Association of Serum 25(OH)D Levels with Incident Type 2 Diabetes in Chinese adults: A Prospective Cohort Study

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Abstract

Although many cross-sectional studies that have examined the relationship of Serum 25-hydroxyvitamin D [25(OH)D] levels with incident type 2 diabetes (T2D), there are very few reports for cohort studies. Here, we performed an a prospective cohort study to investigate the effect modification of traditional risk factors on association of serum 25(OH)D with T2D and to determine if addition of serum 25(OH)D improve the risk prediction of T2D. A total of 1926 adults without diabetes were prospectively followed for 36 months in China. During a mean of 36 months of follow-up, 114 participants (5.9%) developed incident T2D. Although no significant relationship was found in total population, stratified analyses indicated that low 25(OH)D levels was associated with increased T2D risk among individuals who were male, overweight/obese, with insufficient physical activity, or normal fasting plasma glucose at baseline. Additive interaction analysis showed that low 25(OH)D levels and insufficient physical activity interacts to increase the risk of T2D (RERI = 0.875, 95% CI: 0.204–1.545). Addition of low serum 25(OH)D significantly improved the predictive performance of T2D beyond conventional risk factors (NRI = 0.205, 95% CI:0.019–0.391). Our results suggest that low serum 25(OH)D and insufficient physical activity interact synergistically to influence the risk of T2D in Chinese adult population. Adding serum 25(OH)D to conventional risk factors improves the risk prediction of T2D.

1. Introduction

As a metabolic disorder characterized by insulin resistance and pancreatic β cell dysfunction, type 2 diabetes (T2D) has become a worldwide epidemic with high mortality and comorbidity because of its macrovascular and microvascular complications. The increasing incidence of T2D has prompted urgent calls for innovative methods to reverse this trend. Recently, vitamin D has attracted increasing public health interest because of its extra-skeletal effects, including its association with the risk of developing T2D. It is biologically plausible that vitamin D may play a role in the pathogenesis of T2D, as reduced insulin resistance and lowered systematic inflammation have been reported with vitamin D in vivo and vitro studies [1–3].

Some human studies have investigated the potential relationship of vitamin D status with T2D. Several meta-analyses [4–6] summarized these studies and suggested a negative association between blood 25-hydroxyvitamin D [25(OH)D] concentrations and T2D risk. However, most previous studies were conducted in individuals of European descent. For individuals from China where both T2D and vitamin D deficiency were prevalent [7, 8], the information is limited. Moreover, evidence indicated that there existed ethnic differences in the risk profiles for T2D between east Asian and European populations [9]. Considering the important public health implications of the diabetes epidemic and the availability of vitamin D supplementation, exploration of the association between vitamin D status and T2D in Chinese is an important first step in the development of preventive measures for T2D.

Overweight/obesity, old age, male gender, and low physical activity are well-established predisposing factors for T2D. Previous studies have performed subgroup analyses to investigate the modifying effect
of traditional risk factors on the relationship between blood 25(OH)D levels and risk of developing T2D [10–12]. For example, the Danish Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) 10 study found that the inverse association between serum 25(OH)D and incident diabetes was only found in overweight-obese and not in normal weight individuals, suggesting that obesity may modify the effect of vitamin D status on the risk of diabetes [12]. Similar findings were also reported in the Nurses’ Health Study [11]. Sex-specific effects of vitamin D in the pathogenesis of T2D were observed in a prospective observational study in the Diabetes Prevention Program (DPP) [10]. Nevertheless, there is a lack of literature regarding the interactions between traditional risk factors and 25(OH)D on T2D. Moreover, few data exist on whether baseline serum 25(OH)D can refine risk prediction for T2D.

Thus, we conducted a prospective cohort study in Chinese adults to assess the independent association of serum 25(OH)D levels with incident T2D and to further evaluate its interaction with traditional risk factors on risk of T2D. We also explored whether serum 25(OH)D may improve the predictive value of T2D beyond conventional risk factors.

2. Results

3.1 Baseline characteristics of study population

Table 1 summarizes baseline characteristics of the study populations. The final study population was comprised of adult residents with an average age of 52.08 ± 13.82 years, and 808 (41.95%) were men. The median serum 25(OH)D concentration was 25.415 ng/ml (IQR: 21.14–29.55 ng/ml). Of 1926 participants, percentage of vitamin D deficiency (< 20 ng/ml) and insufficiency (20–29 ng/ml) was 19.37% and 57.84%, respectively.
Table 1
Baseline characteristics of the study population according to serum 25(OH)D levels (n = 1926)

| Characteristic                        | Total (n = 1926) | Serum 25(OH)D levels (ng/ml) | P     |
|--------------------------------------|------------------|------------------------------|-------|
|                                      |                  | < 25.415 (n = 963)           | ≥ 25.415 (n = 963) |
| 25(OH)D, median (IQR)                | 25.415 (21.14–29.55) | 21.14 (17.93–23.55)         | 29.55 (27.37–32.45) |
| Age (years)                          | 52.08 ± 13.82    | 51.65 ± 14.18                | 52.52 ± 13.44 | 0.164 |
| Sex (men), n (%)                     | 808 (41.95)      | 294 (30.53)                  | 514 (53.37) | < 0.001 |
| District (rural), n (%)              | 754 (39.15)      | 328 (34.06)                  | 426 (44.24) | < 0.001 |
| Education, n (%)                     |                  |                              | 0.118 |
| No school                            | 327 (16.98)      | 164 (16.93)                  | 163 (17.03) |
| Primary school                       | 524 (27.21)      | 256 (26.58)                  | 268 (27.83) |
| Middle school                        | 938 (48.70)      | 461 (47.87)                  | 477 (49.53) |
| Junior college or higher             | 137 (7.11)       | 82 (8.52)                    | 55 (5.71)  |
| Body mass index (kg/m²)              | 23.73 ± 3.32     | 23.94 ± 3.43                 | 23.53 ± 3.18 | 0.007 |
| normal (< 24)                        | 1091 (56.65)     | 516 (53.58)                  | 575 (59.71) | 0.009 |
| overweight (24–28)                   | 640 (33.23)      | 334 (34.68)                  | 306 (31.78) |
| obesity (≥ 28)                       | 195 (10.12)      | 113 (11.73)                  | 82 (8.52)  |
| FPG (mmol/L)                         | 5.65 ± 0.59      | 5.69 ± 0.58                  | 5.61 ± 0.60 | 0.003 |
| IFG, n (%)                           | 996 (51.71)      | 516 (53.58)                  | 480 (49.84) | 0.101 |
| Hypertension, n (%)                  | 632 (32.81)      | 318 (33.02)                  | 314 (32.61) | 0.846 |
| Dyslipidemia, n (%)                  | 832 (43.20)      | 471 (48.91)                  | 361 (37.49) | < 0.001 |
| Current smoker, n (%)                | 415 (21.55)      | 168 (17.45)                  | 247 (25.65) | < 0.001 |
| Current drinker, n (%)               | 585 (30.37)      | 225 (23.36)                  | 360 (37.38) | < 0.001 |
| Sufficient physical activity, n (%)  | 429 (22.27)      | 235 (24.40)                  | 194 (20.15) | 0.025 |

IQR: interquartile range, FPG: fasting plasma glucose, IFG: impaired fasting glucose
As shown in Table 1, participants were divided into two groups according to the median of serum 25(OH)D. Subjects with 25(OH)D levels $\geq 25.415$ ng/ml tended to be men, current smoker, current drinker, to live in rural areas, and to have lower BMI and FPG (all $P$ values $< 0.01$); and were less likely to have dyslipidemia, sufficient physical activity, and family history of diabetes (all $P$ values $< 0.05$). There was no significant difference in age, education level, proportions of IFG and hypertension between the two groups.

### 3.2 Associations of serum 25(OH)D levels with T2D incidence

As shown in Table 2, during the three years of follow-up, 114 participants (5.9%) developed incident T2D. Taking subjects with serum 25(OH)D $\geq 25.415$ ng/ml as reference, those with 25(OH)D $< 25.415$ ng/ml did have an increased risk of developing T2D in model 1 (HR = 1.48, 95% CI: 1.02–2.16; $P = 0.039$) and model 2 (adjusted HR = 1.49, 95% CI: 1.02–2.20; $P = 0.042$). However, after further adjustments for potential confounders, this association did not reach statistical significance in model 3 (adjusted HR = 1.36, 95% CI: 0.92–1.99; $P = 0.120$). Compared with vitamin D sufficient participants, those with vitamin D deficiency and insufficiency had higher risk of developing T2D in model 1 and model 2. But these associations were not significant in model 3. The HRs (95% CIs) were 1.83 (0.96–3.47) and 1.60 (0.92–2.79) for vitamin D deficient and insufficient subjects. No significant linear trend was observed in Model 3 ($P = 0.076$). When considered as a continuous variable, a 10-ng/ml higher 25(OH)D levels was not significantly associated with T2D incidence in model 1–3 (Table 2).
Table 2
Association of serum 25(OH)D with incident type 2 diabetes

| 25(OH)D (ng/ml) | Events (%) | Model 1 | Model 2 | Model 3 |
|------------------|------------|---------|---------|---------|
|                  |            | HR (95% CI) | \(P\) | HR (95% CI) | \(P\) | HR (95% CI) | \(P\) |
| \(\geq 25.415\) | 46 (4.78)  | 1.00 | - | 1.00 | - | 1.00 | - |
| < 25.415         | 68 (7.06)  | 1.48 (1.02–2.16) | 0.039 | 1.49 (1.02–2.20) | 0.042 | 1.36 (0.92–1.99) | 0.120 |
| \(\geq 20\)     | 88 (5.67)  | 1.00 | - | 1.00 | - | 1.00 | - |
| < 20             | 26 (6.97)  | 1.23 (0.79–1.90) | 0.356 | 1.18 (0.75–1.84) | 0.470 | 1.26 (0.80–1.97) | 0.319 |
| \(\geq 30\)     | 16 (3.64)  | 1.00 | - | 1.00 | - | 1.00 | - |
| < 30             | 98 (6.59)  | 1.82 (1.07–3.09) | 0.026 | 1.83 (1.07–3.14) | 0.028 | 1.66 (0.96–2.84) | 0.068 |
| \(\geq 30\)     | 16 (3.64)  | 1.00 | - | 1.00 | - | 1.00 | - |
| 20-29.9          | 72 (6.46)  | 1.79 (1.04–3.07) | 0.036 | 1.81 (1.05–3.14) | 0.034 | 1.60 (0.92–2.79) | 0.094 |
| < 20             | 26 (6.97)  | 1.92 (1.03–3.58) | 0.040 | 1.89 (1.00–3.60) | 0.051 | 1.83 (0.96–3.47) | 0.065 |

\(P\) for trend*: 0.049 0.068 0.076

Continuous #: 114 (5.92) 0.75 (0.56–1.00) 0.052 0.76 (0.57–1.03) 0.072 0.80 (0.59–1.09) 0.155

Model 1: unadjusted; Model 2: adjusted for age, sex and body mass index; Model 3: adjusted for model 2 and district, education level, fasting plasma glucose, hypertension, dyslipidemia, smoking status, alcohol drinking, physical activity and family history of diabetes.

HR: hazard ratio, CI: confidence interval.

*: Test for trend based on variable containing median value for each group.

#: HR was scaled to 10-ng/ml higher serum 25(OH)D levels

Interaction analysis of 25(OH)D with conventional risk factors on T2D risk
When stratified by sex, men with lower 25(OH)D (< 25.415 ng/ml) had a significantly higher risk of T2D compared with those with 25(OH)D ≥ 25.415 (adjusted HR = 1.82, 95% CI: 1.03–3.21; \( P = 0.039 \); Table 3). No significant association was found in women (adjusted HR = 1.04, 95% CI: 0.62–1.76; \( P = 0.877 \)). When stratified by BMI, physical activity or baseline FPG, the significant relationship of lower 25(OH)D with T2D risk was limited to overweight/obese individuals (adjusted HR = 1.64, 95% CI: 1.00–2.68; \( P = 0.048 \)), participants with insufficient physical activity (adjusted HR = 1.58, 95% CI: 1.03–2.42; \( P = 0.037 \)) or those with FPG < 5.6 mmol/L at baseline (adjusted HR = 2.78, 95% CI: 1.18–6.51; \( P = 0.019 \)).
### Table 3
**Interactions between serum 25(OH)D and traditional risk factors on type 2 diabetes**

|                      | Events (%) | HR (95% CI) * | P*   | RERI (95% CI) | P_{interaction} # |
|----------------------|------------|---------------|------|---------------|-------------------|
| **Total**            | 114 (5.92) | 1.36 (0.92–1.99) | 0.120 |               |                   |
| **Age**              |            |               |      |               |                   |
| < 52 years           | 38 (3.93)  | 0.95 (0.49–1.84) | 0.870 |               |                   |
| ≥ 52 years           | 76 (7.92)  | 1.56 (0.96–2.53) | 0.074 |               |                   |
| **Sex**              |            |               |      |               |                   |
| male                 | 53 (6.56)  | 1.82 (1.03–3.21) | 0.039 |               |                   |
| female               | 61 (5.46)  | 1.04 (0.62–1.76) | 0.877 |               |                   |
| **BMI**              |            |               |      |               |                   |
| < 24 kg/m²           | 40 (3.67)  | 1.10 (0.57–2.13) | 0.784 |               |                   |
| ≥ 24 kg/m²           | 74 (8.86)  | 1.64 (1.00–2.68) | 0.048 |               |                   |
| **Physical activity**|            | 0.875 (0.204–1.545) a | 0.087 |               |                   |
| sufficient           | 21 (4.90)  | 0.79 (0.31–2.07) | 0.637 |               |                   |
| insufficient         | 93 (6.21)  | 1.58 (1.03–2.42) | 0.037 |               |                   |
| **FPG**              |            | -1.729 (-4.031–0.574) | 0.005 |               |                   |
| < 5.6 mmol/L         | 29 (3.12)  | 2.78 (1.18–6.51) | 0.019 |               |                   |
| ≥ 5.6 mmol/L         | 85 (8.53)  | 1.07 (0.69–1.66) | 0.763 |               |                   |

BMI: body mass index, FPG: fasting plasma glucose, HR: hazard ratio, CI: confidence interval, RERI: relative excessive risk due to interaction

*: serum 25(OH) was categorized using median (25.415 ng/ml), Model 3: adjusted for age, sex, body mass index, district, education level, fasting plasma glucose, hypertension, dyslipidemia, smoking status, alcohol drinking, physical activity and family history of diabetes.

#: P for multiplicative interaction

a: statistically significant with RERI > 0 indicating additive interaction

Additive interaction analysis showed that lower 25(OH)D levels (< 25.415 ng/ml) and insufficient physical activity interacts to increase the risk of T2D (Table 3). The RERI was 0.875 (95% CI: 0.204–1.545). A significant multiplicative interaction effect existed between 25(OH)D and FPG levels on T2D (P = 0.005). No evidence suggested more or less than additive or multiplicative interaction for age, sex, or BMI (Table 3).
### 3.3 Prediction value of 25(OH)D for type 2 diabetes

The category free NRI of the model including serum 25(OH)D status improved by 0.205 (95% CI: 0.019–0.391, \(P = 0.034\)) for predicting T2D compared to the conventional risk model (Table 4). After 25(OH)D was added to the conventional risk model, 19% more cases were correctly reclassified.

#### Table 4
Reclassification of type 2 diabetes risk after addition of serum 25(OH)D to conventional risk model

|                          | NRI (95% CI) * | \(P^*\)  | % of events correctly reclassified | \(P\)  |
|---------------------------|----------------|----------|------------------------------------|--------|
| **Total**                 | 0.205 (0.019–0.391) | 0.034    | 19%                                | 0.039  |
| **Age**                   |                |          |                                    |        |
| < 52 years                | 0.006 (-0.318-0.330) | 0.973    | -5%                                | 0.746  |
| \(\geq 52\) years        | 0.349 (0.123–0.576) | 0.004    | 26%                                | 0.022  |
| **Sex**                   |                |          |                                    |        |
| male                      | 0.473 (0.199–0.747) | 0.001    | 17%                                | 0.216  |
| female                    | -0.017 (-0.270-0.235) | 0.896    | -21%                               | 0.096  |
| **BMI**                   |                |          |                                    |        |
| < 24 kg/m\(^2\)          | 0.062 (-0.254-0.378) | 0.701    | 0                                  | 1      |
| \(\geq 24\) kg/m\(^2\)   | 0.249 (0.020–0.478) | 0.041    | 30%                                | 0.011  |
| **Physical activity**     |                |          |                                    |        |
| sufficient                | 0.146 (-0.292-0.584) | 0.515    | 5%                                 | 0.827  |
| insufficient              | 0.293 (0.089–0.497) | 0.006    | 25%                                | 0.017  |
| **FPG**                   |                |          |                                    |        |
| < 5.6 mmol/L              | 0.503 (0.171–0.835) | 0.008    | 45%                                | 0.016  |
| \(\geq 5.6\) mmol/L      | 0.076 (-0.145-0.297) | 0.501    | 11%                                | 0.329  |

BMI: body mass index, FPG: fasting plasma glucose, NRI: net reclassification index, CI: confidence interval

*: serum 25(OH) was categorized using median (25.415 ng/ml), conventional risk factors included age, sex, body mass index, district, education level, fasting plasma glucose, hypertension, dyslipidemia, smoking status, alcohol drinking, physical activity and family history of diabetes

The added predictive value of including 25(OH)D were most evident among adults with age \(\geq 52\) years (NRI = 0.349, 95% CI: 0.123–0.576; \(P = 0.004\)), male gender (NRI = 0.473, 95% CI: 0.199–0.747; \(P = 0.001\)), overweight/obesity (NRI = 0.249, 95% CI: 0.020–0.478; \(P = 0.041\)), insufficient physical activity (NRI =
0.293, 95% CI: 0.089–0.497; \( P = 0.006 \)) or normal FPG (NRI = 0.503, 95% CI: 0.171–0.835; \( P = 0.008 \)). Serum 25(OH)D correctly reclassified 26%, 30%, 25%, and 45% more cases among those with age ≥ 52 years, overweight/obesity, insufficient physical activity or normal FPG at baseline (Table 4).

3. Discussion

The relationship between serum 25(OH)D levels and T2D risk have attracted extensive attention during the past decade; and some previous studies, but not all, reported a significant inverse correlation between the two variables. Several meta-analyses summarized these studies and concluded that lower vitamin D levels might be associated with increased risk of T2D \([4–6]\). For instance, Song et al performed a meta-analysis on the data from 21 prospective studies and found that the summary risk ratio for T2D was 0.62 (95% CI: 0.54–0.70) when comparing the highest to the lowest category of 25(OH)D concentrations \([6]\). The China Kadoorie Biobank (CKB) study indicated that a 10-ng/ml higher 25(OH)D level was associated with a 9% (95% CI: 0%-18%) lower risk of incident diabetes after adjustment for age, sex, latitude, season, SBP, physical activity and body fat percentage \([13]\). While the Hong Kong Osteoporosis Study reported no association between serum vitamin D and the risk of incident diabetes in Hong Kong Chinese \([14]\).

In the current study, the association between serum 25(OH)D levels and incident T2D was nonsignificant in an adult population from east central China. The failure to detect significance may be partly due to the small sample size and short follow-up time. For example, the association of vitamin D deficiency with T2D risk was marginally significant after complete adjustment (adjusted HR = 1.83, 95% CI: 0.96–3.47; \( P = 0.065 \)). Another possible explanation for the non-significant association could be the study population which were at high risk for T2D \([15]\). Vitamin D maybe only beneficial in patients with normal glucose tolerance because the development of T2D consist of a progressive insulin resistance, which is initially compensated by enhanced insulin secretion by pancreatic \( \beta \) cells. At the on-set of T2D, the \( \beta \) cell mass is reduced by 25–50% \([16]\). When IFG is already present, the effect of vitamin D maybe not strong enough to reverse the deterioration of glucose metabolism. In the current study, about half (51.71%) of the study individuals had IFG at baseline. Stratified analysis by baseline FPG showed that the effect of lower 25(OH)D (< 25.415 ng/ml) on T2D risk was significant in individuals with FPG < 5.6 mmol/L and not with FPG ≥ 5.6 mmol/L at baseline. However, in a Swedish middled-aged population, high serum 25(OH)D levels predicted a lower risk of T2D in persons with prediabetes, but not normal glucose tolerance \([17]\). Our results showed a significant multiplicative interaction between 25(OH)D and FPG on T2D, but the test of additive interaction was nonsignificant. The effect modification of baseline glucose metabolism status on the association between vitamin D and T2D risk deserve further investigation.

Stratified analyses also showed that the association between low 25(OH)D with a greater risk of developing T2D were limited to overweight/obese individuals, men, or subject with insufficient physical activity. In agreement with our study, the MONICA 10 study showed that significant inverse association of serum 25(OH)D levels with T2D risk was only evident among overweight/obese individuals \([12]\). The Nurses' Health Study also reported that the inverse association was stronger among overweight/obese
women than women with normal BMI \[11\]. For people with prediabetes \[10\], the inverse relationship between plasma 25(OH)D and incident diabetes was also restricted to people with a BMI \[\geq 30 \text{ kg/m}^2\]. However, results from the Copenhagen City Heart Study did not observe any effect modification by BMI \[18\]. Interaction analysis between 25(OH)D and BMI was conducted in some studies. Most of them only did multiplicative interaction and reported negative results \[10, 11, 18\], except the MONICA 10 study which reported significant multiplicative interaction between 25(OH)D and BMI as well as WC \[12\]. No evidence suggested interaction on the multiplicative or additive scale in our study. Consistent with our findings, data from the National Health and Nutrition Examination Survey (NHANES) indicated that there is no multiplicative or additive interaction between overweight/obesity and insufficient 25(OH)D on T2D \[19\].

As for sex and physical activity, two prospective studies reported that the inverse association of 25(OH)D with incident T2D did not differ by sex or physical activity, and no significant multiplicative interaction existed \[12, 18\]. Although the test of multiplicative interaction is nonsignificant, our results revealed an additive interaction between serum 25(OH)D and physical activity on T2D risk. The joint effects of low serum 25(OH)D and insufficient physical activity on T2D were greater than would be expected from the effects of the individual risk factor alone. This finding may have large public health significance because the burden of T2D in persons with insufficient exercise may be decreased by making improvements in serum 25(OH)D levels. Considering the difficulty in keeping sufficient exercise in modern lifestyle, recommendations to maintain high vitamin D status may be a convenient way to reduce the risk of T2D. Mechanism underlying the additive interaction of serum 25(OH)D with physical activity on T2D risk are not clearly understood. In adults at high risk of T2D, moderate to vigorous physical activity was associated with lower levels of trimethylamine N-oxide (TMAO) \[20\], a novel gut-derived metabolite that is associated with insulin resistance and impaired glucose tolerance \[21, 22\]. Animal studies showed vitamin D supplementation greatly reduced plasma TMAO level in mice \[23\], and a cross-sectional study reported high TMAO concentrations were related with vitamin D deficiency \[24\]. It is plausible to speculate that higher TMAO due to insufficient physical activity may be aggravated by low 25(OH)D level. Further studies are needed to clarify our results and explore the underlying mechanism.

In addition, we calculated the category free NRI to measure the incremental predictive value of adding serum 25(OH)D to traditional risk factors for the development of T2D. The addition of serum 25(OH)D significantly improved the NRI for T2D by 0.205 in total population. The improvement in the prediction of T2D, above conventional risk factors, demonstrates the potential of serum 25(OH)D as biomarkers for T2D risk. When stratified by age, sex, BMI, physical activity and FPG at baseline, the added predictive value of including 25(OH)D were most evident among adults with older age, male gender, overweight/obesity, insufficient physical activity or normal FPG. Few studies previously evaluated the impact of adding baseline serum 25(OH)D on net reclassification of T2D risk beyond conventional risk factors. Researchers found that addition of serum 25(OH)D to the Framingham Risk Score could improve coronary heart disease risk estimation in patients with essential hypertension \[25\]. However, considering
25(OH)D in addition to established risk factors only marginally added prognostic value of mortality risk in patients undergoing coronary catheterization [26].

To the best of our knowledge, the present study is the first to report an additive interaction between serum 25(OH)D level and physical activity on T2D risk. Nevertheless, there are several limitations in the present study. First, the relatively small sample size, which limited our statistical power, and the short-term follow-up, lack of information on vitamin D status at follow-up and dietary habits. The follow-up period may not long enough to detect a modest effect and the association of serum 25(OH)D with T2D is likely to be underpowered, especially within subgroup analysis. Second, we did not have data on oral glucose tolerance and HbA1c, which may lead to misclassification of diagnosis of T2D.

4. Conclusions

In conclusion, low serum 25(OH)D levels were independently associated with an increase in T2D incidence among men, overweight/obese individuals, subjects with insufficient physical activity and those with normal FPG at baseline. Significant additive interaction between 25(OH)D level and physical activity was observed. In relation to T2D risk prediction, adding low serum 25(OH)D to model with conventional risk factors may offer incremental predictive power. Our data are of significance to public health and future studies are needed to assess the effect of vitamin D supplementation on prevention of T2D, especially among people with insufficient exercise.

5. Methods

5.1 Ethics Committee approval

The study was reviewed and approved by a human research ethics committee at The Soochow University (Ethics Approval Reference Number: # 20160001), and it was in compliance with the declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study.

5.2 Study population

From 1 September to 14 October 2013, a total of 2072 adults from the Liandu district in Lishui city, in east-central China (see supplementary data Fig S1), of Han ethnicity were invited to participate in the study. Liandu district consists of 16 blocks, and approximately 400,000 people live there. A multistage sampling method was applied to the present study. First, 4 residence communities in each block were sampled by simple random sampling. Then, systematic sampling was adopted to select 33 households in each residence community. Finally, one adult who had lived in the Liandu district for at least 2 years, was randomly selected from each household without replacement. In total, 2072 residents were enrolled. Of these invited residents, 1926 individuals met the inclusion criteria for the study i.e., without diabetes (based on self-assessment questionnaire), malignancy, chronic liver or renal diseases; fasting plasma glucose (FPG) < 7.0 mmol/L and without vitamin D supplementation. A follow-up survey was conducted for all participants in October 2016 to collect data on FPG and development of T2D.
5.3 Primary endpoint

The development of T2D was the primary endpoint in this study. New-onset T2D was defined as a self-reported history of a physician diagnosis during the follow-up period, and/or receiving pharmacological treatment for T2D, and/or a FPG level $\geq 7.0$ mmol/L $^{[27]}$.

5.4 Baseline measurements

At the baseline visit, standardized questionnaires were used to collect information regarding participants’ demographics [i.e., age, sex and residential district (rural or urban)], socio-economic status (including education level), smoking status (current, former, or never smoked), alcohol use (current, former or never drank), physical activity, and family history of diabetes. Education level was categorized into four groups: no school, primary school, middle school, and junior college or higher. According to the WHO recommendations, sufficient physical activity was defined as engaging in at least 150 minutes of moderate-intensity activity per week or equivalent $^{[28]}$. Family history was judged as positive when at least one of the first-degree relatives had diabetes.

Height (in cm) was measured with a wall-mounted stadiometer and weight (in kg) was measured with a balance-beam scale. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m$^2$). $\text{BMI} \geq 28.0$, 24.0-27.9 and $< 24.0$ kg/m$^2$ was classified as obesity, overweight, and normal weight $^{[29]}$. Blood pressure was measured three times while participants were in the relaxed sitting position after 15 min of rest. There was a 5-min rest period between each measurement and the mean value of the three measurements were used for analysis. Hypertension was defined as a mean systolic blood pressure (SBP) $\geq 140$ mmHg and/or diastolic blood pressure (DBP) $\geq 90$ mmHg and/or the use of antihypertensive medications.

5.5 Data collection and definitions

Participants were instructed to take all regular medications, but not aspirin or nonsteroidal anti-inflammatory drugs for 48 hours before the visit. Participants were further requested to refrain from smoking for 1 h and from vigorous physical activity for 12 h before the visit. A venous blood sample was collected from each participant following an 8-h overnight fast for biochemical analysis. Aliquots of serum, plasma and buffy coat were frozen and shipped on dry ice to Lishui Center for Disease Control and Prevention and stored at -80$^\circ$C for future assays. Serum 25(OH)D concentration was determined using an automatic chemiluminescence immunoassay analyzer (ADVIA Centaur XP, Siemens Healthcare diagnostics Inc, Tarrytown NY, USA). According to the Endocrine Society clinical practice guideline $^{[30]}$, vitamin D deficiency, insufficiency and sufficiency were defined as serum 25(OH)D < 20, 20-29.9 and $\geq 30$ ng/ml. FPG concentration was determined by the hexokinase method using an automatic biochemical analyzer (COBAS c702, Roche Diagnostics Gmbh, Mannheim, Germany). Based on one of the diagnostic criteria for prediabetes proposed by the American Diabetes Association $^{[27]}$, impaired fasting glucose (IFG) was defined as 5.6 mmol/L $\leq$ FPG $< 7.0$ mmol/L. The levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were
measured by an enzymatic method on the automatic biochemistry analyzer (COBAS c702). Dyslipidemia was defined as TC ≥ 6.2 mmol/L and/or TG ≥ 2.3 mmol/L and/or HDL-C < 1.0 mmol/L according to Chinese guideline for the management of dyslipidemia in adults [31].

5.6 Statistical analysis

All data were analyzed by using SAS version 9.4 (SAS Institute, Cary, NC, USA), and statistical significance was defined as two-sided P value < 0.05. Data were presented as mean ± standard deviation or median (interquartile range: IQR) for continuous variables and as n (%) for categorical variables. Participants were divided into two groups using the median of serum 25(OH)D. The differences in quantitative variables between two groups were compared by using independent Student t tests. Qualitative variables were tested by chi-square analysis. The Cox proportional hazard regression model was performed to determine the hazard ratio (HR) for the risk of developing T2D and serum 25(OH)D level with T2D incidence as the dependent variable. Serum 25(OH)D was analyzed as continuous variable or categorical variable which was categorized using median, as well as thresholds of vitamin D deficiency and insufficiency. In Model 1, only serum 25(OH)D level was included as the independent variable. Then age, sex, and BMI were included in model 2. In model 3, the following variables were added to model 2: residential district, education level, FPG, hypertension, dyslipidemia, smoking status, alcohol use, physical activity, and family history of diabetes. Tests for trend were performed by entering the median value of each category of serum 25(OH)D as a continuous variable in the models. We further calculated the HRs for serum 25(OH)D and T2D across strata of age, sex, BMI, physical activity, and baseline FPG. Both additive and multiplicative interactions between serum 25(OH)D (categorized using median) and some established risk factors in association with T2D were calculated in the study. Additive interaction better reflects the presence of biological interaction [32]. The relative excess risk due to interaction (RERI) is calculated to test additive interaction [33]. In the absence of additive interaction, RERI is equal to 0. A cross-product interaction term was set in the Cox proportional hazard regression model to assess multiplicative interaction. The improvement of T2D risk prediction by adding serum 25(OH)D (categorized using median) to traditional risk factors (variables adjusted in Model 3) was assessed using the net reclassification index (NRI) [34, 35].

Declarations

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Authors’ Contributions: ZYH and XYZ contributed equally as first authors and were responsible for collection and compilation of data and in writing the manuscript. JFL performed data analysis. JXW and JFL contributed in the collection and compilation of clinical data. ZYH designed and supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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