Vitamin D levels during pregnancy and cardiovascular outcomes in childhood

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Master's Thesis

Zoonosis and One Health

Facultat de Veterinària Universitat Autònoma de Barcelona

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Content

List of abbreviations

(25(OH)D)	25-Hydroxyvitamin D (Vitamin D)
BMI	Body Mass Index
CRAE	Central Retinal Arteriolar Equivalent
CRVE	Central Venular Retina Equivalent
CVD	Cardiovascular Disease
HDL	High-density Lipoprotein
HPLC	High Performance Liquid Chromatography
INMA	INfancia y Medio Ambiente – Environment and Childhood Project
NCCLS	Clinical and Laboratory Standard Institute
PWV	Pulse Wave Velocity
WHO	World Health Organisation

Abstract

Background:

Low levels of vitamin D are associated with cardiovascular diseases in adults; however, it is unclear if there is a relationship between vitamin D in pregnancy and cardiovascular diseases in offspring.

Aim:

To assess the association of maternal circulating 25-hydroxyvitamin D (25(OH)D) at 12 weeks of pregnancy and child concentrations of (25(OH)D) in preschool age with offspring pulse wave velocity, retinal microcirculation and blood pressure at 11 years.

Methods:

We included 401 mother-child pairs from the INMA-Sabadell cohort study. We used plasma (25(OH)D) at first trimester of pregnancy and offspring plasma at 4 years. Main outcomes measured at 11 years were pulse wave velocity, central venular retina equivalent (CRVE), central retinal arteriolar equivalent (CRAE), and systolic and diastolic blood pressures. We used (25(OH)D) as a continuous and categorical variable: deficiency (≤20 ng/ml), insufficiency (21-30 ng/ml), and sufficiency (>30 ng/ml).

Results:

The median plasma levels of 25(OH)D3 were 29.63 and 37.17 ng/ml in pregnancy and childhood, respectively. Higher maternal vitamin D levels were associated with an increase in systolic blood pressure [beta = 1.06 (95% CI=0.14, 1.97)] but not with diastolic blood pressure. Maternal vitamin D levels were not associated with pulse wave velocity or with microcirculation. Vitamin D in pre-scholar age was not associated with any of the cardiovascular outcomes assessed.

Conclusions:

This study finds little evidence of associations between vitamin D levels during pregnancy or in pre-school age with cardiovascular outcomes in children. Further studies will be needed to understand more clearly the influence of prenatal and early life vitamin D levels on cardiovascular disease in childhood.

1. Introduction

Cardiovascular diseases (CVD) are a major public health problem worldwide[1]. In Europe, cardiovascular diseases represent nearly 45% of total mortality (about 4 million deaths) every year[2]. The main risk factors include obesity[3,4], abdominal adiposity[5,4], lipid disorders[6–8], blood pressure and hypertension[9,8]. Lifestyle also plays an important role in CVD risk because people with low levels of physical activity[10,11] and low consumption of fruits and vegetables[12] have an increased risk of CVD.

Low maternal vitamin D levels have been related with poor childhood development including an increased risk of being born premature or small for gestational age, being overweight[13], having more body fat, and higher levels of HDL cholesterol in childhood[14]. Recent studies have shown that low levels of 25-hydroxyvitamin D (25(OH)D) are an important risk factor for CVD in adults and elderly people[15–17]. In children, two studies conducted in Asia showed an association between low levels of vitamin D and CVD risk[18,19]. Other studies in children showed an association between low levels of vitamin D (under 20 ng/ml) and higher risk of CVD onset, regardless of physical activity and body fat[20–22].

In recent years, it has been a growing interest to measure CVD by using early markers of CVD in childhood, including pulse wave velocity[23], retinal microcirculation[24], and blood pressure[25]. Pulse wave velocity (PWV) is also non-invasive method to detect early arterial stiffness, and it is considered an independent cardiovascular risk factor marker[26,27]. Traditionally PWV has been used as a marker of CVD risk in adults and elderly, however, recent studies have proposed this marker as an estimate of arterial stiffness also in pediatric populations[27,28,29,30]. Retinal microcirculation is a non-invasive method used in adults to detect of cardiovascular disease early. This marker is related to the appearance of coronary artery disease[24], because the change in this microcirculation is a predictor of ambulatory blood pressure[24,31,32].This marker has also been suggested for the detection of early cardiovascular dysfunction in pediatric populations[32], as shown in recent studies that reported an association between arteriolar and venular adaptations in the retina during childhood and CVD risk factors later in adult life[25]. Finally, blood pressure can also be a useful early marker of CVD since elevated blood pressure in pediatric population persists over time[33] and long-term oscillation of blood pressure is associated with CVD risk[8]. In pediatric populations, the

association of low serum vitamin D concentrations with elevated systolic blood pressure in children is likely related to others CVD markers[34].

2. Objective

In this study, we aimed to assess the association of maternal circulating 25-hydroxyvitamin D (25(OH)D) at 12 weeks of pregnancy and child concentrations at preschool age with offspring pulse wave velocity, retinal microcirculation and blood pressure in 11 age children.

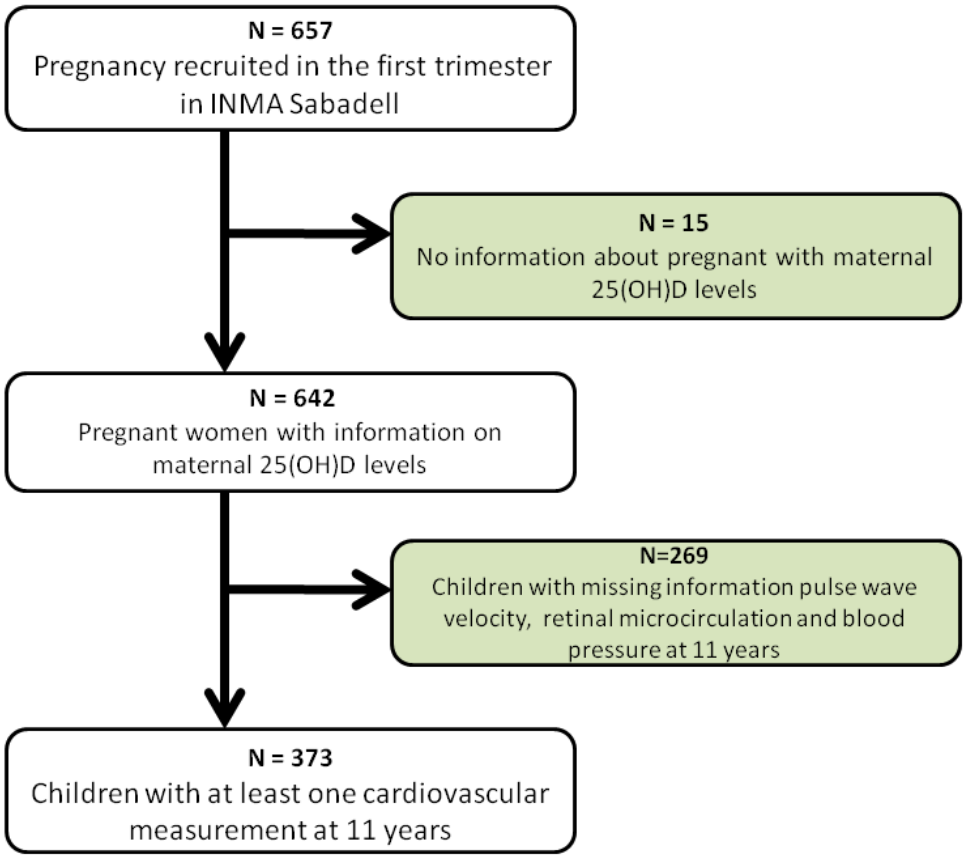
3. Materials and Methods

3.1 Study population

We used data from the INfancia y Medio Ambiente – Environment and Childhood (INMA) Project[35]. The INMA project is a prospective population-based cohort study of mothers and children conducted in 7 Spanish regions. In the present study, we used data from the Sabadell region. Women were recruited between the 10 and 13 weeks of pregnancy in health care centers between July 2004 and July 2006. The inclusion criteria were: a) to be 16 years old or older, b) to have a single pregnancy, c) not having followed an assisted reproduction program, d) to wish to deliver in the reference hospital, and e) to have no communication problems. Women were assessed in the first and third trimesters of pregnancy and their offspring at birth, 14 months, 4, 7, 9 and 11 years of age.

In the present analyses, we included 373 woman-child pairs with data on vitamin D during pregnancy and at least one cardiovascular measurement at 11 years (mean = 11.14 years [standard deviation (SD) = 0 .53]). A flow diagram is shown in Figure 1. All the participants of the study read and signed a written informed consent form. The study was approved by the ethics committee of the reference hospital in the Sabadell region.

Figure 1. Flow chart of study cohort selection.



3.2 Vitamin D assessment

Maternal vitamin D levels were assessed using plasmatic levels from a single maternal fasting blood sample collected in the first trimester of pregnancy (mean = 13.69 [SD = 1.86] weeks of gestation). The childrens' vitamin D was assessed using plasmatic levels from a single blood sample collected at 4 years. Quantification of plasma levels of 25(OH)D3 was performed by High Performance Liquid Chromatography (HPLC) using a BioRAD kit, according to Clinical and Laboratory Standard Institute (NCCLS) protocols, which were validated by the German Program of External Evaluation of Quality (DGKL- RFBReferencezinstutuk fur Bionalytik)[36]. In this study, the cut off for vitamin D in pregnancy and child was defined based on the Endocrine Society clinical practice guidelines[37]: deficiency (≤20ng/ml), insufficiency (21-30ng/ml), and sufficiency (>30ng/ml).

3.3 Cardiovascular measurements

3.3.1 Pulse wave velocity

Cardio-femoral pulse wave velocity was measured in the clinical visit by a VICORDER® - Arterial Stiffness Testing System. Measurements were performed in barefoot children in sternal decubitus, without heavy clothes. A cushion was placed on the neck and shoulders to provide 30º degree inclinations to the heart. Cuffs were placed on the upper right thigh and the neck and connect to the Vicorder. The carotid pulse was detected, the cuff placed around the neck and the cuff then connected to the Vicorder. The thigh cuff was placed on the upper right thigh as high as possible, and the child was requested not to move while measurements were taken. Two measurements were conducted, for systolic and diastolic blood pressures, a measures are were given in m/sec and each measurement was repeated twice consecutively[38–43].

3.3.2 Retinal Microcirculation

A non-myriatic approach was applied in a poorly lit room, seeking to obtain photographs of the retina, an image per eye in each child, with a Canon Cr-DGi Retinal visualization system combined with the Canon D-50 digital camera (Canon INC, Medical Equipment Group, Utsunomiya, Japan). Two trained observers applied the validated computer-assisted program IVAN (Vasculomaticala Nicola, version 1.1, Department of Ophthalmology and Visual Science, University of Wisconsin-Madison, Madison, WI, USA)[44,45], which returns the average of the arteriolar and venous diameters of the retina[46]. Microvascular diameters of the retina are expressed as central retinal arteriolar equivalent (CRAE) and central venular retina equivalent (CRVE), producing measures in μm. For analysis, measurements of left and right eyes were averaged[47].

3.3.3 Blood pressure

Blood pressure was measured with an Omron 705IT (HEM-759-E, Omron Corporation, Kyoto, Japan). A suitable cuff was used for each child; the balloon width was 40% of the circumference of the arm at an intermediate point between the olecranon and the acromion. The length of the cuff bladder was 80% to 100% of the children's arm circumference. The children were seated, with arms and backs supported and feet on the ground. It was preferable that the right arm was exposed without clothing, palm facing up, elbow flexible, and the midpoint of the arm was at the level of the heart. The use of the right arm was preferred. After 5 minutes of rest, 3 consecutive measures

were taken[48]. We used the average between the 2nd and 3rd measurements (systolic and diastolic). This variable was used in two formats, systolic blood pulse and diastolic blood pulse, a measure are were given in mmHg.

3.4 Covariates

We used the following confounders based on the literature. For maternal exposure analyses: pre-pregnancy BMI (kg/m^2), age at delivery (years), smoking during pregnancy (never smokers; partial smokers who quit at the beginning of pregnancy or smoking at some point during pregnancy), gestational age (<37 weeks; > 37 weeks), mother educational level (primary or without education; secondary or university), parity (none; 1 child; and 2 or more children), season at blood drawn in mother (winter; spring; summer or fall), mother ethnicity (caucasian; no caucasian). For child exposure analyses: sex (male; female), height at 11 years (centimeter), child age of assessment (years), BMI Child- 4 years.

3.5 Statistical analysis

We tested all variables of interest for normality using graph and Shapiro–Wilk test. We examined mean, SD or percentages and missing in socio-demographic characteristic of all participants (included and excluded from the study). We also explored mean, SD or percentages of socio-demographic and anthropometric characteristic across the three categories of maternal vitamin D. We assessed differences using *ANOVA* for continuous variables and X^2 for categorical variables. We assessed the distribution of the outcomes (PWV, CRAE, CRVE, and systolic and diastolic blood pressure) according to maternal vitamin D categories and used *ANOVA* or X^2 to explore differences between groups. We used the exposure variables (maternal and child vitamin D levels) as continuous (increase in 10 ng/ml) and categorical (deficiency (≤20ng/ml) – reference, insufficiency (21-30ng/ml), and sufficiency (>30ng/ml)). To assess the association between concentrations of vitamin D (continuous and categorical) and the outcomes, we used multivariate linear regression models with 4 levels of adjustment. For maternal vitamin D models: (a) Model 1, which was minimally adjusted for sex and age of the child at the time of outcome assessment; (b) Model 2, which was model 1 further adjusted for the season when blood was drawn, maternal age, maternal education, parity, smoking during pregnancy and maternal ethnicity; c) Model 3, which was model 2 further adjusted for maternal body mass index and d) Model 4, which was model 3

further adjusted for height of the child at 11 years. Child vitamin D models were the following: a) Model 1, which was minimally adjusted for sex and age of the child at the time of outcome assessment; b) Model 2, which was model 1 further adjusted for the season when blood was drawn, maternal education, and physical activity of the child at 4 years; (c) Model 3, which was model 2 further adjusted for body mass index of the child at 4 years; and (d) Model 4, which was model 3 further adjusted for height of the child at 11 years. All statistical analyses were conducted with STATA 14.2. The threshold for significance was set at 0.05.

4. Results

Study population characteristics

Mothers included in the study were older (mean 31.98 SD 4.12), less likely to smoke during pregnancy (73.33%), higher educated (75.79%), were more likely to be nulliparous (57.37%), Caucasian (98.43%), and had a term delivery (98.17%) than mothers not included in the study (Table 1).

Table 1. Table maternal and child group characteristics of included and excluded populations

Variables	***Included N=373 (58%)***		***Excluded N=269 (42%)***			
	Mean or N	**SD or %**	**Mean or N**	**SD or %**	***p***	**Missing**
Maternal characteristics						
Vitamin D levels in pregnancy (ng/ml)	29.63	11.54	28.57	11.62	*0.256*	2.28%
Pre-pregnancy BMI (kg/m^2)	23.71	4.47	23.69	4.45	*0.968*	0.15%
Age at delivery (years)	31.98	4.12	30.69	4.66	*<0.01*	5.78%
Smoking during pregnancy (N, %)					*0.039*	7.15%
Never	275	73.33	152	64.68		
Partial smokers	53	14.13	37	15.74		
Smoking at some point during pregnancy	47	12.53	46	19.57		
Educational level (N, %)					*<0.01*	0.61%
Primary or without education	92	24.21	97	35.53		
Secondary	161	42.37	118	43.22		
University	127	33.42	58	21.25		
Parity (N, %)					*0.502*	0.61%
No children	218	57.37	147	53.85		
1 child	139	36.58	104	38.10		

2 or more children	23	6.05	22	8.06		
Season at blood drawn mother (N, %)					*0.890*	2.28%
Winter	86	23.06	57	21.19		
Spring	88	23.59	63	23.42		
Summer	92	24.66	73	27.14		
Fall	107	28.69	76	28.25		
Ethnicity (N, %)					*0.003*	0.30%
Caucasian	375	98.43	258	94.16%		
No Caucasian	6	1.57	16	5.84		
Child characteristics						
Vitamin D levels at 4 years (ng/ml)	37.17	15.65	37.83	15.44	*0.763*	55.56%
Sex (N, %)					*0.310*	5.63%
Female	183	47.91	124	52.10		
Male	199	52.09	114	47.90		
Gestational age (N, %)					*0.007*	5.63%
<37 weeks	375	98.17	224	94.12		
> 37 weeks	7	1.83	14	5.88		

The characteristics of the population according to concentrations of maternal vitamin D are shown in Table 2. Mothers has less pre-pregnancy BMI are deficiency levels (mean 22.85 SD 3.45), oldest women`s has more sufficiency (mean 32.32 SD 4.18), mothers with deficiency smoked more (19.54%), most mothers studied secondary education (42.36%), most mothers had no other children (56.84%), and most are Caucasian (98.39%). Children were mostly male (51.47%) and most were over 37 weeks of gestation (98.12%). Lower education level was more likely to have vitamin D deficiency. There were variations in concentration across the seasons when the blood was drawn. Offspring of women with a deficiency of vitamin D had lower concentrations of vitamin D in school age.

Table 2. Table characteristics of the population according to concentrations of maternal vitamin D

Variables	*Continuous*		*Categorized*						
			Deficiency (≤ 20ng/ml)		Insufficiency (21-30ng/ml)		Sufficiency (>30ng/ml)		
	N= 373 (100%)		N=87 (23.32%)		N=93 (24.93%)		N=193 (51.74%)		
	N	**%**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
Variables									
Pre-pregnancy BMI (kg/m^2)	23.7	4.50	22.86	3.45	24.30	5.45	23.79	4.38	*0.092*
Age at delivery (years)	31.96	4.10	31.51	4.08	31.65	3.92	32.32	4.18	*0.215*
Smoking (N, %)									*0.053*
Never	266	71.31	55	63.22	67	75.28	144	75.79	
Partial smokers	53	14.21	15	17.24	16	17.98	22	11.58	
Smoking at some point during pregnancy	47	12.60	17	19.54	6	6.74	24	12.63	
Educational level (N, %)									*0.131*
Primary or without education	91	24.40	26	30.23	26	27.96	39	20.21	
Secondary	158	42.36	39	45.35	39	41.94	80	41.45	
University	123	32.98	21	24.42	28	30.11	74	38.34	
Parity (N, %)									*0.606*
0 Children	212	56.84	54	62.79	48	51.61	110	57.29	
1 Children	137	36.73	27	31.40	40	43.01	70	36.46	
2 Children's	22	5.90	5	5.81	5	5.38	12	6.25	
Season at blood drawn mother (N, %)									*0.000*
Winter	86	23.06	40	45.98	16	17.20	30	15.54	
Spring	88	23.59	25	28.74	17	18.28	46	23.83	
Summer	92	24.66	8	9.20	33	35.48	51	26.42	
Fall	107	28.69	14	16.09	27	29.03	66	34.20	
Ethnicity (N, %)									*0.048*
Caucasian	367	98.39	87	100.00	89	95.70	191	98.96	
No Caucasian	6	1.61	0	0.00	4	4.30	2	1.04	
Child characteristics									
Vitamin D in offspring (N, %)	223	59.79	62	27.80	58	26.01	103	46.19	*0.010*
Sex (N, %)									*0.950*
Female	181	48.53	41	47.13	46	49.46	94	48.70	
Male	192	51.47	46	52.87	47	50.54	99	51.30	
Gestational age (N, %)									*0.891*
<37 weeks	7	1.88	2	2.30	2	2.15	3	1.55	
> 37 weeks	366	98.12	85	97.70	91	97.85	190	98.45	
Waist circumference 11 years	373	100	87	23.32	93	24.93	193	51.74	*0.910*
Height at 11 years	373	100	87	23.32	93	24.93	193	51.74	*0.710*
Age of Assessment	373	100	87	23.32	93	24.93	193	51.74	*0.029*

The distribution of the child cardiovascular outcomes according to the categories of maternal vitamin D concentration is shown in Table 3. The lowest values of CRVE correlated with vitamin D insufficiency (mean 251.76 SD 15.32). The same is true of CRAE (mean 180.79 SD 12.75), pulse wave velocity (mean 4.34 SD 0.52), systolic blood pressure (mean 100.81 SD 8.65) and diastolic blood pressure (mean 58.66 SD 6.92). There were no significant differences between the categories for any of the outcomes of interest.

Table 3. Table description of outcomes according to maternal vitamin D concentrations.

Variables	***Continue***		***Categorized***						
			Deficiency (≤ 20ng/ml)		Insufficiency (20-30ng/ml)		Sufficiency (>30ng/ml)		
	N= 373 (100%)		N= 87 (23,23%)		N=93 (24.93%)		N= 193 (51.74%)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p***
CRVE (µm)	252.24	0.88	253.08	16.85	251.76	15.32	251.82	17.96	*0.830*
CRAE (µm)	181.14	0.65	181.76	12.81	180.79	12.75	181.33	12.54	*0.943*
PWV(m/sec)	4.37	0.02	4.34	0.52	4.35	0.43	4.38	0.44	*0.703*
Systolic (mmHg)	101.69	0.50	101.30	10.53	100.81	8.65	102.29	9.81	*0.441*
Diastolic (mmHg)	59.81	0.38	60.20	7.11	58.66	6.92	60.37	7.59	*0.067*

Abbreviations: Pulse Wave velocity (PWV), Central Retinal Venular Equivalents (CRVE), Central Retinal Arteriolar Equivalents (CRAE), blood pressure systolic (Systolic), blood pressure diastolic (Diastolic)

Maternal Circulating Vitamin D Concentrations and Cardiovascular Outcomes in the Offspring

An association of maternal circulating 25-hydroxyvitamin D concentrations at 12 weeks of pregnancy and offspring arterial stiffness and retinal microcirculation was found. For pulse wave velocity, values close to zero in continuous and categorical vitamin D were found, but not significant to the β (95%CI). Central retinal venular equivalent (CRVE) was found to be negatively associated to continuous and categorical vitamin D levels, but not significant to the β (95%CI). Central retinal arteriolar equivalent (CRAE) was found to be a value close to zero in continuous and categorical vitamin D, but not significant to the β (95% CI). All values are shown in Table 4.

The results of association between maternal 25-hydroxyvitamin D concentrations at 12 weeks of pregnancy and offspring systolic and diastolic blood pressure are shown in Table 5. There was a correlation between systolic blood pressure and vitamin D in model 2 (1.06 (0.14 , 1.98) and model

3 (1.06 (0.14 , 1.97)), however, when categorised, these results are not clear and not significant to β (95% CI).Diastolic blood pressure in continuous vitamin D was found to have values close to zero, but not significant to β (95%CI). The insufficiency category (21 – 30ng/ml) was found to have a negative correlation in all 4 regression models, but not significant to β (95% CI).

Child Circulating Vitamin D Concentrations at preschool age and Cardiovascular Outcomes in childhood

Association of child circulating 25-hydroxyvitamin D concentrations at 4 years and offspring pulse wave velocity in retinal microcirculation was found a values close to zero it was not significant β (95%CI), in central retinal venular equivalent (CRVE) was found a negative values in continuous and categorical vitamin D, but not significative to β (95%CI). Central retinal arteriolar equivalent (CRAE) was found negative values close to zero in continuous vitamin D in categorical category the values decrease, but not significative to β (95%CI). All values show in Table 6.

The results association to child circulating 25-hydroxyvitamin D concentrations at 4 years and offspring, demonstrated in table 7. The systolic and diastolic blood pressure, in continuous and categorical variables was found negative values and close to zero but not significative to β (95%CI). In children at 4 years results show be a no association to vitamin D to cardiovascular markers, pulse wave velocity, retinal microcirculation and blood pressure at 11 years.

Table 4. Association of maternal circulating 25-hydroxyvitamin D concentrations at 12 weeks of pregnancy and offspring arterial stiffness and retinal microcirculation.

		N	Model 1 β (95%CI)	Model 2 β (95%CI)	Model 3 β (95%CI)	Model 4 β (95%CI)
PWV	Continuous 10 ng/ml	373	0.02 (-0.19, 0.06)	0.03 (0.00, 0.07)	0.03 (0.00, 0.07)	0.02 (-0.01, 0.06)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	0.01 (-0.11, 0.15)	0.00 (-0.13, 0.14)	0.00 (-0.14, 0.13)	-0.01 (-0.15, 0.11)
	> 30ng/ml	193	0.06 (-0.05, 0.17)	0.09 (-0.03, 0.21)	0.08 (-0.04, 0.20)	0.05 (-0.06, 0.17)
CRVE	Continuous 10 ng/ml	373	-0.40 (-1.92, 1.11)	-0.39 (-2.06, 1.27)	-0.39 (-2.06, 1.27)	-0.29 (-1.96, 1.36)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-1.42 (-6.42, 3.56)	-1.74 (-7.22, 3.72)	-1.69 (-7.20, 3.82)	-1.55 (-7.04, 3.93)
	> 30ng/ml	193	-1.50 (-5.85, 2.85)	-1.69 (-6.52, 3.13)	-1.66 (-6.51, 3.18)	-1.32 (-6.16, 3.51)
CRAE	Continuous 10 ng/ml	373	0.00 (-1.11, 1.12)	-0.11 (-1.36, 1.12)	-0.11 (-1.35, 1.12)	-0.07 (-1.31, 1.17)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-0.35 (-4.04, 3.33)	0.11 (-3.97, 4.19)	0.34 (-3.76, 4.44)	0.40 (-3.70, 4.50)
	> 30ng/ml	193	0.22 (-2.98, 3.44)	0.21 (-3.38, 3.81)	0.32 (-3.28, 3.93)	0.47 (-3.13, 4.09)

Abbreviations: Pulse Wave velocity (PWV), Central Retinal Venular Equivalents (CRVE), Central Retinal Arteriolar Equivalents (CRAE).

Model 1: Adjusted for sex and age of the child at the time of outcomes assessment.

Model 2: Model 1 further adjusted for the season when blood was drawn, maternal age, maternal education, parity, smoking during pregnancy and maternal ethnicity.

Model 3: Model 2 further adjusted for body mass index at pre pregnancy.

Model 4: Model 3 further adjusted for height at 11 years.

Table 5. Association of maternal circulating 25-hydroxyvitamin D concentrations at 12 weeks of pregnancy and offspring systolic and diastolic blood pressure.

		N	Model 1 β (95%CI)	Model 2 β (95%CI)	Model 3 β (95%CI)	Model 4 β (95%CI)
Systolic	Continuous 10 ng/ml	373	0.58 (-0.25, 1.44)	1.06 (0.14, 1.98)	1.06 (0.14, 1.97)	0.90 (-0.37, 1.78)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-0.39 (-3.19, 2.40)	0.50 (-2.51, 3.52)	-0.15 (-2.86, 3.17)	-0.06 (-2.94, 2.80)
	> 30ng/ml	193	1.55 (-0.88, 3.99)	2.82 (-0.15, 5.48)	2.65 (0.00, 5.30)	2.09 (-0.43, 4.62)
Diastolic	Continuous 10 ng/ml	373	0.34 (-0.31, 1.00)	0.49 (-0.21, 1.19)	0.48 (-0.21, 1.19)	0.42 (-0.27, 1.11)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-1.88 (-4.03, 0.25)	-2.02 (-4.31, 0.27)	-2.17 (-4.48, 0.12)	-2.27 (-4.54, 0.00)
	> 30ng/ml	193	0.37 (-1.49, 2.24)	0.51 (-1.51, 2.53)	0.43 (-1.58, 2.46)	-0.19 (-1.80, 2.20)

Abbreviations: blood pressure systolic (Systolic), blood pressure diastolic (Diastolic).

Model 1: Adjusted for sex and age of the child at the time of outcomes assessment.

Model 2: Model 1 further adjusted for the season when blood was drawn, maternal age, maternal education, parity, smoking during pregnancy and maternal ethnicity.

Model 3: Model 2 further adjusted for body mass index at pre pregnancy.

Model 4: Model 3 further adjusted for height at 11 years.

Table 6. Association of child circulating 25-hydroxyvitamin D concentrations at 4 years and offspring arterial stiffness and retinal microcirculation.

		N	Model 1 β (95%CI)	Model 2 β (95%CI)	Model 3 β (95%CI)	Model 4 β (95%CI)
PWV	Continuous 10 ng/ml	373	0.00 (-0.03, 0.04)	0.00 (-0.03, 0.04)	0.00 (-0.03, 0.03)	0.00 (-0.03, 0.04)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-0.02 (-0.23, 0.17)	-0.03 (-0.24, 0.18)	-0.02 (-0.23, 0.18)	-0.03 (-0.23, 0.17)
	> 30ng/ml	193	0.02 (-0.16, 0.21)	0.03 (-0.16, 0.22)	0.03 (-0.16, 0.22)	0.04 (-0.14, 0.23)
CRVE	Continuous 10 ng/ml	373	-0.18 (-1.72, 1.35)	-0.33 (-1.90, 1.24)	-0.29 (-1.84, 1.24)	-0.38 (-1.93, 1.16)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-1.69 (-10.09, 6.70)	-1.01 (-9.32, 7.29)	-1.06 (-9.38, 7.26)	-0.88 (-9.16, 7.38)
	> 30ng/ml	193	-0.61 (-6.94, 8.17)	1.19 (-6.54, 8.93)	1.17 (-6.57, 8.93)	0.92 (-6.78, 8.63)
CRAE	Continuous 10 ng/ml	373	-0.68 (-1.77, 0.39)	-0.88 (-2.03, 0.26)	-0.74 (-1.88, 0.39)	-0.73 (-1.88, 0.40)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-4.78 (-10.69, 1.12)	-4.73 (-10.81, 1.34)	-4.84 (-10.90, 1.22)	-4.85 (-10.93, 1.22)
	> 30ng/ml	193	-3.01 (-8.32, 2.29)	-3.39 (-9.05, 2.26)	-3.43 (-9.08, 2.21)	-3.42 (-9.08, 2.24)

Abbreviations: Pulse Wave velocity (PWV), Central Retinal Venular Equivalents (CRVE), Central Retinal Arteriolar Equivalents (CRAE).

Model 1: Adjusted for sex and age of the child at the time of outcomes assessment.

Model 2: Model 1 further adjusted for season, maternal education and physical activity of the children at 4 years.

Model 3: Model 2 further adjusted for body mass index at 4 years.

Model 4: Model 3 further adjusted for height at 11 years.

Table 7. Association of child circulating 25-hydroxyvitamin D concentrations at 4 years and offspring systolic and diastolic blood pressure.

		N	**Model 1 β (95%CI)**	**Model 2 β (95%CI)**	**Model 3 β (95%CI)**	**Model 4 β (95%CI)**
Systolic	Continuous 10 ng/ml	373	0.33 (-0.49, 1.16)	0.29 (-0.57, 1.16)	0.34 (-0.45, 1.14)	0.45 (-0.32, 1.23)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-0.25 (-4.28, 4.79)	0.25 (-4.35, 4.86)	0.54 (-3.81, 4.89)	0.34 (-3.83, 4.53)
	> 30ng/ml	193	0.10 (-3.97, 4.18)	-.025 (-4.54, 4.03)	-0.14 (-4.19, 3.91)	0.14 (-3.75, 4.04)
Diastolic	Continuous 10 ng/ml	373	0.02 (-0.60, 0.64)	0.03 (-0.62, 0.69)	0.04 (-0.59, 0.67)	0.07 (-0.56, 0.71)
	≤20ng/ml	87	*Ref*	*Ref*	*Ref*	*Ref*
	21 – 30ng/ml	93	-0.61 (-4.02, 2.79)	-0.87 (-4.35, 2.60)	-0.74 (-4.15, 2.66)	-0.80 (-4.20, 2.59)
	> 30ng/ml	193	0.31 (-2.74, 3.38)	0.14 (-3.09, 3.38)	0.19 (-2.98, 3.37)	0.28 (-2.88, 3.45)

Abbreviations: blood pressure systolic (Systolic), blood pressure diastolic (Diastolic).

Model 1: Adjusted for sex and age of the child at the time of outcomes assessment.

Model 2: Model 1 further adjusted for season, maternal education and physical activity of the children at 4 years.

Model 3: Model 2 further adjusted for body mass index at 4 years.

Model 4: Model 3 further adjusted for height at 11 years.

5. Discussion

In this study of 373 mother-child pairs from the INMA-Sabadell cohort, we observed that maternal vitamin D levels during pregnancy were associated with an increase in systolic blood pressure at 11 years, although the association was attenuated after considering the height of the child. No associations were observed between maternal vitamin D levels and offspring pulse wave velocity and retinal microcirculation at 11 years. Vitamin D levels in pre-school age were not associated with any of the cardiovascular measurements at 11 years.

We did find a small association between concentrations of maternal vitamin D and pulse wave velocity in offspring. Our results are in agreement with the three studies completed prior. First study in Rotterdam, at Netherlands cohort study conducted examined whether 25-hydroxyvitamin D (25(OH)D) concentrations during pregnancy and in cord blood were associated with childhood body composition and cardiovascular outcomes, however the authors found no association between concentrations of maternal vitamin D with pulse wave velocity in offspring[49]. The second study in Swedish, using the Swedish Phenylketonuria (PKU) Register, found no association between concentrations of neonatal vitamin D levels and pulse wave velocity in offspring at the age of 35 years[13]. Last study in a prospective cohort study in south India, the authors used blood samples taken at 21 years and aortic pulse wave velocity at the same time as variables, there is no clear evidence between (25(OH)D3) and aortic pulse wave velocity[50]. Also this according to data found with adult population, as in the study conducted in adults in Korea, which were middle age (55 years), they found an association between vitamin D and the measured variable of brachial pulse wave velocity[51].

We observed no association in preschool age children between (25(OH)D3) and pulse wave velocity, to the best of our knowledge, no published data have been found to date on this non-association in preschool age.

The retinal microcirculation is a new method to predict CVD in adults[24] and children[32]. To the best of our knowledge, this is the first study exploring the association between vitamin D concentrations in mothers and during early childhood and retinal microcirculation in children at 11 years. Until the moment we find a publication that talks about assessed this association, in adults of ≥45 years of age and authors reported an association between low vitamin D levels (lower than 50nmol/L) and retinal micro vascular damage[52].

A total of nine studies have explored the association between maternal vitamin D and blood pressure of the offspring[13,14,49,53–58] , the majority of them found that higher levels of vitamin D decreased blood pressure. First study conducted in Denmark for example, found an inverse association between cord blood vitamin D levels with systolic blood pressure but only in girls[53]. Another study in North America found an association between vitamin D higher levels in cord blood and lower systolic and diastolic blood pressure in preschool age[54]. In Denmark, a cohort study found the same results, with no association between maternal (25(OH)D3) and diastolic blood pressure in offspring of 19–20 y[14]. The South West study found no association between maternal vitamin D and diastolic blood pressure at two different ages, at 9.9 and 15.4 years[55]. A North American study using cord blood samples, associated low levels (<11 ng/mL) of (25(OH)D3) in cord blood with an increase in the systolic blood pressure for a long time 3 to 18 years[56]. In a Dutch study they used blood samples from the mother at second trimester and umbilical cord blood samples were collected at birth, although no association was found to diastolic blood pressure between (25(OH)D3)[49]. A study in obese children, in Italy, found that low levels of vitamin D were associated with high blood pressure[57]. A cross-sectional study in North American adults USA observed that high levels of vitamin D were associated with low systolic blood pressure[58]. Last study Sweden study in 275 subjects aged 35 years could not deliver an association between (25(OH)D3) to diastolic blood pressure[13].

However, in animal models, results have been somewhat contradictory. In a study using normotensive rats, high doses of cholecalciferol for two months (vitamin D supplementation), were associated with an increase of systolic blood pressure[59]. A study in male rats, found that vitamin D deficiency (mean plasma 25(OH)D level of 30 nmol/l) and toxicity (plasma 25(OH)D level of 561nmol/l) induced an increase in systolic blood pressure[60]. In agreement with our results, none of them observed any association with diastolic blood pressure.

The relationship between (25(OH)D3) at 4 years to systolic blood pressure was not found in this study, unlike others two studies in Korean[61] and Peru[62]. The first study in Korean Study, using children of an average age of 13.74 y, using blood samples collected at the same age, they associated systolic and diastolic blood pressure between (25(OH)D3) and others metabolic syndrome, the study divided the concentrations of (25(OH)D3) into 4 quartiles, I (<13.10 ng/mL), II (≥13.10 to <16.63 ng/mL), III (≥16.63 to <20.86 ng/mL) and IV (≥20.86 ng/mL), in which they

found association that increased (25(OH)D3) reduced both blood pressures[61]. Second study in Peru, using adolescents, aged 13–15 years, they associated deficiency level (< 20 ng/ml) of (25(OH)D3) to increased systolic blood pressure (1.30 mm Hg increase, 95% confidence interval: −0.13 to 2.72; P = 0.08) and diastolic blood pressure (1.09 mm Hg increase, 95% confidence interval: 0.04 to 2.14; P = 0.04)[62].

When we investigated the association between vitamin D at 4 years and diastolic blood pressure, we didn't find association, in agreement with a cross-sectional population-based study with children aged 8 and 9 years in Brazil, children with 25(OH)D deficient levels (< 20 ng/ml) were not associated with diastolic blood pressure[63], a case control examined association to vitamin A and vitamin D to cardiovascular risks in China, age between 6-12 y, they found a correlation between serum 25(OH)D levels and diastolic and systolic blood pressure, hypertensive children had lower vitamin D levels compared to a control[64], but in obese child in Australia, middle age 12 years, there was found an association to lower levels (<50 nmol/L.) of serum (25(OH)D3) to high systolic and diastolic blood pressure[65], the last study a clinical study in Iran, girls only, with supplementation to vitamin D, during 9 weeks, age between 12-18 y, they found association a significative reduction in diastolic blood pressure[66].

The main strengths of this study include the use of different novel non-invasive cardiovascular outcomes in childhood, the relatively large sample size, and the vitamin D measurements available at two times points. The main limitations include the loss of follow-up, and unknown familiar history of cardiovascular diseases.

We found some small associations; that show a non-positive effect on developing children. We know of the importance of vitamin D levels during pregnancy and cardiovascular effects in offspring. Based on this, further research is needed to better understand vitamin D levels during pregnancy, the cardiovascular effects in offspring, and their casual mechanisms.

6. Conclusion

This study finds little evidence of associations of vitamin D levels during pregnancy and in pre-school age with pulse wave velocity, retinal microcirculation and blood pressure in 11 age children. Further studies will be needed to understand more clearly the influence of prenatal vitamin D and early life on cardiovascular disease in childhood.

7. Reference

1. WHO. Global Health Risks. *Who*. 2009:9-27. doi:10.2471/BLT.09.070565
2. Townsend N, Nichols M, Scarborough P, Rayner M. Cardiovascular disease in Europe - Epidemiological update 2015. *Eur Heart J*. 2015;36(40):2696-2705. doi:10.1093/eurheartj/ehv428
3. Mandviwala T, Khalid U, Deswal A. Obesity and Cardiovascular Disease: a Risk Factor or a Risk Marker? *Curr Atheroscler Rep*. 2016;18(5). doi:10.1007/s11883-016-0575-4
4. Chan P, Tomlinson B, Shen Y, et al. Abdominal obesity is strongly associated with Cardiovascular Disease and its Risk Factors in Elderly and very Elderly Community-dwelling Chinese. *Sci Rep*. 2016;6(1):1-9. doi:10.1038/srep21521
5. Larsson SC, Wolk A, Hakansson N, Back M. Overall and abdominal obesity and incident aortic valve stenosis: Two prospective cohort studies. *Eur Heart J*. 2017;38(28):2192-2197. doi:10.1093/eurheartj/ehx140
6. Hong KN, Fuster V, Rosenson RS, Rosendorff C, Bhatt DL. How Low to Go With Glucose, Cholesterol, and Blood Pressure in Primary Prevention of CVD. *J Am Coll Cardiol*. 2017;70(17):2171-2185. doi:10.1016/j.jacc.2017.09.001
7. Toth PP. Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease. *Vasc Health Risk Manag*. 2016;12:171-183. doi:10.2147/VHRM.S104369
8. Stevens SL, Wood S, Koshiaris C, et al. Blood pressure variability and cardiovascular disease: Systematic review and meta-analysis. *BMJ*. 2016;354:14-16. doi:10.1136/bmj.i4098
9. Ma W, Zhang B, Yang Y, et al. Correlating the relationship between interarm systolic blood pressure and cardiovascular disease risk factors. *J Clin Hypertens*. 2017;19(5):466-471. doi:10.1111/jch.12987
10. Jackson D, Nazroo J, Degens H, French DP, Pendleton N, McPhee JS. Physical activity in older age: perspectives for healthy ageing and frailty. *Biogerontology*. 2016;17(3):567-580. doi:10.1007/s10522-016-9641-0
11. Koolhaas CM, Dhana K, Schoufour JD, Ikram MA, Kavousi M, Franco OH. Impact of physical activity on the association of overweight and obesity with cardiovascular disease: The Rotterdam Study. *Eur J Prev Cardiol*. 2017;24(9):934-941. doi:10.1177/2047487317693952
12. Psaltopoulou T, Hatzis G, Papageorgiou N, Androulakis E, Briasoulis A, Tousoulis D. Socioeconomic status and risk factors for cardiovascular disease: Impact of dietary mediators. *Hell J Cardiol*. 2017;58(1):32-42. doi:10.1016/j.hjc.2017.01.022
13. Tornhammar P, Ueda P, Hult M, Simila H, Eyles D, Norman M. Season of birth, neonatal vitamin D status, and cardiovascular disease risk at 35 y of age: A cohort study from Sweden. *Am J Clin Nutr*. 2014;99(3):472-478. doi:10.3945/ajcn.113.072520
14. Rytter D, Bech BH, Halldorsson TI, et al. Maternal Vitamin D status at week 30 of gestation and offspring cardio-metabolic health at 20 years: A prospective cohort study

over two decades. *PLoS One*. 2016;11(10):1-12. doi:10.1371/journal.pone.0164758

15. Zhang R, Li B, Gao X, et al. Serum 25-hydroxyvitamin D and the risk of cardiovascular disease: dose-response meta-analysis of prospective studies. *Am J Clin Nutr*. 2017;105(4):810-819. doi:10.3945/ajcn.116.140392

16. Messenger W, Nielson CM, Li H, et al. Serum and dietary vitamin D and cardiovascular disease risk in elderly men: A prospective cohort study. *Nutr Metab Cardiovasc Dis*. 2012;22(10):856-863. doi:10.1016/j.numecd.2010.10.019

17. Skaaby T, Thuesen BH, Linneberg A. Vitamin D, Cardiovascular Disease and Risk Factors. 2017:221-230. doi:10.1007/978-3-319-56017-5_18

18. Murni IK, Sulistyoningrum DC, Oktaria V. Association of vitamin D deficiency with cardiovascular disease risk in children: Implications for the Asia Pacific Region. *Asia Pac J Clin Nutr*. 2016;25(1):S8-S19. doi:10.6133/apjcn.122016.s1

19. Xu WR, Jin HF, Du JB. Vitamin D and Cardiovascular Risk in Children. *Chin Med J (Engl)*. 2017;130(23):2857-2862. doi:10.4103/0366-6999.215500

20. Iqbal A, Dahl A, Lteif A, Kumar S. Vitamin D Deficiency: A Potential Modifiable Risk Factor for Cardiovascular Disease in Children with Severe Obesity. *Children*. 2017;4(9):80. doi:10.3390/children4090080

21. Petersen RA, Dalskov SM, Sorensen LB, et al. Vitamin D status is associated with cardiometabolic markers in 8-11-year-old children, independently of body fat and physical activity. *Br J Nutr*. 2015;114(10):1647-1655. doi:10.1017/S0007114515003372

22. El-Fakhri N, McDevitt H, Shaikh MG, Halsey C, Ahmed SF. Vitamin D and its effects on glucose homeostasis, cardiovascular function and immune function. *Horm Res Paediatr*. 2014;81(6):363-378. doi:10.1159/000357731

23. Moyo K, Porter C, Chilima B, et al. HHS Public Access. 2016;4(1):1-19. doi:10.4102/ajlm.v4i1.277.Use

24. Al-Fiadh AH, Farouque O, Kawasaki R, et al. Retinal microvascular structure and function in patients with risk factors of atherosclerosis and coronary artery disease. *Atherosclerosis*. 2014;233(2):478-484. doi:10.1016/j.atherosclerosis.2013.12.044

25. Wong TY, Klaver CCW, Jaddoe VW V., et al. Retinal Microvasculature and Cardiovascular Health in Childhood. *Pediatrics*. 2015;135(4):678-685. doi:10.1542/peds.2014-3341

26. Saeedi P, Shavandi A, Skidmore P. What Do We Know about Diet and Markers of Cardiovascular Health in Children: A Review. *Int J Environ Res Public Health*. 2019;16(4):548. doi:10.3390/ijerph16040548

27. Ben-shlomo Y, Spears M, Boustred C, et al. HHS Public Access. 2015;63(7):636-646. doi:10.1016/j.jacc.2013.09.063.Aortic

28. Baldo MP, Cunha RS, Molina M del CB, et al. Carotid-femoral pulse wave velocity in a healthy adult sample: The ELSA-Brasil study. *Int J Cardiol*. 2018;251:90-95. doi:10.1016/j.ijcard.2017.10.075

29. Ye C, Pan Y, Xu X, et al. Pulse wave velocity in elastic and muscular arteries: Tracking stability and association with anthropometric and hemodynamic measurements. *Hypertens Res*. 2016;39(11):786-791. doi:10.1038/hr.2016.67

30. Tomiyama H, Matsumoto C, Shiina K, Yamashina A. Brachial-Ankle PWV: Current Status and Future Directions as a Useful Marker in the Management of Cardiovascular Disease and/or Cardiovascular Risk Factors. *J Atheroscler Thromb*. 2016;23(2):128-146. doi:10.5551/jat.32979

31. Strain WD, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. *Cardiovasc Diabetol*. 2018;17(1):1-10. doi:10.1186/s12933-018-0703-2

32. Newman AR, Andrew NH, Casson RJ. Review of paediatric retinal microvascular changes as a predictor of cardiovascular disease. *Clin Exp Ophthalmol*. 2017;45(1):33-44. doi:10.1111/ceo.12773

33. Bao W, Threefoot SA, Srinivasan SR, Berenson GS. Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: The Bogalusa heart study. *Am J Hypertens*. 1995;8(7):657-665. doi:10.1016/0895-7061(95)00116-7

34. Moore CE, Liu Y. Elevated systolic blood pressure of children in the United States is associated with low serum 25-hydroxyvitamin D concentrations related to body mass index: National Health and Examination Survey 2007-2010. *Nutr Res*. 2017;38:64-70. doi:10.1016/j.nutres.2017.01.008

35. Guxens M, Ballester F, Espada M, et al. Cohort profile: The INMA-INfancia y Medio Ambiente-(environment and childhood) project. *Int J Epidemiol*. 2012;41(4):930-940. doi:10.1093/ije/dyr054

36. Rodriguez A, García-Esteban R, Basterretxea M, et al. Associations of maternal circulating 25-hydroxyvitamin D3 concentration with pregnancy and birth outcomes. *BJOG An Int J Obstet Gynaecol*. 2015;122(12):1695-1704. doi:10.1111/1471-0528.13074

37. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-1930. doi:10.1210/jc.2011-0385

38. Schiffrin EL, Vice-chair F, Avolio AP, et al. *Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement from the American Heart Association*. Vol 66.; 2015. doi:10.1161/HYP.0000000000000033.Recommendations

39. Thurn D, Doyon A, Sozeri B, et al. Aortic Pulse Wave Velocity in Healthy Children and Adolescents: Reference Values for the Vicorder Device and Modifying Factors. *Am J Hypertens*. 2015;28(12):1480-1488. doi:10.1093/ajh/hpv048

40. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. 2006;27(21):2588-2605. doi:10.1093/eurheartj/ehl254

41. Arden C. The ESH/ESC guidelines for the management of arterial hypertension. *Prim Care Cardiovasc J*. 2014;7(2):85-88. doi:10.3132/pccj.2014.011

42. Kracht D, Melk A, Shroff R, et al. Validating a New Oscillometric Device for Aortic Pulse Wave Velocity Measurements in Children and Adolescents. *Am J Hypertens*. 2011;24(12):1294-1299. doi:10.1038/ajh.2011.147

43. Kotovskaya Y V., Kobalava ZD, Orlov A V. Validation of the integration of technology that measures additional "vascular" indices into an ambulatory blood pressure monitoring system. *Med Devices Evid Res*. 2014;7(1):91-97. doi:10.2147/MDER.S61839

44. Parr JC, Spears GF. General caliber of the retinal arteries expressed as the equivalent width of the central retinal artery. *Am J Ophthalmol*. 1974;77(4):472-477.

45. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology*. 1999;106(12):2269-2280.

46. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27(3):143-149.

47. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet (London, England)*. 1986;1(8476):307-310.

48. Coleman A, Freeman P, Steel S, Shennan A. Validation of the Omron 705IT (HEM-759-E) oscillometric blood pressure monitoring device according to the British Hypertension Society protocol. *Blood Press Monit*. 2006;11(1):27-32. doi:10.1097/01.mbp.0000189788.05736.5f

49. Miliku K, Felix JF, Voortman T, et al. Associations of maternal and fetal vitamin D status with childhood body composition and cardiovascular risk factors. *Matern Child Nutr*. 2019;15(2):1-9. doi:10.1111/mcn.12672

50. Paul Baker C, Kulkarni B, Radhakrishna K V., et al. Is the association between Vitamin D and cardiovascular disease risk confounded by obesity? Evidence from the Andhra Pradesh Children and Parents Study (APCAPS). *PLoS One*. 2015;10(6):1-11. doi:10.1371/journal.pone.0129468

51. Lee JH, Suh HS. Association of Serum 25-hydroxy-vitamin D Concentration and Arterial Stiffness among Korean Adults in Single Center. *J Bone Metab*. 2017;24(1):51. doi:10.11005/jbm.2017.24.1.51

52. Mutlu U, Ikram MA, Hofman A, et al. Vitamin D and retinal microvascular damage. *Medicine (Baltimore)*. 2016;95(49):e5477. doi:10.1097/md.0000000000005477

53. Larsen SD, Dalgård C, Christensen ME, et al. Blood pressure in 3-year-old girls associates inversely with umbilical cord serum 25-hydroxyvitamin D: An odense child cohort study. *Endocr Connect*. 2018;7(12):1236-1244. doi:10.1530/EC-18-0308

54. Sauder KA, Stamatoiu A V., Leshchinskaya E, Ringham BM, Glueck DH, Dabelea D. Cord Blood Vitamin D Levels and Early Childhood Blood Pressure: The Healthy Start Study. *J Am Heart Assoc*. 2019;8(9):1-12. doi:10.1161/JAHA.118.011485

55. Davey Smith G, Williams DM, Hingorani A, et al. Associations of maternal 25-hydroxyvitamin D in in childhood and adolescence: findings from the pregnancy with

offspring cardiovascular risk factors Avon Longitudinal Study of Parents and Children. *Heart*. 2013;99(24):1849-1856. doi:10.1136/heartjnl-2013-303678

56. Wang G, Liu X, Bartell TR, Pearson C, Cheng TL, Wang X. Vitamin D Trajectories From Birth to Early Childhood and Elevated Systolic Blood Pressure During Childhood and Adolescence. *Hypertension*. 2019;74(2):421-430. doi:10.1161/hypertensionaha.119.13120

57. Banzato C, Maffeis C, Maines E, et al. Hypovitaminosis D and nocturnal hypertension in obese children: An interesting link. *J Hum Hypertens*. 2014;28(6):360-366. doi:10.1038/jhh.2013.122

58. Vishnu A, Ahuja V. Vitamin D and Blood Pressure Among U.S. Adults: A Cross-sectional Examination by Race/Ethnicity and Gender. *Am J Prev Med*. 2017;53(5):670-679. doi:10.1016/j.amepre.2017.07.006

59. Santos PP Dos, Rafacho BPM, Gonçalves ADF, et al. Vitamin D Induces increased systolic arterial pressure via vascular reactivity and mechanical properties. *PLoS One*. 2014;9(6):1-9. doi:10.1371/journal.pone.0098895

60. Mirhosseini NZ, Knaus SJ, Bohaychuk K, Singh J, Vatanparast HA, Weber LP. Both high and low plasma levels of 25-hydroxy Vitamin D increase blood pressure in a normal rat model. *Br J Nutr*. 2016;116(11):1889-1900. doi:10.1017/S0007114516004098

61. Lee DY, Kwon AR, Ahn JM, et al. Relationship between serum 25-hydroxyvitamin D concentration and risks of metabolic syndrome in children and adolescents from Korean National Health and Nutrition Examination survey 2008-2010. *Ann Pediatr Endocrinol Metab*. 2015;20(1):46. doi:10.6065/apem.2015.20.1.46

62. Tomaino K, Romero KM, Robinson CL, et al. Association between serum 25-hydroxy Vitamin D levels and blood pressure among adolescents in two resource-limited settings in Peru. *Am J Hypertens*. 2015;28(8):1017-1023. doi:10.1093/ajh/hpu264

63. Milagres LC, Rocha NP, Filgueiras MDS, et al. Vitamin D insufficiency/deficiency is associated with insulin resistance in Brazilian children, regardless of body fat distribution. *Public Health Nutr*. 2017;20(16):2878-2886. doi:10.1017/S136898001700194X

64. Liang X, Chen M, Qu P, et al. The Association of Vitamin A and Vitamin D with Hypertension in Children: A Case-Control Study. *Int J Hypertens*. 2018;2018. doi:10.1155/2018/9295147

65. Kao KT, Abidi N, Ranasinha S, et al. Low Vitamin D is associated with hypertension in paediatric obesity. *J Paediatr Child Health*. 2015;51(12):1207-1213. doi:10.1111/jpc.12935

66. High-dose vitamin D supplementation is associated with an improvement in several cardio-metabolicrisk factors in adolescent girls: a nine-week follow up study. 2017.