Photochemical & Photobiological Sciences

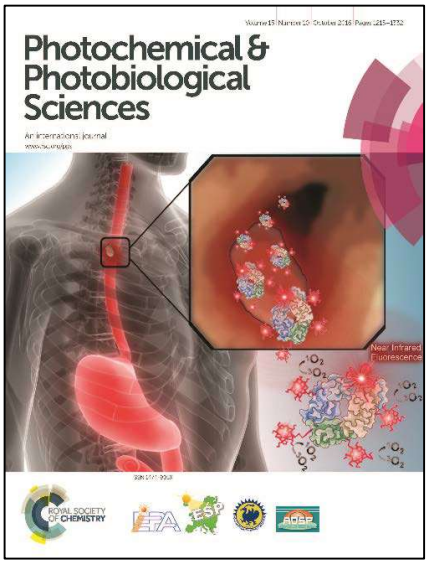
Low wintertime pre-diagnostic vitamin D status is associated with an increased risk of internal malignancies in kidney transplant recipients

Journal:	*Photochemical & Photobiological Sciences*
Manuscript ID	PP-ART-10-2017-000404.R1
Article Type:	Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	de Gruijl, Frank; Leiden University Medical School, Dermatology Wolterbeek, Ron; Leiden University Medical Center, Medical statistics Pavel, Stan; Leiden University Medical Center, Dermatology de Fijter, Hans; Leiden University Medical Center, Nephrology Hamdy, Neveen; Leiden University Medical Center, Endocrinology Bouwes Bavinck, Jan Nico; Leiden University Medical Center,

Photochemical & Photobiological Sciences

Guidelines to Referees

A society-owned journal publishing high quality research on all aspects of photochemistry and photobiology



http://www.rsc.org/pps

Photochemical & Photobiological Sciences aims at publishing high quality articles reporting original research on elemental photochemical and photobiological processes ranging from the interaction of light with molecules, materials and living organisms and the environment, to the applications of light in chemistry, biology and medicine.

For an article to be accepted, it must report new, high-quality research and make a significant contribution to the field.

Communications must report novel scientific work of sufficient importance and impact to justify rapid publication. **Papers** should report more complete studies. **Technical notes** are brief descriptions of novel instruments or methods. **Perspectives** are forward-looking reviews of a topic with emphasis on the current challenges and future outlook. The **Forum** section of the journal offers extremely rapid publication of critical comments or opinions.

Photochemical & Photobiological Sciences' **Impact Factor is 2.344**
(2016 Journal Citation Reports®)

Routine or unnecessarily fragmented work, however competently researched and reported, should not be recommended for publication.

Thank you very much for your assistance in evaluating this manuscript

Dr Dario Bassani
University of Bordeaux, France
Editor-in-Chief (Photochemistry)

Professor Santi Nonell
University Ramon Llull, Spain
Editor-in-Chief (Photobiology)

General Guidance (for further details, see the RSC Refereeing Procedure and Policy)

When preparing your report, please:

- Comment on the **originality**, **importance**, **impact** and **scientific reliability** of the work
- State clearly whether you would like to see the paper accepted or rejected and give detailed comments (with references, as appropriate) that will help both the Editor to make a decision on the paper and the authors to improve it

Please inform the Editor if:

- There is a conflict of interest
- There is a significant part of the work which you are not able to referee with confidence
- If the work, or a significant part of the work, has previously been published, including online publication (*e.g.* on a preprint server/open access server)
- You believe the work, or a significant part of the work, is currently submitted elsewhere
- The work represents part of an unduly fragmented investigation

Low wintertime pre-diagnostic vitamin D status is associated with an increased risk of internal malignancies in kidney transplant recipients

Frank R. de Gruijl[1], Ron Wolterbeek[2], Stan Pavel[1], Johan W. de Fijter[3], Neveen A.T. Hamdy[4], Jan Nico Bouwes Bavinck[1]

[1]Dept. of Dermatology, [2]Dept. of Medical Statistics, [3]Dept. of Nephrology, [4]Dept. of Endocrinology of the Leiden University Medical Center, Leiden, the Netherlands

Short title: “Winter vitamin D status and cancer risk”

Corresponding author: Frank R. de Gruijl, PhD, Dept. of Dermatology, LUMC postal zone S2P, PO Box 9600, 2300 RC, The Netherlands; e-mail: f.r.de_gruijl@lumc.nl

Keywords: vitamin D – cancer – renal transplant recipients – season – winter

Abbreviations: CI, confidence interval; HR, hazard ratio; KTR, kidney transplant recipient; SCC, squamous cell carcinoma; VDR, vitamin D receptor; 25(OH)D, 25-hydroxyvitamin D.

Word count abstract: 199

Word count text: 2997

Abstract

Low serum 25-hydroxyvitamin D (25OHD) concentrations have been associated with increased cancer risk, but the relative importance of seasonality, i.e. high summer concentrations versus low winter concentrations, is unclear. We investigated this issue in a high risk group: kidney transplant recipients with known increased risk of cancer and low vitamin D statuses. We examined the relationship between registered concentrations of 25OHD binned by quarter and subsequent risk of internal malignancy or cutaneous squamous cell carcinoma in 1112 kidney transplant recipients. Hazard ratios for internal malignancies were significantly increased with lower pre-diagnostic 25OHD concentrations in the first quarter of the year (January - March); a 1.4 fold increase (95%CI 1.1;1.7) per 10 nmol/L decrease in 25OHD. This effect was stronger in women, 1.5 (1.2;2.0) per 10 nmol/L decrease, than in men, 1.3 (0.93;1.8 per 10 nmol/L decrease, no gender interaction). Except for women in April - June (1.4 (1.1;1.7) per 10 nmol/L decrease) pre-diagnostic 25OHD concentrations in the other quarters were not statistically significantly associated with internal malignancies. Higher 25OHD concentrations tended to be associated with the development of cutaneous squamous cell carcinomas, independent of the time of the year. Our study indicates that low wintertime 25OHD concentrations are associated with an increased risk of internal malignancies and that transplant recipients may benefit from wintertime vitamin D supplementation. Our findings need further corroboration, but suggest that the lowest concentrations of vitamin D, which occur in winter, are important for the risk of internal malignancies.

Introduction

Kidney transplant recipients have a highly increased risk to develop internal malignancies and cutaneous squamous cell carcinomas (cSCC).[1,2] They are also known to have widespread vitamin D deficiency.[3-5] Vitamin D status, defined as serum concentration of 25-hydroxyvitamin D (25OHD) is commonly low in kidney transplant recipients, particularly at the end of winter with a geometric mean of 27 (95% 21-36) vs. 50 (95% 39-64) nmol/l in healthy controls.[6] This is largely attributed to the common advice given to kidney transplant recipients to avoid sun exposure and to use sunscreens when exposed to the sun, to decrease the risk of developing cSCC.[1] Keyzer et al found that all-cause mortality in kidney transplant recipients was inversely related to vitamin D status, notably also after adjustment for confounders such as renal function.[7]

Vitamin D is predominantly formed in the skin by exposure to (solar) UV radiation (~ 67%), and acquired for a minor part through the (Western) diet.[8,9] The classic function of vitamin D is to maintain calcium and phosphate homeostasis, essential for bone health. However, evidence has been mounting of additional, pleiotropic functions of vitamin D.[10,11] A wide range of non-classic roles for vitamin D has been reported such as immunomodulation, down regulation of cell proliferation, stimulation of cell differentiation and apoptosis through autocrine and paracrine pathways.[10-12] Cancer cells may thus down regulate their growth through an autocrine loop if they carry a functional vitamin D receptor, VDR.[13,14] Recently, our group has shown that UV exposure and corresponding increases in 25OHD impair development of intestinal cancers in mice.[15]

Large studies in the US[17] and in Europe[18] showed that the risk of colon cancer was significantly associated with pre-diagnostic low serum concentrations of 25OHD. With cases and controls properly matched by the month of blood sampling, the former study reported a more pronounced effect on risk from (low) winter concentrations, whereas the latter study corrected computationally for mismatches in months of blood sampling and found no effect on risk from the season in which 25OHD serum concentrations were measured. A recent review concluded that the evidence was not sufficient for “recommendations related to optimal 25OHD

concentrations (and any role for vitamin D supplementation) for primary and secondary prevention of cancer".[20]

We addressed the issue of cancer risk in relation to seasonal differences in 25OHD serum concentrations in a cohort of more than a thousand kidney transplant recipients who have been closely monitored over decades since receiving a kidney transplant and in whom 25OHD concentrations had been measured at some stage after transplantation. Some of these kidney transplant recipients developed internal malignancies and in most cases (70%) pre-diagnostic measurements of 25OHD concentrations had been documented. We also studied the relation between pre-diagnostic concentrations of 25OHD and cSCC risk. Blood sampling was spread evenly over the months of the year, which allowed us to analyze cancer risk in relation to seasonal differences in serum concentrations of 25OHD.

Methods

Patient Cohort

The Leiden Kidney transplant database includes demographic, clinical, laboratory and other data on all patients who received a kidney transplant in the Leiden University Medical Center since 1966. Measurements of 25OHD serum concentrations were available from January 1991. The cohort was also analyzed for elevated cancer risk.[2] All 1906 kidney transplant recipients in the database with transplantations up to June 2007 and at least one documented post-transplantation 25OHD measurement were first identified. Among these kidney transplant recipients we distinguished 196 patients who had histopathologically confirmed internal malignancies or cSCC and 996 patients with neither (Figure 1); the latter group served as "cancer-free" controls. Next, 80 of 196 cases with either form of cancer but without any registered 25OHD concentrations predating the diagnosis of cancer were excluded from the analysis (Figure 1). Of 27 patients who had both internal malignancy and cSCC, 11 had a pre-diagnostic 25OHD concentration only for internal malignancy and 2 only for cSCC. The remaining 7 patients had a pre-diagnostic 25OHD concentration for both internal malignancy and cSCC. These 7 patients were analyzed both in the internal malignancy group and the cSCC group, (Figure 1).

Ethics statement

The study adhered to the ethical principles of the Declaration of Helsinki, and was conducted with the approval of the Medical Ethical Committee of the Leiden University Medical Center. Since we only used archival records and the patients were anonymized, informed consent was legally not required and thus approval of the Medical Ethical Committee was obtained (P07.024).

Immunosuppressive regimen

From 1966 to 1986, immunosuppressive regimen consisted of prednisolone and azathioprine, and from 1986 to 1996 of prednisolone and cyclosporine A. Since 1996, the immunosuppressive regimen of choice has been triple therapy initially using prednisolone, mycophenolate mofetil (MMF) and cyclosporine A and later

prednisolone, MMF and tacrolimus. Most patients initially treated with prednisolone and azathioprine and demonstrating good renal function were not converted to the newer regimen. The patients changed sometimes their immunosuppressive treatment. The immunosuppressive treatment that was used for the longest time was used in those cases. The time of the year did not influence the therapy. Immunosuppressive regimens were grouped into three main categories, all including prednisolone and either azathioprine, MMF or cyclosporine A or tacrolimus.

Laboratory data

Laboratory measurements were performed on freshly collected blood samples by the Central Clinical Chemistry Laboratory of the Leiden University Medical Center. Serum concentrations of 25OHD were determined using standard radioimmunoassays (DiaSorin, Stillwater, Mn, USA), with pre-extraction by protein precipitation in acetonitril.

Longitudinal scatter plots of all the 25OHD measurements showed no evidence of a trend or any systematic variation from 1991 through to 2007 (data not shown). We did not identify any obvious trends to suggest there was a bias. Vitamin D insufficiency was defined as 25OHD concentrations <50 nmol/l and deficiency as 25OHD concentrations <30 nmol/l.

Statistical analyses

All statistical analyses were performed using IBM SPSS statistics for Windows version 23 (SPSS Inc., Chicago, IL). Data are expressed as mean ± SD. Chi-square tests were used for categorical variables and two-tailed Student's T-tests for continuous variables.

Cox proportional hazard analyses were used to calculate hazard ratios for the rates at which internal malignancies or cSCC developed after transplantation, and to adjust for potentially confounding factors such as gender, age at transplantation, time on dialysis and immunosuppressive regimen. *P*-values below 0.05 were considered statistically significant. For the Cox analyses starting time (t = 0) was the date of kidney transplantation and end-time was one of the following milestones: a) diagnosis of a first cancer , b) death, c) lost from follow up or d) end of the study (June 1, 2012).

25OHD concentrations were analyzed in 2 models. Primarily, units of 10 nmol/L decrease in concentrations were used (model 1) and secondary, categorization as deficient, insufficient or sufficient was used (model 2) to analyze the impact of pre-diagnostic 25OHD concentrations. The number of pre-diagnostic 25OHD concentrations varied between 1 and 38 measurements per patient and were introduced in model 1 as a time-varying covariate. For the categorized analyses (model 2) we used the mean value of pre-diagnostic 25OHD concentrations for the specific quarters. All blood measurement results calculated in model 2 were based on mean concentrations, but when using the first, the lowest, the highest, or the median concentrations, respectively similar outcomes were observed (data not shown).

Interaction variables were constructed by using the product of e.g. gender and pre-diagnostic 25OHD concentrations with internal malignancies or cSCC as the outcome. Similarly, interaction variables were constructed for quarter, age and immunosuppressive regimen with pre-diagnostic 25OHD concentrations.

Adjustment for age, gender and immunosuppressive regimen did not importantly change the hazard ratios. We, therefore, only present the non-adjusted hazard ratios.

Results

Baseline characteristics

The baseline characteristics of the kidney transplant recipients with internal malignancies, cSCC and no cancer are depicted in Table 1. Patients with cSCC were more often men compared to the patients without cancer and their follow-up time since transplantation was much longer. The latter was also reflected by the immunosuppressive regimen which, in the patients with cSCC, much more often consisted of azathioprine, because in the earlier days (before 1986) azathioprine was the only available immunosuppressive agent (Table 1).

The characteristics of the patients without cancer according to three different 25OHD concentrations are provided in Table 2. The percentage of women who were 25OHD deficient was significantly higher than the percentage of men. The immunosuppressive regimen was not associated with the 25OHD concentration (Table 2).

Types of cancer

In our patient cohort we identified 65 patients with internal malignancies consisting of 5 cases with head and neck cancer (2 oral cavity, 1 salivary glands, 2 pharynx), 16 with digestive organ cancer (5 colon; 1 cecum; 1 small intestine; 5 stomach, 2 esophagus, 1 gall bladder, 1 pancreas), 12 with lung cancer, 3 with breast cancer; 5 with female genital organ cancer (1 cervix uteri; 2 corpus uteri; 1 ovary; 1 vulva), 6 with male genital organ cancer (5 prostate; 1 testis), 5 with urinary tract cancer (4 kidney; 1 urinary bladder), 11 with hematological cancer (9 lymphoma; 2 leukemia) and 4 with other types of cancer (3 thyroid gland, 1 glioma of the brain) and 58 patients with cSCC.

25OHD concentrations and internal malignancies

In a first straight comparison, seasonal differences in 25OHD concentrations became apparent by plotting average concentrations by quarters for tumor-free controls and transplant recipients who later on developed internal malignancies (Figure 2A). These differences were more pronounced in women than in men (Figure 2C and 2D). We, therefore, also performed the analyses for men and women, separately. We did

not find interactions of quarter, age or immunosuppressive regimen with 25OHD concentrations.

Time-dependent hazard ratios for developing internal malignancies in relation to 10 nmol/L decrease of pre-diagnostic 25OHD concentrations were statistically significant in the first quarter of the year (January – March) for women and for men and women combined but just a trend was observed for men only (model 1, Table 3). The hazard ratio to develop internal malignancies per 10 nmol decrease in 25OHD concentrations measured in the first quarter (January – March) for men and women together was 1.4 (1.1;1.7) if all internal malignancies (n = 35) were included, 1.7 (1.0;3.0) if the analysis was restricted to the digestive tract (cancer of the stomach or intestines, n = 10) and 1.6 (0.9;2.8) for lung cancer (n = 7). With the exception of the second quarter (April – June) for women, pre-diagnostic 25OHD concentrations were not statistically significant associated with internal malignancies in the other quarters (Table 3).

In supplementary Table S1 the association of three different 25-hydroxyvitamin D concentrations (sufficient; insufficient and deficient) with the risk of internal malignancies is presented (model 2) showing largely the same results as presented for model 1.

Winter versus summer concentrations of 25OHD

A scatter plot of winter (January through March) vs. summer (July through September) concentrations (Figure 3) showed a significant ($P < 0.001$) but weak correlation in controls ($R^2 = 0.23$, n = 368) and a somewhat stronger correlation in kidney transplant recipients with internal malignancies ($R^2 = 0.51$, n= 26); the regression in the latter group was steeper but the difference with controls was not significant ($P = 0.31$ for interaction with group).

25OHD concentrations and cSCC

Time-dependent hazard ratios for developing cSCC in relation to 10 nmol/L decrease of pre-diagnostic 25OHD concentrations (model 1) and in relation to the three different 25OHD concentrations (model 2) showed a decrease, i.e., contrary to the association between 25OHD concentrations and internal malignancies, but statistical significance was not reached (Figure 4, Table 4 and supplementary Table S2).

Hence, there was a trend that the risk of cSCC was associated with increased 25OHD concentrations, which was largely independent on the quarter of the year.

Discussion

We found a significant association between pre-diagnostic low winter but not summer 25OHD concentrations and an increased risk of internal malignancy in a large cohort of kidney transplant recipients, which was most pronounced in women. This relationship was mainly attributable to digestive tract and lung cancers (43% of the cancers).

We did not find a statistically significant association between 25OHD concentrations and cSCC, although there was a clear trend that higher 25OHD concentrations were associated with the development of cSCC, independent of the time of the year. It is conceivable that the higher 25OHD concentrations are not causally involved in cSCC carcinogenesis, but merely indicate that these patients had a higher level of UV exposure which can explain the higher 25OHD concentrations in these patients and which is the real carcinogenic factor for cSCC.

Among kidney transplant recipients, a relationship between pre-transplantation 25OHD concentrations and internal malignancy risk has been previously reported (1.12 HR per ng/ml decrease or 4.6% per nmol/l decrease), but, so far, the effect of seasonal variation in vitamin D concentrations has not been studied in this population.[21]

Very much in keeping with our data, some studies on seasonal variation in 25OHD concentrations in immunocompetent subjects also found an increase in risk of neoplasia with lower winter serum concentrations of 25OHD, and no reduction in risk with higher summer concentrations. A study of a Finnish cohort of male smokers showed a significant inverse association between base line serum concentrations of 25OHD in winter and spring at the start of the study and the risk of non-Hodgkin lymphoma in the first 7 years, but not thereafter.[22] A Japanese study found that the risk of colorectal adenoma decreased significantly from the lowest to the highest quartiles in winter concentrations (November -April) of 25OHD.[23]

In our study summer and winter concentrations of 25OHD were not strongly correlated in tumor-free controls ($R^2 = 0.22$), but these concentrations were somewhat more strongly correlated in kidney transplant recipients with internal malignancies ($R^2 = 0.51$) whose winter concentrations were lower. Other factors than sun exposure in summer months were probably important for winter concentrations of

25OHD in cancer-free transplant recipients. In addition to possible genetic differences, diet and other supplementary sources of vitamin D could have specifically contributed to their winter concentrations.[24] In keeping with our findings, other studies[25-27] also found weak correlations between summer and winter concentrations of 25OHD (R^2 = 0.21 and 0.27, and a Spearman rho = 0.08, respectively) although a study from the UK[28] reported a tighter correlation (R^2 = 0.62). Summer and winter concentrations of 25OHD have also been reported to be rather weakly correlated with personal summertime UV doses (R^2 = 0.28 and 0.19, resp.).[27] It thus appears that increases in summer sun exposure are ineffective in ensuring sufficiently high winter concentrations of 25OHD.

In the studies which found colon cancer risk to be also related to summer serum concentrations of 25OHD,[17,18] these concentrations may have been more strongly related to winter concentrations (as in the transplant recipients who develop internal cancers in our study). This could be due to low vitamin D intake in Winter or substantially higher 25OHD concentrations in Summer. As kidney transplant patients are advised to moderate their sun exposure, their summer concentrations of 25OHD may rise to a lesser extent than they otherwise would have done, thus contributing very little to the winter concentrations.

A possible shortcoming of our study is that our clinical archive did not contain information on all relevant data. Because of a lack of data on sun exposure and skin type, we could not test the importance of these factors for the observed associations. Nor could we test for possible confounders like smoking or other factors (such as diet and vitamin supplements) that could have affected 25OHD concentrations. A dominant confounding effect from smoking would, however, probably have affected 25OHD concentrations throughout the year, i.e. over all quarters. Although our main and primary analysis on vitamin D concentrations and internal malignancy risk showed a statically significant inverse association, we did a number of additional statistical tests to check for confounding and interactions (for age, gender and immunosuppressive regimen). The significance of our result would be lost after correction for this additional multiple testing, and therefore the main result needs confirmation in independent studies.

Kidney transplant recipients should continue to be advised to reduce sun exposure in order to decrease the risk of cSCC, but at the same time their 25OHD

concentrations should be monitored in the wintertime and supplementation with vitamin D should be seriously considered in the light of a potential beneficial health effects, in particular reduced cancer risk as suggested by the present study.[29]

In conclusion our data indicate that pre-diagnostic low winter but not summer concentrations of 25OHD are associated with increased internal malignancy risk in our cohort of kidney transplant recipients. This finding suggests that recurrent low winter serum concentrations of 25OHD may enhance internal malignancy development. Since high concentrations of 25OHD in summer are no guarantee for sufficiently high concentrations in winter, one should not rely on summer sun exposure. Wintertime vitamin D supplementation would be the most effective approach to secure sufficiently high winter concentrations of 25OHD (at least > 50 nmol/l). Long-term studies are clearly required to demonstrate a definitive effect of vitamin D supplementation on cancer risk in humans. A recent meta-analysis of randomized control trials (RCTs) failed to show a clear beneficial effect of vitamin D supplementation on cancer risk,[30] although an earlier Cochrane study extracted an overall significant effect on cancer mortality.[31] The mixed bag of RCTs may not have been an adequate base for an assessment of effects on cancer risk; many of them may not have been optimal in design and were not dedicated to assess cancer risk.[29,32] Our data in kidney transplant recipients indicate the primary importance of winter concentrations of 25OHD for cancer risk which may generally apply to people with an overall, year-round, low vitamin D status.

Acknowledgments

This work was supported by a grant of the Foundation for General Light Therapy (the Dutch legacy trust “Stichting voor Algemeene Lichttherapie) without any involvement in the study.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

REFERENCES

1. S. Euvrard, J. Kanitakis and A. Claudy, *N. Engl. J. Med.*, 2003, **348**, 1681-1691.
2. H. C. Wisgerhof, L. G. van der Geest, J. W. de Fijter, G. W. Haasnoot, F. H. Claas, C. S. le, R. Willemze and J. N. Bouwes Bavinck, *Cancer Epidemiol.*, 2011, **35**, 105-111.
3. C. A. Kulak, V. Z. Borba, J. Kulak, Jr. and M. R. Custodio, *Curr Osteoporos Rep*, 2012, **10**, 48-55.
4. S. Mazzaferro, M. Pasquali, F. Pugliese, F. Citterio, A. Gargiulo, S. Rotondi, L. Tartaglione, C. Conte, G. Pirro and F. Taggi, *J Nephrol*, 2012, **25**, 1108-1118.
5. H. Penny, S. Frame, F. Dickinson, G. Garrett, A. R. Young, R. Sarkany, N. Chitalia, G. Hampson and D. Goldsmith, *Clin Transplant*, 2012, **26**, E617-623.
6. K. Querings, M. Girndt, J. Geisel, T. Georg, W. Tilgen and J. Reichrath, *J Clin Endocrinol Metab*, 2006, **91**, 526-529.
7. C. A. Keyzer, I. J. Riphagen, M. M. Joosten, G. Navis, A. C. Muller Kobold, I. P. Kema, S. J. Bakker, M. H. de Borst and N. consortium, *J Clin Endocrinol Metab*, 2015, **100**, 81-89.
8. Health_Council_of_the_Netherlands, *Publication no. 2012/15, The Hague*, 2012.
9. M. F. Holick, *J Cell Biochem*, 2003, **88**, 296-307.
10. D. Bikle, *J Clin Endocrinol Metab*, 2009, **94**, 26-34.
11. A. Verstuyf, G. Carmeliet, R. Bouillon and C. Mathieu, *Kidney Int*, 2010, **78**, 140-145.
12. A. W. Norman, *Am J Clin Nutr*, 2008, **88**, 491S-499S.
13. T. C. Chen, G. G. Schwartz, K. L. Burnstein, B. L. Lokeshwar and M. F. Holick, *Clin Cancer Res*, 2000, **6**, 901-908.
14. K. Colston, M. J. Colston and D. Feldman, *Endocrinology*, 1981, **108**, 1083-1086.
15. H. Rebel, C. D. der Spek, D. Salvatori, J. P. van Leeuwen, E. C. Robanus-Maandag and F. R. de Gruijl, *Int J Cancer*, 2015, **136**, 271-277.
16. C. F. Garland, A. M. Lilienfeld, A. I. Mendeloff, J. A. Markowitz, K. B. Terrell and F. C. Garland, *Gastroenterology*, 1981, **81**, 1115-1124.
17. K. Wu, D. Feskanich, C. S. Fuchs, W. C. Willett, B. W. Hollis and E. L. Giovannucci, *J Natl Cancer Inst*, 2007, **99**, 1120-1129.
18. M. Jenab, H. B. Bueno-de-Mesquita, P. Ferrari, F. J. van Duijnhoven, T. Norat, T. Pischon, E. H. Jansen, N. Slimani, G. Byrnes, S. Rinaldi, A. Tjonneland, A. Olsen, K. Overvad, M. C. Boutron-Ruault, F. Clavel-Chapelon, S. Morois, R. Kaaks, J. Linseisen, H. Boeing, M. M. Bergmann, A. Trichopoulou, G. Misirli, D. Trichopoulos, F. Berrino, P. Vineis, S. Panico, D. Palli, R. Tumino, M. M. Ros, C. H. van Gils, P. H. Peeters, M. Brustad, E. Lund, M. J. Tormo, E. Ardanaz, L. Rodriguez, M. J. Sanchez, M. Dorronsoro, C. A. Gonzalez, G. Hallmans, R. Palmqvist, A. Roddam, T. J. Key, K. T. Khaw, P. Autier, P. Hainaut and E. Riboli, *BMJ*, 2010, **340**, b5500.
19. H. van der Rhee, J. W. Coebergh and E. de Vries, *Eur J Cancer*, 2013, **49**, 1422-1436.
20. A. M. Mondul, S. J. Weinstein, T. M. Layne and D. Albanes, *Epidemiol Rev*, 2017, **39**, 28-48.
21. D. Ducloux, C. Courivaud, J. Bamoulid, A. Kazory, G. Dumoulin and J. M. Chalopin, *Transplantation*, 2008, **85**, 1755-1759.
22. U. Lim, D. M. Freedman, B. W. Hollis, R. L. Horst, M. P. Purdue, N. Chatterjee, S. J. Weinstein, L. M. Morton, A. Schatzkin, J. Virtamo, M. S. Linet, P. Hartge and D. Albanes, *Int J Cancer*, 2009, **124**, 979-986.
23. R. Takahashi, T. Mizoue, T. Otake, J. Fukumoto, O. Tajima, S. Tabata, H. Abe, K. Ohnaka and S. Kono, *Cancer Sci*, 2010, **101**, 1695-1700.
24. H. M. Macdonald, A. Mavroeidi, R. J. Barr, A. J. Black, W. D. Fraser and D. M. Reid, *Bone*, 2008, **42**, 996-1003.
25. H. E. Hanwell, R. Vieth, D. E. Cole, A. Scillitani, S. Modoni, V. Frusciante, G. Ritrovato, I. Chiodini, S. Minisola and V. Carnevale, *J Steroid Biochem Mol Biol*, 2010, **121**, 334-337.
26. K. Nakamura, M. Nashimoto and M. Yamamoto, *Int J Biometeorol*, 2000, **44**, 186-189.

27. E. Thieden, P. A. Philipsen, J. Heydenreich and H. C. Wulf, *Photochem Photobiol*, 2009, **85**, 1480-1484.
28. A. R. Webb, R. Kift, M. T. Durkin, S. J. O'Brien, A. Vail, J. L. Berry and L. E. Rhodes, *Br J Dermatol*, 2010, **163**, 1050-1055.
29. S. J. Wimalawansa, *J Steroid Biochem Mol Biol*, 2018, **175**, 60-81.
30. B. Goulao, F. Stewart, J. A. Ford, G. MacLennan and A. Avenell, *Am J Clin Nutr*, 2018, **107**, 652-663.
31. G. Bjelakovic, L. L. Gluud, D. Nikolova, K. Whitfield, G. Krstic, J. Wetterslev and C. Gluud, *Cochrane Database Syst Rev*, 2014, DOI: 10.1002/14651858.CD007469.pub2, CD007469.
32. L. Rejnmark, L. S. Bislev, K. D. Cashman, G. Eiriksdottir, M. Gaksch, M. Grubler, G. Grimnes, V. Gudnason, P. Lips, S. Pilz, N. M. van Schoor, M. Kiely and R. Jorde, *PLoS One*, 2017, **12**, e0180512.

Author contribution

Frank R. de Gruijl participated in research design, data analysis and in the writing of the paper.

Stan Pavel participated in the writing of the paper.

Ron Wolterbeek participated in research design and analyses of the data.

Johan W. de Fijter participated in the writing of the paper.

Neveen A.T. Hamdy participated in the writing of the paper.

Jan Nico Bouwes Bavinck participated in research design, analyses of the data and in the writing of the paper.

All authors reviewed the manuscript.

Figure Legends:

Figure 1: Flow diagram of selecting kidney transplant recipients (KTR) with recorded 25OHD concentrations from the database. Subgroups of KTR with only internal malignancies or only cutaneous squamous cell carcinoma (cSCC) are depicted next to the subgroup that contracted both, 7 cases remaining after selection for pre-diagnostic vitamin D statuses, resulting in a total of 65 qualifying KTR with internal malignancy and 58 with cSCC.

Figure 2: Mean pre-diagnostic 25OHD concentrations (nmol/L) in four different quarters for all patients with internal malignancies and without cancer (Panel A), and for men (Panel C) and women (Panel D), separately. Panel B shows the 25OHD concentrations of men and women without cancer.

Figure 3. Scatter plots and regression lines of winter versus summer concentrations of 25OHD in kidney transplant recipients who developed no malignancies (N = 368) (Panel A), internal malignancies (N = 26) (Panel B) or cutaneous squamous cell carcinomas (N = 34) (Panel C). The correlations in transplant recipients with internal malignancies (R^2 = 0.51) and in transplant recipients with cutaneous squamous cell carcinoma (R^2 = 0.39) were higher than in controls (R^2 = 0.23, all three $P < 0.001$). Although the regressions were not significantly different (P= 0.31 for internal malignancies and P = 0.21 for cutaneous squamous cell carcinoma compared to controls), the regression lines were steeper in kidney transplant recipients with internal malignancies (0.53 $\pm$ 0.11) and cutaneous squamous cell carcinoma (0.54 $\pm$ 0.12) vs. controls (0.37 $\pm$ 0.04) ($\pm$ SE; all three slopes significantly different from 0, $P < 0.001$). The intercepts were not significantly different from 0 in transplant recipients with internal malignancies (6 $\pm$ 7, P= 0.38) and transplant recipients with cutaneous squamous cell carcinoma (10 $\pm$ 8, P = 0.24), but significantly different from 0 in controls (19 $\pm$ 2, $P < 0.001$). The steeper slope and an intercept not significantly different from 0 suggest that winter concentrations among kidney transplant recipients who developed internal malignancies or cutaneous squamous cell carcinomas depended more on summer concentrations and less on wintertime intake of vitamin D than winter concentrations in controls.

Figure 4: Mean pre-diagnostic 25OHD concentrations (nmol/L) in four different quarters for all patients with cutaneous squamous cell carcinoma and without cancer (Panel A) and for men (Panel B).. The data for women are not shown, because of the low number of women in this analysis.

Table 1 Baseline characteristics of kidney transplant recipients with internal malignancies or cutaneous squamous cell carcinoma and without cancer.*

	No Cancer (N = 996) N (%)	Internal malignancy (N = 65) N (%)	cSCC (N = 58) N (%)
Sex			
Women	398 (40.0)	28 (43.1)	14 (24.1) [a]
Men	598 (60.0)	37 (56.9)	44 (75.9)
Age at transplantation (years)			
05 – 29	177 (17.8)	5 (7.7)	18 (31.0)
30 – 39	204 (20.5)	16 (24.7)	14 (24.1)
40 – 49	223 (22.4)	17 (26.2)	12 (20.7)
50 – 59	240 (24.1)	17 (26.2)	7 (12.1)
60 – 69	131 (13.1)	6 (9.2)	7 (12.1)
70 – 77	21 (2.1)	4 (6.2)	0
Time from transplantation to end of follow-up (years)			
0 – 1	11 (1.1)	0	0 [a]
2 – 7	249 (25.0)	12 (18.5)	2 (3.4)
8 – 12	279 (28.0)	12 (18.5)	5 (8.6)
13 – 17	184 (18.5)	16 (24.5)	9 (15.5)
18 – 22	120 (12.0)	12 (18.5)	8 (13.8)
23 – 40	153 (14.4)	13 (20.0)	34 (58.7)
Time from transplantation to first blood sample (years)			
0 – 1	449 (45.1)	20 (30.8) [a]	8 (13.8) [a]
2 – 7	330 (33.1)	19 (29.2)	10 (17.2)
8 – 12	114 (11.5)	13 (20.0)	13 (22.4)
13 – 17	57 (5.7)	5 (7.7)	16 (27.6)
18 – 22	30 (3.0)	7 (10.8)	5 (8.6)
23 and longer	16 (1.6)	1 (1.5)	6 (10.3)
Immunosuppressive regimen			
Aza in any combination	171 (19.1)	25 (38.5) [a]	37 (63.8) [a]
MMF in any combination	360 (40.2)	4 (6.2)	5 (8.6)
CyA or Tac in any combination	364 (40.7)	36 (55.4)	16 (27.6)
Missing values	101 (10%)	0	0

*In the patients with cancer the 25-hydroxyvitamin D concentrations were recorded before the diagnosis of cancer.
cSCC: cutaneous squamous cell carcinoma; Aza = Azathioprine; MMF = Mycophenolate mofetil; CyA = Cyclosporine A ; Tac = Tacrolimus.
[a]P<0.05 compared to No Cancer.

Table 2. Baseline characteristics according to different 25-hydroxyvitamin D concentrations, restricted to 996 kidney transplant recipients without cancer.

	Deficient < 30 nmol/L (N = 181) N (%)	Insufficient 30-49 nmol/L (N = 389) N (%)	Sufficient ≥50 nmol/L (N = 426) N (%)
Sex			
Women	88 (48.6)	151 (38.8)	159 (37.3) [a]
Men	93 (51.4)	238 (61.2)	267 (62.7)
Age at transplantation (years)			
05 – 29	29 (16.0)	70 (18.0)	78 (18.3)
30 – 39	37 (20.5)	85 (21.8)	82 (19.2)
40 – 49	46 (25.4)	71 (18.3)	106 (24.9)
50 – 59	35 (19.3)	100 (25.7)	105 (24.6)
60 – 69	30 (16.6)	52 (13.4)	49 (11.5)
70 – 77	4 (2.2)	11 (2.8)	6 (1.5)
Immunosuppressive regimen			
Aza in any combination	30 (18.5)	64 (17.4)	77 (21.0)
MMF in any combination	67 (41.4)	143 (39.0)	150 (41.0)
CyA or Tac in any combination	65 (40.1)	160 (43.6)	139 (38.0)
Missing values	19 (10%)	22 (6%)	60 (14%)

Aza = Azathioprine; MMF = Mycophenolate mofetil; CyA = Cyclosporine A ; Tac = Tacrolimus.
[a] $P<0.05$.

Table 3. Time-dependent hazard ratios for developing internal malignancies in relation to 10 nmol/L decrease of personal pre-diagnostic 25-hydroxyvitamin D concentrations in four separate quarters over the year and over the whole year (model 1).

	All patients	Men	Women
	HR (95% CI)	HR (95% CI)	HR (95% CI)
25OHD January - December	1.1 (0.98;1.2)	1.0 (0.89;1.2)	1.2 (0.98;1.4)
	P = 0.132	P = 0.710	P = 0.085
25OHD January - March	**1.4 (1.1;1.7)**	1.3 (0.93;1.8)	**1.5 (1.2;2.0)**
	P = 0.004	P = 0.123	**P = 0.015**
25OHD April - June	1.1 (0.96;1.3)	0.93 (0.76;1.1)	**1.4 (1.1;1.7)**
	P = 0.164	P = 0.459	**P = 0.016**
25OHD July - September	1.1 (0.96;1.2)	1.1 (0.92;1.3)	1.1 (0.91;1.3)
	P = 0.183	P = 0.306	P = 0.348
25OHD October - December	1.1 (0.92;1.3)	0.95 (0.78;1.2)	1.4 (0.99;1.9)
	P = 0.337	P = 0.593	P = 0.061

HR = hazard ratio; CI = confidence interval.
The numbers of patients with and without internal malignancies per category are presented in Table S1.

Table 4. Time-dependent hazard ratios for developing cutaneous squamous cell carcinoma in relation to 10 nmol/L decrease of personal pre-diagnostic 25-hydroxyvitamin D concentrations in four separate quarters over the year and over the whole year (model 1).

	All patients	Men	Women
	HR (95% CI)	HR (95% CI)	HR (95% CI)
25OHD January - December	0.96 (0.87;1.1)	0.98 (0.87;1.1)	0.87 (0.74;1.0)
	P = 0.372	P = 0.732	P = 0.103
25OHD January - March	0.96 (0.83;1.1)	0.95 (0.79;1.1)	0.80 (0.59;1.1)
	P = 0.617	P = 0.604	P = 0.158
25OHD April - June	0.95 (0.82;1.1)	1.1 (0.87;1.4)	**0.75 (0.60;0.94)**
	P = 0.490	P = 0.476	**P = 0.011**
25OHD July - September	0.99 (0.88;1.1)	0.97 (0.83;1.1)	1.1 (0.83;1.4)
	P = 0.934	P = 0.683	P = 0.599
25OHD October - December	0.92 (0.80;1.1)	0.87 (0.75;1.0)	0.94 (0.65;1.4)
	P = 0.277	P = 0.058	P = 0.722

HR = hazard ratio; CI = confidence interval.
The numbers of patients with and without squamous cell carcinoma per category are presented in Table S2.

Table S1. Hazard ratios for developing internal malignancies in relation to personal mean pre-diagnostic 25-hydroxyvitamin D concentrations in four separate quarters over the year and over the whole year (model 2).

	All patients			Men			Women		
	No Cancer N (%)	Internal malignancy N (%)	HR (95% CI)	No Cancer N (%)	Internal malignancy N (%)	HR (95% CI)	No Cancer N (%)	Internal malignancy N (%)	HR (95% CI)
25OHD January - December	(N = 996)	(N = 65)		(N = 598)	(N = 37)		(N = 398)	(N = 28)	
Sufficient (≥50 nmol/L)	426 (42.7)	24 (36.9)	1	267 (44.6)	16 (43.2)	1	159 (39.9)	8 (28.6)	1
Insufficient (30-49 nmol/L)	389 (39.1)	30 (46.2)	1.4 (0.80;23)	238 (39.8)	18 (48.6)	1.2 (0.60;23)	151 (37.0)	12 (42.9)	1.7 (0.70;4.2)
Deficient (<30 nmol/L)	181 (18.2)	11 (16.9)	1.4 (0.68;2.8)	93 (15.6)	3 (8.1)	0/62 (0.18;2.1)	88 (22.1)	8 (28.6)	2.7 (0.98;7.2)
25OHD January - March	(N = 614)	(N = 35)		(N = 375)	(N = 18)		(N = 239)	(N = 17)	
Sufficient (≥50 nmol/L)	158 (25.7)	7 (20.0)	1	82 (21.9)	4 (22.2)	1	76 (31.8)	3 (17.6)	1
Insufficient (30-49 nmol/L)	251 (40.9)	9 (25.7)	1.0 (0.37;2.7)	173 (46.1)	4 (22.2)	0.54 (0.14;2.2)	78 (32.6)	5 (29.4)	1.7 (0.40;7.1)
Deficient (<30 nmol/L)	205 (33.4)	19 (54.3)	**2.5 (1.0; 6.0)**	120 (32.0)	10 (55.6)	1.6 (0.50; 5.1)	85 (35.6)	9 (52.9)	3.7 (0.99;13.9)
25OHD April - June	(N = 561)	(N = 38)		(N = 329)	(N = 19)		(N = 232)	(N = 19)	
Sufficient (≥50 nmol/L)	237 (42.2)	14 (36.8)	1	142 (43.2)	8 (42.1)	1	95 (40.9)	6 (31.6)	1
Insufficient (30-49 nmol/L)	213 (38.0)	14 (36.8)	1.0 (0.50;2.2)	131 (39.8)	10 (52.6)	1.2 (0.48;3.1)	82 (35.3)	4 (21.1)	0.77 (0.22;2.7)
Deficient (<30 nmol/L)	111 (19.8)	10 (26.2)	1.8 (0.79;4.0)	56 (17.0)	1 (5.3)	0.30 (0.04;2.4)	55 (23.7)	9 (47.4)	**3.5 (1.2;10.3)**
25OHD July - September	(N = 576)	(N = 38)		(N = 343)	(N = 22)		(N = 233)	(N = 16)	
Sufficient (≥50 nmol/L)	380 (66.0)	22 (57.9)	1	245 (71.4)	15 (68.2)	1	135 (57.9)	7 (43.8)	1
Insufficient (30-49 nmol/L)	130 (22.6)	13 (34.2)	1.8 (0.92;3.6)	74 (21.6)	6 (27.3)	1.3 (0.52;3.4)	56 (24.0)	7 (43.8)	**3.1 (1.1;9.4)**
Deficient (<30 nmol/L)	66 (11.4)	3 (7.9)	0.77 (0.22;2.6)	24 (7.0)	1 (4.5)	0.65 (0.09;4.9)	42 (18.1)	2 (12.4)	1.3 (0.26;6.5)
25OHD October - December	(N = 549)	(N = 28)		(N = 334)	(N = 17)		(N = 215)	(N = 11)	
Sufficient (≥50 nmol/L)	257 (46.8)	10 (35.7)	1	166 (49.7)	7 (41.2)	1	91 (42.3	3 (27.3)	1
Insufficient (30-49 nmol/L)	196 (35.7)	14 (50.0)	1.9 (0.84;4.3)	115 (34.4)	8 (47.1)	1.6 (0.57;4.3)	81 (37.7)	6 (54.5)	2.8 (0.69;11.2)
Deficient (<30 nmol/L)	96 (17.5)	4 (14.3)	1.2 (0.38;3.9)	53 (15.9)	2 (11.8)	0.94 (0.19;4.5)	43 (20.0)	2 (18.2)	2.1 (0.34;12.6)

HR = hazard ratio; CI = confidence interval.

Table S2. Hazard ratios for developing cutaneous squamous cell carcinoma in relation to personal mean pre-diagnostic 25-hydroxyvitamin D concentrations in four separate quarters over the year and over the whole year (model 2).

	All patients			Men			Women		
	No Cancer N (%)	SCC N (%)	HR (95% CI)	No Cancer N (%)	SCC N (%)	HR (95% CI)	No Cancer N (%)	SCC N (%)	HR (95% CI)
25OHD January - December	(N = 996)	(N = 58)		(N = 598)	(N = 44)		(N = 398)	(N = 14)	
Sufficient (≥50 nmol/L)	426 (42.7)	30 (51.7)	1	267 (44.6)	21 (47.7)	1	159 (39.9)	9 (64.3)	1
Insufficient (30-49 nmol/L)	389 (39.1)	18 (31.0)	0.72 (0.40;1.3)	238 (39.8)	15 (34.1)	0.85 (0.44;1.6)	151 (37.0)	3 (21.4)	0.41 (0.11;1.5)
Deficient (<30 nmol/L)	181 (18.2)	10 (17.3)	1.0 (0.51;2.1)	93 (15.6)	8 (18.2)	1.1 (0.47;2.4)	88 (22.1)	2 (14.3)	0.76 (0.16;3.5)
25OHD January - March	(N = 614)	(N = 34)		(N = 375)	(N = 28)		(N = 239)	(N = 6)	
Sufficient (≥50 nmol/L)	158 (25.7)	14 (41.2)	1	82 (21.9)	10 (35.8)	1	76 (31.8)	4 (66.7)	1
Insufficient (30-49 nmol/L)	251 (40.9)	9 (26.5)	0.48 (0.21;1.1)	173 (46.1)	9 (32.1)	0.46 (0.18;1.1)	78 (32.6)	0	--
Deficient (<30 nmol/L)	205 (33.4)	11 (32.4)	0.72 (0.33;1.6)	120 (32.0)	9 (32.1)	0.55 (0.22;1.4)	85 (35.6)	2 (33.3)	0.81 (0.15;4.5)
25OHD April - June	(N = 561)	(N = 29)		(N = 329)	(N = 21)		(N = 232)	(N = 8)	
Sufficient (≥50 nmol/L)	237 (42.2)	16 (55.2)	1	142 (43.2)	10 (47.6)	1	95 (40.9)	6 (75.0)	1
Insufficient (30-49 nmol/L)	213 (38.0)	10 (34.5)	0.68 (0.31;1.5)	131 (39.8)	9 (42.9)	0.89 (0.36;2.2)	82 (35.3)	1 (12.5)	0.20 (0.02;1.7)
Deficient (<30 nmol/L)	111 (19.8)	3 (10.3)	0.54 (0.16;1.9)	56 (17.0)	2 (9.5)	0.52 (0.11;2.4)	55 (23.7)	1 (12.5)	0.59 (0.07;5.0)
25OHD July - September	(N = 576)	(N = 30)		(N = 343)	(N = 24)		(N = 233)	(N = 6)	
Sufficient (≥50 nmol/L)	380 (66.0)	24 (80.0)	1	245 (71.4)	20 (83.3)	1	135 (57.9)	4 (66.6)	1
Insufficient (30-49 nmol/L)	130 (22.6)	4 (13.3)	0.59 (0.20;1.7)	74 (21.6)	3 (12.5)	0.57 (0.17;1.9)	56 (24.0)	1 (16.7)	0.74 (0.08;6.7)
Deficient (<30 nmol/L)	66 (11.4)	2 (6.7)	0.64 (0.15;2.7)	24 (7.0)	1 (4.2)	0.50 (0.07;3.7)	42 (18.1)	1 (16.7)	1.2 (0.13;10.9)
25OHD October - December	(N = 549)	(N = 26)		(N = 334)	(N = 22)		(N = 215)	(N = 4)	
Sufficient (≥50 nmol/L)	257 (46.8)	15 (57.7)	1	166 (49.7)	12 (54.5)	1	91 (42.3	3 (75.0)	1
Insufficient (30-49 nmol/L)	196 (35.7)	8 (30.8)	0.74 (0.31;1.7)	115 (34.4)	8 (36.4)	0.68 (0.27;1.7)	81 (37.7)	0	--
Deficient (<30 nmol/L)	96 (17.5)	3 (11.5)	0.65 (0.19;2.2)	53 (15.9)	2 (9.1)	0.41 (0.09;1.9)	43 (20.0)	1 (25.0)	1.3 (0.13;13.2)

HR = hazard ratio; CI = confidence interval; SCC = squamous cell carcinoma.

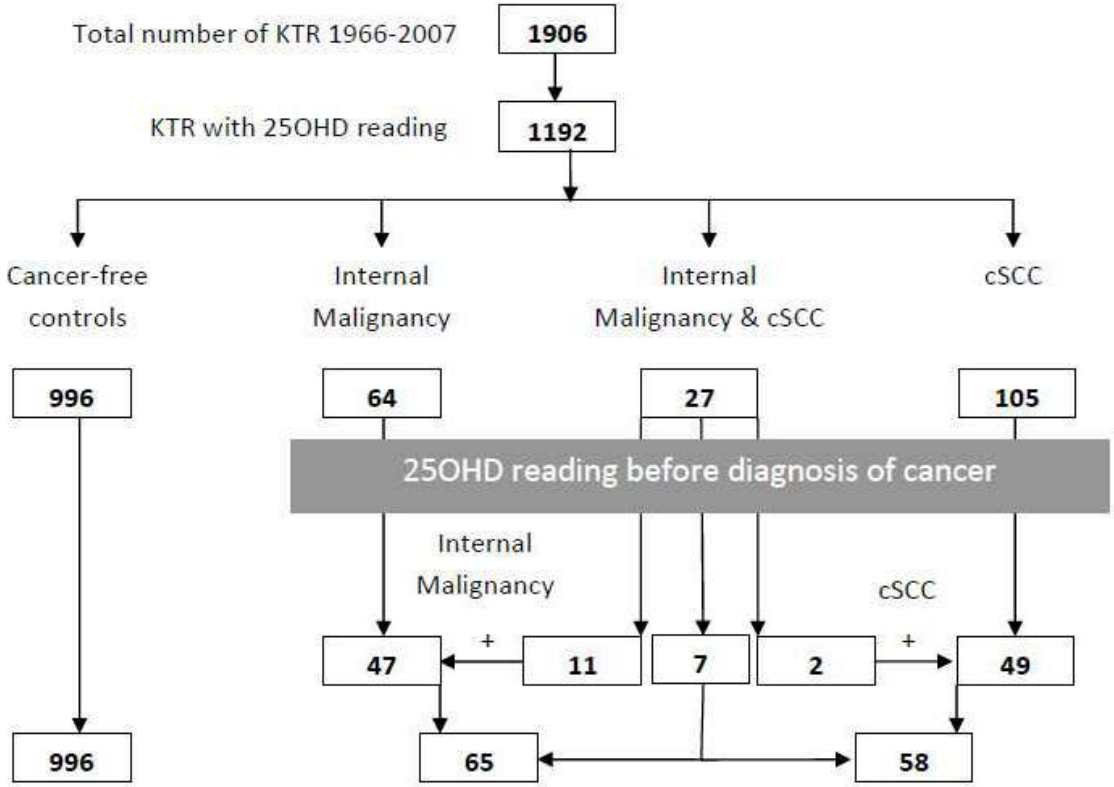

Total number of KTR 1966-2007
1906
KTR with 25OHD reading
1192
Cancer-free controls
Internal Malignancy
Internal Malignancy & cSCC
cSCC
996
64
27
105
25OHD reading before diagnosis of cancer
Internal Malignancy
cSCC
+
+
47
11
7
2
49
996
65
58

Figure 1

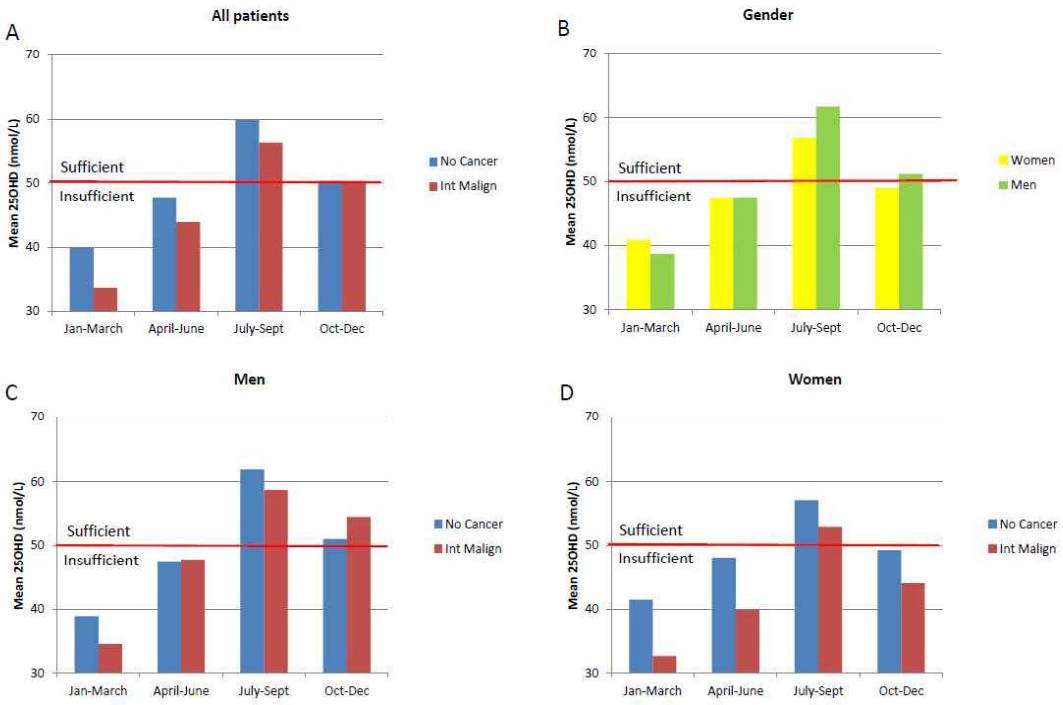

A
All patients
Mean 25OHD (nmol/L)
70
60
50
40
30
Sufficient
Insufficient
Jan-March
April-June
July-Sept
Oct-Dec
No Cancer
Int Malign
B
Gender
Women
Men
C
Men
D
Women

Figure 2

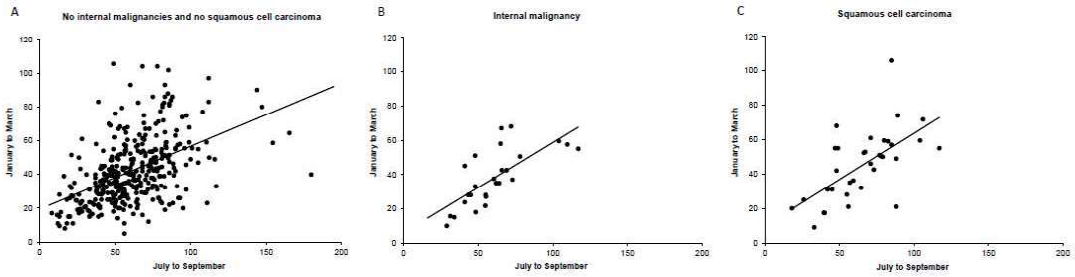

A
No internal malignancies and no squamous cell carcinoma
January to March
July to September
B
Internal malignancy
January to March
July to September
C
Squamous cell carcinoma
January to March
July to September

Figure 3

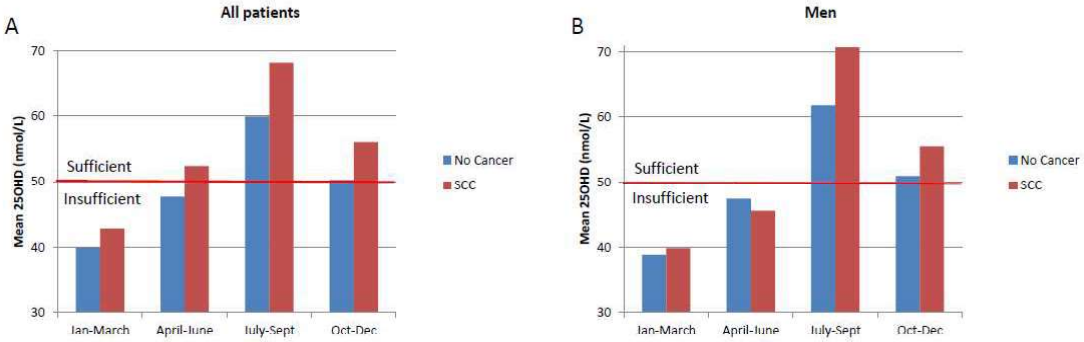
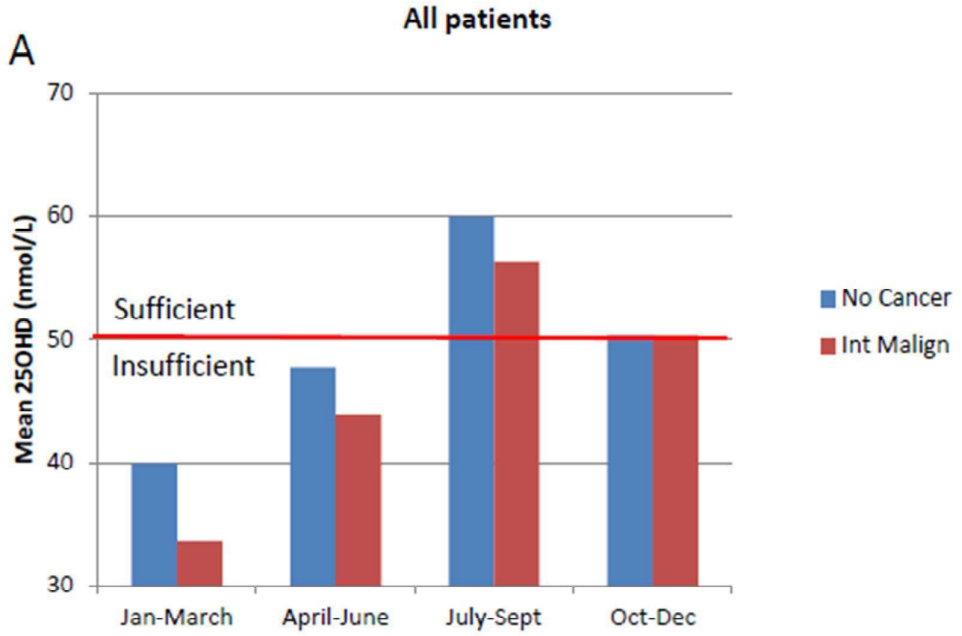

A
All patients
Mean 25OHD (nmol/L)
70
60
50
40
30
Sufficient
Insufficient
Jan-March
April-June
July-Sept
Oct-Dec
No Cancer
SCC
B
Men
Mean 25OHD (nmol/L)
70
60
50
40
30
Sufficient
Insufficient
Jan-March
April-June
July-Sept
Oct-Dec
No Cancer
SCC

Figure 4



Kidney transplant recipients with the lowest wintertime vitamin D levels appear to run the highest risk of internal malignancies. The effect was strongest in women.