# Correlates and family aggregation of vitamin D concentrations in school-aged children and their parents in nine Mesoamerican countries

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# **Abstract**

Objective: To determine the associations of sociodemographic characteristics, diet and outdoor activity as an indicator of sun exposure with serum 25-hydroxyvitamin D (25(OH)D) concentrations in children and their parents from Mesoamerica. We also quantified family aggregation of serum 25(OH)D. Design: Cross-sectional study. Serum 25(OH)D concentrations were quantified using immunoassay. We compared the distribution of 25(OH)D concentrations in adults and children by levels of each correlate with the use of linear regression. Family aggregation was estimated using Pearson and intraclass correlation coefficients.

Setting: Capital cities of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Costa Rica, Panama and Belize, and Tuxtla Gutiérrez in Mexico.

Subjects: Children (n 223) aged 7–12 years and 492 parents.

Results: Mean (sp) 25(OH)D concentrations in adults and children were 81-3 (21-1) and 79-5 (18-1) nmol/l, respectively. Prevalence of vitamin D deficiency (VDD; 25(OH)D <50 nmol/l) was 3-9% among adults and 3-6% among children. In adults, adjusted mean 25(OH)D concentrations were highest in Nicaragua (P<0-0001). Serum 25(OH)D was positively related to time spent gardening (P=0-03). Among children, 25(OH)D concentrations were positively associated with male sex (P=0-005), dairy intake (P=0-03) and mother's serum 25(OH)D concentrations (P<0-0001); and inversely associated with mother's BMI (P=0-02) and number of home assets (P=0-04). Family membership explained 31% of the variability in 25(OH)D concentrations; aggregation was highest between mothers and children. Conclusions: VDD prevalence was low in this study. Sociodemographic characteristics, diet and outdoor activity predict serum 25(OH)D. Family aggregation of serum 25(OH)D is high between mothers and children.

Keywords
Vitamin D
Vitamin D deficiency
Family aggregation
Mesoamerica
Children

Vitamin D deficiency (VDD) is a widespread public health problem, with substantial regional and seasonal variability. The effects of VDD may extend beyond the musculoskeletal system; thus, it is important to characterize the extent of the problem and its determinants at the population level. Few studies have investigated the prevalence of VDD in Mesoamerica. In a study of Guatemalan adolescents, 12·8% had VDD, defined as serum 25-hydroxyvitamin D (25(OH)D) concentration <50 nmol/I<sup>(1)</sup>. In clinic-based studies of adults in Puerto

Rico<sup>(2,3)</sup> and among older adults from Mexico<sup>(4)</sup>, the prevalence of VDD was 36·0 and 36·9%, respectively.

The main source of vitamin D is skin synthesis from exposure to sunlight, although it can also be obtained from some foods including fatty fish, mushrooms, egg yolks and fortified milk. Although skin exposure and time spent outdoors have been investigated as correlates of vitamin D status<sup>(1)</sup>, the relative contributions of both sunlight and diet to the vitamin D status of Mesoamericans have not been examined. The demographic and



socio-economic patterning of vitamin D status in the region is also unknown. Identifying correlates of serum 25(OH)D concentrations in adults and children across the Mesoamerican region is an important initial step to determine which specific subgroups may benefit from public health interventions to prevent and decrease the burden of VDD.

The vitamin D status of family members may be correlated due to shared habits or genetics<sup>(5,6)</sup>. Nevertheless, the level of aggregation of this measure between parents and children has not been estimated. It is relevant to understand whether shared environmental or genetic factors may influence vitamin D status because interventions to improve it might be more effective if they are targeted to families rather than individuals.

The aim of the present study was to examine the associations of sociodemographic characteristics, dietary habits and time spent on outdoor activities with serum 25(OH)D concentrations and the aggregation of 25(OH)D concentrations among mothers, fathers and their children in nine Mesoamerican countries.

#### Methods

## Study population and field methods

The present study was conducted in the context of the Nine Mesoamerican Countries Metabolic Syndrome Study (NiMeCoMeS), a cross-sectional survey investigating dietary and other environmental correlates of metabolic syndrome among school-aged children and their two biological parents. Details on the study design have been described previously<sup>(7)</sup>. Briefly, between July 2011 and November 2013, we identified all public primary schools in peri-urban areas of the capital cities of Guatemala, El Salvador, the Dominican Republic, Honduras, Nicaragua, Costa Rica, Panama and Belize, and from the city of Tuxtla Gutiérrez in Chiapas, Mexico, by contacting each country's Ministry of Education. We considered schools with at least 400 students of both sexes and grouped them by geographic areas roughly representing the four cardinal directions. Next, one to three schools were randomly selected from each geographic area in each city to reach the target enrolment of thirty families per country, a sample size determined by the availability of funding. The study team identified students aged 7-12 years as potentially eligible for recruitment using the list of enrolled students in each school and invited their parents to attend a meeting. The study aims and procedures were explained to the families in attendance and additional eligibility criteria were confirmed. These criteria included living with both biological parents, not being pregnant or having a pregnant mother, and not having a sibling already identified for potential participation in the study. Researchers provided consent forms to families at the end of the meeting and afforded them one week to consider participation. Study personnel collected the signed consent forms at schools and verbally confirmed assent to participate among children of consenting parents. The final sample included 267 families (Guatemala, n 31; El Salvador, n 30; Dominican Republic, n 30; Honduras, n 30; Nicaragua, n 31; Costa Rica, n 27; Panama, n 26; Belize, 31; and Mexico, n 31). The sampling strategy employed did not intend to produce a representative sample of the population in these countries.

Sociodemographic characteristics, food intake and health habits of children and their parents were determined through questionnaires administered during a research visit to the participant's home or at a health centre. The questionnaires inquired on parental age, education level, maternal parity, and socio-economic status indicators including home and household assets ownership. Household food insecurity was assessed with the use of the Latin American and Caribbean Food Security Scale (ELCSA)<sup>(8)</sup>, a survey validated for the region. We evaluated participants' usual diet over the past 12 months using a semi-quantitative ninety-seven-item FFQ that was administered separately to mothers, fathers and children. The FFQ had been previously validated in Costa Rican adults (9,10) and included major sources of energy and micronutrients in food groups such as fruits, vegetables, animal protein (dairy, eggs and meat), carbohydrates (breads, flours, and cereals), beverages (dairy, sugarsweetened beverages, coffee and alcohol), fast foods, desserts, sauces and dressings, cooking fats and oils, and dietary supplements. For each item, research assistants described reference portion sizes in natural units or standard measures for commonly consumed servings among this population. There were nine frequency of intake options: >6 times/d, 4-5 times/d, 2-3 times/d, 1 time/d, 5–6 times/week, 2–4 times/week, 1 time/week, 1–3 times/ month or <1 time/month.

The questionnaires also included questions regarding the time spent on activities typically conducted outdoors as an indicator of sun exposure. For adults, these questions involved the usual frequency of gardening, performing agricultural work or walking outdoors for 1 h during the previous 12 months. Responses were coded into one of ten frequency options: ≥6 times/d, 4–5 times/d, 2–3 times/d, 1 time/d, 5–6 times/week, 2–4 times/week, 1 time/week, 1–3 times/month, <1 time/month or never. For children, mothers reported on their child's average daily time spent playing outdoors on weekdays and weekends.

Trained research assistants obtained anthropometric measures from each participant. Weight was measured in light clothing to the nearest 0·1 kg on digital scales (Tanita, Arlington Heights, IL, USA) and height was measured without shoes to the nearest 1 mm with the use of portable stadiometers (Seca, Hanover, MD, USA). At the end of the visit, a fasting blood sample was collected by venepuncture from each family member.

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## Laboratory methods

Blood samples were transported on ice to each country's collaborating institution where serum was separated and cryopreserved at -20°C until laboratory analysis. Analysis of 25(OH)D was performed at Heartland Assays, Inc. (Ames, IA, USA) with the DiaSorin LIAISON 25-OH Vitamin D Total assay kit, a direct, competitive chemiluminescence immunoassay. This validated method has a sensitivity of 6·25 nmol/l, and an inter- and intra-assay CV of 11·2 and 8·1 %, respectively<sup>(11)</sup>.

# Definition of outcomes

The main outcome of interest was mean serum 25(OH)D concentration. A secondary outcome was the prevalence of serum 25(OH)D <50 nmol/l, a cut-off point recommended by the US Endocrine Society to define deficiency<sup>(12)</sup>. A lower cut-off point of 30 nmol/l, proposed to define deficiency by the US Institute of Medicine<sup>(13)</sup>, was not considered because only one person had 25(OH)D concentration under this threshold.

#### Definition of exposures

#### Adults

Education level was categorized according to the number of completed years of schooling (incomplete elementary, 1-5 years; complete elementary, 6 years; incomplete secondary, 7-11 years; complete secondary, 12 years; or post-secondary, ≥13 years). We categorized height into quartiles separately for men and women. BMI was calculated as kg/m2 and categorized as <25.0, 25.0-29.9, 30.0-34.9 or  $\ge 35.0^{(14)}$ . A category of <18.5 kg/m<sup>2</sup> was not considered because only six people were under this cut-off point. The number of home assets was determined as the total number of affirmative responses to owning a car, bicycle, refrigerator, gas stove, electric stove, blender, microwave, washing machine, colour television, stereo or computer, or having in-house access to the Internet. Household food insecurity was categorized according to the number of affirmative responses to the questions in the ELCSA survey (no insecurity, 0; mild insecurity, 1–5; moderate insecurity, 6–10; severe insecurity,  $\geq$ 11). From the FFQ, we selected foods with a high vitamin D content as dietary exposures according to food composition tables. These included dairy, eggs, and canned tuna or sardines. Dairy intake frequency was calculated from the sum of weighted intake frequencies of milk, cheese, cream and ice cream. Egg intake represented the sum of weighted intake frequencies of one hard boiled or fried egg. Supplement use was not considered as a dietary correlate of vitamin D because fewer than five participants reported use of supplements that may contain vitamin D. Covariates were categorized as presented in Tables 1 and 2.

#### Children

Children's height-for-age Z-score and BMI-for-age Z-score were calculated using sex-specific growth references from

the WHO<sup>(15)</sup>. The parent who had a higher level of educational attainment determined the highest level of parental education. Maternal height in centimetres was categorized into quartiles and maternal BMI was categorized as described for adults. Mother's and father's serum 25(OH)D concentrations were categorized as <50,  $\ge$ 50 to <75 or  $\ge$ 75 nmol/l. Covariates were categorized as presented in Tables 4 and 5.

### Data analysis

Adults and children were analysed separately. Samples of children from Panama were not available, thus the analytic sample comprised 492 adults and 223 children.

We first compared mean and so serum 25(OH)D concentrations by categories of sociodemographic characteristics, food intake frequency and time spent on outdoor activities. For ordinal predictors we conducted tests for linear trend using linear regression models, with 25(OH)D concentration as the continuous outcome and a variable representing ordinal categories of the predictor as a continuous covariate. For nominal predictors, we used  $\chi^2$  score statistics. Next, we estimated the prevalence of serum 25(OH)D <50 nmol/l by levels of sociodemographic, diet and outdoor activity characteristics. For ordinal predictors in adults, we performed tests for linear trend with the use of Poisson regression models, with serum 25(OH)D <50 nmol/l as the dichotomous outcome and a variable representing ordinal categories of the predictor as a continuous covariate. An exchangeable correlation structure was specified in all models of adults to account for intrafamily clustering. For nominal predictors, we used the  $\chi^2$  score test. In children, we used the Cochrane-Armitage exact test for trend for ordinal predictors. For nominal predictors, we used the Fisher's exact test.

We estimated adjusted differences with 95 % CI in mean serum 25(OH)D concentrations by categories of exposure variables with the use of multivariable linear regression. We included predictors that were significantly associated with the outcome in bivariate analysis at P < 0.10; predictors that remained significant (P < 0.05) in multivariable analysis were retained in the final models. Robust estimates of the variance were calculated in all models to overcome potential deviations from the multivariate normality assumption (16).

#### Family aggregation

We estimated Pearson correlations with 95% CI of serum 25(OH)D concentrations between mothers and fathers, mothers and children, and fathers and children. Next, we used linear random-effects models with serum 25(OH)D as the continuous outcome and a random effect for family to partition the variance into within-family and betweenfamily components. We also considered fixed effects for age and sex to account for extraneous between-person variation. The intraclass correlation coefficient (ICC) was calculated as the ratio of the between-family variances to the sum of the between- and within-family variances.

Table 1 Serum 25-hydroxyvitamin D (25(OH)D) concentrations according to sociodemographic characteristics in adults from nine Mesoamerican countries, 2011–2013

		Mean	SD			
Sociodemographic characteristic		(nmol/l)		P†	% <50 nmol/l	P‡
Sex				0.15		0.18
Female (mothers)	248	82.5	21.8		2.8	
Male (fathers)	244	80.1	20.4		4.9	
Age (years)				0.33		0.74
<30	53	83.7	20.6		3.8	
30–34	103	81.0	19.7		3.9	
35–39	152	83.1	23.4		4.0	
40–44	98	78.6	20.1		2.0	
45–54	66	79.0	19⋅8		7.6	
≥55	19	83.6	21.4		0.0	
Education level				0.04		0.90
Incomplete elementary	73	83.9	24.8		4⋅1	
Complete elementary	69	84.0	21.2		2.9	
Incomplete secondary	146	82.3	21.7		4⋅8	
Complete secondary	73	79.2	18∙0		2.7	
Post-secondary	119	78.7	19∙5		4.2	
Height, sex-specific quartile (female/male medians, cm)				0.67		0.33
Q1 (148·8/159·0)	126	82.4	19⋅8		2.4	
Q2 (153·1/165·0)	126	78.9	20.0		3⋅2	
Q3 (157·0/169·7)	121	83.6	22.8		5⋅8	
Q4 (162·7/176·0)	119	80.4	21.9		4.2	
BMI (kg/m²)				0.91		0.07
<25.0	124	79.9	17⋅3		2.4	
25.0–29.9	198	82.6	22.6		3.0	
30-0–34-9	126	81.5	21.3		4.8	
≥35.0	44	79⋅1	23.6		9⋅1	
Number of home assets§				0.04		0.03
0-4	94	85.7	22.4		1.1	
5–7	190	80.7	19.7		3.2	
8–9	89	81.9	24.5		4.5	
10–12	119	78⋅4	19.1		6⋅7	
Food insecurity in the household				0.80		0.24
None	159	81.5	22.4		5.7	
Mild	133	80.9	16.4		2.3	
Moderate	115	80.5	22.5		5.2	
Severe	83	82.9	23.8	0.0004	1.2	0.45
Country, latitude		70.4	444	<0.0001	4.7	0.15
Guatemala, 14.6°	58	72.4	14.1		1.7	
El Salvador, 13·7°	58	81.0	16.8		3.5	
Dominican Republic, 18-5°	60 57	74·2 79·9	16.6		3.3 2.5	
Honduras, 14·1°	62	79·9 97·5	18.9		3⋅5 1⋅6	
Nicaragua, 12·1°	-		28.1		1.6 8.0	
Panama, 9.0°	25	71⋅2 79⋅0	15⋅0 22⋅4			
Costa Rica, 9.6°	53 57	79.0 83.9			9·4 7·0	
Belize, 17:3°	62	85·5	21·2 19·3		7.0 0.0	
Mexico, 16·8°	62	80.0	19.3	0.05	0.0	0.40
Month of measurement	26	71.6	17.1	0.05	7.7	0.48
Jan-Feb Mar Apr	103	71.6 81.6	17·1 18·4		7·7 1·9	
Mar–Apr May–Jun	44	81·6 87·0	18·4 22·5		1.9 4.6	
May-Jun Jul-Sep	126	83·9	23.4		4·6 2·4	
Oct-Dec	193				5·2	
OCI-Dec	193	79.5	20.6		5.∠	

<sup>\*</sup>Totals may be less than 492 because of missing values.

95% CI were obtained using the delta method<sup>(17)</sup>. Higher values for an ICC imply that people from the same family would have more similar serum 25(OH)D concentrations

than people from different families. We then estimated the ICC among mothers and fathers, mothers and children, and fathers and children, and compared them with the ICC

Test for linear trend from linear regression models with serum 25(OH)D as the continuous outcome and a variable representing ordinal categories of each characteristic as the continuous predictor.  $\chi^2$  score test for sex, country and month of measurement. An exchangeable correlation structure was specified in all models to account for intrafamily clustering. ‡Test for linear trend from Poisson regression models with serum 25(OH)D <50 nmol/l as the dichotomous outcome and a variable

<sup>‡</sup>Test for linear trend from Poisson regression models with serum 25(OH)D < 50 nmol/l as the dichotomous outcome and a variable representing ordinal categories of each characteristic as the continuous predictor.  $\chi^2$  score test for sex and month of measurement. An exchangeable correlation structure was specified in all models to account for intrafamily clustering. Fisher's exact test for country. §From a list that included car, bicycle, refrigerator, gas stove, electric stove, blender, microwave, washing machine, colour television, stereo, computer, and having in-home access to the Internet.

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**Table 2** Serum 25-hydroxyvitamin D (25(OH)D) concentrations according to food intake frequency and time spent on outdoor activities in adults from nine Mesoamerican countries, 2011–2013

		Mean	SD			
	n*	nme	ol/l	P†	% <50 nmol/l	<i>P</i> ‡
Food intake frequency						
Dairy§				0.50		0.62
<3/week	129	81.8	21.3		2.3	
3–6/week	117	<b>78</b> ⋅ <b>7</b>	18.4		5⋅1	
1-<1·5/d	134	81⋅2	20.5		3.0	
≥1.5/d	102	83.7	24.4		4.9	
Eggs				0.32		0.54
<3/week	91	83.4	23.9		3.3	
3/week	140	81.0	20.5		5.0	
4-6/week	132	81⋅1	20.7		3⋅8	
≥1/d	119	80.3	20.2		2.5	
Canned tuna/sardines¶				0.65		0.25
<1/month	210	82.3	20.5		2.9	
1–3/month	114	80.3	22.1		3.5	
1/week	94	78.3	18.9		4.3	
>2/week	64	84.1	24.1		6.3	
Outdoor activities						
Gardening				0.02		0.11
Never	298	79.7	20.0		3.7	
1–3 h/month	53	78.8	16.6		0.0	
1-6 h/week	76	83.6	19.4		4.0	
≥1 h/d	61	88.5	29.6		8.2	
Agricultural work	-			0.29		0.30
Never	414	80.9	21.8		4.4	
Any	66	82.4	17.1		1.5	
Walking outdoors		<del></del> -		0.22		0.82
<1 h/week	122	79.3	20.7		4.9	
1–6 h/week	130	80.4	19.5		3.1	
1 h/d	116	84.1	23.4		1.7	
≥1 h/d	116	81.1	21.1		6.0	

<sup>\*</sup>Totals may be less than 492 because of missing values-

Sum of weighted intake frequencies of one hard boiled or fried egg (60 g).

¶Intake of 60 g.

for the whole family to examine which combinations of family membership were associated with greater aggregation. Higher mother–father than mother–child or father–child aggregation could indicate a greater role for shared environment rather than shared genetics in family clustering of 25(OH)D since mothers and fathers are typically not genetically related. In supplemental analysis, we considered whether potential clustering by country could affect the results on family aggregation.

# Results

The mean (sD) age of mothers, fathers and children at enrolment was 37·0 (6·4), 40·6 (8·3) and 10·0 (1·7) years, respectively; 53·4% of children were girls. Sociodemographic characteristics, food intake frequencies and time spent on outdoor activities varied by country (see online supplementary material, Supplemental Table 1).

# Vitamin D correlates in adults

Among adults, mean (sD) serum 25(OH)D concentration was 81·3 (21·1) nmol/l; 82·5 (21·8) nmol/l in mothers and 80·1 (20·4) nmol/l in fathers. The prevalence of serum 25(OH)D < 30, < 50 and < 75 nmol/l was, respectively, 0.2, 3.9 and 39.8 %. Mean serum 25(OH)D concentrations were inversely related to education level and number of home assets. Participants from Nicaragua and those with measurements obtained in May or June had the highest concentrations (Table 1). The prevalence of VDD was higher in adults with more household assets compared with that in adults with fewer home assets. Prevalence of VDD in adults varied across countries, ranging from 0.0% in Mexico to 9.4% in Costa Rica. Mean serum 25(OH)D concentrations were positively associated with time spent gardening (Table 2). In multivariable analysis, mean serum 25(OH)D concentrations varied significantly by country (P < 0.0001); the highest concentrations were in Nicaragua, Mexico and Belize whereas the lowest were in

<sup>†</sup>Test for linear trend from linear regression models with serum 25(OH)D as the continuous outcome and a variable representing ordinal categories of each characteristic as the continuous predictor.  $\chi^2$  score test for agricultural work. An exchangeable correlation structure was specified in all models to account for intrafamily clustering.

<sup>‡</sup>Test for linear trend from Poisson regression models with serum 25(OH)D <50 nmol/l as the dichotomous outcome and a variable representing ordinal categories of each characteristic as the continuous predictor.  $\chi^2$  score test for agricultural work. An exchangeable correlation structure was specified in all models to account for intrafamily clustering.

<sup>§</sup>Sum of weighted intake frequencies of milk (200 g), cheese (30 g), cream (12 g) and ice cream (60 g).

**Table 3** Multivariable-adjusted correlates of serum 25-hydroxyvitamin D (25(OH)D) concentrations in adults from nine Mesoamerican countries, 2011–2013

	Adjusted difference*	95 % CI	
	nm	P†	
Country, latitude	,		<0.0001
Guatemala, 14.6°	Refer	ence	
El Salvador, 13·7°	8.6	3.2, 13.9	
Dominican Republic, 18.5°	1.8	<b>−3.6</b> , 7.1	
Honduras, 14.1°	6.7	0.4, 13.0	
Nicaragua, 12⋅1°	24.7	16.8, 32.5	
Panama, 9.0°	-2.3	-9.0, 4.4	
Costa Rica, 9⋅6°	5.8	–1⋅5, 13⋅2	
Belize, 17⋅3°	9.7	3.4, 15.9	
Mexico, 16.8°	11.7	5.5, 17.8	
Gardening			0.03
Never	Refer	ence	
1–3 h/month	0.4	-4.7, 5.5	
1–6 h/week	4.8	0.0, 9.6	
≥1 h/d	6.4	–0.6, 13.3	

\*From multivariable linear regression with serum 25(OH)D as the continuous outcome. Predictors included country and gardening. An exchangeable correlation structure was specified to account for intrafamily clustering. †For country,  $\chi^2$  score test. For gardening, test for linear trend when a variable representing ordinal categories of gardening time was included as a continuous predictor.

Panama, Guatemala and the Dominican Republic (Table 3). Compared with people who never gardened, adjusted mean serum 25(OH)D concentration was 4-8 nmol/l higher (95 % CI 0-0, 9-6 nmol/l) in people who gardened for 1–6 h/week and 6-4 nmol/l higher (95 % CI -0-6, 13-3 nmol/l) in people who gardened for  $\geq 1$  h/d (P-trend=0-03). Month of measurement was not associated with mean serum 25(OH)D concentrations in multivariable analysis.

#### Vitamin D correlates in children

Among children, mean (sp) serum 25(OH)D concentration was 79.5 (18.1) nmol/l. The prevalence of serum 25(OH)D <30, <50 and <75 nmol/l was, respectively, 0.0, 3.6 and 44.4 %. Mean serum 25(OH)D concentrations were positively related to mother's height, parental 25(OH)D concentrations and Nicaraguan origin (Table 4). The prevalence of VDD was higher in children whose mothers had higher BMI compared with children whose mothers had lower BMI. In addition, the prevalence of VDD was higher in children whose mothers had serum 25(OH)D concentration of <50 or 50-<75 nmol/l compared with children whose mothers' serum 25(OH)D concentration was ≥75 nmol/l. Prevalence of VDD ranged from 0.0 % in Guatemala, the Dominican Republic, Honduras and Mexico to 11.5 % in Costa Rica. Mean serum 25(OH)D concentrations were positively associated with frequency of dairy intake (Table 5). In multivariable analysis, mean serum 25(OH)D concentrations were positively associated with male sex, mother's 25(OH)D concentrations and dairy intake frequency (Table 6). Every 1 nmol/l of maternal serum 25(OH)D was related to an adjusted 0·3 nmol/l higher 25(OH)D concentration in the children (95 % CI 0·2, 0·5 nmol/l; P < 0.0001). Mean 25(OH)D concentration among children with dairy intake  $\geq 2.5$  times/d was 6·8 nmol/l higher than that of children with intake <5 times/week (95 % CI 0·7, 12·9 nmol/l; P = 0.03). Finally, mean serum 25(OH)D concentrations were inversely related to mother's BMI and to having >4 home assets. Maternal height and country of origin were not associated with the children's serum 25(OH)D concentrations in multivariable analysis.

# Family aggregation

Serum 25(OH)D concentrations were positively correlated between family members (Table 7). The mother–child correlation (r=0·49, P<0·0001) was higher than the father–child (r=0·24, P=0·0004) or mother–father (r=0·23, P=0·0004) correlation. Thirty-one per cent of the total variance in serum 25(OH)D was explained by family membership. Adjustment for age and sex did not change the results (adjusted ICC=0·32; 95 % CI 0·24, 0·41). Family membership explained more variability in 25(OH)D concentration than country membership, which only explained 12%. Mother–child aggregation of serum 25(OH)D concentrations was higher than overall family, father–child or mother–father aggregation.

#### Discussion

In the current study of Mesoamerican families, the overall prevalence of serum 25(OH)D concentration <50 nmol/l was relatively low for both adults (3.9%) and children (3.6%). Only one person had a serum 25(OH)D concentration <30 nmol/l, the threshold for deficiency proposed by the US Institute of Medicine<sup>(13)</sup>. Although the burden of VDD in Latin America is not well documented, the prevalence of VDD in our study is among the lowest reported in the region. For example, the prevalence of VDD in the National Survey of Nutrition and Health in Mexican adults was 9.8% in 2006<sup>(18)</sup>. No other country in the Mesoamerican region has conducted a nationwide survey to estimate vitamin D serostatus. Studies of Puerto Rican adults with different underlying health conditions found higher prevalence of VDD, ranging from 14 to 43%<sup>(2,3,19-21)</sup>. Nevertheless, neither these investigations nor our study were representative and the results may not be directly comparable.

Prevalence estimates of VDD among children and adolescents in the Mesoamerican region are variable. In asthmatic school-aged children from Costa Rica<sup>(22)</sup> and among Afro-Caribe Guatemalan adolescents<sup>(1)</sup>, the prevalence of VDD was 3·4 and 5·0%, respectively. These estimates are comparable to ours; however, among children from Southern Mexico (5–11 years old) in 2012 and adolescents (13–19 years old) in 2006 the prevalence of

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Table 4 Serum 25-hydroxyvitamin D (25(OH)D) concentrations according to sociodemographic characteristics in children from eight Mesoamerican countries, 2011-2013

		Mean	SD			
Sociodemographic characteristic	n*	nmo	DI/I	P†	% <50 nmol/l	<i>P</i> ‡
Sex				0.06		0.99
Female	119	77.3	18-0		3.4	
Male	104	81.9	17.9		3.9	
Age (years)				0.06		0.67
<9	72	83.6	20.2		4.2	
9–11 >11	75 76	77∙2 77∙7	16⋅3 17⋅1		4·0 2·6	
Height-for-age Z-score§	70	77.7	17.1	0.87	2.0	0.99
<-1.0	52	78-4	16.5	001	1.9	0.00
-1.0-<0.0	86	81.2	19.3		4.7	
0.0-<1.0	57	77.7	17.1		5.3	
≥1.0	28	79⋅5	19-4		0.0	
BMI-for-age Z-score§				0.22		0.18
<-1.0	33	82.0	14.7		0.0	
-1.0-<0.0	45	79·5	16.3		2.2	
0·0−<1·0 ≥1·0	69 76	80·4 77·4	18⋅9 19⋅7		4·4 5·3	
Parental highest education level	70	77.4	19.7	0.60	5.3	0.10
Incomplete elementary	20	79-6	19.6	0.00	5.0	0.10
Complete elementary	28	75·4	19.4		10.7	
Incomplete secondary	62	82.0	20.8		3.2	
Complete secondary	38	75.0	14.9		2.6	
Post-secondary	75	81⋅1	15.8		1.3	
Mother's height, quartile (median, cm)				0.01		0.34
Q1 (148·8)	58	74.9	15.7		1.7	
Q2 (153·1)	59	77.4	19-1		3.4	
Q3 (157·0)	54 50	85·5	17.7		3.7	
Q4 (162·4) Mother's BMI (kg/m²)	52	80-6	18-3	0.98	5.8	0.003
<25.0	51	77-3	15.7	0.96	0.0	0.003
25.0–29.9	84	81.7	17·2		1.2	
30.0–34.9	63	78·8	19.8		6.4	
≥35.0	25	77.9	20.9		12.0	
Mother's serum 25(OH)D status				<0.0001		0.0004
<50 nmol/l	5	57⋅5	25.6		40.0	
50-<75 nmol/l	74	70.7	15.4		6.8	
≥75 nmol/l	138	84.9	16⋅5	0.04	0.7	0.00
Father's serum 25(OH)D status	10	60 F	00 F	0.01	00.0	0.06
<50 nmol/l 50–<75 nmol/l	10 76	69∙5 76∙5	20⋅5 17⋅8		20·0 4·0	
>75 nmol/l	129	81·3	17.3		2.3	
Number of home assets	123	01.0	17.5	0.45	2.0	0.73
0–4	41	86-3	20.3	0 10	0.0	0.70
5–7	89	76.5	17.5		4.5	
8–9	41	76.8	17.9		7.3	
10–12	52	81⋅2	15.9		1.9	
Food insecurity in the household				0.07		0.25
None	73	76-9	16.2		6.9	
Mild	60	79.7	17.2		1.7	
Moderate Severe	50 39	79·8 84·1	18⋅6 21⋅4		2·0 2·6	
Country, latitude	39	04-1	21.4	0.005	2.0	0.10
Guatemala, 14.6°	28	70.7	10.0	0.003	0.0	0.10
El Salvador, 13·7°	29	79·0	18-2		6.9	
Dominican Republic, 18.5°	29	78.5	13.3		0.0	
Honduras, 14.1°	27	77.8	14.8		0.0	
Nicaragua, 12·1°	31	94.0	23.5		3.2	
Costa Rica, 9.6°	26	76.9	19-6		11.5	
Belize, 17:3°	26	81.0	19.7		7.7	
Mexico, 16·8°	27	76-0	13⋅3	0.67	0.0	0.00
Month of measurement	40	74.0	14.4	0.67	0.0	0.63
Jan–Feb Mar Apr	13 47	74·2	14·4 15.7		0.0	
Mar–Apr May–Jun	20	80⋅3 78⋅1	15⋅7 16⋅0		4⋅3 0⋅0	
	62	80·9	18.7		1.6	
Jul-Sep						

<sup>\*</sup>Totals may be less than 223 because of missing values.

Tosts may be less than 223 because of missing values. Test for trend for ordinal variables.  $\chi^2$  score test for sex, country and month of measurement. ‡Cochrane–Armitage exact test for trend for ordinal variables. Fisher's exact test for sex, country and month of measurement. §According to the WHO reference<sup>(15)</sup>. From a list that included car, bicycle, refrigerator, gas stove, electric stove, blender, microwave, washing machine, colour television, stereo, computer, and having in-home access to the Internet.

**Table 5** Serum 25-hydroxyvitamin D (25(OH)D) concentrations according to food intake frequency and time spent on outdoor activities in children from eight Mesoamerican countries, 2011–2013

		Mean	SD			
	n*	nm	ol/l	P†	% <50 nmol/l	<i>P</i> ‡
Food intake frequency						
Dairy§				0.002		0.63
<5/week	58	75.0	15⋅1		3.5	
5/week to <1.5/d	61	76.0	16⋅5		6.6	
1·5-<2·5/d	50	81.8	18⋅8		2.0	
≥ 2·5/d	43	84.2	17.9		2.3	
Eggs				0.51		0.51
<3/week	41	78⋅5	17.4		2.4	
3/week	50	76.9	19.3		8.0	
4-6/week	67	79.3	16.5		3.0	
≥ 1/d	54	79.9	16-6		1.9	
Canned tuna/sardines¶				0.31		0.74
<1/month	104	77.6	17.3		4.8	
1–3/month	43	81.1	15.3		0.0	
1/week	40	75.2	16.9		7.5	
≥ 2/week	25	85.1	19.9		0.0	
Outdoor activities						
Time spent playing outdoors (h/week)				0.10		0.64
<1	60	74.4	17.3		3.3	
1–10	55	82.3	18.6		3.6	
11–20	58	82.0	18.3		1.7	
≥21	49	79·7	17.5		6·1	

<sup>\*</sup>Totals may be less than 223 because of missing values.

VDD was 29·0 %<sup>(23)</sup> and 11·5 %<sup>(18)</sup>, respectively. These values are substantially higher than the estimates in our study. Similarly, VDD was much more prevalent in indigenous Mayan adolescents living near the coast (21%)<sup>(1)</sup> than in our study from Mesoamerica. These differences suggest that there is high within- and between-country variation in vitamin D status in children from the Mesoamerican region.

In our investigation vitamin D status varied substantially by country, although the country-specific sample sizes were too small to allow for robust statistical inference. Adults and children from Nicaragua had the highest mean serum 25(OH)D concentrations. Reasons to explain this difference are speculative but could be related to socio-economic or dietary differences since Nicaraguans in the present study had fewer home assets and higher dairy intake than participants from other countries and these variables were associated with higher 25(OH)D concentrations.

We found that mean serum 25(OH)D concentrations in adults were positively related to time spent gardening but not to other outdoor activities. Time spent on different outdoor activities may exert distinct effects on serum 25(OH)D levels because they could vary in the factors that ultimately influence the amount of vitamin D produced in the skin, including the amount of skin exposed, the time of day of sun exposure, the use of sunscreen and the

absolute amount of time exposed to the sun. For example, among elderly adults in Europe 25(OH)D concentrations were positively associated with gardening and cycling but not with walking outdoors<sup>(24)</sup>. The body postures during gardening may expose a larger skin surface to the sun compared with walking. In addition, the majority of ambient UV radiation occurs between 10.00 and 16.00 hours<sup>(25)</sup>. Perhaps people tend to garden within these hours, whereas other outdoor activities may occur outside this interval. In a study of Puerto Rican medical residents, time spent outdoors between 10.00 and 16.00 hours was positively associated with serum 25(OH)D<sup>(19)</sup>.

We did not find associations between age, sex or dietary sources of vitamin D and serum 25(OH)D concentrations among adults. A relationship of vitamin D status with age or sex has not been consistently demonstrated in previous studies of Mesoamerican adults  $^{(2,3,18-21,26)}$ . Contrary to our findings, in a study of Puerto Rican adults with BMI  $\geq 25.0 \, \text{kg/m}^2$ , a composite measure representing intake of vitamin D supplements, fish, margarine and milk  $^{(20)}$  was positively related to serum 25(OH)D concentrations. The main source of dietary vitamin D in that study was supplement intake, whereas in our study population supplement use was negligible. Discrepancies in the results might be explained by differences in supplement or food intake between populations.

<sup>†</sup>Test for trend for ordinal variables.

<sup>‡</sup>Cochrane-Armitage exact test for trend for ordinal variables.

<sup>§</sup>Sum of weighted intake frequencies of milk (200 g), cheese (20 g), cream (6 g) and ice cream (60 g).

Sum of weighted intake frequencies of one hard boiled or fried egg (60 g).

<sup>¶</sup>Intake of 25 g.

In children, mean serum 25(OH)D concentrations were positively associated with male sex, frequency of dairy intake, and both maternal height and serum 25(OH)D concentration. On the other hand, maternal BMI and the number of home assets were inversely related to 25(OH)D concentrations. Consistent with previous studies, boys had higher concentrations of serum 25(OH)D than

**Table 6** Multivariable-adjusted correlates of serum 25-hydroxyvitamin D (25(OH)D) concentrations in children from eight Mesoamerican countries, 2011–2013

	Adjusted difference*	95 % CI	
	nm	nol/l	P†
Male v. female	5.7	1.8, 9.7	0.005
Mother's height, quartile			0.26
(median, cm)			
Q1 (148·8)		rence	
Q2 (153·1)	2⋅1	<b>−4.0</b> , 8.2	
Q3 (157·0)	7⋅6	1⋅8, 13⋅5	
Q4 (162·4)	1⋅6	<b>–4</b> ⋅2, 7⋅4	
Mother's BMI, per 1 kg/m <sup>2</sup>	-0.4	–0.7, –0.1	0.02
Mother's serum 25(OH)D,	0.3	0.2, 0.5	<0.0001
per 1 nmol/l			
Number of home assets‡,	<b>-6</b> ⋅4	–12⋅4, –0⋅3	0.04
>4 <i>v</i> . ≤4			
Country, latitude			0.27
Guatemala, 14⋅6°	Refe	rence	
El Salvador, 13.7°	2.0	<b>–</b> 5⋅5, 9⋅6	
Dominican Republic, 18.5°	8.7	2.0, 15.5	
Honduras, 14·1°	4.8	<b>–</b> 2⋅3, 12⋅0	
Nicaragua, 12·1°	8.9	<b>−1.0</b> , 18.8	
Costa Rica, 9.6°	2.8	<b>−5</b> ·2, 10·8	
Belize, 17.3°	6.4	–0.9, 13.6	
Mexico, 16.8°	2.6	<b>−3</b> ·2, 8·4	
Frequency of dairy intake§			0.03
<5/week	Refe	rence	
5/week to <1.5/d	1.0	<b>−4.0</b> , 6.0	
1·5-<2·5/d	3.1	<b>-2</b> ⋅9, 9⋅1	
≥ 2·5/d	6.8	0.7, 12.9	

<sup>\*</sup>From a multivariable linear regression model with serum 25(OH)D as the continuous outcome. Predictors included all variables presented. Empirical variances were specified in the model.

girls<sup>(1,22,23,27)</sup>. Explanations for this difference are speculative; they could be due to sex-related variation in intake of foods with high vitamin D content or in time spent playing outdoors. Boys in the current study may have been less likely to wear sunscreen or may have had greater body surface exposure to sunlight compared with girls. We did not find a relationship between age and mean serum 25(OH)D in children, possibly because the age range in our study was limited. Mother's height was positively related to children's mean serum 25(OH)D concentrations, perhaps reflecting an association with socio-economic status since adult height can be an indicator of socio-economic status<sup>(28)</sup>. There was an inverse association between mothers' BMI and children's mean serum 25(OH)D concentrations. An association between mothers' pre-pregnancy BMI and infants' vitamin D status has been found previously (29). The mechanisms by which maternal BMI may influence children's vitamin D status are largely unknown. Obese women transfer vitamin D to their offspring less effectively at birth<sup>(29)</sup>, but this would not necessarily explain an association during middle childhood. On the other hand, the mother's BMI may be positively correlated with the child's and adiposity is inversely associated with circulating 25(OH)D concentrations<sup>(25)</sup>. Nevertheless, we did not find an association between the children's BMI and vitamin D status. Mothers' and children's serum 25(OH)D concentrations were strongly correlated, consistent with findings from a crosssectional study of Korean adolescents<sup>(30)</sup>. This possibly reflects the effect of genetic and environmental exposures that are shared between mothers and their children. Serum 25(OH)D concentrations were lower in children from households with >4 home assets compared with those from households with <4 assets. This inverse association between a marker of socio-economic status and 25(OH)D is consistent with results from other studies in the region<sup>(18,23,27)</sup>. Finally, in children, frequency of dairy intake was positively related to 25(OH)D concentrations. Fortification of milk with vitamin D is required in Guatemala, Honduras and Mexico<sup>(31)</sup> and in other countries some fortified milk is available. Randomized controlled trials of vitamin D-fortified foods demonstrate that intake of fortified foodstuffs results in higher 25(OH)D concentrations<sup>(32)</sup>. Of note, dairy intake in our study was

Table 7 Family aggregation of serum 25-hydroxyvitamin D (25(OH)D) in eight Mesoamerican countries, 2011–2013

	Mothers, fat	hers, and children	Mothers and fathers		Mothers and children		Fathers and children	
Correlation, r* (95% CI) Intraclass correlation†		_	0.23	0.10, 0.35	0.49	0.38, 0.58	0.24	0.11, 0.36
Within-family variance Between-family variance	282⋅8 127⋅1			345·0 101·7		215·7 191·6		285·3 87·6
ICC‡ (95% CÍ)	0.31	0.23, 0.40	0.23	0.13, 0.37	0.47	0.37, 0.57	0.23	0.13, 0.38

<sup>\*</sup>Pearson correlation coefficient.

<sup>†</sup>For height and frequency of dairy intake, test for linear trend when a variable representing ordinal categories of the characteristic was introduced as a continuous predictor. Wald test for sex, mother's BMI, mother's serum 25(OH)D concentrations and home assets.  $\chi^2$  score test for country.

<sup>‡</sup>From a list that included car, bicycle, refrigerator, gas stove, electric stove, blender, microwave, washing machine, colour television, stereo, computer, and having in-home access to the Internet.

<sup>§</sup>Sum of weighted intake frequencies of milk (200 g), cheese (20 g), cream (6 g) and ice cream (60 g).

<sup>†</sup>Variance partitioned from a linear random-effects model with serum 25(OH)D as the continuous outcome and a random effect for family. Separate models were fitted to partition the variance for each combination of two family members.

related to 25(OH)D concentrations in children but not in adults. This could be because the absolute consumption of dairy is usually higher and more variable in children than in adults<sup>(33)</sup>.

In our study, about a third of the variability in serum 25(OH)D concentration was explained by family membership. Family aggregation of serum 25(OH)D concentrations suggests that it may be effective to target families, rather than individuals, for screening of VDD and to implement interventions designed to improve vitamin D status. Family aggregation can be due to both shared genes and shared environment. Estimates of heritability of circulating 25(OH)D from twin studies range from 37 to  $86\%^{(5,6,3\bar{4}-37)}$ . This could be due to sharing of gene polymorphisms that are associated with 25(OH)D concentration (38). We found that correlation and aggregation between mothers' and fathers' 25(OH)D concentrations were similar to those between fathers and children, but lower than those between mothers and children. Because mothers and fathers are expected to be genetically unrelated, these comparisons could indicate that shared environmental factors play an important role in the vitamin D serostatus of this population, over that played by shared genetics. For example, sun exposure behaviours may aggregate by family. In Denmark, children's sun exposurerelated behaviours were correlated with their mothers' but not with their fathers, (39).

The present study has several strengths. Serum 25(OH) D concentrations are widely used in epidemiological studies as an indicator of vitamin D status since they integrate dietary and sunlight sources of vitamin D. The design involving families allowed for the examination of serum 25(OH)D correlates in adults and children, as well as aggregation within families. The use of standardized methods allowed for comparisons across countries where data were not previously available. There are limitations as well. First, the cross-sectional nature of the study limits the ability to make causal inference. Second, the samples were not representative of the underlying populations. Selection bias might have occurred if the probability of inclusion in the study was not independent of the exposures and outcomes examined. Also, the relatively small within-country samples prevented country-specific analysis. The FFQ that we used has not been validated in children. In addition, information on skin pigmentation, total time spent outdoors or sunscreen use was not available. Assuming that some adult participants use sunscreen, the magnitude of the underlying effect of gardening on mean serum 25(OH)D concentration could have been underestimated. Finally, the DiaSorin LIAISON 25-OH Vitamin D Total assay we used to measure 25(OH)D may underestimate its concentration compared with the gold standard of liquid chromatography-tandem mass spectrometry. This may lead to an overestimation of VDD at the population level<sup>(40)</sup>.

In conclusion, the prevalence of VDD was low in a group of adults and children from the Mesoamerican region. Serum 25(OH)D concentrations vary by country in both adults and children and exhibit a moderate degree of aggregation by family. Nationally representative surveys of vitamin D status are needed to determine individual-and country-level predictors of VDD in the region, including the effects of skin pigmentation, the use of sunscreen, clothing habits, environmental toxicants and air pollution.

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# Supplementary material

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