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Vitamin D status in children and its association with glucose metabolism in northern China

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Abstract

Objectives: The aim of this study was to explore the vitamin D status of children in northern China and the association between vitamin D and glucose metabolism.

Design: Cross-sectional study was conducted among child participants and retrospective study designs was conducted among adult participants.

Setting and participants: The child participants were recruited from the baseline survey of IIVDDC, a total of 326 children were included. The data of adult participants were from the baseline data of the HDNNCDS, a total of 8,469 participants were eligible for our analysis.

Primary and secondary outcome measures: Physical examination, lifestyle and dietary habit data were recorded, and serum levels were measured in all participants. In adults, rickets in childhood was also investigated, which was used to define vitamin D deficiency in childhood. The associations were tested by linear regression and binary logistic regression. The effect of rickets on type 2 diabetes in adulthood was tested by binary logistic regression.

Result: In the pediatric study, only 10.7% of participants were vitamin D sufficient (≥30 ng/mL)v. Inverse correlations between serum 25(OH)D_3 concentration and fasting insulin and HOMA-IR were found, and children with lower serum 25(OH)D_3 concentrations were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, *p* value: 0.027). In an adult study, rickets in childhood increased the risk of type 2 diabetes in male participants (OR = 1.414, 95% CI = 1.013, 1.972; *p* value = 0.042), but this result was not observed in females.

Conclusion: Our findings suggest that vitamin D deficiency is widespread in northern China. Vitamin D deficiency in childhood was associated with IR and increased the risk of type 2 diabetes in male adults.

Keywords: vitamin D deficiency, children, insulin resistance, type 2 diabetes

Introduction

According to IDF statistics, there are 450 million adults with type 2 diabetes worldwide, accounting for approximately 90% of diabetes mellitus cases. Type 2 diabetes has become a global public health problem [1,2]. Some recent population studies have found that risk factors such as obesity, impaired glucose tolerance, or insulin resistance (IR)c in childhood, may also increase the risk of type 2 diabetes in adults [3-6]. These results suggested that controlling risk factors in childhood is an early prevention strategy to reduce the prevalence of type 2 diabetes in adulthood.

Vitamin D is a fat-soluble vitamin with several physiological functions, one of the most important of which is its effect on skeletal health [7]. At present, vitamin D deficiency is still a serious public problem worldwide, particularly in children [8]. The prevalence of vitamin D deficiency was approximately 50% in both developing and developed countries [9-12]. Serum 25(OH)D_3 is less affected by body regulation and is often used to evaluate vitamin D levels. It is usually considered that serum 25(OH)D_3 < 10 ng/mL indicates severe deficiency, 10-20 ng/mL indicates deficiency, 20-29 ng/mL indicates insufficiency, and ≥30 ng/mL indicates sufficiency [13]. Vitamin D deficiency in children is associated with many skeletal diseases, and one of the typical diseases is rickets. The clinical signs of rickets include skeletal deformity, restlessness, motor retardation and bone pain [14]. Recently, the association between vitamin D deficiency and extra-skeletal health has been of great concern, such as its association with glucose metabolism, obesity, respiratory tract infection and atopic dermatitis, among which the association with glucose metabolism has been of particular concern [15-17]. A population study of children and adolescents in Mexico found that low vitamin D levels were associated with IR; when the concentration of serum vitamin D increased, the possibility of presenting IR decreased [18]. Jared P Reis et al. reported that adolescents in the lowest quartile of vitamin D (<15 ng/mL) are more likely to have hyperglycemia compared with those in the highest quartile (>26 ng/mL) [19]. Furthermore, randomized controlled trials showed that vitamin D supplementation could increase insulin sensitivity and decrease IR and fasting glucose concentrations in obese children [20-22]. Since IR in children is a risk factor for type 2 diabetes, we hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting insulin sensitivity. A recent 31-year follow-up prospective study in 3- to 18-year-old young Finns found that high vitamin D levels in childhood could reduce the incidence of type 2 diabetes in adulthood [23]. This study further supported our hypothesis, but current research is very limited.

Harbin is a typical northeast city of China, with a latitude between 44°04′N and 46°40′N, and is a relatively high-latitude area. The winters in Harbin are long, and the sunlight is relatively insufficient year-round, which makes the region's residents vulnerable to vitamin D deficiency. In our study, we first described the vitamin D nutritional status in children and explored the association between vitamin D deficiency and IR from the Investigation and Intervention of Vitamin D Deficiency in Children (IIVDDC). Then, the association between rickets in children and the risk of type 2 diabetes in adults was analyzed using data from the Harbin Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases (HDNNCDS) in a retrospective study. The aim of this study was to provide a theoretical basis for the early prevention and control of adult type 2 diabetes development in children.

Materials and Methods

Design

Cross-sectional study was conducted among child participants and retrospective study designs was conducted among adult participants.

Study population

Part I IIVDDC

The child participants were recruited from the baseline survey of IIVDDC. They were recruited from 4 kindergartens in Nangang District of Harbin, including 2 public kindergartens and 2 private kindergartens, from March to May 2019. The parents and teachers of children were invited to informational meetings at which the study and its procedures were explained to them. 440 children aged 3-7 years who had lived in Harbin for the past 3 years were eligible as participants. The exclusion criteria included spending the last winter vacation in the lower latitude areas of Harbin (n=60), who's information of questionnaire losing > 50% (n=54). A total of 326 children were included, and informed written consent was obtained from all custody holders (**Figure 1**).

Part II HDNNCDS

The data of adult participants were from the baseline data of the HDNNCDS. Residents who had lived in their communities for more than two years and who did not have cancer or type 1 diabetes were recruited. A total of 9,734 people aged 20-74 years completed the in-person baseline survey in 2010 - 2012 [24]. Rickets in childhood were investigated, and participants who had definite answers were included in our study. In the present study, we excluded participants who reported uncertainty about information on rickets in childhood (n=250), who reported extreme values for total energy intake (<500 kcal/d or >4500 kcal, n=291), and who had missing dietary information data (n=724). Finally, a total of 8,469 participants were eligible for our analysis, including 2995 males and 5474 females (**Figure 2**). Written informed consent was provided by all participants.

These study protocols were approved by the Ethics Committee of Harbin Medical University. The methods in this study were conducted in accordance with the approved guidelines.

The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of Harbin Medical University, and written informed consent was provided by all participants. The methods in this study were in accordance the approved guidelines.

Data collection by the questionnaire

Detailed in-person interviews were administered by trained personnel using a structured questionnaire to collect information on demographic characteristics, lifestyle, and dietary intake.

Part I IIVDDC

In the IIVDDC, the questionnaire was completed by parents and teachers in kindergartens, together. The demographic characteristics of the children included age and gender. Lifestyle referred to outdoor physical activity in the past 6 months. Dietary information was collected by using a food-frequency questionnaire (FFQ), and a total of 48 food items were included in the questionnaire, which covered most of the foods in the recipes of the kindergartens included in our study. For each food item, parents and teachers of participants were asked how frequently participants consumed over the preceding year, followed by a question on the amount consumed in lians (a unit of weight equal to 50 g) or mL (for liquid food item) per unit of time. The consumption frequency was transformed to obtain mean consumption a day. Nutrient intakes for each food item consumed were calculated by multiplying the nutrient content listed in the China Food Composition [25]. Before dietary surveys, 60 participants from the IIVDDC were recruited and asked to complete two FFQs (FFQ1 and FFQ2) and a 3-day dietary record to validate the reliability of the FFQs. After adjusting for energy intake, major nutritional factors (staple food, poultry, fish, vegetable, fruit, and milk products), which were assessed by the two FFQs and by the FFQ2 and 3-day dietary records, correlated well. In IIVDDC, the correlation coefficients were 0.49–0.54 and 0.55–0.76.

Part II HDNNCDS

In the HDNNCDS, the demographic characteristics mainly included age, gender, and educational level. Lifestyle referred to smoking, alcohol consumption, and physical activity. Family history of diabetes was also collected. In the FFQ for adults, a total of 103 food items were included in the questionnaire, which covered most of the commonly consumed foods in urban Harbin. The method to validate the reliability of the FFQs in the HDNNCDS was the same as that in the IIVDDC. The correlation coefficients were 0.61–0.70 and 0.61–0.69, respectively[24]. Participants were asked to recall whether they got rickets in childhood. In the questionnaire, the rickets in childhood included the participants' self-report of rickets, diagnosis from the hospital, and/or the existence of rickets signs, such as square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs.

Anthropometric measurement

Anthropometric measurements, including height, weight and waist circumference (WC), were taken by well-trained examiners, with participants wearing light, thin clothing, and no shoes. Body weight, height and waist circumference were measured to the nearest 0.1 kg, 0.1 cm, and 0.1 cm, respectively. Children's sex- and age-adjusted z-scores for body mass index (zBMI) were calculated with the use of WHO Anthro Plus software version 1.0.4 [26]. Adult BMI was calculated as weight (kg) divided by the square of the height in meters (m^2).

Biochemical assessment

Part I IIVDDC

Fasting (more than 10 h) blood samples were collected. Fasting glucose was determined by an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). 25(OH)D_3 was measured using ELISA kits (Mlbio, Shanghai, China). Serum insulin was measured using the immunofluorescence method (Tosoh automated enzyme immunoassay analyzer AIA-2000ST).

Part II HDNNCDS

Fasting and postprandial (2 hours after drinking 75 grams glucose-containing water) blood samples were taken from all participants. Fasting glucose and 2-h postprandial plasma glucose and blood lipids, including triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), were measured using an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured in the same way as children. HOMA-IR was calculated from fasting insulin and glucose using the following formula: fasting insulin concentration (mmol/L) × fasting glucose concentration (mIU/L)/22.5.

Definition of outcomes

Part I IIVDDC

Diagnostic criteria of serum vitamin D status: Serum 25(OH)D_3 < 10 ng/mL was considered severe deficiency, 10-20 ng/mL was considered deficiency, 20-29 ng/mL was considered insufficiency, and ≥30 ng/mL was considered sufficiency[13].

Definition of IR: Children in the highest quartile of HOMA-IR were defined as IR[27].

Part II HDNNCDS

Definition of type 2 diabetes: Type 2 diabetes was defined as fasting glucose ≥7.0 mmol/L and/or 2 h glucose ≥11.1 mmol/L and/or self-report of type 2 diabetes and/or use of hypoglycemic medicine.

The definition of vitamin D deficiency in childhood was as follows: Vitamin D deficiency in childhood was defined as a diagnosis of rickets at the hospital, self-reported rickets, or signs of skeletal deformity during childhood.

Statistical analysis

SPSS v22.0 (Beijing Stats Data Co. Ltd, Beijing China) was used to analyze the data, and a two-sided p value < 0.05 was considered statistically significant. Values are mean ± SD and n (%) per group for all other variables. ANOVA and chi-square test were used to compare the differences in the continuous variables and categorical variables between the groups. The linear regression analysis was used to analyze the association between serum 25(OH)D_3 concentration and fasting glucose, insulin and HOMA-IR in children, expressed as unstandardized β value and Standardized β value. Binary logistic regression analysis was used to analyze the association between serum 25(OH)D_3 concentration and IR in children, and the effect of rickets in childhood on type 2 diabetes in adulthood, expressed as OR value and 95% CI. Bootstrap test of binary logistic regression analysis was used as sensitivity analysis in order to confirm the risk of rickets on diabetes in adulthood male.

Patient and public involvement

participants were not involved in the development of research questions, nor the outcome measures/the design of the study. Also, they were not involved in the recruitment to or conduct of the study. In our study, the participants are informed about their blood parameters, and the results of other examinations are gradually shared with them by text message or phone call. The overall findings and benefits of the study will be disseminated through public media.

Results

Results from IIVDDC

Vitamin D nutrition status in children

There were 21 (6.4%) children with severe vitamin D deficiency, 163 (50%) children with deficiency, 107 (32.8%) children with insufficiency, and only 35 (10.7%) children with sufficiency.

Basic information and diet characteristics of children across HOMA-IR quartiles

Children were grouped by HOMA-IR quartiles. The basic information characteristics are summarized in **Table-1**. Serum 25(OH)D_3 concentration in four quartiles from lowest to highest were 20.82 ± 6.96 ng/mL, 20.96 ± 9.07 ng/mL, 19.94 ± 8.09 ng/mL, and 18.19 ± 6.37 ng/mL. Children in higher quartile group had lower proportion of sport frequency. There were no significant differences for age, gender, zBMI score, or the supplementation of calcium or (and) vitamin D in recent half year among different quartile groups.

Table-1. Characteristics of the subjects in different HOMA-IR quartiles group.

Variable		Q1 (n=81)	Q2 (n=82)	Q3 (n=82)	Q4 (n=81)	*P* Value
Male[1], n (%)		20 (46.5)	68 (49.6)	51 (56.0)	36 (65.5)	0.167
Age, years		5.27 ± 1.59	5.05 ± 1.19	5.22 ± 1.11	5.45 ± 1.33	0.123
25(OH)D_3, ng/mL		20.82 ± 6.96	20.96 ± 9.07	19.94 ± 8.09	18.19± 6.37	0.085
zBMI		-0.08± 2.07	0.74 ± 1.67	0.47 ± 1.47	0.58 ± 1.22	0.112
Exercise frequency, n (%)	Lower	10 (12.3)	20 (24.4)	14 (17.1)	12 (14.8)	0.049
	Higher	60 (74.1)	56 (68.3)	48 (58.5)	39 (48.1)	
	unclear	11 (13.6)	6 (7.3)	20 (24.4)	30 (37.0)	
Calcium or (and) vitamin D supplements [a], n (%)	Have been supplement, %	38 (46.9)	43 (52.4)	45 (54.9)	39 (48.1)	0.049
	Haven't been supplement, %	40 (49.4)	35 (42.7)	28 (34.1)	29 (35.8)	
	unclear, %	3 (3.7)	4 (4.9)	9 (11.0)	13 (16.0)	

[1]Values are mean ± SD and n (%) per group for all other variables. A two-sided P ≤ 0.05 was considered statistically significant; [a] whether have a supplement of calcium and (or) vitamin D with in recent half year.

Furthermore, there were no significant differences in energy, fruit, vegetable, and livestock intakes among different quartiles of HOMA-IR (**Table-2**). The above analysis results were consistent in boys and girls, and the data were not shown.

Table-2. Characteristics of diet in different HOMA-IR quartiles group.

Variable	Q1	Q2	Q3	Q4	*p* Value
Energy [1], kcal/d	1323.44 ± 644.00	1650.28 ± 855.11	1409.05 ± 868.96	1230.34 ± 882.99	0.009
Vegetable, g/d	77.08 ± 32.55	84.85 ± 36.13	89.09 ± 38.12	81.63 ± 44.13	0.428
Fruit, g/d	113.27 ± 48.71	133.58 ± 59.28	126.42 ± 52.44	123.08 ± 56.41	0.265
Livestock, g/d	76.04 ± 38.58	88.97 ± 38.48	80.77 ± 37.25	77.36 ± 36.12	0.230

[1] Values are mean ± SD. A two-sided P ≤ 0.05 was considered statistically significant.

The association between serum 25(OH)D_3 and fasting glucose, insulin and HOMA-IR

Serum 25(OH)D_3 concentration were negatively correlated with fasting insulin and HOMA-IR after adjusting for age, sex, zBMI, calcium or (and) vitamin D supplements, exercise frequency, dietary intakes of energy, vegetable, fruit, and livestock (fasting insulin: unstandardized β coefficient = -0.178, standardized β coefficient = -0.194, p value = 0.001; HOMA-IR: unstandardized β coefficient = -0.032, standardized β coefficient = -0.161, p value = 0.005) **(Table-3)**. However, the significant association between serum 25(OH)D_3 concentration and fasting glucose had not been observed. There was no difference in above analysis results between boys and girls, data were not shown.

Table-3. Linear regression analysis of the association between serum 25(OH)D_3 concentration and fasting glucose and insulin.

Variable	Crude			Model 1 [1]			Model 2		
	Unstandardized β	Standardized β	*p* Value	Unstandardized β	Standardized β	*p* Value	Unstandardized β	Standardized β	*p* Value
Glucose (mmol/L)	0.004	0.065	0.245	0.007	0.107	0.066	0.008	0.115	0.052
Insulin (mIU/L)	-0.169	-0.184	0.001	-0.170	-0.184	0.001	-0.178	-0.194	0.001
HOMA-IR	-0.030	-0.152	0.006	-0.031	-0.157	0.005	-0.032	-0.161	0.005

[1]Model 1: Adjusted for age, gender, zBMI, energy, exercise frequency, calcium or (and) vitamin D supplements. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

The association of serum 25(OH)D_3 concentration with IR

Children in the highest quartile of HOMA-IR were defined as IR, the cut-off point of HOMA-IR was 4.59. As shown in **Table-4**, after adjusting for age, sex, zBMI, calcium or (and) vitamin D supplements, exercise frequency, dietary intakes of energy, vegetable, fruit, and livestock, children with low serum 25(OH)D_3 concentration were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, *p* value: 0.027).

Table-4. Binary logistic regression analysis of the association between serum 25(OH)D_3 concentration and IR.

Variable	Model 1 [1]		Model 2	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Serum 25(OH)D3 concentration (ng/mL)	0.964 (0.928, 1.001)	0.059	0.955 (0.917, 0.995)	0.027

[1]Model 1: Adjusted for age, gender, zBMI, energy, exercise frequency, calcium or (and) vitamin D supplements. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Results from HDNNCDS

Basic information, diet and blood biochemical characteristics of adult participants

The basic characteristics of adult participants are shown in **Table-5**. In both males and females, participants with type 2 diabetes had older age, larger BMI, WC and higher proportion of family history of diabetes, compared with participants without type 2 diabetes. Additionally, the proportion of regular exercise in participants with type 2 diabetes was higher than those without type 2 diabetes. In females, the proportion of drinking was lower in participants with type 2 diabetes and they had lower education levels, whereas the above phenomenon was not observed in males. Furthermore, type 2 diabetes participants had higher T-CHO, TG, and LDL-c concentrations and lower HDL-c concentration in both males and females.

Table-5. Characteristics of the subject in T2D and non-T2D group, by gender.

Variable		Male			Female		
		T2D (n = 773)	Non-T2D (n = 2222)	*p* Value	T2D (n = 1048)	Non-T2D (n = 4426)	*p* Value
Age [1], years		52.46 ± 9.33	49.04 ± 10.99	<0.001	55.64 ± 9.42	49.44 ± 10.06	<0.001
BMI, kg/m^2		26.12 ± 3.37	25.57 ± 3.43	<0.001	25.88 ± 3.74	24.22 ± 3.32	<0.001
WC, cm		92.27 ± 9.21	90.48 ± 9.55	<0.001	86.94 ± 9.79	81.96 ± 9.12	<0.001
Rickets, n (%)		59 (7.6)	132 (5.9)	0.104	81 (7.7)	354 (8.0)	0.700
Education, n (%)	Primary school education or below	28 (3.6)	86 (3.9)	0.038	116 (11.1)	283 (6.4)	<0.001
	Junior or high school education	430 (55.6)	1102 (49.6)		666 (63.5)	2437 (55.1)	
	Bachelor degree or	296 (35.7)	903 (40.6)		202 (19.3)	1434 (32.4)	

	above						
	The situation of education unclear	39 (5.0)	131 (5.9)		66 (6.1)	272 (6.1)	
Smoking, n (%)	Smokers	310 (40.1)	870 (39.2)	0.836	37 (3.5)	175 (4.0)	0.059
	Non-smokers	391 (50.6)	1156 (52.0)		984 (93.9)	4188 (94.6)	
	Former smokers	66 (8.5)	175 (7.9)		11 (1.0)	28 (0.6)	
	The situation of smoking unclear	6 (0.7)	22 (0.9)		16 (1.5)	35 (0.8)	
Drinking, n (%)	Drinking	462 (59.8)	1404 (63.2)	0.219	145 (13.8)	909 (20.5)	<0.001
	Non-drinking	304 (39.3)	796 (35.8)		890 (84.9)	3453 (78.0)	
	The situation of drinking unclear	7 (0.9)	22 (1.0)		13 (1.2)	64 (1.4)	
Exercise, n (%)		418 (54.1)	1039 (46.8)	0.001	558 (53.2)	1949 (44.0)	<0.001
Family history of diabetes, n (%)		171 (22.1)	226 (10.2)	<0.001	268 (25.6)	676 (15.3)	<0.001
T-CHO, mmol/L		5.28 ± 1.19	5.00 ± 0.99	< 0.001	5.50 ± 1.04	5.18 ± 1.00	<0.001
TG, mmol/L		2.73 ± 3.32	2.07 ± 2.09	< 0.001	2.01 ± 1.62	1.41 ± 1.08	<0.001
HDL-c, mmol/L		1.10 ± 0.29	1.15 ± 0.30	< 0.001	1.25 ± 0.31	1.35 ± 0.32	<0.001
LDL-c, mmol/L		3.06 ± 0.91	2.96 ± 0.81	0.003	3.31 ± 0.90	3.00 ± 0.85	<0.001

[1] Values are mean ± SD, or frequency (%). A two-sided P ≤ 0.05 was considered statistically significant.

In both males and females, participants with type 2 diabetes had less consumption of fruit, but the difference of energy intake and consumption of vegetable and livestock were not observed (**Table-6**).

Table-6. Characteristics of energy vegetable, fruit, livestock intake in T2D and non-T2D group, by gender.

Variable	Male			Female		
	T2D (n = 773)	Non-T2D (n = 2222)	*p* Value	T2D (n = 1048)	Non-T2D (n = 4426)	*p* Value
Energy [1], kcal/d	2507.81 ± 790.83	2551.96 ± 775.41	0.175	2187.03 ± 702.46	2201.33 ±679.62	0.543
Vegetable, g/d	136.04 ±52.72	134.81 ± 51.24	0.570	132.93 ± 52.36	130.29 ± 51.30	0.136
Fruit, g/d	123.32 ± 47.85	129.00 ± 49.01	0.005	123.16 ± 50.34	133.55 ± 50.68	<0.001

Livestock, g/d	107.20 ± 45.41	107.90 ± 45.78	0.712	93.31 ± 39.11	92.77 ± 39.59	0.695

[1] Values are mean ± SD. A two-sided P ≤ 0.05 was considered statistically significant.

The association of rickets in childhood with type 2 diabetes in adulthood

Binary logistic regression analysis showed that rickets in childhood was significantly associated with an increased risk of type 2 diabetes in adult males (OR = 1.420, 95% CI = 1.017, 1.983; *p* value = 0.040), after adjusting for age, BMI, WC, exercise, smoking, alcohol consumption, education level, family history of diabetes, and dietary intakes of energy, vegetable, fruit and livestock. However, there was no significant association of rickets in childhood with type 2 diabetes in adult females (**Table-7**). Result of the bootstrap test was consistent with the result of the binary logistic regression analysis (*p* value = 0.041) (**Table-8**).

Table-7. Binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood with different sex.

Variable		Model 1 [1]		Model 2	
		OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Male	Rickets	1.414 (1.013, 1.972	0.042	1.420 (1.017, 1.983)	0.040
Female	Rickets	1.062 (0.813, 1.388)	0.658	1.065 (0.814, 1.392)	0.646

[1]Model 1: Adjusted for age, BMI, WC, education, smoking, drinking, energy, exercise, family history of diabetes. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Table-8. The bootstrap test of binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood male.

Variable	Model 1[1]		Model 2	
	β (95% CI)	*p* Value	β (95% CI)	*p* Value
Rickets	0.346 (0.004, 0.673)	0.039	0.351 (0.002, 0.680)	0.041

[1]Model 1: Adjusted for age, BMI, WC, education, smoking, drinking, energy, exercise, family history of diabetes. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Discussion

This study examined the association of vitamin D status in childhood with glucose metabolism in the north of China. The results showed that, in children, only 10.7% participants with vitamin D sufficient, there was a negative association between serum 25(OH)D_3 concentration and fasting insulin and HOMA-IR, and children with lower 25(OH)D_3 concentration were likely to develop IR.

In adults, childhood rickets was associated with an increased risk of type 2 diabetes in adulthood.

Vitamin D deficiency in children is widespread in worldwide. Regardless of the economic level, the vitamin D deficiency rate was very high in children from different countries [9-12,28,29]. Vitamin D can be obtained from sunshine and foods, such as meat, eggs, and milk. It was generally thought that exposure of skin to ultraviolet rays in the sunshine was the main source of body obtain vitamin D [30]. Therefore, the status of serum vitamin D can be influenced by several factors, such as skin tone, the latitude of residence, season, or use of sunscreen products [31]. Sunshine in areas with high latitude was insufficient for skin to synthesize vitamin D; a previous study showed that residents living in areas above 37°N were insufficiently synthesizing vitamin D in winter [32]. However, in relatively low latitude areas of China, rates of children's suboptimal vitamin D levels (< 30 ng/mL) was approximately 50%, thus vitamin D deficiency was also able to be observed [33]. In our study, approximately 53% of children participants had vitamin D deficiency, which was similar to the recent reports on vitamin D nutritional status of children. Therefore, we should pay more attention to the health problems caused by vitamin D deficiency.

In addition to skeletal health, the association between vitamin D and glucose metabolism in children and adults has also received wide concerned. In children, vitamin D deficiency was connected to IR and impaired fasting glucose [13,34,35]. However, there were a lack of children studies on the association between vitamin D deficiency and glucose metabolism in the northern area of China. Participants in this study were recruited from Harbin, a typical city in northern China. A negative correlation between serum vitamin D concentration and fasting insulin, HOMA-IR, and IR were found in this study of children; these results were consistent with previous reports. In adults, previous research suggested that low vitamin D status was associated with IR, impaired glucose tolerance, decreased insulin sensitivity, and reduction of insulin secretion [18,36-38]. Observational studies showed that patients with type 2 diabetes had lower level of vitamin D than healthy people; there was a negative correlation between vitamin D and HOMA-IR, and HbA1c levels decreased after vitamin D supplementation [39,40]. Additionally, prospective studies had indicated that vitamin D deficiency might increase the risk of type 2 diabetes [41,42-44]. According to recent years' studies, IR in childhood was considered as a risk factor for type 2 diabetes in adulthood [45]. Accordingly, we speculate vitamin D deficiency in childhood may increase the risk of type 2 diabetes in adulthood by influence IR in childhood. However, this deduction needs a long-term cohort study spanning decades from childhood to adulthood, so there have been no reports of this.

Nutritional rickets is the most common type in rickets; it can be caused by deficiencies of vitamin D, calcium, or phosphate, causing primarily to a widening and delay of mineralization of growth plates in bones [46,47]. The clinical signs of rickets include square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs. Rickets needs to be diagnosed in combination with vitamin D level and clinical signs, and vitamin D deficiency alone cannot be diagnosed as rickets, but children who had serum 25(OH)D_3 level under 10.90 ng/mL were likely to have rickets [48]. A retrospective survey was conducted on the prevalence of rickets in our adult study, rickets was determined by whether participants had a diagnosis of disease, had self-reported disease, or had symptoms. In adult population of this study, the prevalence of rickets in males and females was 6.3% and 7.9%, respectively. An earlier study reported that the prevalence of Chinese infants with rickets in 1980s was approximately 18% [49], it was higher than our study. This difference may be due to the birth year of our participants being approximately 20 years earlier than those in that study, when China was experiencing societal hardships. Poverty and poor health conditions may lead to the lack of awareness of disease. Furthermore, the information on rickets was retrospective, since some participants were uncertain whether they had rickets in childhood, which may contribute to the low prevalence of rickets. In our adult study, participants who had rickets were defined as having vitamin D deficiency in childhood. The association between rickets and type 2 diabetes in adults was analyzed to explore the effect of childhood vitamin D deficiency on type 2 diabetes in adulthood. The results showed that males who had rickets in childhood had a higher risk of type 2 diabetes in adulthood, but this result was not observed in females. However, the reason for this phenomenon is still unclear. In addition, a study from Finland found that individuals with high levels of vitamin D in childhood and adolescence had a significant lower risk of type 2 diabetes in adulthood compared with those who had lower level of vitamin D [23]. These findings collectively suggest that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood, which needs to be further be explored in more cohort studies and intervention studies.

Some scholars have explored the pathogenesis through which vitamin D deficiency might induce IR in children. The results of some studies shown that, vitamin D levels were inversely related to oxidative stress and inflammation, the increase of reactive oxygen species (ROS) and formation of cytokines such as interleukin-6 played major roles in IR [50-52]. In addition, the findings of an obese, African-American adolescent study showed that low vitamin D levels was correlated with low adiponectin levels, which was associated with IR in children and adolescents [53-56]. These results support the finding that vitamin D deficiency in childhood increases the risk of type 2 diabetes in adulthood. At the same time, the results of lab studies also support such pathogenesis: there was a close connection between vitamin D and β-cell function. By regulating cytokines to impact β-cell survival, vitamin D receptor and 1-hydroxylase in β-cell played a role in regulating pancreatic β-cell function, insulin secretion, IR and systemic inflammation [31,57,58]. Furthermore, the mechanism of vitamin D decreased IR might relate to the inhibition of vitamin D on inflammation and activation on insulin receptor [59,60]. In a term of epigenetics, results showed that vitamin D can also affect the occurrence of type 2 diabetes by regulating the expression of methyltransferase to prevent hypermethylation of diabetes-related genes [61]. Above all of these might be the pathogenesis of vitamin D deficiency in childhood increased the risk of type 2 diabetes in adulthood.

An advantage of our study was the combination of children and adult participants. First, the negative association between vitamin D deficiency and IR was observed in children, which was a risk factor for type 2 diabetes in adults. Second, the association between rickets in childhood and type 2 diabetes in adulthood was explored, as well. The findings of two age groups were combined to analyze whether vitamin D deficiency in childhood has an early and long-term effect on glucose metabolism in adulthood. These findings can provide a theoretical basis for the early prevention and treatment of type 2 diabetes. However, there were also some limitations in our study. First, there was no significant difference in calcium or (and) vitamin D supplements between 4 quartile groups in the children's baseline data, which may be because this question was not limited to vitamin D supplements. Calcium supplements contain less vitamin D, and children taking calcium supplements alone were unlikely to have the same effect as those who took vitamin D supplements alone. Secondly, using ELISA kits to detect serum 25(OH)D_3 concentration is not the optimal method and may affect 25(OH)D_3 concentration results. Thirdly, in adult study baseline data, participants with type 2 diabetes had a higher proportion of regular exercise and less consumption of fruits than those without type 2 diabetes. This might be due to the participants', with type 2 diabetes, choice of a healthier lifestyle after got the diagnosis of disease. In addition, there might be a recall bias in the adult retrospective study. The definition of vitamin D deficiency in childhood was rickets, which might have led to some participants, who did not have rickets but vitamin D deficiency, to be classified as non-deficient. Furthermore, rickets caused by vitamin D deficiency is the main type of rickets, but other types of rickets have not been excluded. Therefore, long-term design and cohort studies with stricter vitamin D nutritional status monitoring are needed to further verify our results.

Conclusions

In summary, vitamin D deficiency in childhood was associated with IR and might increase the risk of type 2 diabetes in adult males. Early prevention strategies should be undertaken in children to control the rapid increase in type 2 diabetes worldwide, and management of vitamin D deficiency is probably an effective method.

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Authors' contributions

Lixin Na and Changhao Sun designed the study and acquired funding. Junyi Liu and Lixin Na wrote the paper. Liqun Fu and Jingyi Zhang prepared the original draft. Junyi Liu, Shanshan Jin and Yubing Jia collected the data. Junyi Liu and Liqun Fu analysed and interpreted data. All authors read and approved the final manuscript.

Compliance with Ethics Guidelines

The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of Harbin Medical University, and written informed consent was provided by all participants. The methods in this study were in accordance the approved guidelines.

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Competing interests

None of the authors has any potential conflict of interest associated with this research.

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Data sharing statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figure 1 - The follow chart of the children study.

Figure 2 - The follow chart of the adult study.

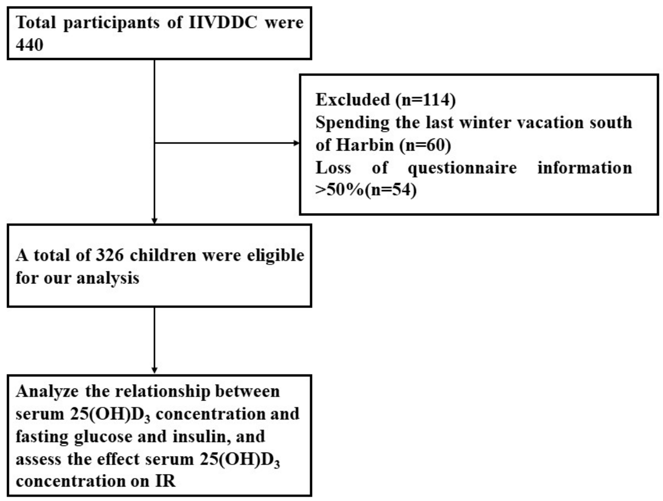

Figure 1. The follow chart of children study

338x190mm (96 x 96 DPI)

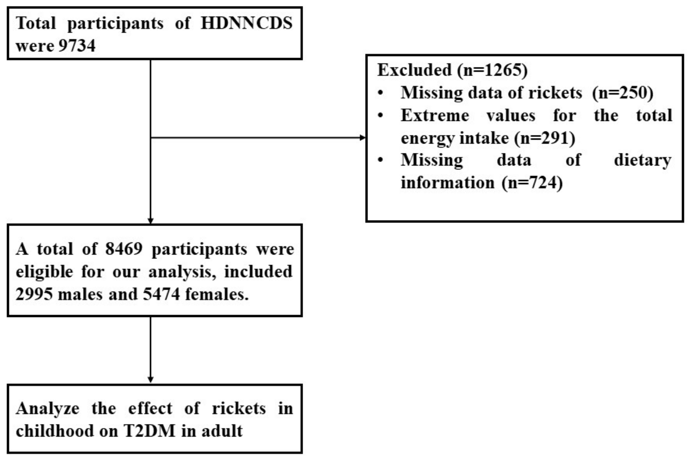

Figure 2. The follow chart of adult study

338x190mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6, 7, 8*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10,12
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	

		(*b*) Report category boundaries when continuous variables were categorized	9, 10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Vitamin D status in children and its association with glucose metabolism in northern China: A combination of a cross-sectional study and a retrospective study.

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Vitamin D status in children and its association with glucose metabolism in northern China: A combination of a cross-sectional study and a retrospective study.

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Abstract

Objectives: The aim of this study was to explore the vitamin D status of children in northern China and the association between vitamin D and glucose metabolism.

Design: Cross-sectional study was conducted among child participants and retrospective study designs was conducted among adult participants.

Setting and participants: Both studies were recruited from Harbin, 326 children were included in children study, 8,469 adults were included in adult study.

Primary and secondary outcome measures: Physical examination, lifestyle and dietary habit data were recorded in all the participants. Serum insulin, glucose, 25(OH)D_3 concentrations in children and serum glucose and lipids levels in adults were measured. In adults, rickets history was also investigated, which was used to define vitamin D deficiency in childhood. The associations were tested by linear regression and binary logistic regression.

Result: In the children study, only 10.7% of participants were vitamin D sufficient (≥30 ng/mL). Inverse correlations between serum 25(OH)D_3 concentration and fasting insulin and HOMA-IR were found, and children with lower serum 25(OH)D_3 concentrations were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, *p* value: 0.027). In an adult study, rickets in childhood increased the risk of type 2 diabetes in male participants (OR = 1.414, 95% CI = 1.013, 1.972; *p* value = 0.042), but this result was not observed in females.

Conclusion: Our findings suggest that vitamin D deficiency is widespread in northern China. Vitamin D deficiency in childhood was associated with IR and increased the risk of type 2 diabetes in male adults.

Keywords: vitamin D deficiency, children, insulin resistance, type 2 diabetes

Strengths and limitations of this study:

- Two studies from Harbin, a city with a high rate of vitamin D deficiency, have important implications for studying vitamin D deficiency in high latitudes and the long-term effects of vitamin D on non-skeletal health.
- The analysis of children's study and the analysis of adults' study with similar environments and dietary habits were included to support each other's findings on the association between vitamin D deficiency and glucose metabolism in the two populations.
- There was possible selection bias and we did not do the external validity of the sample due to a limitation of external sample. Information on rickets that was used to define vitamin D deficiency in childhood was obtained from self-report, the recall bias was not avoidable although we excluded the uncertain participants.
- The sample size of children was small and it was a cross-sectional study. We used ORs to interpret the association of vitamin D deficiency with IR and diabetes, which may overstate effect sizes.

Introduction

According to IDF statistics, there are 450 million adults with type 2 diabetes worldwide, accounting for approximately 90% of diabetes mellitus cases. Type 2 diabetes has become a global public health problem [1,2]. Some recent population studies have found that risk factors such as obesity, impaired glucose tolerance, or insulin resistance (IR) in childhood, may also increase the risk of type 2 diabetes in adults [3-6]. These results suggested that controlling risk factors in childhood is an early prevention strategy to reduce the prevalence of type 2 diabetes in adulthood.

Vitamin D is a fat-soluble vitamin with several physiological functions, one of the most important of which is its effect on skeletal health [7]. At present, vitamin D deficiency is still a serious public problem worldwide, particularly in children [8]. The prevalence of vitamin D deficiency was approximately 50% in both developing and developed countries [9-12]. Serum 25(OH)D_3 is less affected by body regulation and is often used to evaluate vitamin D levels. It is usually considered that serum 25(OH)D_3 < 10 ng/mL indicates severe deficiency, 10-20 ng/mL indicates deficiency, 20-29 ng/mL indicates insufficiency, and ≥30 ng/mL indicates sufficiency [13]. Vitamin D deficiency in children is associated with many skeletal diseases, and one of the typical diseases is rickets. The clinical signs of rickets include skeletal deformity, restlessness, motor retardation and bone pain [14].

Recently, the association between vitamin D deficiency and extra-skeletal health has been of great concern, such as its association with glucose metabolism, obesity, respiratory tract infection and atopic dermatitis, among which the association with glucose metabolism has been of particular concern [15-17]. A population study of children and adolescents in Mexico found that low vitamin D levels were associated with IR; when the concentration of serum vitamin D increased, the possibility of presenting IR decreased [18]. Jared P Reis et al. reported that adolescents in the lowest quartile of vitamin D (<15 ng/mL) are more likely to have hyperglycemia compared with those in the highest quartile (>26 ng/mL) [19]. Furthermore, randomized controlled trials showed that vitamin D supplementation could increase insulin sensitivity and decrease IR and fasting glucose concentrations in obese children [20-22]. Since IR in children is a risk factor for type 2 diabetes, we hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting insulin sensitivity. A recent 31-year follow-up prospective study in 3- to 18-year-old young Finns found that high vitamin D levels in childhood could reduce the incidence of type 2 diabetes in adulthood [23]. This study further supported our hypothesis, but current research is very limited.

Harbin is a typical northeast city of China, with a latitude between 44°04′N and 46°40′N, and is a relatively high-latitude area. The winters in Harbin are long, and the sunlight is relatively insufficient year-round, which makes the region's residents vulnerable to vitamin D deficiency. In our study, we first described the vitamin D nutritional status in children and explored the association between vitamin D deficiency and IR from the Investigation and Intervention of Vitamin D Deficiency in Children (IIVDDC). Then, the association between rickets in children and the risk of type 2 diabetes in adults was analyzed using data from the Harbin Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases (HDNNCDS) in a retrospective study. The aim of this study was to provide a theoretical basis for the early prevention and control of adult type 2 diabetes development in children.

Materials and Methods

Design

Cross-sectional study was conducted among child participants and retrospective study designs was conducted among adult participants.

Study population

The child participants were from the baseline survey of IIVDDC from March to May 2019. They were recruited from 4 kindergartens in Nangang District of Harbin by convenient sampling method, including 2 public kindergartens and 2 private kindergartens based on the consideration of different economic levels. The sample size required of children was determined by Events Per Variable criterion (EPV) ≥10, the minimum sample size was calculated to be 90 participants. The parents and teachers of children were invited to informational meetings at which the study and its procedures were explained to them. A total of 440 children aged 3-7 years who had lived in Harbin for the past 3 years were eligible as participants. The exclusion criteria included spending the last winter vacation in the lower latitude areas of Harbin (n=60), who's information of questionnaire losing > 50% (n=54). A total of 326 children were included, and informed written consent was obtained from all custody holders (**Figure 1**).

The adult participants were from the baseline of the HDNNCDS [24]. Seven urban administrative regions of Harbin were covered in HDNNCDS. According to their financial situation, each region was divided into 3 strata and a total of 42 communities were randomly selected from each stratum in each administrative region by employing a stratified multistage random cluster sampling design. Residents were eligible to participate in the study if they: 1) were between 20 and 74 years old, 2) have been living in Harbin for at least two years, 3) were without cancer or type 1 diabetes mellitus. A total of 9,734 people aged 20-74 years completed the in-person baseline survey in 2010 - 2012. The sample size required in our analysis was calculated by $N = \frac{Z^2_{1-\alpha/2}(1-P)}{\varepsilon^2 p}$, $Z_{1-\alpha/2} = 1.96$, p = 12.8% [25], ε = 10%. By calculating N= 2,617, design efficiency value was 2, the final calculated minimal sample size was 5,234. Rickets in childhood were investigated in the survey, and participants who had definite answers were included in our study. In the present study, we excluded participants who reported uncertainty about information on rickets in childhood (n=250), who reported extreme values for total energy intake (<500 kcal/d or >4500 kcal, n=291), and who had missing dietary information data (n=724). Finally, a total of 8,469 participants were eligible for our analysis, including 2,995 males and 5474 females (**Figure 2**). Written informed consent was provided by all participants.

The study protocols of IIVDDC and HDNNCDS were approved by the Ethics Committee of Harbin Medical University, and written informed consent was provided by all participants. The methods in the study were in accordance with the approved guidelines (The registered ethical number: ChiCTR1800020294 for IIVDDC, ChiCTR-ECH-12002721 for HDNNCDS).

Data collection by the questionnaire

Detailed in-person interviews were administered by trained personnel using a structured questionnaire to collect information on demographic characteristics, lifestyle, and dietary intake. In the IIVDDC, the questionnaire was completed by parents and teachers in kindergartens, together. The demographic characteristics of the children included age and gender. Outdoor physical activity in the past 6 months was investigated, children who had more than 60 minutes of daily activity or more than 3 days of weekly activity were considered as high exercise frequency, otherwise they were considered as low exercise frequency. Children who took calcium or vitamin D orally or intravenously in the past 6 months were considered as supplementation with calcium and (or) vitamin D. Dietary information was collected by using a food-frequency questionnaire (FFQ), and a total of 48 food items were included in the questionnaire, which covered most of the foods in the recipes of the kindergartens included in our study. For each food item, parents and teachers of participants were asked how frequently participants consumed over the preceding year, followed by a question on the amount consumed in lians (a unit of weight equal to 50 g) or mL (for liquid food item) per unit of time. The consumption frequency was transformed to obtain mean consumption a day. Nutrient intakes for each food item consumed were calculated by multiplying the nutrient content listed in the China Food Composition [26]. Before dietary surveys, 60 participants from the IIVDDC were recruited and asked to complete two FFQs (FFQ1 and FFQ2) and a 3-day dietary record to validate the reliability of the FFQs. After adjusting for energy intake, major nutritional factors (staple food, poultry, fish, vegetable, fruit, and milk products), which were assessed by the two FFQs and by the FFQ2 and 3-day dietary records, correlated well. In IIVDDC, Cronbach's α coefficient of major nutritional factors between FFQ1 and FFQ2 were 0.67-0.72 and major nutritional factors between

FFQ1 and 3-day dietary record were 0.62-0.76. Seventeen factors were extracted, and the cumulative variance contribution rate was 0.649, which suggested the good construct validity.

In the HDNNCDS, the demographic characteristics mainly included age, gender, and educational level. Lifestyle referred to smoking, alcohol consumption, and physical activity. Current smokers were defined as those who have smoked at least 100 cigarettes lifetime and smoke every day or some days now. Participants who consumed more than 100ml of white wine, highland barley wine, rice wine, grape wine per day or more then 250ml beer per day were considered to have a drinking habit. And regular exercise was defined as any kind of recreational or sport physical activity other than walking for work or life performed at least 30 minutes for three or more days per week. Family history of diabetes was also collected. In the FFQ for adults, a total of 103 food items were included in the questionnaire, which covered most of the commonly consumed foods in urban Harbin. The method to validate the reliability of the FFQs in the HDNNCDS was the same as that in the IIVDDC. The correlation coefficients of major nutritional factors between FFQ1 and FFQ2 were 0.61–0.70 and major nutritional factors between FFQ1 and 3-day dietary record were 0.61–0.69, respectively[24]. Fifteen factors were extracted, and the cumulative variance contribution rate was 0.832, which suggested the good construct validity. Participants were asked to recall the history of rickets in childhood by specific questions in the questionnaire. The questions included whether they had been diagnosed of rickets at the hospital, had the signs of rickets, such as square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs. The answer options included definitely yes, definitely no and uncertain. Participants who chose one or more "definitely yes" were considered to have rickets in childhood. Participants who reported "uncertain" were excluded from our analysis.

Anthropometric measurement

177 Anthropometric measurements, including height, weight and waist circumference (WC), were taken by well-trained examiners, with participants wearing light, thin clothing, and no shoes. Body weight, height and waist circumference were measured to the nearest 0.1 kg, 0.1 cm, and 0.1 cm, respectively. BMI was calculated as weight (kg) divided by the square of the height in meters (m^2). Children's sex- and age-adjusted z-scores for body mass index (zBMI) were calculated as the calculated value of child BMI minus the median BMI of children of the same age and sex, and then divided by the standard deviation of BMI for children of the same age and sex with the use of WHO Anthro Plus software version 1.0.4 [27].

Biochemical assessment

Fasting (more than 10 h) blood samples were collected in children in the IIVDDC. Fasting glucose was determined by an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). 25(OH)D_3 was measured using ELISA kits (Mlbio, Shanghai, China). Serum insulin was measured using the immunofluorescence method (Tosoh automated enzyme immunoassay analyzer AIA-2000ST).

Fasting and postprandial (2 hours after drinking 75 grams glucose-containing water) blood samples were taken from all adult participants in the HDNNCDS. Fasting glucose and 2-h postprandial plasma glucose, blood lipids, including triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), were measured using an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured in the same way as children.

Definition of variables

Exposures

In the IIVDDC, serum 25(OH)D_3 < 10 ng/mL was considered severe deficiency, 10-20 ng/mL was considered deficiency, 20-29 ng/mL was considered insufficiency, and ≥30 ng/mL was considered

200 sufficiency[13]. In the HDNNCDS, Vitamin D deficiency in childhood was defined as a diagnosis of rickets at the hospital, self-reported rickets, or signs of skeletal deformity during childhood.

Outcomes

In the IIVDDC, HOMA-IR was calculated from fasting insulin and glucose using the following formula: fasting insulin concentration (mmol/L) × fasting glucose concentration (mIU/L)/22.5. Children in the highest quartile of HOMA-IR were defined as IR[28], the cutoff value of IR was HOMA-IR ≥ 4.58 in our study. In the HDNNCDS, type 2 diabetes was defined as fasting glucose ≥7.0 mmol/L and/or 2 h glucose ≥11.1 mmol/L and/or self-report of type 2 diabetes and/or use of hypoglycemic medicine.

Potential confounders

In the IIVDDC, age, gender, zBMI, intake of energy, vegetable, fruit and livestock, exercise frequency, calcium or (and) vitamin D supplements, were included in the analysis as potential confounders of IR. In the HDNNCDS, age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit and livestock, and family history were included as potential confounders of type 2 diabetes.

Statistical analysis

SPSS v22.0 (Beijing Stats Data Co. Ltd, Beijing China) was used to analyze the data, and a two-sided *p* value < 0.05 was considered statistically significant. Values are mean ± SD and n (%) per group for all other variables. ANOVA and chi-square test were used to compare the differences in the continuous variables and categorical variables between the groups. The linear regression analysis was used to analyze the association between serum 25(OH)D_3 concentration and fasting glucose, insulin and HOMA-IR in children, expressed as unstandardized β value and Standardized *β* value. Binary logistic regression analysis was used to analyze the association between serum 25(OH)D_3 concentration and IR in children, adjust for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements, expressed as OR value and 95% CI. The data from children were finally analyzed and presented in general as no gender difference was observed after stratified by sex. Binary logistic regression analysis was used to analyze the association between rickets in childhood with type 2 diabetes in adulthood stratified by sex, adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit and livestock, and family history. Bootstrap test of binary logistic regression analysis was used as sensitivity analysis in order to confirm the risk of rickets on diabetes in adulthood male.

Patient and public involvement

participants were not involved in the development of research questions, nor the outcome measures/the design of the study. Also, they were not involved in the recruitment to or conduct of the study. In our study, the participants are informed about their blood parameters, and the results of other examinations are gradually shared with them by text message or phone call. The overall findings and benefits of the study will be disseminated through public media.

Results

Results from IIVDDC

Basic information and diet characteristics of children across HOMA-IR quartiles

A total of 326 children were included, 53% were boys and 47% were girls, aged 3 y-7 y, the average age is 5.24 ± 1.32 y. There were 21 (6.4%) children with severe vitamin D deficiency, 163 (50%) children with deficiency, 107 (32.8%) children with insufficiency, and only 35 (10.7%) children with sufficiency.

Children were grouped by HOMA-IR quartiles. The basic characteristics are summarized in **Table-1**. Serum 25(OH)D_3 concentration in four quartiles from lowest to highest were 20.82 ± 6.96 ng/mL, 20.96 ± 9.07 ng/mL, 19.94 ± 8.09 ng/mL, and 18.19 ± 6.37 ng/mL. Children in higher quartile group had lower proportion of higher sport frequency. There were no significant differences for age, gender, zBMI score, or the supplementation of calcium or (and) vitamin D in recent half year among different quartile groups. The characteristics across quartiles were consistent in boys and girls, and the data were not shown.

Table-1. Characteristics of the subjects in different HOMA-IR quartiles group.

Variable		Q1 (n=81)	Q2 (n=82)	Q3 (n=82)	Q4 (n=81)	*p* Value
Male[1], n (%)		20 (46.5)	68 (49.6)	51 (56.0)	36 (65.5)	0.167
Age, years		5.27 ± 1.59	5.05 ± 1.19	5.22 ± 1.11	5.45 ± 1.33	0.123
25(OH)D_3, ng/mL		20.82 ± 6.96	20.96 ± 9.07	19.94 ± 8.09	18.19± 6.37	0.085
zBMI		-0.08± 2.07	0.74 ± 1.67	0.47 ± 1.47	0.58 ± 1.22	0.112
Exercise frequency, n (%)	Lower	10 (12.3)	20 (24.4)	14 (17.1)	12 (14.8)	0.049
	Higher	60 (74.1)	56 (68.3)	48 (58.5)	39 (48.1)	
	unclear	11 (13.6)	6 (7.3)	20 (24.4)	30 (37.0)	
Calcium or (and) vitamin D supplements [a], n (%)	Have been supplement, %	38 (46.9)	43 (52.4)	45 (54.9)	39 (48.1)	0.049
	Haven't been supplement, %	40 (49.4)	35 (42.7)	28 (34.1)	29 (35.8)	
	unclear, %	3 (3.7)	4 (4.9)	9 (11.0)	13 (16.0)	

[1]Values are mean ± SD and n (%) per group for all other variables. A two-sided *p* Value < 0.05 was considered statistically significant; [a] whether have a supplement of calcium and (or) vitamin D with in past 6 months.

Furthermore, a significant difference in energy intake among quartiles of HOMA-IR was observed, with a slightly higher level in quartile 2 than that in other quartiles (**Table-2**). There were no differences in fruit, vegetable, and livestock intakes among different quartiles of HOMA-IR. The above analysis results were consistent in boys and girls, and the data were not shown.

Table-2. Characteristics of diet in different HOMA-IR quartiles group.

Variable	Q1	Q2	Q3	Q4	*p* Value
Energy [1], kcal/d	1323.44 ± 644.00	1650.28 ± 855.11	1409.05 ± 868.96	1230.34 ± 882.99	0.009
Vegetable, g/d	77.08 ± 32.55	84.85 ± 36.13	89.09 ± 38.12	81.63 ± 44.13	0.428
Fruit, g/d	113.27 ± 48.71	133.58 ± 59.28	126.42 ± 52.44	123.08 ± 56.41	0.265
Livestock, g/d	76.04 ± 38.58	88.97 ± 38.48	80.77 ± 37.25	77.36 ± 36.12	0.230

[1] Values are mean ± SD. A two-sided *p* Value < 0.05 was considered statistically significant.

The association between serum 25(OH)D_3 and fasting glucose, insulin and HOMA-IR

Serum 25(OH)D_3 concentration were negatively correlated with fasting insulin and HOMA-IR after adjusting for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements (fasting insulin: unstandardized β coefficient = -0.178, standardized β coefficient = -0.194, p value = 0.001; HOMA-IR: unstandardized β coefficient = -0.032, standardized β coefficient = -0.161, p value = 0.005) **(Table-3)**. However, the significant association between serum 25(OH)D_3 concentration and fasting glucose had not been observed. There was no difference in above analysis results between boys and girls, data were not shown.

Table-3. Linear regression analysis of the association between serum 25(OH)D_3 concentration and fasting glucose and insulin.

Variable	Crude			Model 1 [1]			Model 2		
	Unstandardized β	Standardized β	*p* Value	Unstandardized β	Standardized β	*p* Value	Unstandardized β	Standardized β	*p* Value
Glucose (mmol/L)	0.004	0.065	0.245	0.007	0.107	0.066	0.008	0.115	0.052
Insulin (mIU/L)	-0.169	-0.184	0.001	-0.170	-0.184	0.001	-0.178	-0.194	0.001
HOMA-IR	-0.030	-0.152	0.006	-0.031	-0.157	0.005	-0.032	-0.161	0.005

[1]Model 1: Adjusted for age, gender, zBMI, exercise frequency, intake of energy, calcium or (and) vitamin D supplements. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

The association of serum 25(OH)D_3 concentration with IR

Children in the highest quartile of HOMA-IR were defined as IR, the cut-off point of HOMA-IR was 4.59. As shown in **Table-4**, after adjusting for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements, children with low serum 25(OH)D_3 concentration were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, p value: 0.027). This association was consistent in boys and girls, and the data were not shown.

Table-4. Binary logistic regression analysis of the association between serum 25(OH)D_3 concentration and

IR.

Variable	Model 1 [1]		Model 2	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Serum 25(OH)D3 concentration (ng/mL)	0.964 (0.928, 1.001)	0.059	0.955 (0.917, 0.995)	0.027

[1]Model 1: Adjusted for age, gender, zBMI, exercise frequency, intake of energy, calcium or (and) vitamin D supplements. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Results from HDNNCDS

Basic information, diet and blood biochemical characteristics of adult participants

The basic characteristics of adult participants are shown in **Table-5**. A total of 8,469 adult participants, 2,999 male and 5,474 female were included in our analysis. The average age was 49.58 ± 10.51 y. In both males and females, participants with type 2 diabetes had older age, larger BMI, WC and higher proportion of family history of diabetes, compared with participants without type 2 diabetes. Additionally, the proportion of regular exercise in participants with type 2 diabetes was higher than those without type 2 diabetes. In females, the proportion of drinking was lower in participants with type 2 diabetes and they had lower education levels, whereas the above phenomenon was not observed in males. Furthermore, type 2 diabetes participants had higher T-CHO, TG, and LDL-c concentrations and lower HDL-c concentration in both males and females.

Table-5. Characteristics of the subject in T2D and non-T2D group, by gender.

Variable		Male			Female		
		T2D (n = 773)	Non-T2D (n = 2222)	*p* Value	T2D (n = 1048)	Non-T2D (n = 4426)	*p* Value
Age [1], years		52.46 ± 9.33	49.04 ± 10.99	<0.001	55.64 ± 9.42	49.44 ± 10.06	<0.001
BMI, kg/m^2		26.12 ± 3.37	25.57 ± 3.43	<0.001	25.88 ± 3.74	24.22 ± 3.32	<0.001
WC, cm		92.27 ± 9.21	90.48 ± 9.55	<0.001	86.94 ± 9.79	81.96 ± 9.12	<0.001
Rickets, n (%)		59 (7.6)	132 (5.9)	0.104	81 (7.7)	354 (8.0)	0.700
Education, n (%)	Primary school education or below	28 (3.6)	86 (3.9)	0.038	116 (11.1)	283 (6.4)	<0.001
	Junior or high school education	430 (55.6)	1102 (49.6)		666 (63.5)	2437 (55.1)	
	Bachelor degree or above	296 (35.7)	903 (40.6)		202 (19.3)	1434 (32.4)	
	The	39 (5.0)	131 (5.9)		66 (6.1)	272 (6.1)	

	situation of education unclear						
Smoking, n (%)	Smokers	310 (40.1)	870 (39.2)	0.836	37 (3.5)	175 (4.0)	0.059
	Non-smokers	391 (50.6)	1156 (52.0)		984 (93.9)	4188 (94.6)	
	Former smokers	66 (8.5)	175 (7.9)		11 (1.0)	28 (0.6)	
	The situation of smoking unclear	6 (0.7)	22 (0.9)		16 (1.5)	35 (0.8)	
Drinking, n (%)	Drinking	462 (59.8)	1404 (63.2)	0.219	145 (13.8)	909 (20.5)	<0.001
	Non-drinking	304 (39.3)	796 (35.8)		890 (84.9)	3453 (78.0)	
	The situation of drinking unclear	7 (0.9)	22 (1.0)		13 (1.2)	64 (1.4)	
Exercise, n (%)		418 (54.1)	1039 (46.8)	0.001	558 (53.2)	1949 (44.0)	<0.001
Family history of diabetes, n (%)		171 (22.1)	226 (10.2)	<0.001	268 (25.6)	676 (15.3)	<0.001
T-CHO, mmol/L		5.28 ± 1.19	5.00 ± 0.99	< 0.001	5.50 ± 1.04	5.18 ± 1.00	<0.001
TG, mmol/L		2.73 ± 3.32	2.07 ± 2.09	< 0.001	2.01 ± 1.62	1.41 ± 1.08	<0.001
HDL-c, mmol/L		1.10 ± 0.29	1.15 ± 0.30	< 0.001	1.25 ± 0.31	1.35 ± 0.32	<0.001
LDL-c, mmol/L		3.06 ± 0.91	2.96 ± 0.81	0.003	3.31 ± 0.90	3.00 ± 0.85	<0.001

[1] Values are mean ± SD, or frequency (%). A two-sided p Value < 0.05 was considered statistically significant.

In both males and females, participants with type 2 diabetes had less consumption of fruit, but the difference of energy intake and consumption of vegetable and livestock were not observed (**Table-6**).

Table-6. Characteristics of energy vegetable, fruit, livestock intake in T2D and non-T2D group, by gender.

Variable	Male			Female		
	T2D (n = 773)	Non-T2D (n = 2222)	*p* Value	T2D (n = 1048)	Non-T2D (n = 4426)	*p* Value
Energy [1], kcal/d	2507.81 ± 790.83	2551.96 ± 775.41	0.175	2187.03 ± 702.46	2201.33 ±679.62	0.543
Vegetable, g/d	136.04 ±52.72	134.81 ± 51.24	0.570	132.93 ± 52.36	130.29 ± 51.30	0.136
Fruit, g/d	123.32 ± 47.85	129.00 ± 49.01	0.005	123.16 ± 50.34	133.55 ± 50.68	<0.001
Livestock, g/d	107.20 ± 45.41	107.90 ± 45.78	0.712	93.31 ± 39.11	92.77 ± 39.59	0.695

[1] Values are mean ± SD. A two-sided p Value < 0.05 was considered statistically significant.

The association of rickets in childhood with type 2 diabetes in adulthood

Binary logistic regression analysis showed that rickets in childhood was significantly associated with an increased risk of type 2 diabetes in adult males (OR = 1.420, 95% CI = 1.017, 1.983; p value = 0.040), after adjusting for sex, adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit and livestock, and family history. However, there was no significant association of rickets in childhood with type 2 diabetes in adult females (**Table-7**). Result of the bootstrap test was consistent with the result of the binary logistic regression analysis (p value = 0.041) (**Table-8**).

Table-7. Binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood with different sex.

Variable		Model 1 [1]		Model 2	
		OR (95% CI)	p Value	OR (95% CI)	p Value
Male	Rickets	1.414 (1.013, 1.972	0.042	1.420 (1.017, 1.983)	0.040
Female	Rickets	1.062 (0.813, 1.388)	0.658	1.065 (0.814, 1.392)	0.646

[1]Model 1: Adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, family history of diabetes. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Table-8. The bootstrap test of binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood male.

Variable	Model 1[1]		Model 2	
	β (95% CI)	p Value	β (95% CI)	p Value
Rickets	0.346 (0.004, 0.673)	0.039	0.351 (0.002, 0.680)	0.041

[1]Model 1: Adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, family history of diabetes. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Discussion

This study examined the association of vitamin D status in childhood with glucose metabolism in the north of China. The results showed that, in children, only 10.7% participants with vitamin D sufficient, there was a negative association between serum 25(OH)D_3 concentration and fasting insulin and HOMA-IR, and children with lower 25(OH)D_3 concentration were likely to develop IR. In adults, childhood rickets was associated with an increased risk of type 2 diabetes in adulthood.

Vitamin D deficiency in children is widespread in worldwide. Regardless of the economic level, the vitamin D deficiency rate was very high in children from different countries [9-12,29,30]. Vitamin D can be obtained from sunshine and foods, such as meat, eggs, and milk. It was generally thought that exposure of skin to ultraviolet rays in the sunshine was the main source of body obtain vitamin D [31]. Therefore, the status of serum vitamin D can be influenced by several factors, such as skin tone, the latitude of residence, season, or use of sunscreen products [32]. Sunshine in areas with high latitude was insufficient for skin to synthesize vitamin D; a previous study showed that residents living in areas above 37°N were insufficiently synthesizing vitamin D in winter [33]. In relatively low latitude areas of China, 50% of preschooler children had sufficient vitamin D nutritional status (≥ 30 ng/mL), [34]. In our study, only 10.7% of children had sufficient vitamin D nutritional status, which was significantly lower than children at low latitudes, vitamin D insufficiency was even more serious. Therefore, we should pay more attention to the health problems caused by vitamin D deficiency and insufficiency at high latitude.

In addition to skeletal health, the association between vitamin D and glucose metabolism in children and adults has also obtained wide concerned. In children, vitamin D deficiency was connected to IR and impaired fasting glucose [13,35,36]. However, there were a lack of children studies on the association between vitamin D deficiency and IR in the northern area of China. A negative correlation between serum vitamin D concentration and fasting insulin, HOMA-IR, and IR were found in this study of children. In adults, previous research suggested that low vitamin D status was associated with IR, impaired glucose tolerance, decreased insulin sensitivity, and reduction of insulin secretion [18,37-39]. Observational study showed that patients with type 2 diabetes had lower level of vitamin D than healthy people[40]. An intervention studies have shown that HbA1c levels decreased after vitamin D supplementation[41]. Additionally, prospective studies had indicated that vitamin D deficiency in adults might increase the risk of type 2 diabetes [42,43-45]. According to recent years' studies, IR in childhood was considered as a risk factor for type 2 diabetes in adulthood [46]. Therefore, we speculate vitamin D deficiency in childhood may increase the risk of type 2 diabetes in adulthood by influence IR in childhood. However, this deduction needs a long-term cohort study spanning decades from childhood to adulthood, there have been no reports about this so far.

Nutritional rickets is the most common type in rickets, caused by deficiencies of vitamin D, calcium, or phosphate [47,48]. The clinical signs of rickets include square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs. Rickets needs to be diagnosed in combination with vitamin D level and clinical signs, and vitamin D deficiency alone cannot be diagnosed as rickets, but children who had serum 25(OH)D_3 level under 10.90 ng/mL were likely to have rickets [49]. A retrospective survey was conducted on the prevalence of rickets in our adult study, rickets was determined by whether participants had a diagnosis of disease, or had symptoms. In adult population of this study, the prevalence of rickets in males and females was 6.3% and 7.9%, respectively. An earlier study reported that the prevalence of Chinese infants with rickets in 1980s was approximately 18% [50], it was higher than our study. This difference may be due to the birth year of our participants being approximately 20 years earlier than those in that study, when China was experiencing societal hardships. Poverty and poor health conditions may lead to the lack of awareness of disease. Furthermore, the information on rickets was retrospective, since some participants were uncertain whether they had rickets in childhood, which may contribute to the low prevalence of rickets. In our adult study, participants who had rickets were defined as having vitamin D deficiency in childhood. The association between rickets and type 2 diabetes in adults was analyzed to explore the effect of childhood vitamin D deficiency on type 2 diabetes in adulthood. The results showed that males who had rickets in childhood had a higher risk of type 2 diabetes in adulthood, but this result was not observed in females. However, the reason for this phenomenon is still unclear. In addition, a study from Finland found that individuals with high levels of vitamin D in childhood and adolescence had a significant lower risk of type 2 diabetes in adulthood compared with those who had lower level of vitamin D [23]. These findings collectively suggest that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood, which needs to be further be explored in more cohort studies and intervention studies.

Some scholars have explored the pathogenesis through which vitamin D deficiency might induce IR in children. The results of some studies shown that, vitamin D levels were inversely related to oxidative stress and inflammation, the increase of reactive oxygen species (ROS) and formation of cytokines such as interleukin-6 played major roles in IR [51-53]. In addition, the findings of an obese, African-American adolescent study showed that low vitamin D levels was correlated with low adiponectin levels, which was associated with IR in children and adolescents [54-57]. These results support the finding that vitamin D deficiency in childhood increases the risk of type 2 diabetes in adulthood. At the same time, the results of lab studies also support such pathogenesis: there was a close connection between vitamin D and β-cell function. By regulating cytokines to impact β-cell survival, vitamin D receptor and 1-hydroxylase in β-cell played a role in regulating pancreatic β-cell function, insulin secretion, IR and systemic inflammation [32,58,59]. Furthermore, the mechanism of vitamin D decreased IR might relate to the inhibition of vitamin D on inflammation and activation on insulin receptor [60,61]. In a term of epigenetics, results showed that vitamin D can also affect the occurrence of type 2 diabetes by regulating the expression of methyltransferase to prevent

374 hypermethylation of diabetes-related genes [62]. Above all of these might be the pathogenesis of vitamin D deficiency in childhood increased the risk of type 2 diabetes in adulthood.

376 An advantage of our study was the inclusion of both children study analysis and adult study analysis. The children and adults were both from Harbin, a northeast city of China, was chosen with high prevalence of vitamin D deficiency due to its geographic location. Basically, they had similar geographic and climatic environment and dietary habits. In children, we observed the serum $25(OH)D_3$ concentration was reversely associated with HOMA-IR. We hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting insulin sensitivity, based on theoretical speculation. We defined rickets in adult study as a vitamin D deficiency condition in childhood to simulate the vitamin D deficiency in children study. The growth environment related to vitamin D status in the adults was similar with the children. The adults might simulate children's future growth trajectories to some extent, in terms of the association of childhood vitamin D deficiency with adult type 2 diabetes. The results of the two populations provided mutual support. However, there were also some limitations in our study. First, there was possible selection bias and we did not do the external validity of the sample due to a limitation of external sample. Second, the sample size of children was small and it was a cross-sectional study. We used ORs to interpret the association of vitamin D deficiency with IR and diabetes, which may overstate effect sizes. Third, the rickets information was obtained from self-report, the recall bias was not avoidable although we excluded the uncertain participants. The proportion of self-report rickets was 6.3% and 7.8% for male and female, respectively. We assumed that there some missing reports of rickets in their recalling based on the medical and nutritional condition, and lack of health awareness in China 40 years ago. In addition, the definition of vitamin D deficiency in childhood was rickets, which

might have led to some participants, who did not have rickets but vitamin D deficiency, to be classified as non-deficient. Therefore, long-term design and cohort studies with stricter vitamin D nutritional status monitoring are needed to further verify our results.

Conclusions

In summary, vitamin D deficiency in childhood was associated with IR and might increase the risk of type 2 diabetes in adult males. Early prevention strategies should be undertaken in children to control the rapid increase in type 2 diabetes worldwide, and management of vitamin D deficiency is probably an effective method.

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Authors' contributions

Lixin Na and Changhao Sun designed the study and acquired funding. Junyi Liu and Lixin Na wrote the paper. Liqun Fu and Jingyi Zhang prepared the original draft. Junyi Liu, Shanshan Jin and Yubing Jia collected the data. Junyi Liu and Liqun Fu analysed and interpreted data. All authors read and approved the final manuscript.

Compliance with Ethics Guidelines

The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of Harbin Medical University, and written informed consent was provided by all participants. The methods in this study were in accordance the approved guidelines.

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Competing interests

None of the authors has any potential conflict of interest associated with this research.

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Data sharing statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figure 1 - The follow chart of the children study.

Figure 2 - The follow chart of the adult study.

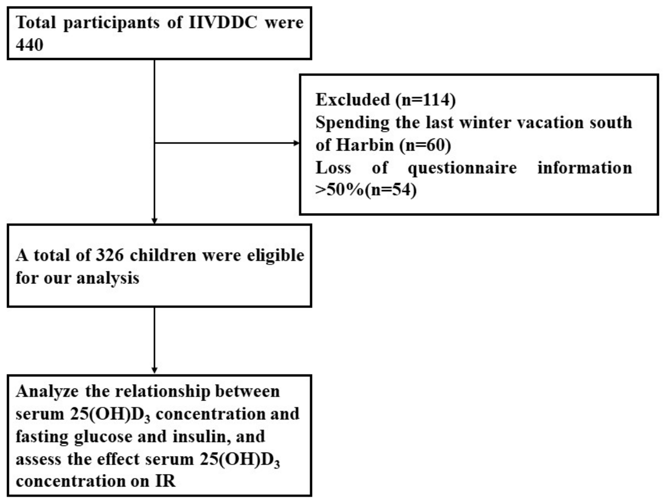

Figure 1. The follow chart of children study

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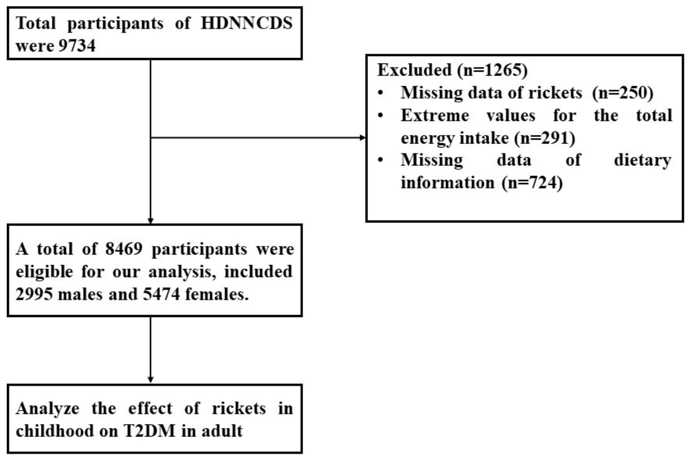

Figure 2. The follow chart of adult study

338x190mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6, 7, 8*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10,12
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	

		(*b*) Report category boundaries when continuous variables were categorized	9, 10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Vitamin D status in children and its association with glucose metabolism in northern China: A combination of a cross-sectional and retrospective study.

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Vitamin D status in children and its association with glucose metabolism in northern China: A combination of a cross-sectional and retrospective study.

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Abstract

Objectives: This study aimed to explore the vitamin D status of children in northern China and the association between vitamin D and glucose metabolism.

Design: Cross-sectional study was conducted among child participants and retrospective study designs was conducted among adult participants.

Setting and participants: Both studies were recruited from Harbin, 326 children were included in children study, 8,469 adults were included in adult study.

Primary and secondary outcome measures: Physical examination, lifestyle and dietary habit data were recorded in all the participants. Serum insulin, glucose, 25(OH)D_3 concentrations in children and serum glucose and lipids levels in adults were measured. Rickets history was also investigated in adults, which was used to define vitamin D deficiency in childhood. The associations were tested by linear regression and binary logistic regression.

Result: In the children study, only 10.7% of participants were vitamin D sufficient (≥30 ng/mL). Inverse correlations between serum 25(OH)D_3 concentration and fasting insulin and HOMA-IR were found, and children with lower serum 25(OH)D_3 concentrations were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, *p* value: 0.027). In an adult study, rickets in childhood increased the risk of type 2 diabetes in male participants (OR = 1.414, 95% CI = 1.013, 1.972; *p* value = 0.042), but this result was not observed in females.

Conclusion: Our findings suggest that vitamin D deficiency is widespread in northern China. Vitamin D deficiency in childhood was associated with IR and increased the risk of type 2 diabetes in male adults.

Keywords: vitamin D deficiency, children, insulin resistance, type 2 diabetes

Strengths and limitations of this study:

- Two studies from Harbin, a city with a high rate of vitamin D deficiency, have important implications for studying vitamin D deficiency in high latitudes and the long-term effects of vitamin D on non-skeletal health.
- The analysis of children's study and the analysis of adults' study with similar environments and dietary habits were included to support each other's findings on the association between vitamin D deficiency and glucose metabolism in the two populations.
- There was possible selection bias and we did not do the external validity of the sample due to a limitation of external sample. Information on rickets that was used to define vitamin D deficiency in childhood was obtained from self-report, the recall bias was not avoidable although we excluded the uncertain participants.
- The sample size of children was small and it was a cross-sectional study. We used ORs to interpret the association of vitamin D deficiency with IR and diabetes, which may overstate effect sizes.

Introduction

According to IDF statistics, there are 450 million adults with type 2 diabetes worldwide, accounting for approximately 90% of diabetes mellitus cases. Type 2 diabetes has become a global public health problem [1,2]. Some recent population studies have found that risk factors such as obesity, impaired glucose tolerance, or insulin resistance (IR) in childhood, may also increase the risk of type 2 diabetes in adults [3-6]. These results suggested that controlling risk factors in childhood is an early prevention strategy to reduce the prevalence of type 2 diabetes in adulthood.

Vitamin D is a fat-soluble vitamin with several physiological functions, one of the most important of which is its effect on skeletal health [7]. At present, vitamin D deficiency is still a serious public problem worldwide, particularly in children [8]. The prevalence of vitamin D deficiency was approximately 50% in both developing and developed countries [9-12]. Serum 25(OH)D_3 is less affected by body regulation and is often used to evaluate vitamin D levels. It is usually considered that serum 25(OH)D_3 < 10 ng/mL indicates severe deficiency, 10-20 ng/mL indicates deficiency, 20-29 ng/mL indicates insufficiency, and ≥30 ng/mL indicates sufficiency [13]. Vitamin D deficiency in children is associated with many skeletal diseases, and one of the typical diseases is rickets. The clinical signs of rickets include skeletal deformity, restlessness, motor retardation and bone pain [14].

Recently, the association between vitamin D deficiency and extra-skeletal health has been of great concern, such as its association with glucose metabolism, obesity, respiratory tract infection and atopic dermatitis, among which the association with glucose metabolism has been of particular concern [15-17]. A population study of children and adolescents in Mexico found that low vitamin D levels were associated with IR; when the concentration of serum vitamin D increased, the possibility of presenting IR decreased [18]. Jared P Reis et al. reported that adolescents in the lowest quartile of vitamin D (<15 ng/mL) are more likely to have hyperglycemia compared with those in the highest quartile (>26 ng/mL) [19]. Furthermore, randomized controlled trials showed that vitamin D supplementation could increase insulin sensitivity and decrease IR and fasting glucose concentrations in obese children [20-22]. Since IR in children is a risk factor for type 2 diabetes, we hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting insulin sensitivity. A recent 31-year follow-up prospective study in 3- to 18-year-old young Finns found that high vitamin D levels in childhood could reduce the incidence of type 2 diabetes in adulthood [23]. This study further supported our hypothesis, but current research is very limited.

89 Harbin is a typical northeast city of China, with a latitude between 44°04′N and 46°40′N, and is a relatively high-latitude area. The winters in Harbin are long, and the sunlight is relatively insufficient year-round, which makes the region's residents vulnerable to vitamin D deficiency. In our study, we first described the vitamin D nutritional status in children and explored the association between vitamin D deficiency and IR from the Investigation and Intervention of Vitamin D Deficiency in Children (IIVDDC). Then, the association between rickets in children and the risk of type 2 diabetes in adults was analyzed using data from the Harbin Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases (HDNNCDS) in a retrospective study. This study aimed to provide a theoretical basis for the early prevention and control of adult type 2 diabetes development in children.

Materials and Methods

Design

A cross-sectional study was conducted among child participants and retrospective study design was conducted among adult participants.

Study population

The child participants were from the baseline survey of IIVDDC from March to May 2019. They were recruited from 4 kindergartens in Nangang District of Harbin by convenient sampling method, including 2 public kindergartens and 2 private kindergartens based on the consideration of different economic levels. The sample size required of children was determined by Events Per Variable criterion (EPV) ≥10, the minimum sample size was calculated to be 90 participants. The parents and teachers of children were invited to informational meetings at which the study and its procedures were explained to them. A total of 440 children aged 3-7 years who had lived in Harbin for the past 3 years were eligible as participants. The exclusion criteria included spending the last winter vacation in the lower latitude areas of Harbin (n=60), who's information of questionnaire losing > 50% (n=54). A total of 326 children were included, and informed written consent was obtained from all custody holders (**Figure 1**).

The adult participants were from the baseline of the HDNNCDS [24]. Seven urban administrative regions of Harbin were covered in HDNNCDS. According to their financial situation, each region was divided into 3 strata and a total of 42 communities were randomly selected from each stratum in each administrative region by employing a stratified multistage random cluster sampling design. Residents were eligible to participate in the study if they: 1) were between 20 and 74 years old, 2) have been living in Harbin for at least two years, 3) were without cancer or type 1 diabetes mellitus. A total of 9,734 people aged 20-74 completed the in-person baseline survey in 2010 - 2012. The sample size required in our analysis was calculated by $N = \frac{Z_{1-\alpha/2}^2(1-P)}{\varepsilon^2 p}$, $Z_{1-\alpha/2} = 1.96$, p = 12.8% [25], $\varepsilon = 10\%$. By calculating N= 2,617, design efficiency value was 2, the final calculated minimal sample size was 5,234. Rickets in childhood were investigated in the survey, and participants who had definite answers were included in our study. In the present study, we excluded participants who reported uncertainty about information on rickets in childhood (n=250), who reported extreme values for total energy intake (<500 kcal/d or >4500 kcal, n=291), and who had missing dietary information data (n=724). Finally, a total of 8,469 participants were eligible for our analysis, including 2,995 males and 5474 females (**Figure 2**). Written informed consent was provided by all participants.

The study protocols of IIVDDC and HDNNCDS were approved by the Ethics Committee of Harbin Medical University, and written informed consent was provided by all participants. The methods in the study were in accordance with the approved guidelines (The registered ethical number: ChiCTR1800020294 for IIVDDC, ChiCTR-ECH-12002721 for HDNNCDS).

133 *Data collection by the questionnaire*

134 Detailed in-person interviews were administered by trained personnel using a structured questionnaire to collect information on demographic characteristics, lifestyle, and dietary intake. In the IIVDDC, the questionnaire was completed by parents and teachers in kindergartens, together. The demographic characteristics of the children included age and gender. Outdoor physical activity in the past 6 months was investigated, children who had more than 60 minutes of daily activity or more than 3 days of weekly activity were considered as high exercise frequency, otherwise they were considered as low exercise frequency. Children who took calcium or vitamin D orally or intravenously in the past 6 months were considered as supplementation with calcium and (or) vitamin D. Dietary information was collected by using a food-frequency questionnaire (FFQ), and a total of 48 food items were included in the questionnaire, which covered most of the foods in the recipes of the kindergartens included in our study. For each food item, parents and teachers of participants were asked how frequently participants consumed over the preceding year, followed by a question on the amount consumed in lians (a unit of weight equal to 50 g) or mL (for liquid food item) per unit of time. The consumption frequency was transformed to obtain mean consumption a day. Nutrient intakes for each food item consumed were calculated by multiplying the nutrient content listed in the China Food Composition [26]. Before dietary surveys, 60 participants from the IIVDDC were recruited and asked to complete two FFQs (FFQ1 and FFQ2) and a 3-day dietary record to validate the reliability of the FFQs. Major nutritional factors (staple food, poultry, fish, vegetable, fruit, and milk products) were assessed by the two FFQs and the FFQ2 and 3-day dietary records, and they correlated well after adjusting for energy intake. In IIVDDC, Cronbach's α coefficient of major nutritional factors between FFQ1 and FFQ2 were 0.67-0.72 and major nutritional factors between FFQ1 and 3-

day dietary record were 0.62-0.76. Seventeen factors were extracted, and the cumulative variance contribution rate was 0.649, which suggested the good construct validity.

In the HDNNCDS, the demographic characteristics mainly included age, gender, and educational level. Lifestyle referred to smoking, alcohol consumption, and physical activity. Current smokers were defined as those who have smoked at least 100 cigarettes lifetime and smoke every day or some days now. Participants who consumed more than 100ml of white wine, highland barley wine, rice wine, grape wine per day or more then 250ml beer per day were considered to have a drinking habit. And regular exercise was defined as any kind of recreational or sport physical activity other than walking for work or life performed at least 30 minutes for three or more days per week. Family history of diabetes was also collected. In the FFQ for adults, a total of 103 food items were included in the questionnaire, which covered most of the commonly consumed foods in urban Harbin. The method to validate the reliability of the FFQs in the HDNNCDS was the same as that in the IIVDDC. The correlation coefficients of major nutritional factors between FFQ1 and FFQ2 were 0.61–0.70 and major nutritional factors between FFQ1 and 3-day dietary record were 0.61–0.69, respectively[24]. Fifteen factors were extracted, and the cumulative variance contribution rate was 0.832, which suggested the good construct validity. Participants were asked to recall the history of rickets in childhood by specific questions in the questionnaire. The questions included whether they had been diagnosed of rickets at the hospital, had the signs of rickets, such as square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs. The answer options included definitely yes, definitely no and uncertain. Participants who chose one or more "definitely yes" were considered to have rickets in childhood. Participants who reported "uncertain" were excluded from our analysis.

Anthropometric measurement

177 Anthropometric measurements, including height, weight and waist circumference (WC), were taken
178 by well-trained examiners, with participants wearing light, thin clothing, and no shoes. Body weight,
179 height and waist circumference were measured to the nearest 0.1 kg, 0.1 cm, and 0.1 cm, respectively.
180 BMI was calculated as weight (kg) divided by the square of the height in meters (m^2). Children's sex-
181 and age-adjusted z-scores for body mass index (zBMI) were calculated as the calculated value of
182 child BMI minus the median BMI of children of the same age and sex, and then divided by the
183 standard deviation of BMI for children of the same age and sex with the use of WHO Anthro Plus
184 software version 1.0.4 [27].

185 *Biochemical assessment*

186 Fasting (more than 10 h) blood samples were collected in children in the IIVDDC. Fasting glucose
187 was determined by an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). 25(OH)D_3 was
188 measured using ELISA kits (Mlbio, Shanghai, China). Serum insulin was measured using the
189 immunofluorescence method (Tosoh automated enzyme immunoassay analyzer AIA-2000ST).
190 Fasting and postprandial (2 hours after drinking 75 grams glucose-containing water) blood samples
191 were taken from all adult participants in the HDNNCDS. Fasting glucose and 2-h postprandial plasma
192 glucose, blood lipids, including triglycerides, total cholesterol (TC), low-density lipoprotein
193 cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), were measured using an
194 automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured in the same
195 way as children.

196 *Definition of variables*

197 *Exposures*

198 In the IIVDDC, serum 25(OH)D_3 < 10 ng/mL was considered severe deficiency, 10-20 ng/mL was
199 considered deficiency, 20-29 ng/mL was considered insufficiency, and ≥30 ng/mL was considered

sufficiency[13]. In the HDNNCDS, Vitamin D deficiency in childhood was defined as a diagnosis of rickets at the hospital, self-reported rickets, or signs of skeletal deformity during childhood.

Outcomes

In the IIVDDC, HOMA-IR was calculated from fasting insulin and glucose using the following formula: fasting insulin concentration (mmol/L) × fasting glucose concentration (mIU/L)/22.5. Children in the highest quartile of HOMA-IR were defined as IR[28], the cutoff value of IR was HOMA-IR ≥ 4.58 in our study. In the HDNNCDS, type 2 diabetes was defined as fasting glucose ≥7.0 mmol/L and/or 2 h glucose ≥11.1 mmol/L and/or self-report of type 2 diabetes and/or use of hypoglycemic medicine.

Potential confounders

In the IIVDDC, age, gender, zBMI, intake of energy, vegetable, fruit and livestock, exercise frequency, calcium or (and) vitamin D supplements, were included in the analysis as potential confounders of IR. In the HDNNCDS, age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit and livestock, and family history were included as potential confounders of type 2 diabetes.

Statistical analysis

SPSS v22.0 (Beijing Stats Data Co. Ltd, Beijing China) was used to analyze the data, and a two-sided *p* value < 0.05 was considered statistically significant. Values are mean ± SD and n (%) per group for all other variables. ANOVA and chi-square test were used to compare the differences in the continuous variables and categorical variables between the groups. The linear regression analysis was used to analyze the association between serum 25(OH)D_3 concentration and fasting glucose, insulin and HOMA-IR in children, expressed as unstandardized β value and Standardized *β* value. Binary logistic regression analysis was used to analyze the association between serum 25(OH)D_3 concentration and IR in children, adjust for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements, expressed as OR value and 95% CI. The data from children were finally analyzed and presented in general as no gender difference was observed after stratified by sex. Binary logistic regression analysis was used to analyze the association between rickets in childhood with type 2 diabetes in adulthood stratified by sex, adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit and livestock, and family history. Bootstrap test of binary logistic regression analysis was used as sensitivity analysis in order to confirm the risk of rickets on diabetes in adulthood male.

Patient and public involvement

Participants were not involved in developing research questions, nor the outcome measures/the study's design. Also, they were not involved in the recruitment to or conduct of the study. In our study, the participants are informed about their blood parameters, and the results of other examinations are gradually shared with them by text message or phone call. The overall findings and benefits of the study will be disseminated through public media.

Results

Results from IIVDDC

Basic information and diet characteristics of children across HOMA-IR quartiles

A total of 326 children were included, 53% were boys and 47% were girls, aged 3 y-7 y, the average age is 5.24 ± 1.32 y. There were 21 (6.4%) children with severe vitamin D deficiency, 163 (50%) children with deficiency, 107 (32.8%) children with insufficiency, and only 35 (10.7%) children with sufficiency.

The basic characteristics of HOMA-IR quartiles grouped children are summarized in Table-1. Serum 25(OH)D_3 concentration in four quartiles from lowest to highest were 20.82 ± 6.96 ng/mL, 20.96 ±

9.07 ng/mL, 19.94 ± 8.09 ng/mL, and 18.19 ± 6.37 ng/mL. Children in higher quartile group had lower proportion of higher sport frequency. There were no significant differences for age, gender, zBMI score, or the supplementation of calcium or (and) vitamin D in recent half year among different quartile groups. The characteristics across quartiles were consistent in boys and girls, and the data were not shown.

Table-1. Characteristics of the subjects in different HOMA-IR quartiles group.

Variable		Q1 (n=81)	Q2 (n=82)	Q3 (n=82)	Q4 (n=81)	*p* Value
Male[1], n (%)		20 (46.5)	68 (49.6)	51 (56.0)	36 (65.5)	0.167
Age, years		5.27 ± 1.59	5.05 ± 1.19	5.22 ± 1.11	5.45 ± 1.33	0.123
$25(OH)D_3$, ng/mL		20.82 ± 6.96	20.96 ± 9.07	19.94 ± 8.09	18.19± 6.37	0.085
zBMI		-0.08± 2.07	0.74 ± 1.67	0.47 ± 1.47	0.58 ± 1.22	0.112
Exercise frequency, n (%)	Lower	10 (12.3)	20 (24.4)	14 (17.1)	12 (14.8)	0.049
	Higher	60 (74.1)	56 (68.3)	48 (58.5)	39 (48.1)	
	unclear	11 (13.6)	6 (7.3)	20 (24.4)	30 (37.0)	
Calcium or (and) vitamin D supplements [a], n (%)	Have been supplement, %	38 (46.9)	43 (52.4)	45 (54.9)	39 (48.1)	0.049
	Haven't been supplement, %	40 (49.4)	35 (42.7)	28 (34.1)	29 (35.8)	
	unclear, %	3 (3.7)	4 (4.9)	9 (11.0)	13 (16.0)	

[1]Values are mean ± SD and n (%) per group for all other variables. A two-sided *p* Value < 0.05 was considered statistically significant; [a] whether have a supplement of calcium and (or) vitamin D with in past 6 months.

Furthermore, a significant difference in energy intake among quartiles of HOMA-IR was observed, with a slightly higher level in quartile 2 than that in other quartiles (**Table-2**). There were no differences in fruit, vegetable, and livestock intakes among different quartiles of HOMA-IR. The above analysis results were consistent in boys and girls, and the data were not shown.

Table-2. Characteristics of diet in different HOMA-IR quartiles group.

Variable	Q1	Q2	Q3	Q4	*p* Value
Energy [1], kcal/d	1323.44 ± 644.00	1650.28 ± 855.11	1409.05 ± 868.96	1230.34 ± 882.99	0.009
Vegetable, g/d	77.08 ± 32.55	84.85 ± 36.13	89.09 ± 38.12	81.63 ± 44.13	0.428
Fruit, g/d	113.27 ± 48.71	133.58 ± 59.28	126.42 ± 52.44	123.08 ± 56.41	0.265
Livestock, g/d	76.04 ± 38.58	88.97 ± 38.48	80.77 ± 37.25	77.36 ± 36.12	0.230

[1] Values are mean ± SD. A two-sided *p* Value < 0.05 was considered statistically significant.

The association between serum 25(OH)D_3 and fasting glucose, insulin and HOMA-IR

Serum 25(OH)D_3 concentration were negatively correlated with fasting insulin and HOMA-IR after adjusting for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements (fasting insulin: unstandardized β coefficient = -0.178, standardized β coefficient = -0.194, p value = 0.001; HOMA-IR: unstandardized β coefficient = -0.032, standardized β coefficient = -0.161, p value = 0.005) **(Table-3)**. However, the significant association between serum 25(OH)D_3 concentration and fasting glucose had not been observed. There was no difference in above analysis results between boys and girls, data were not shown.

Table-3. Linear regression analysis of the association between serum 25(OH)D_3 concentration and fasting glucose and insulin.

Variable	Crude			Model 1 [1]			Model 2		
	Unstandardized β	Standardized β	p Value	Unstandardized β	Standardized β	p Value	Unstandardized β	Standardized β	p Value
Glucose (mmol/L)	0.004	0.065	0.245	0.007	0.107	0.066	0.008	0.115	0.052
Insulin (mIU/L)	-0.169	-0.184	0.001	-0.170	-0.184	0.001	-0.178	-0.194	0.001
HOMA-IR	-0.030	-0.152	0.006	-0.031	-0.157	0.005	-0.032	-0.161	0.005

[1]Model 1: Adjusted for age, gender, zBMI, exercise frequency, intake of energy, calcium or (and) vitamin D supplements. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

The association of serum 25(OH)D_3 concentration with IR

Children in the highest quartile of HOMA-IR were defined as IR, the cut-off point of HOMA-IR was 4.59. As shown in **Table-4**, after adjusting for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements, children with low serum 25(OH)D_3 concentration were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, p value: 0.027). This association was consistent in boys and girls, and the data were not shown.

Table-4. Binary logistic regression analysis of the association between serum 25(OH)D_3 concentration and

IR.

Variable	Model 1 [1]		Model 2	
	OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Serum 25(OH)D3 concentration (ng/mL)	0.964 (0.928, 1.001)	0.059	0.955 (0.917, 0.995)	0.027

[1]Model 1: Adjusted for age, gender, zBMI, exercise frequency, intake of energy, calcium or (and) vitamin D supplements. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Results from HDNNCDS

Basic information, diet and blood biochemical characteristics of adult participants

The basic characteristics of adult participants are shown in **Table-5**. A total of 8,469 adult participants, 2,999 male and 5,474 female were included in our analysis. The average age was 49.58 ± 10.51 y. In both males and females, participants with type 2 diabetes had older age, larger BMI, WC and higher proportion of family history of diabetes, compared with participants without type 2 diabetes. Additionally, the proportion of regular exercise in participants with type 2 diabetes was higher than those without type 2 diabetes. In females, the proportion of drinking was lower in participants with type 2 diabetes and they had lower education levels, whereas the above phenomenon was not observed in males. Furthermore, type 2 diabetes participants had higher T-CHO, TG, and LDL-c concentrations and lower HDL-c concentration in both males and females.

Table-5. Characteristics of the subject in T2D and non-T2D group, by gender.

Variable		Male			Female		
		T2D (n = 773)	Non-T2D (n = 2222)	*p* Value	T2D (n = 1048)	Non-T2D (n = 4426)	*p* Value
Age [1], years		52.46 ± 9.33	49.04 ± 10.99	<0.001	55.64 ± 9.42	49.44 ± 10.06	<0.001
BMI, kg/m^2		26.12 ± 3.37	25.57 ± 3.43	<0.001	25.88 ± 3.74	24.22 ± 3.32	<0.001
WC, cm		92.27 ± 9.21	90.48 ± 9.55	<0.001	86.94 ± 9.79	81.96 ± 9.12	<0.001
Rickets, n (%)		59 (7.6)	132 (5.9)	0.104	81 (7.7)	354 (8.0)	0.700
Education, n (%)	Primary school education or below	28 (3.6)	86 (3.9)	0.038	116 (11.1)	283 (6.4)	<0.001
	Junior or high school education	430 (55.6)	1102 (49.6)		666 (63.5)	2437 (55.1)	
	Bachelor degree or above	296 (35.7)	903 (40.6)		202 (19.3)	1434 (32.4)	
	The	39 (5.0)	131 (5.9)		66 (6.1)	272 (6.1)	

	situation of education unclear						
Smoking, n (%)	Smokers	310 (40.1)	870 (39.2)	0.836	37 (3.5)	175 (4.0)	0.059
	Non-smokers	391 (50.6)	1156 (52.0)		984 (93.9)	4188 (94.6)	
	Former smokers	66 (8.5)	175 (7.9)		11 (1.0)	28 (0.6)	
	The situation of smoking unclear	6 (0.7)	22 (0.9)		16 (1.5)	35 (0.8)	
Drinking, n (%)	Drinking	462 (59.8)	1404 (63.2)	0.219	145 (13.8)	909 (20.5)	<0.001
	Non-drinking	304 (39.3)	796 (35.8)		890 (84.9)	3453 (78.0)	
	The situation of drinking unclear	7 (0.9)	22 (1.0)		13 (1.2)	64 (1.4)	
Exercise, n (%)		418 (54.1)	1039 (46.8)	0.001	558 (53.2)	1949 (44.0)	<0.001
Family history of diabetes, n (%)		171 (22.1)	226 (10.2)	<0.001	268 (25.6)	676 (15.3)	<0.001
T-CHO, mmol/L		5.28 ± 1.19	5.00 ± 0.99	< 0.001	5.50 ± 1.04	5.18 ± 1.00	<0.001
TG, mmol/L		2.73 ± 3.32	2.07 ± 2.09	< 0.001	2.01 ± 1.62	1.41 ± 1.08	<0.001
HDL-c, mmol/L		1.10 ± 0.29	1.15 ± 0.30	< 0.001	1.25 ± 0.31	1.35 ± 0.32	<0.001
LDL-c, mmol/L		3.06 ± 0.91	2.96 ± 0.81	0.003	3.31 ± 0.90	3.00 ± 0.85	<0.001

[1] Values are mean ± SD, or frequency (%). A two-sided p Value < 0.05 was considered statistically significant.

In males and females, participants with type 2 diabetes had less consumption of fruit, but the difference in energy intake and consumption of vegetable and livestock were not observed (Table-6).

Table-6. Characteristics of energy vegetable, fruit, livestock intake in T2D and non-T2D group, by gender.

Variable	Male			Female		
	T2D (n = 773)	Non-T2D (n = 2222)	p Value	T2D (n = 1048)	Non-T2D (n = 4426)	p Value
Energy [1], kcal/d	2507.81 ± 790.83	2551.96 ± 775.41	0.175	2187.03 ± 702.46	2201.33 ±679.62	0.543
Vegetable, g/d	136.04 ±52.72	134.81 ± 51.24	0.570	132.93 ± 52.36	130.29 ± 51.30	0.136
Fruit, g/d	123.32 ± 47.85	129.00 ± 49.01	0.005	123.16 ± 50.34	133.55 ± 50.68	<0.001
Livestock, g/d	107.20 ± 45.41	107.90 ± 45.78	0.712	93.31 ± 39.11	92.77 ± 39.59	0.695

[1] Values are mean ± SD. A two-sided p Value < 0.05 was considered statistically significant.

The association of rickets in childhood with type 2 diabetes in adulthood

Binary logistic regression analysis showed that rickets in childhood was significantly associated with an increased risk of type 2 diabetes in adult males (OR = 1.420, 95% CI = 1.017, 1.983; *p* value = 0.040), after adjusting for sex, adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit and livestock, and family history. However, there was no significant association of rickets in childhood with type 2 diabetes in adult females (**Table-7**). Result of the bootstrap test was consistent with the result of the binary logistic regression analysis (*p* value = 0.041) (**Table-8**).

Table-7. Binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood with different sex.

Variable		Model 1 [1]		Model 2	
		OR (95% CI)	*p* Value	OR (95% CI)	*p* Value
Male	Rickets	1.414 (1.013, 1.972	0.042	1.420 (1.017, 1.983)	0.040
Female	Rickets	1.062 (0.813, 1.388)	0.658	1.065 (0.814, 1.392)	0.646

[1]Model 1: Adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, family history of diabetes. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Table-8. The bootstrap test of binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood male.

Variable	Model 1[1]		Model 2	
	β (95% CI)	*p* Value	β (95% CI)	*p* Value
Rickets	0.346 (0.004, 0.673)	0.039	0.351 (0.002, 0.680)	0.041

[1]Model 1: Adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, family history of diabetes. Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

Discussion

This study examined the association of vitamin D status in childhood with glucose metabolism in the north of China. The results showed that, in children, only 10.7% participants with vitamin D sufficient, there was a negative association between serum 25(OH)D_3 concentration and fasting insulin and HOMA-IR, and children with lower 25(OH)D_3 concentration were likely to develop IR. Childhood rickets were associated with an increased risk of type 2 diabetes in adulthood.

Vitamin D deficiency in children is widespread in worldwide. Regardless of the economic level, the vitamin D deficiency rate was very high in children from different countries [9-12,29,30]. Vitamin D can be obtained from sunshine and foods, such as meat, eggs, and milk. It was generally thought that exposure of skin to ultraviolet rays in the sunshine was the main source of body obtain vitamin D [31]. Therefore, the status of serum vitamin D can be influenced by several factors, such as skin tone, the latitude of residence, season, or use of sunscreen products [32]. Sunshine in areas with high latitude was insufficient for skin to synthesize vitamin D; a previous study showed that residents living in areas above 37°N were insufficiently synthesizing vitamin D in winter [33]. In relatively low latitude areas of China, 50% of preschooler children had sufficient vitamin D nutritional status (≥ 30 ng/mL), [34]. In our study, only 10.7% of children had sufficient vitamin D nutritional status, which was significantly lower than children at low latitudes, vitamin D insufficiency was even more serious. Therefore, we should pay more attention to the health problems caused by vitamin D deficiency and insufficiency at high latitude.

In addition to skeletal health, the association between vitamin D and glucose metabolism in children and adults has also obtained wide concerned. In children, vitamin D deficiency was connected to IR and impaired fasting glucose [13,35,36]. However, there were a lack of children studies on the association between vitamin D deficiency and IR in the northern area of China. This study found a negative correlation between serum vitamin D concentration and fasting insulin, HOMA-IR, and IR. In adults, previous research suggested that low vitamin D status was associated with IR, impaired glucose tolerance, decreased insulin sensitivity, and reduction of insulin secretion [18,37-39]. Observational study showed that patients with type 2 diabetes had lower level of vitamin D than healthy people[40]. An intervention studies have shown that HbA1c levels decreased after vitamin D supplementation[41].

Additionally, prospective studies had indicated that vitamin D deficiency in adults might increase the risk of type 2 diabetes [42,43-45]. According to recent years' studies, IR in childhood was considered as a risk factor for type 2 diabetes in adulthood [46]. Therefore, we speculate vitamin D deficiency in childhood may increase the risk of type 2 diabetes in adulthood by influence IR in childhood. However, this deduction needs a long-term cohort study spanning decades from childhood to adulthood, there have been no reports about this so far.

Nutritional rickets is the most common type in rickets, caused by deficiencies of vitamin D, calcium, or phosphate [47,48]. The clinical signs of rickets include square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs. Rickets needs to be diagnosed in combination with vitamin D level and clinical signs, and vitamin D deficiency alone cannot be diagnosed as rickets, but children who had serum 25(OH)D_3 level under 10.90 ng/mL were likely to have rickets [49]. A retrospective survey was conducted on the prevalence of rickets in our adult study, rickets was determined by whether participants had a diagnosis of disease, or had symptoms. In adult population of this study, the prevalence of rickets in males and females was 6.3% and 7.9%, respectively. An earlier study reported that the prevalence of Chinese infants with rickets in 1980s was approximately 18% [50], it was higher than our study. This difference may be due to the birth year of our participants being approximately 20 years earlier than those in that study, when China was experiencing societal hardships. Poverty and poor health conditions may lead to the lack of awareness of disease. Furthermore, the information on rickets was retrospective, since some participants were uncertain whether they had rickets in childhood, which may contribute to the low prevalence of rickets. In our adult study, participants who had rickets were defined as having vitamin D deficiency in childhood. The association between rickets and type 2 diabetes in adults was analyzed to explore the effect of childhood vitamin D deficiency on type 2 diabetes in adulthood. The results showed that males who had rickets in childhood had a higher risk of type 2 diabetes in adulthood, but this result was not observed in females. However, the reason for this phenomenon is still unclear. In addition, a study from Finland found that individuals with high levels of vitamin D in childhood and adolescence had a significant lower risk of type 2 diabetes in adulthood compared with those who had lower level of vitamin D [23]. These findings collectively suggest that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood, which needs to be further be explored in more cohort studies and intervention studies.

Some scholars have explored the pathogenesis through which vitamin D deficiency might induce IR in children. The results of some studies shown that, vitamin D levels were inversely related to oxidative stress and inflammation, the increase of reactive oxygen species (ROS) and formation of cytokines such as interleukin-6 played major roles in IR [51-53]. In addition, the findings of an obese, African-American adolescent study showed that low vitamin D levels was correlated with low adiponectin levels, which was associated with IR in children and adolescents [54-57]. These results support the finding that vitamin D deficiency in childhood increases the risk of type 2 diabetes in adulthood. At the same time, the results of lab studies also support such pathogenesis: there was a close connection between vitamin D and β-cell function. By regulating cytokines to impact β-cell survival, vitamin D receptor and 1-hydroxylase in β-cell played a role in regulating pancreatic β-cell function, insulin secretion, IR and systemic inflammation [32,58,59]. Furthermore, the mechanism of vitamin D decreased IR might relate to the inhibition of vitamin D on inflammation and activation on insulin receptor [60,61]. In a term of epigenetics, results showed that vitamin D can also affect the occurrence of type 2 diabetes by regulating the expression of methyltransferase to prevent hypermethylation of diabetes-related genes [62]. Above all of these might be the pathogenesis of vitamin D deficiency in childhood increased the risk of type 2 diabetes in adulthood.

An advantage of our study was the inclusion of both children study analysis and adult study analysis. The children and adults were both from Harbin, a northeast city of China, was chosen with high prevalence of vitamin D deficiency due to its geographic location. Basically, they had similar geographic and climatic environment and dietary habits. In children, we observed the serum 25(OH)D_3 concentration was reversely associated with HOMA-IR. Based on theoretical speculation, we hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting insulin sensitivity. We defined rickets in adult study as a vitamin D deficiency condition in childhood to simulate the vitamin D deficiency in children study. The growth environment related to vitamin D status in the adults was similar with the children. The adults might simulate children's future growth trajectories to some extent, in terms of the association of childhood vitamin D deficiency with adult type 2 diabetes. The results of the two populations provided mutual support. However, there were also some limitations in our study. First, there was possible selection bias and we did not do the external validity of the sample due to a limitation of external sample. Second, the sample size of children was small and it was a cross-sectional study. We used ORs to interpret the association of vitamin D deficiency with IR and diabetes, which may overstate effect sizes. Third, the rickets information was obtained from self-report, the recall bias was not avoidable although we excluded the uncertain participants. The proportion of self-report rickets was 6.3% and 7.8% for male and female, respectively. We assumed that there some missing reports of rickets in their recalling based on the medical and nutritional condition, and lack of health awareness in China 40 years ago. In addition, the definition of vitamin D deficiency in childhood was rickets, which might have led to some participants, who did not have rickets but vitamin D deficiency, to be classified as non-deficient. Therefore, long-term design and cohort studies with stricter vitamin D nutritional status monitoring are needed to further verify our results.

Conclusions

In summary, vitamin D deficiency in childhood was associated with IR and might increase the risk of type 2 diabetes in adult males. Early prevention strategies should be undertaken in children to control the rapid increase in type 2 diabetes worldwide, and management of vitamin D deficiency is probably an effective method.

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Authors' contributions

Lixin Na and Changhao Sun designed the study and acquired funding. Junyi Liu and Lixin Na wrote the paper. Liqun Fu and Jingyi Zhang prepared the original draft. Junyi Liu, Shanshan Jin and Yubing Jia collected the data. Junyi Liu and Liqun Fu analysed and interpreted data. All authors read and approved the final manuscript.

Competing interests

None of the authors has any potential conflict of interest associated with this research.

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Data sharing statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of Harbin Medical University, and written informed consent was provided by all participants. The methods in this study were in accordance the approved guidelines.

Investigation and Intervention of Vitamin D Deficiency in Children: ChiCTR1800020294; Harbin Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases: ChiCTR-ECH-12002721.

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Figure 1 - The follow chart of the children study.

Figure 2 - The follow chart of the adult study.

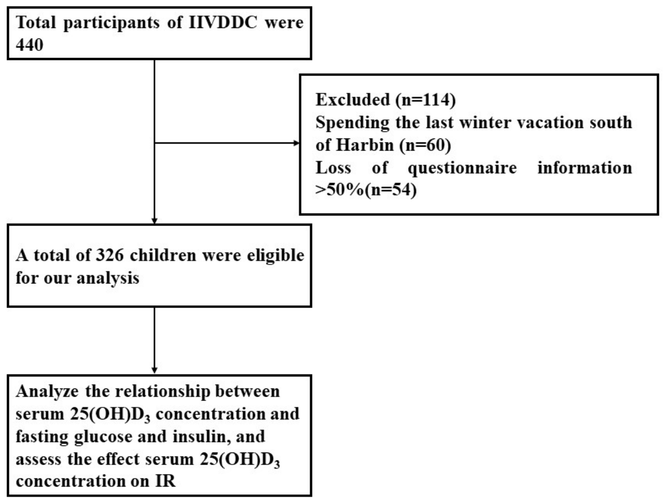

Figure 1. The follow chart of children study

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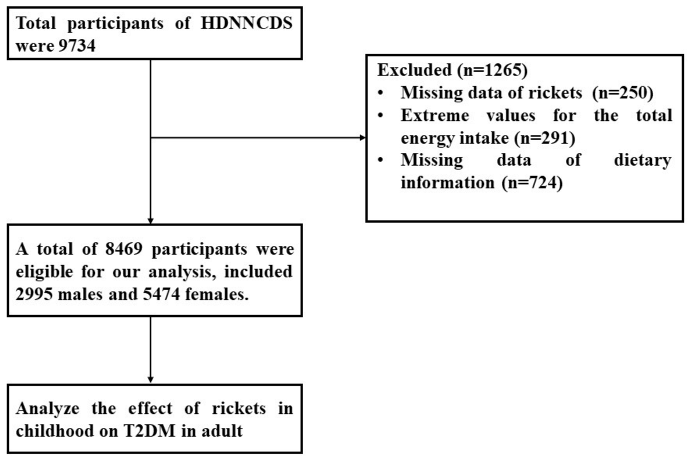

Figure 2. The follow chart of adult study

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6, 7, 8*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10,12
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	

		(*b*) Report category boundaries when continuous variables were categorized	9, 10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.