Research Article

The Effect of Vitamin D Supplementation on Cardiometabolic Risk Factors and Mental Health Symptoms in Obese Children

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Abstract

Objective: Decreased 25(OH)-vitamin D concentrations represent a risk factor for the development of cardiometabolic and mental health disorders in adults. We investigated the effect of vitamin D supplementation on cardiometabolic risk factors and mental health symptoms in overweight and obese children, and adolescents with vitamin D deficiency or insufficiency. Patients and Methods: Two hundred and twenty (n=220) overweight and obese children and adolescents [Mean age ± SEM: 10.24±0.17 years; BMI ± SEM: 26.69±0.28 kg/m2; BMI z-score ± SEM: 2.42±0.07; males: 114 (51.8%), females: 106 (48.2%)] with vitamin D deficiency or insufficiency were studied prospectively and randomly assigned into the supplementation (n=109) or the control group (n=111). Participants in the supplementation group received 50,000 IU cholecalciferol once a week for 6 weeks followed by a maintenance dose. Blood samples for determination of 25(OH)-vitamin D, endocrinologic and cardiometabolic parameters were obtained at baseline and 12 months later. Systolic and diastolic blood pressure was determined twice and the mean value was calculated. Mental health was assessed by questionnaires at baseline and 12 months later. Results: Subjects in the supplementation group had significantly lower BMI (p=0.010), hsCRP (p=0.048) and total cholesterol (p=0.015), and higher HDL (p<0.001) concentrations than the control group. In addition, they demonstrated significantly lower scores in anxiety, attention problems, aggressive behavior, externalizing problems, attention deficit hyperactivity problems and oppositional defiant problems than the control group. Conclusions: Vitamin D supplementation may prevent the development of cardiometabolic risk manifestations and may improve anxiety-related, externalizing (behavioral) and internalizing (emotional) problems in overweight and obese children and adolescents with vitamin D deficiency or insufficiency.

Introduction

Vitamin D is a steroid pro-hormone that plays a fundamental role in bone and mineral metabolism (Holick 2007). Through binding to its ubiquitously expressed vitamin D receptor (VDR), vitamin D also influences the homeostasis of virtually all tissues and organs; it is involved in several important biologic functions, such as the immune response, carbohydrate and lipid metabolism, and endothelial function (Bouillon *et al*. 2008), and its deficiency is associated with the development of skeletal, autoimmune, metabolic and cardiovascular diseases (Pilz *et al*. 2001).

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Obesity in childhood and adolescence represents a major health problem of our century, leads to obesity in adulthood, and is associated with significant morbidity, such as dyslipidemia, hypertension, insulin resistance, glucose intolerance and diabetes mellitus type 2 (Cunnungham *et al*. 2014, Crocker & Yanovski 2009). These cardiometabolic risk factors tend to cluster not only in adults but also in children, leading to metabolic syndrome. Vitamin D deficiency is highly prevalent among overweight and obese subjects (Al-Daghri *et al*. 2016, Turer *et al*. 2013) and is associated with the development of cardiometabolic risk factors in childhood, adolescence and adulthood (Aypak *et al*. 2014, Petersen *et al*.2015, Peterson *et al*. 2014). However, the findings of prospective clinical studies investigating the effect of vitamin D supplementation on cardiometabolic risk factors remain controversial (Pilz *et al*. 2016).

Vitamin D deficiency may also play an important role in the development of mental health problems. The above association is support ed by recent cross-sectional and longitudinal studies in children and adolescents (Grung *et al*. 2017, Lerner *et al*. 2018). Lower 25(OH)-vitamin D concentrations are associated with higher incidence of depression in childhood and adolescence. The association between 25(OH)-vitamin D concentrations and depression emerges in childhood and becomes stronger with greater time separation between assessment of 25(OH)-vitamin D concentrations and assessment of symptoms of depression (Tolppanen *et al*. 2012). In addition, recent large-scale studies in children and adolescents demonstrated inverse associations between emotional/behavioral symptoms (as reported by parents and adolescents themselves) and 25(OH)-vitamin D concentrations, with the strongest association noted in adolescence (Husmann *et al*. 2017). The same inverse associations have also been noted in children and adolescents with neurodevelopmental disorders, such as attention deficit hyperactivity (Mossin *et al*. 2017) and autism spectrum disorders (Saad *et al*. 2016). These findings are further supported by the beneficial effects of vitamin D supplementation on mental health problems (Focker *et al*. 2017), and in particular depressive symptoms (Grung *et al*. 2017, Hogberg *et al*. 2012), general well-being (Schafer *et al*. 2016) and autistic symptoms (Saad *et al*. 2016). However, there are not enough prospective clinical studies in young populations to support the hypothesis that vitamin D supplementation could alleviate mental health problems (Focker *et al*. 2017).

The aim of our study was to investigate prospectively the effect of vitamin D supplementation on cardiometabolic risk factors and mental health symptoms in overweight and obese children and adolescents with vitamin D deficiency or insufficiency.

Materials & Methods

Patients

Two hundred and twenty (n=220) overweight and obese children and adolescents [Mean age ± standard error of the mean (SEM): 10.24 ± 0.17 years, age range: 5.12 – 15.73 years; mean BMI ± SEM: 26.69 ± 0.28kg/m2; mean BMI z-score ± SEM: 2.42 ± 0.07; males: 114 (51.8%), females: 106 (48.2%); pubertal: 55 (25%), prepubertal: 165 (75%)], who had 25(OH)-vitamin D deficiency [serum 25(OH)-vitamin D concentrations < 20 ng/mL] or insufficiency [serum 25(OH)-vitamin D concentrations = 21-29 ng/mL] (Holick *et al*. 2011) were studied prospectively. All participants were recruited from a large number of children and adolescents who were followed-up at the 'Out -patient Clinic for the Prevention and Management of Overweight and Obesity in Childhood and Adolescence', 'Aghia Sophia' Children's Hospital, Athens, Greece, as part of an integrated weight-loss program. Patients were excluded from the study if they had a disorder affecting calcium and/or phosphate metabolism or if they were receiving medications affecting vitamin D metabolism. The study was approved by the 'Aghia Sophia' Children's Hospital Committee on the Ethics of Human Research. Written informed consent was obtained by the parents of all participants.

Methods

All participants were admitted to the Endocrine Unit early in the morning on the day of the study. A detailed medical history, complete physical examination, including Tanner pubertal staging (Marshall & Tanner 1969, Marshall & Tanner 1970), and standard anthropometric measurements were obtained by a single trained observer. Systolic and diastolic blood pressure was determined twice and the mean value was calculated. Furthermore, each participant underwent bioelectrical impedance analysis (BIA) (TANITA MC-780U Multi Frequency Segmental Body Composition Analyzer, Amsterdam, The Netherlands). Blood samples for baseline hematologic, biochemical and endocrinologic investigations were obtained

at 08:00h after a 12-hour overnight fast. Samples were centrifuged and separated immediately after collection, and were stored at –80^{o}C until assayed.

Subsequently, subjects were randomly assigned, using random numbers provided by an online generator (http:www.random.org) into the supplementation group (n=109, 56 with vitamin D deficiency and 53 with vitamin D insufficiency) or the control group (n=111). Subjects in the supplementation group received 50,000 IU cholecalciferol once weekly for 6 weeks. Serum 25(OH)-vitamin D concentrations were determined after 6 weeks and a 6-week high-dose cholecalciferol treatment was repeated if vitamin D sufficiency had not been achieved (Holick *et al*. 2011). Following high-dose cholecalciferol treatment, participants received a maintenance dose of cholecalciferol daily (obese: 2,000 IU; overweight: 1,600 IU). Participants were not blind to the study, however, no information was provided to them or their parents regarding the beneficial effects of vitamin D on cardiometabolic parameters or mental health symptoms, thereby excluding the possibility of bias at the end of the study. All subjects were assessed by a pediatric dietician, and were advised to follow a healthy diet and to avoid the consumption of foods fortified with vitamin D or calcium. Participants were also advised to maintain their usual physical activity levels for the whole duration of the study. Obese subjects were followed-up every month and overweight subjects every two months. At the end of the study, 12 months later, all subjects were re-evaluated as described at baseline assessment.

Mental health assessment

The following questionnaires were completed by all subjects at baseline and 1 year later. For the parent-completed questionnaires, the mental health assessments were done by the same parent at the beginning and at the end of the study. All the psychometric instruments used for the purpose of this study have been translated into Greek, standardized and validated proportionally.

Screen for Child Anxiety Related Disorders (SCARED)

The SCARED is a 41-item inventory rated on a 3 point Likert-type scale used to screen for childhood anxiety disorders (Birmaher *et al*. 1999). There are two versions; one asks questions to parents about their child and the other asks the same questions to the child directly. The instrument measures anxiety using five domains: panic/somatic, separation anxiety, generalized anxiety, social phobia and school phobia. The instrument has generally good internal consistency and discriminant validity (both between anxiety and depressive and disruptive disorders and within anxiety disorders) (Birmaher *et al*. 1999).

Child Depression Inventory (CDI)

The CDI is a widely used and self-administered questionnaire for children and adolescents assessing symptoms of depression (Kovacs 1985). It has good internal consistency, test-retest reliability, and sensitivity to change, but the evidence for discriminant validity is more limited. The child/adolescent is asked to select one of three sentences that best describes him/her during the last 2 weeks (0–2 point scale). An example of an item is as follows: “I am sad once in a while = 0”, “I am sad many times=1”, “I am sad all the time=2”.

Child Behavior Checklist (CBCL)

The CBCL is completed by parents and is used to detect internalizing and externalizing problems in children and adolescents (Achenbach 1991a).

Youth Self Report (YSR)

The YSR is a popular self-report instrument assessing adolescent internalizing and externalizing problems (Achenbach 1991b).

The CBCL includes 113 items and the YSR 118 items. The first pages of the CBCL record demographic information and ratings of positive behaviors, academic functioning, and social competence. The last two pages refer to behaviors and feelings that respondents rate on a 3-point scale, as ‘‘0=not true’’, ‘‘1=somewhat or sometimes true’’ or ‘‘2=very true or often true’’. Examples of items are the following: “Cries a lot”, “Too fearful or anxious”, “Runs away from home”. Ratings are combined to form eight narrow band Scales or clinical syndromes: Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behavior and Aggressive Behavior. There are two ‘broad band’ scales that combine several of the syndrome scales: Internalizing problems sums the Anxious/depressed, Withdrawn-depressed, and Somatic complaints scores; Externalizing problems combines Rule-breaking and Aggressive behavior. There is also a Total problems score, which is the sum of the scores of all the problem items. The instrument has generally good to excellent internal consistency (Cronbach's alpha), adequate inter-rater and test-retest reliabil-

ity. Both CBCL and YSR have been standardized in Greek nationwide populations (Roussos *et al*. 1999, Roussos *et al*. 2001).

Assays

The concentrations of glucose, Total cholesterol (t -CHOL), triglycerides (TG) and High Density Lipoprotein Cholesterol (HDL-C) were determined using the ADVIA 1800 Siemens analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Apolipoproteins A1 (ApoA1), B (ApoB) and Lipoprotein (a) (Lp(a)) concentrations were determined using latex particle-enhanced immunonephelometric assays on the BN ProSpec nephelometer (Dade Behring, Siemens Healthcare Diagnostics, Liederbach, Germany).

Insulin concentrations were determined using automated Electrochemiluminescense immunoassays (ECLIA) on the Cobas e411 analyzer (Roche Diagnostic, Mannheim, Germany). High sensitivity C-reactive protein (hs-CRP) were determined using automated chemiluminescence immunoassays on an IMMULITE 2000 Immunoassay System (Siemens Healthcare Diagnostics Products Ltd, UK). HbA1C was determined using reversed-phase cation exchange High Performance Liquid Chromatography (HPLC) on an automated glycohemoglobin analyzer HA-8160 (Arkray, Kyoto Japan).

The total 25(OH)-vitamin D was measured using Electro-chemiluminescence binding assay (ECLIA) on the Cobas e411 analyzer (Roche Diagnostic, Mannheim, Germany). The intra-assay CV was <6.5% at >15ng/mL and the inter-assay CV <11.5% at >15ng/mL, respectively, with a sensitivity of 3ng/mL. Serum intact PTH concentrations were determined by a sandwich Electrochemiluminescence immunoassay (ECLIA) using the Elecsys PTH assay (Roche Diagnostic, Mannheim, Germany). The intra-assay CVs were <7%, <5% and <6% at 11-75, 76-1000 and >1000pg/mL, respectively. The inter-assay were <9%, <6% and <7% at 11-75, 76-1000 and >1000pg/mL, respectively. The sensitivity of the assay was 1.2pg/mL.

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Normality was tested graphically according to histograms and Q-Q plots in order to determine whether or not to use parametric methods for the analysis of the sample data. Characteristics of participants were compared before and after the intervention by using independent samples t-test or Mann-Whitney U test according to normality. The comparison of pre- and post-intervention within each group was calculated by paired samples t-test or Wilcoxon signed rank test. Data analyses were performed using the SPSS statistical package version 23.0 (SPSS, Chicago, IL).

After assumptions were checked, between-group changes from baseline were analyzed for each outcome variable by mixed models, with time (within), group (between), and time x group interaction serving as independent variables after adjustment for potential confounders, such as age, gender and puberty (p_{time}, p_{group} and $p_{time\ x\ group}$ were calculated for all variables). Evaluable analyses were performed on outcome variables for participants completing the two visits (baseline and 12 months). Mixed regression analyses were carried out by using STATA statistical software version 12.0 (StataCorp, College Station, TX). All the aforementioned statistical tests were two-sided and were performed at a 0.05 significance level.
Insulin resistance was calculated using the homeostasis model assessment (HOMA) method as follows: HOMA-IR = [fasting glucose (mg/dL) x fasting insulin (mU/L)] / 405. The quantitative insulin sensitivity check index (QUICKI) derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose as follows: 1 / [log(fasting insulin μU/mL) + log(fasting glucose mg/dL)].

Results

The clinical characteristics of all subjects are listed in Table 1. Participants in the supplementation with vitamin D group had mean (± SEM) age of 10.36 (±0.23) years, the females were 59 (54.1%) and the males were 50 (45.9%); there were 26 (23.9%) pubertal and 83 (76.1%) pre-pubertal subjects at baseline, and 62 (56.9%) pubertal and 47 (43.1%) pre-pubertal at 12 months. Participants in the control group had mean age (± SEM) of 10.12±0.25 years, the females were 47 (42.3%) and the males were 64 (57.7%); there were 29 (26.1%) pubertal and 82 (73.9%) pre-pubertal subjects at baseline, and 58 (52.3%) pubertal and 53 (47.7%) pre-pubertal at 12 months. The intra-group and inter-group differences in all variables at the beginning and at the end of the study are shown in Tables 1, 2, 3 and 4.

25(OH)-vitamin D concentrations, BMI and body composition

Serum 25(OH)-vitamin D concentrations in the

Table 1. Clinical characteristics of all participants

Variables	Vitamin D group (N=109)			Control group (N=111)			$P_{between\_baseline}$	$P_{between\_12\ months}$	$P_{timex\ group}$
	Baseline	12 months	p_{within}	Baseline	12 months	p_{within}			
Weight (kg)	57.61 ± 1.79	66 ± 1.92	**<0.001***	58.36 ± 1.72	65.77 ± 1.72	**<0.001***	0.669•	0.832•	0.177
Height (cm)	145.78 ± 1.34	155.66 ± 1.76	**<0.001**	144.96 ± 1.49	155.61 ± 1.38	**<0.001**	0.683	0.980	0.120
BMI (kg/m^2)	26.32 ± 0.43	26.19 ± 0.91	0.272*	26.94 ± 0.37	26.87 ± 0.39	0.182*	0.059•	0.132•	0.592
SBP (mmHg)	111.30 ± 1.07	114.53 ± 1.15	**0.015**	110.71 ± 1.19	114.55 ± 1.10	**0.004**	0.713	0.992	0.300
DBP (mmHg)	64.30 ± 1.00	68.31 ± 0.78	**0.001**	63.08 ± 1	68.78 ± 0.93	**0.004**	0.391	0.694	0.277
MAP (mmHg)	79.96 ± 0.87	83.63 ± 0.76	**<0.001**	78.92 ± 0.90	84.04 ± 0.89	**0.001**	0.405	0.727	0.198
Waist (cm)	83.23 ± 1.12	85.64 ± 1.08	**0.006***	83.94 ± 1.30	86.51 ± 1.25	**0.011***	0.475•	0.480•	0.419
Hip (cm)	88.87 ± 1.42	94.50 ± 1.46	**<0.001***	90.44 ± 1.33	95.37 ± 1.32	**<0.001***	0.317•	0.985•	0.661
WHratio	0.94 ± 0.01	0.91 ± 0.07	**0.001***	0.93± 0.01	0.91 ± 0.01	**0.004***	0.837•	0.491•	0.999
Fat Mass (kg)	20.08 ± 0.97	22.05 ± 0.84	**0.003***	21.50 ± 0.82	22.74 ± 0.83	**0.012***	0.070•	0.435•	0.247
Muscle Mass (kg)	35.11 ± 1.11	40.69 ± 1.18	**<0.001***	35.34 ± 1.09	40.57 ± 1.14	**<0.001***	0.793•	0.994•	0.264
Bone Mass (kg)	1.91 ± 0.06	2.27 ± 0.08	**<0.001**	1.94 ± 0.05	2.48 ± 0.28	0.074	0.721	0.466	0.411

Abbreviations: BMI: Body Mass Index, DBP: Diastolic Blood Pressure, MAP: Mean Arterial Pressure, SBP: Systolic Blood Pressure, WHratio: Waist-to-Hip ratio; Variables are presented as mean ± standard error of the mean (SEM); p values were derived by paired samples t-test (p_{within}); *Wilcoxon signed rank test (p_{within}); independent samples t-test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); •Mann-Whitney U test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); mixed models adjusted for age, gender, puberty and time x group interaction term ($p_{time\ x\ group}$ computed by analysis of covariance); Statistically significant associations are shown in bold.

supplementation group increased from 19.19 ± 0.57 ng/mL at the beginning of the study to 24.59 ± 0.70 ng/mL 12 months later (p<0.001). An increase in serum 25(OH)-vitamin D concentrations from 19.35 ± 0.54 ng/mL to 21.81 ± 0.72 ng/mL (p=0.002) was observed in the control group. At the end of the study, a significant inter-group increase was noted (p=0.006), confirming the compliance of subjects with vitamin D supplementation during the study. Vitamin D concentrations rose significantly over time and the supplementation group had significantly higher concentrations than the control group (p=0.005).

A significant decrease in BMI (p<0.001) was observed over the study period and the supplementation group had significantly lower BMI than the control group (p=0.010). The waist to hip ratio demonstrated a significant intra-group decrease both for the supplementation and the control group (p=0.001 and p=0.004, respectively). Significant reductions in the concentrations of SGOT (p<0.001), SGPT (p=0.016) and γ-GT (p<0.001) were also noted over time. Muscle mass increased significantly during the study period in both groups (p<0.001), while there were no statistically significant changes in bone mass and fat mass between groups over time (Table 1).

Cardiometabolic risk factors

The supplementation group demonstrated a statistically significant decrease in total cholesterol concentrations (p=0.015). LDL cholesterol concentrations decreased significantly both in the supplementation and the control group (p<0.001) and over time (p=0.024). The HDL cholesterol concentrations showed a significant increase over time (p<0.001), and the supplementation group had significantly higher HDL concentrations than the control group at the end of the study (p=0.032). A borderline statistically significant decrease over time was noted in ApoA1 concentrations (p=0.050), while the concentrations of lipoprotein Lp(a) were reduced significantly in both groups (p=0.013 in the supplementation group and p=0.011 in the control group) (Table 2).

A significant decrease in hsCRP was observed over the study period in the supplementation group (p=0.048) but not in the control group, and the supplementation group had significantly lower hsCRP than the control group at the end of the study (p=0.049) (Table 2).

HbA1C concentrations decreased significantly from baseline to 12 months in both groups (p<0.001), while a significant decrease in the

Table 2. Biochemical and endocrinologic parameters in all participants

Variables	Reference Range	Vitamin D group (N=109)			Control group (N=111)			$P_{between\_baseline}$	$P_{between\_12\ months}$	$P_{time\ x\ group}$
		Baseline	12 months	p_{within}	Baseline	12 months	p_{within}			
Glucose (mg/dL)	**70-100**	78.10 ± 0.86	81.91 ± 0.90	**0.001**	79.22 ± 0.75	82.27 ± 0.73	**0.002**	0.328	0.759	0.601
Insulin (µUI/mL)	**2.5-25**	15.95 ± 0.92	16.78 ± 0.94	**0.032***	16.14 ± 0.88	16.28 ± 0.84	0.623*	0.759•	0.183•	0.065
HbA1c (%)	**3.8-5.4**	5.30 ± 0.02	5.21 ± 0.02	**<0.001**	5.31 ± 0.02	5.23 ± 0.03	**<0.001**	0.778	0.433	0.284
HOMA-IR	**-**	3.09 ± 0.19	3.59 ± 0.23	**0.002***	3.19 ± 0.18	3.40 ± 0.19	0.771*	0.544•	0.288•	0.126
QUICKI	**-**	0.34 ± 0.005	0.32 ± 0.002	**0.003***	0.33 ± 0.003	0.32 ± 0.003	0.308*	0.544•	0.288•	0.191
SGOT (U/L)	**10-60**	24.66 ± 0.56	20.42 ± 0.52	**<0.001**	24.54 ± 0.58	20.86 ± 0.53	**<0.001**	0.881	0.550	0.797
SGPT (U/L)	**5-45**	22.20 ± 1.07	18.18 ± 0.72	**<0.001***	22.87 ± 1.21	18.60 ± 0.77	**<0.001***	0.728•	0.784•	0.698
γ-GT (U/L)	**2-50**	15.48 ± 0.56	15.12 ± 0.62	0.939	14.94 ± 0.58	13.07 ± 0.48	**<0.001**	0.498	0.219	0.632
Cholesterol (mg/dL)	**120-200**	160.69 ± 2.32	155.24 ± 2.51	**0.015**	154.77 ± 2.58	151.53 ± 2.34	0.134	0.090	0.281	0.590
Triglycerides (mg/dL)	**30-130**	80.39 ± 3.89	77.70 ± 4.72	0.146*	78.85 ± 3.67	73.08 ± 3.34	**0.045***	0.575•	0.703•	0.418
HDL (mg/dL)	**35-65**	50.81 ± 1.21	54.50 ± 1.18	**<0.001**	47.76 ± 0.94	53.41 ± 1.11	**<0.001**	0.081	0.497	0.264
LDL (mg/dL)	**<130**	93.84 ± 2.16	86.68 ± 2.07	**<0.001**	91.23 ± 2.25	84.20 ± 2.02	**<0.001**	0.402	0.392	0.906
ApoA1 (mg/dL)	**115-210**	143.20 ± 1.86	138.35 ± 2.09	**0.009**	141.18 ± 1.65	138.21 ± 2.06	0.100	0.419	0.961	0.720
ApoB (mg/dL)	**55-135**	74.49 ± 1.40	74.34 ± 1.56	0.777	73.35 ± 1.61	72.11 ± 1.47	0.317	0.598	0.297	0.874
Lpa (mg/dL)	**<30**	16.30 ± 2.27	15.29 ± 2.22	**0.013***	15.93 ± 2.25	15.11 ± 2.14	**0.011***	0.580•	0.644•	0.880
Calcium (mg/dL)	**8.2-11.2**	9.94 ± 0.04	11.10 ± 0.38	0.405	9.93 ± 0.04	9.69 ± 0.04	**<0.001**	0.884	0.305	0.896
PTH (pg/mL)	**2.5-37**	34.91 ± 1.14	37.12 ± 1.33	0.109*	33.92 ± 1.25	39.36 ± 1.18	**<0.001***	0.591•	0.125•	0.219
Total 25(OH)-Vitamin D (ng/mL)	**>30**	19.19 ± 0.57	24.59 ± 0.70	**<0.001**	19.35 ± 0.54	21.81 ± 0.72	**0.002**	0.839	**0.006**	**0.011**
hsCRP (mg/dL)	**<0.3**	0.36 ± 0.09	0.15 ± 0.01	**0.048***	0.31 ± 0.04	0.23 ± 0.03	0.074*	0.083•	**0.049•**	0.554

Abbreviations: ApoA1: Apolipoprotein A1, ApoB: Apolipoprotein B, HbA1c: Haemoglobin A1c, HDL: High Density Lipoprotein, HOMA -IR: Homeostatic Model Assessment- Insulin Resistance, LDL: Low Density Lipoprotein, Lpa: lipoprotein a, PTH: Parathohormone, QUICKI: Quantitative insulin sensitivity check index, SGOT: Serum Glutamil Oxaloacetic Transaminase, SGPT: Serum Glutamic Pyruvic Transaminase, γ-GT: Gamma-Glutamyl Transferase. Variables are presented as mean ± standard error of the mean (SEM); p values were derived by paired samples t-test (p_{within}); *Wilcoxon signed rank test (p_{within}); independent samples t-test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); •Mann-Whitney U test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); mixed models adjusted for age, gender, puberty and time x group interaction term ($p_{time\ x\ group}$ computed by analysis of covariance); Statistically significant associations are shown in bold.

QUICKI index was noted only in the supplementation (p=0.003) but not in the control group. Insulin concentrations increased in both groups, however, a significant increase was observed only in the supplementation group. No statistically significant differences were noted in HOMA IR index. At the end of the study, there were no significant differences in insulin, HbA1C, HOMA-IR, QUICKI, systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure

Table 3. Scores of Youth Self Report (YSR), Screen for Child Anxiety Related Disorders (SCARED) and Child Depression Inventory (CDI) questionnaires

Variables	Vitamin D group (N=109)			Control group (N=111)			$P_{between\_baseline}$	$P_{between\_12\ months}$	$P_{time\ x\ group}$
	Baseline	12 months	p_{within}	Baseline	12 months	p_{within}			
Competence Scale T Scores									
Activities	44.35 ± 1.70	38.87 ± 1.50	**0.015**	42.45 ± 1.32	40.80 ± 1.37	**0.040**	0.380	0.342	0.248
Social	45.90 ± 1.49	45.62 ± 1.14	0.848*	46.49 ± 1.40	44.35 ± 1.37	0.074*	0.677•	0.617•	0.186
Total Competence	44.63 ± 1.75	39.62 ± 1.46	0.071*	43.81 ± 1.33	41.61 ± 1.47	**0.018***	0.703•	0.190•	0.439
Syndrome Scale T Scores									
Anxious/ Depressed	56.28 ± 1.16	54.83 ± 0.91	0.959*	56.61 ± 0.94	56.57 ± 1.14	0.588*	0.433•	0.409•	0.702
Withdrawn/ Depressed	54.95 ± 0.96	54.09 ± 0.79	0.681*	55.25 ± 1.31	53.59 ± 0.92	0.670*	0.750•	0.269•	0.982
Somatic Complaints	55.14 ± 1.11	53.13 ± 0.66	0.277*	56.80 ± 1.08	54.42 ± 0.89	0.087*	0.299•	0.626•	0.344
Social Problems	55 ± 0.99	54.11 ± 0.90	0.977*	56.68 ± 1.19	55.06 ± 0.97	0.129*	0.283•	0.352•	0.405
Thought Problems	53.47 ± 0.70	52.30 ± 0.52	**0.022***	54.43 ± 1.08	53.71 ± 0.94	0.637*	0.780•	0.394•	0.994
Attention Problems	53.63 ± 0.75	53.34 ± 0.80	0.488*	54.73 ± 1.03	54.18 ± 0.94	0.843*	0.633•	0.897•	0.788
Rule-Breaking Behavior	52.26 ± 0.58	53.06 ± 0.57	0.114*	53.20 ± 0.71	53.86 ± 0.88	0.323*	0.460•	0.444•	0.840
Aggressive Behavior	53.65 ± 0.81	53.57 ± 0.70	0.484*	55.11 ± 1.27	54.69 ± 1.05	0.720*	0.481•	0.543•	0.832
Internalizing, Externalizing and Total Problems T Scores									
Internalizing Problems	51.81 ± 1.75	48.47 ± 1.63	0.648*	52.91 ± 1.55	49.53 ± 1.90	0.559*	0.628•	0.682•	0.906
Externalizing Problems	48.84 ± 1.25	48.91 ± 1.28	0.560	50 ± 1.55	49.78 ± 1.54	0.540	0.561	0.668	0.856
Total Problems	51.05 ± 1.46	48.17 ± 1.51	0.584	52.23 ± 1.59	50.37 ± 1.59	0.643	0.586	0.320	0.545
DSM-Oriented Scales T Scores									
Affective Problems	56.33 ± 0.98	54.57 ± 0.82	0.866*	56.53 ± 1.13	55.18 ± 1.14	0.831*	0.979•	0.745•	0.680
Anxiety Problems	55.30 ± 1	53.53 ± 0.83	0.383*	55.74 ± 1	55.47 ± 0.95	0.764*	0.732•	0.105•	0.537
Somatic Problems	54.84 ± 1	53.30 ± 0.65	0.499*	56.05 ± 0.94	53.92 ± 0.78	0.122*	0.281•	0.788•	0.347
Attention Deficit/ Hyperactivity Problems	53.49 ± 0.65	53.06 ± 0.57	0.475*	54.60 ± 0.98	53.92 ± 0.79	0.583*	0.877•	0.922•	0.577
Oppositional Defiant Problems	53.86 ± 0.77	53.87 ± 0.77	0.206*	54.44 ± 0.98	54.22 ± 0.77	0.613*	0.699•	0.725•	0.911
Conduct Problems	53.12 ± 0.72	53.30 ± 0.67	0.618*	54.49 ± 1	54.20 ± 0.93	0.767*	0.403•	0.903•	0.818
Scales & Positive Qualities Scale T Scores									
Obsessive-Compulsive Problems	55.72 ± 0.97	53.23 ± 0.64	**0.008***	55.77 ± 0.97	55 ± 1	0.663*	0.898•	0.344•	0.497
Post-traumatic Stress Problems	55.16 ± 1.10	53.98 ± 0.76	0.270*	55.37 ± 1	54.94 ± 0.99	0.583*	0.395•	0.532•	0.816
Positive Qualities	50.40 ± 1.77	52.64 ± 1.56	**0.045***	50.91 ± 2.09	50.02 ± 1.69	0.990*	0.456•	0.201•	0.127
SCAREDChild	2.07 ± 0.17	1.63 ± 0.20	0.100*	2.13 ± 0.21	2.04 ± 0.17	0.179*	0.734•	**0.044•**	0.141
CDI	7.64 ± 0.56	6.85 ± 0.75	0.659*	7.91 ± 0.60	8.32 ± 0.69	0.706*	0.717•	0.057•	0.183

Variables are presented as mean ± standard error of the mean (SEM); p values were derived by paired samples t-test (p_{within}); *Wilcoxon signed rank test (p_{within}); independent samples t-test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); •Mann-Whitney U test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); mixed models adjusted for age, gender, puberty and time x group interaction term ($p_{time\ x\ group}$ computed by analysis of covariance); Statistically significant associations are shown in bold.

Table 4. Scores of Child Behavior Checklist (CBCL) and Screen for Child Anxiety Related Disorders (SCARED) questionnaires for Parents

Variables	Vitamin D group (N=109)			Control group (N=111)			$P_{between\_baseline}$	$P_{between\_12\ months}$	$P_{time\ x\ group}$
	Baseline	12 months	p_{within}	Baseline	12 months	p_{within}			
Competence Scale T Scores									
Activities	39.36 ± 1.10	39.51 ± 1.18	0.952*	37.90 ± 1.06	37.46 ± 1	0.886*	0.296•	0.142•	0.715
Social	46.28 ± 1.01	45.66 ± 1.20	0.639*	42.59 ± 1.17	43.87 ± 1.04	0.198*	**0.016•**	0.173•	0.701
Total Competence	41.70 ± 1.25	41.45 ± 1.28	0.990*	38.95 ± 1.18	38.71 ± 0.99	0.628*	0.146•	0.052•	0.794
Syndrome Scale T Scores									
Anxious/ Depressed	57.59 ± 0.97	55.93 ± 0.86	0.088*	56.35 ± 0.80	56.05 ± 0.89	0.427*	0.542•	0.809•	0.569
Withdrawn/ Depressed	57 ± 1.04	55.06 ± 0.79	0.136*	56.06 ± 0.83	53.92 ± 0.68	0.458*	0.696•	0.440•	0.296
Somatic Complaints	56.77 ± 0.82	54.75 ± 0.86	0.111*	56.18 ± 0.78	54.38 ± 0.71	**0.032***	0.766•	0.979•	0.862
Social Problems	57.15 ± 0.97	55.04 ± 0.82	**0.014***	56.91 ± 0.83	55.70 ± 0.84	0.645*	0.723•	0.277•	0.397
Thought Problems	54.39 ± 0.64	53.61 ± 0.75	0.175*	54.35 ± 0.78	53.86 ± 0.59	0.496*	0.381•	0.074•	**0.009**
Attention Problems	54.05 ± 0.62	52.36 ± 0.46	**0.018***	55.18 ± 0.75	54.03 ± 0.56	0.340*	0.365•	**0.003•**	0.431
Rule-Breaking Behavior	54.68 ± 0.69	52.99 ± 0.52	**0.002***	54.78 ± 0.67	54.03 ± 0.69	0.909*	0.749•	0.468•	0.865
Aggressive Behavior	54.39 ± 0.69	52.69 ± 0.51	0.084*	55.68 ± 0.78	54.86 ± 0.74	0.910*	0.285•	**0.019•**	0.499
Internalizing, Externalizing and Total Problems T Scores									
Internalizing Problems	55.39 ± 1.36	51.24 ± 1.43	0.057*	54.43 ± 1.22	51.20 ± 1.27	0.135*	0.724•	0.967•	0.826
Externalizing Problems	51.42 ± 1.39	47.10 ± 1.43	**0.012**	50.54 ± 1.67	50.97 ± 1.65	0.827	0.482	**0.029**	0.118
Total Problems	53.47 ± 1.54	46.60 ± 1.95	**0.002**	52.49 ± 1.76	51.38 ± 1.77	0.611	0.788	0.059	0.177
DSM-Oriented Scales T Scores									
Affective Problems	58.36 ± 0.90	55.70 ± 0.87	**0.014***	57.55 ± 0.89	56.67 ± 0.84	0.307*	0.462•	0.242•	**0.025**
Anxiety Problems	56.68 ± 0.91	56.16 ± 0.89	0.468*	56.29 ± 0.82	56.52 ± 0.91	0.340*	0.918•	0.640•	0.922
Somatic Problems	56.03 ± 0.83	54.15 ± 0.83	0.098*	55.65 ± 0.79	53.91 ± 0.70	**0.006***	0.785•	0.915•	0.852
Attention Deficit/ Hyperactivity Problems	54 ± 0.65	52.40 ± 0.52	**0.018***	55.23 ± 0.78	54 ± 0.65	0.053*	0.353•	**0.016•**	0.773
Oppositional Defiant Problems	54.42 ± 0.65	52.69 ± 0.44	0.073*	55.08 ± 0.69	54.75 ± 0.71	0.688*	0.547•	**0.046•**	0.337
Conduct Problems	54.56 ± 0.67	52.46 ± 0.49	**0.006***	54.88 ± 0.73	53.67 ± 0.71	0.627*	0.761•	0.696•	0.999
Scales T Scores									
Sluggish Cognitive Tempo	54.40 ± 0.67	53.12 ± 0.55	**0.009***	54.82 ± 0.78	53.84 ± 0.67	0.837*	0.960•	0.568•	0.238
Obsessive-Compulsive Problems	55.56 ± 0.82	54.69 ± 0.78	0.496*	54.63 ± 0.81	54.64 ± 0.74	0.426*	0.206•	0.395•	0.471
Post-traumatic Stress Problems	56.03 ± 0.93	54.18 ± 0.72	0.437*	54.72 ± 1.03	54.70 ± 0.71	0.828*	0.853•	0.571•	0.722
SCARED_Parent	1.70 ± 0.16	1.05 ± 0.20	**0.010•**	1.74 ± 0.16	1.35 ± 0.18	**0.018•**	0.967•	0.106•	**0.022**

Variables are presented as mean ± standard error of the mean (SEM); p values were derived by paired samples t-test (p_{within}); *Wilcoxon signed rank test (p_{within}); independent samples t-test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); •Mann-Whitney U test ($p_{between\_baseline}$ and $p_{between\_12\ months}$); mixed models adjusted for age, gender, puberty and time x group interaction term ($p_{time\ x\ group}$ computed by analysis of covariance); Statistically significant associations are shown in bold.

(MAP) between the supplementation and the control group (Tables 1 and 2).

Mental health assessment

The Youth Self Report (YSR) showed significant decreases in social scale (p=0.038), thought problems (p=0.028) and affective problems (p=0.013) scores over the study period. The supplementation group demonstrated a significant decrease in activities (p=0.015), thought problems (p=0.022) and obsessive-compulsive problems (p=0.008), and a significant increase in positive qualities (p=0.045) scores, while the control group demonstrated a significant decrease in activities (p=0.040) and total competence (p=0.018) scores. The supplementation group demonstrated a significantly lower score in the SCARED questionnaire for children than the control group (p=0.044), while no differences between the two groups were noted in the Child Depression Inventory (CDI) scores at the end of the study (Table 3).

Both groups had significantly lower scores in the SCARED questionnaire for parents, while a significant reduction in this score was noted in the supplementation group over time (p=0.022). The Child Behavior Checklist (CBCL) indicated significant decreases in somatic complaints (p=0.009) and somatic problems (p=0.003) scores over the 12 months in the supplementation group. In addition, the supplementation group demonstrated significant decreases in thought (p=0.009) and affective problems (p=0.025) compared to the control group over time. Subjects in the supplementation group showed statistically significant decreases in social problems (p=0.014), attention problems (p=0.018), rule-breaking behavior (p=0.002), externalizing problems (p=0.012), total problems (p=0.002), affective problems (p=0.014), attention deficit hyperactivity problems (p=0.018), conduct problems (p=0.006) and sluggish cognitive tempo (p=0.009) scores, while subjects in the control group showed significant decreases in somatic complaints (p=0.032) and somatic problems (p=0.006) scores. Finally, participants in the supplementation group had significantly lower scores in attention problems (p=0.003), aggressive behavior (p=0.019), externalizing problems (p=0.029), attention deficit hyperactivity problems (p=0.016) and oppositional defiant problems (p=0.046) than participants in the control group at the end of the study (Table 4).

Discussion

In this study, we investigated the effect of vitamin D supplementation on cardiometabolic risk factors and mental health symptoms in overweight and obese children and adolescents with vitamin D deficiency or insufficiency. We demonstrated that vitamin D supplementation according to the most recent guidelines of the Endocrine Society (Holick *et al*. 2011) resulted in a significant increase in serum 25(OH)-vitamin D concentrations at the end of the 12-month period in the supplemental group compared with the control group. The increase in serum 25(OH)-vitamin D concentrations was associated with a significant decrease in BMI, hsCRP and total cholesterol concentrations, and a significant increase in HDL cholesterol concentrations in the supplementation group compared with the control group. In addition, children and adolescents in the supplementation group demonstrated significantly lower scores in anxiety, attention problems, aggressive behavior, externalizing problems, attention deficit hyperactivity problems and oppositional defiant problems compared with their control counterparts. These findings indicate that vitamin D supplementation may prevent the development of cardiometabolic risk factors and mental health symptoms in overweight and obese children and adolescents with vitamin D deficiency or insufficiency.

A large body of evidence has demonstrated increased prevalence of vitamin D deficiency or insufficiency in overweight and obese children and adolescents (Alemzadeh *et al*. 2008, Censani *et al*. 2018) and an inverse relation between adiposity and serum 25(OH)-vitamin D concentrations (Parikh *et al*. 2004). Although vitamin D supplementation in adults has been shown to result in a significant decrease in BMI (Al-Daghri *et al*. 2012), the findings of the few similar studies in children and adolescents are not consistent (Nader *et al*. 2014, Belenchia *et al*. 2013). In our study, we demonstrated a significant decrease in BMI in the supplementation group compared with the control group, findings that are likely to arise as a result of our significantly larger sample size, the longer duration of vitamin D supplementation in our study, and the use of higher vitamin D doses according to the Endocrine Society guidelines (Holick *et al*. 2011).

We also demonstrated significantly lower hsCRP and total cholesterol concentrations, and significantly higher HDL cholesterol concentrations in the supplementation group than the con-

trol group at the end of the study. No significant difference in triglyceride concentrations was noted between groups. These results concur with those of several epidemiological studies in both children and adults (Kim *et al*. 2008, Forrest & Stuhldreher 2011, Kelishadi *et al*. 2014), which indicate an inverse relation between serum 25(OH)-vitamin D concentrations and the atherogenic lipid profile. Two randomized controlled trials in obese adolescents failed to detect similar changes in the lipid profile following vitamin D supplementation, however, the duration of those studies was shorter than our study and the increase in 25(OH)-vitamin D concentrations following supplementation was modest (Nader *et al*. 2014, Javed *et al*. 2015). Once again, the significant improvement in hCRP, total and HDL cholesterol concentrations following vitamin D supplementation in our study is most likely the result of our larger sample size, longer duration of vitamin D supplementation and higher doses of vitamin D supplementation (Holick *et al*. 2011).

The relationship of vitamin D deficiency with insulin resistance, diabetes mellitus type 2 and metabolic syndrome was first documented in obese adults. Similar observational studies have been conducted subsequently in obese children and adolescents, with the majority of them demonstrating an association between serum 25 (OH)-vitamin D concentrations and indices of insulin sensitivity/resistance (Peterson *et al*. 2014). However, only few clinical studies have examined this relationship in the pediatric population. Our findings of significantly lower HbA1C from baseline to 12 months in both groups, and significantly lower QUICKI in the supplementation group at the end of the study concur with the findings of previous studies that demonstrated an improvement in insulin sensitivity and cardiometabolic risk factors in obese children and adolescents (Belenchia *et al*. 2013, Kelishadi *et al*. 2014). No statistically significant differences in plasma glucose and serum insulin concentrations, and HOMA-IR index were observed between the two groups at the end of our study.

The most powerful evidence regarding the effect of vitamin D on cardiometabolic risk factors comes from studies on hypertension. Cross-sectional studies in adults (Judd *et al*. 2008, Martins *et al*. 2007, Scragg *et al*. 2007) reported a negative association between serum 25(OH)-vitamin D concentrations and the risk of hypertension. However, the findings of observational studies and randomized controlled trials in children and adolescents provide conflicting results (Kelishadi *et al*. 2014, Ashraf *et al*. 2011). Similar to previous studies (Ashraf *et al*. 2011), we did not demonstrate significant differences in systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) between the supplementation groups and the control group at the end of the study. The increase in blood pressure parameters at the end of the study compared to baseline in both groups may be attributed to the gradual increase in normal blood pressure during childhood and adolescence.

Vitamin D deficiency has been largely associated with depressive symptoms in epidemiological studies in adults (Brouwer-Brolasma *et al*. 2013, Jaddou *et al*. 2012), possibly revealing a link between low serum 25(OH)-vitamin D concentrations and depressive disorder (Milaneschi *et al*. 2014). Studies in children and adolescents, also suggest a strong inverse relation between serum 25(OH)-vitamin D concentrations and attention deficit and hyperactivity disorders (Kamal *et al*. 2014). Our study demonstrated that vitamin D supplementation resulted in a significant decrease in anxiety and affective symptoms, attention problems, and externalizing and conduct problems in the supplementation group compared with the control group. These findings concur with those of previous studies and underline the beneficial effects of vitamin D supplementation on mental health parameters (Grung *et al*. 2017, Husmann *et al*. 2017, Hogberg *et al*. 2012, Schafer *et al*. 2016).

To our knowledge, this is the first prospective clinical study that investigated the effect of vitamin D supplementation in overweight and obese children and adolescents with vitamin D deficiency or insufficiency, where the duration of the vitamin D supplementation was 12 months. Our sample size, which was significantly larger than other similar studies in pediatric and/or adolescent population, increases the statistical power of our study and the validity of our results. Another important strength of our study was the fact that the determination of substitution and maintenance doses was based on the Endocrine Society recommendations on vitamin D supplementation (Holick *et al*. 2011). Furthermore, vitamin D concentrations were determined after 6 weeks of treatment, and vitamin D supplementation was administered again in subjects that sufficient concentrations of vitamin D had not been achieved.

Although this research study was carefully conducted, there were a few limitations. The study

participants joined the same weight-loss program from the beginning of the study that could be partly responsible for the improvement noted in various cardiometabolic risk factors. However, it is worth underscoring that subjects in the supplementation group demonstrated significantly lower BMI, hsCRP and total cholesterol concentrations, and higher HDL cholesterol concentrations than those in the control group. The total sun exposure, which is the main source of vitamin D for all participants, was not determined. Finally, no placebo was administered, therefore both participants and researchers were not blind to the intervention.

We conclude that vitamin D supplementation may have a beneficial effect on BMI, hsCRP, total cholesterol and HDL cholesterol concentrations in overweight and obese children and adolescents with vitamin D deficiency or insufficiency, and may prevent the development of cardiometabolic risk manifestations in these subjects. Furthermore, vitamin D supplementation resulted in significantly lower scores in anxiety, attention problems, aggressive behavior, externalizing problems, attention deficit hyperactivity problems and oppositional defiant problems, indicating that it may improve anxiety-related, externalizing (behavioral) and internalizing (emotional) problems in overweight and obese children and adolescents. Future studies in obese children and adolescents are required to provide further evidence on the role of vitamin D supplementation in preventing or reversing the development of cardiometabolic risk factors and mental health symptoms.

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Statement of Ethics

The study was approved by the 'Aghia Sophia' Children's Hospital Committee on the Ethics of Human Research. Written informed consent was obtained by the parents of all participants.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

Authors' Contributions

Christos Giannios (CG) and Evangelia Charmandari (EC) designed the research and wrote the protocol. CG, Ioanna Farakla (IF), Georgios Papadopoulos (GP), Sofia Gennitsaridi (SG) and Sofia Karampatsou (SK) collected the samples and performed the measurements. Ifigeneia Papageorgiou (IP) performed the statistical analysis and interpretation of the data. Penelope Papadopoulou and Gerasimos Kolaitis performed the analysis and interpretation of the psychometric data. EC supervised the research and helped with data interpretation. Penio Kassari (PK) coordinated the research. CG wrote the manuscript. Nicolas C. Nicolaides (NCN) and EC edited and reviewed the manuscript.

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