

Sunlight Exposure and Vitamin D Status in Breastfed Infants

PINKY MEENA, AASHIMA DABAS, DHEERAJ SHAH, #RAJEEV KUMAR MALHOTRA, *SV MADHU AND PIYUSH GUPTA

From the Departments of Pediatrics, #Biostatistics and Medical Informatics, and *Medicine (Division of Endocrinology); University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden, Delhi, India.

Correspondence to: Dr Piyush Gupta, Professor of Pediatrics, University College of Medical Sciences and GTB Hospital, Dilshad Garden, Delhi 110 095, India. prof.piyush.gupta@gmail.com

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Objective: To correlate the sunlight exposure in first 6 months to vitamin D status at 6 months of age in predominantly breastfed infants; and to quantify the sunlight exposure required to achieve serum 25(OH)D level >20 ng/mL, by 6 months of age

Design: Prospective cohort.

Setting: Tertiary-care hospital predominantly catering to urban poor population in Delhi.

Participants: 132 healthy infants, delivered at term, and predominantly breastfed were enrolled at 6-8 weeks of age. Of these, 100 infants were available for final evaluation at 6 months of age (mean (SD) follow-up: 126 (17) days).

Methods: Baseline maternal vitamin D (serum 25(OH)D) levels were obtained at enrolment. The mothers were asked to maintain a daily record of duration of sunlight exposure, timing of exposure, and body surface area exposed, for the infant, on a pre-designed proforma, till the child was 6 months of age. Infant's serum 25(OH)D was measured at 6 months of age.

Main outcome measures: Cumulative Sun Index was calculated as a composite measure of overall duration/time/body surface area exposed to sunlight; and correlated with the infant serum 25(OH)D after adjusting for baseline maternal serum 25(OH)D

levels, season of exposure, and skin color of the infant. Sun index for exposure in morning (before 10 am) and afternoon (10 am-3 pm) were also correlated to vitamin D status.

Results: Of 100 mother-infant pairs completing the study, 90 mothers had vitamin D deficiency (serum 25(OH)D <12 ng/mL). The median duration of exposure of infants to sunlight was 17 min per week, on 6% of body surface area. Vitamin D levels of 67 (67%) infants at 6 months were less than 12 ng/mL and another 23% had insufficient levels (12-20 ng/mL). Cumulative sun index correlated positively to infant's serum 25(OH)D level at 6 months of age ($r=0.461$, $P<0.001$). Increment in afternoon sun index by 1 unit increased the serum 25(OH)D level by 1.07 ng/mL (95% CI 0.37, 1.78; $P=0.003$). A minimum 30 minute weekly afternoon sunlight exposure, between 10 am and 3 pm, over 40% body area (infant clothed in diapers, in prone position) for at least 16 weeks, was estimated requirement to achieve sufficient vitamin D levels (>20 ng/mL) by 6 months of age.

Conclusions: There is a significant positive correlation between afternoon sunlight exposure and infant's vitamin D levels, independent of maternal vitamin D status. Randomized controlled trials are suggested to explore the effectiveness of this simple intervention to prevent or treat vitamin D deficiency in children.

Keywords: Sun index, Rickets, Treatment, Vitamin D deficiency.

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Vitamin D deficiency has emerged as a pandemic affecting all ages including infants [1]. The prevalence of vitamin D deficiency in Indian neonates is reported between 86 to 100% [2], despite adequate availability of sunlight and adequate maternal calcium intake during antenatal period. The sources of vitamin D for infants include cutaneous vitamin D production and breastmilk, with the later usually deficient in vitamin D. Natural vitamin D synthesis remains ineffective mostly due to modern lifestyle where infants remain confined indoors during daytime, which is the prime time for exposure to ultraviolet B rays [1]. Therefore, the American Academy of Pediatrics recommends routine supplementation of vitamin D (400 IU daily) to all infants till 1 year of age [5].

It is not clear whether vitamin D deficiency in Indian infants is due to lack of exposure to sunlight or some

other factors also play a role. Genetic polymorphisms of vitamin D receptor and high melanin content of skin may influence the cutaneous production of vitamin D in Indian infants [6,7]. We conducted this study to ascertain whether any correlation exists between sunlight exposure and vitamin D in Indian infants, and if yes, how much of sunlight exposure is required to achieve sufficient serum 25(OH)D levels (> 20 ng/mL) [8] by 6 months of age.

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METHODS

This prospective observational study was conducted in the Department of Pediatrics and Division of Endocrinology, Department of Medicine, University College of Medical Sciences and GTB Hospital, Delhi after approval from the Ethical Committee of the Institute

and obtaining written informed consent from the caregivers.

We enrolled predominantly breastfed, healthy infants aged 6-8 weeks, born at term, from the immunization clinic of our hospital. Only those born in a health facility with documented birth weight and gestation record were included. Low birth weight (birth weight <2500 g), small for gestational age infants, and NICU graduates were excluded. Infants with congenital malformations, history of seizures, clinical evidence of rickets, chronic systemic disorders, past hospitalization, history of receiving calcium or vitamin D supplements were also excluded. We also excluded infants born to mothers who had received supplemental vitamin D (in excess of 1000 IU/day) in antenatal or postpartum period. Children with a skin disorder such as ichthyosis or atopic dermatitis or any other condition where topical drug was applied were also excluded.

The delivery details of the infant – birth weight, gestational age, and mode of delivery – were noted from the birth record. Age at enrolment was calculated (in days), from the date of birth record. Mothers were asked to provide complete details of intake of calcium/other supplements during antenatal/postnatal period. Weight and length of all infants were recorded at enrolment, as per standard techniques. The baby's skin color was graded according to Fitzpatrick skin color scale [9]. The season of enrolment was stratified into (a) March to May, and (b) June to August.

Mothers were asked to maintain a weekly chart to quantify sunlight exposure. The Lund and Browder Chart [10] was provided to the mothers to mark all areas that were exposed to sunlight in the day. The marking was done daily with mention of exact duration (minutes) and timing (as per clock time) of sun exposure in the performa (**Fig. 1**). Data of one week were recorded on one sheet. Mothers were provided with 6 such sheets at a time. The filled performa were collected from them at 2.5 mo, 3.5 mo, 4.5 mo, and 6 months of age. Compliance to fill the forms was ensured telephonically on a weekly basis. The

mothers were also counseled to continue exclusive breastfeeding.

At enrolment, 3 mL of maternal venous blood sample was collected for estimation of serum 25 hydroxy-vitamin D [25(OH)D]. The venous sample from the infant for estimation of serum 25(OH)D was obtained at the end of study, at 6 months of age. All samples for 25(OH)D were centrifuged and the sera were stored in a deep freeze at -20°C . Serum 25(OH)D was estimated by radioimmunoassay (RIA) with kits manufactured by DiaSorin, USA (interassay variation: 11%; intra-assay variation: 12.5%; sensitivity: at or below 1.5 ng/mL). Serum 25(OH)D values were interpreted as per the following cutoffs – sufficient $\geq 20\text{ ng/mL}$, insufficient 12-20 ng/mL, and deficient $< 12\text{ ng/mL}$ [8].

Previous studies [11,12] showed the correlation between sunlight exposure and vitamin D levels to be around 0.34. For testing the significant correlation over null correlation taken as 0.1 with 80% power and 95% confidence level (one-sided: $r_a > r_0$), a sample of 98 subjects was sufficient. Adding 30% as follow-up loss during the follow-up study period, 130 healthy infants were needed to be enrolled for this study.

Statistical analysis: The data were entered in an Excel sheet. For each participant, total duration of sun exposure (minutes) was calculated for the whole day and also separately for morning hours (before 10 am), and afternoon hours (10 am to 3 pm). Based on the weekly duration of exposure and skin area exposed to sunlight, sun index was calculated for each infant as cumulative sun index (for the whole day exposure), morning sun index (for exposure before 10 am), and afternoon sun index (exposure between 10 am – 3 pm); as per the following formula [11]:

$$\text{Sun index} = (\text{minutes of sun exposure per week}) \times (\text{fraction of body surface area (BSA) exposed to sunlight})$$

Normality of continuous data was checked using skewness and kurtosis test. The strength of correlation between sun index and infant's serum 25(OH)D levels was quantified by Spearman/Pearson correlation depending upon the distribution pattern. Multiple linear regression using 'Enter' method was performed with infants' serum 25(OH)D level as the dependent variable. Independent variables included cumulative sun index, maternal serum 25(OH)D level, season during which exposure occurred, skin color of the infant, and maternal antenatal calcium intake [Model 1]. The analysis was repeated with morning sun index [Model 2] and afternoon sun index [Model 3], replacing the cumulative sun index in the Model 1. The models were aimed at identifying the independent variable that best predicted the infant's

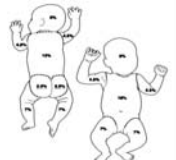
| Body surface area | Time of day | Duration | | |
|---|-------------|----------|-----------|---------|
| | | <30 Min | 30-60 Min | >60 Min |
|  | 7 AM–10 AM | | | |
| | 10 AM–3 PM | | | |
| | 3 PM–6 PM | | | |

FIG. 1 Performa for documentation of sun exposure in infants.

vitamin D status. Based on the results, the duration of sunlight exposure and body surface area of exposure required to achieve sufficient levels of vitamin D (>20 ng/mL) was calculated. Normality of model residuals was tested using skewness and kurtosis test. Data were analyzed using IBM SPSS version 20 statistical software.

RESULTS

Of 300 infants approached, 132 fulfilled the inclusion criteria and were enrolled between March to August 2015. A total 100 mother-baby pairs completed the 6 months follow-up (**Fig. 2**). Of these, 90 mothers had vitamin D deficiency (serum 25(OH)D <12 ng/mL). **Table I** compares the baseline characteristics of the infants who completed the study and those who were lost to follow-up. Infants who were lost to follow-up were older and had better anthropometric indices than those who completed the study. The duration of maternal calcium intake was less among those lost to follow-up without any difference in maternal serum vitamin D as compared to the former group. Of children who completed the study, 50 were enrolled between March to May 2015 (exposed to sun between March to October 2015) and the rest 50 between June to August 2015 (exposed to sun between June 2015 and January 2016).

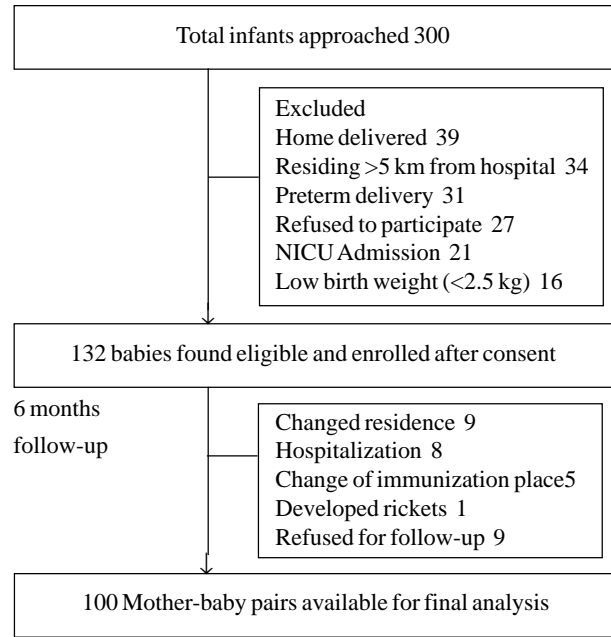


FIG. 2 Study flow chart.

Median (IQR) number of days for which data on sunlight exposure (in 100 infants) were available, was 130

TABLE I BASELINE CHARACTERISTICS OF STUDY POPULATION

| Parameter | Infants completed study (n=100) | Infants lost to follow up (n=32) | P value |
|---|---------------------------------|----------------------------------|---------|
| Age (d) | 48 (46-52) | 51 (50-56) | 0.001 |
| Parity | 2 (1-2) | 2 (1-2.8) | 0.12 |
| Gestation (wks) | 39 (38-40) | 38 (38-40) | 0.34 |
| Birth weight (kg) | 2.8 (2.5-3.0) | 2.8 (2.6-2.8) | 0.78 |
| <i>Anthropometric characteristics*</i> | | | |
| Weight (kg) | 4.2 (3.8-4.5) | 4.8 (4.6-5.0) | <0.001 |
| Length (cm) | 55 (54-56) | 57 (56.5-57.5) | <0.001 |
| Weight-for-age Z-score | -1.0 (-1.64 to -0.46) | -0.32 (-0.55 to 0.07) | <0.001 |
| Length-for-age Z-score | -0.35 (-0.97 to 0.19) | 0.28 (-0.20 to 0.68) | <0.001 |
| Weight-for-length Z-score | -1.14 (-1.61 to -0.63) | -0.55 (-1.09 to -0.06) | 0.002 |
| <i>Skin Fitzpatrick Score</i> | | | |
| Score 3 | 74 (74%) | 26 (81%) | 0.48 |
| Score 4 | 26 (26%) | 6 (19%) | |
| Received antenatal calcium supplementation | 93 (93%) | 31 (97%) | 0.68 |
| Duration of antenatal calcium supplementation (d) | 75 (45-90) | 45 (30-60) | <0.001 |
| Maternal 25(OH)D (ng/mL) | 6.30 (4.39 – 8.06) | 3.56 (2.24 – 7.91) | 0.19 |
| <i>Season of recruitment</i> | | | |
| June to August | 50 (50%) | 21 (65.6) | 0.155 |
| March to May | 50 (50%) | 11 (34.4) | |

All values in median (IQR).

(131,136) days. The median (IQR) weekly sunlight exposure in these infants was 17 (13,23) minutes, including 11 (9,15) minutes of sun exposure before 10 am, and 5 (3,9) minutes between 10 am to 3 pm. Mothers did not expose the infant to sunlight after 3 pm. Average fraction of body surface area (BSA) exposed to sunlight was 6.8% (median 6%; IQR: 4.6%, 7.4%). Overall fraction BSA exposed to sunlight ranged from 2- 40%.

The mean infant serum 25(OH)D level at 6 months of age was 10.9 (SD 5.66) ng/mL (median 9.2, IQR: 7.34,13.36; range: 1.41 to 27.5 ng/mL). Of 100 infants completing the study, 67 (67%) had serum 25(OH)D levels below 12 ng/mL (deficient), 23 (23%) had insufficient levels (12-20 ng/mL); and 10 (10%) had levels above 20 ng/mL (sufficient). The infants' serum 25(OH)D correlated positively and significantly with cumulative sun index (Spearman correlation co-efficient 0.461, $P < 0.001$). Duration of sun exposure and fraction of body surface area exposed to sunlight, independently also correlated significantly with infant serum 25(OH)D at 6 months of age ($r = 0.40$ and 0.459 , respectively; $P < 0.001$).

For Model 1, cumulative sun index, maternal vitamin D levels, and season of exposure were the major determinants of infant's vitamin D concentrations. Every unit increase in cumulative sun index increased the infant's serum 25(OH)D levels by 0.25 units. Maximum R^2 (0.367) was achieved in Model 3 when afternoon sun index replaced cumulative sun index in the model (**Table II**). Compared to maternal vitamin D concentration, the afternoon sun index was also a better predictor of infant's vitamin D level at 6 months of age. A change in afternoon sun index by 1 unit was able to increase the infant's 25(OH)D level by 1.1 units.

The 25th percentile of infant's serum 25(OH)D concentrations was 7 ng/mL. To achieve an additional 13 ng/mL (to attain sufficient level of 20 ng/mL), additional

12 units of afternoon sun index would be required. Assuming minimum fraction of body surface exposed as 0.4 (if the child lies prone exposed to sun required with diapers on), the duration of afternoon sunlight to achieve a sun index of 1 will be 2.5 minutes. Thus, to achieve an increase in sun index by 13 units, one would require to have afternoon sunlight exposure of approximately 30 minutes per week for at least 16-18 weeks. For the winter months, if the child is fully clothed with only face and hands exposed (approximately 10% body surface area), the required exposure is calculated as 2 hours per week. **Table III** summarizes the estimated sun exposures required to achieve sufficient serum 25 (OH)D level (>20 ng/mL) for different baseline levels.

DISCUSSION

The present study establishes a correlation between sun exposure during early infancy and the serum 25(OH)D levels in infants at 6 months of age from Northern India. Sun exposure between 10 am to 3 pm emerged as the best predictor of infant's vitamin D status, ahead of maternal serum 25(OH)D levels. In this study, we could also estimate the duration of sun exposure required to achieve sufficient vitamin D levels in breastfed infants at 6 months of age.

These results were obtained in infants born to vitamin D deficient mothers (90/100 had serum level of 25(OH)D <12 ng/mL). These children were probably born with a poor vitamin D status. We did not measure the infant's vitamin D status at enrolment but presumed it to be a surrogate reflection of maternal vitamin D status. Earlier studies have shown a good correlation between maternal vitamin D status and cord serum 25(OH)D levels at birth and up to 6 months of age [13,14]. Also, the observed sun exposure during the afternoon (5-6 minutes per week on 6% of body surface area) was markedly deficient as compared to that required (30 minutes per week on 40%

TABLE II REGRESSION ANALYSIS OF SUN INDEX AS PREDICTOR OF SERUM 25(OH)D LEVEL OF INFANT

| Independent factors | Cumulative sun index model (Model 1) | Morning sun index model (Model 2) | Afternoon sun index model (Model 3) |
|-----------------------------------|--------------------------------------|-----------------------------------|-------------------------------------|
| Sun Index | 0.25 (0.062, 0.440) [#] | 0.23 (0.01, 0.45)* | 1.07 (0.37, 1.78) [#] |
| Maternal serum 25OHD | 0.67 (0.44, 0.89) [#] | 0.68 (0.45, 0.91) [#] | 0.65 (0.42, 0.87) [#] |
| Season of exposure | 2.02 (0.12, 3.91)* | 2.10 (0.18, 4.01)* | 2.24 (0.39, 4.09)* |
| Antenatal calcium supplementation | 0.10 (-3.56, 3.77) | 0.18 (-3.54, 3.90) | -0.43 (-4.07, 3.21) |
| Skin color | 0.33 (-1.82, 2.48) | 0.33 (-1.90, 2.45) | 0.68 (-1.45, 2.81) |
| R^2 | 0.337 | 0.367 | 0.354 |

Serum 25(OH)D of the infant is the dependent variable in all models. Model 1, 2, and 3 represent cumulative sun index, morning sun index and afternoon sun index, respectively. Rest of the independent variables remain same in all 3 models; Residuals of all models approximated normal distribution; Values expressed as: unstandardized coefficient- B (95% confidence interval); * $P < 0.05$, [#] $P < 0.01$.

TABLE III SUNLIGHT REQUIREMENT TO ACHIEVE SUFFICIENCY LEVELS FOR DIFFERENT BASELINE SERUM 25 (OH) D LEVELS

| Baseline serum 25 (OH) D levels (ng/mL) | Sun index (SI) required* to attain sufficient level of 20 ng/mL | Duration of sun exposure per week (min) | | Increase in serum 25 (OH)D levels (95% CI) |
|---|---|---|---------------------|--|
| | | at 40% surface area | at 10% surface area | |
| 5 | 14 | 35 | 140 | 14.98 (12.96-17.00) |
| 7 | 12 | 30 | 120 | 12.84 (11.11-14.57) |
| 9 | 10 | 25 | 100 | 10.70 (9.26-12.14) |
| 11 | 8 | 20 | 80 | 8.46 (7.30-9.61) |
| 14 | 6 | 15 | 60 | 6.42 (5.55-7.29) |
| 16 | 4 | 10 | 40 | 4.28 (3.67-4.89) |

*Sun index 1.07 raises serum 25 (OH) D by 1 unit. We assumed that relationship between the 6 months serum 25(OH)D levels and sun index is same as relation between the change (6 months minus baseline vitamin D) and sun index. This assumption is made because it may not be ethical to follow an infant having low vitamin D level without giving the supplement.

of body surface area) to achieve sufficient serum level of vitamin D, in the infant, by 6 months of age. Our results showed that only the afternoon sun exposure could offset the disadvantages set up by low maternal vitamin D levels.

McCarty [15] raised concerns over the poor correlation on sunlight exposure questionnaire with serum 25(OH)D levels, owing to recall bias, interviewee fatigue due to long sessions, and not taking into account of other factors influencing vitamin D levels; like age variation, sunscreen application, skin color, dye, clothing and latitude. We tried to neutralize many of these factors by taking a cohort of same age (infant up to six months) with similar diet (breastmilk) and of same geographical location. The predesigned sun exposure charts consisted of easily understandable picture of infant's body to mark body surface area exposed to sunlight. The mothers were asked to fill the chart daily at the time of exposure itself and their compliance was ensured on telephonic call weekly, and direct visit on monthly basis. Ideally, to document sun exposure exactly, these charts should be validated on a daily basis, which was not possible in our study due to logistic constraints. Moreover, we did not verify the reported exposure by infant adapted ultraviolet dosimetry. Documentation of exact UVB exposure and simultaneous correlation with the infant's serum 25(OH)D would have also improved the validity of our analysis on sunlight exposure. Millen, *et al.* [1] have now shown that the validity of self-administered questionnaires on sun exposures closely matches the UV exposure measured by UVB solarmeter.

Earlier studies have also suggested tapping the potential of exposure to sunlight for increasing vitamin D production in the body. Few adult studies have also reported similar results and quantified adequate sunlight exposure required [11,12,16]. Hollick [17]

recommended one minimal erythemal dose (MED) of sunlight to whole body in young adults to increase vitamin D production to reach 20 ng/mL, which is comparable to taking oral dose of 10000-25000 IU of ergocalciferol. In another paper, he suggested exposure of 20% body surface area to 0.5 MED of sunlight for similar results [18]. Specker, *et al.* [19] found that infant serum 25(OH)D was significantly related to UV exposure and maternal serum 25(OH)D. They also concluded that an infant wearing a diaper would require 30 minutes outdoor exposure or 2 hours a week when fully clothed without hat to raise serum 25(OH)D to a level above 11 ng/mL (UV exposure score 2.0) [19]. Despite a geographical and racial variation, we got similar estimates in Delhi. Our advantage over the Specker study was a larger cohort size (100 vs 48 participants) and longer period of follow up (mean 126 days vs 7 days). Hall, *et al.* [20] demonstrated that sufficient exposure to sunlight was present during routine daily activities, to produce enough vitamin D in young college students, not considering vacations. Nurbazlin, *et al.* [11] found significant correlation of sun index with serum 25(OH)D levels ($r=0.180$; $P<0.001$) in rural and urban Malaysian women.

The afternoon sun exposure was found to be most critical in our study instead of morning sun exposure. Alshahrani, *et al.* [21] reported maximum UVB exposure during early morning (8-9 AM) or afternoon hours (2-3 PM) in Riyadh. These differences may be due to Zenith angle, which is responsible for variable cutaneous UVB absorption [18].

To conclude, our study reported significant positive correlation between sunlight exposure and infant's serum vitamin D, irrespective of maternal vitamin D levels. This finding holds importance in the present scenario where much stress is being laid upon infant/maternal

WHAT IS ALREADY KNOWN?

- Endogenous vitamin D synthesis occurs due to ultraviolet B rays of sunlight, which is affected by season, latitude, timing of day and melanin content.

WHAT THIS STUDY ADDS?

- There is a significant correlation between sunlight exposure and serum vitamin D in breastfed infants at 6 months of age.
- Afternoon sun exposure of 30 minutes per week for 16-18 weeks (starting from 6 weeks) over 40% exposed body surface can achieve sufficient vitamin D (20 ng/mL) in infants, at 6 months of age.

vitamin D supplementation. Though UVB radiation has been linked to increased risk of skin damage and skin cancer, it is highly unlikely that the modest exposure suggested in our study can be considered to increase the risk of skin malignancies. Our study has estimated the amount of sun exposure required to maintain sufficient vitamin D levels in infants. Whether this translates into effectiveness, can only be answered by randomized trials on controlled sun exposure for adequate duration as the intervention. Further research may highlight optimal sunlight exposure and minimize excessive commercial and rampant misuse of unwarranted vitamin D supplementation as a routine in otherwise healthy children.

Contributors: The study was conceived by PG, AD, PM, DS, RKM, and SVM contributed to the study design. Data collection was handled by PM and supervised by AD, DS, SVM and PG. SVM also supervised the laboratory work-up for vitamin D status. Statistical analysis was carried by RKM and PG. Literature search was conducted by AD, PM, and PG. Initial draft of the manuscript was written by PM and AD which was edited and refined by PG, DS, SVM, and RKM provided critical inputs to the draft manuscript. The manuscript was approved by all authors.

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Competing interests: None stated.

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