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1 **Cord serum 25-hydroxyvitamin D and risk of early childhood transient**
2 **wheezing and atopic dermatitis**

3

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20

1 **Abstract**

2 **Background:** There is increasing evidence of the effect of maternal vitamin D intake during
3 pregnancy on the risk of asthma and allergic outcomes in the offspring. However, studies on
4 the relationship between cord levels of 25-hydroxyvitamin D (25[OH]D) and asthma and
5 allergic diseases are very few.

6 **Objective:** Our aim was to investigate the associations between cord serum 25(OH)D levels
7 and asthma, wheezing, allergic rhinitis and atopic dermatitis in the offspring from birth to 5
8 years.

9 **Methods:** Cord blood samples were collected at birth and were analyzed for levels of
10 25(OH)D in 239 newborns from the EDEN birth cohort. The children were followed up until
11 age 5 years by symptom ISAAC-based questionnaires (International Study of Asthma and
12 Allergies in Childhood).

13 **Results:** The median cord serum level of 25(OH)D was 17.8 ng/mL (interquartile range: 15.1
14 ng/mL). Using multivariable-adjusted logistic regression models, a significant inverse
15 association was observed between cord serum 25(OH)D and risk of transient early wheezing
16 and early and late-onset atopic dermatitis, as well as atopic dermatitis by the age of 1, 2, 3 and
17 5. We found no association between cord serum 25(OH)D levels and asthma and allergic
18 rhinitis at 5 years of age.

19 **Conclusions:** Cord serum 25(OH)D level was inversely associated with the risk of transient
20 early wheezing and atopic dermatitis by the age of 5 years, but no association was found with
21 asthma and allergic rhinitis.

22

1 **Clinical implications:** The risk of transient wheezing and atopic dermatitis in childhood is
2 inversely associated with cord 25(OH)D levels. Interventions to increase vitamin D levels *in*
3 *utero* and in early life are potential public health interventions to reduce transient wheezing
4 and atopic dermatitis in young children.

5
6 **Capsule summary:** Although research is required to establish the optimal level, timing and
7 route of exposure, recommendations for adequate vitamin D intake in pregnant women may
8 prevent transient early childhood wheezing and atopic dermatitis.

9
10 **Keywords:** Vitamin D; 25-hydroxyvitamin D; Cord blood; Allergic diseases; Wheezing;
11 Atopic dermatitis; Mother-child cohort

12
13 **Abbreviations:** 25(OH)D, 25-hydroxyvitamin D; EDEN, Etude des Déterminants pré et post
14 natals du développement et de la santé de l'Enfant ; ISAAC, International Study of Asthma
15 and Allergies in Childhood ; OR, odds ratio ; CI, confidence interval ; SD, standard deviation.

16

17

1 **Introduction**

2 Over the last decade, the prevalence of allergies and asthma in childhood has increased
3 worldwide, particularly in westernized societies. There is increasing evidence that fetal life
4 and early life exposures, along with associated epigenetic changes (1), are important
5 determinants of the future development of asthma and allergies throughout life (2). Among
6 other environmental factors, prenatal and early life dietary exposures, including vitamin D,
7 have been suggested to play an important role in the development of allergies and asthma in
8 childhood (3).

9 Vitamin D deficiency, defined as a circulating level of < 20 ng/mL of 25-
10 hydroxyvitamin D (25(OH)D), has become a public health issue (4). It has been largely
11 attributed to dietary, lifestyle and behavioral changes during recent decades (4), especially in
12 Western countries. Children as well as pregnant and lactating women are identified as groups
13 vulnerable to vitamin D deficiency (5).

14 While the relationship of vitamin D deficiency and rickets is well established (6), and its
15 association with a wide variety of medical conditions including microbial infections (7),
16 cardiovascular diseases (8), cancers (9), autoimmune diseases (10) and diabetes (11) is well
17 documented, only more recently has the role of vitamin D deficiency and insufficiency in
18 asthma (12-14) and allergic diseases (15) beginning in infancy or childhood (16-18) been
19 debated. In the last couple of decades in the twentieth century and early in the twenty-first
20 century, the vitamin D receptor has been found in a variety of cells, including keratinocytes
21 and numerous cells of the immune system (e.g., dendritic cells, natural killer cells) (19).
22 Several lines of evidence demonstrate the effects of vitamin D on pro-inflammatory
23 cytokines, regulatory T cells, and immune responses (20).

24 Vitamin D is found naturally in very few dietary sources, the only really significant
25 being fatty fish (21) and vitamin D-fortified foods. The latter are scarce in France. The main

1 source of vitamin D in humans is synthesis from 7-dehydrocholesterol in the skin upon
2 exposure to ultraviolet B solar radiation and conversion to a readily-measurable circulating
3 metabolite, 25-hydroxyvitamin D (25(OH)D) in the liver (21). Circulating 25(OH)D is the
4 best index of vitamin D nutritional status (21). Low intake of vitamin D and inadequate
5 exposure to sunlight constitute the main causes of vitamin D deficiency. A number of factors
6 may influence the cutaneous synthesis and dietary sources of vitamin D (22), especially in
7 pregnant women (23). These factors include season of the newborn's birth, maternal vitamin
8 D intake, body mass index, constitutive skin pigmentation, latitude, clothing, sunscreen-use,
9 outdoors activities, age (22,23) and genetic factors (24).

10 During pregnancy the fetus is exposed to vitamin D through cord blood supply and the
11 ability of 25(OH)D to cross the placenta (25). Over the past 2 years, the number of studies,
12 both cross-sectional and prospective, that have investigated the effects of maternal vitamin
13 D intake on the inception and severity of asthma and allergies in the offspring have increased
14 significantly. Most studies have shown that low maternal vitamin D levels (assessed by food
15 frequency questionnaires) increase the risk for asthma (26), wheeze (18,27,28) allergic
16 rhinitis (26) and atopic dermatitis (18), but a few suggest an increased risk with high levels
17 (29-30).

18 However, the majority of these studies did not use objective measures of maternal
19 vitamin D status. In addition, studies examining the associations between cord blood
20 25(OH)D concentrations and asthma and allergic diseases (16) are still very scarce.

21 The purpose of the present study was to investigate whether the levels of cord serum
22 25(OH)D, which is considered the best indicator of newborns' vitamin D status, are
23 associated with risk of asthma, wheeze, allergic rhinitis and atopic dermatitis in offspring. Our
24 study was conducted in the French EDEN mother-child cohort.

25

1

2 **Methods**

3 *Study design and participants*

4 Mother-child pairs were recruited in the EDEN (Etude des Déterminants pré et post
5 natals du développement et de la santé de l'Enfant) prospective Birth Cohort Study
6 (<http://eden.vjf.inserm.fr>). The primary aim of the EDEN Cohort is to identify prenatal and
7 early postnatal nutritional, environmental and social determinants associated with children's
8 health and their normal and pathological development. Pregnant women seen for a prenatal
9 visit at the departments of Obstetrics and Gynecology of the University Hospital of Nancy
10 and Poitiers before their twenty-fourth week of amenorrhea were invited to participate.
11 Enrolment started in February 2003 in Poitiers and September 2003 in Nancy; it lasted 27
12 months in each centre. Women with speaking and writing abilities in French, who did not
13 suffer from type 2 diabetes diagnosed prior to pregnancy, and did not plan to deliver outside
14 the university hospital or move out of the region within 3 years, were included in the study.
15 Multiple pregnancies were excluded. Among eligible women, 55% (2002 women) agreed to
16 participate (1034 women in Nancy and 968 in Poitiers). Of the 2002 mother-child pairs
17 included in the EDEN study, 1140 of them had complete data by the child's age of 5 years.
18 Among the 1140 participants, measurements of cord serum 25(OH)D performed at birth were
19 available in 239 children. The final sample for analysis consisted of 239 mother-child pairs.
20 This sample appeared representative of the overall study population.

21 Women were given an appointment with a study midwife, planned to take place
22 between 24 and 28 gestational weeks, during which an interview on behavioral factors was
23 conducted and biological samples were collected. Further information on the mothers and
24 their newborns, including parity, mode of delivery, newborn's sex and birth weight (measured
25 with electronic Seca scales, Hamburg, Germany [Seca 737 in Nancy and Seca 335 in Poitiers

1 with a 10 g precision]), gestational age and season of birth, were either collected by a
2 questionnaire after birth or extracted from obstetrical and pediatric records. All children were
3 equally receiving vitamin D supplementation during the first three years of life.

4

5 *Ethics Statement*

6 The ethical committees who approved the study is: Comité Consultatif pour la
7 Protection des Personnes dans la Recherche Biomédicale, Le Kremlin-Bicêtre University
8 hospital, and Commission Nationale de l'Informatique et des Libertés. The study was
9 approved on 12 December 2002. Written consent was obtained from the mother for herself at
10 the beginning of the study and from both parents for the newborn child after delivery.

11

12 *Cord serum 25(OH)D measurement*

13 Immediately after delivery, cord blood serum samples were collected by research
14 midwives. In order to prevent any contamination with maternal blood, the cord was doubly
15 clamped immediately after birth (vaginal delivery) or after extraction of the fetus through the
16 uterine incision (elective caesarean section); repeatedly rinsed and venous cord blood serum
17 was sampled between the two clamps. Blood samples were centrifuged within 24 hours of
18 collection. The serum was separated and stored at -80°C . Standard operating procedures for
19 each assay were established. Serum 25(OH)D, which is considered to be the indicator of
20 vitamin D status (31), was measured by immunochemiluminescent immunoassays performed on
21 the LIAISON platform manufactured by DiaSorin (Sallugia, Italy). This assay measures both
22 25(OH)D₂ and 25(OH)D₃ and provides the sum of these two metabolites and does not cross-
23 react with the C3 25(OH)D epimer, which has been shown to complicate the measurement of
24 25(OH)D in neonates. The intra- and interassay coefficient of variation was <10% whatever
25 the measured concentration.

1

2 *Health outcomes*

3 Children were followed up from birth to age 5 years. At the age of 1, 2, 3 and 5 years,
4 parents completed questionnaires including questions on asthma, wheeze, allergic rhinitis and
5 atopic dermatitis, based on the validated International Study of Asthma and Allergies in
6 Childhood (ISAAC) phase-I questionnaire (32,33). For each year, asthma was defined as
7 parental report of doctor-diagnosis of asthma plus either one or more attacks of wheeze or
8 asthma medication in the last 12 months. Wheeze was defined as present if the parents
9 answered “yes” to the question “Has your child had wheezing or whistling in the chest in the
10 preceding 12 months?”. Parental report of wheezing has been shown to be a sensitive and
11 specific outcome when using physician assessment as the criterion standard (34). Allergic
12 rhinitis was defined as sneezing, nasal congestion, or rhinitis, other than with respiratory
13 infections, accompanied by eye itching and tearing during the previous 12 months (35). And
14 atopic dermatitis was defined as atopic dermatitis diagnosed by a doctor in the last 12 months.
15 Given the uncertainty of allergic rhinitis and asthma diagnoses in early childhood (36),
16 analyses were performed on allergic rhinitis and asthma from age 4 to 5 (positive answers to
17 the previous questions in the 5 year-questionnaire). Lifetime prevalence of wheeze and atopic
18 dermatitis at 1, 2, 3 and 5 years were analyzed.

19 Additionally, the data on wheezing from 1 to 5 years of age were used to categorize the
20 children as never wheezers, early transient wheezers, late-onset wheezers, or persistent
21 wheezers according to Martinez et al. (37). Similarly, we distinguished between early- and
22 late-onset atopic dermatitis, e.g. atopic dermatitis up to age 2 years and thereafter.

23

24 *Other Variables*

1 We collected information on potential confounders related to offspring health outcomes,
2 including newborn sex, birth weight, gestational age, season at birth, number of older siblings
3 at birth (0, 1 – 2, ≥ 3), exclusive breastfeeding for ≥ 4 months (yes or no), maternal age at
4 delivery (< 25 years, 25–34 years, or > 34 years), pre-pregnancy maternal body mass index
5 (18.5 – 24.9, 25.0 – 29.9, 30.0 – 34.9 and 35.0 – 39.9 kg/m²), maternal and paternal history of
6 allergies (yes or no), maternal and paternal education level (primary or less, secondary, and
7 university degree or higher), household income (≤ 2300 versus > 2300 Euros per month,
8 median income of the study population), city of residence (Nancy or Poitiers), any smoking
9 during pregnancy (yes or no), environmental tobacco smoke exposure of the child from birth
10 to age of 3 (yes or no), dampness in housing (0 – 3 years) (yes or no), and day care attendance
11 in the first year of life (yes or no). Maternal and paternal allergic history was obtained
12 regarding physician-diagnosed allergic diseases such asthma, rhinitis, eczema and food
13 allergies.

14

15 *Statistical analyses*

16 The sample was described using frequencies of categorical variables and means and
17 standard deviations of continuous variables. The characteristics of the mother-newborn pairs
18 in our study sample (n = 239) were compared to the sample of mother-newborn pairs of
19 children without measurement of cord serum 25(OH)D (n = 901). We used Chi-square tests
20 for categorical data, T-tests for normally-distributed data and Mann-Whitney U tests for
21 skewed data.

22 We fitted logistic regression models to examine the associations between cord serum
23 25(OH)D level as a continuous variable and asthma and allergic rhinitis reported from 4 to 5
24 years and wheeze and atopic dermatitis by the age of 1, 2, 3 and 5 years, as well as the
25 wheezing and atopic dermatitis patterns.

1 In order to identify confounding factors, bivariate analysis between potential
2 confounders and each health outcome were performed. In a first step, all variables associated
3 with one health outcome with a $p < 0.30$ were retained. In a second step, confounders affecting
4 at least 20% of the coefficient estimates of the investigated association between vitamin D
5 and the health outcome were selected and included in the multivariate models. In addition to
6 the inclusion of confounders based on their statistically significant association with health
7 outcomes, we selected other adjustment variables, on the basis of their known relationship to
8 25(OH)D concentrations, independently of any association with exposures and regardless of
9 whether they changed the effect estimates significantly. They included: child's sex (38), pre-
10 pregnancy body mass index (39), and birthweight (40). As a result, the adjustments included:
11 city, maternal age at delivery, maternal pre-pregnancy body mass index (BMI), any smoking
12 during pregnancy, any passive smoke exposure during the first 3 years of life, number of
13 siblings, household income, newborn's sex and weight, season of birth (calendar-based), and
14 exclusive breastfeeding for ≥ 4 months. Multiple logistic regression analysis was used to
15 adjust for confounding factors. Estimations of crude and adjusted odds ratios (ORs) and their
16 95% confidence interval (CI) of asthma, wheeze, allergic rhinitis and atopic dermatitis, at
17 different time point, per 5 ng/mL increase of cord serum 25(OH)D concentration were made
18 by means of logistic regression analysis.

19 To further explore the associations between asthma and allergic rhinitis and cord
20 vitamin D, we examined effect modification of the association of these outcomes with cord
21 serum 25(OH)D, by stratifying the cohort according to maternal history of allergy and testing
22 the interaction term (namely continuous 25(OH)D*maternal history of allergy). All statistical
23 analyses were performed using SAS statistical software version 9.2 (SAS Institute Inc, Cary,
24 North Carolina). P-values < 0.05 were considered statistically significant for all analyses.

25

1 **Results**

2 *Characteristics of the mother-child pairs*

3 Children with both cord serum 25(OH)D data and complete questionnaires from birth to
4 5 years of age were included in this analysis (n = 239). Included children did not differ
5 significantly from other children with complete questionnaires up to 5 years of age but
6 without measurement of cord serum 25(OH)D (n = 901) with respect to all characteristics,
7 except for season of birth and city of residence (Table I).

8 In our study population, no mothers reported type 2 diabetes diagnosed prior to
9 pregnancy or chronic illnesses apart from asthma, eczema and other allergic diseases, such as
10 rhinitis and food allergies.

11

12 *Health outcomes in the offspring*

13 By the age of 1, 2, 3 and 5 years, 24.7%, 32.6%, 35.1% and 38.9% of all children had
14 experienced wheezing, respectively, and 10%, 13.4%, 14.2% and 25% had had atopic
15 dermatitis, respectively. From age 4 to 5, 8% of all children had asthma and 7.5% had allergic
16 rhinitis. The prevalences of early transient, late-onset and persistent wheezing patterns were
17 28.5%, 11.7% and 4.2%, respectively. The prevalences of early-onset and late-onset atopic
18 dermatitis were 34.7% and 20.5%, respectively.

19

20 *Newborns' cord serum levels of 25(OH)D*

21 Figure 1 shows the distribution of cord blood serum 25(OH)D concentrations. The mean
22 concentration and the interquartile range (IQR) in cord blood serum were 17.8 ng/mL and
23 15.1 ng/mL.

24

25 *Relationship of cord serum 25(OH)D and outcomes by the age of 5 years*

1 The associations between cord serum 25(OH)D levels and risk of different wheezing
2 patterns, atopic dermatitis by the age of 1, 3 and 5 years, different atopic dermatitis patterns,
3 and asthma and allergic rhinitis at 5 years of age are presented in table II. Without adjustment,
4 a significant inverse association was observed between cord serum 25(OH)D concentration
5 (per 5 ng/mL increase) and the risk of early transient wheezing and atopic dermatitis by the
6 age of 3 and 5 years and early and late-onset of atopic dermatitis. After multivariable
7 adjustment, the previously observed significant inverse associations persisted (OR for early
8 transient wheezing, per 5 ng/mL increase of cord serum 25(OH)D concentration: 0.67
9 (0.54–0.81); OR for atopic dermatitis by the age of 1, 3 and 5 years : 0.84 (0.71–1.00), 0.82
10 (0.68–0.97), 0.75 (0.63–0.88), respectively; and OR for early and late-onset atopic dermatitis:
11 0.73 (0.62–0.90), 0.75 (0.60–0.94), respectively). The adjusted inverse association along with
12 the confidence intervals and the regression line, between cord serum 25(OH)D concentration
13 and risk of atopic dermatitis by the age of 5 years is shown in Figure 2. The graph represents
14 the predicted probability of atopic dermatitis by the age of 5 years for each observed value of
15 cord serum 25(OH)D concentration in the multivariate model.

16 In contrast, no significant association was observed between cord serum 25(OH)D level
17 and asthma and allergic rhinitis prevalence. To further investigate this null finding, we
18 stratified each of these outcomes according to maternal history of allergy. In all models, cord
19 serum 25(OH)D levels had no association according to either atopic or non-atopic maternal
20 status (all $P > 0.20$).

21

22 **Discussion**

23 *Main findings*

24 In this prospective birth cohort study of French mother-child pairs, we found that cord
25 serum concentration of 25(OH)D was negatively associated with early transient wheezing,

1 atopic dermatitis by the age of 1, 3 and 5 and early and late-onset atopic dermatitis. However,
2 cord serum 25(OH)D levels were not associated with risk of other wheezing phenotypes,
3 asthma and allergic rhinitis at 5 years of age.

4

5 *In literature*

6 To date, there is sparse information on vitamin D status and wheezing and atopic
7 dermatitis. The few studies that have investigated the association between maternal vitamin D
8 status and risk of wheezing (41) and atopic dermatitis (18) in childhood are limited by the fact
9 that vitamin D intake was based only on food frequency questionnaires (18,28). Although
10 such methods have been validated and are representative of intake over a period of time, most
11 of these observational studies did not have direct serological measures of 25(OH)D levels to
12 accurately confirm vitamin D status. However, all studies adjusted for total energy intake and
13 for other nutrients associated with healthy diets.

14 Additionally, a study in 2008 found that vitamin D supplementation significantly
15 improved skin symptoms in children with winter-related atopic dermatitis (42). And in adults,
16 Oren *et al.* (43) also found a protective effect of vitamin D on atopic dermatitis. These results
17 raise interesting questions about the potential role of vitamin D in the pathogenesis of atopic
18 dermatitis.

19

20 Regarding the relationship between early transient wheezing and vitamin D deficit,
21 Martinez *et al.* (37) found evidence that, in most cases, infant wheeze is a transient condition,
22 not associated with increased risk of asthma or allergies later in life. In transient wheezer
23 infants, a reduced airway caliber seems to be the predisposing factor to wheeze in association
24 with viral infections. In our study, the inverse association between cord serum 25(OH)D

1 levels and early transient wheezing is consistent with Camargo et al. findings (16) of an
2 inverse relationship between cord blood 25(OH)D levels and respiratory infections.

3

4 *Potential mechanisms*

5 Cord vitamin D may play a protective role against transient wheezing through its effects
6 on the immune function (44,45). The molecular effects of vitamin D in the skin have been
7 well documented in experimental studies. Vitamin D receptor (VDR) expression in the skin
8 was first confirmed after rats injected with radio-labeled 1,25-dihydroxyvitamin D
9 (1,25(OH)₂D), the active form of 25(OH)D, demonstrated radioactivity concentrated in the
10 nuclei of the epidermis along with a variety of other tissues (46). 1,25(OH)₂D enhances
11 keratinocyte differentiation, as well as have either stimulatory or suppressive effects on
12 keratinocyte growth that is concentration dependent (47).

13 In addition, there is evidence to suggest that vitamin D decreases inflammatory
14 responses in epidermal keratinocytes (48-50). *In vitro* treatment of dendritic cells (DCs) with
15 1,25(OH)₂D or vitamin D analogues led to decreased IL-12 and enhanced IL-10. These
16 cytokine effects, along with inhibitory effects on DC maturation, promote tolerogenic
17 properties and suppressor T cell induction (51). A short treatment course of 1,25(OH)₂D in
18 mice induced tolerogenic DCs and increased regulatory T cells (52). As a result, vitamin D
19 may promote regulatory T cells that are known to play an important role in the prevention of
20 atopic dermatitis development (53) by playing a pivotal role in immune suppression and being
21 crucial to the control of allergic responses.

22 Vitamin D also has a beneficial effect on the permeability barrier in the epidermis. Bikle
23 et al. examined mice null for the expression of 25(OH)D-1 α -hydroxylase. Lower levels of
24 multiple proteins necessary for formation of the stratum corneum, including filaggrin, were
25 observed in the null mice compared to the wild type controls (54). Following skin barrier

1 disruption, null mice had a significantly delayed barrier recovery compared to wild type mice
2 (54).

3 Another potential role for vitamin D is suggested by the work of Schaubert *et al.* (55) ,
4 who found that 1,25(OH)₂D enables keratinocytes to recognize and respond to microbes
5 through action on the Toll-like receptor 2 and leads to upregulation of cathelicidin, an
6 antimicrobial peptide. As a result, vitamin D may promote the production of antimicrobial
7 peptides that have the ability to enhance immunity and protect skin from bacterial infections.

8 Consequently, given the fact that the pathogenesis of atopic dermatitis involves both
9 epidermal barrier and immunologic dysfunction, vitamin D may have a protective role against
10 atopic dermatitis and the development of a variety of other skin disorders as well.

11

12 *Strengths and limitations*

13 First, this is one of the few birth cohort studies to have measured vitamin D levels in
14 cord blood as opposed to estimating infant levels based on vitamin D levels in pregnant
15 women (30) or maternal intake of vitamin D during pregnancy (18,26,28,41). Additionally,
16 newborns's vitamin D status was assessed by measuring serum levels of 25-hydroxyvitamin
17 D, which is considered the best circulating biomarker of vitamin D metabolic status. The
18 analyses were performed on the continuous 25(OH)D variable, since the often used
19 categorization of vitamin D levels is still arbitrary and without consensus. An additional
20 strength of our prospective investigation consists in the robustness of the results as children
21 were drawn from a cohort and their parents were able to provide information on possible
22 confounders, thus diminishing the probability of biased or confounded results. In addition, we
23 collected outcome data at different ages, providing more robust measures of potential health
24 effects than single time-point outcomes could provide. The definition of health outcomes was
25 based on the validated ISAAC questions.

1 Our study also has some limitations. Information on offspring health outcomes was
2 obtained from parental report through self-administered questionnaires, which could result in
3 misclassification. We could not obtain vitamin D data from all eligible newborns with
4 complete information at age 5 years, which may have introduced selection bias. Because of
5 the sample size of our study, although the change-in-estimate (CE) criterion $\geq 10\%$ is a
6 conventional one (56), a more conservative criterion $\geq 20\%$ was used in order to select
7 potential confounders. In addition, the absence of significant associations between asthma,
8 allergic rhinitis and 25(OH)D concentrations may be due to the low prevalences of these
9 outcomes, consequently limiting the power to detect a relationship between 25(OH)D and the
10 outcome. An often-cited limitation of serologic studies is the reliance on a single 25(OH)D
11 measurement per subject. It would have been interesting to measure serum 25(OH)D levels
12 during childhood. The half-life of serum 25(OH)D is approximately 2 to 3 weeks (57), which
13 suggests that the measured concentrations reflect maternal-fetal status during the final months
14 of pregnancy and first months of life. All children in our study were receiving vitamin D
15 supplement during the first three years of life, so difference between children vitamin D levels
16 may depend on diet along with food diversification and on sun-related behaviors, despite
17 heightened awareness of the carcinogenic effect of sun exposure (58). Finally, subjects were
18 followed only to age 5 years, at which age asthma may be evolving from being infection-
19 related to being predominantly related to allergy. Similarly, wheezing in the preschool years
20 is not necessarily indicative of allergic disease as we know that wheeze may be induced by
21 viral infection (59).

22

23 **Conclusion**

24 In summary, our data indicate an inverse association between cord serum 25(OH)D
25 levels and the risk of early transient wheezing and atopic dermatitis in young children. In

1 contrast, we found no apparent association between cord serum 25(OH)D concentrations and
2 other wheezing phenotypes, asthma and allergic rhinitis at 5 years of age.

3 If these associations are causal, interventions to increase vitamin D levels *in utero* and
4 in early life could reduce the risk of transient wheezing and atopic dermatitis in early
5 childhood. Increased exposure to sunlight and increased intake of foods high in vitamin D
6 (not necessarily supplementation) in both pregnant women and infants are potential public
7 health interventions.

8 In light of recent findings on the role of vitamin D, and its effects on asthma and
9 allergic diseases, studies of vitamin D may enhance the understanding, prevention and
10 treatment of these increasingly common conditions in childhood. Further research is required
11 to establish the optimal level (or range) of vitamin D that decreases both the risk for
12 development and severity of these disorders, but also the timing and route of exposure to
13 vitamin D.

1 **References**

- 2 1. Bollati V, Baccarelli A. Environmental epigenetics. *Heredity (Edinb)*. 2010
3 Jul;105(1):105-12.
- 4 2. Gillman MW. Developmental origins of health and disease. *N Engl J Med*. 2005 Oct
5 27;353(17):1848-50.
- 6 3. Robison R, Kumar R. The effect of prenatal and postnatal dietary exposures on
7 childhood development of atopic disease. *Curr Opin Allergy Clin Immunol*. 2010
8 Apr;10(2):139-44.
- 9 4. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007 Jul 19;357(3):266-81.
- 10 5. Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D
11 deficiency in pregnancy and lactation. *Am J Obstet Gynecol*. 2010 May;202(5):429 e1-9.
- 12 6. Thandrayen K, Pettifor JM. Maternal vitamin D status: implications for the
13 development of infantile nutritional rickets. *Endocrinol Metab Clin North Am*. 2010
14 Jun;39(2):303-20, table of contents.
- 15 7. Ginde AA, Mansbach JM, Camargo CA, Jr. Association between serum 25-
16 hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and
17 Nutrition Examination Survey. *Arch Intern Med*. 2009 Feb 23;169(4):384-90.
- 18 8. Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis
19 D in cardiovascular diseases (from the National Health and Nutrition Examination Survey
20 2001 to 2004). *Am J Cardiol*. 2008 Dec 1;102(11):1540-4.
- 21 9. Trump DL, Deeb KK, Johnson CS. Vitamin D: considerations in the continued
22 development as an agent for cancer prevention and therapy. *Cancer J*. 2010 Jan-Feb;16(1):1-9.
- 23 10. Kinder B, Hagaman, JT. Could combating vitamin D deficiency reduce the incidence
24 of autoimmune disease? . *Expert Rev Clin Immunol*. 2011;7(3):255-7.
- 25 11. Ozfirat Z, Chowdhury TA. Vitamin D deficiency and type 2 diabetes. *Postgrad Med J*.
26 2010 Jan;86(1011):18-25; quiz 4.
- 27 12. Lange NE, Litonjua A, Hawrylowicz CM, Weiss S. Vitamin D, the immune system
28 and asthma. *Expert Rev Clin Immunol*. 2009 Nov;5(6):693-702.
- 29 13. Mak G, Hanania NA. Vitamin D and asthma. *Curr Opin Pulm Med*. 2011 Jan;17(1):1-
30 5.
- 31 14. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al.
32 Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma
33 Management Program study. *J Allergy Clin Immunol*. 2010 Jul;126(1):52-8 e5.
- 34 15. Annesi-Maesano I. Perinatal events, vitamin D, and the development of allergy.
35 *Pediatr Res*. 2002 Jul;52(1):3-5.
- 36 16. Camargo CA, Jr., Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, et al.
37 Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and
38 asthma. *Pediatrics*. 2011 Jan;127(1):e180-7.
- 39 17. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and
40 respiratory infections: the vitamin D hypothesis. *Allergy*. 2011 Sep 21.
- 41 18. Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium and vitamin D intake in
42 pregnancy, and wheeze and eczema in infants. *Eur Respir J*. 2010 Jun;35(6):1228-34.
- 43 19. Rudzki E, Samochocki Z, Rebandel P, Saciuk E, Galecki W, Raczka A, et al.
44 Frequency and significance of the major and minor features of Hanifin and Rajka among
45 patients with atopic dermatitis. *Dermatology*. 1994;189(1):41-6.
- 46 20. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf)*.
47 2012 Mar;76(3):315-25.
- 48 21. Brown AJ, Dusso A, Slatopolsky E. Vitamin D. *Am J Physiol*. 1999 Aug;277(2 Pt
49 2):F157-75.

- 1 22. Tsiaras WG, Weinstock MA. Factors influencing vitamin D status. *Acta Derm*
2 *Venereol.* 2011 Mar;91(2):115-24.
- 3 23. Dror DK, King JC, Durand DJ, Allen LH. Association of modifiable and
4 nonmodifiable factors with vitamin D status in pregnant women and neonates in Oakland,
5 CA. *J Am Diet Assoc.* 2011 Jan;111(1):111-6.
- 6 24. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al.
7 Common genetic determinants of vitamin D insufficiency: a genome-wide association study.
8 *Lancet.* 2010 Jul 17 2010;376(9736):180-8.
- 9 25. Salle BL, Delvin EE, Lapillonne A, Bishop NJ, Glorieux FH. Perinatal metabolism of
10 vitamin D. *Am J Clin Nutr.* 2000 May;71(5 Suppl):1317S-24S.
- 11 26. Erkkola M, Kaila M, Nwaru BI, Kronberg-Kippila C, Ahonen S, Nevalainen J, et al.
12 Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic
13 rhinitis in 5-year-old children. *Clin Exp Allergy.* 2009 Jun;39(6):875-82.
- 14 27. Camargo CAJR-S, S.L.; Litonjua, A.A.; Rich-Edwards, J.W.; Weiss, S.T.; Gold, D.R.;
15 Kleinman, K.; Gillman, M.W. Prospective study of maternal intake of vitamin D during
16 pregnancy and risk of wheezing illness in children at age 2 years. *J Allergy Clin Immunol*
17 2006;117:721-2.
- 18 28. Camargo CA, Jr., Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold
19 DR, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in
20 children at 3 y of age. *Am J Clin Nutr.* 2007 Mar;85(3):788-95.
- 21 29. Hypponen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, et al. Infant
22 vitamin d supplementation and allergic conditions in adulthood: northern Finland birth cohort
23 1966. *Ann N Y Acad Sci.* 2004 Dec;1037:84-95.
- 24 30. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, et al. Maternal
25 vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr.* 2008 Jan;62(1):68-
26 77.
- 27 31. Holick MF. Vitamin D status: measurement, interpretation, and clinical application.
28 *Ann Epidemiol.* 2009 Feb;19(2):73-8.
- 29 32. Worldwide variations in the prevalence of asthma symptoms: the International Study
30 of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J.* 1998 Aug;12(2):315-35.
- 31 33. Williams H, Robertson C, Stewart A, Ait-Khaled N, Anabwani G, Anderson R, et al.
32 Worldwide variations in the prevalence of symptoms of atopic eczema in the International
33 Study of Asthma and Allergies in Childhood. *J Allergy Clin Immunol.* 1999 Jan;103(1 Pt
34 1):125-38.
- 35 34. Chong Neto HJ, Rosario N, Dela Bianca AC, Sole D, Mallol J. Validation of a
36 questionnaire for epidemiologic studies of wheezing in infants. *Pediatr Allergy Immunol.*
37 2007 Feb;18(1):86-7.
- 38 35. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International
39 Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J.*
40 1995 Mar;8(3):483-91.
- 41 36. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of
42 Asthma-Summary Report 2007. *J Allergy Clin Immunol.* 2007 Nov;120(5 Suppl):S94-138.
- 43 37. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma
44 and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J*
45 *Med.* 1995 Jan 19;332(3):133-8.
- 46 38. Almqvist C, Worm M, Leynaert B. Impact of gender on asthma in childhood and
47 adolescence: a GA2LEN review. *Allergy.* 2008 Jan;63(1):47-57.
- 48 39. Patel SP, Rodriguez A, Little MP, Elliott P, Pekkanen J, Hartikainen AL, et al.
49 Associations between pre-pregnancy obesity and asthma symptoms in adolescents. *J*
50 *Epidemiol Community Health.* 2011 Aug 15.

- 1 40. Agosti M, Vegni C, Gangi S, Benedetti V, Marini A. Allergic manifestations in very
2 low-birthweight infants: a 6-year follow-up. *Acta Paediatr Suppl.* 2003 Sep;91(441):44-7.
- 3 41. Devereux G, Litonjua AA, Turner SW, Craig LC, McNeill G, Martindale S, et al.
4 Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr.*
5 2007 Mar;85(3):853-9.
- 6 42. Sidbury R, Sullivan AF, Thadhani RI, Camargo CA, Jr. Randomized controlled trial of
7 vitamin D supplementation for winter-related atopic dermatitis in Boston: a pilot study. *Br J*
8 *Dermatol.* 2008 Jul;159(1):245-7.
- 9 43. Oren E, Banerji A, Camargo CA, Jr. Vitamin D and atopic disorders in an obese
10 population screened for vitamin D deficiency. *J Allergy Clin Immunol.* 2008 Feb;121(2):533-
11 4.
- 12 44. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor
13 triggering of a vitamin D-mediated human antimicrobial response. *Science.* 2006 Mar
14 24;311(5768):1770-3.
- 15 45. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the
16 regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab.* 2008
17 Feb;4(2):80-90.
- 18 46. Stumpf WE, Sar M, Reid FA, Tanaka Y, DeLuca HF. Target cells for 1,25-
19 dihydroxyvitamin D₃ in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid.
20 *Science.* 1979 Dec 7;206(4423):1188-90.
- 21 47. Gurlek A, Pittelkow MR, Kumar R. Modulation of growth factor/cytokine synthesis
22 and signaling by 1alpha,25-dihydroxyvitamin D(3): implications in cell growth and
23 differentiation. *Endocr Rev.* 2002 Dec;23(6):763-86.
- 24 48. Zhang JZ, Maruyama K, Ono I, Iwatsuki K, Kaneko F. Regulatory effects of 1,25-
25 dihydroxyvitamin D₃ and a novel vitamin D₃ analogue MC903 on secretion of interleukin-1
26 alpha (IL-1 alpha) and IL-8 by normal human keratinocytes and a human squamous cell
27 carcinoma cell line (HSC-1). *J Dermatol Sci.* 1994 Feb;7(1):24-31.
- 28 49. Komine M, Watabe Y, Shimaoka S, Sato F, Kake K, Nishina H, et al. The action of a
29 novel vitamin D₃ analogue, OCT, on immunomodulatory function of keratinocytes and
30 lymphocytes. *Arch Dermatol Res.* 1999 Sep;291(9):500-6.
- 31 50. Fukuoka M, Ogino Y, Sato H, Ohta T, Komoriya K, Nishioka K, et al. RANTES
32 expression in psoriatic skin, and regulation of RANTES and IL-8 production in cultured
33 epidermal keratinocytes by active vitamin D₃ (tacalcitol). *Br J Dermatol.* 1998 Jan;138(1):63-
34 70.
- 35 51. Adorini L, Penna G, Giarratana N, Roncari A, Amuchastegui S, Daniel KC, et al.
36 Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. *J Steroid*
37 *Biochem Mol Biol.* 2004 May;89-90(1-5):437-41.
- 38 52. Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L.
39 Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D₃ and mycophenolate mofetil
40 treatment mediate transplantation tolerance. *J Immunol.* 2001 Aug 15;167(4):1945-53.
- 41 53. Agrawal R, Wisniewski JA, Woodfolk JA. The role of regulatory T cells in atopic
42 dermatitis. *Curr Probl Dermatol.* 2011;41:112-24.
- 43 54. Bikle DD, Pillai S, Gee E, Hincenbergs M. Regulation of 1,25-dihydroxyvitamin D
44 production in human keratinocytes by interferon-gamma. *Endocrinology.* 1989
45 Feb;124(2):655-60.
- 46 55. Schaubert J, Gallo RL. Antimicrobial peptides and the skin immune defense system. *J*
47 *Allergy Clin Immunol.* 2008 Aug;122(2):261-6.
- 48 56. Tong IS, Lu Y. Identification of confounders in the assessment of the relationship
49 between lead exposure and child development. *Ann Epidemiol.* 2001 Jan;11(1):38-45.

- 1 57. Haddad JG, Jr., Rojanasathit S. Acute administration of 25-hydroxycholecalciferol in
2 man. *J Clin Endocrinol Metab.* 1976 Feb;42(2):284-90.
- 3 58. Wright CY, Reeder AI, Bodeker GE, Gray A, Cox B. Solar UVR exposure, concurrent
4 activities and sun-protective practices among primary schoolchildren. *Photochem Photobiol.*
5 2007 May-Jun;83(3):749-58.
- 6 59. Jartti T, Kuusipalo H, Vuorinen T, Soderlund-Venermo M, Allander T, Waris M, et al.
7 Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in
8 children. *Pediatr Allergy Immunol.* 2010 Nov;21(7):1008-14.

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1 **TABLE I.** Comparison of basic characteristics of mother-newborn pairs with (n = 239) and without
 2 (n = 901) measurement of cord serum 25(OH)D

	Study sample with measurement (n = 239)	Sample without measurement (n = 901)	P-value
City, %			
Poitiers	47.7	57.3	
Nancy	52.3	42.7	0.008
Maternal age, Mean ± SD* (years)	30.8 ± 4.6	30.5 ± 4.7	0.6
< 25, %	9.2	11.6	
25-34, %	69.5	71.1	
> 34, %	21.3	17.4	0.3
BMI, Mean ± SD (kg/m²)	26.4 ± 4.4	26.3 ± 4.5	0.6
Normal, %	46.8	45.9	
Overweight, %	36.7	37.1	
Moderate obesity, %	11.4	11.6	
Severe obesity, %	5.1	5.4	0.9
Maternal allergies, %	31.4	31.0	0.9
Paternal allergies, %	23.0	22.3	0.8
Maternal educational level, %			
Primary school or less	3.3	4.9	
Secondary school	56.2	59.3	
University degree or higher	40.5	35.9	0.1
Paternal educational level, %			
Primary school or less	8.2	7.2	
Secondary school	64.3	65.0	
University degree or higher	27.5	27.8	0.7
Smoking during pregnancy (active, passive), %	31.9	31.8	0.5
Environmental tobacco smoke exposure of the child (0–3 years), %	44.8	48.5	0.3
Dampness in housing (0–3 years), %	5.9	7.8	0.3
Attending daycare (0–1 years), %	15.9	12.0	0.1
Household income (Euros), %			
≤ 2300	36.0	43.0	
> 2300	64.0	57.0	0.06
Older siblings, %			
0	45.6	49.0	
1-2	48.1	47.5	
≥3	6.3	3.6	0.1
Birth weight, Mean (kg) ± SD	3.4 ± 0.5	3.3 ± 0.5	0.06
Gestational age at birth, Mean (weeks) ± SD	39.5 ± 1.5	39.2 ± 1.8	0.3
Sex of the newborn, %			
Female	41.8	48.0	
Male	58.2	52.1	0.1
Season of birth, %			
Summer	23.4	26.9	
Autumn	20.5	23.1	
Winter	16.7	23.8	
Spring	39.3	23.3	0.0008
Exclusively breastfed for ≥ 4 months, %	16.3	14.8	0.6

*SD = standard deviation

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1 **TABLE II.** Association between cord blood 25(OH)D levels (per 5 ng/mL increase) and risk
 2 of wheeze and atopic dermatitis outcomes, and asthma and allergic rhinitis(n = 239)
 3

Outcome	OR (95% CI) *	
	Unadjusted	Adjusted †
Wheeze		
<i>Early transient</i>	0.77 (0.69–0.95), $P_{\ddagger} = 0.01$	0.67 (0.54–0.81), $P = 0.0002$
<i>Late-onset</i>	0.94 (0.77–1.16), $P = 0.58$	0.86 (0.67–1.11), $P = 0.24$
<i>Persistent</i>	1.10 (0.70–0.95), $P = 0.50$	1.15 (0.79–1.691), $P = 0.46$
Atopic dermatitis		
<i>By the age of 1</i>	0.88 (0.76–1.00), $P = 0.08$	0.84 (0.71–1.00), $P = 0.05$
<i>By the age of 2</i>	0.86 (0.77–1.00), $P = 0.05$	0.82 (0.70–0.95), $P = 0.02$
<i>By the age of 3</i>	0.87 (0.76–1.00), $P = 0.05$	0.82 (0.68–0.97), $P = 0.02$
<i>By the age of 5</i>	0.82 (0.71–0.91), $P = 0.003$	0.75 (0.63–0.88), $P = 0.0005$
<i>Early-onset</i>	0.82 (0.70–0.95), $P = 0.01$	0.73 (0.62–0.90), $P = 0.002$
<i>Late-onset</i>	0.82 (0.68–0.98), $P = 0.03$	0.75 (0.60–0.94), $P = 0.01$
Asthma at 5	1.09 (0.87–1.38), $P = 0.42$	1.07 (0.78–1.45), $P = 0.69$
Allergic rhinitis at 5	1.06 (0.84–1.34), $P = 0.62$	0.99 (0.72–1.38), $P = 0.98$

4 *OR (95 % CI): odd ratio and 95% confidence interval. † Adjusted for city, mother's age,
 5 maternal history of allergy, pre-pregnancy body mass index, any smoking during
 6 pregnancy, any passive smoke exposure during the first 3 years of life, number of
 7 siblings, household income, newborn's sex and weight, season of birth, and exclusive
 8 breastfeeding for ≥ 4 months. $\ddagger P = P$ -value.
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1 **Figure legends**

FIG 1. Distribution of vitamin D levels in cord blood (ng/mL). Vitamin D levels are indicated on the x-axis and percentages of newborns are indicated on the y-axis. IQR = interquartile range. To convert ng/mL 25-hydroxyvitamin D to nmol/L, multiply ng/mL by 2.496.

2 **FIG 2.** Adjusted associations between cord serum 25(OH)D levels and predicted probabilities
3 of atopic dermatitis by age of 5 years.