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Parathyroid hormone-ionized calcium dynamics over the first year of life

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Abstract

Background: Ionized calcium (iCa) is believed to be the principle determinant of parathyroid hormone concentration (PTH). However, previous studies contained few infants.
Methods: This ancillary study from our vitamin D_3 dose-response trial in healthy, breastfed infants measured calcium, phosphorus, PTH and 25(OH)D (25-hydroxyvitamin D) at 1, 2, 3, 6, 9 and 12 months of age. The relationship between iCa and PTH was assessed by Pearson correlation and a mixed effects regression model to account for repeated measures.
Results: No significant correlations were observed between iCa and PTH at individual visits ($p>0.2$). After accounting for repeated measures, PTH decreased with increasing iCa (slope −5.25; 95% confidence intervals (CI) −8.78 to −1.73), decreased with increasing 25(OH)D (slope −0.006; 95% CI −0.009 to −0.002, and increased with later visits (6–12 months, $p<0.001$),
Conclusions: We observed a weak negative relationship between iCa and PTH and an increase with age consistent with physiologic maturation.

Keywords: dynamics; infant; ionized calcium; parathyroid hormone.

Introduction

Previous cross-sectional studies have explored the relationship of ionized calcium (iCa) and parathyroid hormone (PTH) across most ages [1–3]. No correlation was found over the first ~7 days of life in neonates, despite this being a period of important physiologic adaptation with marked changes in both analytes [4]. High ionized calcium in utero and immediately postnatally in healthy infants is associated with suppressed PTH. Over the course of ~7 days, there is a nadir in ionized calcium with a concomitant increase in PTH, thus avoiding symptomatic hypocalcemia [4, 5]. In older children and adults, there is a significant negative association between ionized calcium and PTH with a correlation coefficient (r) of ~−0.4 [3, 6]. PTH concentrations have also been associated with phosphorus, sex, 1,25-dihydroxy vitamin D ($1,25(OH)_2D$) or 25-hydroxy vitamin D (25(OH)D) concentrations [1, 2, 6, 7].

From our vitamin D dose response trial, we recently published normative data filling important gaps in the understanding of mineral status in infancy [8, 9]. Of note, iCa, total calcium and phosphorus all declined over to the first year of life, during which PTH concentrations doubled. The latter rose from almost suppressed levels to the mid-normal childhood range [8, 9]. As expected, 25(OH)D concentrations (measuring the storage form of the vitamin) increased with supplementation dose, but declined after 6 months of age. Neither iCa nor PTH were correlated with vitamin D dosage [9].

Our primary objective here is to explore whether the decline in ionized calcium was a major determinant of PTH concentrations over the first year of life. Additional objectives were to assess the effect of time (developmental changes), phosphorus, sex and 25(OH)D on the concentrations of PTH.

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Materials and methods

This was an ancillary study from a randomized dose-response trial in 132 primarily breastfed, healthy, term, appropriate-for-gestation age (AGA) infants [9]. Infants of ~1 month of age were randomized to either 400, 800, 1200 or 1600 IU of vitamin D_3 and followed until 12 months of age. At recruitment (~1 month of age), infants were consuming at least 80% of total milk intake as breast milk. Almost 90% of the infants were breastfed until 6 months and ~35% were breastfed until 12 months. All infants had normal renal function.

All were recruited from the Montreal, QC region; the study was performed between 2007 and 2011. Infants were excluded if their mothers had gestational diabetes, hypertension in pregnancy, malabsorption, or chronic alcohol ingestion. See Gallo et al. for additional details [9].

A capillary sample was collected from infants at inception (1 month), 2, 3, 6, 9, and 12 months of age. All infants were in the fed state at the time of blood sampling. Plasma clinical chemistry – including total calcium and phosphorus (Beckman Coulter DxC600, Mississauga, ON, Canada), whole blood iCa (ABL 725 series blood gas analyzer, Radiometer, London, ON, Canada), parathyroid hormone (enzyme-linked immunosorbent assay, Immutopics International, San Clemente, CA, USA) and total 25(OH)D (LC-MS/MS, Warnex Bioanalytical Services, Laval, QC, Canada). All vitamin D_2 metabolites were below the limit of quantification. Intra-assay coefficients of variations were <15% for all analyses as previously described [9]. The C-3 epimer of 25(OH)D was excluded from the present analyses.

Statistical analyses

Medians, minimum and maximum range of minerals, PTH and 25(OH)D were calculated at each visit. Because 25(OH)D concentrations varied by both time and vitamin D dose, these were cross-tabulated by dose and visit [9]. A loess fit (with a smoother span of 2/3) was used to assess the linearity of the iCa and PTH plots. The proportion (%) of ionized to total calcium was also calculated at each visit. The association between iCa and PTH was measured by Pearson correlation. A mixed effects regression model with a random subject effect to account for repeated measures was also used to examine the relationships between iCa and PTH with adjustment for sex, phosphorus, time (study visit) and 25(OH)D. A similar regression model was used to assess the ratio iCa:total calcium over time and changes in other analytes. Appropriate regression diagnostics were performed. All analyses were performed with STATA (Version 12, College Station, TX, USA). Statistical significance was a p-value <0.05.

Ethics

Research Ethics Board approvals were obtained from McGill University, George Mason University and the University of Manitoba.

Sample size calculations

In a previous study of older children and young adults aged 0.5–20 years, the correlation between PTH and iCa was approximately −0.40 [1]. Using the 132 infants enrolled in the dose-response trial, a power of 0.80, and alpha of 0.05, we would be able to detect a correlation as small as −0.24.

Results

There was a small male predominance of the 132 infants enrolled (Table 1). Overall, the percentage of infants who completed the study at 12 months was not different among the groups [9]. The ionized and total calcium and phosphorus concentrations declined significantly over time, as previously reported [8, 9]. Trends in PTH and ionized calcium showed an approximate linear relationship, confirmed by loess (non-parametric) fits (not shown). Table 2 provides descriptive statistics for the observed concentrations (median, range, sample size) at each study visit. The percentage of ionized to total calcium was robust, but declined significantly over time (slope=−0.12 per month, $p<0.0001$). No analyte concentrations varied with 25(OH)D concentrations.

PTH concentrations in infants did not exceed the manufacturer reference range (Immutopics International, San Clemente, CA, USA) at the 1 and 2 months visits. Compared to initiation, there was a doubling of values between 6 through 12 months with these values all significantly higher than baseline. Few PTH values fell outside the recommended reference range (Immutopics International, San Clemente, CA, USA) at any time. Conversely, 25(OH)D concentrations rose with increasing dose of vitamin D_3 (Table 1).

Simple Pearson correlations assessing the association of PTH and ionized calcium did not demonstrate any associations at any of the six study visits (all p-values >0.20). However, a mixed effects model-with a random intercept to account for the within-subject correlations from the repeated measures-confirmed that PTH was negatively associated with ionized calcium in the pooled data, after adjusting for sex, study visit, phosphorus and 25(OH)D concentrations (Table 3). Moreover, PTH was negatively associated with 25(OH)D. Additionally, there was a significant increase in PTH concentrations with study visit in older infants (6–12 months). A borderline ($p=0.06$) positive association with phosphorus concentration was also noted. More detailed assessments failed to demonstrate any linear interaction between ionized calcium and age ($p=0.19$), suggesting that the strength of the association between PTH and ionized calcium did not vary with age after adjustment for study visit, sex, serum phosphorus, and 25(OH)D concentrations.

Of note, there was attrition in the number of samples available for analysis out to study completion. About 50%

Table 1: Number of infants and sex distribution and 25-hydroxy-vitamin D concentrations at each visit.

	Vitamin D, IU/day	Visit, months					
		1	2	3	6	9	12
Sex (F/Total)	400	17/39	15/35	16/31	15/31	14/30	13/29
	800	16/39	15/36	14/33	13/33	10/29	9/28
	1200	16/38	14/33	14/32	14/31	12/28	13/29
	1600	7/16	7/15	7/15	7/14	6/12	6/12
25(OH)D	400	53.5	71.9	79.0	82.5	78.0	71.3
nmol/L		24.8–99.5	18.9–89.0	34.8–111.3	6.3–130.5	29.3–104.0	49.3–96.3
(n)[a]		(35)	(24)	(29)	(29)	(29)	(29)
[ng/mL]		[21.4	[28.8	[31.6	[33.0	[31.2	[28.5
		9.9–39.8]	7.6–35.6]	13.9–44.5]	2.5–52.2]	11.7–41.6]	19.7–38.5]
	800	59.5	87.4	98.0	103.6	88.1	84.0
		6.3–100.5	30.5–144.5	41.8–184.0	41.8–164.0	40.0–120.1	38.8–113.5
		(29)	(30)	(32)	(30)	(28)	(27)
		[23.8	[35.0	[39.2	[41.4	[35.2	[33.6
		2.5–40.2]	12.2–57.8]	16.7–73.6]	16.7–65.6]	16.0–48.0]	15.5–45.5]
	1200	60.3	111.6	136.3	121.4	98.3	89.9
		24.9–113.5	48.0–210.8	39.5–212.3	57.0–202.5	51.8–312.5	50.5–130.2
		(29)	(30)	(27)	(30)	(27)	(28)
		[24.1	[44.6	[54.5	[48.6	[39.3	[36.0
		10.0–45.4]	19.2–84.3]	15.8–84.9]	22.8–81.0]	20.7–125.0]	20.2–52.1]
	1600	68.2	121.8	186.3	172.3	115.6	95.1
		20.1–92.5	6.3–240.3	102.8–244.5	100.5–219.8	72.8–196.3	54.3–150.5
		(11)	(13)	(13)	(10)	(12)	(12)
		[27.3	[48.7	[74.5	[68.9	[46.2	[38.0
		8.0–37.0]	2.5–96.1]	41.1–97.8]	40.2–87.9]	29.1–78.5]	21.7–60.2]

[a]Median, (range) for 25(OH)D. In the mixed effects regression model, 25(OH)D concentrations were significantly different by dose and visit.

of these were attributable to insufficient blood volume for the analyses and the other half to children lost to follow-up for 25(OH)D [9].

Discussion

From this prospective dataset of infants, we were able to carefully explore the association between PTH and ionized calcium; data are scarce in this population. Despite a near doubling of PTH from relatively low concentrations in association with a significant decline in ionized calcium, there was a relatively weak negative association between PTH and ionized calcium after adjusting for visit, phosphorus, sex and 25(OH)D concentrations between 1 and 12 months. This suggests quite early parathyroid gland maturation in infancy. In fact, simple correlations at each time point (1, 2, 3, 6, 9, and 12 months) were not able to detect this association, which is well accepted in older children and young adults [1]. An a priori sample size calculation demonstrated that with 132 subjects, one would have been able to detect correlations of the magnitude previously described [1]. The absence of a significant interaction term involving age and iCa suggests that the ionized calcium-PTH association is developed by 1 month of age, but is weaker than that seen in older children [1]. Moreover, this association was identified despite relatively low concentrations of PTH compared to older children [1]. This was particularly true of PTH concentrations in the first few months of life. This manuscript appears to be the first to examine the determinants of PTH concentrations vis à vis ionized calcium, phosphorus and vitamin D status in infants. Moreover, we were also able to incorporate new normative data for analytes involved in mineral homeostasis, particularly for ionized calcium [1, 4, 8]. Since 25(OH)D immunoassays are problematic in infants, we also benefited from the gold-standard assay (LC-MS/MS), which can better distinguish vitamin D isoforms in this age group [8, 9].

Earlier studies had examined this potential association in 89 healthy neonates (cord blood and out to 6.5 days of life) and no correlation was found either with phosphorus or ionized calcium and PTH [4]. These negative results might be related to a time of rapid change in physiologic adaptations, i.e. moving from a high ionized calcium in utero environment with suppressed PTH to hypocalcemia

Table 2: Plasma analytes for infants over time.

Analyte	Visit, months					
	1	2	3	6	9	12
PTH[a]	1.67	1.83	1.68	2.77[a]	3.87[a]	3.33[a]
pmol/L	0.13–6.78	0.00–6.74	0.05–9.02	1.05–14.77	0.97–11.78	1.46–10.17
(n)	(126)	(115)	(113)	(109)	(99)	(98)
[pg/mL]	[15.90	[17.42	[15.99	[26.37	[36.84	[31.70
	1.23–64.55]	0.00–64.16]	0.48–85.87]	10.00–140.61]	9.23–112.15]	13.90–96.82]
Ionized calcium	1.40	1.39	1.38	1.35	1.33	1.33
mmol/L	1.29–1.52	1.22–1.48	1.30–1.49	1.16–1.42	1.26–1.43	1.24–1.39
(n)[b]	(127)	(111)	(109)	(109)	(96)	(92)
[mg/dL]	[5.60	[5.56	[5.52	[5.40	[5.32	[5.32
	5.16–6.08]	4.88–5.92]	5.20–5.96]	4.64–5.68]	5.04–5.72]	4.96–5.56]
Total calcium	2.53	2.54	2.55	2.51	2.48	2.47
mmol/L	2.32–2.69	2.37–2.72	2.32–2.76	2.32–2.71	2.18–2.65	2.25–2.65
(n)[b]	(132)	(116)	(114)	(108)	(97)	(98)
[mg/dL]	[10.12	[10.16	[10.20	[10.04	[9.92	[9.88
	9.28–10.76]	9.48–10.88]	9.28–11.04]	9.28–10.84]	8.72–10.60]	9.00–10.60]
Percentage ionized	55.2	54.9	54.0	53.3	53.9	53.9
calcium relative to	51.2–59.3	51.4–58.7	50.7–60.3	47.9–58.0	51.0–61.0	49.0–58.2
total calcium[b]	(127)	(109)	(107)	(107)	(96)	(92)
Phosphorus	2.06	1.95	1.87	1.77	1.73	1.77
mmol/L	1.75–2.39	1.61–2.25	1.56–2.31	1.29–2.18	1.44–2.14	1.32–2.20
(n)[b]	(132)	(111)	(110)	(108)	(96)	(98)
[mg/dL]	[6.39	[6.05	[5.80	[5.49	[5.36	[5.49
	5.43–7.41]	4.99–6.98]	4.84–7.16]	4.00–6.76]	4.46–6.63]	4.09–6.82]

Median, (range). PTH reference range (Immutopics International) for older children and adults 1.47–7.58 pmol/L. Whole blood ionized calcium. Radiometer reference range (adult): Ionized calcium 1.15–1.29 nmol/L. Beckman Coulter reference ranges (age not specified): Total calcium 2.23–2.58 mmol/L; Phosphate 0.78–1.53 mmol/L. [a]PTH – values at 6, 9 and 12 months were significantly higher than at 1 month. [b]Denotes a significant decline with visit in the mixed effects regression model, $p<0.05$.

Table 3: Mixed effects (repeated measures) regression model examining the association between parathyroid hormone and various predictors.

Predictors		Beta coefficient	z-test	95% CI	p-Value
Intercept		8.32	3.1	3.06 to 13.59	0.028
Ionized calcium, mmol/L		−5.25	−2.92	−8.78 to −1.73	0.004
Visit (months)	1 (referent)				
	2	0.16	0.85	−0.22 to 0.63	0.396
	3	0.20	0.91	−0.23 to 0.64	0.361
	6	1.26	5.22	0.79 to 1.74	<0.001
	9	2.15	8.71	1.67 to 2.63	<0.001
	12	1.67	6.61	1.17 to 2.16	<0.001
25(OH)D, nmol/L		−0.006	−3.13	−0.009 to −0.002	0.002
Phosphorus, mmol/L		0.71	1.90	−0.02 to 1.43	0.058
Sex (female referent)		0.07	0.32	−0.34 to 0.48	0.749

There was no evidence for an ionized calcium: age interaction ($p=0.19$, not shown).

and a concurrent upswing in PTH concentrations shortly after birth [1]. Interestingly, these 89 infants were tested 2–4 h post feeding while our infants had been fed more recently, likely providing them with a high mineral load. Although the short half-life of PTH allows for dynamic responses, our demonstration of an association in the post-prandial state may reflect a more stable environment in infants after 1 month of age. Alternatively, the application of multivariate, repeated measure statistical techniques to pooled data improves the statistical power

for detecting weaker associations. Moreover, the cord and neonate data may have been underpowered to see any relationship [1, 4].

The only other study that has examined children in this age range as well as older children and young adults found an overall correlation (r=−0.4) with samples from 66 individuals [1]. Unfortunately, only about 10% of the samples came from 0.5–2 years of age and none from infants in the first 6 months, which limited conclusions for these ages; all children were fasted. In their youngest age group, the authors detected similarly high ionized calcium; however, the percentage ionized calcium to total calcium across children was much lower, ranging from 39% to 47% compared to our 53%–55% range [1]. Another study in adults addressed this same question with multiple sampling over 24 h of PTH and calcium, which found a correlation coefficient of −0.4 in men and a slightly lower value in women [3]. In contrast, the infants in this study did not have a differential sex effect after adjustment for the other variables.

As in older subjects [1], our infants demonstrated a positive association between PTH and phosphorus concentrations, which approached statistical significance (p=0.058). This was not previously observed in neonates, but has been reported in children aged 6 years or older [1, 4]. This difference may be due to small numbers of subjects. Neither of these two earlier manuscripts examining PTH and ionized calcium dynamics in children explored the role of vitamin D status or the impact of $1,25(OH)_2D$ [1, 4]. In adults, the negative associations between PTH and 25(OH)D or $1,25(OH)_2D$ are well established. Interestingly, there were large increases in PTH concentration after the 6 months visit in our infants. One could speculate that these reflect developmental maturation or processes that we do not yet fully understand. Unfortunately, the earlier manuscript examining 0.5–20 years old did not assess impact of time or adjust for age, perhaps due to low numbers [1].

Our manuscript has a number of strengths, including large numbers of infants prospectively evaluated with comprehensive sampling at each time point. Our gold-standard LC-MS/MS assay for 25(OH)D avoids errors [8, 9], and the recent publication of normative mineral and hormone data in breast-fed infants allowed for more comprehensive comparisons. Using appropriate statistical methods, we were able to tease out the impact of time (developmental processes), sex, mineral and vitamin D status on PTH concentrations. Some limitations are lack of $1,25(OH)_2D$ concentrations, the fact that all infants were in the fed state, there was about a loss of about 20% over the study interval for various reasons (insufficient sample, sample clotted, laboratory error etc.) and capillary samples are often hemolyzed, which can alter PTH and mineral concentrations. Additionally, these data may not be directly generalizable to infants fed formula or mixed feedings.

In conclusion, this manuscript provides support for the idea of important maturational processes occurring during a period of critical physiologic adaption that to date has been poorly described. Using an appropriate model to account for repeated measures, the expected negative ionized calcium-PTH association was identified in infants as young as 1 month of age, with no significant iCa:age interaction to suggest changes in the relationship over the first year of life. Changes in PTH concentrations over time were large. The expected associations with phosphorus and vitamin D status were observed.

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