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

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

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21 **Word count: 4511**

22 **Abstract**

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3 23 **Objectives:** The aim of this study was to explore the vitamin D status of children in northern China
4
5 24 and the association between vitamin D and glucose metabolism.
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7
8 25 **Design:** Cross-sectional study was conducted among child participants and retrospective study
9
10 26 designs was conducted among adult participants.
11
12

13 27 **Setting and participants:** The child participants were recruited from the baseline survey of
14
15 28 IIVDDC, a total of 326 children were included. The data of adult participants were from the
16
17 29 baseline data of the HDNNCDS, a total of 8,469 participants were eligible for our analysis.
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19
20

21 30 **Primary and secondary outcome measures:** Physical examination, lifestyle and dietary habit data
22
23 31 were recorded, and serum levels were measured in all participants. In adults, rickets in childhood
24
25 32 was also investigated, which was used to define vitamin D deficiency in childhood. The
26
27 33 associations were tested by linear regression and binary logistic regression. The effect of rickets on
28
29 34 type 2 diabetes in adulthood was tested by binary logistic regression.
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34 35 **Result:** In the pediatric study, only 10.7% of participants were vitamin D sufficient (≥ 30 ng/mL).
35
36 36 Inverse correlations between serum 25(OH)D₃ concentration and fasting insulin and HOMA-IR
37
38 37 were found, and children with lower serum 25(OH)D₃ concentrations were likely to have IR (OR:
39
40 38 0.955, 95% CI: 0.917, 0.995, *p* value: 0.027). In an adult study, rickets in childhood increased the
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42 39 risk of type 2 diabetes in male participants (OR = 1.414, 95% CI = 1.013, 1.972; *p* value = 0.042),
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44 40 but this result was not observed in females.
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49 41 **Conclusion:** Our findings suggest that vitamin D deficiency is widespread in northern China.
50
51 42 Vitamin D deficiency in childhood was associated with IR and increased the risk of type 2 diabetes
52
53 43 in male adults.
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57 44 **Keywords:** vitamin D deficiency, children, insulin resistance, type 2 diabetes
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45 **Introduction**

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

52 Vitamin D is a fat-soluble vitamin with several physiological functions, one of the most important
53 of which is its effect on skeletal health ⁷. At present, vitamin D deficiency is still a serious public
54 problem worldwide, particularly in children ⁸. The prevalence of vitamin D deficiency was
55 approximately 50% in both developing and developed countries ⁹⁻¹². Serum 25(OH)D₃ is less
56 affected by body regulation and is often used to evaluate vitamin D levels. It is usually considered
57 that serum 25(OH)D₃ < 10 ng/mL indicates severe deficiency, 10-20 ng/mL indicates deficiency,
58 20-29 ng/mL indicates insufficiency, and ≥30 ng/mL indicates sufficiency ¹³. Vitamin D deficiency
59 in children is associated with many skeletal diseases, and one of the typical diseases is rickets. The
60 clinical signs of rickets include skeletal deformity, restlessness, motor retardation and bone pain ¹⁴.

61 Recently, the association between vitamin D deficiency and extra-skeletal health has been of great
62 concern, such as its association with glucose metabolism, obesity, respiratory tract infection and
63 atopic dermatitis, among which the association with glucose metabolism has been of particular
64 concern ¹⁵⁻¹⁷. A population study of children and adolescents in Mexico found that low vitamin D
65 levels were associated with IR; when the concentration of serum vitamin D increased, the
66 possibility of presenting IR decreased ¹⁸. Jared P Reis et al. reported that adolescents in the lowest

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3 67 quartile of vitamin D (<15 ng/mL) are more likely to have hyperglycemia compared with those in
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5 68 the highest quartile (>26 ng/mL)¹⁹. Furthermore, randomized controlled trials showed that vitamin
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8 69 D supplementation could increase insulin sensitivity and decrease IR and fasting glucose
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11 70 concentrations in obese children²⁰⁻²². Since IR in children is a risk factor for type 2 diabetes, we
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13 71 hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in
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15 72 adulthood by affecting insulin sensitivity. A recent 31-year follow-up prospective study in 3- to 18-
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18 73 year-old young Finns found that high vitamin D levels in childhood could reduce the incidence of
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21 74 type 2 diabetes in adulthood²³. This study further supported our hypothesis, but current research is
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23 75 very limited.

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26 76 Harbin is a typical northeast city of China, with a latitude between 44°04'N and 46°40'N, and is a
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29 77 relatively high-latitude area. The winters in Harbin are long, and the sunlight is relatively
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32 78 insufficient year-round, which makes the region's residents vulnerable to vitamin D deficiency. In
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34 79 our study, we first described the vitamin D nutritional status in children and explored the
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37 80 association between vitamin D deficiency and IR from the Investigation and Intervention of
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39 81 Vitamin D Deficiency in Children (IIVDDC). Then, the association between rickets in children and
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42 82 the risk of type 2 diabetes in adults was analyzed using data from the Harbin Cohort Study on Diet,
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45 83 Nutrition and Chronic Non-communicable Diseases (HDNNCDS) in a retrospective study. The aim
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47 84 of this study was to provide a theoretical basis for the early prevention and control of adult type 2
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50 85 diabetes development in children.

51 86 **Materials and Methods**

52 87 *Design*

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55 88 Cross-sectional study was conducted among child participants and retrospective study designs was
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3 89 conducted among adult participants.
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5 90 *Study population*
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8 91 *Part I IIVDDC*
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10 92 The child participants were recruited from the baseline survey of IIVDDC. They were recruited
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13 93 from 4 kindergartens in Nangang District of Harbin, including 2 public kindergartens and 2 private
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15 94 kindergartens, from March to May 2019. The parents and teachers of children were invited to
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18 95 informational meetings at which the study and its procedures were explained to them. 440 children
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21 96 aged 3-7 years who had lived in Harbin for the past 3 years were eligible as participants. The
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24 97 exclusion criteria included spending the last winter vacation in the lower latitude areas of Harbin
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26 98 (n=60), who's information of questionnaire losing > 50% (n=54). A total of 326 children were
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28
29 99 included, and informed written consent was obtained from all custody holders (**Figure 1**).
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32 100 *Part II HDNNCDS*
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35 101 The data of adult participants were from the baseline data of the HDNNCDS. Residents who had
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38 102 lived in their communities for more than two years and who did not have cancer or type 1 diabetes
39
40 103 were recruited. A total of 9,734 people aged 20-74 years completed the in-person baseline survey in
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43 104 2010 - 2012²⁴. Rickets in childhood were investigated, and participants who had definite answers
44
45 105 were included in our study. In the present study, we excluded participants who reported uncertainty
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47
48 106 about information on rickets in childhood (n=250), who reported extreme values for total energy
49
50
51 107 intake (<500 kcal/d or >4500 kcal, n=291), and who had missing dietary information data (n=724).
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53 108 Finally, a total of 8,469 participants were eligible for our analysis, including 2995 males and 5474
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56 109 females (**Figure 2**). Written informed consent was provided by all participants.
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58 110 These study protocols were approved by the Ethics Committee of Harbin Medical University. The
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111 methods in this study were conducted in accordance with the approved guidelines.

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

115 *Data collection by the questionnaire*

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

118 *Part I IIVDDC*

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

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3 133 correlated well. In IIVDDC, the correlation coefficients were 0.49–0.54 and 0.55–0.76.
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5 134 *Part II HDNNCDS*
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8 135 In the HDNNCDS, the demographic characteristics mainly included age, gender, and educational
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10 136 level. Lifestyle referred to smoking, alcohol consumption, and physical activity. Family history of
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13 137 diabetes was also collected. In the FFQ for adults, a total of 103 food items were included in the
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16 138 questionnaire, which covered most of the commonly consumed foods in urban Harbin. The method
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18 139 to validate the reliability of the FFQs in the HDNNCDS was the same as that in the IIVDDC. The
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21 140 correlation coefficients were 0.61–0.70 and 0.61–0.69, respectively²⁴. Participants were asked to
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23
24 141 recall whether they got rickets in childhood. In the questionnaire, the rickets in childhood included
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26 142 the participants' self-report of rickets, diagnosis from the hospital, and/or the existence of rickets
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29 143 signs, such as square head, delay in tooth development, rachitic chest, bow legs or X-shaped legs.
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31 144 *Anthropometric measurement*
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34 145 Anthropometric measurements, including height, weight and waist circumference (WC), were taken
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36 146 by well-trained examiners, with participants wearing light, thin clothing, and no shoes. Body
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39 147 weight, height and waist circumference were measured to the nearest 0.1 kg, 0.1 cm, and 0.1 cm,
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41
42 148 respectively. Children's sex- and age-adjusted z-scores for body mass index (zBMI) were calculated
43
44 149 with the use of WHO Anthro Plus software version 1.0.4²⁶. Adult BMI was calculated as weight
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47 150 (kg) divided by the square of the height in meters (m²).
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49 151 *Biochemical assessment*
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52 152 *Part I IIVDDC*
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55 153 Fasting (more than 10 h) blood samples were collected. Fasting glucose was determined by an
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57 154 automatic biochemistry analyzer (Hitachi, Tokyo, Japan). 25(OH)D₃ was measured using ELISA
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3 155 kits (Mlbio, Shanghai, China). Serum insulin was measured using the immunofluorescence method
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5 156 (Tosoh automated enzyme immunoassay analyzer AIA-2000ST).

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8 157 *Part II HDNNCDS*

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10 158 Fasting and postprandial (2 hours after drinking 75 grams glucose-containing water) blood samples
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12
13 159 were taken from all participants. Fasting glucose and 2-h postprandial plasma glucose and blood
14
15 160 lipids, including triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c),
16
17
18 161 and high-density lipoprotein cholesterol (HDL-c), were measured using an automatic biochemistry
19
20
21 162 analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured in the same way as children.
22
23 163 HOMA-IR was calculated from fasting insulin and glucose using the following formula: fasting
24
25
26 164 insulin concentration (mmol/L) × fasting glucose concentration (mIU/L)/22.5.

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29 165 *Definition of outcomes*

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31 166 *Part I IIVDDC*

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33 167 Diagnostic criteria of serum vitamin D status: Serum 25(OH)D₃ < 10 ng/mL was considered severe
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35 168 deficiency, 10-20 ng/mL was considered deficiency, 20-29 ng/mL was considered insufficiency,
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38 169 and ≥30 ng/mL was considered sufficiency¹³.

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40 170 Definition of IR: Children in the highest quartile of HOMA-IR were defined as IR²⁷.

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43 171 *Part II HDNNCDS*

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46 172 Definition of type 2 diabetes: Type 2 diabetes was defined as fasting glucose ≥7.0 mmol/L and/or 2
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48 173 h glucose ≥11.1 mmol/L and/or self-report of type 2 diabetes and/or use of hypoglycemic medicine.

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50
51 174 The definition of vitamin D deficiency in childhood was as follows: Vitamin D deficiency in
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53 175 childhood was defined as a diagnosis of rickets at the hospital, self-reported rickets, or signs of
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56 176 skeletal deformity during childhood.

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59 177 *Statistical analysis*

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3 178 SPSS v22.0 (Beijing Stats Data Co. Ltd, Beijing China) was used to analyze the data, and a two-
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5 179 sided p value < 0.05 was considered statistically significant. Values are mean \pm SD and n (%) per
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8 180 group for all other variables. ANOVA and chi-square test were used to compare the differences in
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11 181 the continuous variables and categorical variables between the groups. The linear regression
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13 182 analysis was used to analyze the association between serum 25(OH)D₃ concentration and fasting
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15
16 183 glucose, insulin and HOMA-IR in children, expressed as unstandardized β value and Standardized β
17
18 184 value. Binary logistic regression analysis was used to analyze the association between serum
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20
21 185 25(OH)D₃ concentration and IR in children, and the effect of rickets in childhood on type 2 diabetes
22
23
24 186 in adulthood, expressed as OR value and 95% CI. Bootstrap test of binary logistic regression
25
26 187 analysis was used as sensitivity analysis in order to confirm the risk of rickets on diabetes in
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28
29 188 adulthood male.

31 189 **Patient and public involvement**

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34 190 participants were not involved in the development of research questions, nor the outcome
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36
37 191 measures/the design of the study. Also, they were not involved in the recruitment to or conduct of
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39 192 the study. In our study, the participants are informed about their blood parameters, and the results of
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41
42 193 other examinations are gradually shared with them by text message or phone call. The overall
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44 194 findings and benefits of the study will be disseminated through public media.

47 195 **Results**

49 196 *Results from IIVDDC*

52 197 *Vitamin D nutrition status in children*

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55 198 There were 21 (6.4%) children with severe vitamin D deficiency, 163 (50%) children with
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57 199 deficiency, 107 (32.8%) children with insufficiency, and only 35 (10.7%) children with sufficiency.
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3 200 *Basic information and diet characteristics of children across HOMA-IR quartiles*

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5 201 Children were grouped by HOMA-IR quartiles. The basic information characteristics are

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8 202 summarized in **Table-1**. Serum 25(OH)D₃ concentration in four quartiles from lowest to highest

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10 203 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.

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13 204 Children in higher quartile group had lower proportion of sport frequency. There were no

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16 205 significant differences for age, gender, zBMI score, or the supplementation of calcium or (and)

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18 206 vitamin D in recent half year among different quartile groups.

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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)	

¹Values 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.

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43 208 Furthermore, there were no significant differences in energy, fruit, vegetable, and livestock intakes

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46 209 among different quartiles of HOMA-IR (**Table-2**). The above analysis results were consistent in

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48 210 boys and girls, and the data were not shown.

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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.

59

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212 *The association between serum 25(OH)D₃ and fasting glucose, insulin and HOMA-IR*

213 Serum 25(OH)D₃ concentration were negatively correlated with fasting insulin and HOMA-IR after
 214 adjusting for age, sex, zBMI, calcium or (and) vitamin D supplements, exercise frequency, dietary
 215 intakes of energy, vegetable, fruit, and livestock (fasting insulin: unstandardized β coefficient = -
 216 0.178, standardized β coefficient = -0.194, p value = 0.001; HOMA-IR: unstandardized β
 217 coefficient = -0.032, standardized β coefficient = -0.161, p value = 0.005) (**Table-3**). However, the
 218 significant association between serum 25(OH)D₃ concentration and fasting glucose had not been
 219 observed. There was no difference in above analysis results between boys and girls, data were not
 220 shown.

221 **Table-3. Linear regression analysis of the association between serum 25(OH)D₃ concentration and fasting**
 222 **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

¹Model 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.

223 *The association of serum 25(OH)D₃ concentration with IR*

224 Children in the highest quartile of HOMA-IR were defined as IR, the cut-off point of HOMA-IR
 225 was 4.59. As shown in **Table-4**, after adjusting for age, sex, zBMI, calcium or (and) vitamin D
 226 supplements, exercise frequency, dietary intakes of energy, vegetable, fruit, and livestock, children
 227 with low serum 25(OH)D₃ concentration were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995,

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3 228 *p* value: 0.027).

4
5 **Table-4. Binary logistic regression analysis of the association between serum 25(OH)D₃ concentration and IR.**

Variable	Model 1 ¹		Model 2	
	OR (95% CI)	<i>p</i> Value	OR (95% CI)	<i>p</i> Value
Serum 25(OH)D ₃ concentration (ng/mL)	0.964 (0.928, 1.001)	0.059	0.955 (0.917, 0.995)	0.027

11¹Model 1: Adjusted for age, gender, zBMI, energy, exercise frequency, calcium or (and) vitamin D supplements.

12 Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

14
15 229 *Results from HDNNCDS*

17 230 *Basic information, diet and blood biochemical characteristics of adult participants*

20 231 The basic characteristics of adult participants are shown in **Table-5**. In both males and females,
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22 participants with type 2 diabetes had older age, larger BMI, WC and higher proportion of family
23 232 history of diabetes, compared with participants without type 2 diabetes. Additionally, the proportion
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25 233 of regular exercise in participants with type 2 diabetes was higher than those without type 2
26
27 diabetes. In females, the proportion of drinking was lower in participants with type 2 diabetes and
28 234 they had lower education levels, whereas the above phenomenon was not observed in males.
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30 235 Furthermore, type 2 diabetes participants had higher T-CHO, TG, and LDL-c concentrations and
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32 lower HDL-c concentration in both males and females.
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40 239 **Table-5. Characteristics of the subject in T2D and non-T2D group, by gender.**

Variable	Male			Female		
	T2D (n = 773)	Non-T2D (n = 2222)	<i>p</i> Value	T2D (n = 1048)	Non-T2D (n = 4426)	<i>p</i> 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 $P \leq 0.05$ 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		<i>p</i> Value	Female		<i>p</i> Value
	T2D (n = 773)	Non-T2D (n = 2222)		T2D (n = 1048)	Non-T2D (n = 4426)	
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

1						
2	Livestock, g/d	107.20 ±	107.90 ±	0.712	93.31 ±	92.77 ±
3		45.41	45.78		39.11	39.59
4						0.695

5 246 ¹ Values are mean ± SD. A two-sided $P \leq 0.05$ was considered statistically significant.

6 247 *The association of rickets in childhood with type 2 diabetes in adulthood*

8
9 248 Binary logistic regression analysis showed that rickets in childhood was significantly associated
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11 249 with an increased risk of type 2 diabetes in adult males (OR = 1.420, 95% CI = 1.017, 1.983; p
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13 value = 0.040), after adjusting for age, BMI, WC, exercise, smoking, alcohol consumption,
14 250 education level, family history of diabetes, and dietary intakes of energy, vegetable, fruit and
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16 251 livestock. However, there was no significant association of rickets in childhood with type 2 diabetes
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19 252 in adult females (Table-7). Result of the bootstrap test was consistent with the result of the binary
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21
22 253 logistic regression analysis (p value = 0.041) (Table-8).

26
27 **Table-7. Binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood**
28 **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

33 ¹Model 1: Adjusted for age, BMI, WC, education, smoking, drinking, energy, exercise, family history of diabetes.

34 Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

37
38 255 **Table-8. The bootstrap test of binary logistic regression analysis of the association of rickets on the risk of**
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40 256 **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

45 ¹Model 1: Adjusted for age, BMI, WC, education, smoking, drinking, energy, exercise, family history of diabetes.

46 Model 2: Adjusted for the consumption of vegetable, fruit and livestock based on model 1.

48 257 **Discussion**

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51 258 This study examined the association of vitamin D status in childhood with glucose metabolism in
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53 259 the north of China. The results showed that, in children, only 10.7% participants with vitamin D
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56 260 sufficient, there was a negative association between serum 25(OH)D₃ concentration and fasting
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58 261 insulin and HOMA-IR, and children with lower 25(OH)D₃ concentration were likely to develop IR.

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3 262 In adults, childhood rickets was associated with an increased risk of type 2 diabetes in adulthood.
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5 263 Vitamin D deficiency in children is widespread in worldwide. Regardless of the economic level, the
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8 264 vitamin D deficiency rate was very high in children from different countries^{9-12,28,29}. Vitamin D can
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11 265 be obtained from sunshine and foods, such as meat, eggs, and milk. It was generally thought that
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13 266 exposure of skin to ultraviolet rays in the sunshine was the main source of body obtain vitamin D³⁰.
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16 267 Therefore, the status of serum vitamin D can be influenced by several factors, such as skin tone, the
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18 268 latitude of residence, season, or use of sunscreen products³¹. Sunshine in areas with high latitude
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21 269 was insufficient for skin to synthesize vitamin D; a previous study showed that residents living in
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24 270 areas above 37°N were insufficiently synthesizing vitamin D in winter³². However, in relatively
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26 271 low latitude areas of China, rates of children's suboptimal vitamin D levels (< 30 ng/mL) was
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29 272 approximately 50%, thus vitamin D deficiency was also able to be observed³³. In our study,
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31 273 approximately 53% of children participants had vitamin D deficiency, which was similar to the
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34 274 recent reports on vitamin D nutritional status of children. Therefore, we should pay more attention
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36 275 to the health problems caused by vitamin D deficiency.
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39 276 In addition to skeletal health, the association between vitamin D and glucose metabolism in children
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42 277 and adults has also received wide concerned. In children, vitamin D deficiency was connected to IR
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44 278 and impaired fasting glucose^{13,34,35}. However, there were a lack of children studies on the
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47 279 association between vitamin D deficiency and glucose metabolism in the northern area of China.
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50 280 Participants in this study were recruited from Harbin, a typical city in northern China. A negative
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52 281 correlation between serum vitamin D concentration and fasting insulin, HOMA-IR, and IR were
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55 282 found in this study of children; these results were consistent with previous reports. In adults,
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57 283 previous research suggested that low vitamin D status was associated with IR, impaired glucose
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3 284 tolerance, decreased insulin sensitivity, and reduction of insulin secretion^{18,36-38}. Observational
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5 285 studies showed that patients with type 2 diabetes had lower level of vitamin D than healthy people;
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7
8 286 there was a negative correlation between vitamin D and HOMA-IR, and HbA1c levels decreased
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11 287 after vitamin D supplementation^{39,40}. Additionally, prospective studies had indicated that vitamin D
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13 288 deficiency might increase the risk of type 2 diabetes^{41,42-44}. According to recent years' studies, IR in
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16 289 childhood was considered as a risk factor for type 2 diabetes in adulthood⁴⁵. Accordingly, we
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18 290 speculate vitamin D deficiency in childhood may increase the risk of type 2 diabetes in adulthood
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21 291 by influence IR in childhood. However, this deduction needs a long-term cohort study spanning
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24 292 decades from childhood to adulthood, so there have been no reports of this.

25
26 293 Nutritional rickets is the most common type in rickets; it can be caused by deficiencies of vitamin
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29 294 D, calcium, or phosphate, causing primarily to a widening and delay of mineralization of growth
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31 295 plates in bones^{46,47}. The clinical signs of rickets include square head, delay in tooth development,
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34 296 rachitic chest, bow legs or X-shaped legs. Rickets needs to be diagnosed in combination with
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37 297 vitamin D level and clinical signs, and vitamin D deficiency alone cannot be diagnosed as rickets,
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39 298 but children who had serum 25(OH)D₃ level under 10.90 ng/mL were likely to have rickets⁴⁸. A
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42 299 retrospective survey was conducted on the prevalence of rickets in our adult study, rickets was
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45 300 determined by whether participants had a diagnosis of disease, had self-reported disease, or had
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47 301 symptoms. In adult population of this study, the prevalence of rickets in males and females was
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50 302 6.3% and 7.9%, respectively. An earlier study reported that the prevalence of Chinese infants with
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52 303 rickets in 1980s was approximately 18%⁴⁹, it was higher than our study. This difference may be
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55 304 due to the birth year of our participants being approximately 20 years earlier than those in that
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57 305 study, when China was experiencing societal hardships. Poverty and poor health conditions may
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3 306 lead to the lack of awareness of disease. Furthermore, the information on rickets was retrospective,
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5 307 since some participants were uncertain whether they had rickets in childhood, which may contribute
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8 308 to the low prevalence of rickets. In our adult study, participants who had rickets were defined as
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10 309 having vitamin D deficiency in childhood. The association between rickets and type 2 diabetes in
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13 310 adults was analyzed to explore the effect of childhood vitamin D deficiency on type 2 diabetes in
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16 311 adulthood. The results showed that males who had rickets in childhood had a higher risk of type 2
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18 312 diabetes in adulthood, but this result was not observed in females. However, the reason for this
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21 313 phenomenon is still unclear. In addition, a study from Finland found that individuals with high
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24 314 levels of vitamin D in childhood and adolescence had a significant lower risk of type 2 diabetes in
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26 315 adulthood compared with those who had lower level of vitamin D ²³. These findings collectively
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29 316 suggest that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in
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31 317 adulthood, which needs to be further be explored in more cohort studies and intervention studies.
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34 318 Some scholars have explored the pathogenesis through which vitamin D deficiency might induce IR
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36 319 in children. The results of some studies shown that, vitamin D levels were inversely related to
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39 320 oxidative stress and inflammation, the increase of reactive oxygen species (ROS) and formation of
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42 321 cytokines such as interleukin-6 played major roles in IR ⁵⁰⁻⁵². In addition, the findings of an obese,
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44 322 African-American adolescent study showed that low vitamin D levels was correlated with low
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47 323 adiponectin levels, which was associated with IR in children and adolescents ⁵³⁻⁵⁶. These results
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50 324 support the finding that vitamin D deficiency in childhood increases the risk of type 2 diabetes in
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52 325 adulthood. At the same time, the results of lab studies also support such pathogenesis: there was a
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55 326 close connection between vitamin D and β -cell function. By regulating cytokines to impact β -cell
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57 327 survival, vitamin D receptor and 1-hydroxylase in β -cell played a role in regulating pancreatic β -cell
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3 328 function, insulin secretion, IR and systemic inflammation ^{31,57,58}. Furthermore, the mechanism of
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5 329 vitamin D decreased IR might relate to the inhibition of vitamin D on inflammation and activation
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8 330 on insulin receptor ^{59,60}. In a term of epigenetics, results showed that vitamin D can also affect the
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11 331 occurrence of type 2 diabetes by regulating the expression of methyltransferase to prevent
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13 332 hypermethylation of diabetes-related genes ⁶¹. Above all of these might be the pathogenesis of
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15
16 333 vitamin D deficiency in childhood increased the risk of type 2 diabetes in adulthood.

17
18 334 An advantage of our study was the combination of children and adult participants. First, the
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20
21 335 negative association between vitamin D deficiency and IR was observed in children, which was a
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23
24 336 risk factor for type 2 diabetes in adults. Second, the association between rickets in childhood and
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26 337 type 2 diabetes in adulthood was explored, as well. The findings of two age groups were combined
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29 338 to analyze whether vitamin D deficiency in childhood has an early and long-term effect on glucose
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31 339 metabolism in adulthood. These findings can provide a theoretical basis for the early prevention and
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34 340 treatment of type 2 diabetes. However, there were also some limitations in our study. First, there
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36
37 341 was no significant difference in calcium or (and) vitamin D supplements between 4 quartile groups
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39 342 in the children's baseline data, which may be because this question was not limited to vitamin D
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41
42 343 supplements. Calcium supplements contain less vitamin D, and children taking calcium
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44 344 supplements alone were unlikely to have the same effect as those who took vitamin D supplements
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47 345 alone. Secondly, using ELISA kits to detect serum 25(OH)D₃ concentration is not the optimal
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49
50 346 method and may affect 25(OH)D₃ concentration results. Thirdly, in adult study baseline data,
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52 347 participants with type 2 diabetes had a higher proportion of regular exercise and less consumption
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55 348 of fruits than those without type 2 diabetes. This might be due to the participants', with type 2
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57 349 diabetes, choice of a healthier lifestyle after got the diagnosis of disease. In addition, there might be
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3 350 a recall bias in the adult retrospective study. The definition of vitamin D deficiency in childhood
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5 351 was rickets, which might have led to some participants, who did not have rickets but vitamin D
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8 352 deficiency, to be classified as non-deficient. Furthermore, rickets caused by vitamin D deficiency is
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10
11 353 the main type of rickets, but other types of rickets have not been excluded. Therefore, long-term
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13 354 design and cohort studies with stricter vitamin D nutritional status monitoring are needed to further
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16 355 verify our results.

17 18 356 **Conclusions**

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21 357 In summary, vitamin D deficiency in childhood was associated with IR and might increase the risk
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24 358 of type 2 diabetes in adult males. Early prevention strategies should be undertaken in children to
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26 359 control the rapid increase in type 2 diabetes worldwide, and management of vitamin D deficiency is
27
28
29 360 probably an effective method.

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33
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35 36 363 **Authors' contributions**

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39 364 Lixin Na and Changhao Sun designed the study and acquired funding. Junyi Liu and Lixin Na
40
41
42 365 wrote the paper. Liqun Fu and Jingyi Zhang prepared the original draft. Junyi Liu, Shanshan Jin and
43
44 366 Yubing Jia collected the data. Junyi Liu and Liqun Fu analysed and interpreted data. All authors
45
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47 367 read and approved the final manuscript.

48 49 368 **Compliance with Ethics Guidelines**

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51
52 369 The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of
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54
55 370 Harbin Medical University, and written informed consent was provided by all participants. The
56
57 371 methods in this study were in accordance the approved guidelines.
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3 372 Investigation and Intervention of Vitamin D Deficiency in Children: ChiCTR1800020294; Harbin
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5 373 Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases: ChiCTR-ECH-
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10
11 375 **Competing interests**

12
13 376 None of the authors has any potential conflict of interest associated with this research.

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19
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21 379 81872614).

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23 380 **Data sharing statement**

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25 381 The datasets used and/or analysed during the current study are available from the corresponding
26
27
28 382 author on reasonable request.

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516 Figure 1 - The follow chart of the children study.

517 Figure 2 - The follow chart of the adult study.

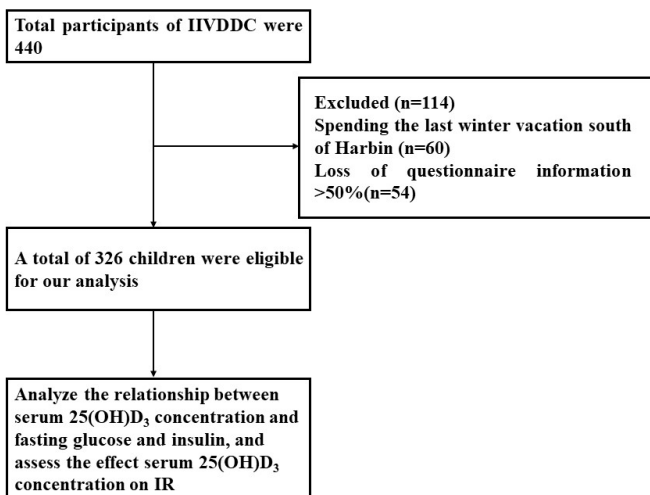


Figure 1. The follow chart of children study

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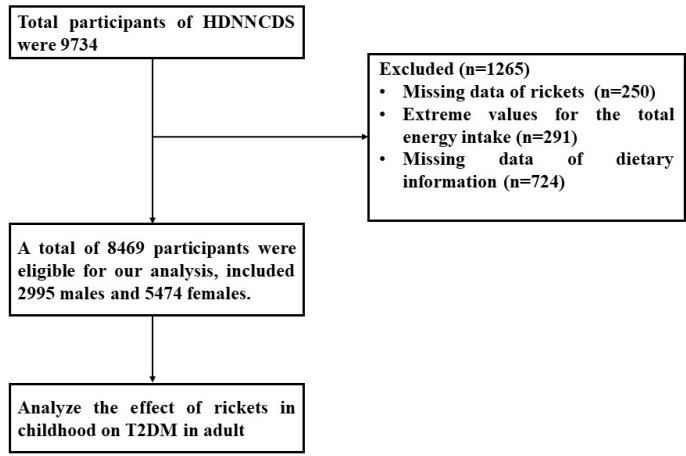


Figure 2. The follow chart of adult study

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 study and a retrospective study.

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3 1 **Vitamin D status in children and its association with glucose metabolism in northern China: A**
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5 2 **combination of a cross-sectional study and a retrospective study.**
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57 22 **Word count: 5,242**
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2
3 **Abstract**

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5 24 **Objectives:** The aim of this study was to explore the vitamin D status of children in northern China
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8 25 and the association between vitamin D and glucose metabolism.

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10 26 **Design:** Cross-sectional study was conducted among child participants and retrospective study
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13 27 designs was conducted among adult participants.

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15 28 **Setting and participants:** Both studies were recruited from Harbin, 326 children were included in
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18 29 children study, 8,469 adults were included in adult study.

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21 30 **Primary and secondary outcome measures:** Physical examination, lifestyle and dietary habit data
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23 31 were recorded in all the participants. Serum insulin, glucose, 25(OH)D₃ concentrations in children
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26 32 and serum glucose and lipids levels in adults were measured. In adults, rickets history was also
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29 33 investigated, which was used to define vitamin D deficiency in childhood. The associations were
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31 34 tested by linear regression and binary logistic regression.

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34 35 **Result:** In the children study, only 10.7% of participants were vitamin D sufficient (≥ 30 ng/mL).
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36 36 Inverse correlations between serum 25(OH)D₃ concentration and fasting insulin and HOMA-IR were
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39 37 found, and children with lower serum 25(OH)D₃ concentrations were likely to have IR (OR: 0.955,
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42 38 95% CI: 0.917, 0.995, *p* value: 0.027). In an adult study, rickets in childhood increased the risk of
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45 39 type 2 diabetes in male participants (OR = 1.414, 95% CI = 1.013, 1.972; *p* value = 0.042), but this
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47 40 result was not observed in females.

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49 41 **Conclusion:** Our findings suggest that vitamin D deficiency is widespread in northern China. Vitamin
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52 42 D deficiency in childhood was associated with IR and increased the risk of type 2 diabetes in male
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55 43 adults.

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57 44 **Keywords:** vitamin D deficiency, children, insulin resistance, type 2 diabetes
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45 **Strengths and limitations of this study:**

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

59 **Introduction**

60 According to IDF statistics, there are 450 million adults with type 2 diabetes worldwide, accounting
61 for approximately 90% of diabetes mellitus cases. Type 2 diabetes has become a global public health
62 problem^{1,2}. Some recent population studies have found that risk factors such as obesity, impaired
63 glucose tolerance, or insulin resistance (IR) in childhood, may also increase the risk of type 2 diabetes
64 in adults³⁻⁶. These results suggested that controlling risk factors in childhood is an early prevention
65 strategy to reduce the prevalence of type 2 diabetes in adulthood.

66 Vitamin D is a fat-soluble vitamin with several physiological functions, one of the most important of

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3 67 which is its effect on skeletal health⁷. At present, vitamin D deficiency is still a serious public problem
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5 68 worldwide, particularly in children⁸. The prevalence of vitamin D deficiency was approximately 50%
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8 69 in both developing and developed countries⁹⁻¹². Serum 25(OH)D₃ is less affected by body regulation
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11 70 and is often used to evaluate vitamin D levels. It is usually considered that serum 25(OH)D₃ < 10
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13 71 ng/mL indicates severe deficiency, 10-20 ng/mL indicates deficiency, 20-29 ng/mL indicates
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15 72 insufficiency, and ≥30 ng/mL indicates sufficiency¹³. Vitamin D deficiency in children is associated
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18 73 with many skeletal diseases, and one of the typical diseases is rickets. The clinical signs of rickets
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21 74 include skeletal deformity, restlessness, motor retardation and bone pain¹⁴.
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24 75 Recently, the association between vitamin D deficiency and extra-skeletal health has been of great
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26 76 concern, such as its association with glucose metabolism, obesity, respiratory tract infection and
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29 77 atopic dermatitis, among which the association with glucose metabolism has been of particular
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31 78 concern¹⁵⁻¹⁷. A population study of children and adolescents in Mexico found that low vitamin D
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34 79 levels were associated with IR; when the concentration of serum vitamin D increased, the possibility
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37 80 of presenting IR decreased¹⁸. Jared P Reis et al. reported that adolescents in the lowest quartile of
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39 81 vitamin D (<15 ng/mL) are more likely to have hyperglycemia compared with those in the highest
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42 82 quartile (>26 ng/mL)¹⁹. Furthermore, randomized controlled trials showed that vitamin D
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44 83 supplementation could increase insulin sensitivity and decrease IR and fasting glucose concentrations
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47 84 in obese children²⁰⁻²². Since IR in children is a risk factor for type 2 diabetes, we hypothesized that
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50 85 vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting
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52 86 insulin sensitivity. A recent 31-year follow-up prospective study in 3- to 18-year-old young Finns
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55 87 found that high vitamin D levels in childhood could reduce the incidence of type 2 diabetes in
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57 88 adulthood²³. This study further supported our hypothesis, but current research is very limited.
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3 89 Harbin is a typical northeast city of China, with a latitude between 44°04'N and 46°40'N, and is a
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5 90 relatively high-latitude area. The winters in Harbin are long, and the sunlight is relatively insufficient
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8 91 year-round, which makes the region's residents vulnerable to vitamin D deficiency. In our study, we
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10 92 first described the vitamin D nutritional status in children and explored the association between
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13 93 vitamin D deficiency and IR from the Investigation and Intervention of Vitamin D Deficiency in
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15 94 Children (IIVDDC). Then, the association between rickets in children and the risk of type 2 diabetes
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18 95 in adults was analyzed using data from the Harbin Cohort Study on Diet, Nutrition and Chronic Non-
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21 96 communicable Diseases (HDNNCDS) in a retrospective study. The aim of this study was to provide
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24 97 a theoretical basis for the early prevention and control of adult type 2 diabetes development in children.

25 26 98 **Materials and Methods**

27 28 29 99 *Design*

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31 100 Cross-sectional study was conducted among child participants and retrospective study designs was
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34 101 conducted among adult participants.

35 36 102 *Study population*

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39 103 The child participants were from the baseline survey of IIVDDC from March to May 2019. They
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42 104 were recruited from 4 kindergartens in Nangang District of Harbin by convenient sampling method,
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44 105 including 2 public kindergartens and 2 private kindergartens based on the consideration of different
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47 106 economic levels. The sample size required of children was determined by Events Per Variable
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49 107 criterion (EPV) ≥ 10 , the minimum sample size was calculated to be 90 participants. The parents and
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52 108 teachers of children were invited to informational meetings at which the study and its procedures
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55 109 were explained to them. A total of 440 children aged 3-7 years who had lived in Harbin for the past
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57 110 3 years were eligible as participants. The exclusion criteria included spending the last winter vacation
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3 111 in the lower latitude areas of Harbin (n=60), who's information of questionnaire losing > 50% (n=54).
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5 112 A total of 326 children were included, and informed written consent was obtained from all custody
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8 113 holders (**Figure 1**).
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11 114 The adult participants were from the baseline of the HDNNCDS ²⁴. Seven urban administrative
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13 115 regions of Harbin were covered in HDNNCDS. According to their financial situation, each region
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16 116 was divided into 3 strata and a total of 42 communities were randomly selected from each stratum in
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18 117 each administrative region by employing a stratified multistage random cluster sampling design.
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21 118 Residents were eligible to participate in the study if they: 1) were between 20 and 74 years old, 2)
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24 119 have been living in Harbin for at least two years, 3) were without cancer or type 1 diabetes mellitus.
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26 120 A total of 9,734 people aged 20-74 years completed the in-person baseline survey in 2010 - 2012.
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29 121 The sample size required in our analysis was calculated by $N = \frac{Z_{1-\alpha/2}^2(1-P)}{\epsilon^2p}$, $Z_{1-\alpha/2} = 1.96$, $p = 12.8\%$
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31 122 ²⁵, $\epsilon = 10\%$. By calculating $N = 2,617$, design efficiency value was 2, the final calculated minimal
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34 123 sample size was 5,234. Rickets in childhood were investigated in the survey, and participants who
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37 124 had definite answers were included in our study. In the present study, we excluded participants who
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39 125 reported uncertainty about information on rickets in childhood (n=250), who reported extreme values
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42 126 for total energy intake (<500 kcal/d or >4500 kcal, n=291), and who had missing dietary information
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44 127 data (n=724). Finally, a total of 8,469 participants were eligible for our analysis, including 2,995
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47 128 males and 5474 females (**Figure 2**). Written informed consent was provided by all participants.
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50 129 The study protocols of IIVDDC and HDNNCDS were approved by the Ethics Committee of
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52 130 Harbin Medical University, and written informed consent was provided by all participants. The
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55 131 methods in the study were in accordance with the approved guidelines (The registered ethical
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57 132 number: ChiCTR1800020294 for IIVDDC, ChiCTR-ECH-12002721 for HDNNCDS).
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3 133 *Data collection by the questionnaire*
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5 134 Detailed in-person interviews were administered by trained personnel using a structured
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8 135 questionnaire to collect information on demographic characteristics, lifestyle, and dietary intake.
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10 136 In the IIVDDC, the questionnaire was completed by parents and teachers in kindergartens, together.
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13 137 The demographic characteristics of the children included age and gender. Outdoor physical activity
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15 138 in the past 6 months was investigated, children who had more than 60 minutes of daily activity or
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18 139 more than 3 days of weekly activity were considered as high exercise frequency, otherwise they were
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21 140 considered as low exercise frequency. Children who took calcium or vitamin D orally or
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23 141 intravenously in the past 6 months were considered as supplementation with calcium and (or) vitamin
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26 142 D. Dietary information was collected by using a food-frequency questionnaire (FFQ), and a total of
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28 143 48 food items were included in the questionnaire, which covered most of the foods in the recipes of
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31 144 the kindergartens included in our study. For each food item, parents and teachers of participants were
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33 145 asked how frequently participants consumed over the preceding year, followed by a question on the
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36 146 amount consumed in lians (a unit of weight equal to 50 g) or mL (for liquid food item) per unit of
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39 147 time. The consumption frequency was transformed to obtain mean consumption a day. Nutrient
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41 148 intakes for each food item consumed were calculated by multiplying the nutrient content listed in the
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44 149 China Food Composition²⁶. Before dietary surveys, 60 participants from the IIVDDC were recruited
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47 150 and asked to complete two FFQs (FFQ1 and FFQ2) and a 3-day dietary record to validate the
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49 151 reliability of the FFQs. After adjusting for energy intake, major nutritional factors (staple food,
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52 152 poultry, fish, vegetable, fruit, and milk products), which were assessed by the two FFQs and by the
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54 153 FFQ2 and 3-day dietary records, correlated well. In IIVDDC, Cronbach's α coefficient of major
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57 154 nutritional factors between FFQ1 and FFQ2 were 0.67-0.72 and major nutritional factors between
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3 155 FFQ1 and 3-day dietary record were 0.62-0.76. Seventeen factors were extracted, and the cumulative
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5 156 variance contribution rate was 0.649, which suggested the good construct validity.
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8 157 In the HDNNCDS, the demographic characteristics mainly included age, gender, and educational
9
10 158 level. Lifestyle referred to smoking, alcohol consumption, and physical activity. Current smokers
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13 159 were defined as those who have smoked at least 100 cigarettes lifetime and smoke every day or some
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15 160 days now. Participants who consumed more than 100ml of white wine, highland barley wine, rice
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17
18 161 wine, grape wine per day or more than 250ml beer per day were considered to have a drinking habit.
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21 162 And regular exercise was defined as any kind of recreational or sport physical activity other than
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23 163 walking for work or life performed at least 30 minutes for three or more days per week. Family history
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26 164 of diabetes was also collected. In the FFQ for adults, a total of 103 food items were included in the
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28
29 165 questionnaire, which covered most of the commonly consumed foods in urban Harbin. The method
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31 166 to validate the reliability of the FFQs in the HDNNCDS was the same as that in the IIVDDC. The
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34 167 correlation coefficients of major nutritional factors between FFQ1 and FFQ2 were 0.61–0.70 and
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36 168 major nutritional factors between FFQ1 and 3-day dietary record were 0.61–0.69, respectively²⁴.
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39 169 Fifteen factors were extracted, and the cumulative variance contribution rate was 0.832, which
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41
42 170 suggested the good construct validity. Participants were asked to recall the history of rickets in
43
44 171 childhood by specific questions in the questionnaire. The questions included whether they had been
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46
47 172 diagnosed of rickets at the hospital, had the signs of rickets, such as square head, delay in tooth
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50 173 development, rachitic chest, bow legs or X-shaped legs. The answer options included definitely yes,
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52 174 definitely no and uncertain. Participants who chose one or more “definitely yes” were considered to
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54
55 175 have rickets in childhood. Participants who reported “uncertain” were excluded from our analysis.
56

57 176 *Anthropometric measurement*

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3 177 Anthropometric measurements, including height, weight and waist circumference (WC), were taken
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5 178 by well-trained examiners, with participants wearing light, thin clothing, and no shoes. Body weight,
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8 179 height and waist circumference were measured to the nearest 0.1 kg, 0.1 cm, and 0.1 cm, respectively.
9
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11 180 BMI was calculated as weight (kg) divided by the square of the height in meters (m²). Children's sex-
12
13 181 and age-adjusted z-scores for body mass index (zBMI) were calculated as the calculated value of
14
15
16 182 child BMI minus the median BMI of children of the same age and sex, and then divided by the
17
18 183 standard deviation of BMI for children of the same age and sex with the use of WHO Anthro Plus
19
20
21 184 software version 1.0.4²⁷.

23 185 *Biochemical assessment*

25
26 186 Fasting (more than 10 h) blood samples were collected in children in the IIVDDC. Fasting glucose
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28
29 187 was determined by an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). 25(OH)D₃ was
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31 188 measured using ELISA kits (Mlbio, Shanghai, China). Serum insulin was measured using the
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33
34 189 immunofluorescence method (Tosoh automated enzyme immunoassay analyzer AIA-2000ST).
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37 190 Fasting and postprandial (2 hours after drinking 75 grams glucose-containing water) blood samples
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39 191 were taken from all adult participants in the HDNNCDS. Fasting glucose and 2-h postprandial plasma
40
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42 192 glucose, blood lipids, including triglycerides, total cholesterol (TC), low-density lipoprotein
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44 193 cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), were measured using an
45
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47 194 automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured in the same
48
49
50 195 way as children.

52 196 *Definition of variables*

54 197 *Exposures*

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56 198 In the IIVDDC, serum 25(OH)D₃ < 10 ng/mL was considered severe deficiency, 10-20 ng/mL was
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59 199 considered deficiency, 20-29 ng/mL was considered insufficiency, and ≥30 ng/mL was considered
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2 200 sufficiency¹³. In the HDNNCDS, Vitamin D deficiency in childhood was defined as a diagnosis of
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4 201 rickets at the hospital, self-reported rickets, or signs of skeletal deformity during childhood.

6
7 202 *Outcomes*

8
9 203 In the IIVDDC, HOMA-IR was calculated from fasting insulin and glucose using the following
10
11 204 formula: fasting insulin concentration (mmol/L) × fasting glucose concentration (mIU/L)/22.5.
12
13 205 Children in the highest quartile of HOMA-IR were defined as IR²⁸, the cutoff value of IR was HOMA-
14
15 206 IR ≥ 4.58 in our study. In the HDNNCDS, type 2 diabetes was defined as fasting glucose ≥ 7.0 mmol/L
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17 and/or 2 h glucose ≥ 11.1 mmol/L and/or self-report of type 2 diabetes and/or use of hypoglycemic
18 207
19
20 208 medicine.

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23 209 *Potential confounders*

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25 210 In the IIVDDC, age, gender, zBMI, intake of energy, vegetable, fruit and livestock, exercise
26
27 211 frequency, calcium or (and) vitamin D supplements, were included in the analysis as potential
28
29 confounders of IR. In the HDNNCDS, age, education, BMI, WC, smoking, drinking, exercise, intake
30 212
31 of energy, vegetable, fruit and livestock, and family history were included as potential confounders
32 213
33 of type 2 diabetes.
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37 215 *Statistical analysis*

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40 216 SPSS v22.0 (Beijing Stats Data Co. Ltd, Beijing China) was used to analyze the data, and a two-sided
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42 217 p value < 0.05 was considered statistically significant. Values are mean ± SD and n (%) per group for
43
44 all other variables. ANOVA and chi-square test were used to compare the differences in the
45 218
46 continuous variables and categorical variables between the groups. The linear regression analysis was
47
48 219 used to analyze the association between serum 25(OH)D₃ concentration and fasting glucose, insulin
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50 220 and HOMA-IR in children, expressed as unstandardized β value and Standardized β value. Binary
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52 221 logistic regression analysis was used to analyze the association between serum 25(OH)D₃
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54 222 concentration and IR in children, adjust for age, gender, zBMI, exercise frequency, intake of energy,
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3 224 vegetable, fruit and livestock, calcium or (and) vitamin D supplements, expressed as OR value and
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5 225 95% CI. The data from children were finally analyzed and presented in general as no gender
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7
8 226 difference was observed after stratified by sex. Binary logistic regression analysis was used to analyze
9
10
11 227 the association between rickets in childhood with type 2 diabetes in adulthood stratified by sex,
12
13 228 adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit
14
15
16 229 and livestock, and family history. Bootstrap test of binary logistic regression analysis was used as
17
18 230 sensitivity analysis in order to confirm the risk of rickets on diabetes in adulthood male.

21 231 **Patient and public involvement**

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23 232 participants were not involved in the development of research questions, nor the outcome
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25
26 233 measures/the design of the study. Also, they were not involved in the recruitment to or conduct of the
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28
29 234 study. In our study, the participants are informed about their blood parameters, and the results of other
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31 235 examinations are gradually shared with them by text message or phone call. The overall findings and
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33
34 236 benefits of the study will be disseminated through public media.

36 237 **Results**

39 238 *Results from IIVDDC*

42 239 *Basic information and diet characteristics of children across HOMA-IR quartiles*

44 240 A total of 326 children were included, 53% were boys and 47% were girls, aged 3 y-7 y, the average
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47 241 age is 5.24 ± 1.32 y. There were 21 (6.4%) children with severe vitamin D deficiency, 163 (50%)
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50 242 children with deficiency, 107 (32.8%) children with insufficiency, and only 35 (10.7%) children with
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52 243 sufficiency.

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55 244 Children were grouped by HOMA-IR quartiles. The basic characteristics are summarized in **Table-**
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57 245 **1**. Serum 25(OH)D₃ concentration in four quartiles from lowest to highest were 20.82 ± 6.96 ng/mL,
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3 246 20.96 ± 9.07 ng/mL, 19.94 ± 8.09 ng/mL, and 18.19 ± 6.37 ng/mL. Children in higher quartile group
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5 247 had lower proportion of higher sport frequency. There were no significant differences for age, gender,
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8 248 zBMI score, or the supplementation of calcium or (and) vitamin D in recent half year among different
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11 249 quartile groups. The characteristics across quartiles were consistent in boys and girls, and the data
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13 250 were not shown.

15 251 **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)	<i>p</i> 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)	

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

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39 252 Furthermore, a significant difference in energy intake among quartiles of HOMA-IR was observed,
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42 253 with a slightly higher level in quartile 2 than that in other quartiles (**Table-2**). There were no
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44 254 differences in fruit, vegetable, and livestock intakes among different quartiles of HOMA-IR. The
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46
47 255 above analysis results were consistent in boys and girls, and the data were not shown.

49 256 **Table-2. Characteristics of diet in different HOMA-IR quartiles group.**

Variable	Q1	Q2	Q3	Q4	<i>p</i> 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

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

257 *The association between serum 25(OH)D₃ and fasting glucose, insulin and HOMA-IR*

258 Serum 25(OH)D₃ concentration were negatively correlated with fasting insulin and HOMA-IR after
 259 adjusting for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock,
 260 calcium or (and) vitamin D supplements (fasting insulin: unstandardized β coefficient = -0.178,
 261 standardized β coefficient = -0.194, p value = 0.001; HOMA-IR: unstandardized β coefficient = -
 262 0.032, standardized β coefficient = -0.161, p value = 0.005) (**Table-3**). However, the significant
 263 association between serum 25(OH)D₃ concentration and fasting glucose had not been observed. There
 264 was no difference in above analysis results between boys and girls, data were not shown.

265 **Table-3. Linear regression analysis of the association between serum 25(OH)D₃ concentration and fasting**
 266 **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

¹Model 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.

267 *The association of serum 25(OH)D₃ concentration with IR*

268 Children in the highest quartile of HOMA-IR were defined as IR, the cut-off point of HOMA-IR was
 269 4.59. As shown in **Table-4**, after adjusting for age, gender, zBMI, exercise frequency, intake of
 270 energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements, children with low
 271 serum 25(OH)D₃ concentration were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, p value:
 272 0.027). This association was consistent in boys and girls, and the data were not shown.

273 **Table-4. Binary logistic regression analysis of the association between serum 25(OH)D₃ 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

¹Model 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	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)	<i>p</i> Value	T2D (n = 1048)	Non-T2D (n = 4426)	<i>p</i> 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

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2 291 ¹ Values are mean \pm SD. A two-sided *p* Value < 0.05 was considered statistically significant.

3
4 292 *The association of rickets in childhood with type 2 diabetes in adulthood*

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6 293 Binary logistic regression analysis showed that rickets in childhood was significantly associated with
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9 294 an increased risk of type 2 diabetes in adult males (OR = 1.420, 95% CI = 1.017, 1.983; *p* value =
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12 295 0.040), after adjusting for sex, adjusted for age, education, BMI, WC, smoking, drinking, exercise,
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14 296 intake of energy, vegetable, fruit and livestock, and family history. However, there was no significant
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17 297 association of rickets in childhood with type 2 diabetes in adult females (**Table-7**). Result of the
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20 298 bootstrap test was consistent with the result of the binary logistic regression analysis (*p* value = 0.041)
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22 299 (**Table-8**).

23
24 **Table-7. Binary logistic regression analysis of the association of rickets on the risk of diabetes in adulthood**
25 **with different sex.**

Variable		Model 1 ¹		Model 2	
		OR (95% CI)	<i>p</i> Value	OR (95% CI)	<i>p</i> 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

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

34
35 300 **Table-8. The bootstrap test of binary logistic regression analysis of the association of rickets on the risk of**
36 **diabetes in adulthood male.**

Variable	Model 1 ¹		Model 2	
	β (95% CI)	<i>p</i> Value	β (95% CI)	<i>p</i> Value
Rickets	0.346 (0.004, 0.673)	0.039	0.351 (0.002, 0.680)	0.041

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

44
45 302 **Discussion**

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48 303 This study examined the association of vitamin D status in childhood with glucose metabolism in the
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51 304 north of China. The results showed that, in children, only 10.7% participants with vitamin D sufficient,
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53
54 305 there was a negative association between serum 25(OH)D₃ concentration and fasting insulin and
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56 306 HOMA-IR, and children with lower 25(OH)D₃ concentration were likely to develop IR. In adults,
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59 307 childhood rickets was associated with an increased risk of type 2 diabetes in adulthood.

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3 308 Vitamin D deficiency in children is widespread in worldwide. Regardless of the economic level, the
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5 309 vitamin D deficiency rate was very high in children from different countries^{9-12,29,30}. Vitamin D can
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8 310 be obtained from sunshine and foods, such as meat, eggs, and milk. It was generally thought that
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10 311 exposure of skin to ultraviolet rays in the sunshine was the main source of body obtain vitamin D³¹.
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13 312 Therefore, the status of serum vitamin D can be influenced by several factors, such as skin tone, the
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16 313 latitude of residence, season, or use of sunscreen products³². Sunshine in areas with high latitude was
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18 314 insufficient for skin to synthesize vitamin D; a previous study showed that residents living in areas
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21 315 above 37°N were insufficiently synthesizing vitamin D in winter³³. In relatively low latitude areas
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23 316 of China, 50% of preschooler children had sufficient vitamin D nutritional status (≥ 30 ng/mL),³⁴. In
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26 317 our study, only 10.7% of children had sufficient vitamin D nutritional status, which was significantly
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29 318 lower than children at low latitudes, vitamin D insufficiency was even more serious. Therefore, we
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31 319 should pay more attention to the health problems caused by vitamin D deficiency and insufficiency
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34 320 at high latitude.

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36 321 In addition to skeletal health, the association between vitamin D and glucose metabolism in children
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39 322 and adults has also obtained wide concerned. In children, vitamin D deficiency was connected to IR
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42 323 and impaired fasting glucose^{13,35,36}. However, there were a lack of children studies on the association
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44 324 between vitamin D deficiency and IR in the northern area of China. A negative correlation between
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47 325 serum vitamin D concentration and fasting insulin, HOMA-IR, and IR were found in this study of
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50 326 children. In adults, previous research suggested that low vitamin D status was associated with IR,
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52 327 impaired glucose tolerance, decreased insulin sensitivity, and reduction of insulin secretion^{18,37-39}.
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55 328 Observational study showed that patients with type 2 diabetes had lower level of vitamin D than
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57 329 healthy people⁴⁰. An intervention studies have shown that HbA1c levels decreased after vitamin D
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3 330 supplementation⁴¹. Additionally, prospective studies had indicated that vitamin D deficiency in adults
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5 331 might increase the risk of type 2 diabetes ^{42,43-45}. According to recent years' studies, IR in childhood
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8 332 was considered as a risk factor for type 2 diabetes in adulthood ⁴⁶. Therefore, we speculate vitamin D
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11 333 deficiency in childhood may increase the risk of type 2 diabetes in adulthood by influence IR in
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13 334 childhood. However, this deduction needs a long-term cohort study spanning decades from childhood
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16 335 to adulthood, there have been no reports about this so far.

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18 336 Nutritional rickets is the most common type in rickets, caused by deficiencies of vitamin D, calcium,
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21 337 or phosphate ^{47,48}. The clinical signs of rickets include square head, delay in tooth development,
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24 338 rachitic chest, bow legs or X-shaped legs. Rickets needs to be diagnosed in combination with vitamin
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26 339 D level and clinical signs, and vitamin D deficiency alone cannot be diagnosed as rickets, but children
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29 340 who had serum 25(OH)D₃ level under 10.90 ng/mL were likely to have rickets ⁴⁹. A retrospective
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31 341 survey was conducted on the prevalence of rickets in our adult study, rickets was determined by
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34 342 whether participants had a diagnosis of disease, or had symptoms. In adult population of this study,
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37 343 the prevalence of rickets in males and females was 6.3% and 7.9%, respectively. An earlier study
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39 344 reported that the prevalence of Chinese infants with rickets in 1980s was approximately 18% ⁵⁰, it
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42 345 was higher than our study. This difference may be due to the birth year of our participants being
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45 346 approximately 20 years earlier than those in that study, when China was experiencing societal
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47 347 hardships. Poverty and poor health conditions may lead to the lack of awareness of disease.
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50 348 Furthermore, the information on rickets was retrospective, since some participants were uncertain
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52 349 whether they had rickets in childhood, which may contribute to the low prevalence of rickets. In our
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55 350 adult study, participants who had rickets were defined as having vitamin D deficiency in childhood.
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57 351 The association between rickets and type 2 diabetes in adults was analyzed to explore the effect of
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3 352 childhood vitamin D deficiency on type 2 diabetes in adulthood. The results showed that males who
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5 353 had rickets in childhood had a higher risk of type 2 diabetes in adulthood, but this result was not
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8 354 observed in females. However, the reason for this phenomenon is still unclear. In addition, a study
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11 355 from Finland found that individuals with high levels of vitamin D in childhood and adolescence had
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13 356 a significant lower risk of type 2 diabetes in adulthood compared with those who had lower level of
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16 357 vitamin D ²³. These findings collectively suggest that vitamin D deficiency in childhood might
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18 358 increase the risk of type 2 diabetes in adulthood, which needs to be further be explored in more cohort
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21 359 studies and intervention studies.

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23 360 Some scholars have explored the pathogenesis through which vitamin D deficiency might induce IR
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26 361 in children. The results of some studies shown that, vitamin D levels were inversely related to
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29 362 oxidative stress and inflammation, the increase of reactive oxygen species (ROS) and formation of
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31 363 cytokines such as interleukin-6 played major roles in IR ⁵¹⁻⁵³. In addition, the findings of an obese,
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34 364 African-American adolescent study showed that low vitamin D levels was correlated with low
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37 365 adiponectin levels, which was associated with IR in children and adolescents ⁵⁴⁻⁵⁷. These results
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39 366 support the finding that vitamin D deficiency in childhood increases the risk of type 2 diabetes in
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42 367 adulthood. At the same time, the results of lab studies also support such pathogenesis: there was a
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44 368 close connection between vitamin D and β -cell function. By regulating cytokines to impact β -cell
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47 369 survival, vitamin D receptor and 1-hydroxylase in β -cell played a role in regulating pancreatic β -cell
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50 370 function, insulin secretion, IR and systemic inflammation ^{32,58,59}. Furthermore, the mechanism of
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52 371 vitamin D decreased IR might relate to the inhibition of vitamin D on inflammation and activation on
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55 372 insulin receptor ^{60,61}. In a term of epigenetics, results showed that vitamin D can also affect the
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57 373 occurrence of type 2 diabetes by regulating the expression of methyltransferase to prevent
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3 374 hypermethylation of diabetes-related genes⁶². Above all of these might be the pathogenesis of vitamin
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5 375 D deficiency in childhood increased the risk of type 2 diabetes in adulthood.
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8 376 An advantage of our study was the inclusion of both children study analysis and adult study analysis.
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11 377 The children and adults were both from Harbin, a northeast city of China, was chosen with high
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13 378 prevalence of vitamin D deficiency due to its geographic location. Basically, they had similar
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16 379 geographic and climatic environment and dietary habits. In children, we observed the serum
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18 380 25(OH)D₃ concentration was reversely associated with HOMA-IR. We hypothesized that vitamin D
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21 381 deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting insulin
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24 382 sensitivity, based on theoretical speculation. We defined rickets in adult study as a vitamin D
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26 383 deficiency condition in childhood to simulate the vitamin D deficiency in children study. The growth
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29 384 environment related to vitamin D status in the adults was similar with the children. The adults might
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31 385 simulate children's future growth trajectories to some extent, in terms of the association of childhood
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34 386 vitamin D deficiency with adult type 2 diabetes. The results of the two populations provided mutual
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37 387 support. However, there were also some limitations in our study. First, there was possible selection
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39 388 bias and we did not do the external validity of the sample due to a limitation of external sample.
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42 389 Second, the sample size of children was small and it was a cross-sectional study. We used ORs to
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44 390 interpret the association of vitamin D deficiency with IR and diabetes, which may overstate effect
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47 391 sizes. Third, the rickets information was obtained from self-report, the recall bias was not avoidable
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50 392 although we excluded the uncertain participants. The proportion of self-report rickets was 6.3% and
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52 393 7.8% for male and female, respectively. We assumed that there some missing reports of rickets in
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55 394 their recalling based on the medical and nutritional condition, and lack of health awareness in China
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57 395 40 years ago. In addition, the definition of vitamin D deficiency in childhood was rickets, which
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3 396 might have led to some participants, who did not have rickets but vitamin D deficiency, to be
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5 397 classified as non-deficient. Therefore, long-term design and cohort studies with stricter vitamin D
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8 398 nutritional status monitoring are needed to further verify our results.
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10 399 **Conclusions**

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13 400 In summary, vitamin D deficiency in childhood was associated with IR and might increase the risk
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16 401 of type 2 diabetes in adult males. Early prevention strategies should be undertaken in children to
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18 402 control the rapid increase in type 2 diabetes worldwide, and management of vitamin D deficiency is
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20
21 403 probably an effective method.
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23 404 **Acknowledgements**

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25
26 405 We are grateful to all participants who took part in this study and the research team.
27

28 406 **Authors' contributions**

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31 407 Lixin Na and Changhao Sun designed the study and acquired funding. Junyi Liu and Lixin Na wrote
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33
34 408 the paper. Liqun Fu and Jingyi Zhang prepared the original draft. Junyi Liu, Shanshan Jin and Yubing
35
36 409 Jia collected the data. Junyi Liu and Liqun Fu analysed and interpreted data. All authors read and
37
38
39 410 approved the final manuscript.
40

41 411 **Compliance with Ethics Guidelines**

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44 412 The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of Harbin
45
46
47 413 Medical University, and written informed consent was provided by all participants. The methods
48
49
50 414 in this study were in accordance the approved guidelines.
51

52 415 Investigation and Intervention of Vitamin D Deficiency in Children: ChiCTR1800020294; Harbin
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55 416 Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases: ChiCTR-ECH-12002721.
56

57 417 **Competing interests**

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3 418 None of the authors has any potential conflict of interest associated with this research.

4
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7
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9
10 421 **Data sharing statement**

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12 422 The datasets used and/or analysed during the current study are available from the corresponding
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14
15 423 author on reasonable request.

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18 424 **Reference**

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556 Figure 1 - The follow chart of the children study.

557 Figure 2 - The follow chart of the adult study.

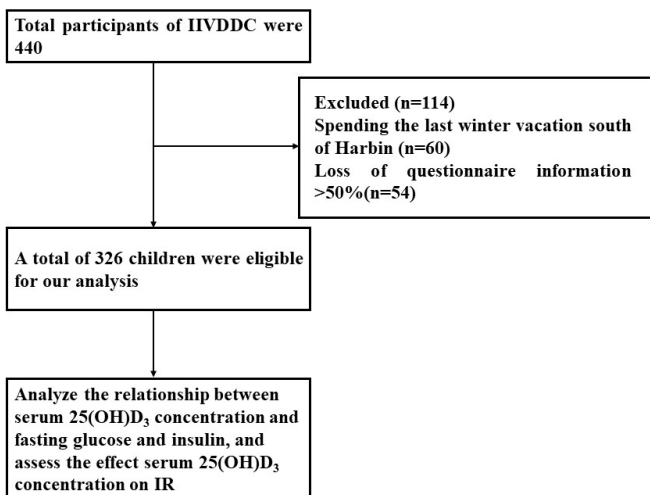


Figure 1. The follow chart of children study

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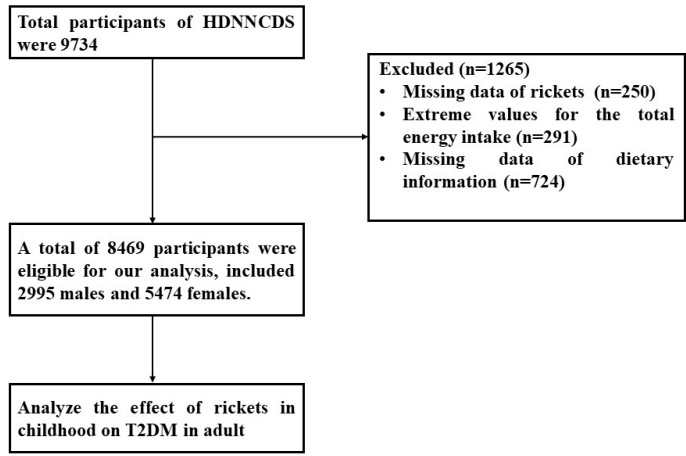


Figure 2. The follow chart of adult study

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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3 1 **Vitamin D status in children and its association with glucose metabolism in northern China: A**
4
5 2 **combination of a cross-sectional and retrospective study.**

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57 22 **Word count: 5,227**
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2
3 **Abstract**

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5 24 **Objectives:** This study aimed to explore the vitamin D status of children in northern China and the
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8 25 association between vitamin D and glucose metabolism.

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10 26 **Design:** Cross-sectional study was conducted among child participants and retrospective study
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13 27 designs was conducted among adult participants.

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15 28 **Setting and participants:** Both studies were recruited from Harbin, 326 children were included in
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18 29 children study, 8,469 adults were included in adult study.

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21 30 **Primary and secondary outcome measures:** Physical examination, lifestyle and dietary habit data
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24 31 were recorded in all the participants. Serum insulin, glucose, 25(OH)D₃ concentrations in children
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26 32 and serum glucose and lipids levels in adults were measured. Rickets history was also investigated in
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29 33 adults, which was used to define vitamin D deficiency in childhood. The associations were tested by
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31 34 linear regression and binary logistic regression.

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34 35 **Result:** In the children study, only 10.7% of participants were vitamin D sufficient (≥ 30 ng/mL).
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36 36 Inverse correlations between serum 25(OH)D₃ concentration and fasting insulin and HOMA-IR were
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39 37 found, and children with lower serum 25(OH)D₃ concentrations were likely to have IR (OR: 0.955,
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42 38 95% CI: 0.917, 0.995, *p* value: 0.027). In an adult study, rickets in childhood increased the risk of
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44 39 type 2 diabetes in male participants (OR = 1.414, 95% CI = 1.013, 1.972; *p* value = 0.042), but this
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47 40 result was not observed in females.

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49 41 **Conclusion:** Our findings suggest that vitamin D deficiency is widespread in northern China. Vitamin
50
51
52 42 D deficiency in childhood was associated with IR and increased the risk of type 2 diabetes in male
53
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55 43 adults.

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57 44 **Keywords:** vitamin D deficiency, children, insulin resistance, type 2 diabetes
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45 **Strengths and limitations of this study:**

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

59 **Introduction**

60 According to IDF statistics, there are 450 million adults with type 2 diabetes worldwide, accounting
61 for approximately 90% of diabetes mellitus cases. Type 2 diabetes has become a global public health
62 problem^{1,2}. Some recent population studies have found that risk factors such as obesity, impaired
63 glucose tolerance, or insulin resistance (IR) in childhood, may also increase the risk of type 2 diabetes
64 in adults³⁻⁶. These results suggested that controlling risk factors in childhood is an early prevention
65 strategy to reduce the prevalence of type 2 diabetes in adulthood.

66 Vitamin D is a fat-soluble vitamin with several physiological functions, one of the most important of

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3 67 which is its effect on skeletal health⁷. At present, vitamin D deficiency is still a serious public problem
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5 68 worldwide, particularly in children⁸. The prevalence of vitamin D deficiency was approximately 50%
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8 69 in both developing and developed countries⁹⁻¹². Serum 25(OH)D₃ is less affected by body regulation
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11 70 and is often used to evaluate vitamin D levels. It is usually considered that serum 25(OH)D₃ < 10
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13 71 ng/mL indicates severe deficiency, 10-20 ng/mL indicates deficiency, 20-29 ng/mL indicates
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15 72 insufficiency, and ≥30 ng/mL indicates sufficiency¹³. Vitamin D deficiency in children is associated
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18 73 with many skeletal diseases, and one of the typical diseases is rickets. The clinical signs of rickets
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21 74 include skeletal deformity, restlessness, motor retardation and bone pain¹⁴.
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24 75 Recently, the association between vitamin D deficiency and extra-skeletal health has been of great
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26 76 concern, such as its association with glucose metabolism, obesity, respiratory tract infection and
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29 77 atopic dermatitis, among which the association with glucose metabolism has been of particular
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31 78 concern¹⁵⁻¹⁷. A population study of children and adolescents in Mexico found that low vitamin D
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34 79 levels were associated with IR; when the concentration of serum vitamin D increased, the possibility
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37 80 of presenting IR decreased¹⁸. Jared P Reis et al. reported that adolescents in the lowest quartile of
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39 81 vitamin D (<15 ng/mL) are more likely to have hyperglycemia compared with those in the highest
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42 82 quartile (>26 ng/mL)¹⁹. Furthermore, randomized controlled trials showed that vitamin D
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44 83 supplementation could increase insulin sensitivity and decrease IR and fasting glucose concentrations
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47 84 in obese children²⁰⁻²². Since IR in children is a risk factor for type 2 diabetes, we hypothesized that
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50 85 vitamin D deficiency in childhood might increase the risk of type 2 diabetes in adulthood by affecting
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52 86 insulin sensitivity. A recent 31-year follow-up prospective study in 3- to 18-year-old young Finns
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55 87 found that high vitamin D levels in childhood could reduce the incidence of type 2 diabetes in
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57 88 adulthood²³. This study further supported our hypothesis, but current research is very limited.
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3 89 Harbin is a typical northeast city of China, with a latitude between 44°04'N and 46°40'N, and is a
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5 90 relatively high-latitude area. The winters in Harbin are long, and the sunlight is relatively insufficient
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8 91 year-round, which makes the region's residents vulnerable to vitamin D deficiency. In our study, we
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10 92 first described the vitamin D nutritional status in children and explored the association between
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13 93 vitamin D deficiency and IR from the Investigation and Intervention of Vitamin D Deficiency in
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15 94 Children (IIVDDC). Then, the association between rickets in children and the risk of type 2 diabetes
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18 95 in adults was analyzed using data from the Harbin Cohort Study on Diet, Nutrition and Chronic Non-
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21 96 communicable Diseases (HDNNCDS) in a retrospective study. This study aimed to provide a
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24 97 theoretical basis for the early prevention and control of adult type 2 diabetes development in children.

25 26 98 **Materials and Methods**

27 28 29 99 *Design*

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31 100 A cross-sectional study was conducted among child participants and retrospective study design was
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34 101 conducted among adult participants.

35 36 102 *Study population*

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39 103 The child participants were from the baseline survey of IIVDDC from March to May 2019. They
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42 104 were recruited from 4 kindergartens in Nangang District of Harbin by convenient sampling method,
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44 105 including 2 public kindergartens and 2 private kindergartens based on the consideration of different
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47 106 economic levels. The sample size required of children was determined by Events Per Variable
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49 107 criterion (EPV) ≥ 10 , the minimum sample size was calculated to be 90 participants. The parents and
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52 108 teachers of children were invited to informational meetings at which the study and its procedures
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55 109 were explained to them. A total of 440 children aged 3-7 years who had lived in Harbin for the past
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57 110 3 years were eligible as participants. The exclusion criteria included spending the last winter vacation
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3 111 in the lower latitude areas of Harbin (n=60), who's information of questionnaire losing > 50% (n=54).
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5 112 A total of 326 children were included, and informed written consent was obtained from all custody
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8 113 holders (**Figure 1**).
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10 114 The adult participants were from the baseline of the HDNNCDS ²⁴. Seven urban administrative
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13 115 regions of Harbin were covered in HDNNCDS. According to their financial situation, each region
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16 116 was divided into 3 strata and a total of 42 communities were randomly selected from each stratum in
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18 117 each administrative region by employing a stratified multistage random cluster sampling design.
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21 118 Residents were eligible to participate in the study if they: 1) were between 20 and 74 years old, 2)
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24 119 have been living in Harbin for at least two years, 3) were without cancer or type 1 diabetes mellitus.
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26 120 A total of 9,734 people aged 20-74 completed the in-person baseline survey in 2010 - 2012. The
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29 121 sample size required in our analysis was calculated by $N = \frac{Z_{1-\alpha/2}^2(1-P)}{\epsilon^2p}$, $Z_{1-\alpha/2} = 1.96$, $p = 12.8\%$ ²⁵, ϵ
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31 122 = 10%. By calculating N= 2,617, design efficiency value was 2, the final calculated minimal sample
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34 123 size was 5,234. Rickets in childhood were investigated in the survey, and participants who had
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37 124 definite answers were included in our study. In the present study, we excluded participants who
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39 125 reported uncertainty about information on rickets in childhood (n=250), who reported extreme values
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42 126 for total energy intake (<500 kcal/d or >4500 kcal, n=291), and who had missing dietary information
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44 127 data (n=724). Finally, a total of 8,469 participants were eligible for our analysis, including 2,995
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46
47 128 males and 5474 females (**Figure 2**). Written informed consent was provided by all participants.
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50 129 The study protocols of IIVDDC and HDNNCDS were approved by the Ethics Committee of
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52 130 Harbin Medical University, and written informed consent was provided by all participants. The
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55 131 methods in the study were in accordance with the approved guidelines (The registered ethical
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57 132 number: ChiCTR1800020294 for IIVDDC, ChiCTR-ECH-12002721 for HDNNCDS).
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3 133 *Data collection by the questionnaire*
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5 134 Detailed in-person interviews were administered by trained personnel using a structured
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8 135 questionnaire to collect information on demographic characteristics, lifestyle, and dietary intake.
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10 136 In the IIVDDC, the questionnaire was completed by parents and teachers in kindergartens, together.
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13 137 The demographic characteristics of the children included age and gender. Outdoor physical activity
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15 138 in the past 6 months was investigated, children who had more than 60 minutes of daily activity or
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18 139 more than 3 days of weekly activity were considered as high exercise frequency, otherwise they were
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21 140 considered as low exercise frequency. Children who took calcium or vitamin D orally or
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23 141 intravenously in the past 6 months were considered as supplementation with calcium and (or) vitamin
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26 142 D. Dietary information was collected by using a food-frequency questionnaire (FFQ), and a total of
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28 143 48 food items were included in the questionnaire, which covered most of the foods in the recipes of
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31 144 the kindergartens included in our study. For each food item, parents and teachers of participants were
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33 145 asked how frequently participants consumed over the preceding year, followed by a question on the
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36 146 amount consumed in lians (a unit of weight equal to 50 g) or mL (for liquid food item) per unit of
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39 147 time. The consumption frequency was transformed to obtain mean consumption a day. Nutrient
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41 148 intakes for each food item consumed were calculated by multiplying the nutrient content listed in the
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44 149 China Food Composition ²⁶. Before dietary surveys, 60 participants from the IIVDDC were recruited
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47 150 and asked to complete two FFQs (FFQ1 and FFQ2) and a 3-day dietary record to validate the
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49 151 reliability of the FFQs. Major nutritional factors (staple food, poultry, fish, vegetable, fruit, and milk
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52 152 products) were assessed by the two FFQs and the FFQ2 and 3-day dietary records, and they correlated
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54 153 well after adjusting for energy intake. In IIVDDC, Cronbach's α coefficient of major nutritional
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57 154 factors between FFQ1 and FFQ2 were 0.67-0.72 and major nutritional factors between FFQ1 and 3-
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3 155 day dietary record were 0.62-0.76. Seventeen factors were extracted, and the cumulative variance
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5 156 contribution rate was 0.649, which suggested the good construct validity.
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8 157 In the HDNNCDS, the demographic characteristics mainly included age, gender, and educational
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10 158 level. Lifestyle referred to smoking, alcohol consumption, and physical activity. Current smokers
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13 159 were defined as those who have smoked at least 100 cigarettes lifetime and smoke every day or some
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16 160 days now. Participants who consumed more than 100ml of white wine, highland barley wine, rice
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18 161 wine, grape wine per day or more than 250ml beer per day were considered to have a drinking habit.
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21 162 And regular exercise was defined as any kind of recreational or sport physical activity other than
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24 163 walking for work or life performed at least 30 minutes for three or more days per week. Family history
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26 164 of diabetes was also collected. In the FFQ for adults, a total of 103 food items were included in the
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29 165 questionnaire, which covered most of the commonly consumed foods in urban Harbin. The method
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31 166 to validate the reliability of the FFQs in the HDNNCDS was the same as that in the IIVDDC. The
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34 167 correlation coefficients of major nutritional factors between FFQ1 and FFQ2 were 0.61–0.70 and
35
36 168 major nutritional factors between FFQ1 and 3-day dietary record were 0.61–0.69, respectively²⁴.
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39 169 Fifteen factors were extracted, and the cumulative variance contribution rate was 0.832, which
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42 170 suggested the good construct validity. Participants were asked to recall the history of rickets in
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44 171 childhood by specific questions in the questionnaire. The questions included whether they had been
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47 172 diagnosed of rickets at the hospital, had the signs of rickets, such as square head, delay in tooth
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50 173 development, rachitic chest, bow legs or X-shaped legs. The answer options included definitely yes,
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52 174 definitely no and uncertain. Participants who chose one or more “definitely yes” were considered to
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55 175 have rickets in childhood. Participants who reported “uncertain” were excluded from our analysis.
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57 176 *Anthropometric measurement*
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3 177 Anthropometric measurements, including height, weight and waist circumference (WC), were taken
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5 178 by well-trained examiners, with participants wearing light, thin clothing, and no shoes. Body weight,
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8 179 height and waist circumference were measured to the nearest 0.1 kg, 0.1 cm, and 0.1 cm, respectively.
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11 180 BMI was calculated as weight (kg) divided by the square of the height in meters (m²). Children's sex-
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13 181 and age-adjusted z-scores for body mass index (zBMI) were calculated as the calculated value of
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16 182 child BMI minus the median BMI of children of the same age and sex, and then divided by the
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18 183 standard deviation of BMI for children of the same age and sex with the use of WHO Anthro Plus
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20
21 184 software version 1.0.4²⁷.

23 185 *Biochemical assessment*

26 186 Fasting (more than 10 h) blood samples were collected in children in the IIVDDC. Fasting glucose
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29 187 was determined by an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). 25(OH)D₃ was
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31 188 measured using ELISA kits (Mlbio, Shanghai, China). Serum insulin was measured using the
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34 189 immunofluorescence method (Tosoh automated enzyme immunoassay analyzer AIA-2000ST).
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37 190 Fasting and postprandial (2 hours after drinking 75 grams glucose-containing water) blood samples
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39 191 were taken from all adult participants in the HDNNCDS. Fasting glucose and 2-h postprandial plasma
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42 192 glucose, blood lipids, including triglycerides, total cholesterol (TC), low-density lipoprotein
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44 193 cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), were measured using an
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47 194 automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured in the same
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49
50 195 way as children.

52 196 *Definition of variables*

54 197 *Exposures*

56 198 In the IIVDDC, serum 25(OH)D₃ < 10 ng/mL was considered severe deficiency, 10-20 ng/mL was
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59 199 considered deficiency, 20-29 ng/mL was considered insufficiency, and ≥30 ng/mL was considered
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2 200 sufficiency¹³. In the HDNNCDS, Vitamin D deficiency in childhood was defined as a diagnosis of
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4 201 rickets at the hospital, self-reported rickets, or signs of skeletal deformity during childhood.

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7 202 *Outcomes*

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9 203 In the IIVDDC, HOMA-IR was calculated from fasting insulin and glucose using the following
10
11 204 formula: fasting insulin concentration (mmol/L) × fasting glucose concentration (mIU/L)/22.5.
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13 205 Children in the highest quartile of HOMA-IR were defined as IR²⁸, the cutoff value of IR was HOMA-
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15 206 IR ≥ 4.58 in our study. In the HDNNCDS, type 2 diabetes was defined as fasting glucose ≥ 7.0 mmol/L
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17 and/or 2 h glucose ≥ 11.1 mmol/L and/or self-report of type 2 diabetes and/or use of hypoglycemic
18 207
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20 208 medicine.

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23 209 *Potential confounders*

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25 210 In the IIVDDC, age, gender, zBMI, intake of energy, vegetable, fruit and livestock, exercise
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27 211 frequency, calcium or (and) vitamin D supplements, were included in the analysis as potential
28
29 confounders of IR. In the HDNNCDS, age, education, BMI, WC, smoking, drinking, exercise, intake
30 212
31 of energy, vegetable, fruit and livestock, and family history were included as potential confounders
32 213
33 of type 2 diabetes.
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37 215 *Statistical analysis*

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40 216 SPSS v22.0 (Beijing Stats Data Co. Ltd, Beijing China) was used to analyze the data, and a two-sided
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42 217 p value < 0.05 was considered statistically significant. Values are mean ± SD and n (%) per group for
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44 all other variables. ANOVA and chi-square test were used to compare the differences in the
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46 continuous variables and categorical variables between the groups. The linear regression analysis was
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48 219 used to analyze the association between serum 25(OH)D₃ concentration and fasting glucose, insulin
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50 220 and HOMA-IR in children, expressed as unstandardized β value and Standardized β value. Binary
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52 221 logistic regression analysis was used to analyze the association between serum 25(OH)D₃
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54 222 concentration and IR in children, adjust for age, gender, zBMI, exercise frequency, intake of energy,
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3 224 vegetable, fruit and livestock, calcium or (and) vitamin D supplements, expressed as OR value and
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5 225 95% CI. The data from children were finally analyzed and presented in general as no gender
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8 226 difference was observed after stratified by sex. Binary logistic regression analysis was used to analyze
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11 227 the association between rickets in childhood with type 2 diabetes in adulthood stratified by sex,
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13 228 adjusted for age, education, BMI, WC, smoking, drinking, exercise, intake of energy, vegetable, fruit
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16 229 and livestock, and family history. Bootstrap test of binary logistic regression analysis was used as
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18 230 sensitivity analysis in order to confirm the risk of rickets on diabetes in adulthood male.

21 231 **Patient and public involvement**

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23 232 Participants were not involved in developing research questions, nor the outcome measures/the
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26 233 study's design. Also, they were not involved in the recruitment to or conduct of the study. In our
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29 234 study, the participants are informed about their blood parameters, and the results of other
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31 235 examinations are gradually shared with them by text message or phone call. The overall findings and
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34 236 benefits of the study will be disseminated through public media.

36 237 **Results**

39 238 *Results from IIVDDC*

42 239 *Basic information and diet characteristics of children across HOMA-IR quartiles*

44 240 A total of 326 children were included, 53% were boys and 47% were girls, aged 3 y-7 y, the average
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46
47 241 age is 5.24 ± 1.32 y. There were 21 (6.4%) children with severe vitamin D deficiency, 163 (50%)
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49
50 242 children with deficiency, 107 (32.8%) children with insufficiency, and only 35 (10.7%) children with
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52 243 sufficiency.

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55 244 The basic characteristics of HOMA-IR quartiles grouped children are summarized in Table-1. Serum
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57 245 $25(\text{OH})\text{D}_3$ concentration in four quartiles from lowest to highest were 20.82 ± 6.96 ng/mL, $20.96 \pm$
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3 246 9.07 ng/mL, 19.94 ± 8.09 ng/mL, and 18.19 ± 6.37 ng/mL. Children in higher quartile group had
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5 247 lower proportion of higher sport frequency. There were no significant differences for age, gender,
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7
8 248 zBMI score, or the supplementation of calcium or (and) vitamin D in recent half year among different
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11 249 quartile groups. The characteristics across quartiles were consistent in boys and girls, and the data
12
13 250 were not shown.

15 251 **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)	<i>p</i> 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)	

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

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39 252 Furthermore, a significant difference in energy intake among quartiles of HOMA-IR was observed,
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42 253 with a slightly higher level in quartile 2 than that in other quartiles (**Table-2**). There were no
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44 254 differences in fruit, vegetable, and livestock intakes among different quartiles of HOMA-IR. The
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46
47 255 above analysis results were consistent in boys and girls, and the data were not shown.

49 256 **Table-2. Characteristics of diet in different HOMA-IR quartiles group.**

Variable	Q1	Q2	Q3	Q4	<i>p</i> 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

58 ¹ Values are mean \pm SD. A two-sided *p* Value < 0.05 was considered statistically significant.

257 *The association between serum 25(OH)D₃ and fasting glucose, insulin and HOMA-IR*

258 Serum 25(OH)D₃ concentration were negatively correlated with fasting insulin and HOMA-IR after
 259 adjusting for age, gender, zBMI, exercise frequency, intake of energy, vegetable, fruit and livestock,
 260 calcium or (and) vitamin D supplements (fasting insulin: unstandardized β coefficient = -0.178,
 261 standardized β coefficient = -0.194, p value = 0.001; HOMA-IR: unstandardized β coefficient = -
 262 0.032, standardized β coefficient = -0.161, p value = 0.005) (**Table-3**). However, the significant
 263 association between serum 25(OH)D₃ concentration and fasting glucose had not been observed. There
 264 was no difference in above analysis results between boys and girls, data were not shown.

265 **Table-3. Linear regression analysis of the association between serum 25(OH)D₃ concentration and fasting**
 266 **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

¹Model 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.

267 *The association of serum 25(OH)D₃ concentration with IR*

268 Children in the highest quartile of HOMA-IR were defined as IR, the cut-off point of HOMA-IR was
 269 4.59. As shown in **Table-4**, after adjusting for age, gender, zBMI, exercise frequency, intake of
 270 energy, vegetable, fruit and livestock, calcium or (and) vitamin D supplements, children with low
 271 serum 25(OH)D₃ concentration were likely to have IR (OR: 0.955, 95% CI: 0.917, 0.995, p value:
 272 0.027). This association was consistent in boys and girls, and the data were not shown.

273 **Table-4. Binary logistic regression analysis of the association between serum 25(OH)D₃ 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

¹Model 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	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)	<i>p</i> Value	T2D (n = 1048)	Non-T2D (n = 4426)	<i>p</i> 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.

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

292 Binary logistic regression analysis showed that rickets in childhood was significantly associated with
 293 an increased risk of type 2 diabetes in adult males (OR = 1.420, 95% CI = 1.017, 1.983; p value =
 294 0.040), after adjusting for sex, adjusted for age, education, BMI, WC, smoking, drinking, exercise,
 295 intake of energy, vegetable, fruit and livestock, and family history. However, there was no significant
 296 association of rickets in childhood with type 2 diabetes in adult females (**Table-7**). Result of the
 297 bootstrap test was consistent with the result of the binary logistic regression analysis (p value = 0.041)
 298 (**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

¹Model 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

¹Model 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.

301 **Discussion**

302 This study examined the association of vitamin D status in childhood with glucose metabolism in the
 303 north of China. The results showed that, in children, only 10.7% participants with vitamin D sufficient,
 304 there was a negative association between serum 25(OH)D₃ concentration and fasting insulin and
 305 HOMA-IR, and children with lower 25(OH)D₃ concentration were likely to develop IR. Childhood
 306 rickets were associated with an increased risk of type 2 diabetes in adulthood.

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307 Vitamin D deficiency in children is widespread in worldwide. Regardless of the economic level, the
308 vitamin D deficiency rate was very high in children from different countries^{9-12,29,30}. Vitamin D can
309 be obtained from sunshine and foods, such as meat, eggs, and milk. It was generally thought that
310 exposure of skin to ultraviolet rays in the sunshine was the main source of body obtain vitamin D³¹.
311 Therefore, the status of serum vitamin D can be influenced by several factors, such as skin tone, the
312 latitude of residence, season, or use of sunscreen products³². Sunshine in areas with high latitude was
313 insufficient for skin to synthesize vitamin D; a previous study showed that residents living in areas
314 above 37°N were insufficiently synthesizing vitamin D in winter³³. In relatively low latitude areas
315 of China, 50% of preschooler children had sufficient vitamin D nutritional status (≥ 30 ng/mL),³⁴. In
316 our study, only 10.7% of children had sufficient vitamin D nutritional status, which was significantly
317 lower than children at low latitudes, vitamin D insufficiency was even more serious. Therefore, we
318 should pay more attention to the health problems caused by vitamin D deficiency and insufficiency
319 at high latitude.

320 In addition to skeletal health, the association between vitamin D and glucose metabolism in children
321 and adults has also obtained wide concerned. In children, vitamin D deficiency was connected to IR
322 and impaired fasting glucose^{13,35,36}. However, there were a lack of children studies on the association
323 between vitamin D deficiency and IR in the northern area of China. This study found a negative
324 correlation between serum vitamin D concentration and fasting insulin, HOMA-IR, and IR. In adults,
325 previous research suggested that low vitamin D status was associated with IR, impaired glucose
326 tolerance, decreased insulin sensitivity, and reduction of insulin secretion^{18,37-39}. Observational study
327 showed that patients with type 2 diabetes had lower level of vitamin D than healthy people⁴⁰. An
328 intervention studies have shown that HbA1c levels decreased after vitamin D supplementation⁴¹.

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3 329 Additionally, prospective studies had indicated that vitamin D deficiency in adults might increase the
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5 330 risk of type 2 diabetes ^{42,43-45}. According to recent years' studies, IR in childhood was considered as
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8 331 a risk factor for type 2 diabetes in adulthood ⁴⁶. Therefore, we speculate vitamin D deficiency in
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10 332 childhood may increase the risk of type 2 diabetes in adulthood by influence IR in childhood.
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13 333 However, this deduction needs a long-term cohort study spanning decades from childhood to
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16 334 adulthood, there have been no reports about this so far.

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18 335 Nutritional rickets is the most common type in rickets, caused by deficiencies of vitamin D, calcium,
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21 336 or phosphate ^{47,48}. The clinical signs of rickets include square head, delay in tooth development,
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24 337 rachitic chest, bow legs or X-shaped legs. Rickets needs to be diagnosed in combination with vitamin
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26 338 D level and clinical signs, and vitamin D deficiency alone cannot be diagnosed as rickets, but children
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29 339 who had serum 25(OH)D₃ level under 10.90 ng/mL were likely to have rickets ⁴⁹. A retrospective
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31 340 survey was conducted on the prevalence of rickets in our adult study, rickets was determined by
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34 341 whether participants had a diagnosis of disease, or had symptoms. In adult population of this study,
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37 342 the prevalence of rickets in males and females was 6.3% and 7.9%, respectively. An earlier study
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39 343 reported that the prevalence of Chinese infants with rickets in 1980s was approximately 18% ⁵⁰, it
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42 344 was higher than our study. This difference may be due to the birth year of our participants being
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44 345 approximately 20 years earlier than those in that study, when China was experiencing societal
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47 346 hardships. Poverty and poor health conditions may lead to the lack of awareness of disease.
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50 347 Furthermore, the information on rickets was retrospective, since some participants were uncertain
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52 348 whether they had rickets in childhood, which may contribute to the low prevalence of rickets. In our
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55 349 adult study, participants who had rickets were defined as having vitamin D deficiency in childhood.

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57 350 The association between rickets and type 2 diabetes in adults was analyzed to explore the effect of
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3 351 childhood vitamin D deficiency on type 2 diabetes in adulthood. The results showed that males who
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5 352 had rickets in childhood had a higher risk of type 2 diabetes in adulthood, but this result was not
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8 353 observed in females. However, the reason for this phenomenon is still unclear. In addition, a study
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10 354 from Finland found that individuals with high levels of vitamin D in childhood and adolescence had
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13 355 a significant lower risk of type 2 diabetes in adulthood compared with those who had lower level of
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15 356 vitamin D ²³. These findings collectively suggest that vitamin D deficiency in childhood might
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18 357 increase the risk of type 2 diabetes in adulthood, which needs to be further be explored in more cohort
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21 358 studies and intervention studies.

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23 359 Some scholars have explored the pathogenesis through which vitamin D deficiency might induce IR
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26 360 in children. The results of some studies shown that, vitamin D levels were inversely related to
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29 361 oxidative stress and inflammation, the increase of reactive oxygen species (ROS) and formation of
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31 362 cytokines such as interleukin-6 played major roles in IR ⁵¹⁻⁵³. In addition, the findings of an obese,
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34 363 African-American adolescent study showed that low vitamin D levels was correlated with low
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36 364 adiponectin levels, which was associated with IR in children and adolescents ⁵⁴⁻⁵⁷. These results
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39 365 support the finding that vitamin D deficiency in childhood increases the risk of type 2 diabetes in
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42 366 adulthood. At the same time, the results of lab studies also support such pathogenesis: there was a
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44 367 close connection between vitamin D and β -cell function. By regulating cytokines to impact β -cell
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47 368 survival, vitamin D receptor and 1-hydroxylase in β -cell played a role in regulating pancreatic β -cell
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50 369 function, insulin secretion, IR and systemic inflammation ^{32,58,59}. Furthermore, the mechanism of
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52 370 vitamin D decreased IR might relate to the inhibition of vitamin D on inflammation and activation on
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55 371 insulin receptor ^{60,61}. In a term of epigenetics, results showed that vitamin D can also affect the
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57 372 occurrence of type 2 diabetes by regulating the expression of methyltransferase to prevent
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3 373 hypermethylation of diabetes-related genes⁶². Above all of these might be the pathogenesis of vitamin
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5 374 D deficiency in childhood increased the risk of type 2 diabetes in adulthood.
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8 375 An advantage of our study was the inclusion of both children study analysis and adult study analysis.
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10 376 The children and adults were both from Harbin, a northeast city of China, was chosen with high
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13 377 prevalence of vitamin D deficiency due to its geographic location. Basically, they had similar
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16 378 geographic and climatic environment and dietary habits. In children, we observed the serum
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18 379 25(OH)D₃ concentration was reversely associated with HOMA-IR. Based on theoretical speculation,
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21 380 we hypothesized that vitamin D deficiency in childhood might increase the risk of type 2 diabetes in
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24 381 adulthood by affecting insulin sensitivity. We defined rickets in adult study as a vitamin D deficiency
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26 382 condition in childhood to simulate the vitamin D deficiency in children study. The growth
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29 383 environment related to vitamin D status in the adults was similar with the children. The adults might
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31 384 simulate children's future growth trajectories to some extent, in terms of the association of childhood
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34 385 vitamin D deficiency with adult type 2 diabetes. The results of the two populations provided mutual
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37 386 support. However, there were also some limitations in our study. First, there was possible selection
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39 387 bias and we did not do the external validity of the sample due to a limitation of external sample.
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42 388 Second, the sample size of children was small and it was a cross-sectional study. We used ORs to
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44 389 interpret the association of vitamin D deficiency with IR and diabetes, which may overstate effect
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47 390 sizes. Third, the rickets information was obtained from self-report, the recall bias was not avoidable
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50 391 although we excluded the uncertain participants. The proportion of self-report rickets was 6.3% and
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52 392 7.8% for male and female, respectively. We assumed that there some missing reports of rickets in
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55 393 their recalling based on the medical and nutritional condition, and lack of health awareness in China
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57 394 40 years ago. In addition, the definition of vitamin D deficiency in childhood was rickets, which
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3 395 might have led to some participants, who did not have rickets but vitamin D deficiency, to be
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5 396 classified as non-deficient. Therefore, long-term design and cohort studies with stricter vitamin D
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8 397 nutritional status monitoring are needed to further verify our results.
9

10 398 **Conclusions**

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13 399 In summary, vitamin D deficiency in childhood was associated with IR and might increase the risk
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16 400 of type 2 diabetes in adult males. Early prevention strategies should be undertaken in children to
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18 401 control the rapid increase in type 2 diabetes worldwide, and management of vitamin D deficiency is
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21 402 probably an effective method.
22

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25
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27

28 405 **Authors' contributions**

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30
31 406 Lixin Na and Changhao Sun designed the study and acquired funding. Junyi Liu and Lixin Na wrote
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34 407 the paper. Liqun Fu and Jingyi Zhang prepared the original draft. Junyi Liu, Shanshan Jin and Yubing
35
36 408 Jia collected the data. Junyi Liu and Liqun Fu analysed and interpreted data. All authors read and
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39 409 approved the final manuscript.
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41 410 **Competing interests**

42
43
44 411 None of the authors has any potential conflict of interest associated with this research.
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48
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50

51 414 **Data sharing statement**

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54 415 The datasets used and/or analysed during the current study are available from the corresponding
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57 416 author on reasonable request.
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59 417 **Ethics Approval**

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3 418 The study protocol of IIVDDC and HDNNCDS was approved by the Ethics Committee of Harbin
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5 419 Medical University, and written informed consent was provided by all participants. The methods
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8 420 in this study were in accordance the approved guidelines.
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11 421 Investigation and Intervention of Vitamin D Deficiency in Children: ChiCTR1800020294; Harbin
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13 422 Cohort Study on Diet, Nutrition and Chronic Non-communicable Diseases: ChiCTR-ECH-12002721.
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555 Figure 1 - The follow chart of the children study.

556 Figure 2 - The follow chart of the adult study.

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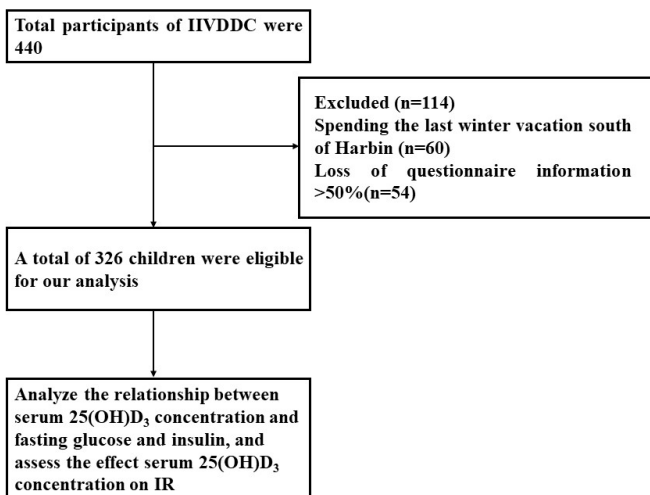


Figure 1. The follow chart of children study

338x190mm (96 x 96 DPI)

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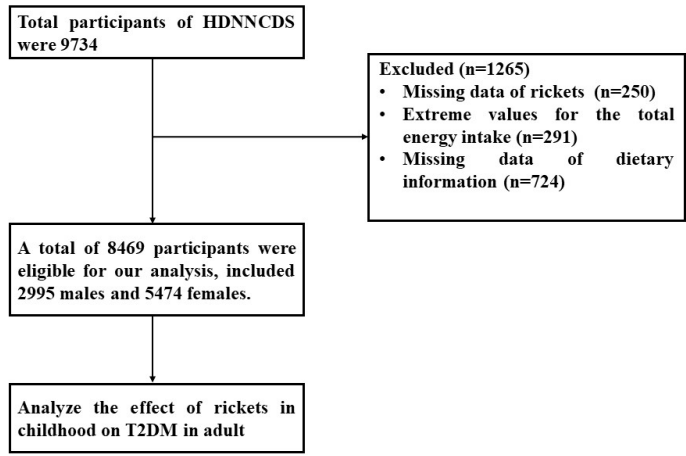


Figure 2. The follow chart of adult study

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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.