Failure of national antenatal vitamin D supplementation programme puts dark skinned infants at highest risk: A newborn bloodspot screening study

Suma Uday[1,2], Sunia Naseem[3], Jamie Large[3], Russell Denmeade[4], Philippa Goddard[4], Mary Anne Preece[4], Rachel Dunn[5], William Fraser[5,6], Jonathan C Y Tang[5], Wolfgang Högler[2,7]

1. Department of Endocrinology and Diabetes, Birmingham Women's and Children's Hospital, Steelhouse lane, Birmingham, B4 6NH, UK
2. Institute of Metabolism and Systems Research, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
3. College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
4. Department of Newborn Screening and Biochemical Genetics, Birmingham Women's and Children's Hospital, Steelhouse lane, Birmingham, B4 6NH, UK
5. Department of Medicine, Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich NR4 7UQ, UK
6. Departments of Diabetes and Endocrinology and Clinical Biochemistry, Norfolk and Norwich University Hospital, Norwich NR4 7UY
7. Department of Paediatrics and Adolescent Medicine, Johannes Kepler University Linz, 4020 Linz, Austria

Corresponding author:

Dr. Suma Uday

Department of Endocrinology and Diabetes,

Birmingham Women's and Children's Hospital,

Steelhouse lane, Birmingham, B4 6NH

Tel: 0121333999 **E-mail: suma.uday@nhs.net**

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ABSTRACT

Objectives: To determine the prevalence of vitamin D deficiency on dried blood spots (DBS) obtained at newborn bloodspot screening (NBS) and thereby test the efficacy of the UK national antenatal supplementation programme in an increasingly ethnically diverse English population. To evaluate the seasonal and ethnic variation in neonatal plasma 25 hydoxyvitamin D (25OHD) and its determinants.

Design: Three thousand random DBS samples received at a single regional newborn screening laboratory (52° N) over two one-week periods, one in winter (February 2019) and one in summer (August 2019), were collected. Data was collected from NBS cards on birth weight, gestational age, maternal age, ethnicity, and post code which was replaced with index of multiple deprivation (IMD). 25OHD concentrations were measured on 6mm sub-punch from DBS using quantitative liquid chromatography tandem mass spectrometry adjusted to equivalent plasma values. 25OHD variation with season was assessed using Mann-Whitney U test and ethnic groups compared using Kruskal-Wallis test. Linear regression was used to assess the determinants of 25OHD concentrations.

Results: 25OHD measurements were available in 2999 (1580 males) subjects [1499 winter-born and 1500 summer-born]. The majority were white British (59.1%) and born at term (mean ±SD gestational age of 38.8±1.8 weeks) with a mean (±SD) birth weight of 3306 (±565) grams. The overall prevalence of vitamin D deficiency [25OHD<30 nmol/L (12 µg/L)] was 35.7% (n=1070) and insufficiency [30-50 nmol/L (12-20 µg/L)] 33.7% (n=1010). The median (IQR) 25OHD concentration was significantly lower in the winter-born compared to summer-born [29.1 (19.8, 40.6) vs 49.2 (34.3, 64.8) nmol/L respectively; p<0.001]. Across both seasons, when compared to white British babies (41.6 nmol/L), the median 25OHD concentrations were significantly lower in babies of black (30.3 nmol/L; p<0.001), Asian (31.3 nmol/L; p<0.001), any other mixed (32.9 nmol/L; p<0.001), mixed white and black (33.7 nmol/L; p<0.05) and

any other white (37.7 nmol/L; $p<0.05$) ethnicity. The proportion of deficiency was also higher in babies of Asian (48%), black (47%) and mixed ethnicity (38-44%) compared to any other white (34%) or white British (30%) ethnicity. Season of birth, ethnicity, gestation and maternal age accounted for almost 24% of the variation in 25OHD concentrations.

Conclusion: The current UK antenatal supplementation programme fails to protect newborns from vitamin D deficiency, especially those from minority ethnic groups who are at high risk of vitamin D deficiency. Nearly 70% of all newborns and 85% of winter-borns had 25OHD concentrations below 50 nmol/L (20 µg/L). Almost 50% of babies of Black or Asian origin were deficient at birth, which explains their high risk of hypocalcaemic complications and rickets if left unsupplemented. Our findings call for an immediate review of the delivery of antenatal and infant vitamin D supplementation programmes and implementation of food fortification in the long term.

Keywords: women, neonate, micronutrient, vitamin, cholecalciferol, fortification

INTRODUCTION

Neonates with vitamin D deficiency can develop serious life-threatening complications such as hypocalcaemic seizures, dilated cardiomyopathy, cardiac failure and, rarely, death in the first months of life.[1] Additional features include poor feeding, craniotabes, hypotonia and delayed motor milestones.[2] In infants over 6 months, vitamin D deficiency can present with hypocalcaemic (seizure, tetany) and hypophosphataemic complications (rickets with bowing deformities of legs, delayed development).

Due to the high calcium demands of rapid growth in infancy,[3] hypocalcaemia can manifest as early as in the first week of life,[4-6] when the mother was severely vitamin D deficient and did not receive adequate replacement in pregnancy. There is robust evidence base now established which suggests that adequate vitamin D status in pregnancy is essential not only to maintain optimal skeletal health in the mother and her newborn,[7] but also to prevent hypocalcaemia,[8] and rickets in the newborn,[9] and to optimise future bone health of the offspring.[10] There is growing evidence on the effect of vitamin D deficiency on non-skeletal pregnancy-related adverse health outcomes such as gestational diabetes, pre-eclampsia and small for gestation birth.[11-12] It is therefore imperative to ensure adequate vitamin D status during pregnancy and infancy. Given the very few dietary sources of vitamin D, there is reliance on sunlight or supplements, in high latitude countries with limited sunshine. Most developed countries therefore have vitamin D supplementation policies in place for high-risk groups which includes pregnant women (antenatal) and infants (postnatal). Infants are particularly at high risk of vitamin D deficiency not only due to reduced sun-exposure but also because the requirements are not met through feeds alone, especially in breastfed infants.[9] We have previously reported on the ineffectiveness of the UK infant vitamin D supplementation policies compared to other European countries.[13] To date, the success and effectiveness of the UK antenatal supplementation programme remains unknown. The UK

guidance recommends a 400 IU/day vitamin D supplement in all pregnant women,[14-15] and a 1000 IU/day supplement in high-risk women, including those with darker skin pigmentation.[15]

Vitamin D status in apparently healthy children, particularly infants, is poorly studied due to practical difficulties in obtaining blood samples.[16] In recent years, measuring 25 hydroxyvitamin D (25OHD) on dried blood spot (DBS), obtained through minimally invasive techniques,[17-18] has evolved significantly enabling reliable assessment of population vitamin D status.[19] Measuring neonatal 25OHD on DBS samples collected as part of the national newborn bloodspot screening (NBS) programme is a suitable way of assessing the population prevalence of vitamin D deficiency in this most vulnerable group. This public health study was designed to assess the effectiveness of the UK antenatal supplementation programmes in preventing vitamin D deficiency in the newborn and provide direction to infant supplementation programmes.

AIMS

1. Determine the prevalence of 25OHD deficiency [<30 nmol/L (12µg/L)] in newborns as a key performance indicator of the UK antenatal vitamin D supplementation programme.
2. Assess seasonal and ethnic variation in DBS 25OHD in newborns
3. Study the determinants of 25OHD in newborns

RESEARCH DESIGN AND METHODS

Study Design:

To collect a representative population sample in an increasingly ethnically diverse population, 3000 DBS samples sent to Birmingham Women's and Children's Hospital's regional newborn

screening laboratory, in the Midlands region of England (52° N), were collected after obtaining ethical and relevant regulatory approvals. Data submitted on the NBS cards were gathered following anonymisation. 25OHD concentration on the DBS was measured by quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) and plasma equivalent values derived.

Study population:

All babies born in England have a blood spot sample collected on day 5 of life as part of the national NBS programme to screen for a specific set of metabolic diseases. To assess seasonal effects, samples were collected at the end of winter and summer months to capture the anticipated nadir and peak in 25OHD concentrations, respectively. All DBS samples received over one week in winter (last week of February 2019) and one week in summer (last week of August 2019) were retrieved after all routine NBS testing had been completed. Exclusion criteria included: samples marked high risk of infection (e.g. HIV), insufficient samples, second tests including neonates >21 days and cases with any missing information.

DBS Samples:

The samples are collected on a standard Whatman 903 filter paper through a heel prick by an appropriately trained healthcare professional. DBS sample were couriered from maternity services to the screening laboratory on a daily basis with; 90% of samples being received on the day and all samples within 3 days of collection. Samples were stored at room temperature and away from direct sunlight at all stages (in maternity service, during transportation and on receipt in the screening laboratory). Study samples were obtained within 7 days of receiving in the screening laboratory, stored at -20°C and analysed at the completion of recruitment (October 2019 - December 2019).

Source Data

Personal data submitted alongside the NBS card is routinely entered and stored by the newborn screening laboratory team in an electronic format. Data provided to the research team was

anonymised by a member of the newborn screening team. Anonymised data gathered included: birth weight, gestational age, maternal age and ethnic group codes. For complete anonymity, postcodes were replaced by Lower-layer Super Output Area (LSOA) that each postcode falls within, and the deprivation indices for that LSOA.[20] LSOA is a geographic hierarchy designed to improve the reporting of small area statistics and includes a mean population of 1500. The Index of Multiple Deprivation (IMD) is obtained by combining seven domains of deprivation: income, employment, education, health, crime, barriers to housing and services and living environment.[20] Each domain and the IMD can be represented in ranks (1 to 32,844) or deciles which are calculated by ranking the 32,844 small areas in England from most deprived to least deprived and dividing them into 10 equal deciles, where 1 is the most deprived and 10 the least deprived.[21]

Ethnicity group codes prescribed by the UK Office of National Statistics were used.[22] The independent ethnic group codes were clubbed and grouped as follows:

Group 1: White British

Group 2: Any other white (white Irish + Any other white)

Group 3: Asian (Indian, Pakistani, Bangladeshi, Chinese and any other Asian background)

Group 4: Black (African, Caribbean, any other black background)

Group 5: Mixed white and black (White and black African and white and black Caribbean)

Group 6: Mixed white and Asian

Group 7: Any other mixed (any other mixed background and any other ethnicity)

Analysis of DBS 25OHD and plasma equivalent values

Dried blood spot 25OHD3 and 25OHD2 concentrations were measured at the Bioanalytical facility in The University of East Anglia, Norwich, UK, by quantitative LC-MS/MS following extraction methods previously detailed.[18,23] In brief, a 6mm sub-punch was made from each

blood spot sample using a pneumatic DBS card puncher (Analytical Sales & Services, NJ, USA). Samples were extracted using 300µL of 50:50 (v/v) isopropanol to water solution containing carbon-13 labelled 25OHD3-$^{13}C_5$ internal standard; in an ultrasonic water bath at 35°C for 30 minutes. The extracts were transferred onto Supported Liquid Extraction (SLE+) plate (Biotage, Uppsala, Sweden) for further clean up, then eluted with 1.5 mL of heptane. The eluent was dried to completeness under nitrogen gas heated at 60°, followed by derivatisation with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). The PTAD-derivatised 25OHD3 and 25OHD2 were separated using a C18 2.7µm. 50 x 2.1mm chromatographic column (Restek, PA, USA) and detected using the Micromass Quattro Ultima Pt tandem mass spectrometer (Waters Corp., Milford, MA, USA) according to analyte precursor to product transitions; 607>298 (25OHD3) and 613>298 (25OHD2). Each batch of sample analysis was performed with matrix-matched calibration standards and quality controls prepared in vitamin D and its hydroxylated metabolite-free packed red cells. The inter/intra-assay coefficient of variation (CV) was between 3.9-9.4%, across the concentration range of the assay, with linearity from the lower limit of quantification (LLoQ) of 1 nmol/L up to 150 nmol/L.

Data are reported as plasma equivalent total 25OHD, which was calculated using the following formula: Plasma equivalent total 25OHD nmol/L = [(DBS 25OHD3 + DBS 25OHD2 nmol/L) -1.2607]/ (1 – 0·60); where 1.2607 is to adjust for the difference in slope between plasma and DBS value,[18] and 0·60 is the mean haematocrit (Hct). Given that 25OHD exists primarily in the extracellular fluid compartment in blood, blood spot measurements for 25OHD must be adjusted for Hct. Owing to the higher Hct in the neonatal period,[24] and specifically in capillary samples,[25] a value of 0.60 was used.

Ethics and consent:

The study was approved by the UK Health Research Authority (HRA) and the East Midlands - Leicester South Research Ethics Committee (REC Reference 19/EM/0019). The study was also approved by the Antenatal and Newborn Research Advisory Committee of Public Health England, UK. Parental consent was not required as all data acquired were anonymised and compliance with the code of practice for the retention and storage of residual newborn blood spots was ensured.[26]

Statistical analysis:

Descriptive statistics are presented as medians with interquartile ranges (IQR) or mean with standard deviation (SD) for continuous variables, and frequencies with percentages for categorical variables. 25OHD concentrations were used both as a continuous variable and categorical variable, defining deficiency as <30 nmol/L (12µg/l), insufficiency as 30-50 nmol/L (12-20µg/l) and sufficiency as >50 nmol/L (>20µg/l) according to the Institute Of Medicine,[27] and Global consensus recommendation classifications.[9] Non-parametric tests were used as 25OHD concentrations were skewed. Whilst normal distribution for 25OHD overall was achieved following square root transformation, the results obtained on parametric tests were comparable and are therefore not reported here.

Mann-Whitney U test was used to compare 25OHD concentrations between the winter and summer-born groups. The Kruskal-Wallis test was used to compare 25OHD concentrations between the different ethnic groups. The factors that were significant ($p<0.05$) on a bivariate analysis were included in the multiple linear regression model. The significant factors included were season of birth, ethnicity, gestational age, maternal age and IMD decile. Ethnicity, gestational age and IMD deciles (1 to 10) were used as categorical variables. Gestational age at birth was categorised as term (>37-41 weeks), extreme pre-term (<28 weeks), pre-term (28-37 weeks) and post-term (>41 weeks). All analyses were performed using SPSS statistical software v25.0 (IBM, Armonk, NY).

RESULTS

25OHD measurements were available on 2999 (1580 males) subjects; 1499 were winter-born and 1500 summer-born. Neonates were between 5 and 19 days old at the time of sample collection. Ninety nine percent of the samples were collected in the first week of life between day 5 and day 7 [93.3% on D5 (n=2800), 5.1% on D6 (n=154) and 0.6% on D7 (n=19)]. The baseline characteristics are presented in **Table 1**. The majority were white British (59.1%) and born at term (mean ± SD gestational age of 38.8 ± 1.8 weeks) with a mean (± SD) birth weight of 3306 (± 565) grams. The mean IMD decile was 4.0 (± 2.7) and nearly a quarter of the cohort (24%, n= 720) were from the most deprived IMD decile (decile 1). The baseline characteristics did not differ significantly between the winter-born and summer-born groups **(Table 1)**.

	Whole study group	**Winter-born**	**Summer-born**	**P value**
Numbers	2999	1499 *	1500	
Number of males (% of total)	1580 (52.7%)	771 (51.4%)	809 (53.9%)	0.16
Birth weight in g	3306 (565)	3313 (563)	3299 (566)	0.49
Gestational age in weeks	38.8 (1.8)	38.8 (1.8)	38.8 (1.7)	0.57
Maternal age in years	30.4 (5.5)	30.4 (5.6)	30.5 (5.4)	0.61
Ethnic groups n (%)				0.64
White British	1774 (59.1%)	877 (58.5%)	897 (59.8%)	
Any other white	264 (8.8%)	134 (8.9%)	130 (8.7%)	
Asian	494 (16.5%)	249 (16.6%)	245 (16.4%)	
Black	173 (5.8%)	90 (6.0%)	83 (5.5%)	
Mixed white and black	94 (3.1%)	53 (3.5%)	41 (2.7%)	
Mixed white and Asian	45 (1.5%)	21 (1.4%)	24 (1.6%)	
Any other mixed	156 (5.2%)	76 (5.1%)	80 (5.3%)	
Index of Multiple Deprivation decile (1=most deprived to 10=least deprived)	4.0 (2.7)	4.1 (2.7)	4.0 (2.7)	0.34

* One missing sample

Table 1: Baseline characteristics of the whole study group and the winter and summer-born sub-groups. Numbers are reported as mean (± SD) or n (%).

Prevalence of Vitamin D deficiency

The median (IQR) 25OHD concentration was 37.8 (24.8, 54.8) nmol/L. Vitamin D deficiency (<30 nmol/L) was present in 35.7% (n= 1070) of the cohort, of whom 6.2% (n= 186) were severely deficient with concentrations below 12.5 nmol/L (5µg/L) . Vitamin D insufficiency (30-50 nmol/L) and sufficiency (>50 nmol/L) were noted in 33.7% (n=1010) and 30.6% (n=919) respectively. Hence, only 30.6% of the neonates had levels in the sufficiency range. Using a higher threshold for sufficiency, 6.7% (n=200) had 25OHD concentrations > 75 nmol/L.

Seasonal and ethnic variation in 25OHD

The median (IQR) 25OHD concentration was significantly lower in winter-born compared to summer-born [29.1 (19.8, 40.6) nmol/L vs 49.2 (34.3, 64.8) nmol/L respectively; p<0.001] **Figure 1a**. Vitamin D deficiency was also more prevalent in the winter-born babies compared to summer-born babies [52.9% (n=794) vs 18.4% (n=276) respectively; p<0.001] **Figure 1b**.

The median (IQR) 25OHD concentrations differed significantly among ethnic groups, as presented in **box 1** below. Compared to white British, the 25OHD concentrations were significantly lower in babies of black (p<0.001), Asian (p<0.001), any other mixed (p<0.001), mixed white and black (p<0.05) and any other white (p<0.05) background. Babies of Mixed white and Asian background also had lower median 25OHD concentrations compared to babies of white British background, but this was not statistically significant. The median 25OHD concentrations for various ethnic groups in winter and summer are shown in **Figure 2** .

Box 1: Median (IQR) 25OHD for various ethnic groups.

Ethnic group (n)	Median (IQR) 25OHD nmol/L	Median (IQR) 25OHD nmol/L	Median (IQR) 25OHD nmol/L
	Total	**Summer**	**Winter**
White British (1774)	41.6 (27.6, 59.3)	56.3 (42.1, 69.7)	29.3 (20.8, 40.3)
Mixed white and Asian (45)	39.1 (22.3, 54.9)	52.0 (34.0, 62.8)	25.6 (14.4, 39.6)
Any other white (264)	37.7 (24.7, 53.2)*	48.4 (33.7, 61.1)	30.4 (20.7, 41.0)
Mixed white and black (94)	33.7 (21.6, 50.3)*	45.1 (33.8, 59.9)	25.3 (16.9, 36.4)
Any other mixed (156)	32.9 (20.1, 47.3)**	36.9 (24.7, 51.9)	26.8 (16.8, 44.1)
Asian (493)	31.3 (20.1, 44.0)**	31.8 (23.7, 46.7)	29.9 (18.3, 42.6)
Black (173)	30.3 (19.4, 43.2)**	38.3 (26.3, 49.1)	24.4 (15.0, 34.4)

**$p<0.001$ and *$p<0.05$ when compared to white British

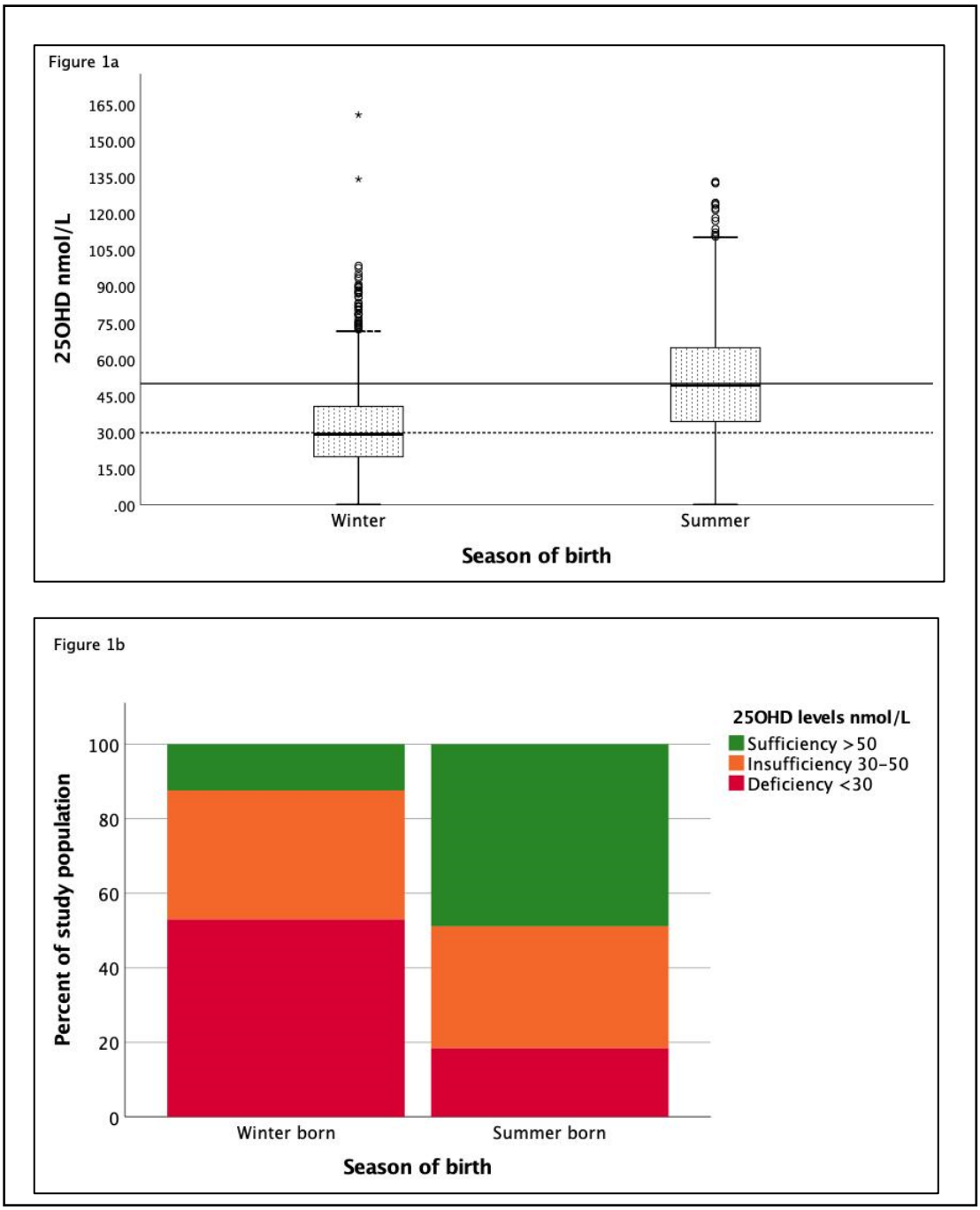

Figure 1: Figure 1a) Box plot demonstrating significantly lower median 25OHD concentrations in winter-born babies compared to summer-born babies [29.1 nmol/L vs 49.2 nmol/L respectively; $p<0.001$]. Horizontal lines are drawn at 30nmol/L (deficiency cut-off) and 50nmol/L (sufficiency cut-off).

Figure 1b) The proportion of deficiency, insufficiency and sufficiency in winter was 52.9%, 34.6% and 12.4%, respectively and in summer 18.4%, 32.7% and 48.9%, respectively.

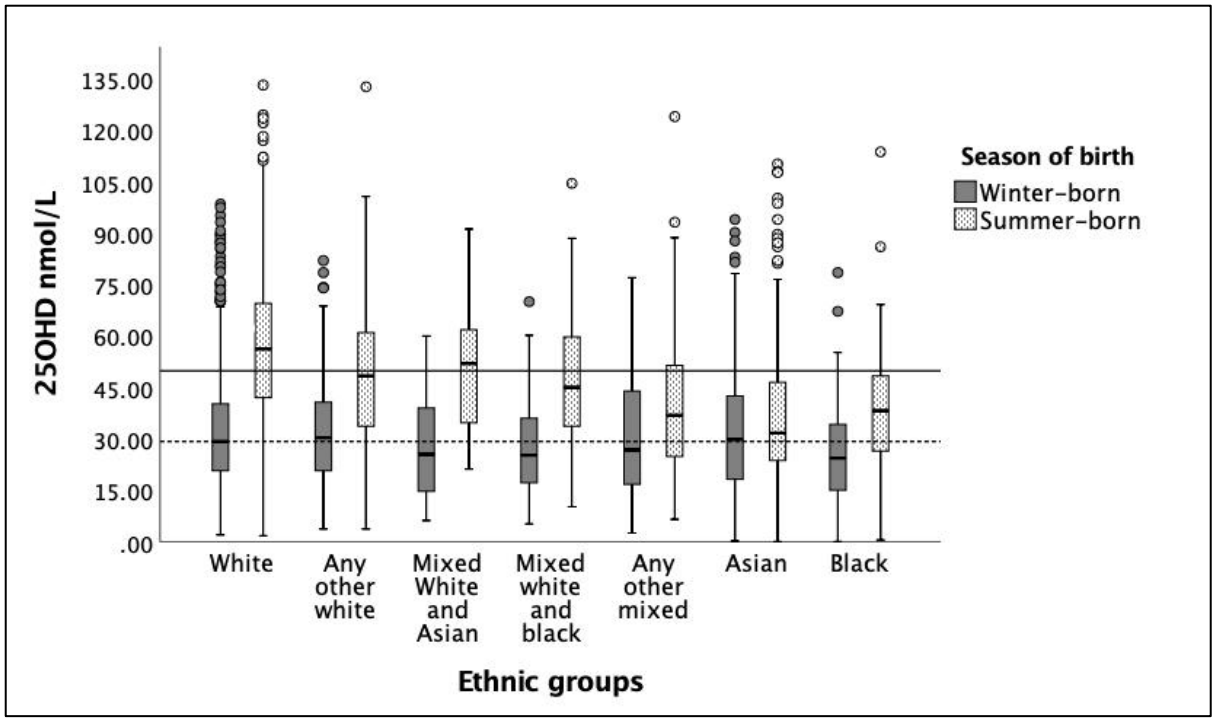

Figure 2: Median (IQR) 25OHD concentrations based on ethnicity and season of birth. When compared to white British the 25OHD concentrations were significantly lower in babies of any other white ($p<0.05$), mixed white and black ($p<0.05$), any other mixed ($p<0.001$), Asian ($p<0.001$) and black ($p<0.001$) background.

The percentage of the study group who were vitamin D deficient across both seasons was lowest in white British at 30.3% and highest in babies of Asian and black ethnic background at 47.7% and 47.4%, respectively. Seasonal differences in the proportion of deficiency and insufficiency in various ethnic groups are presented in **box 2** below and **Figure 3a** (winter) and **Figure 3b** (summer).

Box 2: Seasonal variation in the proportion of 25OHD concentrations <25 nmol/L and ≤50 nmol/L based on ethnicity

Ethnicity	**Summer**		**Winter**	
	% <25nmol/L	**% ≤50nmol/L**	**% <25 nmol/L**	**% ≤50nmol/L**
White	3.8	39.2	36.5	86.7
Any other white	14.6	52.3	35.1	89.6
Black	21.7	75.9	53.3	95.6
Asian	30.2	78.4	40.6	86.3
Mixed white and Asian	8.3	41.7	47.6	90.5
Mixed white and Black	17.1	56.1	49.1	88.7
Any other mixed	25	73.8	48.7	88.2

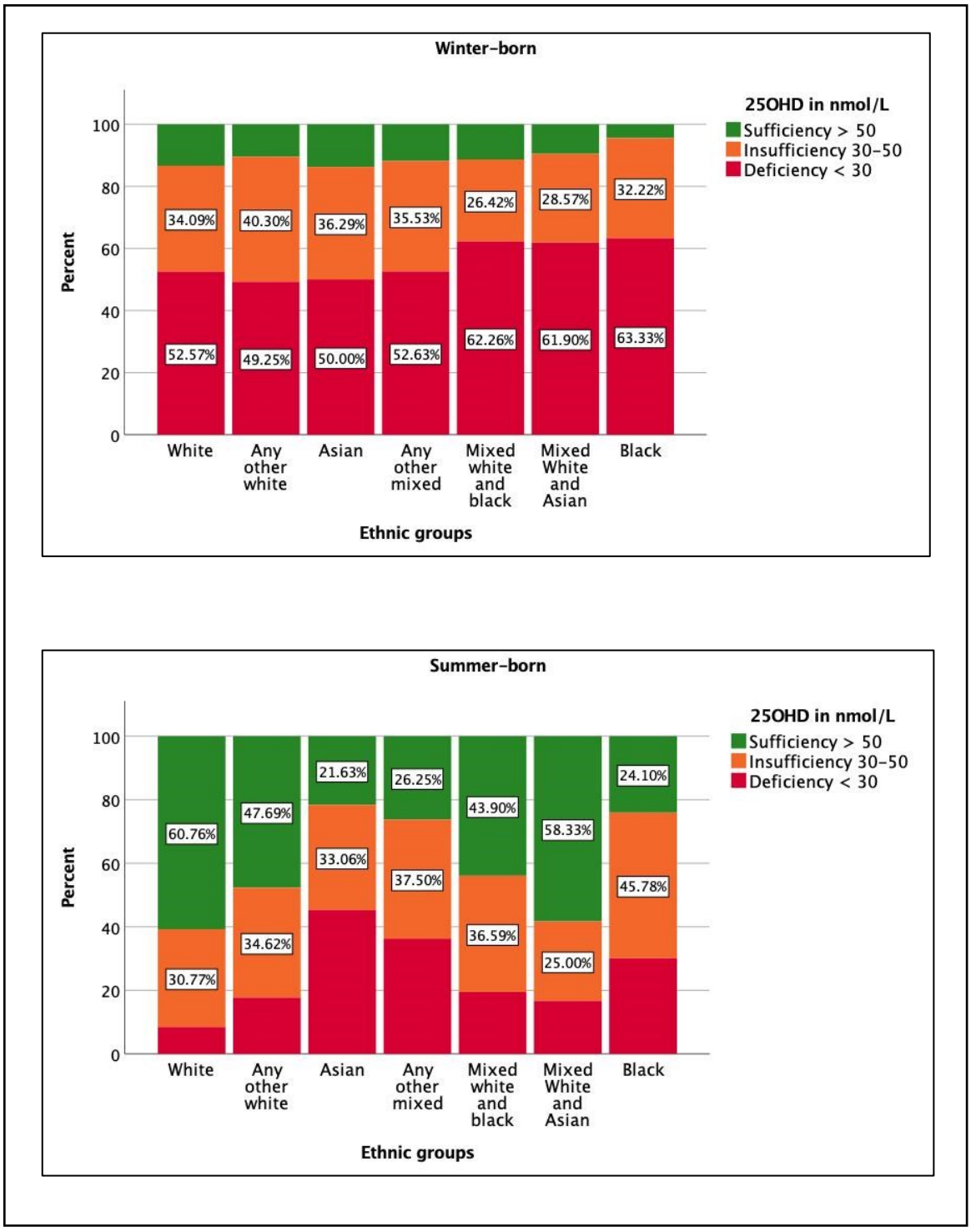

Figure 3: Bar graph representing the proportion of study population that were deficient, insufficient and sufficient according to ethnic groups in winter (3a) and summer (3b) . Vertical bars are colour-coded by vitamin D status and ordered in according to decreasing sufficiency compared to white and any other white.

Determinants of 25OHD concentrations

Parameters which were significant ($p<0.05$) on a bivariate linear regression and included in the multiple regression model were season of birth, ethnicity, gestational age, maternal age, and IMD decile. Birth weight and gender were not associated with 25OHD concentrations ($p>0.05$).

The results of the regression model are presented in **Table 2**. The statistically significant determinants of 25OHD were season of birth, ethnicity (all ethnic groups except mixed white and Asian compared to white British), gestation (prematurity and extreme prematurity compared to term gestation) and maternal age. The mean (±SD) 25OHD concentration in term, pre-term, extremely pre-term and post-term babies was 40.7 (±21.7), 45.9 (±25.2), 64.3 (±26.9) and 38.6 (±16.8) nmol/L respectively.

Nearly 24% (adjusted R^2 0.242) of the variation in newborn 25OHD was explained by the model used, with season of birth being the major contributor at 19% (adjusted R^2 0.19).

Table 2: Determinants of 25OHD concentrations in a linear regression model.

Determinant	β Standardised Co-efficient	95% Confidence interval	P value
Season (Winter/Summer)	0.43	17.70, 20.46	0.000
Ethnicity			
White British	Reference		
Asian	-0.17	-12.37, -8.34	0.000
Black	-0.13	-15.56, -9.35	0.000
Mixed white and black	-0.05	-11.17, -3.19	0.000
Any other mixed	-0.09	-12.86, -6.51	0.000
Any other white	-0.05	-6.54, -1.53	0.002
Mixed white and Asian	-0.02	-10.60, 0.74	0.089
Gestation in weeks			
Term >37- 41 (n=2,422)	Reference		
Extreme pre-term <28 (n=7)	0.07	3.13, 8.50	0.000
Pre-term 28-37 (n=502)	0.05	10.10, 39.43	0.001
Post-term >41 (n=68)	-0.01	-6.74, 2.49	0.367
Maternal age	0.06	0.13, 0.38	0.000
IMD deciles			
Decile 10 (least deprived) (n=136)	Reference		
Decile 1 (n=720)	0.00	-3.55, 3.68	0.972
Decile 2 (n=501)	0.02	-2.33, 5.07	0.469
Decile 3 (n=331)	0.00	-3.82, 3.92	0.982
Decile 4 (n=260)	-0.01	-4.82, 3.19	0.690
Decile 5 (n=309)	0.00	-3.59, 4.19	0.879
Decile 6 (n=217)	0.00	-3.90, 4.33	0.919
Decile 7 (n=201)	0.03	-1.40, 6.96	0.192
Decile 8 (n=175)	0.00	-3.63, 4.97	0.760
Decile 9 (n=136)	0.00	-4.49, 4.40	0.985

DISCUSSION

Nearly 70% of the babies in our multi-ethnic cohort had low vitamin D status (25OHD <50 nmol/L). Vitamin D deficiency, insufficiency and sufficiency were noted in approximately a third each. Winter-born babies are at significant risk of deficiency, with only 15% achieving sufficient vitamin D status. The proportion of deficiency, when compared to white race (52.5% in winter, 8.4% in summer), was much higher in the Black, Asian and Minority Ethnic (BAME) (63.3%, 30.1%), mixed (62.2%, 36.2%) and other white (49.2%, 17.6%) ethnic groups. Season of birth, ethnicity, gestation at birth and maternal age contributed to 24% of the variation in newborn 25OHD concentrations.

The main strength of this study is the large, ethnically diverse newborn cohort from a high latitude country with complete data on birth weight, gestation, maternal age and ethnicity. Very few studies have assessed 25OHD on DBS obtained from NBS.[28-30] To our best knowledge, our study is the first to ascertain prevalence of neonatal vitamin D deficiency in non-archived DBS, eliminating the concern of 25OHD degradation with prolonged storage.[31] The potential limitation is the lack of data on maternal and early infant supplementation, especially since compliance with national policy on vitamin D supplementation is known to be poor.[15] In the UK, the uptakes of both infant,[13,32] and antenatal vitamin supplements,[10] remain <20%.

The high prevalence of vitamin D deficiency and insufficiency in our multi-ethnic newborn cohort, of 70%, is comparable to the pooled global prevalence of 75%.[33] Other studies at comparable latitude also report mean/median 25OHD concentrations below 50 nmol/L.[34] We report a higher prevalence of deficiency (52.9% in winter and 18.4% in summer) compared to the pooled European data (19.7-31.8% in winter and 5.7-15.3% in summer),[35] which is explained by the underrepresentation of ethnic minority children and the absence of newborn data in the latter.[35] The well-recognised seasonal,[35,36] and ethnic,[37,38] variations in

25OHD concentrations were confirmed. Most importantly, the plasma equivalent 25OHD concentrations derived from DBS reported here are comparable to other reports from DBS,[28,39] and also cord serum.[29,40] These agreements across studies confirm the utility of blood spot assays in accurately determining the vitamin D status of the newborn.

The major determinant of newborn 25OHD concentration was the season of birth as previously reported.[39] All ethnicities, except white British and mixed white and Asian, contributed to low vitamin D status. Although vitamin D status in the UK South Asian community has been widely studied, [38,41-44] other vulnerable ethnic groups such as the black and mixed race remain less well studied, despite the high incidence of rickets in these groups.[1,45-47] The mean 25OHD concentration in pre-term babies was around 5 nmol/L higher than the term babies, likely owing to the use of supplements or pre-term formula feeds. No significant effect on 25OHD concentrations was observed in the post-term babies, similar to previous reports.[30,48] Low socio-economic status has been linked to vitamin D deficiency and insufficiency in children.[49-51] Socio-economic status, determined by IMD decile, was independently associated with 25OHD concentrations but did not contribute to the final model confirming the predominant role of sunlight and skin colour.

Our findings have profound implications for public health policies in high latitude countries. Whilst sunlight exposure is a modifiable factor, the lack of UVB at high latitude is hardly modifiable,[52] necessitating robust supplementation and/or food fortification policies. A high proportion of deficiency in our cohort suggests shortfalls in the current UK supplementation policy,[53] likely owing to lack of clarity and inconsistency among UK policy makers. Pregnant women were classed as high risk and recommended 400 IU (10 μg)/day supplement in the letter issued by the UK Chief Medical Officers in 2012,[54] and the National Institute for Health and care excellence (NICE) guidance in 2014.[14] In addition to the general recommendation above, The Royal College of Obstetricians and Gynaecologists specifically

recommend a higher dose of 1000 IU (25μg)/day in dark skinned individuals.[15] Conversely, the most recent Public Health England guidance in 2016,[55] which was based on the Scientific Advisory Committee on Nutrition report on vitamin D and health,[7] did not make any specific supplement recommendations for pregnant women or regard them as high risk. We have previously also elaborated on the complexities of infant supplementation policy in the UK.[56] Clarity in public health policies and a unified approach is crucial for its success.

Adult vitamin D requirements have been extended to pregnant women due to the lack of pregnancy-specific data. Since these requirements cannot be met through diet, the IOM,[27] and global consensus for prevention of rickets,[9] recommend 600 IU (15μg)/day supplements in pregnancy. A study of pregnant women in Denmark (n=107) reported cord blood 25OHD concentrations <50 nmol/L in 61% of newborns (of whom 15% had concentrations <25 nmol/L) despite the use of 400 IU/day supplements in 79% of the women.[57] Antenatal supplementation should aim at preventing vitamin D deficiency in the newborn, bearing in mind that 25OHD concentrations in the neonate are estimated to be 50-70% of maternal concentrations.[57] A supplementation trial of pregnant white women (n=144) residing in northern latitude reported that 1200 IU(30μg)/day supplement achieved sufficiency (25OHD ≥50 nmol/L) in almost all women and prevented deficiency (cord 25OHD <30 nmol/L) in 95% of neonates.[34] Similar dose-finding studies in ethnic minority population are warranted.

Supplement use in the UK population is low,[58] as seen in the case of folic acid supplementation in pregnancy.[59] Only 27% of women aged 19-64 years reported taking vitamin D supplements in the National Diet and Nutrition Survey.[58] In a longitudinal study of pregnant women in Southampton, UK, only 15% (n=30/198) reported using vitamin D supplements.[10] Uptake of infant supplementation is equally poor,[13,32] and unsurprisingly the unsupplemented newborn with low reserve, in particular from BAME background, likely goes on to develop hypocalcaemic complications and rickets.[1,45,46,60-63] Countries with

poor adherence to supplements,[13,62] and/or a high proportion of immigrant population, [56,64,65] have therefore seen a resurgence in nutritional rickets.[66,67] A 90-166 fold higher incidence of rickets seen in the UK children of BAME background, compared to their white counterparts,[46,68] is consistent with our observation of lower 25OHD concentrations seen at birth reported here and the lack of rigid infant supplementation system.[56] Universal supplementation (irrespective of feeding mode) and greater adherence (>90%) to infant supplementation can mitigate these risks as demonstrated by a Danish study (n=108) where the proportion of newborns with 25OHD concentrations <50 nmol/L decreased from 61% at birth to 4% at 4 months; with none having concentrations below 25 nmol/L.[57] Universal supplementation of pregnant women (irrespective of risk factors) also enhances adherence.[69] Clear operational policies,[14] and national monitoring of supplement uptake,[13] are warranted in the UK. Additionally, measuring 25OHD on DBS samples from NBS at regular intervals could be considered to monitor the effect of these interventions.

Vitamin D deficiency in the UK BAME residents and immigrants is a long-standing problem and considered a symbol of health inequality.[56] We have previously elaborated on the UK's progression from eradication of rickets through rationing, food fortification and supplementation of infants and pregnant women with cod-liver oil during world war 2 to its subsequent resurgence ever since the 1960s.[56] The higher prevalence of deficiency in the UK population is in stark contrast to reports from Nordic countries where supplement use is generally high,[70,71] and/or widespread fortification is adopted.[70] Finland serves as a prime example demonstrating a steady improvement in population 25OHD status [from 48 nmol/L in 2000 (n=6134) to 65 nmol/L in 2011 (n=4051)] following systematic fortification of fluid milk products (0.5 μg/ 100 g in 2003, doubled to 1μg/ 100 g in 2010) and increased supplement use (11% to 41%).[72] Even among non-supplement users, 91% achieved sufficiency.[72] The 2011 Finnish health survey reported high-normal 25OHD concentrations [>125 nmol/L (>50

µg/L)] in only 0.2% (n=8/4051), where the majority (n=7/8) were supplement users.[72] The prevalence of maternal and newborn vitamin D deficiency in Finland has reduced from 60-70% in 2007 (n=125) to 1% in 2016 (n=584) due to enhanced supplement use (increase from 40 to 80%) and consumption of fortified food.[73] Hence, a combined approach with fortification and supplementation of high risk groups is the cost-effective way forward for the UK,[74] to improve population health and eliminate social inequalities.[75] Urgent review of the UK public health policies relating to vitamin D supplementation and their implementation, [56,65] is warranted to address the preventable health consequences of vitamin D deficiency in infancy.

CONCLUSIONS

Vitamin D deficiency is highly prevalent in all babies born in the UK, especially in winter months. The high proportion of dark-skinned infants with low vitamin D status, demonstrates the failure of the UK's national antenatal supplementation programme in protecting these ethnic groups, who are well recognised to be at a high risk of vitamin D deficiency. Evidently, the combination of poor antenatal and infant supplementation has resulted in the resurgence of the "English disease" in the UK, where hypocalcaemic seizures and rickets in dark-skinned infants are not a rarity. We conclude that vitamin D supplementation programmes should be delivered and monitored like immunisation programmes, and food fortification implemented. The successful example of the effect of vitamin D food fortification in other high latitude countries, such as Finland, should guide the design of a national fortification programme. We demonstrate that analysis of DBS 25OHD can serve as a key performance indicator for the success of antenatal supplementation programmes and thereby inform preventative public health interventions.

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