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ORIGINAL ARTICLE

25-Hydroxyvitamin D insufficiency discriminates cardiovascular risk factors accumulation in peri-pubertal boys undergoing overweight screening

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Abstract The aim of this study was to evaluate the possible association between cardiometabolic risk factors accumulation and vitamin D status in a cohort of Italian normal weight and overweight male children. 108 boys enrolled in an andrological health prevention project underwent physical examination, anthropometric measurements, and fasting blood sampling. Serum blood glucose, HDL-cholesterol, triglycerides, parathyroid hormone, and 25-hydroxyvitamin D (25(OH)D) were measured. Cardiovascular risk factors were defined according to the National Cholesterol Education Program Adult Treatment Panel III modified for age. Lean and overweight subjects differed in terms of waist circumference ($P < 0.001$), HDL-cholesterol ($P = 0.001$), triglycerides ($P = 0.001$), systolic blood pressure ($P < 0.001$) and diastolic blood pressure ($P = 0.002$). Both groups had similar mean 25(OH)D levels ($P = 0.160$) and were below the sufficiency threshold: indeed only 24 % of normal weight had 25(OH)D $\geq$30 ng/ml, and even less in the overweight/obese group (8 %, $P = 0.03$ vs. normal weight). A significant accumulation of risk factors in course of 25(OH)D insufficiency was detected in both the whole cohort and in the normal weight group ($P = 0.003$ and $P = 0.04$, respectively) with odd ratios of 1.31 (1.16–1.49 95%CI) and 1.41 (1.18–1.69 95%CI), respectively. In course of vitamin D deficiency, the odd ratios were 2.24 (1.34–3.77 95%CI, $P = 0.003$) in the whole cohort and 2.40 (1.27–4.82 95%CI, $P = 0.03$) in lean subjects. We reported a considerable occurrence of cardiovascular risk factors in course of hypovitaminosis D in overweight/obese boys and even in lean subjects, which normally would not have been further evaluated by considering the sole BMI-related parameters. In this regard, 25(OH)D levels appear as a potential discriminating parameter able to identify male children at higher health risk.

Keywords Vitamin D · Children · Obesity · Adolescents · Cardiovascular risk · Metabolic syndrome

Introduction

Since its discovery, decades of research on vitamin D have documented a myriad of potential biological effects. Vitamin D is a secosteroid hormone, primarily produced by the action of sunlight on the skin and secondarily gained from dietary sources. This fat-soluble vitamin needs to be activated to 1α,25-dihydroxyvitamin D3 by a two-step hydroxylation process, passing through the 25-hydroxyvitamin D (25(OH)D) form [1]. In particular, according to the 1997 reference publication for vitamin D intake, serum 25(OH)D is considered as the functional indicator of vitamin D status [2]. In the past, vitamin D deficiency was defined as 25(OH)D concentrations less than 20 ng/ml, as these 25(OH)D levels were associated with rickets and

Andrea Di Nisio and Luca De Toni have been contributed equally to this work.

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osteomalacia, the classical clinical status of vitamin D deficiency [2]. However, recent evidence suggests that higher 25(OH)D, up to 30 ng/ml may be required for optimal health (reviewed in 3). Vitamin D insufficiency, defined as a serum 25(OH)D level of 21–29 ng/ml, may contribute to the burden of chronic diseases, particularly osteomalacia, osteoporosis, and decreased physical performance. Also cancer, cardiovascular disease, type 2 diabetes, and infectious and autoimmune disorders have been associated with hypovitaminosis D [4–6]. These recent findings led to the publication of the 2011 US IOM report [7] (discussed in [8–11]) and a new Clinical Practice Guideline from the US Endocrine Society [4].

The main vitamin D action is on bone mineralization and calcium-phosphorous metabolism, but more recent studies have uncovered its potential role in the pathophysiology of cardiovascular disease. Vitamin D receptors (VDRs) are present on a large variety of cell types, including myocytes, cardiomyocytes, pancreatic beta-cells, vascular endothelial cells, neurons, immune cells, and osteoblasts [5], even though the molecular mechanisms underlying this link are still unclear [12–14]. Recent research has linked inadequate vitamin D status to nonskeletal major chronic diseases, especially cardiovascular diseases (CVD) [15]. Vitamin D deficiency seems to predispose to hypertension, diabetes and metabolic syndrome, left ventricular hypertrophy, congestive heart failure, and chronic vascular inflammation [5, 16]. In addition, epidemiologic studies have linked vitamin D deficiency with increased risk of major adverse cardiovascular events [15]. In adults, cross-sectional data support a slight but significant inverse relationships between 25(OH)D levels, systolic blood pressure [14], and increased prevalence of the clinical signs of metabolic syndrome, such as reduced HDL-cholesterol (HDL-C) levels [12, 13]. Food-based strategies for enhancement of vitamin D status in the population could therefore lower cardiovascular risk [17]. In addition, our group has recently shown reduced levels of 25(OH)D in a group of overweight–obese patients, featured by glucose intolerance, compared to lean controls [18], but the association between low 25(OH)D levels and prevalent cardiometabolic risk factors extends even to the pediatric population: in US children, 25(OH)D insufficiency was shown to be associated with increased blood pressure and insulin resistance. Notably, these associations remained significant even after correcting for obesity, age, sex, and ethnicity [19]. As observed in adulthood, the risk for children to develop cardiovascular and metabolic diseases, such as dyslipidemia, elevated blood pressure and impaired glucose metabolism, is strictly linked to weight gain and obesity [20]. Waist circumference, skin-fold thickness and body mass index (BMI) are common non-invasive clinical measures to define obesity. In particular, BMI represents the most used parameter that sufficiently correlates with direct measures of body adiposity [21]. Accordingly, age- and sex-specific BMI percentiles, such as those based on the U.S. Centers for Disease Control and Prevention (CDC) growth reference curves [22], are typically used for the identification of obesity-related health risks in children and adolescents. It is worth noting that, according to this definition, overweight–obese adolescents are at substantially higher risk of developing obesity in adulthood than normal weight adolescent [23]. Moreover, an increased risk to develop CVD in adulthood has been found in subjects whose CDC-BMI in childhood was greater than the 85th percentile [24]. On this basis, a correct screening for vitamin D status, CVD risk, and overweight status in the pediatric population might be essential for preventing vitamin D-related diseases in adulthood. To this end, we investigated the association between serum 25(OH)D levels and cardiovascular risk factors in a cohort of male children undergoing an obesity and andrological health prevention project. We found that 25(OH)D insufficiency is highly predictive for cardiovascular risk factors accumulation even in a cohort of apparently healthy lean boys.

Methods

Exclusion criteria

The exclusion criteria for entering this study were (1) age below 11 years and above 14 years; (2) personal history of chronic diseases as reported in interviews with individual subjects together with their parents; (3) previous or current treatments with drugs known to interfere with carbohydrates and lipids metabolism (insulin, oral hypoglycemic agents: metformin, sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, statins, fibrates, and ion-exchange resins); (4) current 25(OH)D replacement and/or drugs that affect vitamin D and/or calcium metabolism (steroids, Vitamin D2 and/or D3, Vitamin K, calcitonin, and biphosphonates); and (5) parathyroid disorders. Of initial 200 subjects, 92 were excluded because of lack of informed consent for blood sampling ($n = 53$), documented chronic diseases ($n = 19$), ongoing 25(OH)D replacement ($n = 21$).

Participants and serum assays

A total of 200 male subjects were consecutively recruited in the Operative Unit of Medicine, District of Salerno, section of Sapri (SA, Italy), in agreement with the Secondary School Screening Programme for Obesity Surveillance and Andrological Health Prevention Project in the local children population. All parents of children gave an

informed consent to the study. The investigation was conformed to the principles of the Declaration of Helsinki. The study was approved by the hospital ethics committee. Subjects were equally distributed within a residential range of 79.5 km, at a mean latitude of 40°21″ North. The study was conducted between October 1st and October 31st 2013, to avoid any confounding effect of seasonal variation in 25(OH)D levels, and consisted of a physical examination, including measurements of anthropometrics and blood pressure, and the collection of a venous blood sample after overnight fasting, which was stored at –80 °C after plasma separation. Serum biochemical markers, as fasting blood glucose (FBG), HDL-C, and triglycerides were measured by standard biochemical methods. Serum levels of parathyroid hormone (PTH) and 25(OH)D were also measured in all subjects with, respectively, direct, two sites, sandwich-type chemiluminescent immunoassay (LIAISON N-TACT PTH, DiaSorin Inc., Stillwater MN) and with direct, competitive chemiluminescent immunoassay (LIAISON 25 OH vitamin D TOTAL Assay, DiaSorin Inc.).

Definitions

Pubertal stage was assessed in each patient by the same pediatrician, according to the criteria of Tanner and Whitehouse [25], by means of physical examination and assessment of testicular volume. For this study, cardiovascular risk factors were defined according to the National Cholesterol Education Program Adult Treatment Panel III definition modified for age [26, 27]. Risk factors were defined as follows: (1) waist circumference ≥90th percentile for age and gender [28]; (2) HDL-C ≤40 mg/dL; (3) triglycerides ≥110 mg/dL; (4) systolic blood pressure (SBP) ≥130 mmHg diastolic blood pressure (DBP) ≥85 mmHg; and (5) BFG ≥5.6 nmol/L [29]. Cardiovascular risk factors accumulation was defined as the occurrence of at least 1 risk factor. The BMI percentiles were calculated with respect to age and gender based on the Centers for Disease Control and Prevention growth curves [22]. The study cohort was further split into two main groups according to normal weight and overweight status, associated to the risk of developing CVD in adult age (CDC-BMI <85th percentile and ≥85th percentile, respectively) [22, 24]. A CDC-BMI ≥95th percentile for age and gender was used to define "severe obese" based on growth charts published by the Centers for Disease Control and Prevention in 2000 [22]. According to previous literature [3–5, 30–33], we used the following cut-offs of serum 25(OH)D levels to define vitamin D status: deficiency ≤20 ng/ml; insufficiency 21–29 ng/ml; sufficiency ≥30 ng/ml.

Statistical analysis

Data analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard error means (SEM). The Levene's test was used to test the homogeneity of variance among groups prior to data analysis. If homogeneity of variance assumption was violated, Welch test was performed and the respective P value was reported. Differences between two groups were analyzed using Student's t test. Differences between three or more groups were analyzed using ANOVA, and Fisher's least significant difference (LSD) adjustment for multiple comparisons of groups was applied to the pairwise post hoc comparisons of groups. Multivariate logistic regression was performed to adjust for potential confounders, the final model included variables significant at $P < 0.10$ on univariate analysis. Proportions and discrete variables were compared using the Chi-square test and Fisher's exact test when expected cell size was smaller than five. Odd ratios for relative risk assessment are reported as mean and 95 % CI. Statistical significance was defined at the $P < 0.05$ level using 2-sided tests; highly statistical significance was defined for values of $P < 0.01$.

Results

The clinical characteristics of the study cohort are summarized in Table 1. Assessment of pubertal status by Tanner's method showed 29 subjects in stage II (27 %), 66 (61 %) in stage III, and 13 (12 %) in stage 4. According to

Table 1 Clinical features of the study cohort

$N = 108$[a]	Mean ± SEM
Age (years)	12.2 ± 0.1
Tanner stage I/II/III/IV/V (%)	0/27/61/12/0
Weight (kg)	57.9 ± 1.5
Height (m)	1.6 ± 0.0
BMI (kg/m^2)	23.1 ± 0.5
Waist circumference (cm)	80.5 ± 1.4
HDL-C (mg/dL)	59.4 ± 1.4
Triglycerides (mg/dL)	74.4 ± 3.6
FBG (mmol/L)	4.9 ± 0.1
SBP (mmHg)	105.0 ± 1.5
DBP (mmHg)	64.6 ± 0.8
25(OH)D (ng/ml)	24.6 ± 0.5
PTH (ng/L)	42.1 ± 2.0

SEM standard error of the mean, *BMI* body mass index, *HDL-C* high-density lipoprotein cholesterol, *FBG* fasting blood glucose, *SBP* sistolic blood pressure, *DBP* diastolic blood pressure, *25(OH)D* 25-hydroxyvitamin D, *PTH* parathyroid hormone

[a] N indicates sample size

the definition of U.S. Centers for Disease Control and Prevention, the overall prevalence of overweight subjects (CDC-BMI ≥85th percentile) was 45 % (49/108), whilst the occurrence of severe obesity (CDC-BMI ≥95th percentile) was 27 % (29/108). None of the enrolled subject fulfilled the criteria for underweight classification (CDC-BMI <10th percentile). Clinical features were subsequently analyzed throughout the CDC-BMI classification (Table 2). As expected, there was a substantial difference among groups for those parameters typically associated with metabolic derangements such as waist circumference ($P < 0.001$), HDL-C ($P = 0.013$), triglycerides ($P = 0.039$), SBP ($P = 0.009$), and DBP ($P = 0.032$). No significant difference emerged for FBG ($P = 0.412$). However, when we considered the mean occurrence of cardiovascular risk factors across CDC-BMI groups, we found a significant increase of risk factors only in the severe obesity group ($P < 0.01$. Supplemental Fig. S1). Furthermore, mean serum 25(OH)D levels progressively decreased along with the cumulating number of risk factors within the whole study cohort ($P < 0.01$. Supplemental Fig. S2). Serum 25(OH)D levels were differentially distributed across CDC-BMI groups ($P = 0.046$). In particular, the main differences were ascribable between 10th and 25th percentile group and ≥95th percentile group, and between percentiles from 25th to 95th and ≥95th (Fig. 1).

When the study cohort was split into two main groups according to normal weight (CDC-BMI >10th and <85th percentile) and overweight status (CDC-BMI ≥85th percentile), associated to the risk of developing CVDs in adulthood, a difference in terms of waist circumference ($P < 0.001$), HDL-C ($P = 0.001$), triglycerides ($P = 0.001$), SBP ($P < 0.001$), and DBP ($P = 0.002$) was detected (Table 3), whereas serum 25(OH)D did not differ and was below the cut-off of vitamin D sufficiency (30 ng/ml) in both groups (Table 3, $P = 0.160$). Indeed, a considerable fraction of subjects featured by 25(OH)D insufficiency or deficiency (25(OH)D <30 ng/ml) was observed in both groups (Normal weight: 45/59, 76 %; overweight–obese: 45/49, 92 %, $P = 0.03$). No significant difference was observed in pubertal stage distribution of subjects according to vitamin D status (Chi-square test: $P = 0.36$ for 30 ng/ml insufficiency cut-off; $P = 0.78$ for 20 ng/ml deficiency cut-off).

Finally, we plotted the number of cardiovascular risk factors occurring in each CDC-BMI percentile in relation to serum 25(OH)D levels (Fig. 2). We observed a significant clustering of risk factors only in those patients featured by 25(OH)D insufficiency or deficiency (serum 25(OH)D <30 ng/ml), both in the whole study cohort and in the normal weight group alone (Fisher's exact test: $P = 0.003$ and $P = 0.04$, respectively). In this regard, the odd ratios for cardiovascular risk factors' accumulation in course of 25(OH)D levels <30 ng/ml were 1.31 (1.16–1.49 95%CI) and 1.41 (1.18–1.69 95%CI) in the whole cohort and for normal weight subjects, respectively. Taking into account the vitamin D deficient group (serum 25(OH)D <20 ng/ml), the clustering of risk factors in course of hypovitaminosis D was conserved in both the whole cohort and in the normal

Table 2 Distribution of anthropometric and metabolic parameters of the study cohort within CDC-BMI percentiles

	CDC-BMI percentiles						
	10th–25th ($N = 9$)	25th–50th ($N = 21$)	50th–85th ($N = 29$)	85th–90th ($N = 8$)	90th–95th ($N = 12$)	>95th ($N = 29$)	P value[a]
Age (years)	12.3 ± 0.3	12.3 ± 0.3	11.9 ± 0.2	11.8 ± 0.2	11.8 ± 0.2	12.7 ± 0.2	0.057
Weight (kg)	41.2 ± 1.9	46.9 ± 2.0	51.0 ± 1.6	63.4 ± 4.2	61.4 ± 2.4	74.9 ± 2.4	**<0.001**
Height (m)	1.53 ± 0.03	1.56 ± 0.02	1.55 ± 0.02	1.62 ± 0.04	1.58 ± 0.02	1.61 ± 0.02	0.120
BMI (kg/m^2)	17.4 ± 0.2	19.1 ± 0.3	21.1 ± 0.2	24.0 ± 0.6	24.6 ± 0.7	28.9 ± 0.8	**<0.001**
Waist circumference (cm)	67.4 ± 1.9	69.8 ± 1.9	74.8 ± 1.1	82.4 ± 4.5	82.2 ± 3.3	96.3 ± 2.0	**<0.001**
HDL-C (mg/dL)	64.0 ± 3.6	64.0 ± 3.1	63.0 ± 2.6	63.5 ± 3.3	52.6 ± 3.4	52.8 ± 3.0	**0.013**
Triglycerides (mg/dL)	58.1 ± 5.7	60.6 ± 7.4	66.8 ± 4.2	71.9 ± 7.0	82.2 ± 13.4	94.5 ± 8.9	**0.039**
FBG (mmol/L)	5.0 ± 0.1	4.9 ± 0.1	5.1 ± 0.2	4.7 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	0.412
SBP (mmHg)	98.1 ± 3.4	103.4 ± 2.6	98.3 ± 2.6	114.1 ± 7.6	108.0 ± 3.9	111.0 ± 3.1	**0.009**
DBP (mmHg)	60.0 ± 2.1	62.4 ± 1.7	62.6 ± 0.9	71.0 ± 3.4	62.0 ± 2.0	68.6 ± 2.0	**0.032**
25(OH)D (ng/mL)	27.3 ± 1.9	23.9 ± 1.0	25.3 ± 0.9	26.5 ± 1.6	25.2 ± 1.2	22.6 ± 0.8	**0.046**
PTH (ng/L)	48.5 ± 8.2	38.1 ± 3.2	42.6 ± 4.8	38.4 ± 5.3	47.6 ± 6.8	41.3 ± 3.2	0.737

P values are significant at the 0.05 level are given in bold

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *FBG* fasting blood glucose, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *25(OH)D* 25-hydroxyvitamin D, *PTH* parathyroid hormone

[a] *P* values are for the difference among all groups and LSD adjusted for multiple comparisons: fixed factor: CDC-BMI percentiles, random factor: cohort characteristics

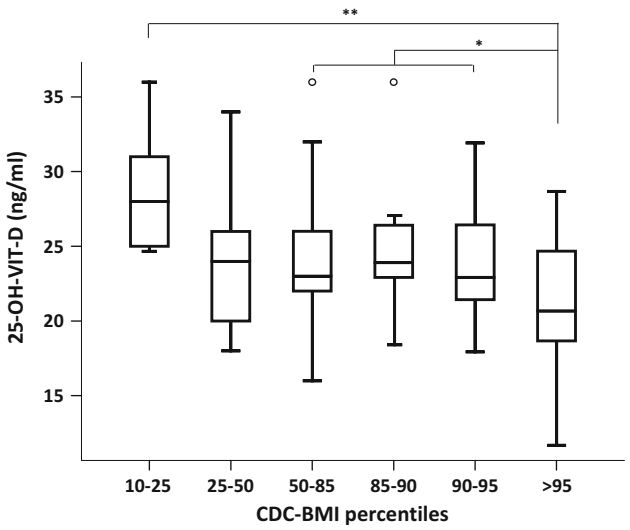

Fig. 1 *Box* plot describing the distribution of serum 25(OH)D levels of the study cohort, within the CDC-BMI percentiles. The *five horizontal lines* of each *box* plot identify, respectively, the minimum, the lower quartile, the median, the upper quartile, and the maximum value. $*P < 0.05$; $**P < 0.01$

weight group (Fisher's exact test: $P = 0.003$ and $P = 0.03$, respectively), with odd ratios of 2.24 (1.34–3.77 95%CI) and 2.40 (1.27–4.82 95%CI), respectively.

Discussion

The main finding of this study is that cardiovascular risk factors are highly prevalent in male children featured by hypovitaminosis D. This evidence persists even in lean subjects that would not have been considered at high risk for CVD in adulthood, according to CDC-BMI classification.

Serum 25(OH)D concentration is widely held to be a better indicator of vitamin D status than 1, 25-dihydroxyvitamin D, or calcitriol, the formally considered active metabolite [2]. Recent studies suggest a tight relationship between vitamin D status and cardiovascular function, but the exact mechanisms subtending this association are still unclear. Suboptimal vitamin D status is associated with increased mortality for cardiovascular disease, coronary heart disease, and various cardiovascular risk factors [34]. In particular, it was suggested that obese individuals are more likely than normal weight individuals to suffer of vitamin D insufficiency because of several important factors such as increased sequestration of vitamin D in fat tissues, low dietary vitamin D intake due to poor nutritional habits, and minimal sun exposure due to sedentary indoor lifestyle [35, 36]. Very recently, a large proportion of young males and females aged 11–20 years old, living in the same area of our study, has been found to suffer from severe hypovitaminosis D [30]. The considerable prevalence of obesity and cardiometabolic disorders, together with vitamin D insufficiency, reported in the pediatric population [37], lead clinicians to begin testing and supplementing with low levels of vitamin D in an effort to prevent and treat cardiometabolic diseases in overweight–obese children. In this study, we evaluated whether vitamin D status could be associated with cardiovascular risk factors in an Italian cohort of normal weight and overweight male children. In agreement with previous studies [12–14,

Table 3 Differences in anthropometric and metabolic parameters between normal weight and overweight subjects

	Normal weight (10th–85th percentiles) ($N = 59$)	Overweight/obese (>85th percentiles) ($N = 49$)	P value[a]
Age (years)	12.12 ± 0.14	12.33 ± 0.14	0.304
Weight (kg)	48.02 ± 1.15	69.73 ± 1.89	**<0.001**
Height (m)	1.55 ± 0.01	1.60 ± 0.01	**0.006**
BMI (kg/m^2)	19.85 ± 0.24	27.07 ± 0.60	**<0.001**
Waist Circumference (cm)	71.92 ± 0.99	90.74 ± 1.86	**<0.001**
HDL-C (mg/dL)	63.49 ± 1.74	54.47 ± 2.07	**0.001**
Triglycerides (mg/dL)	63.27 ± 3.45	87.78 ± 6.32	**0.001**
FBG (mmol/L)	5.00 ± 0.11	4.78 ± 0.05	0.078
SBP (mmHg)	100.00 ± 1.69	110.91 ± 2.43	**<0.001**
DBP (mmHg)	62.14 ± 0.80	67.62 ± 1.48	**0.002**
25(OH)D (ng/ml)	25.14 ± 0.63	23.86 ± 0.64	0.160
PTH (ng/L)	41.90 ± 2.91	42.34 ± 2.66	0.914

P values are significant at the 0.05 level are given in bold

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *FBG* fasting blood glucose, *SBP* sistolic blood pressure, *DBP* diastolic blood pressure, *25(OH)D* 25-hydroxyvitamin D, *PTH* parathyroid hormone

[a] *P* values are for the difference between the two groups in the multivariate analysis, adjusted for multiple comparisons: fixed factor: normal weight or overweight status according to CDC-BMI percentiles, dependent factors: cohort characteristics; covariate: Tanner stage

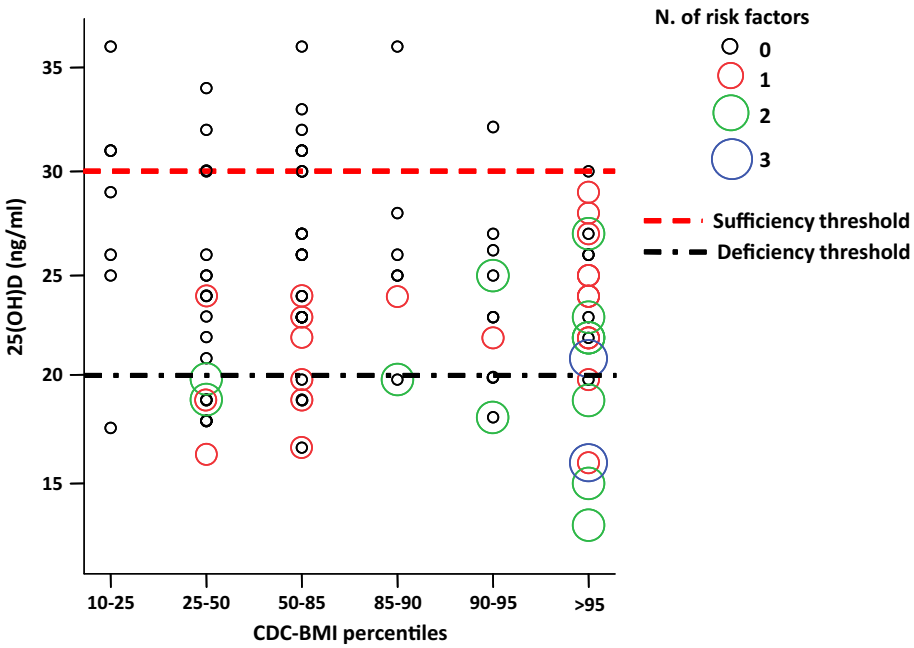

Fig. 2 Accumulation of cardiovascular risk factors ($N \geq 1$), within CDC-BMI percentiles, according to serum 25(OH)D levels of the study cohort. *Black*, *red*, *green*, and *blue dots* represent serum levels of subjects featured by the accumulation of, respectively, none, one, two, and three cardiovascular risk factors, defined according to National Cholesterol Education Program Adult Treatment Panel III definition modified for age. *Red dotted line* represents the vitamin D sufficiency threshold of 30 ng/ml, *black dotted line* represents vitamin D deficiency threshold of 20 ng/ml

19, 20], we found a progressive alteration of cardiometabolic parameters concomitant with increasing CDC-BMI percentiles, together with a reduction of serum 25(OH)D levels. Importantly, no variation in PTH levels was observed across CDC-BMI groups, excluding any confounding effect of this hormone on cardiovascular function [37]. When the study cohort was split into two groups according to normal weight and overweight–obese status, cardiometabolic parameters showed a significant difference, as expected. On the other hand, 25(OH)D levels were equally low in both groups, suggesting that also a considerable fraction of normal weight subjects might suffer of vitamin D insufficiency. Indeed, only 24 % of normal weight children had 25(OH)D levels ≥30 ng/ml, and even less in the obese/overweight group, where only 8 % of children was above the sufficiency threshold. The very high prevalence of vitamin D insufficiency in our study cohort, independently of BMI status, is in agreement with data from ''the Healthy Lifestyle in Europe by Nutrition in Adolescence study (HELENA study)'' where about 80 % of the sample had suboptimal vitamin D levels [38], and also with data from Italian adolescents [30, 31, 33]. Moreover, vitamin D insufficiency status was not correlated with puberty stages, in agreement with previous studies [30, 39]. This prompted us to widen the interaction between 25(OH)D levels and cumulative risk factors occurrence also in those subject that normally would not have been assessed for cardiovascular risks only on the basis of their BMI. Inferring the mean risk factors' accumulation across CDC-BMI percentiles, no significant difference emerged between groups, with the only exception of severely obese subjects. On the other hand, serum 25(OH)D levels underwent a progressive decrease along with cardiovascular risk factors accumulation. Taken together these results suggest that 25(OH)D levels could be predictive of cardiovascular risk factors' summation also in lean subjects. On this basis, by plotting the cumulating number of risk factors across serum 25(OH)D levels in each CDC-BMI percentile, the occurrence of cardiovascular risk factors was detectable only in vitamin D insufficient and/or deficient subjects, and even within the normal weight group alone. We found an increased risk to develop at least one cardiovascular risk factor in the whole cohort, in course of vitamin D insufficiency and deficiency, (OR 1,31 and 2,24, respectively) and in the normal weight group alone (OR 1,41 and 2,40). This result is particularly important when we consider that the utility of CDC-BMI percentiles to identify children at higher risk is not well studied [40]. In fact, there is general agreement in considering the 95th CDC-BMI percentile as the cut-off for surveillance and screening of children and adolescents at higher health risk, while minimizing over- and under-diagnosis [41], and our data are actually in accordance with these diagnostic criteria. Nonetheless, we highlighted a considerable occurrence of cardiovascular risk factors in subjects that normally would not have been further evaluated by considering the sole BMI-related parameters. In this regard, vitamin D status appears as a potential discriminating parameter able to identify children at higher health risk. This study indirectly suggests that vitamin D supplementation may be effective in optimizing vitamin D status in course of cardiometabolic disorders, as shown in a cohort of black youths where vitamin D supplementation was effective in counteracting the progression of aortic stiffness [42].

Our findings must be interpreted in the light of acknowledged limitations. First, it was a retrospective cross-sectional study; therefore, causality cannot be inferred. Second, the sample size is relatively small for the results to be extended to the general population; in addition, it is limited by the fact that vitamin D was measured during one single month of the year (October) and does not reflects the vitamin D status over a longer period (1 year). However, since vitamin D shows seasonal variation [43], our data were homogeneous and between-subjects variation was not ascribable to seasonal fluctuation. In particular, 25(OH)D serum levels at this time of the year (October) are likely to be the result of sunlight exposure during summer, and therefore we could speculate an even worse scenario during winter/spring seasons. Finally, this study is limited only to male subjects. To this end, further analysis on larger cohorts would be required. Nonetheless, it is possible that males and females differ in the degree of CVD risk, associated with vitamin D levels. In fact, male children are more susceptible to metabolic syndrome and its associated cardiovascular risk factors [19]; in particular, an inverse association between 25(OH)D levels and insulin resistance was recently shown in a cohort of apparently healthy male children, but not in female adolescents [44]. These findings suggest that female adolescents, even in course of vitamin D insufficiency, maintain their insulin sensitivity. The developmental and hormonal differences may explain, at least in part, the sex difference in the associations between serum 25(OH)D level and insulin resistance [44]. These sex differences may be reflected also during adulthood: a study on male subjects found a 2-fold risk of myocardial infarction (MI) in males who were vitamin D deficient compared with those in the sufficient range [45].

In conclusion, to our knowledge, this is the first study that suggests vitamin D as a potential discriminating factor useful to identify cardiovascular risk factors together with available health screening parameters in male children.

Author Contribution ADN, LDT, and CF wrote the manuscript, EDA, PS collected the data, MRP performed laboratory assessment, ADN analyzed the data.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest and financial relationships with any organizations that might have an interest in the submitted work. No external funding has been secured for this study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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