Vitamin D affects insulin sensitivity and β -cell function in obese non-diabetic youths

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Abstract

Objective: Vitamin D may potentially play a central role in glucose homeostasis and β -cell function (BCF), although studies are not consistent. Aim of our study was to test the hypotheses of a direct relationship between vitamin D, insulin sensitivity (IS) and BCF in overweight and obese non-diabetic children.

Design and methods: Cross-sectional study carried out at the Childhood Obesity Outpatient Clinic, University Hospital of Verona. One hundred twenty-two Caucasian overweight and obese children (age: 12.8 ± 0.2 years) were enrolled. Exclusion criteria: genetic or endocrine causes of obesity, chronic diseases or therapies. Patients underwent oral glucose tolerance test. HOMA-IR, Matsuda index and insulinogenic index were calculated. BCF was reconstructed by mathematical modeling and described by Derivative and Proportional Control. Total 25-hydroxyvitamin D and vitamin D-binding protein (VDBP) were measured. Two SNPs (rs4588 and rs7041) in the VDBP gene were studied, and bioavailable vitamin D (BVD) was calculated.

Results: Hypovitaminosis D was documented in 90% of patients. Forty-seven subjects were homozygous for both SNPs. Total vitamin D was positively correlated with Matsuda index ($P = 0.002$), VDBP ($P = 0.045$), and negatively with BMI SDS ($P = 0.043$), HOMA-IR ($P = 0.008$), HOMA-B ($P = 0.001$), IGI ($P = 0.007$), derivative control ($P = 0.036$) and proportional control ($P = 0.018$). Total vitamin D, adjusted for age, gender, BMI SDS, puberty and seasonality of vitamin D measurement, was a predictor of Matsuda index, HOMA-IR, HOMA-B, IGI, proportional control (all $P < 0.05$). BVD was positively correlated with total vitamin D ($P < 0.001$) and negatively with BMI SDS ($P = 0.041$).

Conclusions: Hypovitaminosis D negatively influences BCF and IS, suggesting that vitamin D levels might be implicated in glucose metabolism impairment in overweight and obese individuals.

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Introduction

Vitamin D plays a central role in calcium and phosphorus homeostasis and bone metabolism, but it might exert also extra-skeletal actions, affecting pancreatic β -cell function (BCF) and glucose homeostasis. Vitamin D circulating levels are mainly influenced by geographic location of

residency, season and ethnicity (1, 2). Hypovitaminosis D is widely diffuse worldwide and is highly prevalent in children and adolescents (1, 3, 4, 5, 6).

A higher incidence of low total vitamin D levels has been reported in obese compared to the general

population, in both adults and children (2, 7, 8, 9, 10, 11). In a large cohort of US children Ganji *et al.* reported a prevalence of vitamin D deficiency of 21% in normal-weight children, 29–34% in overweight and obese children and 49% in severely obese children (12). Several mechanisms have been considered to explain the lower concentration of vitamin D in the obese, including inadequate diet consumption, decreased sun exposure due also to sedentary lifestyle, 25-hydroxy-vitamin D (25-OHD) trapping in adipose tissue, although a combination of these factors is likely (10). Moreover, it has been suggested that, in spite of their low levels of total vitamin D, obese children would have bioavailable vitamin D (BVD) levels similar to those of normal-weight children, due to reduced concentration of vitamin D-binding protein (VDBP) (13).

Consistent evidence suggests that vitamin D plays a central role in glucose metabolism. Vitamin D promotes the release of insulin, both directly by vitamin D receptors localized in pancreatic β -cells, as well as indirectly, by influencing plasma concentration of calcium and, consequently, calcium-dependent release of insulin (14). Furthermore, 1,25-dihydroxy-vitamin D (1,25-OH₂D) promotes the transcription of insulin and its receptor genes, increasing its expression in liver, muscle and fat tissues and promotes the expression of glucose transporter GLUT4 in muscles and its translocation into adipocytes (15, 16, 17, 18). *In vivo* studies in animals documented that vitamin D deficiency causes an altered release of insulin in response to glucose (19). Furthermore, a protective effect of vitamin D on BCF, through a renin angiotensin system-suppressing action, has been documented in murine models (20). However, a causal effect relationship between hypovitaminosis D and glycemic control, IR or diabetes risk is not completely accepted; indeed, randomized controlled studies, carried out in human cohorts, demonstrated no effect of vitamin D supplementation or maintenance of adequate vitamin D levels in glycemic control and beta-cell function in T2D and suggested that the association between vitamin D concentration and T2D might not be causal (21, 22, 23, 24).

Moreover, notwithstanding a role of vitamin D supplementation in modulation of hepatic insulin resistance (IR) through the effect of vitamin D-regulated pathways on hepatic lipogenesis and gluconeogenesis has been suggested a relationship between IR and vitamin D concentrations is not clearly demonstrated (9, 13, 21, 25, 26).

The aim of our study was to test the hypotheses of a direct relationship between vitamin D, both total vitamin

D and bioavailable vitamin D (BVD) concentrations, and insulin sensitivity (IS) and BCF in a sample of non-diabetic, overweight/obese children and adolescents.

Subjects and methods

Subjects

Study population included 122, 12.8 ± 2.3 years old overweight and obese children and adolescents enrolled at the Pediatric Diabetes and Metabolic Disorders Unity, University Hospital, Verona (Italy). Inclusion criteria were Caucasian ethnicity, gender- and age-specific BMI cut-off for obesity reported in the WHO growth references (27). Exclusion criteria were genetic or endocrine causes of obesity, diabetes (American Diabetes Association criteria) (28), either pre-term or post-term birth, associated chronic diseases or chronic pharmacological therapies.

The protocol was approved by the Institutional Ethics Committee of Verona (Italy). Informed consent was obtained from children and their parents.

Physical examination

At recruitment, physical examination was performed according to standard procedures, as previously described (29). Weight was measured to the nearest 0.5 kg on standard physician's beam scales, with the child wearing only underwear and no shoes. Height was measured to the nearest 0.5 cm on a stadiometer without shoes, with the child's heels, buttocks, shoulders and head against the vertical wall with line of sight aligned horizontally. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. BMI values were standardized using age and sex-specific median, standard deviation (s.d.) and power of the Box–Cox transformation (least mean square method) based on WHO growth references (27). Waist circumference was measured to the nearest 0.5 cm while the subjects were standing, after gently exhaling, as the minimal circumference measurable on the horizontal plane between the lowest portion of the rib cage and the iliac crest (30). Waist-to-height ratio (WHtR), an index of body fat distribution, was calculated as previously described (31). Pubertal stage was assessed according to Tanner criteria (32). Subjects were categorized into pre-pubertal (Tanner stage 1), pubertal (Tanner stage 2–4) and post-pubertal (Tanner stage 5) categories. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded three times on the right arm in mmHg using a

manual sphygmomanometer; for analysis, the average of three blood pressure values was used (33).

Biochemical measurements

Within 15 days from recruitment visit, all patients underwent fasting blood tests for measuring plasma glucose, serum insulin concentration, glycated hemoglobin (HbA1c), lipid profile, liver enzymes and albumin. Plasma glucose was measured with glucose oxidase method and HbA1c by immunoturbidimetric assay (Accu-Chek Inform II and Cobas B101, Roche). Insulin and C-peptide levels were measured by enzyme-immunoassay (Mercodia AB, Sweden). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL-c) were measured by standard methods. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald's equation. Serum albumin levels were measured in a spectrophotometer at 620 nm through colorimetric method (Sclavo Diagnostics International, Italy). Seasonality of blood collection was recorded as summer/fall and spring/winter.

Furthermore, patients underwent a prolonged oral glucose tolerance test (modified-OGTT), performed with standard method (1.75 g/kg of body weight, up to a maximum of 75 g) according to the ADA guidelines (28), with sampling at times of -10, 0, +10, +20, +30, +45, +60, +90, +120, +150, +180 min for measurements of glucose, insulin and C-peptide. Blood samples were collected in SST II or EDTA tubes and centrifuged at 1300g for 10 min after collection; serum and plasma were stored at -80°C until analyzed. No subjects had diabetes according on ADA definition.

BCF was reconstructed by mathematical modeling, as described by Bonadonna *et al.* (34). By this method, BCF is described by two parameters: derivative (or dynamic) control, i.e. the response of the β -cell to the rate of glucose increase of 1 mmol/L per minute that lasts for 1 min (expression of 1st phase of insulin secretion) and proportional (or static) control, i.e. the stimulus-response curve linking of the β -cell to glucose concentration per se (expression of 2nd phase of insulin secretion).

OGTT-derived indices

The following OGTT-derived indices were calculated:

- Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as $(\text{insulin (mU/L)} \times \text{glucose$

$(\text{mg/dL}))/405$, and used as a fasting biomarker of IR (35).

- Homeostasis model assessment for BCF (HOMA-B) was performed as $((360 \times \text{insulin (mU/L)})/(\text{glucose (mg/dL)} - 63))$ and considered as a measure of BCF (36).
- Insulinogenic index (IGI), assessed as $(\text{insulin}_{30} (\text{mU/L}) - \text{insulin}_0 (\text{mU/L})) / (\text{glucose}_{30} (\text{mg/dL}) - \text{glucose}_0 (\text{mg/dL}))$, was selected as a biomarker of insulin bioavailability in response to oral glucose (37).
- Matsuda index: $10\,000 / ((\text{glucose}_0 (\text{mg/dL}) \times \text{insulin}_0 (\text{mU/L})) \times (\text{mean OGTT glucose concentration (mg/dL)}) \times (\text{mean OGTT insulin concentration (mU/L)}))^{1/2}$; it was used as a postprandial biomarker of IS (38).
- Mathematical modeling of glucose and C-peptide curves following the OGTT was performed and derivative and proportional controls of BCF were computed (39).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using salting-out procedures. Genotyping was carried out by a predesigned TaqMan probe (Applied Biosystem), according to the manufacturers' protocol, with a >99% positive call-rate. Polymorphism genotyping was performed using 7900 HT Real Time PCR (Applied Biosystem). We genotyped two common SNPs (rs4588 and rs7041) in the coding region of the *VDBP* gene.

Vitamin D measurements

Total vitamin D and parathyroid hormone (PTH) were measured through chemiluminescent method (IDS-iSYS Multi Discipline automated analyzer; IDS-iSYS, Immunodiagnostic System, Boldon, UK). Vitamin D deficiency, insufficiency and sufficiency were defined as a 25(OH)D levels <20, 20–29 and ≥ 30 ng/mL, respectively (40). Vitamin D-binding protein was measured by ELISA method (R&D Systems) (13, 41).

Forty-seven out of 122 subjects were in homozygotes for the two analyzed SNPs. A single genotype-specific binding affinity constant on the basis of the presence of a single *VDBP* variant according to the equation reported by Powe *et al.* was applied (41). In these subjects BVD-dependent concentration was calculated.

Statistical analysis

Patients' baseline characteristics are reported as mean $\pm$ s.d. Kolmogorov–Smirnov test was used to assess

normal distribution of variables. Skewed variables were transformed (natural log transformed or square root transformed, if and as needed) to correct for non-Gaussian distribution, unless deviations from the Gaussian distribution could not be corrected by transformation. Spearman correlation was applied to normal and skewed distributed variables. Student's *t*-test, Mann–Whitney or chi-squared test were used to detect the differences among patients stratified by gender or total vitamin D groups.

Multiple regression analyses, using HOMA-IR, HOMA-B, Matsuda index, IGI, derivative control or proportional control as dependent variables, were run. Two models were calculated: in the first, total vitamin D was considered as independent variable together with age, gender, pubertal stage, BMI SDS and season of vitamin D measurement, in the second one, total vitamin D was replaced by BVD. Covariates included in regression models were selected as potential confounding factors based on their biological plausibility.

A *P* value <0.05 was considered as a nominal statistical significance. Benjamini–Hochberg (BH) step-up procedure was applied for multiple test adjustment (42). All analyses were performed using SPSS v.22.0 (SPSS).

Results

A sample of 122 subjects (69 males, 53 females) with a mean age of 12.8 ± 2.3 years and a mean BMI SDS of 2.9 ± 0.8 was recruited. Anthropometric and metabolic characteristics of the study population are showed in Table 1. Males had higher waist circumference ($P=0.009$), WHtR ($P=0.004$), 2-h-plasma insulin ($P=0.039$), ALT ($P=0.009$), AST ($P=0.015$) and HOMA-B ($P=0.033$) than females. No other nominal significant differences for others variables between genders were found.

Vitamin D insufficiency (defined as total vitamin D 20–29 ng/mL) and deficiency (defined as total vitamin D <20 ng/mL) were found in 50% ($n=61$) and 40.2% ($n=49$) of children, respectively. No significant differences between genders were found.

Splitting subjects into two groups (group A: subjects with vitamin D deficiency; group B: subjects with normal vitamin D and vitamin D insufficiency), we found nominal significant differences between groups in BMI ($P=0.007$), PTH ($P=0.023$), HOMA-IR ($P=0.002$), HOMA-B ($P=0.008$), IGI ($P=0.041$), Matsuda index ($P=0.001$) and proportional control ($P=0.035$) (Table 2).

None of these differences stayed statistically significant after multiple test adjustment.

Genotyping for two common single-nucleotide polymorphisms in the coding region of the *VDBP* gene (rs4588 and rs7041), we detected 47 subjects homozygous for rs4588 and rs7041 *VDBP* polymorphisms: 25 homozygous for Gc1S/Gc1S alleles, 4 for Gc1F/Gc1F and 18 for Gc2/Gc2. Bioavailable vitamin D in this group of 47 subjects was 4.30 ± 0.31 ng/mL.

Correlation analysis

Results of the correlation analyses are reported in Table 3. Total vitamin D was positively correlated with BVD, also after multiple test adjustment ($r=0.68$, $P\text{-adj}=0.0009$), whereas was nominally correlated with Matsuda index ($r=0.28$, $P=0.002$), and negatively with BMI SDS ($r=-0.18$, $P=0.043$), HOMA-IR ($r=-0.24$, $P=0.008$), IGI ($r=-0.25$, $P=0.007$), derivative control ($r=-0.20$, $P=0.036$), proportional control ($r=-0.22$, $P=0.018$) and fasting plasma insulin.

Bioavailable vitamin D was positively correlated with Matsuda index ($r=0.25$, $P=0.026$) and negatively with BMI SDS ($r=-0.29$, $P=0.041$), IGI ($r=-0.38$, $P=0.007$), derivative control ($r=-0.29$, $P=0.05$), proportional control ($r=-0.31$, $P=0.036$) and plasma insulin at time 30' ($r=-0.43$, $P=0.026$) of the modified OGTT.

Vitamin D-binding protein did not significantly correlate with any other variable except BVD, that remained significant after multiple test adjustment ($r=-0.51$, $P\text{-adj}=0.023$), and total vitamin D ($r=0.17$, $P=0.045$).

Total vitamin D and BVD did not significantly correlate with waist circumference, WHtR, systolic and DBP, HbA1c, lipid profile and plasma glucose concentration during modified OGTT.

Regression analysis

Multivariate regression analysis (Table 4) showed that total vitamin D, independently from age, gender, BMI SDS, pubertal stage, season of vitamin D measurement, was positively associated with Matsuda index ($P=0.020$) and negatively with HOMA-IR ($P=0.038$), HOMA-B ($P=0.012$), IGI ($P=0.042$) and proportional control ($P=0.045$).

Bioavailable vitamin D, independently from age, gender, BMI SDS, pubertal stage, season of vitamin D measurement, was inversely associated with IGI ($P=0.034$) (Table 5). None of these associations remained statistically significant after multiple test adjustment.

Table 1 Physical and biochemical features of the total sample and comparison analysis according to genders. Data are expressed as means $\pm$ s.d. or number and percentage, n (%). Differences between gender were tested by Student's t -test for normally distributed variables and the Mann-Whitney test for skewed distributed variables or using χ^2 test for categorical variables. Nominal P values were reported.

Variables	Total	Male	Female	P value
n	122	69	53	
Age (years)	12.8 $\pm$ 2.3	13.0 $\pm$ 2.4	12.6 $\pm$ 1.9	0.375
Weight (kg)	83.3 $\pm$ 25.2	85.1 $\pm$ 28.3	80.9 $\pm$ 20.6	0.370
BMI (kg/m ²)	31.9 $\pm$ 7.0	31.8 $\pm$ 7.7	32.0 $\pm$ 5.9	0.891
BMI SDS	2.93 $\pm$ 0.77	2.97 $\pm$ 0.85	2.87 $\pm$ 0.66	0.483
Waist circumference (cm)	98.8 $\pm$ 15.9	103.2 $\pm$ 17.7	93.6 $\pm$ 11.6	0.009
WHtR	0.62 $\pm$ 0.07	0.65 $\pm$ 0.08	0.60 $\pm$ 0.06	0.004
Puberty status, n (%)				0.039
Pre-pubertal status	24 (19.8)	15 (21.7)	9 (17.0)	
Pubertal status	49 (40.1)	34 (49.3)	15 (28.3)	
Post-pubertal status	49 (40.1)	20 (29.0)	29 (54.7)	
Season of vitamin D measurement, n (%)				0.672
Summer/fall	59 (48.4)	34 (50)	25 (46.3)	
Winter/spring	63 (51.6)	34 (50)	29 (53.7)	
SBP (mmHg)	119.4 $\pm$ 13.7	121.1 $\pm$ 16.6	117.3 $\pm$ 8.6	0.085
DBP (mmHg)	70.2 $\pm$ 10.4	70.3 $\pm$ 11.8	70.1 $\pm$ 8.3	0.679
Triglycerides (mg/dL)	85.9 $\pm$ 69.2	82.8 $\pm$ 67.7	90.0 $\pm$ 71.6	0.370
Total cholesterol (mg/dL)	155.0 $\pm$ 31.1	156.1 $\pm$ 34.3	153.5 $\pm$ 26.4	0.672
HDL cholesterol (mg/dL)	44.0 $\pm$ 9.9	44.9 $\pm$ 9.8	42.7 $\pm$ 10.1	0.293
LDL cholesterol (mg/dL)	90.0 $\pm$ 29.0	92.4 $\pm$ 31.7	86.9 $\pm$ 25.1	0.351
ALT (UI/L)	31.8 $\pm$ 19.8	35.6 $\pm$ 20.7	26.7 $\pm$ 17.5	0.009
AST (UI/L)	21.8 $\pm$ 9.9	23.2 $\pm$ 9.6	19.8 $\pm$ 9.9	0.015
PTH (pg/mL)	35.2 $\pm$ 12.9	34.5 $\pm$ 12.7	36.0 $\pm$ 13.3	0.526
Albumin (g/dL)	5.29 $\pm$ 0.30	5.30 $\pm$ 0.27	5.28 $\pm$ 0.33	0.538
VDBP (μ g/mL)	201.8 $\pm$ 22.5	199.6 $\pm$ 21.4	204.6 $\pm$ 23.9	0.227
Total vitamin D (ng/mL)	19.66 $\pm$ 7.8	20.59 $\pm$ 7.63	18.45 $\pm$ 7.82	0.132
BVD (ng/mL)*	4.30 $\pm$ 2.06	4.52 $\pm$ 2.23	4.00 $\pm$ 1.79	0.408
Fasting plasma glucose (mg/dL)	91.0 $\pm$ 7.9	92.0 $\pm$ 6.6	89.7 $\pm$ 7.2	0.072
2-h plasma glucose (mg/dL)	113.4 $\pm$ 19.6	113.8 $\pm$ 22.0	112.9 $\pm$ 16.1	0.963
HbA1c (%)	5.54 $\pm$ 0.35	5.54 $\pm$ 0.33	5.53 $\pm$ 0.38	0.872
Fasting plasma insulin (mU/L)	23.8 $\pm$ 13.2	22.6 $\pm$ 13.0	25.3 $\pm$ 13.4	0.262
2-h plasma insulin (mU/L)	137.6 $\pm$ 116.9	122.5 $\pm$ 86.2	156.8 $\pm$ 145.7	0.039
HOMA-IR	5.41 $\pm$ 3.10	5.21 $\pm$ 3.14	5.66 $\pm$ 3.05	0.258
HOMA-B	313.8 $\pm$ 186.1	282.4 $\pm$ 160.2	355.6 $\pm$ 210.3	0.033
IGI	3.43 $\pm$ 2.28	3.34 $\pm$ 2.41	3.54 $\pm$ 2.12	0.466
Matsuda index	2.38 $\pm$ 1.76	2.55 $\pm$ 2.04	2.17 $\pm$ 1.32	0.369
Derivative control (pmol/m ² BSA)/(mmol/L/min)	2252.4 $\pm$ 2049.1	2351.4 $\pm$ 2474.6	2132.5 $\pm$ 1383.8	0.669
Proportional control (pmol/min/m ² BSA)	135.3 $\pm$ 82.4	131.8 $\pm$ 86.9	139.6 $\pm$ 77.3	0.310

* n = 47 subjects (M = 28, F = 19).

BMI, body mass index; BVD, bioavailable vitamin D in 47 subjects genotyped for VDBP variants; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HOMA-B, homeostasis model assessment for β -cell function; HOMA-IR, homeostasis model assessment for insulin resistance; IGI, insulinogenic index; PTH, parathyroid hormone; SBP, systolic blood pressure; VDBP, vitamin D-binding protein; WHtR, waist-to-height ratio.

Discussion

The main finding of this study is the significant association between total vitamin D and IS, IR and insulin secretion indices, adjusting for confounders, supporting the hypothesis of a potential direct effect of vitamin D status on IS, IR and BCF, in non-diabetic, overweight and obese children and adolescents.

Vitamin D affects BCF through several pathways: directly, through interaction with vitamin D receptor localized on β -cells, and indirectly, via regulation of serum calcium concentration and calcium flux through the β cell, therefore influencing both insulin synthesis and secretion (14). Vitamin D promotes also insulin receptor gene transcription and the expression of this receptor in peripheral insulin-target tissues (liver, skeletal muscle,

Clinical Study	D Corica, C Zusi and others	Vitamin D and glucose metabolism in obese youth	181:4	444
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Table 2 Comparison analysis of physical and biochemical characteristics among subjects with vitamin D deficiency (Group A) vs subjects with normal and insufficiency vitamin D (Group B). Patients were divided in the two groups according to total vitamin D value. Data are expressed as means $\pm$ s.d. or number and percentage *n* (%). Differences between gender were tested by Student's *t*-test for normally distributed variables and the Mann-Whitney test for skewed distributed variables or using χ^2 -test for categorical variables. Nominal *P* values were reported.

Variables	Group A (<i>n</i> = 61)	Group B (<i>n</i> = 61)	<i>P</i> value
Age (years)	13.3 $\pm$ 2.3	12.4 $\pm$ 2.2	0.075
BMI (kg/m ²)	33.6 $\pm$ 7.3	30.2 $\pm$ 6.2	0.007*
BMI SDS	3.05 $\pm$ 0.73	2.81 $\pm$ 0.80	0.088
Waist circumference (cm)	100.6 $\pm$ 15.2	96.7 $\pm$ 16.6	0.306
WHtR	0.63 $\pm$ 0.08	0.61 $\pm$ 0.07	0.437
Puberty status, <i>n</i> (%)			0.022
Pre-pubertal status	10 (16.4)	14 (23.0)	
Pubertal status	23 (37.7)	26 (42.6)	
Post-pubertal status	28 (45.9)	21 (34.4)	
Season of vitamin D measurement, <i>n</i> (%)			0.001*
Summer/fall	21 (33.3)	38 (64.4)	
Winter/spring	42 (66.7)	21 (35.6)	
SBP (mmHg)	120.6 $\pm$ 14.9	118.2 $\pm$ 12.4	0.394
DBP (mmHg)	71.6 $\pm$ 12.4	68.8 $\pm$ 7.7	0.180
Triglycerides (mg/dL)	83.40 $\pm$ 59.5	88.46 $\pm$ 78.2	0.691
Total cholesterol (mg/dL)	153.5 $\pm$ 34.3	156.7 $\pm$ 27.4	0.600
HDL cholesterol (mg/dL)	42.1 $\pm$ 9.1	46.0 $\pm$ 10.5	0.052
LDL cholesterol (mg/dL)	90.8 $\pm$ 31.7	89.2 $\pm$ 26.1	0.781
PTH (pg/mL)	37.8 $\pm$ 12.7	32.5 $\pm$ 12.7	0.023
Fasting plasma glucose (mg/dL)	91.2 $\pm$ 7.3	90.9 $\pm$ 6.7	0.814
2-h plasma glucose (mg/dL)	113.4 $\pm$ 19.3	113.3 $\pm$ 20.1	0.980
HbA1c (%)	5.51 $\pm$ 0.34	5.56 $\pm$ 0.37	0.424
Fasting plasma insulin (mU/L)	27.2 $\pm$ 13.3	20.3 $\pm$ 12.2	0.004*
2-h plasma insulin (mU/L)	166.0 $\pm$ 139.9	108.2 $\pm$ 77.7	0.007
HOMA-IR	6.20 $\pm$ 3.22	4.60 $\pm$ 2.78	0.002*
HOMA-B	358.5 $\pm$ 189.8	268.3 $\pm$ 172.3	0.008
IGI	3.79 $\pm$ 2.19	3.06 $\pm$ 2.33	0.041
Matsuda index	1.91 $\pm$ 1.15	2.87 $\pm$ 2.12	0.001*
Derivative control (pmol/m ² BSA)/(mmol/L/min)	2392.6 $\pm$ 1691.3	2114.6 $\pm$ 2355.6	0.151
Proportional control (pmol/min/m ² BSA)	151.5 $\pm$ 90.4	119.4 $\pm$ 71.0	0.035

*Significant *P* values after further adjustment for multiplicity (by using the Benjamini-Hochberg step-up procedure).

BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HOMA-B, homeostasis model assessment for β -cell function; HOMA-IR, homeostasis model assessment for insulin resistance; IGI, insulinogenic index; SBP, systolic blood pressure; WHtR, waist-to-height ratio.

and adipose tissue) (15, 16, 17, 18). Moreover, vitamin D would affect insulin levels reducing inflammation-induced β -cell apoptosis by an immune-regulation effect (43). Accordingly, low vitamin D levels seems to be related to defective insulin secretion, reduced glucose homeostasis, and increased risk of metabolic syndrome and T2D (44, 45).

Several studies investigated mechanisms through vitamin D could influence β -cell function and IS, different from direct effects on calcium metabolism. In two studies including murine models, a suppressing effect of vitamin D on pancreatic renin angiotensin system, that is involved in islet cell proliferation and apoptosis, seems to determine a protective effect on BCF (20, 46, 47). Particularly, mice with hypovitaminosis D presented increased expression

of renin angiotensin system components, impaired β -cell gene transcription and impaired glucose tolerance (47). Accordingly, isolated murine pancreatic islets, incubated *ex vivo* under high-glucose conditions, exhibited an increase in renin angiotensin system components expression and production, prevented by calcitriol administration that contemporarily promoted the β -cell glucose-stimulated insulin secretion (20).

Considering the close link between IR and the pathogenesis of steatohepatitis in obese and diabetic patients, vitamin D appears to be also involved in the regulation of hepatic insulin resistance and development of nonalcoholic fatty liver disease (NAFLD) (48). Hypovitaminosis D and NAFLD frequently coexist in obese models, and growing evidences supported a potentially

Table 3 Spearman correlation analysis among total vitamin D, BVD and clinical and biochemical variables.

Variables	Total vitamin D		BVD	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age (years)	−0.21	0.020	−0.13	0.385
Weight (kg)	−0.19	0.036	−0.18	0.225
BMI (kg/m ²)	−0.24	0.007	−0.23	0.118
BMI SDS	−0.18	0.043	−0.29	0.041
WC (cm)	−0.07	0.557	−0.12	0.557
WHtR	−0.04	0.722	0.06	0.752
SBP (mmHg)	0.03	0.786	0.01	0.972
DBP (mmHg)	−0.11	0.269	0.08	0.637
Triglycerides (mg/dL)	0.06	0.564	−0.22	0.704
Total-c (mg/dL)	0.04	0.713	−0.04	0.764
HDL-c (mg/dL)	0.17	0.092	0.19	0.497
LDL-c (mg/dL)	−0.09	0.512	−0.10	0.743
AST (UI/L)	−0.06	0.653	0.13	0.825
ALT (UI/L)	−0.01	0.917	0.05	0.743
PTH (pg/mL)	−0.31	0.001*	−0.07	0.666
HOMA-IR	−0.24	0.008	−0.17	0.140
HOMA-B	−0.31	0.001*	−0.26	0.084
MATSUDA index	0.28	0.002*	0.25	0.026
IGI	−0.25	0.007	−0.38	0.007
Derivative control	−0.20	0.036	−0.29	0.050
Proportional control	−0.22	0.018	−0.31	0.036
FPG (mg/dL)	0.06	0.512	0.18	0.558
FPI (mU/L)	−0.25	0.004*	−0.22	0.091
HbA1c (%)	0.12	0.110	0.13	0.396
Total vit D (ng/mL)	—	—	0.68	<0.001*
BVD (ng/mL)	0.68	<0.001*	—	—
VDBP (μg/mL)	0.17	0.045	−0.51	<0.001*

Nominal *P* values were reported.

*Significant *P* values after further adjustment for multiplicity (by using the Benjamini–Hochberg step-up procedure).

BMI, body mass index; BVD, bioavailable vitamin D in 47 subjects genotyped for VDBP variants; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, glycated hemoglobin; HOMA-B, homeostasis model assessment for β -cell function; HOMA-IR, homeostasis model assessment for insulin resistance; IGI, insulinogenic index; Total-c, total cholesterol; VDBP, vitamin D-binding protein; WC, waist circumference; WHtR, waist-to-height ratio.

causative relationship between these two conditions. In murine models, vitamin D shows a protective effect against liver damage and steatosis, by inhibiting lipid accumulation through autophagy and by attenuating oxidative stress (49, 50). Treatment with 1,25-OH₂D seemed to improve abnormal hepatic lipid and glucose production in T2D mice and seemed to reduce, through activation of Ca²⁺/calcium-calmodulin protein kinase-beta/AMP-activated protein kinase pathways, hepatic triglyceride storage and glucose output under *in vitro* insulin-resistant condition (51). These results support the regulatory action of vitamin D on glucose and lipid metabolism and, consequently, on IR and sensitivity.

Accordingly, hypovitaminosis D was significantly associated with histologically proven NAFLD and vitamin

D seems to be protective against progression from NAFLD to nonalcoholic steatohepatitis (NASH), inhibiting the proliferation hepatic stellate cells, responsible for collagen deposition and fibrosis (52, 53). However, conversely, other studies have not demonstrated an association between vitamin D deficiency and histological characteristics or severity of NAFLD neither positive effect of vitamin D supplementation on hepatic fat content or markers of hepatic injury or IS (54, 55, 56, 57). Therefore, on the light of these conflicting results, further prospective randomized clinical trials in humans will be necessary to establish the role of vitamin D concentration and supplementation in NAFLD and in hepatic IR.

In our cohort of overweight and obese children and adolescents, we found a high prevalence of hypovitaminosis D (90%), according with others (2, 9). However, we found a direct correlation between total vitamin D and BVD, supporting the hypothesis that bioavailability of vitamin D is reduced proportionally to the reduction of total vitamin D. In our study, the first that simultaneously investigated the association between total vitamin D, BVD and IS, IR and BCF in overweight and obese, non-diabetic, children and adolescents, we found that total vitamin D was significant predictor of IS, IR and BCF, in term of both HOMA-B and the 2nd phase of insulin secretion (proportional control). These relationships were independent from age, gender, pubertal stage, season of vitamin D measurement and BMI SDS. Our data confirm previous studies demonstrating an inverse association between total vitamin D and BMI SDS (2, 7, 9). Moreover, for the first time, we found that also the bioavailable form of vitamin D is inversely associated with BMI SDS, suggesting a direct relationship between the severity of overweight and the degree of hypovitaminosis D.

IR and an altered BCF are two conditions that usually preceded the development of abnormal glucose tolerance and type 2 diabetes (T2D). Our data contribute to support the hypothesis that hypovitaminosis D might be implicated in the pathogenesis of T2D in overweight and obese individuals, who are at risk of glucose metabolism impairment (37, 38). Previous studies in adults provided evidences on the relationships between vitamin D and T2D, sustaining the positive effect of normal vitamin D concentrations in reducing risk of T2D (39, 40, 41, 42), as well as the favorable effects of vitamin D supplementation both in reducing the risk of developing IR and T2D and in improving the glycemic control in patients with T2D (24, 43, 44, 45). In a meta-analysis, Parker *et al.* reported a significant association between high levels of vitamin D and a 55% of reduction on the risk of having T2D

Clinical Study	D Corica, C Zusi and others	Vitamin D and glucose metabolism in obese youth	181:4	446
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Table 4 Multivariate linear regression analysis in the total sample for HOMA-IR, HOMA-B, Matsuda index, IGI, derivative control and proportional control using total vitamin D, age, gender, BMI SDS, pubertal stage and seasonality of vitamin D measurement as independent variables.

Dependent variables	Independent variables	β coefficient (95% CIs)	P value	R ²
HOMA-IR	Total vitamin D	−0.01 (−0.01 to 0.00)	0.038	0.239
	Age	0.01 (−0.03 to 0.04)	0.392	
	Gender	0.07 (−0.03 to 0.17)	0.076	
	BMI SDS	0.15 (0.09–0.21)	<0.001	
	Pubertal stage	0.01 (−0.09 to 0.10)	0.215	
	Seasonality	0.05 (−0.04 to 0.13)	0.556	
HOMA-B	Total vitamin D	−0.02 (−0.03 to −0.004)	0.012	0.306
	Age	0.03 (−0.04 to 0.09)	0.471	
	Gender	0.24 (0.04–0.44)	0.018	
	BMI SDS	0.30 (0.17 to 0.43)	<0.001	
	Pubertal stage	0.08 (−0.12 to 0.28)	0.804	
	Seasonality	−0.10 (−0.31 to 0.11)	0.331	
Matsuda index	Total vitamin D	0.01 (0.001–0.01)	0.020	0.238
	Age	0.004 (−0.03 to 0.03)	0.761	
	Gender	−0.04 (−0.14 to 0.05)	0.281	
	BMI SDS	−0.14 (−0.20 to −0.08)	<0.001	
	Pubertal stage	−0.01 (−0.01 to 0.08)	0.851	
	Seasonality	−0.04 (−0.13 to 0.06)	0.464	
IGI	Total vitamin D	−0.02 (−0.03 to −0.001)	0.042	0.102
	Age	−0.01 (−0.09 to 0.07)	0.901	
	Gender	0.04 (−0.21 to 0.29)	0.691	
	BMI SDS	0.17 (0.02–0.33)	0.025	
	Pubertal stage	0.02 (−0.21 to 0.25)	0.902	
	Seasonality	0.06 (−0.18 to 0.30)	0.595	
Derivative control	Total vitamin D	−0.31 (−0.92 to 0.30)	0.315	0.046
	Age	0.27 (−2.73 to 3.27)	0.859	
	Gender	−0.51 (−9.87 to 8.85)	0.914	
	BMI SDS	2.94 (−2.94 to 8.81)	0.323	
	Pubertal stage	3.08 (−5.53 to 11.69)	0.480	
	Seasonality	−3.83 (−12.93 to 5.28)	0.406	
Proportional control	Total vitamin D	−0.01 (−0.01 to 0.00)	0.045	0.039
	Age	−0.02 (−0.05 to 0.02)	0.437	
	Gender	0.03 (−0.08 to 0.13)	0.351	
	BMI SDS	0.01 (−0.06 to 0.08)	0.677	
	Pubertal stage	0.033 (−0.06 to 0.13)	0.142	
	Seasonality	−0.04 (−0.15 to 0.06)	0.342	

Statistical test performed on log- or root-square transformed data.

BMI, body mass index; HOMA-B, homeostasis model assessment for β -cell function; HOMA-IR, homeostasis model assessment for insulin resistance; IGI, insulinogenic index.

(58). Deleskong *et al.* reported a positive and significant effect of higher vitamin D concentration in reducing risk of T2D in individuals with prediabetes, but not in those subjects with normal glucose tolerance (59). Chiu *et al.*, in a cohort of healthy glucose-tolerant adults, demonstrated a positive correlation between 25(OH)D and IS, and a negative effect of hypovitaminosis D on BCF, assessed by an independent, negative, relationship between 25(OH)D and plasma glucose during a hyperglycemic clamp (60). Moreover, in a large cohort of children and adolescents, Ganji *et al.* reported an inverse correlation between 25(OH)D levels and HOMA-IR (12). Conversely, other studies, carried out both in children and adults, did not document

association between vitamin D levels and IR or glycemic control (9, 21, 61, 62). A causal effect relationship between hypovitaminosis D and glycemic control, IR or diabetes risk (22) as well as not even favorable effects of vitamin D supplementation on plasma glucose, plasma insulin and IR or BCF were reported (24, 63, 64). These contrasting results suggest that several factors, including genetic background (i.e. *VDBP* polymorphisms), baseline vitamin D concentration and measurement methods, dose and routes of administration of vitamin D supplementation, influence the activity of vitamin D.

Although available data of the literature are not consistent, the results of our study suggest that, as an

Clinical Study	D Corica, C Zusi and others	Vitamin D and glucose metabolism in obese youth	181:4	447
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Table 5 Multivariate linear regression analysis in the total sample for HOMA-IR, HOMA-B, Matsuda index, IGI, derivative control and proportional control using BVD, age, gender, BMI SDS, pubertal stage and seasonality of vitamin D measurement as independent variables.

Dependent variables	Independent variables	β coefficient (95% CI)	P value	R ²
HOMA-IR	BVD	-0.05 (-0.23 to 0.13)	0.441	0.316
	Age	-0.02 (-0.08 to 0.03)	0.973	
	Gender	0.01 (-0.15 to 0.18)	0.480	
	BMI SDS	0.18 (0.09-0.27)	<0.001	
	Pubertal stage	0.07 (-0.08 to 0.23)	0.449	
	Seasonality	0.05 (-0.11 to 0.21)	0.864	
HOMA-B	BVD	-0.15 (-0.54 to 0.24)	0.440	0.348
	Age	-0.02 (-0.14 to 0.10)	0.709	
	Gender	0.12 (-0.24 to 0.48)	0.502	
	BMI SDS	0.40 (0.20-0.61)	<0.001	
	Pubertal stage	0.15 (-0.19 to 0.48)	0.387	
	Seasonality	-0.06 (-0.41 to 0.29)	0.732	
Matsuda index	BVD	0.13 (-0.05 to 0.30)	0.108	0.228
	Age	0.03 (-0.03 to 0.08)	0.614	
	Gender	0.004 (-0.16 to 0.17)	0.647	
	BMI SDS	-0.15 (-0.25 to -0.06)	0.001	
	Pubertal stage	-0.04 (-0.19 to 0.12)	0.971	
	Seasonality	-0.04 (-0.20 to 0.12)	0.902	
IGI	BVD	-0.43 (-0.83 to -0.02)	0.034	0.227
	Age	0.03 (-0.11 to 0.17)	0.485	
	Gender	-0.24 (-0.65 to 0.18)	0.108	
	BMI SDS	0.27 (0.02-0.52)	0.025	
	Pubertal stage	-0.05 (-0.17 to 0.63)	0.988	
	Seasonality	0.23 (-0.17 to 0.63)	0.392	
Derivative control	BVD	-10.4 (-30.9 to 10.1)	0.504	0.140
	Age	1.76 (-4.54 to 8.16)	0.540	
	Gender	-9.72 (-28.7 to 9.2)	0.358	
	BMI SDS	4.83 (-6.9 to 16.6)	0.244	
	Pubertal stage	3.5 (-14.3 to 21.4)	0.735	
	Seasonality	-10.5 (-28.9 to 7.9)	0.761	
Proportional control	BVD	-0.17 (-0.35 to 0.02)	0.082	0.132
	Age	-0.01 (-0.06 to 0.05)	0.657	
	Gender	-0.10 (-0.27 to 0.07)	0.171	
	BMI SDS	0.02 (-0.09 to 0.12)	0.716	
	Pubertal stage	-0.04 (-0.20 to 0.12)	0.752	
	Seasonality	-0.04 (-0.35 to 0.02)	0.426	

Statistical test performed on log- or root-square transformed data.

BMI, body mass index; BVD, bioavailable vitamin D in 47 subjects genotyped for VDBP variants; HOMA-B, homeostasis model assessment for β -cell function; HOMA-IR, homeostasis model assessment for insulin resistance; IGI, insulinogenic index.

attempt to reduce the risk to develop glucose metabolism impairment, a periodic monitoring of vitamin D concentration in overweight and obese children and adolescents may be justified as well as the treatment with 25(OH)D in subjects with vitamin D deficiency or insufficiency (65). Of course, the role of vitamin D supplementation as a potential intervention strategy for the management of IR and T2D in obese children and adolescents needs to be assessed by long-term randomized controlled studies.

The study has some potential limitations: (i) the cross-sectional design of the study, that did not allow to verify the causal relationships between vitamin D levels and IS,

IR and BCF; (ii) the lack of data on dietary intake vitamin D and sun exposure. Nevertheless, the potential impact of vitamin D intake with diet on circulating total vitamin D should be negligible, due to the really modest intake of vitamin D in the diet usually reported by overweight and obese children living the same geographical area (66). Moreover, the season of blood test sampling for measuring total vitamin D was considered in the statistical analysis, as an attempt to take indirectly into account sun exposition, higher in spring and summer and lower in autumn and winter; (iii) ethnicity; this study was conducted in subjects with European ancestry, so that results are not directly exportable to subjects with

other ethnic backgrounds; (iv) vitamin D dosage was not detected by gold standard metabolite test (HPLC with MS/MS); (v) serum calcium was not available in our cohort despite no phenotype signs of hypocalcemia were found.

This study has also some strengths: (i) the sample set, including glucose-tolerant, healthy subjects, who were not taking chronic medication, that allows to explore relationships between variables avoiding potential confounders due to co-morbidity, so common in obese adult samples; (ii) the glucose metabolism assessment: in this study, differently from other studies (67, 68, 69, 70) in which the relationship between vitamin D and glucose metabolism in children was investigated considering just indices derived from fasting insulin and glucose levels, we also considered indices of IS and BCF derived from a modified OGTT, a reliable and little invasive test to evaluate glucose tolerance; (iii) vitamin D status assessment: in this study not only the total vitamin D measurement was provided but also BVD was estimated by the VDBP polymorphisms, according to Powe *et al.* equations (35); (iv) PTH analysis lets us establish a significant correlation with total vitamin D in order to exclude that adiposity could be the only cause of hypovitaminosis D.

In conclusion, overweight and obese children and adolescents have a high rate of vitamin D deficiency and insufficiency and hypovitaminosis D is associated with lower BCF and IS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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