The relationship between vitamin D status and the risk of cancer among older adults

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The relationship between vitamin D status and the risk of cancer among older adults

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
BMI	Body mass index
CHANCES	Consortium on Health and Ageing: Network of Cohorts in Europe and the United States
CI	Confidence Interval
CYP24A1	1,25-dihydroxyvitamin D 24-hydroxylase
CYP27A1	Sterol 27-hydroxylase
CYP27B1	25-hydroxyvitamin D 1α-hydroxylase
CYP2R1	Vitamin D 25-hydroxylase
DBP	Vitamin D binding protein
DHRC7	7-dehydrocholesterol reductase
EPIC	European Prospective Investigation into Cancer
ESTHER	Epidemiologie Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung
GC	Group specific component or gc-globulin / vitamin D binding protein
HR	Hazard Ratio
IA	Immunoassay
IDS	Immunodiagnostic Systems
IOM	Institute of Medicine
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
OR	Odds Ratio
RFLP	Restriction Fragment Length Polymorphism
RR	Risk Ratio
SAS	Statistical Analysis System
SNP	Single nucleotide polymorphism
UVB	Ultraviolet B radiation
VDP	Vitamin D binding protein
VDR	Vitamin D Receptor

1 INTRODUCTION

1.1 The vitamin D pathway

Vitamin D is a secosteroid hormone produced by the human body in the skin when interacting with the ultraviolet B (UVB) radiation from sunlight. Several factors influence the synthesis of vitamin D in the skin, among others are skin pigmentation, sunscreen use, ageing, time of the day, clothing coverage, season and latitude [60, 87, 101]. Vitamin D can also be obtained through the diet since it is also produced in other living organisms. There are two main forms of vitamin D that we can obtain through intake of foods and supplements, these are vitamin D_2 (or ergocalciferol) and vitamin D_3 (or cholecalciferol) [86]. Although there are some slight chemical differences between both compounds, the main and most relevant difference between both compounds is their origin. Vitamin D_2 is of plant origin and can be found in larger amounts in mushrooms but can also be produced in yeasts for its commercial use. Vitamin D_3 on the other hand is of animal origin and can be found in foods such as fatty fish (salmon, tuna, sardines), beef liver, eggs and in some countries also in fortified milk and juices [135]. Additionally, vitamin D_3 is the type that can be synthesized in our organism by interaction with UVB (290-315 nm) radiation from sunlight. Several studies have looked at vitamin D intake as a marker of vitamin D status. These studies have assessed associations of vitamin D intake with several disease endpoints such as cardiovascular disease [169], diabetes [2], or cancer [175]. Although the results from these studies are very helpful in understanding how vitamin D influences disease risk, it should be considered that diet accounts for only a small percentage of the variation in circulating 25(OH)D levels, these depending mostly on the sunlight exposure [117]. Vitamin D intake may be really critical in conditions where sunlight exposure is minimal such as in northern latitudes, especially during winter, because sunlight radiation reaching the earth is not sufficient to produce vitamin D; or in sedentary populations, where large periods of time are spent indoors.

The precursor of vitamin D_3 or provitamin D_3 is a molecule termed 7-dehydrocholesterol, also a precursor of cholesterol [72]. The enzyme 7-dehydrocholesterol reductase (DHCR7) regulates the conversion of 7-

dehydrocholesterol to cholesterol. Genetic variation in the DHCR7 gene has been shown to affect concentrations of the provitamin D_3 [149]. Other factors affecting provitamin D_3 levels as well as DHCR7 activity are statin use, vitamin D_3, LDL, HDL and total cholesterol levels [25, 72, 200].

After its production in the skin, or absorption through the gut, both vitamin D_2 and vitamin D_3 (from now on grouped as vitamin D) are transported in the blood to the liver (mainly), but also to other tissues for further conversion to other vitamin D metabolites. The vitamin D binding protein (DBP) or group-specific component (GC) is the main carrier of vitamin D in the circulation, with nearly 90% of the total vitamin D binding to the DBP, and the rest to albumin or being free in the circulation [23]. Transportation of vitamin D is very important because it makes its metabolism possible and permits the delivery of its biological effects to many tissues. In fact, lower DBP levels and genetic variability in the GC gene have shown to be clinically and biologically relevant by modulating associations of vitamin D status with cancer, and by influencing the affinity for vitamin D metabolites, respectively [96, 148, 195].

The first step in the activation of vitamin D begins in the liver, where vitamin D is hydroxylated at the 25 position. This hydroxylation is done mainly by a cytochrome P450 enzyme named 25-hydroxylase (CYP2R1) to form 25-hydroxyvitamin D (25(OH)D), the main circulating form [17, 35]. A study in CYP2R1-knockout mice has suggested that the 25(OH)D synthesis may also be regulated by other hepatic enzymes that remain to be discovered [199]. Another 25-hydroxylase that can be found in the mitochondria, sterol 27-hydroxylase (CYP27A1), has been found to be present not only in the liver but also in many other tissues, thus suggesting effects of vitamin D in tissues other than the bone and intestine [76, 156, 174]. Both enzymes are capable of synthetizing 25(OH)D, but CYP2R1 seems to be more critical, since mutations in the gene encoding this enzyme can lead to vitamin D insufficiency [17]. Circulating 25(OH)D concentration is regarded as the most reliable vitamin D metabolite and also the easiest to measure, thus it is almost always employed as a marker of vitamin D status [197]. Total circulating 25(OH)D concentration integrates vitamin D from the diet as well as that produced in the skin by interacting with UVB light. Genetics also contribute to 25(OH)D levels, but only a few genes may be

involved [83] and the contribution to the total variation in 25(OH)D levels may be less than that from environmental factors [20, 101]. Nevertheless, it has also been suggested that genetic variation can also condition the response to vitamin D supplementation, with some higher doses needed among those with a genetic predisposition to low 25(OH)D levels [186].

The last step in the activation of vitamin D is the hydroxylation of 25(OH)D at the carbon 1 by the cytochrome P450 enzyme named 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) to form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active vitamin D form [27]. This activation of vitamin D takes place mainly in the kidney, but local activation in other tissues of the body such as the colon, as well as in cancer cells, has been reported [51, 91]. As opposed to the renal conversion, which depends mainly on bone homeostasis, the extra-renal $1,25(OH)_2D$ synthesis depends directly on the 25(OH)D concentrations [51]. In addition, genetic variability in the CYP27B1 gene has been shown to affect the enzyme activity [91]. Some studies have employed $1,25(OH)_2D$ as a marker of vitamin D status, particularly in relation to cancer outcomes with overall inconsistent findings [66, 112]. However, due to its relatively short half-life and the tightly regulated production by the calcium levels in the kidney, a measurement of circulating $1,25(OH)_2D$ levels is of little utility for assessing long term vitamin D status [197].

In the kidney, the cytochrome P450 24-hydroxylase enzyme (CYP24A1) can also convert both 25(OH)D and $1,25(OH)_2D$ (preferentially the latter) to the inactive vitamin D metabolites 24, 25-dihydroxyvitamin D ($24,25(OH)_2D$) and 1,24,25-trihydroxyvitamin D ($1,24,25(OH)_3D$), respectively [38, 94]. The CYP24A1 enzyme is responsible mainly for the degradation of vitamin D metabolites, thus avoiding excessive accumulation of the active $1,25(OH)_2D$ metabolite that could lead to excessive calcium levels. Genetic variation in the CYP24A1 gene has been shown to affect the enzyme activity which suggests that vitamin D homeostasis is complex and influenced by genetic factors [91].

Vitamin D exerts its biological functions mainly through the binding of the active compound $1,25(OH)_2D$ to the vitamin D receptor (VDR) [38]. VDRs are found in most tissues and are potential targets for the active vitamin D [22]. The VDR regulates the expression of the promoters of many genes containing DNA

sequences called vitamin D response elements (VDREs). By these mechanisms of gene transcription control vitamin D can fully exert its biological activity by, for example, promoting insulin secretion, inhibiting adaptive immunity but promoting adaptive immunity, and inhibiting cell proliferation and angiogenesis but stimulating cell differentiation and apoptosis [22, 53].

1.2 Associations of circulating 25(OH)D concentration with total and site-specific cancer incidence

The first epidemiological studies on vitamin D and the risk of cancer appeared after ecological studies hypothesizing that geographical differences in cancer rates were to be attributed to variations in sunlight exposure and to vitamin D status, which is closely related to UVB exposure [64]. Since then, several ecological studies have been conducted and associations between UVB, vitamin D and cancer have been suggested for as many as fifteen anatomical sites [73]. Plausible biological mechanisms have been proposed to explain in which way vitamin D could influence carcinogenesis. The biologically active vitamin D pathway metabolite $1,25(OH)_2D$ binds to the VDR regulating gene transcription and thereby influencing cancer development by reducing cell proliferation, angiogenesis and invasion, and by inducing cell differentiation and apoptosis [37]. Alternative mechanisms, such as anti-inflammatory effects, could also provide a basis for the influence of vitamin D in tumorigenesis [53, 98]. The VDR has been found in human cancer cell lines throughout the body thus providing biological explanations to the associations between vitamin D and several types of cancer found in ecological studies. However, ecological studies are prone to potentially severe biases.

In recent years a rapidly increasing number of individual level epidemiological studies have assessed the relationship between serum vitamin D levels and the risk of various forms of cancer. In the following, an overview of the epidemiologic literature on the association of serum vitamin D levels with cancer risk will be provided.

1.2.1 Total cancer

Few studies have assessed the association of 25(OH)D on the total burden of cancer. Most of these studies evaluated associations with total cancer mortality, while only a few assessed cancer incidence [140]. The earliest study on the subject was published in 2006. With data from the Health Professionals Follow-Up

Study (HPFS), a large cohort study from the United States, the investigators predicted low 25(OH)D levels and assessed their association with higher cancer incidence and mortality [69]. A further re-analysis of the data also showed variation according to ethnicity, with black men being at higher risk of developing cancer compared to white men [70]. Several studies followed but the findings have been diverse. Some studies have observed an inverse association [144], others lack of associations [57, 130] and even increased total cancer risk with higher 25(OH)D levels [131]. Only a single study specifically focused on the association of 25(OH)D with total cancer mortality in older adults and no significant association of 25(OH)D with cancer mortality was observed [159]. Moreover, the different studies were conducted in a variety of settings, ethnics and sex groups; also variation in 25(OH)D measurement and confounder adjustment was seen.

Overall, the evidence on the association between serum vitamin D and total cancer incidence and mortality remains inconclusive. Because it seems plausible that some cancers may be more vitamin-D-sensitive than others, in the following subsections evidence on the association of serum 25(OH)D levels with the risk of the most frequent cancers will be presented.

1.2.2 Lung cancer

Lung cancer is the most common incident cancer and also cancer cause of death globally. More than 1.8 million new lung cancer cases were diagnosed in 2012, with almost 1.6 million lung cancer deaths in the same year [52]. The biological plausibility of a potential association of vitamin D and lung cancer is supported by the effect of vitamin D on anti-inflammatory processes in the lung [79]. These mechanisms could not only have an influence on inflammatory lung diseases such as asthma or COPD but could also ultimately influence carcinogenesis [40, 53, 98].

In contrast to other major cancers, only few epidemiological studies have assessed the association of serum 25(OH)D levels with lung cancer risk, and none of the few studies did find an association in the

overall population [36, 55, 57, 69, 100, 187]. However, looking at specific population subgroups, a significant inverse association with lung cancer incidence has been noted among women and young subjects in a Finnish cohort [100], an increased lung cancer mortality for higher 25(OH)D concentrations was found in men in the NHANES III study [57], a lower risk of lung cancer was found among Finish smokers whose blood was drawn during darker months in the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study [187], and a decreased lung cancer mortality was observed among smokers with high serum 25(OH)D concentrations also in the NHANES III study [36].

In summary, evidence for the role of vitamin D in lung cancer risk is rather limited and inconclusive. However, indications of associations between 25(OH)D and lung cancer risk observed in subgroups of the general population deserve further study. In addition, there is a need of studies assessing the influence of vitamin D intake on lung cancer risk.

1.2.3 Colorectal cancer

Colorectal cancer is the fourth most common cancer in the developed world [52]. In 2012, more than 1.3 million new colorectal cancer cases were diagnosed (9.7% of all incident cancer) globally and nearly 700,000 patients died from colorectal cancer (8.5% of the total cancer deaths) [52]. Several modifiable risk factors, such as cigarette smoking, alcohol consumption or obesity, have been identified [29]. A large number of epidemiological studies have suggested inverse associations of vitamin D status with colorectal cancer risk. Most of the studies focused on colorectal cancer incidence, except one study which addressed colorectal cancer mortality [63, 189]. A number of meta-analyses of longitudinal studies have been conducted which consistently found an inverse relationship between 25(OH)D and colorectal cancer risk [63, 112, 122, 175, 189]. The most comprehensive of these meta-analyses was conducted by Ma and colleagues [122] and included 9 studies, most of them from the United States, covering data from more than 1 million study participants. The quality of the included studies was generally high, even though

some studies did not adjust for relevant confounders. Overall, higher 25(OH)D serum levels were significantly associated with a 33% reduction in colorectal cancer risk (HR, for the highest versus lowest 25(OH)D quartile: 0.67; 95% CI, 0.54-0.80). When stratifying by anatomical site, the risk reduction was similar for colon and rectum cancer. However, no significant risk reduction was observed for proximal and distal colon subsites. A stronger inverse association of 25(OH)D and colorectal cancer was noted in studies from the United States, as compared to European studies, whereas no significant association was observed in studies from Asia [122]. A more recent prospective study not included in the before-mentioned meta-analyses found no association between 25(OH)D and colon or rectal cancer in a population of male Americans participating in the Physicians' Health Study (PHS). However, when results from this newer investigation were pooled with previous studies of 25(OH)D and colorectal cancer, the authors still observed an inverse linear association [112].

In summary, there is substantial evidence for an inverse association between 25(OH)D and colorectal cancer risk and for a potential protective effect of vitamin D, although most of the evidence arises from United States populations and there is a heterogeneity in confounder adjustment and in the categorization of individuals according to 25(OH)D levels. A more comprehensive analysis avoiding these methodological constraints is therefore paramount.

1.2.4 Breast cancer

Breast cancer is the most common cancer of women globally. More than 1.6 million of breast cancer cases were diagnosed in 2012 (12% of all incident cancers), with more than 500,000 breast cancer deaths [52]. Several risk factors for breast cancer have been identified, but few of them are really modifiable [128]. Many epidemiological studies have observed associations of higher vitamin D status with decreased breast cancer risk. A meta-analysis by Yin and colleagues published in 2010 summarized the results of 10 studies on the association of 25(OH)D serum levels and breast cancer risk [191]. Overall, a significant inverse

association was found (HR for an increase of serum 25(OH)D by 20 ng/ml, 0.73; 95% CI, 0.60-0.88). Stratification by type of study indicated that the association was much stronger and statistically significant only in case-control studies with measurement of 25(OH)D levels close to the time of diagnosis whereas no significant association was found in cohort studies or nested case-control studies assessing 25(OH)D levels years before diagnosis. A potential role of vitamin D in breast cancer prevention is therefore uncertain and should be clarified in further larger scale prospective analyses.

1.2.5 Prostate cancer

Among men, prostate cancer is the second most frequently diagnosed cancer and the most prevalent cancer in the world. In 2012, nearly 4 million patients had prostate cancer (25% of all prevalent cancers among men), more than 1 million new prostate cancer cases were diagnosed (7.8% of all incident cancers) and more than 300,000 men died from prostate cancer (3.7% of the total cancer deaths) [52]. Prostate cancer is less fatal than other cancers (mortality/incidence ratio=0.28) [52] but its treatment often substantially impairs quality of life. Efforts of prevention should particularly aim for reduction of aggressive disease which is associated with detriments in quality of life and lower survival. A common definition of aggressive prostate cancer is a combination of advanced stage (T3-T4) and high-grade (Gleason score >7).

A systematic review of observational studies on the association of 25(OH)D levels with prostate cancer risk identified 14 prospective studies focusing on 25(OH)D and total prostate cancer risk (4,353 cases); 6 of these studies also included information on aggressive prostate cancer (871 cases) [66]. Despite being a highly powered investigation and inverse associations having been previously observed, the summary risk estimates suggested no association of 25(OH)D concentrations with either total or aggressive prostate cancer [63, 66]. More recently, a case-control study nested within the Malmö Diet and Cancer study (943 prostate cancer cases, 943 controls) suggested a weak nonlinear association between 25(OH)D levels and

the risk of prostate cancer [28]. Moreover, a recent analysis from the "Prostate Testing for cancer and Treatment" (ProtecT) study did not find an association of 25(OH)D with overall prostate cancer risk, but suggested that a decrease in 25(OH)D concentrations may be associated with occurrence of aggressive prostate cancer [67].

In conclusion, although several epidemiological studies have been conducted for prostate cancer, their results have been summarized, and overall no effect of vitamin D status on prostate cancer risk was suggested, newer studies keep adding uncertainty to this important but unresolved issue. There is a need for sufficiently large epidemiological studies that can help explain the disagreeing findings for prostate cancer among the different study populations.

1.2.6 Other cancers

Whereas the evidence for an inverse association of vitamin D and colorectal cancer risk is quite robust and supported by a large number of studies, available evidence is much more limited for the remaining digestive tract cancers [140]. In the NHANES III study population, a combined endpoint of digestive cancers (esophageal, gastric, hepatic and pancreatic) was not significantly associated with 25(OH)D serum levels [57]. On the other hand, in the ULSAM cohort of Swedish men very strong multivariate-adjusted associations with a combined endpoint of pancreas, liver and biliary duct cancer mortality were observed for high (HR, 10.30; 95%CI, 1.81-58.56) and low (HR, 5.71; 95%CI, 1.81-18.03) 25(OH)D serum levels [131]. There have been also several studies examining the association of 25(OH)D levels with risk of digestive tract cancers separately.

For gastric cancer, two studies did not find associations with predicted [69] and measured circulating vitamin D levels [34]. In the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers" (VDPP), data were pooled from 10 prospective cohort studies from the US, China and Finland [61]. Overall, 784 gastric cancer cases were included in this consortium of which 135 were located in the gastric cardia.

Overall, no association of 25(OH)D with total gastric cancer was observed. However, an increased risk of gastric noncardia cancer was observed for the highest vitamin D concentrations. Unexpectedly, among several population subgroups (Asians, never smokers and subjects with low alcohol consumption) low vitamin D levels (<50 nmol/L) as compared to normal vitamin D levels (50-75 nmol/L) were associated with a decreased risk of upper gastrointestinal cancer (no separate analyses were reported for gastric cancer) [4]. In addition, low 25(OH)D serum levels have also been associated with worse gastric cancer survival in Chinese patients [152]. In summary, the association of vitamin D and gastric cancer is still unclear and inconclusive, and requires further study.

Abnet and colleagues have repeatedly assessed the association of 25(OH)D levels with esophageal cancer in a high-risk Chinese rural population with unclear findings [3, 34]. An increased risk of esophageal squamous dysplasia for high 25(OH)D concentrations [3] and an increased risk of esophageal squamous cell carcinoma for low 25(OH)D concentrations were observed [34]. Moreover, Giovannucci and colleagues observed a strongly decreased esophageal cancer risk (RR, per increase by 25nmol/L: 0.37; 95% CI, 0.17-0.80) with increasing levels of predicted 25(OH)D [69]. On the other hand, in the VDPP study no association was observed between circulating 25(OH)D serum concentration and risk of total esophageal cancer (N=265 cases), adenocarcinoma (N=104) or small cell carcinoma (N=142 cases) [4]. Current evidence for esophageal cancer seems conflicting and more studies are needed to clarify these contradictory results.

Results of studies analyzing the relationship of predicted 25(OH)D levels and pancreatic cancer are more consistent. In both the HPFS and the Nurses' Health Study (NHS), higher predicted 25(OH)D levels were associated with significantly lower pancreatic cancer risk [16, 69]. Stolzenberg-Solomon and colleagues have repeatedly analyzed pancreatic cancer risk in relation to 25(OH)D levels in diverse populations with diverse findings. In the ATBC study population of Finnish smokers, higher vitamin D levels were associated with an increase in pancreatic cancer risk [166]. This finding was confirmed in the VDPP study, which included nearly 1,000 pancreatic cancer cases [168]. However, this apparent increment in

pancreatic cancer risk with higher vitamin D concentration has recently been attributed to a statistical artifact by critics [14]. In the "Prostate, Lung, Colorectal and Ovarian Screening Trial" (PLCO), Stolzenberg and colleagues did not find an association between vitamin D and pancreatic cancer [167]. More recently, a pooled analysis of five large American cohorts (451 pancreatic cancer cases and 1,167 controls) found a significantly decreased pancreatic cancer risk for the higher versus lower plasma 25(OH)D levels [188]. Overall, the evidence remains conflicting and further investigations are needed to clarify the association between vitamin D and pancreatic cancer risk.

Vitamin D levels have also been associated with incidence of other rarer cancers although the number of studies is considerably smaller. Thus, 25(OH)D levels have also been related to the risk of bladder cancer with diverse findings: a significant inverse association among male smokers in the ATBC study [132], a non-significant inverse association among men in the HPFS [69] and no association in the PLCO for non-smokers and women [133]. In the VDPP study no association of serum 25(OH)D levels with endometrial cancer was observed [196]. In a meta-analysis of 10 studies of 25(OH)D levels and ovarian cancer reported in 2011, a tentative, statistically non-significant inverse association was observed [192]. Additional analyses from the VDPP consortium likewise did not find evidence for an association [198]. A number of further studies have assessed associations of vitamin D with head and neck cancer [12, 142], kidney cancer [62], non-Hodgkin lymphoma [114, 150], brain cancer [69] and thyroid cancer [154] with diverse findings.

1.3 Association of vitamin D genetic polymorphisms with cancer risks

The first epidemiological studies that assessed cancer risk according to vitamin D-related cancer genetic polymorphisms focused on the VDR [179]. DNA sequence variations or single nucleotide polymorphisms (SNPs) in the VDR have been associated with incidence of lung [45], colorectal [15], breast [171], prostate [19, 194], skin [43] and other cancer sites [104] with overall inconclusive findings. The majority of SNPs investigated were restriction fragment length polymorphisms (RFLPs) which usually convey some limitations: RFLPs are measured with somewhat insensitive techniques that focus only on small areas of the VDR gene [179]. The majority of these SNPs are non-functional, although they are assumed to be in linkage disequilibrium with other functional polymorphisms such as FokI which alters the length of the VDR and its transcriptional activity [104]. In addition, these VDR RFLPs only address a small part of the vitamin D pathway [19] and they are in most cases independent of the vitamin D metabolites' concentrations and vitamin D receptor expression [80].

In recent years, several genome-wide associations studies (GWAS) have uncovered new SNPs associated with 25(OH)D concentrations [7, 31, 127, 184]. In fact up to 53% of the variability in vitamin D status has been attributed to heritability [184]. These SNPs are located within or around genes encoding for key enzymes in the vitamin D pathway i.e., DHCR7, GC, CYP2R1 and CYP24A1 among others. SNPs within these genes may be of more biological and also etiological relevance because first, they may be associated with different gene phenotypes [137]; and second, they are independent of changes in the environment, and therefore could be regarded as an unbiased estimator of predisposition to vitamin D insufficiency, and subsequently of the association with cancer endpoints [178].

1.3.1 Total cancer

To my knowledge, only two investigations have assessed the association of 25(OH)D-related SNPs with total cancer incidence and both were done in the TROMSO study [95, 97]. In a first study, 25(OH)D-

related SNPs in DHCR7, GC, CYP2R1 and CYP24A1 were overall not associated to total cancer incidence, except for subjects with the minor homozygote allele of a polymorphism in CYP24A1 [95]. In a more recent publication in the same study population, two other polymorphisms in GC were genotyped: rs7041 and rs4588 [39]. A combination of genotypes for these two SNPs defines different DBP phenotypes, one of which (1f/1f) was significantly associated with a 25% reduced risk of total cancer incidence. Due to the sparse data for total cancer incidence and the importance of this outcome for the assessment of public health impact, future studies are needed.

On the other hand, many more studies assessing the association of 25(OH)D-related SNPs with the incidence of different cancers have been published. For the four most common cancers, the available studies will be summarized in the following subsections.

1.3.2 Lung cancer

Very few studies have assessed lung cancer risk in relation to vitamin D polymorphisms. Two early studies have suggested that rare VDR genetic variants may be associated with increased lung cancer risk [45], and with worse survival after lung cancer diagnosis [78, 120]. On the other hand, regarding genetic variants associated with 25(OH)D concentration, only two studies have been published, but these may not be comparable with each other due to the different study populations involved. In the TROMSØ study, 25(OH)D-related SNPs in DHCR7, GC, CYP2R1 and CYP24A1 were not associated with lung cancer risk [95]. In a case-control study from Thailand published recently, the rare alleles of the GC polymorphisms rs4588 and rs7041 were associated with lower 25(OH)D levels and also with increased lung cancer risk [124]. Taking into account that many studies have observed associations of 25(OH)D levels with lung cancer risk, there is clearly a need for more studies with 25(OH)D-related SNPs in order to elucidate whether there is really an association with lung cancer risk, or simply an insufficient adjustment for smoking exposure.

1.3.3 Colorectal cancer

A potential role of VDR SNPs on the risk of colorectal cancer has also been assessed in some studies [15, 50, 175]. A recent review and meta-analysis by Bai and colleagues identified 23 studies analyzing the risk of colorectal cancer in relation to several VDR polymorphisms [15]. Among the many VDR polymorphisms assessed, only one (BsmI) was significantly associated with colorectal cancer risk. Further sensitivity analyses showed that this association was restricted to colon and not to rectal cancer. Although this polymorphism is not functional, it has been reported to be in linkage disequilibrium with the FokI VDR polymorphism, which is thought to shorten the VDR protein and alter its transcriptional activity [15]. However, in a more recent, and sufficiently powered analysis of the EPIC data, neither BsmI, nor FokI genotypes were associated with colorectal cancer risk, or modified the association of 25(OH)D levels and colorectal cancer [49].

Considerably less studies have assessed colorectal cancer risk according to 25(OH)D-related SNPs [95, 124, 172]. As opposed to the results of studies of circulating 25(OH)D concentrations and colorectal cancer risk, the results from studies with 25(OH)D-related polymorphisms suggest no link with colorectal cancer, with the exception of the results of a recently published case-control study among Thai [124]. Before we can accept or neglect the importance of vitamin D on colorectal cancer, more and larger studies of 25(OH)D-related SNPs and colorectal cancer risk need to be conducted.

1.3.4 Breast cancer

Associations of VDR SNPs with breast cancer risk have been examined in as many as 21 case-control studies, the results of which have been summarized in meta-analysis [171]. Only the FokI SNP was significantly associated with increased risk of breast cancer, with no evidence of high heterogeneity among studies. According to the authors of the meta-analysis, a limitation of the majority of the published studies may be an insufficient adjustment for confounders (i.e., age, sun exposure and vitamin D intake

were not included as covariates) [171], however, it is not clear how these variables could affect the genotypes' distributions. More recently, a large study of Chinese could not confirm the association of the FokI polymorphism with breast cancer risk [46].

On the other hand, the results from studies assessing breast cancer risk according 25(OH)D-related genetic variation have been more inconclusive. Significant associations of polymorphisms in CYP24A1 and GC with breast cancer risk have been observed in several studies [1, 9, 46, 59, 95], whereas non-significant associations have also been observed for SNPs in DHCR7, GC, CYP2R1 and CYP24A1 in other studies [9, 46, 95, 125]. The discrepancy in the findings across the different studies could be attributed to the different populations involved and the different genotypes' distributions.

1.3.5 Prostate cancer

Associations of VDR gene SNPs with prostate cancer risk have been examined in several studies. A systematic review and meta-analysis of 26 studies published in 2006 did not find any significant association for the most commonly assessed VDR SNPs [19]. More recently, significant associations of VDR SNPs with lethal prostate cancer have been observed [160].

Many studies have assessed prostate cancer risk according to 25(OH)D-related SNPs with overall inconclusive findings. Polymorphisms in genes encoding for the 25-hydroxylases (CYP2R1 and CYP27A1) have been associated with decreased total [134] and lethal [160, 161] prostate cancer incidence. However, there have been also studies with null findings for SNPs in these two genes [95]. On the other hand, for SNPs located in other genes of the vitamin D pathway (GC, DHCR7 and CYP24A1) the results were not so promising [6, 21, 84, 88, 95, 134, 160]. Only one study has assessed a potential effect modification of vitamin D pathway SNPs on the association of 25(OH)D levels with prostate cancer risk [161]. Although significant interactions were observed for the majority of genes, these did not remain significant after adjustment for multiple testing. The different findings for the various vitamin D pathway

genes and the assessment of interaction with 25(OH)D concentrations may well deserve further investigation in large and adequately designed epidemiological studies.

1.4 Vitamin D and Ageing

In the last few decades, with the increases in life expectancy, particularly seen in developed countries, the absolute numbers and the percentage of the population considered as elderly (that is older than 65 years old) has increased greatly. This demographic change towards population ageing conveys substantial increases in the maintenance cost of the health care system, which in the future may even result in significant socioeconomic and political changes [58]. Therefore, there is a clear need to adapt to this ageing phenomenon by investing in aging research and public health policies aimed to enhance the prevention of aging-related diseases, to find cost-effective treatments/solutions for ameliorating the burden of disease related to aging, and to improve the overall well-being/health status of older adults.

Epidemiological studies often observed lower circulating levels of 25(OH)D with increasing age. In fact, vitamin D deficiency among elderly can be as high as to affect 80% of the population in some European countries [143]. There are several factors that could explain this decline in 25(OH)D levels with age [60]. Malabsorption is common at old age which can lead to low levels of calcium, increased parathyroid hormone levels, and thus increased bone turnover to re-absorb calcium. This mechanism is regulated by $1,25(OH)_2D$ whose production is increased leading to a decrease in 25(OH)D levels [10, 138]. With ageing the intake of foods rich in calcium is reduced. In order to counter this, the synthesis of $1,25(OH)_2D$ is increased so that the body is able to obtain more calcium from the intestine and bones, and therefore 25(OH)D levels are decreased [116]. Moreover, at old age the intake of vitamin D is also reduced and the skin is less able to produce vitamin D. Lastly, it has also been reported that with ageing, the intestine becomes more resistant to the $1,25(OH)_2D$ signal to increase calcium absorption, possibly because of a lower VDR expression in the intestine [60].

It is generally accepted that vitamin D deficiency is a key risk factor for skeletal diseases and disorders occurring at old age such as osteoporosis, hip fractures and falls [24, 71]. More recently, vitamin D has also been associated with other diseases occurring in the elderly such as Alzheimer's disease [8, 11, 119], Parkinson's disease [102], depression [165], hypertension [181], cancer [51] and more [129, 173]. Cancer

is also among the most common chronic diseases affecting the elderly. Its incidence sharply increases with age and cancer is soon expected to surpass cardiovascular disease as the major cause of death [18]. The burden of cancer in old adulthood presents several challenges affecting the areas of prevention, screening, treatment and care. It is possibly in the prevention of cancer where there are most opportunities to reduce the burden of cancer among elderly, especially since cancer is a disease with such a large latency period. A key question in this context is: can cancer still be prevented at old age?

Many epidemiological studies have shown that higher vitamin D levels measured during early adulthood are associated with significant reductions in the incidence of cancer many years later [65]. Many cancers take many years to develop, therefore maintaining an adequate vitamin D status during the entire adulthood may probably be relevant for reducing cancer risk as shown in the epidemiological studies. But, what happens to older adults? Is it still relevant to maintain an adequate vitamin D status when the cancer is already developing? Can vitamin D still be of help in slowing down or even stopping cancer development? And, if the answer to these questions is yes, how high must vitamin D levels be in order to reduce cancer risk? These many and highly important questions need timely answers, especially bearing in mind the ongoing process of population ageing and the tendency towards increasing cancer rates in the general population [92].

1.5 Aims of the dissertation

The present doctoral dissertation has the aim to assess the association of vitamin D status with total and site-specific cancer incidence among older adults. In order to address this research question, the following objectives were defined:

1) To conduct a systematic review and meta-analysis of epidemiological studies assessing the association of circulating 25(OH)D concentrations with total cancer incidence and mortality.

2) To carry out statistical analyses using a large dataset of cohort studies of older adults in order to assess the association of 25(OH)D concentrations with total and site-specific cancer incidence.

3) To assess the influence of vitamin D pathway SNPs with circulating 25(OH)D concentrations, and further with total and site-specific cancer incidence in a large cohort of older adults.

2 METHODS

2.1 Systematic review and meta-analysis: 25(OH)D concentration and total cancer incidence and mortality

2.1.1 Literature search and selection of studies

A literature search was conducted to identify prospective cohort studies assessing the association of serum 25(OH)D concentration with total cancer incidence or mortality in subjects without cancer at baseline. We excluded studies conducted among cancer patients. We employed Ovid Medline (Ovid Technologies, Inc., New York, 1946-October 22, 2012), EMBASE (Elsevier, Amsterdam, the Netherlands, 1980-October 22, 2012) and ISI Web of Knowledge (Thomson Scientific Technical Support, New York, 1956- October 19, 2012) databases for relevant articles by various combinations of terms in the article including 25-OH-D, cholecalciferol, calcidiol, calcitriol, 25-hydroxyvitamin D, hydroxycholecalciferol, 25-hydroxyvitamin D3 1-alpha-hydroxylase, vitamin D, 1,25 dihydroxyvitamin D, cancer, tumor, neoplasm, mortality, incidence, risk, and occurrence (see **APPENDIX A**). Duplicate publications were deleted. Each title and abstract was reviewed to determine whether the paper was potentially relevant for the review topic. The full text was reviewed if the abstract indicated that the paper reported on the association between serum 25(OH)D and cancer risk. If a full text article was not published but the abstract provided sufficient data to characterize the study and include the results in the meta-analysis, then the abstract was also considered. Only original studies conducted among humans were considered for the review. Cross-referencing was employed to complement the study identification process. Additionally, relevant articles published during the writing of the manuscript were also added to the meta-analysis.

2.1.2 Data extraction

From eligible studies, three scientists (including myself) extracted the following data independently in a standardized manner, and any initial disagreement was resolved by further review and discussion: authors, publication year, country, study design, characteristics of the study population, duration of follow-up, total cancer incidence and mortality according to serum vitamin D status and the respective measures of association (see below), as well as covariates adjusted for in the analysis.

Main outcome variables of interest were measures of association between circulating 25(OH)D serum concentration and total cancer incidence or mortality. When such data were not explicitly reported, they were derived from data provided in the articles or requested from the authors through personal contacts. The latter was the case for obtaining sex-specific data from the study by Afzal et al [5]. For consistency, serum concentrations of 25(OH)D given in ng/ml were converted to nmol/L, using the pertinent conversion factor (1 nmol/L=0.4 ng/ml). In most studies, total cancer incidence or mortality were reported stratified by various categories of 25(OH)D.

2.1.3 Statistical analyses

The analysis included two major steps: First, a comprehensive graphical analysis of dose-response patterns was employed by plotting relative incidence and mortality for groups of study participants according to circulating 25(OH)D serum concentration, using identical scales for the graphs across studies. Depending on available information, medians, means or midpoints of the categories were used. Second, due to the different categorization of 25(OH)D concentration across studies, all results were recalculated for an increase of serum 25(OH)D by 50 nmol/L, both within studies (taking possible correlations resulting from a common reference category into account) [74], as well as across studies. This approach assumes linearity of the dose-response relationship. Because the assumption of linearity may not

necessarily be true, additional meta-analyses using within-study estimates of high versus low 25(OH)D concentration were carried out.

Summary risk ratios (RRs) from fixed and random effects models were calculated using standard meta-analysis methods [81]. In a conservative approach, the random effects estimates, which allow for variation of true effects across studies, were taken as "main results" [139]. Random effects estimates were derived using both the DerSimonian-Laird method [44, 118], and the maximum likelihood method (more appropriate if the number of included studies is small) [180]. In addition, heterogeneity was assessed by the Tau^2 test, the Cochran's Q test and the I^2 statistic. Standardized deleted residuals analysis was performed to identify outliers. Because the year and the country from which a study is published or the type of assay that is used in a study to measure 25(OH)D concentration may confound the association between 25(OH)D concentration and cancer mortality, meta-regression was used to examine the relationship between publication year (published in 2010 or later vs. before 2010), country (USA vs. others), 25(OH)D assay employed (immunoassay vs. others), and the sizes of effect observed in the studies for cancer mortality. Subgroup analyses were also performed to estimate the association between 25(OH)D serum concentration and total cancer among men and women separately. In sensitivity analyses, only studies with a minimum level of confounder adjustment (sex, age, season of blood draw and smoking) were included. The funnel plot, Begg and Mazumdar rank correlation test and Egger's test of the intercept were employed to assess publication bias [155]. The R/S plus software, version 2.15.0, and the statistics software SAS©, version 9.3 (SAS Institute Inc., Cary, N.C., USA), were used for the analyses.

2.2 Analyses on the association of circulating 25(OH)D concentration with total and site-specific cancer incidence in the CHANCES Consortium

2.2.1 Study design and study population

The Consortium on Health and Aging: Network of Cohorts in Europe and the United States (CHANCES) is a collaborative large scale integrating project funded by the European Commission within the Seventh Framework Programme (http://www.chancesfp7.eu). This consortium combines and integrates data from many cohort studies with a focus on chronic conditions and health determinants among the elderly.

From all participating studies in the CHANCES consortium, three European cohort studies (EPIC-Elderly, ESTHER and TROMSØ), had available plasma or serum 25(OH)D measurements and complete follow-up for cancer incidence, and were included in this investigation. Participants with prevalent cancer at baseline were excluded. A summary description of included studies can be found in **Table 1.**

Briefly, EPIC is a multicenter, prospective cohort study of healthy volunteers (aged 35 to 70 years) recruited between 1992 and 2000 from 10 European countries [153]. Four nested case-control studies of colorectal, breast and prostate cancer and lymphoid malignancies cases and matched controls were conducted and have already been published [93, 107, 121, 176]. Controls were matched by age, sex, study center, time of the day and fasting status at blood collection and among women further by menopausal status, phase of menstrual cycle and use of hormone replacement therapy at blood collection. From these data, participants aged 60 or over at recruitment were included in the EPIC-Elderly study. However, data from only 5 countries of EPIC-Elderly were available for the CHANCES consortium (Denmark, Greece, Netherlands, Spain and Sweden).

ESTHER is an ongoing population based-cohort study conducted in Saarland (Germany). Overall 9,949 older adults (median age = 63 years) were recruited between 2000 and 2002 during a routine health check-up by their general practitioners and 25(OH)D was measured in the whole cohort [158].

TROMSØ is a repeated population-based cohort study conducted in the municipality of the same name, in Norway. The data included in the present investigation correspond to the 4th survey in which 10,262 participants (median age = 63 years) were recruited. 25(OH)D was measured in the entire cohort but smokers from the TROMSØ study (N= 2,112) were excluded because the assay used resulted in smokers having 15-20% higher 25(OH)D than non-smokers which was not reproducible with other assays [75].

Table 1 Description of CHANCES cohorts included in the analyses on the association of circulating 25(OH)D concentrations and cancer incidence.

Study Characteristics	**EPIC-Elderly** [a]	**ESTHER**	**TROMSØ**
Design	Nested case-control	Cohort	Cohort
Location	Greece, Denmark, Netherlands, Spain and Sweden	Germany	Norway
Recruitment	1992-2000	2000-2002	1994-1995 (4^{th} survey)
25(OH)D			
Median (Q_1 - Q_3) (nmol/L)	54 (40 - 69)	46 (34 – 62)	54 (43 - 65)
Assay (Manufacturer)	IA (IDS)	IA (Men: IDS; Women: DiaSorin) Both: standardized to LC-MS/MS (Waters)	IA (Roche)
Study population			
Cohort size	100,442	9,949	10,262
Available for analyses	681 cases (626 controls)	8,928	4,307 [b]
Age, median (Q_1 - Q_3) (years)	63 (61 - 65)	63 (57 – 67)	62 (56 – 68)
Women, n (%)	726 (55)	5383 (56)	2754 (60)
Cancer incidence			
Median follow-up (years)	4	10	16
Follow-up until	1999-2006	2011	2010
Number of cases			
Total	n.a.	1,082	806
Lung	n.a.	134	58
Colorectal	303 (271 controls)	152	161
Breast	126 (126 controls)	163	89
Prostate	71 (45 controls)	189	132
Lymphoma	181 (184 controls)	55	43

[a] Recruitment, median and date of end of follow-up varied for the different nested-case control studies within the EPIC-Elderly. For more details, please refer to the original publications [93, 107, 121, 176].

[b] Current smokers in the TROMSØ study were excluded due to overestimation of 25(OH)D concentrations.

Further details for the three cohorts have also been described previously [75, 153, 158]. All included cohorts were approved by local ethics committees, obtained written informed consent from all study participants and were conducted according to the declaration of Helsinki.

2.2.2 Endpoint definition

Cancer incidence was ascertained by active follow-up and record linkage with national/regional cancer registries [93, 95, 141]. Total cancer incidence was the main endpoint, defined only in the ESTHER and TROMSØ cohorts, and included all malignant neoplasms according to the 10th revision of the International Classification of Disease (ICD-10) codes C00-97 except non-melanoma skin cancers (C44). Lung cancer (C34) incidence was assessed for ESTHER and TROMSØ only because 25(OH)D measurements among lung cancer cases in EPIC-Elderly were not available. Colorectal (C18-21), breast (C50) and prostate (C61) cancer were included in all studies. Lymphoma incident cases were identified in ESTHER according to the International Classification of Diseases for Oncology, Second Edition (ICD-O-2) codes: 9590-95, 9650-67, 9670-77, 9680-88, 9690-98, 9700-17, 9731-32, 9760-62, 9764, 9820-28, 9850, 9940 and 9941. In ESTHER, whenever the ICD-O-2 codes were not available, the ICD-10 codes C81-91 were employed to define lymphomas. In EPIC-Elderly lymphomas were originally classified according to the ICD-O-2 codes but were subsequently reclassified according to ICD-O-3 codes as described elsewhere [121]. In EPIC-Elderly and TROMSØ the International Classification of Diseases for Oncology, Third Edition (ICD-O-3) codes were used: 9590-91, 9670-71, 9673, 9675, 9679, 9680, 9684, 9687, 9689, 9690-91, 9695, 9698-99, 9700-02, 9705, 9708-09, 9714, 9716, 9718-19, 9727-29, 9761, 9765, 9823, 9826-27, 9831-37, 9940, 9948, 9650-55, 9659, 9664-67, 9731-32 and 9734. For the analyses, due to small number of cases, no distinctions were made between Hodgkin's lymphoma, non-Hodgkin's lymphoma, multiple myeloma, chronic lymphocytic leukemia and other rare or unclassified lymphoid malignancies.

2.2.3 Measurement of 25(OH)D concentrations

All studies employed immunoassay methods as previously shown in Table 1 and as described in earlier publications [75, 93, 157, 176]. Due to seasonal fluctuations in 25(OH)D concentrations (**Figure 1**), 25(OH)D concentration was categorized into season-specific quintiles (upper quintile as referent) for all studies since this method is regarded as the most appropriate to reduce bias due to seasonal variation [185]. Season was categorized as winter (December-February), spring (March-May), summer (June-August) and autumn (September-November). Cut-offs for season-specific quintiles can be found in the **Supplementary Table 1**. Additionally, in order to assess the suitability for cancer risk of the American Institute of Medicine (IOM) cut-points [90], we also defined the following clinical categories of vitamin D status: vitamin D deficiency (<30 nmol/L), insufficiency (30-50 nmol/L) and sufficiency (>50 nmol/L). For this analysis, season was introduced in the multivariate model as a covariate.

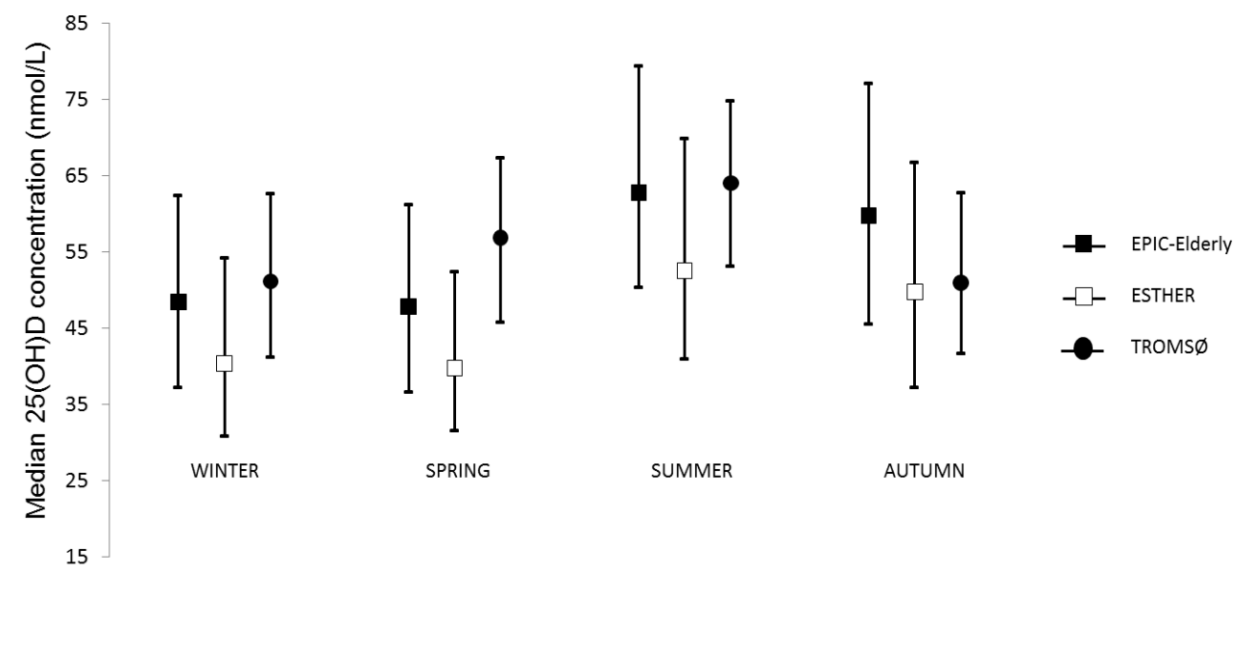

Figure 1 Median and interquartile range of 25(OH)D concentrations (nmol/L) across season of blood draw for EPIC-Elderly controls, ESTHER and TROMSØ, respectively.

2.2.4 Covariates assessment

Information on socio-demographic and lifestyle covariates was obtained from self-administered questionnaires passed to participants at baseline and included age, sex, highest level of education (primary or less, more than primary but less than university or college, university or college) and smoking status (never, former and current smoker). Height and weight were measured in EPIC-Elderly and TROMSØ and self-reported in ESTHER. Body mass index (BMI) was calculated by dividing body weight (in kilograms) by the square of height (in meters). Information regarding duration and intensity of physical activity was self-reported in all studies [77, 136, 151]. Vigorous physical activity (dichotomous: yes, no) was defined as at least one hour per week of physical activity intense enough to cause perspiration, out of breath or faster heart beating.

2.2.5 Statistical analyses

Significant differences in baseline characteristics across clinical 25(OH)D categories were tested with Kruskal-Wallis test for continuous and Chi-Square test for categorical variables. The association between circulating 25(OH)D concentrations and risk of cancer was assessed with Cox proportional hazards models in the ESTHER and TROMSØ cohorts and with conditional logistic regression models in the EPIC-Elderly nested case-control sub-study. Hazard ratios (HR, from Cox regression) and odds ratios (OR, from logistic regression), numerically approximate each other for short follow-ups, rare diseases, risk estimates near to no effect [170] and under the assumption that the exposure distribution is stable under time [103] which may apply for 25(OH)D [164].

Both risk estimates were calculated with their respective 95% confidence intervals for two models with different level of confounder adjustment. Model 1 was adjusted for age (continuous) and sex (categorical) and, in the analysis with clinical 25(OH)D categories, was additionally adjusted for season of blood draw (categorical). Model 2 was additionally adjusted for vigorous physical activity (categorical: yes, no),

highest level of education (ordinal: primary or less, between primary and university, university), BMI (continuous) and smoking status (categorical: never, former, current). Moreover, in the analyses with EPIC-Elderly data, adjustment for country was performed. Further confounder variables such as fish, red meat, vegetables and fruits intakes were considered, but only those variables that were common to all studies were chosen in order to reduce heterogeneity between studies. Subjects with missing values for the confounders adjusted for in model 2 (BMI (0.3%), highest level of education (1.8%), vigorous physical activity (3.0%) and smoking status (1.8%)) were excluded. Risk estimates according to clinical categories of vitamin D status are shown as main results. Dose-response graphs were created by plotting risk estimates across season-specific quintiles of 25(OH)D concentrations.

In a sensitivity analysis for total cancer incidence, subjects developing cancer during the initial 1, 2, 3 and 4 years of follow-up were excluded. The rationale for this sensitivity analysis was to exclude potential bias by reverse causality from cancers that might have been already present but not yet clinically manifest at the time of recruitment. We further modeled 25(OH)D concentration continuously in order to test a potential linear association. Effect modification by sex, age ($<$ or $\geq$ 65 years), BMI ($<$ or $\geq$ 30 kg/m²) and vigorous physical activity (yes, no) was tested for statistical significance by creating product terms of the continuous 25(OH)D variable by the potential effect modifier of interest, and then adding them to the multivariate model 2 for total cancer incidence only.

All statistical tests were two sided with an alpha level of 0.05. Cohort-specific analyses were conducted with SAS, version 9.3 (Cary, North Carolina, USA). Cohort-specific risk ratios were pooled with meta-analysis using random-effects models in a conservative approach in order to account for the variation of the true effects between studies [44]. Heterogeneity was tested for significance with Cochran's Q test [82]. Meta-analyses, tests of heterogeneity, forest plots and dose-response graphs were derived in Microsoft Excel 2010 (Redmond, Washington, USA) using the formulas described by Borenstein et al. [26]. This report was prepared in accordance to standard guidelines for reporting of observational studies [182].

2.3 Analyses on the association of 25(OH)D-related SNPs with total and site-specific cancer incidence in the ESTHER study

2.3.1 Study design and study population

The ESTHER study was described in the previous section. For the present analyses, participants from the baseline survey (N=9,949) were excluded if they had missing genotype data at all given loci (N=1,470), leaving total sample size of 8,479 subjects. Additionally subjects with missing 25(OH)D concentration (N=368) were excluded for analyses with 25(OH)D; subjects with prevalent cancer (N=671) were excluded for analyses with cancer incidence; and subjects with prevalent cancer of colon/rectum (N=88), breast (N=111), prostate (N=68) and lung (N=16) were excluded for analyses with cancer incidence in the respective cancer sites.

2.3.2 SNP selection and genotyping

DNA samples were extracted from baseline full blood samples obtained from the participants. In the context of an international collaboration, a total of 16 SNPs were measured in order to validate the findings of a GWAS of 25(OH)D-related SNPs. Details for the genotyping methods employed have been mentioned elsewhere [184]. The measured SNPs were: rs1461105, rs2463963, rs2972516, rs3755967, rs4387287, rs4762651, rs4861475, rs6469626, rs7308827, rs10797959, rs11195965, rs11603330, rs12696304, rs12794714, rs17163911 and rs17216707.

The final choice of SNPs to be analyzed with respect to cancer risk was done by assessing 1) the association of SNPs with 25(OH)D as a continuous variable, 2) the association of SNPs with risk of low vitamin D status, 3) whether SNPs are in Hardy-Weinberg equilibrium and 4) whether SNPs are located within, or in the vicinity of relevant genes of the vitamin D pathway.

2.3.3 Statistical analyses

Linear regression was employed to assess the association of candidate SNPs with 25(OH)D concentration modeled as a continuous variable under a co-dominant model (with major homozygotes as reference). Subjects with missing genotype for a given polymorphism were excluded from the analysis. Intercepts (β) were obtained and their 95% confidence intervals were calculated to assess statistical significance. The linear regression model included sex, age (continuous), BMI (continuous) and season of blood draw (winter: December-February; spring: March-May; summer: June-August; autumn: September-November). The definition of season categories was based on the similarity of 25(OH)D values for these months [141].

For those SNPs that were significantly associated with 25(OH)D concentration, we investigated whether these SNPs were associated with the different definitions of low vitamin D status [85]: vitamin D severe deficiency (< 30 nmol/L), deficiency (< 50 nmol/L) and insufficiency (< 80 nmol/L). Logistic regression models, adjusted by the covariates above-mentioned, were applied to estimate odds ratios (OR) and 95% confidence intervals (CI) for the minor homozygotes and heterozygotes (major homozygotes as reference) with respect to categories of low vitamin D status. The association with a genotype score was additionally assessed. This score was created by summing the risk alleles for those polymorphisms which were significantly associated with low vitamin D status and additionally were located in genes relevant to the metabolism of vitamin D. When creating a genotype score, subjects with missing genotype for any given polymorphism included in the genotype score were excluded.

Cox proportional hazards models adjusted by age, sex, BMI, smoking status (never, former, current) and physical activity (>2h of vigorous and >2h of light physical activity/week; <1h of physical activity/week; other) were employed to calculate hazard ratios (HR) and 95%CI for the association of 25(OH)D-related SNPs and genotype score with total, colorectal, breast, prostate and lung cancer incidence. To assess whether the association of a given polymorphism with cancer incidence was mediated by vitamin D status, serum 25(OH)D concentration (continuous) was also included in the model as a covariate.

To deal with missing covariate values (2.64%), the multiple imputation SAS procedure was employed to create 5 datasets with imputed values. The analyses were then performed 5 times and later pooled into one for producing the final tables.

Hardy-Weinberg equilibrium for each SNP was assessed with Chi-Squared tests. Linkage disequilibrium was investigated with the r^2 statistic. Statistical significance was defined by a two-sided p-value lower than 0.05. In order to correct for multiple testing, we employed the method of Bonferroni which divides the two sided p-value by a correction factor, in this case equal to the number of polymorphisms under analysis. All analyses were conducted with the statistical analysis software (SAS), version 9.2.

3 RESULTS

3.1 Systematic review and meta-analysis

3.1.1 Literature search

A flow diagram of the search process is given in **Figure 2**. Total searches yielded 11,019 entries. Following removal of 2,838 duplicates, 8,181 titles and abstracts were assessed and 175 articles appeared to be potentially relevant for inclusion in the review. From these, 162 articles were excluded for the following reasons: no original articles but editorials, comments, reviews (N=45), only results for site-specific cancer or adenoma reported (N=95), only all-cause mortality reported (N=10), RCTs on vitamin D supplementation (N=5), repeated studies from the same study population (N=3), only cancer patients assessed (N=3), and only cardiovascular disease mortality reported (N=1). The references of excluded studies are provided in **APPENDIX B**. In addition, during the writing of the manuscript and review process, 3 more studies published after the period covered by the literature search, were included [5, 141, 158]. Finally, 16 articles were included in the review [5, 32, 41, 42, 47, 57, 69, 89, 105, 115, 131, 141, 144, 158, 162, 163]. Cross-referencing in the included studies did not result in the identification of further studies that met the inclusion criteria of this review.

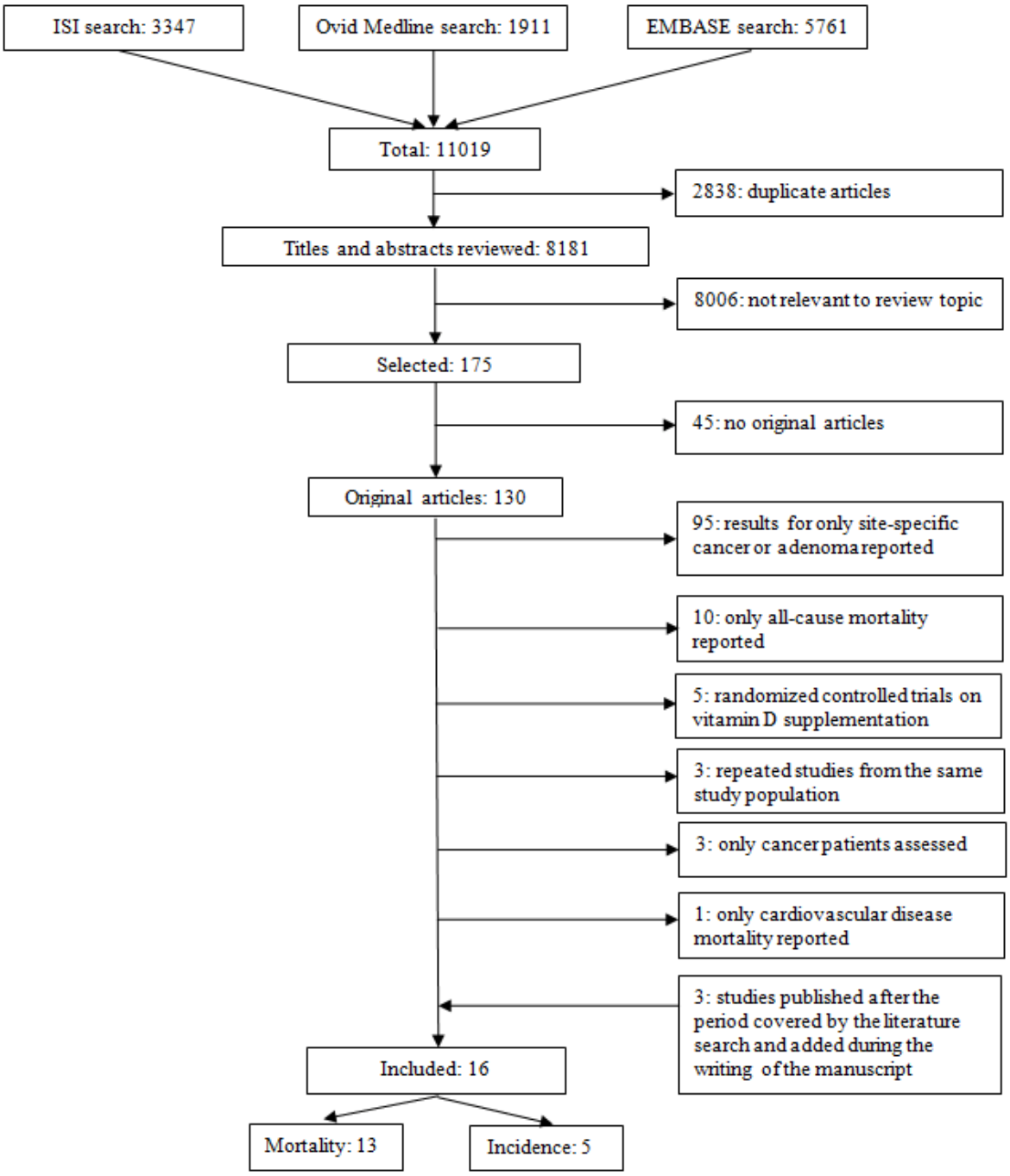

Figure 2 Flow diagram of the literature search process for identifying studies.

3.1.2 Description of included studies

Among the included studies, 5 provided data on cancer incidence [5, 42, 69, 131, 141] and 13 on cancer mortality [32, 41, 47, 57, 69, 89, 105, 115, 131, 144, 158, 162, 163]. Details on the 16 articles that were included in this meta-analysis in terms of study design, study populations, type of 25(OH)D assay employed, study results and covariates adjusted for are summarized in **Table 2** (incidence) and **Table 3** (mortality). Only two studies were reported prior to 2010, the first one in 2006 [69]. Six studies were conducted in the United States [32, 42, 47, 57, 69, 162], four in Germany [105, 141, 144, 158], two in Denmark [5, 163], and one each in Norway [89], Sweden [131], Australia [41] and China [115]. Three studies were restricted to men [32, 69, 131], three other reported gender-specific results for men [57, 115, 141] and one reported results for men by personal communication [5]. One study reported results on post-menopausal women [47] whereas gender-specific results for women were reported in three other studies [57, 115, 141] and in another study by personal communication [5]. Among the 16 included studies, 14 were prospective cohort studies and 2 were nested case-control studies [105, 162]. The majority of studies measured 25(OH)D serum concentration with immunoassays whereas high-performance liquid chromatography-tandem mass spectrometry was employed in two studies [42, 131]. In one study 25(OH)D concentration was determined by immunoassay in a subsample of 1095 subjects free of cancer at baseline and results were later employed to predict 25(OH)D for the complete population from relevant covariates [69].

Dose-response patterns for the studies on total cancer incidence and mortality are illustrated in **Figure 3** and **Figure 4**, respectively. Results were overall inconsistent, but a clear inverse dose-response relationship between 25(OH)D and total cancer incidence and mortality was observed in some studies.

Table 2 Prospective studies reporting on the association of circulating 25(OH)D serum concentration with total cancer incidence.

Author(s), year	Study population					25(OH)D Measurement	RR (95% CI) of cancer incidence according to 25(OH)D (range or median) (nmol/L) [a]		Adjusted for
	Country (blood sampling, follow-up)	No. participants (Sex)		Age (mean)	Setting				
		Cases	Total						
Giovannucci, et al. (2006)	USA (1986; 1987-2000)	4286 (M)	47800 (M)	40-75 (54)	Male health professionals [b]	IA [c]	Per 25 units increase	0.84 (0.72, 0.98)	Age, height, smoking history and intake of total calories, alcohol, red meat, calcium, retinol, total fruits and vegetables, and physical activity.
Michaelsson et al. (2010)	Sweden (1991-1995; 1992-2007)	328 (M)	1194 (M)	≥50 (71)	Adult men	LC-MS/MS	<46: 45-93: >93:	1.39 (0.99, 1.97) 1.00 1.27 (0.89, 1.81)	Age, weight, height, calcium intake, season of blood collection, social class, smoking status, leisure physical activity.
De Boer et al. (2012)	USA (1992-1993; 1992-2006)	335 (M: 30%; F: 70%)	1621 (M: 30%; F: 70%)	≥65 (74)	Older adults	LC-MS/MS	39.8 77.8	1.13 (0.90-1.42) 1.00	Age, sex, season, clinical site, smoking, body mass index and physical activity.
Ordonez Mena et al. (2013)	Germany (2000-2002; 2001-2010)	873 (All)	9580 (All)	50-74 (63)	Population-based	IA standardized to LC-MS/MS	29.5 45.5 76.3	1.10 (0.93, 1.30) 1.00 1.12 (0.95, 1.32)	Age, sex, season, BMI, education, physical activity, smoking, family history of cancer, multivitamin-use, fish, red meat, fruits and vegetables consumption.
		496 (M)	4197 (M)				27.3 47.3 78.5	1.33 (1.06, 1.68) 1.00 1.20 (0.97, 1.47)	
		377 (F)	5383 (F)				30.0 44.5 73.0	0.95 (0.75, 1.20) 1.00 1.07 (0.80, 1.44)	

(*continues on the following page*)

Table 2 (Continued) Prospective studies reporting on the association of circulating 25(OH)D serum concentration with total cancer incidence.

Author(s), year	Study population					25(OH)D Measurement	RR (95% CI) of cancer incidence according to 25(OH)D (range or median) (nmol/L) [a]		Adjusted for
	Country (blood sampling, follow-up)	No. participants (Sex)		Age (mean)	Setting				
		Cases	Total						
Afzal et al. (2013)	Denmark (1981-1983; 1981-2008)	2488 (All)	9791 (All)	20-80 (58)	Population-based	IA	11.0	1.35 (1.12, 1.62)	Age, sex, education, calendar month of blood sampling, cumulated tobacco consumption in pack-years, BMI, alcohol consumption, level of leisure time and work-related physical activity.
							14.0	1.20 (0.99, 1.45)	
							23.0	1.02 (0.91, 1.14)	
							40.0	1.02 (0.93, 1.13)	
							66.1	1.00	
		1121 (M)	4363 (M)				11.0	1.65 (1.24, 2.19)	
							14.0	1.32 (1.00, 1.76)	
							23.0	1.06 (0.89, 1.25)	
							40.0	1.20 (1.03, 1.39)	
							66.1	1.00	
		1367 (F)	5428 (F)				11.0	1.17 (0.91, 1.49)	
							14.0	1.11 (0.86, 1.44)	
							23.0	1.00 (0.86, 1.16)	
							40.0	0.90 (0.79, 1.03)	
							66.1	1.00	

Abbreviations: M: male; F: female; RR: risk ratio; CI: confidence interval; IA: immunoassay; HPLC-MS/MS: High Performance Liquid Chromatography Tandem Mass Spectrometry.

[a] For consistency, serum concentrations of 25(OH)D in ng/ml were converted to nmol/L using the conversion factor, 1 nmol/L=0.4 ng/ml.

[b] Participants included male dentists, optometrists, osteopaths, podiatrists, pharmacists and veterinarians.

[c] 25(OH)D concentration was determined by immunoassay in a subsample of 1095 subjects free of cancer at baseline and results were later employed to predict 25(OH)D for the complete population from relevant covariates.

Table 3 Prospective studies reporting on the association of circulating 25(OH)D serum concentration with total cancer mortality.

Author(s), year	Study population					25(OH)D measurement	RR (95% CI) of cancer mortality according to 25(OH)D (range or median) (nmol/L) [a]	Adjusted for
	Country (blood sampling, follow-up)	No. participants (Sex)		Age (mean)	Setting			
		Deaths	Total					
Giovannucci, et al. (2006)	USA (1986; 1987-2000)	2025 (M)	47800 (M)	40-75 (54)	Male health professionals [b]	IA [c]	Per 25 units increase 0.69 (0.55, 0.86)	Age, height, smoking history and intake of total calories, alcohol, red meat, calcium, retinol, total fruits and vegetables, and physical activity
Pilz et al. (2008)	Germany (1997-2000; 1998-2007)	95 (M: 70%; F: 30%)	3257 (M: 77%; F: 23%)	56-70 (63)	Cardiovascular hospital-based	IA	<25.5: 1.00 25.5-39.0: 0.87 (0.50, 1.52) 39.1-57.5: 0.73 (0.40, 1.32) >57.5: 0.45 (0.22, 0.93)	Age, gender, season, BMI, active smokers, retinol, exercise tertiles, beer and wine consumption, and diabetes mellitus.
Michaelsson et al. (2010)	Sweden (1991-1995; 1992-2007)	164 (M)	1194 (M)	≥50 (71)	Adult men	LC-MS/MS	<46: 2.20 (1.44, 3.38) 46-93: 1.00 >93: 1.54 (0.94, 2.54)	Age, weight, height, calcium intake, season of blood collection, social class, smoking status, leisure physical activity.
Cawthon et al. (2010)	USA (2000-2002; 2001-2009)	97 (M)	1490 (M)	≥65 (74)	Older men	LC-MS/MS	<49.8: 0.52 (0.27, 1.00) 49.8-63.0: 0.90 (0.51, 1.60) 63.0-75.0: 0.80 (0.45, 1.41) ≥75.0: 1.00	Age, clinic, season of blood collection, serum calcium and phosphate, GFR, percentage body fat, weight, race, health status, presence of at least one medical condition, alcohol use, education, activity level (PASE score), marital status, and presence of a functional or mobility limitation.
Hutchinson et al. (2010)	Norway (1994-1995; 1994-2007)	225 (M: 41%; F: 59%)	2410 (M:41%; F: 59%)	25-84 (57)	Smokers	IA	49.2: 0.82 (0.56. 1.21) 64.7: 0.86 (0.59, 1.26) 76.4: 1.02 (0.70, 1.48) 97.5: 1.00	Age, gender, season, BMI, physical activity score, diabetes, hypertension, serum creatinine, prior cardiovascular disease and prior cancer.
		273 (M: 38%; F: 62%)	4751 (M:38%; F: 62%)	25-84 (59)	Non-smokers		33.8: 1.14 (0.80, 1.63) 46.7 1.13 (0.80, 1.61) 56.2 1.23 (0.87, 1.75) 72.3 1.00	

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Table 3 (Continued) Prospective studies reporting on the association of circulating 25(OH)D serum concentration with total cancer mortality.

Author(s), year	Study population					25(OH)D measurement	RR (95% CI) of cancer mortality according to 25(OH)D (range or median) (nmol/L) [a]	Adjusted for
	Country (blood sampling, follow-up)	No. participants (Sex)		Age (mean)	Setting			
		Deaths	Total					
Freedman et al. (2010)	USA (1988-1994; 1989-2006)	884 (All)	16819 (All)	≥17 (44)	Population-based	IA	<37.5: 1.00 37.5-50.0: 1.04 (0.77, 1.41) 50.0-62.5: 1.23 (0.89, 1.69) 62.5-80.0: 1.19 (0.86, 1.65) 80.0-100: 1.12 (0.80, 1.57) ≥100: 1.15 (0.79, 1.68)	Age, race/ethnicity, smoking history, and BMI. Stratification according to season of blood draw.
		513 (M)	7905 (M)				<37.5: 1.00 37.5-50.0: 1.66 (0.98, 2.80) 50.0-62.5: 1.43 (0.90, 2.26) 62.5-80.0: 1.52 (0.82, 2.80) 80.0-100: 1.66 (1.06, 2.61) ≥100: 1.85 (1.02, 3.35)	
		371 (F)	8914 (F)				<37.5: 1.00 37.5-50.0: 0.85 (0.59, 1.22) 50.0-62.5: 1.25 (0.82, 1.90) 62.5-80.0: 1.11 (0.69, 1.79) 80.0-100: 0.86 (0.50, 1.46) ≥100: 0.64 (0.35, 1.18)	
Daly et al. (2011)	Australia (1999-2000; 2000-2007)	213 (All)	10542 (All)	≥25	Population-based	IA	<47.0: 1.86 (1.13, 3.04) 48.0-61.0: 1.88 (1.16, 3.04) 62.0-77.0: 1.79 (1.11, 2.88) >77.0: 1.00	Age, sex, season, latitude, ethnicity, education, smoking, waist circumference, exercise, diabetes status, hypertension, use of lipid-lowering medication, serum cholesterol, triglycerides, HDL-C, history of cardiovascular disease and eGFR.
Eaton et al. (2011)	USA (1993-1998; 1998-2008)	62 (F)	2429 (F)	50-79 (65.8)	Post-menopausal women	IA	3.3-36.5: 1.39 (0.88, 2.19) 36.5-50.0: 1.22 (0.79, 1.89) 50.0-65.4: 1.12 (0.72, 1.72) 65.4-146.7: 1.00	Age, season, ethnicity, CaD trial indicator, education, smoking status, current aspirin use, history of fracture at ≥55 y of age, waist circumference, BMI, physical activity, and use of vitamin D supplements.

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Table 3 (Continued) Prospective studies reporting on the association of circulating 25(OH)D serum concentration with total cancer mortality.

Author(s), year	Study population					25(OH)D measurement	RR (95% CI) of cancer mortality according to 25(OH)D (range or median)(nmol/L) [a]	Adjusted for
	Country (blood sampling, follow-up)	No. participants (Sex)		Age (mean)	Setting			
		Deaths	Total					
Krause et al. (2012)	Germany (1997-2006; 1998-2006)	289	6518 (M: 59%; F: 41%)	19-98 (71)	Hemodialysis patients	IA	<31.3: 1.51 (1.09, 2.08) 31.3-50: 1.05 (0.70, 1.57) 50-75: 1.13 (0.75, 1.69) >75: 1.00	Gender, year of incidence, age at incidence, diabetes types I/II as primary renal disease.
Lin et al. (2012)	China (1986; 1987-2010)	217 (All)	1101 (All)	40-69 (56.5)	Population-based	IA	Per 15 units increase 0.97 (0.89, 1.05)	Age, sex, hypertension, tobacco smoking, BMI and alcohol consumption.
		141 (M)	608 (M)				Per 15 units increase 1.00 (0.91, 1.10)	
		76 (F)	493 (F)				Per 15 units increase 0.88 (0.75, 1.03)	
Signorello et al. (2012)	USA (2002-2009; 2003-2010)	1852 (M: 58%; F: 42%)	1852 (M: 58%; F: 42%)	40-79 (N/A)	Population-based	IA	<25.5: 1.28 (0.78, 2.11) 25.5-37.9: 1.03 (0.66, 1.59) 37.9-54.1: 0.79 (0.52, 1.21) >54.1: 1.00	Gender, race, age at enrollment, community health center enrollment site, date of blood collection, BMI, smoking, physical activity, and household income.
Skaaby et al. (2012)	Denmark (1993-1999; 1994-2009)	301	9146 (M: 50%; F: 50%)	30-71 (49.5)	Population-based	IA/ LC-MS/MS	10-45: 1.00 33-61: 1.10 (0.82, 1.5) 48-81: 1.10 (0.78, 1.5) 65-255: 0.81 (0.57, 1.2)	Study group, lifestyle counseling, lifestyle and group counseling, gender, education, season of blood sample, intake of fish, physical activity, smoking, BMI and alcohol consumption.
Schöttker et al. (2013)	Germany (2000-2002; 2001-2010)	433	9580 (M:44%; F: 56%)	50-74 (63)	Population-based	IA standardized to LC-MS/MS	<30: 1.42 (1.08, 1.87) 30-50: 1.04 (0.83, 1.29) >50: 1.00	Age, sex, season of blood draw, regularly intake of multi-vitamin supplements, fish consumption less than once a week, BMI, scholarly education, physical activity, smoking, systolic blood pressure, chronic kidney disease, serum CRP concentrations and total cholesterol

Abbreviations: M: male; F: female; RR: risk ratio; CI: confidence interval; IA: immunoassay; HPLC-MS/MS: High Performance Liquid Chromatography Tandem Mass Spectrometry; BMI: Body Mass Index; eGFR: estimated glomerular filtration rate; PASE: the Physical Activity Scale for the Elderly; HDL-C: high-density lipoprotein cholesterol;

[a] For consistency, serum concentrations of 25(OH)D in ng/ml were converted to nmol/L using the conversion factor, 1 nmol/L=0.4 ng/ml.

[b] Participants included male dentists, optometrists, osteopaths, podiatrists, pharmacists and veterinarians.

[c] 25(OH)D concentration was determined by immunoassay in a subsample of 1095 subjects free of cancer at baseline and results were later employed to predict 25(OH)D for the complete population from relevant covariates.

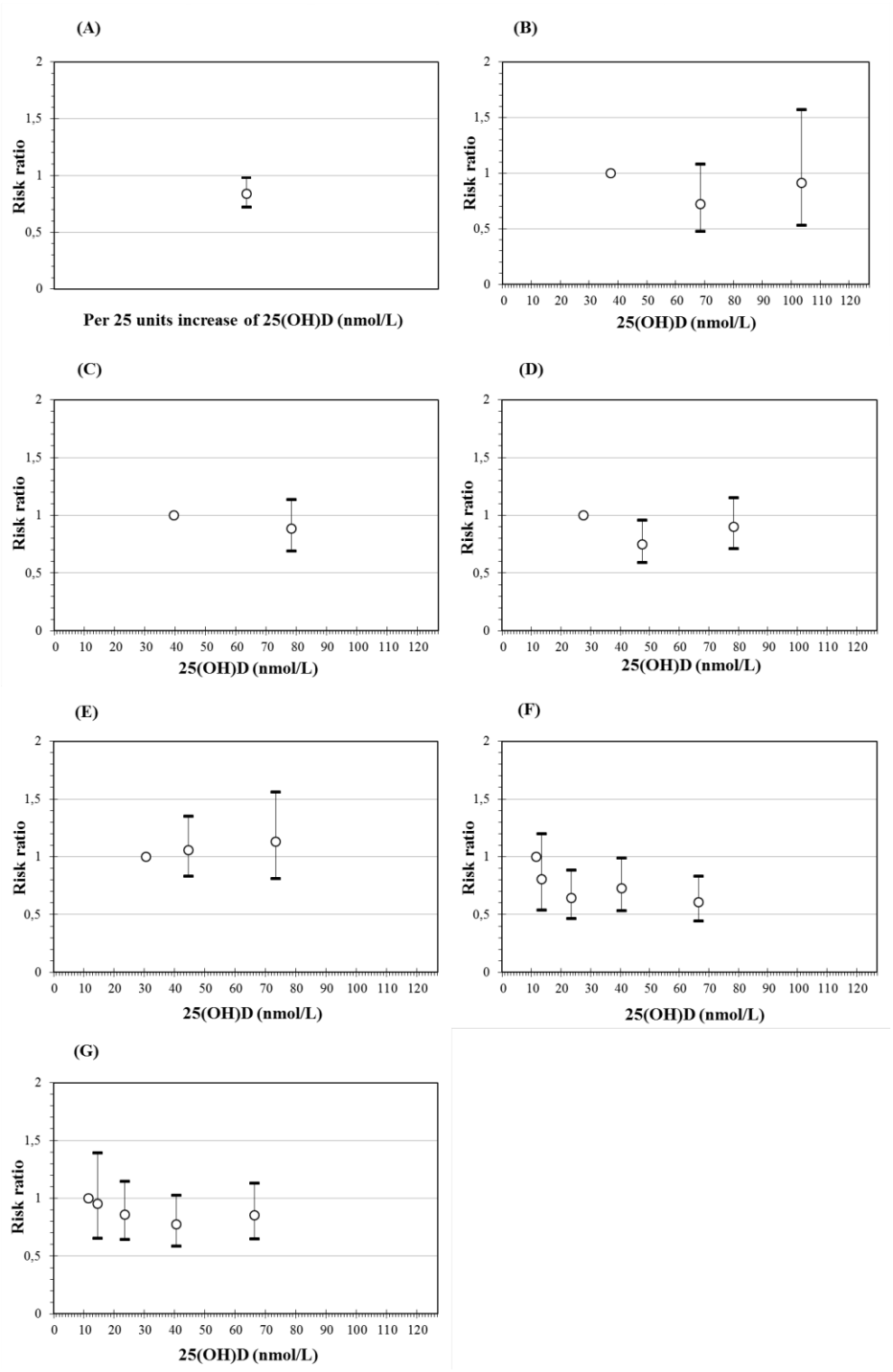

Figure 3 Risk ratios and 95% confidence intervals of total cancer incidence according to circulating 25(OH)D serum concentration.

Depending on available information, medians, midpoints or means of the categories were used for definition of study specific concentrations of serum 25(OH)D categories. **(A)** Giovannucci et al., 2006, USA, men **(B)** Michaelsson et al., 2010, Sweden, men **(C)** De Boer et al., 2012, USA, all **(D)** Ordonez Mena et al., 2013, Germany, men **(E)** Ordonez Mena et al., 2013, Germany, women **(F)** Afzal et al., 2013, Denmark, men **(G)** Afzal et al., 2013, Denmark, women.

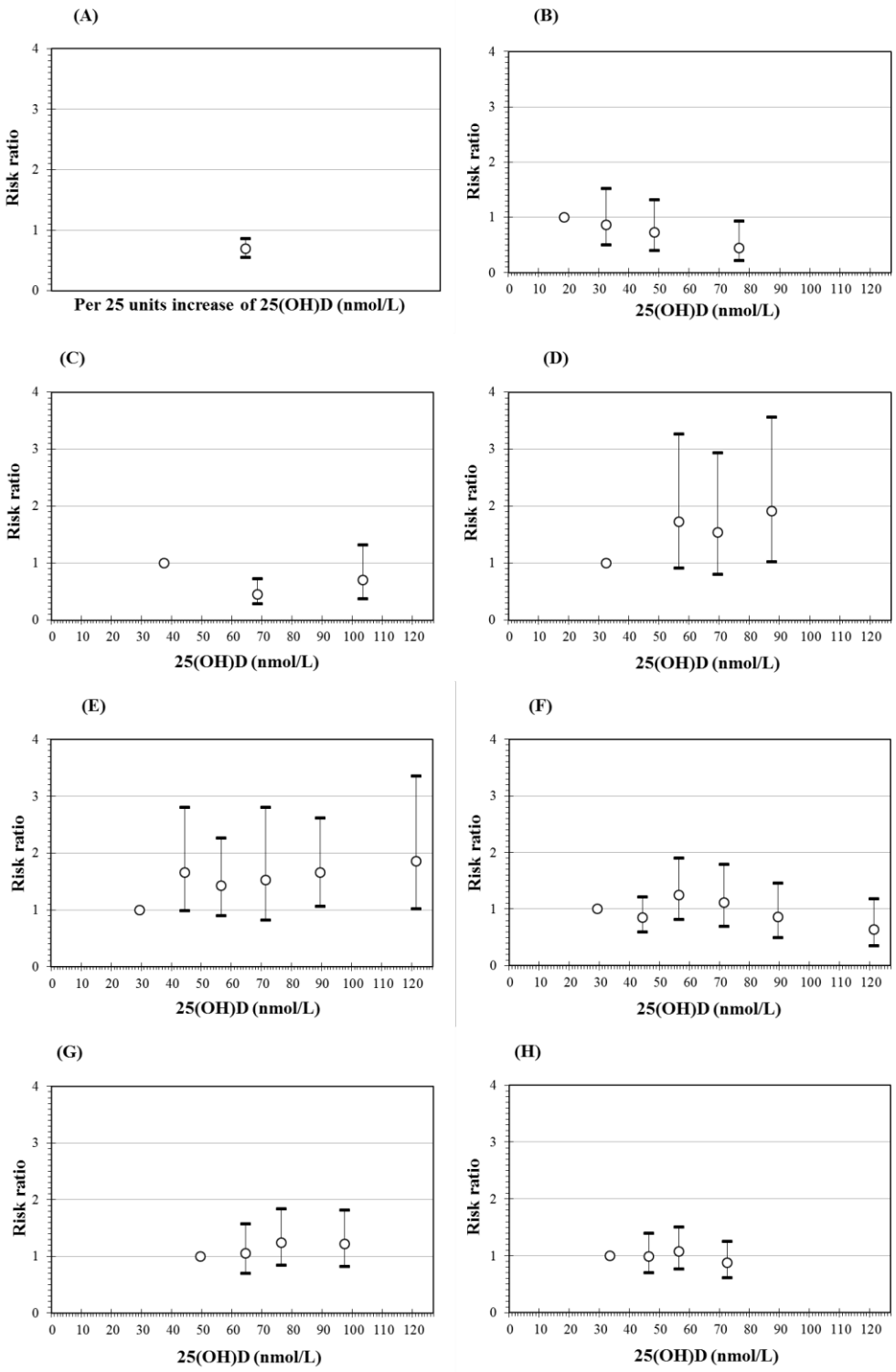

Figure 4 (Part 1) Risk ratios and 95% confidence intervals of total cancer mortality according to circulating 25(OH)D serum concentration.

Depending on available information, medians, midpoints or means of the categories were used for definition of study specific concentrations of serum 25(OH)D categories. **(A)** Giovannucci et al., 2006, USA, men **(B)** Pilz et al., 2008, Germany, all **(C)** Michaelsson et al., 2010, Sweden, men **(D)** Cawthon et al., 2010, USA, men **(E)** Freedman et al., 2010, USA, women **(F)** and men **(G)** Hutchinson et al., 2010, Norway, smokers **(H)** and non-smokers **(I)** Daly et al., 2011, Australia, all **(J)** Eaton et al., 2011, USA, women **(K)** Krause et al., 2012, Germany, all **(L)** Lin et al., 2012, China, men **(M)** and women **(N)** Signorello et al., 2012, USA, all **(O)** Skaaby et al., 2012, Denmark, all **(P)** Schöttker et al., 2013, Germany, all.

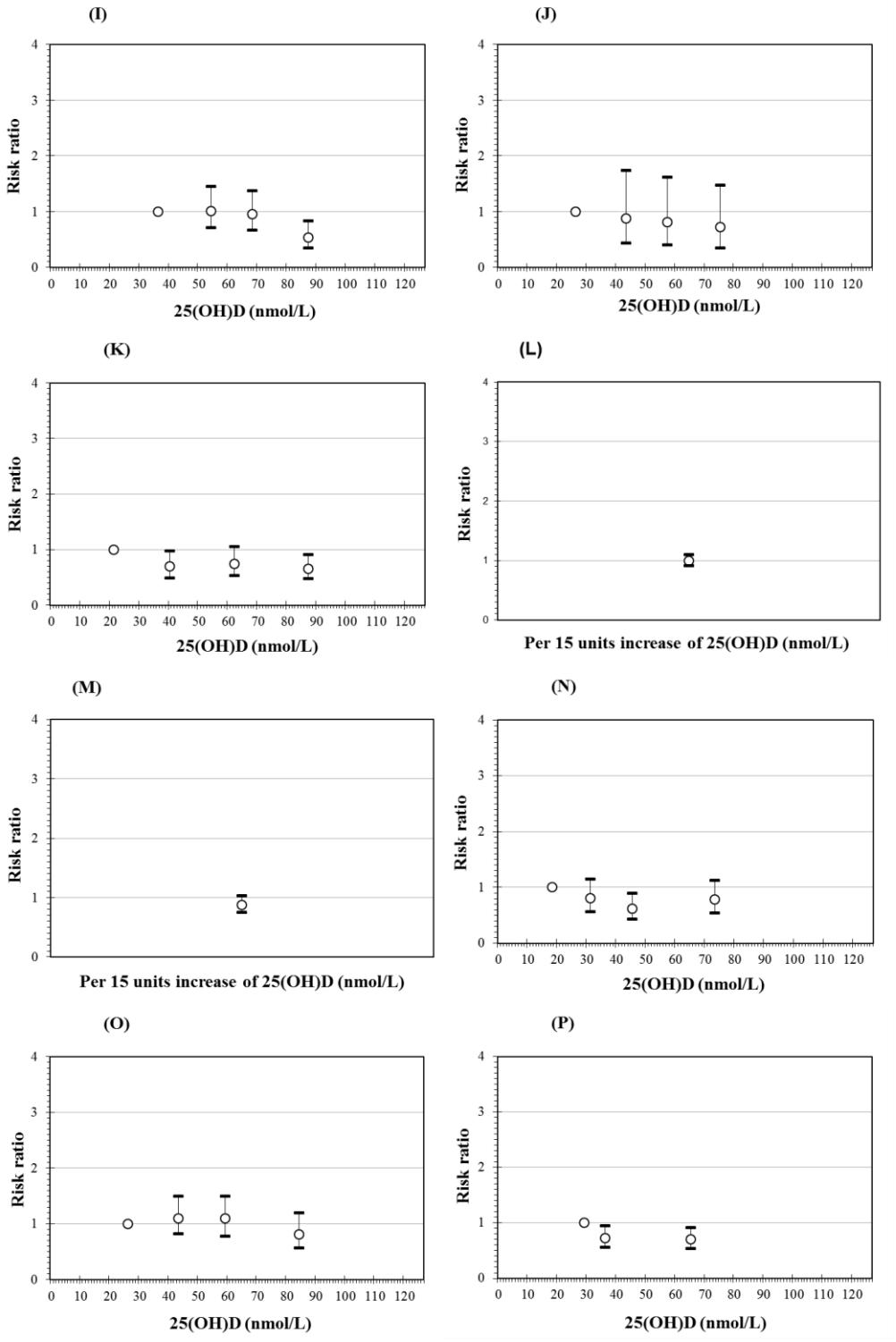

Figure 4 (Part 2) Risk ratios and 95% confidence intervals of total cancer mortality according to circulating 25(OH)D serum concentration.

Depending on available information, medians, midpoints or means of the categories were used for definition of study specific concentrations of serum 25(OH)D categories. **(A)** Giovannucci et al., 2006, USA, men **(B)** Pilz et al., 2008, Germany, all **(C)** Michaelsson et al., 2010, Sweden, men **(D)** Cawthon et al., 2010, USA, men **(E)** Freedman et al., 2010, USA, women **(F)** and men **(G)** Hutchinson et al., 2010, Norway, smokers **(H)** and non-smokers **(I)** Daly et al., 2011, Australia, all **(J)** Eaton et al., 2011, USA, women **(K)** Krause et al., 2012, Germany, all **(L)** Lin et al., 2012, China, men **(M)** and women **(N)** Signorello et al., 2012, USA, all **(O)** Skaaby et al., 2012, Denmark, all **(P)** Schöttker et al., 2013, Germany, all.

3.1.3 Meta analyses

The results of the meta-analyses of studies on total cancer incidence and mortality are shown in **Figure 5** and **Figure 6**, respectively. All RRs refer to an increase in 25(OH)D concentration by 50 nmol/L.

Regarding total cancer incidence, five individual studies found an inverse association. The inverse association was statistically significant in the study of Giovannucci et al. which used predicted rather than measured 25(OH)D concentration [69], but also in the study of Afzal et al. which employed measured 25(OH)D [5]. In meta-analysis (random effects model) using the maximum likelihood estimates, a statistically significant reduction in total cancer incidence with increasing 25(OH)D concentration was observed (RR, 0.89; 95% CI, 0.81-0.97; P=0.01). No significant heterogeneity was evident across studies (Tau^2=0, Q=6.08, p=0.41, I^2=0%). Excluding the study of Giovannucci et al. slightly attenuated the risk estimate (RR, 0.91; 95% CI, 0.82-0.99; P=0.04). Two studies [42, 131] did not specifically report excluding non-melanoma skin cancers (ICD-10 code C44) from the total cancer incidence endpoint. Nevertheless, exclusion of these two studies did not significantly alter the overall estimate. A statistically non-significant decrease in cancer incidence with increasing 25(OH)D concentration was also observed among men (RR, 0.84; 95% CI, 0.70-1.02; P=0.08) pooling data from three studies. Combined data from two studies suggested no association of 25(OH)D with total cancer incidence in women (RR, 0.98; 95% CI, 0.85-1.13; P=0.78). In the meta-analysis for within study risk estimates of high versus low 25(OH)D concentration, similar estimates were found (See **APPENDIX C**, **Supplementary Figure C.1**).

Regarding total cancer mortality, several studies found significant inverse associations. The association was most pronounced in the two earliest studies [69, 144]. In meta-analysis combining all studies, a statistically significant inverse association between serum 25(OH)D concentration and total cancer mortality was observed (RR, 0.83; 95% CI, 0.71-0.96; P<0.01). In sex-specific analyses, a clear inverse association was found among women (RR, 0.76; 95% CI, 0.60-0.98; P=0.03) but not among men (RR, 0.92; 95% CI, 0.65-1.32; P=0.66). However, the sex-specific meta-analyses were based only on sex-specific results reported from three (women) and five studies (men). Large statistical

heterogeneity was observed among the thirteen studies with data for cancer mortality (Tau^2=0.05, Q=38.47, P<0.01, I^2=61%). In standardized deleted residuals analysis, no single study was identified as outlier. Nevertheless, the association was attenuated, although still statistically significant, when the study by Giovannucci et al. [69] which used predicted rather than measured 25(OH)D serum concentration was excluded (RR, 0.86; 95% CI, 0.74-0.98; P=0.02). In the meta-analysis using within study estimates of high versus low 25(OH)D concentration, similar associations were observed (See **APPENDIX C**, **Supplementary Figure C.2**).

Three factors were investigated as potential sources of between-study heterogeneity in meta-regression: significant variation was found for publication year (published in 2010 vs. before 2010: P=0.01), explaining most of the heterogeneity, but not for country (USA vs. others: P=0.36) or type of 25(OH)D assay (immunoassay vs. others: P=0.73). Excluding studies with insufficient confounder adjustment did not significantly alter the overall estimate. The funnel plot did not show an indication of publication bias (Kendall tau= -0.13; P=0.50; Egger's t value= -1.02, P=0.31).

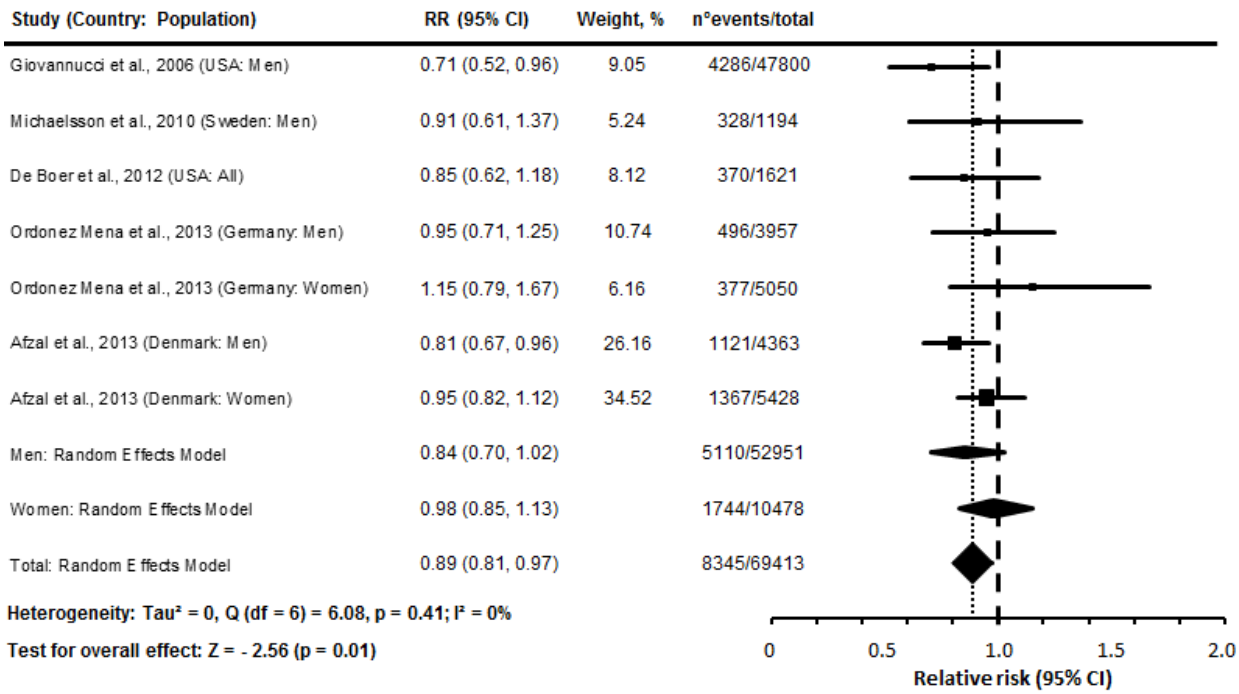

Figure 5 Meta-analyses: Risk ratios of total cancer incidence per 50 nmol/L increase in circulating 25(OH)D serum concentration.

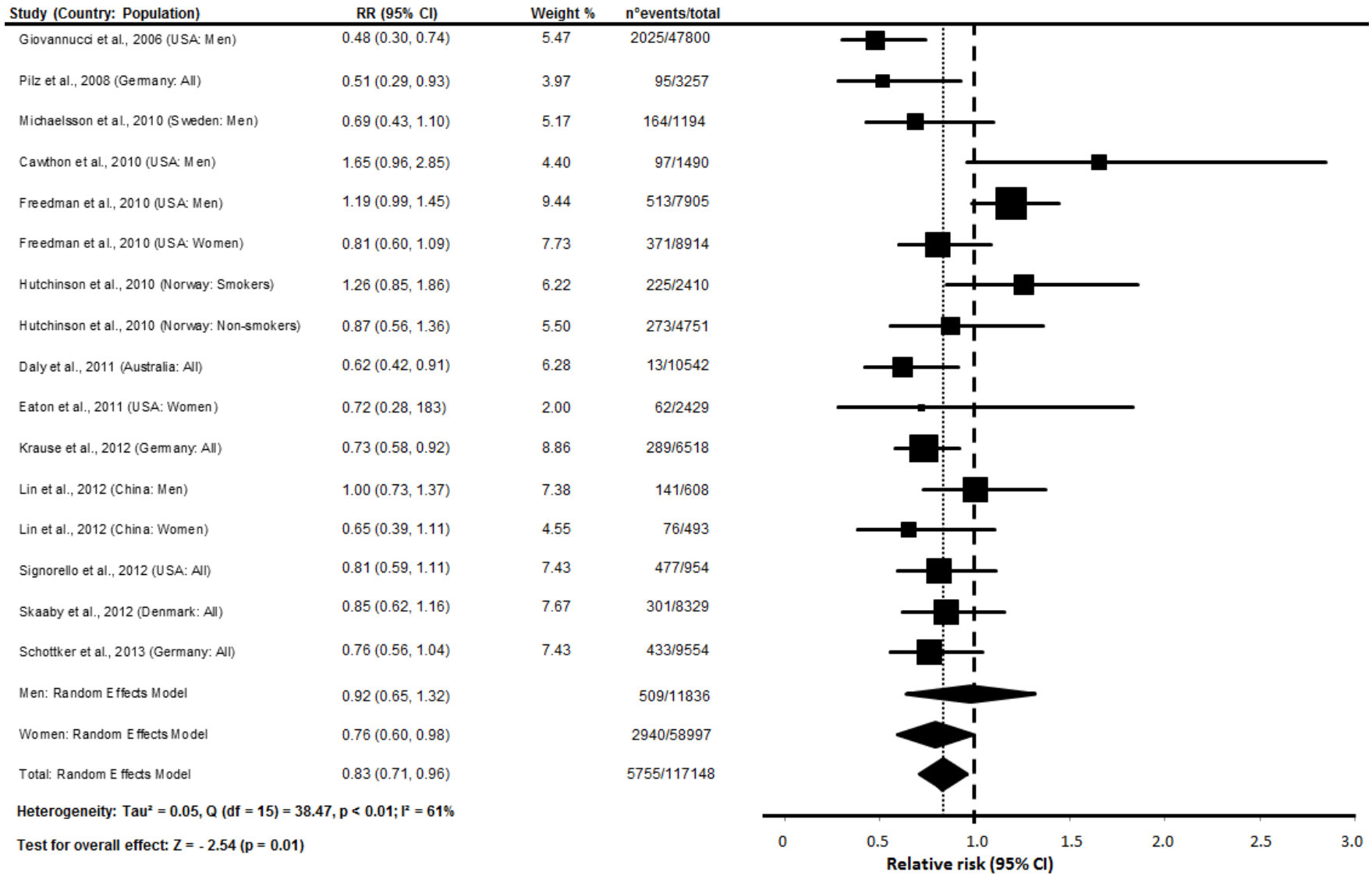

Figure 6 Meta-analyses: Risk ratios of total cancer mortality per 50 nmol/L increase in circulating 25(OH)D serum concentration.

3.2 Association of circulating 25(OH)D concentration with total and site-specific cancer incidence in the CHANCES Consortium

3.2.1 Socio-demographic characteristics according to 25(OH)D levels

Baseline characteristics for the three participating studies according to clinical categories of 25(OH)D concentration are presented in **Table 4** (and according to season-specific 25(OH)D quintiles in the **Supplementary Table 2**). The prevalence of vitamin D deficiency (25(OH)D < 30 nmol/L) was significantly higher in ESTHER (15.1%) as compared to EPIC-Elderly (7.8% in controls) and particularly TROMSØ (5.3%). Suboptimal 25(OH)D concentrations (i.e. below 50 nmol/L) were observed in 40.7% of controls in EPIC-Elderly, and in 58.9% and 40.9% of the ESTHER and TROMSØ participants, respectively. In all studies, vitamin D deficient participants were more often women, significantly older, had significantly higher BMI, and in ESTHER and TROMSØ significantly less often performed vigorous physical activity and were more likely to be never smokers. Higher concentrations of 25(OH)D were associated with higher levels of education in ESTHER and TROMSØ, but not in EPIC-Elderly.

Table 4 Baseline characteristics for each CHANCES cohort across clinical categories of 25(OH)D concentration.

	Study	**EPIC-Elderly** [a]				**ESTHER**				**TROMSØ**			
Characteristic	25(OH)D (nmol/L)	<30	30-50	>50		<30	30-50	>50		<30	30-50	>50	
% total size		7.8	32.9	59.3		15.1	43.8	41.1		5.3	35.6	59.1	
25(OH)D, median (nmol/L)		25.6	41.6	65.8	***	29.5	39.4	65.5	***	25.3	42.3	62.7	***
Season of blood draw (%)					***				***				***
Winter		35.6	30.4	19.2		42.0	29.1	20.6		46.5	36.0	28.8	
Spring		46.7	36.1	24.1		27.4	24.8	14.2		26.9	28.8	40.6	
Summer		6.7	9.4	21.8		11.4	20.9	32.5		1.2	1.7	5.4	
Autumn		11.1	24.1	34.9		19.2	25.2	32.7		25.3	33.6	25.2	
Age, median (years)		63.4	63.3	62.5	**	63.0	63.0	62.0	***	65.0	63.0	61.0	***
Women (%)		64.4	58.1	54.9		58.7	67.4	43.3	***	78.4	63.6	56.0	***
BMI, median (kg/m^2)		27.1	27.2	25.7	**	27.7	27.5	26.8	***	27.7	26.7	25.8	***
Education (%) [b]									**				***
Primary or less		44.4	52.9	48.5		74.0	74.6	71.2		63.3	59.9	48.3	
Primary to university		40.0	31.4	39.5		18.4	18.8	21.2		22.0	24.9	31.1	
University		15.6	14.7	11.3		4.9	3.8	5.5		13.1	14.5	20.2	
Vigorous physical activity (%) [b]		35.6	30.9	34.0		33.8	38.6	48.0	***	24.5	28.9	40.2	***
Smoking status (%) [b]									***				**
Never		42.2	44.5	46.2		47.3	53.8	44.9		55.5	49.7	44.6	
Former		26.7	29.3	31.4		25.6	27.1	38.6		44.5	50.2	55.3	
Current		28.9	25.7	21.8		23.3	16.2	14.2			Excluded		

[a] Data shown for matched controls only.

[b] The sum of the percentages may not add up to 100% due to missing values. The total number of participants with missing values for the variables BMI, highest level of education, vigorous physical activity and smoking status was 55 (0.3%), 276 (1.8%), 463 (3.0%) and 277 (1.8%), respectively.

* p<0.05; ** p<0.01; *** p<0.0001 P-values for testing the statistical significance of differences in baseline characteristics among season-specific 25(OH)D quintiles.

3.2.2 Association of circulating 25(OH)D concentrations with total cancer incidence

During an average of 12 years of follow-up, a total of 1,082 and 806 total cancer cases were identified in the ESTHER and TROMSØ studies, respectively. The association of clinical categories of 25(OH)D concentrations with cancer risk adjusted for age, sex, season of blood draw, highest level of education, smoking status, BMI, and vigorous physical activity is shown in **Table 5** (the results according to season-specific 25(OH)D quintiles can be found in the **Supplementary Table 3**). The results for the age, sex, and season adjusted model were very similar and therefore only the results for the multivariate models were shown.

Overall, clinical categories of 25(OH)D concentrations were not significantly associated with total cancer risk (Table 5). Plotted season-specific 25(OH)D quintiles with respect to total cancer risk suggested an U-shaped association with a statistically significant decreased total cancer risk in the second quintile (HR: 0.81, 95% CI: 0.69;0.95) and higher total cancer risk in the extreme quintiles (**Figure 7 A**). No significant heterogeneity was observed in all meta-analyses for total cancer incidence with both categorizations. No significant variation in the risk estimates across quintiles was observed when excluding cancer diagnoses occurring during the initial 1, 2, 3 and 4 years (data not shown). No significant effect modification (**Table 6**) was observed when stratifying by sex ($P_{interaction}$ = 0.62), age ($P_{interaction}$ = 0.56), BMI ($P_{interaction}$ = 0.66) and vigorous physical activity ($P_{interaction}$ = 0.55).

3.2.3 Associations of circulating 25(OH)D concentrations with site-specific cancer incidence

In ESTHER and TROMSØ cohorts, individually and in combination, low 25(OH)D concentrations were associated with a statistically non-significant increased lung cancer risk (Table 5). There was no significant evidence of a linear increase in lung cancer risk with higher 25(OH)D concentration (p=0.32).

Tentatively increased colorectal cancer risk with low 25(OH)D concentrations was also observed in EPIC-Elderly and TROMSØ, but not in ESTHER. The meta-analysis yielded no significant association of 25(OH)D concentrations with colorectal cancer risk, which was also visible in the dose-

response graph (Figure 7 C). There was no evidence of a linear increase in colorectal cancer risk with higher 25(OH)D concentration (p=0.39).

A significant breast cancer risk reduction was observed for 25(OH)D concentrations between 30 and 50 nmol/L (HR: 0.67, 95% CI: 0.52;0.87). Such decrease in breast cancer risk with lower 25(OH)D concentrations compared to the highest quintile was also apparent across season-specific quintiles, with the exception of the third quintile (Figure 7 D). There was significant evidence of a linear increase in breast cancer risk with higher 25(OH)D (p<0.01).

Overall, no statistically significant association of 25(OH)D with prostate cancer risk was observed, even though a tentatively reduced risk was seen for 25(OH)D concentrations between 30-50 nmol/L (HR: 0.81, 95% CI: 0.63;1.04). No sign of linearity was observed.

Statistically significantly higher lymphoma risk was observed with 25(OH)D concentrations < 30 nmol/L (HR: 1.76, 95% CI: 1.00;3.11). The dose-response relationship showed a tendency towards decreasing lymphoma risk with higher 25(OH)D season-specific quintiles, but the confidence intervals were wide and included the null value for each category (Figure 7 F). Furthermore, increases in 25(OH)D concentrations were not significantly linearly associated with decreased lymphoma risk (p=0.10).

Table 5 Association of clinical categories of vitamin D status with total and site-specific cancer incidence across CHANCES cohorts. [a,b]

Cancer site	EPIC-Elderly			ESTHER			TROMSØ			Summary		
25(OH)D (nmol/L)	cases / controls	OR (95% CI)	w%	events / total	HR (95% CI)	w%	events / total	HR (95% CI)	w%	events / total	RR (95% CI) [c]	P heterogeneity
Total												
< 30			0	183/1348	1.06 (0.88; 1.27)	59	35/229	0.74 (0.51;1.06)	41	218/1577	0.92 (0.64; 1.30)	0.08
30-50		n.a.	0	431/3883	0.90 (0.78; 1.03)	53	290/1523	0.99 (0.85;1.15)	47	721/5406	0.94 (0.85; 1.04)	0.35
> 50				468/3697	1.00 (Ref)		481/2555	1.00 (Ref)		949/6252	1.00 (Ref)	
Lung												
< 30			0	25/1348	1.16 (0.69; 1.94)	72	5/229	2.21 (0.83; 5.91)	28	30/1577	1.39 (0.78; 2.46)	0.25
30-50		n.a.	0	58/3883	1.24 (0.84; 1.85)	66	25/1523	1.57 (0.90; 2.73)	34	83/5406	1.35 (0.98; 1.86)	0.50
> 50				51/3697	1.00 (Ref)		28/2555	1.00 (Ref)		79/6252	1.00 (Ref)	
Colorectal												
< 30	30/25	1.24 (0.64; 2.42)	25	22/1348	0.99 (0.60; 1.65)	44	15/229	1.33 (0.73; 2.44)	31	67/1632	1.15 (0.82; 1.61)	0.74
30-50	114/83	1.35 (0.89; 2.04)	27	66/3883	1.09 (0.76; 1.57)	35	57/1523	1.00 (0.71; 1.41)	39	237/5603	1.12 (0.90; 1.38)	0.54
> 50	159/163	1.00 (Ref)		64/3697	1.00 (Ref)		89/2555	1.00 (Ref)		312/6574	1.00 (Ref)	
Breast												
< 30	12/6	1.37 (0.41; 4.53)	15	23/790	0.74 (0.44; 1.13)	69	3/179	0.38 (0.12; 1.22)	16	38/987	0.73 (0.45; 1.18)	0.32
30-50	41/50	0.62 (0.34; 1.13)	18	79/2605	0.73 (0.52; 1.05)	53	25/964	0.61 (0.38; 0.98)	29	145/3660	0.67 (0.52; 0.87)	0.78
> 50	73/70	1.00 (Ref)		61/1595	1.00 (Ref)		61/1428	1.00 (Ref)		195/3166	1.00 (Ref)	
Prostate												
< 30	4/5	1.43 (0.27; 7.67)	6	30/558	1.12 (0.73; 1.71)	86	2/50	0.51 (0.12; 2.07)	8	36/617	1.06 (0.71; 1.58)	0.54
30-50	19/16	0.54 (0.19; 1.53)	6	55/1278	0.81 (0.58; 1.15)	52	39/559	0.85 (0.58; 1.25)	43	113/1872	0.81 (0.63; 1.04)	0.73
> 50	48/24	1.00 (Ref)		104/2102	1.00 (Ref)		91/1127	1.00 (Ref)		243/3301	1.00 (Ref)	
Lymphoma												
< 30	25/17	1.85 (0.81; 4.21)	48	12/1348	1.69 (0.77; 3.70)	52	0/229	n.a.	0	37/1619	1.76 (1.00; 3.11)	0.88
30-50	57/59	1.15 (0.65; 2.04)	38	24/3883	1.30 (0.69; 2.44)	31	16/1523	0.96 (0.51; 1.81)	31	97/5522	1.13 (0.79; 1.61)	0.80
> 50	99/108	1.00 (Ref)		19/3697	1.00 (Ref)		27/2555	1.00 (Ref)		145/6459	1.00 (Ref)	

OR odds ratio, **HR** hazard ratio, **RR** risk ratio, **CI** confidence interval, **w%** study weight in meta-analysis in percentage, **n.a.** not available.

[a] Risk estimates correspond to those derived from the multivariate model 2 which was adjusted for sex, age, season of blood draw, highest level of education, smoking status, BMI and vigorous physical activity.

[b] HRs were calculated for ESTHER and TROMSØ whereas for the EPIC-Elderly study ORs were calculated due to the nested-case control design. In the EPIC-Elderly study, circulating 25(OH)D serum levels were only measured in colorectal, breast, prostate cancer and lymphoma cases and controls.

[c] In a conservative approach, in order to allow for the variation of true effects between studies, pooled RRs were calculated with meta-analyses using random-effects models.

Table 6 Association of clinical categories of vitamin D status with total cancer incidence stratified by relevant covariates. [a]

Stratification variable ($P_{interaction}$)	25(OH)D (nmol/L)	ESTHER HR (95% CI)	TROMSØ HR (95% CI)	Summary HR (95% CI) [b]
Sex	**Men**			
(0.62)	< 30	1.18 (0.93;1.50)	0.67 (0.34;1.31)	0.97 (0.57;1.65)
	30-50	0.88 (0.73;1.07)	0.98 (0.79;1.20)	0.92 (0.80;1.06)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Women			
	< 30	0.96 (0.71;1.28)	0.80 (0.52;1.23)	0.90 (0.71;1.15)
	30-50	0.90 (0.73;1.12)	1.01 (0.82;1.26)	0.95 (0.82;1.11)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Age	**< 65 years**			
(0.56)	< 30	1.17 (0.91;1.52)	0.47 (0.24;0.92)	0.79 (0.32;1.91)
	30-50	1.00 (0.82;1.21)	0.88 (0.71;1.09)	0.94 (0.82;1.09)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	≥ 65 years			
	< 30	1.00 (0.77;1.30)	0.97 (0.63;1.50)	0.99 (0.79;1.24)
	30-50	0.83 (0.67;1.02)	1.13 (0.91;1.39)	0.97 (0.71;1.31)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
BMI	**< 30 kg/m²**			
(0.66)	< 30	1.16 (0.94;1.43)	0.75 (0.48;1.17)	0.98 (0.64;1.48)
	30-50	0.85 (0.72;1.00)	0.95 (0.80;1.13)	0.90 (0.79; 1.01)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	≥ 30 kg/m²			
	< 30	0.82 (0.57;1.20)	0.71 (0.38;1.31)	0.79 (0.57;1.09)
	30-50	1.01 (0.76;1.34)	1.07 (0.79;1.44)	1.03 (0.84;1.27)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Vigorous Physical Activity	**No**			
(0.55)	< 30	1.10 (0.88;1.38)	0.84 (0.56;1.26)	1.01 (0.80;1.29)
	30-50	0.85 (0.71;1.02)	1.03 (0.85;1.24)	0.93 (0.77;1.12)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
	Yes			
	< 30	0.94 (0.68;1.30)	0.50 (0.22;1.13)	0.77 (0.43;1.38)
	30-50	0.98 (0.79;1.22)	0.95 (0.73;1.22)	0.97 (0.82;1.14)
	> 50	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)

HR hazard ratio, **BMI** body mass index.

[a] Hazard ratios and 95% confidence intervals shown correspond to those derived from model 2 which was adjusted for sex, age, season of blood draw, highest level of education, smoking status, BMI and vigorous physical activity.

[b] In a conservative approach, in order to allow for the variation of true effects between studies, pooled HRs were calculated with meta-analyses using random-effects models.

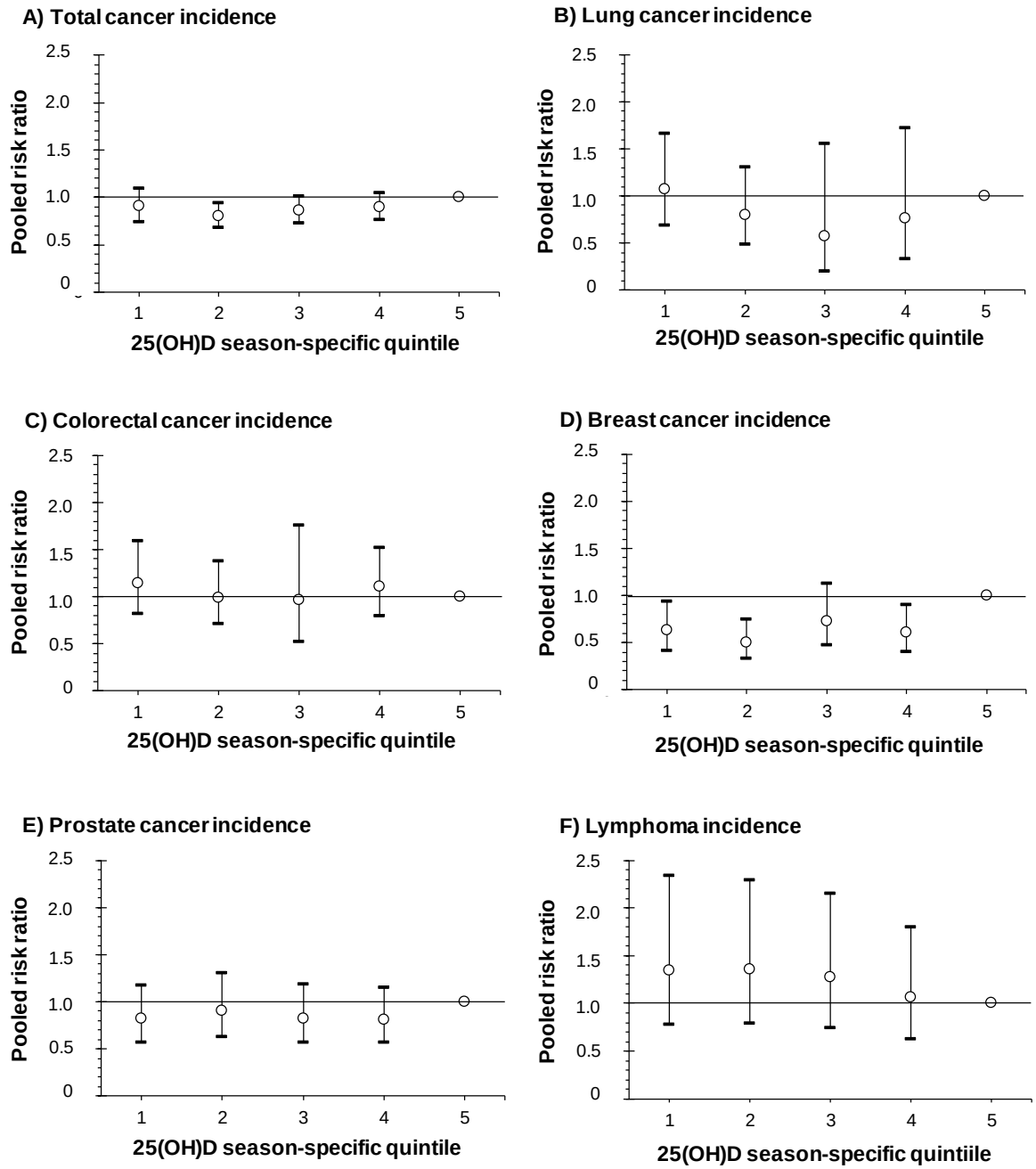

Figure 7 Pooled risk ratios and 95% confidence intervals for risk of **A)** total cancer, **B)** lung cancer, **C)** colorectal cancer, **D)** breast cancer, **E)** prostate cancer and **F)** lymphoma across 25(OH)D study- and season-specific quintiles with the top quintile (higher 25(OH)D) as reference. For total and lung cancer incidence only data from the ESTHER and TROMSØ study were combined.

3.3 Association of 25(OH)D-related SNPs with total and site-specific cancer incidence in the ESTHER study

3.3.1 Association of vitamin D SNPs and genotype score with vitamin D

Overall, only 8 SNPs, out of the 16 SNPs that were genotyped in the complete population, were significantly associated with 25(OH)D concentration: rs2972516, rs3755967, rs4387287, rs4762651, rs7308827, rs11603330, rs12794714 and rs17216707. Differences in mean 25(OH)D ranged from 0.02 to 6.57 nmol/L, with the largest difference observed for rs3755967. Out of the 8 SNPs associated with 25(OH)D concentration, 7 were in Hardy Weinberg Equilibrium (P>0.05) and only one was not: rs4762651. This SNP was therefore excluded from following analyses. To confirm the selection of SNPs, we additionally assessed the association of 25(OH)D related SNPs with the risk of having low vitamin D status.

Three SNPs: rs3755967 (GC), rs11603330 (DHCR7) and rs12794714 (CYP2R1) were significantly associated with clinical definitions of low vitamin D status (**Table 7**). The remaining SNPs were excluded from the next analyses since they were not consistently associated with low vitamin D status and moreover not located within known genes of the vitamin D pathway. Rs1721607 although not consistently associated with low vitamin D status, it was exceptionally considered for following analyses since this SNP is located within the CYP24A1 gene, a relevant gene for the vitamin D pathway which has also been widely investigated in literature. However, this last SNP was not considered for the creation of the genotype score, which added up the number of risk alleles for the 3 SNPs in GC, DHCR7 and CYP2R1. None of the selected 4 polymorphisms were in linkage disequilibrium (r^2 between 0.26 and 0.38). Subjects in the upper tertile of the genotype score (higher number of risk alleles) had lower 25(OH)D concentration and therefore increased the odds of low vitamin D status. Significant variation according to season of blood draw and sex was observed (**Table 8**). Overall, differences in 25(OH)D were significantly greater for subjects with rare risk alleles in summer and autumn than in winter and spring seasons. In summer and autumn, men with rare risk alleles had lower 25(OH)D concentrations than women, whereas in winter and spring this sex difference was not evident.

3.3.2 Association of 25(OH)D related SNPs and genotype score with total cancer

The association of 25(OH)D related SNPs and total cancer incidence is shown in **Table 9**. Overall, no significant association with total cancer incidence was observed for rs3755967 (GC), rs11603330 (DHCR7) and rs17216707 (CYP24A1). On the other hand, rs12794714 (CYP2R1) was associated with increased total cancer risk among heterozygous with the rare allele, however, correction for multiple testing resulted in statistical non-significance of the estimates. The genotype score was not associated with total cancer risk. Excluding cancers occurring during the first 1, 2, 3 and 4 years did not significantly alter the estimates or the direction of the association. Introducing 25(OH)D in the model did not significantly alter the estimates.

3.3.3 Association of 25(OH)D related SNPs and genotype score with site-specific cancer incidence

Overall, there was no statistically significant association of 25(OH)D related SNPs or genotype score with colorectal cancer incidence. Nevertheless, although not statistically significant, an increased risk of colorectal cancer was suggested for the minor homozygotes with the rs11603330 (DHCR7) SNP (HR 1.64, 95% CI 0.94-2.85).

No statistically significant association was observed between SNPs or genotype score with breast cancer.

For prostate cancer, the SNPs in GC, CYP2R1 and CYP24A1 were not significantly associated to prostate cancer risk. On the other hand, the minor homozygotes for the rs11603330 (DHCR7) SNP were at statistically significantly increased prostate cancer risk (HR 1.72, 95% CI 1.05-2.82). However, this estimate was no longer significant after adjusting for multiple testing. Additionally, a non-significant linear trend was observed for increasing number of alleles in the genotype score and higher risk of prostate cancer (HR in Tertile 3: 1.37, 95% CI 0.97-1.93, P-trend 0.07).

Statistically significantly higher lung cancer risk was observed for the heterozygotes (HR 1.56, 95% CI 1.03-2.37) and homozygotes (HR 1.63, 95% CI 1.01-2.63) with the rare allele of the SNP in the

CYP2R1. However, risk estimates were no longer significant after correction for multiple testing. A significantly higher lung cancer risk was observed for subjects with increasing number of rare alleles in the genotype score (HR in Tertile 3: 1.59, 95% CI 1.05-2.41, P-trend 0.03).

Table 7 Association of SNPs and genotype score with continuous 25(OH)D serum concentration and with different clinical definitions of low vitamin D status. [a,b]

SNP ID *(Gene)* Genotype	25(OH)D				Definition of low vitamin D status					
					< 30 nmol/L		< 50 nmol/L		< 80 nmol/L	
	N	Mean	(SE)	ß (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
Genotype score										
Tertile 1	2814	52.96	(0.47)	0.00 Reference	423	1.00 Reference	1599	1.00 Reference	2430	1.00 Reference
Tertile 2	2725	51.16	(0.45)	**-2.10 (-3.36 ; -0.85)**	409	1.01 (0.87 ; 1.17)	1597	**1.11 (1.00 ; 1.24)**	2444	**1.44 (1.21 ; 1.70)**
Tertile 3	2545	48.02	(0.51)	**-4.90 (-6.18 ; -3.63)**	422	1.12 (0.96 ; 1.30)	1643	**1.41 (1.26 ; 1.59)**	2381	**2.38 (1.95 ; 2.89)**
RS3755967 (GC)										
CC	4247	52.38	(0.41)	0.00 Reference	622	1.00 Reference	2413	1.00 Reference	3743	1.00 Reference
CT	3289	49.52	(0.39)	**-3.06 (-4.12 ; -1.99)**	540	**1.17 (1.03 ; 1.33)**	2042	**1.31 (1.18 ; 1.44)**	2999	**1.45 (1.24 ; 1.70)**
TT	617	46.39	(0.88)	**-6.55 (-8.52 ; -4.57)**	100	1.19 (0.94 ; 1.50)	429	**1.94 (1.60 ; 2.35)**	579	**2.23 (1.57 ; 3.17)**
RS11603330 (DHCR7)										
AA	4469	52.0	(0.36)	0.00 Reference	647	1.00 Reference	2593	1.00 Reference	3948	1.00 Reference
AC	3136	49.52	(0.46)	**-2.56 (-3.64 ; -1.49)**	495	1.13 (0.99 ; 1.29)	1949	**1.23 (1.11 ; 1.36)**	2870	**1.49 (1.27 ; 1.75)**
CC	538	47.83	(0.91)	**-3.91 (-6.01 ; -1.81)**	120	**1.70 (1.36 ; 2.13)**	333	1.17 (0.96 ; 1.42)	495	**1.51 (1.08 ; 2.11)**
RS12794714 (CYP2R1)										
GG	2364	51.77	(0.52)	0.00 Reference	389	1.00 Reference	1391	1.00 Reference	2075	1.00 Reference
GA	4030	50.82	(0.36)	-1.00 (-2.19 ; 0.19)	599	0.89 (0.77 ; 1.02)	2396	1.03 (0.93 ; 1.15)	3602	**1.18 (1.00 ; 1.39)**
AA	1732	49.36	(0.67)	**-2.48 (-3.94 ; -1.02)**	270	0.93 (0.78 ; 1.10)	1077	**1.17 (1.03 ; 1.34)**	1617	**2.03 (1.61 ; 2.56)**

[a] Multivariate models were adjusted by age, sex, BMI and season of blood draw (winter: December-February; spring: March-May; summer: June-August; autumn: September-November).

[b] Genotype score was created for those subjects without missing genotype for the SNPs rs3755967 (GC), rs11603330 (DHCR7) and rs12794714 (CYP2R1).

Table 8 Associations of SNPs and genotype score with continuous 25(OH)D serum concentration stratified by season of blood draw and sex. [a]

SNP ID *(Gene)* Genotype	**Adjusted change (ß and 95% CI) in serum 25(OH)D concentration (nmol/L)**					
	Winter/Spring			**Summer/Autumn**		
	Men	**Women**	**Both**	**Men**	**Women**	**Both**
Genotype Score [b]						
Tertile 1	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference
Tertile 2	1.18 (-1.92 ; 4.28)	-0.51 (-2.09 ; 1.07)	0.14 (-1.46 ; 1.73)	**-8.50 (-12.0 ; -5.01)**	-1.20 (-2.93 ; 0.52)	**-4.18 (-6.08 ; -2.28)**
Tertile 3	-2.69 (-5.86 ; 0.49)	**-2.58 (-4.19 ; -0.96)**	**-2.57 (-4.21 ; -0.93)**	**-11.4 (-15.0 ; -7.74)**	**-4.29 (-6.01 ; -2.58)**	**-7.43 (-9.35 ; -5.50)**
RS3755967 *(GC)*						
CC	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference
CT	-2.45 (-5.19 ; 0.28)	-1.15 (-2.51 ; 0.22)	**-1.80 (-3.19 ; -0.41)**	**-5.25 (-8.31 ; -2.20)**	**-3.66 (-5.14 ; -2.19)**	**-4.33 (-5.92 ; -2.75)**
TT	-3.80 (-9.03 ; 1.42)	**-5.04 (-7.65 ; -2.43)**	**-4.34 (-7.00 ; -1.68)**	**-11.3 (-17.0 ; -5.57)**	**-6.37 (-8.98 ; -3.77)**	**-8.38 (-11.3 ; -5.52)**
RS11603330 *(DHCR7)*						
AA	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference
AC	**-3.26 (-6.03 ; -0.49)**	**-2.00 (-3.37 ; -0.63)**	**-2.56 (-3.96 ; -1.16)**	**-4.74 (-7.80 ; -1.67)**	-1.18 (-2.67 ; 0.30)	**-2.75 (-4.34 ; -1.16)**
CC	**-5.83 (-11.1 ; -0.60)**	-1.49 (-4.14 ; 1.15)	**-3.48 (-6.17 ; -0.80)**	**-5.60 (-11.9 ; 0.70)**	**-3.40 (-6.33 ; -0.47)**	**-4.27 (-7.46 ; -1.08)**
RS12794714 *(CYP2R1)*						
GG	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference	0.00 Reference
GA	-1.27 (-4.32 ; 1.79)	0.50 (-1.02 ; 2.02)	-0.36 (-1.91 ; 1.20)	**-3.96 (-7.38 ; -0.53)**	0.42 (-1.24 ; 2.08)	-1.53 (-3.31 ; 0.25)
AA	0.73 (-2.97 ; 4.43)	-1.01 (-2.88; 0.87)	-0.35 (-2.24 ; 1.55)	**-6.66 (-10.8 ; -2.48)**	**-2.60 (-4.64 ; -0.57)**	**-4.40 (-6.57 ; -2.23)**

[a] Multivariate models were adjusted by age, sex, BMI and season of blood draw (winter: December-February; spring: March-May; summer: June-August; autumn: September-November).

[b] Genotype score was created for those subjects without missing genotype for the SNPs rs3755967 (GC), rs11603330 (DHCR7) and rs12794714 (CYP2R1).

Table 9 Associations of 25(OH)D-related SNPs and genotype score with total and site-specific cancer incidence. [a,b]

SNP ID *(Gene)*	**Total cancer**			**Colorectal cancer**			**Breast cancer**			**Prostate cancer**			**Lung cancer**		
Genotype	**N**	**cases**	**HR (95% CI)**	**N**	**cases**	**HR (95% CI)**	**N**	**cases**	**HR (95% CI)**	**N**	**cases**	**HR (95% CI)**	**N**	**cases**	**HR (95% CI)**
Genotype Score															
Tertile 1	2694	328	1.00 Reference	2866	56	1.00 Reference	1567	58	1.00 Reference	1253	62	1.00 Reference	2887	38	1.00 Reference
Tertile 2	2600	327	1.06 (0.91-1.23)	2762	46	0.86 (0.58-1.27)	1487	43	0.78 (0.53-1.16)	1247	65	1.11 (0.78-1.58)	2789	58	**1.64 (1.09-2.47)**
Tertile 3	2427	291	1.03 (0.88-1.20)	2587	42	0.86 (0.58-1.29)	1457	46	0.85 (0.58-1.25)	1106	71	1.37 (0.97-1.93)	2611	53	**1.59 (1.05-2.41)**
p for trend			0.72			0.46			0.39			0.07			**0.03**
RS3755967 *(GC)*															
CC	4060	499	1.00 Reference	4320	84	1.00 Reference	2328	79	1.00 Reference	1904	103	1.00 Reference	4356	67	1.00 Reference
CT	3125	392	1.06 (0.92-1.20)	3333	55	0.87 (0.62-1.22)	1820	58	0.94 (0.67-1.32)	1439	80	1.05 (0.78-1.41)	3367	72	**1.44 (1.03-2.01)**
TT	601	62	0.86 (0.66-1.12)	631	7	0.60 (0.28-1.30)	363	10	0.84 (0.44-1.63)	263	15	1.04 (0.61-1.79)	634	10	0.96 (0.50-1.88)
RS11603330 *(DHCR7)*															
AA	4281	534	1.00 Reference	4542	78	1.00 Reference	2462	82	1.00 Reference	1999	102	1.00 Reference	4581	80	1.00 Reference
AC	2987	351	0.95 (0.83-1.09)	3184	51	0.95 (0.67-1.35)	1748	56	0.96 (0.68-1.34)	1374	77	1.10 (0.82-1.48)	3217	57	1.03 (0.73-1.45)
CC	509	66	1.08 (0.84-1.40)	547	15	1.64 (0.94-2.85)	301	9	0.86 (0.43-1.72)	233	19	**1.72 (1.05-2.82)**	549	12	1.39 (0.76-2.56)
RS12794714 *(CYP2R1)*															
GG	2265	250	1.00 Reference	2401	44	1.00 Reference	1310	37	1.00 Reference	1050	53	1.00 Reference	2422	31	1.00 Reference
GA	3846	494	**1.20 (1.03-1.39)**	4104	73	0.99 (0.68-1.44)	2242	81	1.29 (0.88-1.91)	1781	103	1.19 (0.85-1.66)	4136	81	**1.56 (1.03-2.37)**
AA	1650	206	1.16 (0.96-1.40)	1752	29	0.91 (0.57-1.45)	959	29	1.07 (0.66-1.74)	775	42	1.15 (0.76-1.72)	1772	37	**1.63 (1.01-2.63)**
RS17216707 *(CYP24A1)*															
TT	4986	624	1.00 Reference	5294	95	1.00 Reference	2868	90	1.00 Reference	2314	131	1.00 Reference	5346	103	1.00 Reference
TC	2489	297	0.96 (0.84-1.11)	2658	46	0.97 (0.68-1.38)	1452	54	1.17 (0.83-1.64)	1149	59	0.91 (0.67-1.23)	2678	41	0.83 (0.58-1.20)
CC	308	31	0.81 (0.57-1.17)	329	5	0.88 (0.36-2.18)	183	3	0.51 (0.16-1.62)	136	8	1.19 (0.58-2.44)	330	5	0.76 (0.31-1.88)

[a] Multivariate models were adjusted by age, sex, BMI, smoking status (never, former, current), physical activity (>2h of vigorous and >2h of light physical activity/week; <1h of physical activity/week; other), 25(OH)D serum concentration (continuous) and season of blood draw (winter: December-February; spring: March-May; summer: June-August; autumn: September-November). HRs and 95% CI were derived from the combination of the results for 5 imputed datasets.

[b] Genotype score was created for those subjects without missing genotype for the SNPs rs3755967 (GC), rs11603330 (DHCR7) and rs12794714 (CYP2R1).

4 DISCUSSION

4.1 Systematic review and meta-analysis of prospective studies on the association of circulating 25(OH)D concentration with total cancer incidence and mortality

This is first systematic review with meta-analyses summarizing the results of longitudinal studies on the association of circulating 25(OH)D with total cancer incidence and mortality. These analyses comprised 137,567 subjects and were based on 8,345 diagnoses of incident cancer and 5,755 deaths from cancer. Combined data from five studies suggest that circulating 25(OH)D serum concentration may be inversely related to total cancer incidence. Findings from 13 studies suggested an inverse association of circulating 25(OH)D serum concentration with cancer mortality.

4.1.1 Total cancer incidence

Although a growing number of studies have assessed the relationship between 25(OH)D serum concentration and site-specific cancer incidence, only five studies focused on total cancer incidence, among which two studies were restricted to men [69, 131]. This meta-analysis with comprehensive trend estimation from summarized dose-response data suggested a 11% reduction of overall cancer incidence for an increase of 25(OH)D serum concentration by 50 nmol/L. These results have to be interpreted with caution, given the study population was mainly composed by men and the number of studies was rather small, one of which used predicted rather than measured 25(OH)D concentration.

4.1.2 Total cancer mortality

In the meta-analysis on total cancer mortality, most heterogeneity across the thirteen studies included could be attributed to year of publication. Two earlier studies published before 2010 [69, 144] reported a strong, significant, inverse association of circulating 25(OH)D serum concentration with total cancer mortality. The strongest risk reduction was found in the first prospective study among male health professionals in the USA [69], which used predicted rather than measured 25(OH)D concentration.

Exclusion of this study attenuated the estimated association with total cancer mortality. The other early study was restricted to German patients referred for coronary angiography and included only 95 cancer deaths. It observed a 55% lower cancer mortality for those in the highest compared to those in the lowest quartile of 25(OH)D serum concentration [144]. It is uncertain to what extent results for this special patient population can be generalized. The majority of the eleven more recent studies published since 2010 still found an inverse association of 25(OH)D concentration with total cancer mortality [41, 47, 105, 158, 162, 163], even though this association was statistically significant in two studies only [41, 105]. A significant U-shaped association was reported in only one study [131], with significantly higher cancer mortality at low and high 25(OH)D serum concentration compared to medium concentrations. Two studies suggested higher cancer mortality with increasing 25(OH)D concentrations in dose-response graphs [32, 57]. Potential differences of the study populations and time of recruitment may account for the inconsistent results, as suggested by others [145]. Overall, nonetheless, the results from the meta-analysis suggested an inverse association of 25(OH)D concentration with cancer mortality. This is also in line with findings among cancer patients for whom cancer mortality was decreased with higher 25(OH)D concentrations [147].

While this review and meta-analysis focused on observational studies, few RCTs evaluated a potential preventive effect of supplementation with vitamin D on cancer incidence and/or mortality, the results of which were summarized in a recent systematic review [111]. Three RCTs failed to show a statistically significant decrease in total cancer mortality associated with supplementation of low vitamin D doses plus calcium [13, 30, 177]. However, supplied vitamin D doses (about 800 IU per day) may have been insufficient to raise 25(OH)D at concentrations needed to improve health outcomes. Another RCT among postmenopausal women who received daily 1400-1500 mg calcium supplementation only, supplemental calcium plus 1100 IU vitamin D, or placebo, found that the risk of incident cancer was reduced by 60% in the vitamin D plus calcium group, compared to the placebo group [110]. Risk reduction by calcium alone was 47%. A greater difference of the effects of vitamin D and calcium and calcium alone was observed 4 years after diagnosis. Unfortunately, this study did

not provide data on cancer mortality. Overall, evidence from RCTs is not robust enough to affirm that vitamin D reduces cancer risk [111]. However, newer RCTs are under way with a focus on chronic disease prevention, providing higher vitamin D doses, and without adjunct calcium supplements [108]. Although there is some concern as to whether the new RCTs are aimed to the right study population [146], the results from these studies are expected to further clarify whether and if so, to what extent vitamin D is relevant for the prevention of cancer.

Genetic studies provide a different but also valuable approach by examining the risk of cancer according to polymorphisms which are related to serum 25(OH)D concentration. A possible advantage of these studies is that they may be less prone to confounding [95, 113]. While evidence from such studies is still sparse and inconclusive, further genetic studies are warranted.

4.2 Analyses on the association of circulating 25(OH)D concentration with total and site-specific cancer incidence in the CHANCES Consortium

The CHANCES consortium provides the largest study population up to date examining the association of measured 25(OH)D concentrations and total cancer risk among older adults. Additionally, we provide an assessment of the association of low 25(OH)D concentrations with the incidence of major site-specific cancer endpoints. Overall, we observed no significant association of lower 25(OH)D concentrations with increased total or site-specific cancer incidence. In fact, 25(OH)D concentrations in the range of the IOM's recommendations were associated with the lowest risk of total cancer and breast cancer.

4.2.1 Total cancer

In the previous meta-analysis, the summarized findings from 5 previously published prospective studies assessing the relationship between 25(OH)D concentrations and total cancer incidence suggested a 11% reduction of total cancer incidence for a 50 nmol/L increase in 25(OH)D [193]. Shortcomings of this meta-analysis were the study populations (consisting mainly of men and specific occupational groups) and the restricted data provided by the original publications. Whereas inverse associations of vitamin D with total cancer risk had been more often observed in male than in female study populations [5, 69, 141], no significant differences were observed between males and females in our meta-analysis with individual participant data. We also did not observe any age differences regarding total cancer incidence, but the data for the age stratification was limited to only two cohorts. Our analyses, based mainly on a population of older adults (mean age: 63 years) could suggest that vitamin D may not be relevant for cancer development at old age. It is worth noting that the strongest effect of vitamin D on cancer risk was observed by Giovannucci and colleagues, who employed a statistical algorithm for predicting 25(OH)D rather than measuring it in the complete population [69]. Even though our study, despite its large overall size, had limited power to detect potential weak inverse associations between 25(OH)D and total cancer risk, moderate or strong inverse associations

would appear highly unlikely in the light of the confidence intervals estimated for vitamin D insufficiency and deficiency.

4.2.2 Lung cancer

Previous reports of associations between 25(OH)D and lung cancer risk have observed comparable magnitudes of association i.e., approximately a 20% decrease in lung cancer risk for higher, compared to lower, 25(OH)D concentrations (or a 20% increase in risk for lower, compared to higher, 25(OH)D concentrations) [5, 69, 100, 141, 187]. Although these reported associations were not statistically significant, they are in line with the not statistically significant higher lung cancer risk for vitamin D deficient participants observed in this investigation. Adjustment for other measures of smoking such as the duration or intensity of smoking were not performed, but as pointed out in previous studies that corrected for these variables, the influence of residual confounding is expected be minimal [5, 187]. Therefore, it can neither be confirmed nor rejected that vitamin D plays a role in lung cancer development. Given the low number of observational studies conducted and the potential mechanisms of vitamin D in lung tumorigenesis suggested by in vivo and in vitro studies [54], further investigations are warranted.

4.2.3 Colorectal cancer

Earlier epidemiologic studies had suggested an inverse association of 25(OH)D concentrations with colorectal cancer risk [63, 112, 122, 175, 189] even though few of the included studies, most of which were conducted in the US, individually showed statistically significant results. In line with these findings we observed non-significantly higher colorectal cancer incidence at low 25(OH)D concentrations. However, the magnitude of the association observed in our study was smaller than that of previous studies, which could be due to our population being of older age. These findings suggest that a possible protective role of vitamin D against colorectal cancer among older adults may be rather small. Nevertheless, a number of biological mechanisms by which vitamin D can induce cell

differentiation, cell cycle arrest and apoptosis in colorectal cancer tumors have been suggested [109]. Additionally, several studies have consistently suggested that vitamin D status may also be relevant for the survival of colorectal cancer patients [123].

4.2.4 Breast cancer

For breast cancer, a previous meta-analysis suggested the possibility of reverse causality since the associations of vitamin D with breast cancer risk were observed mainly in case-control studies with measurements of 25(OH)D after diagnosis [191]. Yet, an updated summary including only prospective studies still showed higher 25(OH)D concentrations measured at baseline to be associated with reduced breast cancer incidence during follow-up [183]. Unexpectedly, the present analyses showed an increased breast cancer risk with higher 25(OH)D concentrations. Still, similar trends have been observed in a number of epidemiologic studies [48, 56, 126]. Different findings between the present investigation and the latest meta-analysis could be explained by the different settings, study populations involved, and level of adjustment. Most of the studies included in the previous meta-analysis were nested case-control studies conducted in the US, with limited and heterogeneous adjustment for confounders. In contrast, the present analyses included cohort data from European older adult populations only, and employed consistent adjustment for the most important confounder variables common to all included studies. Nevertheless, it is also possible that the anticancer properties of vitamin D in the breast tissue may pertain more to the progression of the disease since various studies have observed increases in survival of breast cancer patients with high vitamin D status [123].

4.2.5 Prostate cancer

Previous epidemiologic studies on the association of 25(OH)D with prostate cancer risk have suggested either no association, increased risk with higher 25(OH)D concentrations [63, 66, 190], but also U-shaped associations, especially for high-grade disease [106]. In agreement with most single studies conducted hitherto, our data showed no evidence of an association. It has been hypothesized

that circulating $1,25(OH)_2D$ concentrations may be more relevant since the enzymatic conversion of 25(OH)D to $1,25(OH)_2D$ is impaired in prostate cancer cells [68]. In fact, an association of low $1,25(OH)_2D$ with aggressive prostate cancer risk has been suggested [66]. Measurements of $1,25(OH)_2D$ were not available in our study, and aggressive prostate cancer incidence could not be assessed. Given the findings from laboratory studies describing a link of vitamin D with prostate cancer [68], the possibility that the physiologically active vitamin D metabolite may be more relevant for aggressive prostate cancer deserves further study.

4.2.6 Lymphoma

Considerably less studies have assessed the risk of developing lymphoma according to 25(OH)D concentrations, overall showing no effect [69, 114, 121, 150]. The present data showed an increased lymphoma risk for vitamin D deficiency. In comparison to a larger pooled analysis [150], 25(OH)D concentrations were overall lower in the current study population, which could explain why an increased lymphoma risk at very low 25(OH)D concentrations could be discerned in the present investigation. Additionally, all lymphoid malignancies were combined due to the low number of cases for some of them. However, previous studies have observed variations in risk associated to high 25(OH)D concentrations for some of these malignancies, such as increased risk for multiple myeloma [69] or reduced risk for Non-Hodgkin's lymphoma [69, 114] and chronic lymphocytic leukemia [121]. These discrepancies in the findings for different lymphoid malignancies may deserve further study, particularly in older populations.

4.3 Analyses on the association of 25(OH)D-related vitamin D polymorphisms with total and site-specific cancer incidence in the ESTHER study

4.3.1 Total cancer

It has been previously shown that increasing 25(OH)D concentration may reduce the risk of total cancer incidence [193]. Whether this association is causal remains unknown. An association between 25(OH)D-associated SNPs and risk of total cancer would strengthen the causality of the association between 25(OH)D concentration and total cancer risk. To my best knowledge, only one other cohort study has investigated this association [95]. In the TROMSO study, polymorphisms in the GC, CYP2R1, NADSYN1/DHCR7, and CYP24A1 genes were genotyped in 2,924 colorectal cancer cases and 3,264 controls [95]. The investigators of this study measured two polymorphisms that were also genotyped in our study: rs3755967 (GC) and rs12794714 (CYP2R1). However, they did not assess risk of cancer for these two polymorphisms since they were highly correlated to other polymorphisms within the same genes that accounted for larger differences in 25(OH)D. The selected polymorphisms were instead rs2298850 (GC) and rs10741657 (CYP2R1) which were not associated with total cancer incidence. This finding is in line with the lack of association between the SNPs and cancer risk observed in our study. In addition, in the TROMSO study, polymorphisms in DHCR7 and CYP24A1, different from the ones genotyped in our study, were also not associated with total cancer incidence.

4.3.2 Lung cancer

Associations of SNPs and genotype score with lung cancer risk were observed here, although they did not retain statistical significance after correction for multiple testing. To my knowledge, this potential association has been assessed only in two study populations [95, 124]. In the TROMSO study, 25(OH)D-related polymorphisms have been related to lung cancer risk and overall no significant association was observed for neither 25(OH)D related polymorphisms nor the genotype score [95]. In a case-control study based in Thailand, those with the minor alleles for SNPs in the GC were at increased lung cancer risk [124]. These SNPs in GC (rs4588 and rs7041) were not assessed in our

study population, but may deserve further study in relation to lung cancer since they have been shown to affect the affinity of the vitamin D binding protein for the vitamin D metabolites [39].

4.3.3 Colorectal cancer

In a study comprising 2,001 colorectal cancer cases and 2,237 controls from Scotland, 25(OH)D related polymorphisms (located within GC, DHCR7, CYP2R1 and CYP24A1 genes) were unrelated to colorectal cancer risk [172]. Similarly, in the TROMSO study, genetic variants located within the same genes, were overall not significantly associated to colorectal cancer risk, although increased risk was observed for the homozygotes carrying the risk allele of the variant rs2298850 (GC) [95]. In a recently published case-control study based in Thailand, genetic variants in GC were associated with higher colorectal cancer risk [124]. As in most of the earlier studies, we did not observe any significant association between 25(OH)D related polymorphisms and colorectal cancer risk. This is in contrast to our expectations since high 25(OH)D concentrations have been linked to a reduction in colorectal cancer risk [122]. Further studies are need in particular with a focus on SNPs in the GC gene.

4.3.4 Breast cancer

Overall, SNPs and genotype score were not associated with breast cancer risk among participants of the ESTHER study. In literature, findings from studies assessing the relationship of 25(OH)D related polymorphisms and the risk of breast cancer are inconclusive. Three studies found CYP24A1 polymorphisms to be associated with breast cancer in case-control studies conducted in China (rs6091822, rs8124792, and rs6097809) [46], United States (rs34043203, rs2762934, rs1570669) [59] and Norway (rs6013897) [95]. On the other hand, two other investigations reported no association for CYP24A1 polymorphisms and breast cancer among postmenopausal women from the United States [125] or among Canadian women [9]. Regarding polymorphisms in the GC gene, rs7041 [9] and a haplotype of rs7041 and rs4588 22 were associated with breast cancer risk, whereas in three different

studies no association of GC polymorphisms and breast cancer risk was observed [46, 95, 125]. To my knowledge, no study observed a significant effect of polymorphisms in CYP2R1 and DHCR7 on breast cancer risk [46, 95]. Despite the large number and size of the studies that have been conducted for breast cancer, evidence remains inconclusive but suggests CYP24A1 and GC as possible relevant genes for breast cancer risk mediation.

4.3.5 Prostate cancer

An increased, although not statistically significant, risk of prostate cancer for those with higher number of risk alleles (i.e. with lower 25(OH)D concentration) was observed here, especially among those subjects with the CC genotype for rs11603330 (DHCR7). Several studies have assessed risk of prostate cancer according to polymorphisms in the CYP2R1 [95, 160], DHCR7 [95, 134], GC [6, 95, 99, 134, 160] and CYP24A1 [6, 21, 84, 88, 95, 134] genes, overall finding no association. In the largest of these studies, a decrease in overall prostate cancer risk was observed with the genotype of the variant rs10741657 (CYP2R1) associated with lower 25(OH)D concentration [134]. The genotype score in this study also suggested a protective effect of low 25(OH)D. The difference between the findings in this study and our data could be explained firstly by the different polymorphisms under analysis, and secondly by the difference in 25(OH)D concentrations, being lower in our study. A possible U-shaped dose-response of 25(OH)D and prostate cancer risk could also explain both opposite findings, with their data referring to the higher 25(OH)D concentrations, and our data representing the lower part of the 25(OH)D continuum. Future studies should assess associations with SNPs stratifying for low or high 25(OH)D levels.

4.4 Strengths and limitations

4.4.1 Systematic review and meta-analysis: circulating 25(OH)D concentration and total cancer incidence and mortality

A major strength is the application of advanced techniques of statistical analysis that allowed to summarize adjusted associations across studies and over the entire range of serum 25(OH)D values, despite the very heterogeneous categorization of 25(OH)D serum concentration in the individual studies. The hereby included meta-analysis comprised prospective studies in which 25(OH)D was assayed in stored blood samples collected years before the occurrence of cancer, therefore reverse causality is unlikely to be a major issue here. Nevertheless, associations seen in observational studies, as compared to RCTs, are subject to confounding which may limit causal inference. In particular, adjustment for important confounders varied between studies and, although this adjustment did not affect the overall estimate, the possibility of an unmeasured confounder spurring the association observed cannot be discarded.

This systematic review also has important limitations. Although we searched three databases, i.e., Ovid Medline, EMBASE, and ISI Web of Knowledge, and extensive checks for completeness by cross-referencing were employed, the possibility of having missed a relevant study cannot be excluded. Meta-analyses are limited by the data provided by the individual studies. Depending on the results reported, median, midpoints and mean 25(OH)D concentration of the study subgroups had to be used for pooling. As a result, estimates of risk may be less accurate than if individual-level data had been available. Also, different studies used different methods of measuring serum 25(OH)D (and one even used predicted rather than measured 25(OH)D concentration), which might have affected comparability of studies, given the well-known variation of results according to method of measurements [157]. However, meta-regression did not show that varying assay choices had an impact on between-study heterogeneity. Furthermore, despite the lack of indication of major publication bias in the formal evaluations employed, potential publication bias is impossible to be excluded completely, especially in the light of the low number of studies for cancer incidence.

Even though studies on total cancer incidence and mortality may be more relevant from a public health point of view and for prevention than cause specific cancer incidence and mortality, they are uninformative with respect to potential cancer site specific vitamin D effects. For example, previous studies have suggested clear inverse associations of vitamin D with breast [33, 63, 183] and colorectal cancer risk [63, 112, 122, 175, 189], but not with other cancers. The apparently stronger association with total cancer mortality among women than among men observed in the present meta-analysis may be determined to a large part by the strong association with breast cancer, the by far most common cancer among women and the lack of an association with prostate cancer that is common among men.

4.4.2 Analyses on the association of circulating 25(OH)D concentration with total and site-specific cancer incidence in the CHANCES consortium

This is the largest investigation with 25(OH)D measurements assessing total and site-specific cancer risk in a population of older adults. This meta-analysis with individual participant data also ranks among the largest investigations on the association of 25(OH)D with incidence of lung, colorectal, prostate, breast, and lymphoid cancers in older adults. Furthermore, the availability of individual participant data, harmonization of covariates and common multivariable models, permitted to minimize the heterogeneity between studies and avoid misclassification of exposure, outcome and covariates, a common issue in meta-analysis employing data reported by individual studies. In fact, the tests for heterogeneity were not statistically significant for all of the meta-analyses performed, thus suggesting consistency of the effects estimated by the different studies involved. Alternatively, it could also be that the test for heterogeneity was underpowered due to the low number of studies.

Several limitations also need to be addressed. First, although these analyses are among the largest on the topic, sample size may still have not been sufficient to detect a significant small effect of vitamin D on cancer development. Second, current smokers were excluded from the TROMSØ study because the assay employed resulted in smokers having 15-20% higher 25(OH)D than non-smokers which was not reproducible with other assays [75]. This could have attenuated the pooled effect estimates

observed since in the ESTHER data a stronger association with total cancer was observed among current smokers [141]. Third, data from the EPIC-Elderly study were not available for total and lung cancer incidence so that estimates for these two outcomes were based on two cohorts only. Fourth, 25(OH)D was measured only at baseline and thus it may not adequately represent a long term vitamin D status. Nevertheless, the correlations between the measurements at baseline and at follow-up are rather high [164]. Fifth, 25(OH)D was measured with immunoassay methods in all studies (although in the ESTHER study a standardization to the LC-MS/MS was performed), while LC-MS/MS is regarded as the most reliable but also the most costly method. Finally, the findings of this investigation are restricted to European older adults, while associations of 25(OH)D concentrations with cancer risk have often been observed in observational studies conducted among American younger adults.

4.4.3 Analyses on the association of 25(OH)D-related vitamin D polymorphisms with total and site-specific cancer incidence in the ESTHER study

Advantages include the population-based and prospective design of the study, the availability of validated cancer registry data and the standardized 25(OH)D measurement for all subjects. This is the first investigation with cohort design to assess the association of 25(OH)D related polymorphisms with total and site-specific cancer risk.

The main limitation of these analyses was that only four relevant vitamin D pathway SNPs were available, whereas GWAS have identified larger number of polymorphisms influencing 25(OH)D concentrations [7, 31, 184]. Nevertheless, our polymorphisms in GC and CYP2R1 have been previously reported to be in linkage disequilibrium with other variants within the same genes [95] and significantly associated with 25(OH)D, a finding that validates the representativeness of the findings for these SNPs. In the present analyses, linkage disequilibrium between polymorphisms in different genes was not observed, thus one could think that the effect (or lack of it) observed for each SNPs is independent of the effect of other SNPs. Including 25(OH)D concentration in the model did not alter the estimates. This suggests that the effect of these SNPs (if any) may be independent of 25(OH)D

concentrations. Little is understood about the functionality and biological relevance of SNPs in vitamin D pathway genes [39, 91]. Further research should aim to study the phenotype of the relevant enzymes with regards to genetic variation in the respective gene. This would help to distinguish whether the effect of these polymorphisms concerns only the vitamin D metabolism or whether it also involves other biological mechanisms such as those for the development of cancer. The fact that overall we did not find significant associations with the majority of cancer outcomes should not be surprising since that is the conclusion from most of the studies that have been conducted until now.

4.5 Conclusions

Previously, many epidemiological studies have suggested associations of circulating 25(OH)D concentrations, the biomarker of vitamin D status, with the incidence of cancer in many anatomical sites. The findings for the different types of cancer have shown large variation, being suggestive in some cases, and inconclusive for the rest. The strongest level of epidemiological evidence for an association of vitamin D status with cancer can be attributed to colorectal cancer, and to a lower extent to breast cancer. For other cancer sites the epidemiological literature has suggested no association or it is not conclusive, mainly due to the difficulty in achieving a sufficient statistical power. The biological effects of vitamin D on carcinogenesis disclosed in laboratory studies suggest that vitamin D could influence cancer development not only in those sites for which an association with vitamin D status seems more robust, but also in many other sites. Assessing, the overall impact of vitamin D on the development of cancer may be a more suitable way to assess the whole effect of vitamin D on cancer prevention. There have been only a few studies but their results have been inconclusive and not summarized in any way. In addition, no study has hitherto asked the question whether vitamin D status at older age is still relevant for the prevention of cancer.

The results of the systematic review and meta-analysis included in this doctoral dissertation provide suggesting evidence of the importance of a higher vitamin D status in reducing total cancer incidence and mortality: Disparities were observed between males and females which could be explained by the dissimilar findings observed in the literature for prostate and breast cancer, respectively. The findings of this systematic review and meta-analysis may be limited by the rather small number of studies, especially for total cancer incidence.

The findings from the above mentioned systematic review and meta-analysis could not be replicated in the CHANCES Consortium, since no statistically significant association of circulating 25(OH)D concentrations and total cancer incidence could be observed in the entire population or among relevant subgroups. The results of these analyses suggest that vitamin D status at older age may not be associated with the incidence of cancer, but this may also apply to younger adults because no effect modification by age could be appreciated. Most likely, our study was not sufficiently powered to

detect a probable small effect of vitamin D on reducing the risk of cancer. In addition, no significant risk reductions in the incidence of the major cancers (lung, colorectal, breast and prostate) was observed for higher circulating 25(OH)D concentrations, although a increase in lymphoma risk with lower vitamin D status was seen. Surprisingly, vitamin D status in the range of the recommendations issued by the IOM was associated with the lowest risk of total and breast cancer incidence.

Genetic polymorphisms located within or around vitamin D pathway genes involved in the synthesis of the provitamin D (DHCR7), vitamin D hydroxylation (CYP2R1), transport (GC), and degradation (CYP24A1), were associated with circulating concentrations of 25(OH)D in the ESTHER study. However, in the same study, the same polymorphisms were not consistently associated with total and site-specific cancer incidence, thus providing at best, little evidence that genetic variation in the vitamin D pathway may have an effect in cancer development.

A number of clinical trials on the effect of vitamin D on cancer are currently underway and they will probably clarify whether there is a potential role of vitamin D for cancer prevention. However, it should not be forgotten that many limitations affect the design of these trials and that they may not be easy to extrapolate to the general population due to the restricted inclusion criteria. Thus, it is also possible that RCTs are not going to bring the definitive answer. Epidemiological investigations on vitamin D and cancer risk therefore remain important in resolving this important issue. Larger consortia of epidemiological studies with vitamin D measurements and assessment of cancer incidence are desirable to investigate this subject. Last but not least, genetic polymorphisms associated with vitamin D status are also a great avenue of investigating a potential effect of vitamin D on carcinogenesis, but ideally these should also be studied within the context of consortia of large epidemiological studies due to the rather small effects for each given SNP. Also larger numbers of SNPs within each relevant gene, for instance using genotype scores or pathway analysis, should be considered in order to more comprehensively assess the impact of the different vitamin D pathway genes on the development of cancer.

5 SUMMARY

It is generally accepted and also well known that vitamin D plays an important role in bone homeostasis and contributes to an optimal bone health. Many ecological, epidemiological and clinical investigations have claimed that vitamin D may also be involved in cancer development, but overall there is still a lack of consensus for these claims. The epidemiological evidence is more consistent for an association of 25(OH)D concentrations, the best marker of vitamin D status, with colorectal and breast cancer risk. However, biological mechanisms of inhibition of cell proliferation and angiogenesis and induction of cell differentiation and apoptosis attributed to vitamin D may not only apply to the colorectal and breast cancer development. Only a few studies have assessed the association of vitamin D with total cancer risk. Their findings have been inconclusive and limited to selected study populations. An overall assessment of the association of vitamin D status with total cancer incidence in a more representative setting has been missing. Additionally, no investigation has assessed whether vitamin D status at old age may still be relevant with regards to cancer prevention. Genetic polymorphisms associated with 25(OH)D concentrations have also been suggested as a new marker of vitamin D status unaffected by environmental changes in sunlight exposure, diet and supplement intake. Studies assessing associations of these genetic polymorphisms with total cancer incidence are few and their results have been inconclusive.

This dissertation aimed to understand what role vitamin D plays on carcinogenesis from a global perspective looking at total cancer incidence, but also focusing on cancer incidence in major sites (lung, colorectal, breast and prostate), with a special focus as well on the elderly population. This was done in three stages.

In the first stage, a systematic review and meta-analysis of epidemiological studies assessing the association of circulating 25(OH)D concentrations with total cancer incidence and mortality was conducted. Higher, compared to lower, 25(OH)D concentrations were significantly associated with a reduction in total cancer incidence and mortality.

In the second stage, an analysis was performed of the association of circulating 25(OH)D concentrations with total and site-specific cancer incidence among older adults. No significant reductions in cancer risk were observed for higher 25(OH)D concentrations. Overall, increasing 25(OH)D concentrations at older age may not reduce cancer risk. On the other hand, vitamin D status in the range of the IOM's recommendations for vitamin D sufficiency (50 nmol/L) was associated with the lowest risk of total and breast cancer incidence.

In the third stage, genetic polymorphisms within or around genes of the vitamin D pathway (DHCR7, GC, CYP2R1 and CYP24A1) were significantly associated with 25(OH)D concentrations, but not significantly associated with total and site-specific cancer incidence. Genetic polymorphisms associated with 25(OH)D concentrations provide at best little evidence that vitamin D may be involved in cancer development.

To conclude, the research performed within the scope of this doctoral dissertation suggests that vitamin D may play a role (possibly small) in cancer development, although this could not be verified in the analyses conducted among older adults in the CHANCES Consortium and ESTHER study. Improving vitamin D status beyond the IOM's recommendation for vitamin D sufficiency (50 nmol/L) at older age may not bring any additional benefits in reducing cancer risk, but it may be nonetheless very relevant for maximizing bone health.

Future research should make big efforts towards bringing epidemiological data on vitamin D and cancer together in order to form large consortia where the influence of vitamin D status (be it as expressed as 25(OH)D concentrations or as genetic polymorphisms) on the global burden of cancer can be adequately studied. Lastly, ongoing randomized controlled trials, although with methodological limitations, will probably contribute in the future to clarify whether cancer can or cannot be partially prevented by improving vitamin D status.

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7 LIST OF OWN PUBLICATIONS

As a first author:

1) **Ordóñez-Mena JM**, Schöttker B, Fedirko V, Jenab M, Olsen A, Halkjær J, Kampman E, de Groot L, Jansen E, Bueno-de-Mesquita HB, Peeters PH, Siganos G, Wilsgaard T, Perna L, Holleczek B, Pettersson-Kymmer U, Orfanos P, Trichopoulou A, Boffetta P, Brenner H. Pre-diagnostic vitamin D concentrations and cancer risks in older individuals: an analysis of cohorts participating in the CHANCES consortium. Eur J Epidemiol [In Press]
2) **Ordóñez-Mena JM**, Brenner H. Vitamin D and cancer: an overview on epidemiological studies Adv Exp Med Biol 2014;810:17-32.
3) Maalmi H*, **Ordóñez-Mena JM***, Schöttker B, Brenner H. Serum 25-hydroxyvitamin D levels and survival in colorectal and breast cancer patients: systematic review and meta-analysis of prospective cohort studies. Eur J Cancer 2014;50:1510-21.
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5) **Ordóñez-Mena JM***, Schöttker B*, Haug U, Müller H, Köhrle J, Schomburg L, Holleczek B, Brenner H. Serum 25-hydroxyvitamin d and cancer risk in older adults: results from a large German prospective cohort study. Cancer Epidemiol Biomarkers Prev 2013;22:905-16.

***** Equal contributions as first author.

As a co-author:

1) Zhang Y, Schöttker B, **Ordóñez-Mena J**, Holleczek B, Yang R, Burwinkel B, Butterbach K, Brenner H. F2RL3 methylation, lung cancer incidence and mortality. Int J Cancer. 2015 Mar 26. doi: 10.1002/ijc.29537.
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M, Kee F, Bobak M, Trichopoulou A, Boffetta P, Brenner H; Consortium on Health and Ageing: Network of Cohorts in Europe and the United States. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. BMJ 2014;348:g3656.

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8 APPENDICES

APPENDIX A

Supplementary Table A.1 Search strategy and number of studies found in Ovid MEDLINE(R) from 1946 to 2012-10-22.

#	Searches	Results
1	Cholecalciferol.mp. or Cholecalciferol/	5,623
2	Calcifediol.mp. or Calcifediol/	2,780
3	Vitamin D/ or Vitamin D.mp.	43,367
4	Calcitriol/ or Calcitriol.mp.	16,542
5	25 Hydroxyvitamin D.mp.	5,837
6	25-OH-D.mp.	2,848
7	Hydroxycholecalciferols.mp. or Hydroxycholecalciferols/	3,615
8	25-Hydroxyvitamin D3 1-alpha-Hydroxylase.mp. or 25-Hydroxyvitamin D3 1-alpha-Hydroxylase/	844
9	1,25 dihydroxyvitamin D.mp.	3,694
10	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9	55,503
11	cancer.mp.	879,684
12	tumor.mp.	1,007,621
13	Neoplasm.mp. OR Neoplasms/	707,897
14	11 OR 12 OR 13	1,822,085
15	risk.mp. OR Risk/	1,321,135
16	Mortality/ OR mortality.mp.	418,687
17	incidence.mp. OR Incidence/	515,796
18	occurrence.mp.	202,746
19	15 OR 16 OR 17 OR 18	2,048,881
20	10 AND 14 AND 19	1,911

Supplementary Table A.2 Search strategy and number of studies found in EMBASE from 1980 to 2012-10-22.

#	Searches	Results
1	cholecalciferol.mp. OR cholecalciferol/	12,191
2	calcidiol.mp.	1,031
3	vitamin D/ OR vitamin D.mp.	63,952
4	calcitriol.mp. OR calcitriol/	24,409
5	25 hydroxyvitamin D.mp. OR 25 hydroxyvitamin D/	8,949
6	25-OH-D.mp.	4,055
7	hydroxycolecalciferol.mp. OR hydroxycolecalciferol/	1,581
8	25-Hydroxyvitamin D3 1-alpha-Hydroxylase.mp. OR calcidiol 1 monooxygenase/	649
9	1,25 dihydroxyvitamin D.mp.	3,260
10	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9	82,817
11	cancer.mp.	1,827,095
12	tumor.mp. OR tumor/	1,773,089
13	Neoplasm.mp. OR neoplasm/	284,962
14	11 OR 12 OR 13	2,853,880
15	risk.mp. OR risk/	1,874,038
16	mortality/ OR mortality.mp.	764,581
17	incidence/ OR incidence.mp.	655,227
18	occurrence.mp.	249,350
19	15 OR 16 OR 17 OR 18	2,926,700
20	10 AND 14 AND 19	5,761

211 duplicates were deleted.

Supplementary Table A.3 Search strategy and number of studies found in ISI database from 1956 to 2012-10-19.

#	Searches	Results
1	vitamin D	51,547
2	Cholecalciferol	1,608
3	Calcidiol	279
4	Calcitriol	4,962
5	25-hydroxyvitamin D	6,841
6	25-OH-D	2,958
7	Hydroxycholecalciferol	6
8	25-Hydroxyvitamin D3 1-alpha-Hydroxylase	6
9	1,25 dihydroxyvitamin D	11,023
10	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9	57,992
11	Neoplasms	107,528
12	Cancer	1,207,994
13	Tumor	1,014,697
14	11 OR 12 OR 13	1,833,721
15	Risk	1,396,554
16	Mortality	467,273
17	Incidence	399,369
18	Occurrence	301,006
19	15 OR 16 OR 17 OR 18	2,202,058
20	10 AND 14 AND 19	3,347

5 duplicates were deleted.

APPENDIX B

Studies excluded from this review because of:

(A) No original articles but editorials, comments, reviews

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(B) Only site-specific cancer or adenoma reported

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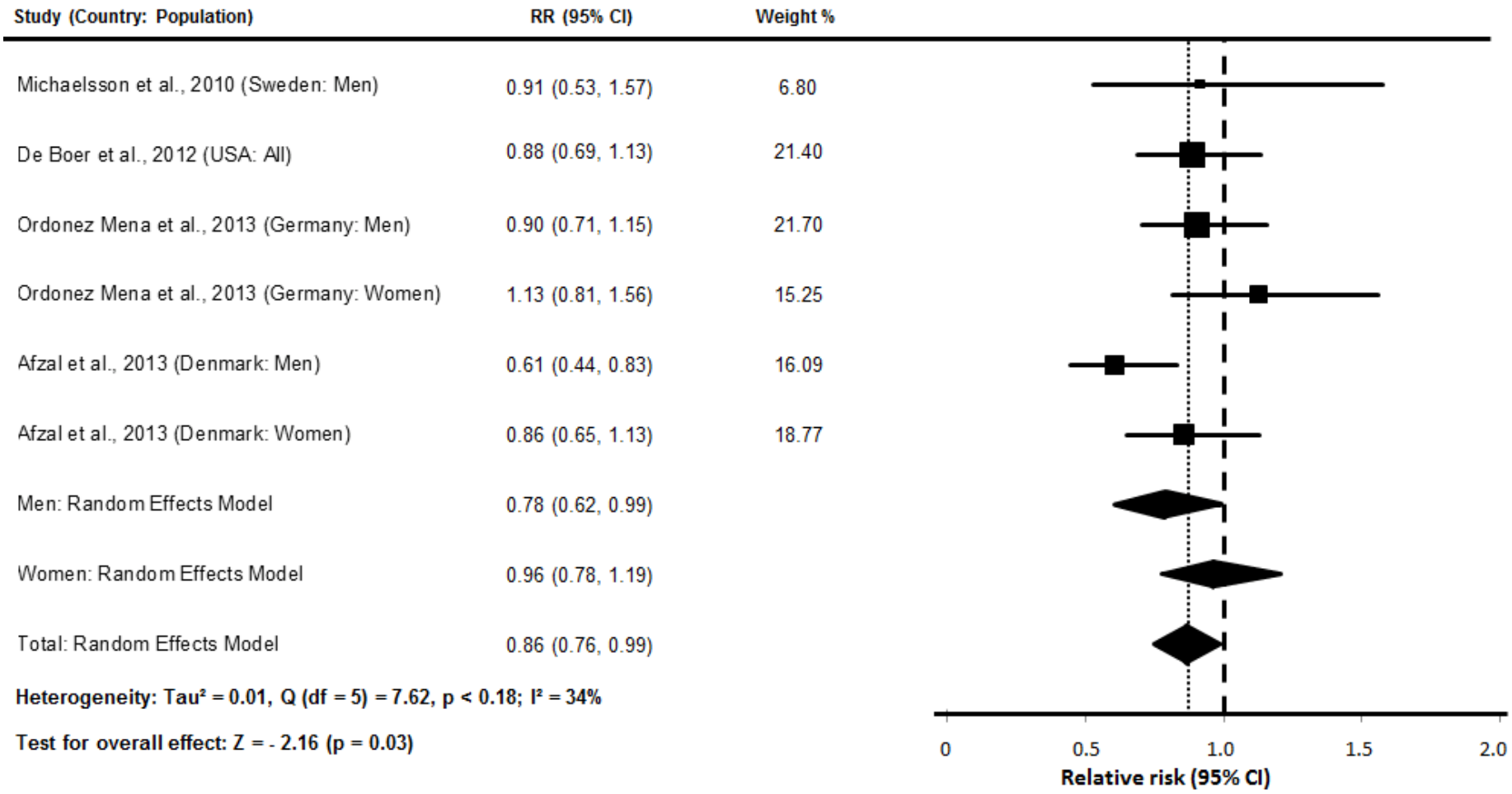
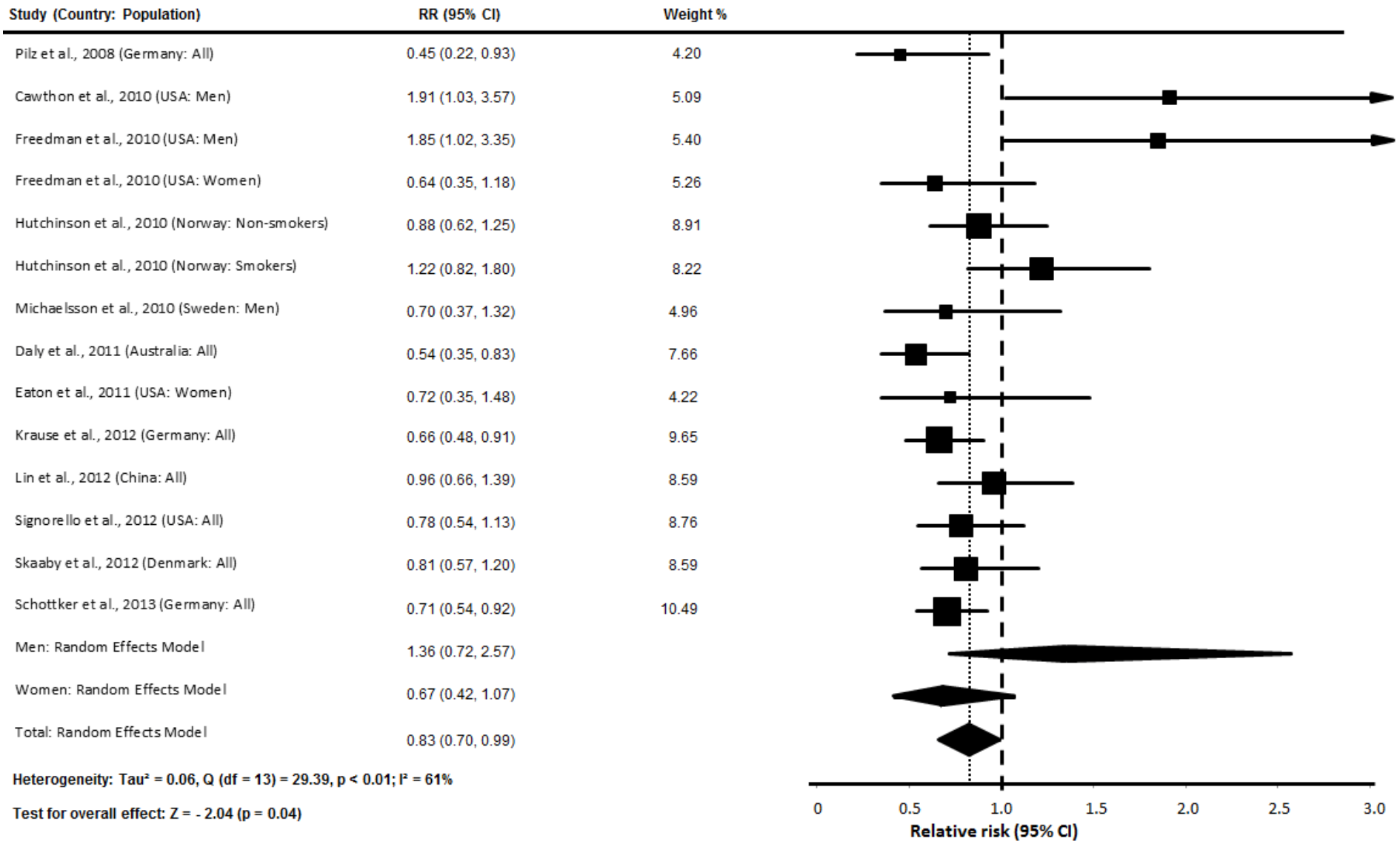
APPENDIX C



Supplementary Figure C.1 Meta-analysis of within-studies risk estimates for total cancer incidence in subjects with high versus low 25(OH)D concentrations.



Supplementary Figure C.2 Meta-analysis of within-studies risk estimates for total cancer mortality in subjects with high versus low 25(OH)D concentrations.

APPENDIX D

Supplementary Table 1 Cut-offs for season-specific 25(OH)D quintiles for each CHANCES cohort.

		25(OH)D quintile (nmol/L)				
Season	**Cohort**	**1**	**2**	**3**	**4**	**5**
Winter	EPIC-Elderly [a]	<37.2	<46.7	<54.5	<66.9	≥66.9
	ESTHER	<29.5	<36.3	<44.9	<59.1	≥59.1
	TROMSØ	<37.9	<47.1	<55.4	<65.4	≥65.4
Spring	EPIC-Elderly [a]	<34.2	<42.6	<52.2	<62.0	≥62.0
	ESTHER	<30.1	<36.3	<43.9	<56.3	>56.3
	TROMSØ	<43.4	<52.6	<60.5	<69.9	≥69.9
Summer	EPIC-Elderly [a]	<46.5	<56.3	<66.6	<81.2	≥81.2
	ESTHER	<38.3	<47.7	<58.1	<74.9	≥74.9
	TROMSØ	<51.1	<59.1	<68.5	<77.7	≥77.7
Autumn	EPIC-Elderly [a]	<43.9	<56.4	<67.5	<83.3	≥83.3
	ESTHER	<34.7	<44.9	<54.7	<72.2	≥72.2
	TROMSØ	<40.1	<47.7	<55.3	<65.6	≥65.6

[a] Cut-offs for the quintiles in the EPIC-Elderly study were obtained from the controls distribution of 25(OH)D.

Supplementary Table 2 Baseline characteristics for each CHANCES cohort across season-specific 25(OH)D quintiles.

	Study	**EPIC-Elderly** [a]						**ESTHER**						**TROMSØ**					
Characteristic	Quintile	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
25(OH)D, median (nmol/L)							**						**						**
Winter		28	42	50	60	76		30	33	40	51	71		35	47	57	68	83	
Spring		28	37	47	56	74		30	33	40	49	68		39	52	61	71	86	
Summer		39	54	60	74	109		32	43	53	65	90		48	59	68	80	96	
Autumn		38	50	62	76	101		30	40	50	62	88		37	48	57	68	82	
Age, median (years)		63	63	63	63	62	*	63	63	63	62	62	**	64	62	61	61	60	**
Women (%)		59	66	56	55	47		62	70	63	52	35	**	67	62	55	55	54	**
BMI, median (kg/m^2)		28	27	27	25	25	**	28	28	27	27	27	**	27	26	26	26	25	**
Education (%) [b]													*						**
Primary or less		53	45	55	54	42		74	74	74	75	69		61	54	50	50	42	
Primary to university		30	39	35	34	45		19	19	20	19	23		24	28	31	29	36	
University		17	14	8.4	12	13		4.4	4.3	4.3	4.6	5.7		14	18	19	20	22	
Vigorous physical activity (%) [b]		27	36	29	35	38	*	33	39	40	45	52	**	28	32	40	41	44	**
Smoking status (%) [b]													**						**
Never		43	46	47	54	37		48	56	51	48	43		51	50	46	42	38	
Former		26	30	32	27	37		25	25	30	36	42		49	50	54	57	61	
Current		29	22	21	19	26		23	16	16	14	13		Excluded					

[a] Results for matched controls combined.

[b] The sum of the percentages may not add up to 100% due to missing values. The total number of participants with missing values for the variables BMI, highest level of education, vigorous physical activity and smoking status was 55 (0.3%), 276 (1.8%), 463 (3.0%) and 277 (1.8%), respectively.

* p<0.01; ** p<0.0001 P-values for testing the statistical significance of differences in baseline characteristics among season-specific 25(OH)D quintiles.

Supplementary Table 3 Association of season-specific 25(OH)D quintiles with total and site-specific cancer incidence across CHANCES cohorts.[a,b]

Cancer site	EPIC-Elderly			ESTHER			TROMSØ			Summary		
25(OH)D quintile	cases / control	OR (95% CI)	w%	events / total	HR (95% CI)	w%	events / total	HR (95% CI)	w%	events / total	RR (95% CI) [c]	P heterogeneity
Total												
1			0	238/1817	0.98 (0.81; 1.19)	60	212/1170	0.80 (0.62; 1.04)	40	450/2987	0.91 (0.74; 1.10)	0.21
2			0	176/1726	**0.77 (0.62; 0.95)**	61	205/1059	0.87 (0.67; 1.13)	39	381/2785	**0.81 (0.69; 0.95)**	0.46
3		n.a.	0	212/1786	0.91 (0.75; 1.10)	66	165/949	0.79 (0.60; 1.03)	34	377/2735	0.86 (0.74; 1.01)	0.41
4			0	213/1805	0.90 (0.75; 1.09)	68	144/754	0.88 (0.67; 1.16)	32	357/2559	0.89 (0.76; 1.04)	0.88
5				243/1794	1.00 (Ref)		80/375	1.00 (Ref)		323/2169	1.00 (Ref)	
Lung												
1			0	39/1817	1.23 (0.73; 2.06)	73	18/1170	0.75 (0.32; 1.74)	27	57/2987	1.07 (0.69; 1.67)	0.32
2			0	17/1726	0.78 (0.42; 1.45)	65	18/1059	0.83 (0.36; 1.92)	35	35/2785	0.80 (0.48; 1.31)	0.91
3		n.a.	0	23/1786	0.96 (0.55; 1.68)	50	7/949	**0.34 (0.12; 0.95)**	50	30/2735	0.57 (0.21; 1.56)	**<0.0001**
4			0	27/1805	1.05 (0.61; 1.80)	63	7/754	0.44 (0.16; 1.22)	37	34/2559	0.76 (0.34; 1.72)	0.14
5				28/1794	1.00 (Ref)		8/375	1.00 (Ref)		36/2169	1.00 (Ref)	
Colorectal												
1	78/53	1.46 (0.81; 2.56)	32	31/1817	1.26 (0.73; 2.15)	36	51/1170	0.82 (0.46; 1.46)	32	157/3108	1.15 (0.82; 1.59)	0.36
2	57/51	1.04 (0.58; 1.88)	32	28/1726	1.23 (0.71; 2.14)	36	39/1059	0.74 (0.41; 1.33)	32	119/2893	0.99 (0.71; 1.38)	0.45
3	52/52	1.27 (0.69; 2.35)	33	32/1786	1.33 (0.79; 2.27)	36	22/949	**0.50 (0.26; 0.95)**	32	106/2839	0.96 (0.53; 1.76)	0.05
4	53/56	0.97 (0.55; 1.71)	32	35/1805	1.37 (0.82; 2.29)	39	33/754	0.95 (0.52; 1.74)	29	121/2668	1.11 (0.80; 1.52)	0.57
5	63/59	1.00 (Ref)		26/1794	1.00 (Ref)		16/375	1.00 (Ref)		105/2291	1.00 (Ref)	
Breast												
1	29/22	0.62 (0.23; 1.68)	16	32/1130	**0.58 (0.35; 0.99)**	58	26/781	0.75 (0.35; 1.64)	26	87/1962	**0.63 (0.42; 0.94)**	0.87
2	23/32	0.41 (0.16; 1.04)	18	33/1193	**0.54 (0.32; 0.90)**	59	15/657	0.51 (0.22; 1.18)	23	71/1905	**0.50 (0.35; 0.76)**	0.89
3	28/29	0.42 (0.16; 1.13)	17	41/1116	0.71 (0.43; 1.17)	55	26/520	1.11 (0.52; 2.38)	28	95/1693	0.73 (0.47; 1.13)	0.31
4	23/26	0.53 (0.21; 1.33)	20	28/928	0.59 (0.35; 1.01)	58	13/411	0.72 (0.31; 1.68)	23	64/1388	**0.60 (0.40; 0.91)**	0.89
5	23/17	1.00 (Ref)		29/623	1.00 (Ref)		9/202	1.00 (Ref)		61/865	1.00 (Ref)	
Prostate												
1	11/10	1.08 (0.18; 6.54)	4	32/687	0.89 (0.57; 1.38)	68	20/389	0.65 (0.33; 1.28)	28	63/1097	0.82 (0.57; 1.18)	0.71
2	11/7	0.65 (0.14; 2.97)	6	26/533	0.87 (0.54; 1.40)	60	34/402	1.03 (0.55; 1.93)	34	71/953	0.91 (0.63; 1.31)	0.83
3	16/8	0.90 (0.16; 5.18)	4	30/670	0.72 (0.45; 1.15)	61	35/429	1.04 (0.56; 1.93)	35	81/1123	0.82 (0.57; 1.19)	0.64
4	17/14	0.87 (0.14; 5.36)	4	34/877	0.72 (0.47; 1.10)	67	29/343	1.08 (0.57; 2.05)	30	80/1251	0.81 (0.58; 1.15)	0.57
5	16/6	1.00 (Ref)		67/1171	1.00 (Ref)		14/173	1.00 (Ref)		97/1366	1.00 (Ref)	
Lymphoma												
1	50/41	1.66 (0.77; 3.56)	47	14/1817	1.63 (0.66; 4.06)	34	8/1170	0.60 (0.18; 2.03)	19	72/3078	1.35 (0.78; 2.35)	0.34
2	35/33	1.42 (0.66; 3.08)	47	11/1726	1.51 (0.59; 3.85)	32	12/1059	1.03 (0.33; 3.24)	21	58/2853	1.35 (0.82; 2.30)	0.87
3	29/37	1.06 (0.48; 2.36)	44	13/1786	1.74 (0.71; 4.27)	35	12/949	1.12 (0.36; 3.48)	22	54/2801	1.27 (0.75; 2.16)	0.70
4	31/30	1.11 (0.53; 2.32)	51	9/1805	1.14 (0.44; 2.96)	30	7/754	0.84 (0.25; 2.89)	18	47/2620	1.06 (0.63; 1.80)	0.92
5	36/43	1.00 (Ref)		8/1794	1.00 (Ref)		4/375	1.00 (Ref)		48/2248	1.00 (Ref)	

[a] Risk estimates correspond to those derived from the multivariate model 2 which was adjusted for sex, age, highest level of education, smoking status, BMI and vigorous physical activity.

[b] HRs were calculated for ESTHER and TROMSØ cohorts whereas for the EPIC-Elderly study ORs were calculated due to the nested-case control design. In the EPIC-Elderly study, circulating 25(OH)D serum levels were only measured in colorectal, breast, prostate cancer and lymphoma cases and matched controls.

[c] In a conservative approach, in order to allow for the variation of true effects between studies, pooled RRs were calculated with meta-analyses using random-effects models.

9 CURRICULUM VITAE

GENERAL INFORMATION

Last name:	Ordóñez Mena
First name:	José Manuel
Gender:	Male
Date of birth:	07.09.1985
Place of birth:	Sevilla
Nationality:	Spanish

EDUCATION

2012	Erasmus Internship	**Epidemiology and Public Health** Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Germany.
2010-2012	MSc	**Nutrition and Health, specialization in Epidemiology and Public Health** Wageningen University, Wageningen, the Netherlands. MSc Thesis on "dietary patterns and all-cause mortality in European older adults participating in the SENECA Study".
2008-2009	Erasmus Exchange	**Physical Education & Health Promotion** University of Physical Education, Semmelweis University, Budapest, Hungary.
2004-2010	*Licenciatura*	**Physical Activity and Sports Sciences** Sports Faculty, Pablo de Olavide University, Sevilla, Spain.

WORKING EXPERIENCE

2012-currently	**Epidemiology** Network Aging Research (NAR), Heidelberg University. Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany.
2013-currently	**Peer reviewer** Cancer Epidemiology, Biomarkers & Prevention, Journal of Internal Medicine, Experimental Gerontology, Journal of the American Geriatrics Society, Nutrition Research

SCIENTIFIC CONFERENCES AND MEETINGS ATTENDED

2015, Delft, Netherlands	18th Vitamin D Workshop
2014, Seattle, United States	47th Annual Meeting of the Society of Epidemiological Research (SER)
2014, Chicago, United States	17th Vitamin D Workshop
2014, Krefeld, Germany	5th Symposium Vitamin D & Analogs in Cancer Prevention and Therapy
2013, Granada, Spain	20th International Congress of Nutrition

LANGUAGES Spanish (native), English (proficiency), German (upper intermediate)

OTHER Member of the PhD Council 2015

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