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Vitamin D levels at birth and risk of type 1 diabetes in childhood: a case-control study

Short running title: Vitamin D and risk of Type 1 diabetes

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ABSTRACT

Aims: to assess whereas vitamin D levels at birth were associated with risk of having type 1 diabetes up to 10 years of age and the potential modifier effect of ethnic group.

Methods: The Piedmont Diabetes Registry and the Newborn Screening Regional data were linked to identify cases (n=67 incident children aged ≤10 years at diabetes onset, 2002-2012) and up to 5 controls (n=236) matched for birthday and ethnic group. Cards with neonatal blood spot were used and 25-hydroxyvitamin D(3) assessed with tandem mass spectroscopy.

Results: In conditional logistic regression, OR for unit increment of log-vitamin D was 0.78 (95% CI 0.56-1.10). Vitamin D was significantly lower in migrant than in Italian control newborn babies ($p<0.0001$) and interaction between vitamin D and migrant status statistically significant ($p=0.04$). Compared to migrant newborns babies with vitamin D ≥ 2.14 ng/ml, migrants with lower levels had an OR of 14.02 (1.76-111.70), whereas no association was evident in Italians.

Conclusions: our case-control study within the Piedmont Diabetes Registry showed no association between vitamin D levels at birth and risk of having type 1 diabetes up to 10 years of age, apart from the subgroup of migrant babies, which might have clinical implications if confirmed.

Environmental changes are involved in the worldwide increasing temporal trend of type 1 diabetes, but the identification of factors acting either as determinants or risk factors of the disease still remains elusive (1). Among these factors, vitamin D has gained particular interest (2-3) as it might have an immunomodulator role in the pathogenesis of autoimmune diseases and an association between its deficiency and type 1 diabetes has been also suggested (4-7). Most of case-control studies have pointed out that lower intake of vitamin D during early life is associated with higher risk of type 1 diabetes, and a metanalysis including studies published up to 2011 (7) has estimated a pooled odds ratio (OR) of 0.71 (95% CI 0.51-0.98) for vitamin D supplementation with respect to non-supplementation. Results, however, might have been hampered by misclassification of vitamin D intake, generally assessed through questionnaires (8).

Heterogeneities among studies might be due to differences among populations in levels of solar ultraviolet B radiation at different latitudes, dietary supplementation, genetic factors and age of examined people. Both the fetal period and the early infancy might be vulnerable phases for the immunomodulator role of vitamin D, but studies examining this issue are very limited and results not consistent (4-6). Finally, ethnic differences might act as a modifier of the association between vitamin D and risk of type 1 diabetes and differences in circulating vitamin D binding protein have been pointed out (9-10). We performed a case-control study within the Piedmont Childhood Diabetes Registry, Italy (11) to test whereas variations in vitamin D concentrations at birth were associated with risk of having type 1 diabetes up to age 10 years and the potential modifier effect of ethnic group on this association.

Subjects and Methods

The Piedmont Childhood Diabetes Registry is monitoring temporal trend of type 1 diabetes in childhood since 1990, using as primary data source the list of all new cases who had been identified by the diabetes pediatric clinics, and as secondary data source the regional administrative list of children who obtained exemption from payment of drugs due to diabetes (11). The diagnosis of type 1 diabetes is performed according to international diagnostic criteria, including markers of

β-cells autoimmunity, and the estimated completeness of ascertainment is 98%. The Piedmont Region has also set up a newborn screening program to identify at birth genetic and metabolic disorders, and a random sample of cards with neonatal blood spot has been kept for research purposes by the Newborn Screening Regional Center, allowing us to perform a case-control study through a linkage of data with the Piedmont Childhood Diabetes Registry. The Regional Ethical Committee has approved the study. Cases were children aged 0-10 years at diabetes onset in period 2000-2012 among residents in Piedmont Region who were included in the Registry. Up to 5 non diabetic control children were matched to each case for birthday (± 30 days), place of birth and ethnic group, defined by the country of birth of parents (Northern Africa, Eastern Europe and Asia). Current data from the Piedmont Childhood Diabetes Registry were used to exclude that control children had diabetes onset up to 2013.

In all newborns dried blood samples were collected at the third day of life, according to the neonatal screening procedures of the Piedmont Region and analysed according to standardized technique, using tandem mass spectroscopy (LC-MS/MS) (12-14). The sample was a cardboard disk soaked in blood, five disks of 3 mm were cut out from a punching machine, corresponding to a content of 50 microliters of blood. The content was converted into corresponding values with a previously tested formula (12): serum [25(OH)D3] ng/ml = dried blood spot [25(OH)D3]ng/ml / (1-0,61). The levels of 25(OH)D3 and 25(OH) D2 were separately determined, with standards based on known amounts. As 25(OH)D2 levels were not detectable, 25(OH)D3 values were described as vitamin D throughout the text.

Vitamin D levels were non normally distributed and they were analyzed after logarithmic transformation (base e). Data are shown as geometric means and interquartile range (IQR). Conditional logistic regression was used to determine the association between vitamin D levels and diabetes. Data were also stratified by median vitamin D levels in control subjects. All statistical analyses were performed using STATA 10.0.

Results

The study base included 67 incident cases of type 1 diabetes aged 10 years and lower at diabetes onset in period 2000-2012 and 236 non diabetic matched control children. Among them, 23 cases were born to migrant families (20 from Northern Africa and 3 from Eastern Europe) and were matched to 57 children of the same ethnic group.

Age was similar in cases and control children (7.0 ± 0.25 vs 7.2 ± 0.49 years, p=0.61) and 48% of them were males. Age at diagnosis in diabetic children was 4.2 ± 3.4 years (IQR 1.5-6.9).

As shown in table 1, geometric means of vitamin D at birth were similar in cases and control newborn babies. Vitamin D levels were significantly lower in migrant than in Italian control newborn babies ($p<0.0001$). In conditional logistic regression, OR of having diabetes up to 10 years of age was 0.78 (95% CI 0.56-1.10) for each unit increment in log vitamin D. As interaction between vitamin D and migrant status was statistically significant (p=0.04), Italian and migrants were separately analyzed. Compared to newborm migrant babies with vitamin D $\geq$ 2.14 ng/ml (median value), migrants with lower levels had an OR of 14.02 (1.76-111.70). Current age did not add significantly to the model nor modified estimated ORs.

Discussion

In this study we found no association between vitamin D in newborn babies and risk of having diabetes up to 10 years of age. Our study was performed within the Piedmont Diabetes Registry and had the unique opportunity to analyze cards with neonatal blood spot which were randomly collected for research purposes over a decade by the Newborn Regional Screening Program on genetic and metabolic disorders. Although we could link a quite limited number of cards referring to diabetic people of our Registry, we could both increase the study power selecting up to 5 control children who were matched to cases on birthday, and reduce the confounding effect of ethnicity by matching cases and control children on ethnic group. The latter finding is relevant, as both environmental and genetic factors influence vitamin D levels and differences among

populations might be due to levels of solar ultraviolet B at different latitudes, dietary and supplemental vitamin D intakes, skin pigmentation as well as genetic factors (9-10). Polymorphism in vitamin D pathway related genes are related to increased likelihood of having vitamin D deficiency in infancy (9) and higher predisposition to autoimmune type 1 diabetes (15). Moreover, black individuals have lower vitamin D binding protein levels than white individuals, whereas the bioavailable 25hydroxyvitamin D levels are similar (10). Among our control newborn babies, migrants had almost two-fold lower vitamin D levels than Italian babies and this difference was highly statistically significant. In conditional logistic regression a significant interaction between migrant status and vitamin D levels was found and stratified analysis was then conducted. In migrants, vitamin D levels below the median value were associated with a statistically significant higher risk of having diabetes up to ten years of age. Our subgroup of babies born to migrant families included mainly babies born to families from Northern Africa, and both genetic and environmental factors are likely to be involved in our results. An increasing number of children with type 1 diabetes among migrants has recently been observed in Italy (16), and the hypothesis that the high prevalence of vitamin D deficiency in migrant children might be involved in this temporal trend deserves consideration in future studies.

Our findings add to current knowledge on this issue, as previous studies assessing the role of vitamin D in the perinatal phase as risk factor for childhood diabetes have been performed in homogeneous Northern European populations (4-6), whereas no data are available in Mediterranean populations. A study performed in Norway showed that low concentration of vitamin D in mothers during pregnancy was associated with an increased risk of type 1 diabetes in the offspring (5), whereas a Finnish study (6) with a similar nested case-control study design did not find any association. However, about 70% of the Finnish mothers had vitamin D insufficiency during first trimester of pregnancy, which may have lead to difficulties to discern any differences between the groups. Another study showed that, compared to period 1998-2003, vitamin D levels increased in Finland after 2003, and this finding might be related to the leveling off of type 1

diabetes incidence that occurred after 2006 (17). In general, people with type 1 diabetes have high prevalence of vitamin D deficiency, even in a subtropical area with abundant sunlight like Australia (18), but its role in disease progression is unclear (19-21). Type 1 diabetes has many epidemiological similarity with multiple sclerosis (22). Recently, among patients with multiple sclerosis mainly treated with interferon, low vitamin D levels early in the disease course were a strong risk factor for disease progression (23). In contrast, the DAISY study (21) showed that although vitamin D levels were lower in children with multiple islet autoantibodies and in children with type 1 diabetes than in autoantibody-negative children, vitamin D deficiency was not associated with faster progression to type 1 diabetes. These findings were more recently confirmed in European children (19). As regards adults, two prospective studies performed in the United States in cohorts of military personnel have consistently shown that lower vitamin D levels were associated with higher risk of having insulin-requiring diabetes (24-25). Vitamin D might merely act as a marker of autoimmunity rather than being directly involved in the pathogenesis of autoimmune diseases. The finding of an association between inherited variations in vitamin D genes and predisposition to type 1 diabetes would support this hypothesis (15). Recent experimental studies would support a protective role of vitamin D. Indeed, lifelong high doses of vitamin D prevented diabetes in NOD mice (26) and was able to promote the generation of tolerogenic mature dentritic cells with an impaired ability to activate autoreactive T cells (27).

Limitation of present study is the low number of cases and control children, so that statistical significant result in our migrant subgroup might be merely due to chance. However, our result should be considered as a working hypothesis, which need to be confirmed by larger studies. Unfortunately, at present the availability of both biobank collected at birth and population-based diabetes registries of type 1 diabetes are limited, and properly design prospective studies are required. Finally, in our study levels of vitamin D at birth were very low in both cases and controls. Dried blood samples were obtained at birth and kept on cards until our assessment, and this procedure has probably provided biased downward estimates of vitamin D levels. However, our

case-control analysis was focused on the potential role of low vitamin D levels at birth on type 1 diabetes risk up to 10 years of age rather than on the frequency of its deficiency. As similar procedures were used in both cases and control subjects, selection bias and detection bias should have not affected our data.

In conclusion, our case-control study within the Piedmont Diabetes Registry showed no association between vitamin D levels at birth and risk of having type 1 diabetes up to 10 years of age. A significant association was found in migrant babies, which might have clinical implications if confirmed.

Guarantor: Francesco Cadario

Conflict of interest: The authors declare that they have no conflict of interest.
Author contributions: FC designed the study, researched data and wrote the manuscript, SS researched data, VP researched data, MP researched data, MV researched data, FC researched data, IR researched data, FF researched data, RL researched data, VdeD researched data, AV researched data, GG researched data, GB contributed to discussion, reviewed/edited manuscript, data, GB designed the study and wrote the manuscript.
Statement of Human and Animal Rights: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).
Statement of Informed Consent: Informed consent of patients included in the study was obtained by the Piedmont Diabetes Registry and the Newborn Screening Regional Center. The study was reviewed and approved by the Ethics Committee of the S.Giovanni Battista Hospital, Turin.
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References

1. Forlenza GP, Rewers M. The epidemic of type 1 diabetes: what is it telling us? Curr Opin Endocrinol Diabetes Obes 2011;18:248-51
2. Mohr SB, Garland FC, Garland CF, Gorham ED, Ricordi C. Is there a role of vitamin deficiency in type 1 diabetes of children? Am J Prev Med 2010;39:189–190
3. Hyppönen E. Vitamin D and increasing incidence of type 1 diabetes-evidence for an association? Diabetes Obes Metab 2010;12:737-743
4. Stene LC, Joner G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case–control study. Am J Clin Nutr 2003;78:1128-1134
5. Sørensen IM, Joner G, Jenum PA, Eskild A, Torjesen PA, Stene LC. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. Diabetes 2012;61:175-178
6. Miettinen ME1, Reinert L, Kinnunen L, Harjutsalo V, Koskela P, Surcel HM, Lamberg-Allardt C, Tuomilehto J. Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. Diabetologia 2012;55:1291-4
7. Dong JY, Zhang WG, Chen JJ, Zhang ZL, Han SF, Qin LQ. Vitamin D intake and risk of type 1 diabetes: a meta-analysis of observational studies. Nutrients 2013;5:3551-62
8. Lehtonen E, Ormisson A, Nucci A, Cuthbertson D, Sorkio S, Hyytinen M, Alahuhta K, Berseth C, Salonen M, Taback S, Franciscus M, González-Frutos T, Korhonen TE, Lawson ML, Becker DJ, Krischer JP, Knip M, Virtanen SM; TRIGR Investigators. Use of vitamin D supplements during infancy in an international feeding trial. Public Health Nutr. 2014;17:810-22
9. Carter GD, Phinney KW. Assessing vitamin D status: time for a rethink? Clin Chem. 2014;60:809-11

10. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369:1991-2000
11. Bruno G, Novelli G, Panero F, Perotto M, Monasterolo F, Bona G, Perino A, Rabbone I, Cavallo-Perin P, Cerutti F; Piedmont Study Group for Diabetes Epidemiology. The incidence of type 1 diabetes is increasing in both children and young adults in Northern Italy: 1984-2004 temporal trends. Diabetologia 2009;52:2531-5
12. Eyles DW, Morley R, Anderson C, Ko P, Burne T, Permezel M, Mortensen PB, Nørgaard-Pedersen B, Hougaard DM, McGrath JJ. The utility of neonatal dried blood spots for the assessment of neonatal vitamin D status. Paediatr Perinat Epidemiol 2010;24:303-308
13. Eyles D, Anderson C, Ko P, Jones A, Thomas A, Burne T, Mortensen PB, Nørgaard-Pedersen B, Hougaard DM, McGrath J. A sensitive LC/MS/MS assay of 25OH vitamin D3 and 25OH vitamin D2 in dried blood spots. Clin Chim Acta 2009;403:145-151
14. Heath AK, Williamson EJ, Ebeling PR, Kvaskoff D, Eyles DW, English DR. Measurements of 25-hydroxyvitamin d concentrations in archived dried blood spots are reliable and accurately reflect those in plasma. J Clin Endocrinol Metab. 2014;99:3319-24
15. Cooper JD, Smyth DJ, Walker NM, Stevens H, Burren OS, Wallace C, Greissl C, Ramos-Lopez E, Hyppönen E, Dunger DB, Spector TD, Ouwehand WH, Wang TJ, Badenhoop K, Todd JA. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. Diabetes 2011;60:1624-1631
16. Cadario F, Cerutti F, Savastio S, Rabbone I, Tumini S, Bruno G; Italian Society of Pediatric Endocrinology and Diabetology Study Group (SIEDP). Increasing burden, younger age at onset and worst metabolic control in migrant than in Italian children with type 1 diabetes: an emerging problem in pediatric clinics. Acta Diabetol. 2014;51:263-7

17. Mäkinen M, Simell V, Mykkänen J, Ilonen J, Veijola R, Hyöty H, Knip M, Simell O, Toppari J, Hermann R. An increase in serum 25-hydroxyvitamin D concentrations preceded a plateau in type 1 diabetes incidence in Finnish children. J Clin Endocrinol Metab 2014;99:E2353-6
18. Greer RM, Portelli SL, Hung BSM, Cleghorn GJ, McMahon SK, Batch JA, Conwell LS. Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes 2013;14:31-41
19. Raab J, Giannopoulou EZ, Schneider S, Warncke K, Krasman M, Winkler C, Ziegler AG. Prevalence of vitamin D deficiency in pre-type 1 diabetes and its association with disease progression. Diabetologia 2014;57:902-908
20. Littorin B, Blom P, Schölin A, Arnqvist HJ, Blohmé G, Bolinder J, Ekbom-Schnell A, Eriksson JW, Gudbjörnsdottir S, Nyström L,Ostman J, Sundkvist G. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). Diabetologia 2006;49:2847-2852
21. Simpson M, Brady H, Yin X, Seifert J, Barriga K, Hoffman M, Bugawan T, Barón AE, Sokol RJ, Eisenbarth G, Erlich H. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia 2011;54:2779-88
22. Multiple Sclerosis International Federation. Atlas of MS 2013. http://www.msif.org/includes/documents/cm_docs/2013/m/msif-atlas-of-ms-2013-report.pdf?f=1. Last accessed October 13, 2014
23. Ascherio A, Munger KL, White R, Köchert K, Simon KC, Polman CH, Freedman MS, Hartung HP, Miller DH, Montalbán X, Edan G, Barkhof F, Pleimes D, Radü EW, Sandbrink R, Kappos L, Pohl C. Vitamin D as an early predictor of multiple sclerosis activity and progression. JAMA Neurol. 2014;71:306-14

24. Gorham ED1, Barrett-Connor E, Highfill-McRoy RM, Mohr SB, Garland CF, Garland FC, Ricordi C. Incidence of insulin-requiring diabetes in the US military. Diabetologia 2009;52:2087-91

25. Bizzarri C1, Pitocco D, Napoli N, Di Stasio E, Maggi D, Manfrini S, Suraci C, Cavallo MG, Cappa M, Ghirlanda G, Pozzilli P; IMDIAB Group.No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. Diabetes Care 2010;33:1962-3

26. Takiishi T, Ding L, Baeke F, Spagnuolo I, Sebastiani G, Laureys J, Verstuyf A, Carmeliet G, Dotta F, Van Belle TL, Gysemans CA, Mathieu C. Dietary supplementation with high doses of regular vitamin D3 safely reduces diabetes incidence in NOD mice when given early and long term. Diabetes 2014;63:2026-36

27. Ferreira GB, Gysemans CA, Demengeot J, da Cunha JP, Vanherwegen AS, Overbergh L, Van Belle TL, Pauwels F, Verstuyf A, Korf H, Mathieu C. 1,25-Dihydroxyvitamin D3 promotes tolerogenic dendritic cells with functional migratory properties in NOD mice. J Immunol. 2014 192:4210-20

Table 1: geometric means, interquartile range and odds ratio (OR) of having type 1 diabetes up to 10 years of age, by vitamin D levels at birth

	Cases	**Control children**
All subjects (n=303)	n=67	n=236
Geometric mean (IQR)	1.42 (0.80-3.00)	1.81 (0.96-3.40)
Log Vitamin D OR (95% CI)	0.78 (0.56-1.10)	
Italians (n=223)	n=44	n=179
Geometric mean (IQR)	2.07 (1.40-3.54)	2.09 (1.30-3.68)
Log Vitamin D OR (95% CI)	0.90 (0.57-1.41)	
Migrants (n=80)	n=23	n=57
Geometric mean (IQR)	0.70 (0.30-1.70)	1.16 (0.60-3.25)
Log Vitamin D OR (95% CI)	0.65 (0.37-1.12)	

Table 2: Results of conditional logistic regression on the association between vitamin D levels at birth and risk of having type 1 diabetes by 10 years of age, by median vitamin D level.

Vit D (ng/ml)	**All subjects (n=303)**			**Italians (n=223)**			**Migrants (n=80)**		
	Cases (n)	**Controls (n)**	**OR (95% CI)**	**Cases (n)**	**Controls (n)**	**OR (95% CI)**	**Cases (n)**	**Controls (n)**	**OR (95% CI)**
<2.14	36	103	1.76 (0.92-3.38)	16	72	1.03 (0.47-2.26)	20	31	**14.02 (1.76-111.7)**
≥2.14	31	133	1.00	28	107	1.00	3	26	1.00