25-Hydroxyvitamin D serum levels and melanoma risk: a case–control study and evidence synthesis of clinical epidemiological studies

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There is accumulating evidence that the vitamin D pathway may play a role in melanoma. The aim of this study was to investigate the association between 25-hydroxyvitamin D [25(OH)D] serum levels and the risk of cutaneous melanoma. A case–control study with 137 incident cases of melanoma (serum samples collected at the time of diagnosis) and 99 healthy controls (serum samples collected between October and April) was carried out and evaluated in the framework of an evidence synthesis of clinical epidemiological studies on the topic to facilitate comparisons and summarize the scientific evidence produced so far. There was a statistically significant difference in the median levels of serum vitamin D between melanoma patients and healthy controls (18.0 vs. 27.8 ng/ml, $P < 0.001$). Among melanoma patients, 66.2%, compared with 15.2% of healthy controls, had vitamin D deficiency (≤ 20 ng/ml), whereas vitamin D sufficiency (≥30 ng/ml) was observed in only 7.4% of melanoma patients and in 37.4% of the healthy controls ($P < 0.001$). A multivariate model including age, sex, and BMI showed a statistically significant inverse association between melanoma and vitamin D sufficiency versus deficiency (odds ratio = 0.04; 95% confidence interval: 0.02–0.10, $P < 0.001$). Also, vitamin D insufficiency versus deficiency was significantly inversely associated with melanoma (odds ratio = 0.13; 95% confidence interval: 0.06–0.27, $P < 0.001$). These results suggest that both deficient and insufficient serum levels of vitamin D are associated with melanoma and that a trend seems to be present with a reduced risk of melanoma when vitamin D approaches normal values. ***European Journal of Cancer Prevention*** **00:000–000**

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Introduction

Vitamin D, mostly derived from sunlight on the skin, is fat-soluble and essential for several physiological functions (Holick, 2007). Epidemiological and experimental studies have highlighted its role in cancer and mortality (Guerrieri-Gonzaga and Gandini, 2013; Pilz *et al.*, 2013). Its biological active metabolites suppress tumor cell proliferation in different cell systems and induce differentiation of cancer cells in different tumor models *in vitro* and *in vivo*, suggesting that high levels of vitamin D metabolites may be protective against cancer. It has also been shown that populations with substantial deficiency of vitamin D are at a higher risk of neoplastic diseases (Teleni *et al.*, 2013). The protective role played by 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in different epithelial tumors (breast, colon, prostate) has been reported widely (Vuolo *et al.*, 2012) and there is accumulating evidence that it may also be involved in melanoma (Eisman *et al.*, 1987; Yudoh *et al.*, 1999; Osborne and Hutchinson, 2002; Albert *et al.*, 2004; Pandolfi *et al.*, 2017; Spath *et al.*, 2017).

1,25$(OH)_2$D-mediated antitumor activity is mostly dependent on the abilities to regulate cell proliferation and differentiation, to induce apoptosis in different cellular systems, and to inhibit angiogenesis in human tumors (Chiang and Chen, 2013).

Recently, thanks to advances in molecular biology studies (Hutchinson *et al.*, 2000; Li *et al.*, 2007; Nemazannikova *et al.*, 2014), more scientific evidence has been collected on the risk of developing melanoma (Egan, 2009) in the presence of vitamin D alterations, but the existing scientific literature provides conflicting results (Gandini *et al.*, 2009; Caini *et al.*, 2014) and additional research is needed.

Evaluation of 25-hydroxyvitamin D [25(OH)D] serum level is recommended as the best indicator of overall vitamin D status because it reflects total vitamin D from

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dietary intake and sunlight exposure, as well as the conversion of vitamin D from adipose stores in the liver (Rosen, 2011).

Two categories are usually used in clinical practice to classify low levels of vitamin D: 'vitamin D deficiency' as a severe form and 'vitamin D insufficiency' as a mild form, without a clear consensus on the threshold levels that have not been subjected to a systematic, evidence-based development process.

Thus, in 2011, the Endocrine Society published a clinical practice guideline (Holick *et al.*, 2011) not recommending but only suggesting that vitamin D deficiency, insufficiency, and sufficiency be defined as 25(OH)D less than or equal to 20 ng/ml, between 21 and 29 ng/ml, and between 30 and 100 ng/ml, respectively. These reference ranges were used in this study.

On the basis of these recent findings, we investigated the association between vitamin D status and the risk of cutaneous melanoma in patients and healthy controls.

Patients and methods

This case–control study, carried out at the Dermatology Clinic of 'Sant'Andrea Hospital' Rome, Italy, enrolled 137 incident cases of melanoma, diagnosed from 2007 to 2012 and histologically confirmed, and 99 healthy controls recruited, on a voluntary basis, from among the medical and paramedical staff of the hospital. Eligible criteria were age between 18 and 85 years and no history of cancer or acute/chronic diseases at the time of enrollment.

As the level of 25(OH)D may depend on BMI, other treatments, and supplementations, exclusion criteria for cases and controls were as follows: advanced age (>85 years); obesity (BMI > 30); and taking any of the following treatments: corticosteroids, calcium antagonists, bisphosphonates, calcium, or vitamin D supplementations because of their influence on vitamin D metabolism.

All patients affected by melanoma and healthy controls included in the study were resident in the Lazio region (Central Italy).

The study was reviewed and approved by the institutional review board of the Hospital and all study participants provided written informed consent.

Laboratory examinations

Serum samples were collected at the time of diagnosis in the patients and between October and April in the healthy controls to assess levels of calcium, 25(OH)D and parathyroid hormone (PTH). PTH was assayed using an electrochemiluminescence immunoassay kit (DPC Immulite 2000 intact PTH assay; Diagnostic Products Corporation (DPC), Los Angeles, California, USA) and 25(OH)D concentrations were determined using an automated chemiluminescence assay kit (Liaison 25-hydroxy vitamin D Total; Diasorin, Stillwater, Minnesota, USA); the intra-assay and interassay coefficients of variation were 8.1 and 10.2%, respectively. The Liaison 25(OH)D assay is cospecific for $25(OH)D_2$ and $25(OH)D_3$; thus, it reports a total 25(OH)D concentration. Participants were classified according to their 25(OH)D concentrations into three groups ('deficient' ≤ 20 ng/ml, 'insufficient' 21–29 ng/ml, and 'sufficient' ≥ 30 ng/ml) using cutoff values suggested by the Endocrine Society Clinical Practice Guideline published in 2011 (Holick *et al.*, 2011).

Statistical analysis

We adhered to the recommendations issued by the Strengthening the Reporting of Observational Studies Initiative (von Elm *et al.*, 2007).

Using an online sample size calculator to compute the sample size required to achieve a desired statistical power of 0.8 with an α level of 0.05, the recommended sample size per group was 97.

The distributions of the laboratory parameters among the cases and controls were examined, and their medians and interquartile ranges were compared using the Mann–Whitney test.

Percentages and the χ^2-test were used to compare categorical variables.

Multivariate unconditional logistic regression models were used to assess the association between 25(OH)D serum levels and melanoma after adjusting for some possible confounders. Vitamin D deficiency was chosen as the reference group for vitamin D status. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were computed.

Results

There were 137 melanoma patients, aged between 21 and 85 years, and 99 control participants, aged between 25 and 69 years. There were 61 (44.5%) and 32 (32.3%) men in the patient and control groups, respectively ($P = 0.058$). Table 1 shows the demographic and biochemical results for both patients and controls, and Table 2 summarizes the histological features of the surgically resected primary melanomas. There was a statistically significant difference in the median levels of serum vitamin D between melanoma patients and healthy controls (18.0 vs. 27.8 ng/ml, $P < 0.001$). Histological type of melanoma had the following median levels of serum vitamin D (ng/ml): superficial spreading melanoma 18.4, nodular melanoma 17.4, and acral lentiginous melanoma 17.9 (differences were not statistically significant). No significant difference was found in age, PTH, and calcium serum levels, whereas we observed a small but statistically significant difference in BMI medians between melanoma patients and healthy controls (24.0 vs. 22.0, $P < 0.001$).

Figure 1 shows the distribution of serum 25(OH)D levels in melanoma patients and healthy controls. As shown in Table 3, 66.2% of melanoma patients showed vitamin D

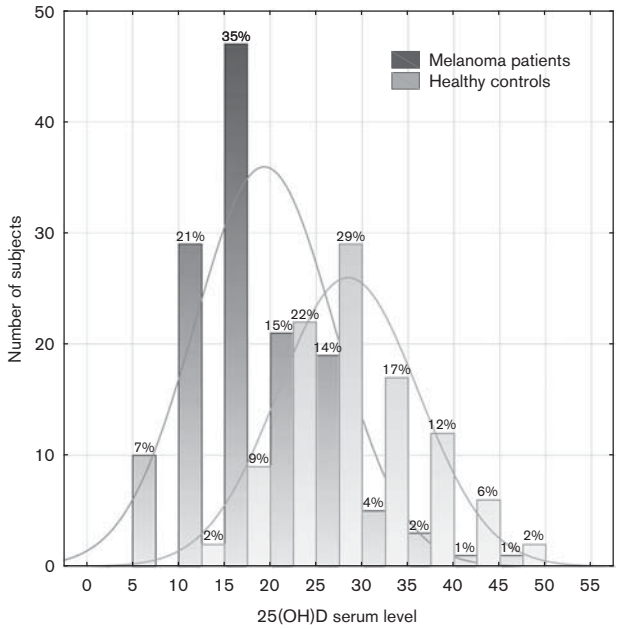
Table 1 Demographic and biochemical results for melanoma patients and healthy controls

	Melanoma patients ($n=137$)			Healthy controls ($n=99$)			P value
	Mean ± SD	Median	Interquartile range	Mean ± SD	Median	Interquartile range	Mann–Whitney test
Age	52.0 ± 15.1	51.0	40.0–63.0	51.6 ± 9.6	54.0	47.0–60.0	0.813
BMI	23.9 ± 2.9	24.0	22.0–26.2	22.7 ± 2.6	22.0	21.0–24.2	0.001
Vitamin D (ng/ml)	19.2 ± 7.6	18.0	14.5–23.5	28.3 ± 7.6	27.8	22.3–33.3	< 0.001
Parathyroid hormone (pg/ml)	39.7 ± 19.3	35.9	26.1–50.8	42.4 ± 17.1	39.7	28.6–51.0	0.102
Calcium (mg/dl)	9.3 ± 0.5	9.3	8.9–9.7	9.3 ± 0.42	9.3	9.1–9.6	0.843

Table 2 Histological characteristics of melanoma

	n (%)
Histologic type	
Superficial spreading melanoma, spitz, and spitzoid superficial spreading melanoma	89 (65.0)
Nodular melanoma, spitzoid nodular melanoma	18 (13.1)
Acral lentiginous melanoma	12 (8.8)
Melanoma of unknown primary origin	18 (13.1)
Clark level	
1	28 (21.4)
2	32 (24.4)
3	48 (36.6)
4	21 (16.0)
5	2 (1.5)
Breslow thickness (%)	
< 0.75 mm	77 (58.3)
0.75–0.99 mm	11 (8.3)
1.00–1.99 mm	23 (17.4)
2.00–2.99 mm	8 (6.1)
≥ 3.00 mm	13 (9.8)

Fig. 1



Distribution of 25-hydroxyvitamin D [25(OH)D] serum levels (ng/ml) in healthy controls and melanoma patients.

deficiency and only 7.4% had vitamin D sufficiency compared with healthy controls, among whom, only 15.2% were vitamin D deficient whereas 37.4% were the sufficient ($P<0.001$). Vitamin D insufficiency was observed in 26.5% of melanoma patients and 47.5% of healthy controls.

ORs computed by logistic regression from a multivariate model including age, sex, and BMI are reported in Table 4. Age and sex were not associated significantly with melanoma. A statistically significant inverse association between melanoma and vitamin D sufficiency (≥30 ng/ml) versus deficiency (≤ 20 ng/ml) was observed (OR = 0.04, 95% CI: 0.02–0.10, $P<0.001$). Also, vitamin D insufficiency (21–29 ng/ml) versus deficiency (≤ 20 ng/ml) was found to be significantly inversely associated with melanoma (OR = 0.13, 95% CI: 0.06–0.27, $P<0.001$). These ORs imply that within the defined categories of levels of vitamin D, the following trend can be observed: the more vitamin D approaches normal values, the lower is the OR and thus the greater seems to be the protection.

BMI was also associated independently with melanoma (OR = 2.34, 95% CI: 1.09–5.02, $P=0.030$ for individuals with BMI between 25.0 and 30.0).

Discussion

Our study shows that the median value of 25(OH)D is significantly lower in melanoma patients than in controls and that the distribution of 25(OH)D in melanoma patients is shifted toward lower values compared with the controls.

Also, over 90% of melanoma patients have deficient or insufficient 25(OH)D serum levels at the time of diagnosis, whereas only 7.4% had sufficient values (≥30 ng/ml). Both sufficiency and insufficiency are associated significantly with a lower risk of melanoma compared with vitamin D deficiency.

These results are in agreement with Gambichler *et al.* (2013), who found that only 7.2% of patients in a large German cohort of 764 melanoma patients had sufficient vitamin D values (≥30 ng/ml).

In our logistic model, vitamin D sufficiency is associated inversely with melanoma, but also insufficient levels imply significantly lower risk compared with vitamin D deficiency (reference group).

Previous studies have found both conflicting and similar results to ours (Table 5).

Table 3 Comparison between melanoma patients and healthy controls according to vitamin D status ($P < 0.001$)

	Melanoma patients ($n = 137$) [n (%)]	Healthy controls ($n = 99$) [n (%)]
Vitamin D deficiency (≤ 20 ng/ml)	91 (66.4)	15 (15.2)
Vitamin D insufficiency (21–29 ng/ml)	36 (26.3)	47 (47.5)
Vitamin D sufficiency (30 + ng/ml)	10 (7.3)	37 (37.4)

Table 4 Melanoma risk estimate from multivariate logistic regression

Variables	Odds ratio	95% confidence interval	*P*-value
Age	0.98	0.96–1.01	0.206
Sex (female vs. male)	0.73	0.38–1.40	0.346
BMI ≥ 25.0 vs. BMI < 25.0	2.34	1.09–5.02	0.03
Vitamin D insufficiency (21–29 ng/ml) vs. vitamin D deficiency (≤ 20 ng/ml)	0.13	0.06–0.27	< 0.001
Vitamin D sufficiency (≥30 ng/ml) vs. vitamin D deficiency (≤ 20 ng/ml)	0.04	0.02–0.10	< 0.001

Some studies found an increased risk of melanoma associated with the highest 25(OH)D serum levels.

The study by van der Pols *et al.* (2013) was prospective and involved 1191 Australian adults who participated from 1992 to 1996 in the Nambour Skin Cancer Prevention Trial of daily sunscreen use and β-carotene supplementation; they were followed for 11 years to assess the association between baseline serum 25(OH)D levels and the risk of skin cancer. In the subgroup of 17 patients with melanoma, dichotomizing 25(OH)D serum concentration as less than 30 ng/ml and at least 30 ng/ml, the authors reported a nonsignificant increased risk of melanoma (OR = 2.71, 95% CI: 0.98–7.48) for the only eight patients with baseline 25(OH)D levels above or equal to 30 ng/ml (75 nmol/l) after adjustment for several factors (Table 5).

In a nested case–control study of 50–69-year-old Finnish male smokers (within the α-Tocopherol β-Carotene Cancer Prevention Study), Major *et al.* (2012) found no overall association between serum 25(OH)D and melanoma. Modeling serum 25(OH)D as a categorical variable (< 25, 25, 37.50, and 50 + nmol/l), a lower risk of melanoma was suggested for serum vitamin D levels between 15 and 19.9 ng/ml (37.50–49.99 nmol/l) (OR = 0.60, 95% CI: 0.25–1.44), and an increased risk for serum vitamin D levels of at least 20 ng/ml (≥50 nmol/l) (OR = 1.32, 95% CI: 0.64–2.72), compared with men whose prediagnostic levels were less than 10 ng/ml (< 25 nmol/l). These results were not statistically significant. It is noteworthy that these findings cannot be extended to the whole population because the study only included male smokers from Finland.

In the prospective study by Afzal *et al.* (2013), 10 060 White individuals from the Danish general population were followed up for 28 years; 78 individuals developed melanoma. Multivariable adjusted hazard ratios for melanoma were 4.72 (95% CI: 0.96–23.3) for serum 25(OH)D of at least 20 ng/ml (50 nmol/l) versus less than 10 ng/ml (25 nmol/1) and 6.3 (95% CI: 1.38–28.8) for the top (67th–100th) versus the bottom (0th–34th) percentile. Despite the large cohort, the study found only 78 melanoma patients and thus the 95% CI are wide and the results are inconsistent.

In contrast, other studies have found an inverse association with high 25(OH)D levels, as we have.

In a sun exposure and melanoma risk, case–control study in Northern England (Newton-Bishop *et al.*, 2011), the authors also investigated the correlation between reported sun exposure and serum vitamin D levels in a subsample of the cases ($n = 805$) and population controls ($n = 187$). They found that serum vitamin D level was not independently protective (OR = 0.89, 95% CI: 0.76–1.04/8 ng/ml, 20 nmol/l increase), but comparing cases and matched siblings ($n = 128$) using conditional logistic regression adjusting for age, sex, season, and hair color, significant protective effects for serum vitamin D levels were observed (OR = 0.64, 95% CI: 0.46–0.87/8 ng/ml increase, 20 nmol/l).

From the study of Nürnberg *et al.* (2009), calculating risk estimates from their published raw data, we found a significant inverse association with melanoma risk on comparing more than 20 ng/ml versus less than or equal to 20 ng/ml categories (OR = 0.48; 95% CI: 0.29–0.80).

In a case–control study investigating vitamin D receptor polymorphisms and melanoma susceptibility, Randerson-Moor *et al.* (2009) compared serum vitamin D levels for the 941 cases and 114 controls from North England and found a nonsignificant adjusted OR of 0.94 (95% CI: 0.79–1.12) for an increment of 8 ng/ml (20 nmol/l) of 25(OH)D serum level.

Four studies (Nurnberg *et al.*, 2009, Major *et al.*, 2012, Afzal *et al.*, 2013, van der Pols *et al.*, 2013, for a total of 392 cases overall) were included in the meta-analysis by Caini *et al.* (2014), yielding a nonsignificant summary relative risk of 1.46 (95% CI: 0.60–3.53) for the highest versus the lowest categories ($I^2 = 54\%$). However, Caini *et al.* (2014) calculated the OR for the study by

Table 5 Evidence synthesis of clinical epidemiological studies

References	Country	Characteristics of studied population	Study design	Time elapsing from blood draw to diagnosis	Number of melanoma cases	Number of controls	Vitamin D serum levels	Risk (95% confidence interval)	Adjustments	Statistical significance
Studies which found an increased risk of melanoma associated with highest 25(OH)D serum levels										
Van der Pols *et al.* (2013)	Australia	1191 adults (range: 29–79 years) from NAMBOUR skin cancer prevention trial of daily sunscreen use and β-carotene supplementation from 1992 to 1996	Prospective cohort study	All blood samples in 1996 (in August = end of winter in Australia). Follow-up for detecting incident cases from 1996 to 2007.	17	–	≥30 vs. <30 ng/ml	OR = 2.71 (0.98–7.48)	OR from logistic regression adjusted for: age, sex, β-carotene, and sunscreen allocations during the trial, personal history of skin cancer before 1996, family history of skin cancer, skin color, usual time spent outdoors	NS
Major *et al.* (2012)	Finland	368 Finnish male smokers (range: 50–69 years) from the α-Tocopherol β-Carotene Cancer Prevention study from 1986 to 2005	Nested case–control study	Median time from baseline blood draw to diagnosis was 8.9 years. Median follow-up time for controls was 18.2 years. Serum collected at study entry and stored at −70°C. Incident cases from 1986 to 2005.	92	276	10–14.9 vs. <10 ng/ml	OR = 1.04 (0.52–2.12)	OR from conditional logistic regression model adjusted for age at randomization, date of blood draw, height, weight, dietary cholesterol, and skin behavior (e.g. skin burns easily in prolonged direct sunlight (no/yes/missing). Serum 25(OH)D was modeled as distinct clinically-defined categories (< 25, 25–37.49, 37.50–49.99, and 50+ nmol/l).	NS
							15–19.9 vs. <10 ng/ml	OR = 0.60 (0.25–1.44)		NS
							≥20 vs. <10 ng/ml	OR = 1.32 (0.64–2.72)		NS
Afzal *et al.* (2013)	Denmark	10 060 White individuals from the Danish general population (range: 20–100 years) – Copenhagen City Heart study from 1981 to 1983	Prospective cohort study	Plasma samples collected at baseline in 1981–1983 and stored at −20°C until 2009–2010. Patients developed melanoma during up to 28 years of follow-up.	78	–	≥20 vs. <10 ng/ml	HR = 4.72 (0.96–23.3)	HR from Cox proportional hazards regression adjusted for age, year of birth, sex, cumulated tobacco consumption in pack-years, BMI, income, occupational physical exertion, physical intensity of leisure-time activities, running and cycling habits, calendar month of blood draw	NS
							Top vs. bottom tertile	HR = 6.3 (1.38–28.8)		Significant
Studies which found a decreased risk of melanoma associated with highest 25(OH)D serum levels										
Newton-Bishop *et al.* (2011)	UK	1647 individuals from North England (range: 18–76 years). Incident melanoma cases and two different types of controls:	Case–control study	–	805	187 (population)	Increase by 8 ng/ml	OR = 0.89 (0.76–1.04)	OR from unconditional logistic regression adjusted for age, sex, weekend sun exposure	NS

Table 5 (*continued*)

References	Country	Characteristics of studied population	Study design	Time elapsing from blood draw to diagnosis	Number of melanoma cases	Number of controls	Vitamin D serum levels	Risk (95% confidence interval)	Adjustments	Statistical significance
		population and sibling controls.								
					128	128 (siblings)	Increase by 8 ng/ml	OR = 0.64 (0.46–0.87)	OR from conditional logistic regression adjusted for age, sex, season of year sampled and hair color	Significant
Nürnberg *et al.* (2009)	Germany	346 individuals (range: 14–65+ years). Patients from Departments of Dermatology, University of Homburg and Mannheim, from 1997 to 2007 and healthy controls.	Case–control study	–	205	141	> 20 vs. <10 ng/ml	OR = 0.82 (0.44–1.55)	This OR was calculated by Caini *et al.* (2014), from Nürnberg *et al.* (2009) published row data to make meta-analysis	NS
					86	91	> 20 vs. ≤ 20 ng/ml	OR = 0.48 (0.29–0.80)	This OR was calculated by the authors of this study from Nürnberg *et al.* (2009) published row data to make comparison with our results	Significant
Randerson-Moor *et al.* (2009)	UK	1451 Individuals from North England (range: 18–75 years). Incident melanoma cases from 2000 to 2006 and controls matched by age and sex.	Case–control study	–	941	114	Increase by 8 ng/ml	OR = 0.94 (0.79–1.12)	OR from logistic regression adjusted for age, sex BMI, deprivation score	NS
This study	Italy	236 individuals (range: 21–85 years). Incident melanoma cases from Dermatology Unit, Sant'Andrea Hospital, from 2007 to 2012 and healthy controls.	Case–control study	–	137	99	≥ 30 vs. ≤ 20 ng/ml	OR = 0.04 (0.02–0.10)	ORs from logistic regression adjusted for age, sex, BMI	Significant
							21–29 vs. ≤ 20 ng/ml	OR = 0.13 (0.06–0.27)		Significant

CI, confidence interval; HR, hazard ratio; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio.

Nürnberg *et al.* (2009) from raw data, comparing serum levels less than 10 ng/ml with more than 20 ng/ml, obtaining a conservative estimate, whereas considering at least 20 ng/ml versus less than 20 ng/ml in 205 cases and 141 controls, we found an OR of 0.48 (95% CI: 0.29–0.80), implying a 50% significant reduction in risk.

No definite conclusion can be drawn from these studies, but some aspects need consideration.

First, there is no universal agreement on the choice of the cutoffs for defining vitamin D deficiency and insufficiency, and the lack of a shared definition confuses the comparisons among the studies (International Agency for research on Cancer, 2008; Ross *et al.*, 2011).

Second, the studies reviewed did not primarily aim to assess the association between vitamin D status and melanoma.

Third, as the incidence of melanoma is low, even large prospective studies identify only a few patients (Afzal *et al.*, 2013; van der Pols *et al.*, 2013) and thus conclusions may not reach statistical significance.

Fourth, case–control studies cannot verify that the relationship between 25(OH)D and melanoma patients is causal. There is an intense debate on this topic. Indeed, because of the discrepancies observed between observational and intervention studies, vitamin D was hypothesized to be a marker, not a cause, of 'ill health' because of the 'reverse causation' bias (Robsahm *et al.*, 2013; Autier *et al.*, 2014; Guessous, 2015).

A limitation of our study is that we have not adjusted for sun exposure, skin phototype, and season, even though the entire spectrum of ultraviolet is recognized as a risk factor for melanoma (El Ghissassi *et al.*, 2009). However, sun exposure and season are correlated with vitamin D levels. To adjust for sun exposure is, at least partially, adjusting for vitamin D. Furthermore, if melanoma patients are sun seekers, they should have high levels of vitamin D, which is not observed. Individuals from the same small geographical area (in this study, Latium region, Central Italy) should be highly homogeneous with respect to skin phototype, avoiding this bias. Furthermore, comparisons with other studies should take account of the heterogeneous prevalence of different phototypes in Europe. Current evidence is still contradictory about whether skin pigmentation influences vitamin D photosynthesis (Xiang *et al.*, 2015).

We collected all our control serum samples between October and April and all patient serum samples at the time of diagnosis. Therefore, even in the worst possible scenario in which vitamin D levels could be overestimated in all patients, because of summer season collection, we still found lower vitamin D levels in cases than in controls; thus, lack of adjustment for season should not confound the results.

The interpretation of the results of case–control studies requires caution because of the possible occurrence of 'reverse causation' bias, but also that of cohort studies is difficult because the exposure (the level of vitamin D) is measured long before the onset of the disease. Indeed, the cohort studies produced little evidence of an association with vitamin D unless evaluated during follow-up. The well-designed cohort study by Saiag *et al.* (2015) showed that 25(OH)D variation during follow-up is an independent melanoma prognostic marker.

The association between serum vitamin D levels and melanoma was only noted for BMI as serum vitamin D levels vary with the proportion of body fat and vitamin D has complex metabolic interactions with fat cells (Li *et al.*, 2008; Sun and Zemel, 2008). The significant difference between the means of BMI patients and controls is small. In the multivariate logistic regression, the inverse association between vitamin D and the risk of melanoma remained after adjustment for BMI. Furthermore, BMI of at least 25 was found to be independently and significantly associated with melanoma risk. Excess body weight has been investigated as a potential risk factor for melanoma in a meta-analysis. A significant pooled effect estimate (1.31, 95% CI: 1.18–1.45) was reported for overweight men (Sergentanis *et al.*, 2013). To explain the role of BMI in melanoma pathogenesis, some investigators (de Giorgi *et al.*, 2013) have hypothesized that chronic hyperinsulinemia, hyperestrogen levels, high plasma leptin levels, and low level of vitamin D may be risk factors for tumor growth. Inflammation, fibrosis, and neoangiogenesis, typical of adipose tissue, constitute microenvironments that could promote the development and progression of cancer.

Recent studies (Newton-Bishop *et al.*, 2015; Fang *et al.*, 2016) have shown that lower vitamin D levels are associated independently with poorer outcomes even after controlling for systemic inflammation.

To conclude, even if no clear conclusions can be drawn, several epidemiological studies support a protective effect of 'normal' vitamin D levels not only on melanoma risk but also on prognosis, stage, and survival. Several authors have reported an association between low levels of vitamin D and higher Breslow thickness. Wyatt *et al.* (2015) found that serum 25(OH)D less than 20 ng/ml (50 nmol/l) was associated with a statistically significant quadrupling risk of the thicker tumor; Newton-Bishop *et al.* (2009), in a prospective study of survival, reported that higher $25(OH)D_3$ levels were associated with lower Breslow thickness at diagnosis ($P=0.002$) and were independently protective against relapse and death. Randerson-Moor *et al.* (2009) showed that thinner tumors were associated with higher serum levels of vitamin D at recruitment, after adjusting for age, sex, season of serum collection, deprivation score, and BMI. Nürnberg *et al.* (2009) confirmed that there was a trend toward greater

tumor thickness in patients with lower vitamin D levels, that these patients had earlier distant metastatic diseases, and that vitamin D levels are reduced in stage IV melanoma patients.

Several cell-based studies investigated the molecular events that may account for the role of vitamin D in melanoma growth and progression (Ahonen *et al.*, 2000; Spath *et al.*, 2017). These showed as plausible vitamin D-induced mechanisms growth arrest, apoptosis of tumor cells or their non-neoplastic progenitors, chemoprotective mechanisms including enhancing DNA repair, anti-oxidant protection, and immunomodulation.

Finally, the Women's Health Initiative Randomized Controlled Trial (Tang *et al.*, 2011) showed that in women with a history of no-melanoma skin cancer (NMSC), vitamin D supplementation reduced melanoma risk, suggesting a role for vitamin D supplements in this high-risk group. Although these data are from post-hoc subgroup analyses, the results suggest that increasing vitamin D serum levels may prevent the development of melanoma in women at high risk. A role for vitamin D supplementation in preventing melanoma in women with a history of NMSC warrants further investigation. Patients with a history of NMSC should avoid sun exposure and have very low 25(OH)D levels; therefore, this subgroup of patients could benefit from vitamin D supplementation.

Some evidence supports the hypothesis that low vitamin D increases melanoma risk and other tumors (Garland *et al.*, 1989; Ahonen *et al.*, 2000; Engel *et al.*, 2010). Our study highlights 'normal' vitamin D status as a favorable condition to reduce the risk of melanoma (Zittermann *et al.*, 2012). However, more rigorous and randomized clinical trials are necessary to shed more light on the association between 25(OH)D and melanoma risk and prognosis (Raimondi *et al.*, 2016).

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Conflicts of interest

There are no conflicts of interest.

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