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

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

3

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

5

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

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21 Conflict of Interest statement.

22 NONE

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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

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

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

84

85 **PATIENTS AND METHODS**

86 This prospective study took place in the University Hospital of Ioannina, the
87 regional hospital that accommodates the majority of deliveries (85%) in a well-
88 defined area in Northwest Greece. It aimed to include all the formula fed preterm
89 infants born in the hospital at between 32 and 36 weeks GA during a two-year period
90 (2004-2006) and subsequently followed up in the neonatal outpatient clinic. The main
91 outcome variable was serum vitamin D and additional variables were PTH and ALP.
92 Birth weight, catch-up growth velocity, season and diet were the surrounding factors.
93 Prior to enrolment of the infants, the aim of the study was explained to the parents and
94 their written informed consent was obtained. The study was approved by the
95 Scientific Ethical Committee of the University Hospital of Ioannina.

96 All preterm neonates in this hospital are monitored up to at least the second
97 year of life. For the first year of life appointments are scheduled at the chronological
98 age of 2 and 6 weeks, and 3, 6, 9 and 12 months, which were accordingly also
99 designated as study points for this project. Infants with congenital anomalies or
100 chronic diseases and those with follow up of less than 12 months were excluded from
101 the study. All the infants studied were of Greek descent. Details of the antenatal and
102 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

127 enzyme-immunoassay (EIA) method using the kit by IDS Systems Ltd, UK. The
128 sensitivity of the method was 2 ng/ml. The intra- and inter-assay CVs were 5.3% and
129 4.6% respectively. The biologically intact molecule of PTH (iPTH) was measured by
130 a two-site enzyme linked immunosorbent assay (ELISA) using the kit by
131 BIOMERICA Inc (USA). The sensitivity of the method was 0.9 pg/ml. The intra- and
132 inter-assay CVs were 3.2% and 7.7% respectively. The sample required for each
133 determination for either assay was 25 μ l. ALP activity, total Ca and inorganic P levels
134 were determined on Beckman-Coulter AU 2700/5400 biochemistry analyzers. An
135 upper normal range of ALP: 570IU/L was used for infants.

136 **Statistical Analysis**

137 Differences in biochemical parameters among the six study points and
138 between subgroups were evaluated using repeated measures analysis of variance
139 (ANOVA). When this analysis yielded significant results it was followed by simple
140 ANOVA followed by the Fisher's PLSD test. Simple and multiple regression analyses
141 were performed at each study point to investigate the association between 25(OH)D
142 level and the other variables, specifically, PTH, Ca, P, ALP, season (summer or
143 winter period), gender, BW, GA, and postnatal growth velocity in either height or
144 weight. Logistic regression analysis was used to examine the relationship of vitamin
145 D with variables that are by nature non continuous. For purposes of logistic
146 regression, 25(OH)D values were grouped into quintiles and the first quintile was
147 correlated with the other parameters in a binary manner. Values are expressed as
148 mean plus/minus standard deviation (SD). Logarithmic transformation was made for
149 parameters that did not show normal distribution. A level of significance of $p < 0.05$
150 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.,

199 2010; Weng et al., 2007; Willis et al., 2007; Chapuy et al., 1997; Kinyamu et
200 al.,1998; Heaney, 2004; Harkness et al., 2005;) there is a general consensus to
201 increase the reference values of vitamin D in infants and children (18 Vieth et al.,
202 2007; Agostoni et al., 2010). The levels of 20ng/ml and 32ng/ml, respectively, were
203 applied as cutoff values for vitamin D deficiency and insufficiency for this study.
204 Using the cutoff of 20ng/ml, over the study period 120 (18.5%) of vitamin D
205 measurements were low, in 92 (72%) infants. Vitamin D insufficiency (20- 32 ng/ml)
206 was observed in a further 190 (29.2%) measurements in 99 (77.3%) infants, on a
207 mean of 2 occasions. Most measurements of vitamin D below 32ng/ml, specifically
208 202 (65%), were observed at the first 2 study points (i.e., at 2 and 6 weeks of life).
209 From 3rd month onwards, 35% of measurements (108) were below 32ng/ml and
210 between 6 and 12 months of age 18.4% (57) (Figure 1). No infant had a level of
211 25(OH)D below 20 ng/ml for more than two consecutive points during the study
212 period. SGA children had levels of 25(OH)D <20 ng/ml in 21.9% measurements and
213 20-32ng/ml in 34.1%.

214 Variation in the levels of PTH during the study period exhibited a pattern
215 inverse to that of 25(OH)D, being high at the first 2 study points and decreasing
216 thereafter (Table 3). No differences in PTH levels between SGA and AGA infants
217 were detected. As normal PTH levels have not been defined for this age group, we
218 considered as upper normal limit the 95th percentile for PTH in the infants with
219 vitamin D>32ng/ml, which was 48pg/ml. Using this criterion, 86/310 measurements
220 (28%) in infants with vitamin D level <32ng/ml showed levels of PTH above the 95th
221 percentile. A PTH level of above 60pg/ml (the “euparathyroid” cutoff value in older
222 individuals) was found in 68 cases, mostly during the first 3 study periods. The

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

226 Levels of ALP were consistently higher in SGA infants (Table 3). Mean Ca
227 and P levels did not differ between study points or groups and no infant had a level of
228 $Ca < 8.5 \text{ mg/dl}$ or $P < 5 \text{ mg/dl}$ throughout the study period.

229 In regression analysis at each study point, a significant association of vitamin
230 D was found with Ca at 2 weeks ($r=0.21$, $p=0.04$), 6 weeks ($r=0.20$, $p=0.05$) and 3
231 months ($r=0.19$, $p=0.05$), with ALP ($r=-0.26$, $p=0.02$) at 6 weeks and with P ($r=0.22$,
232 $p=0.02$) at 6 months. Among the other variables (season; summer or winter period,
233 gender, BW, GA, and postnatal growth velocity in either height or weight), only body
234 weight growth was inversely correlated with 25(OH)D at 2 and 6 weeks and at 6
235 months ($r=-0.20$, -0.25 , -0.24 , respectively, $p < 0.05$). At the 2 weeks and 6 months
236 study points this relationship was independent of the other examined factors: odds
237 ratio (OR): 0.84 confidence interval (CI):0.74-0.95 and OR: 0.85 CI:0.76-0.97,
238 $p < 0.05$ respectively.

239

240 **DISCUSSION**

241 Recent studies have extended the significance of vitamin D well beyond bone
242 health (Hypponen et al., 2001; Krause et al., 1998; Lappe et al., 2007). Levels of
243 25(OH)D have been linked with other biomarkers such as PTH and indices of insulin
244 resistance, bone mineralization and Ca absorption, in efforts to define the optimal
245 serum levels (Bischoff-Ferrari et al., 2006; Viljakainen et al., 2006; Roth et al.,
246 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 (Evangelidou 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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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 ⁴⁹⁷
Birth weight (g)	2,371±307	1,620±218	0.001 ⁴⁹⁸
BW percentile	45±17	3±5	0.001 ⁴⁹⁹
Birth weight z-score	-0.14±1.1	-2.22±0.67	0.001 ⁵⁰⁰
CHL(cm)	46.5±2.8	41.6±2.3	0.001 ⁵⁰¹
Head circumference (cm)	31.5±1.7	29.8±1.8	0.001 ⁵⁰²
Gender/F (%)	47(46%)	15(58%)	NS ⁵⁰³
Season/summer (%)	58(57%)	15(58%)	NS ⁵⁰⁴
Admission in the NICU	33(32%)	16(61.5%)	0.01 ⁵⁰⁵
Sepsis (%)	4(3.9%)	2(7.7%)	NS ⁵⁰⁶
RDS 3-4grade (%)	2(1.96%)	1(3.8%)	NS ⁵⁰⁷
NEC (%)	2(1.96%)	2(7.7%)	NS ⁵⁰⁸
12 months			
Body weight (g)	9,559±1026	8,693±1094	0.01 ⁵⁰⁹
CHL (cm)	77±2.8	74.4±3.9	0.01 ⁵¹⁰
Head circumference (cm)	46±1.3	44.5±1.8	0.01 ⁵¹¹

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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±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±12*	26±16	37±15	45±14	45±15	46±16
	winter	19±8	24±9	33±11	45±13	43±10	48±15
SGA	summer	22±7	24±7	38±10	49±9	40±9	36±6
	winter	20±8	22±8	35±13	46±13	37±12	37±7

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

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539 **Table 3** Levels of serum parameters (mean values±SD) in late-preterm infants
 540 appropriate (AGA n=102) and small for gestational age (SGA, n=26) in the first year
 541 of life
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	Parameter	2 weeks	6 weeks	3 months	6 months	9 months	12 months	Statistics
AGA	25(OH)D(ng/ml)	21±11	25±14	35±13	45±14	45±14	47±16	P<0.01
	Range	(6-85)	(4.9-53)	(7.5-95)	(7.7-91)	(21-90)	(19-91)	
SGA		20±7	23±8	36±12	47±10	38±13*	37±13*	P<0.01
	Range	(8-36)	(7-47)	(6-70)	(19-54)	(19-53)	(22-57)	
AGA	PTH (pg/ml)	40±36	42±29	21±15	16±12	16.5±13	16±15	¶P<0.01
SGA		46±40	46±36	20±13	14±9	12.9±11	13±6.4	P<0.01
AGA	ALP (IU/L)	273±88	323±97	340±199	299±70	283±56	287±63	ns
SGA		328±103*	370±153*	385±139*	348±95**	393±99***	369±90***	ns
AGA	Ca (mg/dl)	10.1±0.5	10.0±0.5	10.5±0.4	10.5±0.5	10.5±0.3	10.5±0.3	ns
SGA		10.1±0.6	10.2±0.4	10.5±0.4	10.5±0.4	10.8±0.5	10.7±0.5	ns
AGA	P (mg/dl)	7.1±0.7	7.0±0.5	6.8±0.6	6.4±0.5	6.1±0.5	6.0±0.5	ns
SGA		7.0±0.8	6.8±0.9	6.9±0.7	6.4±0.4	6.3±0.4	6.0±0.5	ns

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

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

545 || Within groups statistics: 25(OH)D: AGA: 2weeks vs 6weeks,p<0.05 2,6weeks vs

546 3,6,9,12months p<0.01, 3months vs 6,9,12 months p<0.05 SGA: 2,6weeks vs

547 3,6,9,12months p<0.05, 3months vs 6 months p<0.05. PTH: AGA: 2,6 weeks vs

548 3,6,9,12 months p<0.01, 3months vs 6,9,12,months p<0.05. SGA: 2,6 weeks vs

549 3,6,9,12 months p<0.05, 3months vs 9,12,months p<0.05



