


RESEARCH NOTE

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A method to correct for the influence of bovine serum albumin-associated vitamin D metabolites in protein extracts from neonatal dried blood spots

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Abstract

Objective: We developed an assay to measure the concentration of 25 hydroxyvitamin D₂ and D₃ in protein extracts derived from stored neonatal dried blood spots. During this study, we postulated that these samples had been contaminated with exogenous vitamin D metabolites because of the addition of bovine serum albumin (BSA) as part of an extraction step undertaken 7 years earlier. The aim of the current study was to develop methods in order to adjust for this contamination.

Results: We identified between-plate variations in 25 hydroxyvitamin D₂ and D₃ concentrations which suggested the presence of three different BSA batches. Based on repeat extraction (without the addition of BSA) and testing of 395 samples, we developed models to correct for the exogenous 25 hydroxyvitamin D₂ and D₃. The regression models were $\text{Diff}_{25\text{OHD}_3} = -8.2 + 1.8 * \text{Diff}_{25\text{OHD}_2}$ for low contamination, $\text{Diff}_{25\text{OHD}_3} = 23.8 + 1.7 * \text{Diff}_{25\text{OHD}_2}$ for middle contamination, and $\text{Diff}_{25\text{OHD}_3} = 14.3 + 3.0 * \text{Diff}_{25\text{OHD}_2}$ for high contamination. After these corrections, the three subsamples had comparable distributions within the expected range for both 25 hydroxyvitamin D₂ and D₃.

Keywords: 25 hydroxyvitamin D, Bovine serum albumin, Assay methodology, LC–MS/MS

Introduction

Our group has published several epidemiological studies that have involved the measurement of vitamin D status based on stored neonatal dried blood spots (DBS) [1–3]. In contrast to standard clinical tests for vitamin D status (which are based on several millilitres of serum from venous blood), neonatal DBS provide a very small volume of whole blood (one 3.2 mm DBS punch is equivalent to 1.64 µL whole blood). Because these samples are

collected for routine neonatal screening, there is often very little material available for subsequent research studies. Fortunately, with advances in tandem mass spectroscopy and refinements in assays methods, it is now possible to reliably and accurately assess vitamin D status based on small samples from DBS [4–6].

We recently commenced a large project that involved the assessment of neonatal vitamin D status in approximately 76,000 Danish neonates. The Lundbeck Foundation Initiative for Integrated Psychiatric Research (iPSYCH) study is a case-cohort sample, with cases selected on the presence of selected mental disorders, and a randomly selected population-based cohort [7]. Seven years prior to our study, the neonatal DBS from the iPSYCH samples were retrieved and processed in

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order to extract DNA suitable for genotyping [8–10]. The protein extracts kept after the DNA study were available for future approved research. Our group developed a sensitive and robust assay to measure the concentration of 25 hydroxyvitamin D₃ (25OHD₃) and 25 hydroxyvitamin D₂ (25OHD₂) in the left over protein extracts from the iPSYCH sample [11]. During our analysis, we identified that the iPSYCH samples were returning: (a) very high concentrations of 25OHD₃ (often over 100 nmol/L), and (b) the presence of appreciable concentrations of 25OHD₂ in the majority of samples (while previous studies had identified 25OHD₂ in less than 10% of neonatal DBS, [2, 12]). Here we describe our steps to explore the reasons for these unexpected findings, and explore ways to improve the precision of our previously published assay [11].

As part of the investigations into these findings, we noted that bovine serum albumin (BSA) had been included in the DNA extraction process. BSA is not required (nor recommended) for assays related to 25OHD₂ and 25OHD₃ assays, but has been recommended as an aid to optimize DNA extraction suitable for amplification [9]. To the best of our knowledge, there have been no prior studies that have tested for the presence of 25OHD₂ and 25OHD₃ in BSA. However, it is known that cattle produce vitamin D from bright sunshine [13] and are also often given vitamin D supplements in order to reduce post-weaning hypocalcaemia [14]. We speculated that the unexpectedly high concentrations of 25OHD₂ and 25OHD₃ identified in our assays were the results of BSA-related contamination with exogenous vitamin D metabolites.

We note that other large population-based studies have used modelling strategies to provide corrected 25OHD₃ estimates. The National Health and Nutrition Examination Survey (NHANES) issued corrected values to account for changes in assay accuracy over different waves [15, 16]. The UK Biobank study (n ~ 500,000) used modelling to correct values for a range of biomarkers (including the vitamin D metabolites assessed in the current study) after a dilution artefact during an early wash phase of aliquot handling was detected [17].

The aims of this study were to (a) measure if 25OHD₂ and 25OHD₃ were present in recently-purchased batches of BSA; (b) re-assay 25OHD₂ and 25OHD₃ in a subset of the iPSYCH neonatal DBS with the identical extraction steps but without BSA, in order to compare contaminated versus uncontaminated in pairwise samples; and (c) based on the findings from the pairwise comparisons, apply a set of corrections to the full iPSYCH sample in order to derive estimates adjusted for the influence of BSA contamination. These methods

can guide researchers to reproduce our steps if BSA contamination has influenced related assays.

Main text

Material and methods

In the following section we concisely outline (a) the methods we used to determine if BSA contains 25OHD₂ and 25OHD₃, (b) the selection of a subsample of DBS for repeat testing without the addition of BSA, and (c) based on the paired contaminated versus uncontaminated samples, the modelling steps we used to apply corrections across the entire sample.

We first assayed the concentration of 25OHD₂ and 25OHD₃ in 11 individuals using our standard (non-BSA exposed) laboratory protocols (full details are provided in the Supplement). For the high-throughput assessment of 25OHD₂ and 25OHD₃ concentration in the large iPSYCH sample, the protein extracts were prepared and subsequently assayed in 96-well plates (full details of this assay are available in Boelt et al. [11]). We inspected the mean 25OHD₂ and 25OHD₃ concentration for each of the 1050 plates, when ordered by the sequence of testing routinely used in the iPSYCH sample (i.e. based on date of birth of the neonates). If the results from the entire iPSYCH sample were uniformly increased, it would suggest that contamination was from one batch of BSA across the entire sample. If there were abrupt between-plate changes in mean 25OHD₂ and 25OHD₃ concentration, it would suggest that contamination was from more than one BSA batch.

In order to quantify the extent of BSA-related contamination with 25OHD₂ and 25OHD₃, we chose a subsample of the iPSYCH sample for repeat testing using the identical methods for the preparation of the protein extract (and subsequent assay for 25OHD₂ and 25OHD₃), but without the addition of BSA (henceforth, the uncontaminated results). By comparing the contaminated versus uncontaminated results in these pairs of samples, we deduced the contribution of the BSA-related 25OHD₂ and 25OHD₃ to the contaminated results. Finally, based on the repeat-tested subsample, we derived statistical models that adjusted for BSA contamination in the entire iPSYCH sample (henceforth, the corrected results).

Results

The concentration of 25OHD₂ and 25OHD₃ in recently purchased BSA batches

Based on routine laboratory assays of 11 individuals, none of the samples had detectable 25OHD₂, while 25OHD₃ based on full blood concentration ranged from 11.2 to 58.4 nmol/L. We then assessed the

contribution of 17 different batches of recently purchased BSA to the extraction buffer used in the preparation of the DBS protein extract. We found that only two BSA batches had appreciable concentrations of 25OHD2 and 25OHD3. The contribution of these two batches to the samples is shown in the Additional file 1: Table. For example, one BSA batch contributed a mean (standard deviation) of 20.9 (3.0) nmol/L of 25OHD2 and 27.7 (3.0) nmol/L of 25OHD3 in addition to the endogenous 25OHD2 and 25OHD3. Of interest, previous studies by our group had found that fewer than 10% of neonatal dried blood spots had detectable concentrations of 25OHD2 [1, 2, 12, 18–21]. Thus, we concluded that the presence of measurable 25OHD2 in our iPSYCH sample could serve as a proxy marker for probable BSA-related contamination.

The pattern of contaminated 25OHD2 and 25OHD3 concentration across all samples

A total of 76,611 samples were assessed across 1,050 96-well plates. Figure 1 shows the concentration of mean 25OHD2 and 25OHD3 per plate ordered in the sequence of testing (left sided panels), and density plots to show the distribution of these values (right sided panels). Firstly, for the majority of the samples, the concentration of 25OHD3 was generally higher than previous DBS-based studies [18]. Secondly, there was a small but distinct subsample with low (but clinically appropriate) 25OHD3 concentrations (across plate numbers 24–42). Finally, there was a third subsample (across plate numbers 735–804) with middle-range 25OHD3 concentrations. A strikingly similar between-plate pattern was also found for 25OHD2 concentrations. In the absence of documentation of (a) the number of BSA batches that

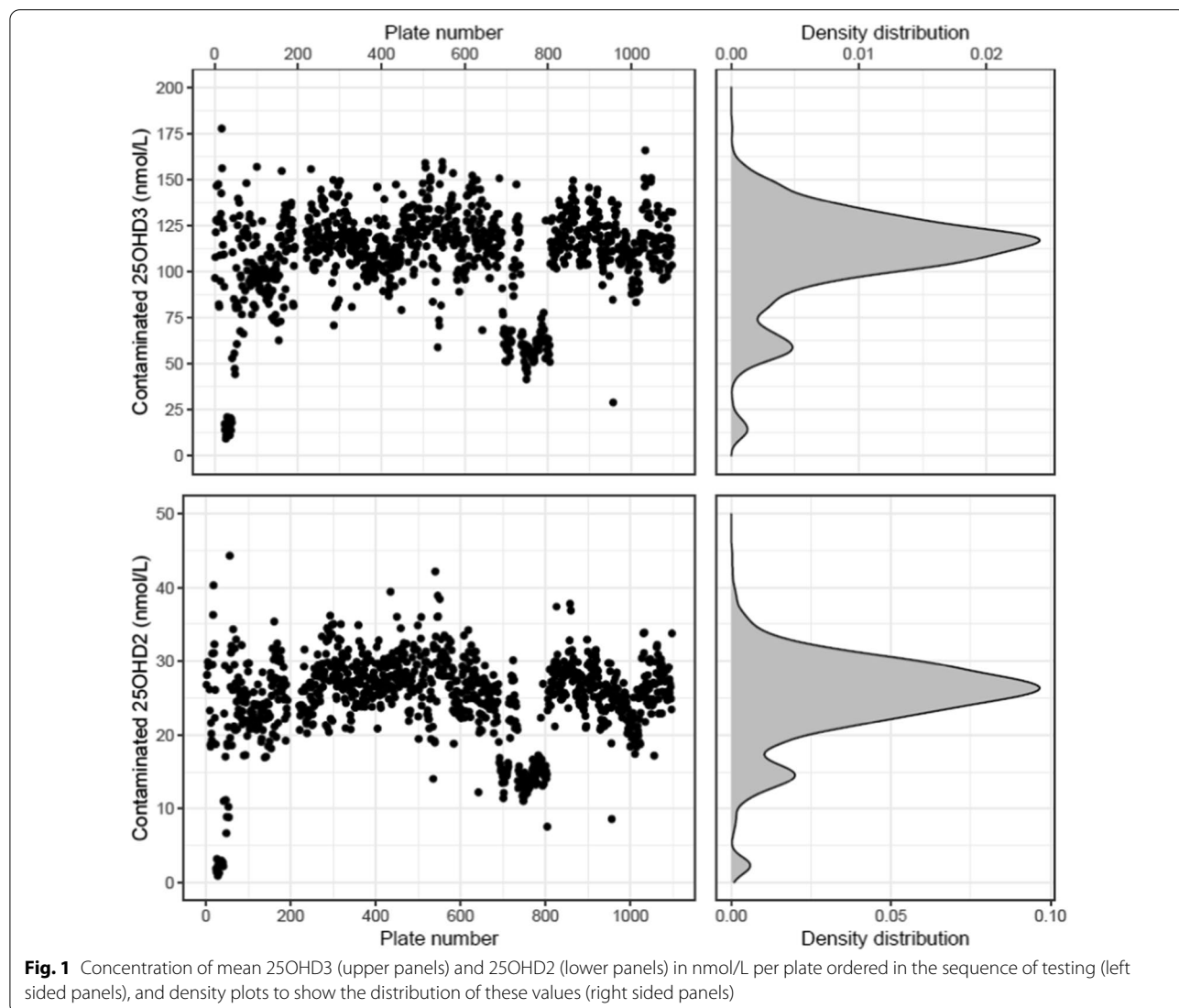


Fig. 1 Concentration of mean 25OHD3 (upper panels) and 25OHD2 (lower panels) in nmol/L per plate ordered in the sequence of testing (left sided panels), and density plots to show the distribution of these values (right sided panels)

were originally used for DNA extraction, and (b) what concentrations of 25OHD2 and 25OHD3 were in BSA batches used seven years prior to our study, we assumed that there were three different BSA batches used in the prior DNA extraction analyses. We classified all plates into those with low-, middle-, or high-contamination if the contaminated 25OHD2 was ≤ 10 , 10–20, or ≥ 20 nmol/L, respectively.

Comparing paired contaminated versus uncontaminated samples

Based on the three low-, middle- and high-contamination subgroups, we randomly selected 395 individuals for repeat testing. We went back to the original stored DBS to collect samples for protein extraction but without BSA contamination. We used the same protocol for the assessment of 25OHD2 and 25OHD3 as for the full iPSYCH sample [11]. Within this repeat-test sample, two participants with very high concentrations were excluded (25OHD2 = 267 nmol/L; 25OHD3 = 501 nmol/L).

Consistent with our previous, independent studies based on Danish neonatal dried blood spots which found 25OHD2 in a minority of the samples, only 18 of the 393 (4.6%) samples had non-zero 25OHD2 concentrations (Fig. 2a). We plotted the contaminated versus uncontaminated measures of 25OHD3 according to the low-, middle- and high-contamination subgroups. There was a clear correlation between these values in each of the subgroups (Fig. 2b). We found a linear relationship between contaminated 25OHD2 and 25OHD3 values ($25OHD3_{[BSA]} = 12.2 + 3.3 \times 25OHD2_{[BSA]}$; $R^2 = 0.91$;

$p\text{-value} < 0.001$; Fig. 2c). The 4.6% of the repeat testing (not exposed to BSA) sample that had non-zero 25OHD2 concentration were the values furthest below the contaminated 25OHD2 versus 25OHD3 regression line. Based on this finding, we identified the samples with expected non-zero 25OHD2 (this in-sample analysis correctly identified 98.7% of the true negatives and 66.7% of the true positives for non-zero 25OHD2).

Correcting for the BSA-related 25OHD2

We returned to the full sample ($n = 76,611$) and plotted the contaminated 25OHD2 versus contaminated 25OHD3, and identified 4.6% ($n = 3508$) of the sample that was furthest below the line of regression between these two values (Fig. 2c). We assumed that these observations would have had non-zero 25OHD2 concentrations despite the BSA contamination. Within this set, we predicted their corrected 25OHD2 using a linear regression model based on repeat-test (contaminated 25OHD2 and 25OHD3) subset ($\text{corrected } 25OHD2_{[Corr]} = 2.86 + 0.84 \times 25OHD2_{[BSA]} - 0.23 \times 25OHD3_{[BSA]}$; $R^2 = 0.81$; $p\text{-value} < 0.001$). Apart from this subset, the remainder of the samples were recoded as 25OHD2 = 0 nmol/L.

Correcting for the BSA-related 25OHD3

Next, within the subsample with repeat testing, we plotted (a) the difference between contaminated minus uncontaminated 25OHD2, versus (b) the difference between contaminated minus uncontaminated 25OHD3. In light of our finding that 25OHD2 concentration could act as a proxy marker for BSA contamination, the

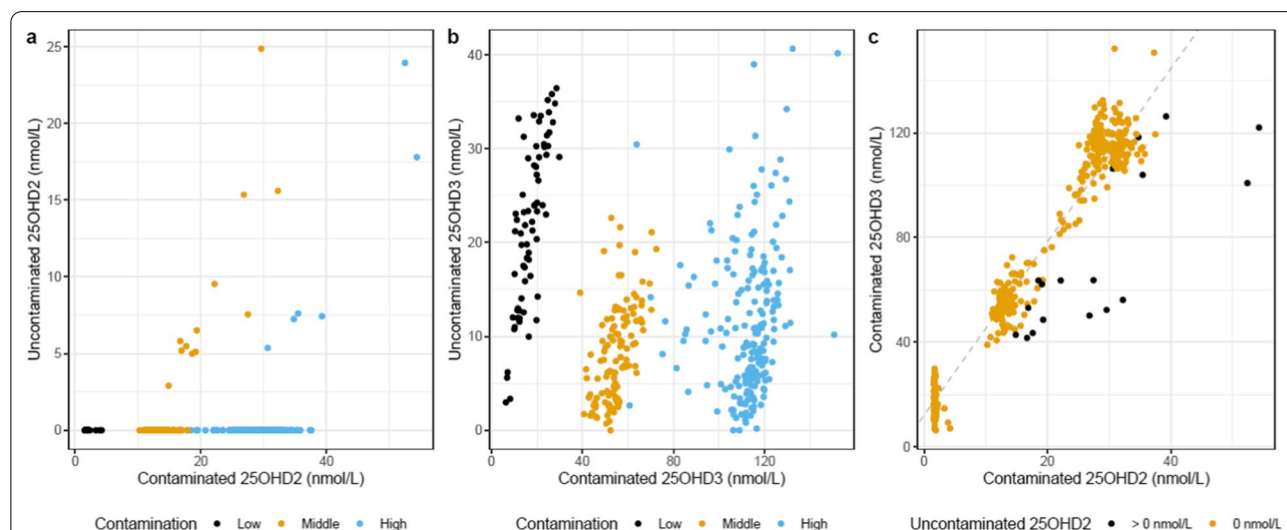
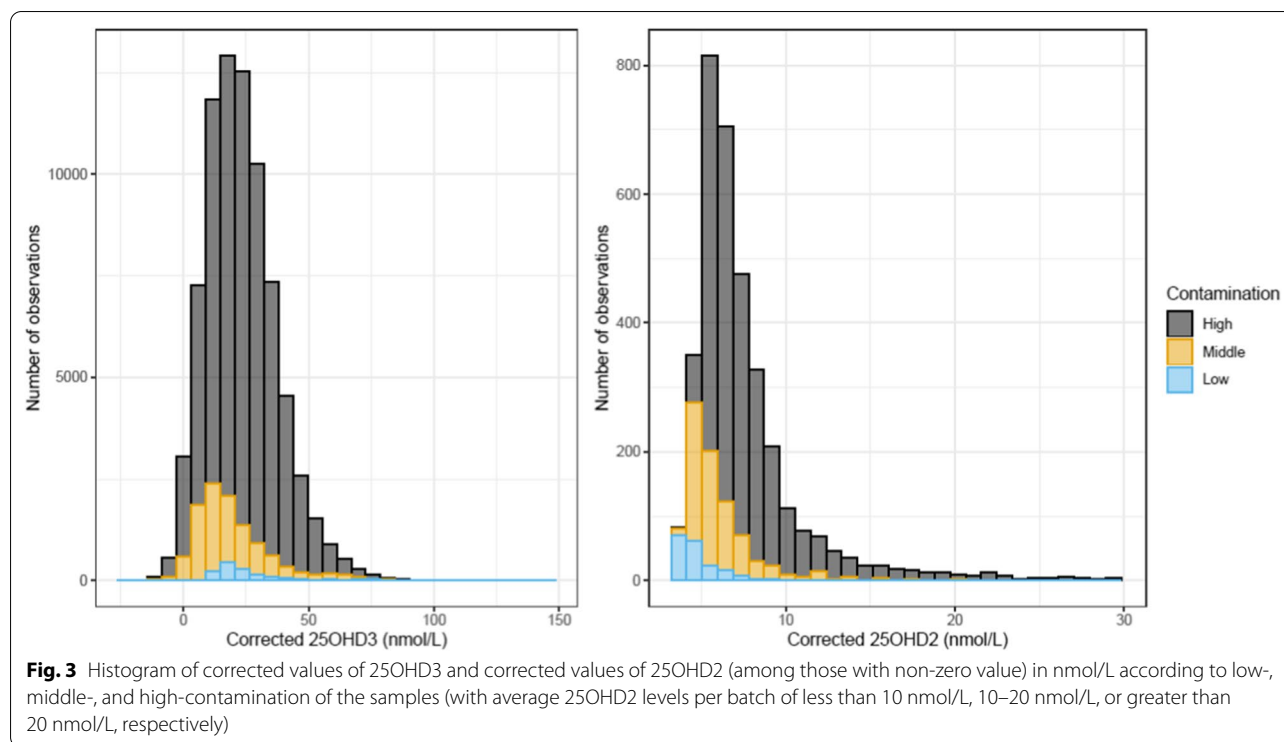


Fig. 2 Scatter plots for contaminated versus uncontaminated 25OHD2 (a) and 25OHD3 (b) in nmol/L in the entire sample according to low-, middle-, and high-contamination of the samples (with average 25OHD2 levels per batch of less than 10 nmol/L, 10–20 nmol/L, or greater than 20 nmol/L, respectively). c shows a scatter plot of contaminated 25OHD2 versus contaminated 25OHD3 in nmol/L (with a dashed regression line) according to uncontaminated 25OHD2 being zero or non-zero



difference scores were stratified into the low-, middle- and high- contamination subgroups. Based on the slopes and intercept of these difference scores (Additional file 1: Figure), we were then able to derive corrected values for 25OHD3 for the entire sample. The regression models were $\text{Diff}_{25\text{OHD}3} = -8.2 + 1.8 * \text{Diff}_{25\text{OHD}2}$ for low contamination, $\text{Diff}_{25\text{OHD}3} = 23.8 + 1.7 * \text{Diff}_{25\text{OHD}2}$ for middle contamination, and $\text{Diff}_{25\text{OHD}3} = 14.3 + 3.0 * \text{Diff}_{25\text{OHD}2}$ for high contamination. The distributions for the corrected 25OHD2 and 25OHD3 for the three subgroups are shown in Fig. 3. Mindful that the two lower concentration subgroups only contributed 15% of the total sample, the corrected distributions were within expectations for dried blood spots based on samples previously analysed in our laboratory [1, 3, 19]. The values for 25OHD2 are all very low and the modes of these three subgroups are below the lowest level of quantification (less than 5 nmol/L) for similar assays based on dried blood spots [2, 12].

Limitations

BSA could also contain other biologically interesting analytes which could bias results of future biomarker studies that have included BSA during sample preparation. The method outlined above was optimized for 25OHD2 and 25OHD3, it is not clear how BSA could impact on the precision of other assays. The use of BSA

can influence the accurate measurement of vitamin D metabolites, however methods are available to use targeted repeat assays in order to develop models to correct the influence of this agent.

Abbreviations

BSA: Bovine serum albumin; 25OHD2: 25-Hydroxyvitamin D₂; 25OHD3: 25-Hydroxyvitamin D₃.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13104-022-06077-1>.

Additional file 1: Table S1. Summary of the contribution of 25OHD2, 25OHD3 from BSA results. Mean of the concentration (nmol/L), standard deviation (\pm SD) and coefficient of variation (%CV) are calculated for each sample. None of the donors had detectable 25OHD2. **Figure S1.** Scatter plots of the difference (contaminated–uncontaminated) values of 25OHD3 and 25OHD2 in nmol/L according to low-, middle-, and high-contamination of the samples (with average 25OHD2 levels per batch of less than 10 nmol/L, 10–20 nmol/L, or greater than 20 nmol/L, respectively). These models based on the subsample with repeated measures were used to predict corrected values in the entire sample ($R^2 = 0.97$; p -value < 0.001). The regression models were $\text{Diff}_{25\text{OHD}3} = -8.2 + 1.8 * \text{Diff}_{25\text{OHD}2}$ for low contamination, $\text{Diff}_{25\text{OHD}3} = 23.8 + 1.7 * \text{Diff}_{25\text{OHD}2}$ for middle contamination, and $\text{Diff}_{25\text{OHD}3} = 14.3 + 3.0 * \text{Diff}_{25\text{OHD}2}$ for high contamination.

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Author contributions

The laboratory work was designed and undertaken by SGB and ASC. The modelling steps was done by OPR. SGB, OPR, CA, BV, JJM and AC were involved in the interpretation of the data. The manuscript was prepared by JMcG, SGB and OPR. SGB, OPR, CA, BV, JJM and AC approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Owing to the sensitive nature of these data, individual level data can be accessed only through secure servers where download of individual level information is prohibited. Each scientific project must be approved before initiation, and approval is granted to a specific Danish research institution. International researchers may gain data access through collaboration with a Danish research institution. More information about getting access to the iPSYCH data can be obtained at <https://ipsych.au.dk/about-ipsych/>.

Declarations

Ethics approval and consent to participate

The collection of the 11 samples for the BSA-related analyses were conducted in accordance with the Declaration of Helsinki and the protocol complies with the Danish Ethical Committee law by not being a health research project (§2,1) but a method development study not requiring an ethical approval. Concerning the assay of the iPSYCH samples, the Danish Scientific Ethics Committee, the Danish Health Data Authority, the Danish data protection agency and the Danish Neonatal Screening Biobank Steering Committee provided approval for this study. By Danish law, this register-based study did not require informed consent. Permission has been granted to study genetic and environmental factors for the development and prognosis of mental disorders, and the development and refinement of assays related to this research.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests.

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References

- Keller A, Handel MN, Frederiksen P, Jacobsen R, Cohen AS, McGrath JJ, et al. Concentration of 25-hydroxyvitamin D from neonatal dried blood

- spots and the relation to gestational age, birth weight and Ponderal index: the D-tect study. *Br J Nutr.* 2018;119(12):1416–23.
- Eyles DW, Trzaskowski M, Vinkhuyzen AAE, Mattheisen M, Meier S, Gooch H, et al. The association between neonatal vitamin D status and risk of schizophrenia. *Sci Rep.* 2018;8(1):17692.
- Nielsen NM, Munger KL, Koch-Henriksen N, Hougaard DM, Magyari M, Jorgensen KT, et al. Neonatal vitamin D status and risk of multiple sclerosis: a population-based case-control study. *Neurology.* 2017;88(1):44–51.
- Eyles D, Anderson C, Ko P, Jones A, Thomas A, Burne T, et al. A sensitive LC/MS/MS assay of 25OH vitamin D-3 and 250H vitamin D-2 in dried blood spots. *Clin Chim Acta.* 2009;403(1–2):145–51.
- Kvaskoff D, Heath AK, Simila HA, Ko P, English DR, Eyles DW. Minimizing matrix effects for the accurate quantification of 25-hydroxyvitamin D metabolites in dried blood spots by LC-MS/MS. *Clin Chem.* 2016;62(4):639–46.
- Kvaskoff D, Ko P, Simila HA, Eyles DW. Distribution of 25-hydroxyvitamin D3 in dried blood spots and implications for its quantitation by tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2012;901:47–52.
- Pedersen CB, Bybjerg-Grauholm J, Pedersen MG, Grove J, Agerbo E, Baekvad-Hansen M, et al. The iPSYCH2012 case-cohort sample: new directions for unravelling genetic and environmental architectures of severe mental disorders. *Mol Psychiatry.* 2018;23(1):6–14.
- Hollegaard MV, Grauholm J, Borglum A, Nyegaard M, Norgaard-Pedersen B, Orntoft T, et al. Genome-wide scans using archived neonatal dried blood spot samples. *BMC Genomics.* 2009;10:297.
- Hollegaard MV, Sorensen KM, Petersen HK, Arnardottir MB, Norgaard-Pedersen B, Thorsen P, et al. Whole genome amplification and genetic analysis after extraction of proteins from dried blood spots. *Clin Chem.* 2007;53(6):1161–2.
- Poulsen JB, Lescai F, Grove J, Baekvad-Hansen M, Christiansen M, Hagen CM, et al. High-quality exome sequencing of whole-genome amplified neonatal dried blood spot DNA. *PLoS ONE.* 2016;11(4):e0153253.
- Boelt SG, Melgaard L, Thorbek MJ, MacSween NSJ, McGrath JJ, Cohen AS. Sensitive and robust LC-MS/MS assay to quantify 25-hydroxyvitamin D in leftover protein extract from dried blood spots. *Int J Neonatal Screen.* 2021;7(4):82.
- McGrath JJ, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, et al. Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. *Arch Gen Psychiatry.* 2010;67(9):889–94.
- Nelson CD, Powell JL, Price DM, Hersom MJ, Yelich JV, Drenowski ME, et al. Assessment of serum 25-hydroxyvitamin D concentrations of beef cows and calves across seasons and geographical locations. *J Anim Sci.* 2016;94(9):3958–65.
- Weiss WP, Azem E, Steinberg W, Reinhardt TA. Effect of feeding 25-hydroxyvitamin D3 with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves. *J Dairy Sci.* 2015;98(8):5588–600.
- Schleicher RL, Sternberg MR, Lacher DA, Sempos CT, Looker AC, Durazo-Arvizu RA, et al. A method-bridging study for serum 25-hydroxyvitamin D to standardize historical radioimmunoassay data to liquid chromatography-tandem mass spectrometry. *Natl Health Stat Report.* 2016;93:1–16.
- Statistics CNCfH. Analytical note for 25-hydroxyvitamin D data analysis using NHANES III (1988–1994), NHANES 2001–2006, and NHANES 2007–2010 (October 2015): center for disease control; 2015. <https://wwwn.cdc.gov/nchs/nhanes/vitamind/analyticalnote.aspx>.
- Allen NE, Arnold M, Parish S, Hill M, Sheard S, Callen H, et al. Approaches to minimising the epidemiological impact of sources of systematic and random variation that may affect biochemistry assay data in UK Biobank. *Wellcome Open Res.* 2020;5:222.
- Handel MN, Frederiksen P, Cohen A, Cooper C, Heitmann BL, Abrahamsen B. Neonatal vitamin D status from archived dried blood spots and future risk of fractures in childhood: results from the D-tect study, a population-based case-cohort study. *Am J Clin Nutr.* 2017;106(1):155–61.
- Jacobsen R, Thorsen SU, Cohen AS, Lundqvist M, Frederiksen P, Pipper CB, et al. Neonatal vitamin D status is not associated with later risk of type 1 diabetes: results from two large Danish population-based studies. *Diabetologia.* 2016;59(9):1871–81.
- Keller A, Frederiksen P, Handel MN, Jacobsen R, McGrath JJ, Cohen AS, et al. Environmental and individual predictors of 25(OH)D concentrations

measured from neonatal dried blood spots in Denmark: the D-tect study. *Br J Nutr.* 2018;121:1–25.

21. Thorsteinsdottir F, Cardoso I, Keller A, Stougaard M, Frederiksen P, Cohen AS, et al. Neonatal vitamin D status and risk of asthma in childhood: results from the D-tect study. *Nutrients.* 2020;12(3):842.

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