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Vitamin D and parathormone levels of late-preterm formula fed infants during the first year of life

SHORT TITLE: Vitamin D in large -preterm formula fed infants

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Abbreviations: 25(OH)D: vitamin D, PTH: parathormone, BW: birth weight, CHL: crown to heel length, ALP: alkaline phosphate

Conflict of Interest statement.

NONE

31 **ABSTRACT**

32 **Objective:** Preterm infants are at risk for low vitamin D but documentation on late-
33 preterm infants is sparse. This prospective study monitored longitudinally vitamin D
34 and parathormone (PTH) levels in late-preterm, formula fed infants during the first
35 year of life, taking into consideration in-utero and postnatal growth, season and diet.

36 **Population and methods:** The study population comprised 128 infants of gestational
37 age (GA) 32-36 weeks; 102 were appropriate (AGA) and 26 small for GA (SGA).
38 Serum levels of vitamin D [25(OH)D], PTH calcium (Ca), phosphate (P) and alkaline
39 phosphate (ALP) were estimated at 2 and 6 weeks and at 3,6,9 and 12 months of age.

40 **Results:** The 25(OH)D levels were relatively low at 2 and 6 weeks in both AGA and
41 SGA infants (21 ± 11 , 20 ± 7 ng/ml and 25 ± 16 , 23 ± 8 ng/ml respectively), but increased
42 at 6 months (45 ± 14 , 47 ± 10 ng/ml) and remained stable thereafter. SGA infants had
43 lower 25(OH)D levels at 9 and 12 months (AGA 45 ± 14 , 47 ± 18 ng/ml vs SGA 38 ± 13 ,
44 37 ± 13 ng/ml, $p<0.05$). 25(OH)D deficiency (<20 ng/ml) was found in 18.5% of
45 measurements in 92 (72%) infants and 25(OH)D insufficiency (20-32ng/ml) in 29.2%
46 of measurements in 99 (77.3%) infants. Most measurements with vitamin D <32 ng/ml
47 were observed at the first 3 study points, where PTH showed an inverse association
48 with 25(OH)D, reaching a plateau thereafter. **Conclusion:** Late-preterm, formula fed
49 infants may have suboptimal vitamin D levels and elevated PTH, especially during
50 the first 3 months. Those born SGA may have lower vitamin D levels up to the end of
51 the first year of life.

52 **Keywords:** vitamin D, parathormone, late-preterm infant, small-for-gestational age,
53 catch-up growth

54

55 INTRODUCTION

56 Most of the studies on vitamin D status in infants have focused on full-term,
57 exclusively breastfed infants and the American Academy of Pediatrics (AAP) has
58 recently increased the recommended vitamin D daily dose for breast-fed infants,
59 aiming to ensure vitamin D levels of above 20ng/ml (Challa et al., 2005; Specker et
60 al., 1985; Ala-Houhala, 1985; Ziegler et al., 2006; Wagner and Greer, 2008). Other
61 studies have investigated preterm infants with gestational age (GA) < 32 weeks (or
62 birth weight < 1800g), which are regarded to be at higher risk for vitamin D
63 deficiency (Henriksen et al., 2006; Delvin et al., 2005; Lévy et al., 2005;
64 Backström et al., 1999; Koo et al., 1995; Koletzko et al., 2005). In contrast, studies
65 of larger preterm infants, with GA 32-36 weeks, have been few, although these infants
66 comprise more than 9% of the entire neonatal population (377,000 births in the USA
67 in 2005) and about 70% of all premature deliveries, with an increasing trend over
68 recent years (Engle et al., 2007). These infants may have different nutritional needs
69 from either full term infants or preterm infants of lower GA. Only recently have
70 research efforts turned to this large neonatal group which appears to be susceptible to
71 a variety of adverse perinatal and long term outcomes (Engle et al., 2007; Petrini et
72 al., 2009).

73 Another neonatal population at risk is that of small for gestational age (SGA)
74 neonates who are deprived in-utero of adequate nutrient supply. The accelerated
75 growth pattern after birth (catch-up) experienced by most SGA infants may further
76 increase their nutritional needs and deplete their vitamin D stores. One recent clinical
77 trial examined the effects of vitamin D supplementation in SGA breastfed Indian
78 infants (Kumar et al., 2011). To the best of our knowledge no studies have examined

the adequacy of vitamin D status in late-preterm appropriate for gestational age (AGA) or SGA formula fed infants during the first year of life.

This prospective study aimed to monitor longitudinally the vitamin D levels in preterm, formula fed infants of 32-36 weeks GA during the first year of life, taking into consideration in-utero and postnatal growth patterns.

PATIENTS AND METHODS

This prospective study took place in the University Hospital of Ioannina, the regional hospital that accommodates the majority of deliveries (85%) in a well-defined area in Northwest Greece. It aimed to include all the formula fed preterm infants born in the hospital at between 32 and 36 weeks GA during a two-year period (2004-2006) and subsequently followed up in the neonatal outpatient clinic. The main outcome variable was serum vitamin D and additional variables were PTH and ALP. Birth weight, catch-up growth velocity, season and diet were the surrounding factors. Prior to enrolment of the infants, the aim of the study was explained to the parents and their written informed consent was obtained. The study was approved by the Scientific Ethical Committee of the University Hospital of Ioannina.

All preterm neonates in this hospital are monitored up to at least the second year of life. For the first year of life appointments are scheduled at the chronological age of 2 and 6 weeks, and 3, 6, 9 and 12 months, which were accordingly also designated as study points for this project. Infants with congenital anomalies or chronic diseases and those with follow up of less than 12 months were excluded from the study. All the infants studied were of Greek descent. Details of the antenatal and postnatal history along with information about maternal vitamin and mineral

103 supplementation during pregnancy were recorded. The GA was estimated from early
 104 antenatal ultrasonography (US) (12-16 weeks of gestation). Children with BW below
 105 the 10th percentile on the Alexander growth charts were classified as SGA (Alexander
 106 et al., 1999). Feeding information, whether breast milk, formula or a combination, the
 107 timing of introduction of solid foods and their type, the possible supplementary
 108 vitamins administration was collected at each study point for all infants. The milk
 109 formulas used were specified for fullterm infants because, following the current
 110 protocol of the Neonatal Unit, special transitional preterm formulas are given only to
 111 neonates with GA<32 weeks or BW<1500g. The vitamin D content of the formulas
 112 used by the study population, as reported by the manufacturers, varied between 400
 113 and 600IU per milk liter for the early infancy formulas (0-6 months) and was 600IU
 114 per milk liter for the late infancy formulas (6-12 months). Vitamin D enriched infant
 115 cereal and fruit preparations given in the latter period contained 100-120 IU vitamin
 116 D per serving. For purposes of analysis, summer season was regarded as the period
 117 May to October and winter as November to April.

118 Body weight, CHL and HC were examined at each study point, using standard
 119 techniques, by two of the authors (SA,VS). Postnatal growth velocity was estimated
 120 as the difference in weight (in grams) or height (in cm) between two study points
 121 divided by the time elapsed in days. At each follow-up appointment routine morning
 122 venepuncture is performed, according to the standard hospital protocol for follow-up
 123 of preterm infants, for estimation of metabolic and nutritional parameters [blood
 124 count, ferritin, calcium (Ca), phosphate (P), alkaline phosphate (ALP)]. At this routine
 125 venepuncture extra blood was collected from the study infants for the measurement of
 126 serum 25(OH)D and parathormone (PTH). Serum 25(OH)D was determined by an

enzyme-immunoassay (EIA) method using the kit by IDS Systems Ltd, UK. The sensitivity of the method was 2 ng/ml. The intra- and inter-assay CVs were 5.3% and 4.6% respectively. The biologically intact molecule of PTH (iPTH) was measured by a two-site enzyme linked immunosorbent assay (ELISA) using the kit by BIOMERICA Inc (USA). The sensitivity of the method was 0.9 pg/ml. The intra- and inter-assay CVs were 3.2% and 7.7% respectively. The sample required for each determination for either assay was 25µl. ALP activity, total Ca and inorganic P levels were determined on Beckman-Coulter AU 2700/5400 biochemistry analyzers. An upper normal range of ALP: 570IU/L was used for infants.

Statistical Analysis

Differences in biochemical parameters among the six study points and between subgroups were evaluated using repeated measures analysis of variance (ANOVA). When this analysis yielded significant results it was followed by simple ANOVA followed by the Fisher's PLSD test. Simple and multiple regression analyses were performed at each study point to investigate the association between 25(OH)D level and the other variables, specifically, PTH, Ca, P, ALP, season (summer or winter period), gender, BW, GA, and postnatal growth velocity in either height or weight. Logistic regression analysis was used to examine the relationship of vitamin D with variables that are by nature non continuous. For purposes of logistic regression, 25(OH)D values were grouped into quintiles and the first quintile was correlated with the other parameters in a binary manner. Values are expressed as mean plus/minus standard deviation (SD). Logarithmic transformation was made for parameters that did not show normal distribution. A level of significance of $p < 0.05$ was set. It was estimated that a sample size of 128 children was sufficient to

151 demonstrate a 20% difference in all the parameters examined, with a power > 80% at
 152 a significance level of 0.05 (Altman,1991). In this estimation the documented drop-
 153 out trends on follow up and the cases where samples would be unsuitable for
 154 evaluation were taken into consideration.

155

156 **RESULTS**

157 A total of 216 infants with 32-36 weeks GA were eligible for the study, of
 158 which were 48 excluded; for 43 infants, the parents were unable to keep most of the 6
 159 follow-up appointments during the study period or were unwilling to continue follow-
 160 up and for 5 the laboratory data collected were inadequate. Also excluded from the
 161 study were a further 40 infants, 14 of which were exclusively breastfed and 26
 162 partially breastfed, because it was difficult to quantify the average proportion of
 163 breast milk and formula. The characteristics of the non participating infants did not
 164 differ otherwise from those of the study group. The participating infants were all
 165 formula fed during the first six months of life. Among them 102 were classified as
 166 AGA and 26 as SGA. The characteristics of the study neonates are depicted in Table
 167 1. The majority of the infants (79%) were fed milk formulas containing vitamin D 480
 168 IU per liter during the first 6 months. Only 5 neonates (3.9%) received a transitional
 169 formula containing vitamin D 980 IU per liter for a 3 month period because of
 170 BW<1500g. For all infants, solid food was introduced after the 5th month of age,
 171 based on the standard infant nutrition protocols, and no major deviation was reported
 172 by the parents. According to parental reporting no study infant received a supplement
 173 of vitamin D during the study period. No differences between AGA and SGA infants
 174 were reported regarding the type or duration of formula feeding or the time of

175 introduction and type of solid foods. Formula milk consumption was reported to be
176 higher than 1000 ml/d at 3 and 6 months for all study infants. At 9 and 12 months it
177 was higher than 600ml/d in all infants, including the milk used in preparing foods.
178 Milk intake did not differ between AGA and SGA groups at any study point. Two
179 feedings of vitamin D enriched cereal or fruit baby foods (100-120 IU vitamin D per
180 serving) were given in the later period to the majority (92%) of the infants, and with
181 the exception of egg yolk no other food containing vitamin D was identified in their
182 diet. All the mothers had received Ca but no mother had received vitamin D
183 supplement throughout pregnancy. Although no deliberate exposure of the infants to
184 sun was reported by any parent, as they are advised to avoid direct sunlight during the
185 first year of life, seasonality was taken into consideration.

186 A total number of 650 measurements in 128 infants were made (mean 5.1
187 measurements per infant) over the 6 time points. No statistically significant
188 differences in vitamin D level according to season (summer or winter) were observed
189 except for the first study period (Table 2), and thus the serum parameters of AGA and
190 SGA infants are depicted in Table 3 independent of season.

191 The levels of 25(OH)D in AGA infants were lower at the 2 and 6 weeks study
192 points and increased significantly thereafter (Table 3). At the 6 months study point the
193 mean 25(OH)D level was double the levels at 2 and 6 weeks and remained stable up
194 to 12 months. The SGA infants followed a similar pattern, but at the 9 and 12 months
195 study points their mean 25(OH)D levels were lower than those of the AGA children
196 (Table 3). This difference was independent of GA, body weight, season and gender.
197 Based on recent data from adult and pediatric populations (Hathcock et al., 2007;
198 Holick, 2007; El-Hajj Fuleihan et al., 2006; Vieth et al., 2007; Agostoni et al.,

2010; Weng et al., 2007; Willis et al., 2007; Chapuy et al., 1997; Kinyamu et al., 1998; Heaney, 2004; Harkness et al., 2005;) there is a general consensus to increase the reference values of vitamin D in infants and children (18 Vieth et al., 2007; Agostoni et al., 2010). The levels of 20ng/ml and 32ng/ml, respectively, were applied as cutoff values for vitamin D deficiency and insufficiency for this study. Using the cutoff of 20ng/ml, over the study period 120 (18.5%) of vitamin D measurements were low, in 92 (72%) infants. Vitamin D insufficiency (20- 32 ng/ml) was observed in a further 190 (29.2%) measurements in 99 (77.3%) infants, on a mean of 2 occasions. Most measurements of vitamin D below 32ng/ml, specifically 202 (65%), were observed at the first 2 study points (i.e., at 2 and 6 weeks of life). From 3rd month onwards, 35% of measurements (108) were below 32ng/ml and between 6 and 12 months of age 18.4% (57) (Figure 1). No infant had a level of 25(OH)D below 20 ng/ml for more than two consecutive points during the study period. SGA children had levels of 25(OH)D <20 ng/ml in 21.9% measurements and 20-32ng/ml in 34.1%.

Variation in the levels of PTH during the study period exhibited a pattern inverse to that of 25(OH)D, being high at the first 2 study points and decreasing thereafter (Table 3). No differences in PTH levels between SGA and AGA infants were detected. As normal PTH levels have not been defined for this age group, we considered as upper normal limit the 95th percentile for PTH in the infants with vitamin D>32ng/ml, which was 48pg/ml. Using this criterion, 86/310 measurements (28%) in infants with vitamin D level <32ng/ml showed levels of PTH above the 95th percentile. A PTH level of above 60pg/ml (the “euparathyroid” cutoff value in older individuals) was found in 68 cases, mostly during the first 3 study periods. The

relationship between PTH and vitamin D levels during these 3 study periods is depicted in Figure 2. PTH levels were higher than the “euparathyroid” cutoff value almost exclusively in infants with 25(OH)D below the insufficiency limit (32ng/ml).

Levels of ALP were consistently higher in SGA infants (Table 3). Mean Ca and P levels did not differ between study points or groups and no infant had a level of $\text{Ca} < 8.5 \text{ mg/dl}$ or $\text{P} < 5 \text{ mg/dl}$ throughout the study period.

In regression analysis at each study point, a significant association of vitamin D was found with Ca at 2 weeks ($r=0.21$, $p=0.04$), 6 weeks ($r=0.20$, $p=0.05$) and 3 months ($r=0.19$, $p=0.05$), with ALP ($r=-0.26$, $p=0.02$) at 6 weeks and with P ($r=0.22$, $p=0.02$) at 6 months. Among the other variables (season; summer or winter period, gender, BW, GA, and postnatal growth velocity in either height or weight), only body weight growth was inversely correlated with 25(OH)D at 2 and 6 weeks and at 6 months ($r=-0.20$, -0.25 , -0.24 , respectively, $p<0.05$). At the 2 weeks and 6 months study points this relationship was independent of the other examined factors: odds ratio (OR): 0.84 confidence interval (CI):0.74-0.95 and OR: 0.85 CI:0.76-0.97, $p<0.05$ respectively.

239

240 **DISCUSSION**

Recent studies have extended the significance of vitamin D well beyond bone health (Hypponen et al., 2001; Krause et al., 1998; Lappe et al., 2007). Levels of 25(OH)D have been linked with other biomarkers such as PTH and indices of insulin resistance, bone mineralization and Ca absorption, in efforts to define the optimal serum levels (Bischoff-Ferrari et al., 2006; Viljakainen et al., 2006; Roth et al., 2005; Sichert-Hellert et al., 2006). Based on the latest information, $>30 \text{ ng/ml}$ has

247 been suggested as the optimal level of vitamin D for adults, a level associated with
 248 maximal suppression of PTH (Bischoff-Ferrari et al., 2006). There is a general
 249 consensus to increase the threshold level of circulating vitamin D in infants, as in
 250 adults, with a target value for 25(OH)D of >32ng/ml (80nmol/L) (Hathcock et
 251 al.,2007, Holick, 2007, El-Hajj Fuleihan et al.,2006, Vieth et al.,2007, Agostoni et
 252 al.,2010, Weng et al., 2007, Willis et al., 2007, Chapuy et al.,1997, Kinyamu et al.,
 253 1998, Heaney, 2004, Harkness et al.,2005). Applying the recently suggested cutoff
 254 values to the population of the present study, 72% of the infants were found to be
 255 vitamin D deficient on at least one occasion, and 78% were found to be vitamin D
 256 insufficient on a mean of 2 occasions throughout the study period, which covered the
 257 first year of life. Suboptimal vitamin D levels were documented, not only early after
 258 birth (mainly reflecting maternal levels) but throughout the study period, with 35% of
 259 low levels being observed between 3 and 12 months of age. The stability of levels
 260 after the 6 months point may be attributed mainly to the continuing consumption of
 261 fortified milk formula and cereal or fruit preparations enriched with vitamin D.

262 The inverse relationship between vitamin D and PTH shown in Figure 2
 263 implies a threshold effect for 25(OH)D above which PTH remains at a lower, steady-
 264 state level. The point at which PTH reaches its nadir is around 32 ng/mL, which
 265 justifies the use of this value as the cutoff point for insufficiency in this study. This
 266 phenomenon has already been observed in studies in adulthood and adolescence, with
 267 a lower PTH plateau value at around 30 ng/mL (Chapuy et al., 1997; Kinyamu et al.,
 268 1998; Heaney, 2004; Harkness et al.,2005). The present study for the first time
 269 provides support for operation of the same physiological mechanism in an infant
 270 cohort at least during the first 3 months of life, and also implies physiological
 271 consequences at vitamin D levels above 20 ng/mL. PTH is the principal determinant

272 of bone remodeling (Harkness et al., 2005) and increased levels may lead to
273 secondary hyperparathyroidism and bone mineral depletion, especially in periods of
274 accelerated bone growth as the infancy.

275 Despite breastfeeding promotion policies, most children in Greece (latitude
276 39⁰N) are not breastfed after the first few months and for their supply of vitamin D
277 they rely on the milk formulas fortified with vitamin D. Late-preterm infants are
278 usually fed with formulas specific to the needs of fullterm infants and they have not
279 been well studied for vitamin D status, probably on the assumption of adequacy of
280 vitamin D supplementation. Shorter GA and lower fat content are two factors that
281 may influence the placental supply and stores of vitamin D in this group of infants at
282 birth. The reported vitamin D content of the formulas given to the study infants is the
283 same as that of formulas currently given to full-term neonates in Europe and the USA,
284 adhering to published recommendations (Koletzko et al., 2005). ESPGHAN, in a
285 recent commentary aimed at increasing the vitamin D supply, recommends giving a
286 vitamin D supplement to formula fed preterm infants in the first months (Agostoni et
287 al., 2010). As this study demonstrated, this may also be applicable to late-preterm
288 infants, possibly on an individual basis after measurement of serum levels of
289 25(OH)D. We were unable to find studies on late-preterm formula fed infants of
290 similar design with which to compare our results. Percentages of vitamin D
291 insufficiency similar to those found in the infants in the present study have been
292 demonstrated recently in children and adolescences (Weng et al., 2007; Willis et al.,
293 2007).

294 A lower mean vitamin D level was found in the SGA than in the AGA infants
295 at the end of the first year, specifically at the 9 and 12 months study points. This
296 difference was independent of GA, body weight, season and other confounders. It

297 could be attributable to the reduced trans-placental transfer of nutrients experienced
298 by SGA infants, which may include lower vitamin D transport. Low reserves in the
299 SGA infants because of the relative reduction of fat tissue at birth available to store
300 vitamin D may also have played a role. A further additive factor could be the
301 increased postnatal needs due to catch-up growth that is experienced by the SGA
302 infants after birth. Lower vitamin D levels were recently related to insulin resistance
303 in adults (Reis et al., 2007) and it has been shown that SGA children show evidence
304 of insulin resistance very early after birth (Evagelidou et al., 2007, Mericq et al.,
305 2005). These possible relationships need further studies for verification of the possible
306 role of vitamin D in SGA populations. The higher mean ALP levels found in the SGA
307 infants might reflect an effect on bone mass, which would imply the need for even
308 higher vitamin D supplementation than those supplied by formula in this rapidly
309 growing group.

310 A negative relationship between postnatal growth velocity and vitamin D level
311 was shown in this study at the 3 earlier study points. Late-preterm children with
312 accelerated postnatal growth may need closer observation for optimal vitamin D
313 serum level, irrespective of whether they are AGA or SGA.

314 It is important to note that there were a number of limitations in this study.
315 One limitation was that the information on consumption of milk and solids was
316 collected according to parental reporting at each visit so lacked precision in
317 calculation of dietary constituents. All the infants, however, received formula milk in
318 the recommended quantities, and no group differences were found. Ultraviolet light
319 exposure was not included as a variable in the analysis. Although all the parents
320 reported absence of exposure of their infants to direct sunlight, and this is reflected by

321 the similar seasonal vitamin D levels found in the infants, brief periods of exposure
322 cannot be excluded. The lack of a control group of full-term infants for comparison
323 limits the findings of this study to the late-preterm population. Finally the number of
324 SGA infants was rather small and further verification of vitamin D levels in this group
325 is needed.

326 **CONCLUSIONS**

327 It is concluded that late-preterm, formula fed infants may have suboptimal serum
328 levels of vitamin D during the first year of life and increased PTH levels, especially
329 during the first 3 months. Among this group, those born SGA may have lower
330 vitamin D levels at the end of the first year. Vitamin D supplementation for formula
331 fed late-preterm infants at the first months of age may be an option, possibly on an
332 individual basis after measurement of serum levels of 25(OH)D. Further studies on
333 late-preterm infants in other geographical locations are needed to confirm the findings
334 of the present study.

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346 REFERENCES

- 347 1. Agostoni C, Buonocore G, Carnielli VP, et al. ESPGHAN Committee on
 348 Nutrition. Enteral nutrient supply for preterm infants: commentary from the
 349 European Society of Paediatric Gastroenterology, Hepatology and Nutrition
 350 Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2010;**50**:85-91.
- 351 2. Ala-Houhala M. 25-hydroxyvitamin D levels during breastfeeding with or
 352 without maternal or infantile supplementation of vitamin D. *J Pediatr*
 353 *Gastroenterol Nutr* 1985;**4**:220–226
- 354 3. Alexander GR, Kogan MD, Himes JH: 1994-1996 U.S. singleton birth weight
 355 percentiles for gestational age by race, Hispanic origin, and gender. *Matern*
 356 *Child Health J* 1999; **3**:225-231
- 357 4. Altman D. Practical Statistics for Medical Research. London: Chapman and
 358 Hall, 1991:456–460.
- 359 5. Backström MC, Mäki R, Kuusela AL, Sievänen H, Koivisto AM, Ikonen RS
 360 et al. Randomised controlled trial of vitamin D supplementation on bone
 361 density and biochemical indices in preterm infants. *Arch Dis Child Fetal*
 362 *Neonatal Ed* 1999;**80**:F161-66.
- 363 6. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes
 364 B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for
 365 multiple health outcomes. *Am J Clin Nutr* 2006;**84**:18–28
- 366 7. Challa A, Ntourntoufi A, Cholevas V, Bitsori M, Galanakis E, Andronikou S.
 367 Breastfeeding and vitamin D status in Greece during the first 6 months of life.
 368 *Eur J Pediatr* 2005 **164**:724-729

- 369 8. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S et al.
 370 Prevalence of vitamin D insufficiency in an adult normal population.
 371 *Osteoporos Int* 1997;**7**:439-443
- 372 9. Delvin EE, Salle BL, Claris O, Putet G, Hascoet JM, Desnoulez L, et al. Oral
 373 vitamin A, E and D supplementation of pre-term newborns either breast-fed or
 374 formula-fed: a 3-month longitudinal study. *J Pediatr Gastroenterol Nutr*
 375 2005;**40**:43-47.
- 376 10. El-Hajj Fuleihan E, Nabulsi M, Tamim H, et al. Effect of vitamin D
 377 replacement on musculoskeletal parameters in school children: a randomized
 378 controlled trial. *J Clin Endocrinol Metab* 2006;**91**:405–412
- 379 11. Engle WA, Tomashek KM, Wallman C; Committee on Fetus and Newborn,
 380 American Academy of Pediatrics. "Late-preterm" infants: a population at risk.
 381 *Pediatrics* 2007;**120**:1390-1401.
- 382 12. Evagelidou E, Giapros VI, Challa AS, Kiortsis DN, Tsatsoulis AA,
 383 Andronikou SK. Serum adiponectin levels, insulin resistance, and lipid profile
 384 in children born small for gestational age are affected by the severity of
 385 growth retardation at birth. *Eur J Endocrinol* 2007; **156**: 271-277
- 386 13. Harkness L, Cromer B. Low levels of 25-hydroxy vitamin D are associated
 387 with elevated parathyroid hormone in healthy adolescent females. *Osteoporos*
 388 *Int* 2005;**16**:109-113
- 389 14. Hathcock JN, Shao A, Vieth R, Heaney RP. Risk assessment for vitamin D.
 390 *Am J Clin Nutr* 2007;**85**:6–18
- 391 15. Heaney RP. Functional indices of vitamin D status and ramifications of
 392 vitamin D deficiency *Am J Clin Nutr* 2004;**80**(suppl):1706S–1709S

- 393 16. Henriksen C, Helland IB, Rønnestad A, Grønn M, Iversen PO, Drevon CA.
 394 Fat-soluble vitamins in breast-fed preterm and term infants *Eur J Clin Nutr*
 395 2006;**60**:756-762.
- 396 17. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;**357**: 266–281
- 397 18. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of
 398 vitaminD and risk of type 1 diabetes: a birth-cohort study. *Lancet*
 399 2001;**358**:1500-1503.
- 400 19. Specker BL, Valanis B, Hertzberg V, Edwards N, Tsang RC. Sunshine
 401 exposure and serum 25-hydroxyvitamin D concentrations in exclusively
 402 breast-fed infants. *J Pediatr* 1985;**107**: 372–376
- 403 20. Kinyamu HK, Gallagher JC, Rafferty KA, Balhorn KE. Dietary calcium and
 404 vitamin D intake in elderly women: effect on serum parathyroid hormone and
 405 vitamin D metabolites. *Am J Clin Nutr* 1998;**67**:342-48.
- 406 21. Koletzko B, Baker S, Cleghorn G et al. Global standard for the composition of
 407 infant formula: recommendations of an ESPGHAN coordinated international
 408 expert group. *J Pediatr Gastroenterol Nutr* 2005;**41**:584-599.
- 409 22. Koo WW, Krug-Wispe S, Neylan M, Succop P, Oestreich AE, Tsang RC.
 410 Effect of three levels of vitamin D intake in preterm infants receiving high
 411 mineral-containing milk. *J Pediatr Gastroenterol Nutr* 1995;**21**:182-189.
- 412 23. Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet
 413 B and blood pressure. *Lancet* 1998;**352**:709-710.
- 414 24. Kumar GT, Sachdev HS, Chellani H, Rehman AM, Singh V, Arora H, Filteau
 415 S. Effect of weekly vitamin D supplements on mortality, morbidity, and
 416 growth of low birthweight term infants in India up to age 6 months:
 417 randomised controlled trial. *BMJ* 2011;**342**: d2975. doi: 10.1136/bmj.d2975.

- 418 25. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP.
 419 Vitamin D and calcium supplementation reduces cancer risk: results of a
 420 randomized trial. *Am J Clin Nutr* 2007;**85**:1586-1591.
- 421 26. Mericq V, Ong KK, Bazaes R et al. Longitudinal changes in insulin sensitivity
 422 and secretion from birth to age three years in small- and appropriate-for-
 423 gestational-age children. *Diabetologia* 2005;**48**: 2609-2614
- 424 27. Petrini JR, Dias T, McCormick MC, Massolo ML, Green NS, Escobar GJ.
 425 Increased risk of adverse neurological development for late preterm infants *J*
 426 *Pediatr* 2009;**154**:169-176.
- 427 28. Reis JP, von Mühlen D, Kritz-Silverstein D, Wingard DL, Barrett-Connor E.
 428 Vitamin D, parathormone levels, and the prevalence of metabolic syndrome in
 429 community-dwelling older adults. *Diabetes Care* 2007 ;**30**:1549-1555.
- 430 29. Roth DE, Martz P, Yeo R, Prosser C, Bell M, Jones AB. Are national vitamin
 431 D guidelines sufficient to maintain adequate blood levels in children? *Can J*
 432 *Public Health* 2005;**96**:443–449
- 433 30. Sichert-Hellert W, Wenz G, Kersting M. Vitamin intakes from supplements
 434 and fortified food in German children and adolescents: results from the
 435 DONALD study. *J Nutr* 2006; **136**:1329–1333
- 436 31. Vieth R, Bischoff-Ferrari H, Boucher BJ, et al. The urgent need to recommend
 437 an intake of vitamin D that is effective. *Am J Clin Nutr* 2007;**85**:649–650
- 438 32. Viljakainen HT, Natri AM, Karkkainen MM, et al. A positive dose-response
 439 effect of vitamin D supplementation on site specific bone mineral
 440 augmentation in adolescent girls: a double- blinded randomized placebo-
 441 controlled 1-year intervention. *J Bone Miner Res* 2006;**21**:836–844

33. Wagner CL, Greer FR; Section on Breastfeeding and Committee on Nutrition, American Academy of Pediatrics. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008;**122**:1142–1152
34. Weng FL, Shults J, Leonard MB, Stallings VA, Zemel BS. Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. *Am J Clin Nutr* 2007 Jul;**86**:150-158.
35. Wilkins CH, Sheline YI, Roe CM, Birge SJ, Morris JC. Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am J Geriatr Psychiatry* 2006;**14**:1032-1040.
36. Willis CM, Laing EM, Hall DB, Hausman DB, Lewis RD. A prospective analysis of plasma 25-hydroxyvitamin D concentrations in white and black prepubertal females in the southeastern United States. *Am J Clin Nutr* 2007;**85**:124-130.
37. Ziegler EE, Hollis BW, Nelson SE, Jeter JM. Vitamin D deficiency in breastfed infants in Iowa *Pediatrics* 2006;**118**: 603-610.

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467 **Figure legends**

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470 **FIGURE 1** Serum level of vitamin D [(25(OH)D] (box and plots) in late preterm infants
471 (n=128) during the first year of life. Horizontal lines represent the cutoff levels for vitamin D
472 deficiency (20ng/ml) and insufficiency (32ng/ml)

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476 **FIGURE 2** The relationship between serum levels of intact parathormone (PTH) and vitamin
477 D [25(OH)D] level in late preterm infants (n=128) during the first 3 study periods (2 weeks
478 R=0.23, 6 weeks R=0.20, 3 months R=0.23, p<0.05) (Note: A single outlier value of vitamin
479 D:85 ng/ml is not depicted in the '6 weeks of age' figure)

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492 **Table 1.** Characteristics of the late-preterm study infants (n=128) appropriate (AGA
 493 n=102) and small for gestational age (SGA, n=26)

Characteristics	AGA (n= 102)	SGA (n= 26)	P
Birth			
Gestational age (weeks)	34.6±1.4	34.5±1.5	NS
Birth weight (g)	2,371±307	1,620±218	0.001
BW percentile	45±17	3±5	0.001
Birth weight z-score	-0.14±1.1	-2.22±0.67	0.001
CHL(cm)	46.5±2.8	41.6±2.3	0.001
Head circumference (cm)	31.5±1.7	29.8±1.8	0.001
Gender/F (%)	47(46%)	15(58%)	NS
Season/summer (%)	58(57%)	15(58%)	NS
Admission in the NICU	33(32%)	16(61.5%)	0.05
Sepsis (%)	4(3.9%)	2(7.7%)	NS
RDS 3-4grade (%)	2(1.96%)	1(3.8%)	NS
NEC (%)	2(1.96%)	2(7.7%)	NS
12 months			
Body weight (g)	9,559±1026	8,693±1094	0.01
CHL (cm)	77±2.8	74.4±3.9	0.01
Head circumference (cm)	46±1.3	44.5±1.8	0.01

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518 RDS=respiratory distress syndrome, NEC= necrotizing enterocolitis

519 CHL= crown to heel length, NS=non significant

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523 **Table 2.** Vitamin D levels (ng/ml) (mean $\pm$ SD) in the two study groups of
 524 appropriate for gestational age (AGA) and small for gestational age (SGA) infants
 525 divided according to season

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		2 weeks	6 weeks	3 months	6 months	9 months	12 months
AGA	summer	23 $\pm$ 12*	26 $\pm$ 16	37 $\pm$ 15	45 $\pm$ 14	45 $\pm$ 15	46 $\pm$ 16
	winter	19 $\pm$ 8	24 $\pm$ 9	33 $\pm$ 11	45 $\pm$ 13	43 $\pm$ 10	48 $\pm$ 15
SGA	summer	22 $\pm$ 7	24 $\pm$ 7	38 $\pm$ 10	49 $\pm$ 9	40 $\pm$ 9	36 $\pm$ 6
	winter	20 $\pm$ 8	22 $\pm$ 8	35 $\pm$ 13	46 $\pm$ 13	37 $\pm$ 12	37 $\pm$ 7

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529 *p<0.05 between summer and winter period in AGA group

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Table 3 Levels of serum parameters (mean values $\pm$ SD) in late-preterm infants appropriate (AGA n=102) and small for gestational age (SGA, n=26) in the first year of life

	Parameter	2 weeks	6 weeks	3 months	6 months	9 months	12 months	Statistics
AGA	25(OH)D(ng/ml)	21 $\pm$ 11	25 $\pm$ 14	35 $\pm$ 13	45 $\pm$ 14	45 $\pm$ 14	47 $\pm$ 16	P<0.01
	Range	(6-85)	(4.9-53)	(7.5-95)	(7.7-91)	(21-90)	(19-91)	
SGA		20 $\pm$ 7	23 $\pm$ 8	36 $\pm$ 12	47 $\pm$ 10	38 $\pm$ 13*	37 $\pm$ 13*	P<0.01
	Range	(8-36)	(7-47)	(6-70)	(19-54)	(19-53)	(22-57)	
AGA	PTH (pg/ml)	40 $\pm$ 36	42 $\pm$ 29	21 $\pm$ 15	16 $\pm$ 12	16.5 $\pm$ 13	16 $\pm$ 15	¶P<0.01
SGA		46 $\pm$ 40	46 $\pm$ 36	20 $\pm$ 13	14 $\pm$ 9	12.9 $\pm$ 11	13 $\pm$ 6.4	P<0.01
AGA	ALP (IU/L)	273 $\pm$ 88	323 $\pm$ 97	340 $\pm$ 199	299 $\pm$ 70	283 $\pm$ 56	287 $\pm$ 63	ns
SGA		328 $\pm$ 103*	370 $\pm$ 153*	385 $\pm$ 139*	348 $\pm$ 95**	393 $\pm$ 99***	369 $\pm$ 90***	ns
AGA	Ca (mg/dl)	10.1 $\pm$ 0.5	10.0 $\pm$ 0.5	10.5 $\pm$ 0.4	10.5 $\pm$ 0.5	10.5 $\pm$ 0.3	10.5 $\pm$ 0.3	ns
SGA		10.1 $\pm$ 0.6	10.2 $\pm$ 0.4	10.5 $\pm$ 0.4	10.5 $\pm$ 0.4	10.8 $\pm$ 0.5	10.7 $\pm$ 0.5	ns
AGA	P (mg/dl)	7.1 $\pm$ 0.7	7.0 $\pm$ 0.5	6.8 $\pm$ 0.6	6.4 $\pm$ 0.5	6.1 $\pm$ 0.5	6.0 $\pm$ 0.5	ns
SGA		7.0 $\pm$ 0.8	6.8 $\pm$ 0.9	6.9 $\pm$ 0.7	6.4 $\pm$ 0.4	6.3 $\pm$ 0.4	6.0 $\pm$ 0.5	ns

*p<0.05, ** p<0.01, *** p<0.001 between group statistics AGA vs SGA

¶P<0.01 denotes the overall ANOVA significance within each group

|| Within groups statistics: 25(OH)D: AGA: 2weeks vs 6weeks, p<0.05 2,6weeks vs 3,6,9,12months p<0.01, 3months vs 6,9,12 months p<0.05 SGA: 2,6weeks vs 3,6,9,12months p<0.05, 3months vs 6 months p<0.05. PTH: AGA: 2,6 weeks vs 3,6,9,12 months p<0.01, 3months vs 6,9,12,months p<0.05. SGA: 2,6 weeks vs 3,6,9,12 months p<0.05, 3months vs 9,12,months p<0.05