Vitamin D serum levels and non-melanoma skin cancer risk

Carolina Morgado-Águila1∗, Guadalupe Gil-Fernández2, Orlando Rafael Dávila-Villalobos3, Jesús Pérez-Rey4, Purificación Rey-Sánchez5 and Francisco José Rodríguez-Velasco2∗

1Department of Plastic and Reconstructive Surgery, Cáceres University Hospital Complex, Extremadura Health Service, Cáceres, Extremadura, Spain
2Department of Nursing, Faculty of Medicine and Health Sciences, University of Extremadura, Badajoz, Extremadura, Spain
3Department of Gynecology and Obstetrics, Cáceres University Hospital Complex, Extremadura Health Service, Cáceres, Extremadura, Spain
4Department of Public Health, Extremadura Health Service, Extremadura Health Service, Cáceres, Extremadura, Spain
5Department of Nursing, Faculty of Nursing and Occupational Therapy, University of Extremadura, Cáceres, Extremadura, Spain

∗These authors contributed equally to this work.

ABSTRACT

Background. Skin cancer is one of the common malignancies. There is sufficient evidence that sunlight (ultraviolet radiation) contributes to the development of skin cancer, but there is also evidence that relates adequate serum levels of vitamin D produced on the skin by the action of ultraviolet radiation with the decreased risk of various types of cancers, including skin cancer. The aim of this study was to investigate the association of vitamin D serum levels among patients with non-melanoma skin cancers (basal cell carcinoma and squamous cell carcinoma) and controls.

Methods. A prospective observational case-control study was conducted in a sample of 84 subjects in Extremadura (Spain). Forty-one patients with histologically diagnosed basal cell carcinomas and squamous cell carcinomas and 43 healthy controls were randomly chosen to assess whether vitamin D (25(OH)D3) serum level, age and sex were related to non-melanoma skin cancer and to determine the possible risk of this type of skin cancer for these variables.

Results. When analysing serum vitamin D levels, we ensured that all our subjects, both cases and controls, had normal or low serum vitamin D levels, even though the samples were taken during months with the highest solar irradiance in our region. It is striking in our results that there was a higher percentage of subjects with deficits of vitamin D who did not have skin cancer (66%) than patients with deficits with these types of skin cancers (34%). When adjusting the model for age and sex, vitamin D values above 18 ng/ml increased the risk of suffering from non-melanoma skin cancer by nearly 7-fold (aOR: 6.94, 95% CI [1.55–31.11], p = 0.01).

Conclusions. Despite the controversial data obtained in the literature, our results suggest that lower levels of vitamin D may be related to a reduced incidence of non-melanoma skin cancer.
INTRODUCTION

Non-melanoma skin cancer (NMSC) refers to all types of cancer that occur in the skin and are not melanoma. Several types of skin cancer fall within the broader category of NMSC, with the most common types being basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (Griffin, Ali & Lear, 2016; Lomas, Leonardi-Bee & Bath-Hextall, 2012).

Non-melanoma skin cancers are considered the most frequent cancers (American-Cancer-Society, 2021; Griffin, Ali & Lear, 2016; Siegel et al., 2021), but many countries do not record the incidence of BCC or SCC, recording them only as a part of NMSC. Unfortunately, this category can include other skin tumours. Moreover, of those that do register and record incidence of non-melanoma skin cancers, some rely on notification, with or without histological proof. BCC and SCC commonly occur as multiple tumours in a single person. Studies may count the number of people with lesions or the number of lesions, so care must be taken when using these data. In addition, most studies are performed in predominantly white populations, so the incidence and risk factors for NMSC in black populations are even less clear. However, the incidence is rising by 3–7% per year in most countries (Lomas, Leonardi-Bee & Bath-Hextall, 2012; Lucas et al., 2006). The primary difficulty in measuring the incidence arises from poor registration practices in the majority of countries, as happens in Spain (Tejera-Vaquero et al., 2016).

The etiopathogenesis of these skin cancers seems to be multifactorial: both intrinsic and environmental factors are involved. There is evidence supporting the hypothesis that ultraviolet radiation causes skin cancer (Narayanan, Saladi & Fox, 2010), but at the same time, there is sufficient scientific evidence indicating the protective effect of vitamin D produced in the skin by ultraviolet radiation against different types of cancers, including NMSC (Reichrath, Saternus & Vogt, 2017). This paradoxical effect can be explained by evidence suggesting that the skin’s synthesis of vitamin D is self-limited and fades after five to ten minutes, while longer durations of sun exposure elevate the risk of skin cancer (IARC, 2008; Reichrath, 2006).

BCC and SCC are directly correlated with the accumulation of sun exposure on the skin over years. In contrast, cases of melanoma (and some BCCs) are slightly different. The pattern believed to induce melanoma is one of brief, intense sun exposure rather than damage from years of tanning (Ananthaswamy, 2001).

Apart from the classical actions in mineral metabolism, vitamin D performs other non-classical functions, such as the regulation of cell proliferation and differentiation in many cell types. Specifically, there are numerous functions of the skin regulated by vitamin D and/or its receptor (vitamin D receptor: VDR). Among them, we found inhibition of proliferation (Cordero et al., 2002), promotion of immunosuppression (Dusso, Brown & Slatopolsky, 2005), regulation of the follicular cycle (Bikle et al., 2006; Hochberg et al., 1985; Marx, Bliziotes & Nanes, 1986), suppression of tumour formation (Bikle, 2011) and stimulation of differentiation (Bikle, 2012; Bikle, Oda & Xie, 2004).

Laboratory studies suggest that vitamin D and its metabolites may reduce the risk of skin cancer. However, only a few epidemiological studies have assessed the association between vitamin D serum levels and NMSC risk, and these have shown controversial...
results: some report an association between higher vitamin D levels and an increased risk of developing skin cancer (Asgari et al., 2010; Eide et al., 2011; Liang et al., 2012; Soares et al., 2018; van der Pols et al., 2013; Vojdeman et al., 2019), while others report a decreased risk (Tang et al., 2011; Tang et al., 2010), and others found no association (van Dam et al., 2000; van Deventer, Kannenberg & du Toit, 2018). In 2014, Caini et al. (2014) found a statistically significant positive association with an increased risk of NMSC for high values of 25(OH)D$_3$ in their review and meta-analysis. Their findings seem to suggest that vitamin D intake via foods and/or supplements is neither associated with elevated skin cancer risk nor exerts any protective effect against skin cancer development; however, it is unclear whether an amount of UVB radiation exists that could ensure a health benefit without increasing the risk of skin cancer, suggesting that further research is needed.

Serum levels of 25(OH)D$_3$, and not 1,25(OH)$_2$D$_3$, are the best indicator of vitamin D status. Optimal serum levels of 25(OH)D$_3$ range between 30 and 60 ng/ml. Levels between 20 and 30 ng/ml are considered low, and levels between 60 and 100 ng/ml are considered high but still within the normal range. Values less than 20 ng/ml indicate a deficit of vitamin D, while values between 100 and 150 ng/ml are considered an excess of vitamin D (Holick, 2007).

The National Institutes of Health (NIH) in the United States (US) concludes that vitamin D levels equal to or greater than 30 ng/ml are adequate for cancer prevention (Bischoff-Ferrari et al., 2006; Gorham et al., 2005). However, according to clinical practice guidelines of the American Society for Endocrinology (Endocrine Society Practice Guidelines), serum vitamin D levels should not be investigated in patients without risk factors, as there is no scientific evidence for the benefit of screening for vitamin D in the general population (Tang et al., 2012). Efforts are currently being made to determine the optimal 25(OH)D$_3$ serum levels (and the intake of vitamin D needed to obtain them) for the prevention of cancer, as the pharmacological modulation of the endocrine system of vitamin D represents a promising strategy for cancer prevention and treatment and more specifically for skin cancer (Neale et al., 2016; Waterhouse et al., 2019). In this regard, the International Agency for Research on Cancer (IARC), which is part of the World Health Organization (WHO), considers that setting a lower limit of 20 or 30 ng/ml is currently inappropriate, as there are no randomized studies suggesting that maintaining those levels of 25(OH)D$_3$ can prevent any type of cancer or any other pathology. For this reason, they consider it premature to change the recommendations on the intake of vitamin D, added to the fact that there is insufficient evidence regarding the lack of harm due to prolonged intake vitamin D (IARC, 2008).

Apart from understanding the genetic basis of skin cancer, being able to modify environmental factors, such as vitamin D intake or sun exposure, is extremely important for the prevention and prognosis of this pathology. This is why we designed a study in which we assessed the relationship between NMSC and serum vitamin D levels. The objective of our study was to evaluate whether vitamin D levels have a differential effect in patients with NMSC versus healthy subjects and to determine the possible risk of NMSC for the variables sex, age and vitamin D levels and their model fit. Our study aims to provide knowledge
so that in future research, it could be possible to propose new measures regarding optimal levels of vitamin D that could help prevent or improve the prognosis of NMSC.

**MATERIALS & METHODS**

**Study design and ethics**

This was a prospective observational case-control study performed in Extremadura (a region located in southwestern Spain) at the Plastic Surgery Service of the Cáceres University Hospital Complex (the only reference Centre in Extremadura for Plastic Surgery).

Each subject provided written informed consent before entering the study, which met the requirements of the World Medical Association and the Declaration of Helsinki. This study was approved by the local Ethics Committee and the Institutional Review Board (IRB) of the University of Extremadura (protocol ID number: 74/2015 - approved on May 26th, 2015). The correspondence between codes and subjects' identification data was kept in a separate location that was only accessible to the coordinating investigator and/or investigators responsible for the centre or to the corresponding authorities of the hospital Clinical Research Ethics Committees (CREC, approved on April 29th, 2015). This study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies (von Elm et al., 2007).

**Study procedures**

We performed intentional non-probabilistic sampling. The recruited subjects were those who attended our outpatient plastic surgery consultation. The cases included patients suspected of suffering from NMSC. Apart from skin cancer, these subjects were otherwise healthy. They were recruited when surgery was proposed, after the clinical diagnosis. The suspected skin cancers were excised 2 weeks later, and at the same time peripheral venous blood samples were drawn. We also enrolled the control subjects in our outpatient plastic surgery consultation. They were healthy patients that consulted for other medical consultations, such as traumatic wounds or burns, and peripheral venous blood samples were drawn during this first visit after being thoroughly examined - photoexposed and non-photoexposed areas - by an experienced plastic surgeon to confirm that they did not have any type of skin cancer. Data were collected as previously described in other study from our research group (Morgado-Águila et al., 2020). Subsequently, of all the recruited patients, the selection was randomized using the SPSS program to generate random numbers (SPSS, Chicago, IL, USA).

Both cases and controls had similar sun exposure habits, and they all come from Extremadura, a mostly rural region in Spain with high solar radiation (latitude 40°).

Only patients over 18 years of age with BCC or SCC were included. Exclusion criteria for cases and controls included history of treatment with immunosuppressive drugs, having received an organ transplant or taking vitamin supplements in any form. We also excluded subjects with non-malignant lesions or other types of skin cancer or if we identified the coexistence of BCC or SCC.
Calculations of minimally detectable effect sizes, as well as of sample sizes for the present study, were performed using G*Power software (version 3.1.9, Universität Düsseldorf, Düsseldorf, Germany) (Faul et al., 2009). For our sample size (controls, \( n = 43 \), cases, \( n = 41 \)), we calculated the statistical power to detect medium effect sizes (anticipated Cohen’s \( d = 0.60/\text{OR} = 3 \) \( \alpha = 0.05 \), obtaining an output of 0.88.

We chose to divide the age variable in \( \leq 70 \) years old and >70 years old (\( \leq 70 \) age/>70 age) according to the closest cut-off value in relation to the mean (66.90) and median (66.50) of that variable in our study sample.

**Study population and sample size**

We initially evaluated 93 subjects. Cases and controls were recruited during 2016 and 2017 in the Plastic Surgery Department in Cáceres (Spain). The patient group consisted of 50 randomly selected Caucasian subjects with suspected non-melanoma skin cancer, and additionally 43 healthy Caucasian individuals were enrolled to serve as controls.

To avoid selective pressure (selection bias) in our sample, given that sex and age are variables related to the occurrence of the disease and that these variables were intended to be studied, we adopted the criterion of random selection of subjects with the same inclusion and exclusion criteria in both groups. A total of 33 subjects were males (controls, \( n = 9 \), cases, \( n = 24 \)), and 51 were females (controls, \( n = 34 \), cases, \( n = 17 \)).

Twenty-four patients had BCC and 17 had SCC. A total of 9 subjects were excluded, 5 of them because they had a non-malignant lesion or other type of skin cancer, and 4 of them because they had or previously had both skin cancers (BCC and SCC). Figure 1 illustrates the participant selection process.

**Skin samples**

Surgical excision was always performed by the same surgeon according to previous protocols (Brodland & Zitelli, 1992; Gulleth et al., 2010), and the skin samples were sent to the Pathology Laboratory for Histologic Study in a tertiary-level hospital. Pathologists studied the samples as part of their routine work, and they did not know that the patients were being studied for any reason other than clinical management.

**Vitamin D measurement**

Patients and controls were randomly chosen. However, to avoid biases regarding the measurement of vitamin D due to seasonal variations in serum vitamin D levels and possible differences in terms of solar radiation, all samples were recruited between May and August (2016 and 2017), that is, during the four months of the year with the highest solar radiation in our region (Fig. 2).

Blood specimens in both cases and controls were drawn in red stopper tubes, which contained no anticoagulant or preservative, and were subsequently frozen at \(-90^\circ\text{C}\).

An independent laboratory (STAB—Servicio de Técnicas Aplicadas a la Biociencia) in the nearby city of Badajoz (Spain) assessed the vitamin D levels in the peripheral venous blood samples. The aim of our study and the sample identification and categorization (case or control) were unknown to the laboratory personnel.
To determine plasma levels of vitamin D (25(OH)D$_3$), they used the Human 25(OH) Vitamin D ELISA kit from Abbexa Ltd., and 50 µl of undiluted sample were used for each point.

First, we stratified the absolute plasma levels of vitamin D provided by the laboratory into groups according to values commonly used (Holick, 2007): <20 ng/ml (deficit), 20–30 ng/ml (low limit), 30–60 ng/ml (normal), 60–100 ng/ml (high limit), 100–150 ng/ml (excess), and >150 ng/ml (intoxication). However, in our sample, the upper levels of the variable were not reached. For this reason, the vitamin D variable was re-stratified post hoc into two categories: ≤18 ng/ml and >18 ng/ml. We chose to divide the vitamin D variable according to the nearest rounded cut-off value in relation to the mean (19.28) and median (18.08) of that variable in our study sample, which was 18 ng/ml (≤18 ng/ml/>18 ng/ml).

**Study outcomes**

The aim of this study was to assess whether vitamin D levels have a differential effect in patients with NMSC versus healthy subjects and to evaluate their possible protective or risk effects. The primary outcome of the study was to assess differences in vitamin D levels between the study groups (cases vs. controls). Secondary outcomes were to determine the possible risk of NMSC for the variables sex, age and vitamin D levels and their model fit.
Figure 2  Average global irradiance in the provinces of Cáceres and Badajoz (Spain). Average global irradiance in the province of Cáceres (Spain) (A) and average global irradiance in the province of Badajoz (Spain) (B). The radiative flux is expressed in kWh m$^{-2}$ día$^{-1}$. The source of data was obtained from the State Meteorological Agency of Spain (SanchoÁvila et al., 2012).

Statistical methods
Initially, a descriptive analysis of serum vitamin D levels was performed in relation to the variables gender, age, and tumour type. The normality of the data was tested using the Shapiro–Wilk test, and vitamin D and age were not normally distributed. Likewise, the $\chi^2$ test was used to evaluate the relationship between the type of tumour and vitamin D levels.
Binary logistic regression was employed to calculate the odds ratios (OR) and 95% confidence intervals (95% CI) to assess the risk of skin cancer for vitamin D serum level, age, and sex among the subjects.

For other analyses, Fisher’s test was used for categorical data, Student’s t-test was used for continuous parametric data, and the Mann–Whitney and Kruskal-Wallis tests were used for continuous non-parametric data. The significance threshold was set at two-sided alpha = 0.05 for all analyses.

All statistical analyses were performed using SPSS for Windows, version 24.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Study sample
A total of 84 subjects were included in our study. The patient group consisted of 41 subjects with NMSC, and 43 healthy individuals were enrolled to serve as controls. A total of 33 (39.3%) were males, and 51 (60.7%) were females.

Our subjects were between 38 and 93 years old with a mean age of 66.9 ± 14.81 years (males: 70.55 ± 13.67; females: 64.55 ± 15.16). The mean age of the control subjects was 56.79 ± 10.78 years and that of the BCC and SCC patients was 76.50 ± 8.57 and 78.94 ± 12.63 years, respectively.

Vitamin D levels in cases and controls
Table 1 shows baseline serum vitamin D levels of the study groups. A total of 59.5% of our sample had serum vitamin D levels below 20 ng/ml, placing them in deficit according to the prescribed standards; 22 patients (26.2%) had serum vitamin D levels in the range of 20-30 ng/ml (low limit), and 12 patients (14.3%) had levels between 30–60 ng/ml (normal). Levels above stratum 30–60 ng/ml were not observed in our sample. A total of 67.4% of the subjects in the control group had serum vitamin D levels below 18 ng/ml, while 70.7% of the cases (BCC/SCC) had levels above 18 ng/ml.

Serum vitamin D levels for each group (controls, BCC cases and SCC cases) are shown in Table 2. The mean and standard error of the mean (SEM) vitamin D levels in the study sample were 19.28 ± 1.03 ng/ml (range 43.4). For sex and age, there were no statistically significant intra-group differences (Controls and Cases) in vitamin D levels. The highest serum vitamin D levels were observed in the case group. No statistically significant differences in serum vitamin D levels were observed in the case group (BCC/SCC). The mean and SEM of serum vitamin D levels in the case and control groups were 23.48 ± 1.31 ng/ml and 15.28 ± 1.34 ng/ml, respectively.

However, as shown in Fig. 3, we observed that there were statistically significant differences when comparing BCC (23.02 ± 1.68 ng/ml) and SCC (24.11 ± 2.12 ng/ml) cases to controls (15.28 ± 1.34 ng/ml) with respect to serum vitamin D levels in our sample (p = 0.00).

Vitamin D, age and sex and non-melanoma skin cancer risk
We used the binary logistic regression test to assess whether vitamin D serum level, age or sex were related to skin cancer risk. The vitamin D variable was dichotomized (according to...
Table 1  Baseline characteristics of the sample groups.

| Vitamin D^a | Cases (%) | Controls (%) | Total sample (%) |
|-------------|-----------|--------------|------------------|
| <20 ng/ml (Deficit) | 17 (41.5) | 33 (76.7) | 50 (59.5) |
| 20-30 ng/ml (Low Limit) | 15 (36.6) | 7 (16.3) | 22 (26.2) |
| 30-60 ng/ml (Normal) | 9 (22) | 3 (7) | 12 (14.3) |
| Vitamin D^b | Cases (%) | Controls (%) | Total sample (%) |
| ≤18 ng/ml | 12 (29.3) | 29 (67.4) | 41 (48.8) |
| >18 ng/ml | 29 (70.7) | 14 (32.6) | 43 (51.2) |
| Sex | Cases (%) | Controls (%) | Total sample (%) |
| Male | 24 (58.5) | 9 (20.9) | 33 (39.3) |
| Female | 17 (41.5) | 34 (79.1) | 51 (60.7) |
| Age | Cases (%) | Controls (%) | Total sample (%) |
| ≤70 age | 8 (19.5) | 39 (90.7) | 47 (56) |
| >70 age | 33 (80.5) | 4 (9.3) | 37 (44) |

Notes.
Data are expressed as frequencies (percentages) within the control and case groups.
Abbreviations: BCC, Basal Cell Carcinoma; SCC, Squamous Cell Carcinoma.
^aStandard vitamin D stratification (Holick 2007), values above stratum 30–60 ng/dl were not found in our sample.
^bStratification carried out according to the cut-off values defined in our sample.

Table 2  Vitamin D levels in sample groups.

| Controls | BCC | SCC |
|----------|-----|-----|
| Mean | SEM | p-Value | Mean | SEM | p-Value | Mean | SEM | p-Value |
| Sex | | | | | | | | | |
| Male | 15.82 | 3.12 | 0.92 | 22.25 | 2.08 | 0.49 | 26.16 | 3.33 | 0.36 |
| Female | 15.14 | 1.50 | | 24.91 | 2.88 | | 22.68 | 2.80 | |
| Age | | | | | | | | | |
| ≤70 age | 15.53 | 1.43 | 0.51 | 22.15 | 4.73 | 0.95 | 24.35 | 5.81 | 0.77 |
| >70 age | 12.93 | 3.58 | | 23.26 | 1.80 | | 24.06 | 2.36 | |

Notes.
25(OH)D_3 mean levels are expressed in ng/ml. p-Values were performed using the Mann–Whitney U-test.
Abbreviations: BCC, Basal Cell Carcinoma; SCC, Squamous Cell Carcinoma; SEM, Standard Error of the Mean.

-----
the cut-off values defined in our sample). Serum vitamin D levels above 18 ng/ml increased the risk of suffering from NMSC up to 5-fold (OR: 5.01, 95% CI [1.99–12.65], p = 0.00) compared to levels below 18 ng/ml. Moreover, when adjusting for vitamin D levels, sex and age, vitamin D accounted for nearly a 7-fold increase in the risk for developing NMSC (aOR: 6.94, 95% CI [1.55–31.11], p = 0.01) (Table 3).

It was also observed that being over 70 years of age increased the risk of developing NMSC, with this variable being the one that predicted the strongest association (OR: 40.22, 95% CI [11.19–145.62], p = 0.00). The sex variable was associated with a protective factor for females over males in the occurrence of NMSC (OR: 0.19, 95% CI [0.07–0.49], p = 0.00). In our sample, when adjusting for vitamin D levels, sex and age, data implied that the female sex was protected against NMSC (aOR 0.11, 95% CI [0.02–0.54], p = 0.00), and

Morgado-Águila et al. (2021), PeerJ, DOI 10.7717/peerj.12234
Figure 3  Error bars for mean 25(OH)D, levels of cases and controls (95% CI). There were statistically significant differences when comparing BCC and SCC cases to controls with respect to serum vitamin D levels in our sample. No statistically significant differences in serum vitamin D levels were observed between cases (BCC and SCC). Abbreviations: BCC, Basal Cell Carcinoma; SCC, Squamous Cell Carcinoma. 

$p$-Values were performed using the Mann–Whitney $U$-test.

Table 3  Association between vitamin D level, sex, age, and non-melanoma skin cancer.

|                          | OR (95% CI) | $p$-Value | aOR (95% CI) | $p$-Value |
|--------------------------|-------------|-----------|--------------|-----------|
| Vitamin D (>18 ng/ml reference) | 5.01 (1.99–12.65) | 0.00 | 6.94 (1.55–31.11) | 0.01 |
| Sex (Female reference)   | 0.19 (0.07–0.49) | 0.00 | 0.11 (0.02–0.54) | 0.00 |
| Age (>70 age reference)  | 40.22 (11.19–145.62) | 0.00 | 58.59 (11.26–304.90) | 0.00 |

Notes. Data shown as ORs (95% CIs) and $p$-values were obtained from binary logistic regression.

Abbreviations: 95% CI, 95% confidence intervals; OR, odds ratio; aOR, odds ratio adjusted for vitamin D levels, sex, and age; the reference category is the "control group".

being over 70 years of age increased the risk of NMSC by up to 58-fold (aOR 58.59, 95% CI [11.26–304.90], $p = 0.00$) (Table 3).

Omnibus tests of coefficients of the binary logistic regression model were significant for the variables vitamin D serum level, sex, and age. The model obtained a good fit of the variables, with a Nagelkerke $R$-squared of 0.72. The OR values increased in the adjusted model (aOR), as shown in Table 3.

**DISCUSSION**

It is well known that sex and age are related to NMSC (Garcovich et al., 2017; Joseph, Mark & Mueller, 2001; Oberszyn, 2008; Pascual et al., 2004; Scrivener, Grosshans & Cribier, 2002). Consistently with previous studies, our study parallely illustrates the fact that female sex serves as a protective factor NMSC, conversely, age above 70 augments the risk of NMSC.

Vitamin D levels in our sample were normal or low in any case, regardless of seasonal influence. This may be part of the so-called vitamin D deficiency pandemic. Vitamin
D deficiency and insufficiency are global health issues that need to be addressed, such as a pandemic, since they afflict more than one billion children and adults worldwide. They have become a major public health interest due to their association with acute and chronic illnesses, including cardiovascular disease, infectious diseases, diabetes mellitus, neurological disorders, osteoporosis, fractures, autoimmune diseases, and cancer. Improving vitamin D status worldwide would have remarkable effects on public health (Cashman et al., 2016; Holick, 2010; Holick, 2017; Holick & Chen, 2008).

There is controversy as to the optimal serum vitamin D levels that could be protective against cancer (Bischoff-Ferrari et al., 2006; Gorham et al., 2005; IARC, 2008). Low vitamin D serum levels were related to a lower incidence of NMSC in our sample. Vitamin D serum levels greater than 18 ng/ml may increase the risk of NMSC.

We want to emphasize that, as derived from the studies performed for skin cancer or even for other types of tumours, such as breast, colorectal or prostate cancer, vitamin D serum levels cannot be considered an independent risk factor for NMSC. It seems probable that other factors, such as ethnicity, phenotype, 25(OH)D₃ plasma levels, VDR gene polymorphisms and UV radiation exposure, are confounding factors that introduce heterogeneity (Giovannucci, 2005; Gnagnarella et al., 2020; Mahamat-Saleh, Aune & Schlesinger, 2020).

The best indicator of vitamin D status is 25(OH)D₃ serum level. If it is not possible to assess this variable in a vacuum due to the factors that could influence 25(OH)D₃ status, such as diet or dietary supplements, sun exposure (UV radiation) or body mass index (Wortsman et al., 2000), which should also be assessed. Even though the measurement thus far accepted is the measurement of 25(OH)D₃, news online (no scientific publications thus far) includes medical personalities stating that determining only vitamin D₃ values can lead physicians to make incorrect decisions. This could happen in the case of people who are vegetarians, for example, who tend to have low levels of D₃ and yet high vitamin D₂ levels. The same can happen in people who suffer some type of pathology as in patients with biliary cirrhosis, intestinal problems or in cases of celiac disease (Organización Médical Colegial, 2009). In our study, we considered the best variable in terms of biases to be the plasma levels of 25(OH)D₃ for trying to assess the relationship between vitamin D plasma levels and the risk of BCC or SCC in our population.

As stated in the previous sections that there are few published works linking vitamin D levels and NMSC, with inconsistent results (Asgari et al., 2010; Caini et al., 2014; Eide et al., 2011; Liang et al., 2012; Soares et al., 2018; Tang et al., 2011; Tang et al., 2010; van Dam et al., 2000; van der Pols et al., 2013; van Deventer, Kannenberg & du Toit, 2018; Vojdeman et al., 2019). We consider this to be a limitation for our study since we cannot design it from a more generalized hypothesis. In this regard, before accomplishing our work, we expected to find, based on information obtained from the bibliography collected and that had been exposed thus far in this text, studies that supported the protection of vitamin D against skin cancer.

In our bibliographic search, we found very few articles focusing on the possible relationship between vitamin D serum levels and NMSC risk and with very contradictory results.
For example, in a case-control study performed in older American men, a lower incidence of NMSC was found in subjects with higher concentrations of pre-vitamin D compared to subjects with lower concentrations (Tang et al., 2010). Lesiak et al. (2011) also found that mean serum 25(OH)D$_3$ was significantly higher in a control group of Polish origin than in cases of BCC.

Nevertheless, similar to the results obtained in our study, we also found opposing observations to this previous study. For example, a study in a Brazilian sample has been recently published in which it is concluded that patients with NMSC presented higher 25(OH)D$_3$ serum levels than controls, although even with those higher levels, they theoretically still had deficits of vitamin D (Soares et al., 2018). A similar finding was published by Eide et al. in a study of a sociodemographically diverse population in Detroit (Eide et al., 2011), who observed a relationship between higher (but not increased) 25(OH)D$_3$ serum levels and the risk of developing NMSC. Vojdeman et al. (2019) conducted a study on the Danish population and found higher levels of vitamin D in relation to an increased incidence of melanoma and NMSC.

In 2009, the authors of one of the multiple meta-analyses on vitamin D and skin cancer performed a bibliographic search looking for publications that studied the relationship between melanoma, NMSC, VDR gene polymorphisms, intake of vitamin D and serum levels of 25(OH)D$_3$ (Gandini et al., 2009). Specifically, in this meta-analysis, they found interesting results regarding polymorphisms and NMSC. In particular, VDR gene FokI and BsmI polymorphisms were associated with NMSC, even if in different directions. The FokI f allele exhibited a significant positive association with NMSC, while the BsmI B allele displayed a significant negative association with NMSC. However, they concluded that the association of vitamin D intake was less clear and that more studies were needed to clarify the role of diet in skin cancer.

Initially, we hypothesized that vitamin D serum levels would be normal or elevated, not deficient, in our patients due to the seasonal influence of sampling. However, despite living in a country with high solar radiation (40° latitude), all subjects had normal or low vitamin D levels, and no subject had elevated levels. The highest percentage of subjects (59.5%) was conspicuously deficient in vitamin D, despite having been studied in the four months with the highest solar radiation in our region of Extremadura (Fig. 2). This may be due to the so-called vitamin D deficiency pandemic (Cashman et al., 2016; Holick, 2010; Holick & Chen, 2008).

Indeed, we did not expect to observe more patients with deficiency of vitamin D in the three subgroups, but it is especially striking that, considering the supposed protective role of vitamin D in skin cancer, we found a greater number of patients with deficit that did not present tumours than even the sum of patients in deficit with either of the two types of tumours (Table 1). In fact, there was a significant difference between the levels of vitamin D and whether the subject suffered from skin cancer: the lowest levels of vitamin D were related to a lower incidence of NMSC in our sample.

Currently, based on IARC recommendations (IARC, 2008), physicians do not prescribe NMSC patients vitamin D supplements. In fact, according to the Endocrine Society Practice Guidelines, serum vitamin D levels should not be investigated in patients without
risk factors, as there is no scientific evidence for the benefit of vitamin D screening in the general population (Tang et al., 2012). To change recommendations on vitamin D levels across heterogeneous populations in terms of phototype, latitudes, intake of vitamin D from the diet and habits of sun exposure, clinical trials are needed, so that these recommendations will be truly evidence-based (Caini et al., 2014).

Based on our bibliographic search and results, we believe it is necessary to develop more epidemiological and experimental studies to elucidate the intricate relationship between vitamin D levels and the development of cancer, specifically skin cancer, in terms of sunscreen use, dietary changes, or possible use of vitamin D supplements or their analogues. Despite the striking results obtained in our work, we cannot propose any other new preventive or therapeutic measures regarding non-melanoma skin cancers in relation to vitamin D optimal serum levels.

We are aware that our study is not without limitations: we could only collect a limited number of participants, we were unable to match the ages in cases and controls, solar irradiiances were measured indirectly using data from the Spanish Meteorological Agency (AEMET), and we did not control for individual irradiiances or personal or professional factors that could alter sun exposure, and therefore vitamin D levels, of individuals.

However, we believe it is necessary to continue the current line of investigation, since it could lead to a radical change in the prevention and prognosis of this type of cancer.

**CONCLUSIONS**

The present study demonstrates that low vitamin D levels are associated with a lower incidence of BCC and SCC. Vitamin D serum levels above 18 ng/ml may increase the risk of NMSC.

Our results provide useful information for acquiring further knowledge on the relationship between vitamin D and NMSC. Further studies with a larger number of participants and more information about possible confounding factors are needed to confirm or reject our results.

**ACKNOWLEDGEMENTS**

The authors thank STAB (Servicio de Técnicas Aplicadas a la Biociencia, University of Extremadura, Badajoz, Spain) for the laboratory analyses. The authors also thank the Plastic Surgery Service in Cáceres (Spain) for their commitment in collecting blood from the patients and its maintenance and the Territorial Delegation in Extremadura for the Spanish Meteorological Agency (AEMET) for allowing us to use their data.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**

The publication of this work is financed by a grant of the Regional Government of Extremadura. European Regional Development Fund (FEDER) - “UNA MANERA DE HACER EUROPA” (No. GR18189). There was no additional external funding received for
this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**
The following grant information was disclosed by the authors:
Regional Government of Extremadura.
European Regional Development Fund (FEDER) - “UNA MANERA DE HACER EUROPA”: GR18189.

**Competing Interests**
The authors declare there are no competing interests.

**Author Contributions**
- Carolina Morgado-Águila conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Guadalupe Gil-Fernández, Orlando Rafael Dávila-Villalobos and Jesús Pérez-Rey analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Purificación Rey-Sánchez and Francisco José Rodríguez-Velasco conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

**Human Ethics**
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):
The study has been approved by the local Ethics Committee and the Institutional Review Board (IRB) of the University of Extremadura (protocol ID number: 74/2015 - approved on May 26th, 2015). The study has also been approved by the hospital Clinical Research Ethics Committees (CREC, approved on April 29th, 2015).

**Data Availability**
The following information was supplied regarding data availability:
The raw measurements are available in the Supplementary File.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.12234#supplemental-information.

**REFERENCES**

American-Cancer-Society. 2021. *Cancer facts & figures 2021*. Atlanta: American Cancer Society, 24–25.

Ananthaswamy HN. 2001. Sunlight and skin cancer. *Journal of Biomedicine and Biotechnology* 1:49 DOI 10.1155/S1110724301000122.
Asgari MM, Tang J, Warton ME, Chren MM, QuesenberryJr CP, Bikle D, Horst RL, Orentreich N, Vogelman JH, Friedman GD. 2010. Association of prediagnostic serum vitamin D levels with the development of basal cell carcinoma. *Journal of Investigative Dermatology* **130**:1438–1443 DOI 10.1038/jid.2009.402.

Bikle DD. 2011. The vitamin D receptor: a tumor suppressor in skin. *Discovery Medicine* **11**:7–17.

Bikle DD. 2011. Vitamin D receptor: a tumor suppressor in skin. *Discovery Medicine* **11**:7–17.

Bikle DD. 2012. Vitamin D and the skin: physiology and pathophysiology. *Reviews in Endocrine and Metabolic Disorders* **13**:3–19 DOI 10.1007/s11154-011-9194-0.

Bikle DD, Elalieh H, Chang S, Xie Z, Sundberg JP. 2006. Development and progression of alopecia in the vitamin D receptor null mouse. *Journal of Cellular Physiology* **207**:340–353 DOI 10.1002/jcp.20578.

Bikle DD, Oda Y, Xie Z. 2004. Calcium and 1, 25(OH)2D: interacting drivers of epidermal differentiation. *Journal of Steroid Biochemistry and Molecular Biology* **89-90**:355–360 DOI 10.1016/j.jsbmb.2004.03.020.

Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *American Journal of Clinical Nutrition* **84**:18–28 DOI 10.1093/ajcn/84.1.18.

Brodland DG, Zitelli JA. 1992. Surgical margins for excision of primary cutaneous squamous cell carcinoma. *Journal of the American Academy of Dermatology* **27**:241–248 DOI 10.1016/0190-9622(92)70178-I.

Caini S, Boniol M, Tosti G, Magi S, Medri M, Stanganelli I, Palli D, Assedi M, Marmol VD, Gandini S. 2014. Vitamin D and melanoma and non-melanoma skin cancer risk and prognosis: a comprehensive review and meta-analysis. *European Journal of Cancer* **50**:2649–2658 DOI 10.1016/j.ejca.2014.06.024.

Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, Moreno L, Damsgaard CT, Michaelsen KF, Molgaard C, Jorde R, Grinnnes G, Moschonis G, Mavrogianni C, Manios Y, Thamm M, Mensink GB, Rabenberg M, Busch MA, Cox L, Meadows S, Goldberg G, Prentice A, Dekker JM, Nijpels G, Pilz S, Swart KM, Van Schoor NM, Lips P, Eiriksdottir G, Gudnason V, Cotch MF, Koskinen S, Lamberg-Allardt C, Durazo-Arvizu RA, Sempos CT, Kiely M. 2016. Vitamin D deficiency in Europe: pandemic? *American Journal of Clinical Nutrition* **103**:1033–1044 DOI 10.3945/ajcn.115.120873.

Cordero JB, Cozzolino M, Lu Y, Vidal M, Slatopolsky E, Stahl PB, Barbieri MA, Dusso A. 2002. 1, 25-Dihydroxyvitamin D down-regulates cell membrane growth- and nuclear growth-promoting signals by the epidermal growth factor receptor. *Journal of Biological Chemistry* **277**:38965–38971 DOI 10.1074/jbc.M203736200.

Dusso AS, Brown AJ, Slatopolsky E. 2005. Vitamin D. *American Journal of Physiology-Renal Physiology* **289**:F8–28 DOI 10.1152/ajprenal.00336.2004.

Eide MJ, Johnson DA, Jacobsen GR, Krajenta RJ, Rao DS, Lim HW, Johnson CC. 2011. Vitamin D and nonmelanoma skin cancer in a health maintenance organization cohort. *Archives of Dermatology* **147**:1379–1384 DOI 10.1001/archdermatol.2011.231.
Faul F, Erdfelder E, Buchner A, Lang AG. 2009. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behavior Research Methods 41:1149–1160 DOI 10.3758/BRM.41.4.1149.

Gandini S, Raimondi S, Gnagnarella P, Dore JF, Maisonneuve P, Testori A. 2009. Vitamin D and skin cancer: a meta-analysis. European Journal of Cancer 45:634–641 DOI 10.1016/j.ejca.2008.10.003.

Garcovich S, Colloca G, Sollena P, Andrea B, Balducci L, Cho WC, Bernabei R, Peris K. 2017. Skin cancer epidemics in the elderly as an emerging issue in geriatric oncology. Aging and Disease 8:643–661 DOI 10.14336/AD.2017.0503.

Giovannucci E. 2005. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control 16:83–95 DOI 10.1007/s10552-004-1661-4.

Gnagnarella P, Raimondi S, Aristarco V, Johansson HA, Bellerba F, Corso F, Gandini S. 2020. Vitamin D Receptor Polymorphisms and Cancer. Advances in Experimental Medicine and Biology 1268:53–114 DOI 10.1007/978-3-030-46227-7_4.

Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, Newmark HL, Giovannucci E, Wei M, Holick MF. 2005. Vitamin D and prevention of colorectal cancer. Journal of Steroid Biochemistry and Molecular Biology 97:179–194 DOI 10.1016/j.jsbmb.2005.06.018.

Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, Newmark HL, Giovannucci E, Wei M, Holick MF. 2005. Vitamin D and prevention of colorectal cancer. Journal of Steroid Biochemistry and Molecular Biology 97:179–194 DOI 10.1016/j.jsbmb.2005.06.018.

Griffin LL, Ali FR, Lear JT. 2016. Non-melanoma skin cancer. Clinical Medicine 16:62–65 DOI 10.7861/clinmedicine.16-1-62.

Gulleth Y, Goldberg N, Silverman RP, Gastman BR. 2010. What is the best surgical margin for a Basal cell carcinoma: a meta-analysis of the literature. Plastic and Reconstructive Surgery 126:1222–1231 DOI 10.1097/PRS.0b013e3181eaa50.

Hochberg Z, Gilhar A, Haim S, Friedman-Birnbaum R, Levy J, Benderly A. 1985. Calcitriol-resistant rickets with alopecia. Archives of Dermatology 121:646–647 DOI 10.1001/archderm.1985.01660050098023.

Holick MF. 2007. Vitamin D Deficiency. New England Journal of Medicine 357:266–281 DOI 10.1056/NEJMr070553.

Holick MF. 2010. The vitamin D deficiency pandemic: a forgotten hormone important for health. Public Health Reviews 32:267–283 DOI 10.1007/BF03391602.

Holick MF. 2017. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. Reviews in Endocrine and Metabolic Disorders 18:153–165 DOI 10.1007/s11154-017-9424-1.

Holick MF, Chen TC. 2008. Vitamin D deficiency: a worldwide problem with health consequences. The American Journal of Clinical Nutrition 87:1080S–1086S DOI 10.1093/ajcn/87.4.1080S.

IARC WG. 2008. Vitamin D and cancer. IARC working group reports.

Joseph AK, Mark TL, Mueller C. 2001. The period prevalence and costs of treating nonmelanoma skin cancers in patients over 65 years of age covered by medicare. Dermatologic Surgery 27:955–959 DOI 10.1046/j.1524-4752.2001.01106.

Lesiak A, Norval M, Wodz-Naskiewicz K, Pawliczak R, Rogowski-Tylman M, Sysa-Jedrzejowska A, Sobjanek M, Wlodarkiewicz A, Narbutt J. 2011. An
enhanced risk of basal cell carcinoma is associated with particular polymorphisms in the VDR and MTHFR genes. *Experimental Dermatology* **20**:800–804 DOI 10.1111/j.1600-0625.2011.01328.

Liang G, Nan H, Qureshi AA, Han J. 2012. Pre-diagnostic plasma 25-hydroxyvitamin D levels and risk of non-melanoma skin cancer in women. *PLOS ONE* **7**:e35211 DOI 10.1371/journal.pone.0035211.

Lomas A, Leonard-Bee J, Bath-Hextall F. 2012. A systematic review of worldwide incidence of nonmelanoma skin cancer. *British Journal of Dermatology* **166**:1069–1080 DOI 10.1111/j.1365-2133.2012.10830.x.

Lucas R, McMichael T, Smith W, Armstrong BK, Prüss-Üstün A, World Health. 2006. *Solar ultraviolet radiation: global burden of disease from solar ultraviolet radiation / Robyn Lucas. [others ]*. Geneva: World Health Organization.

Mahamat-Saleh Y, Aune D, Schlesinger S. 2020. 25-Hydroxyvitamin D status, vitamin D intake, and skin cancer risk: a systematic review and dose–response meta-analysis of prospective studies. *Scientific Reports* **10**:13151 DOI 10.1038/s41598-020-70078-y.

Marx SJ, Bliziotes MM, Nanes M. 1986. Analysis of the relation between alopecia and resistance to 1, 25-dihydroxyvitamin D. *Clinical Endocrinology* **25**:373–381 DOI 10.1111/j.1365-2265.1986.tb01703.x.

Morgado-Aguila C, Rey-Sanchez P, Gil-Fernandez G, Costa-Fernandez MC, Rodriguez-Velasco FJ. 2020. Vitamin D receptor polymorphisms and non-melanoma skin cancer risk: a case-control study. *Journal of Clinical Medicine* **9**(12) DOI 10.3390/jcm9123819.

Narayanan DL, Saladi RN, Fox JL. 2010. Ultraviolet radiation and skin cancer. *International Journal of Dermatology* **49**:978–986 DOI 10.1111/j.1365-4632.2010.04474.x.

Neale RE, Armstrong BK, Baxter C, Romero BDuarte, Ebeling P, English DR, Kimlin MG, McLeod DS, OC RL, van der Pols JC, Venn AJ, Webb PM, Whiteman DC, Wockner L. 2016. The D-health trial: a randomized trial of vitamin D for prevention of mortality and cancer. *Contemporary Clinical Trials Communications* **48**:83–90 DOI 10.1016/j.cct.2016.04.005.

Oberyszyn TM. 2008. Non-melanoma skin cancer: importance of gender, immunosuppressive status and vitamin D. *Cancer Letters* **261**:127–136 DOI 10.1016/j.canlet.2008.01.009.

Organización Médico Colegial. 2009. Los niveles de Vitamina D Total, un nuevo marcador para indicar si una persona sufrirá un cáncer, una enfermedad inflamatoria o inmunológica. Available at [http://www.medicosypacientes.com/articulo/los-niveles-de-vitamina-d-total-un-nuevo-marcador-para-indicar-si-una-persona-sufrir%C3%A1-un%231](http://www.medicosypacientes.com/articulo/los-niveles-de-vitamina-d-total-un-nuevo-marcador-para-indicar-si-una-persona-sufrir%C3%A1-un%231) (accessed on 21 July 2021).

Pascual JC, Belinchon I, Ramos JM, Blanes M, Betlloch I. 2004. Skin tumors in patients aged 90 years and older. *Dermatologic Surgery* **30**:1017–1019 discussion 1019-1020 DOI 10.1111/j.1524-4725.2004.30307.x.

Reichrath J. 2006. The challenge resulting from positive and negative effects of sunlight: how much solar UV exposure is appropriate to balance between risks of vitamin D
deficiency and skin cancer? *Progress in Biophysics and Molecular Biology* 92:9–16 DOI 10.1016/j.pbiomolbio.2006.02.010.

Reichrath J, Saternus R, Vogt T. 2017. Endocrine actions of vitamin D in skin: relevance for photocarcinogenesis of non-melanoma skin cancer, and beyond. *Molecular and Cellular Endocrinology* 453:96–102 DOI 10.1016/j.mce.2017.05.001.

Sancho Ávila JM, Martín JRiesco, Jiménez Alonso C, Sánchez De Cos Escuin MC, Montero Cadalso J. 2012. Atlas De Radiación Solar en España utilizando datos del SAF De Clima De EUMETSAT. Available at [http://www.aemet.es/documentos/es/serviciosclimaticos/datosclimatologicos/atlas_radiacion_solar/atlas_de_radiacion_24042012.pdf](http://www.aemet.es/documentos/es/serviciosclimaticos/datosclimatologicos/atlas_radiacion_solar/atlas_de_radiacion_24042012.pdf).

Scrivener Y, Grosshans E, Cribier B. 2002. Variations of basal cell carcinomas according to gender, age, location and histopathological subtype. *British Journal of Dermatology* 147:41–47 DOI 10.1046/j.1365-2133.2002.04804.x.

Siegel RL, Miller KD, Fuchs HE, Jemal A. 2021. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians 71:7–33 DOI 10.3322/caac.21654.

Soares AM, Szefnfeld VL, Enokiara MY, Michalany N, Castro CH. 2018. High serum 25-hydroxyvitamin D concentration in patients with a recent diagnosis of non-melanoma skin cancer: a case-control study. *European Journal of Dermatology* 28:649–653 DOI 10.1684/ejd.2018.3401.

Tang JY, Fu T, Lau C, Oh DH, Bikle DD, Asgari MM. 2012. Vitamin D in cutaneous carcinogenesis: part I. *Journal of the American Academy of Dermatology* 67:803.e801–812 quiz 815-806 DOI 10.1016/j.jaad.2012.05.044.

Tang JY, Fu T, Leblanc E, Manson JE, Feldman D, Linos E, Vitolins MZ, Zeitouni NC, Larson J, Stefanick ML. 2011. Calcium plus vitamin D supplementation and the risk of nonmelanoma and melanoma skin cancer: post hoc analyses of the women’s health initiative randomized controlled trial. *Journal of Clinical Oncology* 29:3078–3084 DOI 10.1200/jco.2011.34.5967.

Tang JY, Parimi N, Wu A, Boscardin WJ, Shikany JM, Chren MM, Cummings SR, EpsteinJr EH, Bauer DC. 2010. Inverse association between serum 25(OH) vitamin D levels and non-melanoma skin cancer in elderly men. *Cancer Causes Control* 21:387–391 DOI 10.1007/s10552-009-9470-4.

Tejera-Vaquerizo A, Descalzo-Gallego MA, Otero-Rivas MM, Posada-Garcia C, Rodriguez-Pazos L, Pastushenko I, Marcos-Gragera R, García-Doval I. 2016. Skin cancer incidence and mortality in spain: a systematic review and meta-analysis. *Actas Dermo-Sifiliográficas* 107:318–328 DOI 10.1016/j.adengl.2016.02.015.

van Dam RM, Huang Z, Giovannucci E, Rimm EB, Hunter DJ, Colditz GA, Stampfer MJ, Willett WC. 2000. Diet and basal cell carcinoma of the skin in a prospective cohort of men. *The American Journal of Clinical Nutrition* 71:135–141 DOI 10.1093/ajcn/71.1.135.

van Deventer L, Kannenberg SMH, du Toit J. 2018. Vitamin D status in adult patients with nonmelanoma skin cancer in Cape Town. *South Africa: A Cross-Sectional Study* 57:922–927 DOI 10.1111/ijd.14068.
von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, Initiative S. 2007. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Epidemiology* 18:800–804 DOI 10.1097/EDE.0b013e3181577654.

van der Pols JC, Russell A, Bauer U, Neale RE, Kimlin MG, Green AC. 2013. Vitamin D status and skin cancer risk independent of time outdoors: 11-year prospective study in an Australian community. *Journal of Investigative Dermatology* 133:637–641 DOI 10.1038/jid.2012.346.

Vojdeman FJ, Madsen CM, Frederiksen K, Durup D, Olsen A, Hansen L, Heegaard AM, Lind B, Tjonneland A, Jorgensen HL, Schwarz P. 2019. Vitamin D levels and cancer incidence in 217, 244 individuals from primary health care in Denmark. *International Journal of Cancer* 145:338–346 DOI 10.1002/ijc.32105.

Waterhouse M, English DR, Armstrong BK, Baxter C, Romero BDuarte, Ebeling PR, Hartel G, Kimlin MG, McLeod DSA, O’Connell RL, vanderPols JC, Venn AJ, Webb PM, Whiteman DC, Neale RE. 2019. A randomized placebo-controlled trial of vitamin D supplementation for reduction of mortality and cancer: statistical analysis plan for the D-Health Trial. *Contemporary Clinical Trials Communications* 14:100333 DOI 10.1016/j.conctc.2019.100333.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. 2000. Decreased bioavailability of vitamin D in obesity. *American Journal of Clinical Nutrition* 72:690–693 DOI 10.1093/ajcn/72.3.690.