Childhood 25-OH Vitamin D Levels and Carotid Intima-Media Thickness in Adulthood: The Cardiovascular Risk in Young Finns Study

Markus Juonala, Atte Voipio, Katja Pahkala, Jorma S. A. Viikari, Vera Mikkilä, Mika Kähönen, Nina Hutri-Kähönen, Antti Jula, David Burgner, Matthew A. Sabin, Jukka Marniemi, Britt-Marie Loo, Tomi Laitinen, Eero Jokinen, Leena Taittonen, Costan G. Magnussen, and Olli T. Raitakari

Research Centre of Applied and Preventive Cardiovascular Medicine (M.J., A.V., K.P., C.G.M., O.T.R.) and the Departments of Medicine (M.J., J.S.A.V.), University of Turku, 20520 Turku, Finland; Division of Medicine (M.J., J.S.A.V.) and Department of Clinical Physiology and Nuclear Medicine (O.T.R.), Turku University Hospital, 20520 Turku, Finland; Murdoch Children's Research Institute and Royal Children's Hospital (M.J., D.B., M.A.S.), 3052 Parkville, Victoria, Australia; Paavo Nurmi Centre, Sports and Exercise Medicine Unit, Department of Physical Activity and Health (K.P.), University of Turku and Turku University Hospital, 20520 Turku, Finland; Department of Food and Environmental Sciences (V.M.) and Hospital for Children and Adolescents (E.J.), University of Helsinki, 00014 Helsinki, Finland; Departments of Clinical Physiology (M.K.) and Pediatrics (N.H-K.), University of Tampere and Tampere University Hospital, 33520 Tampere, Finland; National Institute of Health and Welfare, Department of Chronic Disease Prevention (A.J., J.M., B-M.L.), 20720 Turku, Finland; Department of Paediatrics (D.B., M.A.S.), University of Melbourne, 3010 Melbourne, Victoria, Australia; Department of Paediatrics (D.B.), Monash University, 3800 Clayton, Victoria, Australia; Department of Pediatrics (L.T.), Vaasa Central Hospital, 65130 Vaasa, Finland; Department of Pediatrics (L.T.), University of Oulu, 90014 Oulu, Finland; Department of Clinical Physiology, University of Eastern Finland and Kuopio University Hospital (T.L.), 70029 Kuopio, Finland; and Menzies Research Institute (C.G.M.), 7000 Hobart, Tasmania, Australia

Context: Low vitamin D levels in adulthood have been associated with cardiovascular disease.

Objective: To investigate if low vitamin D levels in childhood are related with increased carotid artery intima-media thickness (IMT) in adulthood.

Design, Setting, and Participants: The analyses included 2148 subjects from the Cardiovascular Risk in Young Finns Study, aged 3–18 years at baseline (in 1980). Subjects were re-examined at age 30–45 years (in 2007). Childhood levels of 25-hydroxy-vitamin D were measured from stored serum in 2010.

Main Outcome Measure: The carotid artery IMT from 2007 was used.

Results: When adjusted for age, sex, and childhood risk factors, continuous data of childhood 25-OH vitamin was inversely associated with adulthood carotid IMT levels among females ($\beta \pm$ SE -0.006 ± 0.003, $P = 0.03$), but not among males (0.001 ± 0.004, $P = 0.88$). Children with 25-OH vitamin D levels in the lowest quartile (<40 nmol/L) had significantly increased odds of having high-risk IMT (highest decile of common carotid or carotid bulb IMT or carotid plaque) as adults, in analyses adjusted for age, sex and either childhood risk factors (odds ratio 1.70 [95 % CI 1.15–2.31], $P = 0.0007$) or adult risk factors, including adult vitamin D levels (odds ratio 1.80 [1.30–2.48], $P = 0.0004$). In sex-specific analyses, these associations were significant both in females and males (P always <0.05). In sensitivity analyses, those with childhood vitamin D levels in the lowest quintile (<37 nmol/L), gave similar results to those using a quartile cut-point.

Conclusions: Low 25-OH vitamin D levels in childhood were associated with increased carotid IMT in adulthood. (***J Clin Endocrinol Metab*** **100: 0000–0000, 2015)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received October 28, 2014. Accepted January 7, 2015.

Abbreviations: BMI, body mass index; BP, blood pressure; CV, coefficient of variation; IMT, intima-media thickness; MI, myocardial infarction.

The importance of vitamin D in bone metabolism is well known. There is increasing interest in the association between vitamin D and atherosclerotic disease (1, 2). For example, low levels of vitamin D have been shown to be related to increased risk of stroke, myocardial infarction (MI), and total cardiovascular events (3–8). However a meta-analysis of 51 trials showed that vitamin D-raising interventions were not associated with significant beneficial effects on MI or stroke (9).

Increased carotid intima-media thickness (IMT) is a marker of structural atherosclerosis, which correlates with cardiovascular risk factors (10), and predicts cardiovascular events (11). Carotid IMT has been widely used as a surrogate measure of atherosclerosis in epidemiological studies. A number of childhood risk factors, including dyslipidemia, elevated blood pressure (BP), smoking, and increased body mass index (BMI) are associated with increased carotid IMT in adulthood (10, 12–15). Results from adult cohorts have been controversial concerning the association between vitamin D levels and carotid IMT. Some studies have observed significant associations (16, 17), whereas in others vitamin D has not been independently related with IMT (18, 19). However, there is paucity of information concerning the association between childhood vitamin D and subclinical atherosclerosis in adulthood. In addition, recent research has suggested sex-specific effects of vitamin D on cardiometabolic risk markers (20), but there is a need for additional prospective data.

Vitamin D deficiency and insufficiency are highly prevalent among children worldwide (21). We therefore examined the relationship between low childhood vitamin D levels and adult carotid IMT. The 2148 subjects (1,187 females, 961 males) were participants of the prospective Cardiovascular Risk in Young Finns Study with serum concentrations of 25-hydroxy-vitamin D measured from stored frozen samples taken at the age of 3–18 years in 1980 (and analyzed in 2010), and carotid ultrasound studies performed 27 years later in adulthood (22).

Materials and Methods

Subjects

The Cardiovascular Risk in Young Finns Study is a multicenter follow-up study of atherosclerosis precursors of Finnish children and adolescents (22). The first cross-sectional survey was conducted in 1980, when 3596 participants, aged 3, 6, 9, 12, 15, and 18 years, were randomly chosen from the five study areas on the basis of the national population register. In the 27-year follow-up in 2007, we performed vascular ultrasound studies in 2204 of these individuals, aged 30–45 years. For this study, 2148 individuals who had data on 25-OH vitamin D from baseline and carotid IMT at the 27-year follow-up were included. The study has been approved by local Ethics committees and all subjects and/or their parents gave written informed consents. The authors have had full access to the data and take full responsibility for their integrity.

Vitamin D measurements

Childhood serum samples were taken in 1980, stored at −20°C and analyzed in 2010. The follow-up serum samples were taken in 2007, stored at −70°C and analyzed in 2008. Serum 25-OH vitamin D was analyzed by radioimmunoassay (RIA) (DiaSorin, Inc.) at both time points. The limit of detection was 3.8 nmol/L. The interassay coefficient of variation (CV) was 8.5% (n = 128) at the level of 35.7 nmol/L, and 8.8% (n = 113) at the level of 135.3 nmol/L. We categorized individuals into the lowest quartile (<40 nmol/L) or quintile (<37 nmol/L) for low 25-OH vitamin D.

Cardiovascular risk factors

In childhood and adulthood, height and weight were measured, and BMI calculated as weight, kg/(height, m)^2. At baseline, BP was measured from the brachial artery using a standard mercury sphygmomanometer. From 3-year-olds, BP was measured with an ultrasound device. At the 2007 assessment, BP was measured using a random-zero sphygmomanometer. At each time point, the average of three measurements was used in the analysis. For the determination of serum lipid levels, venous blood samples were drawn after an overnight fast (22). Information on diet in childhood was obtained with a questionnaire on food choices and dietary behavior, including a short 19-item nonquantitative food frequency question. In adulthood, the participants completed a more comprehensive 128-item food frequency questionnaire that provided an estimate of food consumption in grams per day. In childhood, at age 12–18 years, smoking data were collected using the questionnaire, together with a confidential medical history that was taken with the parents absent. Smoking was defined as smoking cigarettes on a weekly basis or more often. In adulthood, those smoking daily were considered smokers. Physical activity was assessed with questions concerning the frequency and intensity of physical activity and a physical activity index was calculated based on the variables as previously described (23). There were two different kinds of physical activity questionnaires for the younger (3- to 6-year olds, a parent-completed questionnaire) and older children (9- to 18-year-olds, self-completed questionnaire). The calculated physical activity indices were age-standardized to allow comparison across age groups. In childhood, the length of time that parents spent in education was considered an indicator of socio-economic status, whereas in adulthood, the participant's own duration of study was used in the analyses.

Carotid artery studies

Ultrasound studies were performed using Sequoia 512 ultrasound mainframes (Acuson) with 13.0 MHz linear array transducers (10). Carotid IMT was measured on the posterior (far) wall of the left carotid artery. At least four measurements were taken ≈10 mm proximal to the bifurcation to derive mean carotid IMT. The digitally stored scans were manually analyzed by one reader blinded to the subject's details. The between-visit CV of IMT measurements was 6.4% and the intra-observer CV was 3.4% in our laboratory (10). In addition to continuous IMT measurements, we used a dichotomous IMT variable, which categorized subjects as having "high-risk IMT," if the mean IMT of

the common carotid artery or carotid bulb area was ≥90th percentile of the study population or having a carotid plaque, ie, a distinct area of the vessel wall protruding into the lumen >50% of the adjacent intima-media layer (24). Using this definition, 321 participants (14.9%) had high-risk IMT.

Statistical methods

Group comparisons were performed with *t*-tests and χ^2 tests, as appropriate. Linear regression analyses were used to analyze cross-sectional determinants of childhood vitamin D concentrations, and the associations of continuous variables of childhood vitamin D and adult carotid IMT. As a number of previous studies have suggested, a nonlinear association between vitamin D levels and carotid IMT (16, 25, 26) explored the possibility of a nonlinear relationship between vitamin D and IMT using statistical multivariate models that included IMT as the dependent variable and vitamin D, and higher order vitamin D terms as independent variables (27). Because prior research has suggested sex differences in the associations of vitamin D and cardiovascular risk markers (20) and we have earlier observed within Young Finns cohort that the associations of childhood cardiovascular risk factors with carotid IMT are different between males and females (10), the analyses were also performed sex-specifically. We additionally investigated the effect of low childhood vitamin D levels on the occurrence of high-risk IMT using logistic regression analyses, adjusted for age, sex (sex-combined analyses), and either childhood or adult risk factors. Analyses were performed using SAS software version 9.2. Statistical significance was inferred at a two-tailed *P* value ≤ .05.

Results

Baseline characteristics of the 2148 participants are shown in Table 1. Girls had lower childhood vitamin D concentrations than boys (50.0 vs 53.3 nmol/L, $P < .0001$). In both sexes, there was a significant correlation between baseline and follow-up concentrations of 25-OH vitamin D ($r = 0.30$, $P < .0001$ in females, $r = 0.19$, $P < .0001$ in males, Supplemental Figures 1 and 2). Thirty nine percent of males and 31% of females in the lowest vitamin D quartile in childhood remained in the same quartile in

Table 1. Characteristics of Study Subjects in Childhood and Adulthood

Variable	All	Female	Male	*P* Value[a]
N	2148	1187	961	
Childhood (in 1980)				
Age (y)	10.7 (5.0)	10.7 (5.0)	10.6 (5.1)	.59
25-OH vitamin D (nmol/L)	51.4 (15.6)	50.0 (16.0)	53.3 (15.0)	<.001
Prevalence having 25-OH vitamin D < 40 nmol/L(%)	22.8	26.7	17.9	<.001
Prevalence having 25-OH vitamin D < 50 nmol/L(%)	47.1	52.1	40.9	<.001
LDL-cholesterol (mmol/L)	3.44 (0.81)	3.49 (0.82)	3.36 (0.80)	.0006
HDL-cholesterol (mmol/L)	1.56 (0.31)	1.57 (0.30)	1.55 (0.31)	.35
Triglycerides (mmol/L)	0.66 (0.31)	0.68 (0.30)	0.64 (0.31)	.002
Systolic blood pressure (mmHg)	113 (12)	112 (11)	114 (13)	.002
BMI (kg/m^2)	17.9 (3.1)	17.8 (3.0)	18.0 (3.1)	.31
Fruit consumption (freq/week)	6.9 (2.8)	7.0 (2.8)	6.7 (2.9)	.05
Vegetable consumption (freq/week)	6.3 (2.9)	6.4 (2.8)	6.2 (3.0)	.09
Fish consumption (freq/week)	1.1 (1.1)	1.1 (1.1)	1.1 (1.1)	.86
Butter users (%)	65.9	67.1	64.4	.20
Physical activity score				
Among 3–6 year olds (range)	16.1 (2.3)	15.7 (2.3)	16.6 (2.3)	<.0001
Among 9–18 year olds (range)	9.0 (1.8)	8.7 (1.6)	9.5 (1.9)	<.0001
Smoking prevalence (%, among 12 to 18 year olds)	18.0	14.0	21.0	.01
Parental study years	10.0 (3.2)	9.9 (3.1)	10.2 (3.2)	.04
Study month (%, Sep/Oct/Nov/Dec)	5/61/30/4	4/60/30/6	5/64/30/1	<.0001
Adulthood (in 2007)				
Age (y)	37.7 (5.0)	37.7 (5.0)	37.6 (5.1)	.59
25-OH vitamin D (nmol/L)	59.2 (19.1)	61.0 (20.0)	56.9 (16.9)	<.0001
LDL-cholesterol (mmol/L)	3.10 (0.79)	2.95 (0.72)	3.29 (0.82)	<.0001
HDL-cholesterol (mmol/L)	1.34 (0.32)	1.44 (0.33)	1.21 (0.28)	<.0001
Triglycerides (mmol/L)	1.39 (0.91)	1.19 (0.64)	1.64 (1.31)	<.0001
Lipid lowering medication prevalence (%)	2.1	1.2	3.1	.002
Systolic blood pressure (mmHg)	121 (14)	117 (14)	126 (13)	<.0001
Blood pressure lowering medication prevalence (%)	6.9	6.5	7.4	.41
BMI (kg/m^2)	26.0 (4.8)	25.4 (5.1)	26.8 (4.2)	<.0001
Smoking prevalence (%)	18.3	15.2	22.1	<.0001
Carotid IMT (mm)	0.627 (0.096)	0.613 (0.085)	0.644 (0.105)	<.0001
Carotid plaque prevalence (%)	2.5	1.7	3.5	.006
Prevalence of high-risk IMT (N/%)	321/15.0	122/10.3	199/20.7	<.0001

Values are mean (SD) unless stated otherwise.

[a] *P* values from *t*-tests and χ-square test for comparisons between females and males.

Table 2. Multivariable Cross-Sectional Correlates of Childhood 25-OH Vitamin D Concentration

Variable	β	SE.	P Value
Male sex	1.8	0.7	.008
Age (y)	−1.0	0.1	<.0001
Triglycerides (mmol/L)	−4.7	1.2	<.0001
Vegetable consumption (freq/week)	0.4	0.1	.001
Butter use (no/yes)	−1.7	0.7	.01
Study month (Sep/Oct/Nov/Dec)	−5.8	0.5	<.0001
Physical activity (z-score)	0.7	0.3	.04
Smoking (no/yes)	−2.3	1.2	.06
LDL-cholesterol (mmol/L)	0.8	0.4	.06
HDL-cholesterol (mmol/L)	−0.3	1.2	.82
Systolic BP (mmHg)	0.1	0.2	.93
BMI (kg/m^2)	0.3	0.2	.06
Fruit consumption (freq/week)	0.1	0.1	.58
Parental study years	0.1	0.1	.46
Fish consumption (freq/week)	0.5	0.3	.12

Results are from linear regression analysis. β-values indicate vitamin-D level change for 1 U increase in continuous variables.

adulthood. There was no significant difference in baseline concentrations of 25-OH vitamin D in those followed to adulthood and those lost to follow-up (51.5 vs 51.0 nmol/L, respectively, $P = .40$). At follow-up, mean ± SD carotid IMT levels were 0.644 ± 0.105 mm in men and 0.613 ± 0.086 mm in women ($P < .0001$). A total of 54 (2.5%) individuals had carotid plaque.

Cross-sectional determinants of baseline vitamin D levels

Male sex, vegetable consumption, and physical activity were associated with higher levels of 25-OH vitamin D, whereas age, serum triglycerides, butter consumption, and study month were associated with lower values (Table 2).

Childhood vitamin D and adult carotid IMT

In the total cohort, as well as in sex-specific analyses, childhood levels of 25-OH vitamin D had a significant inverse association with adult IMT levels in unadjusted analyses (Table 3). After adjustment for age and conventional childhood or adulthood cardiovascular risk factors, a significant relation was observed only in females. There was no evidence for a significant sex/vitamin D interaction term in a logistic regression model ($P = .46$). However, we observed a significant second order term for child vitamin D × child vitamin D ($P = .01$), supporting a nonlinear relationship between vitamin D and IMT. In addition, an interaction term for second order vitamin D and sex (child vitamin D × child vitamin D × sex) was significant ($P = .01$). In sex-specific analyses, the second order term interaction was significant in males ($P = .002$), but not females ($P = .40$).

In the univariate analyses, both adult vitamin D ($P = .05$) and the change in vitamin D levels between childhood and adulthood ($P = .01$) were significantly associated with adult carotid IMT, but their effects became insignificant after adjustment for age and sex (adjusted $P = .22$ and $P = .80$, respectively).

Low childhood vitamin D levels and high-risk IMT

Those individuals with 25-OH vitamin D levels in the lowest quartile (< 40 nmol/L) in childhood had a significantly higher prevalence of high-risk IMT as adults (21.9% vs 12.7%, $P < .001$). This difference remained statistically significant following adjustment for age, sex, and either childhood (Figure 1) or adult cardiovascular risk factors, including adult 25-OH vitamin D levels (Figure 2), and was observed in both males and females. In this

Table 3. Associations Between Childhood Levels of Serum 25-OH Vitamin D (in 1980) and Adult Carotid IMT (in 2007)

	Females	P Value	Males	P Value	All	P Value
Unadjusted	−0.015 (0.002)	<.001	−0.012 (0.003)	<.001	−0.012 (0.002)	<.001
Adjusted with age and sex (combined analysis)	−0.006 (0.002)	.03	0.001 (0.004)	.77	−0.003 (0.002)	0.22
Adjusted with age, sex (combined analysis), and childhood BMI	−0.006 (0.002)	.02	0.001 (0.004)	.70	−0.003 (0.002)	.19
Adjusted with age, sex (combined analysis), childhood lipids, blood pressure, BMI, smoking, butter use, physical activity, study month, vegetable, fruit and fish consumption, and parental school years	−0.006 (0.003)	.03	0.001 (0.004)	.88	−0.003 (0.002)	.19
Adjusted with age, sex (combined analysis), adulthood lipids, BMI, systolic blood pressure, fruit consumption, vegetable consumption, school years (own), smoking, physical activity and 25-OH vitamin D concentration and childhood study month	−0.006 (0.002)	.01	0.001 (0.004)	.89	−0.003 (0.002)	.19

Results are β(SE.) derived from regression analyses for 1-SD change in vitamin D levels.

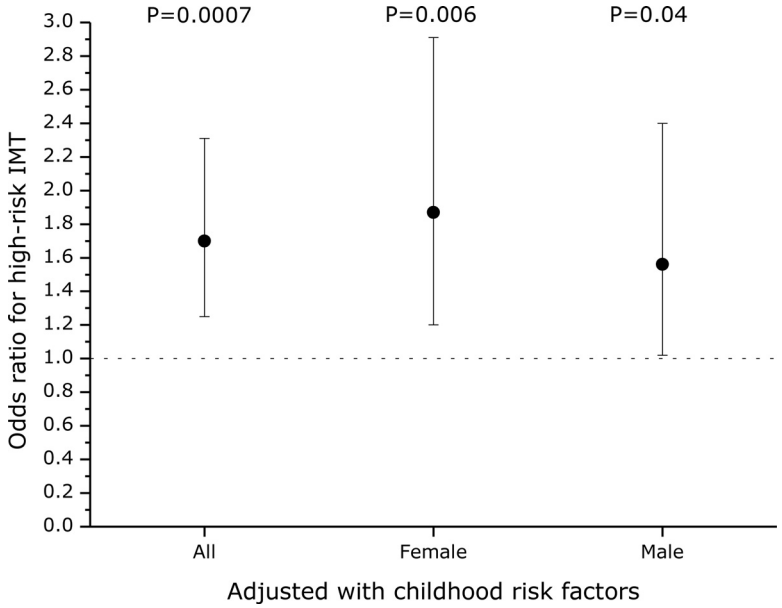

Figure 1. Odds ratios (OR) and 95% confidence intervals for adulthood high-risk IMT (common carotid or bulb area IMT ≥90th percentile or carotid artery plaque) among individuals with childhood 25-OH vitamin D levels in the lowest quartile (<40 nmol/L). The results are from logistic regression analyses adjusted with age, sex (combined analysis), and childhood risk factors including BMI, LDL-cholesterol, HDL-cholesterol, triglycerides, systolic BP, fruit consumption, vegetable consumption, fish consumption, butter use, physical activity, study month, parental school years, and smoking.

analysis, adult 25-OH vitamin D levels were not associated with high-risk IMT ($P = .10$). There was no sex/childhood vitamin D interaction in the overall logistic model ($P = .20$ for interaction term).

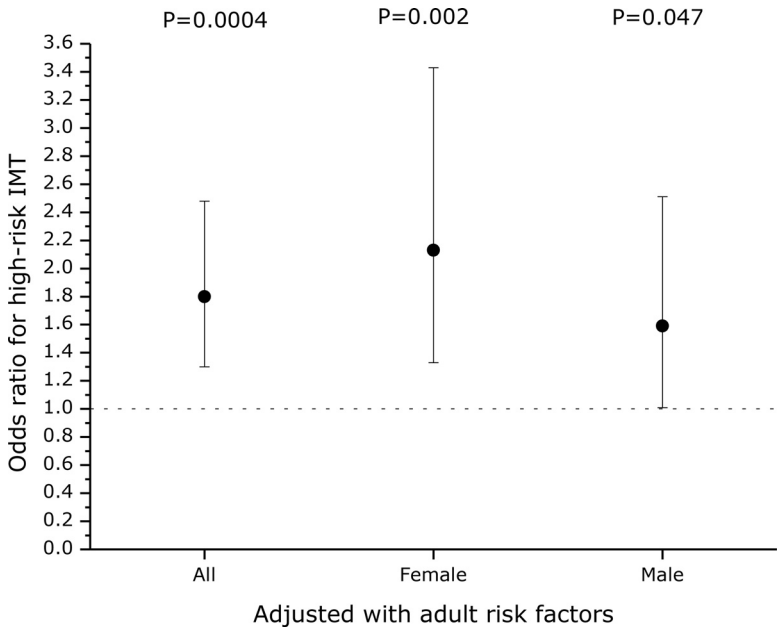

Figure 2. Odds ratios (OR) and 95% confidence intervals for adulthood high-risk IMT (common carotid or bulb area IMT ≥90th percentile or carotid artery plaque) among individuals with childhood 25-OH vitamin D levels in the lowest quartile (<40 nmol/L). The results are from logistic regression analyses adjusted with age, sex (combined analysis), and adult risk factors including BMI, LDL-cholesterol, HDL-cholesterol, triglycerides, systolic BP, fruit consumption, vegetable consumption, school years (own), smoking, physical activity, and 25-OH vitamin D concentration and childhood study month.

In addition to the main analyses, we performed several sensitivity analyses: (1) using sex-specific 90th percentile cut-points to define high-risk IMT, (2) excluding individuals who received lipid-lowering or BP-lowering medication, (3) using non-HDL cholesterol as the only lipid measure to avoid multicollinearity. In all these analyses the results were similar to those shown in Figure 1 and 2 ($P < .05$, all comparisons).

Analysis using varying cut-points to define low childhood vitamin D levels were consistent in males and females In males, significant associations were observed using the cut-points of 35, 37, 39, and 43 nmol/L, and in females, significant associations were observed at the same cut-points, apart from 35 nmol/L (Table 4).

Discussion

The importance of vitamin D for cardiovascular health has been the focus of increasing interest. In the current study, we show an association between low 25-OH vitamin D levels in childhood and increased occurrence of subclinical atherosclerosis in adulthood. This relationship remained significant after adjustment for conventional childhood or adult risk factors, including adult 25-OH vitamin D levels. Conversely, low levels of adult vitamin D were not associated with subclinical atherosclerosis.

Previous epidemiological data have shown that individuals with low vitamin D levels have an increased risk of incident MI or overall cardiovascular events (3–6, 8). However, the association between vitamin D levels and carotid IMT from adult cohorts has been controversial. Some studies have observed significant associations (16, 17), whereas in keeping with the present data, others have not shown an independent association between vitamin D and carotid IMT (18, 19). In addition, random-

Table 4. Odds Ratios (OR) and 95% Confidence Intervals for Adulthood High-Risk IMT (common carotid or bulb area IMT ≥90th percentile or carotid artery plaque) Among Individuals With Low Childhood 25-OH Vitamin D Levels According to Different Cut-Points

	OR (95% CI)		
Cut-Point Level	**Females**	**Males**	**All**
35 nmol/L	1.49 (0.91–2.43)	1.73 (1.05–2.86)	1.64 (1.15–2.31)
37 nmol/L	1.65 (1.04–2.62)	1.63 (1.02–2.61)	1.65 (1.19–2.30)
39 nmol/L	1.76 (1.13–2.76)	1.77 (1.14–2.74)	1.78 (1.30–2.42)
41 nmol/L	1.63 (1.05–2.53)	1.51 (0.99–2.29)	1.55 (1.15–2.10)
43 nmol/L	1.57 (1.01–2.44)	1.51 (1.01–2.25)	1.52 (1.13–2.03)
45 nmol/L	1.43 (0.92–2.22)	1.21 (0.82–1.76)	1.29 (0.97–1.72)
47 nmol/L	1.22 (0.79–1.90)	1.16 (0.79–1.72)	1.18 (0.89–1.56)

Data from logistic regression analyses adjusted for age, sex, and childhood risk factors including BMI, LDL-cholesterol, HDL-cholesterol, triglycerides, systolic blood pressure, fruit consumption, vegetable consumption, fish consumption, butter use, physical activity, study month, parental school years, and smoking.

ized trials and meta-analysis of the effects of vitamin D supplementation have not demonstrated a significant reduction in cardiovascular risk with increases in serum vitamin D (9, 28). This suggests that the effects of vitamin D on cardiovascular risk may operate earlier in the life-course.

In the current prospective cohort study, we showed that when vitamin D levels were considered a continuous variable, there was an independent association between childhood vitamin D and adult IMT in females, whereas among males the association became insignificant following adjustment with other child risk factors. Moreover, our study revealed that low 25-OH-vitamin levels in childhood were associated with high-risk carotid IMT in adulthood in analyses adjusted with several potential confounding factors (age, BMI, socioeconomic status, smoking, diet, season), among both sexes and using different cut-points. However, results of overall and sex-specified analyses differed in linear and nonlinear models. There was no evidence of sex/vitamin D interaction on carotid IMT. Instead, we observed a significant second order term for child vitamin D × child vitamin D supporting a nonlinear relationship between vitamin D and IMT, especially among males. In addition, there was a significant sex difference in childhood vitamin D levels. Other possible explanations for different findings between males and females in linear and nonlinear models are sex differences in IMT levels and carotid plaque prevalence observed within the study cohort.

There are several potential pathophysiological mechanisms linking low childhood vitamin D with adult atherosclerosis. Calcitriol (1,25-dihydroxyvitamin D3), the biologically active form of vitamin D, contributes to vascular proliferation (29), and also inhibits vascular calcification (30). Calcitriol is a potent immune modulator, and contributes to innate immune responses, critical to host defense in children (31). Low vitamin D levels in early life are associated with increased susceptibility to common infections (32), and there is evidence on the role of childhood infection in the early development of vascular pathology and cardiovascular risk (33). In addition, low vitamin D levels are associated with increased parathyroid hormone (PTH) levels, which have been shown to contribute to atherosclerosis development through several mechanisms (34, 35). Finally, vitamin D may be a negative endocrine regulator of renin-angiotensin system (36).

From a clinical perspective, our findings suggest that suboptimal vitamin D levels in childhood should be considered a possible risk factor for adult cardiovascular disease, although the therapeutic implications are unknown. This is in keeping with current dietary recommendations supporting the use of supplemental vitamin D during childhood (37). US guidelines suggest that optimal vitamin D levels in childhood were ≥ 50 nmol/L (37). In the present study, we observed that vitamin D levels below 43 nmol/L were associated with increased IMT (Table 4). In the intervening period since collection of childhood samples used in this study, the legislation and national policies on the nutrient fortification of foods has been liberalized in Finland. At present, most milks, sour milks and yoghurts are fortified with vitamin D at the level of 1 μg/100 g and margarines and spreads at 10 μg/100 g, leading to higher serum vitamin D levels in children and adolescents (38). However, children whose diet is poor in natural or fortified sources of vitamin D (fish, margarines, milk products), who are not regular users of vitamin D supplements, and have inadequate sunlight exposure, may still be at risk of low serum levels.

The main strength of the present study was a large, randomly selected, cohort prospectively followed for up to 27 years from childhood. In addition to 25-OH vitamin D levels, extensive data were available on other possible determinants of early atherosclerosis, allowing for adjustment for many potential confounding factors.

The present study has a number of potential limitations. Because baseline 25-OH vitamin D is the key variable in this analysis, the potential for measurement error is not trivial. We analyzed childhood 25-OH vitamin D from serum samples that had been collected in 1980 and stored for 30 years in −20°C. Thus, it is possible that the levels of 25-OH vitamin D from stored samples may be inaccurate and erroneously low, although this would not have introduced a systematic bias. Moreover only a minimal decline has been reported for plasma 25-OH vitamin D level for up to 4 years of storage at −20°C (39), and 25-OH-vitamin has been suggested to be a stable compound in a clinical environment (40). Furthermore, we observed that several factors, such as winter season, smoking, physical inactivity and diet, known to correlate with 25-OH vitamin D levels in other studies (1) had a significant association in the present cohort suggesting our measurements are robust and the general patterns of association are likely to be true. In addition, the values and distribution of vitamin D levels in childhood and adulthood were similar. Other limitations of this study include the loss of original participants during the long-term follow-up. However, we have previously shown that the follow-up cohort is representative of the original sample (15). In addition, there was no significant difference in baseline levels of 25-OH vitamin D between participants and those lost to follow-up. As the study population is comprised of young adults, we were not able to study associations with clinical cardiovascular events. Instead, we measured carotid IMT, a widely used intermediate cardiovascular risk phenotype, as the outcome measure. Detailed data on childhood dietary supplementation are not available, so we were unable to investigate whether socioeconomic status and other variables are correlated with vitamin D supplementation. Our study cohort was racially homogenous, and the generalizability of our results may be limited to Caucasians. Finally, observational studies are prone to bias when trying to establish causality.

In summary, we found that low levels of 25-OH vitamin D in childhood, but not adulthood, were associated with subclinical atherosclerosis in adults. This association was independent of conventional cardiovascular risk factors, including serum lipids, BP, smoking, diet, physical activity, obesity indices and socioeconomic status, as well as 25-OH vitamin D levels in adulthood. These observations suggest that low 25-OH vitamin D levels in childhood might have deleterious effects on vasculature.

Acknowledgments

Address all correspondence and requests for reprints to: Markus Juonala, P.O. Box 52, 20521 Turku, Finland. E-mail: markus.juonala@utu.fi.

This study was financially supported by the Academy of Finland (Grants No. 126 925, 121 584, 124 282, 129 378, 117 797, and 41 071), the Social Insurance Institution of Finland, the Turku University Foundation, Paulo Foundation, Paavo Nurmi Foundation, Juho Vainio Foundation, Sigrid Juselius Foundation, Maud Kuistila Foundation, Research funds from the Kuopio, Turku, and Tampere University Hospitals, the Finnish Foundation of Cardiovascular Research, the Finnish Medical Foundation, the Orion-Farmos Research Foundation, and the Finnish Cultural Foundation. C.G.M. holds a National Health and Medical Research Council Early Career Fellowship (Public Health Fellowship, APP1037559). D.B. holds a National Health and Medical Research Senior Research Fellowship (APP1064629) and National Heart Foundation Future Leader Fellowship (100369). Research at Murdoch Childrens Research Institute is supported by the Victorian Government's Operational Infrastructure Support Program. The sponsors had no role in preparing the manuscript.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Lavie CJ, Lee JH, Milani RV.** Vitamin D and cardiovascular disease will it live up to its hype? *J Am Coll Cardiol*. 2011;58:1547–1556.
2. **McGreevy C, Williams D.** New insights about vitamin D and cardiovascular disease: a narrative review. *Ann Intern Med*. 2011;155:820–826.
3. **Marniemi J, Alanen E, Impivaara O, et al.** Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. *Nutr Metab Cardiovasc Dis*. 2005;15:188–197.
4. **Wang TJ, Pencina MJ, Booth SL, et al.** Vitamin D deficiency and risk of cardiovascular disease. *Circulation*. 2008;117:503–511.
5. **Giovannucci E, Liu Y, Hollis BW, Rimm EB.** 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med*. 2008;168:1174–1180.
6. **Kilkkinen A, Knekt P, Aro A, et al.** Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol*. 2009;170:1032–1039.
7. **Brøndum-Jacobsen P, Benn M, Jensen GB, Nordestgaard BG.** 25-hydroxyvitamin D levels and risk of ischemic heart disease, myocardial infarction, and early death: population-based study and meta-analyses of 18 and 17 studies. *Arterioscler Thromb Vasc Biol*. 2012;32(11):2794–2802.
8. **Kassi E, Adamopoulos C, Basdra EK, Papavassiliou AG.** Role of vitamin D in atherosclerosis. *Circulation*. 2013;128(23):2517–2531.
9. **Elamin MB, Abu Elnour NO, Elamin KB, et al.** Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2011;96:1931–1942.
10. **Raitakari OT, Juonala M, Kähönen M, et al.** Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA*. 2003;290(17):2277–2283.
11. **O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr.** Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med*. 1999;340(1):14–22.
12. **Davis PH, Dawson JD, Riley WA, Lauer RM.** Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. *Circulation*. 2001;104(23):2815–2819.

13. **Li S, Chen W, Srinivasan SR, et al.** Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMA*. 2003;290(17):2271–2276.
14. **Magnussen CG, Venn A, Thomson R, et al.** The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood evidence from the cardiovascular risk in Young Finns study, the Bogalusa Heart study, and the CDAH (Childhood Determinants of Adult Health) study. *J Am Coll Cardiol*. 2009;53:860–869.
15. **Juonala M, Magnussen CG, Berenson GS, et al.** Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med*. 2011;365:1876–1885.
16. **Targher G, Bertolini L, Padovani R, et al.** Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. *Clin Endrinol (Oxf)*. 2006;65:593–597.
17. **Carrelli AL, Walker MD, Lowe H, et al.** Vitamin D deficiency is associated with subclinical carotid atherosclerosis: the Northern Manhattan study. *Stroke*. 2011;42:2240–2245.
18. **Deleskog A, Piksasova O, Silveira A, et al.** Serum 25-hydroxyvitamin D concentration in subclinical carotid atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2013;33(11):2633–2638.
19. **Blondon M, Sachs M, Hoofnagle AN, et al.** 25-Hydroxyvitamin D and parathyroid hormone are not associated with carotid intima-media thickness or plaque in the multi-ethnic study of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2013;33(11):2639–2645.
20. **Moore A, Hochner H, Sitlani CM, et al.** Plasma vitamin D is associated with fasting insulin and homeostatic model assessment of insulin resistance in young adult males, but not females, of the Jerusalem Perinatal Study. *Public Health Nutr*. 2014;22:1–8.
21. **Holick MF.** Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–281.
22. **Raitakari OT, Juonala M, Rönnemaa T, et al.** Cohort Profile: The Cardiovascular Risk in Young Finns Study. *Int J Epidemiol*. 2008;37:1220–1226.
23. **Telama R, Viikari J, Välimäki I, et al.** Atherosclerosis precursors in Finnish children and adolescents. X. Leisure-time physical activity. *Acta Paediatr Scand*. 1985;Suppl 318:169–181.
24. **Juonala M, Viikari JS, Kähönen M, et al.** Childhood levels of serum apolipoproteins B and A-I predict carotid intima-media thickness and brachial endothelial function in adulthood: the cardiovascular risk in young Finns study. *J Am Coll Cardiol*. 2008;52:293–299.
25. **van Dijk SC, Sohl E, Oudshoorn C, et al.** Non-linear associations between serum 25-OH vitamin D and indices of arterial stiffness and arteriosclerosis in an older population. *Age Ageing*. 2015;44:136–142.
26. **Atabek ME, Eklioglu BS, Akyürek N, Alp H.** Association between vitamin D level and cardiovascular risk in obese children and adolescents. *J Pediatr Endocrinol Metab*. 2014;27(7–8):661–666.
27. **Juonala M, Viikari JS, Laitinen T, et al.** Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation*. 2004;110:2918–2923.
28. **Pittas AG, Chung M, Trikalinos T, et al.** Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med*. 2010;152:307–314.
29. **Davies MR, Hruska KA.** Pathophysiological mechanisms of vascular calcification in end-stage renal disease. *Kidney Int*. 2001;60:472–479.
30. **Fraser JD, Otawara Y, Price PA.** 1,25-Dihydroxyvitamin D3 stimulates the synthesis of matrix gamma-carboxyglutamic acid protein by osteosarcoma cells. Mutually exclusive expression of vitamin K-dependent bone proteins by clonal osteoblastic cell lines. *J Biol Chem*. 1988;263:911–916.
31. **Müller K, Haahr PM, Diamant M, Rieneck K, Kharazmi A, Bendtzen K.** 1,25-Dihydroxyvitamin D3 inhibits cytokine production by human blood monocytes at the post-transcriptional level. *Cytokine*. 1992;4:506–512.
32. **Science M, Maguire JL, Russell ML, Smieja M, Walter SD, Loeb M.** Low serum 25-hydroxyvitamin D level and risk of upper respiratory tract infection in children and adolescents. *Clin Infect Dis*. 2013;57(3):392–397.
33. **Liuba P, Persson J, Luoma J, Ylä-Herttuala S, Pesonen E.** Acute infections in children are accompanied by oxidative modification of LDL and decrease of HDL cholesterol, and are followed by thickening of carotid intima-media. *Eur Heart J*. 2003;24(6):515–521.
34. **Rostand SG, Drüeke TB.** Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. *Kidney Int*. 1999;56:383–392.
35. **Hagström E, Hellman P, Larsson TE, et al.** Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation*. 2009;119:2765–2771.
36. **Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP.** 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*. 2002;110:229–238.
37. **Wagner CL, Greer FR.** Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*. 2008;122:1142–1152.
38. **Tylavsky FA, Cheng S, Lyytikäinen A, Viljakainen H, Lamberg-Allardt C.** Strategies to improve vitamin D status in northern European children: exploring the merits of vitamin D fortification and supplementation. *J Nutr*. 2006;136:1130–1134.
39. **Ockè MC, Shrijver J, Obermann-de Boer GL, Bloemberg BP, Haenen GR, Kromhout D.** Stability of blood (pro)vitamins during four years of storage at -20 degrees C: consequences for epidemiologic research. *J Clin Epidemiol*. 1995;48:1077–1085.
40. **Hollis BW.** Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. *Am J Clin Nutr*. 2008;88(Suppl 2):507S–510S.