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Vitamin D status and cardiometabolic risk markers in young Swedish children: a double-blind randomized clinical trial comparing different doses of vitamin D supplements

Inger Öhlund,¹ Torbjörn Lind,¹ Olle Hernell,¹ Sven-Arne Silfverdal,¹ Per Liv,² and Pia Karlsland Åkeson³

¹Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden; ²Section of Sustainable Health, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden; and ³Department of Clinical Sciences, Pediatrics, Lund University, Lund, Sweden

ABSTRACT

Background: Observational studies have linked low vitamin D status to unfavorable cardiometabolic risk markers, but double-blinded vitamin D intervention studies in children are scarce.

Objectives: The aim was to evaluate the effect of different doses of a vitamin D supplement on cardiometabolic risk markers in young healthy Swedish children with fair and dark skin.

Methods: Cardiometabolic risk markers were analyzed as secondary outcomes of a double-blind, randomized, milk-based vitamin D intervention trial conducted during late fall and winter in 2 areas of Sweden (latitude 63°N and 55°N, respectively) in both fair- and dark-skinned 5- to 7-y-old children. During the 3-mo intervention, 206 children were randomly assigned to a daily milk-based vitamin D₃ supplement of either 10 or 25 µg or placebo (2 µg; only at 55°N). Anthropometric measures, blood pressure, serum 25-hydroxyvitamin D [25(OH)D], total cholesterol, HDL cholesterol, apoA-I, apoB, and C-reactive protein (CRP) were analyzed and non-HDL cholesterol calculated at baseline and after the intervention.

Results: At baseline, serum 25(OH)D was negatively associated with systolic and diastolic blood pressure ($\beta = -0.194$; 95% CI: $-0.153, -0.013$; and $\beta = -0.187$; 95% CI: $-0.150, -0.011$, respectively). At follow-up, there was no statistically significant difference in any of the cardiometabolic markers between groups.

Conclusions: We could not confirm any effect of vitamin D supplementation on serum lipids, blood pressure, or CRP in healthy 5- to 7-y-old children. The study was registered at clinicaltrials.gov (NCT01741324). *Am J Clin Nutr* 2020;111:779–786.

Keywords: serum lipids, blood pressure, latitude, skin color, vitamin D supplement

Introduction

Low vitamin D status has been associated with the metabolic syndrome and with cardiovascular diseases (CVDs) in adults (1), and higher concentrations of serum 25-hydroxyvitamin D [25(OH)D] have been associated with a substantial reduction in CVD (2, 3). Cardiometabolic risk markers for disease in adulthood (e.g., unfavorable lipid profile, high blood pressure, and overweight) may originate already in early life (4–6).

Few studies, mostly cross-sectional or observational, have examined associations between serum 25(OH)D and cardiometabolic risk markers in children and adolescents (7–13). In a Danish observational study in healthy children aged 8–11 y (13), higher 25(OH)D was associated with lower diastolic blood pressure, lower total cholesterol (TC), and LDL cholesterol.

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Supplemental Table 1 is available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

Address correspondence to IO (e-mail: inger.ohlund@umu.se).

Abbreviations used: BMIZ, BMI z score; CVD, cardiovascular disease; CRP, C-reactive protein; ITT, intention-to-treat; RCT, randomized controlled trial; TC, total cholesterol; 25(OH)D, 25-hydroxyvitamin D.

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Similarly, in the Canadian TARGETs Kids study (11), higher 25(OH)D was associated with a more favorable lipoprotein profile—in particular, non-HDL cholesterol. However none of these results were in accordance with a recent randomized controlled trial (RCT) in healthy Danish children aged 4–8 y (14). A large intervention study on vitamin D supplements in adolescent girls in Iran resulted in improved 25(OH)D, diastolic blood pressure, LDL cholesterol, and TC, but not systolic blood pressure, triglycerides, or HDL cholesterol (15). On the contrary, neither in healthy adolescents in the United Kingdom (16) nor in obese adolescents in the United States (17) did supplementation with vitamin D affect markers of cardiometabolic risk and the Cardiovascular Risk in Young Finns Study found associations between subclinical atherosclerosis in adults and low concentrations of 25(OH)D in childhood but not in adulthood (18).

Even if some findings (11, 13, 18) point towards associations between cardiometabolic risk factors and vitamin D status, the causal relation remains unclear. One systematic review revealed a weak association between higher concentrations of 25(OH)D and a more favorable lipid profile, but those conclusions were based on both contradictory and nonsignificant associations (19). Another review suggested that low vitamin D status may be a marker of illness rather than a cause of disease. The authors emphasized that many studies had a follow-up time that was too short, as well as vitamin D supplementation that was too low dose or of too short a duration to reveal a difference and thus concluded that better-designed trials are needed to draw firm conclusions (20).

Insufficient vitamin D status in preschool children and adolescents in an area at high latitude (21, 22), and controversy about recommended intakes of vitamin D (23, 24), prompted us to perform a double-blind RCT in young Swedish children. Vitamin D supplements of 10 or 25 $\mu\text{g}/\text{d}$ achieved the primary outcome of 25(OH)D concentrations ≥ 50 nmol/L in almost all children (25, 26). In that trial, data on serum lipids, serum C-reactive protein (CRP), blood pressure, and anthropometric measures were also collected as secondary outcomes. This gave us an opportunity to study relations between vitamin D supplementation and cardiometabolic risk markers. We hypothesized that a milk-based vitamin D₃ supplement provided to Swedish children aged 5–7 y, resulting in increased 25(OH)D concentrations > 50 nmol/L, might affect cardiometabolic risk markers. The objective of this article was to examine baseline associations between vitamin D status and cardiometabolic risk markers, and to evaluate the effect of milk-based vitamin D₃ supplements on these markers in young Swedish children after a 3-mo intervention.

Methods

Study cohort and data collection

This trial used secondary outcomes from a double-blind, randomized, milk-based vitamin D intervention trial (registered at clinicaltrials.gov; identifier: NCT01741324) reported in detail elsewhere (25–27). Briefly, the study was conducted in the northern (Umeå, latitude 63°N) and southern (Malmö, latitude 55°N) areas of Sweden in 206 children aged 5–7 y, half with fair and half with dark skin (Figure 1). The skin color of each child was visually classified by the parents together with a research nurse as “fair skin” or “dark skin” according to Fitzpatrick

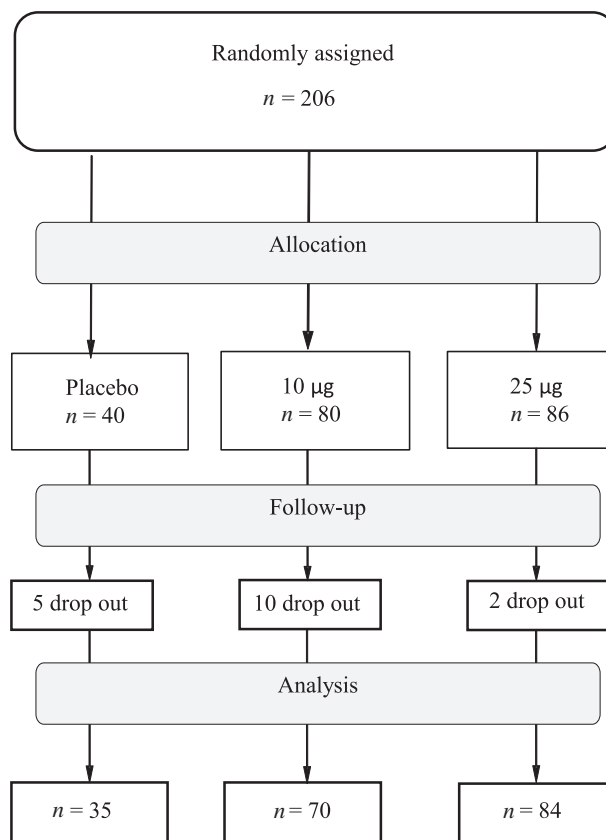


FIGURE 1 Flow diagram of Swedish 5- to 7-y-old study children, randomly assigned to milk-based vitamin D₃ supplements [10 or 25 μg or placebo (2 μg)] during 3 winter months, followed from baseline until analysis.

(28). The children were randomized by a computer-generated scheme prepared by one of the authors and assigned by nurses to a milk-based vitamin D₃ supplement of either 10 or 25 μg (both sites) or the standard fortified milk as placebo (2 μg ; in Malmö only). Due to earlier vitamin D studies in Umeå, parents were expected to condition their participation on active vitamin D supplements for their child. The milk was lactose-free, low-fat (0.5 g/100 mL), ultra-high-temperature treated, in packages of 200 mL for consumption of 1 dose daily for 3 mo (90 packages). The placebo milk was the regular milk from Valio Ltd containing 2 $\mu\text{g}/\text{package}$, whereas in Sweden, low-fat milk but not full-fat milk was fortified at that time. The children drank the study milk instead of their ordinary milk once per day. Total intake of vitamin D from the study product and from the diet was calculated. Packages were labeled A, B, and C, and research staff and parents were blinded until the intervention was completed. Inclusion criteria were healthy children aged 5–7 y, with no known gastrointestinal disease or cow-milk allergy. The intervention was conducted during late fall and winter (November 2012 to March 2013), when cutaneous synthesis of vitamin D is negligible in Sweden.

Of the 206 included children, 189 completed the blood sampling at the follow-up visit. Sixteen of the 17 dropouts were not willing or not able to participate in the follow-up blood sampling, or disliked the study product from the study start, whereas 1 child was unable to carry the study products when travelling abroad (25, 26).

Questionnaire

A questionnaire, described earlier (25, 26), regarding the child's skin type, foreign travel to sunnier destinations, use of sunscreens, time spent outdoors, general health, and socioeconomic data, was used. The questionnaire included a validated short FFQ to describe dietary intake of vitamin D (27) at baseline and follow-up. Parents not fluent in Swedish were assisted by an interpreter at the study visits.

Anthropometric measures and biochemical analyses

Clinical examinations and blood sampling were conducted at baseline and follow-up. Height was measured to the nearest millimeter using a Seca 264 digital scale or a Seca 213 portable stadiometer (Seca) in Umeå and in Malmö, respectively. Weight was measured to the nearest 100 g using a Tanita BWB-800MA and a Seca 878 calibrated digital floor scale in Umeå and Malmö, respectively. BMI (kg/m^2) was calculated and converted to BMI z score (BMIz) using the AnthroPlus-programme (available at <http://www.who.int/growthref/en/>) for children and adolescents aged 5–19 y based on the WHO reference dataset 2007 (29). Waist circumference was measured to the nearest millimeter with a plastic, nonstretchable measuring tape. Systolic and diastolic blood pressure was measured with an automated oscillometric sphygmomanometer (Dinamap V 100; GE Healthcare) in Umeå and with an Omron M6W automatic blood pressure monitor in Malmö.

Anesthetic cream (EMLA; AstraZeneca) was used before blood sampling. Venous blood samples were collected in a nonfasting state but ≥ 2 h after a meal. Samples were immediately protected from light, centrifuged, and serum and plasma were stored at -80°C until analyses.

Serum 25(OH) D_2 (CV: 5–6%) and 25(OH) D_3 (CV: 4–6%) were analyzed by MS on an API 4000 LC/MS/MS system (AB Sciex). Serum lipids (TC, HDL cholesterol, apoA-I, apoB) were assessed on Cobas 6000/8000 analyzers (Roche Diagnostics Ltd; CV: 3–5%), all according to the laboratory routines at the Department of Clinical Chemistry, Malmö University Hospital. Non-HDL cholesterol was calculated as TC minus HDL cholesterol.

CRP was analyzed with a commercial high-sensitivity kit (Quantikine ELISA kit; R&D Systems) at the Umeå University Hospital Pediatric Research Laboratory. The assay was performed according to the instructions of the manufacturer. The difference of absorbance at 570 nm and 450 nm was measured using the SpectraMax 340 Microplate Reader (Molecular Devices) and calculated as mean value of duplicate analyses with SoftMax Pro 2.6.1 software using a 4-parameter logistic equation. Samples with a CV (%CV) $> 10\%$ were reanalyzed. A control serum was analyzed for intra- and interassay variability, resulting in 4% and 7% variability, respectively.

Statistical analyses and calculations

Statistical analyses were performed using SPSS version 24 (SPSS, Inc.) and R 3.4.2 (30). Descriptive data are presented as means (SDs), medians and IQRs, and frequency (%).

At baseline, differences between sex and between fair- and dark-skinned children were assessed by *t* test. Baseline relations between 25(OH)D and cardiometabolic risk markers (i.e., serum

TC, HDL cholesterol, calculated non-HDL cholesterol, apoA-I, apoB, CRP, blood pressure, and BMIz) were investigated using univariate and adjusted regression analyses. In the adjusted model, sex, skin color, study site, and mothers' education were included as potential confounders.

The effect of the intervention was evaluated with respect to the following outcome variables: TC, HDL cholesterol, non-HDL cholesterol, apoA-I, apoB, CRP, and systolic and diastolic blood pressure. For each outcome, an ANCOVA model was fitted with the follow-up measurement as a dependent variable, the corresponding baseline measurement as covariate, and group (intervention or control) as an explanatory variable. The models were also adjusted for sex and study site (Malmö or Umeå), as we considered these 2 factors important predictors with the potential to increase statistical power and precision in effect size estimates. Model assumptions of equality of variance across groups and normality of residuals were assessed and verified from graphical inspections. A markedly non-normal distribution was found for CRP, and this variable was therefore logarithmically transformed, for both baseline and follow-up measurements; thereafter, model assumptions were deemed acceptable. Pairwise comparisons of model-estimated follow-up concentrations between the 3 groups were performed using Tukey's honestly significant difference test. Additionally, explorative post hoc analyses were performed when also including baseline 25(OH)D as a covariate in the ANCOVAs.

As a sensitivity analysis, analyses were also done under an intention-to-treat (ITT) principle, using multiple imputation of missing data from dropouts or other causes, with 100 replications. The analyses were conducted using the R function `aregImpute` from the `Hmisc` package. As a further explorative analysis, we included a group \times skin color interaction in each model to investigate potential heterogeneity in treatment response.

Sample size calculations for the initial vitamin D intervention trial (26) was based on a previous prevalence study in Swedish children (21). Assuming a modest effect (effect size of 0.25), a total of 160 children was needed (power $> 87\%$, $\alpha = 0.05$) to identify a group difference in 25(OH)D of 3.75 nmol/L. In addition, a control group with an additional 40 children was included in southern Sweden. Anticipating a 10% drop-out rate, 220 participants were needed. The level of significance was set at $P \leq 0.05$.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty of Umeå University (Dnr 2012-158-31M) and registered at clinicaltrials.gov (NCT01741324).

Results

Baseline characteristics

As reported in detail elsewhere (25), most children were born in Sweden, but $> 50\%$ of their parents were born elsewhere. Approximately 50% of the parents had a higher educational level (≥ 12 y). During weekdays, nearly all children were at daycare centers, preschool, or school. In addition, during the intervention period, 5 children traveled abroad 1–2 wk, 3 from the northern and 2 from the southern study site, all of whom were dark

TABLE 1 Baseline characteristics of 5- to 7-y-old children with fair and dark skin divided by placebo and vitamin D intervention groups¹

	Placebo: 2 µg	Vitamin D supplement	
		10 µg	25 µg
<i>n</i>	40	80	86
Age, mo	76.8 [72.9, 78.3]	75.30 [70.8, 82.5]	75.6 [70.0, 80.9]
Sex, boys/girls	19 (47.5)/21 (52.5)	42 (52.5)/38 (47.5)	34 (39.5)/52 (60.5)
Skin type, fair/dark	22 (55.0)/18 (45.0)	41 (51.2)/39 (48.8)	45 (52.3)/41 (47.7)
Weight (<i>z</i> score, WHO)	0.31 [−0.46, 0.97]	0.44 [−0.04, 1.16]	0.50 [−0.18, 1.33]
Height (<i>z</i> score, WHO)	0.60 [−0.12, 1.38]	0.74 [−0.05, 1.58]	0.68 [0.15, 1.27]
Waist circumference, cm	52.0 [49.0, 55.0]	53.0 [51.0, 55.6]	54.0 [51.5, 57.0]
BMI (<i>z</i> score, WHO)	−0.22 [−0.79, 0.79]	0.19 [−0.44, 0.79]	0.34 [−0.56, 0.97]
Dietary vitamin D, µg/d	5.2 ± 2.2	6.2 ± 2.6	6.0 ± 2.4
Serum 25(OH)D, nmol/L	49 [34, 65]	57 [42, 69]	59 [41, 71]
TC, mmol/L	3.85 [3.58, 4.40]	4.20 [3.80, 4.60]	4.40 [4.00, 4.90]
Non-HDL cholesterol, mmol/L	2.44 [2.10, 2.85]	2.54 [2.28, 3.09]	2.81 [2.50, 3.30]
HDL cholesterol, mmol/L	1.46 [1.30, 1.61]	1.50 [1.23, 1.77]	1.50 [1.24, 1.80]
apoA-I, g/L	1.34 [1.23, 1.45]	1.40 [1.23, 1.53]	1.45 [1.25, 1.59]
apoB, g/L	0.67 [0.63, 0.73]	0.68 [0.61, 0.80]	0.75 [0.64, 0.83]
CRP, ng/mL	432.5 [185.1, 830.4]	274.3 [137.1, 851.3]	237.8 [110.8, 938.9]
Systolic blood pressure, mm Hg	106 [99, 112]	105 [101, 110]	106 [99, 111]
Diastolic blood pressure, mm Hg	65 [56, 68]	63 [59, 68]	65 [59, 69]

¹Values are shown as medians [IQRs], frequency (%), and means ± SDs. BMIz, BMI *z* score by WHO reference dataset 2007; CRP, C-reactive protein; TC, total cholesterol; 25(OH)D, 25-hydroxyvitamin D.

skinned. They all used high-factor sun protection and their change in 25(OH)D did not differ compared with other children given the same supplement.

The mean ± SD dietary vitamin D intake at baseline was 5.9 ± 2.5 µg/d, with small variations between the study groups (Table 1), and 41% of the children had insufficient concentrations of serum 25(OH)D (i.e., ≤50 nmol/L), whereas deficiency, defined as concentrations <30 nmol/L, was found in 10%, particularly in those with dark skin (25).

Dyslipidemia, defined as TC ≥5.2 mmol/L (31), was found in 6.6% of children, and overweight and obesity, defined by Cole et al. (32), in 14.4% and 4.4% of children, respectively. Neither BMI nor waist circumference were associated with measured serum lipids, or with 25(OH)D (data not shown).

Girls had higher mean ± SD concentrations than boys of non-HDL cholesterol (2.8 ± 0.55 mmol/L vs. 2.6 ± 0.58 mmol/L;

t test, *P* = 0.015) and apoB (0.73 ± 0.12 g/L vs. 0.69 ± 0.14 g/L; *t* test, *P* = 0.047), whereas no differences in anthropometric measures related to sex or skin color were noticed. Children with dark skin had lower mean ± SD 25(OH)D than those with fair skin (47 ± 17 nmol/L vs. 63 ± 17 nmol/L; *P* ≤ 0.001) and higher mean ± SD diastolic blood pressure (65 ± 9 mm Hg vs. 63 ± 7 mm Hg; *P* = 0.05) but with no differences in serum lipid concentrations (data not shown).

Baseline and postintervention associations with 25(OH)D

In the univariate regression analysis of baseline data, 25(OH)D was positively associated with serum lipids (i.e., TC, non-HDL cholesterol, and apoB), but negatively with CRP and diastolic blood pressure (Table 2). Adjusting for sex, skin color, study site,

TABLE 2 Baseline associations between serum 25(OH)D and metabolic markers in Swedish 5- to 7-y-old children assessed by linear unadjusted and adjusted regression analysis¹

	Unadjusted			Adjusted ²		
	β	95% CI ³	<i>P</i>	β	95% CI ³	<i>P</i>
TC, mmol/L	0.170	0.001, 0.010	0.017	0.125	−0.001, 0.001	0.135
Non-HDL cholesterol, mmol/L	0.145	0.000, 0.009	0.042	0.062	−0.003, 0.007	0.454
HDL cholesterol, mmol/L	0.074	−0.001, 0.004	0.302	0.130	−0.001, 0.005	0.122
apoA-I, g/L	0.103	0.000, 0.003	0.150	0.084	−0.001, 0.003	0.325
apoB, g/L	0.178	0.000, 0.002	0.013	0.120	0.000, 0.003	0.153
Systolic blood pressure, mm Hg	−0.134	−0.116, −0.002	0.057	−0.194	−0.153, −0.013	0.021
Diastolic blood pressure, mm Hg	−0.202	−0.145, −0.028	0.004	−0.187	−0.150, −0.011	0.023
Log CRP, ng/mL	−0.210	−0.027, −0.006	0.003	−0.113	−0.022, 0.044	0.167
BMIz	0.037	−0.006, 0.011	0.603	−0.026	−0.011, 0.008	0.754
Waist circumference, cm	0.022	−0.032, 0.044	0.756	0.006	−0.043, 0.046	0.917

¹*n* = 206. BMIz, BMI *z* score by WHO reference dataset 2007; Log CRP, logarithmically transformed C-reactive protein; TC, total cholesterol; 25(OH)D, 25-hydroxyvitamin D.

²All variables were adjusted for sex, skin color, study site, and mothers' education.

³95% CI at baseline.

TABLE 3 Differences in serum lipid concentrations, CRP, and blood pressure after the intervention between placebo and vitamin D intervention groups (10 µg and 25 µg) in children aged 5–7 y (*n* = 189), adjusted for baseline measurements¹

	10 µg vitamin D vs. placebo			25 µg vitamin D vs. placebo			25 µg vs. 10 µg vitamin D		
	Diff	95% CI	<i>P</i> ²	Diff	95% CI	<i>P</i> ²	Diff	95% CI	<i>P</i> ²
TC, mmol/L	0.15	−0.09, 0.38	0.32	0.21	−0.02, 0.44	0.08	0.07	−0.12, 0.25	0.70
Non-HDL cholesterol, mmol/L	0.08	−0.14, 0.30	0.66	0.10	−0.11, 0.32	0.50	0.02	−0.16, 0.20	0.96
HDL cholesterol, mmol/L	0.06	−0.06, 0.19	0.47	0.11	−0.02, 0.23	0.11	0.04	−0.06, 0.15	0.57
apoA-I, g/L	0.00	0.08, 0.08	0.99	0.00	0.08, 0.08	1.00	0.00	−0.06, 0.07	0.98
apoB, g/L	0.05	0.00, 0.09	0.06	0.05	0.01, 0.10	0.02	0.01	−0.03, 0.05	0.88
CRP, ng/mL	0.23	−0.51, 0.98	0.74	0.36	−0.35, 1.08	0.45	0.13	−0.44, 0.71	0.85
Systolic blood pressure, mm Hg	0.01	−4.01, 4.04	1.00	−0.03	−3.93, 3.88	1.00	−0.04	−3.18, 3.10	1.00
Diastolic blood pressure, mm Hg	1.60	−2.58, 5.78	0.64	1.63	−2.43, 5.68	0.61	0.03	−3.23, 3.29	1.00

¹*P* values and 95% CIs were adjusted for multiple pairwise comparisons between the 3 groups using Tukey's honestly significant difference test. Numbers of children were 35, 70, and 84 in the placebo, 10-µg vitamin D, and 25-µg vitamin D groups, respectively. CRP, serum C-reactive protein; Diff, difference; TC, total cholesterol.

²Between-group differences assessed by ANCOVA.

and mothers' education, only blood pressure remained negatively associated with 25(OH)D.

Impact of intervention

As previously reported (26), intervention with a daily vitamin D supplement of either 10 µg or 25 µg resulted in a substantial increase (Δ) in 25(OH)D concentration [Δ = 11 (95% CI: 10, 12) µg and Δ = 25 (95% CI: 21, 28) µg, respectively], and particularly among those with lower baseline concentrations, but with no change (Δ = 0.1; 95% CI: −3, 3 µg) in the placebo group. After the intervention, insufficient concentrations of 25(OH)D (\leq 50 nmol/L) remained in 16.4% of the children and deficiency ($<$ 30 nmol/L) remained in 2.6%, mainly in the placebo group.

Differences in cardiometabolic markers between the intervention and placebo groups after the intervention, as estimated from the ANCOVA models, are presented in Table 3. The concentration of apoB (g/L) was higher in the 25-µg group compared with placebo, but other differences in serum lipids at follow-up were small and nonsignificant. However, when reanalyzing the ANCOVAs under the ITT principle using multiple imputation, apoB was no longer statistically significant (*P* = 0.41). No other changes compared with the per-protocol analysis were observed. Cardiometabolic markers (mean, 95% CI) at baseline and follow-up in all groups are shown in Figure 2. Note that the randomization resulted in substantial group differences at baseline for several outcome variables (Figure 2). Results from unadjusted analyses (i.e., not adjusted for sex and residence region) are presented in Supplemental Table 1.

Discussion

In the present study, all differences in cardiometabolic markers at follow-up were small and statistically nonsignificant. No effect of clinical importance on cardiometabolic markers in healthy children with fair and dark skin was found after a 3-mo vitamin D supplementation period, despite a significant increase (Δ) in 25(OH)D, both within and between the intervention groups from baseline to follow-up (25).

These results are in accordance with a recent RCT in 4- to 8-y-old healthy children in Denmark (14) and a similar RCT in healthy adolescents aged 14–18 y in the United Kingdom

(16), but with some differences. First, the Swedish study groups comprised both fair- and dark-skinned children. Second, the increase in 25(OH)D after the vitamin D intervention was higher in Swedish than in Danish fair-skinned children and UK adolescents, and highest in those Swedish children with dark skin. Contrary to the Danish and UK studies, no decrease in 25(OH)D occurred in the Swedish placebo group. Moreover, daily dietary vitamin D intake, excluding the daily vitamin D supplement, was higher in the Swedish than in the Danish children and UK adolescents, which is probably explained by the fortification of many Swedish foods with vitamin D. Neither the present study nor the Danish or UK RCTs (14) could confirm results from observational studies in which adverse cardiometabolic risk markers have been suggested to be associated with lower concentrations of 25(OH)D (9–13).

Adjusting for baseline values in the ANCOVA was particularly important in the present analyses due to the group differences at baseline in some of the outcome variables, generated by the randomization (33) (Figure 2). All differences at follow-up were small and statistically nonsignificant, with 1 exception: apoB remained higher in the 25-µg group compared with the placebo group. However, since this analysis was based on secondary outcomes from a previously published RCT, adjustments for multiple comparisons were only performed to compensate for post hoc between-group comparisons within each outcome, and not to compensate for an increased familywise error rate due to tests performed on 8 outcomes. This calls for caution when interpreting results from single statistical tests. In fact, using ITT analysis revealed no statistically significant difference in apoB between groups, and hence observed differences in the per-protocol analysis might have been affected by unbalanced drop-out rates. apoB is an important marker of cardiovascular risk and may be a favorable alternative to LDL cholesterol (34). Vitamin D interventions in Argentinian children (35) and Saudi females resulted in decreased concentrations of apoB (36), but this could not be confirmed by us.

In our baseline analysis, 25(OH)D was negatively associated with CRP and systolic and diastolic blood pressure, consistent with a Danish observational study (13), but not associated with BMI_z or waist circumference. However, at follow-up, the association between 25(OH)D and blood pressure was no longer present and we could not confirm any significant effect of

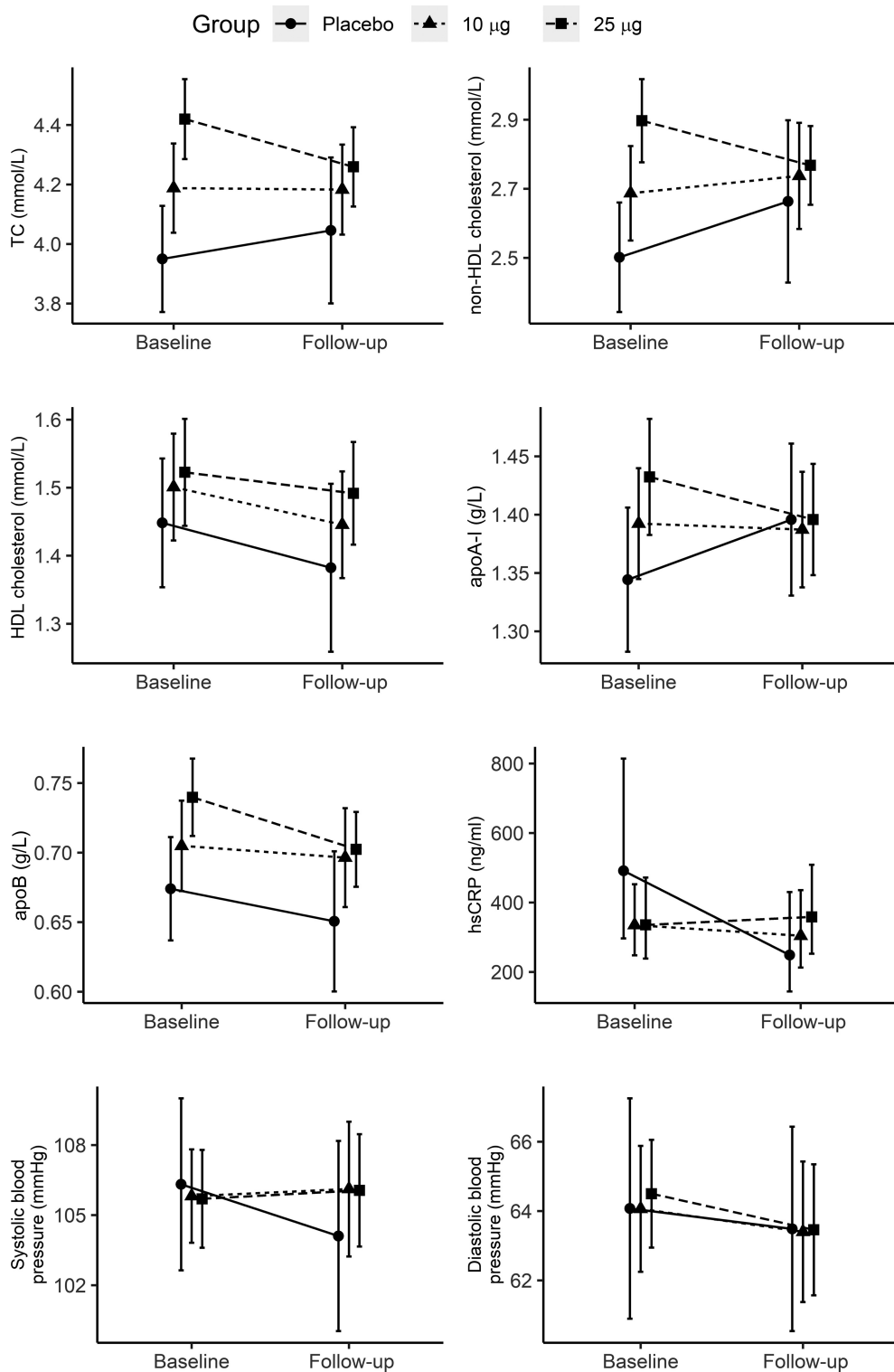


FIGURE 2 Profile plot of observed mean values of cardiometabolic markers at baseline and follow-up for placebo ($n = 35$), the 10- μg group ($n = 70$), and the 25- μg group ($n = 84$), with corresponding 95% CIs. Note that the randomization solely by chance resulted in spurious group differences that may appear as dose-response relations already at baseline for TC, non-HDL cholesterol, HDL cholesterol, apoA-I, and apoB. hsCRP, high-sensitivity C-reactive protein; TC, total cholesterol.

vitamin D supplements on blood pressure, in accordance with a large meta-analysis (37). In addition, and contrary to our baseline results, the Danish observational study also reported a negative association between vitamin D status and BMIz,

waist circumference, TC, and LDL cholesterol (13), whereas the Canadian TARGets Kids study (11) found higher 25(OH)D to be associated with lower non-HDL cholesterol in young children.

Diverse, or opposite, results may be caused by differences in concentrations of 25(OH)D and cardiometabolic markers. A recent systematic review on cardiometabolic risk score in children revealed a weak association between higher concentrations of 25(OH)D and more favorable lipid profiles in children, albeit partly based on contradictory and nonsignificant associations (19). Even though meta-analyses (20) of observational studies and randomized trials found some suggestive relations between vitamin D and CVD, hypertension, high BMI, and the metabolic syndrome, only significant associations of vitamin D with birth weight and dental caries for children could be confirmed. In addition, a systematic review of 13 observational studies and 18 trials in healthy adults could not confirm any significant effect on cardiometabolic outcomes (38). In fact, it has been hypothesized that low vitamin D status may be a consequence of ill health rather than its cause (39). Note that most meta-analyses of published RCTs are from studies in adult populations with sufficient 25(OH)D concentrations and most of them showed no beneficial effect on cardiometabolic markers. The hypothesis suggested by observational findings on adverse health outcomes of low vitamin D status could therefore not be rejected (40).

In the present study, there were 18.8% of children with overweight and obesity (i.e., comparable to young Swedish children in general) (41). Obesity is a cardiovascular risk factor in young children (42) and has been associated with low concentrations of 25(OH)D, and as mentioned, the latter has been associated with cardiometabolic risk factors (9–13). We earlier reported higher BMI to be associated with higher concentrations of 25(OH)D, which was explained by higher dietary vitamin D intake (21). In the present RCT, BMIz was not associated with 25(OH)D, in contrast to a recent Danish study in obese children and adolescents (43), and vitamin D supplementation did not affect serum lipids in accordance with an RCT in obese adolescents supplemented with a daily dose of 50 µg vitamin D (17).

Reasons for the comorbidity between obesity, cardiovascular risk markers, and 25(OH)D have been suggested to be multifactorial, including behavioral differences regarding quality of diet, extent of outdoor activities, genetics, and adipose tissue metabolism (44–46).

The strength of this study is its prospective, double-blind, randomized controlled design within a relatively large cohort, conducted during the winter season without interference of sun exposure on vitamin D status. Furthermore, our study comprised both fair- and dark-skinned children, adding an additional aspect to the impact of vitamin D supplements. In addition, a daily log for adherence to the study product was filled out and the food-frequency questionnaire was validated (27), together allowing us to calculate total vitamin D intake.

One limitation is the difference in baseline concentrations of serum lipids, with significantly higher baseline concentrations of TC in the study group randomly assigned to 25 µg, and another is that markers of insulin or glycated hemoglobin were not included. Moreover, no placebo group was recruited from Umeå. We do not know if a longer period of vitamin D supplementation would have changed our results, but we can speculate that a longer duration of supplementation may have had a larger impact on serum lipids, although studies in adults could not find any additional benefit after intervention periods of 1–5 y (40). Furthermore, as the outcomes of the present study are secondary outcomes, they were

not taken into account in the sample size estimation of the power analysis.

The present study, as well as the Danish double-blind, placebo-controlled trial with similar results, both comprised healthy children with no known cardiovascular risk factors. Thus, our finding that vitamin D supplementation did not seem to affect normal concentrations of serum lipids does not exclude that additional vitamin D would be beneficial to a risk-group population of children (i.e., with the metabolic syndrome or with a high risk of CVDs, particularly if having insufficient vitamin D status at baseline). This remains to be investigated in future trials.

In conclusion, in this clinical trial, no effect of vitamin D supplementation on serum lipids, blood pressure, or CRP in healthy 5- to 7-year-old children could be confirmed despite a substantial increase in 25(OH)D concentration in the intervention groups, particularly among those with insufficient baseline vitamin D status.

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The authors' responsibilities were as follows—IÖ, TL, OH, SAS, and PKÅ: contributed to design, conception, and development of overall research plan and study oversight of the project; IÖ and PKÅ: were primary investigators at the respective sites (hands-on conduct of data collection); IÖ: analyzed data and performed statistical analysis together with TL and PL and drafted the manuscript; and all authors: reviewed and participated in writing and read and approved the final manuscript. OH is a previous member of Valio Baby Food Scientific Advisory Board. The other authors report no conflicts of interest.

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