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EDITORIAL

Maurizio Rossini

Department of Medicine, Section of Rheumatology, University of Verona

Dear Colleagues,
As you will see, this issue features two contributions relative to the debate on the possible extra skeletal effects of vitamin D supplementation, in particular on type-2 diabetes (T2DM) and in the field of cancer treatment.
You will note that both authors correctly conclude that in general available trials have not found significant results on these fronts: rather, because they were conducted on populations which on the whole were not vitamin D deficient, they are not able to exclude a protective effect of vitamin D supplementation in subjects who are deficient, especially if we consider that sub-analyses of these subjects actually suggest a positive effect.
We see, for example, that a post-hoc analysis of the randomized clinical trial by Pittas et al. [1] on a small number of participants that had baseline circulating levels of 25-hydroxyvitamin D <12 ng/mL (< 30 nmol/L) showed that the risk of developing T2DM was reduced by 60% in subjects treated with cholecalciferol with respect to those given the placebo (hazard ratio [HR] 0.38, 95% IC 0.18-0.80).
We further find that in the study conducted on patients affected by lung tumors vitamin D supplementation did not on the whole produce the expected results. Yet when patients with early stage adenocarcinoma and low vitamin D levels were selected, supplementation in fact reduced mortality by over 60% with respect to the placebo (HR = 0.37; 95% IC 0.15-0.95). [2]
The time required to assess an outcome may also be fundamental: you will see, for example, that the negative conclusion of the VITAL trial [3] would change if the follow-ups of the first 1-2 years were excluded: such an exclusion, in my opinion, would be reasonable, given the biological latency. In that case, vitamin D supplementation shows a significant – 25% – reduction of death by cancer (HR = 0.75; 95% IC 0.59-0.96).
With regard to the documentation on a significant effect of vitamin D supplementation only in subjects with low baseline 25-hydroxyvitamin D3 levels, it is worth remembering that the literature contains numerous other examples, both skeletal and extra skeletal [4]. Figure 1 shows several examples of different effects of supplementation on some extra skeletal risks with respect to baseline serum levels – low and not low – in supplemented patients.
This should not surprise us [5], in view of the fact that vitamin D acts as a nutrient: it is beneficial when lacking, though not so when it is not lacking…
To conclude, I do not believe that we can affirm today that we are overestimating the possible extra skeletal benefits of vitamin D supplementation. Neither do I think that we can deny them, given that the design and results of clinical trials conducted thus far do not allow us to exclude such benefits.
What do you think?
I hope you enjoy reading this issue.

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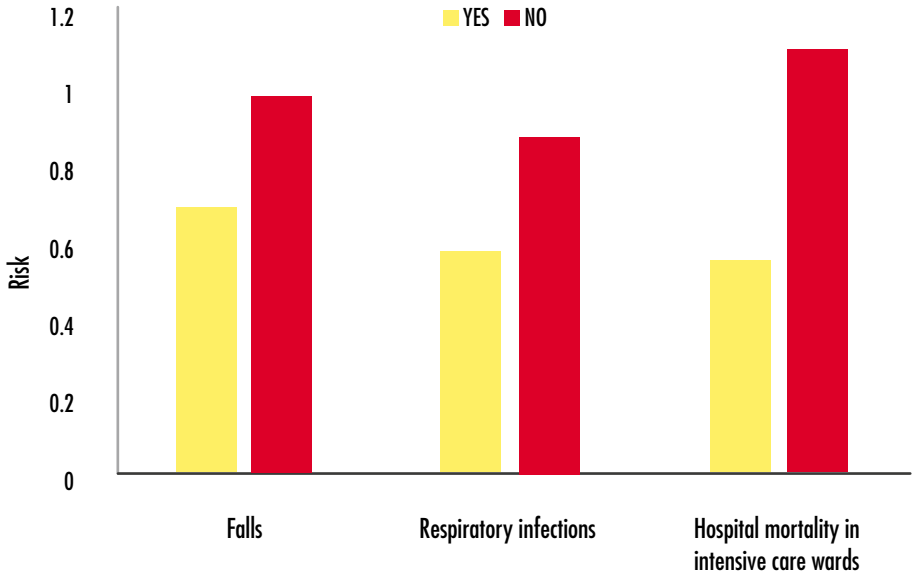

FIGURE 1.
Effects of vitamin D supplementation on extra skeletal risks (relative risk, odds ratio or hazard ratio) with respect to baseline serum levels of 25-hydroxyvitamin D3, either low (YES) or not low (NO) ($p < 0.05$ among the groups).

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THE EFFECT OF VITAMIN D_3 SUPPLEMENTATION ON THE RISK OF THE ONSET OF TYPE 2 DIABETES: are we overestimating its possible extra skeletal benefits?

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Vitamin D deficiency has been associated with the presence of multiple chronic non-skeletal pathologies (including cardiovascular diseases, hypertension, non-alcoholic hepatic steatosis, some neoplasms and diabetes), suggesting the possibility that this vitamin can have numerous pleiotropic effects at the extra skeletal level, thanks to the ubiquitous distribution of its receptor [1-3].

Among these non-skeletal chronic pathologies which are potentially associated with reduced circulating levels of vitamin D, type-2 diabetes mellitus (T2DM) has represented one of the most important focuses of scientific research in the last decade [4].

Several epidemiological studies have shown that patients with T2DM have reduced circulating levels of vitamin D with respect to the non-diabetic population (comparable for age, gender and degree of obesity) and that low vitamin D levels are associated with a greater prevalence of micro- and macro-vascular chronic diabetic complications [4-6]. Experimental models have further demonstrated that reduced vitamin D levels are associated with increased insulin resistance and impaired insulin secretion on the part of the beta-cell, in addition to high levels of various procoagulant factors and inflammatory markers, and that most of these disorders improve after vitamin D_3 administration [2, 4, 7].

On the basis of this evidence, various prospective observational studies have successively shown the existence of a significant correlation between reduced circulating levels of vitamin D and increased risk of developing T2DM (especially in patients with reduced glucose tolerance) [8], thus confirming the biological plausibility of the involvement of vitamin D in the onset of T2DM. Nonetheless, until now available findings have been exclusively based on data that do not allow us to define a possible causal role of vitamin D in the development of diabetes. In particular, it is still not clear whether vitamin D_3 supplementation is able to reduce the risk of developing diabetes.

A recent randomized clinical trial, published by Pittas et al. in the August issue of the *New England Journal of Medicine* [9], aims to provide an answer to this question. In this broad RCT, called the "D2d trial," the authors assembled a sample of over 2,400 adults (45% females, 67% Caucasian, average age = 60 years, average BMI = 32 kg/m^2) with a high risk of developing diabetes (that is, individuals with at least two of the following disorders: fasting glycemia between 100 and 125 mg/dL, glycemia two hours after OGTT between 140 and 199 mg/dL, and glycated hemoglobin between 5.7 and 6.4%) but who were not selected based on their baseline vitamin D status. Indeed, their average circulating 25-hydroxyvitamin D levels were 28 ± 10 ng/mL; only 21.7% of the sample had 25-hydroxyvitamin D baseline values < 20 ng/mL. By means of a randomized, double-blind study, these subjects were later assigned either to an active treatment group with high doses of vitamin D_3 (cholecalciferol 4,000

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IU daily, n = 1,211) or to treatment with a placebo (n = 1,212). Subjects underwent a follow-up exam after 2.5 years on average. The principal outcome of the study was the appearance of new cases of T2DM. During the trial, circulating vitamin D levels more than doubled in those subjects treated with cholecalciferol (average baseline values increased from 27.7 ng/mL to 54.3 ng/mL by the end of the study), while levels remained nearly unchanged in the group treated with the placebo (with average baseline values going from 28.2 ng/mL to 28.8 ng/mL by the end of the trial). The authors of the study observed that the risk of developing T2DM during the follow-up was substantially comparable in the group treated with cholecalciferol with respect to the group given the placebo (9.4 and 10.7 events every 100 persons per year; hazard ratio 0.88, 95% CI 0.75-1.04; p = 0.12) (Fig. 1). Overall adherence of the participants to the treatment was quite high (~86%), while the incidence of adverse events (including hypercalcemia, eGFR reduction and nephrolithiasis) was low, and perfectly comparable between subjects treated with daily doses of cholecalciferol and those taking the placebo.

Statistical analysis conducted on the above-mentioned subgroups of subjects did not show the presence of any significant differences between the two treatment groups (Fig. 2). In particular, the results of the study did not vary when the population was divided by gender, race, geographic latitude, incidence of obesity or even by baseline circulating levels of 25-hydroxyvitamin D (< 20 ng/mL vs. ≥ 20 ng/mL) [9]. Nonetheless, a post-hoc analysis of the data of a smaller group of participants (n = 103, 4.3% of the total) with baseline circulating 25-hydroxyvitamin D levels < 12 ng/mL (< 30 nmol/L) revealed that the risk of developing T2DM was significantly lower in subjects treated with cholecalciferol with respect to those given the placebo (hazard ratio 0.38, 95% CI 0.18-0.80). By contrast, in participants (n = 2,319, 95.7% of the total) with baseline 25-hydroxyvitamin D levels ≥ 12 ng/mL, the risk of developing T2DM was comparable in the two treatment groups (hazard ratio 0.92, 95% CI 0.78-1.08) [9].

The results of this broad RCT show that vitamin D_3 supplementation in high doses (4,000 IU/day of cholecalciferol per os) in pre-diabetic subjects (that is, with a high risk of developing diabetes) who were not selected for baseline vitamin D deficiency is well tolerated (with no toxicity risk for excessive cholecalciferol intake); yet such supplementation cannot be associated with any significant reduction in the onset of T2DM during the 2.5-year follow-up exam [9].

For the most part, these results confirm what had already been observed in a previous RCT with a smaller sample, which was published in 2016 [9]. In this Norwegian study, called the "Tromso Vitamin D and T2DM trial", 511 subjects with prediabetes (61% male, average age = 62 years, average BMI = 30 kg/m^2, and average 25-hydroxyvitamin D values = 24 ± 8 ng/mL) were randomized and treated with either a placebo or 20,000 IU cholecalciferol per week (roughly 2,900 IU/day) for a period of 5 years [9]. Similarly to what was observed in the "D2d trial", the authors of this study did not find any significant benefit of vitamin D_3 supplementation with respect to the onset of T2DM during the study's follow-up exam (hazard ratio 0.90; 95% CI 0.69-1.18) [9].

Based on the results of these two RCTs, we can conclude that vitamin D_3 supplementation in high doses (with daily doses of cholecalciferol varying between 2,900 and 4,000 IU) in adults with a high risk of diabetes who are not selected on the basis of circulating levels of 25-hydroxyvitamin D do not seem to have a significant protective effect on the risk of developing T2DM. In both clinical trials, such supplementation is only associated with an average reduction of 10-12% of the risk for developing T2DM during a follow-up period of between 2 and 5 years [9, 10].

This conclusion, of course, does not exclude the possibility that future randomized clinical trials with larger samples may be able to obtain statistically significant findings showing long-term benefits of treatment with cholecalciferol with regard to T2DM development

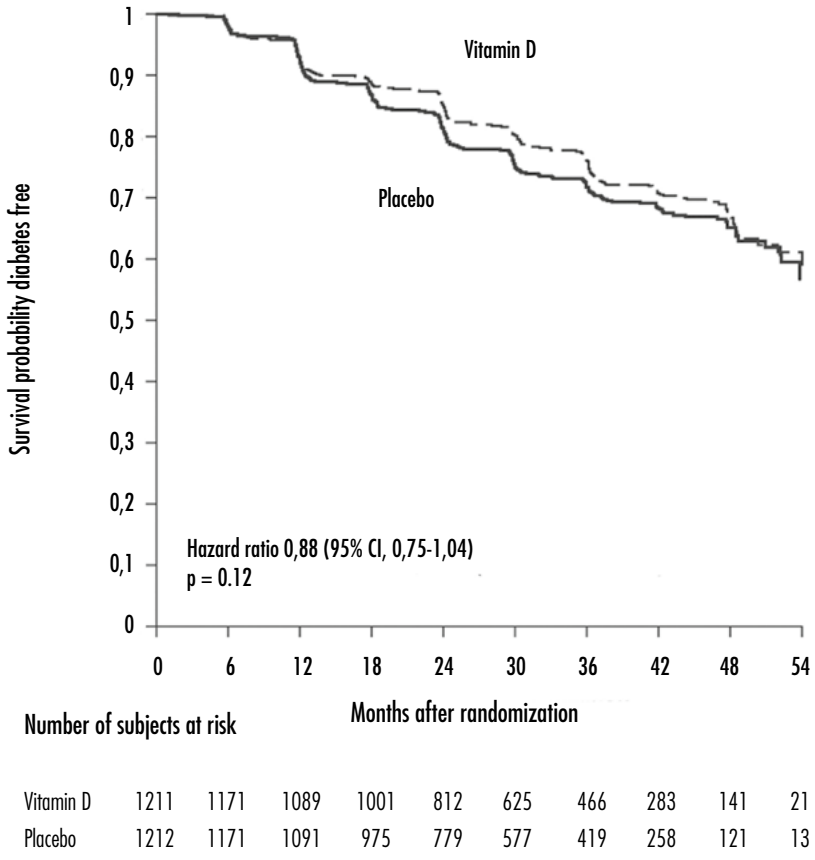

FIGURA 1.
Kaplan-Meier curve on effect of treating subjects with high doses of cholecalciferol (4,000 IU/day) vs. placebo on risk of developing T2DM in 2,423 prediabetic adult subjects. Data published in and taken from "D2d trial" [9].

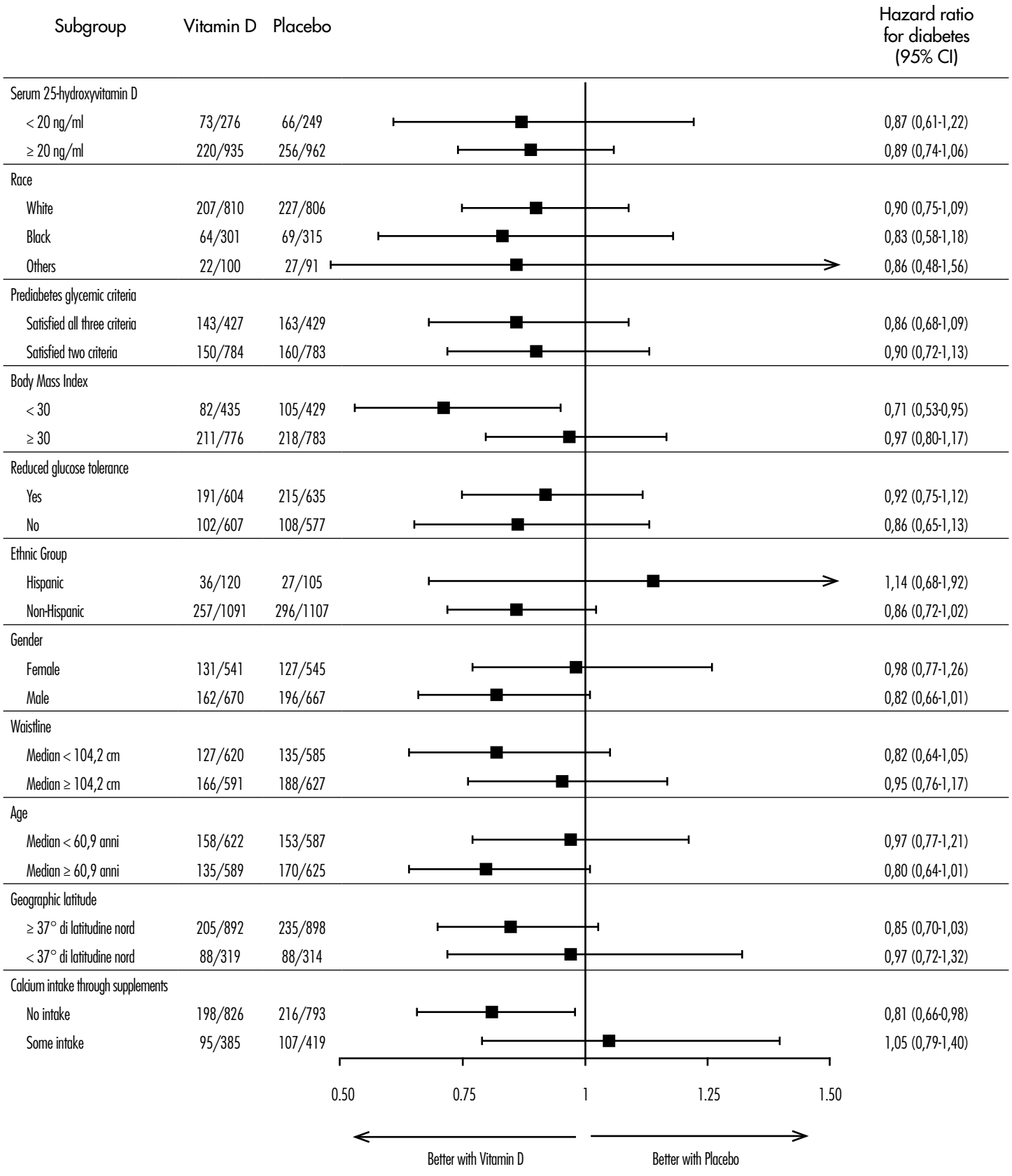

FIGURA 2.
Effect of treatment with high doses of cholecalciferol (4,000 IU/day) vs. placebo on risk of developing T2DM in various subgroups of specified prediabetic subjects. Data published in and taken from "D2d trial" [9].

(given that neither of the two clinical trials in question had a sample large enough to show a significant reduction in diabetes risk of 10-12%). Another factor that is even more important to emphasize is that the majority of subjects included in the two RCTs had optimal levels of circulating vitamin D levels [9, 10]. Indeed, in the "D2d trial", 42.2% of the participants had 25-hydroxyvitamin D levels ≥ 30 ng/mL, 36.1% had values between 20 and 29 ng/mL, while only 21.7% of the participants had 25-hydroxyvitamin D levels < 20 ng/mL [9]. It is, then, possible to hypothesize that the high percentage of subjects with adequate vitamin D levels who were included in these two RCTs may have reduced the researchers' ability to find benefits of cholecalciferol supplementation with regard to the risk of T2DM onset in the two treated groups.

It is furthermore useful to recall, as we have already seen, that a post-hoc analysis of data from the "D2d trial", conducted on participants (n = 103, 4.3% of the total) who had extremely low baseline circulating levels of 25-hydroxyvitamin D (< 12 ng/mL), itself suggested that the risk of developing T2DM was reduced by over 60% in subjects treated with cholecalciferol compared to those given the placebo (hazard ratio 0.38, 95% CI 0.18-0.80) [9]. In an increasingly evident manner, this fact underlines the need for researchers who plan future RCTs that evaluate the possible benefits of oral supplementation of vitamin D_3 on the risk of T2DM onset (and quite probably other important skeletal and extra skeletal outcomes as well, as has been shown in recent trials and meta-trials) [11-13] to take into account the vitamin D status of the participants enlisted in such trials. Such a measure seems justifiable given that it is reasonable to believe that the benefits of vitamin D_3 supplementation at high doses with regard to the long-term risk of developing T2DM may be greater in patients with vitamin D deficiency, compared to those who have adequate levels of circulating vitamin D [14].

CONFLICT OF INTEREST

The Author declares that he has no conflict of interest.

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VITAMIN D IN ONCOLOGY

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INTRODUCTION

Studies in vitro and in vivo demonstrated that the physiologically active vitamin D metabolite (1,25(OH)D or calcitriol), which exerts its action via the vitamin D Receptor (VDR), has antiproliferative effects in various cell types and was found to regulate the expression of tumor-related genes, mediate inhibition of cell growth, adhesion, migration, metastases and angiogenesis. Furthermore, a number of epidemiologic showed inverse associations of cancer incidence with high 25-hydroxycholecalciferol (25(OH)D). However, observational studies suffer from reverse causation bias and intervention studies did not confirm these associations. Discrepancies with randomised clinical trials (RCTs) suggest that low 25(OH)D could be just a marker of ill health. Inflammatory processes involved in disease occurrence and clinical course would reduce 25(OH)D, which would explain why low vitamin D status (measured by 25OHD) is reported in a wide range of disorders.

More convincing results were found for mortality, in fact evidence comes not only from observational studies but also from clinical trials. A meta-analysis of observational studies showed a nonlinear relationship of overall mortality risk with increasing circulating 25(OH)D, with optimal concentrations around 30-35 ng/ml. A meta-analysis of randomized clinical trials in healthy subjects showed that current doses of vitamin D supplements are associated with a significant decrease in overall mortality for vitamin D_3 supplementation, whereas no association with vitamin D_2 supplementation was found.

Recent evidence suggests to investigate the link of vitamin D with cancer survival and mortality, identifying this topic as one of the most promising area of research.

OBSERVATIONAL STUDIES

A meta-analysis of cohort studies (1) showed that people with high baseline 25(OH)D were at significant decreased risk of cancer deaths. Summary risk estimates were: Summary Relative Risk (SRR) = 0.91 (95% CI: 0.85-0.98) and 0.69 (95% CI: 0.61-0.78) for primary prevention cohorts (participants not selected on the basis of pre-existing chronic disease) and secondary prevention cohorts (pre-existing baseline conditions) respectively, adjusting for several potential confounding factors. Subgroup analyses indicated that the inverse associations of 25(OH)D with cancer specific mortality were significantly stronger in the populations with low prevalence of vitamin D supplement use (< 10%).

In an individual-patients pooled analysis of 8 cohorts studies a consistent increase in mortality was observed for subjects with 25(OH)D concentrations below 40 nmol/L. The prevalence of 25(OH)D concentrations below 40 nmol/L was estimated to be about 20%. No clear linear relationship between 25(OH)D and cancer mortality (2) was demonstrated, however in a previous pooled analysis a significant association with cancer mortality was observed among subjects with a history of cancer (risk ratio = 1.70 (95% CI: 1.00-2.88)) (3).

A mendelian pooled analysis of the UK Biobank evaluated whether genetically predicted 25(OH)D concentrations are associated with cancer mortality summarising data of 438 870 healthy subjects and 6998 cancer-specific deaths. Results showed that genetically low plasma 25(OH)D concentrations were not associated with cancer mortality (4). More consistent results suggesting inverse association of 25(OH)D with cancer mortality were found by meta-analyses for patients with some specific cancer sites: Pancreas, Breast, Lung, Prostate, Colorectal and Haematological (Table I). Some single cohort studies also found a significant decreased cancer mortality risk for the upper aerodigestive tract and Gastric cancer (Table II).

Since sun exposure is a recognised risk factor for melanoma, the commonly given advice to melanoma patients to reduce their sun exposure after diagnosis could further exacerbate their vitamin D insufficiency. In fact, in a pro-

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TABLE I.
Recent meta-analysis on 25OHD and survival/cancer mortality.

	Cancer sites	First author, Publication year	N. studies	No. subjects	Endpoint	Summary Risk estimate (95%CI)	Contrasts
Meta-analysis	Pancreas	Zhang, 2017	8	2166	Mortality	0.81 (0.68-0.96)	High *vs* low
	Breast	Hu, 2018	6	5984	Overall survival	0.67 (0.56-0.79)	Highest with lowest
	Lung	Huang, 2017	8	2166	Overall survival	0.80 (0.59-1.08)	High *vs* low
		Feng, 2017	4	17919	Mortality	0.76 (0.61-0.94)	High *vs* low
			5		Overall survival	1.01 (0.88-1.16)	High *vs* low
	Prostate	Song, 2018	7	7808	Overall survival	0.91 (0.87-0.97)	20 nmol/L increase
	Colorectal	Maalmi, 2018	11	7718	Overall survival	0.67 (0.57-0.78)	Highest with lowest
	Hematological	Wang, 2015	7	2643	Overall survival	0.54 (0.45-0.65)	Normal *vs* low
	Any	Chowdhury, 2014	17	120735	Mortality	0.80 (0.70-0.91)	High *vs* low
Pooled-analysis any		Ong, 2018*		6998	Mortality	0.97 (0.84-1.11)	20 nmol/L increase
		Gaksch, 2017	8	26916	Mortality	0.79 (0.60-1.04)	> 100 *vs* 75-99 nmol/L
		Schöttker, 2014	8	26018	Mortality	0.60 (0.35-1.00)	Top *vs* bottom quintiles

* Mendelian randomisation.

spective cohort of 1171 melanoma patients the variation of 25(OH)D from baseline was found associated with risk of relapse: an increased risk was found with a reduction and an increase in 25(OH)D (5). Patients who did not change their habitudes and had sunny holidays after melanoma diagnosis likely correspond to the reference category of no change in 25(OH)D in the study by Saiag et al. (5). In a cohort of 691 melanoma patients we found that the risk of melanoma recurrence was significantly lower in patients who had holidays in the sun after melanoma diagnosis avoiding sun exposure during peak hours (6). Furthermore, sunny holidays before melanoma diagnosis were found to be significantly associated with lower Breslow thickness, the main prognostic factor of melanoma. Number of weeks of sunny holidays was also significantly and inversely associated with thickness in a dose-dependent manner (6).

A big prospective cohort of 1,042 melanoma patients after a median follow-up time of seven years showed that low vitamin D was significantly associated with worse melanoma prognostic factors (high tumor thickness, ulcerated tumor and advanced melanoma stage). Multivariable hazards ratios confirmed a significant reduced risk of relapse, overall survival and melanoma specific survival for increasing values of 25OHD (7), adjusting for markers of inflammation.

RANDOMIZED CLINICAL TRIALS

Few RCTs investigated effect of vitamin D supplementation on cancer mortality or survival in cancer patients (Table III).

The Cochran collaboration in 2014 reviewed 18 clinical trials and showed that Vitamin D_3 (cholecalciferol), given singly (with no calcium), is associated with decreased cancer mortality and all-cause mortality, even if limitations were outlined due to low statistical power and risk of attrition bias (8).

A nationwide, randomized, placebo-controlled trial (VITAL), with vitamin D_3 at a dose of 2000 IU per day, conducted on 25,871 participants, showed overall no results on all main endpoints such as cancer incidence. However when first 1-2

TABLE II.
Cohort studies on 25OHD and survival/cancer mortality for cancer sites.

Cancer site	First author, PY	Country	No. subjects	Risk estimate (95% CI)	Contrasts*
Melanoma	Fang, 2016			0.71 (0.55-0.93)	> 20 *vs* < 20
Upper aerodigestive tract	Gugatschka, 2011	Austria	88	0.89 (0.83-0.97)	> 10 *vs* < 10
Gastric	Ren, 2012 (NHANES)	China	197	0.59 (0.37-0.91)	≥ 50 *vs* < 50
Head and neck	Meyer, 2011	Canada	522	0.85 (0.57-1.28)	> 78 *vs* < 48

PY: publication year; * ng/mL.

TABLE III.
Randomised clinical trial (CRT) on vitamin D and survival/cancer mortality.

Study design	Cancer site	First author, PY	Arms	N. trials	Endpoint	N. deaths	HR (95% CI)
RTC	Breast	Chlebowski, 2008	Vitamin D + calcium Placebo	1	Mortality	46	0.99 (0.55-1.76)
Meta-analysis of RCT	Prostate	Shahvazi, 2019	Vitamin D *vs* control	3	Survival	477	1.05 (0.81-1.36)
RTC	Any	Trivedi, 2003	Vitamin D Placebo	1	Mortality	63	0.86 (0.61-1.20)
RTC	Any	Wactawski-Wende, 2006; Brunner, 2011	Vitamin D + calcium Placebo	1	Mortality	744	0.90 (0.77-1.05)
RTC	Any	Avenell, 2011 (RECORD)	Vitamin D Calcium Vitamin D+ Calcium Placebo	1	Mortality	329	0.85 (0.68-1.06)
Meta-analysis of RCT	Any	Keum, 2019	Vitamin D *vs* control	5	Mortality	1591	0.87 (0.79-0.96)

PY: publication year.

years of follow-up were excluded to take into account of latency effect, a significant decreased risk of cancer death was estimated for vitamin D arm *vs* placebo: Hazard Ratio (HR) = 0.75 (95% CI: 0.59-0.96) (9).

A randomized, double-blind trial in 155 lung cancer patients, who received vitamin D supplements (1,200 IU/day) for 1 year after surgery or placebo, found overall no results. However, selecting patients with early-stage adenocarcinoma with low 25(OH)D, vitamin D supplementation was found to be significantly associated with 63% decrease risk of death (HR = 0.37; 95% CI: 0.15-0.95) (10)

A randomised clinical trial in 417 digestive tract cancer patients assessed the effect of vitamin D (2000IU/d) versus placebo on relapse free survival (AMATERASU trial). Overall no effect was found, however in patients with medium baseline serum 25(OH)D levels (between 20 and 40 ng/mL), supplementation was found to be associated with a significant decreased risk of relapse (HR = 0.46; 95% CI, 0.24-0.86). No association was found for the patients with 25(OH)D below 20 ng/mL. The dose of vitamin D could have been insufficient to increase vitamin D levels in that subgroup (11).

The SUNSHINE study, a phase 2 randomised clinical trial of 139 advanced/metastatic colorectal cancer patients, assessed the efficacy of high-dose vitamin D3 vs standard dose (+standard chemotherapy): 8,000 IU/d for 14 days, then 4,000 IU a day thereafter versus 400 IU/d during all cycles. Multivariable analysis showed a significant reduced risk of relapse: HR = 0.64 (95%CI: 0-0.90). The effect of high-dose vitamin D3 on progression-free survival appeared to be greater among patients with a lower BMI, more metastatic sites and KRAS wild-type cancers (p = 0.04, p = 0.02 and p = 0.04 respectively for interaction). Furthermore, vitamin D was associated with fewer grade 3 or higher diarrhea events (12).

In 2019 a meta-analysis summarised 5 clinical trials and included 1591 cancer deaths. The 25(OH)D levels attained was between 54 and 135 nmol/l in the intervention group and the summary risk estimate indicated a significant reduced risk of cancer death: SRR = 0.87 (95% CI: 0.79-0.96), with no heterogeneity. Interestingly the effect was largely attributable to interventions with daily dosing (as opposed to infrequent bolus dosing). No statistically significant heterogeneity was observed by attained levels of circulating 25(OH)D (13).

DISCUSSION

Findings from observational studies constitute suggestive pieces of evidence of a relationship between vitamin D and cancer survival and mortality but they are insufficient to establish causality. Main results of RCTs showed overall no effect on cancer mortality and survival, however subgroup analyses are suggestive and strong enough to consider that RCTs may not have correctly addressed the question. Several issues were raised on the validity of the conclusions. First of all, RCTs included study participants irrespective of their 25(OH)D level and may thus have failed to detect significant treatment effects in vitamin D deficient individuals. The doses used in the majority of trials are ordinary doses of vitamin D supplements as for the prevention of fractures and we do not know the exact dose that could be effective for cancer mortality and survival.

A particular case is the one of melanoma patients. Given that ultraviolet exposure is a recognized risk factor for melanoma, a common advice after melanoma diagnosis is to stop sun exposure. Thus, the UK melanoma guidelines recommend checking vitamin D levels in all melanoma patients at diagnosis and offer supplementation if necessary. However, there are some concerns that oral supplementation of vitamin D may not be as

efficient as limited controlled sun exposure and more studies are needed in this area.
Obese subjects are usually vitamin D deficient because of "trapping" of the vitamin D parent compound, cholecalciferol, in adipose tissue. Moreover obesity is inversely associated with physical activity that is positively associated with 25OHD among people with normal- and overweight BMI but not in people with obese BMI. The association between physical activity and vitamin D status has often been attributed to physical activity being a surrogate for sun exposure; however, in the few studies in which both estimates are adjusted for sun exposure, the physical activity-vitamin D relationship persisted (14).
It has also been speculated that immune-modulating ability of vitamin D could offer indications for a novel application in cancer patients receiving immunotherapy, to reinforce the anti-tumoral response and to prevent and/or limit the onset of immune related adverse events (15).
Evidence from RTCs do not allow definitive answers but it raises the hypothesis that combination therapy is required for cancer survival/mortality. New RCTs should be organized in particular in this setting because we need more information on dose of vitamin D supplementation, per specific cancer sites and stages, and to assess the benefits in patients with low vitamin D status at baseline. New studies should also take into account BMI and have good follow-up of all participants, in order to reduce attrition bias, better evaluate compliance and the effect of vitamin D on cancer therapy toxicity.

Conflicts of Interest

The author declare no conflict of interest.

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