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Vitamin D supplementation trial in infancy: body composition effects at 3 years of age in a prospective follow-up study from Montréal

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Summary

Background: The impact of vitamin D status on body composition is not well understood.

Objectives: Evaluate how vitamin D supplementation in infancy affects body composition at 3 years of age.

Methods: Double-blind randomized trial of 132, 1-month-old healthy, breastfed infants randomly assigned to receive oral vitamin D_3 supplements of 400, 800, 1200 or 1600 IU d^{-1} for 11 months. In the present analysis, 87 (66%) returned at 3 years of age. Body composition was measured using dual-energy x-ray absorptiometry and plasma 25-hydroxyvitamin D [25(OH) D] concentrations by liquid chromatography tandem mass spectrometry.

Results: Anthropometry, body composition, diet, activity and demographics were similar across dosage groups at 3 years. Mean 25(OH)D concentration from 1 month to 3 years was higher ($P < 0.001$) in the 1200 IU group than 800 and 400 IU groups. Children with 25(OH)D concentrations above 75 nmol L^{-1} had lower fat mass (~450 g; $P = 0.049$). In multiple linear regression, mean 25(OH)D was associated with lean mass percent ($\beta = 0.06$; CI: 0.00, 0.12; $P = 0.042$), fat mass ($\beta = -11.29$; CI: −22.06, −0.52; $P = 0.048$) and body fat percent ($\beta = -0.06$; CI: −0.12, −0.01; $P = 0.045$).

Conclusions: Higher vitamin D status from infancy through to 3 years of age associates with leaner body composition.

Keywords: 25-hydroxyvitamin D, dose response, fat mass, lean muscle mass, paediatrics.

Introduction

Circulating 25-hydroxyvitamin D [25(OH)D] concentrations positively associate with lean body composition in infants (1) and adolescents (2). Correcting vitamin D deficiency in pre-pubertal girls increased lean mass compared with placebo (3). Whether vitamin D positively relates to lean body composition in younger children is unknown. Addressing this knowledge gap could improve understanding of the nutritional and environmental factors associated with healthy growth and body composition from birth to early childhood (4).

Vitamin D status is routinely assessed through total circulating 25(OH)D concentrations (5). Health guidelines recommend a standard-of-care supplemental intake of 400 IU d^{-1} for infants to establish a healthy 25(OH)D concentration in the range of 50 to 125 nmol L^{-1} (20–50 ng mL^{-1}) (6,7) and to continue

supplementation until a dietary source can provide this amount (8). Our previous dose-response study demonstrated that vitamin D_3 dosages of at least 400 IU d^{-1} in breastfed infants from 1 to 12 months of age increased 25(OH)D concentrations above 50 nmol L^{-1} (20 ng mL^{-1}) (9) and was associated with leaner body composition (1). The objective of the present study was to evaluate whether vitamin D supplementation in the first year of life (1,9) affects body composition at 3 years of age. Exploratory analyses tested for predictors of lean and fat mass to clarify the importance of vitamin D against known factors in the acquisition of a healthy body composition.

Methods

Study design and participants

This report is on an observational follow-up of children 3 years of age who participated in a published trial (1,9). Briefly, 132 infants at 1 month of age were randomized to receive one of four dosages of vitamin D_3 (400, 800, 1200 or 1600 IU d^{-1}) until 12 months with measurements conducted at 3, 6, 9 and 12 months of age. After 12 months, all families received the same brief education on using food and supplements for achieving the recommended vitamin D intake of 400 IU d^{-1} (7). Ethical approval was obtained from all associated University Institutional Review Boards. At baseline, demographic information for both parents was self-reported, including indicators of socio-economic status, education and race. Study visits included anthropometry, blood biochemistry and body composition measures. At the 3-year visit, parents completed 24 h food recall, sun index and physical activity surveys. The Peabody motor assessment (10) was completed by a certified occupational therapist.

Anthropometry

Height was measured using a stadiometer (Seca 213, Seca Medical Scales and Measuring Systems, California, USA) and body mass using a calibrated scale (Detecto, Missouri, USA). Body mass index (BMI; kg/m^2) and weight-for-age (WAZ), height-for-age (HAZ) and BMI-for-age (BAZ) Z-scores were derived using World Health Organization (WHO) software (WHO AnthroPlus, Switzerland) and the WHO 2007 growth standards (11).

Biochemistry analysis

A non-fasted capillary blood sample (heparinized) was collected via finger lance and plasma stored at −80 °C until analysis. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to measure 25(OH)D_3 as well as 3-epimer-25(OH)D_3, 24,25$(OH)_2D_3$ by Warnex Bioanalytical Services. This method is described in detail elsewhere (9); intra-assay coefficients of variation (CV) were <15% for all vitamin D metabolites with certification obtained from the Vitamin D External Quality Assessment Scheme. The measured 25(OH)D_3 concentrations of the National Institute of Standards and Technology standard reference materials (SRM 968e), were within 7.0% and 2.5% of the certified values for levels 2 and 3, respectively. Other biochemical measures included blood-ionized calcium (ABL80 FLEX Radiometer Medical A/S, Denmark) and intact PTH using ELISA (Immutopics International, California, USA) sensitive to 1.26 pmol L^{-1} (12 ng mL^{-1}) and had an intra-assay CV of <8%.

Body composition

Body composition was assessed using dual-energy x-ray absorptiometry (DXA; APEX version 13.2 or 13.2.3, Hologic 4500A Discovery Series, Massachusetts, USA). Infant whole-body mode was used between 0–12 months, and whole body with paediatric software was used at the 3-year visit. Output included lean mass (g; LM), LM as a percentage of total body mass (LM%), fat mass (g; FM), body fat percentage and total body mass. The CV for quality control measurements was 1% for bone mineral content and 0.327% for bone mineral density using a lumbar spine phantom (Hologic phantom No. 14774). Values for LM and FM accretion (g $month^{-1}$) were calculated as change in LM/FM from 1 month to 3 years, while LM index (LMI) and FM index (FMI) were calculated with LM and FM relative to height^2 (kg/m^2).

Habitual activity estimation scale questionnaire

All parents completed the validated Habitual Activity Estimation Scale (HAES) questionnaire for a weekday (Tuesday, Wednesday or Thursday) and weekend (Saturday) over the previous 2 weeks (12). For each time interval in the HAES (wake-up to breakfast, breakfast to lunch, lunch to dinner and dinner to bedtime), the percent time spent in each activity level was used to determine their child's overall level of physical activity: 'inactive' (sleeping, resting), 'somewhat inactive' (watching television), 'somewhat active' (playing with toys) and 'very active' (activities that make the subject 'breathe hard', including running and skipping).

Peabody developmental motor assessment

The Peabody assessment provides an assessment of gross and fine motor skills composed of six subtests (reflexes, stationary, locomotion, object manipulation, grasping and visual-motor integration) that measure interrelated motor abilities that develop early in life (10).

Dietary intake

Dietary intakes were determined using three 24 h recalls (one in person, two telephone) and nutrient intake generated using NUTRITIONIST PRO software version 4.7.0 (Axxya Systems LLC, Washington, USA) using the 2010b Canadian Nutrient File database (Health Canada). A validated 13-item food frequency questionnaire (FFQ) for preschool children, completed by the primary caregiver, was used to capture calcium and vitamin D intake during the month prior to the 3-year visit (13).

Sun index

Sun exposure, winter travel to more equatorial latitudes, and use of sunscreen were assessed using recall questionnaires that provided: percentage of body surface area exposed, frequency of sunscreen use, and total hours spent in direct sunlight per day. Sun index for each child was calculated by multiplying the percent body surface area exposed by the time spent outside (minutes per day), although this index does not consider the use of sun block (14). Season was defined at the time of visit using the equinox and solstice dates (15).

Skin pigmentation

Constitutive upper underarm and facultative forehead, lateral forearm shin skin pigmentation, were measured using a spectrophotometer (CM-600D, Konica Minolta, New Jersey, USA). The individual typological angle (ITA) of the forehead site was calculated (ITA° = [arc tangent (L* – 50)/b*)] 180/3.14159) (16). Infants were classified into five skin types: dark (≤10°), olive (10–28°), medium (28–41°), fair (41–55°) and very fair (>55°).

Statistical methods

Baseline and 3-year differences among groups were tested using ANOVA and X^2. The mean 25(OH)D concentration across all time points was calculated using the area under the curve based on the trapezoidal method. This allowed the examination of each participant's 25(OH)D concentration over the entire study without looking at individual time points (the interpretation was similar when each 25(OH)D assessment was included). The effect of vitamin D dosage on mean 25(OH)D concentration and body composition was evaluated. The relationship between vitamin D status and body composition at 3 years was then explored as a categorical variable. The Canadian Paediatric Society (CPS) defines a 25(OH)D status of $<75\,nmol\,L^{-1}$ ($30\,ng\,mL^{-1}$) as insufficient for normal growth and development of 6–11 year-old children (6), whereas the Institutes of Medicine (IOM) defines $<50\,nmol\,L^{-1}$ ($20\,ng\,mL^{-1}$) as an insufficient concentration (7). Participants above or below these cut-offs were compared for body composition using *t*-tests.

Regression models were constructed with variables important to childhood development including sex, mother's race (white or non-white), mother's education, family income (above or below $60 000), mean 25(OH)D concentration, energy intake ($kcal\,d^{-1}$) and relative protein intake ($g\,kg^{-1}\,d^{-1}$). Other variables explored included 3-epimer-25(OH)D_3, 24,25$(OH)_2D_3$, PTH, iCa, HAES (weekdays and weekends), Peabody scores (stationary, locomotion, and object manipulation), macronutrient, vitamin D ($IU\,d^{-1}$) and calcium ($mg\,d^{-1}$) intakes, and sun exposure (sun index and skin phototype). For models where mean plasma 25(OH)D accounted for a significant amount of the variation, we substituted 25(OH)D with the IOM cut-off ($50\,nmol\,L^{-1}$, $20\,ng\,mL^{-1}$) or CPS cut-off ($75\,nmol\,L^{-1}$, $30\,ng\,mL^{-1}$) but neither improved the model. Thus, models are presented with plasma 25(OH)D as a linear variable. Standard diagnostic procedures were performed. To check for multicollinearity, we used variance inflation factor using three as the cut-off and eigan values below 1.55. No colinearity was detected in the models. Significance for all statistical analyses was set at $P < 0.05$ and performed using SAS(version 9.2, North Carolina, USA).

Results

Eighty-seven children (66%) who completed the original trial returned for the 3-year follow-up (49 boys, 38 girls). Anthropometric, dietary, activity, Peabody and demographic variables at 3 years did not differ by vitamin D supplementation group (Table 1). There were no differences in treatment group allocation among returners vs. non-returners; however, mothers of returners were significantly older ($P = 0.05$) and of white ethnicity ($P = 0.01$) than non-returners (Table S1). All children grew in lean mass and fat mass over the duration of the study (Fig. 1).

Table 1 Characteristics of participants at follow-up (36 months) by treatment group

	400 IU *n* = 25	800 IU *n* = 25	1200 IU *n* = 26	1600 IU *n* = 11	All *n* = 87
Variable					
Age (month)	36.6 ± 0.7	36.6 ± 1.0	36.5 ± 0.6	37.9 ± 1.9	36.7 ± 1.1
Sex – male (*n*/%)	12/48%	16/64%	15/58%	6/55%	49/56%
Skin color – fair/very fair (n/%)	21/84%	21/84%	25/96%	9/90%	76/87%
Mother's ethnicity – White (*n*/%)	22/88%	20/80%	24/92%	6/55%	72/83%
Mother's with college/ university education (*n*/%)	22/88%	23/92%	24/92%	11/100%	80/91%
Family income – ≥$75 000 CND	19/76%	15/60%	16/62%	5/45%	55/63%
Anthropometry					
Height (m)	0.95 ± 0.03	0.95 ± 0.04	0.95 ± 0.03	0.97 ± 0.04	0.96 ± 0.03
HAZ	−0.12 ± 0.77	−0.27 ± 1.04	−0.21 ± 0.78	−0.16 ± 0.87	−0.20 ± 0.86
Weight (kg)	14.9 ± 1.4	14.5 ± 1.7	15.3 ± 1.8	15.4 ± 1.9	15.0 ± 1.9
WAZ	0.37 ± 0.75	0.06 ± 0.89	0.50 ± 0.87	0.33 ± 0.87	0.31 ± 0.85
BMI (kg/m^2)	16.4 ± 1.1	16.0 ± 1.0	16.7 ± 1.2	16.2 ± 1.1	16.3 ± 1.1
BAZ	0.63 ± 0.81	0.32 ± 0.75	0.84 ± 0.84	0.59 ± 0.81	0.60 ± 0.82
Lean mass (kg)	9.8 ± 1.0	9.6 ± 1.2	9.9 ± 1.2	10.4 ± 1.8	9.8 ± 1.2
Lean mass %	65.2 ± 4.5	66.4 ± 5.3	65.5 ± 4.2	66.3 ± 3.4	65.8 ± 4.5
Fat mass (kg)	4.6 ± 0.9	4.3 ± 1.0	4.6 ± 0.9	4.6 ± 0.8	4.5 ± 0.9
Body fat percentage	30.8 ± 4.6	29.5 ± 5.5	30.6 ± 4.3	29.6 ± 3.4	30.2 ± 4.6
Δ Lean mass 3 years – 1 month (g)	6045.9 ± 1080.0	5807.3 ± 1332.7	5990.1 ± 1201.4	6573.7 ± 1419.1	6026.8 ± 1231.7
Δ Fat mass 3 years – 1 month (g)	3453.7 ± 944.5	3358.1 ± 1005.1	3595.8 ± 1039.5	3682.6 ± 862.8	3496.9 ± 970.6
LMI (kg/m^2)	10.8 ± 0.7	10.6 ± 0.8	10.9 ± 0.9	10.8 ± 1.1	10.8 ± 0.8
FMI (kg/m^2)	5.1 ± 1.0	4.8 ± 1.0	5.1 ± 1.0	4.8 ± 0.6	4.9 ± 0.9
Biochemistry					
25(OH)D (nmol L^{-1})	71.8 ± 19.0	69.5 ± 14.1	79.8 ± 30.5	72.0 ± 14.4	73.8 ± 22.6
C-3 Epimer (nmol L^{-1})	3.0 ± 2.5	3.1 ± 2.6	4.1 ± 4.1	2.6 ± 2.2	3.3 ± 3.0
24,25(OH)$_2$D (nmol L^{-1})	15.0 ± 13.1	13.6 ± 11.9	16.8 ± 23.2	9.0 ± 2.9	14.3 ± 15.8
PTH (pmol L^{-1})	3.7 ± 1.2	3.7 ± 1.8	3.4 ± 1.0	3.8 ± 1.0	3.6 ± 1.3
Ionized Calcium (mmol L^{-1})	1.29 ± 0.03	1.30 ± 0.05	1.28 ± 0.06	1.27 ± 0.05	1.29 ± 0.05
Dietary Intake (3 × 24 h recall)					
Energy Intake (kcal d^{-1})	1433.8 ± 288.8	1394.2 ± 254.2	1502.4 ± 324.4	1367.0 ± 323.9	1433.9 ± 292.6
Protein Intake (% energy)	16.2 ± 2.7	15.8 ± 2.7	15.2 ± 2.8	18.1 ± 3.0	16.1 ± 2.9
Protein Intake (g/kg/d)	3.9 ± 0.9	4.0 ± 1.1	3.8 ± 0.9	3.9 ± 0.5	3.9 ± 0.9
Carbohydrate (% energy)	57.0 ± 6.6	54.3 ± 6.3	55.9 ± 6.3	51.0 ± 4.0	55.1 ± 6.2
Fat Intake (% energy)	28.8 ± 5.7	31.1 ± 4.9	30.8 ± 5.0	32.3 ± 3.3	30.5 ± 4.9
Habitual Activity Estimation Scale Questionnaire					
Weekday level of physical activity (*n* = 80)					
'Very inactive'	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
'Inactive'	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
'Somewhat inactive'	2 (8.7%)	1 (4.2%)	3 (12.5%)	0 (0%)	6 (7.5%)
'Somewhat active'	12 (52.2%)	9 (37.5%)	11 (45.8%)	5 (55.6%)	37 (46.3%)
'Active'	8 (34.8%)	9 (37.5%)	8 (33.3%)	2 (22.2%)	27 (33.8%)

(*Continues*)

Table 1 (Continued)

	400 IU $n=25$	800 IU $n=25$	1200 IU $n=26$	1600 IU $n=11$	All $n=87$
'Very active'	1 (4.3%)	5 (20.8%)	2 (8.3%)	2 (22.2)	10 (12.5%)
Weekend level of physical activity ($n=80$)					
'Very inactive'	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
'Inactive'	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
'Somewhat inactive'	1 (4.0%)	1 (4.2%)	2 (8.7%)	1 (11.1%)	5 (6.2%)
'Somewhat active'	13 (52.0%)	10 (41.7%)	11 (47.8%)	4 (44.4%)	37 (46.3%)
'Active'	8 (32.0%)	6 (25.0%)	7 (30.4%)	1 (11.1%)	22 (27.5%)
'Very active'	3 (12.0%)	7 (29.2%)	3 (13.0%)	3 (33.3%)	16 (20.0%)
Peabody Developmental Motor Assessment					
Overall Quotient	91.42 ± 17.67	96.16 ± 5.96	94.24 ± 17.40	96.73 ± 6.36	94.33 ± 13.82
Overall Percentile[a]	45.50 ± 20.63	40.68 ± 14.24	51.36 ± 19.77	42.00 ± 15.22	45.35 ± 18.23

[1]Normal range 100 ± 15 (16)
HAZ, height-for-age; WAZ, weight-for-age; BAZ, BMI-for-age; BMI, body mass index; LMI, lean mass index; FMI, fat mass index.

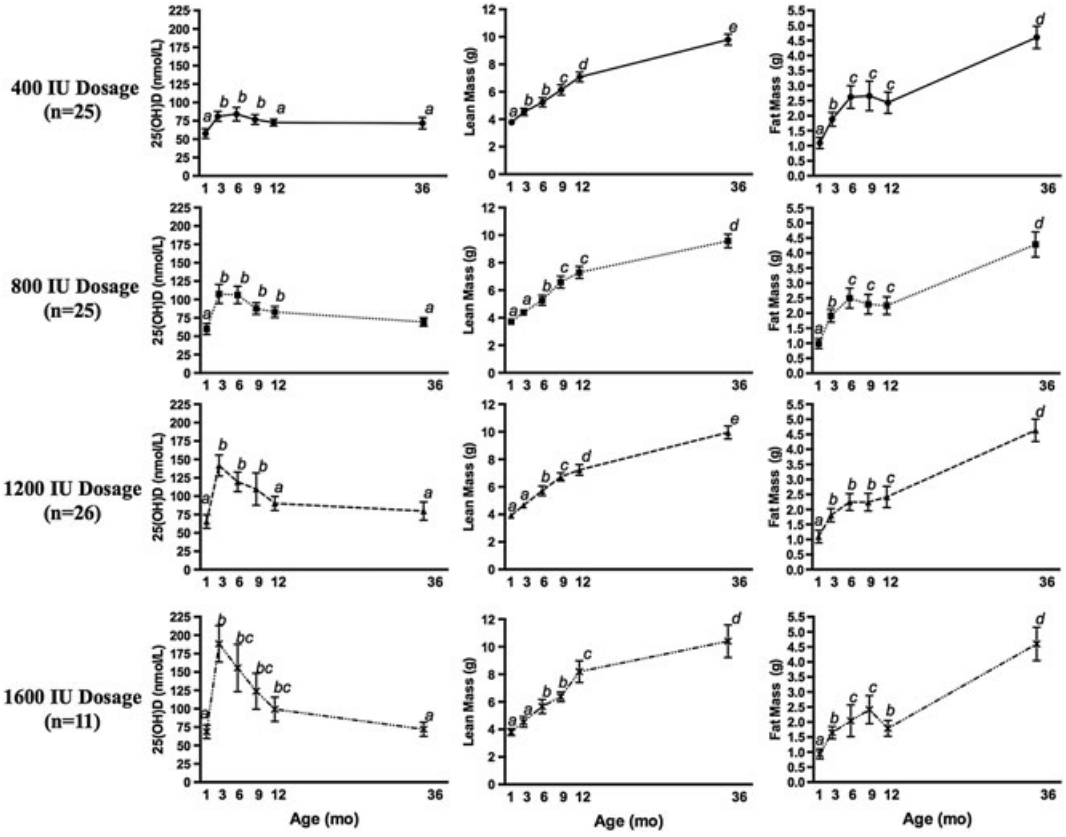

Figure 1 Vitamin D status, lean mass, and fat mass across infancy (1 to 36 months) in all four dosage groups (mean ± 95% confidence intervals).

Effect of vitamin D dosage on mean 25(OH)D concentration

Mean 25(OH)D concentration from 1 to 36 months differed by dosage group ($P<0.001$), where children in the 1200 IU d^{-1} group (102.9 ± 21.6 nmol L^{-1}) had higher values vs both the 800 IU d^{-1} (84.1 ± 25.7 nmol L^{-1}) and 400 IU d^{-1} (73.8 ± 12.6 nmol L^{-1}) groups. No other differences were observed. The 25(OH)D concentrations of each group at each time

point (Fig. 1) show early increases in vitamin D status from 1 to 3 months followed by declining values thereafter in all groups to 36 months. There was no effect of vitamin D dosage on 3-epi-25(OH)D_3 or 24,25$(OH)_2D_3$ ($P = 0.156$; Table 1).

Effect of vitamin D on body composition at 3 years

There was no effect of vitamin D dosage group on any body composition variable ($P > 0.357$; Table 1). Vitamin D status, lean and fat mass accretion over the first 36 months of life are illustrated in Fig. S1. In addition, there was no effect of having 25(OH)D concentration above 50 nmol L^{-1} for any body composition variable ($P > 0.096$; Table S2). Similarly, there was no effect of being above >75 nmol L^{-1} ($P > 0.078$) for LM, LM %, body fat percentage, LMI or FMI. However, children >75 nmol L^{-1} had a lower FM (~450 g; 4.3 ± 0.9 vs. 4.7 ± 1.0 kg) compared with children below ($P = 0.049$).

Effect of level of physical activity on body composition at 3 years

There was a significant effect of level of physical activity on weekends on FM and BF%. Children rated 'very active' (20.0%) had lower FM and BF% ($P < 0.034$) compared with 'somewhat inactive' (6.2%) and 'somewhat active' (46.3%) children. There were no other differences in any body composition variable according to level of physical activity on weekdays ($P > 0.547$) or weekends ($P > 0.255$). The Peabody assessment showed an overall gross motor quotient of 94.3 ± 13.8, and an overall percentile of 94.0 ± 14.6; 92% of the children had a motor quotient within the normal range of 100 ± 15 (17). There was no effect of any Peabody variable on body composition.

Regression analyses/predictors of body composition at 3-year

A significant amount of variation (adjusted R^2) in LM (Table 2) was accounted for by energy and protein (g kg^{-1}) intakes, whereas sex and mean 25(OH)D explained LM% and only sex was associated with LMI. In the models for FM (Table 2), sex, mother's education and protein intake (g kg^{-1} d^{-1}) accounted for a significant amount of the variation (adjusted R^2) with body fat percentage explained by sex and mean 25(OH)D concentration and FMI explained by sex and protein intake (g kg^{-1}).

Discussion

This follow-up study of a vitamin D supplementation trial in healthy term-born infants, who received a daily dose of at least 400 IU from 1 to 12 months of age, is the first to our knowledge to focus on body composition. While there were no differences in vitamin D status or body composition at 3-year across the different vitamin D dosage groups, this study provides novel findings that suggest higher plasma 25(OH)D concentrations early in life are associated with leaner body composition (18). Children with 25(OH)D concentrations above the suggested CPS cut-off of 75 nmol L^{-1} at 3 years of age had lower FM (~450 g) than children below 75 nmol L^{-1}. Children with physical activity levels of 'very active' on weekends as rated by their parents also had lower FM and body fat percentage compared with children rated as 'somewhat active' and 'somewhat inactive'. After accounting for these important correlates, mean 25(OH)D concentration over the first 3 years of life explained a statistically significant amount of variation in LM%, FM and body fat percentage. Interestingly, rapid gains in FM during the first 8 months of life predicted subsequent obesity at 9 years of age (4). Overall, our data suggest that children with higher vitamin D status over their first 3 years have a leaner body composition and that efforts to achieve healthy status targets should be made through ensuring adequate dietary or supplemental vitamin D intakes.

At present, there are two thresholds for evaluating vitamin D status; the IOM (50 nmol L^{-1}) and CPS (75 nmol L^{-1}) suggested cut-offs. Having a 25(OH)D concentration above the 75 nmol L^{-1} cut-off corresponded to lower FM at 3 years of age. Considering our previous results demonstrating 69% of infants receiving 1200 IU d^{-1} were able to achieve 75 nmol L^{-1} by 12 months as opposed to only 38% receiving 400 IU d^{-1} (9) and the results of the present study, there is a growing body of evidence to support the CPS cut-off. Additionally, in line with our previous data in the first year of life (1), it appears that variation in 3-epi-25(OH)D_3 and 24,25$(OH)_2D_3$ are not associated with body composition.

In addition to the mean 25(OH)D concentration over 3 years, other variables important to body composition included sex, mother's education, energy intake and relative protein intake (g kg^{-1} d^{-1}). Although research has suggested excess FM sequesters circulating vitamin D (19), our previous (1) and current work do not support this phenomenon in infancy to 3 years of age as FM increases while 25(OH)D plateaus. Male children were leaner as demonstrated by higher LM% and LMI as well as lower FM, body

Table 2 Correlates of body composition in children 3 years of age

	Regression coefficients	*P*-values	95% Confidence intervals
Lean mass (adjusted $R^2 = 0.326$)	Intercept = 9898.74		
Sex (ref = female)	287.28	0.330	−282.02, 856.58
Mean 25(OH)D_3 (nmol L^{-1})	5.24	0.443	−8.00, 18.48
Mother's Race (ref = white)	658.37	0.248	−438.35, 1755.09
Mother's education (ref = high school)	−1514.10	0.134	−3443.6, 415.46
Family income (ref = <$59 999)	−180.87	0.647	−948.97, 587.23
Energy intake (kcal d^{-1})	1.66	0.013	0.42, 2.90
Relative protein intake (g $kg^{-1} d^{-1}$)	−554.65	0.007	−934.01, −175.29
Lean mass % (adjusted $R^2 = 0.287$)	Intercept = 45.92		
Sex (ref = female)	4.71	0.001	2.21, 7.20
Mean 25(OH)D_3 (nmol L^{-1})	0.06	0.042	0.00, 0.12
Mother's race (ref = white)	2.98	0.233	−1.83, 7.79
Mother's education (ref = high school)	3.15	0.471	−5.31, 11.61
Family income (ref = <$59 999)	0.96	0.580	−2.41, 4.33
Energy intake (kcal d^{-1})	0.01	0.893	−0.01, 0.01
Relative protein intake (g $kg^{-1} d^{-1}$)	−1.52	0.083	−3.18, 0.15
Lean mass index (adjusted $R^2 = 0.303$)	Intercept = 9.324		
Sex (ref = female)	0.64	0.005	0.23, 1.04
Mean 25(OH)D_3 (nmol L^{-1})	0.01	0.445	−0.01, 0.01
Mother's race (ref = white)	0.55	0.182	−0.24, 1.34
Mother's education (ref = high school)	−0.27	0.709	−1.65, 1.12
Family income (ref = <$59 999)	0.05	0.857	−0.50, 0.60
Energy Intake ($kcal^{-1} d^{-1}$)	0.01	0.058	0.00, 0.01
Relative protein intake (g $kg^{-1} d^{-1}$)	−0.22	0.126	−0.49, 0.05
Fat mass (adjusted $R^2 = 0.443$)	Intercept = 9165.18		
Sex (ref = female)	−843.93	0.001	−1360.90, −380.96
Mean 25(OH)D_3 (nmol L^{-1})	−11.29	0.048	−22.06, −0.52
Mother's race (ref = white)	−274.40	0.551	−1166.26, 617.46
Mother's education (ref = high school)	−1749.80	0.036	−3318.94, −180.66
Family income (ref = <$59 999)	−269.83	0.405	−894.46, 354.80
Energy intake (kcal d^{-1})	0.86	0.105	−0.15, 1.87
Relative protein intake (g $kg^{-1} d^{-1}$)	−561.64	0.001	−870.14, −253.14
Body fat % (adjusted $R^2 = 0.306$)	Intercept = 50.84		
Sex (ref = female)	−4.7	0.001	−7.28, −2.23
Mean 25(OH)D_3 (nmol L^{-1})	−0.06	0.045	−0.12, −0.01
Mother's race (ref = white)	−2.83	0.263	−7.70, 2.04
Mother's education (ref = high school)	−3.65	0.411	−12.22, 4.93
Family income (ref = <$59 999)	−1.09	0.535	−4.50, 2.32
Energy intake (kcal d^{-1})	0.01	0.769	0.00, 0.01
Relative protein intake (g $kg^{-1} d^{-1}$)	−1.71	0.055	−3.40, −0.03
Fat mass index (adjusted $R^2 = 0.357$)	Intercept = 9.28		
Sex (ref = female)	−0.82	0.003	−1.31, −0.33
Mean 25(OH)D_3 (nmol L^{-1})	−0.01	0.337	−0.03, 0.00

(*Continues*)

Table 2 (Continued)

	Regression coefficients	*P*-values	95% Confidence intervals
Mother's race (ref = white)	−0.42	0.410	−1.37, 0.53
Mother's education (ref = high school)	−1.07	0.204	−2.74, 0.60
Family income (ref = <$59 999)	−0.20	0.545	−0.87, 0.46
Energy intake (kcal d^{-1})	0.01	0.359	0.00, 0.01
Relative protein intake (g kg^{-1} d^{-1})	−0.46	0.010	−0.79, −0.13

$25(OH)D_3$, plasma 25-hydroxy vitamin D_3; mother's race (white vs non-white); mother's education (high school vs. college/university/vocational); family income (above or below $60 000).

fat percentage, and FMI compared with female children in line with previous research (20,21). Mother's with higher education had leaner children (decreased FM). Family income showed no effect on body composition (22), in contrast with previous research (23), which may be due to the rather homogenous study group. With respect to nutrition, children with higher energy intakes (kcal d^{-1}) had higher amounts of LM but energy intake was not linked to other body composition variables. A higher relative protein intake (g kg^{-1} d^{-1}) was inversely associated with LM, FM and FMI, but not LM% and LMI or body fat percentage suggesting relatively minimal effects. Previous research has demonstrated increased protein intakes can result in lower amounts of FM (24), although no relationship has also been demonstrated (25) in children as young as 3 years. However, these studies did not examine intake relative to body mass (g kg^{-1} d^{-1}).

Interestingly, a child's level of physical activity on weekends as rated by their parent was important for FM and body fat percentage. 'Very active' children had lower FM and body fat percentage than children rated as only 'somewhat active' or 'somewhat inactive' demonstrating the importance of physical activity to a leaner body composition in young children. Although the rating of physical activity on weekdays did not relate to body composition, weekends are less structured implying that families who are more active on the weekend have leaner children (26).

The present study is not without limitations. The study group may have been too homogenous to detect some relationships (all were healthy, term born, appropriate size for gestational age and sex). Infants were mainly (88%) breastfed until 6 months of age (9) and demonstrated no differences between groups in macronutrients or micronutrient intakes. Further, our sample was predominately white and although mother's race had no impact on body composition, whether this effect would have been observed in a more demographically diverse group is unknown. The fact that all children were receiving at least 400 IU d^{-1} of vitamin from 1 to 12 months likely contributed to the very good vitamin D status >50 nmol L^{-1} 25(OH)D and precluded studying children with lower vitamin D status. Although we had a rather large sample for a repeated measures design of over 3-year, because of the variability in body composition variables, a type II error cannot be ruled out. We also did not capture data between 1 year and 3 years; thus, it is not clear whether the mean vitamin D status is fully captured using our follow-up data. Finally, our study cannot provide a possible mechanism for the importance of vitamin D to a leaner body composition, although previous work has demonstrated the vitamin D receptor binds the biologically active form of vitamin D, 1α,25-dihydroxyvitamin D [$1,25(OH)_2D$], to signal gene transcription and sensitize the Akt/mTOR pathway involved in protein synthesis (27,28). More research is certainly warranted, to establish how 25(OH)D contributes to a leaner body composition.

Conclusion

This long-term follow-up of a vitamin D supplementation study in children demonstrates no difference in body composition across any dosage groups (400, 800, 1200 and 1600 IU). However, a higher vitamin D status over the first 3 years of life was associated with a leaner body composition. Further, children with higher levels of physical activity (parent-rated) were also leaner compared with less active children. All children maintained a vitamin D status >50 nmol L^{-1} of 25(OH)D which is common in population surveillance in Canada (29) and the USA (30). This novel study demonstrates higher vitamin D status and physical activity levels associate with leaner body composition in 3-year-old children.

Disclosure statement

The authors have nothing to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Vitamin D status, lean mass, and fat mass across infancy (1 mo to 36 mo). — Different letters denote significant differences within same line ($P>0.05$).
Table S1. Comparison of returners vs. non-returners. Data presented as No. (%) unless otherwise indicated
Table S2. Vitamin D Status Cut-offs and Body Composition.