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ORIGINAL REPORT

Serum 25-Hydroxyvitamin D_3 Levels Are Associated With Breslow Thickness at Presentation and Survival From Melanoma

Julia A. Newton-Bishop, Samantha Beswick, Juliette Randerson-Moor, Yu-Mei Chang, Paul Affleck, Faye Elliott, May Chan, Susan Leake, Birute Karpavicius, Sue Haynes, Kairen Kukalizch, Linda Whitaker, Sharon Jackson, Edwina Gerry, Clarissa Nolan, Chandra Bertram, Jerry Marsden, David E. Elder, Jennifer H. Barrett, and D. Timothy Bishop

From the Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, St James's University Hospital, Leeds; Department of Dermatology, University Hospital Birmingham National Health Service Foundation Trust, Birmingham, United Kingdom; and Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA.

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S.B., J.R.-M., and Y.-M.C. contributed equally to this work.

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Corresponding author: Julia A. Newton-Bishop, MD, Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, St James's Hospital, Beckett St, Leeds LS9 7TF, United Kingdom; e-mail: j.a.newton-bishop@leeds.ac.uk.

The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
A cohort study was carried out to test the hypothesis that higher vitamin D levels reduce the risk of relapse from melanoma.

Methods
A pilot retrospective study of 271 patients with melanoma suggested that vitamin D may protect against recurrence of melanoma. We tested these findings in a survival analysis in a cohort of 872 patients recruited to the Leeds Melanoma Cohort (median follow-up, 4.7 years).

Results
In the retrospective study, self-reports of taking vitamin D supplements were nonsignificantly correlated with a reduced risk of melanoma relapse (odds ratio = 0.6; 95% CI, 0.4 to 1.1; $P = .09$). Nonrelapsers had higher mean 25-hydroxyvitamin D_3 levels than relapsers (49 *v* 46 nmol/L; $P = .3$; not statistically significant). In the cohort (prospective) study, higher 25-hydroxyvitamin D_3 levels were associated with lower Breslow thickness at diagnosis ($P = .002$) and were independently protective of relapse and death: the hazard ratio for relapse-free survival (RFS) was 0.79 (95% CI, 0.64 to 0.96; $P = .01$) for a 20 nmol/L increase in serum level. There was evidence of interaction between the vitamin D receptor (*VDR*) BsmI genotype and serum 25-hydroxyvitamin D_3 levels on RFS.

Conclusion
Results from the retrospective study were consistent with a role for vitamin D in melanoma outcome. The cohort study tests this hypothesis, providing evidence that higher 25-hydroxyvitamin D_3 levels, at diagnosis, are associated with both thinner tumors and better survival from melanoma, independent of Breslow thickness. Patients with melanoma, and those at high risk of melanoma, should seek to ensure vitamin D sufficiency. Additional studies are needed to establish optimal serum levels for patients with melanoma.

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INTRODUCTION

Vitamin D_3 has pleiotropic effects, via the vitamin D receptor (VDR), of relevance to cancer. These include effects on cell growth, differentiation, and apoptosis[1] and the regulation of tumor/immune system interaction.[2] Vitamin D_3 is synthesized in the skin as a result of exposure to sunlight and is present in a few foods and dietary supplements. Suboptimal 25-hydroxyvitamin D_3 levels are widespread,[3] and a role for vitamin D deficiency has been documented for a number of cancers.[4-7]

Known predictors of survival in patients with melanoma relate to histologic characteristics of the tumor,[8] particularly the Breslow thickness[9] (the distance from the granular cell layer of the skin to the deepest part of the tumor in millimeters), ulceration,[10] age,[11] tumor site, and sex,[12] and to social deprivation.[13,14] To identify other environmental predictors, we adopted a two-stage approach, involving a comparison of relapsed and nonrelapsed patients, then took a suggestive lead into a cohort study (hypothesis testing stage). For the initial retrospective study, we reasoned that by recruiting patients 3 or more years beyond initial diagnosis, we would remove some of the dominant effects attributable to the primary tumor, allowing subsequent environmental exposures to become more apparent.

In the pilot retrospective study comparing late relapsers and nonrelapsers, although not statistically

significant, the analyses were consistent with a role for vitamin D in relapse. We have subsequently tested this in an independent prospective study in which lower levels of 25-hydroxyvitamin D_3 were associated both with thicker, poorer-prognosis melanomas and with poorer outcome within Breslow thickness categories.

METHODS

Studies were approved by the Multi-Centre Research Ethics and the Patient Information Advisory Groups.

Pilot Retrospective Study

Melanoma cases had all been diagnosed at least 3 years previously without relapse. Patients were then eligible as "relapsers" if relapse occurred or as "nonrelapsers" if they were free of relapse.[15] Participants had a tumor Breslow thickness greater than 0.75 mm and were recruited between May 2000 and January 2005. Relapsers and nonrelapsers were frequency-matched for age, sex, and Breslow thickness. Participants were asked to complete a questionnaire (data collected on regular use of vitamins, minerals, fish oils, fiber, or other food supplements 1 year before interview) and give a single blood sample for DNA and for serum cryopreservation. In the United Kingdom, multivitamins usually contain 5 μg of vitamin D_3 (range, 2.5 to 10 μg), and most patients who took fish oils took them as cod liver oil containing similar quantities of vitamin D_3. These were therefore grouped in the analysis, which was based on use of any vitamin D supplementation compared with none.

Recruitment to the Prospective Cohort Study

The patients were population-ascertained incident patients with melanoma (stages I to IIIA) recruited in a geographically defined area of northern England from September 2000 through March 2008. Participants completed a questionnaire containing questions on drug intake and use of dietary supplements. Serum (single sample), plasma, lymphocytes, and DNA were stored. Recruitment (and therefore blood sampling) took place wherever possible within a period 3 to 6 months after diagnosis. Histopathology details of the primary tumor were extracted from pathology reports. Relapse/survival data were obtained from the patient by annual patient questionnaire, from the cancer registry, and by examination of clinical notes. Participants self-reported height and weight, and this was used to estimate the body mass index (BMI), as serum vitamin D levels vary with proportion of body fat, and vitamin D has complex metabolic interactions with fat cells.[16,17]

Serum 25-Hydroxyvitamin D_2 and D_3 Measurement

Concentrations of 25-hydroxyvitamin D_2 and D_3 (nmol/L) were measured in 100 μL of cryopreserved serum by liquid chromatography tandem mass spectrometry (see Appendix, online only). D_2 levels were almost uniformly undetectable ($<$ 10 nmol/L), and D_2 and D_3 levels were therefore summed. We shall refer to this henceforth as serum vitamin D levels.

Single Nucleotide Polymorphism Methodology

Known and putative functional polymorphisms in *VDR* were genotyped (Appendix, online only).

Statistical Methodology

Pilot retrospective study. Two-sided *t* tests were used for comparing continuous measures between relapsers and nonrelapsers. Associations between categoric variables were tested for using Pearson χ^2 tests. Unconditional logistic regression models were used to estimate the odds ratio (OR) and 95% CI for the effect of self-reported vitamin D supplementation and *VDR* genotypes on risk of relapse. These analyses were carried out using STATA version 9 (Stata Statistical Software, Release 9, 2005; StataCorp, College Station, TX).

Prospective cohort study. Adjusted means (least squares means in SAS) of serum vitamin D levels corrected for sex, age, BMI, and month sampled were calculated for different Breslow thickness groups using a general linear model. Relapse-free survival (RFS) and overall survival (OS) analyses were performed using Cox proportional hazards models to examine the associations between self-reported vitamin D supplement intake, measured serum vitamin D levels, and *VDR* status and risk of relapse and death from melanoma. Vitamin D supplement intake was categorized as a dichotomous variable according to whether the patient reported regularly taking multivitamins or cod liver oil a year before interview. Serum vitamin D levels were treated as a continuous variable in the analyses. In addition, serum vitamin D levels were categorized into tertiles for Kaplan-Meier survival analysis. Serum vitamin D levels were dichotomized using median levels as a cutoff point to investigate interactions with *VDR* genotype (coded as number of variant allele [0, 1, 2] in the additive models or presence of variant allele [0, 1] in the dominant models) in the survival analyses. Interaction was tested by comparing the model with main effects and an interaction term with a reduced model with only main effects, using the likelihood ratio test. Hazard ratios (HR) and their 95% CIs are presented. These analyses were carried out using the SAS/STAT statistical software version 9.1 for PC (SAS Institute, Cary, NC).

RESULTS

Pilot Retrospective Study

Overall, 131 (79%) of 165 eligible patients participated as relapsers, for whom 91 patients had complete supplement history, serum vitamin D level, and *VDR* data. We recruited 169 nonrelapsers (62% participation proportion) cared for by the same clinicians as the relapsers; 149 patients (88%) had complete data. Median time from

Table 1. Distribution of Dietary Supplement Use by Case-Control Status in the Pilot Retrospective Study

Reported Use of Supplements*	Nonrelapsers		Relapsers		*P*
	No.	%	No.	%	
No supplements taken	79	53.0	56	61.5	
Supplement(s) not containing vitamin D	8	5.4	7	7.7	
Vitamin D only/fish oils/both†	30	20.1	13	14.3	
Multivitamins/multivitamins and fish oils/multivitamins and fish oils and vitamin D†	32	21.5	15	16.5	
Total‡	149	100	91	100	$\chi^2_3 = 3.0$; $P = .4$
No supplements taken	79	53.0	56	61.5	Odds ratio = 0.7
Any supplement taken	70	47.0	35	38.5	95% CI, 0.4 to 1.2
No supplement/supplement(s) not containing vitamin D	87	58.4	63	69.2	Odds ratio = 0.6
Supplement(s) containing vitamin D	62	41.6	28	30.8	95% CI, 0.4 to 1.1

*Numbers vary between 242 and 248 because of missing data.
†Patients may have also taken supplements not containing vitamin D.
‡Supplement use ascertained for 240 participants because of missing data.

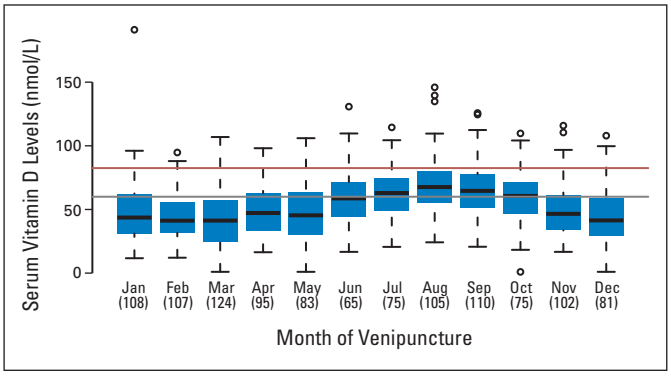

Fig 1. Variation in serum vitamin D levels by season of venipuncture in the prospective cohort study cases (number of patients in parentheses). A conservative optimal level of 60 nmol/L is indicated by the gray line, and a higher optimal level based on stabilization of parathyroid hormone levels at 75 to 90 nmol/L is indicated by the red line.

diagnosis to relapse/interview for the relapsers was 6.6 years (range, 3.1 to 28.1 years) and for nonrelapsers was 7.4 years (range, 3.2 to 31.7 years). There was no effect of season of surgery on case-control status.

A total of 47% (70 of 149) of nonrelapsers and 38% (35 of 91) of relapsers reported regular use of any supplements before relapse (OR = 0.7; 95% CI, 0.4 to 1.2). Sixty-two (42%) of 149 nonrelapsers and 28 (31%) of 91 relapsers reported regular intake of supplemental vitamin D 1 year before interview (OR = 0.6; 95% CI, 0.4 to 1.1; $P = .09$; Table 1). Thirty-seven percent of both female and male patients took vitamin D supplements ($\chi^2_1 = 0.005$, $P = .9$). There was no association between relapse and the postal code–derived Townsend score,[18] a measure of deprivation (*t* test, $P = .5$), or BMI.[15]

There was no effect on vitamin D serum levels of duration of sample storage or of BMI (data not shown). Measured serum vitamin D levels were higher in patients who reported taking vitamin D supplements (mean, 54 nmol/L; 95% CI, 51 to 58 nmol/L) compared with those not taking supplements (mean, 43 nmol/L; 95% CI, 40 to 47 nmol/L; *t* test, $P = .0001$). Nonrelapsers had higher levels (mean, 49 nmol/L; 95% CI, 45 to 52 nmol/L) than relapsers (mean, 46 nmol/L; 95% CI, 41 to 50 nmol/L; $P = .3$), although the difference was not statistically significant (*t* test, $P = .3$).

There was marginal evidence of a main effect of *VDR* alone irrespective of supplementation, as heterozygosity for both Cdx2 and FokI was inversely associated with relapse (OR = 0.5, 95% CI, 0.3 to 1.0, and OR = 0.5, 95% CI, 0.3 to 1.0, respectively) compared with homozygotes for the ancestral allele. However, the effect was not seen for rare homozygotes. None of the other single nucleotide polymorphisms showed a genotype difference by relapse status (data not shown). The sample size was too limited to investigate the joint associations with *VDR* and serum vitamin D levels on relapse.

Prospective Cohort Study Analysis

A total of 1,132 patients with melanoma were recruited (63% participation proportion). The focus of this analysis is on the 872 participants with a tumor thickness in excess of 0.75 mm recruited before October 2006 (median follow-up, 4.7 years). There had been 173 relapses and 141 deaths by November 2008.

Serum vitamin D levels varied with season as expected (Fig 1), and in the majority of patients with melanoma, the levels were suboptimal (64%), particularly in the months of November through June, taking a conservative estimate of 60 nmol/L as optimal. Parathyroid hormone levels are reported to increase at a level of 75 nmol/L,[19] so we took the 60 nmol/L level to be conservative. There was no effect of season of surgery on OS in an unadjusted comparison of winter compared with summer ($P = .62$). Serum vitamin D levels are known to vary with age and BMI, and in this study, levels were shown to be lower in younger individuals (test for trend, $P = .001$, adjusted for sex, month of venipuncture, and BMI) and those with higher BMI (test for trend, $P = .005$, adjusted for age, sex, and month). The mean serum vitamin D levels ranged from 54 nmol/L (95% CI, 51 to 56 nmol/L) for participants with a BMI less than 24.9, to 55 nmol/L (95% CI, 53 to 57 nmol/L) for BMI in the range 24.9 to 29.9, and 48 nmol/L (95% CI, 45 to 51 nmol/L) for BMI more than 29.9. Participants who reported use of vitamin D supplements had significantly higher serum vitamin D levels: 540 participants who were not taking supplements had a mean level of 50 nmol/L (95% CI, 48 to 52 nmol/L) compared with a mean of 60 nmol/L (95% CI, 57 to 63 nmol/L) for the 247 patients who reported taking supplements. The Spearman correlation between Breslow thickness and serum vitamin D levels was −0.10 ($P = .001$). Increased Breslow thickness was associated with lower serum vitamin D levels (test for linear trend $P = .002$, Table 2).

In univariate survival analysis (see Appendix, online only), later age was associated with poorer survival (HR per year of age for RFS was 1.03; 95% CI, 1.01 to 1.04), and RFS was lower for male patients, with an HR of 1.85 (95% CI, 1.37 to 2.49; Appendix Table A2, online only). Patients with limb tumors were at reduced risk of relapse compared with those with tumors on the trunk (HR = 0.65; 95% CI, 0.46 to 0.93). Increases of 20 nmol/L in serum vitamin D levels were associated with a reduced risk of relapse (HR = 0.75; 95% CI, 0.64 to 0.90) and OS (HR = 0.80; 95% CI, 0.68 to 0.96) consistently across seasons (Appendix Table A2 and Fig 2). Reported use of vitamin D supplementation showed no statistically significant effect on outcome in univariate (RFS: HR = 0.81, 95% CI, 0.56 to 1.17; OS: HR = 0.71, 95% CI, 0.47 to 1.09; Appendix Tables A2) or in multivariate analyses

Table 2. Association Between Serum Vitamin D Levels at Recruitment and Breslow Thickness in the Prospective Cohort Study

Breslow Thickness (mm)*	No. of Patients	Crude Mean	95% CI	Adjusted Mean†	95% CI
< 0.75	152	57.2	53.5 to 61.0	55.8	52.5 to 59.0
0.75-1	259	54.1	51.3 to 56.9	54.9	52.0 to 57.8
1-2	381	52.4	50.2 to 54.5	53.7	51.3 to 56.2
2-3	156	50.8	47.1 to 54.4	51.6	47.8 to 55.4
> 3	182	49.6	46.3 to 52.9	48.5	44.8 to 52.2

*Based on 1,130 melanoma cases.
†Adjusted for age, sex, body mass index, and month sampled using a general linear model ($P = .002$ for linear trend).

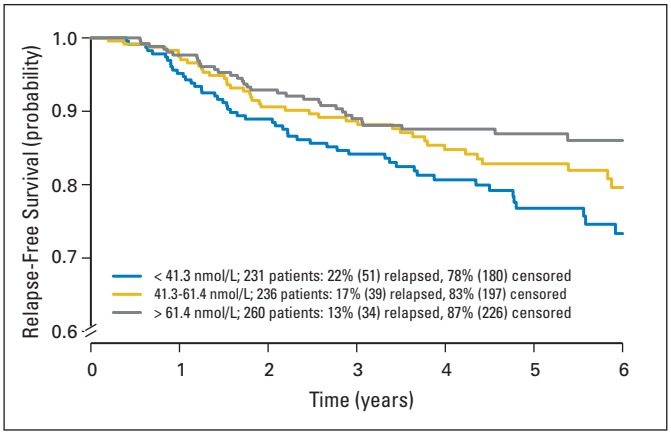

Fig 2. Kaplan-Meier curves of different serum vitamin D levels at interview (categorized based on tertile cutoff points) on relapse-free survival from melanoma in the cohort study.

(data not shown), being statistically consistent with both no effect and the estimate for the initial study.

Table 3 shows the results of multivariate analyses. After adjustment for age, sex, Townsend score, tumor site, Breslow thickness, and BMI, higher serum vitamin D levels were still seen to be protective for RFS and, to a lesser extent, for OS, consistently across the seasons (Table 3). The adjusted HRs for RFS and OS across seasons were 0.79 (95% CI, 0.64 to 0.96) and 0.83 (95% CI, 0.68 to 1.02), respectively, per 20 nmol/L increase in serum vitamin D levels. The inverse association with higher serum vitamin D levels, stratified by season, persisted through the range of levels detected based on tertile analysis. The HRs for RFS were 0.70 (95% CI, 0.42 to 1.14) and 0.57 (95% CI, 0.33 to 0.97) for the upper two tertiles of 41.3 to 61.4 nmol/L and more than 61.4 nmol/L, respectively, compared with the lowest tertile of $\leq$ 41.3 nmol/L group. Thus there was no evidence of an adverse effect of the highest observed serum levels on outcome.

There was no clear effect of *VDR* genotype on outcome in the cohort study, apart from weak evidence that inheritance of the BsmI A allele was adversely associated with RFS. Assuming a dominant model, carriage of the A allele increased risk of relapse (HR = 1.44; 95% CI, 1.02 to 2.03), but there was no evidence of association using a general 2 *df* model (P = .12, Appendix Table A2).

An interaction between serum vitamin D levels and *VDR* single nucleotide polymorphisms on outcome was investigated. Inheritance of the BsmI A allele is associated with poorer outcome from melanoma in those with low vitamin D levels, but not in those with high vitamin D levels (P = .02 for interaction, Table 4).

DISCUSSION

The pilot retrospective study was consistent with the hypothesis that vitamin D reduces the risk of relapse from melanoma. The subsequent cohort study tests and supports this hypothesis.

Most human diets provide little vitamin D, as it is primarily found in fatty fish. In some countries, foods are supplemented with the D_2 form; dietary supplements usually contain the more potent D_3 form.[20] Most people in Europe are reliant on cutaneous synthesis as a result of sun exposure for the majority of their vitamin D, because supplementation of foods is infrequent, and fatty fish does not generally form a large part of the diet. There remains some controversy around ideal levels of vitamin D; in a study of Swedish women aged 75 years or more, vitamin D levels less than 50 nmol/L doubled the fracture risk.[21] It is of note that the mean level among supplement takers in both the pilot and cohort studies was above this value, and the mean for those not taking supplements was lower. A higher level of 75 to 90 nmol/L is generally now thought to be optimal, because at this level, parathyroid hormone levels reach a nadir.[20] That patients with melanoma in the United Kingdom have low levels is not surprising, as suboptimal levels of vitamin D have been reported in many "healthy" populations,[20,22] and patients with melanoma often avoid sun exposure.

Table 3. Risk Factors for Relapse From Melanoma and Overall Death in the Multivariable Analysis From the Prospective Cohort Study

Parameter	Relapse From Melanoma		Overall Death	
	HR	95% CI	HR	95% CI
Age, per year	**1.01**	**1.00 to 1.03**	**1.04**	**1.02 to 1.05**
Sex, male *v* female	**1.69**	**1.10 to 2.61**	1.27	0.81 to 2.00
Townsend score, per quartile increase	1.06	0.89 to 1.26	1.11	0.92 to 1.33
Site				
Head and neck *v* trunk	0.90	0.50 to 1.62	0.85	0.47 to 1.53
Limbs *v* trunk	0.92	0.56 to 1.51	0.72	0.43 to 1.20
Others *v* trunk	1.10	0.52 to 2.32	0.43	0.18 to 1.04
Breslow thickness, per mm	**1.35**	**1.26 to 1.44**	**1.29**	**1.21 to 1.38**
BMI				
24.9-29.9 *v* < 24.9	0.63	0.39 to 1.03	0.82	0.50 to 1.33
> 29.9 9 *v* < 24.9	1.21	0.75 to 1.96	1.18	0.71 to 1.96
25-hydroxyvitamin D_3 level, per 20-nmol/L increase				
January to March	**0.72**	**0.56 to 0.96**	**0.72**	**0.54 to 0.96**
April to June	0.85	0.67 to 1.08	0.80	0.62 to 1.06
July to September	**0.77**	**0.63 to 0.96**	0.85	0.70 to 1.04
October to December	**0.77**	**0.60 to 0.98**	0.82	0.64 to 1.04

NOTE. Findings significant at the 5% level are highlighted in bold.
Abbreviations: HR, hazard ratio; BMI, body mass index.

Table 4. Interaction of *VDR* With Serum Levels of Vitamin D on Relapse From Melanoma, Under Different Inheritance Modes in the Cohort Study

Additive Model*						Dominant Model*					
	< 50.4 nmol/L		≥ 50.4 nmol/L				< 50.4 nmol/L		≥ 50.4 nmol/L		
VDR Genotype	HR	95% CI	HR	95% CI	*P*†	*VDR* Genotype	HR	95% CI	HR	95% CI	*P*†
Cdx2 (G>A)											
GA *v* GG	1.04	0.70 to 1.56	0.71	0.44 to 1.13	.22	GA+AA *v* GG	0.98	0.58 to 1.63	0.68	0.39 to 1.20	.36
AA *v* GG	1.09	0.49 to 2.44	0.50	0.19 to 1.29							
GATA (A>G)											
AG *v* AA	0.87	0.61 to 1.24	1.09	0.74 to 1.62	.40	AG+GG *v* AA	1.19	0.69 to 2.06	0.88	0.50 to 1.55	.45
GG *v* AA	0.75	0.37 to 1.53	1.19	0.54 to 2.63							
FokI (C>T)											
CT *v* CC	1.07	0.75 to 1.53	1.24	0.84 to 1.82	.59	CT+TT *v* CC	0.98	0.59 to 1.62	1.31	0.72 to 2.37	.46
TT *v* CC	1.15	0.57 to 2.35	1.54	0.71 to 3.32							
BsmI (G>A)											
GA *v* GG	1.98	1.39 to 2.83	0.90	0.61 to 1.32	.003	GA+AA *v* GG	2.53	1.39 to 4.59	0.96	0.55 to 1.69	.02
AA *v* GG	3.93	1.93 to 8.02	0.80	0.37 to 1.75							
ApaI (C>A)											
CA *v* CC	1.79	1.25 to 2.57	1.06	0.71 to 1.57	.05	CA+AA *v* CC	2.68	1.26 to 5.69	0.83	0.44 to 1.56	.02
AA *v* CC	3.22	1.57 to 6.61	1.12	0.51 to 2.47							
TaqI (T>C)											
TC *v* TT	1.88	1.33 to 2.67	0.85	0.57 to 1.26	.003	TC+CC *v* TT	2.30	1.30 to 4.05	0.87	0.50 to 1.53	.02
CC *v* TT	3.54	1.76 to 7.11	0.72	0.33 to 1.58							

NOTE. Serum levels of vitamin D ≥ 50.4 *v* < 50.4 nmol/L (cutoff based on median serum vitamin D levels). Genotypes for all six *VDR* single nucleotide polymorphisms were in Hardy-Weinberg equilibrium. The BsmI, ApaI, and TaqI polymorphisms were in linkage disequilibrium (D′ = 0.97 to 0.99) as were Cdx2 and GATA (D′ = 0.97).

*Analyses are adjusted for age, sex, site, and Breslow thickness.

†*P* value for interaction.

The difficulty of designing and implementing prospective studies for melanoma, which could address multiple hypotheses relating exposure to prognosis, prompted the novel retrospective study design in which only patients who had survived at least 3 years were recruited. Although it was itself logistically challenging, the comparatively short time frame of a retrospective study permits prioritization and focus of hypotheses, enabling longer-term cohort studies to be instigated. A major problem associated with interpreting retrospective studies involves recall bias, and in this study, this might potentially relate to reporting of vitamin D supplementation. However, serum vitamin D levels corroborated the history given by the participants, in that supplement takers had significantly higher levels, reducing this concern. The other major problem with the interpretation is confounding, such as from socioeconomic status, as deprivation is potentially related to both prognosis and prevalence of supplement taking; in this analysis, taking vitamin D did not vary by socioeconomic status, as indicated using the Townsend score. Supplement takers ate more fruit and less meat, but there was no effect of these dietary factors on relapse.[15] Other possible sources of bias in a retrospective study are acknowledged, such as changed behavior after relapse and bias of ascertainment. Furthermore, it is possible that other unmeasured exposures, such as concurrent drug therapies, may also have an effect. Many of these concerns are removed in a prospective cohort study, which we then went on to carry out.

The vitamin D levels were higher in nonrelapsers from the pilot retrospective study than in relapsers although the difference was not statistically significant (which may be a consequence of limited power).

In the subsequent prospective cohort study, there was a strong inverse correlation between serum vitamin D levels and Breslow thickness. This relationship persisted when the data were corrected for factors known to be associated with lower vitamin D levels, such as age, although it is possible that the relationship is due to an unmeasured confounder. Serum vitamin D levels and BMI proved independently predictive of relapse even when corrected for Breslow thickness, supportive of the view that tumor progression after removal of the primary is also related to serum vitamin D levels.

Several previous case-control studies have reported an association between BMI and melanoma risk,[23-26] but not all.[27] There are no previously published data suggesting a poorer outcome from melanoma for the obese, but there are for other cancers.[28,29] It is interesting that better survival occurred in patients with an intermediate BMI (between 24.9 and 29.9), in which group serum vitamin D levels were highest.

Although analysis of the cohort study data provides strong evidence to support the hypothesis from the initial retrospective study, the possibility must be considered that serum vitamin D levels might merely be a marker for another causal relationship. Although vitamin D levels and prognosis might be confounded by some unmeasured exposure, the principle of Mendelian Randomization indicates that genotype assortment cannot be due to confounding. Thus the fact that the BsmI *VDR* variant A allele showed evidence of an interaction with serum vitamin D levels in the cohort study supports the suggestion that the association between vitamin D levels and prognosis is not due to confounding.

The level of serum vitamin D that would be most beneficial for patients with cancer is not known. Although there are some data that suggest that very high levels might be harmful as a result of suppression of dendritic cell function,[30] it has been suggested that levels of 75 nmol/L are desirable.[20] The cohort study provides no strong data about optimal levels, but there was no evidence for anything other than a beneficial effect for higher levels.

Sun avoidance after a diagnosis of melanoma is very common, and this study suggests that patients with melanoma have suboptimal levels of serum vitamin D. Protection from sunburn is important and should be continued, but efforts should be made to ensure vitamin D sufficiency, and these data suggest that this can be achieved using supplements. We have shown that vitamin D deficiency is common in patients with melanoma in the United Kingdom and that this may increase the risk of relapse from melanoma. This study suggests that, in the absence of medical contraindications, patients with melanoma who are avoiding sun exposure should take vitamin D_3 supplements sufficient to ensure normal levels. More research is needed to identify optimal levels for patients with melanoma.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Julia A. Newton-Bishop, Samantha Beswick, Paul Affleck, Clarissa Nolan, Jennifer H. Barrett, D. Timothy Bishop

Financial support: Julia A. Newton-Bishop, David E. Elder, D. Timothy Bishop

Administrative support: Julia A. Newton-Bishop, Paul Affleck, Susan Leake, Birute Karpavicius, Sue Haynes, Linda Whitaker, Edwina Gerry, Clarissa Nolan, Chandra Bertram, Jerry Marsden

Provision of study materials or patients: Julia A. Newton-Bishop, Samantha Beswick, Juliette Randerson-Moor, Kairen Kukalizch, Sharon Jackson, Chandra Bertram, Jerry Marsden

Collection and assembly of data: Julia A. Newton-Bishop, Samantha Beswick, Juliette Randerson-Moor, Yu-Mei Chang, Paul Affleck, May Chan, Susan Leake, Birute Karpavicius, Sue Haynes, Kairen Kukalizch, Linda Whitaker, Sharon Jackson, Edwina Gerry, Clarissa Nolan

Data analysis and interpretation: Julia A. Newton-Bishop, Samantha Beswick, Juliette Randerson-Moor, Yu-Mei Chang, Faye Elliott, May Chan, Jennifer H. Barrett, D. Timothy Bishop

Manuscript writing: Julia A. Newton-Bishop, Samantha Beswick, Juliette Randerson-Moor, Yu-Mei Chang, Paul Affleck, Faye Elliott, May Chan, Linda Whitaker, David E. Elder, Jennifer H. Barrett, D. Timothy Bishop

Final approval of manuscript: Julia A. Newton-Bishop, Samantha Beswick, Juliette Randerson-Moor, Yu-Mei Chang, Paul Affleck, Faye Elliott, May Chan, Susan Leake, Birute Karpavicius, Sue Haynes, Kairen Kukalizch, Linda Whitaker, Sharon Jackson, Edwina Gerry, Clarissa Nolan, Chandra Bertram, Jerry Marsden, David E. Elder, Jennifer H. Barrett, D. Timothy Bishop

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