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Predicting ambient ultraviolet from routine meteorological data; its potential use as an instrumental variable for vitamin D status in pregnancy in a longitudinal birth cohort in the UK

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Background Maternal vitamin D status in pregnancy has been postulated to have important effects on intrauterine development. UVB radiation is not commonly measured but is the prime determinant of circulating 25-hydroxyvitamin-D [25-(OH)D] and is highly dependent on regional weather including cloud cover, ozone and sunshine hours.

Methods Using linear regression we described the relationship between estimated ambient-erythemal ultraviolet (eUV) exposure in Oxford (1990–95) and total hours of sunshine and month in order to forecast eUV in nearby regions, whilst adjusting for regional variations in weather. The forecast was validated with empirical data collected from Cornwall and then predicted for the Avon region. Total 98-day prenatal ambient-eUV was then predicted in 355 expectant mothers in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort and its relationship with maternal vitamin D status was determined.

Results Estimated ambient-eUV was strongly associated with measured ambient-eUV ($r^2 = 0.989$) with a near 1:1 prediction for the validation data set [$\beta = 0.99$, 95% confidence interval (CI) 0.913, 1.067 $r^2 = 0.980$]; strong seasonal associations were observed between eUV in the last trimester of pregnancy and maternal serum 25-(OH)D concentrations ($r^2 = 0.40$).

Conclusion This technique of prediction could be applied to existing cohorts allowing the relationship between maternal vitamin D status and the health of the offspring to be studied via instrumental variable analysis.

Keywords Epidemiology, maternal exposure, pregnancy, prenatal exposure delayed effects, seasons, ultraviolet rays, vitamin D, instrumental variable, ALSPAC

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Introduction

Vitamin D is an important pro-hormone and vitamin D insufficiency has been implicated in a number of different non-communicable diseases, including osteoporosis,[1,2] coronary heart disease,[3,4] peripheral arterial disease,[5] colon cancer,[6] prostate cancer,[7] breast cancer,[8] rheumatoid arthritis[9] and type I diabetes.[10] However, it is not clear for how long, or at what stage of life an individual needs to be vitamin D insufficient or deficient, in order to become predisposed to an increased risk of various diseases.

There is some evidence to suggest that *in utero* exposure to vitamin D is important in determining long-term outcomes including bone health,[11,12] prostate growth,[13] diabetes[14] and asthma.[15] However, the typical methods used to study the effects of vitamin D status on health outcomes are either observational,[16] or from an ecological perspective.[8,10] A common problem of observational or ecological data intrinsic to the method of collection is confounding and the 'ecological fallacy', respectively. However, it is difficult to identify the extent of this problem without employing more robust methods.

Although there are no randomized control trials that report long-term health outcomes of vitamin D supplementation in pregnancy, several trials are underway in this area e.g. Maternal Vitamin-D Osteoporosis Study (MAVIDOS) trial in Southampton, UK.[17] However, randomized control trials into the effects of vitamin D intakes on health outcomes can suffer from treatment contamination (confounding) by increased exposure to naturally occurring UVB. Whilst it may be possible to control for the differing effects of exposure by UVB-sensitive badges,[18] it increases the burden for the participant and may introduce another form of bias.

An alternative method of investigating causal effects is via instrumental variable analysis.[19,20] In order for a variable to be considered an 'instrument', it must be (i) independent of measured or unmeasured confounders (e.g. ambient-UVB radiation is unrelated to social economic position or any other confounder); (ii) associated with the exposure of interest (e.g. ambient-UVB radiation is associated with vitamin D status, given that UVB exposure is the prime determinant of vitamin D); and conditionally independent of the health outcome given the exposure and the measured or unmeasured confounders (e.g. ambient-UVB is unrelated to the health outcome after taking into account vitamin D status and the confounders). Because of the requirements of an instrument, the effect of the instrument (ambient-UVB) on the health outcome of interest (e.g. bone mass) is the product of the effect of the instrument (ambient-UVB) on exposure (vitamin D status), and the effect of the exposure (vitamin D status) on the health outcome of interest, therefore providing an unbiased estimate of the effect of the exposure (vitamin D status) independent of confounders.[19] We are unaware of any previous studies applying this technique of instrumental variable analysis to the study of vitamin D status.

25-hydroxyvitamin-D [25-(OH)D] is used as a marker of vitamin D status, and routine assays often assess both 25-(OH)D_3 (cholecalciferol) and 25-(OH)D_2 (ergocalciferol). Vitamin D_3 is primarily synthesized from exposure to UVB radiation (280–315 nm), with small quantities also contained in oily fish and cod liver oil, whereas vitamin D_2 is consumed within the diet. Exposure to UVB is dependent on intrinsic factors (e.g. skin type, age, clothing/skin protection,[21] personal behaviour[22]) and extrinsic factors [e.g. solar zenith angle (reflecting season, time of day and latitude), ozone, cloud cover, pollution and surface reflection]. Knowledge of extrinsic factors can be generalized to a population, whereas knowledge of intrinsic factors relies on accurate self-reporting or compliance with UVB-exposure meters, both of which are subject to confounding and other biases, and can be costly to collect.

Exploring the role of vitamin D on the intrauterine environment is impeded by the lack of availability of study populations in which vitamin D status or UVB exposure in the last trimester of pregnancy has been measured. However, since date and place of birth are widely available in nearly all cohorts, it should be possible to estimate ambient-UVB in pregnancy by combining this information with meteorological data, and subsequently make inferences with regards to the effects of UVB which is well known to be the primary source of vitamin D.[23–27]

We describe a method of estimating ambient-erythemal ultraviolet (eUV) from total hours of sunshine and month of year, which we then validated with data from weather stations of similar latitude and altitude. Subsequently, we confirmed that cumulative ambient-eUV exposure in the last trimester of pregnancy provides a useful estimate of maternal vitamin D status in the last trimester of pregnancy, based on measured concentrations of serum [25-(OH)D] in 355 expectant mothers from the Avon Longitudinal Study of Parents and Children (ALSPAC). We propose that ambient-eUV, derived using the method reported here, can be used to investigate the effect of maternal vitamin D status on a variety of different outcomes through the associations described in Figure 1.

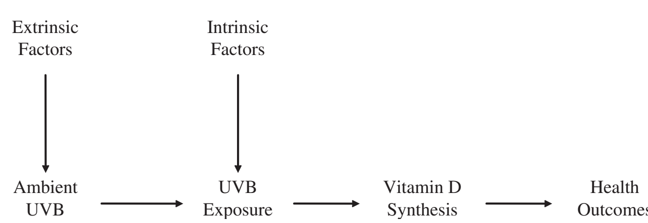

Figure 1 Proposed directed acylic graph linking ambient-UVB, UVB exposure, cutaneous vitamin D synthesis and health outcomes

Materials and methods

Participants

ALSPAC is a geographically-based birth cohort study, investigating factors influencing the health, growth and development of children. All pregnant women resident within a defined part of the former county of Avon in South West England with an expected date of delivery between April 1991 and December 1992 were eligible for recruitment, of whom less than 14 000 were enrolled[28] (http://www.alspac.bristol.ac.uk). Ethical approval was obtained from the ALSPAC Law and Ethics committee and relevant local ethics committees. Data in ALSPAC are collected by self-completion postal questionnaires sent to parents, by linkage to computerized records, by abstraction from medical records and from examination of the children at research clinics.

[25-(OH)D] determination

[25-(OH)D] was measured in serum from blood samples in 355 pregnant mothers using a chemiluminescence immuno-assay technique (DIASORIN 13040 Analyser, Saluggia, Italy) recognizing both 25-(OH)D_2 and 25-(OH)D_3 within and between batch precision for low and high QC (2005–06), 10–12 and 12–15%, respectively. Assay performance was within external-quality-scheme standards (DEQUAS) during this period.

eUV and meteorological data

The National Radiological Protection Board (NRPB), now part of the Health Protection Agency, recorded eUV at a number of different sites around the UK at the time mothers were being recruited (1990–94). eUV is a measure of UV exposure, which weights the wavelength according to its harmful effects (erythema). eUV is reported to the general population as it reflects how long you can stay in the sun (sun burn index). High levels of eUV are principally composed of short wavelengths of UVB, thus eUV is a reasonable proxy for UVB exposure. This relationship has been shown to hold with an error of $<$10% for all solar zenith angles (the position of the sun in the sky, which reflects season, time of day and latitude), where UVB with a wavelength of 280–315 nm is $\sim$7.55 (eUV).[29] Due to the proportionality between eUV and UVB, results will be presented with respect to eUV.

The NRPB measured eUV in Chilton, Oxfordshire ($\sim$60 miles East North East from Avon), from 1990, and Camborne, Cornwall ($\sim$180 miles South West from Avon), from 1993 (partially over the period of interest), but no eUV measurements were taken in the Avon region, which is geographically positioned between Oxford and Cornwall. Archive weather data, which included total hours of sunshine, were recorded at local Meteorological Office weather stations in Oxford, Cornwall and Avon from 1990.

Statistical analyses

Model generation

Due to the extrinsic properties that determine eUV exposure, specifically solar zenith angle and cloud cover, it is possible to derive a prediction model via linear regression using total monthly recorded sunshine (inversely related to cloud cover) and month (an indicator of average solar zenith angle) as a categorical exposure to predict eUV in Oxford. Model 1 assumes all other extrinsic factors such as ozone and surface reflection are constant. Higher-order sun terms were fitted (e.g. hours of sunshine squared and sunshine cubed), and nested models were compared using likelihood ratio tests to determine the most parsimonious model with the best fit. The fitted model may lead to an underestimation of residual variation as total monthly hours of sunshine are used instead of daily recorded sunshine. However, monthly estimates are simple to handle and are more commonly available than daily records.

Model validation

The validity of the model was tested using the eUV and hours of sunshine data collected in Cornwall. The model, derived as above, was used to predict eUV from the sunshine measures. These predicted eUV measures were then compared with the actual eUV measures recorded. This model assumes that any regional weather patterns are encapsulated in the local sunshine measurements.

Local eUV estimation

We then used this validated model to predict eUV exposure in Avon, adjusting for regional weather variations by using measures of local sunshine hours per day.

Individual last trimester prediction

Using locally estimated eUV, we estimated cumulative ambient-eUV in the 98 days pre-birth, i.e. in the last trimester of pregnancy, due to its postulated importance in neonatal bone development,[30] for every mother in the cohort. Monthly totals of predicted ambient-eUV were calculated and proportionally assigned over the 98 days pre-birth. This method of estimation assumes that extrinsic factors that influence eUV are uniform across the region.

Cumulative ambient-eUV in the last trimester of pregnancy and maternal [25-(OH)D]

The association between eUV in the last trimester and measured vitamin D status was investigated using linear regression, evidence of homoscedasticity led to [25-(OH)D] being $\log_e$ transformed and a non-linear model fitted by ordinary least squares (OLS).

Results

Model 1 described above proved to explain most of the residual variation between eUV, sunshine and month. However, the addition of total sunshine^2 (Model 2) resulted in a significant improvement in fit $P < 0.0001$, $r^2 = 0.9889$ and this model was therefore used for all analyses and predictions. Parameter estimates are listed in Table 1. Model 2 was then validated from data collected in Cornwall. There was no evidence of a mean difference between observed and predicted eUV {mean dif. (pred–obs) = 0.19, standard deviation (SD) = 0.87, [95% confidence interval (CI) (pred–obs): −0.25, 0.64.]}; 82.35% of predicted values were within 1 watts h m^{-2} eUV of observed values and 100% of values were within 2.5 watts h m^{-2} eUV of observed values. eUV varies seasonally from 2.4 watts h m^{-2} in the winter to 52.9 watts h m^{-2} in the summer. Pitman's test confirmed that there was no evidence that the measured or estimated standard deviation were different (SD $eUV_{measured} = 6.2$, SD $eUV_{predicted} = 6.2$, $P = 0.99$). The resulting predictions for Oxford, Cornwall and Avon are shown in the top, middle and lower panel of Figure 2, respectively. As has been shown, the model for predicting eUV from sunshine meteorological data in Oxford was able to predict eUV in Cornwall with a small level of error.

Table 1 Linear regression estimates used to estimate cumulative eUV exposure in the last trimester of pregnancy from data collected in Oxford, UK, from January 1990 to December 1994. January was coded as the baseline month

Parameter	Estimate	95% CI
Intercept	1.194	−0.32, 2.70
Monthly sunshine hours	−0.022	−0.05 to 0.01
Quadratic monthly sunshine hours	0.0002	0.0002 to 0.0002
Mean monthly increases in eUV[a] compared with January		
February	0.746	−0.31 to 1.80
March	2.957	1.67 to 4.25
April	5.478	3.93 to 7.03
May	9.549	7.92 to 11.18
June	12.942	11.33 to 14.56
July	13.029	11.33 to 14.73
August	9.606	7.88 to 11.33
September	6.120	4.68 to 7.56
October	2.218	0.92 to 3.52
November	0.449	−0.59 to 1.48
December	−0.050	−1.08 to 0.98

[a]eUV is measured in watts h m^{-2}.

Following the prediction of monthly eUV estimates, the date 98 days pre-birth was calculated for all individuals in the cohort, and the total eUV exposure for each individual obtained by imputation. The imputation resulted in typical seasonal variations, with the peaks and troughs of cumulative eUV exposure delayed approximately one-and-a-half months compared with the summer maximums and winter minimums.

Using linear regression, the association between vitamin D status [serum 25-(OH)D concentration] (the outcome) and imputed cumulative eUV exposure in the 3 months prior to birth (the exposure) was

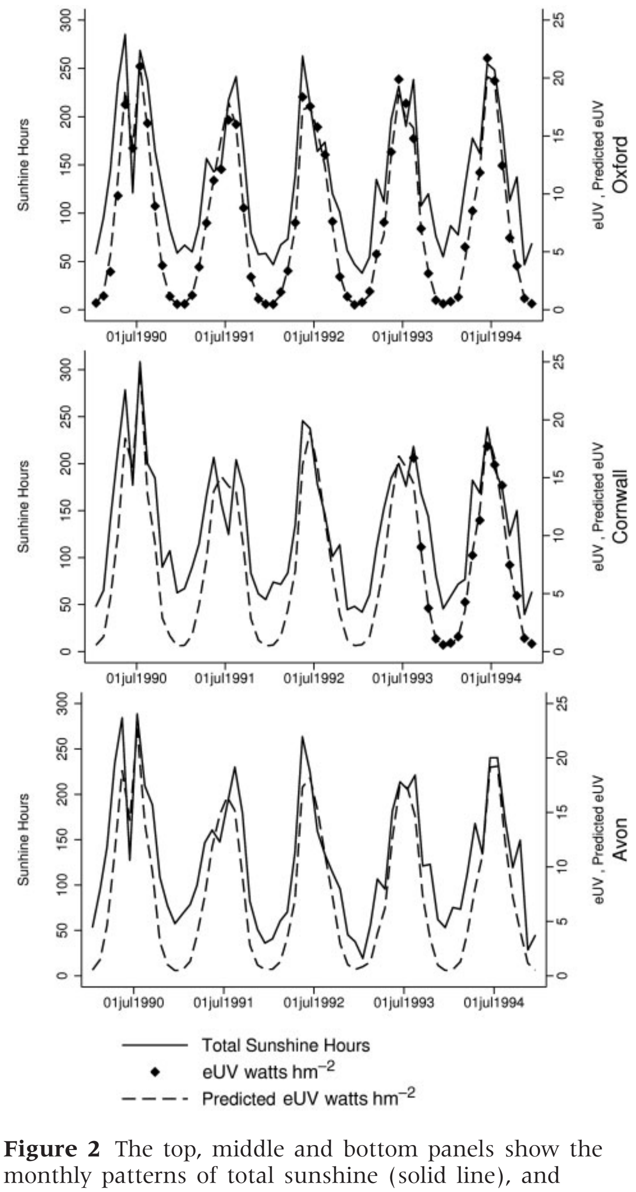

Figure 2 The top, middle and bottom panels show the monthly patterns of total sunshine (solid line), and predicted levels of eUV (dashed lines) at recording sites in Oxford, Cornwall and Avon, respectively. Measured eUV in the Oxford and Cornwall sites (solid diamonds) are displayed with predicted eUV

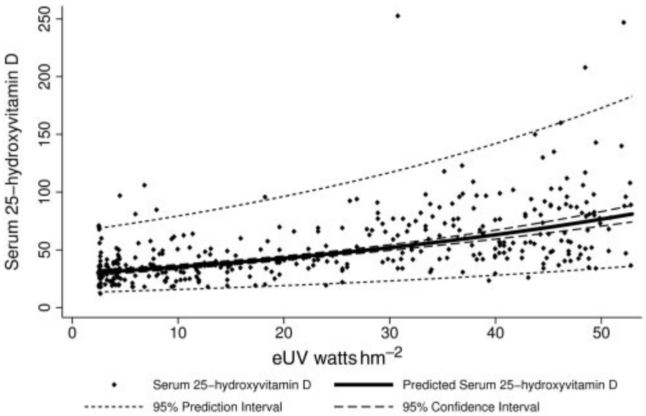

Figure 3 OLS regression was used to fit the following model: $E\{\ln[25\text{-(OH)D}] \mid \text{eUV}\} = \alpha + \beta(\text{eUV})$ where $\alpha = 3.365$ (95% CI 3.290, 3.440) and $\beta = 0.020$ (95% CI 0.017, 0.022), or the non-linear equivalent $E[25\text{-(OH)D} \mid \text{eUV}] = \exp(\alpha) \times \exp(\beta(\text{eUV}))$, eUV measured in watts h m^{-2}

investigated in the 355 study mothers. There was a strong linear association where a one-unit increase in eUV resulted in a 1.04 nmol^{-1} increase in serum [25-(OH)D] (95% CI 0.88, 1.22), $r^2 = 0.311$. However, the model was not homoscedastic and a non-linear model was fitted by OLS regression and $\log_e$ transforming [25-(OH)D]; this model was homoscedastic, and the fit was improved ($r^2 = 0.400$, $P < 0.0001$). The best prediction of serum [25-(OH)D] from eUV is the non-linear model presented in Figure 3. Further analyses controlling for the age of mother at birth and differing time of venopuncture did not affect these findings (as they were orthogonal with eUV exposure).

Discussion

We aimed to determine if retrospective locally recorded measurements of sunlight could be used to estimate UVB exposure, which would then allow us to make inferences about vitamin D status. Pairing measured hours of sunshine and eUV exposure from meteorological collection centres in Oxford enabled us to build a simple prediction model, which was then validated against data collected in Cornwall. This model was then used to predict UVB exposure during inception of the ALSPAC cohort study, based in Avon.

The association between estimated ambient-eUV in Avon and serum [25-(OH)D] in the 355 expectant mothers was strong. Forty per cent of the residual variation was explained by the final model, which did not take into account any behavioural variants,[22] age or skin type,[21] all of which are known to influence the production of vitamin D_3. Introducing mother's age at delivery into the model failed to improve the fit of the model ($P = 0.98$); all the mothers in the random subsample were White, and no information on specific skin tones was available. Due to variability in timing of blood sampling, the model may have been improved by estimating eUV exposure at a fixed time prior to venopuncture. However, the 'a priori' decision was to investigate the association between vitamin D levels and UVB exposure over the last 98 days of pregnancy due to its importance in neonatal bone development.[30]

Interestingly, the observed association between maternal vitamin D status and estimated cumulative eUV availability was non-linear, such that increasing levels of ambient-eUV exposure led to greater increases in vitamin D concentration than expected from a simple linear relationship, which is consistent with previous observations that the potential for synthesizing previtamin D in the winter is greatly reduced.[2] A likely explanation for this finding is that increased levels of background UVB are also associated with a greater proportion of time spent outside, consistent with a previous report that the amount of time outside typically increases in the summer.[22]

The intuitive method used to estimate ambient-UVB, based on routine data for areas where no measurements were taken, is equally applicable to studies in other cohorts where prenatal exposure to UVB is of interest and where there is access to locally collected meteorological data, it may also prove to be applicable in non-pregnant adults as well as those who are pregnant.

Limitations

The model described depends on a number of assumptions, which include month being used as an indicator of solar zenith angle (which controls for seasonal changes in eUV irradiation) and hours of sunshine being inversely related to cloud cover, thus allowing seasonal and local weather adjustment. Whilst both assumptions are plausible, there may be minor violations with respects to cloud cover, since light cloud cover may unduly lower the hours of total sunshine but have very little effect on ambient levels of eUV.[21] In addition, the model takes no account of variation in pollution, ozone, altitude, latitude or surface reflection. In spite of these omissions, the estimates of eUV in Cornwall, based on data collected in Oxford, which is >200 miles away, were very accurate. Since Avon is located 140 miles closer to Oxford than Cornwall, our model should perform as well, if not better for Avon, although we have no data to verify this assertion. In addition, our model for predicting last trimester eUV is the integral of a model estimating total monthly eUV from total hours of sunshine and sunshine,[2] and month as a categorical indicator of solar zenith angle; this may not be the most efficient or parsimonious method, but its simplicity makes the method the most accessible.

Finally we assume that eUV is a good proxy for UVB.[21,29]

Applications of the prediction

The method described has a number of possible applications in elucidating associations between UVB exposure and health outcomes, including its use as an instrument in instrumental variable analysis.

Epidemiological studies often suffer from confounding, and the ability to make unbiased inferences is important. There may be many mechanisms by which true UVB exposure and maternal vitamin D status may be confounded. For example, socio-economic position may influence the diet of the mothers, and their access to safe outside areas where they can be exposed to UVB. Whilst it is possible to adjust for proxies of socio-economic position, there is always the concern that residual confounding may exist, which is why we primarily rely on the use of randomized control trials to indicate causal associations. Recently epidemiology has adopted the use of instrumental variables to make causal inferences.[19,20] For a variable to be considered an instrument, it must be only related to the outcome of interest through the proposed causal path of instrument, exposure and outcome (see Figure 4).

Ambient-UVB radiation satisfies the criteria as an instrument because (i) ambient-UVB in pregnancy is associated with vitamin D status through the causal path of ambient-UVB, actual UVB exposure and vitamin D synthesis; (ii) time of conception and subsequently ambient-UVB levels in the last trimester of pregnancy is unrelated to all measured and unmeasured confounders; and (iii) ambient-UVB radiation is independent of the health outcome of interest given an individual's vitamin D status and confounding factors. This assumes that there is no other functional link between ambient-UVB and the health outcome of interest. If ambient-UVB in the last trimester of pregnancy is an instrument of true maternal UVB exposure, and true UVB exposure is the prime determinant of vitamin D_3, then it may be possible to estimate the causal effect of vitamin D_3 on a number of different health outcomes of the child (Figure 4).

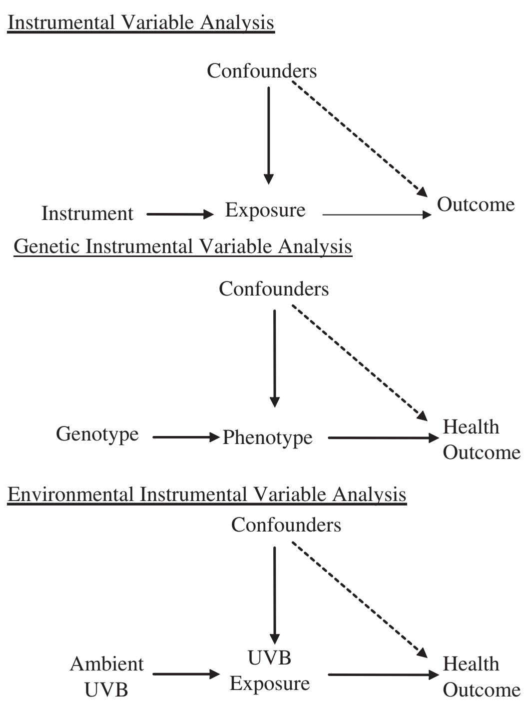

Figure 4 Causal directed acyclic graph illustrating the generic use of instrumental variables, its current application in genetic epidemiology and its proposed use as an environmental instrument

Because randomized control trials are costly and must be prospective by design, it will be many years before causal evidence can be provided about the effects of maternal vitamin D on long-term health outcomes of the child using such an approach. However, as date and location of birth can be collected retrospectively, then, with the judicious collection of meteorological data, it should be possible to reconstruct ambient levels of UVB in many established cohorts. After the instrument has been constructed, it will be possible to detect causal associations between maternal vitamin D concentration in the last trimester of pregnancy and a number of different health outcomes. As ambient-eUV explains 40% of the variation of serum 25(OH)D, we consider this a strong instrument, especially in comparison with many genetic instruments, which may explain <5% of the total variation of the phenotype. Therefore, the potential for unravelling many of the methodological problems associated with studies of maternal vitamin D exposure is great. In addition, if recorded data on actual maternal vitamin D concentrations exist, it will be possible to estimate the magnitude of the causal effect of maternal vitamin D concentration on the health outcome of interest.

The generalizability of this method and using environmental instruments in other cohorts requires careful consideration. Geographic and topographic separation of the cohort is an important factor to consider. ALSPAC is a birth cohort with a small catchment area of ($1340\,km^2$) which is only a little larger than New York City ($1200\,km^2$) and is not divided by mountain ranges, which can isolate weather patterns that influence cloud cover and therefore UVB; this, in turn, yields homogenous weather patterns across the county. However, if the cohort catchment area is large, topographically divided into areas of low and high social economic position, and ambient-eUV significantly differs in both locations; then this may be one way that the instrument assumption may be violated. However, if the populations are heterogeneous, and the location of the individual is known, stratified analyses could be performed, which should appropriately adjusted for any regional differences and still allow generalizable results. Therefore, careful consideration of cohort location is always required before ambient-eUV can automatically be considered an instrument.

In summary, this study illustrates how an environmental exposure (ambient-eUV) may be modelled using simple linear regression from routine meteorological data (sunlight). This method provides an attractive alternative to prospective randomized designs, which can be costly and time consuming, and could make it possible to utilize existing data from established cohort studies. In addition it illustrates how the causal link between ambient-eUV, eUV exposure and the production of vitamin D may be able to provide robust insights into the causal effects between vitamin D and a number of different health outcomes via instrumental variable analysis.

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Conflict of interest: None declared.

References

[1] Nieves JW, Barrett-Connor E, Siris ES *et al*. Calcium and vitamin D intake influence bone mass, but not short-term fracture risk, in Caucasian postmenopausal women from the National Osteoporosis Risk Assessment (NORA) study. *Osteoporos Int* 2008;**19:**673–79.

[2] Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004;**79:**362–71.

[3] Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol* 2006;**92:**39–48.

[4] Wong A. Incident solar radiation and coronary heart disease mortality rates in Europe. *Eur J Epidemiol* 2008; **23:**609–14.

[5] Melamed ML, Muntner P, Michos ED *et al*. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease. Results from NHANES 2001 to 2004. *Arterioscler Thromb Vasc Biol* 2008;**28:**1179–85.

[6] Soerjomataram I, Louwman WJ, Lemmens VE *et al*. Are patients with skin cancer at lower risk of developing colorectal or breast cancer? *Am J Epidemiol* 2008;**167:** 1421–29.

[7] Bodiwala D, Luscombe CJ, Liu S *et al*. Prostate cancer risk and exposure to ultraviolet radiation: further support for the protective effect of sunlight. *Cancer Lett* 2003; **192:**145–49.

[8] Mohr SB, Garland CF, Gorham ED *et al*. Relationship between low ultraviolet B irradiance and higher breast cancer risk in 107 countries. *Breast J* 2008;**14:** 255–60.

[9] Cutolo M, Otsa K, Uprus M *et al*. Vitamin D in rheumatoid arthritis. *Autoimmun Rev* 2007;**7:**59–64.

[10] Mohr SB, Garland CF, Gorham ED *et al*. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia* 2008;**51:**1391–98.

[11] Lanham SA, Roberts C, Cooper C *et al*. Intrauterine programming of bone. Part 1: alteration of the osteogenic environment. *Osteoporos Int* 2008;**19:**147–56.

[12] Lanham SA, Roberts C, Perry MJ *et al*. Intrauterine programming of bone. Part 2: alteration of skeletal structure. *Osteoporos Int* 2008;**19:**157–67.

[13] Konety BR, Leman E, Vietmeier B *et al*. In vitro and in vivo effects of vitamin D (calcitriol) administration on the normal neonatal and prepubertal prostate. *J Urol* 2000;**164:**1812–18.

[14] Fronczak CM, Baron AE, Chase HP *et al*. In utero dietary exposures and risk of islet autoimmunity in children. *Diabetes Care* 2003;**26:**3237–42.

[15] Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;**120:** 1031–35.

[16] Javaid MK, Crozier SR, Harvey NC *et al*. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* 2006; **367:**36–43.

[17] Controlled Clinical Trials Limited. *Maternal Vitamin D Osteoporosis Study: ISRCTN82927713*. http://www.controlled-trials.com/ (20 November 2008, date last accessed).

[18] Milne E, Corti B, English DR *et al*. The use of observational methods for monitoring sun-protection activities in schools. *Health Educ Res* 1999;**14:**167–75.

[19] Greenland S. An introduction to instrumental variables for epidemiologists. *Int J Epidemiol* 2000;**29:** 722–29.

[20] Lawlor DA, Harbord RM, Sterne JA *et al*. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;**27:** 1133–63.

[21] Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. *Prog Biophys Mol Biol* 2006;**92:**17–25.

[22] Diffey B. A behavioral model for estimating population exposure to solar ultraviolet radiation. *Photochem Photobiol* 2008;**84:**371–75.

[23] Lamberg-Allardt C. Vitamin D intake, sunlight exposure and 25-hydroxyvitamin D levels in the elderly during one year. *Ann Nutr Metab* 1984;**28:**144–50.

[24] McKenna MJ, Freaney R, Meade A *et al*. Hypovitaminosis D and elevated serum alkaline phosphatase in elderly Irish people. *Am J Clin Nutr* 1985;**41:**101–9.

[25] Davies M, Mawer EB, Hann JT *et al*. Seasonal changes in the biochemical indices of vitamin D deficiency in the elderly: a comparison of people in residential homes,

long-stay wards and attending a day hospital. *Age Ageing* 1986;**15:**77–83.

[26] Toss G, Almqvist S, Larsson L *et al*. Vitamin D deficiency in welfare institutions for the aged. *Acta Med Scand* 1980; **208:**87–89.

[27] Webb AR, Pilbeam C, Hanafin N *et al*. An evaluation of the relative contributions of exposure to sunlight and of diet to the circulating concentrations of 25-hydroxyvitamin D in an elderly nursing home population in Boston. *Am J Clin Nutr* 1990;**51:**1075–81.

[28] Golding J, Pembrey M, Jones R. ALSPAC – the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol* 2001;**15:** 74–87.

[29] McKenzie R, Smale D, Kotkamp M. Relationship between UVB and erythemally weighted radiation. *Photoch Photobio Sci* 2004;**3:**252–56.

[30] Javaid MK, Cooper C. Prenatal and childhood influences on osteoporosis. *Best Pract Res Clin Endocrinol Metab* 2002; **16:**349–67.