

1 The Effects of Vitamin D Supplementation During Infancy on Growth During
2 the First Two Years of Life

3 Helena H. Hauta-alus^{1,2,3}, Elisa M. Holmlund-Suila^{1,2}, Eero Kajantie^{1,3,4,5}, Jenni Rosendahl^{1,2}, Saara M.
4 Valkama^{1,2}, Maria Enlund-Cerullo^{1,2}, Sture Andersson¹, Outi Mäkitie^{1,6,7}

5

6 ¹ Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University
7 Hospital, Helsinki, Finland

8 ² Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of
9 Helsinki, Helsinki, Finland

10 ³ Finnish Institute for Health and Welfare (THL), Helsinki, Finland

11 ⁴ PEDEGO Research Unit, MRC Oulu, Oulu University Hospital and University of Oulu, Oulu,
12 Finland

13 ⁵ Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology,
14 Trondheim, Norway

15 ⁶ Department of Molecular Medicine and Surgery, Karolinska Institutet, and Clinical Genetics,
16 Karolinska University Laboratory, Karolinska University Hospital, Stockholm, Sweden

17 ⁷ Folkhälsan Institute of Genetics, Helsinki, Finland

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21 Corresponding author and person whom reprint requests should be addressed:

22 Helena Henrietta Hauta-alus, Children's Hospital, University of Helsinki and Helsinki University
23 Hospital, Helsinki, Biomedicum 2 C, P.O. Box 705, 00020 HUS, Finland. Tel +358 947175731.

24 Email helena.hauta-alus@helsinki.fi.

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34

35 **Abstract**

36 Context. The relationship between maternal and infant vitamin D and early childhood growth remains
37 inadequately understood.

38 Objective. To investigate how maternal and child 25-hydroxyvitamin D [25(OH)D] and vitamin D
39 supplementation impact growth during the first 2 years of life.

40 Design. A randomized, double-blinded intervention study.

41 Setting. A single-center study from pregnancy until offspring age 2 years.

42 Participants. Altogether 812 term-born children with complete data, recruited at Maternity Hospital.

43 Intervention. Children received daily vitamin D₃ supplementation 10 µg (Group-10) or 30 µg (Group-
44 30) from age 2 weeks to 2 years.

45 Main outcome measures. Anthropometry and growth rate at age 1 and 2 years.

46 Results. Toddlers born to mothers with Pregnancy 25(OH)D >125 nmol/L were at 2 years lighter and
47 thinner than the reference group with 25(OH)D 50-74.9 nmol/L (P<0.010). Mean 2-year 25(OH)D
48 concentrations were 87 nmol/L in Group-10 and 118 nmol/L in Group-30 (P<0.001). When Group-30
49 was compared with Group-10, difference in body size was not statistically significant (P>0.053), but
50 Group-30 had slower growth in length and head circumference between 6 months and 1 year
51 (P<0.047), and more rapid growth in weight and length-adjusted weight between 1 and 2 years
52 (P<0.043). Toddlers in the highest quartile of 25(OH)D (>121 nmol/L) were shorter (mean difference
53 0.2 SD score (SDS), P=0.021), lighter (mean difference 0.4 SDS, P=0.001) and thinner (in length-
54 adjusted weight) (mean difference 0.4 SDS, P=0.003) compared with the lowest quartile (<81.2
55 nmol/L).

56 Conclusion. Vitamin D and early childhood growth may have an inverse U-shaped relationship.

57

58 Introduction

59 Vitamin D has a vital role in childhood growth and development and chronic and severe vitamin D
60 deficiency leads to rickets, stunted growth and delayed neuromuscular development (1). Maternal
61 vitamin D deficiency may increase the likelihood of pregnancy complications and prenatal growth
62 restriction (2–4). Vitamin D status is defined by blood 25-hydroxyvitamin D concentration
63 [25(OH)D], which is generally considered sufficient at values at or above 50 nmol/L (1,5,6). Vitamin
64 D insufficiency (25(OH)D below 50 nmol/L) is common worldwide (7,8), the prevalence ranging
65 from 7% in Northern Europe to 90% in the Middle East (5). Especially populations with inadequate
66 sunlight exposure are at an increased risk. In addition to endogenously produced vitamin D in the
67 skin, diet and supplements are important sources of vitamin D.

68 A global consensus recommendation for prevention of vitamin D deficiency rickets was published in
69 2016 (1). The recommended vitamin D supplementation was a daily dose of 15 µg for pregnant
70 women and 10 µg for infants (1). The latest guidelines given by the Finnish national health authorities
71 in 2018 recommend a daily total intake of 10 µg for pregnant women and infants (9). WHO does not
72 recommend vitamin D supplementation for pregnant women (10). Some researchers consider the
73 target 25(OH)D level to be much higher than 50 nmol/L, preferably >75-100 nmol/L, and therefore
74 recommend higher supplemental vitamin D intake, up to 100 µg/d, also for pregnant women (11–13).

75 In general, it is presumed that vitamin D enhances childhood growth, although evidence is limited and
76 conflicting, and in particular the linearity of the relationship is not known (3,14–17). The association
77 between 25(OH)D concentrations and growth might be non-linear and hence dissimilar at different
78 ranges of 25(OH)D, which could explain discrepancies between studies.

79 The effect of vitamin D on childhood growth pattern may best be seen during the prenatal period and
80 infancy due to rapid growth rate. Early growth is particularly relevant for later health outcomes as
81 specific growth patterns have been associated with increased risk of chronic diseases, for example
82 through body composition and metabolic changes. While the associations between prenatal growth

83 and adult disease are particularly well established (18), growth during the first years after birth is also
84 important (19,20).

85 Vitamin D intervention in Infants (VIDI) –study is a double-blinded and randomized clinical trial
86 (RCT) comparing the effect of daily vitamin D supplementation of 10 µg or 30 µg from birth until 2
87 years of age, the primary outcomes being bone strength and infection episodes (21,22). We previously
88 reported an unexpected association between higher maternal and infant 25(OH)D with slower infant
89 growth in the VID I cohort; mothers with 25(OH)D above 125 nmol/L had the smallest infants at 6
90 months and 1 year of age (23). In the current study, we aimed to investigate if maternal and child
91 25(OH)D further predict growth parameters at 2 years of age, and whether the dose of vitamin D
92 supplementation in infancy influences childhood growth pattern from 6 months to 2 years of age.

93

94 Materials and Methods

95 Subjects

96 In Helsinki, Finland (60th parallel North), we recruited at Kätilöopisto Maternity Hospital 987 families
97 to the VID I study between January 2013 and June 2014. A description of the recruitment and study
98 protocol has been published previously (21,22). Briefly, according to the inclusion criteria, the
99 mothers were of Northern European origin without regular medication and with singleton pregnancy.
100 Exclusion criteria for the newborns were: nasal continuous positive airway pressure treatment or need
101 for nasogastric tube > one day, intravenous glucose infusion, seizures, and duration of phototherapy >
102 three days. The infants were born between 37 and 42 weeks of gestation with birth weights
103 appropriate for gestational age (standard deviation score [SDS] between -2.0 and +2.0).

104 Infants were randomized to receive daily vitamin D₃ supplementation with either 10 µg [hereafter
105 referred to as Group-10] or 30 µg [hereafter referred to as Group-30] from age 2 weeks to 2 years. The
106 study included three study visits at the age of 6 months, 1 and 2 years, and retrospectively and
107 prospectively collected questionnaires.

108 Written informed consent was obtained from the parents at recruitment. This study was conducted
109 according to the guidelines laid down in the Declaration of Helsinki. Ethical approval was obtained
110 from the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa
111 (107/13/03/03/2012). The project protocol is registered at ClinicalTrials.gov (NCT01723852).

112 Of the recruited 987 families, we excluded 12 who did not meet the inclusion criteria and 1 infant
113 diagnosed with Rieger syndrome, leaving 974 study participants. Further, 126 children were excluded
114 from the present analysis due to lacking data on length and additional 9 due to lacking data on
115 25(OH)D at 2 years' follow-up. This resulted in a final number of 812 study subjects. Number of
116 subjects varies in some analyses and are presented in tables and figures.

117 Family data

118 Parental data were obtained from a self-administered baseline questionnaire, filled out after delivery,
119 and from medical records. Parental heights (cm) and weights (kg) before pregnancy were standardized
120 into sex-specific z-scores. Body mass index (BMI) was calculated (kg/m^2).

121 Parental education level was categorized into 'lower' and 'higher' education (lower =lower or upper
122 secondary or post-secondary non-tertiary education/less than a bachelor degree, higher =first or
123 second stage of tertiary education/at least a bachelor degree), according to the highest received degree
124 of either parent. Parental smoking status was assessed before pregnancy and at infant age of 2 years
125 and applied as a merged previous and current smoking status. Family income level was enquired with
126 a questionnaire completed at infant age of 2 years.

127 Child anthropometrics

128 Birth size was measured by midwives according to standard procedures. The measurements were
129 collected from birth records, and transformed to parity-, gestational age- and sex-specific standard
130 deviation scores (SDS) based on national newborn body size curves (24). Infant weight (kg), length
131 (cm) and head circumference (cm) were measured at 6 months and at 1 and 2 years' follow-up visits
132 by a pediatrician or a research nurse. At 1 and 2 years, mid-upper-arm circumference (MUAC) (mm)
133 was measured. Length was measured with a tabletop meter in a supine position, and weight with an

134 electronic scale (Seca®, Hamburg, Germany). Weight, length, length-adjusted weight and head
135 circumference were expressed as SDS using age- and sex-specific national references (25) and
136 considered normal when between -2.0 and +2.0 SDS. BMI at 2 years of age was calculated and
137 together with MUAC, standardized into sex-specific z-score within the present study population.

138 Study compliance and duration of breastfeeding were determined based on prospectively collected
139 study diaries. Average vitamin D intake from food at 1 year of age was calculated based on 3-day
140 food records (26).

141 Biochemical analyses

142 We analyzed 25(OH)D concentration from maternal serum samples in early pregnancy, at birth from
143 umbilical cord blood (UCB), and from infant serum samples at the age of 1 and 2 years using the IDS-
144 iSYS fully automated immunoassay system with chemiluminescence detection (Immunodiagnostic
145 Systems Ltd., Bolton, UK). Pregnancy samples were collected at prenatal clinics on average at
146 gestational week 11 between June 2012 and February 2014 as part of the mothers' normal follow-up
147 [hereafter referred to as Pregnancy 25(OH)D] (23). UCB for 25(OH)D measurement was obtained at
148 birth (gestational weeks 37 to 42) between January 2013 and June 2014 [hereafter referred to as UCB
149 25(OH)D]. Maternal 25(OH)D refers to both Pregnancy and UCB 25(OH)D. Children's samples at 1
150 year follow-up were obtained between December 2013 and May 2015 [hereafter referred to as Infant
151 25(OH)D], and samples at 2 years follow-up between December 2014 and May 2016 [hereafter
152 referred to as Toddler 25(OH)D].

153 Pregnancy serum and UCB plasma 25(OH)D were analyzed simultaneously and Infant and Toddler
154 serum 25(OH)D in a separate series with intra-assay variation <7% for Pregnancy 25(OH)D and
155 Infant/Toddler 25(OH)D, and <13% for UCB 25(OH)D. The quality and accuracy of the 25(OH)D
156 analyses are validated on an ongoing basis by participation in the vitamin D External Quality
157 Assessment Scheme (DEQAS, Charing Cross Hospital, London, UK). The method showed a $\leq 8\%$
158 positive bias against NIST Reference Measurement Procedure. Detailed information on the 25(OH)D
159 analysis has been previously reported (22).

160 Vitamin D sufficiency was defined as $25(\text{OH})\text{D} \geq 50$ nmol/L (5,6) . Further, we used additional cut-off
161 values for $25(\text{OH})\text{D}$, namely 75 nmol/L, which has been suggested to be a higher threshold value for
162 bone health (11) , and 125 nmol/L, above which values have been related to health risks (5,6).

163

164 Statistical analyses

165 The normality of the variables was visually inspected, and statistical tests were chosen accordingly.

166 Infant and family characteristics were reported as means, standard deviations, and percentages.

167 Covariates were chosen based on literature and consistent association with several growth measures.

168 Missing values of covariates were multiple imputed (5 imputations). The difference between

169 intervention groups was examined with Independent-Samples T-test, Mann-Whitney U-test or

170 Pearson Chi-Square test.

171 Growth rate, referred to here as conditional growth, was investigated by using the residuals from

172 linear regression models in which body size SDS at each successive age was regressed on

173 corresponding body size SDS at all earlier ages (27). These residuals indicate how much a

174 measurement of body size at each time point differs from that predicted by the corresponding

175 measurements at earlier time points.

176 We used univariate and multivariate linear and quadratic regression analysis to determine associations

177 between $25(\text{OH})\text{D}$ and growth measures. We show in the tables unadjusted model 1, and model 2

178 adjusted with corresponding birth size, maternal and paternal height z-scores and intervention group.

179 All analyses were also stratified by intervention group and shown in relevant tables separately.

180 Additional adjustments were conducted with covariates of maternal prepregnancy BMI and paternal

181 BMI, parental smoking status, parental education level, family income level, and duration of

182 breastfeeding. These results are reported in the text only if an effect was observed.

183 Further, we investigated child growth in categories of $25(\text{OH})\text{D}$ with ANCOVA adjusted for

184 corresponding birth size SDS, maternal and paternal height z-scores and intervention group. Maternal

185 $25(\text{OH})\text{D}$ concentrations were categorized into four groups; <50 nmol/L, 50-74.9 nmol/L (reference

186 group), 75-125 nmol/L and >125 nmol/L, and Toddler 25(OH)D into three groups; <75 nmol/L
187 (reference group), 75-125 nmol/L and >125 nmol/L due to only five toddlers having a concentration
188 below 50 nmol/L. In addition, Toddler 25(OH)D concentration was categorized into quartiles; <81.2
189 nmol/L (reference group), 81.2-99.2 nmol/L, 99.3-120.7 nmol/L and >121 nmol/L. Differences in
190 child size between categories were compared with linear regression applying 50-74.9 nmol/L, <75
191 nmol/L or first quartile as a reference group. Additional adjustments were conducted with covariates
192 of maternal prepregnancy BMI and paternal BMI, parental smoking status, parental education level,
193 family income level, and duration of breastfeeding. These results are reported in the text only if an
194 effect on the results was observed.

195 Statistical significance was determined at $P < 0.05$. All statistical analyses were conducted using the
196 IBM SPSS program for Windows, version 25 (IBM, Chicago, IL, USA).

197

198 Results

199 Subject characteristics are shown in Tables 1 and 2 according to intervention groups. Infant 25(OH)D
200 concentrations were higher at the age of 1 and 2 years in Group-30 compared with Group-10 but no
201 difference was observed in mean values of body size parameters (22). However, when we compared
202 mean conditional growth values indicating growth rate, we discovered that growth in length and head
203 circumference were slower between 6 months and 1 year, but growth in weight and length-adjusted
204 weight were accelerated between 1 and 2 years in Group-30 compared with Group-10 (Figures 1 and
205 2). Almost all subjects (>92%) had normal body size (measured values between -2.0 and +2.0 SDS) at
206 all time points.

207 At 1 year, total ($r=0.56$, $p<0.001$) and supplemental vitamin D intake ($r=0.59$, $p<0.001$) correlated
208 with Infant 25(OH)D. Similarly, supplemental vitamin D intake at 2 years correlated with Toddler
209 25(OH)D ($r=0.61$, $p<0.001$). To exclude the possibility that body size as such, by possible dilution or
210 fat mass, affected how vitamin D intake was reflected in 25(OH)D concentration, we tested

211 interactions between supplemental vitamin D intake (compliance-based $\mu\text{g}/\text{day}$) and weight (kg) in all
212 linear models, and no interaction was detected.

213 No linear relation existed between Maternal 25(OH)D and offspring body size at 2 years (Table 3).

214 But the mothers whose Pregnancy 25(OH)D was above 125 nmol/L had lighter (measured in weight)
215 and thinner (measured in length-adjusted weight, MUAC and BMI) children at 2 years of age
216 compared with the reference group of children with Pregnancy 25(OH)D 50-74.9 nmol/L (Figure 3).

217 A quadratic association was confirmed between Pregnancy 25(OH)D and the children's length-
218 adjusted weight and BMI at 2 years ($p < 0.003$) suggesting an inverse U-shaped association (Figure 3).

219 Toddlers at 2 years of age with UCB 25(OH)D below 50 nmol/L at birth were taller than the reference
220 group of 50-74.9 nmol/L (Figure 4) but this association was attenuated after adjustment for maternal
221 prepregnancy BMI, paternal BMI, parental smoking status, parental education level, family income
222 level, and duration of breastfeeding ($p = 0.062$). Toddlers with UCB 25(OH)D above 125 nmol/L were
223 thinner (in BMI) at 2 years compared with the reference group of 50-74.9 nmol/L (Figure 4). Higher
224 Pregnancy 25(OH)D and UCB 25(OH)D associated with accelerated growth in head circumference at
225 2 years, while no association for other growth parameters was observed (Table 4).

226 We have previously reported that across the VIDI cohort, higher Infant 25(OH)D at 1 year associated
227 with slower growth at 1 year in several growth parameters (23). In the present study, we stratified the
228 results according to intervention group, and observed that linear associations between Infant 25(OH)D
229 and growth measures disappeared in Group-10 but were enhanced in Group-30 (Table 5).

230 At 2 years in the whole cohort, higher Toddler 25(OH)D associated linearly with smaller body size in
231 all other parameters except head circumference (Table 6). The association between Toddler 25(OH)D
232 and length attenuated after full adjustment for maternal and paternal factors ($p = 0.054$). After
233 stratification these linear results at 2 years of age by intervention group, associations between Toddler
234 25(OH)D and growth measures attenuated for length and remained for weight, length-adjusted weight
235 and BMI in both groups, while for MUAC the association disappeared in Group-10 and remained in
236 Group-30 (Table 6).

237 In the whole cohort, a quadratic association was observed between Toddler 25(OH)D and head
238 circumference ($p < 0.035$) implying an inverse U-shaped association (Figure 5). Toddler 25(OH)D had
239 no linear relation for conditional growth at 2 years (Table 4).

240 When comparing growth parameters in three groups of Toddler 25(OH)D, those with 25(OH)D above
241 125 nmol/L (highest group) were lighter (in weight) and thinner (in length-adjusted weight and BMI)
242 compared with the reference group with 25(OH)D < 75 nmol/L (Figure 5). Toddlers with 25(OH)D
243 between 75 nmol/L and 125 nmol/L had larger head circumference than the reference group of < 75
244 nmol/L (Figure 5). Figure 6 shows adjusted mean values for growth measures in quartiles of Toddler
245 25(OH)D. Children in the highest quartile of 25(OH)D (> 121 nmol/L) were shorter (in length), lighter
246 (in weight) and thinner (in length-adjusted weight and BMI) than the reference group in the lowest
247 quartile (< 81.2 nmol/L) (Figure 6).

248

249 Discussion

250 We examined the association of vitamin D in pregnancy and in early childhood with child growth in
251 an RCT-based cohort in Northern Europe with low sunlight exposure. VIDJ study is an intervention
252 trial with >800 infants comparing the effect of vitamin D supplementation of 10 µg/d and 30 µg/d
253 during the first 2 years of life.

254 The dose of vitamin D supplementation had little effect on early childhood growth, as the mean body
255 size measures were similar in both intervention groups (22). However, growth in length and head
256 circumference was slower between 6 months and 1 year but growth in weight and length-adjusted
257 weight was more rapid between 1 and 2 years in Group-30 compared with Group-10. Almost all
258 children were vitamin D sufficient (≥ 50 nmol/L) and 21% of the children had 25(OH)D above 125
259 nmol/L at 2 years. The possible effect of vitamin D on growth may be mediated through 25(OH)D
260 concentration, as we observed that higher 25(OH)D in early pregnancy, at birth, and at 1 and 2 years
261 of age associated with smaller body size in the offspring during 2 years' follow-up.

262 Previous studies on vitamin D and growth in early childhood have been inconclusive (15,16,28–30).

263 We have previously reported an inverse association between both Maternal 25(OH)D and Infant
264 25(OH)D and growth measures at age 6 months and 1 year (23). These findings were obtained before
265 the intervention code was opened and were based solely on measured 25(OH)D concentration. In line
266 with a Danish study (28) and contrary to an Equadorian study (30), we now observed that higher
267 Toddler 25(OH)D at age 2 years associated with smaller anthropometric growth parameters.

268 However, at 1 year, the associations were not observed within Group-10 but were enhanced in Group-
269 30. This is consistent with a non-linear relationship, implying that the effect of vitamin D dosage on
270 growth would depend on the attained 25(OH)D. At 2 years, these inverse associations between
271 25(OH)D and most growth measures remained in both intervention groups. This might be explained
272 by differing growth rates between intervention groups and time points. The intervention effect may be
273 smaller at 2 years than at 1 year because other factors such as food intake, physical activity and

274 endocrine factors, especially growth hormone secretion, have a larger role in child growth after
275 infancy.

276 We also applied both clinical cut-off values and quartiles for Toddler 25(OH)D at 2 years. These
277 results demonstrated that toddlers with 25(OH)D above 125 nmol/L or 121 nmol/L were the shortest
278 (in length), lightest (in weight) and thinnest (in length-adjusted weight and BMI) at 2 years of age. In
279 addition to our previous findings (31,32), others have found unfavorable and non-linear relations
280 between vitamin D and child health outcomes (33,34).

281 In longitudinal analysis, maternal 25(OH)D concentration in early pregnancy and at birth had no
282 linear relation to offspring growth anthropometry at 2 years. However, mothers with 25(OH)D above
283 125 nmol/L in early pregnancy, had the lightest and thinnest children at age 2 years, suggesting that
284 maternal 25(OH)D may affect infant growth until age 1 year but the effect diminishes thereafter,
285 possibly due to catch-up growth (23,35,36). Furthermore, other factors at an older age possibly have a
286 larger role than maternal 25(OH)D if it is in the “moderate range”. In line with our findings,
287 Christensen et al. found an inverse relation between UCB 25(OH)D and offspring leg length from age
288 1.5 to 3 years of age (37). Further, U- or J-shaped association have been suggested to exist between
289 maternal 25(OH)D and prenatal growth (38,39). However, several studies have found no relation
290 between maternal 25(OH)D and offspring postnatal growth (40–45).

291 Conflicting results between studies may be related to geographical and genetic differences, leading to
292 e.g. variable response to vitamin D supplementation (46,47), and varying cut-offs applied for
293 25(OH)D. Furthermore, it may be that only severe vitamin D deficiency (<30 nmol/L) (36,48), and, as
294 suggested by our results, high 25(OH)D (>125 nmol/L) impair childhood growth. Vitamin D
295 supplementation without vitamin D deficiency and “moderate” 25(OH)D concentrations would
296 therefore not show associations with growth parameters. In our study, both maternal and child’s
297 25(OH)D concentrations were at exceptionally high level compared with many other study cohorts.
298 This was due to widely used vitamin D supplementation during pregnancy and national vitamin D
299 food fortification (49,50). In the VIDJ cohort we have shown that genotype modifies individual’s
300 25(OH)D and the response to vitamin D supplementation (47,51). This individual dose-response was

301 shown in Group-30 but not in Group-10 (47), which might explain why we in the current study did
302 not observe a similar relation between both vitamin D supplementation and vitamin D concentration
303 and growth.

304 Severe vitamin D deficiency leads to growth impairment. If vitamin D indeed has an inverse U-
305 shaped association with early growth, the mechanism how high 25(OH)D could disturb normal
306 growth is unclear. Its role as a plasma calcium regulator could be one possible pathway. If high
307 25(OH)D leads to high circulating 1,25-dihydroxyvitamin D [1,25(OH)₂D], this enhances calcium and
308 phosphate resorption from bone to increase plasma calcium levels, thus possibly impairing growth
309 (52). Based on one study, maternal 25(OH)D would not increase 1,25(OH)₂D after 25(OH)D reaches
310 the level of 100 nmol/L (53). However, many organs and tissues, like the growth plate, have the
311 ability to produce 1,25(OH)₂D locally (54,55) and thus high 25(OH)D could lead to high local
312 production of 1,25(OH)₂D. In our cohort, at age 1 year, Infant 25(OH)D correlated with plasma
313 calcium (56) and PTH concentrations (23). Furthermore, PTH levels were lower in Group-30 than
314 Group-10 at age 1 year and 2 years. These observations suggest that vitamin D influenced the
315 endocrine system. However, the intervention group did not affect measured bone parameters (22).
316 Vitamin D may also affect growth-regulating hormones, e.g. insulin-like growth factor 1 (IGF-1)
317 which may activate 1,25(OH)₂D production (57, 58) .

318 We have a large and homogenous sample of North-European subjects with longitudinal data from
319 early pregnancy until child age of 2 years covering all seasons. Data were collected and processed in a
320 standardized fashion in a single maternity hospital. However, subjects had more commonly a higher
321 education and normal weight than nationally representative population. The small number of subjects
322 having maternal 25(OH)D values in both extreme ends, and only few vitamin D insufficient children
323 may have constrained our analyses. We applied multiple methods to discover the possible relation
324 between vitamin D and childhood growth and adjusted for potential confounders. As we did not
325 observe a direct effect of vitamin D supplementation but rather consistent associations between
326 25(OH)D and growth parameters, we cannot determine true direction of causality. However,
327 interactions of absolute body size and vitamin D intake were not observed.

328 The debate about the optimal 25(OH)D level for health outcomes is still ongoing (59,60). Studies with
329 subjects of high 25(OH)D concentrations are scarce (61), especially in geographical locations with
330 limited solar radiation, hence our data with exceptionally high 25(OH)D values are of importance in
331 gaining more understanding about the relationship between vitamin D and health.

332

333 Conclusion

334 In this large study, high maternal and child 25(OH)D concentrations were associated with delayed
335 growth in 1- and 2-years old children, but infant vitamin D supplementation in itself had only a minor
336 impact on growth measures. Our results imply that vitamin D may have an inverse U-shaped relation
337 with childhood growth. Therefore, aiming for higher than sufficient 25(OH)D levels with high
338 vitamin D dosages may have undesired consequences on child growth. The clinical relevance of our
339 results, however, remains to be evaluated in future studies.

340

341

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350

351 Data Availability

352

353 Some or all datasets generated during and/or analyzed during the current study are not publicly
354 available but are available from the corresponding author on reasonable request.

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562 **Figure 1** Mean (95% CI) values of conditional growth at 1 year of age, i.e. growth rate,
 563 according to intervention groups. Conditional growth at 1 year refers to the difference of body
 564 size at 1 year with expected based on body size at birth and 6 months, expressed in
 565 standardized residuals, SD units. Statistical difference tested with Independent-Samples T
 566 test. Number of subjects for length and length-adjusted weight: 10 µg, n=401; 30 µg, n=410,
 567 for weight: 10 µg, n=402; 30 µg, n=410, and for head circumference: 10 µg, n=387; 30 µg,
 568 n=394.

569 **Figure 2** Mean (95% CI) values of conditional growth at 2 years of age, i.e. growth rate,
 570 according to intervention groups. Conditional growth at 2 years refers to the difference of body
 571 size at 2 years with expected based on body size at birth, 6 months and 1 year, expressed in
 572 standardized residuals, SD units. Statistical difference tested with Independent-Samples T
 573 test. Number of subjects for length: 10 µg, n=401; 30 µg, n=410, for weight: 10 µg, n=401; 30
 574 µg, n=409, for length-adjusted weight: 10 µg, n=400; 30 µg, n=409, and for head
 575 circumference: 10 µg, n=380; 30 µg, n=387.

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Figure 3 Pregnancy 25(OH)D and offspring growth measures at 2 years of age. Symbols present
 adjusted mean (95% CI) values of growth measures in Pregnancy 25(OH)D categories of <50 nmol/L
 (n=24), 50-74.9 nmol/L (n=220) (reference group), 75-125 nmol/L (n=420) and >125 nmol/L (n=16).
 Adjustments are for corresponding birth size SDS, maternal and paternal height z-scores and
 intervention group. Statistical difference tested with linear regression with 50-74.9 nmol/L applied as a
 reference group. 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex- and age-
 specific normative data for infant growth; Length/weight, length-adjusted weight, HC, head
 circumference; MUAC, mid-upper-arm circumference (in z-score); BMI, body mass index (in z-score).
 The reference group's symbol has been highlighted.

Figure 4 Umbilical cord blood (UCB) 25(OH)D and offspring growth measures at 2 years of age.
 Symbols present adjusted mean (95% CI) values of growth measures in UCB 25(OH)D categories of
 <50 nmol/L (n=27), 50-74.9 nmol/L (n=304) (reference group), 75-125 nmol/L (n=429) and >125
 nmol/L (n=34). Adjustments are for corresponding birth size SDS, maternal and paternal height z-
 scores and intervention group. Statistical difference tested with linear regression with 50-74.9 nmol/L
 applied as a reference group. 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex-
 and age-specific normative data for infant growth; Length/weight, length-adjusted weight, HC, head
 circumference; MUAC, mid-upper-arm circumference (in z-score); BMI, body mass index (in z-score).
 The reference group's symbol has been highlighted.

Figure 5 Toddler 25(OH)D and offspring growth measures at 2 years of age. Symbols present
 adjusted mean (95% CI) values of growth measures in Toddler 25(OH)D categories of <75 nmol/L
 (n=138) (reference group), 75-125 nmol/L (n=502) and >125 nmol/L (n=172). Adjustments are for
 corresponding birth size SDS, maternal and paternal height z-scores and intervention group.
 Statistical difference tested with linear regression with <75 nmol/L applied as a reference group.
 25(OH)D, 25-hydroxy vitamin D; SDS, SD-score, based on Finnish sex- and age-specific normative
 data for infant growth; Length/weight, length-adjusted weight, HC, head circumference; MUAC, mid-
 upper-arm circumference (in z-score); BMI, body mass index (in z-score). The reference group's
 symbol has been highlighted.

578 **Figure 6** Toddler 25(OH)D in quartiles and offspring growth measures at 2 years of age. Symbols
 579 present adjusted mean (95% CI) values of growth measures in Toddler 25(OH)D quartiles of 1. quartile
 580 (<81.2 nmol/L, n=203) (reference group), 2. quartile (81.2-99.2 nmol/L, n=203), 3. quartile (99.3-120.7
 581 nmol/L, n=204) and 4. quartile (>121 nmol/L, n=202). Adjustments are for corresponding birth size
 582 SDS, maternal and paternal height z-scores and intervention group. Statistical difference tested with
 583 linear regression with 1. quartile applied as a reference group. 25(OH)D, 25-hydroxy vitamin D; SDS,
 584 SD-score, based on Finnish sex- and age-specific normative data for infant growth; Length/weight,
 585 length-adjusted weight, HC, head circumference; MUAC, mid-upper-arm circumference; BMI, body
 586 mass index. The reference group's symbol has been highlighted.

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Table 1 Family characteristics according to intervention groups

	Group-10 n=402	Group-30 n=410	P value
Maternal age, year	31.4 (4.0)	31.9 (4.5)	0.10
Paternal age, year ^a	32.9 (5.0)	33.7 (5.8)	0.026
Maternal height, cm	166.3 (6.1)	166.3 (5.9)	0.94
Paternal height, cm ^b	180.7 (6.7)	180.2 (6.6)	0.36
Maternal prepregnancy BMI ^c	23.2 (3.7)	23.3 (3.7)	0.64
Paternal BMI ^d	26.0 (3.5)	25.6 (3.2)	0.048
Pregnancy 25(OH)D, nmol/L ^e	82.9 (21.9)	81.8 (17.8)	0.49
Pregnancy sampling, gestational week	11.3 (2.2)	11.5 (3.3)	0.39
Maternal supplemental vitamin D intake, µg/d ^d	17.1 (19.8)	14.6 (12.8)	0.26
Maternal smoking, yes, % (n)	15 (61/399)	16 (66/409)	0.74
Paternal smoking, yes, % (n)	26 (102/397)	25 (100/404)	0.76
Parental education, higher, % (n)	81 (322/397)	84 (345/410)	0.26
Family income level ^f			0.24
<40 000 €/year, % (n)	16 (64)	18 (70)	
40 000-89 000 €/year, % (n)	59 (231)	54 (216)	
>90 000 €/year, % (n)	20 (79)	20 (81)	
Don't know	5 (18)	8 (31)	

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594 Values are means (SD) and P values are based on Independent-Samples T test, Mann-Whitney U
595 test or Chi-Square.
596 ^a9 missing values; ^b16 missing values; ^c4 missing values; ^d24 missing values; ^e132 missing values; ^f22 missing
597 values.

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Table 2 Infant growth parameters from birth to 2 years of age according to intervention groups

	Group-10 n=402	Group-30 n=410	P value
At birth			
Gestational age, wk	40.1 (1.1)	40.2 (1.1)	0.08
Length, cm	50.3 (1.7)	50.4 (1.8)	0.35
Length, SDS	-0.12 (0.89)	-0.11 (0.92)	0.86
Weight, kg	3.50 (0.37)	3.56 (0.40)	0.027
Weight, SDS	-0.19 (0.79)	-0.12 (0.84)	0.17
Length-adjusted weight, SDS	0.02 (0.93)	0.14 (0.93)	0.08
Head circumference, cm ^a	35.2 (1.4)	35.2 (1.4)	0.45
Head circumference, SDS ^a	0.19 (1.04)	0.08 (1.02)	0.12
UCB 25(OH)D, nmol/L [range] ^b	83.4 (28.2) [36.7-283.7]	81.8 (23.5) [37.8-229.0]	0.39
At 1 year of age			
Age at follow-up, y	1.00 (0.03)	1.00 (0.03)	0.93
Length, cm ^a	75.4 (2.6)	75.2 (2.5)	0.19
Length, SDS ^a	-0.49 (1.0)	-0.59 (0.98)	0.14
Weight, kg	9.8 (1.2)	9.8 (1.1)	0.44
Weight, SDS	-0.19 (1.0)	-0.24 (0.99)	0.48
Length-adjusted weight, SDS ^a	0.04 (1.0)	0.04 (1.0)	0.94
Head circumference, cm ^e	46.6 (1.2)	46.4 (1.2)	0.08
Head circumference, SDS ^e	-0.32 (0.97)	-0.45 (0.93)	0.053
MUAC, mm ^f	152.7 (12.9)	153.0 (11.8)	0.69
MUAC ² , z-score ^f	-0.02 (1.0)	0.02 (0.96)	0.66
Blood 25(OH)D, nmol/L [range] ^g	82.8 (19.9) [37.0-140.0]	116.0 (27.6) [51.8-241.0]	<0.001
Vitamin D intake from food, µg/day ^h	6.3 (3.7)	6.1 (3.7)	0.38
Compliance, % ⁱ	90.1 (10.4)	89.4 (10.6)	0.48
Supplemental vitamin D intake, compliance based µg/day ⁱ	9.0 (1.0)	26.8 (3.2)	<0.001
Energy intake, MJ/day ^h	3.36 (0.9)	3.31 (0.9)	0.26
At 2 years of age			
Age at follow-up, y	1.99 (0.03)	1.99 (0.03)	0.27
Length, cm	87.8 (3.2)	87.7 (3.0)	0.77
Length, SDS	-0.24 (1.04)	-0.27 (1.0)	0.71
Weight, kg ^d	12.5 (1.4)	12.6 (1.4)	0.44
Weight, SDS ^d	-0.19 (0.98)	-0.14 (0.99)	0.47
Length-adjusted weight, SDS ^d	-0.12 (0.98)	-0.02 (0.98)	0.15
Head circumference, cm ⁱ	49.1 (1.3)	49.1 (1.3)	0.60
Head circumference, SDS ⁱ	-0.22 (1.0)	-0.26 (0.98)	0.56
MUAC, mm ^k	161.8 (11.2)	162.5 (12.2)	0.42
MUAC, z-score ^k	-0.03 (0.95)	0.03 (1.04)	0.40
BMI, kg/m ² ^d	16.2 (1.2)	16.3 (1.2)	0.15
BMI, z-score ^d	-0.05 (0.99)	0.05 (1.01)	0.14

Blood 25(OH)D, nmol/L [range]	86.5 (19.7) [42.4-153.5]	117.7 (26.1) [56.5-207.4]	<0.001
Compliance, % ^l	86.6 (16.2)	85.5 (17.7)	0.73
Compliance based supplemental vitamin D intake, µg/day ^l	8.7 (1.6)	25.6 (5.3)	<0.001
Duration of breastfeeding, months ^m	10.7 (5.7)	11.0 (5.6)	0.44

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Values are means (SD). P values are based on Independent-Samples T test or Mann-Whitney U test. SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; UCB, umbilical cord blood; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; BMI, body mass index.
^a1 missing value; ^b18 missing values; ^c22 missing values; ^d2 missing values; ^e7 missing values; ^f34 missing values; ^g58 missing values; ^h107 missing values, breast milk intake not included; ⁱ10 values missing; ^j14 missing values; ^k17 missing values; ^l20 values missing; ^m6 values missing, duration of breastfeeding was set to 2 years if still ongoing at 2 years' follow-up

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Table 3 Associations between Maternal 25(OH)D concentrations and offspring's growth measures at 2-years' follow-up visit

	SDS				z-score	
	Length	Weight	Length-adjusted weight	Head circumference	MUAC	BMI
Pregnancy 25(OH)D, 10 nmol/L, n=680						
Model 1, unadjusted	-0.01 (-0.05, 0.02)	-0.02 (-0.06, 0.02)	-0.02 (-0.05, 0.02)	-0.00 (-0.04, 0.04)	-0.03 (-0.07, 0.01)	-0.02 (-0.05, 0.02)
P value	0.48	0.29	0.38	0.97	0.12	0.42
Model 2, adjusted ^a	-0.02 (-0.05, 0.02)	-0.02 (-0.06, 0.01)	-0.00 (-0.00, -0.00)	0.00 (-0.02, 0.02)	-0.03 (-0.07, 0.00)	-0.02 (-0.05, 0.02)
P value	0.29	0.16	0.33	0.97	0.11	0.37
UCB 25(OH)D, 10 nmol/L, n=794						
Model 1, unadjusted	0.01 (-0.02, 0.03)	-0.01 (-0.04, 0.02)	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.02)	0.00 (-0.02, 0.03)	-0.02 (-0.05, 0.00)
P value	0.67	0.43	0.13	0.40	0.80	0.09
Model 2, adjusted ^a	0.00 (-0.02, 0.03)	-0.01 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.02)	-0.02 (-0.04, 0.01)
P value	0.72	0.60	0.24	0.69	0.65	0.19

Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression.

SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; BMI, body mass index; UCB, umbilical cord blood.

^aModel 2 is adjusted for the corresponding birth size SDS (except for MUAC and BMI; the covariate was length-adjusted birth weight), maternal and paternal height z-scores, and intervention group.

Missing values: in Pregnancy 25(OH)D analyses: 1 value missing in weight, length-adjusted weight and BMI; 12 values missing from head circumference and MUAC, in UCB 25(OH)D analyses: 2 values missing in weight, length-adjusted weight and BMI; 15 values missing from head circumference and MUAC.

Table 4 Associations between Maternal and Toddler 25(OH)D and conditional growth at 2 years' follow-up visit

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Pregnancy 25(OH)D, 10 nmol/L, n=679	SD unit				
	Length	Weight	Length-adjusted weight	Head circumference	MUAC
Model 1, unadjusted	-0.01 (-0.05, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.03, 0.04)	0.04 (0.00, 0.08)	0.00 (-0.04, 0.03)
P value for linear association	0.64	0.83	0.78	0.036	0.83
Model 2, adjusted ^a	-0.01 (-0.05, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.03, 0.04)	0.04 (0.00, 0.08)	0.00 (-0.04, 0.03)
P value for linear association	0.65	0.81	0.74	0.037	0.80
UCB 25(OH)D, 10 nmol/L, n=793					
Model 1, unadjusted	0.02 (-0.01, 0.04)	0.00 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	0.03 (0.01, 0.06)	0.01 (-0.01, 0.04)
P value for linear association	0.23	0.74	0.20	0.015	0.30
Model 2, adjusted ^a	0.01 (-0.01, 0.04)	-0.01 (-0.03, 0.02)	-0.02 (-0.04, 0.01)	0.03 (0.01, 0.06)	0.02 (-0.01, 0.04)
P value for linear association	0.27	0.61	0.19	0.014	0.25
Toddler 25(OH)D at 2 years, 10 nmol/L, n=811					
Model 1, unadjusted	0.01 (-0.01, 0.04)	0.02 (0.00, 0.05)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	-0.01 (-0.03, 0.02)
P value for linear association	0.40	0.072	0.34	0.30	0.59
Model 2, adjusted ^a	0.00 (-0.03, 0.03)	0.01 (-0.02, 0.04)	0.00 (-0.03, 0.03)	0.01 (-0.02, 0.04)	-0.02 (-0.05, 0.01)
P value for linear association	0.82	0.60	0.82	0.48	0.32

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Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression. Conditional growth refers to the difference of body size at 2 years with expected based on body size at birth, 6 months and 1 year, expressed in standardized residuals, SD units.

25(OH)D, 25-hydroxyvitamin D concentration; UCB, umbilical cord blood.

^aModel 2 is adjusted for maternal and paternal height z-scores, and intervention group.

Missing values: in Pregnancy 25(OH)D analyses: 1 value missing in length-adjusted weight; 40 values missing from head circumference; 32 values missing from MUAC, in UCB 25(OH)D analyses: 1 value missing in length; 2 values missing length-adjusted weight; 42 values missing from head circumference; 44 values missing from MUAC, in Toddler 25(OH)D analyses: 1 value missing in length-adjusted weight; 2 values missing from length-adjusted weight; 44 values missing from head circumference; 46 values missing from MUAC.

Table 5 Associations between Infant 25(OH)D concentrations and growth measures at 1-year's follow-up visit stratified by intervention group

Infant 25(OH)D, 10 nmol/L	SDS				z-score
	Length	Weight	Length-adjusted weight	Head circumference	MUAC
Group-10, n=371					
Model 1, unadjusted	0.02 (-0.03, 0.07)	0.01 (-0.04, 0.06)	0.00 (-0.05, 0.05)	-0.01 (-0.06, 0.04)	-0.01 (-0.07, 0.04)
P value	0.47	0.69	0.95	0.79	0.68
Model 2, adjusted ^a	0.02 (-0.03, 0.07)	0.02 (-0.03, 0.07)	0.00 (-0.05, 0.06)	0.00 (-0.05, 0.04)	-0.01 (-0.06, 0.05)
P value	0.39	0.54	0.92	0.96	0.77
Group-30, n=383					
Model 1, unadjusted	-0.04 (-0.07, 0.00)	-0.07 (-0.10, -0.03)	-0.06 (-0.10, -0.02)	-0.04 (-0.07, 0.00)	-0.04 (-0.08, 0.00)
P value	0.047	<0.001	0.001	0.039	0.026
Model 2, adjusted ^a	-0.02 (-0.05, 0.01)	-0.05 (-0.09, -0.02)	-0.06 (-0.10, -0.02)	-0.02 (-0.05, 0.01)	-0.04 (-0.08, -0.01)
P value	0.17	0.001	0.001	0.19	0.022

Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression.

SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference.

^aModel 2 is adjusted for the corresponding birth size SDS (except for MUAC; the covariate was length-adjusted birth weight), maternal and paternal height z-scores and intervention group (except in analyses stratified by intervention groups).

Missing values: 1 value missing from length and length-adjusted weight; 5 values missing from head circumference; 32 values missing from MUAC.

Table 6 Associations between Toddler 25(OH)D concentrations and growth measures at 2-years' follow-up visit stratified by intervention group

Toddler 25(OH)D, 10 nmol/L	SDS				z-score	
	Length	Weight	Length-adjusted weight	Head circumference	MUAC	BMI
All, n=812						
Model 1, unadjusted	-0.02 (-0.05, 0.00)	-0.03 (-0.06, -0.01)	-0.03 (-0.05, 0.00)	-0.01 (-0.04, 0.00)	-0.02 (-0.04, 0.01)	-0.03 (-0.06, 0.00)
P value	0.057	0.009	0.040	0.32	0.13	0.035
Model 2, adjusted ^a	-0.03 (-0.05, 0.00)	-0.04 (-0.07, -0.02)	-0.02 (-0.05, 0.00)	-0.00 (-0.02, 0.02)	-0.02 (-0.04, 0.01)	-0.06 (-0.09, -0.02)
P value	0.038^b	0.001	0.001	0.92	0.030	0.001
Group-10, n=402						
Model 1, unadjusted	-0.04 (-0.09, 0.02)	-0.07 (-0.12, -0.02)	-0.06 (-0.11, -0.02)	0.02 (-0.04, 0.07)	-0.03 (-0.08, 0.02)	-0.06 (-0.11, -0.01)
P value	0.16	0.008	0.009	0.56	0.20	0.011
Model 2, adjusted ^a	-0.03 (-0.07, 0.01)	-0.05 (-0.10, -0.01)	-0.06 (-0.11, -0.01)	0.01 (-0.04, 0.06)	-0.02 (-0.07, 0.02)	-0.06 (-0.11, -0.01)
P value	0.19	0.016	0.018	0.68	0.33	0.018
Group-30, n=410						
Model 1, unadjusted	-0.03 (-0.07, 0.01)	-0.05 (-0.09, -0.01)	-0.04 (-0.08, -0.01)	-0.03 (-0.06, 0.01)	-0.04 (-0.08, -0.00)	-0.04 (-0.08, -0.01)
P value	0.12	0.009	0.018	0.15	0.049	0.021
Model 2, adjusted ^a	-0.02 (-0.06, 0.01)	-0.04 (-0.07, 0.00)	-0.04 (-0.06, -0.02)	-0.01 (-0.03, 0.01)	-0.04 (-0.06, -0.02)	-0.04 (-0.07, -0.01)
P value	0.13	0.034	0.027	0.56	0.062	0.032

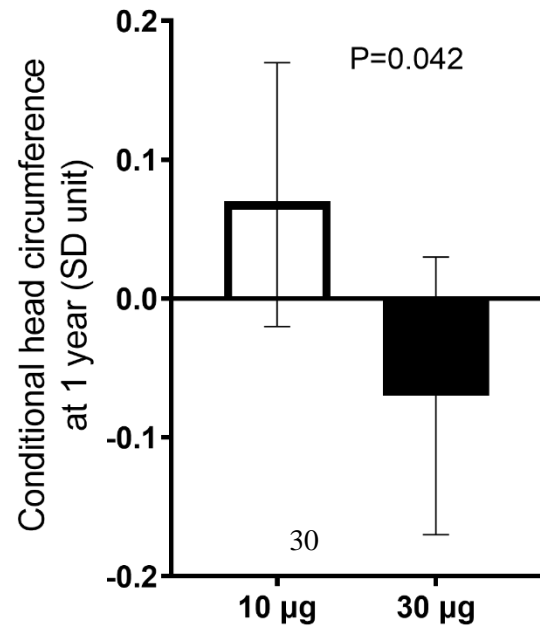
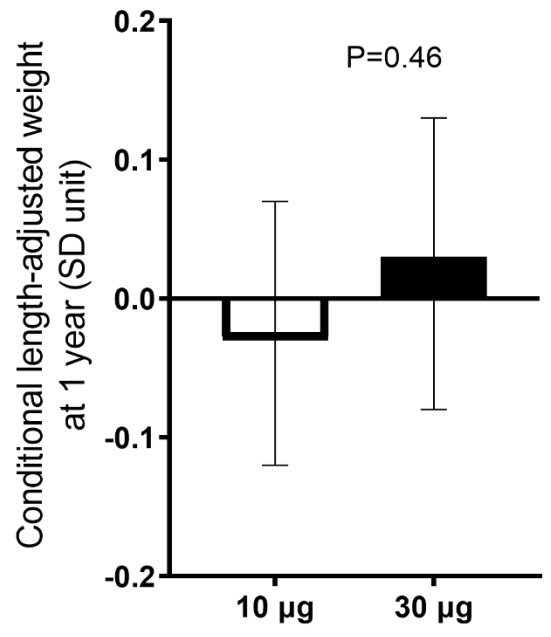
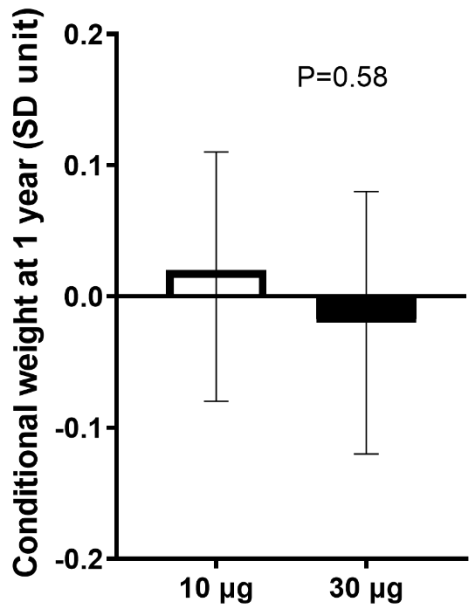
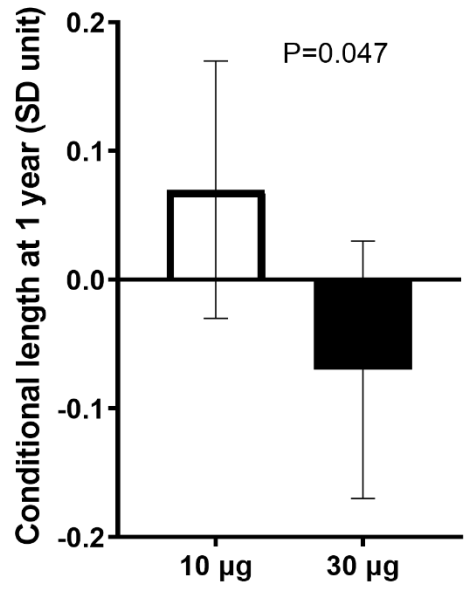
Values are beta coefficients (95% CI) per 10 nmol/L increase in 25(OH)D concentration based on linear regression.

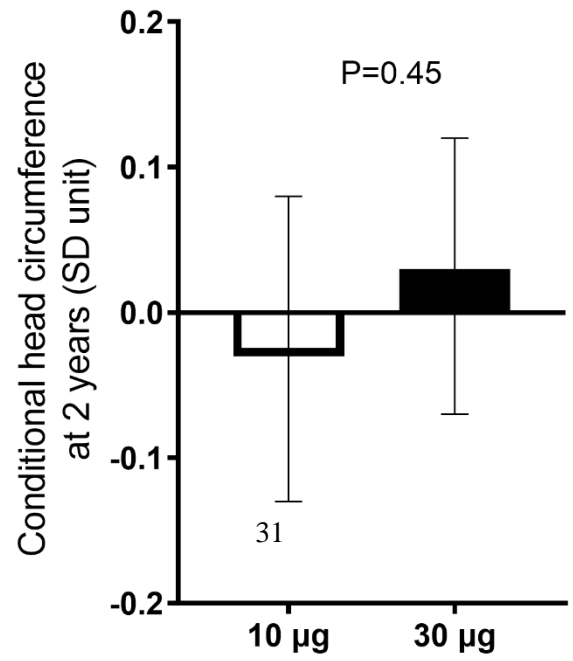
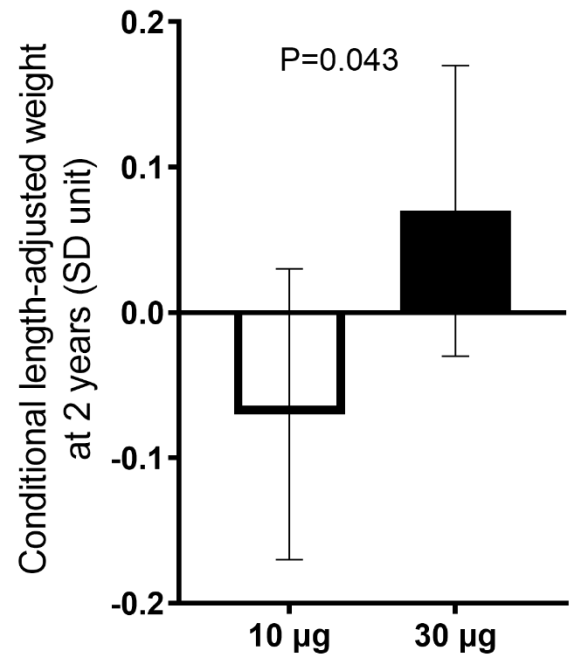
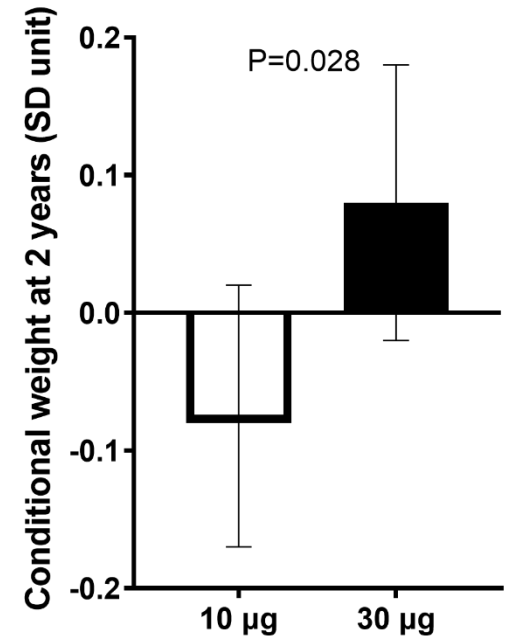
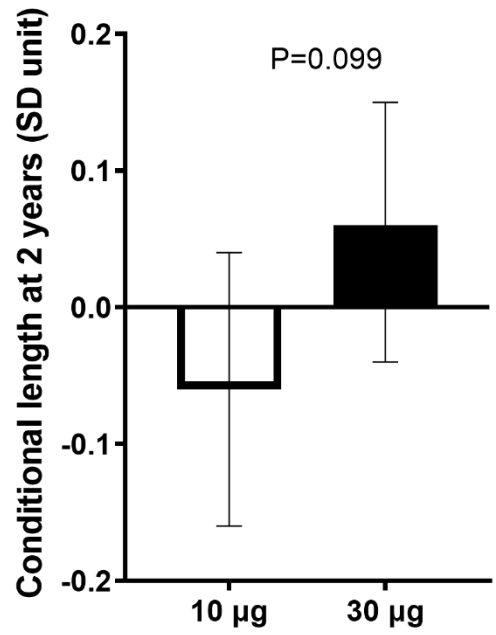
SDS, standard deviation score, based on Finnish sex- and age-specific normative data for infant growth; 25(OH)D, blood 25-hydroxyvitamin D concentration; MUAC, mid-upper-arm circumference; BMI, body mass index.

^aModel 2 is adjusted for the corresponding birth size SDS (except for MUAC and BMI; the covariate was length-adjusted birth weight), maternal and paternal height z-scores and intervention group.

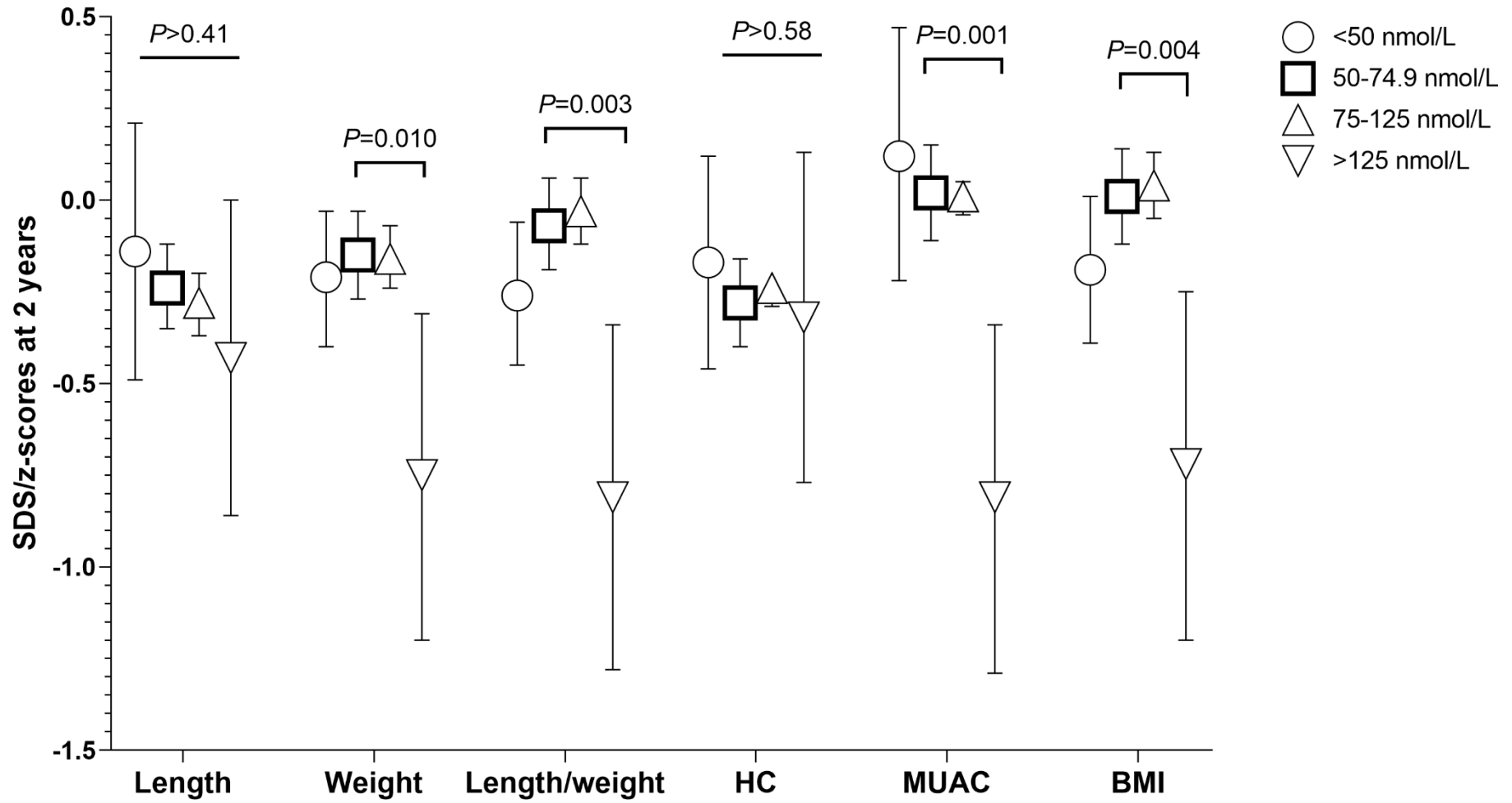
^bAdditional adjustment for maternal prepregnancy BMI, paternal BMI, parental smoking status, parental education level, family income level, and duration of breastfeeding attenuated the association to P=0.054.

Missing values: 2 values missing from weight, length-adjusted weight and BMI; 14 values missing from head circumference; 17 values missing from MUAC.

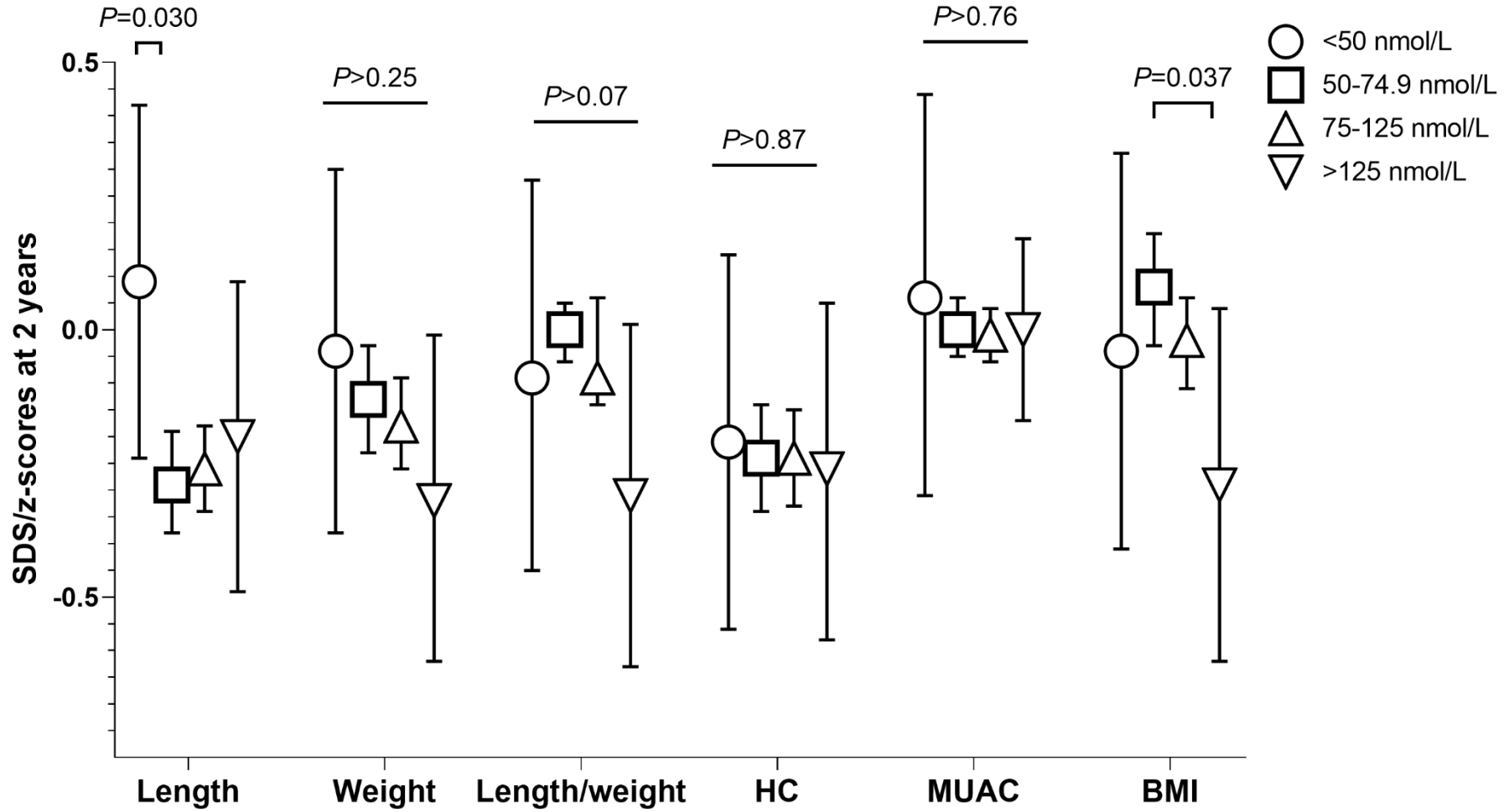




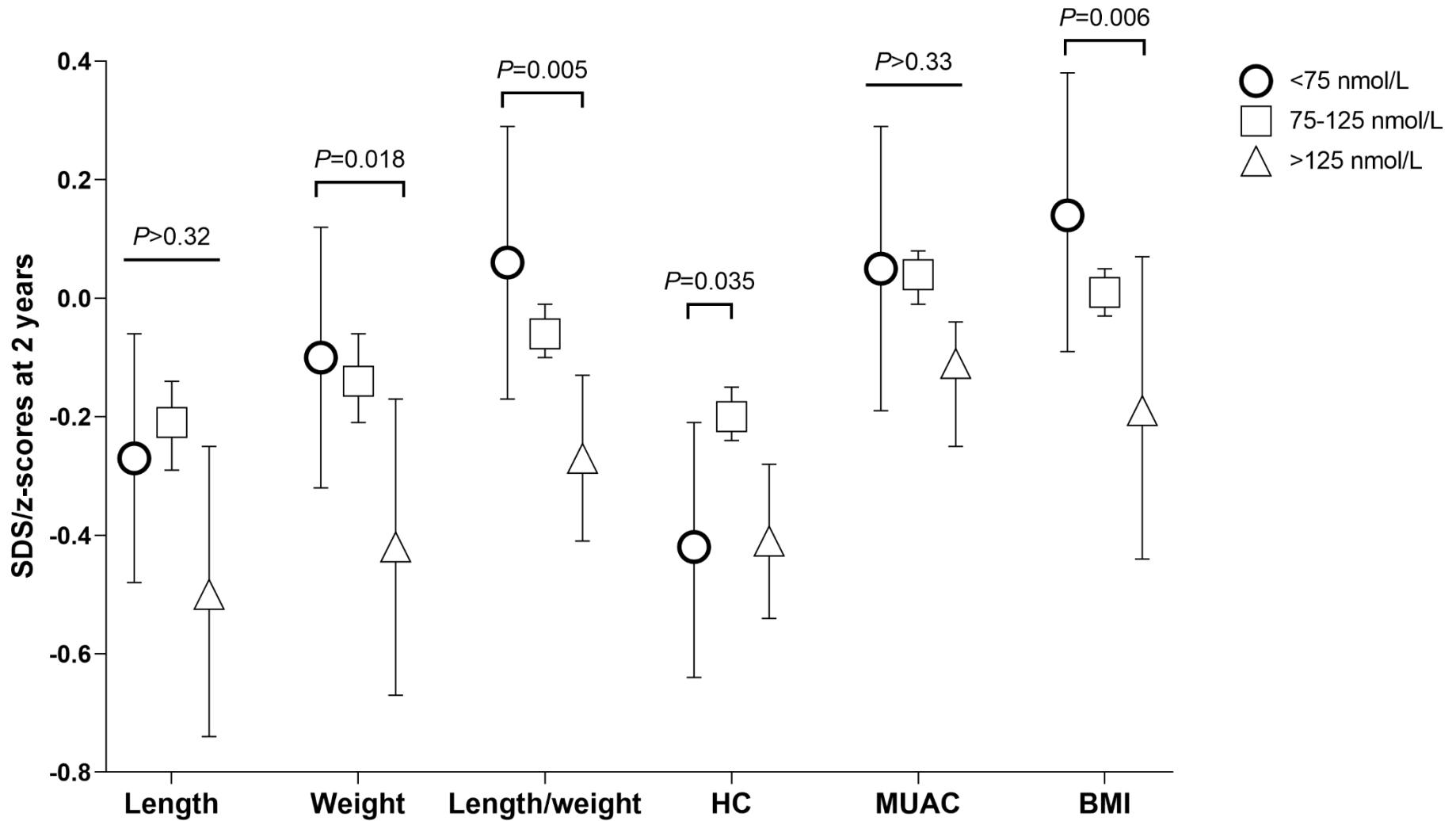
Pregnancy 25(OH)D and offspring growth measures at 2 years (n=680)



UCB 25(OH)D and offspring growth measures at 2 years (n=794)



Toddler 25(OH)D and growth measures at 2 years (n=812)



Toddler 25OHD at 2 years in quartiles (n=812)

