    | 0.001   | 1.15±0.87                        | 0.693   |
| FPG (mmol/L)               | 7.38±1.40                      | 0.023   | 7.59±1.36                        | 0.0045  |
| 2h-PG (mmol/L)             | 7.68±1.98                      | 0.030   | 7.77±2.95                        | 0.026   |
| HbA1c (%)                  | 7.18±2.01                      | 0.001   | 7.29±3.03                        | 0.006   |
| TG (mmol/L)                | 1.54±1.08                      | 0.652   | 1.33±1.12                        | 0.651   |
| TC (mmol/L)                | 5.09±1.06                      | 0.052   | 4.48±1.04                        | 0.067   |
| HDL-C (mmol/L)             | 0.89±0.27                      | 0.051   | 1.08±0.25                        | 0.059   |
| PTH (pg/mL)                | 44.40±19.30                    | 0.049   | 36.37±18.28                      | 0.044   |
| Serum 25OHD (nmol/L)       | 55.09 (38.71–72.01)            | 0.001   | 44.98 (35.47–81.69)              | 0.001   |

FPG, fasting plasma glucose; 2h-PG, 2-h 75 g oral glucose tolerance test; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; PTH, parathyroid hormone. Data are mean ± SD, median (interquartile range), or ratio.

Table 2. Daily intake of macronutrients and calcium in T2D cases and controls by sex.

| Nutrient/d               | Males                          | t/Z-value | p value | Females                          | t/Z-value | p value |
|--------------------------|--------------------------------|-----------|---------|----------------------------------|-----------|---------|
| Total energy (kcal)      | 1,847.46±573.12                | 2.817     | 0.036   | 1,346.67±485.21                 | 4.305     | <0.001  |
| Protein (g)              | 75.88±0.65                     | 0.56      | 0.56    | 52.06±3.86                      | 3.388     | 0.034   |
| Carbohydrate (g)         | 245.21±16.93                   | 4.502     | <0.001  | 184.92±24.87                    | 5.335     | <0.001  |
| Fat (g)                  | 62.72±0.67                     | 2.175     | 0.034   | 44.46±3.32                      | 4.165     | <0.001  |
| Diet-fiber (g)           | 10.58±1.69                     | 0.561     | 0.561   | 9.04±1.89                       | 2.467     | 0.041   |
| Diet-Ca (mg)             | 510.03±34.72                   | 1.924     | 0.56    | 387.70±29.75                    | 0.826     | 0.69    |
| Diet-Mg (mg)             | 394.29±228.77                  | 0.697     | 0.71    | 359.66±228.75                   | 0.308     | 0.97    |
| Diet-P (mg)              | 959.87±241.2                   | 0.436     | 0.86    | 696.79±341.73                   | 2.407     | 0.043   |
| Nutrient (% En)          |                                 |           |         |                                 |           |         |
| Carbohydrate (%En)       | 53.72±18.74                    | -2.199    | 0.023   | 55.87±15.62                     | -3.677    | 0.016   |
| Protein (%En)            | 16.16±4.53                     | -1.604    | 0.34    | 15.13±5.09                      | 2.891     | 0.031   |
| Fat (%En)                | 30.11±18.09                    | 2.676     | 0.034   | 28.99±17.28                     | 2.902     | 0.024   |

T2D, type 2 diabetes mellitus; %En, % of total energy. Data are mean or median. Intake of calcium and phosphorus were adjusted to total energy intake.
were submitted to subgroup analysis based on gender, by multiple regression. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using binary logistic regression models for nutrient intake and clinical data related to T2D.

RESULTS

Clinical data in T2D patients and healthy individuals by sex
A total of 605 individuals (337 males and 268 females) were included in the T2D group, and assessed alongside 724 healthy individuals (control group: 405 males and 319 females). Average ages of male and female T2D patients were 53.16 and 55.69 y, respectively, and showed non-significant difference compared with control values. A family history of T2D in the patient group was more frequent than in controls. No statistically significant differences were found in residence status, smoking status or alcohol consumption between T2D patients and healthy individuals (Table 1).

Significant differences were obtained between the T2D and control groups, subdivided by sex, in glucose administration, 2-h postprandial blood glucose, HbA1c (%), serum total cholesterol, and HDL-cholesterol (all \( p < 0.05 \)). PTH showed a significant difference between the healthy individuals and the patients in males but not females (44.40±19.30 vs. 53.32±13.07, \( p < 0.05 \)). No significant differences were found in triglyceride levels. Over half of the patients had FPG values exceeding the control target for the prevention of diabetes-related complications (<7.0 mmol/L, according to The International Diabetes Federation) (\( p < 0.05 \)).

Daily intake of macronutrients and calcium in T2D cases and controls by sex

Daily intake data for macronutrients, calcium and phosphorus in healthy and diabetic cases subdivided by sex are shown in Table 2. Total energy, carbohydrate, fat, and fat-to-energy ratio were significantly higher in healthy male controls than T2D cases (\( p < 0.05 \)). However, the carbohydrate-to-energy ratio was significantly lower in the male control than the patient subgroup. In females, total energy, protein, protein-to-energy ratio, carbohydrate, fat, fat-to-energy ratio, diet fiber, phosphorus and magnesium were significantly higher in healthy females than the T2D group. Similar to that in males, the carbohydrate-to-energy ratio was significantly lower in female controls compared with their T2D counterparts.

Binary logistic regression analysis for the identification of significant factors

Binary logistic regression analysis was used to identify significant risk factors of T2D, after controlling for age, BMI, history of hypertension, energy-adjusted phosphorus and total energy. In males, alcohol consumption and total energy were risk factors for T2D (OR = 5.01, \( p = 0.002; \) OR = 1.85, \( p = 0.001 \)); however, serum 25OHD was not related to T2D (OR = 1.00, \( p = 0.016 \)) (Table 3). In females, older age and higher daily intake of total energy and fat were significant risk factors when comparing the patients and the healthy (respectively, \( OR = 1.87, \ p = 0.006; \) \( OR = 1.72, \ p = 0.001; \) \( OR = 1.21, \ p = 0.05 \)) (Table 4). Diet calcium and serum 25OHD were two protecting factors against T2D (OR = 0.40, \( p = 0.034; \) OR = 0.50, \( p = 0.019 \)) in women.

DISCUSSION

In this study we present evidence for cross-sectional associations of decreased bone metabolism and T2D with increased dietary intake of calcium and serum 25OHD levels in females. In contrast, there were no significant associations of bone metabolism indices with T2D in males.

Due to rapid economic and social changes, the Chinese diet and lifestyle have changed, and the traditional Chinese dietary pattern, which is recognized to decrease the risk of developing diabetes (19), is not implementd. According to evidence from the 2002 China National Nutrition and Healthy Survey, the new Chinese dietary pattern has a higher intake of animal food but still lacks dairy products and physical activity, which results in substantially higher risk of prediabetes in Chinese adults. Data from the China National Nutrition Survey of 1992 and 2002 showed a drop in average dietary calcium intake from 405–388.8 mg/d, significantly lower than the Chinese dietary reference has recommended (800 mg/d) (34).The major and most well-known function of vitamin D and calcium is to promote mineralization. However, recent evidence suggested that vitamin D and calcium homeostasis may also be important for a variety of non-skeletal outcomes. Indeed, multivariate analyses showed a marginally significant inverse association of T2D with dietary calcium intake in American women (13, 35). In the prospective studies, low

| Variables | Odds ratio | 95% CI | \( p \) value |
|-----------|------------|-------|--------------|
| Age       | 5.005      | 1.79–13.97 | 0.02      |
| Alcohol consumption | 5.01 | 1.79–13.97 | 0.002    |
| Total energy (kcal) | 1.85 | 1.27–2.67 | 0.001    |
| 25OHD     | 1.00       | 0.99–1.00 | 0.016     |

Table 3. Binary logistic regression analysis of factors associated with T2D in males.

| Variables | Odds ratio | 95% CI | \( p \) value |
|-----------|------------|-------|--------------|
| Age       | 1.87       | 1.20–2.92 | 0.006    |
| Total energy (kcal) | 1.72 | 1.19–2.53 | 0.001    |
| Fat (g)   | 1.21       | 1.01–1.79 | 0.05     |
| Diet-Ca (mg) | 0.40 | 0.17–0.93 | 0.034    |
| 25OHD     | 0.50       | 0.28–0.89 | 0.019     |

Table 4. Binary logistic regression analysis of factors associated with T2D in females.

Adjusted further for age, BMI, history of hypertension, energy-adjusted phosphorus and total energy.
calcium intake was consistently found to be inversely associated with T2D incidence (13, 27). Another investigation by Black Women’s Health Study (a prospective cohort study of ~59,000 women aged 21–69 at baseline) found an odds ratio for T2D incidence of 0.82 (0.72–0.93) for highest vs. lowest calcium intake (28). In this study, data from the female group suggested that dietary calcium played a protective role in T2D (OR=0.40, p=0.034, 95%CI 0.17–0.93), corroborating the above findings. Serum 25OHD was also found to protect women from T2D (OR=0.50, p=0.019, 95%CI 0.28–0.89). An Australian study demonstrated that elevated serum 25OHD amounts were correlated to significantly lower risk of diabetes in both adult men and women (12). Similarly, vitamin D deficiency is inversely correlated to insulin resistance, regardless of the glucose tolerance status (18). The discrepant findings indicated that other parameters (e.g. genetic component, ethnicity, and environmental conditions) might be involved in these complex relationships.

Our results, showing a lower prevalence of T2D in females with increasing dietary intake of calcium and serum 25OHD levels, were in line with a meta-analysis that demonstrated an inverse association of vitamin D status with the prevalence T2D in females. Although we have no definite explanation for this finding, some underlying mechanisms are imaginable. The average age of female subjects in our study was above 55 y, i.e. menopausal women. Menopause is associated with changes in bone turnover, weight gain and altered body fat distribution. Postmenopausal women are at increased risk to develop vitamin D and calcium deficiencies. Female patients with T2D have lower estrogen levels during menopause (29); this often increases the risk of many diseases, e.g. accelerating loss of serum calcium (12). However, the exact mechanisms associated with the sex differences deserve further investigation. Thus, one might speculate that the positive effect of calcium results from dietary intake in premenopausal women. The lower protein intake of T2D females compared with that of healthy females suggested less animal-based food consumption in T2D females, while T2D males showed higher protein intake and more animal-based food consumption than of healthy counter-males showed higher protein intake and more animal-based food consumption in T2D females, while T2D compared with that of healthy females suggested less animal-based food consumption in T2D females, while T2D males showed higher protein intake and more animal-based food consumption than of healthy counter-males showed higher protein intake and more animal-based food consumption in T2D females, while T2D males showed higher protein intake and more animal-based food consumption than of healthy counter-males showed higher protein intake and more animal-based food consumption in T2D females, while T2D males showed higher protein intake and more animal-based food consumption than of healthy counter-males showed higher protein intake and more animal-based food consumption in T2D females, while T2D males showed higher protein intake and more animal-based food consumption than.
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9) Gow ML, Garnett SP, Baur LA, Lister NB. 2016. The effectiveness of different diet strategies to reduce type 2 diabetes risk in youth. *Nutrients* **8**: 486.

10) Jensen ME, Ducharme FM, Theoret Y, Belanger AS, Dellvin E. 2016. Data in support for the measurement of serum 25-hydroxyvitamin D (25OHD) by tandem mass spectrometry. *Data Brief* **8**: 925–929.

11) Wood JR, Connor CG, Cheng P, Ruedy KJ, Tamborlane TW, Klingensmith G, Schatz D, Gregg B, Cengiz E, Willi S, Bacha F, Beck RW. 2016. Vitamin D status in youth with type 1 and type 2 diabetes enrolled in the Pediatric Diabetes Consortium (PDC) is not worse than in youth without diabetes. *Pediatr Diabetes* **17**: 584–591.

12) Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, Sikaris K, Grantham N, Ebeling PR, Daly RM. 2011. Serum 25-hydroxyvitamin D, calcium intake, and risk of type 2 diabetes after 5 years: results from a national, population-based prospective study (the Australian Diabetes, Obesity and Lifestyle Study). *Diabetes Care* **34**: 1133–1138.

13) Modi KD, Ahmed MI, Chandwani R, Kumar KV. 2015. Prevalence of vitamin D deficiency across the spectrum of glucose intolerance. *Diabetes Metab Disord* **14**: 54.

14) Tai K, Need AG, Horowitz M, Chapman IM. 2008. Vitamin D, glucose, insulin, and insulin sensitivity. *Nutrition* **24**: 279–285.

15) Palomer X, Gonzalez-Clemente JM, Blanco-Vaca F, Mauricio D. 2008. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obes Metab* **10**: 185–197.

16) Zemel MB. 1998. Nutritional and endocrine modulation of intracellular calcium: implications in obesity, insulin resistance and hypertension. *Mol Cell Biochem* **188**: 129–136.

17) Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. 2007. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* **30**: 980–986.

18) van Dam RM, Hu FB, Rosenberg L, Krishnan S, Palmer JR. 2006. Dietary calcium and magnesium, major food sources, and risk of type 2 diabetes in U.S. black women. *Diabetes Care* **29**: 2238–2243.

19) Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB. 2006. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* **29**: 650–656.

20) Choi HK, Willett WC, Stampfer MJ, Rimm E, Hu FB. 2005. Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. *Arch Intern Med* **165**: 997–1003.

21) Harinarayan C, Arvind S, Joshi S, Thennarasu K, Vedavayas V, Baindur A. 2014. Improvement in pancreatic β-cell function with vitamin D and calcium supplementation in vitamin D-deficient nondiabetic subjects.