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# Vitamin B12 deficiency is associated with geographical latitude and solar radiation in the older population

Sebastián Cabrera [a], David Benavente [b], Miriam Alvo [a], Paola de Pablo [c], Charles J. Ferro [d,*]

[a] Nephrology Division, Clinical Hospital, University of Chile, Chile
[b] Section of Acute Nephrological Procedures, Clínica Las Condes, Chile
[c] Department of Rheumatology, University of Birmingham, Queen Elizabeth Hospital, UK
[d] Department of Nephrology, Queen Elizabeth Hospital, Birmingham, UK

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ABSTRACT

*Background:* Vitamin B12 and folic acid deficiency are common in the older and are associated with several conditions including anaemia, cardiovascular disease, cognitive impairment and cancer. Evidence from *in vitro* studies suggests that solar radiation can degrade both vitamins in the skin. Chile is the longest country in the world running perfectly North–South making it an ideal place to study potential associations of latitude and solar radiation on vitamin B12 and folic acid deficiency.
*Objectives:* The objective was to examine the association between vitamin B12 and folic acid deficiencies and latitude.
*Methods:* Plasma samples were collected from Chileans aged 65+ years ($n = 1013$) living across the whole country and assayed for vitamin B12 and folic acid concentrations as part of the Chilean Health Survey 2009–2010, which is a national representative sample study.
*Results:* Overall, the prevalence of vitamin B12 deficiency was 11.3%, with the prevalence in the North of the country being significantly greater than in the Central and South zones (19.1%,10.5%, and 5.7%, respectively; $P < 0.001$). The prevalence of folic acid deficiency in the whole cohort was 0.7% with no difference between the 3 geographical zones. Using logistic regression analyses, vitamin B12 deficiency was significantly associated with geographical latitude (OR 0.910 [95% confidence intervals 0.890–0.940], $P < 0.001$) and solar radiation (OR 1.203 [95% confidence intervals 1.119–1.294], $P < <0.001$). These associations persisted after adjustments for confounders (OR 0.930, $P < 0.001$ and 1.198, $P = 0.002$, respectively).
*Conclusions:* In the Chilean population of 65+, the prevalence of vitamin B12 deficiency is associated with living closer to the Equator and solar radiation. Although degradation by solar radiation might explain this observation, further work is required to establish the potential mechanisms. In countries that routinely fortify food with folic acid, efforts to identify vitamin B12 deficiency might be more cost-efficiently targeted in areas closest to the Equator.



## 1. Background

The primary source of vitamin B12 in the human diet is animal products (meat, fish, milk, eggs, shellfish, poultry). Vitamin B12 is essential for the synthesis of DNA and myelin sheaths as well as erythropoiesis. The principal cause of vitamin B12 deficiency is malabsorption [1,2]. Humans have low requirements and deficiency can take up to 3–5 years to develop, causing several disorders including megaloblastic anemia and demyelinating disease [1,2].

Folic acid is a water-soluble vitamin and the principal source is fresh fruit and vegetables [3]. Reserves in the body are scarce and folic acid deficiency manifests quickly in a matter of months. Folic acid deficiency is usually caused by poor dietary intake (eg. low vegetable consumption or alcoholism), malabsorption (eg. Coeliac disease) or metabolism problems (eg. polymorphisms of 5-methyltetrahydrofolate or drugs) and results in megaloblastic anaemia and neural tube defects in foetuses of folate-deficient mothers [3]. This has led many countries (eg. USA and Australia) to routinely fortify food with folic acid. In addition, folic acid has an important role in the metabolism of homocysteine, a recognised cardiovascular risk factor, with low folic acid concentrations associated with increased circulating homocysteine [3,4]. Both deficiencies of vitamin B12 and

* Corresponding author. Address: Department of Nephrology, Queen Elizabeth Hospital, Birmingham B15 2WB, UK. Tel.: +44 121 3715859; fax: +44 121 3715858.
*E-mail address:* charles.ferro@uhb.nhs.uk (C.J. Ferro).

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folic acid are common in older people where they are associated with anaemia, cardiovascular disease and cognitive impairment [1,2,4,5].

In recent years, data from *in vitro* studies suggests that solar ultraviolet (UV) radiation could degrade both vitamin B12 and folic acid in the skin [6–8]. Although the exact mechanisms are not well understood, recent evidence suggests that UV radiation can penetrate the dermal circulation and that the active forms of both vitamin B12 and folic acid are directly susceptible to photodegradation [6–8]. Furthermore, UV radiation produces free radicals that have been shown to degrade active forms of vitamin B12 [7]. These observations could have significant clinical implications, especially for older populations close to the Equator, where solar radiation is intense. Indeed, there is a need for clinical and epidemiological studies to examine this [6,7].

Chile is the longest country in the world with 4250 km that run North–South almost perfectly perpendicular to the Equator. In terms of latitude, the southern tip of Chile is equivalent to south Norway or south Alaska with the northern end corresponding to Western Sahara or Guatemala. (Fig. 1) Chile's geography, together with a relatively genetically homogeneous population, makes it an ideal country to investigate the effect of the latitude and solar radiation on human health. In this study we examined the association of latitude and solar radiation with vitamin B12 and folic acid deficiency in a Chilean national representative sample.

## 2. Methods

### 2.1. Study population

The 2009–2010 Chilean National Health Survey (CNHS2009/2010), commissioned by the Chilean Government Ministry for Health, was a cross-sectional survey using a multistage, stratified probability design to produce a representative sample of the Chilean population. A total of 5416 participants were recruited of which 1013 (20%) were 65+ years old [9]. Plasma vitamin B12 and folic acid concentrations were measured only among participants aged 65+. This study was approved by the ethical committee of the Pontificia Universidad Católica de Chile.

Weight and height were measured according to the World Health Organisation recommendations. Body mass index (BMI) was calculated using the formula of weight (kg)/height$^2$ (m). Normal BMI, overweight and obesity were defined as BMI of 18.5–24.9, 25–30 and >30, respectively [10]. Fruit and vegetable consumption was evaluated using portion cards showing different fruit and vegetables [9]. Salt consumption was estimated by measuring urinary sodium excretion from a spot sample [11]. Educational level was classified as low <5 years, medium 5–10 years and high >10 years in full-time education [12]. Alcohol consumption was assessed using a drinking short scale (Escala Breve de Beber Anormal EBBA),


**Fig. 1.** Geographical map of Europe and Chile. Chile has been inverted and placed so that the southern latitude of Chile corresponds to the same northern latitude in Europe. The southern tip of Chile is equivalent to south Norway or south with the northern end corresponding to Western Sahara.

which has been previously validated in the Chilean population. A definite alcohol excess intake is defined as an EBBA score ⩾2 [13].

Monthly family Income was estimated and graded in US dollars, as follows: low <$500, medium $500–1300 and high >$1300. Brachial blood pressure was measured 3 times in the morning after 5 minutes of rest using automated equipment (Omron HEM 742 ®) [14]. The average of these 3 readings are presented. Hypertension was defined as a systolic pressure >140 mm/Hg and/or a diastolic pressure >90 mm/Hg [15].

### 2.2. Biochemical analyses

Vitamin B12 and folic acid plasma concentrations were measured by chemiluminescence using the Advia CentaurMR immunoassay system (Siemens Healthcare, Germany). Vitamin B12 and folic acid deficiency were defined as plasma concentrations of <148 pmol/L and <12 nmol/L [2,6], respectively. Anti-endomysial IgA antibody concentrations were measured using a commercially available ELISA test (IMMCO Diagnostics). A high probability of coeliac disease was defined as an anti-endomysial IgA antibody concentration greater than 20UE/L [16,17]. The 4-variable modification of diet in renal disease (MDRD) equation, with serum creatinine recalibrated to be traceable to an isotope-derived mass spectroscopy method, was used to determine estimated GFR (eGFR). The presence of chronic kidney disease (CKD) was defined as an eGFR of <60 ml/min/1.73 $m^2$ [18].

### 2.3. Latitude

Chile is divided into 3 distinct geographical zones: North, Central and South, which in turn are divided into a total of 15 administrative regions each with a capital city. The latitude of the capital city was used as the index of latitude for participants living in that administrative region. Each geographic zone has a distinct climate (Fig. 1): the North Zone has high temperatures with dry weather similar to North Africa. The Central Zone is temperate with weather similar to the Mediterranean countries of Europe. The South Zone is characterized by weather similar to Scandinavia.

### 2.4. Solar radiation

Solar radiation data was obtained from the Solar Radiation Laboratory of the Republic of Chile [19]. Solar radiation was measured using bimetallic actinography (Robitzch–Fuess Type 58dc) in a standardised method throughout the country [19]. Annual radiation data from all measuring stations in each administrative region were collected and averaged to show average daily regional and geographical zone solar irradiation values. Solar radiation was expressed as $MJ/m^2/day$.

### 2.5. Statistical analyses

Summary statistics for continuous variables are presented as the mean ± standard deviation and median with an interquartile range for normally and non-normally distributed values respectively. Categorical variables are presented as percentages. Differences between categorical variables were compared with the Pearson-$\chi^2$ test. Continuous variables were compared using one-way analysis of variance (ANOVA) and the Kruskal–Wallis test for normally and non-normally distributed values respectively. Correlations were assessed by calculating Spearman's and Pearson's correlation coefficients as appropriate. A $P$ value <0.05 was considered statistically significant. All statistical analyses were performed SPSS software V21.0 (IBM, SPSS Inc, Chicago, IL).

Independent variables were pre-specified for analyses based on previous studies showing a relationship with vitamin B12 or folic acid deficiency (e.g. gender, age, fruit and vegetable consumption, serum liver enzyme concentrations, previous liver disease, family income, and raised anti-endomysial IgA antibody concentrations as a surrogate for coeliac disease) [1,2,4,5]. Independent variables found to have an association with vitamin B12 or folic acid deficiency reaching a significance level of ⩽0.2 on univariate analyses were also entered into selection algorithms. Linear regression was used to examine the relationships between vitamin B12 and folic acid concentrations and solar radiation and geographical latitude. Logistic regression was used to examine the independent determinants of vitamin B12 and folic acid deficiency. When variables were strongly correlated with each other (eg. serum creatinine and eGFR), only the one with the stronger association was entered. The two potential determinants of interest, solar radiation and geographical latitude are strongly correlated with each other thus separate models were created to examine their association with vitamin B12 and folic acid deficiency.

## 3. Results

The different environmental conditions for three geographical zones are shown in Table 1. As expected, there was a North to South decrease in solar radiation. There was a significant inverse correlation between latitude and solar radiation ($r = -0.951$, $P < 0.001$).

Participant characteristics are presented in Table 1 for the whole study population and by geographical zone. Patients in the south were more likely to have a lower educational level, lower average monthly incomes and a lower intake of fruit and vegetables. They also had lower average serum folic acid levels than participants in the north and centre. Patients of the central zone attained a higher educational level. There were no other differences across the three geographical zones.

### 3.1. Vitamin B12

Vitamin B12 concentrations were higher in the South compared with the North (Table 1; $P = 0.001$). In univariate analyses, vitamin B12 concentration was significantly associated with geographical latitude (beta 2.68 95% CI 0.50–4.90, $P = 0.02$). This association persisted after adjustment for factors known to be associated with vitamin B12 deficiency (gender, eGFR and alcohol intake: beta coefficient 95% CI 2.53.313–4.75; $P = 0.03$). There was a tendency for vitamin B12 concentrations to be associated with solar radiation (beta −5.75 95% CI-11.82–0.33) but this did not quite reach statistical significance ($P = 0.06$).

The prevalence of vitamin B12 deficiency in this cohort was 11.3%. Vitamin B12 deficiency was more common in the North than the South (Fig. 2A) (19.1% vs. 5.7%, $P < 0.001$).

In univariate analyses, the prevalence of vitamin B12 deficiency was associated with geographical latitude ($P < 0.001$), geographic zone ($r = -0.2$, $P < 0.001$), solar radiation ($P < 0.001$), eGFR ($P = 0.006$) and alcohol intake ($P = 0.04$). No associations were observed between vitamin B12 deficiency and age, suspected coeliac disease, family income, fruit and vegetables intake, BMI, smoking status, liver enzyme concentrations or previous liver disease.

Logistic regression analyses for vitamin B12 deficiency are presented in Table 2A. Vitamin B12 deficiency was associated with solar radiation and latitude. These associations persisted in all the models adjusted for gender, age, month of sampling, fruit and vegetable consumption, presence of liver disease, liver enzyme concentrations, excessive alcohol intake and estimated glomerular filtration rate with minimal changes in the estimates. A sensitivity analysis was performed defining vitamin B12 "deficiency" as the lowest 10% (<143 pmol/L) and 20% (<169 pmol/L) of the population

**Table 1**
Geographical zone characteristics and participant demographics.

| Geographical zone characteristics | Chile | North | Centre | South |
|---|---|---|---|---|
| Latitude limits (°) | 17.5–56.5°S | 17.5–29.1°S | 29.1–38.5°S | 38.5–56.5°S |
| Average solar radiation (MJ/m²/day) | 14.9 (12.8–15.3) | 19.0 (18.2–19.1) | 14.9 (14.8–15.2) | 11.0 (10.8–12.9) |
| *Participant demographics* | | | | |
| | All ($n$ = 1103) | North ($n$ = 280) | Centre ($n$ = 365) | South ($n$ = 368) |
| Female (%) | 61.5 | 56.4 | 65.2 | 61.7 |
| Age (years) | 73 (68–79) | 73 (68–78) | 74 (68–80) | 72 (68–78) |
| Body mass index (kg/m²) | 28.4 ± 5.3 | 28.1 ± 4.9 | 28.0 ± 5.2 | 29.0 ± 5.6 |
| *Years in education* (%)* | | | | |
| >10 years | 5.7 | 4.7 | 7.8 | 4.4 |
| 5–10 years | 26.7 | 35.1 | 29.9 | 17.1 |
| <5 years | 67.6 | 60.1 | 62.3 | 78.5 |
| None | 2.9 | 2.5 | 2.6 | 3.6 |
| *Monthly family income in US dollars (%)*** | | | | |
| <500 | 55.4 | 53.2 | 55.1 | 57.3 |
| 500–1300 | 30.0 | 26.8 | 31.4 | 31.0 |
| >1300 | 9.4 | 14.6 | 7.2 | 7.6 |
| Fruit and vegetable consumption (g/day) ‡ | 194 (114–320) | 217 (137–400) | 194 (125–320) | 182 (91–320) |
| Salt consumption (g/day) | 9.8 (8.2–11.4) | 9.9 (8.4–11.4) | 9.8 (8.0–11.3) | 9.4 (8.2–11.4) |
| Systolic pressure (mmHg) | 146 (132–164) | 145 (127–164) | 145 (132–162) | 147 (133–166) |
| Diastolic pressure (mmHg) | 76 (69–83) | 76 (63–85) | 76 (65–83) | 76 (69–83) |
| Hypertension (%) | 62.3 | 56.4 | 62.4 | 65.9 |
| eGFR (ml/min/1.73m²) | 76.6 ± 19.5 | 77.6 ± 19.7 | 75.2 ± 20.9 | 77.3 ± 17.9 |
| CKD (%) | 16.6 | 16.1 | 20.1 | 13.8 |
| Diabetes mellitus (%) | 21.7 | 21.8 | 20.8 | 22.6 |
| Alcohol problems (%) | 8.3 | 10.5 | 7.9 | 6.9 |
| Folic acid (ng/mL)† | 21.7 ± 9.5 | 22.1 ± 8.8 | 22.9 ± 10.3 | 20.2 ± 9.2 |
| Vitamin B12 (pmol/mL) | 391 ± 280 | 357 ± 280 | 404 ± 322 | 407 ± 232 |
| Suspected coeliac disease (%) | 2.0 | 1.9 | 0.6 | 3.3 |
| Gamma GT (UE/L) | 34.4 ± 51.8 | 37.9 ± 58.7 | 29.6 ± 34.7 | 36.2 ± 58.3 |
| AST (UE/L) | 21.1 ± 13.1 | 21.5 ± 12.2 | 20.5 ± 15.3 | 21.3 ± 11.7 |

Results for normally and non-normally distributed values are presented as mean ± standard deviation and median with interquartile range respectively.
AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; gamma GT, gamma glutamyl transpeptidase.
CKD was defined as an eGFR of <60 ml/min/1.73 m²; coeliac disease was defined as an anti-endomysial IgA antibody concentration greater than 20UE/L; hypertension was defined as a blood pressure >140/90 mm/Hg or a previous clinical diagnosis of hypertension; alcohol problems was defined as an EBBA score ⩾2; diabetes mellitus was defined as fasting glucose ⩾126 mg/L and/or a haemoglobin A1c ⩾7% or a previous clinical diagnosis of diabetes mellitus.
* Central zone > North and South zones ($P$ = 0.02).
** North zone > Central and South zones ($P$ = 0.02).
‡ South zone < North and Central zones ($P$ < 0.001).
† North and Central zones > South zone ($P$ = 0.02).

in terms of vitamin B12 concentrations. These different cut offs did not materially affect the results. The full results are presented in Supplemental Material 1.

### 3.2. Folic acid

Folic acid concentrations tended to be marginally lower in the South of the country compared with the North (Table 1; $P$ = 0.02). Folic acid concentrations did not decrease with decreasing latitude or increasing solar radiation.

The prevalence of folic acid deficiency was 0.7% with no difference between the 3 geographical zones (Fig. 2B). In univariate analyses, folic acid deficiency was associated with age ($P$ = 0.011) and suspected coeliac disease ($P$ = 0.007). There was no association with geographical latitude ($P$ = 0.42) or solar radiation ($P$ = 0.40). Adjusting for potential confounders did not change the results.

## 4. Discussion

To the best of our knowledge, this is the first study showing that both proximity to the Equator and increased solar radiation are associated with an increase prevalence of vitamin B12 deficiency. We did not find any relationship between either geographical latitude or solar radiation and folic acid deficiency in the same population. However, we cannot confidently exclude that such associations might exist given that flour is fortified with folic acid throughout the country [5]. This probably explains why the overall deficiency of folic acid is so low (0.7%) in this cohort.

In recent years, an increasing body of evidence has linked geographical factors such as latitude and solar radiation with human diseases [20]. Several studies have demonstrated an inverse association between solar radiation and prevalence of hypertension and cardiovascular death [20]. Vitamin D deficiency is frequently proposed as a potential mediator of these associations [21]. However, low concentrations of vitamin B12 and folic acid are also associated with increased cardiovascular risk, especially in the older population [3]. Intriguingly, some recent *in vitro* studies have demonstrated photodegradation of both vitamin B12 and folic acid by UV radiation [6,7]. However, clinical studies have been small and mainly confined to patients with skin diseases, such as vitiligo and psoriasis [22–24].

Vitamin B12 deficiency in the older population is a common (11% in this study) and an important public health issue [1,2]. The manifestation occurs late with significant neurological, cardiovascular and haematological consequences. Our study supports the theory that solar radiation might be an important factor in the development of vitamin B12 deficiency. We found that B12 deficiency was significantly more common in the North of Chile, which is close to the Equator. As an observational study we cannot assign causality and cannot exclude other environmental factors associated with vitamin B12 deficiency. For example, a recently published study reported a north–south gradient of vitamin B12 levels in Europe, and suggested that the difference found may be caused by the different dietary patterns between countries [25], but it did not evaluate the effect of the solar radiation or latitude and did not consider older populations. The mechanisms by which

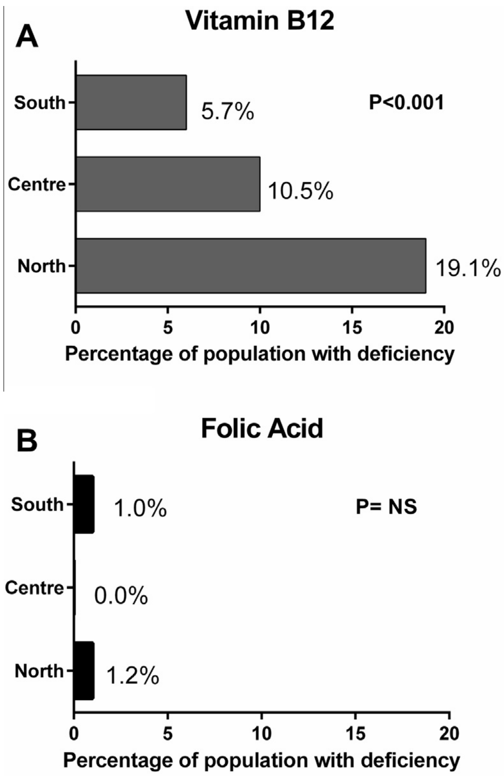


**Fig. 2.** Prevalence of vitamin B12 and folic acid deficiency in the 3 Chilean Geographical Zones. (A) The prevalence of vitamin B12 deficiency decreased from South to North ($P < 0.001$). (B) There were no differences in the prevalence of folic acid deficiency between the 3 zones.

**Table 2**
Binary logistic regression analysis for Vitamin B12 deficiency with (A) geographical latitude and (B) average daily solar radiation.

| | OR (95% confidence intervals) | *P*-value |
|---|---|---|
| *Panel A* | | |
| Unadjusted | 0.910(0.890–0.940) | <0.001 |
| Model 1 | 0.926(0.889–0.965) | <0.001 |
| Model 2 | 0.916(0.890–0.942) | <0.001 |
| Model 3 | 0.930(0.890–0.969) | 0.001 |
| *Panel B* | | |
| Unadjusted | 1.203(1.119–1.294) | <0.001 |
| Model 1 | 1.210(1.078–1.357) | 0.001 |
| Model 2 | 1.227(1.137–1.325) | <0.001 |
| Model 3 | 1.198(1.067–1.344) | 0.002 |

*Model 1:* was adjusted for variables described in the literature that are known to affect the prevalence of vitamin B12 deficiency: gender, age, month of sampling, fruit and vegetable consumption, presence of liver disease, liver enzyme concentrations (aspartate aminotransferase AST and gamma glutamyl transpeptidase gamma GT) and excessive alcohol intake.
*Model 2:* was adjusted for variables found to be associated with the prevalence of vitamin B12 deficiency in univariate analysis ($P \leqslant 0.2$): gender, estimated glomerular filtration rate and excessive alcohol intake.
*Model 3:* was adjusted for all variables in models 1 and 2.

solar radiation may affect levels of vitamin B12 are not well understood. Active forms of vitamin B12 have been shown to be directly susceptible to photodegradation by UV radiation, as well as indirectly by the formation of free radicals [7]. This has led to the hypothesis that skin darkening has occurred to protect vitamin B12 from photodegradation [7].

Folic acid is another important micronutrient that has been postulated by *in vitro studies* to be subject to photodegradation as a consequence of solar radiation [6]. Clinical series have also suggested that radiation from either sunshine or a sunbed, can decrease plasma folic acid concentrations [6,23,24]. Our study did not find any relationship between folic acid deficiency and latitude or solar radiation. However, flour is fortified with folic acid in Chile [5] and this probably explains the very low prevalence (0.7%) of folic acid deficiency found in the study population as previously reported [26].

Interestingly, vitamin B12 deficiency can be masked by normal or high folic acid levels [27]. This is important, because many countries, including Chile, routinely fortify food with folic acid, potentially increasing the risk of symptomatic B12 deficiency presenting late. Thus, although the overall prevalence of vitamin B12 deficiency in Chile is consistent with that reported in other countries [1,3], it is important to know in terms of public health that it is heterogeneous across the country, with areas with less latitude and more solar radiation having a higher prevalence of vitamin B12 deficiency. Indeed, in the North of Chile the prevalence of vitamin B12 deficiency was nearly 4 times higher than in the South suggesting increased efforts to identify and manage vitamin B12 deficiency should be targeted to zones closer to the Equator.

Our study has a number of strengths including a large population of over 1000 participants and a sampling methodology designed to produce a representative sample of the general population, which is relatively genetically and culturally homogeneous, with the majority being of mixed Spanish and aboriginal descent [28] with one of the smallest rates of immigration in Latin America and the world [29]. Another important strength was that all blood samples were processed in the same laboratory using the same methodology, and all data and samples were collected using the same standardised and uniform methods [9]. Moreover, anti-endomysial IgA antibodies were used as a surrogate for coeliac disease, a condition that is often undiagnosed in older people.

However, causality cannot be attributed given the observational nature of the study design. There were some regional differences in the population studied with participants in the South tending to have lower incomes, lower educational attainment and lower fruits and vegetables intake. Despite these differences the prevalence of vitamin B12 deficiency was lower in the South of the country. Interestingly, although food is fortifed with folic acid throughout Chile, folic acid concentrations were lower in the South consistent with a lower dietary quality. One of the main causes of vitamin B12 deficiency in older people is a low intake of animal derived foods. Although possible, this likely does not explain our observations as the multivariate models controlled for family income, which is associated with meat consumption [30]. Further, Chile has the 9th highest meat consumption per population in the world [31], and perhaps most importantly, studies in the elderly Chilean population with vitamin B12 deficiency have demonstrated that most had a more than adequate intake of animal products and vitamin B12 [26]. Furthermore a controlled study of supplementing the diet with foods high in vitamin B12 did not improve the prevalence of patients with vitamin B12 deficiency suggesting that low dietary intake is not a major contribution to vitamin B12 deficiency in the elderly Chilean population [32].

Although interesting, our findings require confirmation in other cohorts/populations. Further research is also required into potential confounding effects of diet and lifestyle especially in relation time spent outdoors and use of tanning machines. The influence of skin colour also requires further investigation. Indeed, it has been suggested that the susceptibility of vitamin B12 to photodegradation may be one of the reasons for skin darkening [7].

In summary, we report the first study showing a positive association between vitamin B12 deficiency with solar radiation and latitude. Although degradation by solar radiation might explain this observation, further work is required to establish the potential mechanisms. In countries that routinely fortify food with folic acid, efforts to identify vitamin B12 deficiency might be more cost-efficiently targeted in areas closest to the Equator.

## Competing interests

None of the authors have any competing interests to declare.

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## Appendix A. Supplementary material

The data used in this study can be obtained from the Chilean Ministry for Health on http://epi.minsal.cl/estudios-y-encuestas-poblacionales/encuestas-poblacionales/encuesta-nacionalde-salud/. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotobiol.2014.07.001.

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