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Associations of 25 Hydroxyvitamin D and High Sensitivity C-reactive Protein Levels in Early Life

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Abstract: Vitamin D deficiency and elevated high sensitivity C-reactive protein (hs-CRP) have been associated with several health outcomes, but knowledge on early life trajectories and association between 25 hydroxyvitamin D (25(OH)D) and hs-CRP is lacking. We investigated the association between longitudinal measurements of 25(OH)D and hs-CRP, respectively, from pregnancy to childhood and throughout childhood in two Danish mother–child cohorts—the $COPSAC_{2010}$ and $COPSAC_{2000}$. In $COPSAC_{2010}$, there was an association between 25(OH)D concentrations at week 24 in pregnancy and at age 6 months in childhood (n = 633): estimate (95% CI); 0.114 (0.041;0.187), p = 0.002, and between 25(OH)D at age 6 months and 6 years (n = 475): 0.155 (0.083;0.228), $p < 0.001$. This was also demonstrated in the $COPSAC_{2000}$ cohort between 25(OH)D concentrations in cord blood and at age 4 years (n = 188): 0.294 (0.127;0.461), $p < 0.001$ and at age 6 months and 4 years (n = 264): 0.260 (0.133;0.388), $p < 0.001$. In $COPSAC_{2000}$, we also found an association between hs-CRP at age 6 months and 12 years in childhood (n = 232): 0.183 (0.076;0.289), $p < 0.001$. Finally, we found a negative association between the cross-sectional measurements of 25(OH)D and hs-CRP at age 6 months (n = 613) in $COPSAC_{2010}$: −0.004 (−0.008;−0.0004), p = 0.030, but this was not replicated in $COPSAC_{2000}$. In this study, we found evidence of associations across timepoints of 25(OH)D concentrations from mid-pregnancy to infancy and through childhood and associations between hs-CRP levels during childhood, although with weak correlations. We also found a negative cross-sectional association between 25(OH)D and hs-CRP concentrations in $COPSAC_{2010}$ proposing a role of vitamin D in systemic low-grade inflammation, though this association was not present in $COPSAC_{2000}$.

Keywords: vitamin D; hs-CRP; low-grade inflammation; COPSAC; 25(OH)D; pregnancy; children

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1. Introduction

High-sensitivity C-reactive protein (hs-CRP) is a known marker of systemic low-grade inflammation in many chronic disorders, including inflammatory bowel disease (IBD) [1], cardiovascular disease [2,3], depression [4] and chronic obstructive pulmonary disease (COPD) [5]. Further, increased concentrations of hs-CRP have been linked to decreased lung function in childhood [6,7], allergic sensitization at school age [8], early life airway microbiota [9] and childhood asthma [10,11], which has led to suggestions of using hs-CRP as a clinical biomarker of low-grade inflammation for grading, diagnosing and preventing disease [11]. We have previously shown an association between hs-CRP levels in pregnant

mothers and their offspring at age 6 months [12]; however, knowledge on the development of low-grade inflammation throughout childhood is lacking.

Vitamin D sufficiency in early life has also been shown to be associated with several health outcomes during childhood, including greater bone mineralization [13], decreased risk of enamel defects [14], asthma [15] and various skin conditions. Experimental studies have suggested reduced replication of virus in bronchial epithelial cells [16], induced antimicrobial production [17] and upregulation in the early life airway immune profile [18] as possible mechanisms for preventing asthma. Since the cutaneous conversion of 7-dehydrocholesterol to pre-vitamin D_3 and then vitamin D_3 occurs only when exposed to sunlight by ultraviolet B radiation [19], human blood concentrations depend on many factors such as pigmentation, lifestyle, skin protection, etc. It is unclear whether vitamin D status remains stable from early to later in life, but it has previously been demonstrated in the Western Australian Pregnancy Cohort (Raine) longitudinal study that 25 hydroxyvitamin D (25(OH)D) concentrations tracked from school age until age 20 years [20]. However, the Raine study did not investigate the relationship between maternal vitamin D concentrations during pregnancy and vitamin D concentrations through early childhood.

In this study, we utilized two Danish mother–child cohorts—the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) 2000 and 2010—to investigate potential association across timepoints of 25(OH)D and hs-CRP concentrations during pregnancy and childhood and examined the possible relationship between 25(OH)D and hs-CRP, which are important for childhood health and disease.

2. Materials and Methods

2.1. Ethics

The studies were approved by the local Ethics Committee (HKF 01-289/96; H-B-2008-093) and the Danish Data Protection Agency (2015-41-3696). Both oral and written informed consent was obtained from the parents during enrollment.

2.2. Study Populations

The Danish $COPSAC_{2000}$ and $COPSAC_{2010}$ clinical, single-center, mother–child cohorts have previously been described in detail including enrollment procedure, baseline characteristics and flow of the participants [13,18,21,22].

In summary, the prospective $COPSAC_{2000}$ cohort is a high-risk asthma cohort of 411 children born to mothers with a history of asthma, which were enrolled during pregnancy at week 36. The children were monitored from age 1 month until age 18 years undergoing a minimum of 18 scheduled and acute care clinical visits [22,23], allowing for deep phenotyping of the children.

The $COPSAC_{2010}$ is a population-based cohort including 700 children of pregnant mothers enrolled at week 24 in pregnancy. The pregnant women participated in two randomized controlled trials of high-dose (2800 IU/day) vs. standard-dose (400 IU/day) vitamin D [18] and fish-oil vs. olive-oil [24] from week 24 gestation until 1 week postpartum. The children were followed longitudinally in the COPSAC research clinic with a minimum of 14 scheduled and acute care visits from age 1 week until age 10 years.

2.3. Measurements of Hs-CRP and 25(OH)D

In $COPSAC_{2000}$, blood samples from the cubital vein of the children at age 6 months, 7 and 12 years were centrifuged and stored at −80 °C until analysis, where hs-CRP concentrations were determined by a high-sensitivity electrochemiluminescence assay from MesoScale Discovery with a lower limit of detection of 0.007 ng/mL. Total serum 25(OH)D concentrations were measured at birth in cord blood and at 4 years of age using the isotope dilution liquid chromatography–tandem mass spectrometry [25]. Total plasma 25(OH)D concentrations were measured at age 6 months using the same technique as above. The laboratories participated in the proficiency testing program Vitamin D External Quality Assessment Scheme (DEQAS).

In $COPSAC_{2010}$, blood samples from the children at age 6 months were analyzed for hs-CRP concentrations using a similar method as in $COPSAC_{2000}$. Total serum 25(OH)D concentrations were measured from maternal blood samples in pregnancy week 24 using the same method as above. Child samples at age 6 months and 6 years were analyzed using the DiaSorin LIAISON 25-OH Vitamin D Total Assay [26]. The laboratory used US National Institute of Standards and Technology (NIST) level 1 protocol.

2.4. Covariates

We included environmental determinants previously shown to be related to hs-CRP and 25(OH)D concentrations in the children from our cohorts [6,23,27], which were sex, season of samples, older children in home at birth and any infection 14 days prior to hs-CRP measurement based on daily diary registrations of symptoms of cold, cough, pneumonia, ear infection, fever or gastric infection [22].

2.5. Statistical Analyses

The analyses of the associations between hs-CRP and 25(OH)D at different timepoints were performed using linear regression models and illustrated by scatter plots. Additionally, the models were adjusted for covariates. The hs-CRP values were log-transformed prior to analyses, given the skewed distribution of data. All analyses were performed using R (version 4.0.3) with $p < 0.05$ considered indicative of significance.

3. Results

3.1. Associations of 25(OH)D from Pregnancy to Childhood and in Childhood

Of the 700 children in the $COPSAC_{2010}$ cohort, 633 (90%) had available serum 25(OH)D measurements at age 6 months (mean (SD): 84.8 (23.8) nmol/L) with mothers with available 25(OH)D measurements at pregnancy week 24. We found an association between concentrations at week 24 in pregnancy and at age 6 months in childhood: crude estimate (95% CI); 0.114 (0.041;0.187), $p = 0.002$, although the correlation was weak ($R^2 = 0.015$). At age 6 years, 475 (75%) of the children with 6 months measurements had available serum 25(OH)D measurements (mean (SD): 64.3 (20.0) nmol/L), which demonstrated an association between these two time points: 0.155 (0.083;0.228), $p < 0.001$, $R^2 = 0.036$ (Figure 1).

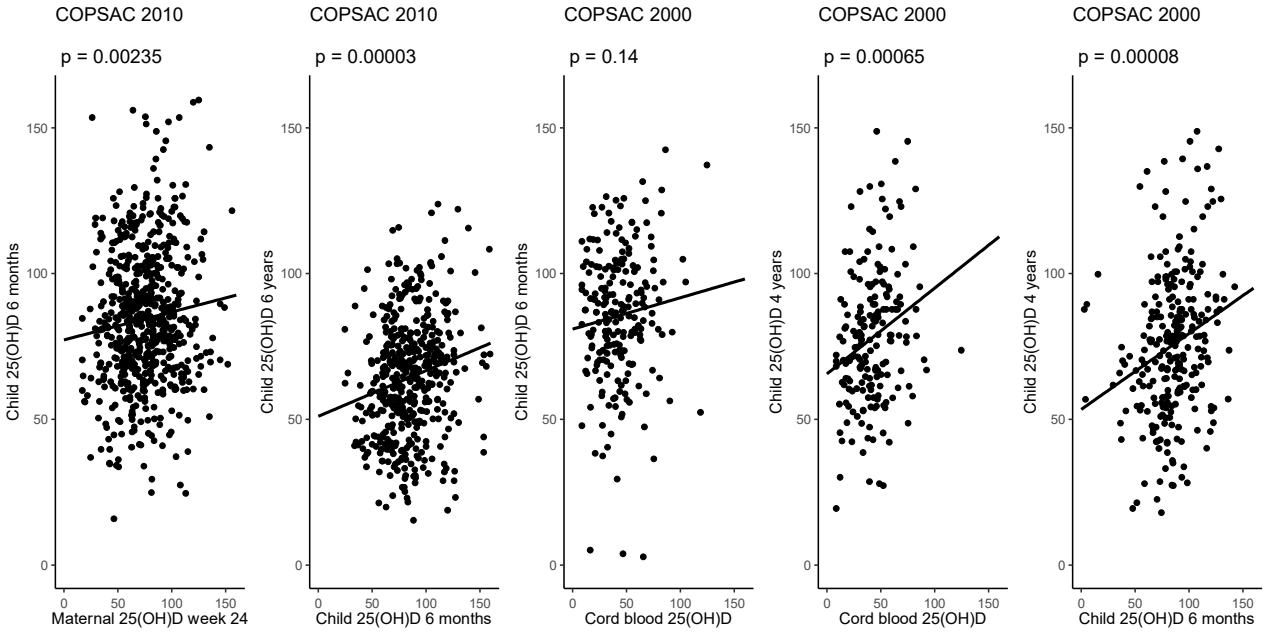

Figure 1. The associations between 25(OH)D at pregnancy week 24, age 6 months and 6 years in $COPSAC_{2010}$ and cord blood, age 6 months and 4 years in childhood in $COPSAC_{2000}$. All values are in nmol/L.

Of the 411 children in the $COPSAC_{2000}$ cohort, 257 (63%) had available cord blood measurements (mean (SD): 43.1 (20.8) nmol/L), 347 (84%) had available measurements at age 6 months (mean (SD): 85.9 (22.7) nmol/L) and 298 (73%) had available measurements at age 4 years of serum 25(OH)D (mean (SD): 76.0 (25.4) nmol/L). Among the 215 children with both cord blood and 6 months 25(OH)D measurements, we did not find a significant association between these time points: estimate (95% CI); 0.101 (−0.034;0.236), $p = 0.143$, $R^2 = 0.010$; however, we found a significant association between 25(OH)D from cord blood and at 4 years during childhood ($n = 188$): 0.294 (0.127;0.461), $p < 0.001$, $R^2 = 0.061$, and a significant association between 25(OH)D at age 6 months and 4 years ($n = 264$): 0.260 (0.133;0.388), $p < 0.001$, $R^2 = 0.058$ (Figure 1).

3.2. Associations of hs-CRP in Childhood

Of the 411 children in $COPSAC_{2000}$, 300 (73%), 276 (67%) and 313 (76%) had available hs-CRP measurements (ng/mL) at age 6 months, 7 and 12 years in childhood, respectively. Among the 211 children with hs-CRP measurements at both 6 months and 7 years, a trend towards an association was observed: crude estimate (95% CI); 0.097 (−0.005;0.200), $p = 0.063$, $R^2 = 0.016$. In children ($n = 232$) with both 6 months and 12 years hs-CRP measurements, we found an association between these two time points: 0.183 (0.076;0.289), $p < 0.001$, $R^2 = 0.047$, which was also significant in the analysis of children ($n = 247$) with hs-CRP at age 7 vs. 12 years: 0.373 (0.246;0.501), $p < 0.001$, $R^2 = 0.120$ (Figure 2).

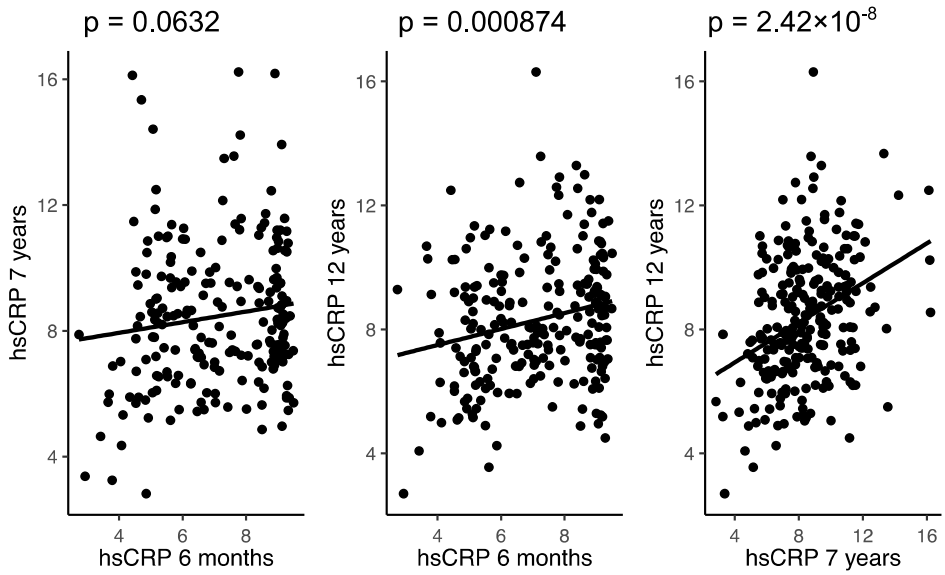

Figure 2. The associations between hs-CRP at age 6 months, 7 and 12 years in childhood in the $COPSAC_{2000}$ cohort. All values are in ng/mL and log transformed.

3.3. Association between Hs-CRP and 25(OH)D in Both Cohorts

Cross-sectional measurements of hs-CRP and serum 25(OH)D at age 6 months were available in 613 (88%) children in $COPSAC_{2010}$. There was a negative association between hs-CRP and 25(OH)D from a linear regression model: crude estimate (95% CI); −0.004 (−0.008;−0.0004), $p = 0.030$. Among the 613 children with both hs-CRP and 25(OH)D measurements at age 6 months, 208 children with any diary registered infection 14 days prior to hs-CRP measurement were excluded in a stratified model, leaving 405 children available for analysis, which still showed a negative association between hs-CRP and 25(OH)D: −0.005 (−0.009;−0.0006), $p = 0.027$. However, in a fully adjusted analysis for sex, sample season, older children in home and any infections 14 days prior to measurement, we did not find an association (Table 1).

Table 1. The association between log hs-CRP and vitamin D at age 6 months in $COPSAC_{2010}$ from uni- and multivariable linear regression models.

	hs-CRP Estimate *	95% CI	*p* Value
Crude (*n* = 613)	−0.004	−0.008; −0.0004	0.030
Adjusted for environmental and demographic factors (*n* = 613) [1]	−0.002	−0.006; 0.001	0.230
Children with no infection (*n* = 405) [2]	−0.005	−0.009; −0.001	0.027

[1] Environmental and demographic factors: Sex, sample season, older children in home and any infection 14 days prior to measurement. [2] Including children with no infections 14 days prior only. * CRP values are in ng/mL and log-transformed.

In $COPSAC_{2000}$, 299 (73%) children had cross-sectional measurements of hs-CRP and plasma 25(OH)D at age 6 months with no significant association between these: 0.003 (−0.003;0.009), $p = 0.401$. In the fully adjusted model of sex, sample season, older children in home and any infection 14 days prior to measurement, there was still no significant association ($n = 299$): 0.004 (−0.002;0.010), $p = 0.157$. In children with no infection 14 days prior to measurement ($n = 208$), there was also no association: 0.003 (−0.004;0.009), $p = 0.436$ (Table 2).

Table 2. The association between log hs-CRP and vitamin D at age 6 months in $COPSAC_{2000}$ from uni- and multivariable linear regression models.

	hs-CRP Estimate *	95% CI	*p* Value
Crude (*n* = 299)	0.003	−0.003; 0.009	0.401
Adjusted for environmental and demographic factors (*n* = 299) [1]	0.004	−0.002; −0.010	0.157
Children with no infection (*n* = 208) [2]	0.003	−0.004; −0.009	0.436

[1] Environmental and demographic factors: Sex, sample season, older children in home and any infection 14 days prior to measurement. [2] Including children with no infections 14 days prior only. * CRP values are log-transformed.

4. Discussion

4.1. Primary Findings

In two Danish mother–child cohorts with close longitudinal follow-up, we found evidence of association across timepoints of serum 25(OH)D concentrations from mid-pregnancy to childhood and throughout childhood. Further, we found association between hs-CRP concentrations measured from early childhood at age 6 months through to age 12 years, suggesting an early trajectory of both 25(OH)D and hs-CRP. We demonstrated a negative association between hs-CRP and serum 25(OH)D concentrations using cross-sectional measurements at age 6 months in the $COPSAC_{2010}$ cohort, proposing a role of vitamin D in systemic low-grade inflammation. However, the inverse association between hs-CRP and 25(OH)D was not apparent in the high-risk $COPSAC_{2000}$ cohort.

4.2. Strengths and Limitations

The main strength of our study is the close longitudinal clinical follow-up of the children from two large-scale cohorts with several blood samples performed both in pregnancy and during childhood, which allows for analyses of correlation of measurements over a long period. Another strength is the thorough, deep phenotyping of the children with daily diary cards filled out by the parents in $COPSAC_{2010}$ with registration of any signs of infections, which is crucial when assessing hs-CRP given its well-established role as a marker of inflammation and infection. Additionally, there is information on a wide range of environmental and demographic exposures, which previously have been used for identification of determinants of hs-CRP and 25(OH)D concentrations [6,23,27]. A limitation of the study is the lack of information on other important factors for 25(OH)D

concentrations such as sunscreen protection, hours spent in the sun and diet in both cohorts and although we adjusted for important covariates based on our previous studies, our findings could be influenced by residual lifestyle confounders given the observational study design. Another limitation is the lack of ethnic diversity in our cohorts consisting primarily of Caucasians, which only allows for generalization of our findings among this ethnic group and, therefore, may not be applicable to other populations. Finally, it was a limitation that our cohorts were not similar in terms of population characteristics and sample sizes, where the $COPSAC_{2010}$ is a larger population-based cohort [21] and the $COPSAC_{2000}$ is a smaller high-risk cohort [22], which may explain why we did not find an association between hs-CRP and 25(OH)D at age 6 months in $COPSAC_{2000}$, despite adjusting for relevant covariates. The difference in measurement methods of 25(OH)D in the two cohorts could possibly also explain why the results differ when analyzing the relationship with hs-CRP; however, it should not influence the 25(OH)D correlations within the cohorts as the same method is used within each cohort.

4.3. Interpretation

Our findings of associations between 25(OH)D concentrations from pregnancy to childhood and through childhood until age 6 years are in line with previous studies among older populations [20,28]. In the Australian Raine study, 25(OH)D concentrations at age 6 years were associated with concentrations measured until age 20 years [20]. Further, 25(OH)D status at age 6 years was characterized as a predictor of peak bone mass around age 20 years in the same cohort, which highlights the clinical importance of early life vitamin D sufficiency, since our findings demonstrated an association across timepoints already from pregnancy week 24 to childhood. The association of 25(OH)D concentrations over time has also been shown in a Norwegian study over a 14-year period in adulthood [28], but was not found in a mixed South African population investigating correlation from age 11 to 20 years [29]. The latter study was limited by the number of subjects (n = 76) and could also reflect that the association over time is diverse across ethnic groups. The clinical importance of vitamin D status has been investigated in relation to several disorders, and low 25(OH)D concentration has been suggested to be related to increased risk of, e.g., bone, inflammatory and infectious diseases [30]. Most notably, the risk of osteoporosis seems dependent on child bone mineralization [31,32], which is suggested to be highly influenced by early life vitamin D status [13]. Further, supplementation with high doses of vitamin D in pregnancy has shown to protect against early asthma development, suggesting a role of vitamin D in asthma prevention.

The association between hs-CRP concentrations at age 6 months and age 12 years is also in line with previous literature [33,34]. The JUPITER study (n = 8901) demonstrated an association between hs-CRP concentrations measured over a 4-year period among a mixed ethnic population of adults [33]. This finding was supported by the Cardiovascular Risk in Young Finns Study where adulthood CRP was predicted by childhood measurements (n = 1617) during a 21-year follow-up [34]. The clinical implications of elevated hs-CRP have been investigated in relation to cardiovascular disease risk in particular and described as a predictor of coronary heart disease [3]. In addition, increased hs-CRP concentrations have been linked to a broad range of diseases, including inflammatory bowel disease (IBD) [1], depression [4], COPD [5], decreased lung function in childhood [6,7], allergic sensitization at school age [8], early life airway microbiota [9] and childhood asthma [10,11].

It was previously shown in the $COPSAC_{2010}$ cohort that hs-CRP concentrations in the pregnant mother at week 24 of gestation were associated with concentrations at age 6 months [12], which adds to the hypothesis of association across timepoints of hs-CRP concentrations beginning in early life similar to the associations between 25(OH)D concentrations from pregnancy through childhood demonstrated in this paper. Interestingly, we also found that these two measures were negatively correlated at age 6 months in the $COPSAC_{2010}$ cohort. A previous meta-analysis (n = 924) showed the beneficial effect of vitamin D supplementation (400–7143 IU/day) on hs-CRP concentrations across different

populations and diseases, suggesting a protective effect of vitamin D against systemic low-grade inflammation, which is linked to the development of disease [35]. Considering the proposed role of vitamin D in the inflammatory response [36], this effect is biologically plausible, which indicates a protective role of maintaining sufficient circulating 25(OH)D concentrations to protect against low-grade inflammation and possibly protect against associated disorders such as cardiovascular disease.

5. Conclusions

We found significant associations between 25(OH)D concentrations from pregnancy to childhood and through childhood, and associations between hs-CRP concentrations through childhood, although with weak correlations. Further, we found a negative cross-sectional association between hs-CRP and 25(OH)D concentrations in early childhood, suggesting a role of vitamin D in systemic low-grade inflammation, though this association was not present in $COPSAC_{2000}$. These findings could potentially lead to the development of new preventive strategies due to the established role of low-grade inflammation in many chronic disorders, which is reflected by concentrations of hs-CRP. As a result of the known immune modulatory effects of vitamin D and the observation of inverse association between 25(OH)D and hs-CRP in this study, supplementation with vitamin D may revert systemic low-grade inflammation and prevent the development of a broad range of health outcomes.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Danish Ethics Committee (H-B-2008-093) and the Danish Data Protection Agency (2008-41-2599). All parents gave written informed consent before enrollment.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. All parents gave written informed consent before enrollment.

Data Availability Statement: Anonymized data available on request by mail to chawes@copsac.com with publication.

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Conflicts of Interest: The authors declare no conflict of interest.

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