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Impact of Vitamin D Supplementation on Gross Motor Development of Healthy Term Infants: A Randomized Dose-Response Trial

Brandy Wicklow[1,2], Sina Gallo[3], Annette Majnemer[4], Catherine Vanstone[3], Kathryn Comeau[3], Glenville Jones[5], Mary L'Abbe[6], Ali Khamessan[7], Atul Sharma[4], Hope Weiler[3], & Celia Rodd[3,4,*]

[1]The Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada, [2]University of Manitoba, Pediatrics and Child Health, Winnipeg, Manitoba, Canada, [3]School of Dietetics and Human Nutrition, McGill University, Ste. Anne de Bellevue, Quebec, Canada, [4]Montreal Children's Hospital, Montreal, Quebec, Canada, [5]Departments of Biomedical and Molecular Sciences and Medicine, Queen's University, Kingston, Ontario, Canada, [6]Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada, [7]EURO-PHARM International Canada Inc., Montreal, Quebec, Canada

ABSTRACT. In addition to benefits for bone health, vitamin D is implicated in muscle function in children and adults. *Aims*: To determine if vitamin D dosage positively correlated with gross motor development at 3 and 6 months of age. We hypothesized that higher doses would be associated with higher scores for gross motor skills. *Methods*: A consecutive sample of 55 healthy, term, and breastfed infants from Montreal, Canada were recruited from a randomized trial of vitamin D supplementation between 2009 and 2012. Infants were randomized to 400 International Units (IU) ($n = 19$), 800 IU ($n = 18$) or 1,200 IU ($n = 18$) vitamin D_3/day. Motor performance at 3 and 6 months was quantified by the Alberta Infant Motor Scale (AIMS). Plasma vitamin D_3 metabolites were measured by tandem mass spectrometry. *Results*: AIMS scores did not differ at 3 months. However, total AIMS scores and sitting subscores were significantly higher at 6 months in infants receiving 400 IU/day compared to 800 IU/day and 1,200 IU/day groups ($p < .05$). There were weak negative correlations with length and C-3 epimer of 25(OH)D. *Conclusions*: In contrast to our hypothesis, gross motor achievements were significantly higher in infants receiving 400 IU/day vitamin D. Our findings also support longer infants being slightly delayed.

KEYWORDS. Alberta Infant Motor Scale, gross motor, infant development, infant motor skills, vitamin D

*co-senior authors.
Address correspondence to: Brandy Wicklow, MD, MSc, The Children's Hospital Research Institute of Manitoba, 513-715 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 3P4. (E-mail: bwicklow@hsc.mb.ca)

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INTRODUCTION

The known effects of vitamin D on increasing bone mineral density and the potential for reducing future fracture risk have led to significant efforts to determine optimal vitamin D status (Golden and Abrams, 2014). This optimal status would provide benefits for bone mass while avoiding the risk of vitamin D toxicity, including hypercalcemia, hypercalciuria, and nephrocalcinosis (Vogiatzi et al., 2014). Typically, vitamin D status is assessed by measuring the storage form of vitamin D – 25-hydroxyvitamin D (25(OH)D) – in serum or plasma. However, other indicators are being explored to help define optimal status (Beaudart et al., 2014), such as muscle mass and function. Debilitating proximal myopathy is a known consequence of vitamin D deficiency (frequently described as <10 ng/mL [conversion ng/mL × 2.5 = nmol/L] 25(OH)D) (Fluss et al., 2014; Faridi and Aggarwal, 2010; Ziambaras and Dagogo-Jack, 1997; Prabhala et al., 2000; Kozanoglu et al., 2005; van der Heyden et al., 2004; Alyaarubi and Rodd, 2005; Institute of Medicine, 2011). In infants, this manifests as significant delays in gross motor development (Alyaarubi and Rodd, 2005; Ward et al., 2007). In children and adults with vitamin D deficiency, restoring plasma vitamin D status dramatically reverses muscle weakness within weeks; repletion is often defined as ≥20 ng/mL 25(OH)D (Flicker et al., 2005; Dukas et al., 2004; Murray et al., 2006). Additionally, a recent study reported a beneficial influence of vitamin D on muscle mass in adults with the implication that this may lead to improved motor function and prevention of falls (Scott et al., 2010).

The timing of attainment of motor skills is an important marker of neuromotor maturation. Otherwise, healthy infants in the lowest 10th percentile for motor milestone achievement may be considered an at risk population requiring further assessment and close monitoring (Piper and Darrah, 1994). Epidemiological evidence suggests that gross and fine motor skills attainment in infancy is important for interaction with the environment and future academic achievements (Murray et al., 2006; Taanila et al., 2005). Despite the recent evidence for the role of vitamin D in motor function in older children and adults, there is a paucity of data on the role of vitamin D on infant motor development.

As noted above, the blood or plasma concentration of the storage form of vitamin D, 25(OH)D, is commonly used to assess vitamin D status. Interestingly, infants also have moderate to high concentrations of C-3-epimer-25(OH)D (Bailey et al., 2014; Gallo et al., 2013; Singh et al., 2006). This epimer has the same chemical structure as 25(OH)D with the exception of the orientation of one hydroxyl group (-OH). This change in orientation is felt to inactivate any vitamin D activity. Surprisingly, C-3-epimer-25(OH)D is found in high concentrations in infants (Bailey et al., 2014; Bailey et al., 2013). Newer tandem mass spectrometry (LC-MS/MS) methods are required to measure both isoforms accurately (Bailey et al., 2014; Gallo et al., 2013). Additionally, the metabolite 24, 25-dihydroxyvitamin D (24, 25(OH)2D) synthesized from 25(OH)D is found in relatively high concentrations when there are robust concentrations of 25(OH)D, it can also be measured by LC-MS/MS.

This pilot study was performed to investigate whether healthy term infants receiving different dosages of vitamin D supplementation differ in their acquisition of motor skills. One validated tool for evaluating Canadian children is the Alberta

Infant Motor Scale (AIMS) (Piper et al., 1992), which assesses developmental milestones in children up to the stage of independent walking. The AIMS tool was chosen as it was the only Canadian based assessment tool at the time of recruitment that was validated in infants up to the age of walking. Infants enrolled in a dose-response trial (ClinicalTrials.gov # NCT00381914) were asked to participate in this ancillary trial. All were healthy, term, and breastfed infants previously randomized in a double-blind manner to 400, 800, or 1,200 IU/day vitamin D_3 supplementation starting at 1 month and continued till 12 months of age. Our hypothesis was that vitamin D dosage and 25(OH)D plasma concentrations would be positively correlated with infant gross motor skills at 3 and 6 months of age. These time points were selected because the infants would be deriving most of their vitamin D from the supplements, given high rates of breastfeeding and limited alternate food sources.

METHODS

Sample Size

Sample size calculation was based on power to detect a clinically significant difference of 1.0 standard deviation (3.5 points) in AIMS motor score between the two extreme treatment groups (400 IU/day and 1,200 IU/day) (Piper and Darrah, 1994). For 80% power, we aimed to enroll 15 infants per treatment arm; 3–4 additional infants were recruited in each arm in case of study dropouts.

Participants

Infants participating in a preexisting randomized control trial of vitamin D_3 supplementation (ClinicalTrials.gov #NCT00381914) were approached on a sequential basis at their baseline study visit and asked to participate in this ancillary study (Gallo et al., 2013). Recruitment for the ancillary component occurred from August 2009 to July 2011. Randomization and treatment allocation were blinded to families and study staff, including the occupational therapist or physiotherapist performing the AIMS assessments. Participating infants were healthy, breastfed and, term infants of an appropriate size for gestation, residing in Montreal and the surrounding area. Infants were recruited to the dose-response study from participating general pediatric clinics during their two-week well-baby visit; see Gallo *et al.* for complete details regarding the main study (Gallo et al., 2013). Infants were excluded from the larger study if they were born to women with gestational diabetes, hypertension in pregnancy, or chronic alcohol use or if infants were shown to have a malabsorption syndrome, were of a multiple birth, or had a neurologic or genetic condition affecting development. Written informed consent for this ancillary study was obtained from the parent(s). The study received ethics approval from the Montreal Children's Hospital Institutional Review Board.

A total of 55 infants were enrolled into the study following randomization to the dose-response trial. Of the 55 infants, 19 were randomized to receive 400 IU, 18 were randomized to 800 IU, and 18 were randomized to receive 1,200 IU vitamin D_3 supplementation daily. Only two mothers who were approached refused participation in the substudy due to the time commitment required for the follow up visits. Two infants did not participate in the 6 months AIMS assessments, as they

were unable to coordinate the last study visit within the 1-week time frame; both were receiving 800 IU vitamin D_3 daily. Nonparticipants did not differ from infants completing the study for baseline demographics or anthropometric measures (data not shown).

Measures

Alberta Infant Motor Scale

The Alberta Infant Motor Scale (AIMS) (Piper et al., 1992; Piper and Darrah, 1994) evaluates the spontaneous movement repertoire of infants (birth to walking) and is a performance-based assessment focusing on what the infant does spontaneously, with minimal handling or facilitation by the examiner. The AIMS is a Canadian norm-referenced tool designed to identify delays or deviations from typical development in young children (Piper and Darrah, 1994). The measures consist of 58 items that assess posture and movement with and against gravity. Items are scored as observed/not observed for each of the positions or movements attained and results in a quantitative score for the level of motor performance in each supine, prone, sitting, and standing position (Piper and Darrah, 1994). Performance scores are tallied to give an overall raw composite score, which is compared to normative data. Delay in gross motor development using the AIMS score is defined as a score $<10^{th}$ percentile (Piper and Darrah, 1994).

Evaluation of Vitamin D Status

Plasma vitamin D metabolites – including 25(OH)D_3, C-3-epimer-25(OH)D_3, and 24, 25(OH)$2D_3$ – were quantified by tandem mass spectroscopy at a single laboratory (LC-MS/MS, Warnex Inc., Laval, QC). No infants had measurable concentrations of vitamin D2 metabolites or epimers. Feeding practices were surveyed by interview at each study visit; intake noted included both breast milk and fortified infant formula intake (Gallo et al., 2013).

Procedure

Infants were administered the AIMS in their home by either a pediatric occupational therapist or physiotherapist. The same therapist carried out both the 3 and 6 months assessments on each individual infant. One therapist performed over 90% of all assessments. All AIMS assessments occurred within one week of the infant turning 3 and 6 months. Infants were seen at 1, 2, 3, and 6 months for anthropometric measurements (length, weight, and head circumference) and plasma biochemistry. Anthropometric measurements at birth were obtained from hospital birth records. Length, weight, and head circumference measures were converted to z-scores based on age and gender-specific normative values using data from the WHO (http://www.who.int/childgrowth/software). At the 3 and 6 months visits, infants underwent dual-energy x-ray absorptiometry (Hologic 4500 A Discovery, APEX software version 13.2:1, Hologic Inc., Waltham, MA) for determination of lean body mass. Data on ethnicity, household income, maternal education, maternal age and parity, and infant feeding practices were obtained through parent interview at the first study visit. Additional details of the larger study have been published elsewhere (Gallo et al., 2013).

Data Analysis

Descriptive statistics were used to characterize the three infant groups (400 IU, 800 IU, and 1,200 IU vitamin D_3); the data were examined graphically for outliers. Data were normally distributed; mean and standard deviation (SD) are provided. The three treatment groups were compared for baseline characteristics, including growth parameters and demographics, with continuous variables assessed by factorial analysis of variance (one-way ANOVA) and dichotomous variables by chi-squared test of proportions (Fisher exact test for small samples). One-way ANOVA was used to compare 25(OH)D concentrations and infant motor scores in the 3 treatment groups at 3 and 6 months follow-up. Significant group differences were localized with post-hoc Tukey tests. Pearson correlations were used to identify other possible associations with motor score outcomes. A linear regression was used to test for the independence of covariates found to be significant in this bivariate screening (length z-score and C-3 epimer) with the 6 months composite AIMS score as the outcome; no interactions were found. Standard diagnostic testing was performed. All data were analyzed on an intention-to-treat basis. The significance level was set at <0.05.

RESULTS

Group Characteristics

At baseline, no differences were found among the three groups of infants in anthropometric measures, sex, maternal age, percentage first born, household income, maternal education, vitamin D metabolites, and C-3-epimer-25(OH)D_3 (Table 1). At 3 and 6 months, these also did not differ; additionally at birth, weight, length, and head circumference were not different among the groups, with mean ($\pm$ SD) z-scores of 0.6 ($\pm$ 0.8), 1.1 ($\pm$ 1.2), and 0.4 ($\pm$ 1.1), respectively. All mothers had a high socioeconomic status (income and education); there was no difference in the percentage of women in the three groups attaining college or university education (90% – 400 IU vs. 94% – 800 IU, vs. 89% – 1,200 IU, $p = .9$).

As expected, plasma 25(OH)D_3 measured at 3 and 6 months were significantly different among the three groups (by ANOVA), with higher concentrations in the 800 IU/day and 1,200 IU/day groups. Pairwise, post-hoc Tukey comparisons are presented in Table 1. Similar differences were noted in C-3-epimer-25(OH)D_3 (3 and 6 months) and 24, 25(OH)2D_3 (3 months only). At both 3 and 6 months, all infants achieved the 20 ng/mL threshold proposed by the Institute of Medicine (Institute of Medicine, 2011)

AIMS Scores

There was no difference in the AIMS scores at 3 months among groups. However at 6 months, total scores and sit subscores were significantly higher in infants receiving 400 IU/day vitamin D_3 compared to the 800 IU and 1,200 IU groups. Infants in the 400 IU/day group attained milestones in the sitting subscore earlier than the other 2 groups at 6 months. Additionally, prone subscores were also higher in infants receiving 400 IU compared to the 800 IU group (Table 2). Chi-square test of proportions comparing the 3 groups at 6 months showed no statistical differences

TABLE 1. Characteristics of Infants in the Three Groups Receiving 400 IU, 800 IU, or 1200 IU Daily of Vitamin D_3 Supplementation at 1, 3, and 6 Months of Age. Data Are Presented as Means ± Standard Deviation, or Percent (Categorical Variables)

Group Characteristics	400 IU Vitamin D_3 ($n = 19$)			800 IU Vitamin D_3 ($n = 18$)[c]			1200 IU Vitamin D_3 ($n = 18$)		
Age (months)	1	3	6	1	3	6	1	3	6
Sex (% male)	47% (9/19)	47% (9/19)	47% (9/19)	61% (11/18)	61% (11/18)	62% (10/16)	61% (11/18)	61% (11/18)	61% (11/18)
Weight z-score	0.27 ± 0.89	0.07 ± 0.76	0.04 ± 0.91	0.20 ± 0.93	0.08 ± 1.00	0.05 ± 1.16	0.43 ± 0.78	0.23 ± 0.78	0.16 ± 0.76
Length z-score	−0.03 ± 1.03	0.22 ± 0.69	−0.19 ± 0.72	−0.04 ± 0.91	0.32 ± 0.92	0.34 ± 1.15	−0.04 ± 0.96	0.22 ± 0.95	0.06 ± 0.92
Head circumference z-score	0.84 ± 1.05	0.51 ± 1.06	0.52 ± 0.92	0.56 ± 0.69	0.77 ± 1.32	0.63 ± 1.08	0.95 ± 0.83	0.75 ± 0.75	0.82 ± 0.80
Weight for length z-score	0.49 ± 1.06	−0.08 ± 0.97	0.28 ± 0.98	0.37 ± 0.86	−0.15 ± 0.94	−0.10 ± 1.23	0.69 ± 0.94	0.14 ± 0.80	0.27 ± 0.70
Maternal age (years)	31.9 ± 4.7	—	—	32.1 ± 4.8	—	32.4 ± 5.0	32.3 ± 4.1	—	—
Parity (% first born)	47% (9/19)	—		44% (8/18)	—	44% (7/16)	22% (4/18)	—	—
Household income (%)(> $75,000 CD/annum)[b]	63% (12/19)	—	—	53% (9/17)	—	60% (9/15)	50% (9/18)	—	—
Breastfed (any) (%)	100% (19/19)	95% (18/19)	95% (18/19)	100% (18/18)	100% (18/18)	100% (16/16)	100% (18/18)	100% (18/18)	89% (16/18)
Birth season[d] (% Born) (spring and summer)	53% (10/19)			44% (8/18)		50% (8/16)	66% (12/18)		
Ethnicity (%) (white/other)	84%, 16/3	—	—	83%, 15/3	—	88%, 14/2	94%, 17/1	—	—
25(OH)D3 (ng/mL)[a]	22.6 ± 8.2	33.1 ± 7.5	32.5 ± 10.2	20.9 ± 9.1	40.6 ± 11.6	40.2 ± 10.3	26.3 ± 10.0	54.3 ± 16.5[e]	46.3 ± 16.2[e]
C-3-epimer-25(OH)D3 (ng/mL)	5.0 ± 3.4	7.0 ± 3.9	5.0 ± 3.0	4.5 ± 3.1	11.1 ± 7.1	6.8 ± 4.8	8.2 ± 5.1	18.5 ± 9.6[e]	9.8 ± 5.6
24,25(OH)^{2}D3 (ng/mL)	2.5 ± 1.5	4.4 ± 1.7	4.3 ± 2.2	2.0 ± 1.3	5.8 ± 3.3	4.9 ± 2.4	2.5 ± 1.3	8.4 ± 4.0[e]	6.2 ± 2.8

[a]Conventional units (ng/mL) ×2.5 can be used to convert to SI units (nmol/L) for all vitamin D metabolites or epimers.
[b]Missing variable income for one family in the 800 IU group.
[c]2 infants were not assessed by the AIMS at 6 months in the 800 IU group ($n = 16$).
[d]Season of Birth: Spring and summer have sufficient levels of ultraviolet sun rays type B (UVB) to allow endogenous vitamin D synthesis; therefore, the synthesizing period defined as the date of birth of April 1st to October 31st (months with the potential for cutaneous vitamin D synthesis) and fall and winter (nonsynthesizing) defined as November 1st to March 31st.
[e]Mean ± SD, $p < .05$ compared to the 400 IU group for that same time period.

TABLE 2. Comparisons of Motor Performance as Determined by the Alberta Infant Motor Scales at 3 and 6 Months of Age in Infants Receiving 400 IU, 800 IU, and 1,200 IU Vitamin D3 Supplementation, Respectively

	3 Months			6 Months		
Motor Performance Alberta Infant Motor Scale	400 IU $n = 19$	800 IU $n = 18$	1200 IU $n = 18$	400 IU $n = 19$	800 IU[a] $n = 16$	1.200 IU $n = 18$
Prone raw score (SD)	4.3(1.7)	4.1(1.6)	3.3(1.2)	10.5(2.3)	8.8(1.7)[b]	9.0(2.0)
Supine raw score (SD)	3.9(0.6)	4.1(0.5)	3.9(0.7)	7.7(1.1)	7.0(0.6)	7.3(0.8)
Sit raw score (SD)	1.6(0.8)	1.4(0.9)	1.4(0.7)	6.6(1.8)	5.4(1.4)[b]	5.3(1.1)[b]
Stand raw score (SD)	2.0(0.0)	2.0(0.0)	2.0(0.0)	2.8(0.4)	2.8(0.5)	2.6(0.5)
Total composite score (SD)	11.7(2.3)	11.5(2.1)	10.7(1.9)	27.6(4.6)	23.9(2.7)[b]	24.2(3.1)[b]
Percentile score (SD)	41.2(24.8)	38.4(21.9)	29.8(19.8)	44.7(27.7)	23.6(13.9)[b]	25.6(17.7)[b]

[a]Missing values for two infants at 6 months in the 800 IU group ($n = 16$).
[b]$p < .05$ compared to 400 IU group Mean (SD).

TABLE 3. Correlations Between AIMS Scores, Vitamin D Metabolite Concentrations and Anthropometry at 6 Months

Alberta Infant Motor Scale	Plasma Vitamin D Metabolite or Epimer		Anthropometry			
6 months assessment	25(OH)D3	C-3-epimer-25(OH)D3	Length z-score	Weight z-score	Head circumference z-score	Lean body mass (grams/%)
Prone raw score	−0.24	−0.30[a]	−0.16	0.03	−0.07	−0.06/ − 0.01
Supine raw score	−0.10	−0.12	−0.20	−0.18	−0.15	−0.01/0.16
Sit raw score	−0.12	−0.25	−0.28[a]	−0.05	−0.03	−0.16/ − 0.05
Total composite score	−0.23	−0.32[a]	−0.28[a]	−0.08	−0.12	−0.10/0.03

Note: r values presented.
[a]$p < .05$.

in the number of infants with "delayed" AIMS scores ($\leq 10^{th}$ percentile) ($p = .13$) (400 IU = 1, 800 IU = 4, and 1,200 IU = 3).

At 6 months, composite AIMS scores were not correlated with lean mass, weight, or head circumference z-scores; there was a modest negative correlation with length z-score ($r = -0.28$, $p = .04$) (Table 3). Total composite AIMS score at 6 months were not associated with $25(OH)D_3$ but was negatively and modestly correlated with the C-3-epimer-$25(OH)D_3$; the prone score was similarly negatively correlated with the C-3 epimer ($r = -0.32$, $p = .03$, $r = -0.30$, $p = .02$, respectively). In a linear regression model, the statistically significant negative correlations between composite AIMS score at 6 months (dependent variable) and concurrent C-3 epimer concentration and length z-score (independent variables) persisted ($p = .012$ and $p = .021$, respectively), confirming the independent nature of these associations.

DISCUSSION

In contrast to our *a priori* hypothesis, infants with 400 IU/day vitamin D_3 performed significantly better in the prone, sitting, and total composite scores compared to infants in the two higher supplement groups, who also had higher concentrations of $25(OH)D_3$. Of note, all infants had robust 25(OH)D concentrations. Longer infants have been reported to be slightly slower in attaining motor milestones, which may be, in part, due to mechanical factors. Our observation of a negative association of motor skill attainment and the C-3-epimer-$25(OH)D_3$ is an unexpected and perplexing finding.

A satisfactory mechanistic explanation remains elusive. Vitamin D is inherently important for muscle development and motor function (Endo et al., 2003; Garcia et al., 2011; Ceglia and Harris, 2013), and several mechanisms have been proposed. Genomic or transcriptional effects occur when calcitriol (1,25-dihydroxyvitamin D) binds to vitamin D receptors, resulting in increased protein synthesis in mus-

cle (Curry et al., 1974; Rachez and Freedman, 2000; Bischoff-Ferrari et al., 2012). Nongenomic effects occur through cell surface receptors that influence intracellular calcium concentrations directly, which may be important for muscle contractility. As a result, vitamin D deficiency is associated with atrophy of type II muscle. Correcting frank vitamin D deficiency improves muscle function, with a concomitant increase in type II muscle fibers (Bischoff-Ferrari et al., 2012). These fast-twitch fibers are the first to be recruited when a quick muscle reaction is required. In adults, there is ongoing debate as to the existence of a 25(OH)D threshold beyond which muscle function ceases to improve further (20–24 ng/mL) (Bischoff-Ferrari et al., 2012).

Although muscle function is intimately integrated with the nervous system, a review of the literature reveals that vitamin D or its metabolites do not appear to impact neural function in children, unlike adults where higher concentrations of 25(OH)D have been associated with better cognitive function (Garcion et al., 2002; Tolppanen et al., 2012). All our infants had achieved at least the 20 ng/mL 25(OH)D threshold, and these arguments are less relevant. Nevertheless, higher concentrations of 25(OH)D may be an explanation for the difference in outcomes.

Precedent has been established from three recent manuscripts (Sanders et al., 2010; Smith et al., 2007; Bleicher et al., 2014). They report that higher concentrations of 25(OH)D are associated with increased fractures and falls in adults. None of the investigators elucidated a potential mechanism for these phenomena but a tentative corollary might be drawn with the infants obtaining higher doses (800 or 1,200 IU/day) with resulting higher 25(OH)D concentrations. Moreover, none assessed concentrations of other vitamin D metabolites and epimers.

The weak, negative association of AIMS scores with C-3-epimer-25(OH)D_3 concentrations is novel. To date there has been no functional significance attributed to this epimer (Singh et al., 2006). It was first described in a minority of infants, albeit in abundance (up to 40% of 25(OH)D concentrations) (Singh et al., 2006). It has recently been found in lower concentrations in some adults (Lensmeyer et al., 2012). Nearly 100% of infants in our dose-response trial had detectable concentrations, mirroring concentrations of 25(OH)D_3 over time (early rise by 3 months and gradual decline) (Gallo et al., 2013). Additional studies regarding its role in muscle strength and development are warranted.

All infants' nutritional intakes were comparable for macro -and micronutrients (Gallo et al., 2013) obviating potential confounders. Despite most infants consuming breast milk until the age of 6 months, not all were exclusively breastfed until that time, instead relying on either soy or cow's milk formula. It is reassuring to note that no developmental differences have been observed in infants up to 1 year of age fed breast milk, soy or cow's milk formula, again eliminating potential confounders (Andres et al., 2012). Given the pilot nature of this project, there are undoubtedly additional confounders that we could not explore. However, because these infants had been randomized to the original dose-response trial and because we had very few refusals for this ancillary study, their effect is likely minimized. Potentially important confounders include the amount of time that parents stimulate their infants or the amount of time that infants spend on their tummies (Majnemer and Barr,

2005). Now that infants sleep on their backs, "tummy time" is important in promoting trunk control; infants in the 400 IU group had well developed tone. All our participants were of higher socioeconomic status than the general population and all infants met Institute of Medicine recommendations for vitamin D status; these factors may limit generalizability.

The strengths of our study include excellent participation rates with low attrition, use of a gender-neutral validated assessment tool, on-time assessment of gross motor skills (within one week of projected date) by experienced therapists, and a sophisticated method (LC-MS/MS) for measuring vitamin D metabolites and C-3-epimer-25(OH)D. Limitations of our study include the lack of data on the date of confinement (due date) and biological age (exact number of weeks post conception) of the infants and therefore infants gestational ages were based on maternal report; we did not anticipate differences owing to the randomized nature of the study. Another drawback of this study is the lack of power to detect if any treatment group could be associated with increased number of infants with motor delay ($<10^{th}$ percentile). Additionally, although the AIMS may be used to define delay on the basis of a total score below the 10^{th} percentile, it was not designed to identify "advanced" motor performance. We also recognize that AIMS is intended for one-time assessment, which does not predict future development.

CONCLUSION

At 6 months of age, infants receiving 400 IU/day had enhanced mean sitting, prone, and composite AIMS scores compared with infants receiving 800 IU/day and 1,200 IU/day. Our findings suggest that higher dosages of vitamin D supplementation in infancy are not required to optimize motor skills acquisition to 6 months of age. Interestingly, there was a modest negative correlation between AIMS score and C-3-epimer-25(OH)D_3; a compound, which is typically felt to be biologically inactive. It is plausible that the elevated levels of the C-3-epimer-25(OH)D_3 in infants compared to older children and adults negatively results in measurable effects on muscle development and or function in infancy. Other mechanistic explanations remain elusive; the modest negative correlation with length z-score has previously been noted (WHO Multicentre Growth Reference Study, 1992). Additional studies are needed to confirm our findings regarding the negative association of infant motor skills attainment with the C-3-epimer-25(OH)D_3; a compound traditionally believed to have no biologic significance and whether 400 IU/day may be the optimal dose for the attainment of gross motor skills at this age.

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ABOUT THE AUTHORS

Brandy Wicklow, MD, MSc, Manitoba Institute of Child Health, 715 McDermot Avenue, Winnipeg, Manitoba, R3E 3P4, Canada; University of Manitoba, Pediatrics and Child Health, 840 Sherbrooke Street, Winnipeg, Manitoba, R3A 1S1, Canada. **Sina Gallo,** MSc, RD, School of Dietetics and Human Nutrition, McGill University, 21, 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada. **Annette Majnemer,** PhD, OT(C), Montreal Children's Hospital, 2300 Tupper, Montreal, Quebec, H3H 1P3, Canada. **Catherine Vanstone,** MSc, RD, School of Dietetics and Human Nutrition, McGill University, 21, 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada. **Kathryn Comeau,** MSc, RD, School of Dietetics and Human Nutrition, McGill University, 21, 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada. **Glenville Jones,** PhD, Departments of Biomedical and Molecular Sciences and Medicine, Queen's University, Botterel Hall, Room 915, 18 Stuart Street, Kingston, Ontario, K7L 3N6, Canada. **Mary L'Abbe,** PhD, Department of Nutritional Sciences, University of Toronto, FitzGerald Building, 150 College Street, Room 316 Toronto, Ontario, M5S 3E2, Canada. **Ali Khamessan,** PhD, EURO-PHARM International Canada Inc., 9400, boul. Langelier Montreal, Quebec, H1P 3H8, Canada. **Atul Sharma,** MD, MSc, Montreal Children's Hospital, 2300 Tupper, Montreal, Quebec, H3H 1P3, Canada. **Hope Weiler,** PhD, RD, School of Dietetics and Human Nutrition, McGill University, 21, 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada. **Celia Rodd,** MD, MSc, School of Dietetics and Human Nutrition, McGill University, 21, 111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, H9X 3V9, Canada; Montreal Children's Hospital, 2300 Tupper, Montreal, Quebec, H3H 1P3, Canada.

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