The Role of Vitamin D and UVB on the Risk and Survival of Oesophageal and Gastric Cancer

A dissertation submitted to the University of Dublin, Trinity College, for the Degree of Doctor of Philosophy in the School of Medicine

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Declaration

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Summary

In recent years there has been a resurgence in vitamin D research. These studies have used numerous methods to estimate vitamin D, such as 25-hydroxyvitamin D (25(OH)D) measurement, UV dose estimation or dietary estimates from food questionnaires. A large number of these studies have found a beneficial relationship between vitamin D and the risk and survival of many diseases and conditions, such as osteoporosis, cardiovascular diseases and cancer [1-6]. There has been some strong evidence supporting an association between vitamin D and the decreased risk and improved survival of various cancers, such as prostate, breast and colorectal cancers [5, 7]. However, the relationship between vitamin D and oesophageal and gastric cancer occurrence and mortality has been scarce and mixed [6, 8-10]; only a handful of studies have been carried out to date. Different study designs, confounder adjustment and follow-up times from these studies lead to significant differences between studies. This may be one reason why there has been no consistent relationship found.

Additionally, the studies which have been undertaken, suffer from a number of limitations such as small sample sizes, study design flaws or not adequately accounting for the various sources of vitamin D, for example, supplementation use. Moreover, as each published study used different vitamin D estimations, comparisons between these are difficult. Therefore, there is a need to explore this relationship further.

This thesis aimed to explore the relationship between vitamin D and oesophageal or gastric cancer risk and survival. It utilised vitamin D concentration in the circulation and developed a method of using ambient UVB measurements along with personal characteristics to capture long term “average” vitamin D status of individuals.

This thesis used detailed UVB doses which were restricted only to wavelengths which can induce synthesis of vitamin D (280-315 nm) and adjusted for ozone level, altitude and cloud cover, with the best temporal and spatial resolution to date. When examining daily UVB doses in Ireland and the UK, strong variations in doses were observed between different latitudes, longitudes, and seasons, despite the small latitude and longitude differential which exists within each of the countries. This has been broadly explored previously [11], however, this study was the first to carry out such detailed analysis within the two countries.

Following exploration of UVB doses between countries, this thesis developed a number of different and simple vitamin D estimates, including: individually calculated D-UVB estimates and a vitamin D score estimates. The D-UVB dose calculated was able to account for both the accumulation and diminution of vitamin D in the body to mimic circulating 25(OH)D status. A vitamin D scoring system was also created. This incorporated UVB information as well as

supplement use, oily fish consumption and sun exposure (as available). Both of these vitamin D estimates were found to be strongly associated with 25(OH)D concentrations and improved the prediction of 25(OH)D deficiency and sufficiency in a large Irish cohort.

These vitamin D estimates were then used, along with 25(OH)D concentrations to examine the relationship between vitamin D and the risk and survival of oesophageal and gastric cancer. An inverse relationship between vitamin D scores, D-UVB doses and the risk of oesophageal and gastric cancer occurrence was found but the relationship with survival was less clear. No associations were observed for mortality in oesophageal cancer, or when oesophageal and gastric cancers were combined as upper gastrointestinal cancers. However, this study may have been under-powdered to detect any associations for mortality.

These findings contributed to the sparse information which is currently available on the topic of vitamin D and oesophageal and gastric cancer risk and survival, and highlight the importance of cancer specific and subtype specific analysis. Furthermore, this study explored a number of different vitamin D estimates and describes why using multiple estimates can offer a broader and more comprehensive view on the topic of vitamin D and slowly developing health outcomes.

Overall, this thesis demonstrated the importance of detailed UVB measurements, the contribution of UVB-induced skin synthesis to vitamin D status, and the potential for its use in exploring the relationship between vitamin D and cancer incidence and survival. Using these UVB doses, along with personal vitamin D related variables, a reduced risk of upper gastrointestinal cancers was found, however, no association between vitamin D and the survival of upper gastrointestinal cancers was observed. Further research is needed in a larger cohort of individuals using a comprehensive set of vitamin D estimates, in order to fully explore the relationship between vitamin D and the risk and survival of oesophageal and gastric cancers.

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Abbreviations

1,25(OH)D	1-25-dihydroxyvitaminD
25(OH)D	25-hydroxyvitamin D
95% CI	95% confidence Interval
AIC	Akaike information criterion
AUC	Area under the curve
BCC	Basal cell carcinoma
BIC	Bayesian information criterion
BMI	Body mass index
CI	Confidence Interval
cw-D-UVB	Cumulative and weighted vitamin D UVB
DF	Degrees of Freedom
DNA	deoxyribonucleic acid
D-UVB	UVB at wavelengths with the ability to synthesis vitamin D
DXA	Dual energy x-ray absorptiometry
E	East
FGF23	Fibroblast growth factor 23
GJAC	Gastric junction adenocarcinoma
HBP	High blood pressure
HPLC	High-performance liquid chromatography
HR	Hazard Ratio
HSE	Health, Safety and Environment
IBD	Inflammatory Bowel Disease
IQR	Interquartile range
IU	International units
LC-MS/MS	liquid chromatography tandem mass spectrometry
MCD	Monthly cumulative dose of UVB
MDDM	Mean daily UVB dose for each month
MEDDM	Median daily UVB dose for each month
MEDDS	Median daily UVB dose per season
mJ/cm^2	Mill joules per square centimetre
MS	Multiple Sclerosis
MSG	Meteostat second generation
N	North
NHS	National Health Service
nm	Nano Metres
nmol/L	Nanomoles/litre
OEAC	Oesophageal adenocarcinoma
OESCC	Oesophageal squamous cell carcinoma
OR	Odds ratio
P21	CDK-interacting protein 1
PTH	Parathyroid Hormone
RCT	Randomised controlled trials
RNA	Ribonucleic acid
ROC	Receiver operator curve

RR	Relative risk
RXR	Retinoid X receptor
S	South
SCC	Squamous cell carcinoma
SD	Standard Deviation
SE	Standard Error
SNPs	Single nucleotide polymorphisms
T1	Time point 1
T2	Time point 2
T3	Time point 3
T4	Time point 4
TEMIS	Tropospheric Emission Monitoring Internet Service
Th1	T-helper cells 1
Th2	T-helper cells 2
TUDA	Trinity, University of Ulster and Department of Agriculture
UK	United Kingdom
UV	Ultra violet
VDR	Vitamin D receptor
VDRE	Vitamin D response elements
VDscore1	Vitamin D scoring calculation method 1
VDscore2	Vitamin D scoring calculation method 2
VDscore3	Vitamin D scoring calculation method 3
VDscore4	Vitamin D scoring calculation method 4
W	West
W.H.O.	World Health Organisation

1. Introduction

1.1. Outline of Topic

1.1.1. Vitamin D

Vitamin D is a fat-soluble vitamin, however, it is often considered a pro-hormone rather than a true vitamin because of its actions in the human body [12]. A pro-hormone is an inactivated hormone which can initiate signalling cascades in the body after it has been activated. Vitamin D can be obtained from two sources; either it is synthetized in the skin following exposure to UVB radiation from sunlight, or it is absorbed from dietary sources (food and supplements). It has two forms, cholecalciferol and ergocalciferol. Vitamin D_3 (cholecalciferol) is mainly produced in the skin following exposure to solar ultraviolet (UV) radiation. It is also present in small amounts in a limited number of animal food sources, most prominently in oily fish, red meat and eggs. Vitamin D_2 (ergocalciferol) on the other hand is primarily found in plant sources, such as mushrooms [13]. UV-induced synthesis in skin remains the most important source of Vitamin D for the majority of people. However, this UV-vitamin D relationship depends on the strength of UV radiation and on the length of exposure.

Currently, when carrying out observational studies, the best method of estimating vitamin D status at a point in time is the measurement of 25-hydroxyvitamin D (25(OH)D) concentration in serum. Vitamin D deficiency is often hard to define due to differing 'cut-off' levels proposed. Some studies outline deficiency as levels <25 Nanomoles/litre (nmol/L), others at <50 nmol/L; similarly, sufficiency of vitamin D can either be classed as >50 nmol/L or >75 nmol/L [8, 14, 15]. The Institute of Medicine has recently suggested that <30 nmol/L defines deficiency, 30-50 nmol/L suggests risk of deficiency while those with levels >50 nmol/L are sufficient [16].

Vitamin D deficiency has been shown to be a prevalent issue, especially in older individuals [17-19]. A recent European wide study found 13% of their total cohort (n=55,844) were deficient in vitamin D, as they had less than 30 nmol/L on average throughout the year [20]. In this study, 46% of the population examined in Ireland and 55% in the UK had insufficient vitamin D (<50 nmol/L); moreover, 12.4% and 22% of the cohorts respectively, were deficient (<30 nmol/L) [20]. A much larger percentage were vitamin D deficient throughout the winter months, with almost 20% of the Irish population, and over 30% of the UK population having 25(OH)D of <30 nmol/L from November to March. This is due to the well-known seasonality of vitamin D. As vitamin D synthesis occurs mainly through the action of solar UVB, seasonal fluctuations in 25(OH)D concentrations occur naturally throughout the year. Higher 25(OH)D concentrations

are normally observed following the summer months, when UVB doses are at their highest, with a decline in vitamin D synthesis, and therefore lower 25(OH)D concentration in the winter and spring months. However, even during the summer months in Ireland and the UK vitamin D deficiency is still prevalent [20]. Due to the high northerly location of these countries, as well as the lack of consistent sunshine due to cloud cover, the availability and strength of UVB does not enable enough vitamin D to allow sufficiency throughout the year, for most individuals [11].

1.1.2. Vitamin D Synthesis in the Skin and Metabolism

Vitamin D synthesis by UV radiation is initiated when UV photons at wavelengths of 280-315 nm are absorbed by 7-dehydrocholesterol in the epidermal layer of the skin. Photolysis of 7-dehydrocholesterol occurs to form an inactive pre-vitamin D. As this inactive pre-vitamin D is unstable, an internal electron shift occurs, moving an electron from C9 to C10 positions. Spontaneous isomerisation subsequently occurs to form an inactive vitamin D [21-23]. This is hydroxylated in the liver by a family of cytochrome P450 enzymes, such as CYP2R1, CYP2D11 and CYP2D25 to form 25(OH)D. 25(OH)D is the main storage form of the vitamin [24]. Further hydroxylation occurs in tissues locally or kidneys by CYP27B1 and forms 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of vitamin D [23]. This hydroxylation step is highly controlled through the regulation of CYP27B1 by a number of hormones, including parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) [23]. PTH activates CYP27B1 and increases $1,25(OH)_2D$ concentration. However increased calcium in the blood supresses PTH activity thereby regulating CYP27B1 activity and reducing $1,25(OH)_2D$ availability. In conjunction with this, FGF23 can inhibit CYP27B1: this can be achieved through increased phosphate and calcium levels. $1,25(OH)_2D$ itself can also regulate CYP27B1 though activation of FGF23, suppression of PTH and also induction of $1,25(OH)_2D$ 24-hydroxylase CYP24A1. This catabolizes $1,25(OH)_2D$ into $1,24,25(OH)_3D3$ and calcitroic acid, to reduce the amount of active vitamin D present in circulation [23, 25] (**Figure 1.1**).

$1,25(OH)_2D$ is the ligand for the vitamin D receptor (VDR). VDR is a nuclear receptor which acts as a transcriptional regulator. Once the ligand ($1,25(OH)_2D$) is bound to VDR, two important functions can occur. One side of the complex is capable of binding with retinoid X receptor (RXR), while the other side is necessary for the recruitment of co-regulators which facilitate gene modulation [26]. RXR is important for deoxyribonucleic acid (DNA) binding, a RXR-VDR heterodimer complex forms and can translocate to the nucleus where it binds to enhancer elements known as vitamin D response elements (VDRE) [26, 27]. These enhancers are short sections of DNA which can be bound to proteins, in this case VDR, and aid in the transcription

of genes. The RXR-VDR-VDRE complex, along with certain cofactors, can next bind ribose nucleic acid (RNA) polymerase II. This can then induce transcription of genes which in turn exert effects on the body, for example the transcription of calcium binding protein, osteocalcin and CDK-interacting protein 1 (P21), a cyclin-dependent kinase inhibitor [28, 29]. Importantly, there is also evidence to suggest that VDR complex is responsible for the activation or repression of a number of proto-oncogenes and tumour-suppressor genes [29-31]

1.1.3. Vitamin D Receptor (VDR)

Once 1,25$(OH)_2$D has bound to the VDR, the complex can have an effect on transcription of important genes. There is some evidence to suggest different polymorphisms in the *VDR* gene (and consequentially variations in the VDR protein) can modify the activity of the vitamin D-VDR complex [32]. For instance, the A-allele of rs11568820 can bind to the DNA more efficiently than the G-allele and therefore can increase transcriptional activity of VDR [33] and rs10735810 has been shown to affect the translational start site of VDR [34]. Therefore, *VDR* polymorphisms can potentially affect and modify associations found for 25(OH)D and various health outcomes [35-38]. For example, some polymorphisms in *VDR* gene have been linked to risk of cancers, including prostate [39], breast [40], skin and colorectal [41, 42], as well as other diseases, such as, diabetes [38] and multiple sclerosis [43]. Furthermore, high VDR expression has for example been linked to increased survival in prostate and breast cancers [44-46].

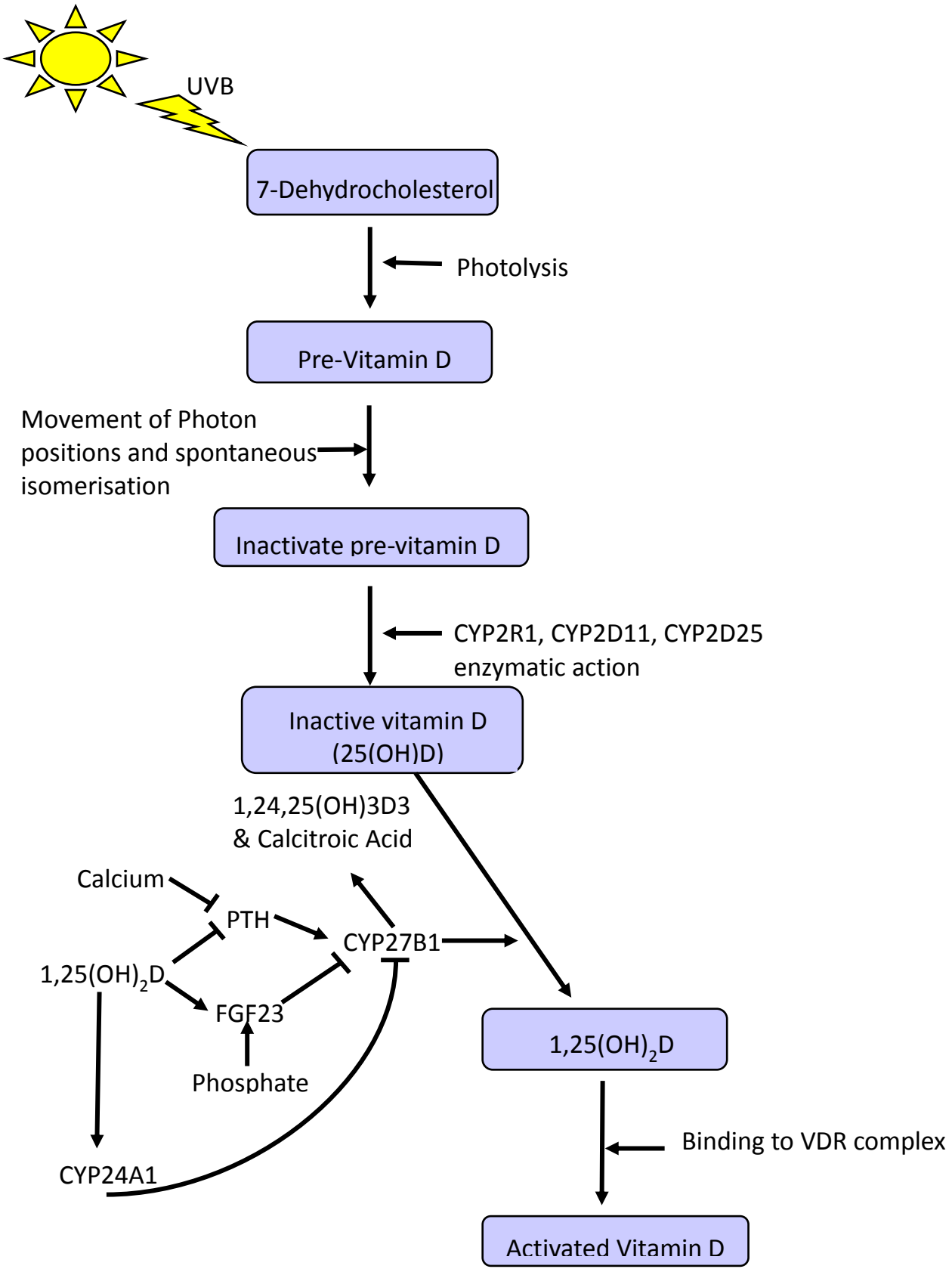


Figure 1.1: Metabolism and pathways of Vitamin D

1.1.4. UVB

UV light is electromagnetic radiation with wavelengths of 10-400 nanometres (nm). This is made up of UVA, UVB and UVC [47]. UVC, which makes up the largest part of the spectrum, has the shortest wavelengths (10-280 nm) and is the most damaging for humans. However, it is blocked by the ozone layer, water vapour and other atmospheric components and does not reach the earth's surface. The earth's atmosphere can also absorb some UVA (315-400 nm) and UVB (280-315 nm), although most can still permeate through. UVA accounts for 95% of radiation reaching earth. UVA is capable of penetrating the deep skin layers such as the dermis. It is considered detrimental for health, being mostly responsible for skin aging, and tanning [48]. Artificial sources of UVA also exist, primarily in sun beds [49]. UVB on the other hand, accounts for 5% of UV reaching the earth and can only penetrate as far as the superficial layers of skin [48]. As UVB effects the top most layers of skin it is also responsible for tanning and burning along with the wrinkling and aging of the skin, if carelessly over-exposed to it. It is also responsible for vitamin D production.

UVB intensity varies greatly depending on latitude, altitude, time of day, season, cloud cover and ozone column [50, 51]. For example, about half of the total UVB radiation can be blocked by heavy cloud cover when compared to clear skies [52]. In addition, UVB varies considerably throughout the day and is at its strongest at midday. For example, around 20-30% of total daily UVB radiation is received between 11am-1pm [52]. UVB also increases with altitude; with every 1 km increase in altitude resulting in a 6% increase in solar UVB flux [52]. Additionally, UVB dramatically decreases with increasing latitude or ozone [51]. As latitude increases, the angle at which UVB rays reach the earth's surface increases. This means the pathway from the Sun to the Earth is longer at higher latitudes than at the equator. This longer distance decreases the proportion of UVB reaching earth, as it has longer to travel, this also results in a longer path through the ozone layer to reach earth, which strongly affects the dose of UVB, as it gets degraded by ozone. Additionally, an increase in cloud cover and air pollution can block much of the UVB from reaching the earth's surface [50]. It has been considered that at latitudes above 45° the dose and intensity of UVB are insufficient to enable synthesis of sufficient amounts of vitamin D for most of the year [51]. It has also been noted that vitamin D in the body has a half-life of around four to six weeks, so for high latitude countries, exposure to adequate doses of UVB in the summer is unlikely to sustain an individual throughout the winter months [53]. Accurate measurements of UVB are difficult to obtain: while latitude and altitude are fixed and the effects of time of year/day can be modelled, the erratic nature of ozone and cloud cover precludes accurate estimation in most cases.

UVB is the most important natural source of vitamin D, however, it is very difficult to measure the UVB dose an individual receives to determine how much UVB contributes to an individual's 25(OH)D concentration. [50]. The doses of UVB absorbed by individuals can differ dramatically from person to person. Sun enjoyment, physical activity, and religious or cultural clothing practices can dramatically affect the doses of UVB received and absorbed.

Furthermore, the amount of 25(OH)D synthesized by individuals can also differ considerably, even with the same ambient UVB dose. This is due to a number of genetic and lifestyle factors which contribute to the rate of vitamin D synthesis by UVB; such as skin pigmentation, age, and sun screen use. Older individuals, those with darker skin, and those who use sun screen often have a decreased ability for cutaneous vitamin D synthesis [22, 54, 55]. It is almost impossible to determine doses of UVB received by free-living individuals due to the above reasons.

1.1.5. Exposure to UVB

UVB is important for vitamin D production, however, UV radiation has also been linked to adverse effects, most notably skin aging and skin cancer; and this is why UV radiation has been classed as a human carcinogen [44, 45]. UVA and UVB penetrate the epidermis and superficial layers of the skin, where the majority of skin cancers originate and it has been shown that both UVA and UVB radiation promote the development of skin cancer which most commonly occurs in fair skinned individuals [56, 57].

Skin cancer can be split into two main types; melanoma and non-melanoma. Non-melanoma can be further subdivided into basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) and it is these which are the most common types of skin cancer [58]. These cancers occur most regularly in areas of the skin which are frequently exposed to sunlight, such as the arms, neck and face [59]. It has been observed in numerous epidemiological studies that there is an increased risk of skin cancer development in those individuals who are exposed to high intensity UV [60-63].

Similarly, UV plays a considerable role in the development of melanoma [64]. Mortality rates for melanoma cancer are much higher than those of non-melanoma cancer. Along with the epidemiological evidence, biological changes induced by UV, such as DNA damage have been reported. UV radiation can cause the formation of cyclobutane pyrimidine dimers; these are formed by cytosine and thymine and can interfere with DNA replication and therefore lead to carcinogenic mutations [65]. This further supports to the theory that UV contributes to the malignant process leading to skin cancer [66].

However, it has also been observed that occupational exposure to sunlight can reduce melanoma risk [67, 68], and lower 25(OH)D has been associated with thicker tumours and a poorer prognosis in those who developed melanoma [69]. Additionally, most melanomas occur in areas of the body not normally exposed to the sun (lower legs, back, soles of feet or nailbeds) [48, 66, 70]. However, these are places which could be exposed to extra-high intensity UV radiation during holidays and prolonged time in the sun while sun bathing. This is why controlled, smart and personalised exposure to UVB may be of paramount importance. Regular exposure for short periods of time when UVB is at mild but sufficient intensity can allow accumulation of sufficient vitamin D without an additional risk of disease.

In stark contrast to what has been observed in skin cancers, UVB exposure, mainly through the action of vitamin D, has been shown to decrease risk and benefit survival of other cancer types [7, 71]. Again, this supports the argument of the importance of controlled exposure to UVB, as deficiency in vitamin D, from too little sun exposure could be detrimental in the development and progression of some cancer types. Exposure to sunshine, especially in countries where consistent sunshine is unavailable, is often confined to holidays in areas with higher UV radiation. During this period, individuals “soak up the sun” to an unhealthy degree, spending long periods outdoors with large exposure to high-intensity UV radiation. It is obvious that what is necessary is personal awareness of a need to expose oneself to sunshine in a sensible way. Like most things in life, quantity of a substance, rather than the substance itself, is an important factor, and one which is often ignored for UV exposure.

1.1.6. Skeletal Effects

Vitamin D has been shown to have many effects on the body. The link between vitamin D and skeletal effects was established and first described in 1822 by Sniadecki [28]. This paper concluded that the difference in incidences of rickets between urban and rural children was due to lack of sunlight exposure experienced by urban children. This theory was further backed by Palm *et al.* who noted the same trend in industrialized Britain [72]. The mechanism behind this relationship involves vitamin D initiating the absorption of calcium and phosphate from the intestines, which can then build and maintain bone. In recent times, there have been numerous randomised controlled trial (RCTs) published outlining the benefit of vitamin D in the prevention of bone disorders and conditions [1, 2]. Vitamin D’s role in calcium absorption and bone strength is still its most well-known and researched role.

1.1.7. Non-skeletal effects

There have been a number of diseases and conditions which have also been linked with the beneficial effects of vitamin D, which are unrelated to the traditional role of vitamin D and skeletal effects; below is a brief outline.

1.1.7.1. Cardiovascular diseases

Several longitudinal studies have found that high 25(OH)D concentrations are associated with a reduced risk of cardiovascular events. For example, Wang *et al.* found a 15% reduced risk of a cardiovascular event when those with >37 nmol/L 25(OH)D were compared to those with <37 nmol/L (hazard ratio (HR)=2.04, 95% confidence interval (95%CI): 1.42-2.94) [3]. Similarly, Giovannucci *et al.* found an increased risk of myocardial infarction in vitamin D deficient men (<37 nmol/L) vs sufficient groups (>75 nmol/L), (relative risk (RR)=2.42, 95%CI: 1.53-3.84) [73]. Furthermore, in a RCT where subjects were exposed to either UVA or UVB light, it was found that those who had been exposed to UVB had reduced blood pressure after six weeks [74] with other studies finding similar results [75, 76]. However, some RCTs have found no association [77, 78], though some may argue that the level of vitamin D supplementation prescribed was low.

1.1.7.2. Metabolic diseases

There has been some research suggesting a link between diabetes or other metabolic conditions and vitamin D. Hyppönen *et al.* carried out a birth cohort study, in which infants were followed for the first year of life and their consumption and frequency of vitamin D received was recorded. This study noted a decreased risk of diabetes among those with who were regularly taking supplements compared to those who were not (RR=0.12, 95%CI: 0.03-0.47). Furthermore, this study also observed an inverse dose-response trend when comparing doses of supplementation; with those taking recommended or high doses having a reduced risk of diabetes [4]. Similarly, a large cross-sectional study found an inverse relationship between metabolic syndrome prevalence and vitamin D concentration [79]. However, in a meta-analysis of studies examining 25(OH)D concentration and type two diabetes, a significant inverse association was only found after the exclusion of non-Hispanic blacks (odds ratio (OR)=0.36, 95%CI: 0.16-0.80) [80]. A number of RCTs have also found an association between vitamin D and a number of insulin sensitivity and secretion biomarkers [81, 82], however others have only

found an association in stratified analysis, when diabetic patients were excluded [83], or have observed no association [84, 85].

1.1.7.3. Autoimmune conditions

There is evidence suggesting that vitamin D can have an effect on multiple aspects of the immune system and is associated with multiple immune disorders such as inflammatory bowel disease (IBD) and multiple sclerosis (MS). $1,25(OH)_2D3$, the active form of vitamin D, directly targets T-helper cells (Th1 and Th2), which are important in balancing an immune response and dysregulation can precipitate the conditions outlined above [86]. Through its suppression of Th1 cells, vitamin D can mediate the release of anti-inflammatory cytokines and therefore dampen the auto-immune response which can lead to the development of IBD and MS. [87]. A relationship between vitamin D and autoimmune conditions was first suggested after researchers noted an increase in the prevalence of these conditions in high latitude areas. As higher latitudes have less UVB and therefore individuals would have lower vitamin D levels a link between the two was suggested [88, 89]. It has consistently been shown that those with Crohn's disease or other IBD conditions have low vitamin D concentrations [87, 90]. In a prospective cohort study, vitamin D supplementation was found to raise 25(OH)D concentrations in patients and reduce their Crohn's disease activity index scores, demonstrating the potential effect of vitamin D on IBD activity [91]. Similarly, in a cohort study of MS patients testing the association between risk of relapse and vitamin D concentration, they found an inverse linear relationship between higher vitamin D and relapse rate (HR=0.91, 95%CI 0.85–0.97) [92]. However, RCTs of vitamin D supplementation testing this hypothesis have not been as successful [93, 94].

1.1.7.4. Cognitive diseases

In conjunction with evidence for an association between vitamin D and cardiovascular, metabolic and immunological conditions it has also been hypothesised that it has a role in neurocognitive decline. It has been found that $1,25(OH)_2D3$ is associated with axon regeneration [95]. This is of paramount importance when coupled with the evidence demonstrating reduced 25(OH)D concentration an older population [96]. In a systematic review and meta-analysis, it was found that those with low vitamin D concentrations have a significantly increased risk of developing cognitive impairment when compared to those with normal vitamin D concentrations (HR=2.39, 95%CI 1.91-3.0). Other studies have also noted an association

between vitamin D deficiency and Alzheimer disease [97, 98]. However, a recent RCT whereby Alzheimer patients were supplemented up to 18,000 IU/d vitamin D and nasal insulin vs 1,000 IU/d, found no difference in cognitive function between the two groups [99].

1.1.7.5. Cancer

Garland and Garland (1980) were the first to report the vitamin D-cancer hypothesis after it was noted that colon cancer mortality was highest in areas of low natural sunlight and a mechanism involving vitamin D was suggested [100]. This theory has since gained much momentum, with multiple vitamin D exposures (UVB exposure, dietary/supplement intake and 25(OH)D measurements) being tested in relation to multiple internal cancers, excluding skin cancer, using many different study designs. The evidence for this relationship varies. However, the majority of studies support the theory that higher vitamin D is associated with a reduced risk of cancer and increased survival.

The most consistent evidence stems from UVB studies. For example; Grant *et al.*, found an inverse correlation between cancer mortality and UVB radiation for numerous cancer sites, including breast, colon, ovarian, prostate, gastric, and oesophageal cancers [101]. Boscoe *et al.* found similar results in ten cancer sites and this study also found an inverse association with UVB and cancer incidences [102]. Further ecological studies have found comparable results [103-107]. Observational studies such as the one from Tran *et al.*, found decreased risk of oesophageal adenocarcinoma (OR=0.49, 95%CI: 0.31-0.79) and oesophago-gastric junction adenocarcinoma (OR=0.52, 95%CI: 0.33-0.81) in individuals with higher lifetime mean daily UV radiation exposure [6]. Similar results have been found for other cancer sites [108-110]. Observational studies looking at multiple exposures, such as one from John *et al.*, found a decreased risk of breast cancer in those with occasional and frequent recreational sun exposure, however no significant association was observed for dietary or supplement intake [111]. On the other hand, Blackmore *et al.* saw a decreased risk in breast cancer tumours in those with increased vitamin D sources (from both solar and diet) [112]. There has been further evidence for dietary intake of vitamin D and risk of breast cancer from Shin *et al.*, who found a decreased risk of cancer in pre-menopausal women who had a high vitamin D intake (RR=0.72, 95% CI: 0.55 to 0.94) [113], similar results were found by Pritchard *et al.* for colorectal cancer [114]. However, there have also been some conflicting evidence suggesting no significant relationship exists [115, 116].

As 25(OH)D takes into account both UVB and dietary (diet and supplementation) sources, it is considered the best method of estimating vitamin D concentration at the time of blood draw.

There have been a large number of studies linking adequate vitamin D status, i.e.: 25(OH)D concentration, with a reduced risk and mortality of various cancers [29, 117-120]. One large study by Giovannucci *et al.* used 25(OH)D concentration from 1095 participants along with other variables such as dietary vitamin D intake, location of residence (regional-UVB), physical activity as a proxy for sun exposure and skin pigmentation to develop a "predicted 25(OH)D" for each participant in their larger cohort (n=47,800). From this they found that a 25 nmol/L increase in 25(OH)D was associated with a 17% reduction in cancer incidence and a 29% decrease in cancer mortality [121]. Similarly, Mohr *et al.* found a reduced risk of breast cancer in a pooled study of 11 observational studies (RR=0.61, 95%CI: 0.47-0.80) [122]. However, some studies found no association between 25(OH)D, cancer risk and survival [123, 124].

The gold standard study design for determining if a drug has a beneficial effect on survival or instances of a particular disease is an RCT. Currently there are a number of ongoing trials looking at vitamin D and cancer incidence and survival, such as the VITAL trial [125], while a small number of trials have been published. The majority of published RCTs have found no effect, however some argue that inadequate vitamin D doses were given, or design flaws such as recruitment of those without low vitamin D concentration were present [126-129]. For example, in a study by Baron *et al.*, where the risk of colorectal adenomas and vitamin D was examined, 2,259 women were supplemented with 1,000 IU vitamin D and 1200 mg calcium. After a follow up period of 3-5 years, this study failed to find any association between vitamin D and colorectal adenomas [130]. However, when subsequent research was carried out on this cohort, it was discovered that vitamin D and calcium had a significant effect on adenoma risk when stratified by VDR genotype. This suggests that the relationship between vitamin D and colorectal adenomas is modified by VDR genotype [131].

There have been two RCTs which have found a beneficial effect. One was a three-arm study in which participants were given 1,450 mg/d calcium only, 1,100 IU/d vitamin D and 1,450 mg/d calcium, or placebo. Those who were supplemented with both calcium and vitamin D had a 77% reduced incidence of any cancer after the four year study [132]. Another study by Bolland *et al.*, reanalysed a previous study (that found no effect) which had supplemented women with 400 IU/d vitamin D and 1 g/d calcium. In a subgroup who were not taking personal calcium and vitamin D supplements, a 14% decreased risk of total cancer and a 20% decreased risk of invasive breast was found [133].

Furthermore, in a recently published systematic review and meta-analysis looking at circulating vitamin D concentrations and progression and survival of cancer, a significant beneficial impact of 25(OH)D was found. When examining any cancer diagnosis, there was a significantly reduced

risk of disease progression (HR=0.84, 95%CI: 0.77-0.91) and increased survival (HR= 0.74, 95%CI: 0.66-0.82) when comparing high versus low vitamin D concentration across all studies [134]. This study also found a significant link between VDR genotypes and survival as variant rs1544410 (BsmI) was associated with overall survival (HR=1.40, 95% CI: 1.05-1.75) and variant rs7975232 (ApaI) was associated with progression-free survival (HR=1.29, 95% CI: 1.02-1.56) [134]

The relationship between vitamin D and cancer is further supported by biological evidence. It has been shown that vitamin D can limit cancer growth and progression. For example, $1,25(OH)_2D$ can regulate apoptotic proteins such as repressing the pro-survival protein, *B-cell lymphoma 2* and increasing the expression of pro-apoptotic proteins such as *BAX [29]*. *Cyclin-Dependent Kinase Inhibitors* such as *P21* cause cell cycle arrest and prevent cancer cell proliferation, this protein is encoded by *Cyclin-Dependent Kinase Inhibitor 1A* gene which contains a functional VDRE and could be a target for VDR-$1,25(OH)_2D$ complex [29, 135]. Furthermore, it has been observed that $1,25(OH)_2D$ can impact other hallmarks of cancer such as increasing cell differentiation and prevention of angiogenesis [29].

1.1.8. Vitamin D Status Assessment

All studies researching vitamin D have the same primary problem: determining how to measure vitamin D status. This is an important issue as there are various approaches used in order to estimate vitamin D status, including assessment of: 25(OH)D concentration, dietary vitamin D intake through food frequency questionnaires [136], supplementation use and dose taken, self-reported sun exposure, and finally ambient UVB, often through latitude, season or satellite measurements. These all contain their own advantages and drawbacks [137].

The variations between these approaches can be substantial due to the numerous sources of vitamin D, while only a few approaches take them all into account. Therefore, using any of these proxy measures on their own could lead to misinformation about the overall vitamin D status of individuals. This leads to great uncertainty when comparing studies.

25(OH)D is currently the best method of estimating vitamin D status of individuals at a specific time point i.e. the day of blood draw [138]. This measurement has many merits; it is easy to measure from a routine blood sample, it takes into account vitamin D from all sources (UVB, dietary and supplements) and it can be used to easily identify deficiency. However, there are also a number of drawbacks to this method. It is often unfeasible for large cohorts to take blood measurements for all participants, due to cost or logistical issues or unavailability of a sample.

Equally, it is often difficult to get ethical approval or consent for blood measurements in community-based studies or in some subpopulations, e.g. babies or children.

Setting cut-off levels for deficiency are possible with 25(OH)D concentration measurement, however, there are still disputes between researchers and agencies about which cut-off level is appropriate, e.g. some argue that deficiency occurs at <25 nmol/L while others argue this occurs at <50 nmol/L [8, 139]. This leads to confusion when describing the prevalence of deficiency, adequacy and sufficiency in research and clinical practice [140]. Additionally, when trying to compare observational studies which use different 25(OH)D cut-off values and a disease outcome it becomes increasingly challenging. For example, one recent meta-analysis examining the association between vitamin D and cancer progression clearly demonstrated how variable these cut-off values can be and how it can lead to significant heterogeneity between studies thus making conclusions difficult [134, 141].

Furthermore, often studies measure 25(OH)D concentration at different points during a disease development process such as before or after diagnosis or treatment, which could affect subsequent results and comparisons between studies.

There are also a number of assays which can be used for 25(OH)D concentration estimation and each of these are subject to their own measurement errors, in addition to an issue with comparing results from different methods and different laboratories [142]. For example, older methods of vitamin D measurement have higher variability associated with them and some may only recognize vitamin D_2 or vitamin D_3, but not both, which could yield much lower concentration estimates than newer methods [143]. This is especially important when comparing results between studies.

Some of these methods of measurement include: competitive protein binding assays, radioimmunoassay, high-performance liquid chromatography (HPLC) and the current gold standard method: liquid chromatography tandem mass spectrometry (LC-MS/MS).

A competitive protein binding assay was the first method developed, this used vitamin D binding protein as the binder and ^{3}H-25(OH)D^{3} as the reporter. However, this method is difficult and a lot of 25(OH)D can be lost in the process.

A radioimmunoassay was the next method developed in the 1980s and became the standard approach for estimating vitamin D deficiency. This method was composed of a radiolabelled 25(OH)D-specific antigen, and a 25(OH)D-specific antibody. When this 25(OH)D antibody-radiolabelled antigen complex was then added to blood, the radiolabelled antigen was released

as the antibody bound to the 25(OH)D in the blood. Therefore the amount of "free" radiolabelled antigen could then be measured to estimate the concentration of 25(OH)D.

However, newer, more consistent methods were soon developed; HPLC and LC-MS/MS. Reverse phase HPLC uses chromatography to separate and quantify both 25(OH)D_2 and 25(OH)D_3, these can then be detected using UV. This method is consistent, however the process is relatively slow and as such the current favoured method is LC-MS/MS. This method uses both chromatography and mass spectrometry in order quantify 25(OH)D with high quality results. However, the equipment for this is expensive.

Many studies use a one-time 25(OH)D concentration measurement per individual, which is highly dependent on a number of factors; such as time of year, recent sun exposure and the method of measurement used. A subsequent measurement of that individual's 25(OH)D concentration, even only a few weeks later could be drastically different depending on personal factor and behaviours, ambient UVB or changes in the measuring instrument used [142].

Some also argue that a one-time measurement of 25(OH)D is not a good representation of a person's long term "average" vitamin D status [144, 145]. This is especially true when determining associations between vitamin D status and a disease outcome [145]. As 25(OH)D can change dramatically depending on season, place of residence, dietary changes and a whole host of other factors over time, using a one-time measurement is not ideal, especially when investigating a disease or condition which develops slowly over time. Using a long-term "average" of 25(OH)D concentration is the best method available for vitamin D assessment, however this is typically not possible as often epidemiology studies only use one measurement of 25(OH)D, which can lead to measurement error of the "true average vitamin D status" of an individual. Using this measurement without proper adjustment for time of year or month of blood draw can lead to poor precision in the estimate which in turn leads to non-differential misclassification of 25(OH)D status. This can have a huge impact on studies which seek to determine an association between 25(OH)D and a health outcome as it would bias the study towards the null, even if an association was present [145, 146].

Using latitude or season as a proxy of vitamin D status is most common in ecological studies [100]. The advantages of these methods are the ease at which vitamin D can be estimated for a large cohort. The problem with this lies, not only with ecological fallacy when relating vitamin D status to a certain disease or condition but also the lack of vitamin D concentration as this can vary dramatically based on dietary vitamin D intake, sun exposure and many other factors. In order to combat this, there have been a number of researchers who use sun exposure diaries

as a proxy [147]. This method is hindered however, by self-reported sunlight exposure and by the lack of validated sun exposure questionnaires [148].

Another disadvantage of these questionnaires is the need for specific questionnaires depending on the cohort being studied. Due to the heterogeneity at which cutaneous vitamin D synthesis may occur (depends on age, skin colour, clothing coverage of the skin), which can differ widely between countries, country-specific validated questionnaires would be needed. For instance different questionnaires are needed if estimating vitamin D status in Qatar and the UK as these countries differ dramatically in terms of time spent outdoors and amount of skin exposed to the sun. These questionnaires also often use generalised ambient UVB information over large regions which does not accurately capture ambient UVB doses.

More sophisticated approaches such as measurement of solar elevation angle, global solar radiation and using satellite or ground measures of UVB have also been used for vitamin D estimation. However, this method typically estimates vitamin D over a large area with low spatial or temporal resolution [7]. Additionally, the use of this method is often carried out without adjustment for important factors which interfere with UVB dose, such as altitude, cloud cover, ozone layer cover and pollution [50, 51, 149]. One advantage of the above UVB/ latitude methods is that it allows retrospective lifetime vitamin D estimation, which is very difficult to capture by using the other methods [150, 151].

Dietary vitamin D estimation is one approach which has been employed in a number of studies [136, 152]. This involves using an individual food frequency questionnaires along with a nutrient database for reference to estimate vitamin D status. These reference databases include for example, the UK composition of foods or the Irish composition of foods database [153, 154]. However, many studies suggest that vitamin D measurement using diet alone is not enough to estimate overall vitamin D status, because quantities found in food are scarce. It has been shown that natural sunlight exposure provides the majority of vitamin D in our body [155] and research has shown that other characteristics, such as BMI and physical activity can be more important in determining vitamin D status than dietary intake [121, 156].

Other dietary sources of vitamin D, such as supplementation are extremely important. These are found in tablets or drops form, or in fortified products. Routine fortification of milk does not occur in Ireland or in the UK, unlike some other parts of the world. However, a recent study found that supplementation was the most important dietary source of vitamin D in Scotland [157]. This is because supplements of vitamin D contain typically far more vitamin D than is available in food. Studies which fail to take into account these supplemental dietary sources in their questionnaires could be seriously underestimating vitamin D intake [136, 158, 159].

Additionally, it has also been observed that the origin of and cooking method used can affect vitamin D, factors which cannot realistically be accurately incorporated into questionnaires [160]. For example, farmed salmon has only 25% of the vitamin D which is present in wild salmon and frying fish can lead to a loss of almost half of the vitamin D, when compared to other cooking methods [161].

Another approach to measuring vitamin D is a more mathematical one. This is a recent method which has been applied in a small number of studies. This method uses a sample number of 25(OH)D measurements, along with other determinants of vitamin D, identified through multivariable linear regression, to predict 25(OH)D for a larger cohort of participants. The advantages of this method are recognizable as it employs the best method of vitamin D status estimation, while removing the issues with logistics and cost. This method has been found to be useful in certain incidences [121, 162], however, there have also been doubts about its use for determining a relationship between vitamin D and health outcomes. It has been shown that variables which are used in the initial linear model and are also associated with the health outcome in question, can significantly bias the relationship between 25(OH)D concentration and the health outcome [163].

As more and more research is now exploring the associations between vitamin D and multiple health outcomes it is imperative that acknowledgment of the weaknesses involved in the measurement of vitamin D is made. Any of these measures of vitamin D alone can have problems associated with them and therefore lead to misinformation about the overall vitamin D status of individuals [144]. This in turn would affect the results of studies which use these measurements. A combined approach using ambient UVB, dietary and supplement information could be useful for an accurate estimation of vitamin D status when 25(OH)D measurement is not feasible, or in addition to 25(OH)D, particularly when investigating outcomes which develop over long periods of time.

1.1.9. Oesophageal and Gastric cancer

Vitamin D has been shown to have a beneficial effect on many cancer sites and types. The relationship between vitamin D and upper gastrointestinal cancers however, is less evident. This is mainly due to the lack of research on the topic. Upper gastrointestinal cancers comprise of cancers of the upper gastrointestinal digestive system such as cancer of oesophagus, stomach, liver, pancreas and gall bladder. Mortality rates of upper gastrointestinal cancers remain very high. Only oesophageal and gastric cancer will be considered in this thesis.

2.1.1.1 Oesophageal Cancer

Oesophageal cancer has two common subtypes, oesophageal adenocarcinoma and squamous cell carcinoma. Oesophageal adenocarcinoma affects the lower third of the oesophagus, and arises due to repetitive gastro-oesophageal reflux causing alterations to the native columnar epithelium. This can result in Barrett's oesophagus, dysplasia and finally cancer. Squamous cell carcinoma on the other hand mainly affects the upper and mid-third of the oesophagus.

It is estimated that 456,000 new oesophageal cancer cases occur annually [164]. In 2014 alone, there were 396 incidence cancer cases diagnosed in Ireland and 8,900 cases in the UK [165, 166]. Oesophageal cancer is the 13th most common cancer in Ireland, 14th most common in the UK and the 15th most common worldwide [164-168]. This cancer is more prevalent in males than females with 64% of oesophageal cancers cases in Ireland in 2014 being diagnosed in males [165]. This trend was also observed in the UK with males accounting for 51% of all oesophageal cancer diagnoses in 2014. 56% of oesophageal cancers diagnosed in the UK between 2012 and 2014 were in those ages 70 or older [166]. A similar trend was observed in Ireland with the majority of those diagnosed from 1994 to 2014 aged over 75 [165].

Over the last 20 years, the prevalence of oesophageal cancer in Ireland has slightly decreased. The age-standardised incidence rate in 1994 was 11.9 in males and dropped to 10.7 in 2014. A similar decrease was observed in females; 6.4 in 1994 and 5.0 in 2014 [165]. This slight decrease was also observed in females in the UK with an 8% reduction in cancer incidence since the 1990s. However, the UK has experienced an 11% increase in male oesophageal cancer incidence in the last 30 years [166].

Survival rates of oesophageal cancer are also poor. In 2014 7,790 deaths were attributed to oesophageal cancer in the UK. 5-year survival rates in Ireland are just over 16% while 10-year survival rates are only 12% in the UK [169].

Both subtypes of oesophageal cancer can be attributed to lifestyle factors. Incidence of oesophageal adenocarcinoma for example, has been associated with obesity, in particular central adiposity, tobacco smoking, and gastrointestinal reflux disease [170-172], while squamous cell carcinoma has been associated with consumption of alcohol, hot mate, pickled vegetables, opium use, tobacco smoking or chewing of nass [173-175].

Western regions have witnessed rapid increases in oesophageal adenocarcinoma incidence; a threefold increase in cancer incidences has been observed since the 1970s [176, 177].

In contrast, incidence rates of oesophageal squamous cell carcinoma appear to be declining in some western areas, such as France, Switzerland and Finland [178, 179]. However, squamous cell carcinoma remains the predominant oesophageal cancer type in some developing countries, and is endemic in parts of Asia, stretching from Northern Iran, through central Asia and North central China, which is known as the "oesophageal cancer belt" [167]. Incidences are also high in Uruguay and Southern Brazil, and the Transkei region in Africa [180-182].

2.1.1.2 Gastric Cancer

Gastric cancer affects the stomach and mainly arise from the glands on the mucosal layer of the stomach. Histologically, adenocarcinomas make up about 90% of stomach cancers [183], however there are two distinct subtypes of stomach cancer; cardia and non-cardia gastric cancer. Cardia cancer occurs in the stomach near the oesophageal-gastric junction while non-cardia cancer arises in more distal regions of the stomach. The rates of gastric cancer vary greatly depending on sex and location, however, incidence is high worldwide. Highest incidences are reported in areas of South America, eastern Europe and East Asia [184].

In the UK, there were 6,682 new gastric cancer cases diagnosed in 2014, making it the 16th most common cancer in the UK. Rates within Ireland are higher; it is the 7th most common cancer and 599 new cases diagnosed in Ireland in 2014 [185]. Overall in Europe, 139,600 cases were diagnosed in 2012 making it the 4th most common cause of cancer in Europe. Gastric cancer is also more common in males. In 2014 for example, 63% of all gastric cancer cases in Ireland were diagnosed in males [164, 165]. Gastric cancer is also more prevalent in older adults with those aged over 75 accounting for the group with the highest level of gastric cancer diagnosis in Ireland [165]. Similarly in the UK, just over half (51%) of all gastric cancer cases diagnosed from 2012-2014 were in those aged over 75 [166].

Rates of gastric cancer have decreased slightly in Ireland in the last 20 years. There was a 4% decrease in male age-standardised incidences rates between 1994 and 2014 [165]. This decrease however was not as dramatic in female cases with only a 1.4% decrease in age standardised rates in 20 years. This decrease was observed to a greater extent in the UK with a 48% reduction in cancer incidence rates since the 1990s [166].

Survival rates of gastric cancer are still very poor [164, 186]. Overall, there were 4,576 gastric cancer deaths in the UK in 2014 and 107,000 deaths in Europe in 2012 making gastric cancer the 4th most common cause of cancer death in Europe [164]. 5-year survival rates in Ireland were 24% in 2014 while 10-year survival rates were only 15% in the UK in 2011 [169].

Risk factors for gastric cancer include; cigarette smoking, salty and smoked foods, low intake of fruit and vegetables [183]. However, some risk factors are cancer-type specific. For example obesity, and gastrointestinal reflux disease are thought to be important risk factors for cardia gastric cancer but not for non-cardia cancer. Similarly, infection with Helicobacter Pylori and lower socioeconomic status have been shown to be associated more with gastric non-cardia cases than cardia cases [187].

Previous attempts to determine an association between vitamin D, and the development and survival of oesophageal and gastric cancer have shown conflicting results with some evidence supporting an inverse relationship, others a positive relationship and others no association [188-190]. Therefore it is necessary to disentangle this relationship in order to determine the best practices for preventing and managing these conditions.

1.2. Rationale

This thesis aimed to explore the relationship between vitamin D and oesophageal or gastric cancer risk and survival. It utilised vitamin D concentration in the circulation and developed a method of using ambient UVB measurements along with personal characteristics to capture long term "average" vitamin D status of individuals.

There has been a recent resurgence in research into vitamin D and its health effects. The importance of vitamin D for health and the role it has shown to play in numerous diseases and conditions has previously been outlined. However, there is a gap in the knowledge; research has been carried out on its effect in many prevalent cancers types, such as breast, colorectal and prostate, but there has been little or no research carried out on its role in rarer cancers including, oesophageal and gastric cancer. As there has been a strong beneficial effect of vitamin D on the risk and survival of another digestive cancer i.e. colorectal cancer, it is hypothesised that a similar effect might be observed in oesophageal and gastric cancer. In the limited research which has been carried out on the topic of vitamin D and upper gastrointestinal cancer risk and survival (which is described in more detail in chapter 2) there have been conflicting results reported. The role of vitamin D and UVB in oesophageal and gastric cancer will be examined in this study. It is hoped that the overall role of vitamin D on upper gastrointestinal cancer and its subtypes can be elucidated.

1.3. Research Questions

There are a number of research questions which need to be addressed in order to achieve the aims this thesis has set out. It is important to determine what factors could influence 25(OH)D dose in order to determine what factors could affect a longer term vitamin D estimate. It is also necessary to investigate UVB doses over Ireland and the UK in order to develop an accurate UVB estimate for this vitamin D estimate. When examining the relationship between vitamin D and upper gastrointestinal cancer risk and survival, it is necessary to review the current literature on the topic. Finally, it is necessary the association between vitamin D and oesophageal and gastric cancer risk and survival using multiple estimates of vitamin D.

1.3.1. Research Objectives

The specific objectives were

- To describe current literature around the topic of vitamin D, UVB, and the risk and survival of oesophageal and gastric cancer and complete a systematic review and meta-analysis of published studies.
- To use the Tropospheric Emission Monitoring Internet Service (TEMIS) database to describe seasonal and annual UVB doses over Ireland and the UK with the highest spatial and temporal resolution to date.
- To investigate if ambient UVB preceding blood sample is associated with 25(OH)D concentration.
- To investigate the contribution of ambient UVB and other determinants to 25(OH)D status in an older Irish cohort.
- To develop a vitamin D estimate using ambient UVB and personal vitamin D related characteristics.
- To develop a simple vitamin D estimate using ambient UVB and personal vitamin D related characteristics.
- To examine the role of ambient UVB, 25(OH)D and a non-seasonally biased vitamin D estimate in oesophageal and gastric cancer risk and survival in different cohorts.

1.4. Outline of Thesis

The thesis is organised as follows.

- **Chapter two**; Describes current literature on the topic of vitamin D and the risk and survival of oesophageal and gastric cancer.
- **Chapter three;** Describes regional and seasonal differences in ambient UVB doses over Ireland and the UK.
- **Chapter four;** Details how individual cumulative and weighted vitamin D UVB doses (cw-D-UVB) were calculated for each individual and their association with 25(OH)D concentration. This chapter also explores the development of a very simple vitamin D scoring system, as a proxy for vitamin D. Finally, this chapter explores the use of cw-D-UVB and the vitamin D scoring system in prediction of 25(OH)D.
- **Chapter five;** Examines 25(OH)D concentration in an older Irish cohort and the importance of cw-D-UVB. This chapter also improves upon the vitamin D scoring system outlined in chapter four using personal characteristics and examines its use in the prediction of vitamin D deficiency in an older cohort.
- **Chapter six;** Investigates the role of annual ambient UVB and the vitamin D score in the risk and survival of oesophageal and gastric cancer in a UK cohort.
- **Chapter seven;** Investigates the role of 25(OH)D and annual ambient UVB in the survival of oesophageal and gastric cancer in an Irish cohort.
- **Chapter eight;** Summarises the main findings of the thesis and discusses findings in relation to current literature and their use in future research.

2 Literature Review

2.1 Aim

The aim of this Chapter was to carry out a systematic review and meta-analysis examining the relationship between different vitamin D exposures and the risk and survival of oesophageal and gastric cancer. The different exposures explored were 25(OH)D, vitamin D intake, UVB dose and vitamin D related variation/molecular expression.

2.2 Structure of Literature Review

Chapter two is structured as follows:

Section 2.3 provides the introduction to the review carried out

Section 2.4 describes the search strategy and selection criteria used to review the literature.

Section 2.5 provides the results and discussion of the four systematic reviews carried out,

- Vitamin D and risk of oesophageal cancer (section 3.5.1)
- Vitamin D and survival of oesophageal cancer (section 3.5.2)
- Vitamin D and risk of gastric cancer (section 3.5.3)
- Vitamin D and survival of gastric cancer (section 3.5.4)

Section 2.6 summarises the literature review chapter

2.3 Introduction

When assessing the role of vitamin D and cancer there are two questions which can be asked, whether vitamin D has an impact on the risk of developing cancer and/or on survival once a person has been diagnosed. As these are two different issues, it was decided to carry out separate systematic reviews on each aspect for each of the cancers being studied, oesophageal and gastric. Therefore, four separate reviews and meta-analysis were conducted; risk of oesophageal cancer, survival of oesophageal cancer, risk of gastric cancer and survival of gastric cancer. Precursor legions were also considered including; Barrett's oesophagus and squamous dysplasia. Barrett's oesophagus, is a pre-cursor condition for adenocarcinoma and squamous dysplasia, is a pre-cursor condition for squamous cell carcinoma. As none of the studies examined precursor conditions for gastric cancer, none were included.

2.4 Methods

2.4.1 Search Strategy

Four databases were searched; Ovid MEDLINE (US National Library of Medicine, Bethesda, Maryland), EMBASE (Reed Elsevier PLC, Amsterdam, Netherlands), Web of Science (Thompson Reuters, Times Square, New York, USA) and Cochrane database (John Wiley & Sons). Initially MEDLINE, EMBASE and Web of science was searched but the Cochrane database was added in a subsequent updated search in December 2017. These databases were searched for studies that contained one of the following key terms i) vitamin D, cholecalciferol, ergocalciferol, 25(OH)D, vitamin D receptor(s), or calcitriol receptor(s), or ii) single nucleotide polymorphism(s) or genetic polymorphism(s), or sun exposure, ultraviolet, UVB, solar radiation, sunlight, latitude or geographic variation, combined with either iii) Barrett's (o)esophagus, (o)esophageal cancer, adenocarcinoma, squamous dysplasia, squamous cell carcinoma, tumour(s) or neoplasm(s) for oesophageal cancer or when examining gastric cancer risk the following terms were used iii) Gastric cancer, stomach cancer, cardia adenocarcinoma, non-cardia adenocarcinoma, tumour(s) and neoplasm(s). Survival outcome in both types of cancer was examined in a similar way with the exclusion of pre-cursor lesions and in combination with iv) death, mortality or survival. Studies were limited to ones conducted on humans and available in English but no date restrictions were specified. Detailed search strategies are shown in **Appendix 1.** Observational (case-control, retrospective and prospective cohort, cross-sectional) were included and case reports and ecological studies were excluded. Review articles found were excluded but checked for references. Grey literature was not searched and hand searching was not carried out. Only studies which examined oesophageal and gastric cancer were included. Studies which combined cancer types were excluded. Overall and cause-specific survival through medical records were deemed eligible, while relative survival was deemed ineligible. Titles were initially screened and full text articles were read when deemed eligible for inclusion. MOOSE guidelines were followed when carrying out these reviews [191]. Corresponding authors were contacted where necessary to obtain information for the review e.g.: OR values for each of the SNPs or which covariates were adjusted for. Methodologic quality for case–control and cohort studies was evaluated using the Newcastle-Ottawa Scale [192] (**Appendix 2**). For cross-sectional studies, an adapted version of the Newcastle–Ottawa Scale was used [193]. Sensitivity analysis based on the quality assessment scores was intended to be carried out but due to the limited number of studies found for each exposure type this was not done. When examining the risk of oesophageal cancer and vitamin D, two independent reviewers undertook the screening process and data extraction (details of what was carried out by whom is in **Appendix 3**). For gastric cancer risk,

gastric cancer survival and oesophageal cancer risk, only one reviewer undertook the screening and data extraction process.

2.4.2 Data Extraction

Article eligibility for this review was determined by PICO criteria [194]

- **P**articipants: Individuals of any age who have received a diagnosis of cancer of the oesophagus or stomach were included in the review. Those with pre-malignant conditions for oesophageal cancer were also included when assessing risk.
- **I**ntervention/Exposure: Assessment of vitamin D: such as 25(OH)D measurements, UVB exposure, vitamin D intake (from foods and/or supplements), VDR expression, and vitamin D-related genetic polymorphisms of the study participants.
- **C**omparators: Study reports association between vitamin D measurements or vitamin D-related exposures outlined above with cancer risk with individuals who have not received a diagnosis of oesophageal/gastric cancer or pre-malignant conditions or reports association between vitamin D measurements or vitamin D-related exposures outlined above with oesophageal or gastric cancer survival.
- **O**utcome: Risk of oesophageal/ gastric cancer and/or pre-malignant lesions of the oesophagus, or cancer-specific mortality.

The following information was extracted from each study included in this review: publication year, study authors, sample size, number of cases (and controls), study design, outcome measured, vitamin D measurement used, mean/median age and sex of participants, follow-up time, proportion Caucasian, country residence of participants, covariates considered, OR/RR and 95% CIs or HR and 95% CIs adjusted for the maximum number of confounding variables.

2.4.3 Statistical Analysis

For both *risk* and *survival* of oesophageal and gastric cancer, a meta-analysis' for each exposure type was carried out, these included 25(OH)D status, dietary assessment, and VDR polymorphisms.

When examining risk of cancer, summary ORs were calculated if at least two studies considered the same exposure; (i) separately for each oesophageal/gastric lesion subtype (adenocarcinoma, squamous cell carcinoma, squamous dysplasia, and Barrett's oesophagus,

cardia-adenocarcinoma, non-cardia adenocarcinoma), and (ii) for any oesophageal/gastric cancer. Highest versus the lowest reported category of exposure (the lowest exposure level was the reference) were compared.

For survival analysis, summary ORs were calculated if at least two studies considered the same exposure (any oesophageal/gastric cancer (adenocarcinoma, squamous cell carcinoma, gastric adenocarcinoma). Survival of cancer in the highest versus the lowest reported category of exposure were compared.

In some studies RR or OR were used, whereas adjusted HR were extracted from cohort studies. These measures were used in the meta-analysis as given, because the HR, OR and RR are approximate to one another when event rates are low, as is the case with oesophageal and gastric cancer [195]. Random-effects models were used to calculate pooled OR from each of the extracted ORs and 95% CIs. Standard errors (SE) were used to calculate weighting for each study. Due to the expected heterogeneity between studies such as population differences in latitude, diet, 25(OH)D concentration and 25(OH)D categories, DerSimonian and Laird random effects models were used [196]. All analyses were performed in R and the R-package 'metafor' was used for meta-analyses [197]. P-value <0.05 was considered statistically significant. The I^2 statistic was calculated to quantify the degree of heterogeneity between studies: larger I^2 values indicate greater heterogeneity [198]. Risk of publication and selection bias was evaluated by checking for asymmetry in the funnel plots of the study OR against the standard error of the logarithm of the OR [199].

2.5 Results and Discussion

2.5.1 Vitamin D and Risk of Oesophageal Cancer

2.5.1.1 Systematic Review

The original flowchart for study selection is shown in **Figure 2.1**. 690 titles were received and abstracts were initially screened (n=475 after removing duplicates). 430 articles were excluded and 45 full text articles were read, from these, 14 articles were identified that examined relationship between vitamin D exposures and oesophageal neoplasms [8, 9, 158, 159, 200-209]. Databases were initially screened in 2014 and studies extracted. An updated search was then conducted in 2015. One extra study was found [210], so a total of 15 articles were included in the systematic review. The Cochrane database was subsequently also searched in 2017 and no new studies were found. These publications related to risk of oesophageal cancer or pre-cursor lesions and: 25(OH)D concentration (N=3), vitamin D intake (N=4), UVB radiation (N=1), and/or vitamin D related genetic variants or molecular expression (N=7), as outlined in **Table 2.1.**

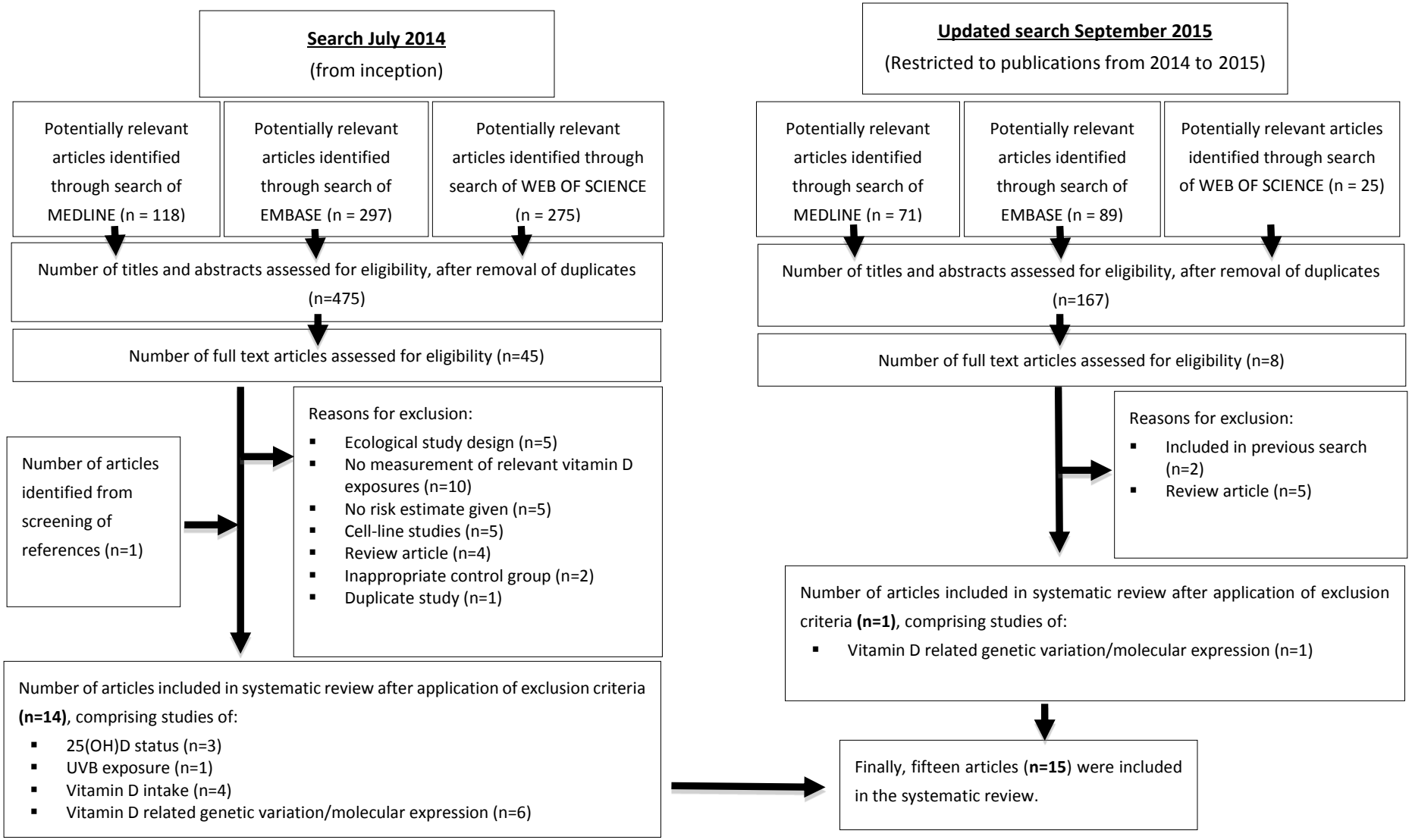


Figure 2.1: Flow chart of Search strategy for oesophageal cancer risk and vitamin D

Table 2.1: Characteristics of studies of vitamin D related exposures and the risk of oesophageal cancer and pre-malignant conditions [1,2].

Study and location	Study design	Study location	NO score	Vitamin D exposure	Outcomes	Cases	Controls / Cohort	Adjusted confounders: Age	Sex	Energy	BMI	Smoking	Alcohol	NSAIDs	Reflux	Education	SE Status	PA	H. pylori	Race
VITAMIN D STATUS																				
Chen *et al.* (2007)	Case-cohort	China	8	Serum 25(OH)D	Squamous cell carcinoma	545	1,105	✓	✓		[4]✓	✓	✓							
Abnet *et al.* (2007)	Cross-sectional	China	8[3]	Serum 25(OH)D	Squamous dysplasia	230	490	✓	✓		✓									
Abnet *et al.* (2010)	Nested case-control	China, Finland, USA	8	Serum/ plasma 25(OH)D	All oesophageal cancer	265	264	✓	✓		[4]✓	✓	✓			✓				✓
					Adenocarcinoma	104	103	✓	✓		[4]✓	✓	✓			✓				✓
					Squamous cell carcinoma	142	142	✓	✓		[4]✓	✓	✓			✓				✓
VITAMIN D INTAKE																				
Launoy *et al.* (1998)	Hospital-based case-control	France	5	Interview diet history	Squamous cell carcinoma	208	399	✓	[5]	✓		✓	✓							
Mayne *et al.* (2001)	Population-based case-control	USA	6	Dietary intake (104-item FFQ)	Adenocarcinoma	282	687	✓	✓	✓	✓	✓	✓			✓				✓
					Squamous cell carcinoma	206	687	✓	✓	✓	✓	✓	✓			✓				✓
Lipworth *et al.* (2009)	Hospital-based case-control	Italy	6	Dietary intake (78-item FFQ)	Squamous cell carcinoma	304	743	✓	✓		✓	✓	✓			✓				
Mulholland *et al.*(2011)	Population-based case-control	Ireland	7	Dietary intake (101-item FFQ)	Adenocarcinoma	218	252	✓	✓	✓	✓	✓	✓	✓	[4]✓	✓			✓	
					Barrett's oesophagus	212	252	✓	✓	✓	✓	✓	✓	✓	[4]✓	✓			✓	

UVB RADIATION																				
Tran *et al.* (2012)	Population-based case-control	Australia	8	Lifetime daily mean ambient UV radiation	Adenocarcinoma	330	1,417	✓	✓		✓	✓	✓		✓	✓		[4]✓	✓	
					Squamous cell carcinoma	279	1,417	✓	✓		✓	✓	✓		✓	✓		[4]✓	✓	
					Junctional tumours	386	1,417	✓	✓		✓	✓	✓		✓	✓		[4]✓	✓	
VITAMIN D RELATED GENETIC VARIANTS/MOLECULAR EXPRESSION																				
De Gottardi *et al.* (2006)	Cross-sectional	Switzerland	4	VDR expression (tissue)	Barrett's oesophagus	6	6	none												
					Adenocarcinoma	6	6	none												
Li *et al.*(2008),	Case-control	China	5	VDR *TaqI* polymorphism	Squamous cell carcinoma	126	169	✓	✓							✓				
Van den Winkel *et al.*(2009)	Case-control	Netherlands[6]	N/A[7]	VDR polymorphisms	Squamous cell carcinoma	64	202	none												
Chang *et al.* (2012)	Population-based case-control	Ireland	8	VDR polymorphisms	Adenocarcinoma	224	256	✓	✓	✓	✓	✓	✓		✓	✓	✓			
Gu *et al*. (2014)	Hospital-based case-control	China	7	VDR polymorphisms	Squamous cell carcinoma	629	686	✓	✓			✓	✓							
Janmaat *et al.* (2015)	Case-control	Netherlands	6	VDR polymorphisms	Barrett's oesophagus	260+150	202	none												
					Adenocarcinoma	141	202	none												
Wang *et al.* (2015)	Case-control	China	7	Vitamin D-related polymorphisms	Squamous cell carcinoma	1942	2111		✓	✓										

Footnote:

[1] Abbreviations: FFQ: Food Frequency Questionnaire, *NO score*: Newcastle-Ottawa quality scale score (maximum score: 9), *BMI*: Body mass index, *Energy*: Energy intake, *Reflux*: Gastro-oesophageal reflux symptoms; *SE Status*: Socioeconomic status; *PA*: Physical activity; *H. Pylori: Helicobacter pylori* infection.

[2] Adjusted confounders: *Energy*; *BM*; *Reflux*; SE; *PA*; *H. Pylori*

[3] An adapted version of the Newcastle-Ottawa quality scale as it was cross-sectional;

[4] Covariate considered but removed from the final model

[5] All male cohort;

[6] Same cohort (shared controls)

[7] Newcastle-Ottawa quality score could not be derived due to insufficient detail (only abstract available);

2.5.1.2 Vitamin D status

Three studies examined the role of 25(OH)D and oesophageal cancer risk (**Figure 2.2**).

Chen *et al.* (2007) studied oesophageal cancer cases (N=545) and a random sample of controls (N=1071) from General Population Trial of Linxian, China [9]. In this prospective study, initial blood samples were taken from a large population of healthy adults (~29,600), and participants were followed up for over five years. An increased risk of squamous cell carcinoma was found for those with higher concentrations of pre-trial 25(OH)D: when comparing the highest with the lowest quartile of 25(OH)D, $HR_{overall}$ was 1.30 (95%CI: 0.97-1.73, p_{trend}=0.013), and in males (HR=1.77, 95%CI: 1.16-2.70), but association was not significant in females.

A cohort consortium pooled data from eight prospective studies hailing from Finland, the USA, and Asia were conducted to ensure a large total population of upper gastrointestinal cancer cases (oesophageal and gastric, N=1,065) and age, sex, race and season-of-blood-draw-matched controls (N=1,066) [8]. 104 oesophageal adenocarcinoma and 142 oesophageal squamous cell carcinoma cases were included. This study found no association between 25(OH)D and risk of oesophageal cancer overall, or with histological subtypes of cancer when 25(OH)D concentration of 50-75 nmol/L was compared to <25 nmol/L ($OR_{Adenocarcinoma}$=1.69, 95%CI: 0.25-2.12 and $OR_{squamous\ cell\ carcinoma}$=0.72, 95%CI: 0.28-1.89). However, in stratified analysis of all upper gastrointestinal cancer (oesophageal and gastric), a positive association for risk of cancer was revealed in Asians (OR=1.88, 95%CI: 1.10-3.22).

Abnet *et al.* (2007) examined the association between 25(OH)D and risk of oesophageal squamous dysplasia, a pre-cursor lesion for squamous cell carcinoma, in a cross-sectional study of healthy residents, also from Linxian (N=724) [202]. In total, 230 (32%) squamous dysplasia cases were found, and the highest quartile of serum 25(OH)D concentrations was associated with significantly elevated risks of squamous dysplasia after adjustment for many variables (RR=1.86, 95%CI: 1.35-2.62).

In this meta-analysis, no significant associations between squamous cell carcinoma risk and 25(OH)D were found (OR=1.20, 95%CI: 0.77-1.63). No meta-analysis could be conducted for other subtypes as only a single study was available [8, 202]. Overall, a statistically significantly increased risk of any oesophageal cancer (adenocarcinoma and squamous cell carcinoma) was observed when comparing high vs. low levels of 25(OH)D concentration in the meta-analysis, (OR=1.39, 95%CI: 1.03-1.74) (**Figure 2.2**).

It is worth mentioning the study of Giovannucci *et al.*, which was not included in this review as it measured *predicted* 25(OH)D concentration. Giovannucci *et al.* measured 25(OH)D from 1095 participants, and used these measurements, along with multiple factors that influence vitamin

D status, such as UVB, diet, supplements, skin pigmentation and BMI to create a predictive model of 25(OH)D concentration for a larger cohort (N=47,800). It was found that higher levels of predicted 25(OH)D were associated with a *decreased* risk of oesophageal cancer (RR=0.37, 95%CI: 0.17-0.80) [121]; however, factors used to predict 25(OH)D could affect cancer risk independent from their association to vitamin D status. For instance BMI was included in the original predictor model, however, this could bias results, as BMI is not only associated with a lower 25(OH)D concentrations but also highly related to the risk of upper gastrointestinal cancers.

An increased risk of oesophageal cancer with high 25(OH)D concentration in this meta-analysis was observed, however, there are a number of limitations in relation to the included studies, along with possible population-specific effects which should be addressed. Primarily, there are very few studies which have examined this topic (N=3) and those which have, contained a large proportion of the Han Chinese population, of which two were in Linxian China. As this region is known for having some of the highest rates of oesophageal squamous cell carcinoma in the world [8, 9, 202], some environmental or genetic exposures could be present in this region which influence the rates of this particular cancer. There have been some suggestion that exposure to coal, which is widely used in the region, has some effect. Vitamin D has been proposed as having a role in the metabolism of polycyclic aromatic hydrocarbons, which are found in coal [202]. This allows for the possibility that a population specific effect of the risk of oesophageal and gastric cancer is possible, which may not exist in other locations [211].

Furthermore, in the study by Chen *et al.*, all 25(OH)D samples were taken in March which has been shown to be the month with the lowest 25(OH)D throughout the year, and also the lowest variability between samples. Some countries experience the vitamin D winter, this is a period of time in which cutaneous synthesis of vitamin D is not possible and can last from October to March. As the majority of an individual's reserve of vitamin D in the body has been depleted by March, this is why one often sees very low 25(OH)D status and more importantly low variability in this month, which affects the power of statistical analysis.

Therefore it could be argued that using 25(OH)D concentration from March, as has been done by Chen *et al*. is a poor predictor of individuals all-year vitamin D rank in the population [212].

Moreover, in the stratified analysis by Abnet *et al.*, the follow up time varied considerably with one of the cohorts having a median follow up time of only 1.7 years.

One of the main limitations of this meta-analysis is the small number of published information on the topic. As such it is difficult to ascertain if there is publication bias present. When examining funnel plots (which are shown in **Figure 2.3**), with only two or three studies present,

the larger studies were observed to be close to the mean towards the top of the funnel, which is to be expected and demonstrates no publication bias. However, with such a small number of studies it is difficult to conclusively establish whether there is no publication bias present. Additionally, as one of the studies included was quite large this would have had a considerable influence on the overall summary OR. Due to this, the observed positive association might not represent the overall association between 25(OH)D and oesophageal cancer risk but rather one study is skewing the results to a positive association.

In order to account for heterogeneity a random effects model was used, however some evidence of heterogeneity may be still present as the I^2 value was suggestively significant (p-value=*0.08*).

Despite the individual limitation of the studies, the studies in this meta-analysis scored well in the quality assessments conducted (all 25(OH)D studies scored 8, maximum score was 9). Overall however the limited number of studies using 25(OH)D mean that further research needs to be carried out before conclusions can be drawn.

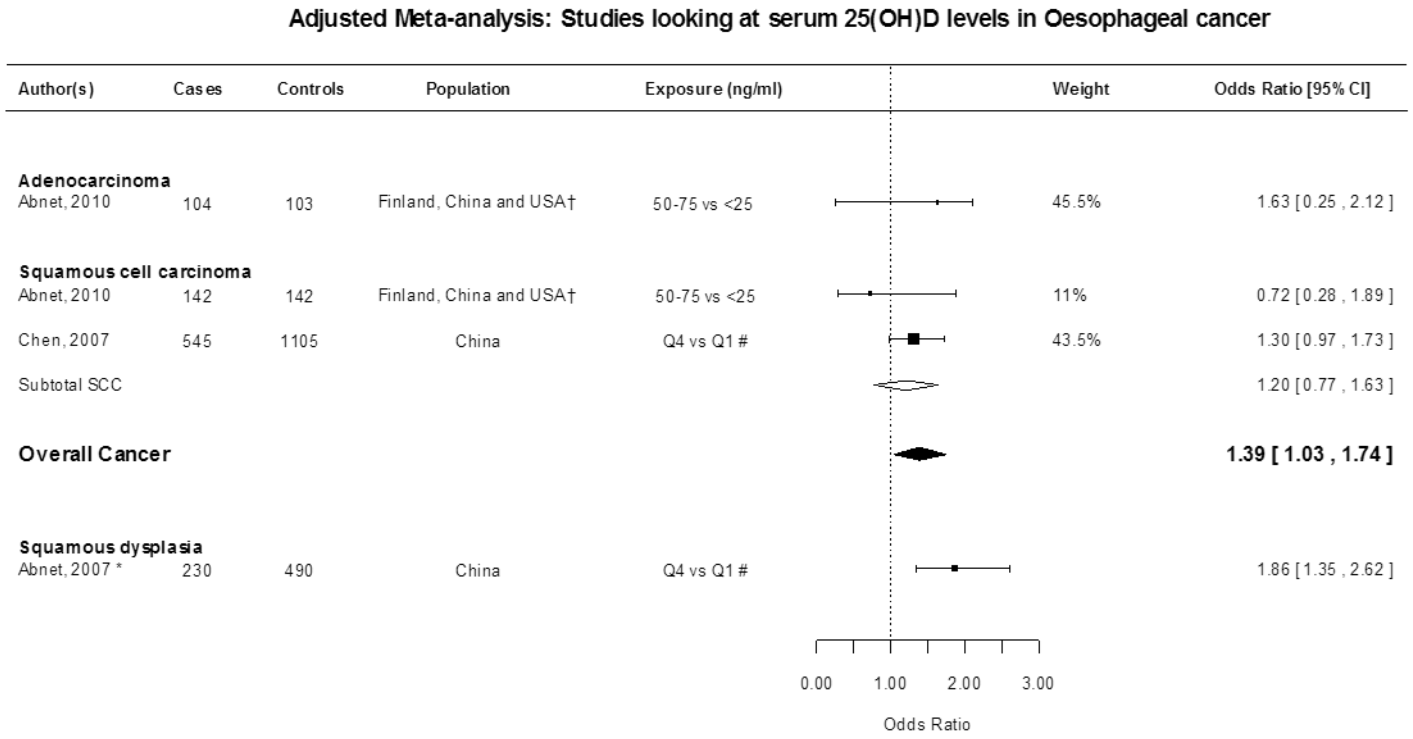


Figure 2.2: Meta-analysis of studies looking at serum 25(OH)D and oesophageal cancer risk

Meta-analysis of studies looking at serum 25(OH)D and oesophageal neoplasia using adjusted OR estimates. Squamous cell carcinoma (squamous cell carcinoma) I^2 = 21.93%, Q-value=1.28, p-value= 0.25. Overall Cancer I^2: 60.54% Q(df = 2) = 5.0679, p-value = 0.079. Weights are shown for overall cancer. * This study was a cross-sectional study, while the others were case-control. † Total number of Upper gastrointestinal cancer cases from each study given for the Cohort Consortium: Finland: 416, China: 313, USA: 296. # Geometric means of vitamin D concentrations per quantiles: Chen *et al.* (2007): 25th: 19.9 nmol/l, 75th: 57.2 nmol/l. and Abnet *et al.* (2007) 25th: 24.1 nmol/l 75th: 48.2 nmol/l. Q1 vs Q4: quartile 1 vs quartile 4

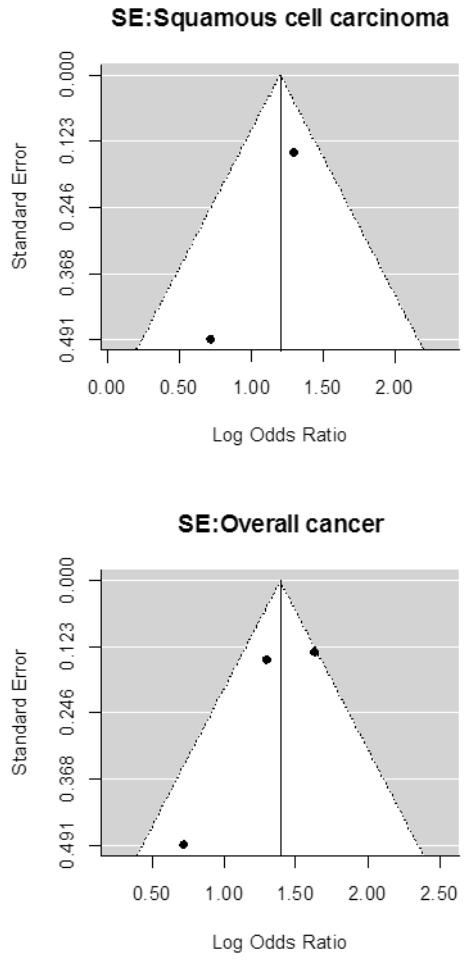


Figure 2.3: Funnel plot for serum 25(OH)D and oesophageal neoplasia

2.5.1.3 Vitamin D Intake

Four studies examined the risk of oesophageal cancer and pre-curser lesions and vitamin D intake: two studies examined risk of adenocarcinoma [200, 201], three examined risk of squamous cell carcinoma [158, 159, 200] and a single study examined risk of Barrett's oesophagus.

A decreased risk in squamous cell carcinoma in those with higher dietary vitamin D intake was found two studies [158, 159]. In a study of 208 squamous cell carcinoma patients and 399 controls, Launoy *et al.* found a significant protective effect of vitamin D intake over four categories (<3, 3-5, 5-7, >7 µg/d, p-trend:<0.001) [159]. However, when comparing those with highest vs. lowest daily intake, a non-significant effect was observed (OR=0.70, 95%CI: 0.32-1.54). Similarly, Lipworth *et al.* found a protective effect of high vitamin D intake on the risk of squamous cell carcinoma (OR=0.58, 95%CI: 0.39-0.86) in an Italian case-control study comprising 304 cases and 743 controls with those with the lowest tertile as reference. The association was strengthened among heavy smokers (OR=8.7, 95%CI: 4.1-18.7) and alcohol consumers (OR=41.9, 95%CI: 13.7-128.6) with those with the highest tertile as reference [158]. Conversely, in a case-control study conducted in USA, no significant associations were found for squamous cell carcinoma (n=206 [cases], OR=1.00, 95%CI: 0.74-1.36). Similar findings were observed in adenocarcinoma (n=282 [cases], OR =1.10, 95%CI: 0.86-1.40) [200]. Contrastingly, an all-Ireland study found an increased risk of oesophageal adenocarcinoma was associated with higher vitamin D intake levels in 218 adenocarcinoma cases (OR=1.92, 95%CI: 0.98-3.76) [201] but not pre cursor lesions; as no association was found in 212 Barrett's oesophagus cases (OR=1.18, 95%CI: 0.61-2.29) [201].

No associations were observed between vitamin D intake and risk of neoplastic lesions of the oesophagus in the meta-analysis (OR=1.03, 95%CI: 0.65-1.42). In a subtype-specific meta-analysis, no significant associations were found for adenocarcinoma (OR=1.45, 95%CI: 0.65-2.24) or squamous cell carcinoma (OR=0.80, 95%CI: 0.48-1.12, **Figure 2.4**).

Limitations for each of the studies included in this meta-analysis are present. Most noticeably, none of these studies collected information on vitamin D supplementation, which has a major contribution to the vitamin D status of individuals choosing to take these [157] and the concentration in these supplements are typically multiple times higher than the vitamin D which is found in food. Without the inclusion of dietary supplements these studies are blind to the most significant dietary source of vitamin D.

Furthermore, there were other specific limitations in each of the studies. Launoy *et al.* for example, looked at various aspects of the diet and did not specifically focus on vitamin D in their

research. As such, the FFQ might not have been tailored to maximise information on vitamin D; this is particularly relevant since food sources of vitamin D are scarce. Indeed, no information on vitamin D or fish oils supplements were collected.

Additionally, hospital controls were used in this study but no other information is given about them which could have impacted the results e.g. these patients may have had vitamin D related conditions such as osteoporosis or inflammation, which are associated with poorer vitamin D status [213]. Similarly in the study by Mayne *et al.* hospital patients who had been admitted for an acute illness were used as controls. However, the majority of these patients were patients with trauma (mostly sprains and fractures) (65%) and orthopaedic disorders (55%). Due to the well-established link between vitamin D and bone health, it may be possible that the control patients in this study could have had particularly low vitamin D concentrations, as lower vitamin D is associated with falls, fractures and osteoporosis [214, 215]. In addition, mean vitamin D intake of the control or patient groups were not given in this study so comparison with other studies is difficult.

In the Irish study by Mulholland *et al.*, participants were asked to relay their dietary habits over a 12 month period, five years prior to their interview which could have led to recall bias. There was also a modest differential between the highest (>3.3 μg/d) versus the lowest intake groups (<2.1 μg/d) in this study and even the "high group" has quite a low concentration of vitamin D recorded compared to other studies, with >7 μg/d being used as the high category in other studies [159].

The limitations above are some of the reasons why these studies scored poorly in the Newcastle-Ottawa scoring system. The use of hospital controls and lack of adjustment for supplementation in almost all studies led to the inferior scores when compared to studies which measured 25(OH)D. Limitations also exist within the meta-analysis procedure; for example, there was significant heterogeneity found when examining oesophageal adenocarcinoma (p-value=0.02) and all cancer types (p-value=0.01). Furthermore there was some evidence of publication bias when investigating the relationship in adenocarcinoma as can be observed form the funnel plots in **Figure 2.5.** This limits the interpretability of the results.

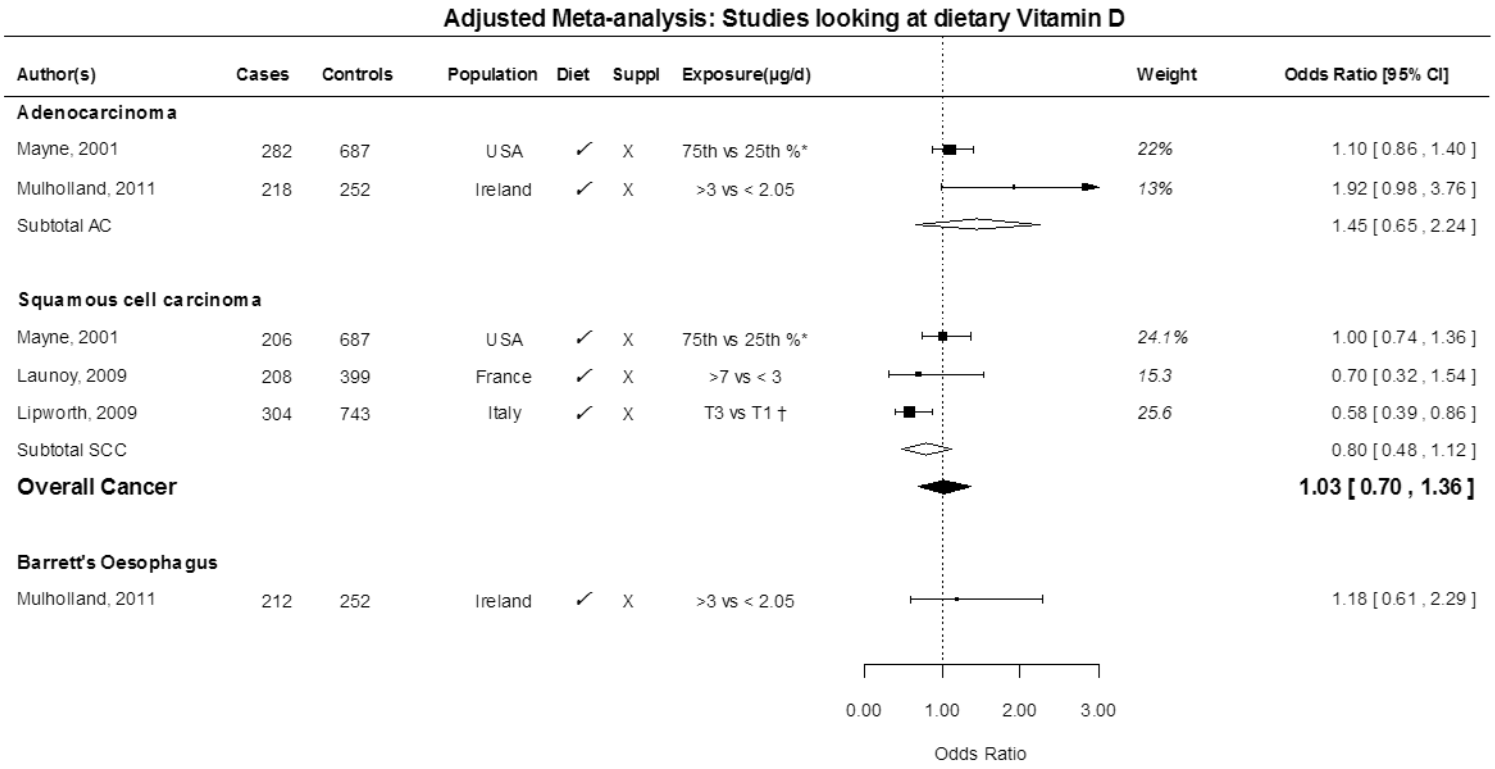


Figure 2.4: Meta-analysis of studies looking at dietary vitamin D intake and oesophageal cancer risk

Meta-analysis of studies looking at dietary vitamin D intake and oesophageal neoplasia using adjusted OR estimates. Adenocarcinoma (AC): I^2 = 80%, Q-value=5.07, p-value= 0.024. Squamous cell carcinoma (squamous cell carcinoma) I^2 = 38%, Q-value=2.8, p-value= 0.24. Overall Cancer I^2: 77%, Q (df = 5) = 12.75, p-value = 0.012. * 75th vs 25th percentiles: Mean vitamin D intake for each quartile not specified in paper, † T1 vs T3: the 33rd and 67th percentiles of vitamin D reported in this paper were 2.51 and 3.51µg/d respectively.

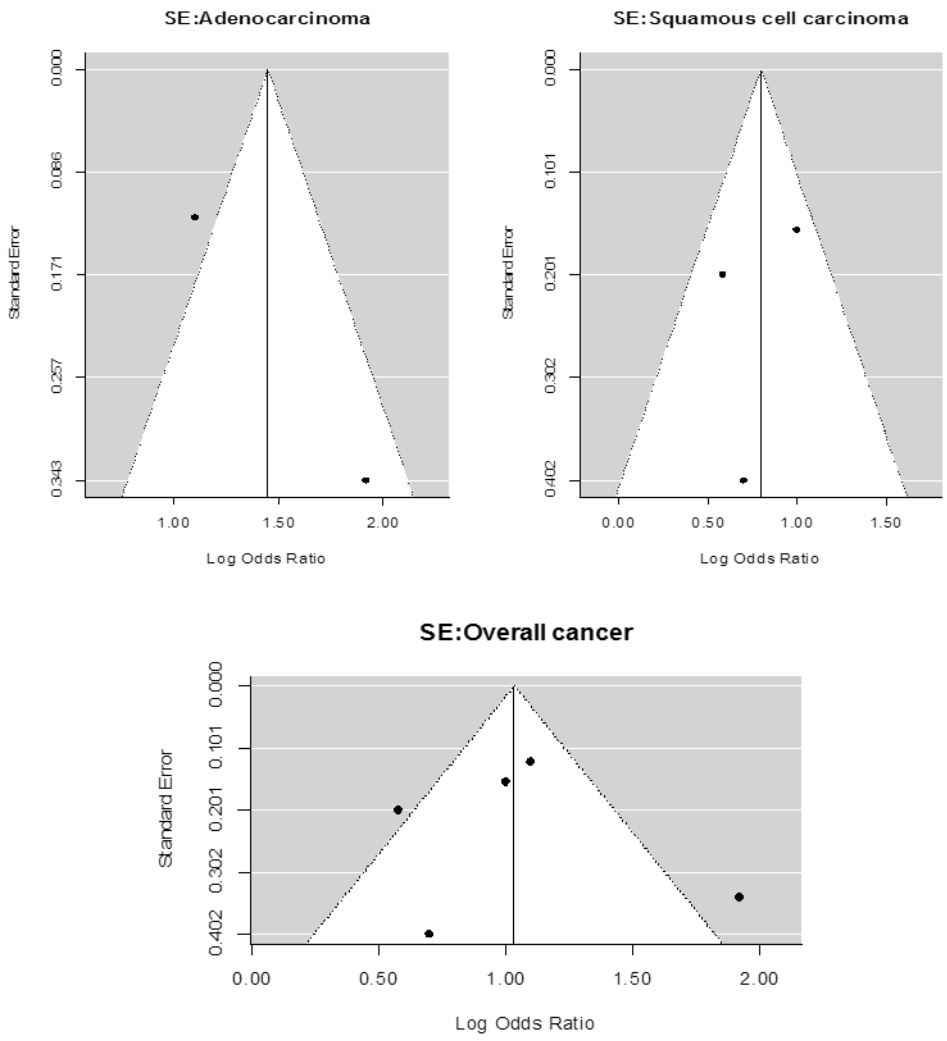


Figure 2.5: Funnel plot for vitamin D intake and oesophageal neoplasia

2.5.1.4 UVB radiation

One study examined the risk of oesophageal cancer and ambient UV radiation. In this study, Tran *et al.* measured lifetime UV radiation exposure in an Australian population-based case-control study [209]. This study used average ambient UV radiation in conjunction with ozone measurements and place of residence to determine lifetime cumulative UV dose and average lifetime daily UV radiation dose for each participant. It found that individuals with the highest tertile of mean lifetime daily ambient UV radiation exposure had a significantly reduced risk of oesophageal adenocarcinoma and oesophago-gastric junctional tumours. Some risk reduction was also suggested for squamous cell carcinoma, although this was not consistently shown between two cancer sites. As only one study was found in the systematic review a meta-analysis on the topic could not be carried out, however a summary OR for the overall cancer was calculated (**Figure 2.6**). A significantly decreased risk in oesophageal cancer was determined when comparing high lifetime UV radiation exposure to low UV radiation exposure (OR=0.63, 95%CI: 0.36-0.91).

Limitations for this study are two-fold, firstly the ambient UV radiation estimate was not adjusted for cloud cover which is known to impact the amount of UV radiation reaching the earth's surface. Secondly, this study was unable to account for personal factors which affect vitamin D synthesis such as time spent outside, clothing worn and sun screen use.

One benefit to using ambient UV radiation however, is that these results are unlikely to be confounded by lifestyle factors. Some argue that the relationship which has been found for vitamin D and various health outcomes, such as cancer and cardiovascular disease, is confounded by healthy lifestyle. This theory suggests that a healthy diet, BMI level and moderate physical activity are not only highly correlated with vitamin D concentration but also with a reduction in the health outcome in question. As individuals who lead a healthy lifestyle are likely to spend time outdoors doing exercise, may have a greater interest in their health and take supplements or eat healthy sources of vitamin D; these individual may have a higher 25(OH)D concentration. Moreover, these individuals may also be less likely to develop conditions such as cancer and cardiovascular disease because they lead a healthy lifestyle. The opposite is also important as those who are overweight have been shown to have poorer health outcomes as well as a lower 25(OH)D concentrations, which is also a confounding factor in the relationship between vitamin D and health outcomes. Some argue that this healthy lifestyle is a much more important factor in the reduction of risk of various conditions, and higher vitamin D exposure is just a marker of this. However, as UV radiation is independent of these "Healthy

lifestyle" factors, it is beneficial when determining if vitamin D concentration does impact oesophageal cancer risk, without the risk of confounding.

In addition, it is possible to estimate ambient UV radiation exposure for longer periods in a person's life, while only a single measurement of 25(OH)D is typically available, and as these measurements are highly seasonal, the UV radiation measurement could give a more accurate estimate of a person's vitamin D status over a longer period.

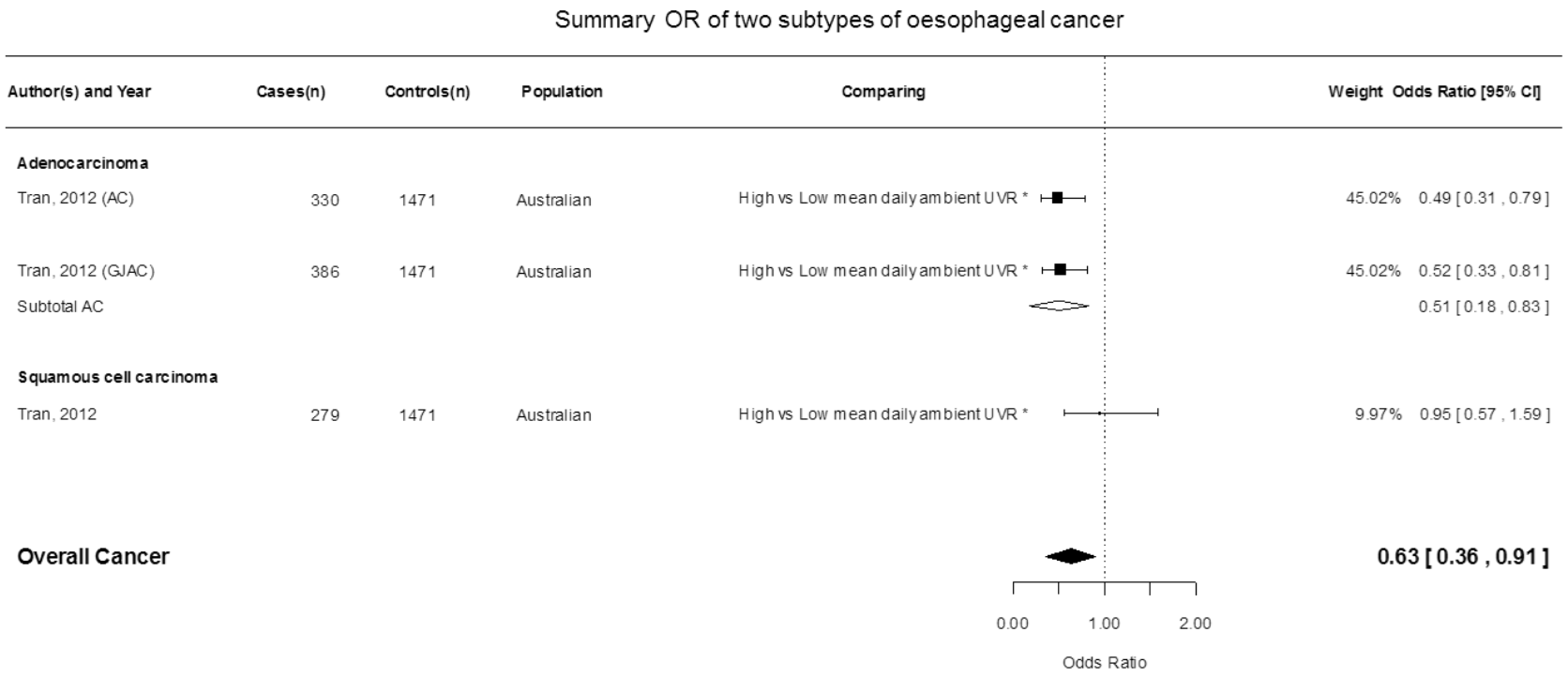


Figure 2.6: Summary OR of oesophageal cancer subtypes in Tran study examining UVB and oesophageal cancer risk

Meta-analysis of studies looking at UVB and oesophageal neoplasia Adenocarcinoma (AC= adenocarcinoma and GJAC= gastric junction adenocarcinoma) I^2: 0%, Q (df = 2) = 0.0081, p-value = 0.92, Overall I^2: 39%, Q (df = 2) = 1.64, p-value = 0.199. * OR values used were mean daily ambient UVR levels which is an age corrected measure of ambient UVR expose, Cumulative lifetime ambient UVR tertiles from all cases and controls were: Highest third: 83,176,460 J/m², Lowest third 66,678,230 J/m².

2.5.1.5 Genetic variants and expression

Seven studies were found from the systematic search. These investigated VDR polymorphisms, molecular expression, or other vitamin D related polymorphisms; five of which examined VDR polymorphisms [203, 205-208], one of which investigated VDR expression [204] and one of which considered vitamin D-related genetic variation [210].

When examining VDR polymorphisms and oesophageal cancer risk, no significant association was found in three studies [203, 205, 207]. Li *et al.* examined the effect of VDR TaqI (rs731236) genotypes and the risk of squamous cell carcinoma and squamous dysplasia in a Chinese population. No association was found for either condition [207]. Similar results were found for the majority of the 27 VDR polymorphisms examined by Chang *et al.* when investigating adenocarcinoma risk in an Irish case-control study. However, some suggestive evidence was observed for two single nucleotide polymorphisms (SNPs); rs2238139 and rs2107301 (OR=0.26, 95%CI: 0.07-0.93 and OR=0.19, 95%CI: 0.06-0.67) [203]. The largest study to date (629 cases) was carried out by Gu *et al.* and it examined four VDR polymorphisms (rs2107301, rs2228570, rs1989969 and rs11568820) in a Chinese population. No association was found between selected SNPs and risk of squamous cell carcinoma, although some suggestive evidence was reported for rs2107301 in patients who were drinking alcohol [205].

The final two studies investigating VDR polymorphisms, were carried out in the same Dutch cohort. Janmaat *et al.* report different distributions of two SNPs in healthy controls versus cases: protective alleles were T for rs1989969 and G for rs2238135 variant. For individuals carrying the rs1989969/rs2238135 haplotype T/G, a two-fold reduction in risk was reported for adenocarcinoma (OR=0.50, 95%CI: 0.27-0.96), and Barrett's oesophagus, (OR=0.46, 95%CI: 0.26-0.80). These results were replicated for Barrett's oesophagus in a separate cohort (OR=0.44; 95%CI: 0.23-0.85) [206]. Van den Winkel *et al.* examined the same haplotype and found a reduced risk of developing squamous cell carcinoma in patients with T/G haplotype (OR=0.43, 95%CI: 0.19-0.97) [208].

It has been hypothesized that VDR expression can regulate apoptosis, which in turn is important for the transformation of Barrett's oesophagus into adenocarcinoma [216]. It has also been previously reported that high expression of VDR can impact disease progression and overall survival of prostate, colon and breast cancers [44, 46, 217]. In the study in which VDR expression was investigated, De Gottardi *et al.* found no difference in VDR expression between oesophageal biopsies from patients with normal mucosa, Barrett's oesophagus or adenocarcinoma, however the sample size for this study was extremely small (n=6 for each condition) [204].

Finally, Wang *et al.* hypothesised that genetic variants associated with vitamin D status may affect the risk of oesophageal neoplasia via their effect on circulating vitamin D concentrations. 12 SNPs which had previously been found to modify vitamin D status through a European GWAS study [204] were tested for their association with the risk of squamous cell carcinoma. This was a large Chinese study comprising just over 4,000 participants (n=4,053; n=1,942 cases), however, no significant associations were found between any of these SNPs and the risk of squamous cell carcinoma [210]. One of the limitations to this study however was the assumption that SNPs from European ancestry will affect vitamin D status from a Chinese population in the same way; there have been studies which have reported different genetic effects between populations. Another limitation from this study is that the SNPs chosen may have only a weak relationship with 25(OH)D and this is why no significant results were observed [211].

The meta-analysis conducted for these studies was limited due to the diverse SNPs used and cancer subtypes studied as well as the small effect size's found for these SNPs. A suggestive association for oesophageal cancer in those with T/G in rs2107301 was found (OR= 0.68 95%CI: 0.32-1.04) (**Figure 2.7**). Contrary to this however, previous articles have found an increased risk, or no association for other cancer types in variant rs2107301 T/G [218, 219]. No association was found for risk of oesophageal cancer and FokI (rs2228570), rs2238135 or TaqI (rs731236) for crude or adjusted estimates (**Figure 2.7, 2.8**). A significant decreased oesophageal cancer risk was found for Haplotypes rs1989969/rs2238135 T/G variants when the two Dutch studies were combined (OR=0.45, 95%CI: 0-0.91) (**Figure 2.7**). In accordance with this, previous studies have also reported a reduced risk in other cancers for individuals who have the more common G allele, when compared with those with the rarer C allele for variant rs2238135 [218]. No evidence of publication bias or heterogeneity was found in this meta-analysis (**Figure 2.9, 2.10**), however as there are only a small number of studies included in this review, caution is advised when interpreting results.

One of the main advantages in examining genetic components in relation to disease is the fact that they can be investigated independently of lifestyle factors such as weight, diet or time spent outdoors. Another benefit to using genetic data is that it is present throughout a person's life and is unchanging; this is unlike other factors which influence vitamin D status such as diet, UVB or age. However limitations to these types of studies exist, for example large population sizes are needed in order for enough statistical power to find an effect. The studies included in this review suffer from this limitation as the sample sizes are moderate to small (median number of cases was 141). Additionally, many of these studies failed to adjust for important cofounders such as age, sex, and BMI which could have impacted the results.

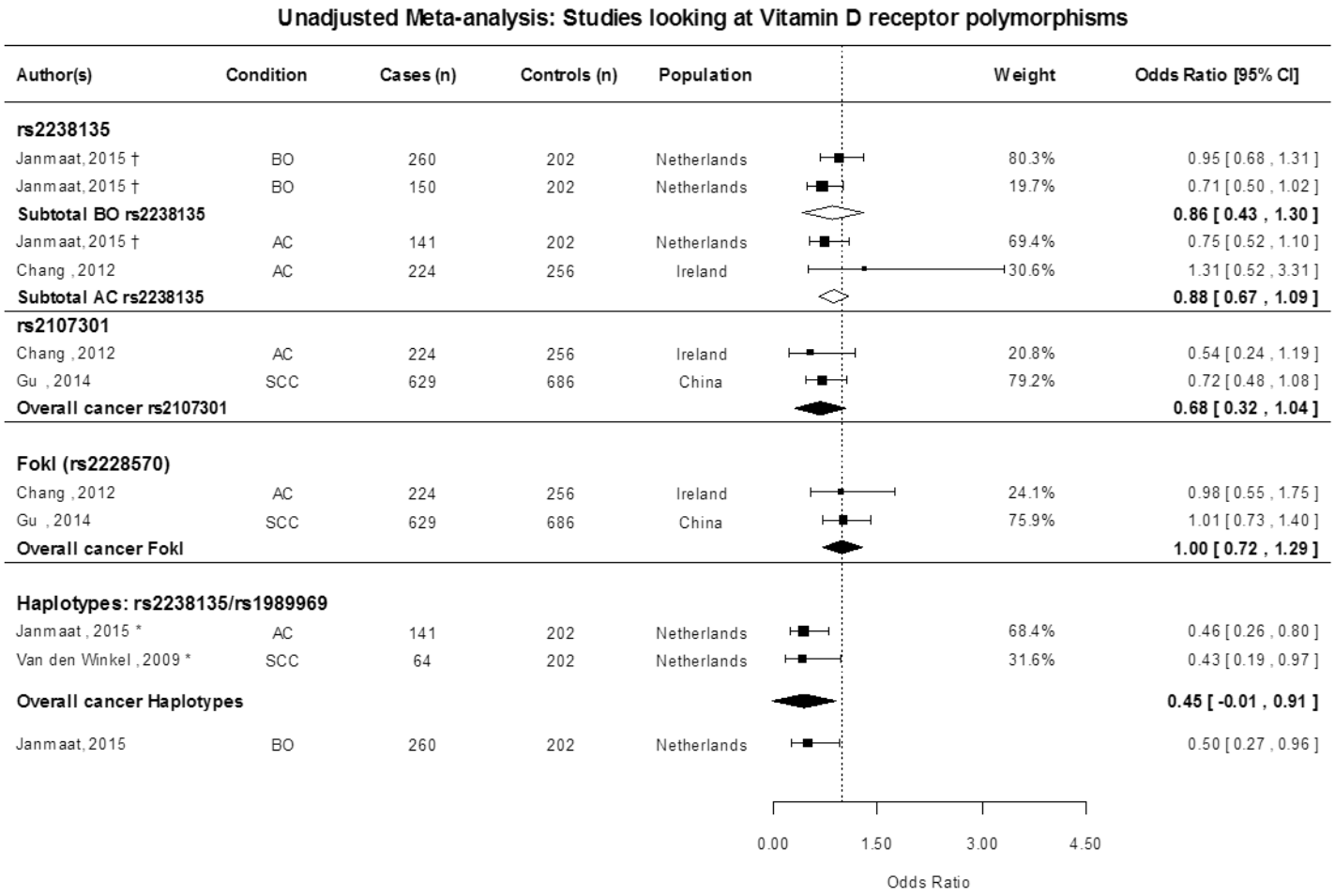


Figure 2.7: Meta-analysis of studies looking at selected VDR polymorphisms and oesophageal cancer risk

Meta-analysis of studies looking at selected VDR polymorphisms and oesophageal neoplasia using crude OR estimates. BO: Barrett's oesophagus, AC: adenocarcinoma, squamous cell carcinoma: squamous cell carcinoma.† OR values calculated from Allele frequencies given in paper, # Replication Barrett's oesophagus cohort used in paper, * same control cohort used in these studies. Weights are shown for overall cancer. rs2238135: Overall I^2: 0%, Q (df = 3) = 2.45, p-value=0.49; rs2107301: Overall I^2: 0%, Q (df = 1) = 0.16, p-value=0.69; FokI: Overall I^2: 0%, Q (df = 1) = 0.008, p-value=0.93; haplotypes: Overall I^2: 0%, Q (df = 1) = 0.0036, p-value=0.95.

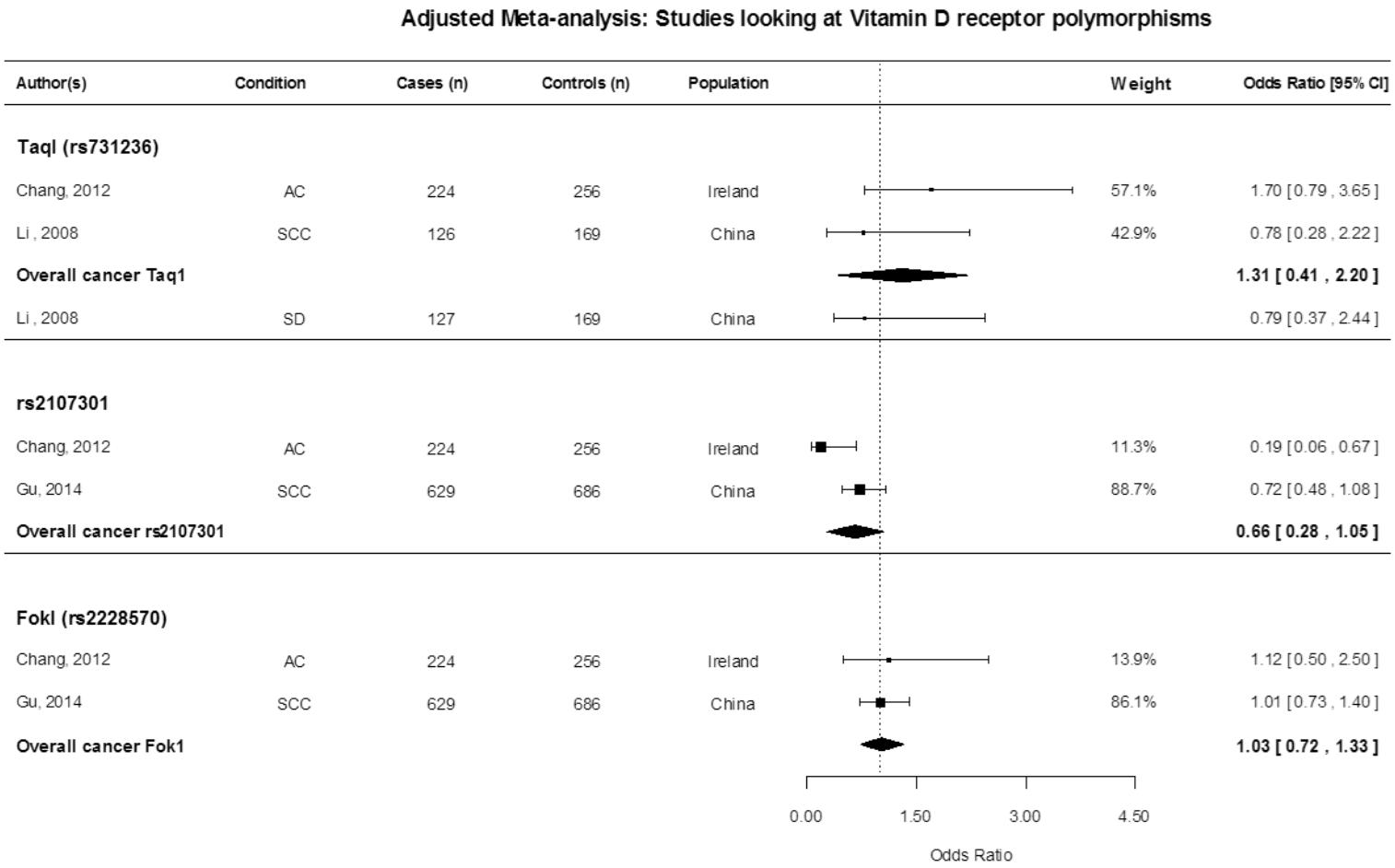


Figure 2.8: Meta-analyses of studies looking at selected VDR polymorphisms and oesophageal cancer risk

Meta-analyses of studies looking at selected VDR polymorphisms and oesophageal neoplasia using adjusted OR estimates. SD: Squamous dysplasia, AC: adenocarcinoma, squamous cell carcinoma: squamous cell carcinoma. Weights are shown for overall cancer. TaqI: Overall I^2: 50%, Q (df = 1) = 1.98, p-value = 0.16; RS2107301: Overall I^2: 0%, Q (df = 1) = 0.73, p-value = 0.39; FokI: Overall I^2: 0%, Q (df = 1) = 0.06, p-value = 0.80.

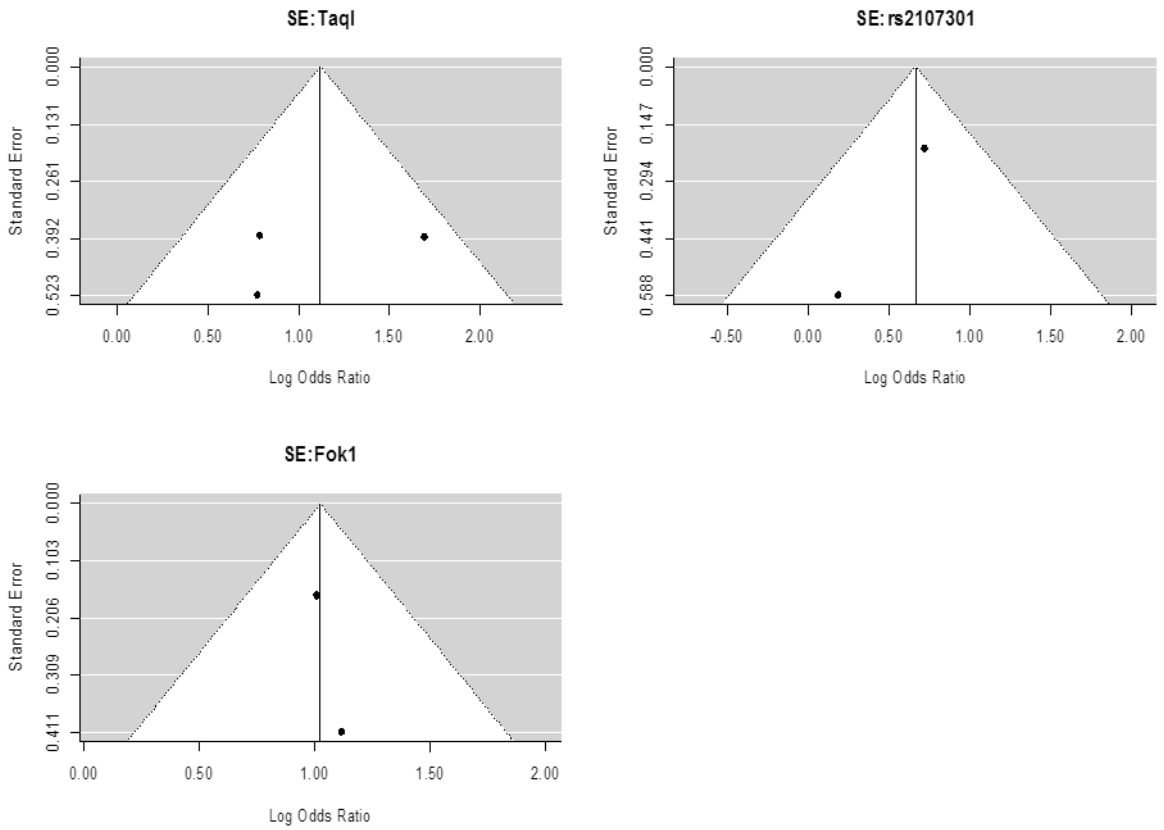


Figure 2.9: Funnel plot for adjusted VDR expression and oesophageal neoplasia

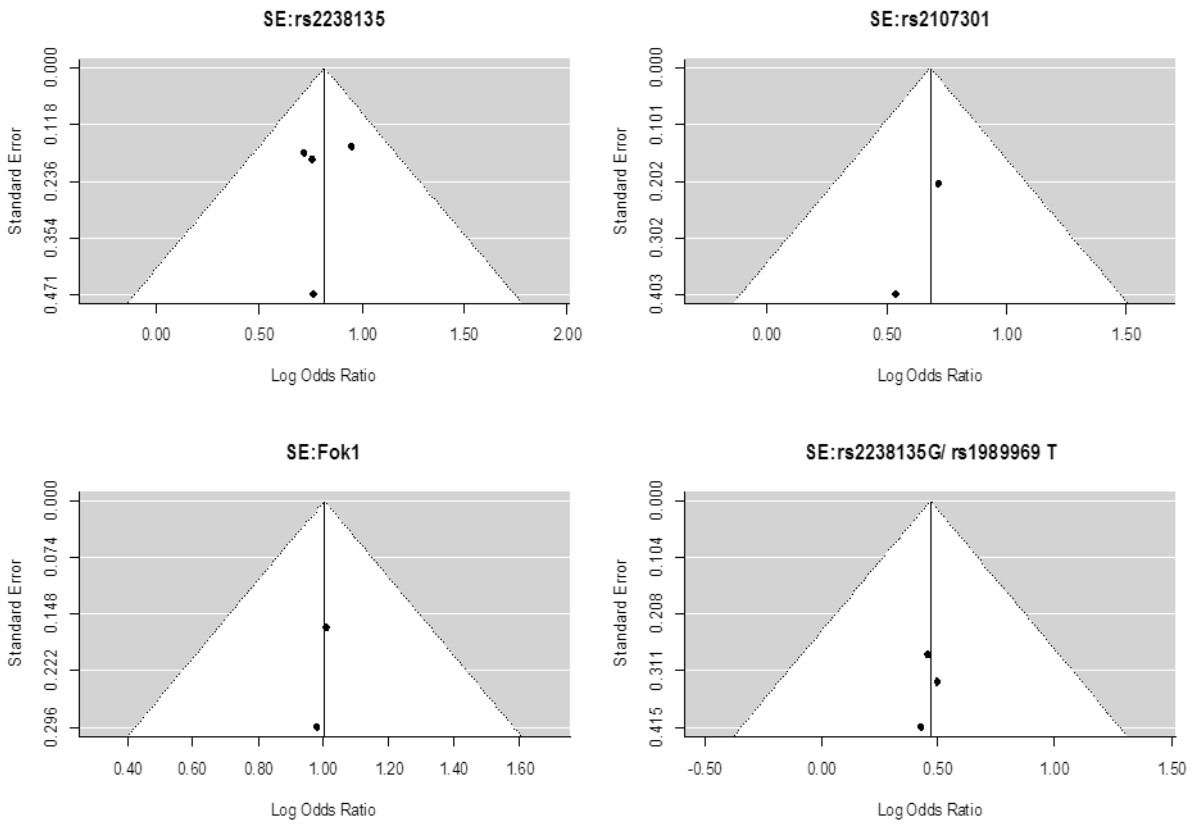


Figure 2.10: Funnel plot for unadjusted VDR expression and oesophageal neoplasia

2.5.1.6 Conclusion

The results in the systematic review and meta-analysis paint a somewhat perplexing picture on the topic of oesophageal cancer and vitamin D. From the results which have been observed, it is not possible to make definite conclusions on the topic. Different results were found depending on the exposure utilised.

The small number of studies in this review led to significant limitations in the meta-analysis methods. Publication bias and heterogeneity between studies are important factors which should be considered when interpreting results. This bias could lead to unreliable results and may be one reason why discrepancies between exposure types were found.

Furthermore, the inconsistencies may also be due to the limitations in each of the studies to capture the average long term vitamin D status for their participants or underlying population differences which were unable to be accounted for and these may also have had an impact on the results. A significant increase in oesophageal cancer when 25(OH)D was measured in three studies was noted. These studies predominately consisted of Chinese participants. This is contrary to what has been observed for the majority of other cancers. Due to the inconsistent results found during this review and meta-analysis no firm conclusions can be made.

2.5.2 Vitamin D and Survival of Oesophageal Cancer

2.5.2.1 Systematic Review

The flowchart for study selection for survival and vitamin D is shown in **Figure 2.11**. 334 titles and abstracts were first identified through the databases, and duplicates were removed (N=302). 15 full text articles were read and no studies was deemed eligible [220]. The Cochrane database was subsequently searched but no new studies were found. The absence of studies precluded advancing with the systematic review or meta-analysis.

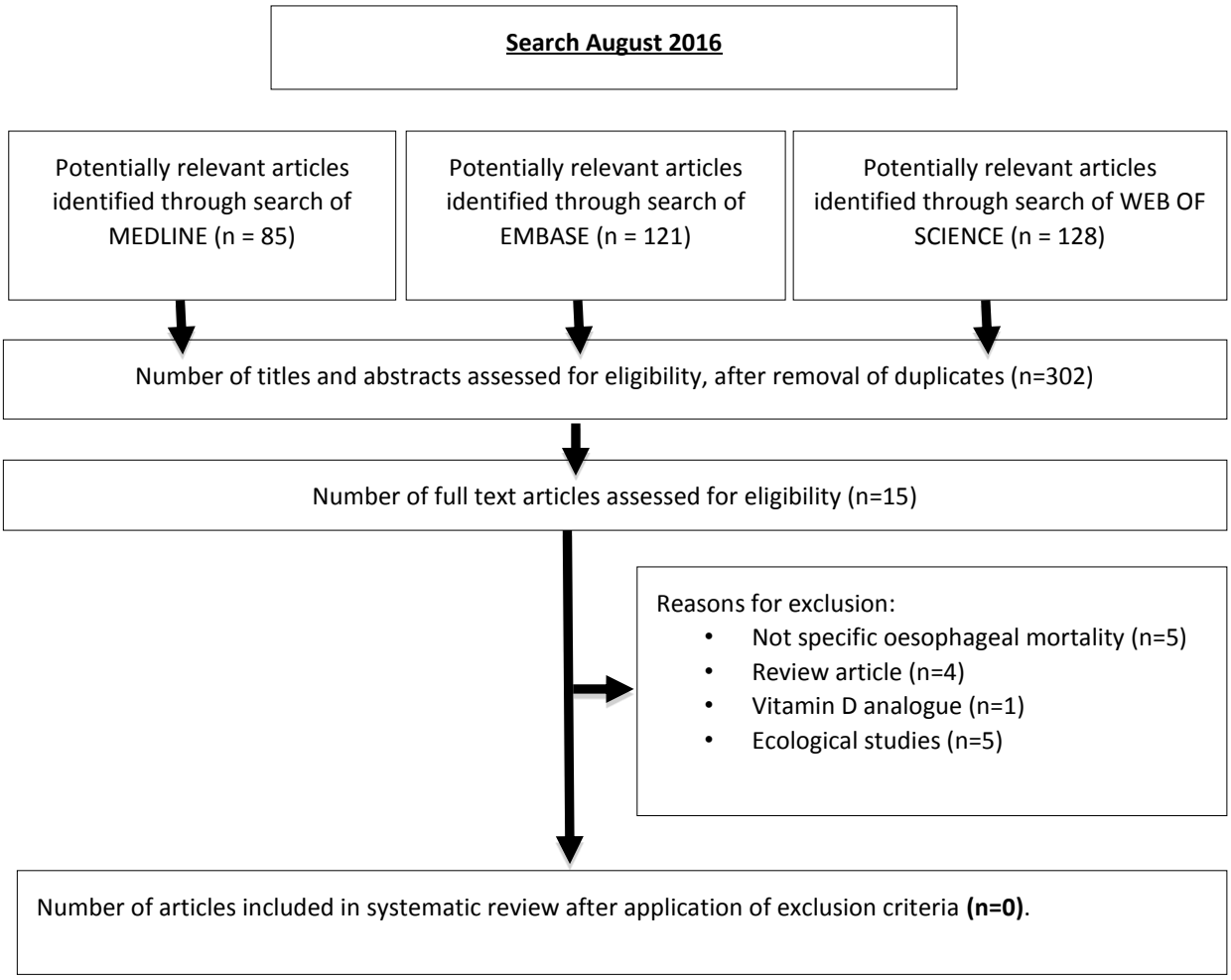


Figure 2.11: Search Strategy for Oesophageal cancer survival and Vitamin D

2.5.3 Vitamin D and Risk of Gastric Cancer

2.5.3.1 Systematic Review

The flowchart for study selection for risk of gastric cancer and vitamin D is shown in **Figure 2.12**. 1,964 titles and abstracts were first identified through the databases, and duplicates were removed (N=1,452). 512 articles were then screened and 25 articles were deemed relevant. These were read in full and eight studies were deemed eligible. The Cochrane database was subsequently searched in December 2017 but no new studies were found. These publications related to risk of gastric cancer and: 25(OH)D concentration (N=2) [8, 9], vitamin D intake (N=4) [200, 221-223], and vitamin D related genetic variants (N=2) [224, 225] as outlined in **Table 2.2**.

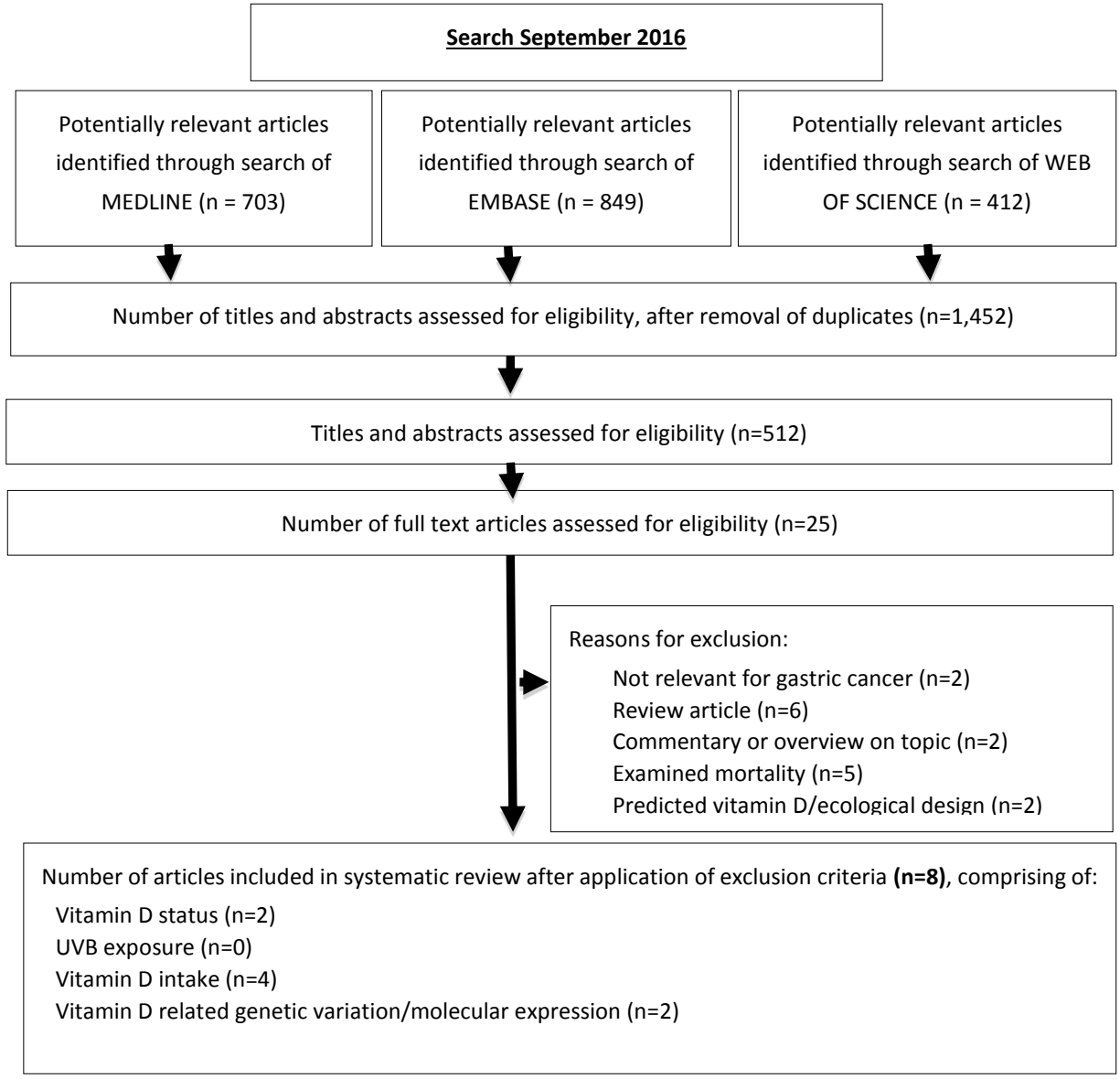


Figure 2.12: Search Strategy for Gastric cancer risk and vitamin D

Table 2.2: Characteristics of studies of vitamin D related exposures and the risk of gastric cancer [1,2].

Study and location	Study design	NO score	Study location	Vitamin D exposure	Outcomes	Cases	Controls/ Cohort	Adjusted confounders: Age	Sex	Energy	BMI	Smoking	Alcohol	NSAIDs	FH	Education	SE Status	Sun Ex	Occup	Race
VITAMIN D STATUS																				
Chen *et al.* (2007)	Case-cohort	8	China	Serum 25(OH)D	Cardia Adenocarcinoma	353	1,086	✓	✓		[3]✓	✓	✓							
					Non-cardia Adenocarcinoma	81	1,096	✓	✓		[3]✓	✓	✓							
Abnet *et al.* (2010)	Nested case-control	8	China, Finland, USA	Serum/ plasma 25(OH)D	Any GA cancer[3]	183	202	✓	✓		[3]✓	✓	✓			✓				✓
					Cardia Adenocarcinoma	34	40	✓	✓		[3]✓	✓	✓			✓				✓
					Non-cardia Adenocarcinoma	103	115	✓	✓		[3]✓	✓	✓			✓				✓
VITAMIN D INTAKE																				
Vecchia *et al.* (1994)	Case-control	3	Italy	Dietary intake (29 IFQ)	Any GA cancer	723	2,024	✓	✓	✓	✓				✓	✓				
Cornée *et al.* (1995)	Case-control	4	France	Dietary History Questionnaire	Any GA cancer	92	128	✓	✓	✓									✓	
Mayne *et al.* (2001)	Population-based case-control	6	USA	Dietary intake (104-item FFQ)	Cardia Adenocarcinoma	255	687	✓	✓	✓	✓	✓	✓			✓				✓
					Non-cardia Adenocarcinoma	352	687	✓	✓	✓	✓	✓	✓			✓				✓
Pelucchi *et al.* (2008)	Case-control	5	Italy	Dietary intake (78-item FFQ)	Any GA cancer	230	547	✓	✓	✓	✓	✓	✓		✓	✓				

Study and location	Study design	NO score	Study location	Vitamin D exposure	Outcomes	Cases	Controls/ Cohort	Adjusted confounders												
								Age	Sex	Energy	BMI	Smoking	Alcohol	NSAIDs	FH	Education	SE Status	Sun Ex	Occup	Race
Shen *et al.* (2014)	Case-control	4	China	VDR polymorphisms[4]	Any GA cancer	564	564	none												
Cong *et al.* (2015)	Case-control	3	China	VDR *FokI* polymorphism	Squamous cell carcinoma	187	212	✓	✓		✓	✓	✓	✓	✓	✓		✓		

Footnote:

1 Adjusted confounders: *Energy*: Energy intake; *BMI*: Body mass index; *Reflux*: Gastro-oesophageal reflux symptoms; *Occup*: Occupation; *FH*: family history of gastric cancer; *SE Status*: Socioeconomic status; *Sun ex*: Sunshine exposure;

2 *IFQ*: indicator food questionnaire; *FFQ*: Food Frequency Questionnaire.

3 any GA cancer; any gastric cancer

4 Polymorphisms used: TaqI and ApaI

2.5.3.2 Vitamin D status

Two studies examined the role of 25(OH)D and gastric cancer risk [8, 9]. The study designs and their limitations have already been discussed in detail in relation to oesophageal cancer risk. In summary, Chen *et al.* found no association between 25(OH)D concentration and gastric cancer risk in cardia and non-cardia sub-groups, (HR_{Cardia}=1.11, 95%CI: 0.80-1.55 and $HR_{Non\text{-}Cardia}$=1.10 95%CI: 0.60-2.01) when comparing the lowest 25(OH)D quartile with the highest [9]. Similarly the cohort consortium by Abnet *et al.* found no association between gastric cancer risk and serum 25(OH)D concentration (HR=0.77, 95%CI: 0.55-1.08) when comparing those deficient (<25 nmol/L) with a sufficient subgroup (50-75 nmol/L) [8]. In the meta-analysis, no association in cardia or non-cardia gastric cancer risk was found when comparing the high vs. low concentrations of circulating 25(OH)D; (OR_{cardia}=1.06, 95%CI: 0.74-1.37 and $OR_{non\text{-}cardia}$=0.87, 95%CI: 0.51-1.24) (**Figure 2.13**). No evidence of publication bias or heterogeneity was observed in this meta-analysis (**Figure 2.14**) (overall gastric i^2= 0%, p-value=0.51)

Similar results were found for any gastric cancer (OR=0.98, 95%CI: 0.74-1.22). A previous systematic review and meta-analysis conducted on this topic found comparable results, (OR=0.92, 95%CI: 0.74-1.14), however they included another study in their meta-analysis [226]. This was omitted from this analysis as it used *predicted* 25(OH)D concentration instead of measured 25(OH)D concentration.

No association was found in this meta-analysis. This may be because association does not exist or due to the limitations of the individual studies (Chen *et al.*, and Abnet *et al.*) which have been previously outlined when discussing oesophageal cancer risk and equally impact the results presented here.

The studies in this meta-analysis scored highly in the quality assessment, however, the overall weight of the study by Chen *et al.* (50%) would have had a large influence on the summary OR and the overall conclusions of the meta-analysis. Further research is needed with studies of high quality and large sample size before conclusions can be made.

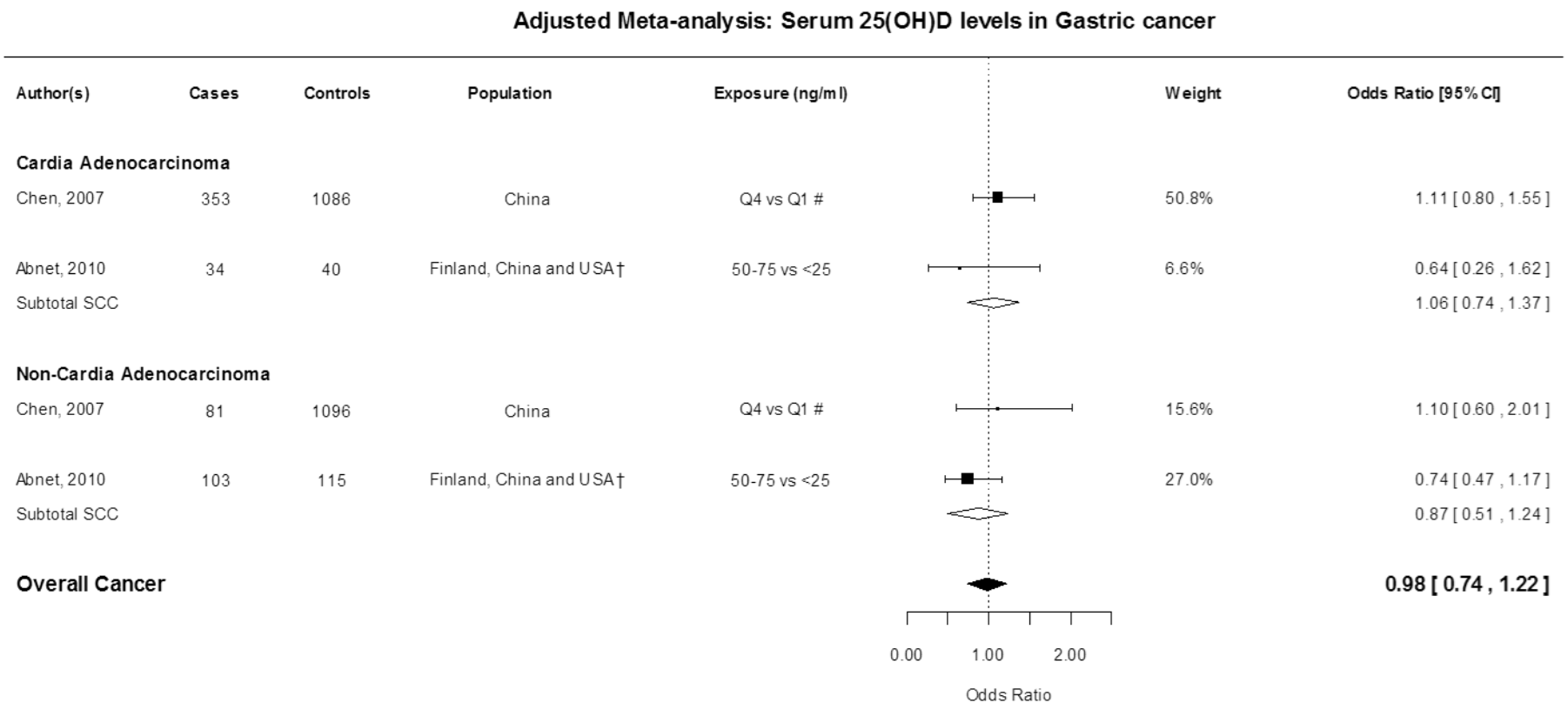


Figure 2.13: Meta-analysis of studies examining at serum 25(OH)D and Gastric cancer risk

Meta-analysis of studies examining at serum 25(OH)D and Gastric cancer risk using adjusted HR estimates. Cardia I^2 = 0%, Q-value= 0.87, p-value= 0.35. Non-Cardia I^2 = o%, Q-value=0.87, p-value= 0.35. Overall Cancer I^2: 0%, Q (df = 3) = 2.30, p-value = 0.51. Weights are shown for overall cancer. † Total number of Upper gastrointestinal cancer cases from each study given for the Cohort Consortium: Finland: 416, China: 313, USA: 296. #Geometric means of vitamin D concentrations per quantiles: Chen *et al.* (2007): 25th: 19.9 nmol/l, 75th: 57.2 nmol/l.

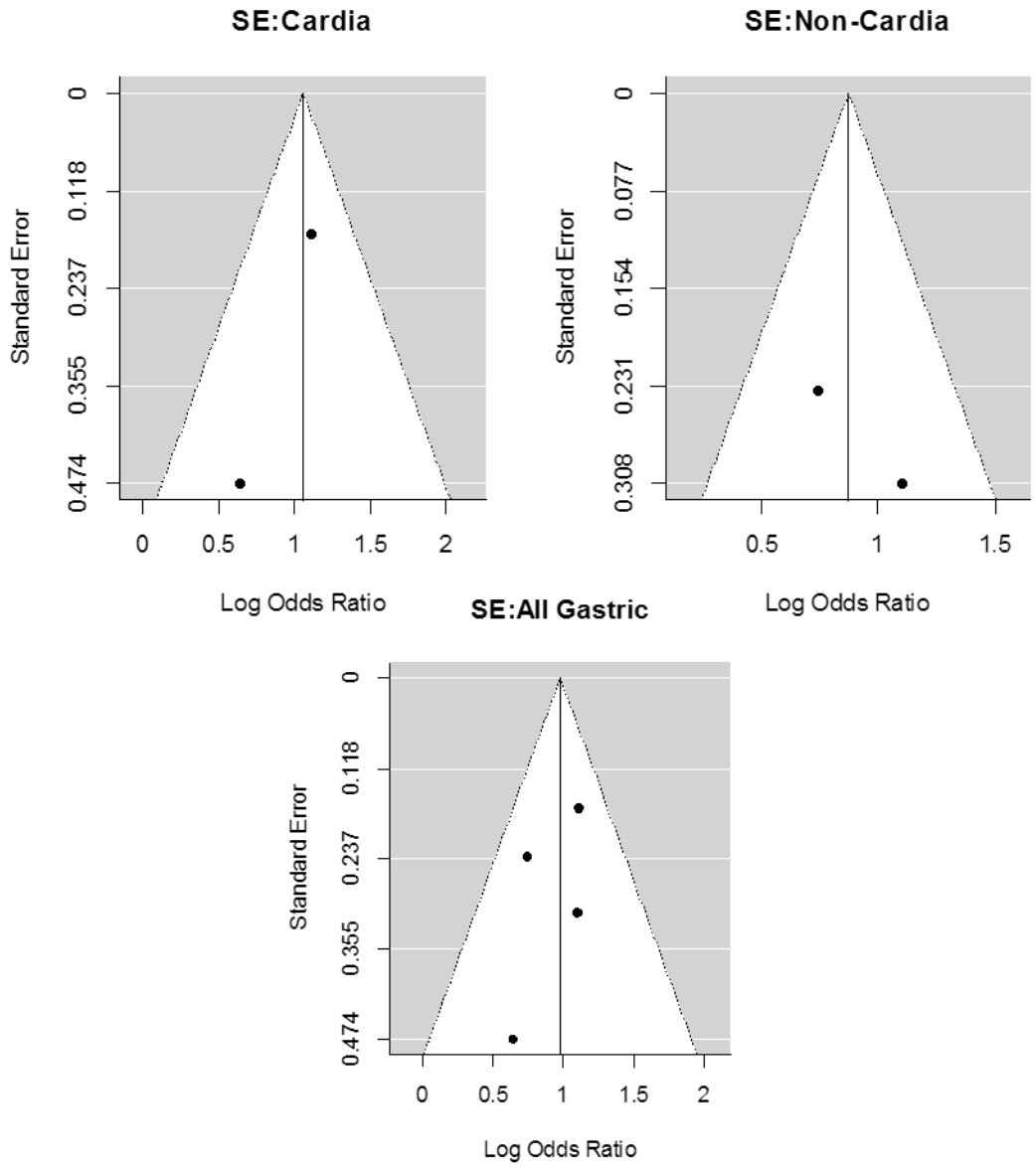


Figure 2.14: Funnel plots for studies examining serum 25(OH)D and Gastric cancer risk

2.5.3.3 Vitamin D intake

There were four studies which examined the association between vitamin D intake and risk of gastric cancer [200, 221-223].

In the first of these La Vecchia *et al.* examined the risk of gastric cancer and intake of a number of micronutrients. This study recruited individuals from 1985-1992 and found no significant trend between quintiles of vitamin D intake. However, when examining those in Quintile five vs Quintile one a suggestive positive association was found (OR=1.35, 95%CI: 1.00-1.83) [223].

In accordance with this, the next study to examine the issue found no association, although sample sizes were small (N_{cases}=92, OR=1.20, 95%CI: 0.60-2.37) [222]. Similarly, in the case-control study by Mayne *et al.*, no link between dietary intake of vitamin D and risk of gastric cancer was found (OR_{cardia}=1.05, 95%CI: 0.81-1.36, and $OR_{non\text{-}cardia}$ =0.92, 95%CI: 0.72-1.16) [200]. This trend was also observed in an Italian study by Pelucchi *et al.*, where 230 cases and 547 controls were examined, (OR=1.33, 95%CI: 0.80-2.21).

When a meta-analysis was conducted no association between vitamin D intake and gastric cancer risk was found (OR=1.12, 95%CI: 0.94-1.30) (**Figure 2.15**). A previous meta-analysis on the topic, which contained the same studies found very similar results OR=1.09, 95%CI: 0.94-1.25). The results may differ from here due to a difference in random effects model employed in the study [226]. This previous meta-analysis also combines serum 25(OH)D and dietary intake of vitamin D together to find an overall effect of vitamin D on gastric cancer risk. It is inappropriate to combine the two different exposures in a meta-analysis, due to the inherent differences between them therefore no such combined analysis was carried out. When examining heterogeneity between studies, no significant I^2 values were found. However, there was some evidence of publication bias with these studies as funnel plots were found to be asymmetrical (**Figure 2.16**).

Limitations for each of the studies in this meta-analysis are evident however, notably, none of these studies collected information on vitamin D supplementation, which makes a major contribution to vitamin D status, especially in high latitude countries [157]. As mentioned previously, limitations of the study by Mayne *et al.* included using hospital patients (who had orthopaedic disorders) as the control group.

Similarly in the study by Vecchia *et al.* and Pelucchi *et al.,* 20% and 23% respectively, of controls were orthopaedic patients, which may bias the control group to lower vitamin D due to the link between bone disorders and low vitamin D. In addition, all of the above studies examined various nutrients and minerals from the diet and were not primarily focused on vitamin D and

therefore their food frequency questionnaires may not have been adequately tailored to assess the dietary vitamin D sources. As natural sources of vitamin D food are scare and levels within these food sources are low, dietary vitamin D often comes from fortified foods such as milk and cereals, which were not included in this questionnaire and the lack of inclusion of these products may have caused an underestimation of vitamin D. These are some of the reasons why the above studies scored poorly in the quality assessment.

2.5.3.4 Genetic variants and expression

There were two studies which examined the association between vitamin D related genetic variants and risk of gastric cancer [224, 225]. Shen *et al.* was the first to examined VDR variants and gastric cancer risk. This case-control study of 564 individuals investigated the VDR variants *TaqI* and *ApaI* and found no associations (OR=1.89, 95%CI: 0.27-2.83, and OR=1.04, 95%CI: 0.87–1.25) [227]. Similarly in a study by Cong *et al.*, no associations were found when investigating VDR *FokI* polymorphism [224]. Sample size in this study was small however (cases=187). A meta-analysis could not be carried out as there were no studies which examined this relationship in the same VDR polymorphism. The quality of these studies were also poor as little information was given about the recruitment of the case and control groups and particularly in the study by Shen *et al.* no adjustment for confounders was carried out.

2.5.3.5 Conclusions

Overall, there is no evidence of an association between gastric cancer risk and vitamin D from all exposures tested to date. This could be attributed to the insufficient research carried out in this area. There was only one individual study which found a marginal positive association between vitamin D intake and risk (La Vecchia *et al.*) but overall there was no evidence of an association. Additionally, a number of these studies had limitations which could have contributed to the null finding.

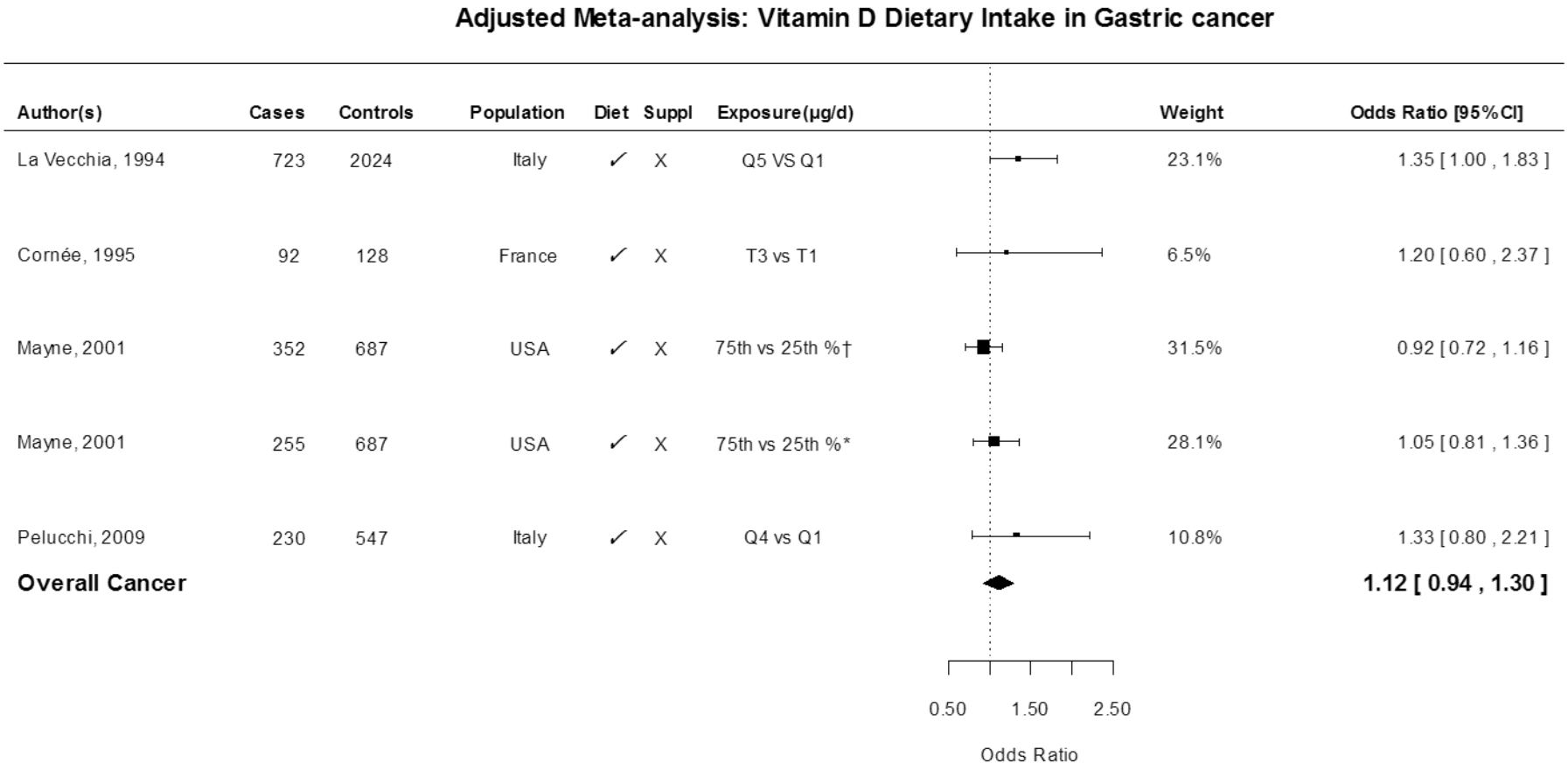


Figure 2.15: Meta-analysis of studies examining at dietary vitamin D and Gastric cancer risk

Meta-analysis of studies examining at dietary vitamin D and gastric cancer risk using adjusted HR estimates. Overall Cancer I^2: 32.4%, Q (degrees of freedom (DF) = 3) = 5.91, p-value = 0.21. † Cardia adenocarcinoma, *non-cardia adenocarcinoma. Q5 vs Q1: quintile 5 vs quintile 1; 75^{th}-25^{th}%: 75^{th} vs 25^{th} percentile; T3 vs T1; Tertile 3 vs tertile 1; Q4 vs Q1; quartile 4 vs quartile 1.

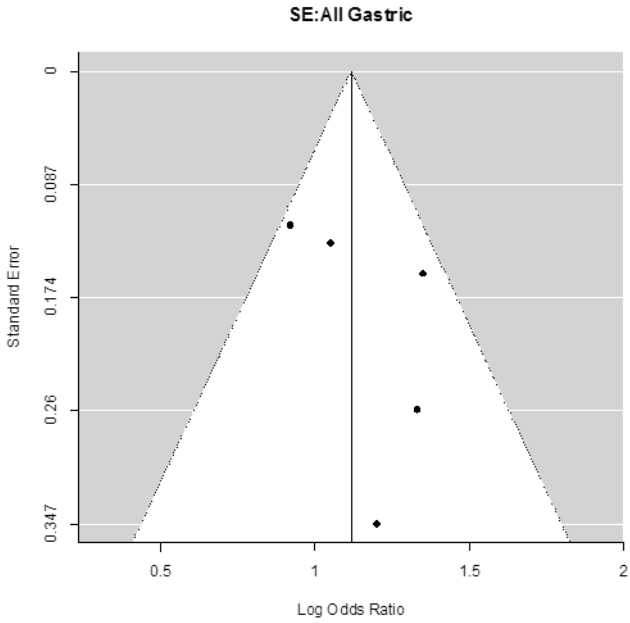


Figure 2.16: Funnel plots for studies examining dietary vitamin D and Gastric cancer risk

2.5.4 Vitamin D and Survival of Gastric Cancer

2.5.4.1 Systematic Review

The flowchart for study selection for survival of gastric cancer and vitamin D is shown in **Figure 2.17**. 335 titles and abstracts were first identified through the databases, and duplicates were removed (N=310). The Cochrane database was subsequently searched in December 2017 but no new studies were found. 19 full text articles were read and one study was deemed eligible. This publication related to risk of gastric cancer and 25(OH)D concentration (N=1) [139, 228] as outlined in **Table 2.3**.

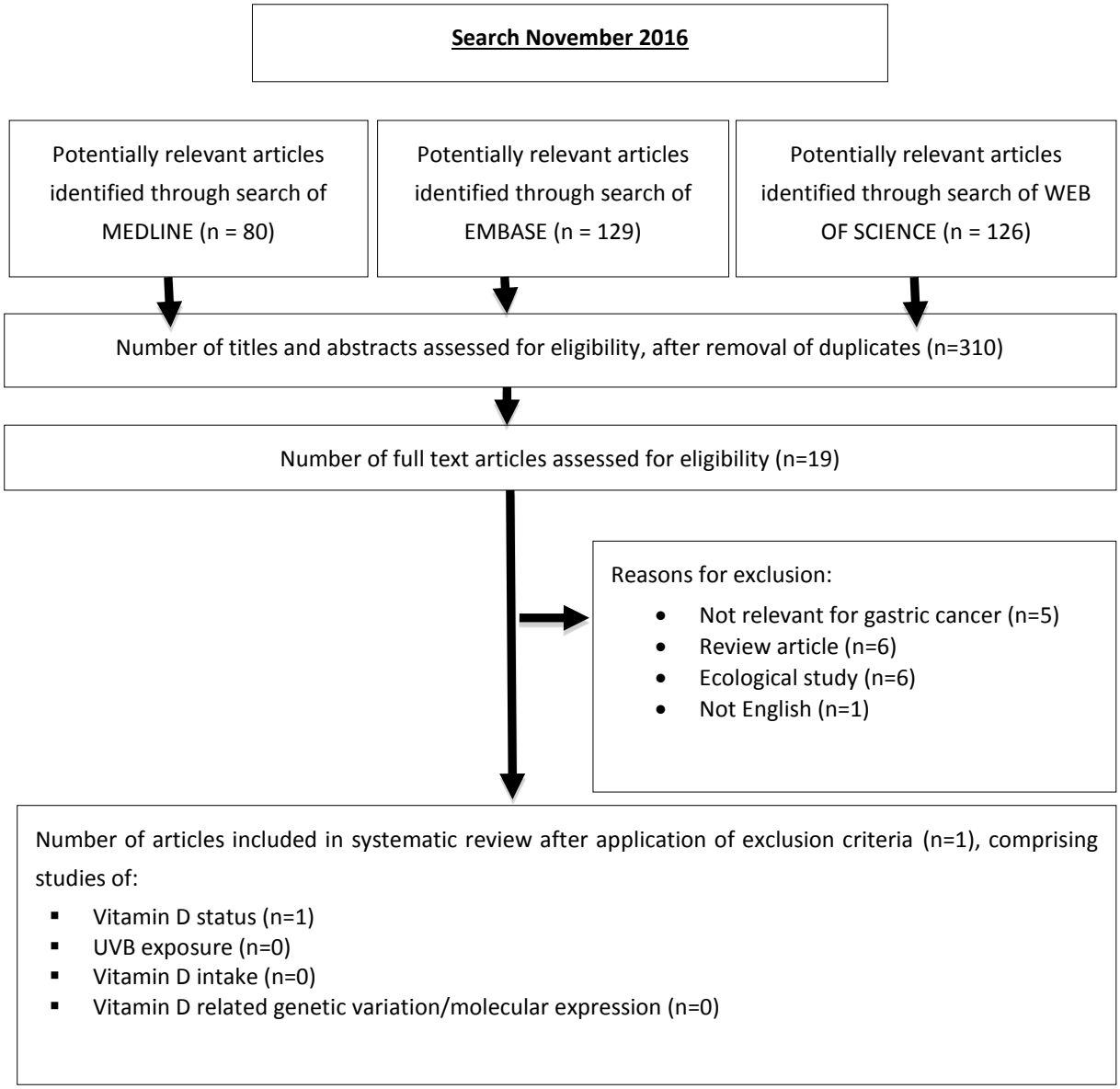


Figure 2.17: Flow-chart of study selection for vitamin D and survival of gastric cancer systematic review[1].

Table 2.3: Characteristics of studies of vitamin D related exposures and oesophageal cancer mortality [1].

Study and location	Study design	NO score	Study location	Vitamin D exposure	Outcomes	Cases	Adjusted confounders											
							Age	Sex	R/U	BMI	Smoking	Alcohol	Out Occ	Air Qual	SE Status	PA	Race	Diet
VITAMIN D STATUS																		
Ren *et al.* (2015)	Retrospective Cohort study	5	China	Serum 25(OH)D	Gastric cancer	197	not specified											

Footnote:

[1] Adjusted confounders: *R/U*: rural vs urban; *BMI*: Body mass index; *Out Occ:* Outdoor occupation; *SE Status*: Socioeconomic status; *PA*: Physical activity; *Air Qual:* Air quality;

2.5.4.2 Vitamin D status

There was only one study which examined the association between 25(OH)D concentration and survival of gastric cancer. Ren *et al.* conducted a study of 197 gastric cancer patients in China and examined the relationship between 25(OH)D and overall survival. Patients were retrospectively analysed after a follow-up of 5 years. This study found an inverse relationship between mortality and those with sufficient concentrations of vitamin D when compared to deficient concentrations (<50 nmol/l vs >50 nmol/L) (HR=0.58; 95%CI: 0.37–0.91) [228]. This paper failed to specify the adjustments made during the multivariate analysis which makes interpretation of results difficult. However, a strength in this study lies with 25(OH)D measurement which was done shortly after diagnosis but before treatment, rather than years prior to diagnosis which has been done in some other studies [8]. This allows an accurate account of vitamin D status at the time of diagnosis.

2.5.4.3 Conclusions

As there is only one study which has examined this topic in detail no meta-analysis could be conducted. Other studies looking at gastric cancer, with reasonable sample sizes, need to be completed before conclusions can be made on the topic.

2.6 Conclusion

Overall the relationship between vitamin D and risk and survival of upper gastrointestinal cancer remains unclear. Due to the severely limited research on the topic with only a few studies published to date there are multiple gaps which need to be addressed.

When examining the association between vitamin D and risk of oesophageal cancer, no consistent relationship was observed. A significant detrimental association was observed for risk of any oesophageal cancer when 25(OH)D was measured, however no effect was observed when dietary intake was examined. However, there was some consistency when examining the risk of gastric cancer in that no significant associations were observed for any of the exposures tested.

When investigating the association between vitamin D and upper gastrointestinal cancer survival, there was a near-complete lack of information on this topic. There are currently no published studies which have investigated an association between vitamin D and oesophageal cancer survival and only one has examined the relationship between gastric cancer and survival. Therefore, evidence is lacking and no firm conclusions about the association between vitamin D and survival of upper gastrointestinal cancers can be made.

The quality of this meta-analysis rests upon the quality of the original studies included in the meta-analysis. If there are limitations with the original studies then these limitations are automatically incorporated into the meta-analysis. For example, study design flaws (such as the measurements of 25(OH)D years prior to diagnosis), small sizes and quality of the studies which have been discussed in the individuals sections have all had an impact on the overall quality of this systematic review and meta-analysis. Furthermore, flawed or undetailed vitamin D estimates used in the original studies would have reduced the quality of the overall meta-analysis. For example, as UVB measurements or dietary estimations are only estimates of vitamin D, rather than exact measurements of vitamin D, as they have some error associated with them, which would be also observed in this meta-analysis. Furthermore, while 25(OH)D is currently the best method of estimating vitamin D status, a one-time measurement taken years before cancer diagnosis may not give a representative account of a person's long term vitamin D status. This is especially important when examining the effects of a long term disease such as the risk or survival of cancer. An estimate which can be measured multiple times or over longer periods of time may be more informative when examining this relationship. As none of the incorporated studies measured 25(OH)D concentration more than once, this could be considered a limitation of this study.

An additional limitation stems from the inclusion of a number of studies which were conducted in the Linxian region of China, population specific effects of the Linxian region of China could be at play in these studies and reaffirmed in this meta-analysis when a combined OR was calculated. This could limit the generalisability of this study.

There are also some limitations which are specific to vitamin D studies. As a number of different vitamin D exposures that have been used (25(OH)D concentration, vitamin D intake, UV radiation), comparisons between studies are difficult and only studies which have examined the same exposure could be compared. This reduced the number of the studies which could be included the same meta-analysis and as such reduced the power of the overall study.

In addition to the limitations observed for individuals studies included in the meta-analysis, there are also some limitations of the meta-analysis process overall. For example, in some of the forest plots significant heterogeneity can be observed. This may be due to the differences in study design, population groups and cancer subtypes examined between individual studies. Even when random effects models were employed the differences between timing of 25(OH)D measurement, adjustment of different confounders and the use of different food frequency questionnaires between studies resulted in some significant heterogeneity. Therefore results may not accurately represent the relationship between oesophageal and gastric cancer risk and vitamin D exposures.

Furthermore, as there is a limited number of studies on the topic, there are some large studies which influenced the overall summary OR more than others, for example, when examining the relationship between 25(OH)D measurements and gastric cancer, 51% of the weight of the overall OR can be attributed to the study by Chen *et al.* In addition, the study design of some of the systematic reviews conducted are flawed as only one reviewer searched and extracted potential studies in the review of risk of gastric cancer and the survival of oesophageal and gastric cancer. This is a further limitation of this study.

Overall, the research on the topic of vitamin D and oesophageal and gastric cancer risk and survival is scarce, and in some cases non-existent. This leads to difficulty in making conclusions on the topic. This review calls for more accurate vitamin D measurements and a considerable amount of further research. This research should contain large sample sizes and incorporate all sources of vitamin D.

3 UVB in Ireland and the UK

3.1 Aim

The aim of this chapter was to investigate UVB in Ireland and the UK using very detailed UVB data from the TEMIS database. This database was used to describe differences in UVB dose due to season, latitude and longitude over the two countries. This chapter hoped to demonstrate that detailed UVB estimates can offer more to vitamin D studies than broader estimates.

3.2 Introduction

UV radiation is key contributor to vitamin D status. Synthesis in the skin following exposure to UVB has been shown to account for approximately 80-100% of vitamin D requirements in some cases [229]. UV dose is measured in J/m^2 and relates to the energy hitting a unit surface area of an irradiated object [230]. The energy which is received at a given time is known as the "exposure dose".

There are many different types of UV radiation which can be measured (**Figure 3.1**); such as global UV radiation, ambient UVB and personal UVB dose. Global radiation is the total solar radiation which hits the earth's surface. Ambient UVB on the other hand is the amount of UVB radiation in a specific area at a specific time. Personal UVB dose is the amount of UVB a person has been exposed to. Global radiation or ambient UVB can be measured using satellite measurements, ground measurements or theoretically calculated using radiative transfer models [231]. Additionally, UVB can be measured using dosimeters. Dosimeters are devices which are attached to the surface of clothing and they can measure the UVB dose reaching the device [232]. Personal UVB dose can be assessed through various sunlight questionnaires, by recalled number of hours spent in sunshine over certain periods of time, outdoor recreational activities or sun burn occasions in the past, or dosimeter reading.

Global UV radiation is of limited value as a vitamin D proxy as it contains all wavelengths of radiation. Ambient UVB on the other hand is much more useful, however it varies dramatically depending on ozone column, cloud cover, altitude and solar zenith angle, season and time of day at a given location [51]. Dosimeters are useful as this measure accounts for how long someone is exposed to UV radiation, and at what times of the day they are exposed. Furthermore, they can account for cloud cover and ozone, as they only measure UVB which reaches the dosimeters at ground level. Personal UVB exposure only accounts for 5-15% of total ambient UVB [233]. This is because there are numerous factors which can affect personal UVB

dose. These primarily include time spent outdoors, time of day spent outdoors, clothing choices, sun screen use, cosmetic use and choosing to walk on the sunny side of the road. For example, unlike the majority of the population, outdoor workers can be exposed to 20%-30% of the total ambient UVB in a given location [233]. Each of these factors in turn can affect the potential cutaneous skin synthesis of vitamin D in an individual. It is often very difficult, if not impossible, to measure accurate personal UVB dose in free-living individuals as these personal factors can vary dramatically within and between individuals. In fact, personal exposure to UVB within a population can vary from $1/10^{th}$ to 10 times the mean ambient UVB dose at that location [233]. An Australian study for example noted an increase in 25(OH)D concentration of 5.2 nmol/L for every 10% decrease in clothing cover; this suggests that personal factors can have dramatic impact on UVB exposure and therefore vitamin D concentration [234]. Dosimeters can be used to estimate personal exposure to UVB, however, these too have their issues, they do not take into account how much skin coverage is exposed to the skin, nor are they suitable for large studies given the costs involved. Furthermore, depending on the type of dosimeter used, there can be some disadvantages, for example chemical dosimeters cannot clearly distinguish between UVA and UVB radiation and can often become saturated in strong sunshine, while electronic dosimeters are restricted to a certain angle of exposure of UVB [235]. Additionally, none of the above estimates can account for how much UVB is absorbed into the skin or how much vitamin D is synthesised. Factors such as age, and skin colour can have a dramatic effect on the absorption of UVB and synthesis of 25(OH)D with darker skin and older age having a reduced ability for absorption and synthesis.

Due to this wide variation, personal UVB can be difficult to measure and ambient UVB is often measured instead. This is then subsequently adjusted for personal and genetic vitamin D related variables such as skin colour, age and time spent outdoors. Unless otherwise specified, the UVB measured in this thesis is ambient UVB (**Figure 3.1**).

There are a number of different estimates which can be calculated from ambient UVB;

- Daily ambient UVB dose is the total daily dose of ambient UVB exposure in a given location.
- Cumulative ambient UVB is the sum of daily ambient UVB for a specified amount of time, e.g.: cumulative ambient UVB dose in a given month or annual ambient UVB.
- Cumulative and weighted UVB dose is similar to cumulative UVB dose except it is also weighted to adjust for the accumulation and diminution of vitamin D synthesised by UVB in the body.

Each of these specific ambient UVB estimates have different functions. For example when examining regional and seasonal differences in UVB dose, using daily and cumulative ambient UVB is useful. When predicting 25(OH)D status in individuals, cumulative and weighted UVB would be appropriate as it accounts for the build-up and break down of vitamin D in the body. Some of these UVB measurements will be discussed further in subsequent chapters.

The use of UVB data as a proxy for vitamin D status has been carried out on multiple occasions when examining the association between vitamin D and health outcomes, as has been discussed previously in chapter one [149]. However, the UVB measures used in epidemiological studies often inadequately adjust for relevant factors, such as cloud cover, ozone column and altitude, which can have a large impact upon the ambient UVB dose reaching the earth's surface [50, 51, 149]. These estimations therefore give an imprecise measure of UVB and in turn an imprecise approximation of vitamin D status. Latitude, altitude and solar angle (determined by the geographical location, time of day and time of year) can be easily modelled, however, other factors such as ozone column and cloud cover can vary dramatically from day to day, or hour by hour.

Previous estimates of ambient UVB dose have been limited in their ability to account for such variability. This limitation is why most epidemiological studies settle for using season as a (poor) proxy of exposure to UVB. Furthermore, even if UVB (and not UV) is used in studies often all wavelengths within the UVB spectrum are included while only a narrow band of wavelengths can actually induce synthesis of cutaneous vitamin D, hence biasing the estimate further. In this chapter, an accurate measure of ambient UVB was used, this measure incorporates altitude, solar zenith angle, cloud cover, ozone column, and restricts UVB to only wavelengths with the ability to synthesize vitamin D, to describe UVB radiation over Ireland and the UK.

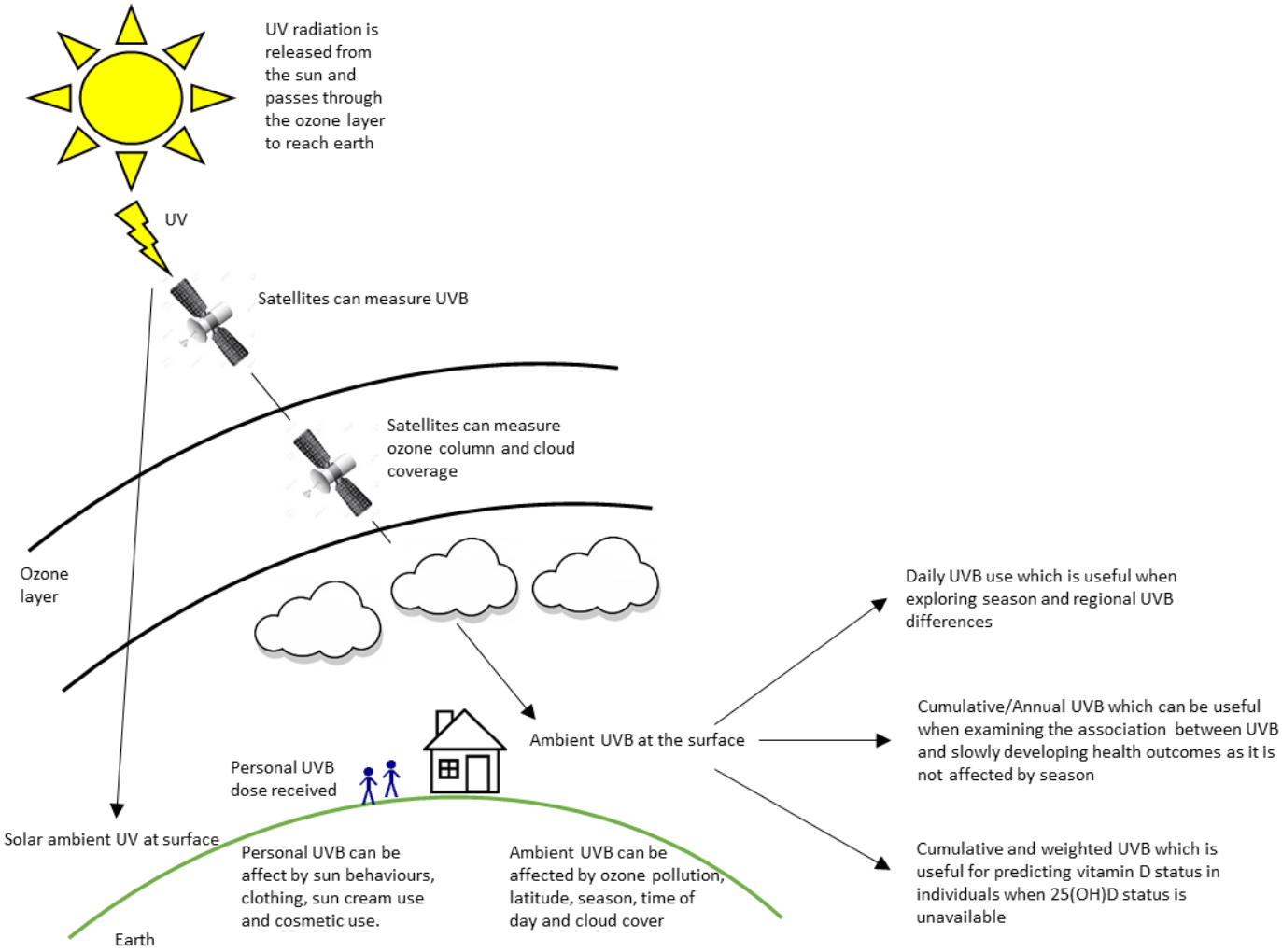


Figure 3.1: Diagram of UV radiation reaching Earth

3.3 Methods

3.3.1 Ambient UVB Data Source

UV dose data from the TEMIS database (www.temis.nl/uvradiation/UVdose.html; version 1.4) was used. This service, provided by the Royal Netherlands Meteorological Institute in conjunction with the European Space Agency provides the amount of UV radiation incident at the surface of the earth in Wm^{-2}, as a function of the total ozone column and the solar zenith angle at a given local solar time (**Appendix 3**) [236]. Using satellites, measurement is carried out every 10 minutes from sunrise to sunset. A correction for cloud cover, surface elevation and surface UV reflectivity (UV albedo) is applied to the estimate. UVB doses were restricted to wavelengths which are relevant for cutaneous vitamin D production (D-UVB, wavelengths of 280 to 315 nm, peak conversion occurs at 295-297 nm). The coefficients of these measurements depend on the action spectrum and the final dose has been weighted for this [237, 238]. Cloud cover attenuation was measured every half an hour from geostationary Meteosat Second Generation (MSG) satellite observations. For the UV dose data used in this study, the action spectrum of the final draft version of ref. [239], dated Sept. 2005, was used. The TEMIS data for D-UVB are given in given in kJ/m^2. For this study, the D-UVB dose data is converted to mJ/cm^2, where 1 kJ/m^2 = 100 mJ/cm^2. The final estimate given is the amount of daily UV radiation hitting the earth's surface in a given areas which is capable of inducing cutaneous synthesis of vitamin D if absorbed by the human skin. The data is provided on a 0.5° × 0.5° (longitude × latitude) grid, each cell covering an area of approximately 55 km (north (N)-south (S)) by 33 km (east (E)-west (W)). When examining D-UVB over the island of Ireland, data from 69 grids from July 2005 to June 2015 were utilised (**Appendix 4: Figure 1**). The grids are labelled latitudinally from south (A) to north (J) and longitudinally from west (1) to east (11), e.g. Dublin city is part of the grid E10 and Malin Head is part of grid J8. When observing D-UVB over the UK, 211 grids covering Scotland, England and Wales were used and were measured from July 2005 to June 2016 **(Appendix 4: Figure 2**). Similarly, the grids are labelled latitudinally from south (A) to north (W) and longitudinally from west (1) to east (20) e.g. London was grid D16. Grids for Ireland and the UK were constructed independently and therefore grid names from each do not represent the same latitude band i.e.; D7 for Ireland corresponds to a latitude of 52.25°N while D7 from England corresponds to a latitude of 51.25°N.

3.3.2 Analysis of Ambient D-UVB in Ireland and the UK

In order to facilitate descriptive analysis of D-UVB over Ireland and the UK, the following measurements were calculated for each location:

By the addition of daily D-UVB in each month cumulative dose for each month was calculated. As data for a number of years was available, the mean monthly cumulative D-UVB dose was found. This was calculated for each calendar month from June 2005-July 2015 for Ireland and from June 2005-July 2016 for the UK. This was recorded as the **monthly cumulative dose (MCD)**.

For each day of the year (e.g. 31st of August), the mean dose for that day over the ten year period was found. These estimates were then grouped by month, and the mean daily D-UVB dose was found for the 12 months, giving **mean daily dose for each month (MDDM)**. **Median daily dose for each month (MEDDM)** was calculated in a similar manner. For each season, the median of MEDDMs was then found to give the **median daily dose per season (MEDDS)**. The seasons were coded as: winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug) and autumn (Sep-Nov).

To examine the differences in latitude in more detail, five different extreme geographic locations on the island of Ireland were chosen along with the eight largest cities and eight large regional areas. The five geographic locations were the most northerly point (Malin Head [J8]) and the most southerly point (Mizen Head [A3]) on the mainland, the most central town (Athlone [E7]), an area to the west (Achill [F2]) and an area to the east (Dublin [E10]). The eight largest cities were Belfast [H11], Derry [H8], Dundalk [F10], Galway [E4], Dublin [E10], Limerick [D5], Waterford [C8] and Cork [B6]. While the eight large regional areas that were chosen are as follows Northern Ireland, North West, Midlands East, Dublin, West, Shannon, South East and South West. Details of the counties and grids for each of these regions are shown in **Appendix 4: Figure 3, table 1**. To examine D-UVB across the UK in more detail, 13 locations were chosen based on the largest cities, these included: Plymouth [B9], Portsmouth [C13], Bristol [D16], Aberystwyth [F9], Birmingham [F14], Coventry [F14], Liverpool [H11], Manchester [H12], Sheffield [H14], Leeds [I14], Newcastle [K14], Glasgow [M7], and Aberdeen [O12] (**Appendix 4: Figure 2**). Four capital cities in the UK; London, Cardiff, Belfast and Edinburgh were also examined in more detail.

3.4 Results

3.4.1 Descriptive Results for Ambient D-UVB over Ireland

Mean daily dose in Ireland was found to be 86.2 mJ/cm² and the mean annual over ten years was found to be 28,138 mJ/cm². Large variation in UVB dose was observed between months, seasons and regional areas. Large variation was also observed between latitudes despite the small latitude differential in the country (51.5°N vs 55.4°N) (**Figure 3.2, 3.3, 3.4**).

Large variation can be easily observed when examining median daily dose for different seasons as spring and summer months have higher D-UVB doses than autumn and winter, (winter MEDDS=5.65 mJ/cm²; spring MEDDS=96.70 mJ/cm²; summer MEDDS=188.50 mJ/cm²; and autumn MEDDS=42.42 mJ/cm², (**Figure 3.3a, Table 3.1**). This is also observed when examining the UVB scale between months in **Figure 3.2,** which differs dramatically. A seasonal effect can also be observed when examining the mean daily dose per month in the different latitude bands (A-J) as fluctuations throughout the year are highly evident (**Figure 3.4**).

Larger variation within the spring, autumn and summer seasons was also noted (**Figure 3.3**). When examining mean daily dose per month a much greater variation in June was observed (range: 203-228 mJ/cm²), July (range: 198-223 mJ/cm²), and August (range: 141-169 mJ/cm²), than that observed for January (range: 2.77-5.26 mJ/cm²), or December (range: 2.16-4.15 mJ/cm²). This variation was also prominently visible when comparing the most northerly point (Malin Head) with the most southerly point (Mizen Head). A 27 mJ/cm² mean daily dose difference between the two points during June was found, while a difference of 2.16 mJ/cm² was observed in December (**Figure 3.4**). A mean monthly difference of 15.75 mJ/cm² was found between Mizen and Malin head. Overall, a 19.8% higher cumulative annual D-UVB dose at Malin Head (6,804 mJ/cm²) was found when compared to Mizen Head (5,694 mJ/cm²).

The highest mean daily D-UVB dose over the ten years was found in the south of Ireland in June and the lowest was found in Northern Ireland in December. When examining the eight largest cities and towns in Ireland in detail, it was observed, unsurprisingly, that Belfast (NE) and Derry (NW) had the lowest median D-UVB, while Cork (SW) and Waterford (S) had the highest (**Table 3.2**). It was also noted that in Belfast and Derry, which are part of the same latitude band, there was little difference in D-UVB between them. However, when cities located further south and in the same latitude band (Galway and Dublin) were examined, slightly higher D-UVB levels in Dublin compared to Galway were found. This demonstrates differences between the East and West, although they are in the same latitude band (**Table 3.2**). This trend was also observed when investigating D-UVB over different regions, with the South East having higher median D-

UVB levels than the Shannon region (SW), although both regions are of similar latitude (**Table 3.3**). A similar trend can be seen, especially in summer and autumn months in **Figure 3.2.**

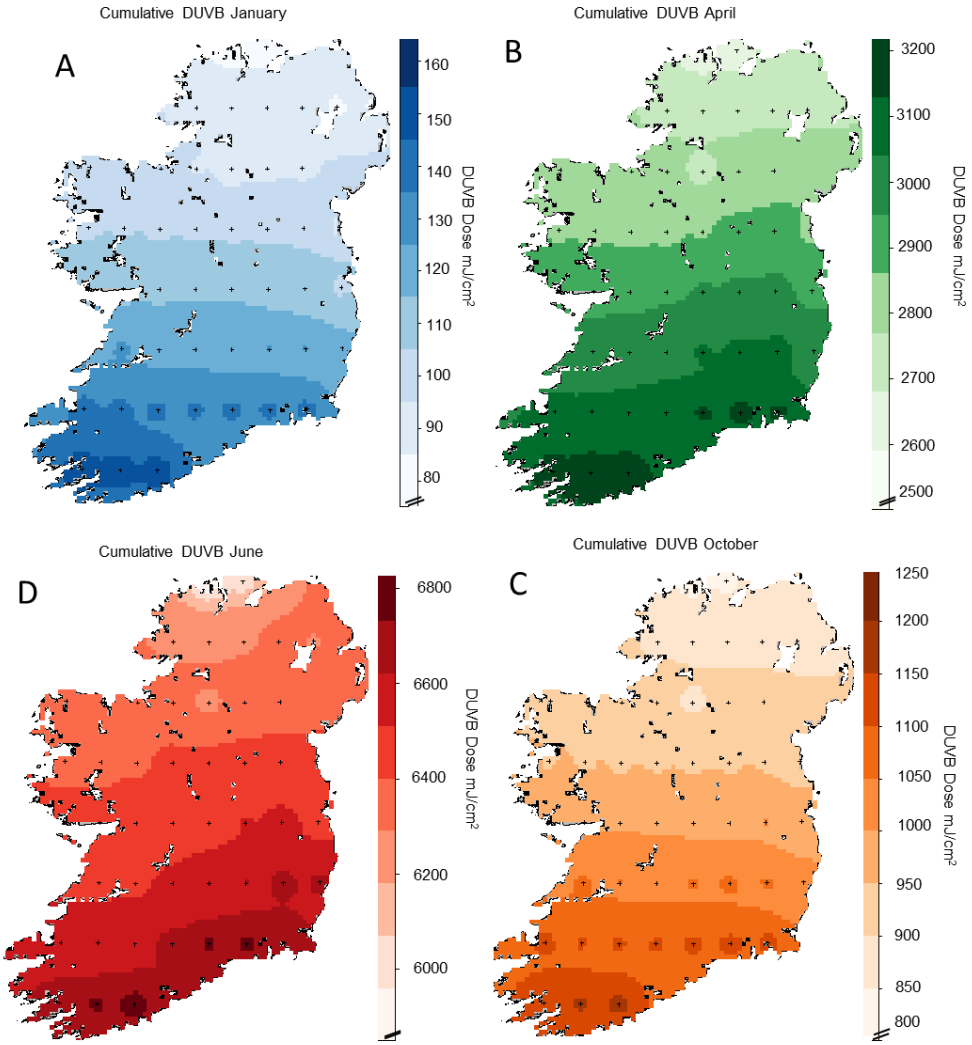


Figure 3.2: Map of ambient D-UVB in Ireland
Cumulative D-UVB level over Ireland in **A)** January, **B)** April **C)** June **D)** October from 2005-2015. D-UVB is measured in mJ/cm². Note the large differences in the scale between months.

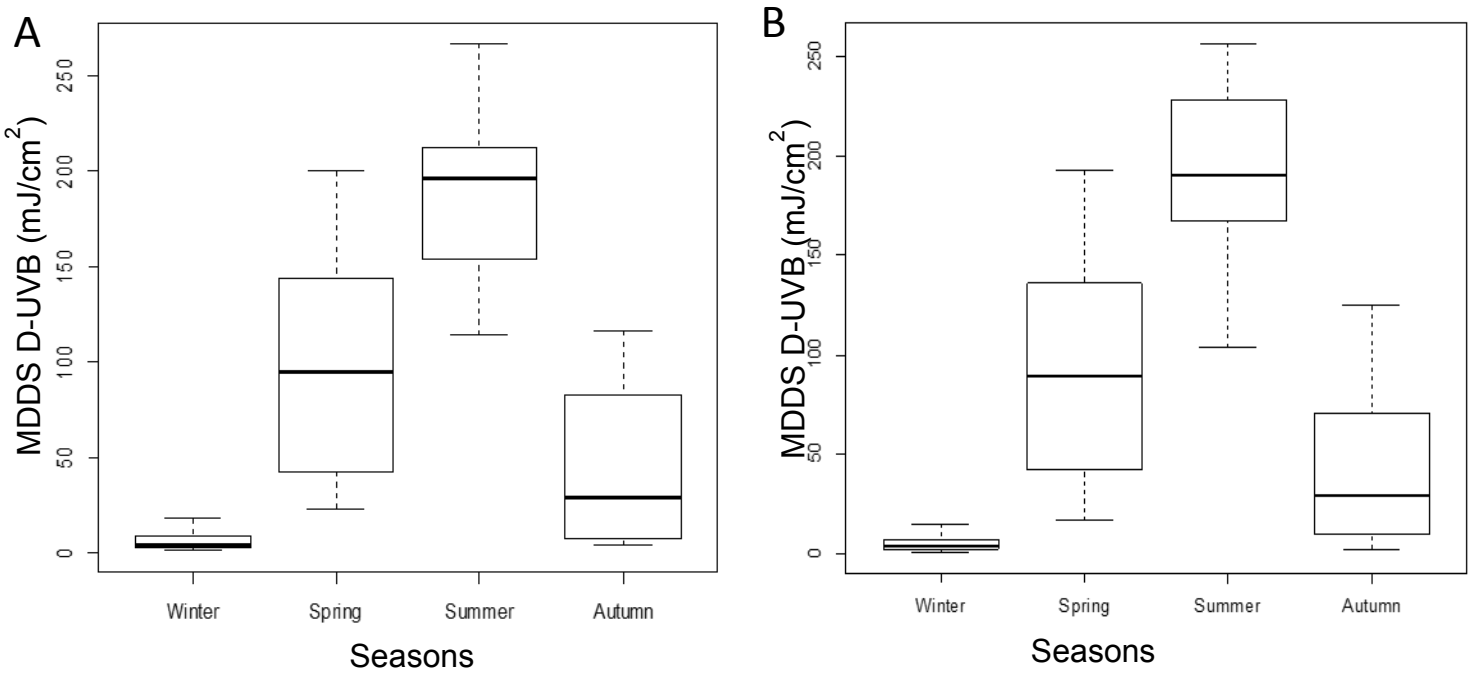


Figure 3.3: Median daily ambient D-UVB for each season in Ireland and the UK

Median daily D-UVB dose over: **A)** Ireland **B)** the UK. Season; winter: Dec-Feb, spring: Mar-May, summer: Jun-Aug, autumn: Sep-Nov**.**

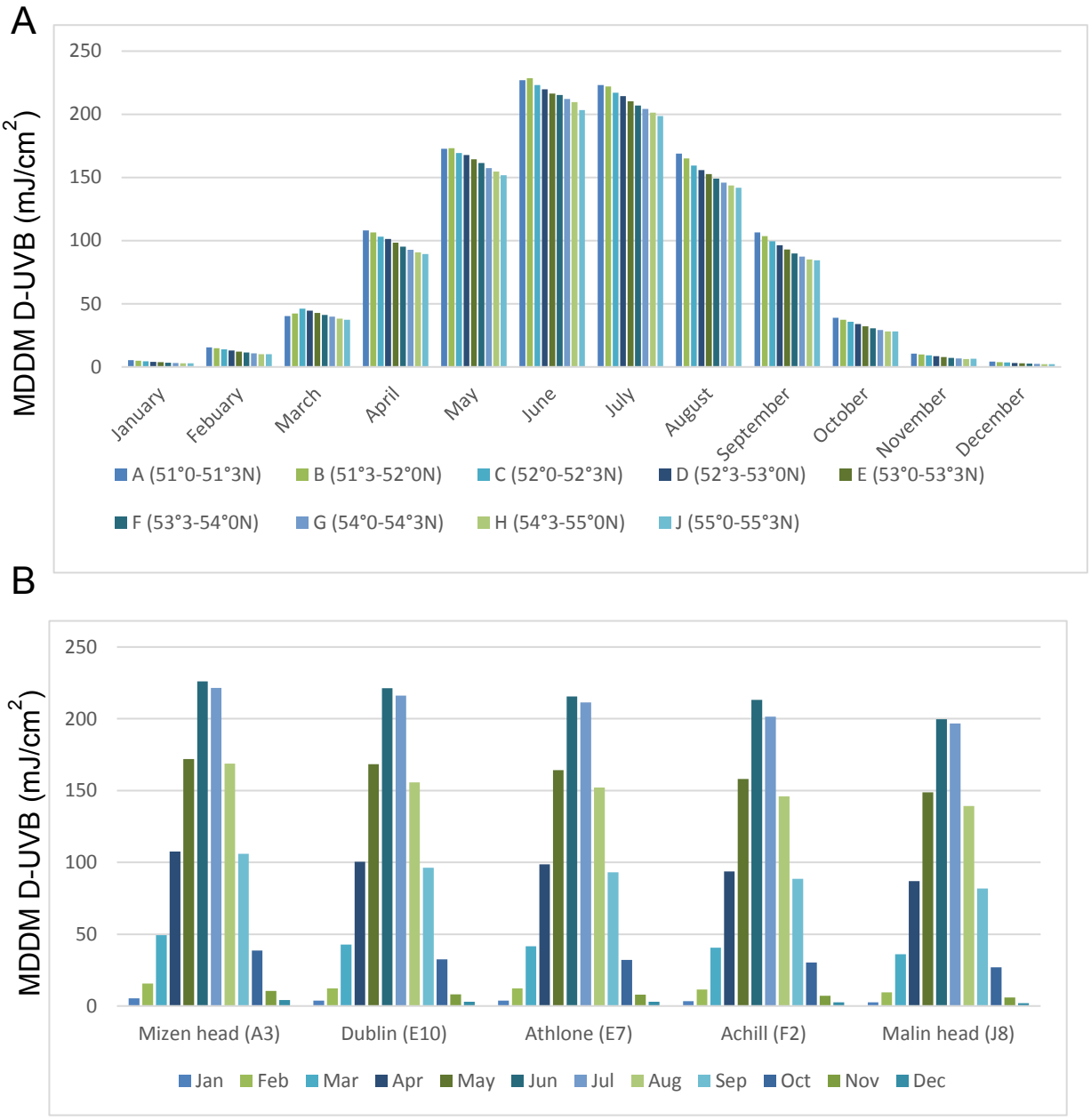


Figure 3.4: Ambient D-UVB over latitude bands and extreme locations in Ireland

Average monthly D-UVB level **A)** over latitude groups in Ireland, A (51°0N-51°3N), B (51°3N-52°0N), C (52°0N-52°3N), D (52°3N-53°0N), E (53°0N-53°3N), F (53°3N-54°0N), G (54°0N-54°3N), H (54°3N-55°0N), and J (55°0N-55°3N) **B)** Over 5 areas in Ireland: Mizen Head (51.45°N, 9.82°W), Dublin (53.35°N, 6.26°W), Athlone (53.43°N, 7.95°W), Achill (53.96°N, 10.00°W), and Malin Head (55.38°N, 7.37°W).

Country	**Winter MEDDS (mJ/cm^2)** (Min-Max)	**Spring MEDDS (mJ/cm^2)** (Min-Max)	**Autumn MEDDS (mJ/cm^2)** (Min-Max)	**Summer MEDDS (mJ/cm^2)** (Min-Max)	**Overall cumulative (mJ/cm^2)**
Ireland	5.7 (1.8-7.6)	96.7 (51.1-159.5)	42.4 (12.4-60.5)	188.5 (135.1-296.8)	28,138
UK	5.1 (2.0-9.18)	91.2 (44.7-153.4)	40.4 (11.6-59.7)	188.8 (123.4-279.5)	30,515

Table 3.1: Examining daily ambient D-UVB dose per season in Ireland and the UK

Footnote:

MEDDS: median daily dose per season

Table 3.2: Median daily ambient D-UVB dose for the eight most populous cities and towns on the island of Ireland[1, 2].

Region	Median Daily[3]	Daily D-UVB dose, median (IQR)											
		Jan	*Feb*	*March*	*April*	*May*	*June*	*July*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
All Ireland	**63** (72-101)	**3** (2-5)	**11** (8-16)	**38** (29-53)	**96** (80-113)	**157** (131-189)	**213** (184-244)	**204** (181-235)	**148** (131-169)	**91** (71-113)	**30** (21-42)	**7** (5-10)	**3** (2-3)
Derry	**55** (68-96)	**2** (2-3)	**10** (6-13)	**34** (25-49)	**89** (74-105)	**146** (125-181)	**209** (175-242)	**197** (173-226)	**140** (125-159)	**82** (63-106)	**26** (18-37)	**5** (4-8)	**2** (2-2)
Belfast	**54** (69-97)	**2** (2-3)	**9** (6-14)	**34** (26-50)	**89** (74-107)	**151** (127-183)	**214** (183-239)	**198** (175-228)	**139** (123-163)	**81** (62-104)	**26** (18-36)	**5** (4-8)	**2** (2-3)
Dundalk	**59** (72-101)	**3** (2-4)	**11** (7-15)	**37** (29-53)	**96** (79-111)	**157** (133-192)	**214** (185-248)	**202** (181-237)	**147** (131-169)	**89** (69-111)	**28** (20-40)	**7** (5-9)	**3** (2-3)
Galway	**60** (72-101)	**3** (2-5)	**11** (8-16)	**38** (29-53)	**95** (80-114)	**159** (131-189)	**210** (183-244)	**203** (180-236)	**150** (131-168)	**89** (70-112)	**30** (21-42)	**7** (5-10)	**3** (2-3)
Dublin	**62** (74-104)	**3** (3-5)	**11** (8-16)	**38** (29-55)	**98** (81-116)	**160** (136-195)	**220** (191-250)	**207** (185-244)	**151** (134-172)	**94** (72-116)	**30** (22-42)	**7** (5-10)	**3** (2-4)
Limerick	**63** (74-103)	**3** (3-5)	**12** (8-17)	**40** (30-54)	**98** (83-115)	**160** (133-194)	**213** (186-244)	**205** (184-235)	**152** (132-169)	**94** (71-114)	**32** (22-43)	**8** (6-11)	**3** (3-4)
Waterford	**66** (77-107)	**4** (3-6)	**13** (9-18)	**41** (32-56)	**105** (87-122)	**163** (139-201)	**220** (191-255)	**218** (190-249)	**156** (137-179)	**98** (77-121)	**34** (24-45)	**8** (6-12)	**3** (3-4)
Cork	**69** (80-109)	**4** (3-6)	**13** (10-19)	**42** (33-57)	**106** (90-122)	**166** (142-200)	**223** (195-256)	**219** (195-250)	**161** (141-185)	**103** (82-123)	**35** (26-48)	**9** (7-12)	**4** (3-5)

Footnote:

[1] Derry (55.0°N, 7.3°W), Belfast (54.6°N, 5.9°W), Dundalk (54.0°N, 6.4°W), Galway (53.3°N, 9.1°W), Dublin (53.3°N, 6.3°W), Limerick (52.7°N, 8.6°W), Waterford (52.3°N, 7.1°W) and Cork (51.9°N, 8.5°W).

[2] Values represent median and IQR in brackets

[3] Median daily D-UVB dose for the whole year

Table 3.3: Median daily ambient D-UVB dose for eight larger geographic regions on the island of Ireland [1, 2].

Region	Median Daily[3]	Daily D-UVB dose, median (IQR)											
		Jan	*Feb*	*March*	*April*	*May*	*June*	*July*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Northern Ireland	**53** (45-78)	**2** (2-3)	**9** (6-13)	**34** (25-49)	**89** (73-105)	**148** (125-180)	**209** (179-238)	**196** (174-226)	**139** (122-160)	**81** (63-105)	**26** (18-36)	**5** (4-8)	**2** (2-3)
North West	**53** (67-94)	**2** (2-3)	**9** (6-13)	**34** (25-49)	**88** (72-105)	**144** (122-177)	**204** (173-233)	**194** (173-223)	**139** (123-157)	**82** (63-105)	**26** (18-37)	**5** (4-8)	**2** (2-3)
West	**58** (48-81)	**3** (2-4)	**10** (7-15)	**37** (27-52)	**94** (77-110)	**155** (128-184)	**210** (182-242)	**200** (179-230)	**145** (128-165)	**88** (68-110)	**28** (20-40)	**7** (5-9)	**3** (2-3)
Midlands East	**61** (72-102	**3** (2-5)	**11** (7-16)	**38** (29-53)	**96** (79-113)	**157** (133-192)	**215** (184-248)	**205** (182-238)	**147** (131-168)	**91** (69-113)	**29** (21-41)	**7** (5-9)	**3** (2-3)
Dublin	**62** (74-104)	**3** (3-5)	**11** (8-16)	**38** (29-55)	**98** (81-116)	**160** (136-195)	**220** (191-250)	**207** (185-244)	**151** (134-172)	**94** (72-116)	**30** (22-42)	**7** (5-10)	**3** (2-4)
South East	**65** (76-106)	**4** (3-5)	**12** (9-17)	**40** (31-56)	**103** (85-119)	**162** (138-198)	**220** (190-252)	**214** (188-246)	**154** (136-176)	**97** (76-119)	**33** (23-45)	**8** (6-11)	**3** (3-4)
Shannon	**63** (74-102)	**4** (3-5)	**12** (8-17)	**40** (30-55)	**98** (83-115)	**160** (133-192)	**213** (185-243)	**205** (182-235)	**153** (134-171)	**93** (73-115)	**32** (22-44)	**8** (6-11)	**3** (3-4)
South West	**66** (77-107)	**4** (3-6)	**13** (9-19)	**43** (33-58)	**103** (87-120)	**164** (138-197)	**221** (190-251)	**214** (189-246)	**160** (141-181)	**101** (80-121)	**34** (25-47)	**9** (7-12)	**4** (3-4)

Footnote:

[1] Details on regions are shown in more detail in Appendices Figure 3.

[2] Values represent median and IQR in brackets

[3] Median daily D-UVB dose for the whole year.

3.4.2 Descriptive Results for Ambient D-UVB over the UK

Mean daily ambient D-UVB dose in the UK was found to be 83.4 mJ/cm² and mean annual ambient D-UVB dose was 30,515 mJ/cm². These are similar to what was observed for Ireland. Again large seasonal variation throughout the year was observed with higher D-UVB being observed in summer months compared to winter (**Figure 3.5, 3.6**). Variation in D-UVB dose was also observed at different latitudes. When examining the most northerly point (Out Stack, Shetland Islands), and the most southerly point (Lizard Point, Cornwall) (49.96°N vs 60.86°N), a 98.46 mJ/cm² mean daily D-UVB dose difference was observed between the two points during June, while a difference of 5.17 mJ/cm² was observed in December (**Figure 3.5**). These were slightly larger in variation compared to Ireland due to the larger latitude and longitude differential in the UK. Seasonal differences were also substantial: winter MEDDS=5.05 mJ/cm²; spring MEDDS=91.24 mJ/cm²; summer MEDDS=188.79 mJ/cm²; and autumn MEDDS=40.39 mJ/cm² (**Table 3.1**). There were however, similar to what was observed for Ireland. Again, large variations throughout the year were observed (**Figure 3.5, 3.6**).

When examining the 13 largest cities in the UK, a clear inverse relationship was observed between D-UVB dose and latitude (**Figure 3.6, Table 3.4**). Similar to what was observed in Ireland, there was an east-west D-UVB gradient noted, for example, Liverpool, Manchester and Sheffield all lie within the same latitude band but median D-UVB dose increases as you move further east; for example in March: Liverpool 35.95 mJ/cm², Manchester; 36.39 mJ/cm² and Sheffield 37.46 mJ/cm² (**Table 3.4**). This was even more apparent when examining median D-UVB dose in the summertime; for example July: Liverpool 224.17 mJ/cm², Manchester; 226.20 mJ/cm² and Sheffield 232.23 mJ/cm² and similarly when examining Birmingham 237.30 mJ/cm² vs Aberystwyth 225.30 mJ/cm² (**Table 3.4**).These trends were also observed when examining the four capital cities of the UK, with differences between east-west being noted for example between London and Cardiff (**Figure 3.7**).

Marginal differences between D-UVB in the same location over the years (2006-2015) were observed (**Figure 3.8**). D-UVB remained relatively constant from 2005-2015 in the same area.

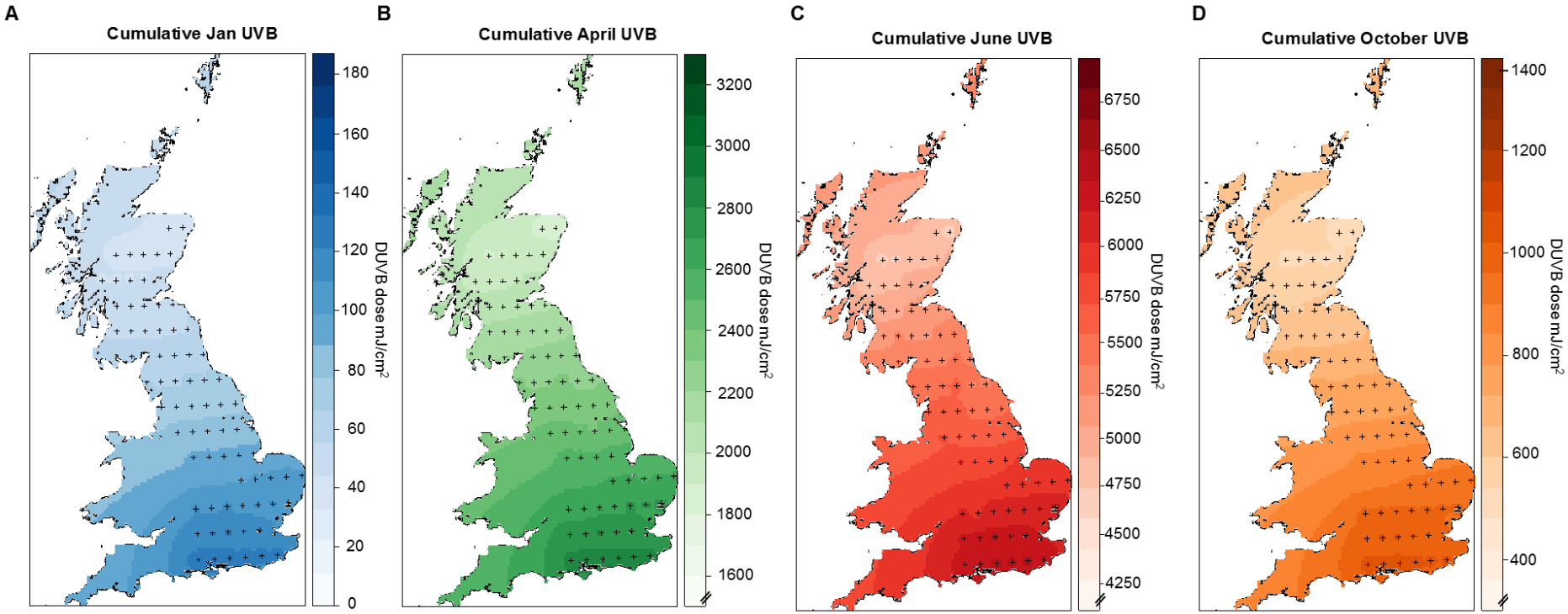


Figure 3.5: Map of ambient D-UVB in the UK

Overall cumulative D-UVB level over the UK for **A)** January, **B)** April, **C)** June **D)** October, from 2005-2016. D-UVB is measured in mJ/cm²

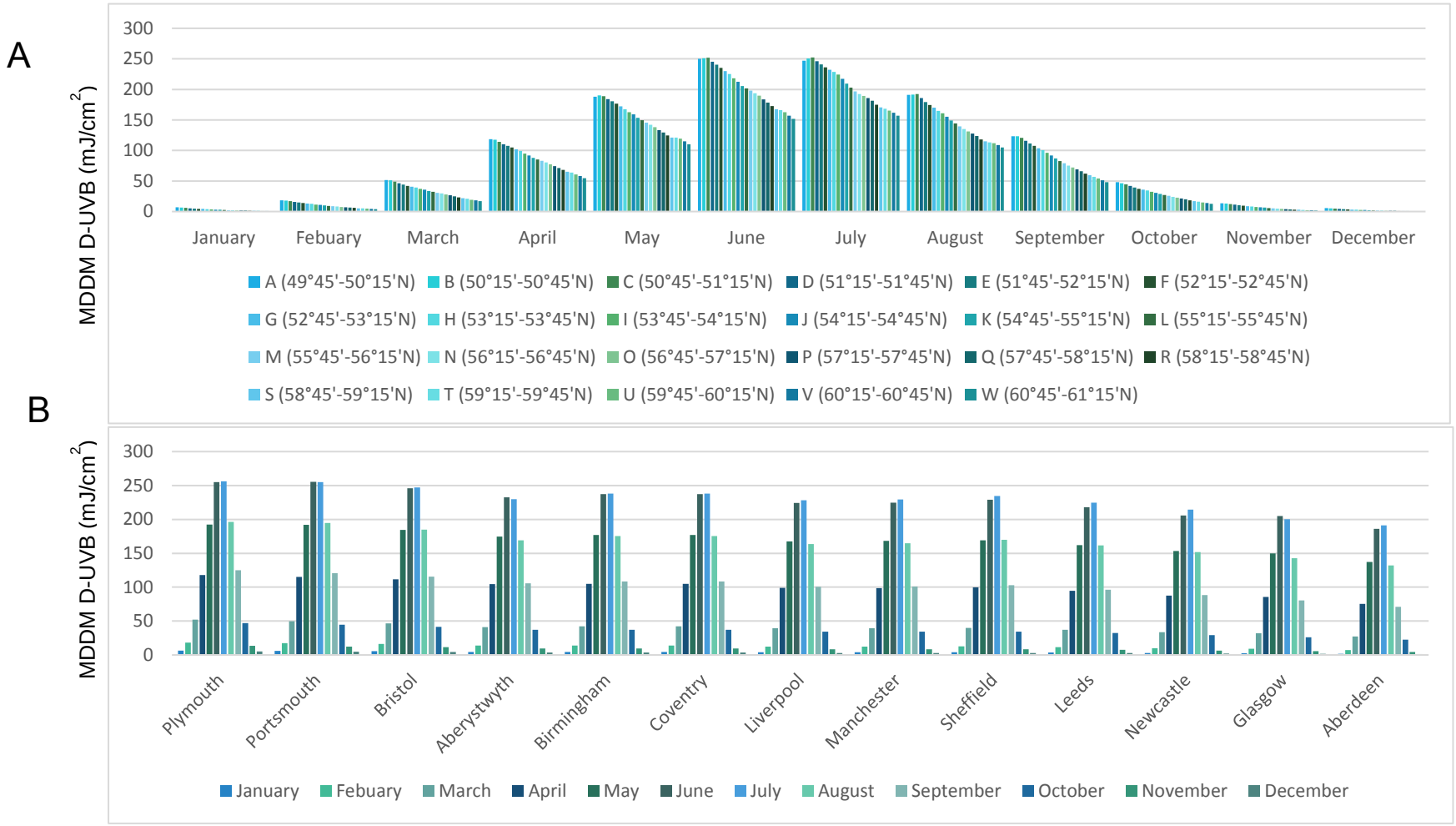


Figure 3.6: A**mbeint D-UVB in the UK over different latitudes and main cities**

Average monthly D-UVB level **A)** over latitude groups in the UK, A-W **B)** Over 13 main cities in the UK: Plymouth (50.37°N, 4.14°W), Portsmouth (50.82°N, 1.08°W), Bristol (51.45°N, 2.59°W), Aberystwyth (52.42°N, 4.08°W), Birmingham (52.48°N, 1.89°W), Coventry (52.40°N, 1.51°W), Liverpool (53.40°N, 2.99°W), Manchester (53.48°N, 2.24°W), Sheffield (53.38°N, 1.47°W), Leeds (53.80°N, 1.55°W), Newcastle (54.98°N, 1.61°W), Glasgow (55.86°N, 4.25°W), Aberdeen (57.15°N, 2.09°).

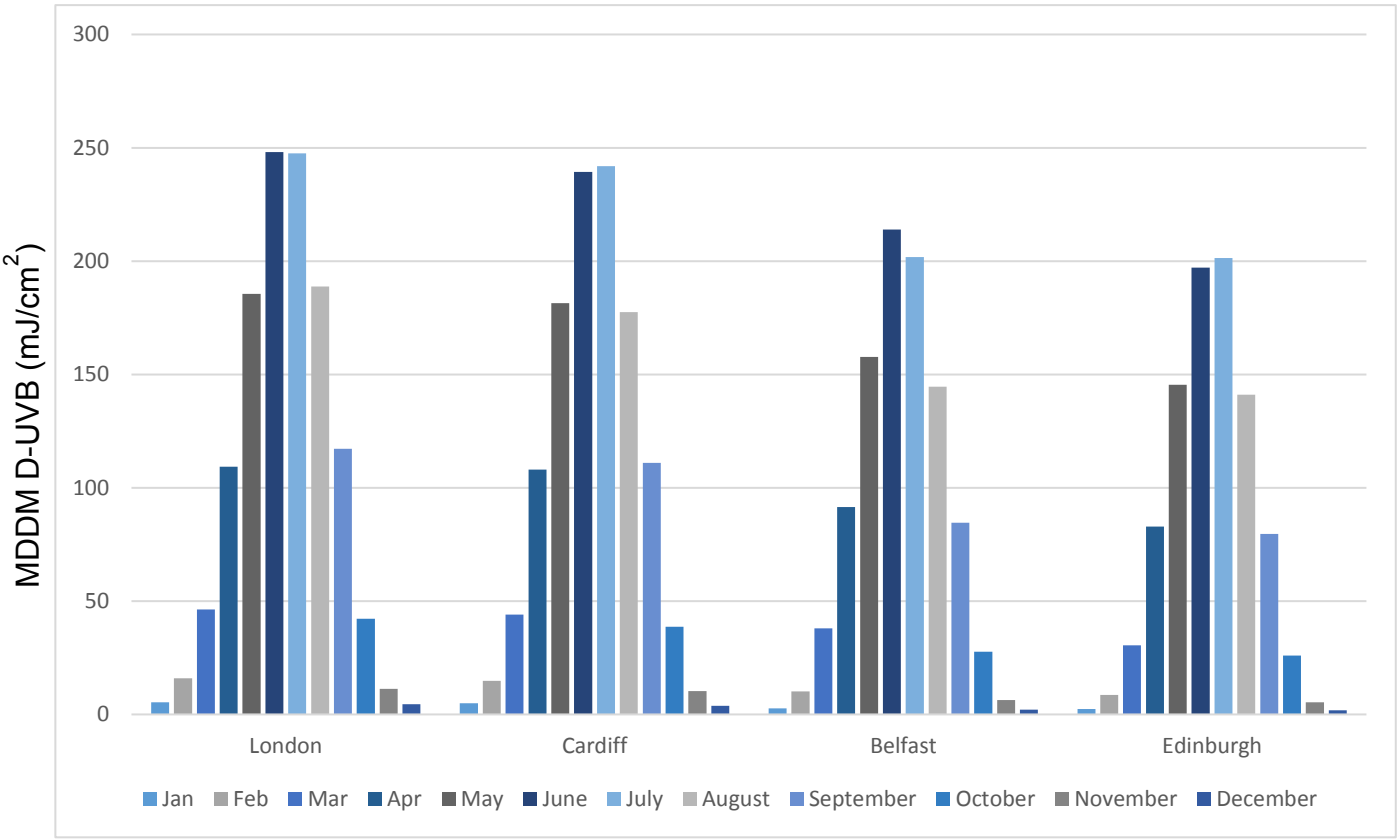


Figure 3.7: Ambient D-UVB for the capital cities of the UK

Mean daily dose of D-UVB (MDDM) over London, Cardiff and Edinburgh was calculated using TEMIS data from June 2005-July 2016, while MDDM for Belfast was calculated using TEMIS data from June 2005-July 2015. London (51.51°N, 0.13°W); Cardiff (51.48°N, 3.18°W); Belfast (54.59°N, 5.93°W); Edinburgh (55.95°N, 3.18°W)

Table 3.4: Median daily ambient D-UVB dose for thirteen cities in the UK.

Region	Median Daily[3]	Daily D-UVB dose, median (IQR)											
		Jan	*Feb*	*March*	*April*	*May*	*June*	*July*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Plymouth	**105** (85-123)	**6** (4-8)	**17** (12-22)	**51** (37-67)	**118** (94-134)	**190** (146-221)	**253** (202-295)	**253** (210-287)	**192** (164-219)	**122** (100-147)	**46** (32-57)	**12** (8-17)	**5** (4-6)
Portsmouth	**104** (80-121)	**5** (3-7)	**16** (10-22)	**47** (33-64)	**114** (89-130)	**187** (142-218)	**255** (198-287)	**252** (201-289)	**192** (152-215)	**117** (91-141)	**43** (28-56)	**11** (7-15)	**5** (3-5)
Bristol	**100** (81-116)	**5** (3-6)	**15** (10-21)	**43** (33-62)	**112** (91-129)	**180** (143-210)	**244** (204-276)	**244** (209-277)	**183** (153-207)	**113** (92-138)	**40** (29-52)	**10** (7-14)	**4** (3-5)
Birmingham	**95** (77-110)	**4** (3-5)	**13** (9-17)	**39** (30-56)	**105** (84-121)	**173** (139-201)	**236** (199-260)	**237** (198-268)	**174** (146-196)	**105** (84-130)	**36** (25-46)	**9** (6-12)	**3** (3-4)
Coventry	**95** (77-110)	**4** (3-5)	**13** (9-17)	**39** (30-56)	**105** (84-121)	**173** (139-201)	**236** (199-260)	**237** (198-268)	**174** (146-196)	**105** (84-130)	**36** (25-46)	**9** (6-12)	**3** (3-4)
Aberystwyth	**91** (76-117)	**4** (3-5)	**13** (9-17)	**38** (28-53)	**104** (84-122)	**170** (140-201)	**230** (195-261)	**225** (193-259)	**166** (142-189)	**100** (83-124)	**34** (25-47)	**8** (6-12)	**3** (3-4)
Liverpool	**88** (72-103)	**4** (2-5)	**11** (8-15)	**36** (28-51)	**99** (78-113)	**163** (130-192)	**221** (189-245)	**224** (188-262)	**161** (132-181)	**97** (80-118)	**34** (22-43)	**7** (5-10)	**3** (2-4)
Manchester	**89** (73-104)	**4** (2-5)	**12** (8-15)	**36** (28-52)	**99** (76-113)	**164** (132-194)	**222** (190-249)	**226** (191-266)	**163** (135-186)	**97** (79-118)	**33** (23-48)	**7** (5-10)	**3** (2-4)
Sheffield	**91** (74-106)	**3** (2-5)	**12** (8-15)	**37** (28-53)	**100** (78-115)	**165** (134-195)	**227** (197-251)	**232** (196-272)	**168** (140-192)	**100** (80-120)	**33** (22-44)	**8** (5-10)	**3** (2-4)
Leeds	**86** (68-100)	**3** (2-4)	**10** (7-14)	**34** (26-48)	**94** (73-108)	**159** (125-185)	**217** (179-244)	**220** (179-257)	**160** (129-180)	**93** (73-112)	**31** (20-40)	**7** (5-9)	**3** (2-3)

Table 3 continued.

Region	Median Daily[3]	Median Daily D-UVB dose per month											
		Jan	*Feb*	*March*	*April*	*May*	*June*	*July*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*
Newcastle	**80** (66-93)	**3** (2-3)	**9** (6-12)	**30** (23-43)	**87** (68-100)	**150** (125-173)	**203** (175-230)	**211** (177-241)	**150** (124-170)	**85** (68-102)	**27** (19-36)	**6** (4-8)	**2** (2-3)
Glasgow	**77** (63-191)	**2** (1-3)	**8** (5-11)	**29** (22-42)	**84** (67-98)	**147** (117-173)	**204** (174-235)	**198** (165-231)	**141** (119-158)	**78** (61-97)	**25** (16-33)	**5** (3-7)	**2** (1-2)
Aberdeen	**70** (55-83)	**2** (1-2)	**7** (4-9)	**25** (19-35)	**74** (55-87)	**134** (104-158)	**183** (149-212)	**188** (153-220)	**131** (106-147)	**68** (52-83)	**21** (14-29)	**4** (3-6)	**1** (1-2)

Footnote:

[1] Plymouth (50.37°N, 4.14°W), Portsmouth (50.82°N, 1.08°W), Bristol (51.45°N, 2.59°W), Aberystwyth (52.42°N, 4.08°W), Birmingham (52.48°N, 1.89°W), Coventry (52.40°N, 1.51°W), Liverpool (53.40°N, 2.99°W), Manchester (53.48°N, 2.24°W), Sheffield (53.38°N, 1.47°W), Leeds (53.80°N, 1.55°W), Newcastle (54.98°N, 1.61°W), Glasgow (55.86°N, 4.25°W), Aberdeen (57.15°N, 2.09°W)

[2] Values represent median and IQR in brackets

[3] Median daily D-UVB dose for whole year.

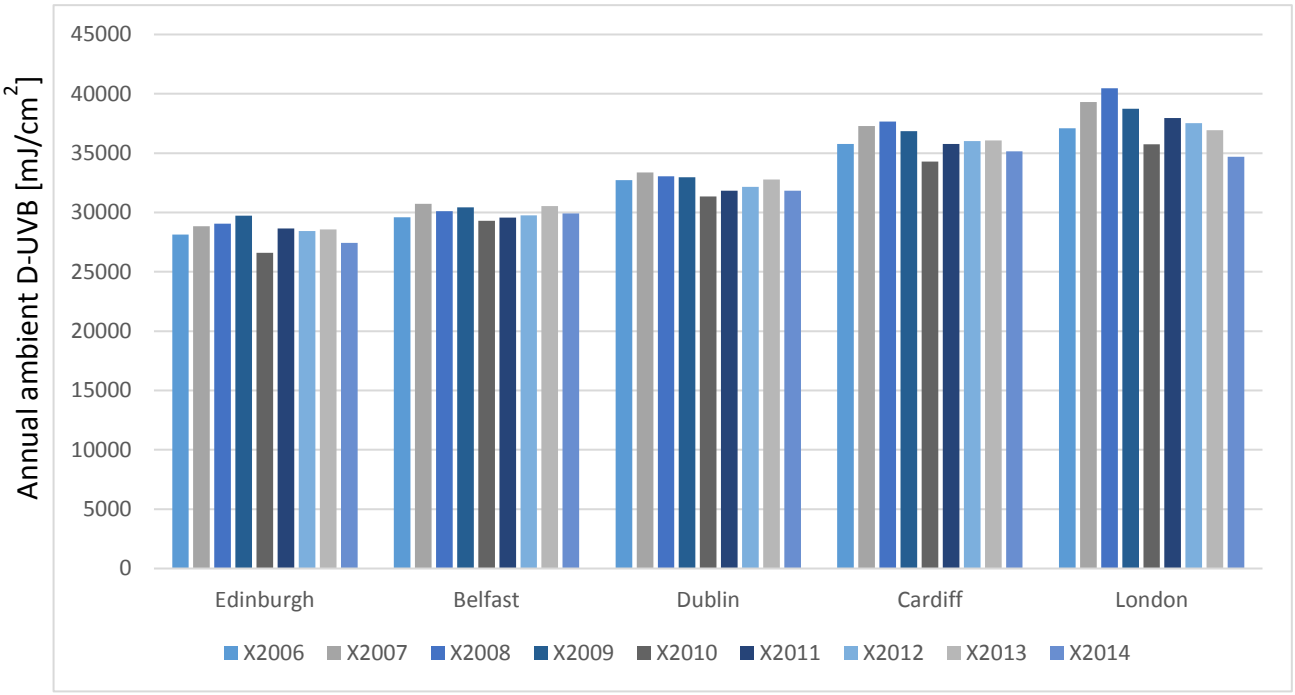


Figure 3.8: Annual ambient D-UVB over time for the capital cities or Ireland and the UK
Relationship between annual ambient D-UVB through the years at different locations

3.5 Discussion

The results outlined in this chapter highlight considerable differences in the D-UVB level across Ireland and the UK, even though these are two high latitude countries which do not cover very large geographical areas. Broadly comparable D-UVB doses were observed across Ireland and the UK, however, there were noticeable seasonal, latitudinal and longitudinal differences between and within the two countries. The differences in D-UVB doses described in chapter may seem like small changes in D-UVB dose, however these differences are accumulative and as such, over long periods of time they can have a large impact on the D-UVB dose received by individuals and the vitamin D dose which can be synthesized. This is discussed further in chapter 8.

3.5.1 Seasonal Variation

Ireland and the UK enjoy a maritime temperate climate due to the influence of the Atlantic Ocean, and do not experience the extreme weather which is observed in other parts of the world at similar latitudes. Nonetheless, substantial differences in D-UVB level were found throughout the year and between seasons in this thesis [240]. Seasonal variation in D-UVB dose is nothing novel. However, in this chapter the seasonal differences which were observed were able to be quantified in greater detail: for example, almost 200 times higher daily D-UVB doses were recorded in the summer compared to winter. This is unsurprising as UV originates from the sun and during the summer, UV rays have a shorter distance to travel from the sun due to the relative position and angle of the Earth, therefore making the radiation stronger. Furthermore, longer days and longer periods of sunshine are found in the summer compared to the winter. Stark differences between autumn and winter were also observed; median daily dose in autumn was found to be almost eight times the median daily dose recorded in winter. Similarly, summer and spring differences were noticeable; a two-fold difference in the median daily dose was found in summer compared to spring. Previous studies examining D-UVB over Scotland have found comparable results [241].

3.5.2 Latitude Variation

A large variation in ambient D-UVB has been shown across Europe [11], however, this study highlights the large D-UVB differences which are observed even despite the small latitude differentials within these two countries, Ireland extends from 51.5°N to 55.4°N and the UK extends from 49.96°N to 60.86°N. Very large variation was noted when examining D-UVB dose

at different latitudes and a clear trend of decreasing D-UVB as you move further north, in both Ireland and the UK. This was further highlighted when extreme points (most northerly vs most southerly) were examined: for instance, a 34% higher mean daily ambient D-UVB dose was observed in July in Plymouth (SW England) when compared to Aberdeen (NE Scotland) during the same month. Many previous studies have noted a decrease in UVB with increasing latitude [51], but this study was able to examine these differences on a more detailed scale and focused on D-UVB which is a unique approach.

Moreover, if these large differences in D-UVB were observed when examining a ten degree change in latitude (as is the case in the UK) one would expect this variation to be magnified greatly when examining D-UVB in countries which span a wider range of latitudes, or are closer to the equator; Chile for example has a latitude difference of almost 38° as it stretches from 17.5°S to 55.8°S, while The United States of America has an even larger latitude difference between its extreme points, a 47° difference, as it extends from 24.3°N to 71.2°N. The expected large differences would therefore need to be taken into account when carrying out research on D-UVB or vitamin D in these countries.

Noteworthy variation in D-UVB was also observed within the same season due to the differences in latitude, for example median daily dose in the UK in spring ranges from 14.7 mJ/cm^2 to 190 mJ/cm^2, while the range in winter is much less dramatic (0.09 mJ/cm^2 to 17.25 mJ/cm^2). Spring and summer months were found to have much greater D-UVB variation than during winter, which is mostly due to the overall higher D-UVB dose and the effect of weather. For example, the highest mean daily dose recorded in the UK in September was 123 mJ/cm^2; this was almost equal (99 mJ/cm^2) to the mean daily *dose difference* between latitude bands in the UK in July (range 151-250). This means that the mean daily D-UVB dose *difference* experienced in July between latitudes groups A to W is as great as the highest daily D-UVB dose experienced at latitude group W alone in September. This highlights the wide variation between latitudes.

3.5.3 Longitudinal Variation

Additionally, this study found variation in D-UVB between areas at similar latitudes but varying longitudes. These results demonstrate that variation in D-UVB exists, not only between latitudes but also longitudes, something which is typically ignored in previous studies. Cities further east were found to have consistently higher D-UVB doses than the west, although the differences were much less striking than what was observed for latitude. For example there was a 2% median daily D-UVB dose increase as you move further east between Athlone [53.3°N] and

Dublin [53.4°N]; a 3% increase between Liverpool [53.48°N] and Sheffield [53.38°N] and a 4% increase between Aberystwyth [52.42°N] and Birmingham [51.45°N] in July. These differences are small when examining daily doses, however, they accumulate over time and larger differences can be observed from season to season or if annual D-UVB is being measured.

This trend has also been reported in a previous study, which utilised ground D-UVB and satellite measurements to model D-UVB doses over Ireland and the UK [242]; they measured UVB every half an hour and cloud cover twice daily [242]. This study improves upon this as the TEMIS database uses a more detailed temporal and spatial resolution; D-UVB was measured every ten minutes, cloud cover every half an hour and D-UVB was restricted to only wavelengths which synthesise vitamin D.

3.5.4 Implications of this Research

These results demonstrate that local geographical and metrological conditions and microclimate can have a much greater impact on D-UVB than previously thought, irrespective of latitude differences. They also highlight the importance of accurate local D-UVB measurements when examining D-UVB in research, as often studies only measure D-UVB dose for large areas at one, or a few locations [241]. However, if these countries extend over a wide range of latitudes, altitudes or climates and only measure UVB at a few locations, the precision of the D-UVB measurement would be reduced and regional differences blurred [102, 243].

For instance, Boscoe *et al.* measured solar UV-exposure to a geographic resolution of one degree, which they describe as being 111 km N to S and between 75 and 101 km E to W [102]. As Ireland is 486 km N/S and 275 km E/W and similarly the UK is 965 km N/S and 485 km E/W, using the above method from Boscoe *et al.* applied to Ireland and the UK; Ireland would be divided into only nine grids and similarly the UK into 45 grids. As the current method applied in this thesis actually divided Ireland in 69 grids and the UK into 211 and variation between each of these grids was observed, it is easy to hypothesize that previous studies may have failed to take into account these regional differences that have been clearly demonstrated.

Furthermore, this thesis highlights that crude adjustment for season and latitude as a proxy for UVB and subsequently a proxy for vitamin D status, as has been done most often previously [105, 106], is not sufficient. This method of adjustment does not adequately address the large variation that exists within seasons and at the same latitude. For example, there was a 58 mJ/cm² difference in median daily doses between July and August in London, although these months are traditionally grouped together in the “summer” season.

Similarly, there was a dramatic variation within each season in this study, overlapping D-UVB was demonstrated between seasons, particularly between spring and summer. This is of particular importance as more often than not epidemiological studies use season as a covariate when examining the association between vitamin D and health outcomes, to account for different times of blood draw between participants. If the covariate used is a weak predictor of a factor is trying to capture, then individuals 25(OH)D would subsequently not be adjusted correctly. This could impact the power of the analysis to detect a relationship between vitamin D and a health outcome, as confounding by time of year when the measurement was taken would not be appropriately accounted for. This creates difficulties when interpreting results from these studies and determining if vitamin D has an effect on health outcomes. For example, there may be an inverse association between vitamin D and the common cold found, however if the vitamin D measurement was poorly adjusted for season, then the relationship found would be severely confounded by time of year, as vitamin D would act as a marker of season and the common cold is more prevalent during certain months of the year.

Using more accurate D-UVB estimates, which is accurately adjusted for altitude, cloud cover, and ozone layer and measured over smaller geographic locations researchers would be able to accurately account for seasonal differences in vitamin D and therefore time of year would be less of a confounding factor in studies. Therefore, this research calls for more detailed UVB measurements to be used so that ambient doses of UVB in studies can be estimated, as accurately as possible.

This research also has important implications for the study design of future vitamin D studies. As vitamin D is highly correlated with UVB, studies which measure 25(OH)D during winter or spring months, when 25(OH)D concentration tends to be very low across the population, may have difficulty discriminating between those with the highest and lowest vitamin D status. As a consequence, statistical power to detect statistical associations with health outcomes might be affected, as incorrect rank within a population is assigned. Therefore, timing of 25(OH)D measurements during the year should be taken into account when designing future studies.

This study is the first to examine in detail D-UVB in Ireland and the UK using the TEMIS database. This is freely available resource which measures UV data daily in great detail over Europe. This under-utilized resource could have a large impact on UVB and vitamin D studies in Europe. As there are many different ways in which UVB can be measured, adjusted and manipulated, comparing UV data from one study to another can be challenging. For instance, if researchers wanted to compare UVB over two different regions in one particular country which were measured and adjusted slightly differently from each other, it could be difficult to ascertain if

the variation in D-UVB which was observed was because of actual regional differences in D-UVB dose, or due to the difference in the measurement approach and adjustments which were made. This study demonstrates the ease at which this comprehensive resource can be used to explore and compare D-UVB in different countries. If this resource was more widely employed, it would not only allow for more accurate and detailed D-UVB measurements within studies, than those currently in use [105, 106], but also improve the ease that which comparison between geographical regions can be made.

3.5.5 Annual UVB

In this chapter it has been shown that ambient annual D-UVB at a given location does not change dramatically from year to year over the period studied. There was no particular increasing or decreasing trend observed over ten years of data. This means that an annual D-UVB for a given year is predictive of annual D-UVB dose in a different year for that location, and correlated with cumulative dose over many years. As the TEMIS data is only available for the past ten years, an average over this time period was all that could be calculated, however, it can be presumed that UVB prior to this would be of a similar level. In accordance with this, a study by Smedley *et al.* found no significant change in UV or UVB in the UK from 1979-2008 [244]. This is important as annual D-UVB would be predictive of a longer-term UVB exposure, and could be useful when examining the relationship between vitamin D and slowly developing conditions such as cancer. However, this may not always be the case in the future, as climate change can have an impact on UVB doses reaching Earth through the depletion of ozone layer which can increase the UVB dose. Additionally, the increase in pollution and smog, due to the burning of fossil fuels could alter UVB doses as these can block UVB reaching Earth.

3.5.6 Sun Exposure Guidelines

The research outlined in this chapter also indirectly impacts for sun exposure guidelines. Currently there are conflicting guidelines that exist for “adequate sun exposure”. Some studies denote that any sun exposure can be dangerous due to the risks associated with skin cancer [245], while multiple others acknowledge the need for sunshine exposure in order to prevent vitamin D deficiency. For example, The Health Service Executive (HSE) which is the Public Health Service in Ireland actively recommends that people remain in the shade, wear sunglasses, cover up and use sun screen when outdoors. Furthermore they note that “most people receive enough vitamin D levels through a healthy diet and sunlight exposure during typical day-to-day

outdoor activities" and indeed they say: "The amount of vitamin D that can be produced in the skin is limited, so to seek UV exposure for the purpose of getting vitamin D is a waste of time" [246]. Contrastingly however, a broad recommendation by the W.H.O. suggests 5-15 minutes of sunlight exposure three times a week is necessary to prevent vitamin D deficiency [247, 248]. This recommendation has been reiterated by The National Health Service (NHS) in the UK [249], The Irish Cancer Society [250] and multiple others [251, 252]. Even the UK Scientific Advisory Committee on Nutrition, who aim to assess current vitamin D reference values are unable to make firm conclusions on sun exposure. They note that UVB is the most important source of vitamin D, however, they fail to make any recommendations about how much sun exposure is needed. As such, there are mixed and even contradictory health messages being communicated to the public, even from agencies within the same country. However, even these broad guidelines of 5-15 minutes of sunlight exposure makes no distinction between the differences in UVB dose in different countries or between seasons. As this research clearly highlights the variation which is possible even within a specific country or between latitudes and seasons it begs the question as to why this "one-size-fits-all" global sun behaviour recommendation is deemed appropriate. These substantial variations observed in this research could help inform public health committees of the importance and need for consistent, clear, and regionalised sun behaviour guidelines.

3.5.7 Strengths and Limitations

This chapter investigated UVB dose covering Ireland and the UK and has carried out detailed analysis of the two countries using the TEMIS database. This gives a detailed measure of the D-UVB dose and takes into account many variables which can considerably alter D-UVB dose, such as cloud cover, ozone layer column, and altitude. Furthermore, it only calculates the UVB dose at wavelengths which are relevant for vitamin D production, all of which are major strengths of this study. This was also the most detailed study to date using the most comprehensive D-UVB measurement on D-UVB doses in Ireland and the UK. However there is one limitation to this dataset which needs to be addressed; TEMIS database from this study was calculated using a peak action spectrum of 295nm which was derived from the final draft version of ref.[239], however the published report of ref.[239] had a peak of 298nm. This leads to a difference in daily UV dose values of a factor of about 2.2 (2.1 and 2.3 higher in summer and winter respectively using 298nm). For example if daily D-UVB dose was 4 mJ/cm^2 then D-UVB using another action spectrum could be 8.8 mJ/cm^2. This might seem like a considerable difference in daily D-UVB dose but it only affects the absolute values of the D-UVB doses. This is because the

absolute value of the daily UV dose changes with the normalisation of the action spectrum. Therefore, the results in this thesis may not be directly comparable to other studies which used a different action spectrum. However, the use of a different action spectrum does not affect the statistical relationships (regressions and correlations) presented in this chapter or subsequent chapters, merely the absolute value of the presented UVB. In accordance with this, statistical relationships using TEMIS data which used a different action spectrum have been examined in subsequent studies and similar correlations have been observed [253].

This chapter uses the most detailed spatial resolution to date, however, this can be further improved. The grids which were examined in this chapter were at a 0.5 x 0.5 degree resolution, which still covers a large area approximately 55 km (north-south) by 33 km (east-west). However, smaller grids could potentially be used in order to give greater detail to this study, for example a 0.25 x 0.25 degree resolution, and the use of large grids could be considered a limitation of this study. The TEMIS data base takes into account cloud cover, altitude and ozone cover, however it does not take into account pollution. This is another limitation of this data base, and therefore this chapter, as pollution has been shown to impact the level of D-UVB which can reach the earth's surface. A further limitation of TEMIS is that the dataset can be difficult to work with as it is quite complex. Data from every day from every grid for the last ten years was obtained and this can be difficult to manipulate. Additionally, there were a number of "blackout days" whereby no cloud cover or ozone cover data was available and as such UV dose could not be estimated. In order to work with a full dataset, average daily UVB for that day and that grid over the other 9 years was calculated and inserted into these blackout days. This not only adds further complexity to this data set but also reduces the precision of the estimate slightly, as D-UVB was not available for every single day over the last ten years. Finally, this chapter examined ambient D-UVB doses in Ireland and the UK, however this chapter was unable to relate this to personal D-UVB dose exposure. This database only takes into account ambient D-UVB dose and not the personal D-UVB dose which would be received by individuals and synthesized into vitamin D. However, it is extremely difficult to calculate personal dose in free living individuals and as such ambient UVB is often used as it is quantifiable.

3.6 Conclusion

Using a readily available and accurate ambient UVB resource, this chapter described the D-UVB doses across the Ireland and the UK over a ten year period and noted regional differences in

both countries. Large differences in D-UVB between months, seasons, latitudes and longitudes were found in Ireland and the UK. Furthermore, this chapter demonstrates why more consideration needs to be given to how D-UVB doses are measured and adjusted in future research as previous attempts to adjust for UVB using latitude, or seasonal estimates does not accurately account for the variation which exists. Additionally, it has been shown that D-UVB dose has the potential to be used in numerous ways such as to describe regional differences between countries and to be used as a more accurate vitamin D proxy, to be used alone or as a covariate in adjusted models.

4 Ambient UVB Dose at Place of Residence, 25(OH)D and Supplementation

4.1 Aim

This chapter hypothesized that a strong relationship between 25(OH)D and D-UVB exists. The aim of this chapter was threefold. Firstly, the association between 25(OH)D concentration and a UVB estimate at multiple time points over a 1 year period was examined. Secondly, this relationship was tested among those taking high dose vitamin D supplements. Finally, this chapter developed a simple scoring system to estimate 25(OH)D concentration using the two most important sources of vitamin D: supplementation and UVB.

4.2 Introduction

In the previous chapter, D-UVB doses over Ireland and the UK were found. Large regional and seasonal differences were noted. This highlighted the importance of detailed UVB measurements when using UVB in research. The first aim of this chapter is to explore the relationship between D-UVB and 25(OH)D concentration.

UVB dose directly impacts the synthesis of vitamin D in the body. Once vitamin D is synthesised, it is stored as 25(OH)D. This can then be transformed into the active form of vitamin D: $1,25(OH)_2D$, which can then regulate transcription of various genes. After exposure to D-UVB, 25(OH)D can accumulate in the body. Accumulation of high levels of 25(OH)D can occur after prolonged D-UVB exposure (or a high intake of supplements). As the constant accumulation of vitamin D in the body can lead toxicity, hypervitaminosis D, and hypercalcemia, levels of circulating vitamin D are tightly controlled. This control is mediated though the action of CYP24A1 which can degrade both 25(OH)D and $1,25(OH)_2D$. Circulating 25(OH)D normally has a half-life of around 15 days, before it is used up or starts to break down [254]. Therefore, 25(OH)D synthesis in the days leading up to blood measurement is more important than the 25(OH)D which was synthesised in the distant past.

As this accumulation and diminution of 25(OH)D is an essential aspect of vitamin D metabolism, when UVB is used to estimate 25(OH)D status, it is necessary to also take this into account. In this chapter the relationship between 25(OH)D and a D-UVB measurement which can take into account this accumulation and diminution will be explored. The measurement which will be used in this study is cumulative and weighted D-UVB (cw-D-UVB). This measurement is weighted so that it mimics the dynamic of 25(OH)D concentrations in the body, in the hope that it gives a more accurate vitamin D proxy measure. Additionally, this cw-D-UVB is calculated based on a

0.5° x 0.5° reference grid. This allows a detailed measure of D-UVB to be calculated which can take into account regional differences in D-UVB.

Furthermore, as 25(OH)D is highly seasonally biased, it is necessary to take time of year into account when developing an estimate of vitamin D. As such, the cw-D-UVB used in this chapter will be based on the date of participant's blood draw, to account for seasonal differences.

25(OH)D status does not only take into account cutaneous synthesis of vitamin D, but also dietary sources such as supplements and food. It has been shown that supplementation is a significant determinant of 25(OH)D status at northern latitudes [255]. Therefore, high dose vitamin D supplementation has an impact on 25(OH)D status independently of D-UVB dose. The second aim of this chapter is to investigate if the cw-D-UVB dose estimate created for individuals is an important determinant of 25(OH)D status in those taking high dose supplements.

Finally, by combining both the cw-D-UVB estimate and supplementation given, this chapter aims to assess the value of a composite vitamin D proxy at estimating 25(OH)D concentration. Very few studies have used combined variables when estimating an individual's vitamin D status, as it is difficult to know how much each source of vitamin D contributes to the overall vitamin D status of an individual. It is hoped that a very simple scoring system which will divide the cohort into groups depending on their supplementation use and cw-D-UVB dose can be developed in this chapter. It is hoped that this scoring system would be a useful predictor of 25(OH)D concentration.

4.3 Methods

4.3.1 Study Population

This study is a secondary analysis of a double-blind randomised placebo-controlled study which investigated the effect of vitamin D supplementation on clinical outcomes in patients with established Crohn's disease who were in remission [256, 257]. This study was conducted at Tallaght Hospital and St James's Hospital, Dublin. The study was approved by the St. James's Hospital and the Adelaide and Meath Hospital Research Ethics Committee (reference 2011/11/04). All participants provided informed, written consent. The enrolment period for this study was from October to December 2011, however baseline blood samples (time point 1: T1) were taken any time between March 2012 and July 2013. The dataset used in this analysis comprised of 92 Crohn's patients. Mean age of the cohort was 43.3 years (Standard Deviation (SD): 12). Each participant was allocated vitamin D_3 2,000 IU/d or placebo for one year with serum 25(OH)D measured every four months. Complete exclusion criteria is described elsewhere [257]. Briefly, patients were excluded if they were alcohol dependent, had a history of hypercalcaemia (corrected serum calcium >2.66 nmol/L), known hypersensitivity to vitamin D or had personal supplemental intakes of vitamin D >1,000 IU/d. Patients were allowed to continue with prescribed vitamin D and calcium supplementation under this threshold if they were taking it. Measurement of 25(OH)D was taken four times during the study period (T1-T4). These were taken at approx. four month intervals so that each individual had measurements which spanned an entire year and seasonal variation could be observed. As baseline samples (T1) were taken throughout the year, subsequent samples also varied by month and year.

4.3.2 Vitamin D Measurement

Total 25(OH)D ($25(OH)D_2$ and $25(OH)D_3$) was measured from serum samples by LC-MS/MS at the Biochemistry Department of St James's Hospital, Dublin, Ireland, which is verified by the Vitamin D External Quality Assessment Scheme and National Institute of Standards and Technology. All samples were assayed at the same laboratory to minimise technical assay variability. Due to drop out and late recruitment, not all four 25(OH)D measurements are available for all participants (N= 41 for treated participants). There were 25(OH)D measurements at all four time points for 70 participants. Baseline 25(OH)D was missing for 10 individuals. 25(OH)D concentration was also missing for seven individuals at T2, six at T3 and one at T4, within this, there were two time points missing for two individuals.

4.3.3 Cumulative and Weighted D-UVB Estimation Calculation

The TEMIS database, as described earlier, contains daily D-UVB doses over Europe. From this, daily ambient D-UVB doses in Ireland from 2005-2016 were retrieved. This data contained regionalised ambient D-UVB information for 69 distinct grid fields covering Ireland. In order to investigate the relationship between cumulative D-UVB and cw-D-UVB, mean daily D-UVB doses for each day of the year from 2005-2016 at one location were found. Daily ambient D-UVB doses 135-days prior to each day of the year were then combined to give a 135 day-cumulative estimate of D-UVB throughout the year.

135 days was chosen as it was determined as optimal in a previous study using a similar method [241]. 135-day period was found as an optimal period as D-UVB contribution prior to that is negligible to 25(OH)D concentration [241]. This method was also used to maximise the percentage of variance explained. This thesis chose to use 135 days in order to remain consistent with previous research carried out. However, sensitivity analysis was carried out at 60-days and 120-days (data not shown) and differences in cw-D-UVB dose between the three measurements were minor after weighting was taken into account.

These mean daily doses were also weighted as per equation 1, to give a 135 day cumulative and weighted D-UVB estimate for each day of the year. This weighting was carried out so that exposures immediately preceding blood sampling contribute more to the estimate than exposures from a more distant past. It has been observed that the half-life of vitamin D in the body is normally 2 months, while circulating 25(OH)D can get used up or broken down after 15 days [254], further supported by analytical confirmation, a half-life of 35 days was chosen as optimal [241]. The weighting equation is shown below; where x = days ago (starting day before and up to 135 days prior to sampling), y = rate of disappearance of effect of D-UVB in days (half-life set at 35 days ([241])), and e(-ln2)(x/y) is the weighing formula applied. This formula allowed the calculation of a 135-day cumulative D-UVB dose and a 135-day cw-D-UVB for each day of the year, and an investigation of the relationship between them.

Cumulative and weighted ambient D-UVB

$$(x) = \sum_{x=1:135} \left(\textit{D-UVB } (\mathrm{x}) \right) * e^{-\left(\frac{ln2}{y}\right)x}$$

Equation 1: Cumulative and weighted D-UVB dose

4.3.4 Cw-D-UVB Calculation for Study Participants

In order to calculate cw-D-UVB for each individual in the cohort, a TEMIS grid field was assigned to each participant in the study based on their residential location. Daily D-UVB doses over 135 days prior to blood draw were then extracted independently for each participant. This measurement was dependent on a participant's grid reference and date of blood draw, in order to account for the seasonal and regional differences in D-UVB. These daily ambient D-UVB doses were then combined to give a cumulative estimate of D-UVB, with each daily dose being weighted as described above. As 25(OH)D accumulates and breaks down in the body, it was necessary to account for this with the D-UVB estimate. This is the reason why the daily estimates were weighted. This calculation provided estimates of cw-D-UVB for each participant prior to blood draw, at their place of residence. As 25(OH)D measurements were taken four times in this cohort (25(OH)D_{T1}, 25(OH)D_{T2}, 25(OH)D_{T3}, and 25(OH)D_{T4}), four individual cw-D-UVB doses (cw-D-UVB_{T1}, cw-D-UVB_{T2}, cw-D-UVB_{T3}, and cw-D-UVB_{T4}) were calculated for each individual prior to the date at which each of the 25(OH)D concentrations were taken.

4.3.5 Vitamin D Score (VDscore1)

A new variable called VDscore1 was created, using a simple scoring system based on an individual's treatment allocation and cw-D-UVB dose, two of the most important sources of vitamin D for humans.

To create this scoring system the cohort was first stratified into those who were and were not taking high dose supplements (namely those who were randomised to take supplements and those who were randomised to placebo). Next, four groups were created according to the quartile of cw-D-UVB dose.

Quartile one contains individuals who belong to the 1st-24th percentile of cw-D-UVB doses, Quartile two: 25th-49th percentile, Quartile three: 50th-74th percentile and finally quartile four: 75th-100th percentile. Depending on which quartile of cw-D-UVB and category of supplementation (supplemented/placebo) a patient belonged to, they were assigned a VDscore1 score (**Table 4.1**). Simply, a score of +4 was given if an individual was part of the supplemented group and +0 if they were part of the placebo group. They were next given a score based on their cw-D-UVB quartile, +1 for each increase in quartile number. This ensured that randomisation group was the most important factor in the score with cw-D-UVB coming second.

These scores ranged from 1-8, with number one being a participant who was in the lowest cw-D-UVB quartile and was not supplemented and number eight being a participant who was supplemented and belonged to the highest quartile of cw-D-UVB (**Table 4.1**). A different VDscore1 was calculated for each time point (T1-T4) due to the differences in cw-D-UVB doses which had previously been calculated for each of the time points. Therefore each participant had four VDscore1s.

	Non-supplemented (placebo)				**Supplemented (2000 IU)**			
cw-D-UVB	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Scores	1	2	3	4	5	6	7	8

Table 4.1: Allocation of VDscore1 to individuals
Four different VDscore1 were calculated for each individual depending on their cw-D-UVB dose for that time point.

4.3.6 Statistical Analysis

All analyses were performed in R (R Development Core Team, 2011). Seasonal differences between 25(OH)D concentration and cw-D-UVB were assessed by looking at the seasons; winter [Dec-Feb], spring [Mar-May], summer [Jun-Aug], autumn [Sep-Nov].

Linear regression models were used to determine if there was an association between cw-D-UVB dose and serum 25(OH)D at baseline (T1), four months (T2), eight months (T3) and 12 months (T4) follow up. The model was originally adjusted for age, sex, smoking status, alcohol consumption, baseline (T1) 25(OH)D concentration [low: <50 nmol/L, medium: 50-74 nmol/L, high: ≥75 nmol/L], and randomisation group. This was then narrowed down by backwards stepwise regression and the final model was adjusted for age, gender, randomisation group, cw-

D-UVB dose and baseline 25(OH)D group (except when examining the association for T1, this model was not adjusted for baseline 25(OH)D level). Final model selection was determined by R^2 number, Akaike information criterion (AIC) and Bayesian information criterion (BIC) (**Table 4.2**). Stratification by randomisation group was also carried out to test associations in supplemented and placebo groups separately. Linear regression was deemed appropriate after examining residuals, r^2, and carrying out diagnostic plots (**Appendix 5**). VIF scores were also checked for multicollinearity but this was not observed.

Multilevel regression models were used to determine if there was an association between cw-D-UVB dose or VDscore1 and serum 25(OH)D for all four time points. Model one was adjusted for age, sex, D-UVB and randomisation group. Model two was adjusted for age, gender and VDscore1.

The Boruta method was then used for classification of 25(OH)D into different categories (<25 nmol/L [deficient] , 25-40 nmol/L [high risk of deficiency] , 40-50 nmol/L [low risk of deficiency], 50-75 nmol/L [sufficient], >75 nmol/l [highly sufficient]) using cw-D-UVB and VDscore1 [258]. This method uses a wrapper approach around a random forest classifier. The method can be used to determine the importance of the variables at classifying the different categories. It does this by creating random probe variables (shadow min/shadow mean/ shadow max) and examines if the variables used in the model are more important classifiers than the random probes [259]. The 'Boruta' package was used for this analysis [260]. P<0.05 was considered statistically significant.

Model	R^2	AIC	BIC
1	0.64	635	660
2	0.62	634	654
3	0.62	632	650
4	0.59	669	685

Table 4.2: Model selection for association analysis.
Model one was adjusted for age, gender, smoking status [current, previous, never], alcohol intake [yes/no], baseline 25(OH)D concentration [high, medium, low] and randomisation. Model two was adjusted for age, gender, alcohol intake, baseline 25(OH)D concentration and randomisation. Model three was adjusted for age, gender, baseline 25(OH)D concentration and randomisation. Model four was adjusted for gender, baseline 25(OH)D concentration and randomisation. Model three was selected as the final model.

4.4 Results

4.4.1 Cw-D-UVB Doses vs Daily D-UVB:

The relationship between mean daily ambient D-UVB doses for each day of the year from 2005-2016 and the cw-D-UVB calculated using daily D-UVB doses were first examined. As expected, a dramatic seasonal fluctuation for both the daily D-UVB and cw-D-UVB was found (**Figure 4.1a**). Both of these estimates were higher during the summer months, however they peak at different times: the peak of daily-D-UVB doses occurs on the 2nd of July, while the peak of cw-D-UVB is approximately one month later, on the 4th of August (**Figure 4.1**).

The relationship between cumulative D-UVB and cw-D-UVB was found not to be linear, but rather elliptical in shape (**Figure 4.1b**). It was noted that for the same cumulative D-UVB dose, cw-D-UVB dose differed depending on whether daily D-UVB in the days leading up to D-UVB estimation were increasing or decreasing, preceding the time-point of interest. Higher cw-D-UVB doses were found when daily D-UVB doses increased and the opposite was true if daily D-UVB were decreasing (**Figure 4.1b**).

The absolute contribution of daily doses to the cumulative and weighted estimate also differed depending on if daily D-UVB doses were increasing or decreasing (**Figure 4.2**). It can be observed that if daily D-UVB doses are increasing prior to sampling then the D-UVB doses on the days leading up to this point, contribute more, than days of the distant past. However, when D-UVB doses are decreasing prior to sampling, days in the distant past, which would have had higher daily D-UVB doses, are contributing more to 25(OH)D concentrations compared to the days directly prior to sampling which would have had lower D-UVB doses. This is why cw-D-UVB between spring and summer differ so much, as although the daily D-UVB around these times would be similar, the actual cumulative and weighted dose are widely different. This demonstrates the importance of time of sampling when calculating cw-D-UVB doses, or indeed taking 25(OH)D measurement.

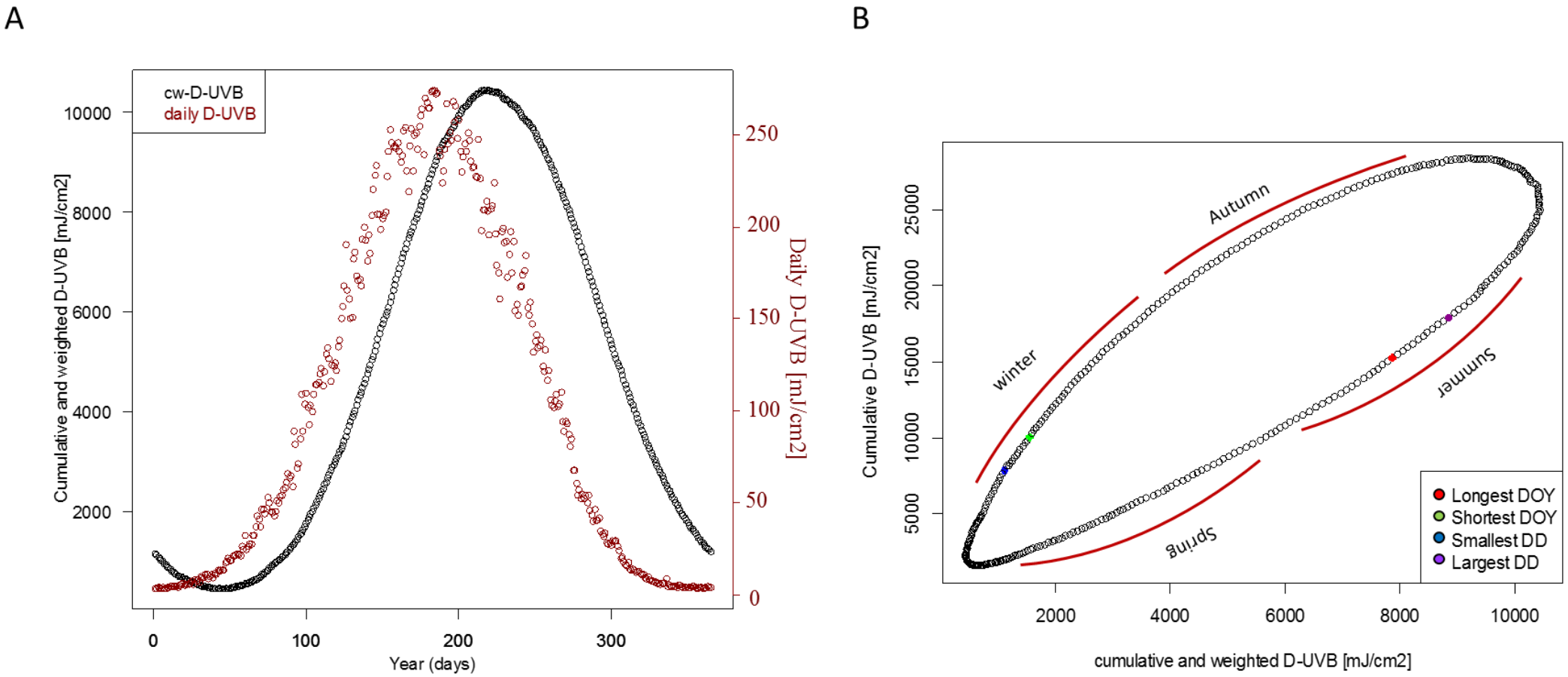
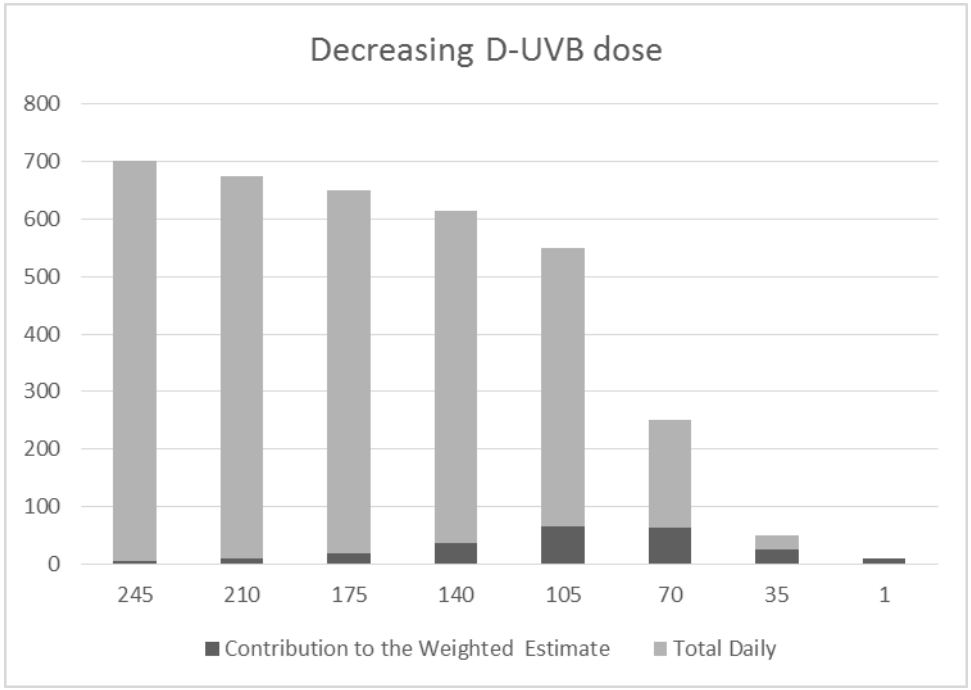


Figure 4.1: Analysis of cw-D-UVB over one year.

A) Cumulative and weighted D-UVB dose (cw-D-UVB) vs mean daily D-UVB dose from 2005-2017 in London **B)** mean 135-day cw-D-UVB D-UVB dose vs 135day-Cumulative D-UVB dose over a one year period in London. DOY: day of year, DD: daily dose.

A



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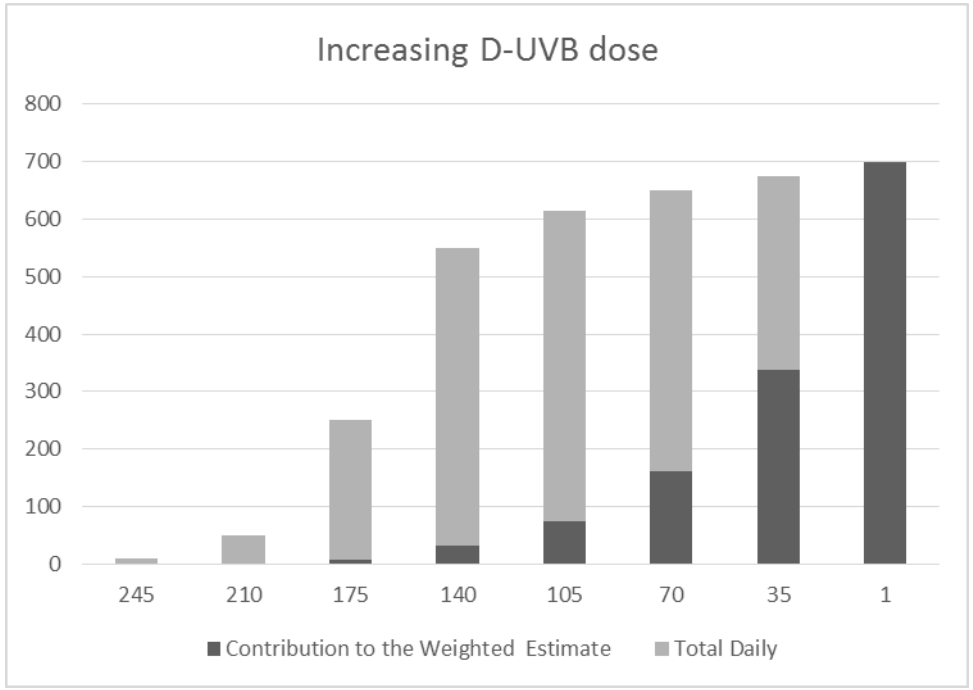


Figure 4.2: Difference in cw-D-UVB dose with increasing and decreasing daily doses

A) Contribution to weight estimate if daily D-VB doses are decreasing **B)** Contribution to weight estimate if daily D-VB doses are increasing

4.4.2 Baseline characteristics

Baseline measurements (T1) were taken at different times from 2012-2013 and therefore the dates of each additional time point are unique to each patient (T2, T3, T4). Median 25(OH)D concentration at T1, T2, T3 and T4 was found to be 65, 65, 61, 62 nmol/L in the placebo patients and 66, 104, 101 and 98 nmol/L in the supplemented patients (**Table 4.3**), however there was wide variation in 25(OH)D concentrations between individuals due to the seasonal effects.

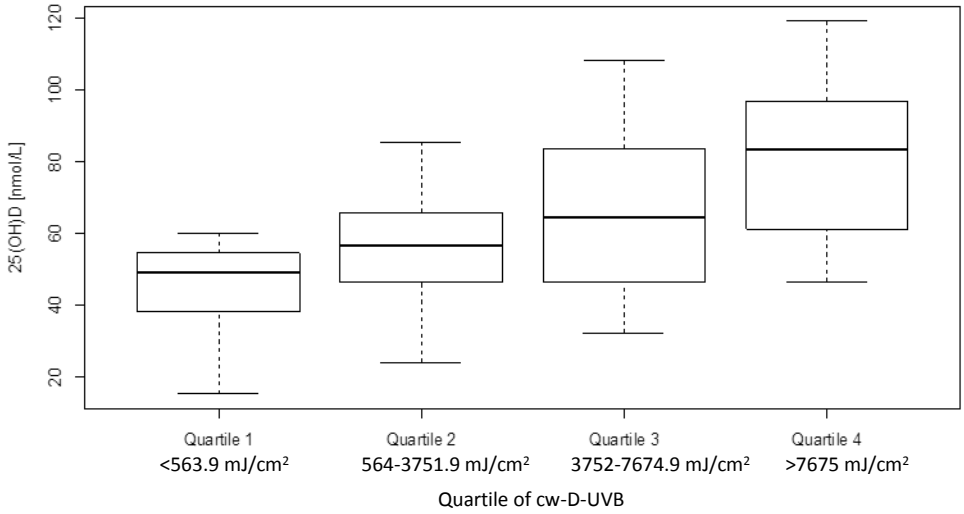
A high correlation between baseline 25(OH)D_{T1} concentration in the Crohn's cohort and the individually calculated cw-D-UVB_{T1} doses was noted (**Figure 4.3**). An increasing linear trend was observed in a scatterplot of 25(OH)D and cw-D-UVB_{T1}, and when 25(OH)D_{T1} was plotted against quartiles of cw-D-UVB (**Figure 4.3**).

Table 4.3: Baseline characteristics of Crohn's cohort

Characteristic	*All* *N (%)*	*Vitamin D* *N (%)*	*Placebo* *N (%)*
No. of patients	92	50 (54)	42 (46)
***Age*[1]**	43.3 (12)	42.4 (12)	44.56 (12)
Sex (female)	45 (49)	22 (45)	23 (55)
***25-OHD (nmol/l)*[1]**			
T1	57.3 (47-84)	57.3 (46-89)	57.2 (51-80)
T2	87.5 (63-109)	100.0 (85-128)	63.7 (64-84)
T3	84.8 (54.9-107.8)	99.0 (79-124)	58.5 (40-77)
T4	82.0 (57-105)	97.5 (81-116)	64.0 (41-77)
***Cw-D-UVB (mJ/cm²)*[1]**			
T1	3752 (564-7675)	3072 (527-7930)	3841 (712-7112)
T2	4801 (1443-8603)	4987 (1429-8284)	4585 (1741-8935)
T3	1483 (521-6364)	1483 (596-6364)	1453 (389-5840)
T4	2556 (563-7313)	2027 (406-7842)	2637 (597-7275)
***VDscore1*[1]**			
T1	3 (2-4)	2 (1-4)	3 (2-3)
T2	5 (3-7)	6 (5-8)	2 (2-4)
T3	5 (3-7)	7 (6-8)	2 (1-4)
T4	5 (3-7)	7 (5-8)	3 (1-3)
Alcohol			
Yes	46 (50)	29 (63)	17 (37)
No	40 (43)	18 (45)	22 (55)
NA	6 (7)	3 (50)	3 (50)
Smoking Status			
Never-smoker	38 (41)	23 (61)	15 (39)
past smoker (> 1year)	39 (42)	18 (46)	21 (54)
Current smoker	9 (9)	6 (66)	3(33)
NA	6 (7)	3 (50)	3 (50)
Season of T1 blood draw (NA=1)			
Winter	14 (15)	10 (71)	4 (29)
Spring	35 (38)	18 (51)	17 (49)
Summer	24 (26)	13 (54)	11 (46)
Autumn	18 (20)	9 (50)	9 (50)

Footnote: [1]values represent median and IQR

A



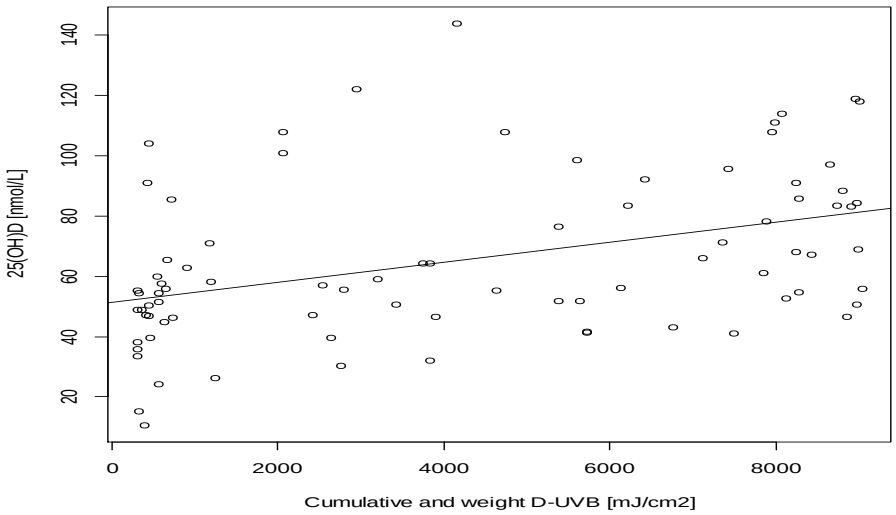


Figure 4.3: Relationship between 25(OH)D and cw-D-UVB

Relationship between 25(OH)D and **A)** quartiles of cw-D-UVB, **B)** Scatter plot of 25(OH)D vs cw-D-UVB at baseline

4.4.3 Seasonal Differences in 25(OH)D and cw-D-UVB

When seasonal differences were examined, both the highest 25(OH)D concentrations and cw-D-UVB doses were found in patients sampled during the summer months (**Figure 4.4**).

Unsurprisingly, there were dramatic differences in the change in serum 25(OH)D concentration over the four time points between the supplemented and placebo patients with vitamin D supplemented individuals having higher 25(OH)D concentrations than placebo participants. (**Figure 4.5, 4.6**).

A seasonal fluctuation of 25(OH)D and cw-D-UVB was observed in the majority of placebo patients (**Figure 4.5**). For example, when examining Patient 33 in detail, one can observe that they were first sampled in November and their baseline 25(OH)D was 74.3 nmol/L; their 25(OH)D concentration then decreased when they were next sampled in March (T2): 30.8 nmol/L; following this it increased in July (T3): 94.3 nmol/L and finally decreased again in November (T4): 77 nmol/L (**Figure 4.5**). Similar fluctuation was also observed when cw-D-UVB was measured examined for Patient 33 (**Figure 4.5**).

Similarly, when examining Patient 25 one can see that this individual was first sampled in September and their 25(OH)D was 41 nmol/L, this decreased in T2 which occurred in January: 21 nmol/L, increased at T3 in May: 43 nmol/L and finally decreased once more at T4 in September the following year: 36 nmol/L. This trend was again observed when cw-D-UVB was estimated (**Figure 4.5**). Due to the fact that this individual's baseline was sampled in September, their 25(OH)D concentration was not taken during the peak of the summer months, this means that relatively consistent 25(OH)D concentrations were found for three of the four time points in this individual (**Figure 4.5**).

Seasonal fluctuations of 25(OH)D concentration were less evident in supplemented participants taking 2,000 IU/d and a steady rise in 25(OH)D was observed between T1 and T2 in the majority of patients in the supplemented group. Unsurprisingly, seasonal effects were still noted when cw-D-UVB was determined for these individuals.

For example, Patient 11 was first sampled in September and had a baseline 25(OH)D of 95.7 nmol/L, this was then increased to 129 nmol/L even though next follow up was in January, then further increased to 133 nmol/L in May and remained relatively steady in September, 123 nmol/L. This is unlike Patient's 11 cw-D-UVB dose which decreased at T2, remained low for T3, and increased for T4, and followed the seasonal trend expected from D-UVB (**Figure 4.6**). A similar effect was observed for Patient 20, with a steady increase in 25(OH)D concentration from T1 to T4, although one would expect 25(OH)D to drop at T2 in November (**Figure 4.6**). In some cases, even in those supplemented, both 25(OH)D and cw-D-UVB followed seasonal patterns.

Patient 83 for example, was first sampled in February and had a 25(OH)D concentration of 35.8 nmol/L. This was then increased in June following supplementation to 85.4 nmol/L, despite continued supplementation 25(OH)D decreased in October (53.5 nmol/L) and again in February (24 nmol/L). Correspondingly, seasonal trends were also observed when cw-D-UVB doses were examined. Similar seasonal behaviours of 25(OH)D and cw-D-UVB were also evident in patients 76 and 80, despite high dose supplementation (**Figure 4.6**).

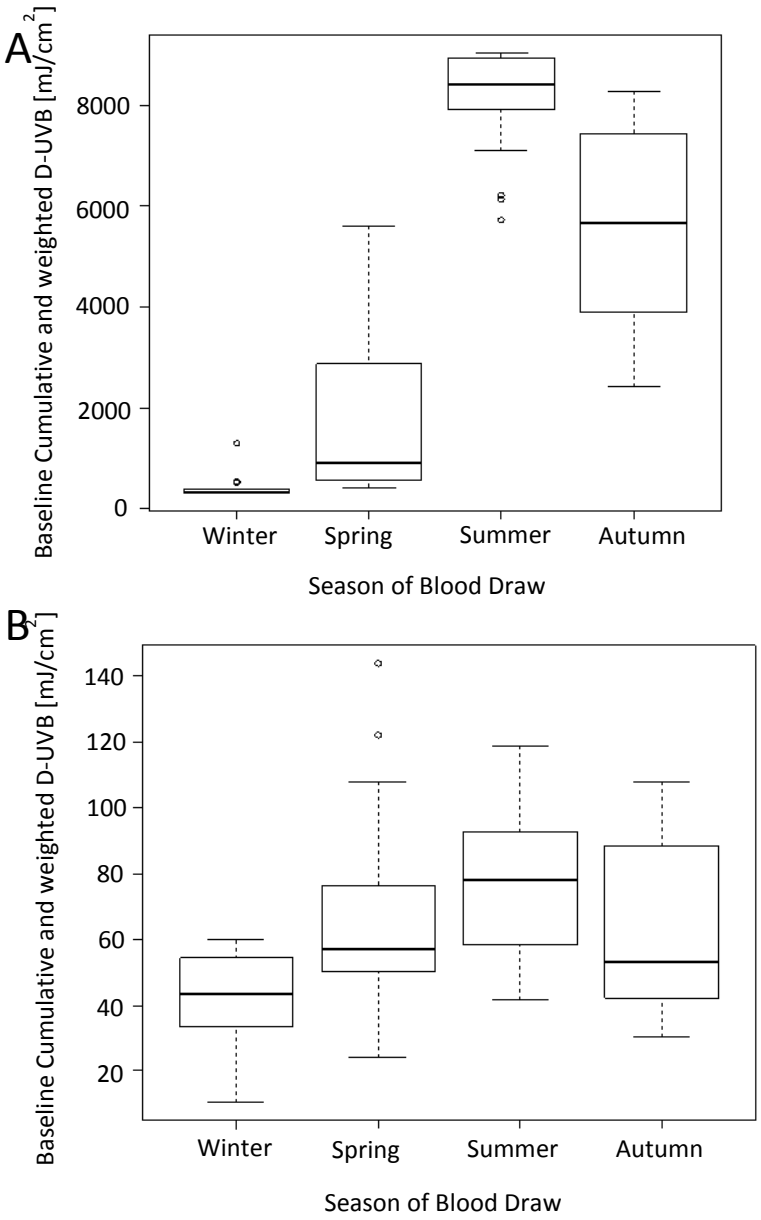
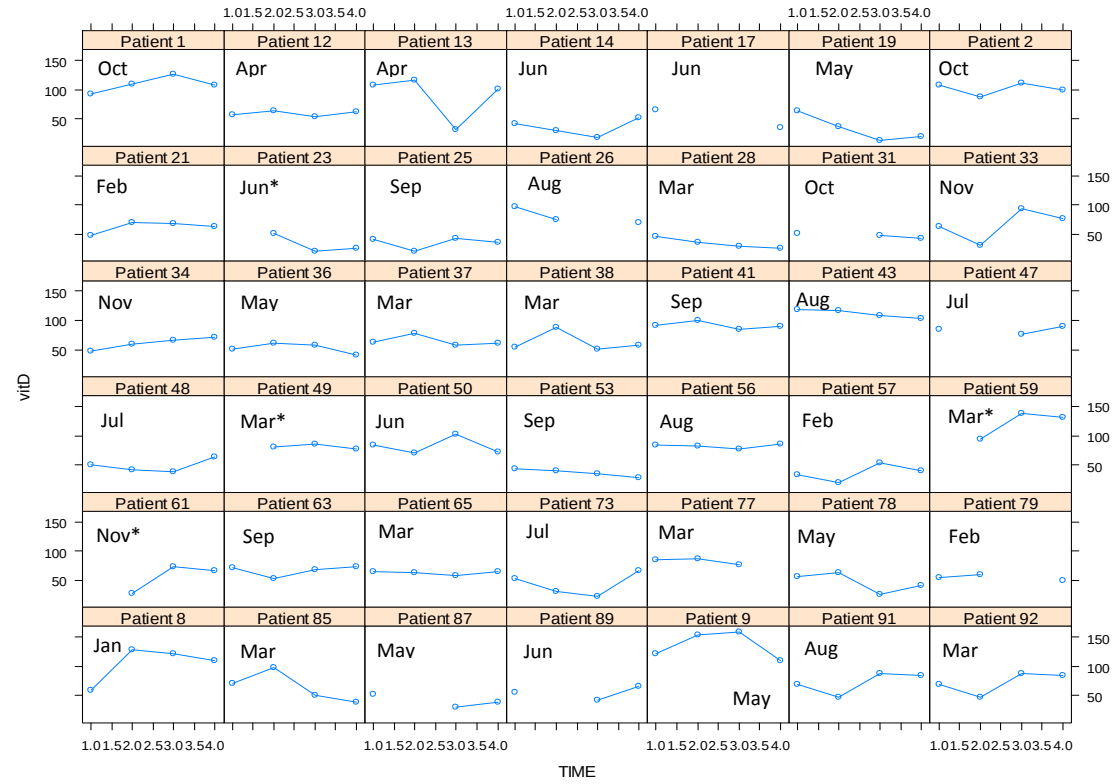


Figure 4.4: Seasonal differences between 25(OH)D and cw-D-UVB
Relationship between season and **A)** cw-D-UVB and **B)** 25(25(OH)D. Season; winter [Dec, Jan, Feb], spring [Mar, Apr, May], summer [Jun, Jul, Aug], autumn [Sep, Oct, Nov].

A



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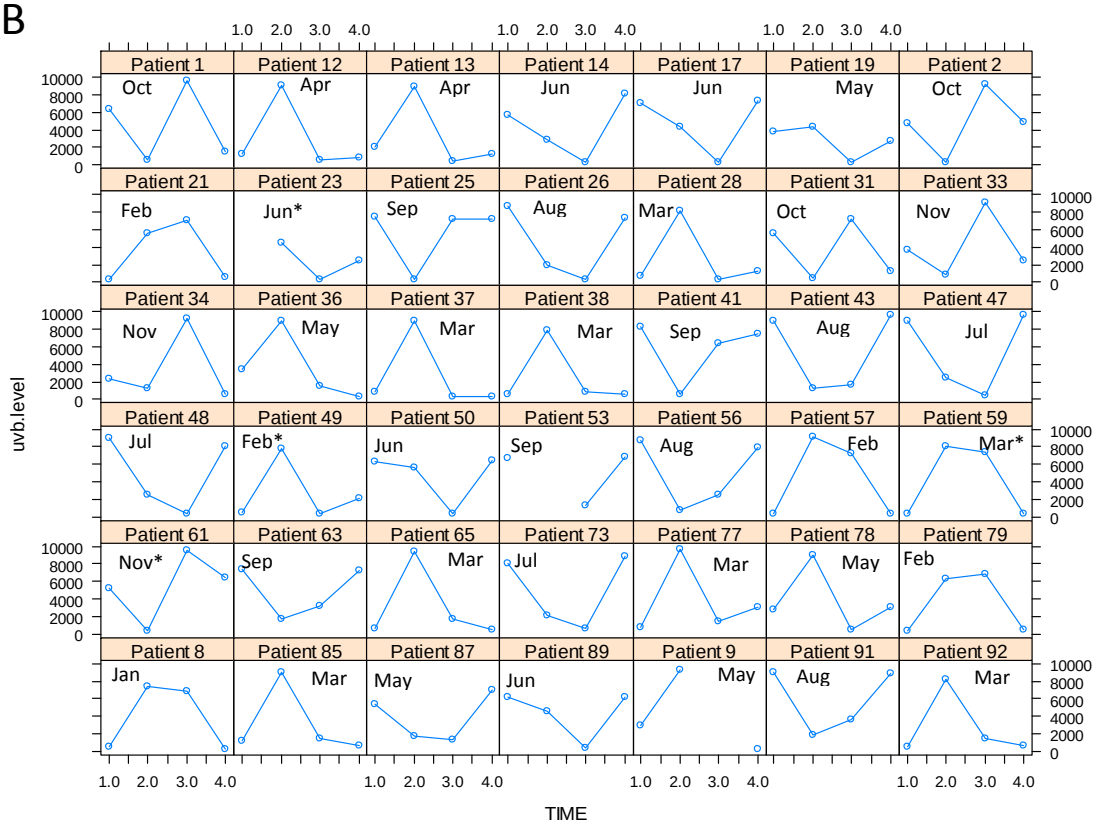


Figure 4.5: 25(OH)D concentration and cw-D-UVB dose for placebo patients for four time points

A) 25(OH)D concentration **B)** cw- D-UVB dose estimates over the one year period for all patients randomised to receive placebo. Time points one to four were not taken at the same time for each individual but instead taken at four month intervals following baseline. * Estimated baseline month due to missing sample

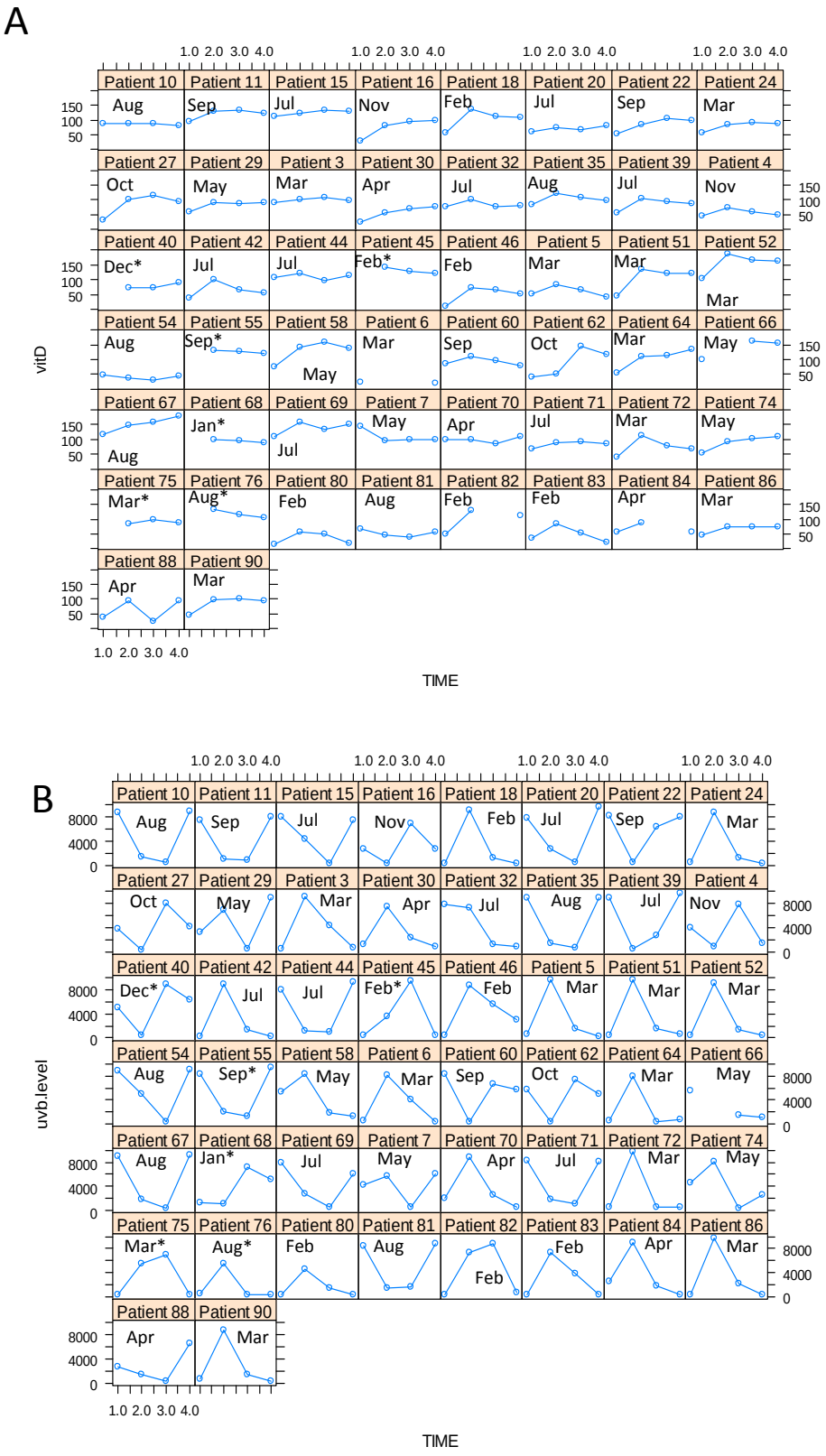


Figure 4.6: cw-D-UVB dose for supplemented patients

A) 25(OH)D concentration **B)** cw-D-UVB dose estimates over the one year period for all patients randomised to receive 2000 IU daily. Time points one to four were not taken at the same time for each individual but instead taken at four month intervals following baseline.* Estimated baseline month due to missing sample

4.4.1 Associations between 25(OH)D and cw-D-UVB

A strong association between cw-D-UVB and serum 25(OH)D concentration in a linear regression model was observed. Individual cw-D-UVB doses were highly associated with the 25(OH)D concentrations at T1, T2, and T3 (**Table 4.4**). For every 1,000 mJ/cm^2 increase in cw-D-UVB dose, an average increase of 3 nmol/L was observed for 25(OH)D concentration. Similar trends were noted when this association was restricted to supplementation only participants and in placebo only patients (**Table 4.4**). Strong associations were also observed between cw-D-UVB and serum 25(OH)D concentration in a multilevel regression model (**Table 4.5**).

It was also observed that 25(OH)D was strongly associated with the VDscore1 developed in all four time points (**Table 4.6**). Individually calculated VDscore1s for each time point were highly associated with 25(OH)D concentrations at that corresponding time point. E.g.: VDscore1 calculated using cw-D-UVB which was estimated for T3 was associated with 25(OH)D concentration at T3. For every unit increase in VDscore1, 25(OH)D concentration increased an average of 9.3 nmol/L. This trend was also observed in a multilevel model (**Table 4.7**).

When the classification ability both vitamin D estimates created was examined, cw-D-UVB was found to be a tentatively important variable when classifying 25(OH)D groups at baseline (T1) and at T2. This means that this variable is as good as the best random probe variable but this algorithm was not able to say with enough confidence that it was better than these random probes. One the other hand randomisation was found to be an important variable when examining models at T3 (**Figure 4.7**). VDscore1, which incorporated both cw-D-UVB dose and randomisation, was also found to be an important variable in at all time points after randomisation (**Figure 4.8**). It was also noted that VDscore1 performed better in this analysis than randomisation alone at T2 and T3 and was just as important as randomisation at T4.

In order to determine if VDscore1 was a stronger predictor of 25(OH)D concentration than supplementation alone, two models were created and compared. These contained information on age, gender and either supplementation use or VDcore1. The difference between the models was examined by looking at the AIC and BIC of the models and the R^2 values. It was found that models which contained VDscore1 at 2 and T3 performed better as they had a lower AIC and BIC number and a higher r^2 value, although this was not the case at T4 (**Table 4.8**).

Table 4.4: Associations between cw-D-UVB and 25(OH)D concentration

Time point	Association with	N	Cw-D-UVB2		
All participants			**Beta**	**SE**	**P-value**
Cw-D-UVB$_{T1}$[1]	25(OH)D$_{T1}$	82	$2.9x10^{-3}$	$8.3x10^{-4}$	**$8.7x10^{-4}$**
Cw-D-UVB$_{T2}$[2]	25(OH)D$_{T2}$	84	$2.7x10^{-3}$	$7.3x10^{-4}$	**$5.6.x10^{-4}$**
Cw-D-UVB $_{T3}$[2]	25(OH)D$_{T3}$	85	$3.7x10^{-3}$	$1.0x10^{-3}$	**$5x10^{-4}$**
Cw-D-UVB $_{T4}$[2]	25(OH)D$_{T4}$	91	$-8x10^{-4}$	$7.7x10^{-4}$	0.31
Vitamin D supplemented					
Cw-D-UVB$_{T1}$[1]	25(OH)D$_{T1}$	44	$3.8x10^{-3}$	$1.2x10^{-3}$	**$2.8x10^{-3}$**
Cw-D-UVB$_{T2}$[3]	25(OH)D$_{T2}$	49	$3.0x10^{-3}$	$1.1x10^{-3}$	**$9x10^{-3}$**
Cw-D-UVB $_{T3}$[3]	25(OH)D$_{T3}$	47	$3.5x10^{-3}$	$1.6x10^{-3}$	**0.03**
Cw-D-UVB $_{T4}$[3]	25(OH)D$_{T4}$	50	$-1.9x10^{-3}$	$1.2x10^{-3}$	0.14
Placebo					
Cw-D-UVB$_{T1}$[1]	25(OH)D$_{T1}$	38	$1.8x10^{-3}$	$1.5x10^{-3}$	0.15
Cw-D-UVB$_{T2}$[3]	25(OH)D$_{T2}$	35	$1.9x10^{-3}$	$9.8x10^{-4}$	*0.058*
Cw-D-UVB $_{T3}$[3]	25(OH)D$_{T3}$	38	$4.5x10^{-3}$	$1.2x10^{-3}$	**$6x10^{-4}$**
Cw-D-UVB $_{T4}$[3]	25(OH)D$_{T4}$	41	$3.6x10^{-3}$	$9.1x10^{-4}$	0.69

Footnote:

[1] Model adjusted for age and sex

[2] Model adjusted for age, sex, baseline 25(OH)D concentration [Low: <50 nmol/L, Medium: 50-74 nmol/L, High ≥75 nmol/L]

[3] Model adjusted for age at diagnosis, sex, baseline 25(OH)D concentration [Low: <50 nmol/L, Medium: 50-74 nmol/L, High ≥75 nmol/L]

Table 4.5: Multilevel associations between cw-D-UVB and 25(OH)D concentration

Model fit: AIC=2866, BIC= 2892

Variable	Estimate	SE	DF	T-value	P-value
All participants					
Sex					
Female	Ref	Ref	Ref	Ref	Ref
Male	2.28	5.73	82	0.39	0.69
Age diagnosis	0.25	0.23	82	1.07	0.29
Vitamin D supplementation	32.0	2.80	228	11.43	**<0.001**
Cw-D-UVB dose	0.002	0.0002	228	6.71	**<0.001**

Table 4.6: Association between VDscore and 25(OH)D

Associations between cw-D-UVB and 25(OH)D concentration over four time points [1].

Time point	Association with	N	VDscore1		
All participants			**Beta**	**SE**	**P-value**
Cw-D-UVB$_{T1}$	25(OH)D$_{T1}$	82	9.68	2.58	**0.0003**
Cw-D-UVB$_{T2}$	25(OH)D$_{T2}$	84	9.79	1.25	**$6x10^{-11}$**
Cw-D-UVB $_{T3}$	25(OH)D$_{T3}$	85	10.78	1.32	**0.001**
Cw-D-UVB $_{T4}$	25(OH)D$_{T4}$	91	7.0	1.32	**$1.5x10^{-6}$**

Footnote:

[1] model adjusted for age, sex, and 25(OH)D at baseline. Significant results are shown in bold.

Table 4.7: Multilevel associations between VDscore1 and 25(OH)D concentration

Model fit: AIC=2833, BIC= 2856

Variable	Estimate	SE	DF	T-value	P-value
All participants					
Sex					
Female	Ref	Ref	Ref	Ref	Ref
Male	-0.09	5.37	82	-0.02	0.99
Age diagnosis	0.39	0.22	82	1.77	0.08
VDscore1	7.68	0.52	229	14.80	**<0.001**

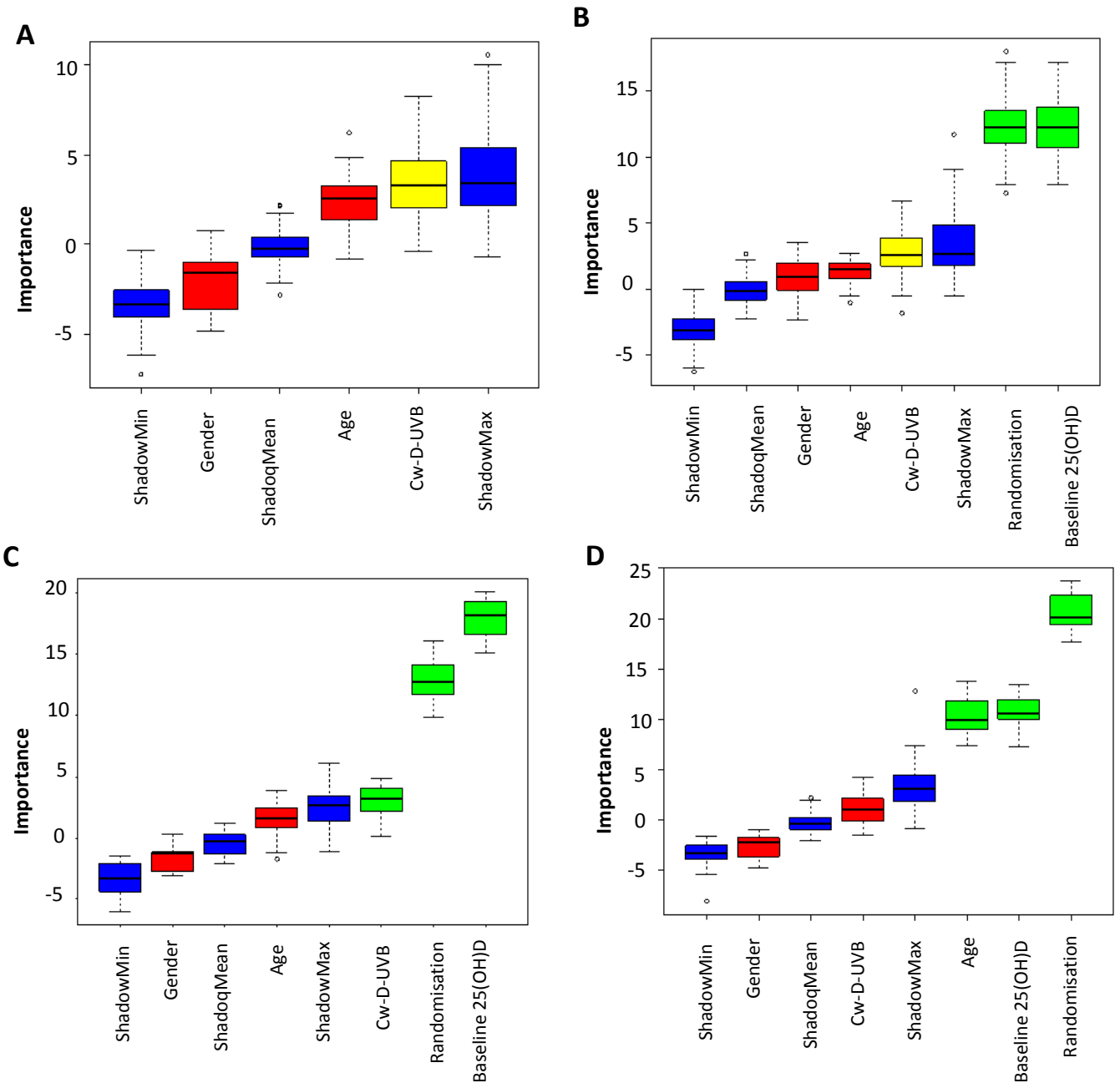


Figure 4.7: Boruta method and cw-D-UVB

Boruta method to determine important variables with the model. Shadow min/ shadow Mean and Shadow max represent random probes and are shown in blue. Those in red are deemed not to be important variables, yellow determines uncertain variables and green demonstrates variables which are deemed important. Cw-D-UVB association with 25(OH)D at **A**) time point T1, **B**) time point T2, **C**) time point T3, **D**) time point T4.

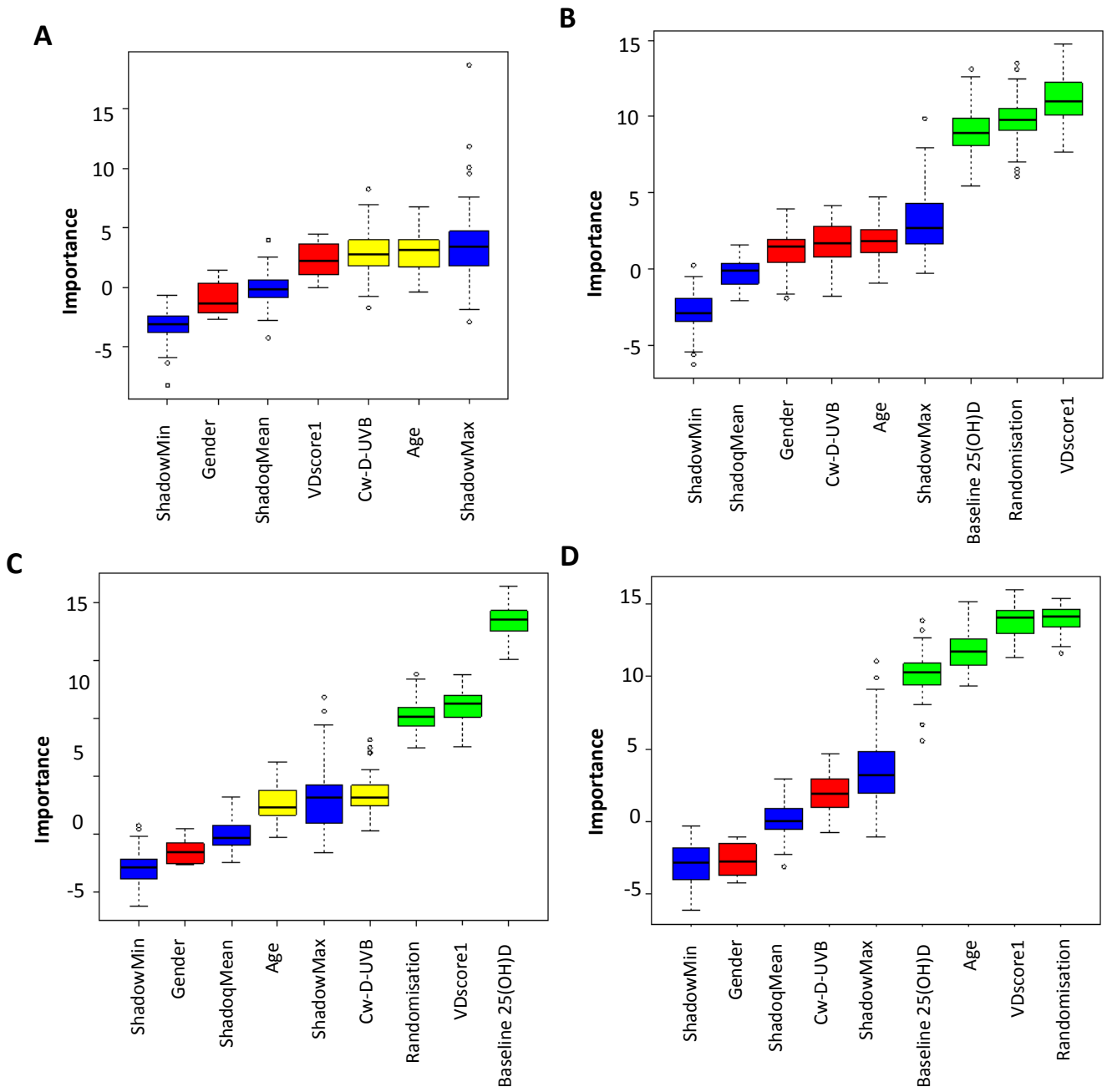


Figure 4.8: Boruta method and VDscore1

Boruta method to determine important variables with the model. Shadow min/ shadow Mean and Shadow max represent random probes and are shown in blue. Those in red are deemed not to be important variables, yellow determines uncertain variables and green demonstrates variables which are deemed important. VDscore1 association with 25(OH)D at **A**) time point T1, **B**) time point T2, **C**) time point T3, **D**) time point T4

Table 4.8: Direct model comparisons of the association with 25(OH)D between randomisation use alone and VDscore1 use alone

Time point	T2		T3		T4	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
R^2	0.41	0.38	0.43	0.35	0.28	0.35
AIC	777.8	791.9	794.1	816.8	811.1	802.8
BIC	789.8	804.0	806.2	829.0	823.3	814.9

Footnote:
Model 1 contained: Age, gender, and VDscore1
Model 2 contained: Age, gender, and randomisation

4.5 Discussion

4.5.1 Daily, cumulative, and cumulative and weighted D-UVB

Differing peak times of ambient daily D-UVB and cw-D-UVB were found. These findings were unsurprising as the cw-D-UVB estimate is weighted to adjust for the accumulation and diminution of UVB in the body, to mimic that of 25(OH)D, and as such it lags behind daily D-UVB doses throughout the year. The peak of daily-D-UVB was found in July, while the peak for cw-D-UVB doses was found a month later, in August. The lowest daily D-UVB dose was found in December, but the lowest cw-D-UVB dose was found in February after months of no or limited skin synthesis. The "lag" between the two measurements is also what one sees between daily D-UVB doses and 25(OH)D concentration. Indeed, there has been many studies demonstrating that 25(OH)D doses are at the lowest during February and March [212].

A non-linear relationship between cumulative D-UVB and cw-D-UVB was also found. This was also unsurprising as the cw-D-UVB dose was created to approximate the accumulation and diminution of vitamin D in the body, while cumulative dose only captures the accumulation over a period of time.

25(OH)D in the body is metabolised after approximately 15 days and as such 25(OH)D synthesised in the days prior to date of sampling are of more importance to vitamin D estimation than the 25(OH)D which was synthesised in the distant past. Therefore, daily D-UVB doses in the lead up to day of sampling are given more weight in cw-D-UVB dose than daily D-UVB doses taken a long time prior to sampling, as vitamin D synthesised in the past would mostly be used up. This is important as it means that even if two individuals have similar cumulative doses, they may have differing cw-D-UVB doses depending on what time of the year they were sampled.

At times when D-UVB dose is decreasing, cw-D-UVB is going to be smaller than at times when D-UVB dose is increasing for the same cumulative dose. For instance, cumulative D-UVB dose on the 1st of August and the 19th of October were similar (24,484 mJ/cm^2 vs 24,602 mJ/cm^2), however, cw-D-UVB doses at these dates were considerably different; for August 1st this was 10,374 mJ/cm^2 and 6,114 mJ/cm^2 for October 19th, due to the fact that the days leading up to the 1st of Aug had higher D-UVB doses than those leading up to the 19th of Oct. This ensures that this cw-D-UVB follows a similar seasonal trend to 25(OH)D concentrations. Additionally, depending on whether daily D-UVB doses are increasing or decreasing at the time of sampling, different days prior to sampling will contribute more to the cw-D-UVB estimate. The dose is weighted to take into consideration the half-life of 25(OH)D. However, if daily D-UVB doses are decreasing and on day 70 for example, daily D-UVB dose would be larger than daily D-UVB dose on day 35, even when weighting is taken into consideration. Therefore, the contribution to the

cw-D-UVB dose would be greater from day 70 than on day 35, due to the large overall amount of D-UVB dose which was received on day 70.

4.5.2 Seasonal Differences in 25(OH)D and cw-D-UVB

When examining the seasonal differences between baseline estimates of 25(OH)D and cw-D-UVB doses, comparable results were found. Greater seasonal variation in 25(OH)D concentration was observed in the seasonal plots when compared to the seasonal variation in cw-D-UVB doses, particularly for those who were sampled in spring and winter. This is unsurprising as 25(OH)D status reflects all sources of vitamin D while cw-D-UVB only takes into account UVB. As dietary and supplementation habits differ from individuals, a larger variation in 25(OH)D concentration is unsurprising. Similarly, there were differences observed when examining baseline measurements of individuals sampled in summer, with cw-D-UVB doses being much higher than in those who were sampled in spring and autumn. These seasonal differences were not as dramatic when 25(OH)D concentrations were examined. This may be due to the large variation which was observed in 25(OH)D concentration within seasons or because cw-D-UVB takes into account the amount of ambient D-UVB in a given location, but it cannot account for the "utilisation" of that D-UVB dose i.e.: personal exposure dose. There were much higher cw-D-UVB doses in summer months than the rest of the year, however, it is not known if individuals in this study spent enough time outdoors to take advantage of these high levels and utilise this for vitamin D synthesis, as detailed information on personal use was not known.

Some studies have shown that seasonal variation is not significant in some populations due to widespread vitamin D supplementation [261], however this study observed seasonal variation within individual patients e.g. Patient 33. As 25(OH)D and cw-D-UVB were measured and estimated at four time points during a one year period, seasonal trends and dramatic differences in 25(OH)D concentration were clearly noticeable between summer and winter months in the same individual. This was most evident in those receiving placebo, however similar trends were also noted in those receiving high dose supplementation. This demonstrates how changeable 25(OH)D concentration is throughout the year within one individual. For example, a 2.5-fold increase in 25(OH)D was noted in one placebo patient (Patient 51) who first sampled in March and subsequently measured again in July. Furthermore, another placebo patient (Patient 88) whose $25(OH)D_{T2}$ measurement was taken in August was found to be highly sufficient at that time (95.5 nmol/L), but was subsequently found to be deficient when 25(OH)D measurement was taken again in December (24 nmol/L).

4.5.3 Associations between 25(OH)D and cw-D-UVB

The correlation between 25(OH)D and cw-D-UVB estimates was noted when scatterplots were examined. This trend was also observed when quartiles of cw-D-UVB were created. Significant associations were found at most time points: it was observed that cw-D-UVB calculated 135 days prior to blood draw was significantly associated with 25(OH)D concentration. These results are comparable to a previous study carried out on a Scottish cohort [241]. However the research carried out in this chapter offers two novel aspects. Firstly, this association was examined in a group of individuals who were sampled at four different time points. Strong associations were observed, including when multilevel modelling was undertaken to take advantage of multiple measures for each individual. Secondly, this association was present irrespective of the high dose supplementation, which had not been described previously. This suggests that UVB is still an important source of vitamin D, even at high latitudes and in those taking large doses of supplements.

4.5.4 VDscore1

As there are multiple sources of vitamin D (UVB, supplementation and dietary sources), using UVB alone, or similarly dietary factors alone is not enough to accurately predict a person's vitamin D status. This becomes an issue when 25(OH)D status is not available in a study as often researchers have to rely on a single vitamin D source for which data is available, which fails to account for all vitamin D sources. Therefore, a simple, inexpensive method of estimating vitamin D status from supplementation and cw-D-UVB dose was developed. These were chosen as these are the two most important contributors to a person's vitamin D status which were available.

Strong associations between the VDscore1 and 25(OH)D status were found at all four time points. Furthermore, this variable was considered important when classifying individuals into 25(OH)D groups, even after individuals were taking high dose supplementation. One could argue that perhaps this was only strongly associated as it contained information on randomisation group which was such as strong predictor of 25(OH)D, however, the inclusion of cw-D-UVB to the score was shown to improve prediction of 25(OH)D more than just randomisation group alone. This was observed in two ways. Firstly, when examining the importance of variables using the Boruta methods, it was found that VDscore1 had greater importance than randomisation alone at classifying individuals at T2 and T3. Secondly, it was shown that models which contained Vscore1 alone instead of randomisation group alone performed better as they had low AIC and

BIC scores and higher r^2 values at both T2 and T3 time points. However, this was not the case T4. It is not known why the model with the inclusion of VDscore1 did not perform better than the model containing supplementation use at T4 but it did for the other time points. The aim of this chapter was to create a simple vitamin D estimate which could incorporate two of the most important sources of vitamin D; supplementation use and D-UVB exposure. This chapter highlighted that a creation of a vitamin D estimate is possible, and that it can perform better than vitamin D related variables alone in some instances. However, this method is only a very simple, preliminary method of vitamin D estimation and perhaps it does not accurately incorporate supplementation dose into this score. This score made the assumption that supplementation was four times as important as cw-D-UVB dose. However, this may not be the case in all instances, and supplementation could be in fact many more times more important than D-UVB at certain times of the year. For example supplementation might contribute more to 25(OH)D during the winter months than the summer months, meaning that this score might be appropriate for summer months but not as accurate during the winter months. The opposite may also be true with an increase D-UVB quartile contributing more than +1 in the summer months. This may be one of the reasons why supplementation was found to be just as important as VDscore1 at T4 but not the other time points. This chapter had information at four time points during the year but unfortunately, the initiation of this RCT was not conducted at the same month or season for all individuals and as such each of the time points contain individuals whose blood samples were taken over the entire year. Due to this and low sample sizes, it was impossible to investigate the relationship between 25(OH)D and VDscore1 by exact month or season.

This type of scoring system has been carried out previously [262], however one benefit of this scoring method is its simplicity. Instead of using regression coefficients to determine which score to assign to which participant, the importance of each variable in a regression model was examined and then the cohort was split into groups based on this e.g.: patients were first split by supplementation and then cw-D-UVB quartile. Regression coefficients change depending on the cohort used; these approaches may not be transferrable from one cohort to another, while the simple score that was created here potentially can. The implications of this research are clearly visible; a simple classification tool which could be used to identify groups of individuals at risk of deficiency cheaply and effectively would be useful in research. It would allow vitamin D deficiency to be estimated within large cohorts of individuals at different locations when 25(OH)D measurement is unavailable. It should be acknowledged that this research was carried

out in a small cohort and details on all sources of vitamin D were unavailable, however, this research demonstrates that it is possible to create a vitamin D scoring system.

4.5.5 Implications for Future Research

Seasonal fluctuations are clearly evident in this study and these results have important implications for future vitamin D research, especially given that studies which are examining the association between vitamin D and health outcomes typically use a one-time measurement of 25(OH)D. If this measurement is not adequately seasonally adjusted, the results of these studies may be biased as participants rank in the population is not correctly captured. For example, one of the highest cited articles demonstrating an association between 25(OH)D and risk of breast cancer failed to adjust for season or time of blood draw in their model [263]. As participant's blood was sampled from June 1989 to October 1990, one can hypothesize that a seasonal effect would have been observed in these samples and perhaps if blood sampling was repeated at a different time of the year, their vitamin D status may have been very different making the vitamin D status estimate unreliable.

Furthermore, one could argue that perhaps a one-time measurement of 25(OH)D (or a seasonally biased proxy estimate such as cw-D-UVB or VDscore1), may not be the best estimate of vitamin D to use for slowly developing health outcomes such as cancer. These measurements only give an estimate of vitamin D status at a particular point in time and as health outcomes such as cancer or cardiovascular disease can take a number of years to develop, a measurement which can estimate average vitamin D may be more appropriate.

4.5.6 Strengths and Limitation

This cohort was chosen as it was a longitudinal design and was therefore an opportunity to examine the variation of 25(OH)D and D-UVB doses over a year. This is an important strength as it not only demonstrates the potential for errors in studies which only use a one-time measurement of 25(OH)D status when exploring the relationship between vitamin D and health outcomes, but also has important implications for the recommendation on the frequency and timing of vitamin D assessment in primary care settings. This cohort was an RCT in design as such the effect of high dose vitamin D supplementation and D-UVB could be jointly examined, throughout the year.

The association between UVB and 25(OH)D has been shown numerous times [6, 264-267]. However, very few previous studies have adjusted their UVB dose estimate for as many factors

(cloud, altitude, ozone column etc.), nor did they have such detailed spatial and temporal daily resolution as carried out in this research. This study also took advantage of the fact that TEMIS data base is a freely available resource which can be used for all countries over Europe. Additionally, these previous studies did not individually calculate D-UVB for each participant or take into account the accumulation and diminution of UVB in the body. These are major strengths of this study.

However, this study is not without its flaws. This data contained pre-collected data and because of this, some important vitamin D related information such as details of utilisation of UVB e.g.: amount of time spent outdoors and time of day spent outdoors), dietary sources of vitamin D and personal supplementation use are missing from this dataset. Due to this, personal sun exposure could not be taken to account in this study. Additionally, the aim of this chapter was to create a simple vitamin D estimate which incorporated two important sources of vitamin D, supplementation and UVB exposure. However, in creating a simple method, some assumptions are made. For example in the creation of VDscore1, it was assumed that supplementation was four times as important as important as UVB exposure, as supplementation dose results in the addition of +4 to a vitamin D score while an increase in D-UVB quartile only results in an increase of +1 in vitamin D score. This is a very simple approach, and perhaps too simple. The relationship between supplementation use and cw-D-UVB may change depending on the time of year or location. For example supplementation may be four times as important as cw-D-UVB in its contribution to 25(OH)D at the majority of time points throughout the year in Ireland, but in countries where D-UVB exposure is much greater, perhaps supplementation would not contribute as much to 25(OH)D concentration as sun exposure would. Unfortunately, this thesis was limited in that it only examined D-UVB in Ireland and the UK and as such this study was unable to examine if this vitamin D score would be an important predictor in areas with higher D-UVB or if it would need to be altered. Furthermore, this study was unable to examine if this score was associated with 25(OH)D to the same extent for all seasons, due to the small sample size at each time point and the large variation in time of year at each time point. This is a further limitation of this study.

Another limitation is that there were some missing time-points for some individuals and only 70 participants had 25(OH)D information for all four time points. There were also limitations with cohort size: this cohort was recruited for a small randomised controlled trial and not a large epidemiology study. This small sample size impacts upon the power of this study, especially when split into randomisation groups and this should therefore be considered when interpreting results. Additionally, there was some missing information in this cohort, this was a limitation of

this cohort as some individuals did not have all four measurements of 25(OH)D, and this further reduced the sample size of this cohort when associations were examined, as these individuals were excluded from the analysis. However, multilevel modelling was conducted in order to compensate for this as this type of analysis can handle missing variables for some individuals. Furthermore, this analysis strengthened the association results shown in this chapter as this analysis takes into account that 25(OH)D and cw-D-UVB were measured and calculated per person over multiple time points, rather than examining the results at four separate time points.

Another limitation of this cohort is that it is not a healthy cohort but a cohort of those in remission from Crohn's disease. The aim of this study was to explore the relationship between 25(OH)D concentration and D-UVB dose. However, it is not known if the relationships found in this study would be the same in a "healthy" cohort or in patients suffering from a different disease. There has been some evidence to suggest that those with active Crohn's disease have lower levels of 25(OH)D than those in remission [268, 269]. However, these studies observed high levels of 25(OH)D concentration in those in remission (mean 25(OH)D concentration in one study was 64 nmol/L) [269]. This is similar to the concentration observed in this study, as mean 25(OH)D concentration was 65.4 nmol/L. Furthermore, it has also been observed that those with Crohn's disease do not have significantly more vitamin D deficiency than healthy controls [270].

Moreover, other studies which have examined 25(OH)D concentrations in healthy Irish adults have found similar vitamin D concentrations to those found in this Crohns disease cohort. For example, in a study by Hill *et al.* mean 25(OH)D concentration was 54.5 nmol/L [271]. Similarly, in cohorts of healthy Irish participants, Andersen *et al.*, Magee *et al.* and Laird *et al.* all found mean 25(OH)D concentrations to be within a similar range to those found in this study; 44 nmol/L, 48 nmol/L and 44 nmol/L respectively [272-274]. Crohns disease sufferers may also have issues with absorption of nutrients in food, however it has been shown that vitamin D and calcium absorption in patients with crohns disease is not significantly different from absorption in healthy adults [275]. However, even though the evidence suggests that vitamin D concentration in those in remission from Crohns disease is not unlike vitamin D from a healthy population, these patients may have reduced vitamin D exposure. For example, those with active crohns disease may spend more time indoors due to their illness, although these patients were in remission, they may be unable to lead very active lives whereby they spend large amounts of time outdoors and as such may have reduced D-UVB exposure. Unfortunately, no information on the utilisation of UVB was available for this cohort, and as such no comparisons can be made with a healthy cohort. This is a limitation of this study and reduces the generalisability of this cohort. This research should be replicated in a large cohort of healthy

individuals to ensure reproducibility and generalisability. Additionally this study was carried out in an Irish population who reside at a high northerly latitude and this study should be repeated in a cohort of participants from southern Europe or areas with lower latitudes to ensure that results are reproducible at all latitudes.

4.6 Conclusion

In this chapter seasonal fluctuations of 25(OH)D were clearly visible in the majority of participants, especially those who were not taking supplements. It was also noted that cw-D-UVB doses calculated in individuals closely followed this same seasonal trend as 25(OH)D. Additionally, a linear trend between baseline 25(OH)D and baseline cw-D-UVB were found. Moreover, strong associations between cw-D-UVB and 25(OH)D were found before and after randomisation. All of these results demonstrate that there is a strong relationship between 25(OH)D and the cw-D-UVB estimate developed. These associations were also found to be still present in those taking high dose supplements demonstrating that UVB dose is still important in these individuals. Supplementation was also strongly associated with 25(OH)D.

Furthermore, when combining both randomisation group and cw-D-UVB doses, a simple vitamin D scoring system, VDscore1, was developed which was also found to be associated with 25(OH)D in a linear and multilevel regression model and an important variable when classifying patients into groups of 25(OH)D. These estimates could potentially be used in the future to identify those at risk of vitamin D deficiency. However, this study was undertaken in a small cohort in those with Crohns disease and therefore more research needs to be carried out in a much larger cohort of healthy individuals to determine if the relationships observed here can be reproduced.

5 Ambient UVB dose at Place of Residence and 25(OH)D in an Older Irish Cohort

5.1 Aim

The aim of this chapter is to build upon previous work and calculate cw-D-UVB for a much larger cohort of Irish individuals and relate this to 25(OH)D concentration. As it has been previously shown that the role of cutaneous vitamin D synthesis decreases with age [22, 54], associations between 25(OH)D concentration and cw-D-UVB in this much larger but older cohort will be investigated. Furthermore, the complexity of the VDscore1 developed will be increased with the addition of two important vitamin D related variables; oily fish consumption and sun enjoyment. This chapter will also investigate the use of cw-D-UVB and VDscore in the prediction of vitamin D deficiency.

5.2 Introduction

It has previously been shown in this thesis that 25(OH)D concentration as measured in blood is strongly associated with individually calculated 135-day cw-D-UVB prior to date of blood draw. This was shown in a small cohort of patients (n=92) who are receiving vitamin supplementation or placebo. A simple VDscore1 for each individual has been calculated previously, depending on ambient cw-D-UVB dose and vitamin D supplementation and determined that this was highly associated with blood 25(OH)D concentrations. This chapter wanted to build upon this knowledge and test both cw-D-UVB and VDscores use in a larger cohort or older individuals, after inclusion of additional factors to VDscore.

5.3 Methods

5.3.1 Study Population

All study participants included in this study were patients who had been recruited from the "Trinity, University of Ulster and Department of Agriculture Study" (TUDA) [276]. This is an all-Ireland cross-sectional study of participants aged over 60 which looked at the role of nutritional, genetic, health and lifestyle factors in the development of common diseases of aging (n=5,138, Median age: 73 years [range: 60-101]). This cohort collected detailed clinical, lifestyle, dietary, genetic and biochemical data with an aim to examine the gene-nutrient interactions in the development of chronic disease of ageing including dementia, osteoporosis and cardiovascular disease.

Recruitment started in Dec 2008 and was completed in Sept 2012. Three categories of participants were recruited, those with cognitive impairment (n=1,699), high blood pressure (HBP) (n=2,073), and osteoporosis (n=1,366).

The osteoporosis cohort were recruited from a specialist bone health service and in St James's Hospital, Dublin. These consisted of individuals who received a diagnosis of osteoporosis or osteopaenia within 3 years prior to recruitment. Osteoporosis was defined as a score of >-2.5 at any site (hip, femoral neck and vertebral column) using a dual energy x-ray absorptiometry (DXA) which is used to measure bone mineral density.

The cognitive cohort were recruited from geriatric clinics and a day hospital service at St James's Hospital, Dublin. A score of ≤80 using the Repeatable Battery for the Assessment of Neuropsychological Status was considered to indicate an impairment of cognitive function [277]. There is no universal normality cut off on psychometric tests including this assessment. However, 2 standard deviations below the mean is often used as a cut off value: this equates to the 7% percentile of the data. In this cohort, the 9% percentile was used as the cut off, and this was equal to a score of 80 or below.

The HBP cohort was recruited from General Practitioners (GP) practises in Northern Ireland. These were individuals who had a diagnosis of hypertension which was confirmed by their GP. Patients were considered hypertensive if systolic blood pressure was above 140 mmHg, diastolic pressure was above 90 mmHg, or if participants were prescribed blood pressure medication.

Inclusion criteria were as follows: aged over 60, no diagnosis of dementia and ethnically Irish parents. All participant underwent a single assessment consisting of a face to face 90-minute interview which was performed by trained researchers. This occurred either on the day of their outpatient attendance or retrospectively. If the assessment occurred retrospectively, the

participant was contacted by telephone in advance of the assessment and sent study information by post. During this interview detailed self-reported sociodemographic, lifestyle and health questionnaires were completed. Information on various factors were collected, including: age, sex, smoking status (never, past, current smoker), alcohol intake (never, past, current drinker), oily fish consumption (yes/no), sun holidays in the past six months (yes/no) [the majority of which were taken in Spain, the Canary Islands and other warmer European countries], vitamin D supplement use (yes/no), BMI, sun enjoyment (enjoy staying in sunshine, sometimes stay in sunshine, avoid sunshine), and sun protection use (always, usually, sometimes, rarely, never). Residential address was needed for calculation of UVB and was not known for 48 participants so these were excluded.

Ethical approval was granted by the relevant authorities in each jurisdiction: the Research Ethics Committee of St. James's Hospital and The Adelaide and Meath Hospital, Dublin, and the Office for Research Ethics Committees Northern Ireland (reference 08/NI/RO3113) with corresponding approvals from the Northern and Western Health and Social Care Trusts, Northern Ireland. All participants provided written informed consent at the time of enrolment. All blood samples and questionnaire data were coded and the identifiers removed prior to analysis.

5.3.2 Vitamin D Measurement

A 50 ml blood sample was taken from participants and samples were refrigerated and centrifuged at 3000 rpm within three hours of collection. Total 25(OH)D ($25(OH)D_2$ and $25(OH)D_3$) was measured by LC-MS/MS (API 4000; AB SCIEX; Chromsystems GmbH) with an inter-assay coefficient of Variation of <5.7% from serum samples, at the Biochemistry Department of St James's Hospital, Dublin, Ireland, which is verified by the Vitamin D External Quality Assessment Scheme and National Institute of Standards and Technology. Concentrations of 25(OH)D equal to or above 50 nmol/L indicated sufficiency; 40-49 nmol/L indicated a low risk of deficiency, while 25-39 nmol/L indicated high risk of deficiency. Those with concentrations below 25 nmol/L were classed as deficient [258].

5.3.3 Cumulative and Weighted D-UVB Estimation Calculation

The TEMIS database was used for calculation of cw-D-UVB for each individual as described in the previous chapter. Briefly, daily ambient D-UVB doses 135 days prior to blood draw at residential location were extracted for each participant. These daily doses were weighted and

summed to give an individual cumulative and weighted 135 day D-UVB dose for each participant. The histogram of this variable within the cohort is shown in **Figure 5.1**

5.3.4 VDscore1

VDscore1 was calculated as described in Chapter 4. Participants were first stratified by supplementation use and then cw-D-UVB quartile. Participants were then assigned a score of between 1 and 8 depending on their stratification. A histogram of this variable within the cohort is shown in **Figure 5.1.**

5.3.5 Vitamin D scoring Calculation Method two (VDscore2)

Building on the previous model of VDscore1, VDscore2 was calculated. In addition to cw-D-UVB dose and supplementation use, there are numerous other factors which impact on 25(OH)D status, such as sun behaviours and diet. Therefore, VDscore2 was calculated for each individual based on their cw-D-UVB dose, supplementation use, sun enjoyment level and if they consume oily fish. As there are three main sources of vitamin D (UVB, supplementation and dietary sources), it was necessary to include variables from all three sources into this new VDscore. Sun enjoyment was also added as it is related to the utilisation of UVB in individuals.

In order to inform the development of this score, the association between each variable of interest and 25(OH)D was examined. Using regression analysis it was determined that supplementation was the strongest predictor of 25(OH)D in this cohort (**Table 5.1**). As this has also been shown in other studies [255], and previously found in the Crohn's cohort in Chapter 4, the TUDA cohort was first stratified into those who supplemented with vitamin D and those who did not. The next most important predictor of 25(OH)D status was found to be cw-D-UVB (**Table 5.1**), therefore each supplement group was further split into four categories based on the quartile of their cw-D-UVB dose. As enjoyment of the sun was found to be the next most important variable, these eight groups were then further divided into three [those who avoided the sun, those who enjoyed it and those who sometimes enjoyed the sun] to give a total of 24 possible groups. Finally, these 24 groups were split based on their dietary intake of vitamin D (in this case if they consumed oily fish). This resulted in 48 categories for each individual (**Table 5.2**). Effectively this VDscore2 was generated by the addition of +24 if an individual took supplements, +0 +6, +12, or +18 for each quartile of cw-D-UVB an individual belongs to, +0, +2 or +4 if an individual avoided the sun, sometimes enjoyed the sun or enjoyed the sun and finally +0, or +1

if an individual did not or did consume oily fish (**Table 5.2**). The histogram of this variable within the cohort is shown in **Figure 5.1.**

5.3.6 Vitamin D Scoring Calculation Method three (VDscore3)

A third vitamin D scoring method was created. In order to calculate the VDscore3 a multivariable regression model was used whereby the relationship between 25(OH)D and a selection of vitamin D related variables (supplementation use, cw-D-UVB quartiles, oily fish intake and enjoyment of the sun) was investigated. Once the relationship between each of the variables and 25(OH)D was calculated, the β coefficient was used as the "score" for a given variable. For example, those who had not been supplemented were given a sub-score of zero while those who had been supplemented were given a sub-score of 32.09, as this was the β coefficient for supplementation. This was then carried out for quartiles of cw-D-UVB (sub-scores= Q1: 0, Q2: 3.3, Q3: 7.35, Q4: 12.97), sun enjoyment (avoid sun: 0, sometimes enjoy: 4.32, enjoy sun: 9.55) and finally oily fish consumption (No: 0, Yes: 3.55). The sub-scores were then summed to give a final VDscore3 score per person. This method was created as to accurately account for the contribution of each of the variables to the final score. The histogram of this variable within the cohort is shown in **Figure 5.1.** These were then split into tertiles.

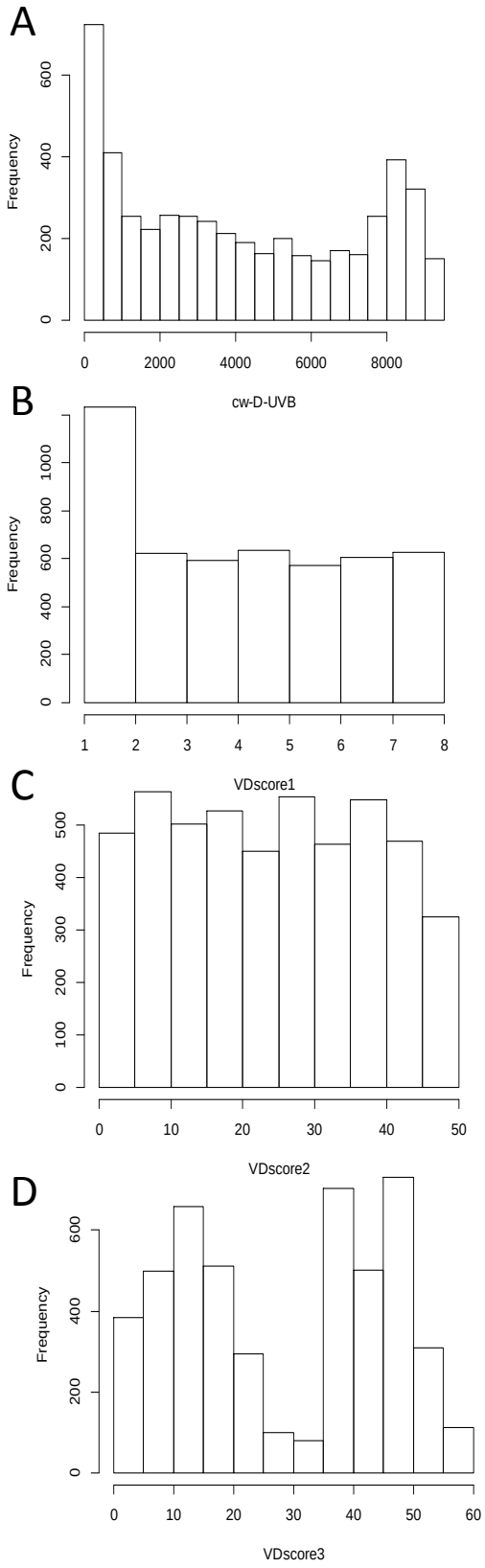


Figure 5.1: Histograms showing frequency of cw-D-UVB dose, VDscore1, VDscore2 and VDscore3 in the TUDA cohort

A) cw-D-UVB **B)** VDscore1, **C)** VDscore2, **C)** VDscore3

Table 5.1: Beta coefficient and stepped increases in VDscore2 and VDscore3

VDscore3 was created using adjustments for supplement use, cw-D-UVB, sun enjoyment, oily fish consumption, age and sex in the linear regression model

Variable	Beta coefficients in regression model for VDscore3	Stepped increase in VDscore2	Difference between VDscore2/VDscore3
Supplement use			
No	0	0	0
Yes	32.09	24	0.75
Quartiles of UVB			
Q1	0	0	0
Q2	3.30	6	1.81
Q3	7.35	12	1.63
Q4	12.97	18	1.39
Sun enjoyment			
Avoid	0	0	0
Sometimes	4.32	2	0.46
Enjoy	9.55	4	0.41
Oily Fish Consumption			
No	0	0	0
Yes	3.55	1	0.28

Table 5.2: Allocation of VDscore2 to individuals

		No Supplementation							
		UVB Q1		**UVB Q2**		**UVB Q3**		**UVB Q4**	
Cohort	**Sun Enjoyment**	**Oily fish**		**Oily fish**		**Oily fish**		**Oily fish**	
		No	Yes	No	Yes	No	Yes	No	Yes
ALL	Avoid sunshine	1	2	7	8	13	14	19	20
	Sometimes enjoy sunshine	3	4	9	10	15	16	21	22
	Enjoy sunshine	5	6	11	12	17	18	23	24

		Supplementation							
		UVB Q1		**UVB Q2**		**UVB Q3**		**UVB Q4**	
Cohort	**Sun Enjoyment**	**Oily fish**		**Oily fish**		**Oily fish**		**Oily fish**	
		No	Yes	No	Yes	No	Yes	No	Yes
ALL	Avoid sunshine	25	26	31	32	37	38	43	44
	Sometimes enjoy sunshine	27	28	33	34	39	40	45	46
	Enjoy sunshine	29	30	35	36	41	42	47	48

5.3.7 Statistical Analysis

Multivariable backwards stepwise linear regression analysis was used to examine the association between cw-D-UVB dose, VDscore1, VDscore2, VDscore3 tertiles and serum 25(OH)D. Final model was chosen based on R^2 value, the number of NAs present in model and AIC and BIC numbers. Adjustments were made for age, sex, patient cohort, smoking status, oily fish consumption, sun holiday in the last six months and BMI. When cw-D-UVB was examined adjustments were also made for supplementation use. Supplementation was not used in models which contained vitamin D scores as there was high co-linearity found between these variables (tested using VIF scores), however, this was not found to be the case with oily fish consumption and therefore this variable was still included in the model. Diagnostic plots were used to ensure linear regression was appropriate (**Appendix 6**). The relationship between 25(OH)D, cw-D-UVB, VDscore1, VDscore2 and VDscore3 and personal baseline characteristics was also carried out using regression analysis. This was carried out to investigate if the relationship between 25(OH)D and personal characteristics would be similar to the relationship between personal characteristics and cw-D-UVB, VDscore1, VDscore2 and VDscore3 in the same cohort. The variables in these models were chosen by backwards stepwise linear regression.

Ambient cw-D-UVB was split into quartiles and the median 25(OH)D in each quartile and in each sun enjoyment category was determined. The cohort was then further split into those who were 60-74y (younger old) and over 75y (older old) to more accurately portray the relationship between cw-D-UVB, sun enjoyment and 25(OH)D, given that cutaneous vitamin D synthesis decreases with age [54].

Mann-Whitney tests were conducted to determine if there was a statistical difference in the 25(OH)D concentration in those who enjoyed the sunshine compared to those who did not, in each of the four UVB quartiles.

Mean 25(OH)D for each of the 48 categories of VDscore2 were determined and a table to demonstrate the increase in mean 25(OH)D with increasing VDscore2 was created.

Random forest analysis were then employed in order to assess the contribution of cw-D-UVB, sun enjoyment, VDscore1, VDscore2 or VDscore3 in predicting 25(OH)D sufficiency or deficiency. Different models were constructed for those under 75 and those aged 75 or older (cut-off was chosen as the mean age of the cohort was just over 74 years). This data was then split into two groups; with part of the cohort randomly selected for the training data set and the remainder for the testing data set. Two types of classification analysis were undertaken using the training data set, this was split into 0 or 1 for those who were deficient (<25 nmol/L), and those who were sufficient (>50 nmol/L). Receiver operating characteristic (ROC) curves were then created,

using the testing data set, to measure the performance of the random forest analysis. The area under the curve (AUC) demonstrates the ability of the test to accurately classify each binary pair from each category. The higher the AUC the better the prediction method is classifying each participant correctly. The models selected were adjusted for the same variables as the association analysis.

Model one included age, sex, BMI, cohort type, smoking status, sun holiday in the last six months, sun enjoyment, oily fish consumption, and supplement use; **model two** included variables from model one in addition to cw-D-UVB quartiles, **model three** included age, sex, BMI, cohort type, smoking status sun holiday in the last six months and VDscore1, while **model four** included age, sex, BMI, cohort type, smoking status sun holiday in the last six months and VDscore2 and **model five** included age, sex, BMI, cohort type, smoking status sun holiday in the last six months and VDscore3.

The 'Boruta' model uses and improves upon the random forest model when determining the most important variables, therefore Boruta analysis was carried out in addition to the random forest analysis to fully explore the differences between models and estimates of vitamin D status. Using this the most important variables when classifying 25(OH)D deficiency (<25 nmol/L) and sufficiency (>50 nmol/L) were determined. Four different models were created, **model one** contained age, sex, BMI, cohort type, alcohol consumption, smoking status; **model two** contained variables from model one, sun enjoyment, oily fish consumption, supplement use and cw-D-UVB; **model three** contained variables from model two plus VDscore1; **model four** contained variables from model two plus VDscore2 and **model five** contained variables form model two plus VDscore3. All analyses were performed in R (R Development Core Team, 2011) using the Random Forest, ROCR and Boruta packages. Differences were considered significant at $P < 0.05$.

5.4 Results

5.4.1 Baseline Characteristics

cw-D-UVB, VDscore1, VDscore2, VDscore3 and 25(OH)D were assessed in 5,138 individuals among. A median serum 25(OH)D of 54.5 nmol/L (Interquartile range: 34-81 nmol/L) was found for this cohort (**Table 5.3**). Overall, 32% of this cohort were deficient or at high risk of deficiency (25(OH)D < 40 nmol/L). This was 38.6% in the cognitive cohort, 39.4% in those with HBP and 11.8% in those with osteoporosis (**Table 5.4**). 47.4% of the entire cohort took supplements. Those whose vitamin D was taken in the summer had the highest 25(OH)D concentration, while the lowest was found in spring, however 25(OH)D concentrations overlapped greatly between all seasons (**Figure 5.2a**). This was unlike seasonal cw-D-UVB doses which did not see the same degree of overlap (**Figure 5.2b**). It was also noted that people whose vitamin D was measured in December, January, February, and March were found to have a higher risk of deficiency (25(OH)D <40 nmol/L) compared to those who's bloods were taken during the summer and autumn months (**Figure 5.2c**).

When examining baseline characteristics between sub-cohorts of the TUDA cohort, it was discovered that there were some differences between cohorts (**Table 5.4**). Unsurprisingly, 25(OH)D concentration and vitamin D supplement use was found to be much higher in the osteoporosis cohort. Median 25(OH)D concentration was 26 nmol/L and 31 nmol/L higher in the osteoporosis cohort when compared to the cognitive and HBP cohorts respectively. Similarly, supplement use 28% and 54% higher in the osteoporosis cohort when compared to the cognitive and HBP cohorts.

There was also some differences in ages between cohorts with the majority of those in the cognitive cohort ages over 75, while the majority in the other two cohorts were aged under 75. A higher percentage of those who were obese were found to be part of the HBP cohort (38% vs 24% and 19%). The majority of those with cognitive disorders did not take sun holidays in the six months prior to their TUDA interview, compared a large proportion of participants in the other two cohorts who did. It was also noted that those in the osteoporosis cohort enjoyed the sun to a greater extent than the other two cohorts (35% vs 29 and 28%) (**Table 5.4**). There was very little difference between cohorts in terms of smoking status, alcohol consumption, oily fish consumption, education and month of recruitment.

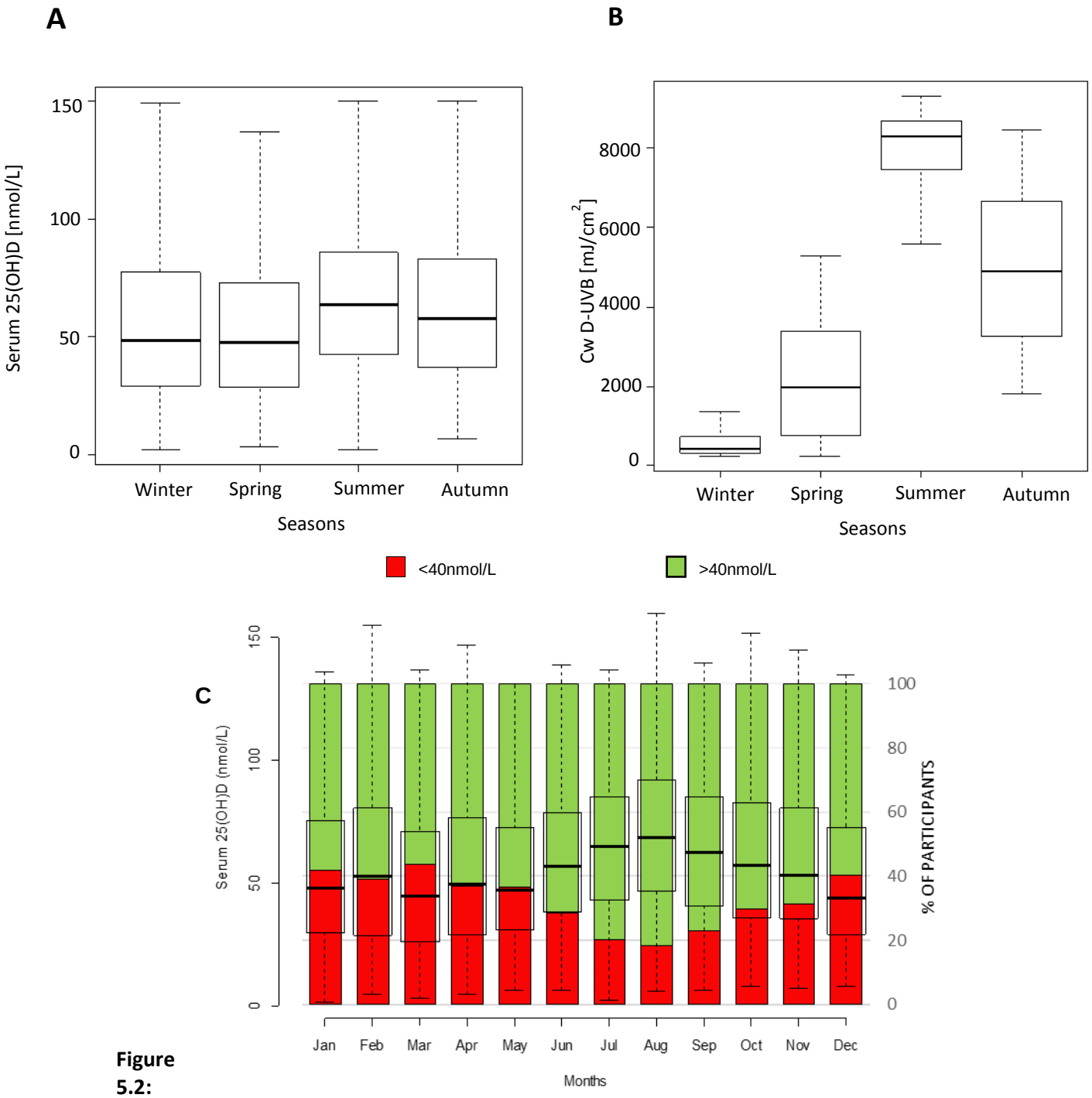


Figure 5.2:

25(OH)D distribution throughout the year in TUDA cohort

Seasonal fluctuations in **A)** Serum 25(OH)D and **B)** cw-D-UVB from TUDA cohort from 2009-2012- (winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug) autumn (Sep-Nov)). **C)** Serum 25(OH)D concentration in participants and percentage of participants at risk of insufficiency (<40 nmol/L) during each month.

Table 5.3: Baseline characteristics of TUDA cohort stratified by cw-DUVB quartile

Baseline characteristics of entire cohort overall and after stratification according to quartiles of cw-D-UVB [1, 2, 3].

		cw-D-UVB			
Characteristics	**All**	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
cw-D-UVB, mJ/cm²	3650 (1216-7182)	431 (326-625)	2420 (1854-3035)	5278 (4415-6211)	8334 (7922-8687)
25(OH)D, nmol/L (NA=15)	54.5 (34-81)	54.3 (28-74)	49.5 (32-76)	60.2 (36-81)	66.6 (43-88)
<40, nmol/L	1634 (32)	539 (33)	450 (27)	375 (23)	270 (17)
≥ 40, nmol/L	3504 (68)	747 (21)	833 (24)	910 (26)	1014 (29)
Sex					
Female	3452 (67)	879 (25)	827 (24)	864 (25)	882 (25)
Male	1686 (33)	407 (24)	456 (27)	421 (25)	402 (24)
Age					
<75, y	2885 (56)	705 (25)	777 (27)	740 (26)	663 (23)
≥ 75, y	2253 (44)	581 (26)	506 (22)	545 (24)	621 (28)
BMI, kg/m², (NA=25)					
Underweight <18.5	109 (2)	26 (24)	25 (24)	23 (20)	35 (32)
Normal weight, 18.6-24.9	1430 (28)	361 (25)	328 (23)	360 (25)	381 (26)
Overweight, 25-29.9	2003 (39)	505 (25)	524 (26)	490 (25)	484 (24)
Obese, 30-39.9	1435 (28)	348 (24)	359 (25)	376 (26)	352 (25)
Extremely Obese, ≥40	136 (3)	37 (28)	38 (28)	33 (24)	28 (20)
Cohort					
Cognitive Impairment	1699 (33)	485 (29)	377 (22)	376 (22)	461 (27)
HBP	2073 (40)	422 (20)	605 (29)	563 (28)	483 (23)
Osteoporosis	1366 (27)	379 (28)	301 (22)	346 (25)	340 (25)
Supplement Users (NA/don't know=254)					
Yes	2437 (47)	633 (26)	571 (23)	605 (25)	628 (26)
No	2447 (48)	582 (24)	650 (27)	624 (25)	591 (24)
Oily Fish Consumption (NA=2)					
Yes	3060 (60)	735 (24)	757 (25)	760 (25)	808 (26)
No	2076 (40)	550 (27)	526 (25)	524 (25)	476 (23)
Sun Holiday in the Last 6 Months (NA=8)					
Yes	894 (17)	201 (23)	225 (25)	235 (26)	234 (26)
No	4235 (83)	1083 (25)	1055 (25)	1049 (25)	1048 (25)
Province[4]					
Ulster	2063 (40)	418 (20)	603 (29)	560 (27)	482 (23)
Leinster	3058 (60)	865 (28)	676 (22)	722 (24)	795 (26)
Munster and Connacht	17 (3)	3 (18)	4 (24)	3 (18)	7 (41)
Season of Blood Draw					
Winter	1044 (20)	830 (80)	214 (20)	0	0
Spring	1290 (25)	456 (35)	580 (45)	254 (20)	0
Summer	1310 (26)	0	0	302 (23)	1008 (79)
Autumn	1494 (29)	0	489 (33)	730 (49)	276 (18)

Year of Blood Draw					
2008	6 (0.1)	2 (40)	4 (60)	0	0
2009	1430 (28)	196 (14)	399 (28)	407 (28)	428 (30)
2010	2309 (45)	522 (23)	615 (27)	601 (25)	571 (25)
2011	1156 (23)	452 (39)	201 (17)	251 (22)	252 (22)
2012	237 (5)	114 (48)	64 (27)	26 (11)	33 (14)
Smoking Status (NA=2)					
Current smoker	615 (12)	155 (25)	159 (26)	157 (26)	144 (23)
Never smoker	2387 (46)	580 (24)	614 (26)	575 (24)	618 (26)
Past smoker	2134 (41)	551 (26)	510 (24)	552 (26)	521 (24)
Alcohol Consumption (NA=2)					
Current drinker	2946 (57)	720 (25)	743 (25)	743 (25)	740 (25)
Past drinker	916 (18)	218 (24)	238 (26)	222 (24)	238 (26)
Never	1274 (25)	353 (28)	295 (23)	324 (25)	302 (24)
Sun Enjoyment (NA=2)					
Avoid Direct sunshine	1679 (33)	389 (23)	376 (22)	450 (27)	464 (28)
Sometimes enjoy sunshine	1965 (38)	492 (24)	524 (27)	480 (25)	469 (24)
Enjoy staying in sunshine	1492 (29)	404 (27)	382 (25)	355 (24)	351 (24)
Sun Protection (NA=3)					
Always	853 (17)	240 (28)	212 (25)	204 (24)	197 (23)
Usually	711 (14)	179 (25)	201 (29)	158 (22)	173 (24)
Sometimes	771 (15)	175 (23)	223 (29)	208 (27)	165 (21)
Rarely	314 (6)	86 (27)	85 (27)	67 (22)	76 (24)
Never	2486 (48)	605 (24)	561 (23)	648 (26)	672 (27)
Age Finished Education (NA=10)					
≤14, y	2187 (43)	544 (25)	514 (24)	573 (26)	556 (25)
15-18.9, y	1787 (35)	466 (26)	480 (27)	426 (24)	415 (23)
19-24.9, y	1057 (21)	255(24)	267 (26)	258 (24)	277 (26)
≥25, y	97 (2)	19 (21)	22 (23)	26 (26)	30 (31)

Footnote:

[1] Abbreviations: cw-D-UVB; Cumulative and weighted UVB dose, HBP; High blood pressure, IQR; interquartile range, NA; not available.

[2] For 25(OH) D, and D-UVB, values represent median and inter quartile range

[3] For all other variables, values represent the number of participants in that D-UVB group and percentage.

[4] Ulster is located in the north of Ireland, Leinster is in the east/south east of Ireland, Connacht is located in the west of Ireland and Munster is located in the south/south west of Ireland.

Table 5.4: Baseline characteristics of TUDA cohort stratified by sub cohort[1, 2, 3].

Characteristics	Cohort		
	Cognitive	**HBP**	**Osteoporosis**
cw-D-UVB, mJ/cm²	3577 (803-7580)	3733 (1505-6793)	3670 (820-7171)
25(OH)D, nmol/L (NA=15)	50.7 (29-78)	45.7 (31-66)	76.7 (56-96)
<40, nmol/L	656 (39)	817 (39)	161 (12)
≥ 40, nmol/L	1043 (61)	1256 (61)	1205 (88)
Sex			
Female	1139 (67)	1149 (55)	1164 (85)
Male	560 (33)	924 (45)	202 (15)
Age			
<75, y	343 (20)	1607 (78)	935 (68)
≥ 75, y	1356 (80)	466 (22)	431 (32)
BMI, kg/m², (NA=25)			
Underweight <18.5	51 (3)	6 (0)	52 (4)
Normal weight, 18.6-24.9	590 (35)	311 (15)	529 (39)
Overweight, 25-29.9	611 (36)	885 (43)	507 (37)
Obese, 30-39.9	402 (24)	778 (38)	255 (19)
Extremely Obese, ≥40	42 (2)	75 (4)	19 (1)
Supplement Users (NA/don't know=254)			
Yes	884 (53)	540 (27)	1013 (81)
No	777 (47)	1428 (73)	242 (19)
Oily Fish Consumption (NA=2)			
Yes	882 (52)	1330 (64)	848 (63)
No	817 (48)	742 (36)	517 (38)
Sun Holiday in the Last 6 Months (NA=8)			
Yes	87 (5)	424 (20)	384 (28)
No	1610 (95)	1647 (80)	978 (72)
Season of Blood Draw			
Winter	360 (21)	399 (19)	285 (21)
Spring	502 (30)	444 (21)	344 (25)
Summer	439 (26)	532 (26)	339 (25)
Autumn	398 (23)	698 (34)	398 (29)
Year of Blood Draw			
2008	6 (<1)	0 (0)	0 (0)
2009	805 (47)	528 (25)	97 (7)
2010	527 (31)	1339 (65)	443 (32)
2011	361 (21)	110 (5)	685 (50)
2012	0 (0)	96 (5)	141 (10)
Smoking Status (NA=2)			
Current smoker	192 (11)	224 (11)	199 (15)
Never smoker	768 (45)	964 (47)	655 (48)
Past smoker	739 (43)	885 (43)	510 (37)
Alcohol Consumption (NA=2)			
Current drinker	787 (47)	1140 (58)	855 (68)
Past drinker	412 (25)	323 (16)	149 (12)
Never	461 (28)	504 (26)	249 (20)

Sun Enjoyment (NA=2)			
Avoid Direct sunshine	641 (39)	639 (32)	336 (27)
Sometimes enjoy sunshine	645 (39)	755 (38)	476 (38)
Enjoy staying in sunshine	375 (23)	573 (29)	442 (35)
Sun Protection (NA=3)			
Always	124 (7)	296 (15)	374 (30)
Usually	115 (7)	322 (16)	240 (19)
Sometimes	119 (7)	437 (22)	165 (13)
Rarely	62 (4)	132 (7)	100 (8)
Never	1240 (75)	780 (40)	375 (30)
Age Finished Education (NA=10)			
≤14, y	900 (54)	679 (35)	509 (41)
15-18.9, y	479 (29)	841 (43)	382 (30)
19-24.9, y	245 (15)	413 (21)	332 (26)
≥25, y	31 (2)	31 (2)	30 (2)

Footnote:

[1] Abbreviations: cw-D-UVB; Cumulative and weighted UVB dose, HBP; High blood pressure, IQR; interquartile range, NA; not available.

[2] For 25(OH) D, and D-UVB, values represent median and inter quartile range

[3] For all other variables, values represent the number of participants in that D-UVB group and percentage.

5.4.2 25(OH)D, VDscore1, VDscore2 and VDscore3

The relationship between 25(OH)D concentration and VDscore1 was first examined. A trend for increasing 25(OH)D concentration with higher tertiles of VDscore1 was noted. This was also observed for VDscore2 tertiles, Vscore2 deciles and VDscore3 deciles. A linear trend was noted in a scatterplot and when 25(OH)D was plotted against VDscore2 and VDscore3 (**Figure 5.3)**. Furthermore, a linear relationship between VDscore2 and VDscore3 was found (**Figure 5.3**).

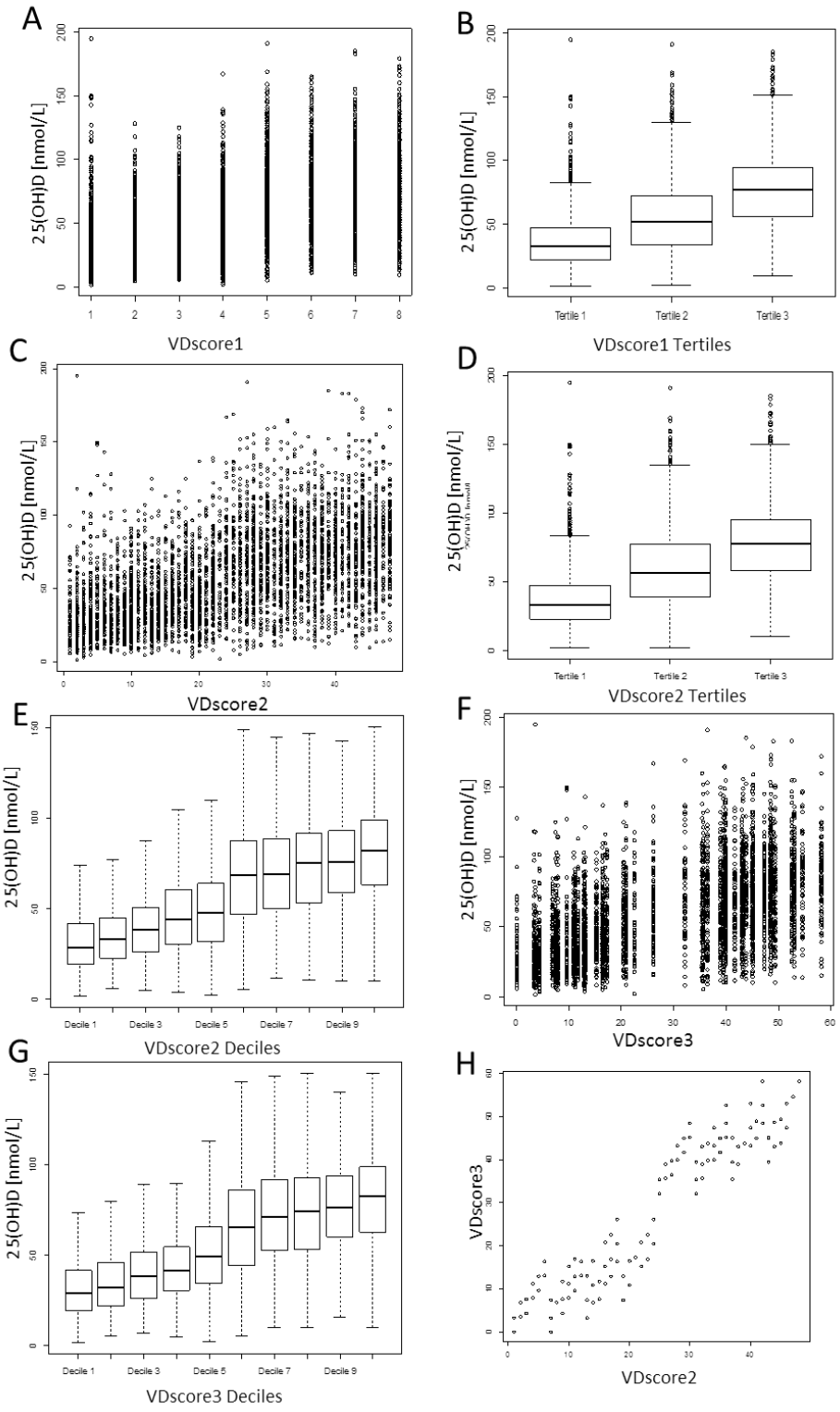


Figure 5.3: Relationship between 25(OH)D, VDscore1, VDscore2 and VDscore3

Relationship between estimates **A)** Scatterplot of 25(OH)D and VDscore1; **B)** association between 25(OH)D and VDscore1 tertiles **C)** scatterplot of 25(OH)D and VDscore2; **D)** tertiles of VDscore2, **E)** deciles of VDscore2 **F)** Scatterplot of 25(OH)D and VDscore3, **G)** association between 25(OH)D and deciles of VDscore3, **H)** relationship between VDscore2 and VDscore3

5.4.3 Cw-D-UVB Quartiles, Sun Exposure and VDscore2

A consistent trend towards higher 25(OH)D concentration in those with higher cw-D-UVB and who enjoy the sun was noted (**Figure 5.4**). In fact, a large majority of those who were in the lower quartiles of cw-D-UVB (Q1 and Q2) and avoided the sun had a median 25(OH)D within the insufficient range (<40 nmol/L), while those who were in the cw-D-UVB higher quartiles (Q3 and Q4) and enjoyed the sun were often in the sufficient range of 25(OH)D (>50 nmol/L), given no supplementation (**Figure 5.4, 5.5, Table 5.5**). These differences between sun enjoyment and 25(OH)D were also largely statistically significant (**Table 5.6**). Very large differences in serum 25(OH)D concentration between cw-D-UVB quartiles were observed; over 20 nmol/L were observed in some instances, particularly among individuals below the age of 75y who were not taking supplements (**Figure 5.4, 5.5**). For example a 21.2 nmol/L difference in median 25(OH)D was observed between Q1 and Q4 in those under 60 who did not take supplements and avoided the sun. This increase in median 25(OH)D was even greater when differences in sun enjoyment were also taken into account (~31nmol/L in those who reported enjoying sunshine).

Mean 25(OH)D in each of the 48 categories of VDscore2 were also noted. When all participants were included in the analysis and in the stratified analysis, it was found that the mean 25(OH)D concentration increased dramatically with increasing VDscore2 (**Figure 5.6, 5.7**). For example, mean 25(OH)D in a participant who had a VDscore2 of 5 (meaning they did not take supplements, were in quartile one of cw-D-UVB, did not eat oily fish and enjoyed the sun) was 41.8 nmol/L. When this is compared to an individual who had a VDscore2 of 24 (meaning they did not take supplements, were in quartile four of cw-D-UVB, did not eat oily fish and enjoyed the sun), they had a mean 25(OH)D of 55 nmol/L. This is a difference of 13.2 nmol/L, even though all variables remained the same apart from quartile of cw-D-UVB. These differences in mean 25(OH)D were even more dramatic when comparing those with different supplementation use, sun enjoyment status and oily fish consumption. There was a mean 25(OH)D difference of 54.1 nmol/L between those with the highest VDscore2 and the lowest (**Figure 5.6, 5.7**).

It was also noted that those who enjoyed the sun had higher mean 25(OH)D concentration than those who avoided the sun, even in the same cw-D-UVB quartile. For example, those with a VDscore2 of seven (meaning they did not take supplements, were in quartile two of cw-D-UVB, did not eat oily fish and avoided the sun) had a mean 25(OH)D of 32.2 nmol/L, this was increased by 10.8 nmol/L to 43 nmol/L in those with a score of 11; the only difference between the two scores is that those with VDscore2 11 enjoyed the sun (**Figure 5.6**).

Furthermore, it was observed that those who ate oily fish had mostly higher mean 25(OH)D than those who did not, even if all other factors were the same, sometimes a difference of up to 8 nmol/L. A

linear trend was also noted in a scatterplot of VDscore2 and median 25(OH)D and a distinct difference in median 25(OH)D concentration could be observed when examining non-supplemented individual's vs supplemented individuals.

		No Supplementation								Supplementation							
		Younger old (60-74)				Older old (60-74)				Younger old (60-74)				Older old (60-74)			
		cw-D-UVB				cw-D-UVB				cw-D-UVB				cw-D-UVB			
Cohort	**Sun enjoyment**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
All	Avoid sunshine	24.3	32.9	37.9	45.5	28.8	31.6	32.3	33.7	65.0	57.2	71.6	77.2	70.1	65.2	77.7	77.9
	Sometimes enjoy sunshine	29.3	38.6	44.9	53.7	28.8	29.2	35.6	44.3	67.1	72.1	75.5	84.2	72.6	75.9	74.2	76.4
	Enjoy sunshine	36.3	43.9	52.1	67.1	28.5	43.9	37.9	40.4	67.7	78.8	83.8	84.5	75.8	74.7	72.8	85.8
Cognitive impairment	Avoid sunshine	21	36	22.8	30.5	30.0	27.6	29.6	31.5	53.7	51.0	54.1	78.1	68.8	58.0	76.0	75.4
	Sometimes enjoy sunshine	22.6	30.2	44.1	44.1	26.1	27.7	33.5	39.6	64.9	93.0	81.2	103.2	70.0	72.0	73.4	75.9
	Enjoy sunshine	35.4	40.1	40	60.8	27.1	37.3	34.7	34.7	58.9	53.7	73.4	74.9	71.4	72.1	73.3	76.8
HBP	Avoid sunshine	24.3	32.0	38.8	45.5	25.8	34.0	34.3	37.2	47.9	44.1	67.8	63.1	57.4	59.5	76.9	80.6
	Sometimes enjoy sunshine	31.3	38.8	44.4	54.9	33.9	32.05	43.5	48.3	50.5	61.0	70.2	63.2	76.0	70.2	73.4	86.4
	Enjoy sunshine	35.7	43.7	51.3	65.4	30.7	44.8	36.8	38.9	54.8	67.8	63.8	75.2	56.7	79.9	50.8	92.0
Osteoporosis	Avoid sunshine	31.5	48.3	46.9	61.2	41.9	35.6	36.9	47.1	72.4	75.5	76.6	86.6	75.2	89	80.2	80.1
	Sometimes enjoy sunshine	28.4	39.9	49.6	49.4	30.0	30.7	39	50.2	71.3	79.2	79.4	87.3	84.5	83	83.0	71.3
	Enjoy sunshine	41.4	52.1	65.4	84.6	47.4	61.2	85.4	69.2	77.0	85.4	87.6	92.9	84.8	75.3	86.8	96.2
Female	Avoid sunshine	24.1	28.9	34	44.4	29.5	31.7	32.3	35.6	68.6	57.3	74.7	77.8	72.1	72.2	79.1	79.6
	Sometimes enjoy sunshine	30.2	34.1	43.8	50.1	27.6	29.5	36.8	36.3	67.5	73.5	78.2	83.2	76.2	77.1	74.1	78.7
	Enjoy sunshine	39.6	41.2	52.7	57.9	28.4	43.7	34.9	42.5	70.2	80.9	86.6	85	78.4	72.6	72.8	85.3
Male	Avoid sunshine	26.3	35.7	39.9	46.3	25.5	30.6	32.4	32.2	54.3	57	60.3	73.5	47.6	55.0	69.6	74.3
	Sometimes enjoy sunshine	28.2	39.9	46.0	59.5	32.7	28.8	34.8	51.4	60.4	64.6	60.7	91.2	68.2	58.2	74.4	70.6
	Enjoy sunshine	34.7	45.1	52.1	69.2	28.6	44.4	39.3	35.7	64.5	66.1	66.6	83.7	60.7	82.5	68.6	85.9

≤30 nmol/L ≤40 nmol/L ≤50 nmol/L ≤75 nmol/L >75 nmol/L

Figure 5.4: Median 25(OH)D stratified by cw-D-UVB Quartiles and enjoyment of sunshine

HBP: high blood pressure cohort

Table 5.5: cw-D-UVB quartiles and sun enjoyment

The number of participants along with the median, inter-quartile range and mean serum 25(OH)D in each of the cw-D-UVB quartiles and by sun enjoyment in the entire cohort and stratified by patient group, sex and supplement use [1].

	25(OH)D, nmol/L	cw-D-UVB Quartile 1 (*n*=1286)			cw-D-UVB Quartile 2 (*n*=1283)			cw-D-UVB Quartile 3 (*n*=1285)			cw-D-UVB Quartile 4 (*n*=1284)		
		Enjoy sun	Sometimes enjoy sun	Avoid sun	Enjoy sun	Sometimes enjoy sun	Avoid sun	Enjoy sun	Sometimes enjoy sun	Avoid sun	Enjoy sun	Sometimes enjoy sun	Avoid sun
All (*n*=5138)	*n*	404	492	389	382	524	376	355	480	450	351	469	464
	%	(32%)	(38%)	(30%)	(30%)	(41%)	(29%)	(28%)	(37%)	(35%)	(28%)	(37%)	(36%)
	Median	52.5	47.8	41.2	55.3	49.5	43.8	64.2	57.7	48.8	75.2	63.3	56.8
	IQR Range	31-79	28-77	24-70	40-81	31-76	27-66	43-88	38-78	31-77	54-94	44-86	38-82
	Mean	58.1	55.4	49.2	61.0	55.6	49.6	66.9	59.4	55.6	74.3	66.1	61.1
Cognitive (*n*=1699)	*n*	112	187	186	87	173	117	82	140	154	106	158	197
	%	(23%)	(39%)	(38%)	(23%)	(46%)	(31%)	(21%)	(37%)	(42%)	(23%)	(34%)	(42%)
	Median	45.4	41.9	47.0	51.1	46.3	44.1	54.2	48.9	47.1	68.4	55.5	53.4
	IQR Range	25-72	23-73	26-72	32-79	27-76	24-64	34-86	29-75	28-82	43-88	37-81	31-82
	Mean	51.1	50.3	51.4	56.3	53.6	49.2	61.6	55.0	56.0	66.6	60.8	58.3
HBP (*n*=1366)	*n*	145	154	123	174	245	186	150	211	202	142	185	156
	%	(35%)	(36%)	(29%)	(29%)	(40%)	(31%)	(27%)	(37%)	(36%)	(30%)	(39%)	(32%)
	Median	39.9	37.3	27.2	46.0	43.5	38	51.7	52.5	41.7	68.6	57.2	47.8
	IQR Range	29-57	27-57	19-37	37-60	30-60	25-54	40-68	38-72	29-59	48-91	41-81	36-66
	Mean	46.6	45.6	32.1	50.6	48.2	41.6	56.0	55.4	45.9	69.9	61.7	53.1
Osteoporosis (*n*=2073)	*n*	151	147	80	121	106	73	123	129	94	103	126	111
	%	(39%)	(40%)	(21%)	(40%)	(35%)	(25%)	(35%)	(38%)	(27%)	(30%)	(37%)	(32%)
	Median	72.6	67.7	67.5	80.7	77.3	72.9	83.9	72.0	76.1	89.9	78.6	74.6
	IQR Range	50-92	45-91	44-89	63-98	55-96	47-93	65-99	54-88	57-93	75-100	60-101	60-98
	Mean	74.7	71.6	70.5	79.5	75.9	70.5	83.7	71.0	75.5	88.1	79.4	77.6

	25(OH)D, (nmol/L)	UVB Quartile 1 (n=1286)			UVB Quartile 2 (n=1287)			UVB Quartile 3 (n=1284)			UVB Quartile 4 (n=1281)		
		Enjoy sun	Sometimes enjoy sun	Avoid sun	Enjoy sun	Sometimes enjoy sun	Avoid sun	Enjoy sun	Sometimes enjoy sun	Avoid sun	Enjoy sun	Sometimes enjoy sun	Avoid sun
Females (n=3452)	*n*	253	322	303	232	339	256	213	312	339	217	318	347
	%	(29%)	(36%)	(35%)	(28%)	(41%)	(31%)	(25%)	(36%)	(39%)	(25%)	(36%)	(39%)
	Median	59.3	54.3	45.1	65.8	54.6	46	72.0	61.4	52.9	75.8	65.5	61.0
	IQR Range	39-86	30-84	26-73	40-87	34-85	26-76	46-91	39-81	32-81	55-95	43-88	39-86
	Mean	64.7	59.8	52.6	65.6	59.9	52.5	72.0	61.4	58.0	75.8	67.0	63.4
Males (n=1686)	*n*	151	170	86	150	185	120	142	168	111	134	151	117
	%	(38%)	(41%)	(21%)	(33%)	(41%)	(26%)	(34%)	(40%)	(26%)	(34%)	(38%)	(28%)
	Median	40.2	39.2	33.25	49.4	43.5	39.7	53.4	52.4	43.6	70.3	62.6	49.8
	IQR Range	26-63	25-65	21-47	40-67	30-60	29-55	42-72	35-73	31-63	49-92	49-82	36-71
	Mean	46.8	46.2	37.7	54.0	47.7	43.3	59.3	55.8	48.0	71.8	64.3	54.5
Supplements (n=2437)	*n*	198	258	177	171	236	163	165	223	217	175	229	224
	%	(31%)	(41%)	(28%)	(30%)	(41%)	(29%)	(27%)	(37%)	(36%)	(28%)	(36%)	(36%)
	Median	69.4	69.7	67.9	77.0	73.8	61.8	82.9	74.4	75.5	84.8	80.5	77.0
	IQR Range	52-87	48-91	47-87	57-93	54-95	44-86	63-101	60-89	51-90	68-100	60-99	55-96
	Mean	71.8	71.1	69.3	76.0	75.1	65.6	82.0	74.9	72.8	83.6	79.6	77.7
No Supplements (n=2447)	*n*	175	211	196	185	269	196	174	228	222	148	222	221
	%	(30%)	(36%)	(34%)	(29%)	(41%)	(30%)	(28%)	(36%)	(36%)	(25%)	(38%)	(37%)
	Median	32.9	29.1	26.2	43.7	34.7	32.9	48.2	42.1	33.8	62.8	51.0	41.7
	IQR Range	23-49	22-43	18-37	32-55	25-47	20-47	35-65	29-56	23-46	38-78	36-66	28-58
	Mean	40.0	34.5	30.7	45.1	38.1	36.2	52.8	44.0	37.8	61.6	52.6	44.3

Footnote: [1]Values represent Mean, Median IQR 25(OH)D concentration for cw-D-UVB quartiles and different levels of sun behaviour

Table 5.6: Mann-Whitney test tests examining differences in 25(OH)D concentration and sun enjoyment status

Investigating the difference in serum 25(OH)D concentration between those who avoided the sun and those who enjoyed the sun, in each of the 4 cw-D-UVB quartiles for entire cohort and those under and over 75y [1]

cw-D-UVB Quartiles	*All cohort* *P-value*	*<75y (n=1720)* *P-value*	*≥ 75y (n=1706)* *P-value*
Entire cohort			
Quartile 1 (*n*=1286)	**$3.06X10^{-05}$**	**$1.91\ X10^{-08}$**	0.47
Quartile 2 (*n*=1287)	**$6.76X10^{-09}$**	**$1.70X10^{-09}$**	**0.04**
Quartile 3 (*n*=1284)	**$4.60\ X10^{-08}$**	**$2.73X10^{-12}$**	0.61
Quartile 4 (*n*=1281)	**$7.37X10^{-11}$**	**$6.700X10^{-08}$**	**$2.5X10^{-04}$**
Supplement users (*n*=2437)			
Quartile 1 (*n*=633)	0.367	0.283	0.52
Quartile 2 (*n*=571)	**$5.22X10^{-04}$**	**$4.0X10^{-04}$**	0.31
Quartile 3 (*n*=605)	**$5.22X10^{-04}$**	**$8.23\ X10^{-05}$**	0.91
Quartile 4 (*n*=628)	**0.014**	**0.029**	0.16
Non supplement users (*n*=2447)			
Quartile 1 (*n*=582)	**$4.30\ X10^{-05}$**	**$3.02X10^{-07}$**	*0.07*
Quartile 2 (*n*=650)	**$2.90X10^{-06}$**	**$4.8X10^{-04}$**	**0.003**
Quartile 3 (*n*=624)	**$2.09X10^{-11}$**	**$9.25X10^{-11}$**	**0.05**
Quartile 4 (*n*=591)	**$3.920X10^{-09}$**	**$2.13X10^{-06}$**	**0.03**

Footnote: [1] Mann-Whitney test tests were carried out. Significant differences, $P < 0.05$

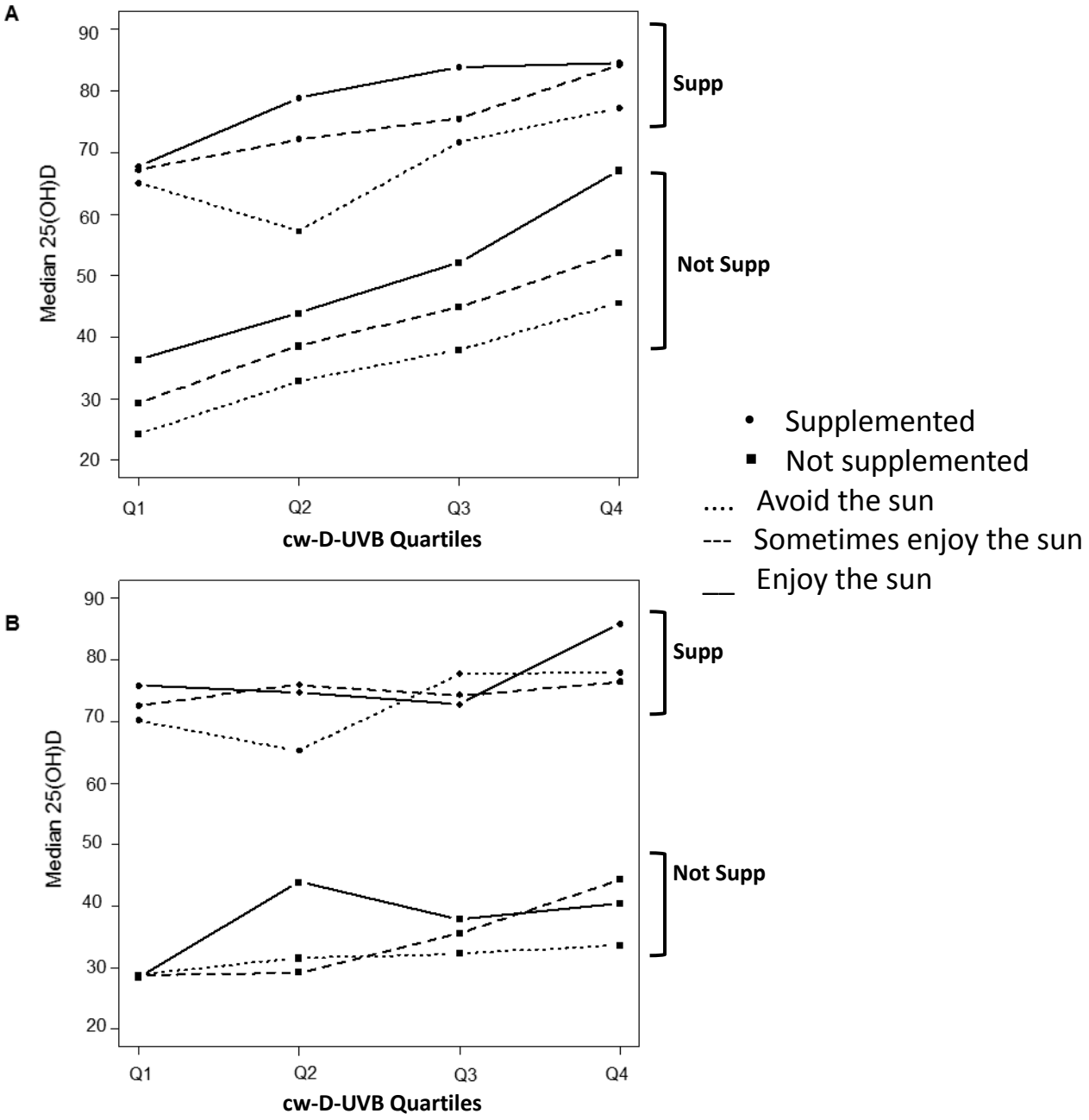


Figure 5.5: Median serum 25(OH)D concentrations in each Quartile of cw-D-UVB stratified by supplement use, sun enjoyment status and age A) 60-74, B) 75+

Median serum 25(OH)D concentration in each quartile of cw-D-UVB. Median 25(OH)D in each quartile is spit by supplementation and sun enjoyment: **A)** 60-74y (*n*=1702), **B)** Aged 75y or over (*n*=1726); circle: supplemented (supp), square: not supplemented (not supp), full line: enjoy the sun, dashed line: sometimes enjoy the sun, dotted line: avoid the sun.

Cohort	UVB Sun Enjoyment	No Supplementation								Supplementation							
		UVB Q1		UVB Q2		UVB Q3		UVB Q4		UVB Q1		UVB Q2		UVB Q3		UVB Q4	
		OFC[1] No	OFC Yes	OFC No	OFC Yes	OFC No	OFC Yes	OFC No	OFC Yes	OFC No	OFC Yes	OFC No	OFC Yes	OFC No	OFC Yes	OFC No	OFC Yes
ALL	Avoid sunshine	29.3	31.6	32.2	40.2	34.9	40.3	43.9	44.7	71.9	67.1	62.4	67.8	70.7	74.3	71.0	82.1
	Sometimes enjoy sunshine	31.7	36.7	36.1	39.2	39.0	47.8	55.0	54.9	68.2	73.1	79.0	72.3	76.2	74.3	77.8	80.6
	Enjoy sunshine	41.8	38.6	43.0	46.7	49.8	54.4	54.9	65.0	66.7	75.9	75.1	76.7	77.5	84.9	84.0	83.4
Cognitive	Avoid sunshine	32.9	32.7	30.1	34.4	33.0	35.0	39.9	34.8	68.9	66.2	61.4	61.8	64.1	74.9	68.5	82.2
	Sometimes enjoy sunshine	31.2	28.2	31.0	30.2	34.6	39.6	41.1	43.2	64.5	69.4	79.8	69.3	78.7	72.5	78.6	75.3
	Enjoy sunshine	28.9	35.2	40.7	44.5	41.9	46.6	49.0	55.1	70.7	70.0	65.3	70.4	75.7	80.3	79.2	74.5
HBP	Avoid sunshine	23.6	27.7	31.5	41.6	34.8	41.9	45.5	49.2	73.9	49.2	52.3	56.6	53.4	68.0	57.7	73.6
	Sometimes enjoy sunshine	30.3	41.1	38.0	42.5	43.1	50.6	52.6	60.4	65.9	59.8	71.6	64.8	74.1	68.2	71.7	72.2
	Enjoy sunshine	49.7	36.4	41.4	45.1	50.6	53.6	56.9	68.3	53.6	57.9	68.0	70.4	66.6	64.4	79.4	77.0
Osteo	Avoid sunshine	42.9	49.6	45.7	53.8	48.0	41.2	52.6	59.5	78.2	77.6	73.9	81.2	85.9	79.1	82.2	86.0
	Sometimes enjoy sunshine	35.1	37.7	55.9	40.7	40.3	49.5	54.2	54.3	73.1	83.0	84.0	79.8	75.6	80.8	79.2	88.0
	Enjoy sunshine	51.9	54.4	59.3	62.9	64.6	67.6	67.0	79.4	71.6	86.5	84.0	81.7	84.7	91.4	91.7	90.5
Female	Avoid sunshine	31.1	32.6	31.8	39.8	35.4	37.4	44.2	45.7	77.0	69.8	63.1	69.2	72.0	76.0	72.8	83.1
	Sometimes enjoy sunshine	35.8	33.9	36.5	37.5	39.7	47.9	42.3	52.4	68.6	75.3	83.8	73.8	72.5	76.3	78.6	80.7
	Enjoy sunshine	43.6	42.9	42.8	46.9	47.6	54.0	50.6	62.4	68.1	79.1	78.0	77.1	77.8	87.3	84.3	84.7
Male	Avoid sunshine	23.9	29.0	32.7	40.7	33.6	45.0	43.1	42.5	51.7	52.3	55.4	62.6	60.4	67.1	63.6	78.1
	Sometimes enjoy sunshine	27.5	39.6	35.5	41.1	38.1	47.7	54.5	58.8	67.4	66.3	67.4	65.6	87.6	68.6	72.1	80.4
	Enjoy sunshine	40.2	35.2	43.2	46.5	51.2	54.8	60.5	66.9	63.0	63.5	68.4	74.9	76.8	74.1	83.0	79.8

≤30 nmol/L ≤40 nmol/L ≤50 nmol/L ≤75 nmol/L >75 nmol/L

Figure 5.6: Mean 25(OH)D concentration in each of the 48 VDscore2 category groups

Heat map of mean 25(OH)D in each of the 48 VDscore2 categories for all participants and split by cohort type and sex[1]

Footnote: [1]OFC: oily fish consumption, HBP: high blood pressure cohort, Osteo: osteoporosis cohort

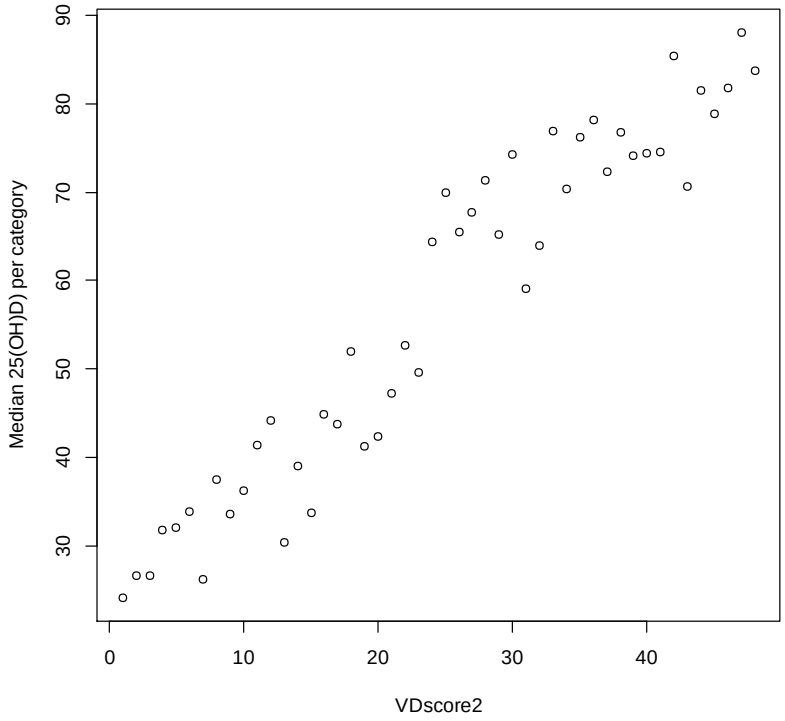


Figure 5.7: Median 25(OH)D concentration and VDscore2
Median 25(OH)D concentration for each VDscore2 possibility (1-48)

5.4.4 Associations between 25(OH)D, cw-D-UVB, VDscore1, VDscore2 and VDscore3

Adjusted linear regression was employed and a significant association between serum 25(OH)D concentration and a number of variables in this model were found. Strong contributions of vitamin D supplementation on circulating 25(OH)D concentration were observed in all instances ($P<2x10^{-16}$) (**Table 5.7**). Serum 25(OH)D concentration was also significantly positively associated with cw-D-UVB; there was a 1.58 nmol/L increase in 25(OH)D concentration for every 1,000 mJ/cm^2 unit increase in cw-D-UVB.

VDscores were next examined. Models were similar to the model shown above with cw-D-UVB, however these models were not adjusted for supplementation dose due to the high degree of collinearity between this variable and the VDscores. 25(OH)D concentration was found to be strongly associated with VDscore1, VDscore2 and VDscore3 (p-value: $< 2x10^{-16}$) (**Table 5.7**)

Every unit increase in VDscore2 resulted in a 1.03 nmol/L increase in 25(OH)D concentration. While this was 0.87 nmol/L when VDscore3 was examined, and 6 nmol/L when VDscore1 was examined. This is due to the difference in scales between the scores.

When examining the r^2, all models had similar r^2 values. On the other hand however, partial eta squared, which is the variance explained by a given variable, was found to be 21% for VDscore1, 22% for VDscore2, 22% for VDscore3, and 2.7% for cw-D-UVB (**Table 5.7**). Furthermore, it was noted that 25(OH)D concentration was also highly associated with VDscore2 tertiles (**Table 5.8**). A number of other variables such as sun holiday in the last six months, sun enjoyment, oily fish consumption and patient cohort were also positively associated with 25(OH)D while BMI, and smoking status was negatively associated (**Table 5.7**).

Next, the relationship between cw-D-UVB, VDscore1, VDscore2, VDscore3 as the dependent variable and personal characteristics were examined (**Table 5.9**)**.** The trend for decreasing vitamin D with increasing BMI status was found for all estimates (25(OH)D status, cw-D-UVB, VDscore1, VDscore2 and VDscore3) (**Table 5.9**). Increasing vitamin D status was also associated with increasing age for all estimates. An increase in vitamin D status was noted in those who went on a sun holiday in the last six months in 25(OH)D, cw-D-UVB, VDscore1, VDscore2 and VDscore3 (**Table 5.9**). Some differences between variables were also observed, for example cw-D-UVB was negatively correlated with an increase in sun protection use, however it was positively associated with 25(OH)D, and the VDscores. (**Table 5.9**).

Table 5.7: Association between serum 25(OH)D concentration and cw-D-UVB, VDscore1, VDscore2 and VDscore3

Association in entire cohort (*N*=5,138)[1].

Variable		*Model 1 (25(OH)D)*[2,3]			*Model 2 (25(OH)D)*			*Model 3 (25(OH)D)*[4,5]			*Model 4 (25(OH)D)*[4,6]		
		Beta	**SE**	**p-value**	**Beta**	**SE**	**p-value**	**Beta**	**SE**	**p-value**	**Beta**	**SE**	**p-value**
Age		0.02	0.05	0.74				0.02	0.05	0.69	0.02	0.05	0.68
Cw-D-UVB [Beta per 1000 mJ/cm^2]		1.58	0.12	**< 2x10^{-16}**	NA	NA	NA	NA	NA	NA	NA	NA	NA
VDscore1		NA	NA	NA	6.15	0.17	**< 2x10^{-16}**	NA	NA	NA	NA	NA	NA
VDscore2		NA	NA	NA	NA	NA	NA	1.03	0.03	**< 2x10^{-16}**	NA	NA	NA
VDscore3		NA	NA	NA	NA	NA	NA	NA	NA	NA	0.87	0.02	**< 2x10^{-16}**
BMI		-0.57	0.07	**8x10^{-16}**	0.58	0.07	**< 2x10^{-16}**	-0.58	0.07	**< 2x10^{-16}**	-0.57	0.07	**8.1x10^{-16}**
Patient Cohort	Osteo	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Cognitive	-10.30	1.00	**< 2x10^{-16}**	-10.95	1.10	**< 2x10^{-16}**	-10.99	1.11	**< 2x10^{-16}**	-10.36	1.10	**< 2x10^{-16}**
	HBP	-8.60	1.00	**< 2x10^{-16}**	-10.34	1.11	**< 2x10^{-16}**	-10.41	1.02	**< 2x10^{-16}**	-8.70	1.02	**< 2x10^{-16}**
Sex	Male	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Female	0.12	0.80	0.88	0.57	0.83	0.49	0.50	0.83	0.49	0.04	0.82	0.93
Smoking Status	Current	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Past	5.10	1.20	**1.9x10^{-5}**	5.28	1.20	**1.0x10^{-5}**	5.28	1.20	**1.1x10^{-5}**	5.11	1.19	**1.8 x10^{-5}**
	Never	6.40	1.20	**8.2 x10^{-8}**	6.71	1.20	**2.6x10^{-8}**	6.54	1.21	**2.6 x10^{-8}**	6.37	1.19	**7.9 x10^{-8}**
Recent Sun holiday	No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Yes	10.50	1.00	**< 2x10^{-16}**	10.32	1.01	**< 2x10^{-16}**	10.31	1.02	**< 2x10^{-16}**	10.50	1.00	**< 2x10^{-16}**
Oily fish consumption	*No*	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Yes	2.05	0.74	**0.005**	1.99	0.74	**0.007**	1.00	0.74	0.17	-1.00	0.74	0.18
Sun enjoyment	Avoid sun	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Sometimes	2.99	0.85	**0.0005**	3.23	0.86	**0.0002**	1.24	0.86	0.15	-0.88	0.86	0.30
	Enjoy sun	6.60	0.94	**2.2x10^{-12}**	6.88	0.95	3.7x10^{-13}	2.84	0.95	**0.003**	-10.36	1.10	*0.06*
Supplement use	*No*	Ref	Ref	Ref	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Yes	27.70	0.85	**< 2x10^{-16}**	NA	NA	NA	NA	NA	NA	NA	NA	NA
R^2		Using cw-D-UVB		0.37	Using VDscore1		0.36	Using VDscore2		0.37	Using VDscore3		0.37

Footnote:

[1] Abbreviations: cw-D-UVB; Cumulative and weighted UVB dose, HBP; High blood pressure, Osteo: osteoporosis, NA; not applicable, SE; standard error.

[2] Multivariable linear regression was used to test associations. Adjusted for the age, BMI, patient cohort, sex, smoking status, recent sun holiday, supplementation, sun enjoyment, oily fish consumption and cw-D-UVB.

[3] Model 1, CW-D-UVB: order of important variables (partial eta^2): supplementation 19.9%; cw-D-UVB: 3.6%; sun holiday 2.2%;

[4] Multivariable linear regression was used to test associations. Adjusted for the age, BMI, patient cohort, sex, smoking status, recent sun holiday, VDscore2 or VDsore3. Supplementation was removed from adjustments due to the high multi-collinearity between supplementation and VDscores (VDscore1: VIF=4.1, VDscore2: VIF=4.5 VDscore3: VIF=13.0). No multi-collinearity was observed between sun enjoyment and oily fish and VDscores and therefore they were kept in the model.

[5] Model 2, VDscore1: order of important variables (partial eta^2): VDscore2; 21%, cohort; 2.7%, Sun holiday 2.1% (VDscore1: stepwise increase depending on supplement use, cw-D-UVB dose)

[6] Model 3, VDscore2: order of important variables (partial eta^2): VDscore2; 22%, sun holiday; 2.3%, BMI 1.5% (VDscore2: stepwise increase depending on supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption)

[7] Model 4, VDscore3: order of important variables (partial eta^2): VDscore3; 21.3%, cohort 2.6%; sun holiday 2.1% (VDscore3: addition of regression coefficients for supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption).

Table 5.8: Association between serum 25(OH)D concentration and VDscore2 tertiles

Association between serum 25(OH)D concentration and selected variables, including VDscore2 tertiles [1, 2,3].

Variable		Model 1 (25(OH)D Tertiles)		
		Beta	**SE**	**p-value**
Age		0.01	0.06	0.885
VDscore2	Tertile 1	Ref	Ref	Ref
	Tertile 2	19.85	0.91	**$< 2x10^{-16}$**
	Tertile 3	33.56	0.97	**$< 2x10^{-16}$**
BMI		-0.63	0.07	**$< 2x10^{-16}$**
Patient Cohort	Osteo	Ref	Ref	Ref
	Cognitive	-12.10	1.10	**$< 2x10^{-16}$**
	HBP	-11.82	1.02	**$< 2x10^{-16}$**
Sex	Female	Ref	Ref	Ref
	Male	-0.59	0.80	0.47
Smoking Status	Current	Ref	Ref	Ref
	Past	5.60	1.20	**$3.9x10^{-6}$**
	Never	6.82	1.20	**$1.4 x10^{-8}$**
Recent Sun holiday	No	Ref	Ref	Ref
	Yes	11.20	1.0	**$< 2x10^{-16}$**
R SQUARED		0.343		

Footnote:

1 Abbreviations: Osteo; osteoporosis, HBP; High blood pressure, SE; standard error.

2 VDscore2: stepwise increase depending on supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption

3 Statistically significant differences are shown in bold

Table 5.9: Vitamin D and personal characteristics

Comparing the determinants of vitamin D status in the cohort using all four proxies of vitamin D status as the dependent variable; 25(OH)D, cw-D-UVB, VDscore1, VDscore2 and VDscore3 [1,2].

Variable	25(OH)D			Cw-D-UVB			VDscore1			VDscore2			VDscore3		
	Beta	**SE**	***P***	**Beta**	**SE**	***P***	**Beta**	**SE**	***P***	**Beta**	**SE**	***P***	**Beta**	**SE**	***P***
Sex															
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Male	-2.47	0.93	0.008	-0.10	0.10	0.32	-0.53	0.07	**$5x10^{-14}$**	-2.73	0.42	$1.4x10^{-10}$	-2.93	0.52	$1.8x10^{-8}$
Age	0.24	0.06	**$1x10^{-4}$**	0.02	0.007	**0.003**	0.03	0.004	**$2x10^{-10}$**	0.17	0.03	**$1.4x10^{-9}$**	0.19	0.03	**$5x10^{-8}$**
BMI															
Underweight	1.67	2.88	0..56	0.21	0.31	0.49	0.33	0.22	0.12	1.63	1.30	0.21	1.34	1.60	0.40
Healthy weight	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Overweight	-4.73	1.03	**$4.2x10^{-6}$**	-0.18	0.11	0.10	-0.24	0.08	**0.002**	-1.51	0.46	**0.001**	-1.84	0.57	**0.001**
Obese	-10.10	1.12	**<$2 x10^{-16}$**	-0.09	0.12	0.45	-0.44	0.08	**$1.6x10^{-7}$**	-2.88	0.50	**$1.4x10^{-8}$**	-3.99	0.62	**$1.7x10^{-10}$**
Morbidly obese	-15.27	2.62	**$6.5x10^{-9}$**	-0.40	0.28	0.16	-0.49	0.20	**0.01**	-3.36	1.18	**0.004**	-4.54	1.45	**0.002**
Smoking															
Never	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Previous	-1.87	0.90	**0.04**	-0.04	0.10	0.70	-0.09	0.07	0.19	-0.53	0.40	0.19	-0.66	0.50	0.19
Current	-8.04	1.34	**$2.3x10^{-9}$**	-0.15	0.14	0.28	-0.34	0.10	**$7x10^{-4}$**	-2.04	0.60	**$8x10^{-4}$**	-2.59	0.74	**$5x10^{-4}$**
Cohort															
Osteoporosis	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
HBP	-21.73	1.10	**<$2 x10^{-16}$**	-0.08	0.12	0.51	-1.22	0.09	**<$2 x10^{-16}$**	-10.91	0.50	**<$2 x10^{-16}$**	-9.57	0.69	**<$2 x10^{-16}$**
Cognitive	-18.05	1.24	**<$2 x10^{-16}$**	-0.37	0.13	**0.005**	-1.79	0.08	**<$2 x10^{-16}$**	-7.44	0.56	**<$2 x10^{-16}$**	-15.00	0.61	**<$2 x10^{-16}$**
Use of Sun screen															
Never	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Rarely	3.31	1.78	*0.06*	-0.47	0.19	**0.01**	0.11	0.13	0.43	1.01	0.80	0.20	2.53	0.99	**0.01**
Sometimes	5.19	1.28	**$4.8x10^{-5}$**	-0.42	0.14	**0.002**	0.09	0.10	0.32	1.10	0.57	*0.06*	2.71	0.71	**$1x1^{-4}$**
Usually	5.21	1.32	$7.7x10^{-5}$	-0.46	0.14	**0.001**	0.12	0.10	0.22	1.46	0.59	**0.01**	3.57	0.73	**$2x1^{-6}$**
Always	5.32	1.29	**$3.8x10^{-5}$**	-0.49	0.14	**$4x10^{-4}$**	0.07	0.10	0.47	1.12	0.58	*0.05*	3.18	0.71	**$9x10^{-6}$**
Sun holiday in last six months															
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Yes	11.54	1.16	**<$2 x10^{-16}$**	0.36	0.12	**0.003**	0.22	0.09	**0.009**	1.76	0.52	**$7x10^{-4}$**	2.37	0.64	**$2x10^{-4}$**

Footnote:

1 VDscore1: stepped increase depending on supplement use and cw-D-UVB dose

2 VDscore2: stepped increase depending on supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption

3 VDscore3: addition of regression coefficients for supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption.

5.4.1 Predicting Deficiency and sufficiency of 25(OH)D using cw-D-UVB

An investigation into whether it was possible to classify participants into different deficiency/ sufficiency categories using either cw-D-UVB, VDscore1, VDscore2 or VDscore3 was carried out.

The addition of supplementation, cw-D-UVB + supplementation, VDscore1, VDscore2 or VDscore3 to the random forest model increased the prediction ability of the model; AUC was improved by 10% when supplementation was added to the model. This was further improved by 15.3% when cw-D-UVB and supplementation was added to the model. Similar improvements were observed when VDscore1, VDscore2 or VDscore3 were included in the models. This trend was also observed when stratified to those under 75 (an increase of 7.2 was observed for supplementation alone, 13.5% for cw-D-UVB + supplementation, 13.9% for VDscore1, 16.1% for VDscore2 and 15.9% for VDscore3 higher for deficiency) (**Figure 5.8, Table 5.10**). Similar results were also observed when predicting sufficiency and when stratified by those over 75. These results suggest that the addition of cw-D-UVB, VDscore1, VDscore2 or VDscore3 to a baseline model results in similar prediction abilities of deficient and sufficient patients.

Using the Boruta method a number of variables were found to be important when classifying deficiency and sufficiency of 25(OH)D; BMI, smoking, age, sex and cohort type (**Figure 5.10**). This is similar to what was observed in the association analysis (**Table 5.7**) but with the addition of age. Vitamin D related variables were next added and it was found that supplementation use, sun enjoyment, oily fish consumption and cw-D-UVB were all considered important variables. Supplement use was the most important when classifying individuals into deficient or sufficient groups (**Figure 5.9**). However, once VDscore1, VDscore2 or VDscore3 was added to this model, it was found to be the most important variable when classifying deficient individuals (**Figure 5.9**).

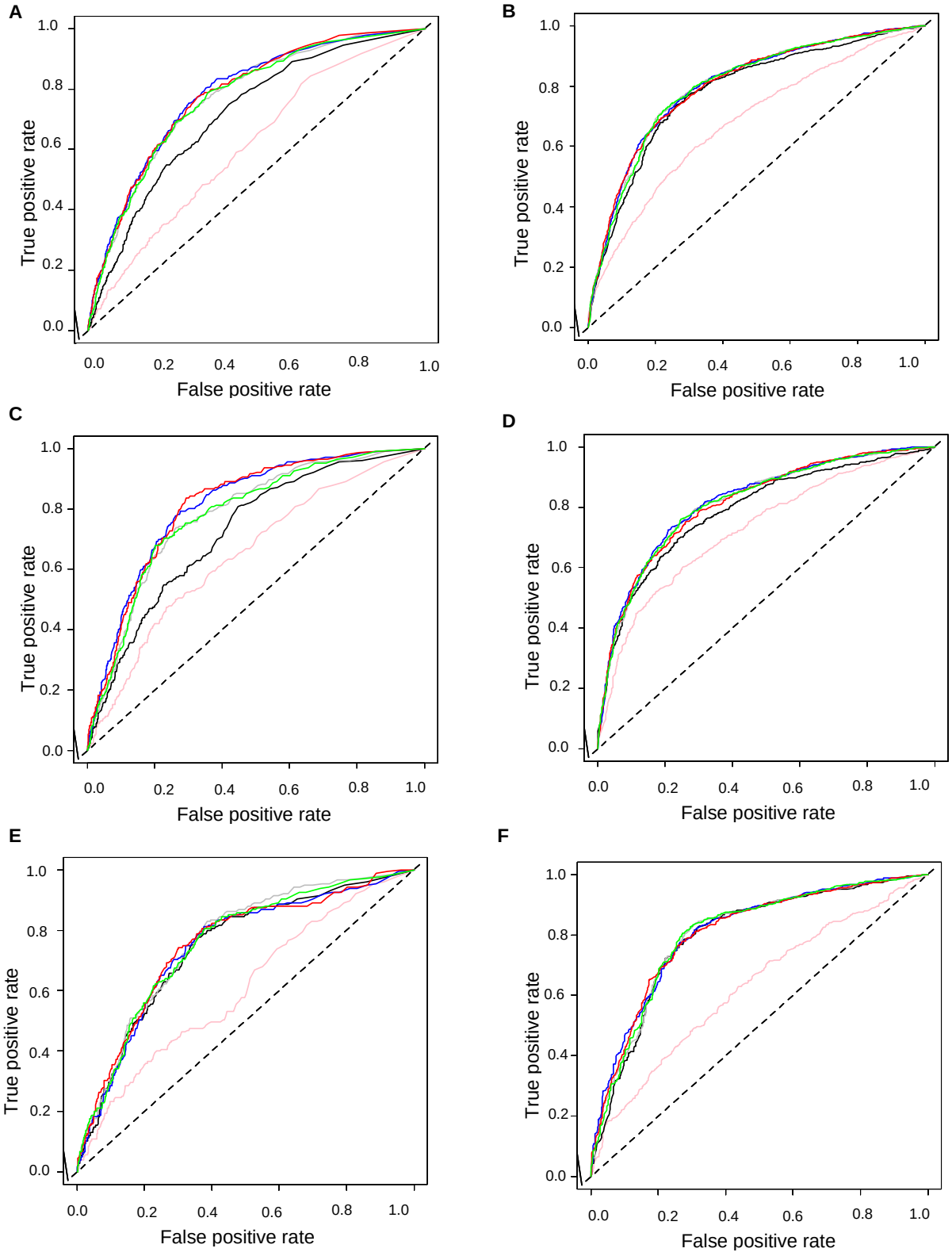


Figure 5.8: ROC curves predicting vitamin D deficiency

ROC curves predicting vitamin D deficiency (< 25 nmol/L) and sufficiency (≥ 50 nmol/L) using models. **A)** Deficiency in entire cohort (total *n*=4,843, <25 nmol/l 25(OH)D *n*= 665); **B)** Sufficiency in entire cohort (total *n*=4,843, ≥50 nmol/l 25(OH)D *n*= 2,664); **C)** Deficiency in those under 75 years old (total

n=2,696, <25 nmol/l 25(OH)D *n*= 326); **D)** Sufficiency in those under 75 years old (total *n*=2,696, ≥50 nmol/l 25(OH)D *n*= 1,478); **E)** Deficiency in those over 75 years old (total *n*=2,147, <25 nmol/l 25(OH)D *n*= 339); **F)** Sufficiency in those over 75 years old (total *n*=2,147, ≥50 nmol/l 25(OH)D *n*= 1,186); Pink is baseline model; Black is the baseline model plus supplementation; Grey is the baseline model plus cw-D-UVB; Green is baseline model plus VDscore1; Red is baseline model plus VDscore2; Blue is baseline plus VDscore3 and green is baseline model plus annual UVB. AUC and model adjustment details for each model shown below in Table 5.10

Table 5.10: AUC from ROC curves

AUC from the ROC curves showing the strength of each of the classification models for entire cohort and split by age (60-74 and ≥75) [1, 2, 3].

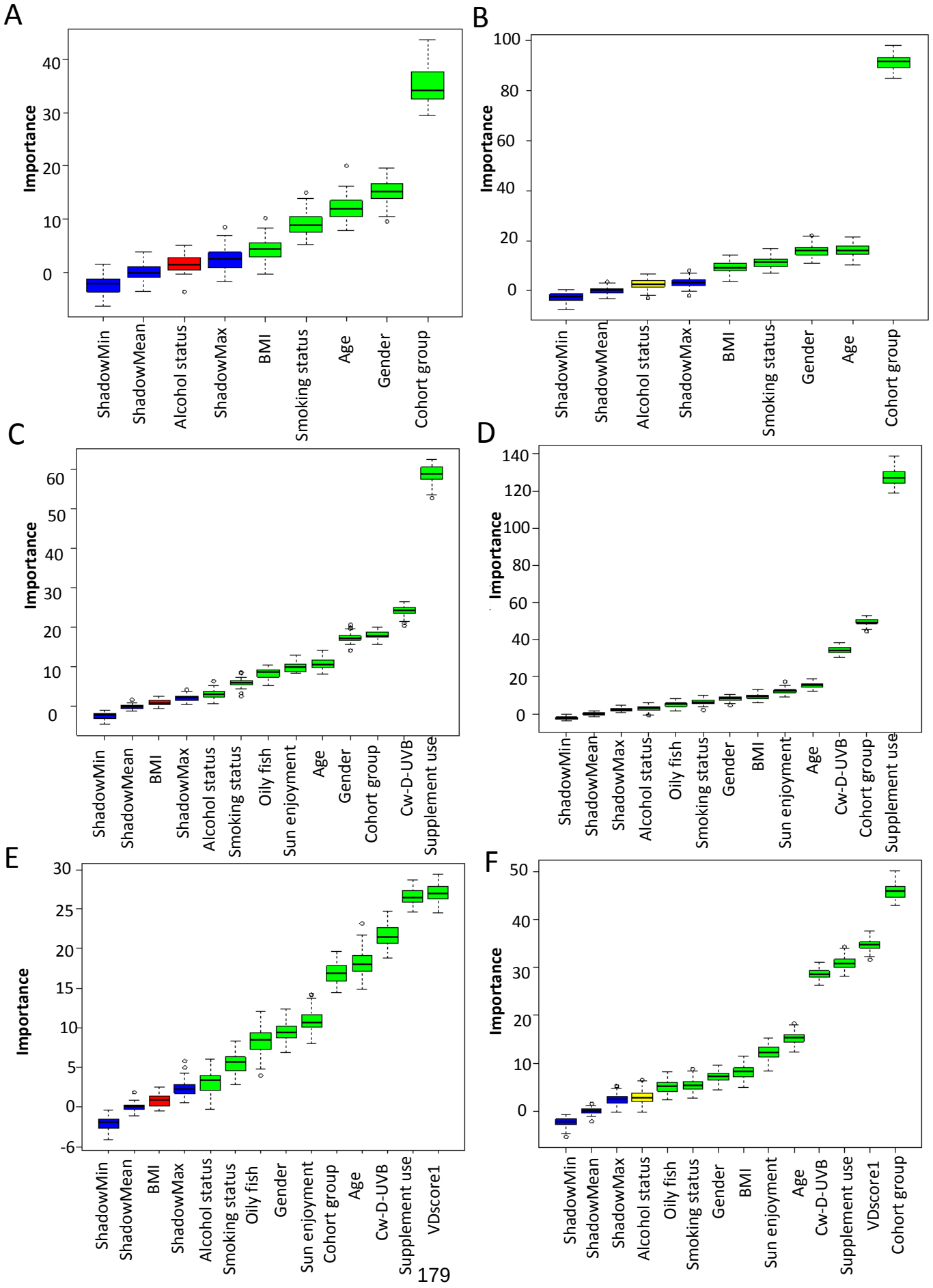
	Variables	AUC	
All cohort		*<25 nmol/l*	*>50 nmol/l*
Model 1 (Pink)	Age, Sex, BMI, Cohort, Sun holiday last six months, smoking status	61.8%	68.0%
Model 2 (Black)	Model 1 + Supplement use	71.8%	78.2%
Model 3 (Grey)	Model 2 + cw-D-UVB quartiles	77.1%	80.0%
Model 4 (Green)	Model 1 + VDscore1	76.9%	80.0%
Model 5 (Blue)	Model 1 + VDscore2	77.9%	80.1%
Model 6 (Red)	Model 1 + VDscore3	78.4%	80.0%
Under 75 years old			
Model 1 (Pink)	Age, Sex, BMI, Cohort, Sun holiday last six months, smoking status	64.1%	70.6%
Model 2 (Black)	Model 1 + Supplement use	71.3%	77.3%
Model 3 (Grey)	Model 2 + cw-D-UVB quartiles	77.6%	80.4%
Model 4 (Green)	Model 1 + VDscore1	78.0%	80.5%
Model 5 (Blue)	Model 1 + VDscore2	80.2%	80.9%
Model 6 (Red)	Model 1 + VDscore3	80.0%	80.4%
Over 75 years old			
Model 1 (Pink)	Age, Sex, BMI, Cohort, Sun holiday last six months, smoking status	62.5%	63.7%
Model 2 (Black)	Model 1 + Supplement use	75.0%	79.6%
Model 3 (Grey)	Model 2 + cw-D-UVB quartiles	76.0%	80.0%
Model 4 (Green)	Model 1 + VDscore1	75.7%	80.1%
Model 5 (Blue)	Model 1 + VDscore2	77.1%	79.6%
Model 6 (Red)	Model 1 + VDscore3	77.2%	79.1%

Footnote:

1 VDscore2: vitmain D scoring system 2, VDscore3: vitamin D scoring system 2

2 VDscore2: stepwise increase depending on supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption

3 VDscore3: addition of regression coefficients for supplement use, cw-D-UVB dose, sun enjoyment and oily fish consumption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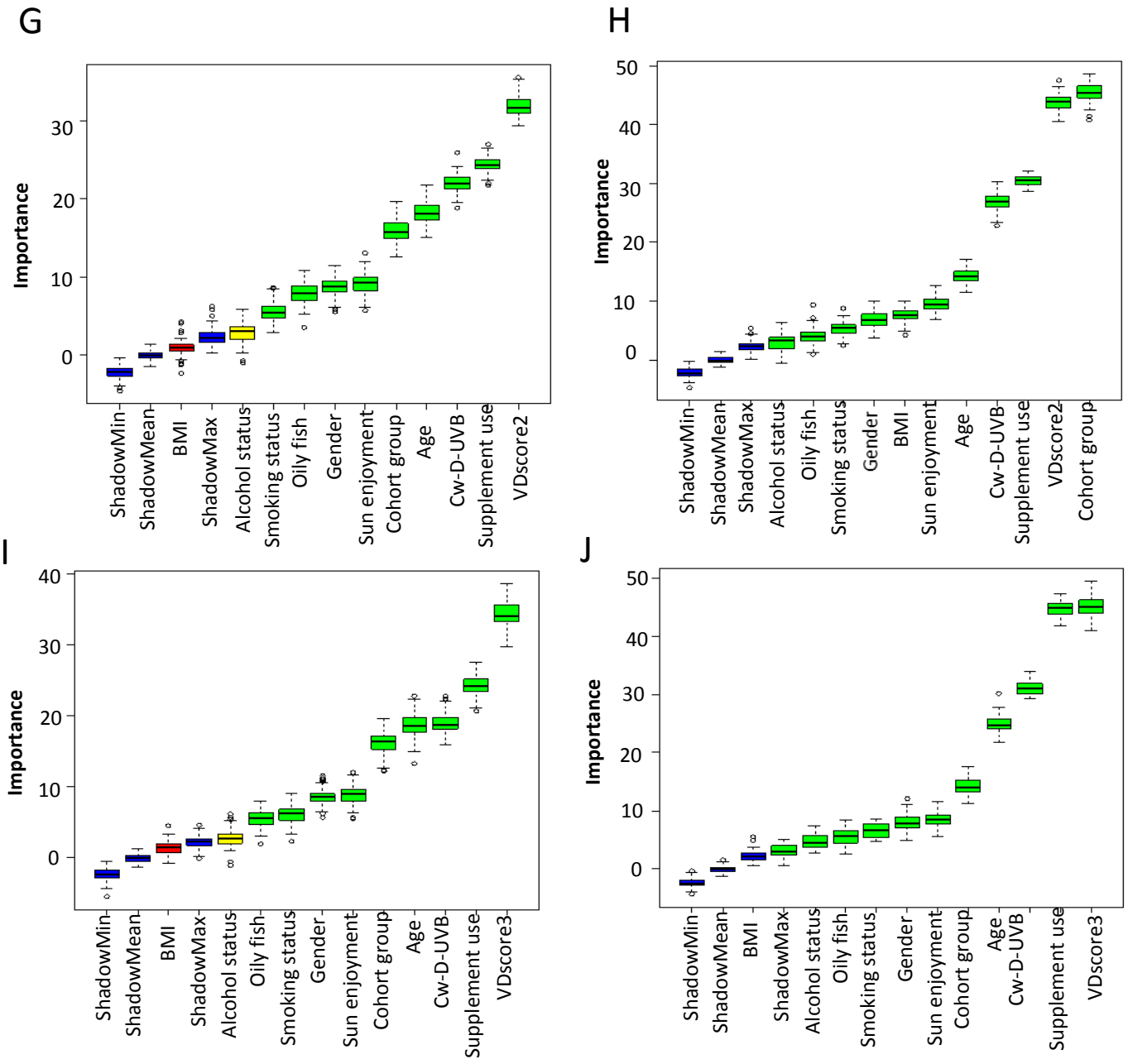

Figure 5.9: Boruta method for 25(OH)D deficiency or sufficiency prediction

Boruta method to determine important variables with the model. Shadow min/ shadow Mean and Shadow max represent random probes. Those in red are deemed not to be important variables, yellow determines uncertain variables and green demonstrates variables which are deemed important. **A)** Deficiency classification with baseline characteristics, **B)** Sufficiency classification with baseline characteristics, **C)** Deficiency classification with cw-D-UVB added, **D)** Sufficiency classification with cw-D-UVB added, **E)** Deficiency classification with VDscore1 added, **F)** Sufficiency classification with VDscore1 added, **G)** Deficiency classification with VDscore2 added, **H)** Sufficiency classification with VDscore2 added **I)** Deficiency classification with VDscore3 added, **J)** Sufficiency classification with VDscore3 added.

5.5 Discussion

5.5.1 Baseline Characteristics

Baseline characteristics were similar at all quartiles of D-UVB, however there were some differences observed when baseline characteristics were stratified by cohort type. 25(OH)D varied considerably from cohort to cohort, while D-UVB doses remained similar. This was mainly due to the inclusion of the osteoporosis cohort in this study. This cohort had been diagnosed with osteoporosis and due to the known relationship between vitamin D, calcium and bone strength, this cohort would have been have been monitored for vitamin D deficiency, as part of their treatment. This resulted in a higher proportion of individuals within that cohort taking supplements (81% vs 53% in the cognitive cohort and 27% in the HBP cohort). This most likely contributed to the higher median 25(OH)D concentrations which was observed within this group. There was also a higher percentage of those who were overweight or obese in the HBP cohort (42%) compared to the other cohorts (cognitive: 26% and osteoporosis: 20%). This is unsurprising as HPB is has been shown to be associated with those who are overweight or obese [278]. Most of the other baseline factors were similar between the three cohorts.

Within the overall cohort, there were seasonal fluctuations observed in both serum 25(OH)D concentrations and cw-D-UVB measurements. However, seasonal variation in cw-D-UVB doses were more noticeable. A reduction in seasonality in the 25(OH)D concentrations may have been due to the large percentage (47%) of the cohort taking supplements. Additionally, other sources, of vitamin D, would have contributed to 25(OH)D concentration during winter and spring months and reduced the seasonal fluctuations. As expected, it was also noted blood samples taken from December-April were more likely to have insufficient 25(OH)D concentrations compared to samples taken in the summer months. The highest risk of deficiency was found in those who were sampled in March- this again is unsurprising, as often, those who are sampled in February and March typically have the lowest 25(OH)D concentrations.

It is worth noting that large differences between median cw-D-UVB doses in each of the cw-D-UVB quartiles were found. For example, there was over a five-fold difference in median cw-D-UVB between quartile one and quartile two; this difference doubled between quartile one and quartile three (10-fold) and doubled again, so that the difference between quartile one and quartile four was almost 20 fold. The dose of cw-D-UVB within quartiles were vary varied and a broad range of doses were observed.

5.5.2 Cutaneous Synthesis of Vitamin D in an Older Cohort

The ability to synthesise vitamin D has been shown to decrease with age [22, 54] through experiments demonstrating a reduction in 25(OH)D concentration following exposure to solar radiation in different age groups, along with suggestions that concentrations of 7-dehydrocholesterol (which is a key substrate) decreases with age. This study highlights the importance of skin synthesis even in older adults at high latitudes, as ambient D-UVB at place of residence was found to be associated with 25(OH)D status. Additionally, this was further confirmed in predictive models focusing on vitamin D deficiency. In accordance with this, direct and indirect evidence suggesting a role of natural sun exposure in older populations can easily be found in the literature: 25(OH)D is higher in community-living adults when compared to institutionalised adults [279]; older community dwelling individuals showed seasonal variation in their 25(OH)D status [279]; and older individuals who undertook outdoor leisure activities such as gardening and cycling had higher 25(OH)D than those who did not [280].

5.5.3 Association between Ambient D-UVB and 25(OH)D Status

Contrary to the prevailing view that cutaneous synthesis of vitamin D is not an important determinant of vitamin D in older individuals and at northern latitudes [51, 155, 281], 25(OH)D status was found to be strongly associated with ambient D-UVB. A Canadian study examined UVB in a similar manner to this current study; that is through satellite measurements which had been adjusted to account for ozone column, medium range weather forecasts, and restricted to vitamin D producing wavelengths. This study also found a strong association between their UVB estimate and 25(OH)D measurements but they failed to find an association in patients over 60 years old [267]; a null finding that may be due to the less detailed UVB exposure estimate and/or a much smaller cohort. Additionally, this association between ambient D-UVB and 25(OH)D was still highly evident in participants who were taking supplements in the TUDA cohort, conversely to what was observed in a previous study examining global solar radiation (the sum of direct and diffuse solar radiation) [243]; this might be explained by the lack of adjustment for a range of important determinants of UVB exposure, resulting in an imprecise ambient UVB dose estimate in this study.

Previous studies have examined the association between 25(OH)D and UVB proxies and have found strong associations, however these studies have not restricted wavelengths to those relevant for vitamin D synthesis, and often presume constant cloud cover or ozone column, or

do not account for those variables at all. For example, Sayers *et al.*, examined the association between ambient erythemal UV in expectant mothers and maternal 25(OH)D. This study assumed that extrinsic factors such as ozone were constant. Furthermore, cloud cover was omitted in their model but monthly recorded sunshine was used as a substitute. Nonetheless, this study found a strong association between their ambient erythemal UV measurement and 25(OH)D, however, they did not adjust for all possible modifying variables associated with UVB measurement (cloud cover, ozone etc.) [264].

Similarly, Nair-Shalliker *et al.*, presumably did not adjust for confounding variables as no mention of ozone or cloud adjustment was given. This study also used recalled hours of sun exposure along with average ambient UV irradiance 16 weeks prior to blood draw which were measured from 8am-5pm daily. As sunshine hours in Australia during the summer months can last from 6am-8pm, this study failed to take into account the additional five hour long exposure that their participants could have been exposed to UV radiation, albeit this would be the weakest time for sun exposure [265]**.**

Other studies have also examined the relationship between UVB and health outcomes, for instance a study by Tran *et al.* used mean lifetime daily UV radiation exposure and found a reduced risk for oesophageal adenocarcinoma in those with the highest UV tertile. They used average UV radiation recorded along with ozone measurements and place of residence to determine lifetime ambient UV but, similar to Sayers and Nair-Shalliker, this study failed to take into account cloud cover [6] and there is no mention if they restricted their UV measurements to UVB or if UVA was also included. If the latter was the case, there could be considerable disparity in the UV measured and UVB dose recorded as 95% of UV radiation reaching earth is UVA which cannot induce vitamin D synthesis [49]. It has previously been shown that without the adjustment of cloud cover, ozone or altitude, UVB level can be altered substantially, and this could limit the interpretability of such results [50]. Furthermore, a report by European cooperation in Science and Technology 713 (E-COST) on UV-forecasting recommends that it is essential to take into account ozone column and cloud cover when forecasting UV index [282] and therefore these adjustments are necessary for accurate UVB exposure assessment.

5.5.4 cw-D-UVB, Supplementation, Sun Enjoyment, and 25(OH)D

It has been previously noted in chapter four that when participants were stratified depending on their ambient cw-D-UVB dose and supplementation status to create VDscore1, strong

associations were found between this newly created variable and 25(OH)D status. This association was re-investigated in the TUDA cohort. A strong relationship between quartiles of ambient cw-D-UVB, supplementation and median 25(OH)D concentrations were found, especially among those who were 60-74 years old. There was almost a 43 nmol/L difference between those supplemented and non-supplemented in the same quartile of cw-D-UVB. This highlights the importance of supplementation within this elderly cohort. Furthermore, the impact of supplementation can be easily be observed when examining the relationship between 25(OH)D and VDscore3, and again between median 25(OH)D per VDscore2. Each of these graphs (**Figure 5.3F and 5.3H**) show a clear separation in the centre of the graph. This gap is due to the large difference in 25(OH)D dose between those who are supplemented and those who are not.

Additionally, an increase of approximately 23 nmol/L was found between those who belonged to Q1 and those who belonged to Q4 of ambient cw-D-UVB dose, highlighting how important ambient UVB is i.e.: the time of year and the location of an individual, when estimating 25(OH)D status. Furthermore, these differences were even more pronounced when stratified by sun enjoyment status, as there was almost a 36 nmol/L difference between median 25(OH)D concentration in those in D-UVB Q1 who avoided the sun and those in Q4 who enjoyed the sun in the non-supplemented group. Overall, those who *avoided the sun* were found to have a lower median 25(OH)D in all four cw-D-UVB quartiles when compared to those who *sometimes enjoy sunshine* and particularly those who *enjoy sunshine*. In summary, there was trend towards higher 25(OH)D status with an increase in sun enjoyment, ambient D-UVB dose and supplementation.

5.5.5 Association between 25(OH)D, cw-D-UVB and VDscores

Currently, there are various methods used in order to estimate vitamin D status, including, 25(OH)D concentration measurement, dietary vitamin D estimation through food frequency questionnaires [136], self-reported sun exposure, supplementation use, estimation of ambient UVB through latitude, season or satellite measurements, and estimation of personal exposure through time spent outdoors or sun burn occasions. These all contain their own advantages and drawbacks [137]. It has been previously highlighted that using any of these measures individually could lead to misinformation about the overall vitamin D status of individuals. A simple vitamin D score which incorporates individually calculated ambient D-UVB, along with other important vitamin D related variables, was created in order to approximate, the overall

vitamin D status in the body. In doing this three vitamin D scoring systems were created, as described earlier. Previous studies have used similar techniques to develop scoring systems examining the risk of various conditions [262, 283]. Furthermore, 25(OH)D has been previously predicated using a similar method of estimation coefficients from a regression model [121].

Other important vitamin D related variables such as sun holiday in the last 6 months or sun protection use were not included in this score for a number of reasons. For example, no information was available about the length and location of the sun holiday and inclusion of this variable could have added heterogeneity in to the score. Similarly, although participants were asked about sun protection, no information about how often this was applied, where on the body it was applied and what sun protection factor (SPF) was used and again this may have added heterogeneity to the score if included. There were also a number of individuals with missing information on these variables and inclusion would have reduced the sample size available for this cohort. Additionally, the contribution of these variables to 25(OH)D has not been as well investigated in previous literature.

In chapter four, it was determined that VDscore1 marginally improved the prediction of 25(OH)D groups. This chapter aimed to build upon the vitamin D score which was created in chapter four and to investigate this score, and its performance when predicting deficiency in a larger cohort. This was investigated using linear models and random forest prediction. In order to determine the best fit model, r^2 values were compared. A model containing supplementation (in addition to age, gender, smoking status, cohort type, sun holiday, BMI and oily fish consumption) was found to have an r^2 value of 0.34. This was improved when cw-D-UVB was added to the model (r^2=0.37). Similar improvement was also observed in the ROC curves; an increase from 61.8% to 71.8% in prediction ability of deficiency was observed when supplementation was added to the baseline model (age, gender, smoking status, cohort, sun holiday, BMI), a further increase to 77.1% was observed when both supplementation and cw-D-UVB was added to the baseline model. This demonstrates that the addition of cw-D-UVB information to the model improves it, albeit marginally, as was shown in chapter four. Next, VDscores were investigated. An improvement was found using the VDscores compared to the baseline model (model 1). However, no improvement was made to the model containing both cw-D-UVB and supplementation as separate variables (cw-D-UVB & supplementation r^2=0.37, VDscore1, r^2=0.36, VDscore2, r^2=0.37, VDscore3, r^2=0.37). Similar results were found when ROC curves were investigated. The addition of cw-D-UVB dose alone or incorporated VDscores increased the AUC by a similar amount. Similarly, it was also noted that the variance explained by the variables within the models were similar for VDscores and for cw-D-UVB+

supplementation (cw-D-UVB + supplementation: 3.6% and 20%; VDscore2: 21% and VDscore3: 22%). As the improvement using VDscores is quite small in comparison to using the individual variables, one might wonder why this scoring system is necessary at all.

One of the main drivers to create a vitamin D score was simplicity. Scoring systems have been used in multiple areas of health and have been found to be beneficial due to their simplicity. For example the BMI score. This score is not the most effective measure of obesity as it is unable to take into account muscle mass. Other measures such as visceral fat area or waist circumference have been shown to be more accurate measures of obesity [284]. However, this score is still clinically used and well understood by the general population. This demonstrates that although simple scores may not achieve the most accurate results, they still have purpose. Another example is "Life's simple 7 score". This scoring system was developed as a simple measure to assess risk of a cardiovascular event in individuals [283]. The questions incorporated into this score are simple and the outcomes of this score are not the most accurate measure of a risk of cardiovascular event. However, this score has been used in multiple studies [285-287], was developed and advertised by the American Heart Association and is used to assess a person's risk of a cardiovascular event in a simple manner. This is a similar approach to what was undertaken in this thesis, as it aimed to create a simple vitamin D estimate which could incorporate multiple sources of vitamin D into one estimate. Although predictive ability was similar when vitamin D variables were included in a model separately and when they were included combined in a VDscore using VDscores, allows an individual to be ranked in a population based on their vitamin D status therefore identifying those at highest risk of deficiency in a simple manner. This would not be possible without a vitamin D score: individuals could be ranked based on information from all relevant factors separately, but this would result in a number of different ranks per person which could become complicated and unfeasible in a research setting. Using supplementation alone to rank individuals is possible but as mentioned previously, prediction was better with the inclusion of other vitamin D related variables. In addition, this approach would be limited in a population with low prevalence of supplementation. In contrast to this, in a population with stronger UVB radiation, one might expect greater contribution of UVB in predictive models.

Similar associations between 25(OH)D and VDscores were observed. However, there was a slight improvement in the model when VDscore2 and VDscore3 were used compared to VDscore1. The r^2 was increased by one (from 0.36 to 0.37) and the relative importance of each variable was increased from 21% using VDscore1 to 22% using VDscore2 and VDscore3. Furthermore, from the ROC curves better prediction using VDscore2 and VDscore3 than

VDscore1 was observed. This is most likely because, although VDscore1 contains information on two of the most important sources of vitamin D, it fails to adjust for utilisation of UVB and dietary sources of vitamin D, which also impact upon 25(OH)D concentration.

A linear correlation found between VDscore2 and VDscore3, demonstrating that these two VDscores were closely related to one another, as expected. Additionally, it was observed that as VDscore2 scores increased, mean 25(OH)D also incrementally increased. This was observed in the entire cohort and when stratified by sub-cohort type and sex. This trend was very similar to what was observed for cw-D-UVB, supplementation and sun enjoyment.

Similar scoring systems have been carried out in the past, for example Deschasaux *et al.*, included physical activity, BMI and skin type in a similar scoring system to estimate vitamin D insufficiency in a scoring model which was created using multivariable logistic regression [288]. This study, which was carried out in France, also found strong associations with 25(OH)D in two separate cohorts [288]. The Vitamin D scoring system used here gives a greater overview and more comprehensive portrayal of a person's vitamin D status compared to the method employed by Deschasaux *et al.*, as it uses a superior estimate of ambient D-UVB. Additionally, Deschasaux's research also failed to take into account supplementation or any dietary sources of vitamin D [255]. The scoring models (VDscore2, VDscore3), were not adjusted for skin colour as individuals in this cohort were only recruited if they had ethnically Irish parents (the majority of which are shown to have skin types I and II) [289].

Contrary to what was carried out by Deschasaux *et al.*, Bertrand *et al*., used UV flux in addition to other determinants of 25(OH)D such as dietary and supplemental vitamin D to determine a predicted 25(OH)D concentration in a study in the USA. This annual average UV flux took into account latitude, altitude and cloud cover, however it fails to adjust for ozone column [266]. Other studies have also employed a similar method [290, 291], however these too have also failed to account for various factors which influence UVB dose estimate or use imprecise measurements to account for UVB.

Furthermore, it has been shown that when variables which are used in the initial linear model to *predict* vitamin D status are also associated with a certain health outcome which is being investigated, the confounding relationship between the variable and the health outcome can significantly bias the relationship between the *predicted* 25(OH)D concentration and the health outcome [163]. For example, there is a strong relationship between BMI and risk of colorectal cancer. If perhaps BMI was used to create a "predicted 25(OH)D" and this "predicted 25(OH)D" was used to examine the association between vitamin D and colorectal cancer, the existing relationship between BMI and colorectal cancer could bias the results. Physical activity, which

was also used in the scoring system outlined by Deschasaux *et al.,* could also be considered a confounder as outlined above, as physical activity has also been shown to be associated with some health outcomes e.g. cardiovascular disease.

As such, the inclusion of variables which could be related to both health outcomes (e.g. age, physical activity, BMI) and 25(OH)D were not included in the scores, this was to ensure that the vitamin D scoring systems could potentially be used not only to classify deficiency in individuals but also for use as a proxy when investigating associations with health outcomes, when 25(OH)D is unavailable or in addition to it.

Moreover, physical activity may have been used by Deschasaux *et al.* as a proxy measure for time spent outdoors, due to the link between physical activity and outdoor activity. This study used sun enjoyment as this is specific variable that captures the tendency for sun exposure, rather than sun exposure, that may occur consequently as a result of physical activity. The above points highlight some of the strengths of the vitamin D scoring systems created in this thesis and how these compare to others which have been developed previously.

5.5.6 Associations between Vitamin D Estimates and Personal Characteristics

When examining relationship between 25(OH)D, cw-D-UVB, VDscore1, VDscore2 or VDscore3 and personal characteristics in an older adult cohort, similar results were found. For example, decreasing BMI, increasing age and sun holidays in the last six months were all associated with increasing 25(OH)D, VDscore1, VDscore2, VDscore3 status or cw-D-UVB dose.

It might seem unusual that the vitamin D scoring systems are also associated with personal factors. This relationship between VDscores and personal characteristics such as BMI, might be in fact an example of a vitamin D causal pathway. As it has been shown that the VDscore1, VDscore2 and VDscore3 are strongly related to 25(OH)D concentration and many studies have previously shown that 25(OH)D concentration is strongly related to BMI, and then this could suggest that the VDscores are at the start of a causal pathway between 25(OH)D and BMI. This theory would mean that as VDscore2 determines 25(OH)D status, and as 25(OH)D is (causally) related to BMI, this is also the reason why there is an (indirect) relationship between VDscores and BMI.

There were also some variables which were not associated with VDscores in the same manner as 25(OH)D was. For example, differences were found in the relationship between cw-D-UVB and sun protection, when compared to other vitamin D estimates. The discrepancies found

between the variables may be due to other confounding variables. For example, those who always used sun protection were found to have less cw-D-UVB compared to those who rarely used sun protection. However those who always used sun protection were found to have increased 25(OH)D, VDscore2 and VDscore3 compared to those who rarely used it. This may be because VDscores take into account enjoyment of the sun as a factor, and perhaps those who always used sun protection were in fact those who enjoyed the sun more, and therefore had higher VDscores. This may also be the case for 25(OH)D concentration as those who had higher 25(OH)D concentrations not only always used sun protection but also spent more time outdoors or spent time abroad. This may also be the reason why no association was found for VDscore1, as this variable does not take sun enjoyment into account. Therefore discrepancies may occur between the estimates due to some other confounding variables, however, the majority of the associations for all estimates were found to be similar.

5.5.7 Sufficiency and Deficiency Prediction using Vitamin D Estimates

It was hypothesised that the addition of the ambient cw-D-UVB or VDscore variables may improve the prediction of 25(OH)D deficiency. A substantial improvement in prediction of 25(OH)D deficient individuals was found with the inclusion of cw-D-UVB, VDscore1, VDscore2 or VDscore3 compared to baseline. There was similar improvement in prediction observed when supplementation + cw-D-UVB, were added into the model individually and when these variables were incorporated as part of VDscore1, VDscore2 and VDscore3. Therefore the hierarchical way in which VDscore1 and VDscore2 was developed, and the mathematical way in which VDscore3 did not decrease the prediction ability of the model, and in fact increased its usability as it allowed individuals to be ranked based on a number of important vitamin D related variables rather than just by individual factors.

5.5.8 VDscore2 vs VDscore3.

When examining the difference between VDscore2 and VDscore3, it was noted that there was little difference between the models. However, when comparing VDscore2 and VDscore3, it can be observed that VDscore2 underestimated the contribution of supplement use and cw-D-UVB in this cohort but overestimated the contribution of sun enjoyment and oily fish consumption. This is a limitation of this simple approach, however, the over and under adjustments made

were small. e.g.: a value of +32 was given in VDscore3 model if a participant takes supplements, while this was +24 in the VDscore2 model, similarly +13 was given in VDscore3 for those who belonged to quartile four of cw-D-UVB dose while +18 was given in VDscore2. One important advantage of this hierarchical model is its simplicity.

Furthermore, very little difference was found when examining the association and prediction of 25(OH)D between two of the vitamin D scoring methods. Both VDscore2 and VDscore3 showed an improvement in prediction compared to baseline model, and similar predictive ability was observed. Both VDscore models were found to be important predictors of vitamin D deficiency and sufficiency, with both VDscore2 and VDscore3 ranking as the most important variable for predicting deficiency.

The method of categorically assigning numbers to participants based on their variables as was done in VDscore2 without prior knowledge of their relationship to 25(OH)D could be considered simpler than the regression coefficient method used in VDscore3 and in some other studies. This is because the contribution of determinants to vitamin D status can vary depending on the cohort used; therefore VDscore2 could be a more versatile approach, as it can be used with any cohort without having to be modified depending on the cohort. Additionally, this method is extremely simple. This model was created in a hierarchical fashion, whereby participants were first classified according to supplementation, which has been found to be the most important determinant of vitamin D status in individuals living at high latitudes. This type of hierarchical classification was continued for other important vitamin D related variables so that individuals could be ranked in the population based on their vitamin D status. This score is also quantifiable so that it can be easily interpreted. For example, in this study those with a VDscore2 score of nine or less could be at risk of deficiency as the median 25(OH)D status within categories 1-9 were <40 nmol/L. The argument of simplicity vs accuracy is an important one and should be addressed on a case by case basis, however, there was a good deal of correlation between the two estimates and accuracy was not disregarded by using VDscore2.

5.5.9 Implications of this Research

Firstly, ambient D-UVB was found to be associated with 25(OH)D status in a large and older cohort. This suggests that the contribution of ambient D-UVB is still an important variable even in a high latitude country and in those over 60y. This result further strengthens the results found in chapter four as it demonstrates that cw-D-UVB is associated with 25(OH)D in a cohort where

the ability for cutaneous synthesis of vitamin D is reduced. Therefore, if cw-D-UVB is still associated with 25(OH)D in two groups, those who do not rely on cutaneous synthesis of vitamin D or those who's ability to synthesise vitamin D is impaired (older individuals), one could expect that cw-D-UVB would be associated with 25(OH)D in most other groups, and potentially to a stronger degree.

Secondly, by combining ambient D-UVB at place of residence and other vitamin related exposures such as sun enjoyment, supplementation, and oily fish, a vitamin D scoring system can be developed which is strongly associated with 25(OH)D (1.03 nmol/L increase in 25(OH)D for every increase in VDscore2). This newly developed scoring system was also found to be an important variable when predicting vitamin D deficiency. This vitamin D scoring system has the potential to be used as a simple vitamin D classification tool.

This tool could be used as a simple estimate of vitamin D deficiency. This tool would be easy to develop as it used freely available and individually calculated ambient D-UVB doses. In order to calculate a vitamin D score for an individual a few questions related to residential location, dietary, supplementation and sun exposure habits would be all that is needed. This VDscore score could be useful for research purposes as it would allow an approximation of vitamin D status in large studies when it might be unfeasible to take blood measurements.

Additionally, this classification tool has the potential to be developed, replicated and finally incorporated into clinical setting. There has been a major increase in vitamin D assessments in primary care settings the last number of years, most likely to do with the increase in awareness about vitamin D deficiency and potential health benefits [292]. However, this increase in testing has also caused an increase in costs, as vitamin D testing is expensive [292]. Furthermore, it has been shown that up to 55% of those in the UK have insufficient vitamin D throughout the year, however, routine vitamin D testing is not recommended [20, 293], in part due to the costs involved. Vitamin D testing is only recommended for at risk groups, such as those over 65y. However, participants within that age group are not necessarily the only ones who are at risk of deficiency, in fact a recent study in the UK found that 22% of this population were deficient in vitamin D although the median age within in this cohort was well below 65y [294].

This demonstrates that vitamin D deficiency is an issue even in those who are not considered at risk, but vitamin D testing is not routinely carried out in this group. If this VDscore2 could be developed further into a clinical tool, it could alleviate this issue with cost and vitamin D deficiency by identification of high risk individuals. If individuals scored low in the VDscore then this would then inform the General Practitioner or nurse if it is advisable to measure 25(OH)D status in this individual.

Personalised medicine is an important factor in health with many health professionals and professional bodies outlining the importance and benefits it can offer, not only to patients themselves, but also in terms of costs, as it allows a more targeted approach. This vitamin D classification tool would be a very simple and innovative way in which personalised care could be incorporated into general practices. This method would not only reduce costs of estimating vitamin D status by reducing unnecessary testing, but also it would allow identification of individuals who may be deficient but not be tested as they wouldn't qualify to be an "at risk" individual.

Another benefit of this classification tool is that it can take into account regional D-UVB differences. For instance, this tool could be used by individuals around the country, but differing results would be given depending on the location of the individual. As those who live in more northerly areas such as Northern Ireland or Scotland are more at risk of deficiency than those who live in areas further south in Ireland and the UK, this method can take this into account.

However, this system would first need to be examined more closely and replicated in a larger cohort before such as classification tool could be implemented.

5.5.10 Strengths and Limitations

The quality of the UVB source used in this study was one of the main strengths of this study. This was a comprehensive dataset which contained information on D-UVB doses every day in multiple locations over the last ten years. This measurement was also adjusted for multiple factors which can impact UVB dose. Furthermore, this dataset restricted wavelengths to only those which are appropriate for vitamin D synthesis. Additionally, the individual calculation of cw-D-UVB based on place of residence and date of blood draw, that accounts for accumulation and diminution of vitamin D in the body offers critical improvements over similar studies [6, 264-267]. The 25(OH)D measurements used in this study were measured using the "gold standard" method of measurement, LC-MS/MS. This was another strength of this study and the quality of data used. Furthermore, only individuals with ethnically Irish parents were recruited and as the majority of Irish people are shown to have skin types I and II [289]. This means that this cohort was homogeneous in terms of skin colour. This is important because those with darker skin are known to have a reduced ability for cutaneous skin synthesis. Further strengths include a large and well-characterised cohort with information on multiple aspects of health.

The lack of other age groups in this study is a limitation. As this cohort consisted of only those aged over 60, this limits the generalisability of the study as it is not known if similar results would be found in a cohort of all ages. However, it has also been shown that the ability for vitamin D synthesis by UV production is reduced as one ages. Therefore, to find such strong associations between UVB and 25(OH)D within an older cohort, with limited ability to synthesise vitamin D, suggests that this association could be even stronger in younger individuals with superior ability for vitamin D synthesis.

Another important limitation of this cohort is that it was made up of three distinct sub-cohorts of older individuals with cognitive disorders, osteoporosis and HBP. There were some differences between in baseline characteristics and this should be taken into account when interpreting results. However, trends for increasing median 25(OH)D with an increase in VDscores were consistent across cohorts. This is an important limitation of this study as it limits the generalisability of the results. These conditions are common in the general population, however, this cohort is does not include healthy individuals, and therefore, it might not be accurately representative of the general population of individuals aged over 60 years. These underlying conditions may have modified results as it is unknown if individuals with these specific condition have a different relationship with vitamin D or UVB than the general population. In particular, osteoporosis has been linked with low vitamin D levels. These individuals would have been diagnosed with osteoporosis and most likely been tested and monitored for vitamin D deficiency, and prescribed supplements. Therefore, these individuals could be expected to have higher 25(OH)D concentrations than members of the general population who would not have been as closely monitored for vitamin D deficiency. Indeed, the median 25(OH)D concentration within this sub-cohort was 76.7 nmol/L which was much higher than the median 25(OH)D which has been reported for other Irish studies. For example, the TILDA study, which is a study of older healthy adults aged over 60 in Ireland found a median 25(OH)D of 51.3 nmol/L [274]. Therefore, the osteoporosis sub-cohort might have contributed to an underestimation of vitamin D deficiency prevalence in this study, this could be observed as cohort type was found to be the most important variable when classifying sufficient individuals. Furthermore, the success of the vitamin D score may have been hampered by the impact of supplementation in this cohort. Supplementation was by far the most important variable in predicting 25(OH)D concentration and as such the impact of other variables included in this score did not measure up to the importance of supplementation. However, this high impact of supplementation may have been due to inclusion of a cohort, where monitoring of vitamin D status was employed. This could have led to an overestimation of supplementation in

the cohort. This high level of supplementation, could have had an impact on the performance of the vitamin D score and in other locations where D-UVB doses are higher, or the impact of supplementation is not as great, the addition of D-UVB doses may have resulted in a better prediction ability, than what was shown here. Although supplementation will always be an important source of vitamin D in the population, perhaps in a cohort where vitamin D monitoring is not occurring, other factors may have more impact on 25(OH)D concentration. Therefore, further research is needed in a population representative of the general population.

Those with dementia and cardiovascular diseases have been shown to have a higher risk of vitamin D deficiency [3, 295]. However, the median 25(OH)D concentration of these sub-cohorts was similar to what has been previously reported in healthy adults in Ireland (median 25(OH)D Cognitive: 50.7 nmol/L, HBP: 45.7 nmol/L). Furthermore, the overall median 25(OH)D within this cohort was found to be similar to levels found in other healthy Irish cohorts [271-274]. In conclusion, there is an issue with generalisability of these findings, and further research needs to be conducted in a large cohort representative of the general population before conclusions are made.

The aim of this chapter was to build upon the previous simple vitamin D estimate which was created in chapter four so that the population could be ranked in terms of their vitamin D status. It was important to maintain the simplicity of the score but it was also necessary to try incorporate the main three sources of vitamin D (D-UVB, diet and supplementation) and take into account utilisation of D-UVB dose. This was carried out using a hierarchical approach, whereby the most important variable was first selected and assigned certain values and then the next most important variable was selected and so on until all important variables were included in the model. This approach has many merits, particularly in its simplicity. This approach combines all sources of vitamin D into one simple estimate, which could allow the a broad estimation of a person's vitamin D rank within a population or the calculation of a threshold value by which participants may be at risk of deficiency. This would not be possible if numerous variables were examined separately, as each individual would have a different rank depending on the variable and their overall vitamin D rank would not be possible to calculate. However, this approach also has a number of flaws. For example, a number of assumptions are made in the creation of a VDscore score. It has been demonstrated that supplementation is the most important source of vitamin D in this population and when examining the regression coefficients it was found that use of supplementation increased 25(OH)D concentration by 32 nmol/L. However, in VDscore2, an increase of 24 nmol/L was assigned to supplementation use. The fact that this simple approach does not equate exactly with the regression coefficients is a

limitation of this study. However, as mentioned previously, the aim of this chapter was to build upon and create a simple vitamin D estimate, and although it might not capture the exact contribution of each variable to the overall 25(OH)D concentration. However, it is almost impossible to achieve this due to the number of factors which can contribute to personal 25(OH)D dose e.g.: time spent outdoors, sun holiday frequency, sun protection use, clothing coverage, and use of fortified products.

Another limitations of these VDscores is that it is assumed that the relationship between supplementation, D-UVB and oily fish consumption is the same throughout the year and at all locations. This might not be the case as supplementation or dietary sources may have a greater contribution to 25(OH)D concentration at some times of the year or in different locations, depending on cohort being examined. Furthermore, this score is limited in that it has only been examined in an Irish cohort and perhaps the contribution of each of the variables associated with 25(OH)D dose is considerably different in areas where there is higher D-UVB exposure, different dietary patterns or where fortification of products is much more commonplace. For example, areas of Norway have been shown to have high 25(OH)D concentrations, despite their high latitude and low UVB doses. This is due to their high consumption of fish [255] and perhaps this high consumption of oily fish contributes more to their 25(OH)D concentrations than supplementation or D-UVB concentration, something which is not captured in the current VDscore. However, it was not possible to examine this relationship as only Irish cohorts were available for this thesis.

It is important to acknowledge the limitations of this vitamin D estimate. However, it was the best simple estimate which could have been achieved in a cohort of northerly residing participants.

There are also additional limitations to this study, firstly, there was some missing data in this cohort and as such, in order to maximise sample sizes some variables were not included in the analysis. Secondly, self-reporting sun enjoyment, should be noted. The fact that sun enjoyment was taken as a proxy of utilisation of UVB should be considered. Furthermore, other personal factors that affect skin synthesis, such as: time spent outdoors, clothing choices, or angle of exposed skin to the sun rays, were not adjusted for, however these variables are largely impossible to capture in free living individuals over a prolonged period. This cohort was also lacking in information on non-supplementary dietary vitamin D intake, such as meat consumption which has been shown to be an important source of dietary vitamin D in Ireland [296].

5.6 Conclusion

This study highlights how ambient UVB and personal sun exposure preferences can have a strong affect 25(OH)D concentration, even in older adults. It also suggests that combining ambient D-UVB, sun enjoyment variables, vitamin D supplementation and oily fish consumption to develop a simple vitamin D score can potentially be used to classify a participants into whether or not they have a high risk of being vitamin D deficient, which could have important implications.

6 UVB, VDscores and Upper Gastrointestinal Cancer in a Large UK Cohort

6.1 Aim

The primary aim of this chapter was two-fold. Firstly, this chapter aimed to investigate the association between two vitamin D exposures and the risk of upper gastrointestinal cancer in a UK cohort. A nested case control study design was used to examine this hypothesis.

Secondly this chapter aimed to investigate the association between two vitamin D exposures and the survival of oesophageal and gastric cancer in UK cohort.

Secondary analysis examining the interaction between vitamin D related variables and vitamin D exposures on the risk of upper gastrointestinal cancer was also conducted.

6.2 Introduction

The prevalence and risk factors associated with oesophageal and gastric cancers have already been discussed in detail in chapter one. The survival rates of these cancers are poor and the incidences of upper gastrointestinal cancer are increasing in some parts of the world [164]. Therefore, more research needs to be carried out in order to determine risk factors, and determinants which impact disease incidence and mortality rates. This way the best practices in preventing or reducing risk in these diseases can be formed. This thesis has previously outlined the limited amount of research which has been carried out to assess the association between vitamin D and oesophageal and gastric cancer. These studies reported differing results, depending on population studied and vitamin D estimate used. Moreover, the previous studies examining the relationship between vitamin D and oesophageal and gastric cancer survival are also lacking, with only one study examining the relationship for gastric cancer alone.

In addition, many of these studies had design flaws associated with them, which have been mentioned previously. Furthermore, the majority of these studies were carried out in Chinese populations with a large proportion of them stemming from the “Linxian region” of China, which is known to have some of the highest rates of oesophageal cancer in the world, perhaps due to the exposure to coal, which is widely used in the region. Vitamin D has been proposed as having a role in the metabolism of polycyclic aromatic hydrocarbons, which are found in coal [202].

Therefore, it is possible that a population specific effect of the risk of oesophageal and gastric cancer exists for this region and is reflected in these studies [8, 9, 202, 211].

In this chapter D-UVB dose and vitamin D scores will be used to investigate the relationship between vitamin D and the risk and survival of oesophageal and gastric cancer. This will be carried out using the UK Biobank Cohort. This is a very large (n=500,000), well characterised and detailed cohort with information on many aspects of health and lifestyle.

6.3 Methods

6.3.1 Study Participants

Data from this study was collected for UK biobank which is a data set of 500,000 community dwelling individuals across England, Scotland and Wales [297]. These participants were recruited from 2006-2010. A number of different methods were used for data collection including a self-completed questionnaire, a computer assisted interview, physical and function measures along with blood, urine and saliva collection [298].

Participants filled in a number of questionnaires, and provided information on socio-demographic characteristics and lifestyle; the variables which were used in this study as well as the categorical response options are given in **Table 6.1.** Information about participants' health was collected. Self-reported presence of different oesophageal or gastric problems was assessed, including: gastro-oesophageal reflux, oesophagitis/Barrett's oesophagus or gastric ulcer. Self-reported information on other conditions, such as osteoporosis, cardiovascular conditions, diabetes etc. was also collected. Cardiovascular condition was defined by previous history of hypertension, heart/cardiac problem, heart attack or heart failure.

Participants had multiple phenotypes measured, such as height and weight. These were used to calculate BMI. W.H.O. classification was used for categorisation into underweight (<18.5), normal weight (18.5-24.99), overweight (25-30) and obese (>30). Further detail about the cohort can be found elsewhere [297-299].

Ethical approval for this study, including secondary data analysis was received by the UK biobank and all participants gave written informed consent [300]. The analysis in this study was conducted under application number 1265. A subset for which the vitamin D scores could be calculated was selected. These were individuals who had completed information on their residential location, oily fish consumption, average time spent outdoors and vitamin D supplementation. The participant's ages ranged from 40-69 years. In order to explore the relationship between D-UVB, Vitamin D scores and the development and survival of oesophageal and gastric cancer a subset of individuals from this cohort who had been diagnosed with upper gastrointestinal cancer were selected. This information was gathered via linkage to the national cancer registry, which registers and collects data on all cancers and provides detailed information on ICD-10-CM diagnosis codes. There were 857 primary upper gastrointestinal cancer cases. These consisted of 235 cases diagnosed before the interview and 622 diagnosed after the interview (**Figure 6.1**). ICD-10-CM diagnosis codes were used to identify cancer location: broadly, cases with ICD-10-CM codes C15.0-C15.9 were classed as oesophageal

cancer cases and C16.0-C16.9 were classed as gastric cancer cases. C15.3/15.4 denoted upper and middle thirds of the oesophagus (typical location for squamous cell carcinoma) and C15.5 denoted lower third (typical location for adenocarcinoma) [301]. Tumour histological information, from the national cancer registry was also used to determine oesophageal cancer subtypes (adenocarcinoma and squamous cell carcinoma). Cancer stage was not available for this cohort at this time. Therefore, no adjustment for cancer stage was made, which would have had a major impact on survival analysis.

Analysis was carried out on two types of upper gastrointestinal cancer and some subtypes of these e.g.: Oesophageal cancer, oesophageal adenocarcinoma, oesophageal SCC and gastric cancer. As oesophageal and gastric cancer have similar risk factors, such as obesity, acid reflux, smoking, age, gender, alcohol use and a diet low in fruit vegetables and high fibre foods, it was decided to analysis the risk and survival of these cancers both together and separately as has been done previously in numerous studies [8, 302-304]. Finally, 25(OH)D concentration was not available for this cohort.

Table 6.1: Information about variables included in this analysis.

Variable Name	Categorical Variable Response
Age at biobank attendance	Continuous variable
Sex	Male/Female
Qualifications	Coded as numeric as follows: 1. None of the above, 2. Certificate of Secondary Education or ordinary level general certificate of education 3. Advanced level general certificate of education 4. National Vocational Qualification or Higher National Diploma/Certificate 5. Other professional qualifications 6. College or university degree
Smoking	current/previous/ never
Alcohol consumption	current/previous/ never
vitamin D supplement use	Yes/No Derived from variable containing information about any supplement or mineral use
Supplement use 24hrs prior to interview	Yes/No Derived from variable containing information about dietary habits 24hours prior to biobank interview
Oily Fish consumption	Derived from variable containing information about the frequency of oily fish consumption. Possible responses were as follows: 1. Never 2. Less than once a week 3. Once a week 4. 2-4 times a week 5. 5-6 times a were 6. Once or more daily 7. Do not know 8. Prefer not to answer This variable was coded in two ways:
	Binary coding: Yes/No Never was coded as "No", Prefer not to answer was coded as "NA" and everything else was coded as "Yes". This variable was used for VDscore4 development
	Frequency coding 1. Less than once a week: "low consumption, 2. 1-4 times a week :"intermediate consumption" 3. 5-6 times per week or greater: "high consumption"
Ease of tanning	Possible answers: 1. Get very tanned 2. Get moderately tanned 3. Get mildly or occasionally tanned 4. Never tan, only burn 5. Do not know

	6. Prefer not to answer Those who answered “do not know” or “prefer not to answer” were coded as “NA”
Skin colour	Possible answers: 1. Black 2. Brown 3. Dark olive 4. Light olive 5. Fair 6. Very fair 7. Do not know 8. Prefer not to answer Those who answered “do not know” or “prefer not to answer” were coded as “NA”
Use of sun protection	Possible answers: 1. Never/rarely 2. Sometimes 3. Most of the time 4. Always 5. Do not go out in sunshine 6. Do not know 7. Prefer not to answer Those who answered “do not know” or “prefer not to answer” were coded as “NA”
Time spent outdoors	This variable was derived from two variables “average number of hours/ day outdoors in the summer” and “average number of hours/ day outdoors in the winter” and was coded as follows: 1. 0-2 hrs/day: Low 2. 2-5 hrs/day: Intermediate 3. >5 hrs/day: High
BMI	This was coded as per WHO recommendations 1. <18.5: Underweight 2. 18.6-24.9: Normal weight 3. 25-29.9: Overweight 4. 30-39.9: Obese
Overall health rating	Self-reported; Possible answers: 1. Poor 2. Fair 3. Good 4. Excellent 5. Do not know
Osteoporosis	Yes/No Derived from list of self-reported non-cancer illnesses
Oesophagitis/Barrett’s oesophagus	Yes/No Derived from list of self-reported non-cancer illnesses
Gastric ulcer	Yes/No Derived from list of self-reported non-cancer illnesses
Gastro-oesophageal reflux	Yes/No Derived from list of self-reported non-cancer illnesses

Cardiovascular disease	Yes/No Derived from list of self-reported non-cancer illnesses Coded yes if one if participant mentioned a previous history of 1. hypertension 2. heart/cardiac problem 3. heart attack 4. heart failure

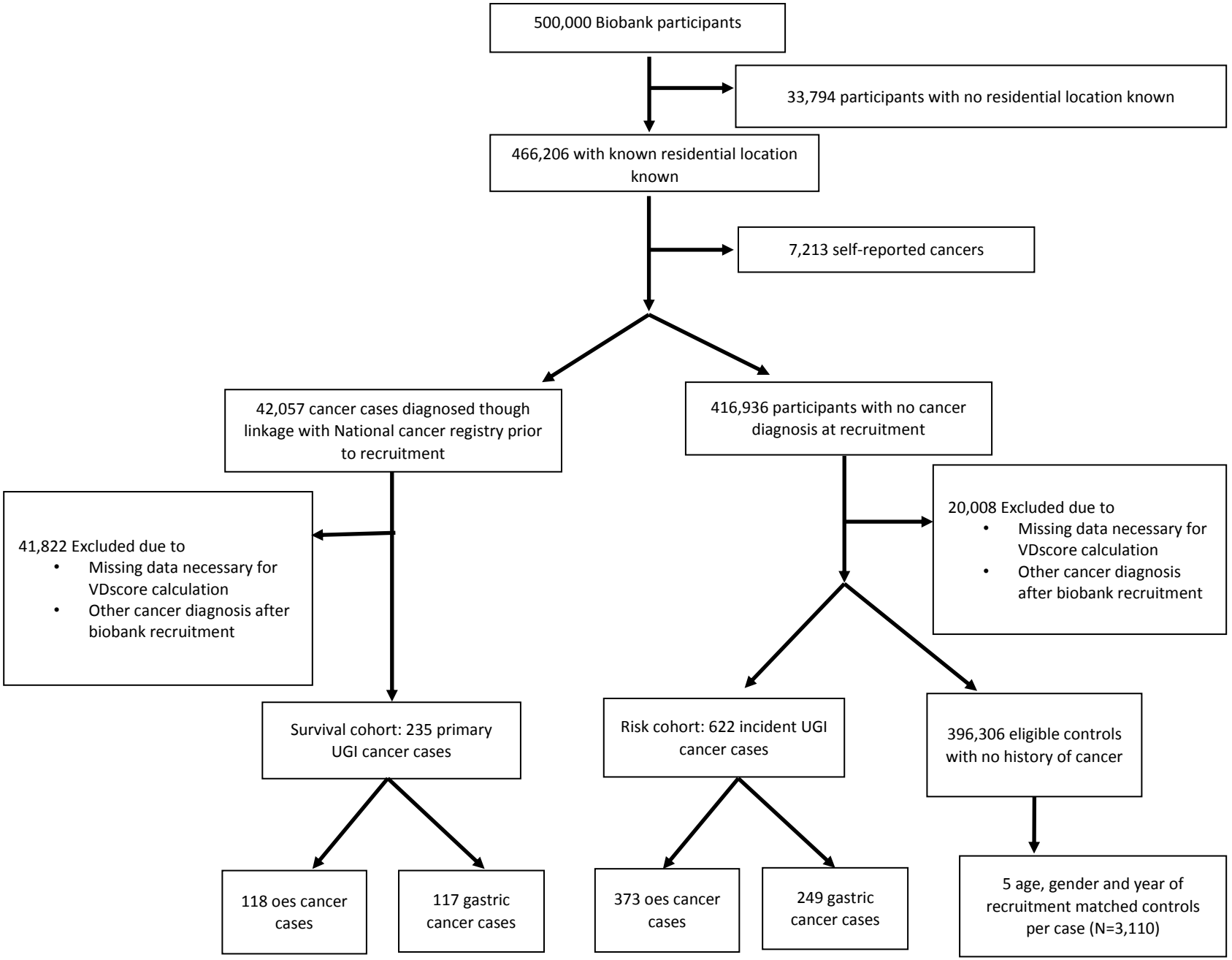

Figure 6.1: Flow chart of survival and risk cohorts selection from UK Biobank cohort.

6.3.1.1 Risk analysis cohort

Odds of developing oesophageal and gastric cancer was assessed using a nested case-control study. Individuals who were diagnosed with a primary oesophageal or gastric cancer after their attendance at biobank (622 incident cases) and had never been diagnosed with a previous cancer were selected. Median time from biobank attendance to upper gastrointestinal cancer diagnosis was 3.09 years. The distribution of time between attendance and cancer diagnosis is shown in **Figure 6.2**.

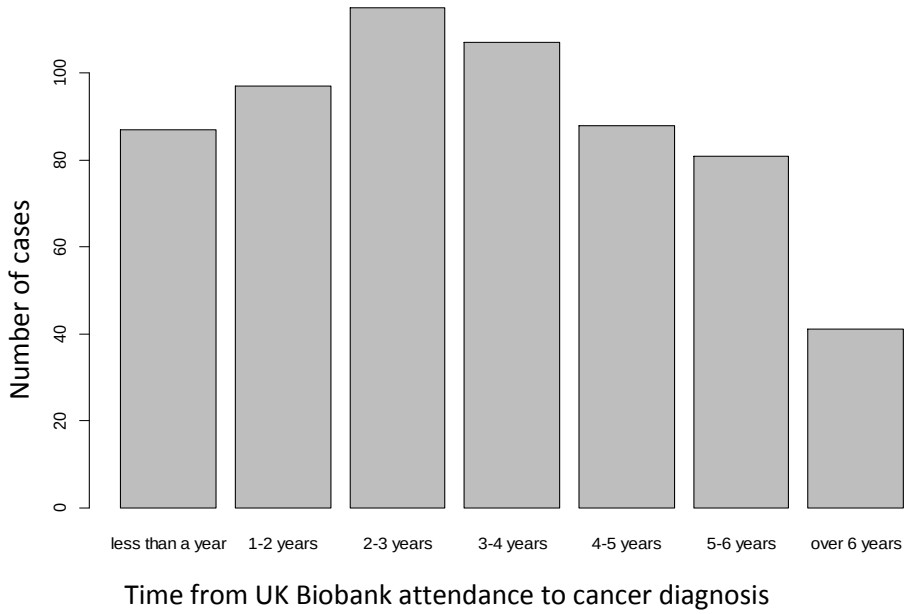

Figure 6.2 Distribution of cases diagnosis used in risk analysis from date of attendance at UK Biobank.

6.3.1.2 Survival analysis cohort

Individuals with an oesophageal or gastric cancer diagnosis prior to biobank recruitment (date of cancer diagnosis recorded from national cancer registry) were selected for survival analysis. Cox-proportional hazard analysis was used to determine an association between D-UVB or vitamin D score and survival of oesophageal and gastric cancer. Kaplan-Meier curves were also constructed. The analysis was carried out in those who received a primary oesophageal or gastric cancer diagnosis prior to their biobank attendance. Incident cancer cases which were diagnosed after biobank attendance and were used in the risk cohort and were not included in the survival analysis due to insufficient follow up information. Some important characteristics of cancer were missing from this analysis, most notably, cancer stage which is known to have a large influence on cancer mortality, therefore, results should be interpreted with care. Median time from cancer diagnosis to biobank interview was 3.5 years. The distribution of time between cancer diagnosis and attendance is shown in **Figure 6.3**.

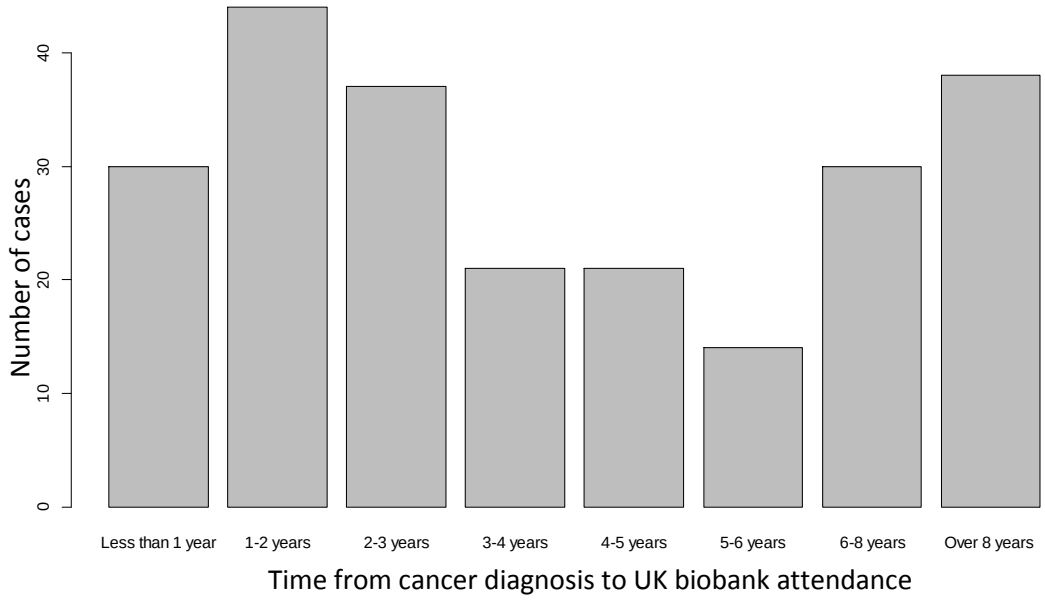

Figure 6.3: Distribution of date of diagnosis in cases used in survival analysis to date of attendance at UK Biobank.

6.3.2 UVB Source

UV dose data from the TEMIS database as described earlier was used in this analysis. Daily D-UVB estimates from the 211 grids that cover the UK, from July 2005 to June 2016 were utilised.

6.3.3 Annual D-UVB Calculation

As cancer is a slowly developing disease, a one-time measure of 25(OH)D or a seasonally biased D-UVB measurement such as cw-D-UVB, may not accurately estimate of an individual's vitamin D status over a long period of time and could easily be confounded by time of year. Therefore, annual D-UVB was calculated to give an estimation of vitamin D in this cohort over a 1 year period. This was calculated for each individual in the Biobank cohort (n=466,206). Residential location was used and daily D-UVB doses at these locations were collected for the 365 days preceding the date of attendance at the UK Biobank. These individual daily estimates were then summed to give an annual D-UVB dose. Annual ambient D-UVB dose is by definition strongly correlated with mean daily D-UVB dose.

6.3.4 Vitamin D Scoring Calculation Method four (VDscore4)

VDscore4 was then calculated in a similar manner to VDscore2, as described in Chapter 5. However, in order to create a non-seasonally biased vitamin D score, annual D-UVB rather than cw-D-UVB was used in its calculation.

As it concluded from association analysis in chapter four and five, that supplementation was the most important factor in determining 25(OH)D concentrations the cohort was first split into those who supplemented with vitamin D and those who did not. These two groups were then further split based on which quartiles of annual D-UVB [quartile one to four] individuals belonged too. These eight separate groups were further divided into 24 groups, by splitting each by the average time spent outdoors [low, medium and high]. Finally, the 24 groups were split into 48 using oily fish consumption [yes vs no]. This resulted in 48 individual categories based capturing information on vitamin D exposures (**Table 6.2**.)

Once categories were defined, a number between one and 48 was assigned to each individual based on their characteristics. Number one consisted of those who do not take supplements, are in the lowest annual D-UVB quartile, have low sun exposure and no oily fish consumption while those with number 48 were taking vitamin D supplements, had the highest annual D-UVB quartile, and had high sun exposure and ate oily fish.

Table 6.2: VDscore4 allocation based on vitamin D related characteristics[1, 2].

		No Supplementation							
		Annual D-UVB Q1		Annual D-UVB Q2		Annual D-UVB Q3		Annual D-UVB Q4	
Cohort	Time spent outdoors	Oily fish		Oily fish		Oily fish		Oily fish	
		No	Yes	No	Yes	No	Yes	No	Yes
ALL	Low	1	2	7	8	13	14	19	20
	Medium	3	4	9	10	15	16	21	22
	High	5	6	11	12	17	18	23	24

		Supplementation							
		Annual D-UVB Q1		Annual D-UVB Q2		Annual D-UVB Q3		Annual D-UVB Q4	
Cohort	Time spent outdoors	Oily fish		Oily fish		Oily fish		Oily fish	
		No	Yes	No	Yes	No	Yes	No	Yes
ALL	Low	25	26	31	32	37	38	43	44
	Medium	27	28	33	34	39	40	45	46
	High	29	30	35	36	41	42	47	48

Footnote:

[1] Annual D-UVB Q1: Annual D-UVB quartile 1, Annual D-UVB Q2: Annual D-UVB quartile 2, etc.

[2] Time spent outdoors: 0-2 hrs/day represented “low” category, 2-5 hrs/day “intermediate” and >5hrs/day “high”.

6.3.5 Statistical Analysis

Firstly, the association, between vitamin D estimates (annual D-UVB and VDscore4) and the risk of upper gastrointestinal cancer occurrence was examined. Secondly, this chapter aimed to investigate the association, between vitamin D estimates (annual D-UVB and VDscore4) and upper gastrointestinal survival.

This large cohort was first described using categorical and continuous variables; categorical variables are presented as numbers and percentages, and continuous variables are presented as medians and IQRs.

Risk analysis: Conditional logistic regression was used for analysis of an association between annual D-UVB and VDscore4 and odds of developing oesophageal and gastric cancer. Odds ratios and confidence intervals were calculated based on annual D-UVB tertiles or VDscore4 tertiles (lowest as reference). Tertiles were created by splitting the data into 3 equal parts, those who were in the 0-33.2 percentile were classed as tertile 1, the 33.3-66.6 percentile were classed as tertile 2 and anything with a higher percentile was classed as tertile 3. Backwards stepwise regression was used to determine the final model. Outline of all models are shown in **Table 6.3**.

Eligible controls were selected from the pool of individuals who had never had a diagnosis of cancer (including skin cancer), either self-reported and not on the national registry or registered in the national cancer registry. All individuals in the cohort who matched in gender, year of recruitment and ± one year of age, for a given case were identified, and five were randomly chosen from that set for a given case. Recruitment for the UK biobank occurred batches over a number of years at different locations. For example the recruitment in Oxford and surrounding areas occurred from April to November 2007. Individuals were also recruited from other areas at different times e.g.: Cardiff from October 2007 to May 2008, Bristol from July 2008 to November 2009, and Sheffield from August 2009 to July 2010. These are just an example of few of the locations. Due to this recruitment procedure, exact recruitment date, i.e. month and year, (and hence follow up duration) was correlated with location. Due to this, this cohort could not be matched by exact recruitment date (month and year) as unwanted matching by UVB would have consequentiality occurred. However, matching by recruitment year was possible as this was not heavily correlated with a specific location as recruitment took place at a variety of locations during each year.

The controls were matched to cases in a 1:5 ratio. Statistical power gained from increasing the case:control ratio generally diminishes when more than four or five controls per case are used

unless there is a reasonably high correlation coefficient for exposure between cases and their matched controls (known as *Phi*), a threshold value of over 0.2 has been suggested [305]. A *Phi* value of less than 0.07 was calculated for this study and therefore five controls per case were used. Analysis was carried out for all upper gastrointestinal cancer types and subtypes unless there were less than 10 cases in a given tertile or less than 5 events, then this subtype analysis was excluded due to small numbers and lack of statistical power.

Covariates used in the final model were: smoking status, alcohol intake, BMI, oesophageal-gastric reflux, qualifications, and gastric ulcers. Other covariates (**Table 6.3**) were also considered but excluded from final model.

Survival analysis: When examining the role of vitamin D on the survival of oesophageal and gastric cancer, tertiles of annual D-UVB and VDscore4 (lowest tertile as reference) were used in the construction of Kaplan-Meier survival curves for cancer specific and all-cause mortality. Cox-proportional hazard models were used to determine hazard ratios after adjustment for important covariates. All hazard ratios were checked for proportionality through examination of Schoenfeld residuals. Backwards stepwise regression was used to determine the final model. Outline of all models are shown in **Table 6.4**. Covariates used in the final model were: age, gender, smoking status, alcohol intake, BMI, cancer type and oesophageal-gastric reflux, weight loss, skin colour, and cardiovascular condition. Cancer stage was not available for this study and therefore was not adjusted for in this regression model. Other covariates considered were; egg consumption, qualifications, use of UV protection, ease of skin tanning, vitamin D supplementation 24hrs prior to biobank interview, and presence of gastric ulcers, but were removed from the final model.

Analysis was carried out for all upper gastrointestinal cancer types and subtypes unless there were less than 10 cases in a given tertile or less than five events, then this subtype analysis was excluded due to small numbers and lack of statistical power. Death information was obtained from the UK biobank which was linked to the "National Death Registry". Cause of death was also derived from the national death registry. This was coded using ICD-10 medical codes. Those who had a primary cause of death from gastric or oesophageal cancer using the ICD1 codes were classed as "died from disease". All-cause mortality was classed as any mortality during the study period. Censoring time was calculated from date of diagnosis to date of death or date of last follow up. Death data censoring date was used for survival analysis and this was the 1st of January 2016 for participants from Wales and England, while in those from Scotland it was 31st of November 2015.

The release of 25(OH)D concentrations for this cohort was delayed by a number of months and will not become available until late 2018. Therefore, the association between 25(OH)D and the risk and survival of oesophageal and gastric cancer could not be determined.

All analyses were performed in R (R Development Core Team, 2011) and using the R-package 'Survival' (Thomas Lumley, 2015). P<0.05 was considered statistically significant.

Table 6.3: Model selection for risk of cancer analysis.

Model	R^2	AIC	BIC	Missing data
1[2]	0.04	1972	2086	206
2[3]	0.04	2023	2124	139
3[4]	0.03	2028	2112	135
4[5]	0.03	2031	2111	130
5[6]	0.03	2030	2105	130
6[7]	0.03	2067	2120	75
7[8]	0.03	2065	2114	75
8[9]	0.03	2063	2108	75
9[10]	0.03	2106	2145	41

Footnote:

1 AIC: Akaike information criterion; BIC: Bayesian information criterion

2 *Model 1* contains: egg consumption, skin colour, qualification, use of sun protection, ease of skin tanning, vitamin D supplement use 24hrs prior to interview, cardiovascular condition, gastric ulcers present, oesophageal-gastric reflux present, BMI, smoking status, alcohol consumption and osteoporosis.

3 *Model 2* contains: Model 1 minus Ease of skin tanning.

4 *Model 3* contains: Model 2 minus use of sun protection.

5 *Model 4* contains: Model 3 minus never eats eggs.

6 *Model 5* contains: Model 4 minus osteoporosis.

7 *Model 6* contains: Model 5 minus skin colour.

8 *Model 7* contains: Model 6 minus vitamin D supplement use 24hrs prior.

9 *Model 8* contains: Model 7 minus cardiovascular condition.

10 *Model 9* contains: Model 8 minus highest qualification.

Model 8 was selected as the final model and contained: smoking status, alcohol intake, BMI, qualifications, gastro-oesophageal reflux, and gastric ulcers.

Table 6.4: Model selection for survival analysis.

Model	R^2	AIC	BIC	Missing data
1[2]	0.27	528	599	25
2[3]	0.25	531	599	25
3[4]	0.23	528	589	25
4[5]	0.23	527	585	24
5[6]	0.21	530	586	24
6[7]	0.21	528	582	24
7[8]	0.21	526	578	24
8[9]	0.18	524	564	21
9[10]	0.17	531	567	12
10[11]	0.17	556	582	5

Footnote:

[1] AIC: Akaike information criterion; BIC: Bayesian information criterion

[2] *Model 1* contains: sex, age, egg consumption, skin colour, qualification, use of sun protection, ease of skin tanning, vitamin D supplement use 24hrs prior to interview, cardiovascular condition, weight loss, gastric ulcers present, oesophageal-gastric reflux present, uGI cancer type, diabetes, BMI, any oesophageal or gastric issue present, smoking status, VDscore4 and alcohol consumption.

[3] *Model 2* contains: Model 1 minus gastric ulcers.

[4] *Model 3* contains: Model 2 minus UV sun protection.

[5] *Model 4* contains: Model 3 minus egg consumption.

[6] *Model 5* contains: Model 4 minus any oesophageal or gastric issue present.

[7] *Model 6* contains: Model 5 minus diabetes.

[8] *Model 7* contains: Model 6 minus qualifications.

[9] *Model 8* contains: Model 7 minus vitamin D supplement used 24hrs prior to interview.

[10] *Model 9* contains: Model 8 minus ease of skin tanning.

[11] *Model 10* contains: Model 9 minus skin colour.

Model 10 was selected as the final model and contained: age, sex, smoking status, alcohol intake, BMI, cancer type and oesophageal-gastric reflux, weight loss, skin colour, and cardiovascular condition

6.4 Results

6.4.1 Baseline Characteristics for UK Biobank Cohort

Baseline characteristics of the UK Biobank cohort are shown in table 6.5. The majority of the cohort were found not to usually take vitamin D supplements (only 4%), and even less took vitamin D supplements 24hrs prior to the biobank interview (1% of overall cohort or <10% of those who took supplements regularly) (**Table 6.5**). It was also interesting to note that a large proportion of participants were overweight, obese or extremely obese (42%, 22% and 2%). Medium consumption of oily fish (2-4 times a week) was reasonably high (55%), and 64% of this cohort spent greater than two hours outdoors per day on average during the year. It was also noted that a large number of individuals in this cohort were overweight or obese (66%) and the majority had fair or very fair skin types. When stratified by quartile of annual D-UVB, it was found that the majority of characteristics were mostly evenly dispersed. However, there were a few differences, for example, there were a higher proportion of brown and black individuals who had higher quartiles of D-UVB.

Table 6.5: Baseline characteristics of cohort (N=466,230).

Characteristics	All	Annual D-UVB			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
	N (%)	*N (%)*	*N (%)*	*N (%)*	*N (%)*
Sex					
Female	252,896 (54)	63,786 (25)	62,331 (25)	62,441 (25)	64,336 (25)
Male	213,335 (46)	52,731 (25)	54,307 (25)	54,037 (25)	52,260 (25)
Age	56.6 (50-63)	56.6 (50-63)	56.6 (50-63)	56.6 (50-63)	56.5 (50-63)
BMI					
Underweight (<18.5)	2,399 (1)	552 (23)	501 (21)	659 (27)	687 (29)
Normal weight (18.6-24.9)	151,952 (33)	36,313 (24)	35,299 (23)	38,756 (26)	41,584 (27)
Overweight (25-29.9)	197,572 (42)	50359 (25)	50402 (26)	48731 (25)	48080 (24)
Obese (30-39.9)	103,277 (22)	26,716 (25)	27,446 (27)	25,399 (25)	23,716 (23)
Extremely Obese (≥40)	8,738 (2)	2,176 (25)	2177 (25)	2224 (25)	2161 (25)
Weight loss (NA=2)					
Yes	70,415 (17)	17,306 (25)	18,316 (26)	17,718 (25)	17,075 (24)
No	388,667 (83)	97,522 (25)	96,670 (25)	96,895 (25)	97,578 (25)
Supplement Users					
Yes	18,877 (4)	3,907 (21)	4,373 (23)	5,030 (27)	5,567 (29)
No	447,352 (96)	112,610 (25)	112,265 (25)	111,448 (25)	111,029 (25)
Supplement use 24hrs prior to interview					
Yes	2240 (1)	154 (7)	571 (25)	985 (44)	530 (24)
No	463,989 (99)	116,363 (25)	116,067 (25)	115,493 (25)	116,066 (25)
Oily Fish Consumption					
Low	204,430 (44)	53,091 (25)	50,204 (25)	50,669 (25)	50,466 (25)
Medium	257,358 (55)	62,498 (25)	65,327 (25)	64,634 (25)	64,899 (25)
High	4,441 (1)	928 (21)	1,107 (25)	1,175 (26)	1,231 (28)
Skin colour (NA=5,521)					
Very fair	35,794 (8)	9,536 (27)	8,945 (25)	8,612 (24)	8,701 (24)
Fair	313,554 (68)	81,581 (27)	79,114 (25)	76,168 (25)	76,691 (25)
Olive	86,422 (19)	20,311 (24)	21,986 (25)	22,080 (26)	22,045 (25)
Dark olive	8,657 (1)	2,027 (23)	2,291 (26)	2,280 (26)	2,059 (25)
Brown	13,022 (3)	1,537 (12)	2,318 (18)	4,615 (35)	4,552 (35)
Black	3,259 (1)	204 (6)	525 (16)	1250 (39)	1280 (39)
Ease of skin tanning (NA=10,897)					
Never tan, only burn	77,917 (17)	21,066 (27)	20301 (26)	18,396 (24)	18,154 (23)
Get mildly tanned	96,451 (21)	25,114 (26)	23,744 (25)	23,982 (25)	23,611 (24)
Get moderately tanned	182,221(40)	44,793 (25)	45,358 (25)	45,842 (25)	46,228 (25)
Get very tanned	98,743 (22)	23,324 (24)	24,632 (25)	25,169 (25)	25,618 (26)

Year of attendance					
2006	4 (0)	2 (50)	2 (50)	0 (0)	0 (0)
2007	46,616 (10)	13743 (17)	9,677 (12)	10,248 (13)	46,189 (58)
2008	174,866 (38)	59,928 (34)	40,673 (23)	28,076 (16)	46,189 (26)
2009	162, 186 (35)	38,042 (23)	37,761 (23)	43,695 (27)	42,688 (26)
2010	82,557 (17)	4,802 (6)	28,525 (35)	34,459 (42)	14,771 (18)
Smoking Status					
Current smoker	48,462 (10)	12,110 (25)	12,370 (26)	12,111 (25)	11,871 (24)
Past smoker	161,741 (35)	39,505 (25)	40,805 (25)	40,448 (25)	40,983 (25)
Never smoker	254,547 (55)	64,538 (25)	63,075 (25)	63,544 (25)	63,390 (25)
Alcohol Consumption					
Current drinker	429,708 (92)	107,831 (25)	107,833 (25)	106,597 (25)	107,447 (25)
Past drinker	16,528 (4)	4,120 (25)	4,142 (25)	4,280 (26)	3,986 (24)
Never	19,732 (4)	4,513 (23)	4,586 (23)	5,540 (28)	5,093 (26)
Time spent outdoors					
Low	166,778 (36)	39,625 (24)	40,601 (24)	42,302 (25)	44,250 (27)
Medium	235,567 (50)	60,039 (25)	59,531 (25)	58,103 (25)	57,894 (25)
High	63,884 (14)	16,853 (26)	16,506 (25)	16,073 (25)	14,452 (24)
Sun Protection use					
Always	96,559 (21)	26,416 (27)	25,428 (26)	22,595 (24)	22,120 (23)
Mostly	165,624 (36)	41,526 (25)	41,603 (25)	40,783 (25)	41,712 (25)
Sometimes	154,260(33)	37,190 (24)	38,214 (25)	39,585 (26)	39,271 (25)
Rarely/Never	46,514 (9)	10,702 (23)	10,677 (23)	12,493 (27)	12,642 (27)
Do not go out in the sun	2,731 (1)	609 (22)	645 (24)	806 (30)	671 (25)
Overall health rating 1 (NA=94)					
Poor	20,350 (3.7)	5,145 (25)	5,683 (28)	5,337 (26)	4,185 (21)
Fair	96,162 (21)	24,324 (25)	25,605 (27)	24,471 (25)	21,761 (23)
Good	270,246 (58)	67,039 (25)	67,610 (25)	67,459 (25)	68,138 (25)
Excellent	77,792 (17)	19,615 (25)	17,296 (23)	18,778 (24)	22,103 (28)
Do not know	1,586 (0.3)	372 (23)	422 (27)	409 (26)	383 (24)
Osteoporosis					
Yes	7,421 (2)	1,918 (26)	2,050 (28)	1,740 (22)	1,713 (22)
No	458,808 (98)	114,599 (25)	114,588 (25)	114,738 (25)	114,883 (25)

6.4.2 Risk Cohort Characteristics

When differences between overall UK Biobank cohort and risk cohort were explored, there were some similarities and some differences (**Table 6.5, Table 6.6**). Overall the risk cohort was similar to the overall UK Biobank cohort when in terms of skin colour, supplement use, ease of skin tanning, time spent outdoors, and BMI. There were less females included in the risk cohort when compared to the overall UK Biobank cohort (26% vs 54%). The median age of the patients in the risk cohort was also older (63y vs 57y). There were slight differences in sun protection use; those in the risk cohort tended to avoid sun-screen use. Unsurprisingly, there were differences in smoking status which is an important risk factor for oesophageal and gastric cancer (54% past/current smokers in risk cohort vs 45% in the overall UK biobank cohort). In the risk cohort alone, there was a higher proportion of male cases (74%), overweight or obese people (47% and 26%) and previous or current smokers (47% and 19%). Some significant characteristic differences were noted between the age/sex/recruitment year matched controls and cases (**Table 6.6**). Cases had a higher percentage of overweight or obese individuals (77% vs 73%), current or previous smokers (66% vs 52%), and previous drinkers (7% vs 4%) (**Table 6.6**).

Table 6.6: Baseline characteristics of cases and controls age, sex and year of recruitment matched in Risk cohort.

Characteristics	*Cases Oes[1] N=373 (10%)*	*Cases Gas[1] N=249 (7%)*	*All cases N=622 (17%)*	*Oes Controls N=1865 (60%)*	*Gas Controls N=1245 (40%)*	*Entire Risk cohort N=3110 (100%)*
Sex						
Female	86 (23)	75 (30)	161 (26)	430 (23)	375 (30)	966 (26)
Male	287 (77)	174 (70)	461 (74)	1435 (77)	870 (70)	2766 (74)
Age (median, IQR)	63 (59-66)	63 (59-67)	63 (59-66)	63(59-66)	63 (58-67)	63 (58-66)
BMI[2] (NA=22)						
Underweight/Normal	78 (21)	65 (26)	143 (23)	518 (28)	356 (29)	1017 (27)
Overweight	123 (33)	120 (48)	290 (47)	857 (46)	590 (48)	1737 (47)
Obese	170 (46)	64 (26)	187 (30)	475 (26)	294 (24)	956 (26)
Skin colour (NA=57)						
Very fair/Fair	292 (79)	188 (76)	480 (78)	934 (76)	1436 (78)	2850 (78)
Light olive/Dark olive	75 (20)	54 (22)	129 (21)	255 (21)	344 (19)	728 (20)
Brown/Black	2 (1)	5 (2)	7 (1)	39 (3)	51 (3)	97 (3)
Smoking Status (NA=16)						
Current smoker	72 (19)	45 (18)	117 (19)	177 (10)	106 (9)	400 (11)
Past smoker	191 (51)	99 (40)	290 (47)	797 (43)	523 (42)	1610 (43)
Never smoked	109 (29)	103 (42)	212 (34)	885 (48)	609 (49)	1706 (46)
Alcohol Consumption (NA=2)						
Current drinker	334 (90)	223 (92)	557 (90)	1749 (94)	1158 (93)	3464 (93)
Past drinker	27 (7)	17 (7)	44 (7)	64 (3)	48 (4)	156 (4)
Never drank	11 (3)	3 (1)	19 (3)	52 (3)	39 (3)	110 (3)
Oily Fish						
Low (0-<1 times/wk.)	160 (43)	95 (38)	255 (41)	747 (40)	498 (40)	1500 (40)
Medium (1-4 times/wk.)	207 (55)	153 (61)	360 (58)	1104 (59)	740 (59)	2204 (59)
High (≥5 times/wk.)	6 (2)	1 (1)	7 (1)	14 (1)	7 (1)	28 (1)
Vitamin D Supplement						
Yes	11 (3)	8 (3)	19 (3)	68 (4)	46 (4)	133 (4)
No	362 (97)	241 (97)	603 (97)	1797 (96)	1199 (96)	3599 (96)
Barrett's oesophagus						
Yes	9 (2)	4 (2)	13 (2)	3 (<1)	1 (<1)	17 (<1)
No	364 (98)	245 (98)	609 (98)	1862 (99)	1244 (99)	3715 (99)
Gastric ulcers						
Yes	6 (2)	7 (3)	13 (2)	20 (1)	15 (1)	48 (1)
No	367 (98)	242 (97)	609 (98)	1845 (99)	1230 (99)	3684 (99)
Oesophageal/Gastric Reflux						
Yes	30 (8)	12 (5)	42 (7)	81 (4)	61 (5)	184 (5)
No	343 (92)	237 (95)	580 (93)	1784 (96)	1184 (95)	3548 (95)
Sun Protection use (NA=4)						
Always	55 (14)	44 (18)	99 (16)	294 (16)	230 (18)	623 (17)
Mostly	104 (28)	78 (31)	182 (29)	608 (33)	394 (32)	1184 (32)
Sometimes	144 (39)	90 (36)	234 (38)	690 (37)	450 (36)	1374 (37)
Rarely/Never	63 (17)	34 (14)	97 (16)	266 (14)	166 (13)	529 (14)
Do not go out in the sun	7 (2)	2 (1)	9 (1)	5 (0)	4 (0)	18 (0)

Characteristics	*Cases Oes*[3] *N=373 (10%)*	*Cases Gas*[3] *N=249 (7%)*	*All cases N=622 (17%)*	*Oes Controls N=1865 (60%)*	*Gastric Controls N=1245 (40%)*	*Entire Risk cohort N=3110 (100%)*
D-UVB (median, IQR)						
D-UVB[2]	34280 (32680-37440)	34380 (31960-37460)	34300 (32340-37460)	34750 (33150-38260	34750 (33100-38510)	34680 (33060-38220)
Tertile 1	136 (36)	94 (38)	230 (37)	594 (32)	420 (34)	1244 (33)
Tertile 2	129 (35)	85 (34)	214 (34)	639 (34)	391 (31)	1244 (33)
Tertile 3	108 (29)	70 (28)	178 (29)	632 (34)	434 (35)	1244 (33)
Time spent outdoors Summer (NA=40)						
Low (0-2 hrs/day)	109 (30)	69 (28)	178 (29)	506 (27)	349 (28)	1033 (28)
Medium (2.1-5 hrs/day)	151 (40)	100 (41)	251 (41)	802 (43)	548 (44)	1601 (43)
High (>5.1hrs/day)	109 (30)	77 (31)	186 (30)	536 (29)	336 (27)	1058 (29)
Time spent outdoors Winter (NA=43)						
Low (0-2 hrs/day)	260 (70)	160 (66)	420 (68)	1250 (68)	838 (28)	2508 (28)
Medium (2.1-5 hrs/day)	81 (22)	64 (26)	145 (24)	441 (24)	311 (44)	897 (43)
High (>5.1hrs/day)	29 (8)	20 (8)	49 (8)	153 (8)	82 (27)	284 (29)
Qualifications (NA=35)						
None	95 (26)	86 (35)	181 (29)	402 (22)	280 (23)	863 (23)
CSE or O-levels[1]	57 (15)	38 (15)	95 (15)	246 (13)	163 (13)	504 (14)
A-levels[1]	16 (4)	12 (5)	28 (5)	89 (5)	60 (5)	177 (5)
NVQ or HND/C[1]	55 (15)	34 (14)	89 (14)	236 (13)	172 (14)	497 (13)
Other prof qualification[1]	56 (15)	23 (9)	79 (13)	299 (16)	180 (15)	558 (15)
University degree	91 (25)	53 (22)	144 (23)	575 (31)	379 (31)	1098 (30)

Footnote:

1. Gas: gastric cancer cases; oes: oesophageal cancer; CSEs: Certificate of Secondary Education; O levels: Ordinary level general certificate of education; A levels: advanced level general certificate of education; NVQ: National Vocational Qualification; HND/C: Higher National Diploma/Certificate; Other prof qualification: Other professional qualification
2. WHO classification was used for categorisation into underweight, normal, overweight and obese; Underweight/Normal (<24.9), Overweight (25-29.9), Obese (>30)
3. D-UVB tertiles: Tertile 1 (<33730 mJ/cm^2), Tertile 2 (33740-36040 mJ/cm^2), Tertile 3 (>36070 mJ/cm^2

6.4.3 Regression Analysis in Risk Cohort

Using conditional logistic regression a strong association between both annual D-UVB and VDscore4 and the development of upper gastrointestinal cancer was found (**Table 6.7, 6.8**). Higher tertiles of VDscore4 and annual D-UVB were both strongly inversely associated with the development of any primary upper gastrointestinal cancers (annual D-UVB: OR=0.73, 95%CI: 0.59-0.91; VDscore4: OR=0.68, 95%CI: 0.55-0.83). This trend was also found after the adjustment for important co-factors (annual D-UVB: OR=0.79, 95%CI: 0.63-0.99; VDscore4: OR=0.69, 95%CI: 0.56-0.85). This trend was further strengthened when restricted to oesophageal cancer only, lower oesophageal cancer cases and adenocarcinoma cases: for instance a 47% decreased odds in lower third oesophageal cancer for tertile three vs tertile one of VDscore4 was observed (**Table 6.8**). Higher tertiles of VDscore4 was also observed to be associated with a reduced odds of any oesophageal or any gastric cancer but this was not observed when annual D-VB was used as an estimate.

Interaction between vitamin D estimates and important vitamin D variables was also carried out. No significant interaction between VDscores or annual D-UVB was found for categorical variables related to BMI, alcohol status, smoking status, and skin colour (**Table 6.9, 6.10**).

In brief, compared to reference, higher tertiles of annual D-UVB and VDscore4 are associated with reduced odds of the development of upper gastrointestinal cancers. This was especially noticeable in oesophageal cancer cases.

Table 6.7: Association between tertiles of annual D-UVB on the odds of developing oesophageal or gastric cancer (age, sex and year of recruitment matched controls)

Conditional logistic regression looking at the association between annual D-UVB on the odds of developing oesophageal or gastric cancer stratified by cancer type and location [1, 2, and 3].

	Analysis	Number of cases	Number of controls	**Tertile 1** (30830-33100)[4]			**Tertile 2** (34000-35230)					**Tertile 3** (38110-39270)					**P-trend**
				N case	**N control**	**OR**	**N case**	**N control**	**OR**	**95% CL**	**p-val**	**N case**	**N control**	**OR**	**95% CL**	**p-val**	
	Analysis 1[6]																
All	*Unadjusted*	622	3110	230	1014	Ref	214	1030	0.91	0.74-1.13	0.40	178	1066	0.73	0.59-0.91	**0.005**	**0.0002**
	Adjusted	622	3110	230	1014	Ref	214	1030	0.91	0.73-1.13	0.38	178	1066	0.79	0.63-0.99	**0.04**	**0.003**
Cancer location	*Oesophageal*	373	1865	136	594	Ref	129	639	0.89	0.67-1.17	0.40	108	632	0.80	0.60-1.07	0.13	**0.06**
	Up/mid third Oes	198	990	17	81	Ref	22	77	1.55	0.70-3.45	0.28	11	92	0.51	0.20-1.30	0.16	**0.05**
	Lower third Oes	50	250	81	319	Ref	64	337	0.69	0.47-1.00	**0.05**	53	334	0.64	0.43-0.96	**0.03**	**0.02**
	Gastric	249	1245	94	420	Ref	85	391	0.94	0.66-1.33	0.72	70	434	0.78	0.55-1.10	0.16	**0.01**
Histology	*OEAC*	243	1215	88	361	Ref	79	395	0.85	0.59-1.21	0.36	60	379	0.74	0.50-1.08	0.12	0.16
	OESCC	76	380	26	116	Ref	26	135	0.71	0.36-1.38	0.31	24	129	0.78	0.41-1.48	0.45	0.25

Footnote:

1 Controls were matched to cases by age, sex and year of recruitment in a 5:1 ratio. Each case was assigned a specific 5 controls so when stratified by oesophageal or gastric cancer the controls were stratified according to their specific case's cancer diagnosis in conditional logistic regression.

2 OEAC; oesophageal adenocarcinoma, OESCC; oesophageal squamous cell carcinoma, Oes; oesophageal

3 Adjusted model has been adjusted for: smoking status, alcohol intake, BMI, oesophageal-gastric reflux, qualifications, and gastric ulcers. Cut offs used were tertiles of annual D-UVB

4 Annual D-UVB (mJ/cm^2) and IQR

Table 6.8: Association between tertiles of VDscore4 on the odds of developing oesophageal or gastric (age, sex and year of recruitment matched controls)

Conditional logistic regression looking at the association between VDscore4 on the odds of developing oesophageal or gastric cancer stratified by cancer type and location[1,2,3,4].

	Analysis	Number of cases	Number of controls	**Tertile 1** (4-6)[5]			**Tertile 2** (10-14)					**Tertile 3** (16-22)					**P-trend**
				N case	**N control**	**OR**	**N case**	**N control**	**OR**	**95% CL**	**p-val**	**N case**	**N control**	**OR**	**95% CL**	**p-val**	
	Analysis 1[6]																
All	*Unadjusted*	622	3110	230	974	Ref	158	751	0.86	0.69-1.08	0.20	234	1412	0.68	0.55-0.83	**0.0002**	**0.0005**
	Adjusted	622	3110	230	974	Ref	158	751	0.85	0.67-1.07	0.16	234	1412	0.69	0.56-0.85	**0.0005**	**0.002**
Cancer location	*Oesophageal*	373	1865	136	563	Ref	103	463	0.95	0.71-1.28	0.73	134	838	0.76	0.51-0.89	**0.005**	**0.005**
	Up/mid third Oes	198	990	18	73	Ref	18	68	1.03	0.47-2.26	0.95	14	109	0.47	0.19-1.13	*0.09*	**0.002**
	Lower third Oes	50	250	82	310	Ref	53	243	0.83	0.56-1.25	0.37	63	437	0.53	0.36-0.78	**0.001**	**0.03**
	Gastric	249	1245	94	384	Ref	55	287	0.72	0.49-1.07	0.10	100	574	0.71	0.52-0.99	**0.04**	0.12
Histology	*OEAC*	243	1215	87	345	Ref	68	287	1.02	0.70-1.49	0.91	72	503	0.63	0.44-0.91	**0.01**	**0.03**
	OESCC	76	380	27	112	Ref	18	101	0.66	0.33-1.34	0.25	31	167	0.69	0.38-1.25	0.22	0.10

Footnote:

1 Controls were matched to cases by age, sex and year of recruitment in a 5:1 ratio. Each case was assigned a specific 5 controls so when stratified by oesophageal or gastric cancer the controls were stratified according to their specific case's cancer diagnosis in conditional logistic regression.

2 OEAC; oesophageal adenocarcinoma, OESCC; oesophageal squamous cell carcinoma

3 VDscore4 [calculated using annual D-UVB, supplement use, oily fish consumption and estimated hourly sunlight exposure] and Tertiles of these were used to explore the relationship

4 Adjusted model has been adjusted for: smoking status, alcohol intake, BMI, oesophageal-gastric reflux, qualifications, and gastric ulcers. Cut offs used were tertiles of VDscore4

5 VDscore4 IQR

Table 6.9: Interaction analysis of the association between annual D-UVB tertiles on the odds of developing primary oesophageal or gastric cancer

Conditional logistic regression looking at the interaction between annual D-UVB tertiles on the odds of developing primary oesophageal or gastric cancer [1, 2,3].

Analysis	Number of cases	Number of controls	Tertile 1 (30830-33100)[4]			Tertile 2 (34000-35230)					Tertile 3 (38110-39270)					P-trend
			N cases	N control	OR	N cases	N control	OR	95% CL	p-val	N cases	N control	OR	95% CL	p-val	
BMI																
Underweight/normal	143	874	44	258	Ref	50	274	Ref	Ref	Ref	49	342	Ref	Ref	Ref	Ref
Overweight	290	1447	103	488	Ref	107	481	1.04	0.60-1.80	0.88	80	478	1.02	0.58-1.79	0.94	0.99
Obese/extremely obese	187	769	83	263	Ref	55	266	0.65	0.35-1.18	0.15	49	240	0.83	0.45-1.51	0.54	0.98
Alcohol																
Never	19	99	8	27	Ref	5	30	Ref	Ref	Ref	6	34	Ref	Ref	Ref	Ref
Previous	44	112	17	33	Ref	13	36	1.31	0.28-6.23	0.73	14	43	0.95	0.21-4.32	0.95	0.85
Current	557	2896	204	954	Ref	195	964	1.80	0.51-6.40	0.36	158	989	1.27	0.37-4.34	0.71	0.72
Smoking																
Never	212	1485	75	489	Ref	80	464	Ref	Ref	Ref	57	541	Ref	Ref	Ref	Ref
Previous	290	1317	107	423	Ref	100	465	0.74	0.46-1.17	0.20	83	432	1.19	0.73-1.95	0.49	0.60
Current	117	291	47	99	Ref	32	94	0.73	0.38-1.41	0.35	38	90	1.41	0.74-2.70	0.29	0.38
Skin colour[5]																
Very fair/fair	480	2375	177	802	Ref	168	773	Ref	Ref	Ref	135	795	Ref	Ref	Ref	Ref
Olive/dark olive	129	606	48	173	Ref	43	216	0.78	0.46-1.33	0.36	38	210	0.86	0.50-1.50	0.60	0.53

Footnote:

1. Controls were matched to cases by age, sex and year of recruitment in a 5:1 ratio
2. annual D-UVB Tertiles were used as cut off values
3. Adjusted model has been adjusted for: smoking status, alcohol intake, BMI, oesophageal-gastric reflux, qualifications, and gastric ulcers
4. annual D-UVB IQR
5. Those with brown/black skin were excluded from the analysis due to very low numbers (n=7 cases)

Table 6.10: Interaction analysis of the association between VDscore4 tertiles on the odds of developing primary oesophageal or gastric cancer

Conditional logistic regression looking at the interaction between VDscore4 and lifestyle variables on the odds of developing oesophageal or gastric cancer[1,2,3,4].

Analysis	Number of cases	Number of controls	**Tertile 1** (4-6)[4]			**Tertile 2** (10-14)					**Tertile 3** (16-22)					P-trend
			N cases	N control	OR	N cases	N control	OR	95% CL	p-val	N cases	N control	OR	95% CL	p-val	
BMI																
Underweight/normal	143	874	43	256	Ref	39	194	Ref	Ref	Ref	61	424	Ref	Ref	Ref	Ref
Overweight	290	1447	80	249	Ref	41	201	0.78	0.43-1.41	0.41	66	319	0.84	0.50-1.43	0.53	0.21
Obese/extremely obese	187	769	80	249	Ref	41	201	0.56	0.29-1.08	0.06	66	319	0.79	0.45-1.42	0.33	0.17
Alcohol																
Never	19	99	8	30	Ref	5	20	Ref	Ref	Ref	6	41	Ref	Ref	Ref	Ref
Previous	44	112	18	29	Ref	10	28	0.55	0.11-2.80	0.47	16	55	0.76	0.18-3.25	0.71	0.41
Current	557	2896	203	888	Ref	142	703	0.95	0.26-3.57	0.95	212	1316	1.27	0.39-4.18	0.68	0.95
Smoking																
Never	212	1485	81	459	Ref	55	348	Ref	Ref	Ref	76	687	Ref	Ref	Ref	Ref
Previous	290	1317	105	391	Ref	70	339	0.91	0.54-1.51	0.70	115	590	1.19	0.76-1.88	0.45	0.66
Current	117	291	43	94	Ref	32	62	1.26	0.64-2.50	0.51	42	127	1.22	0.66-2.25	0.53	0.38
Skin colour[6]																
Very fair/fair	480	2375	177	748	Ref	118	569	Ref	Ref	Ref	185	1053	Ref	Ref	Ref	Ref
Olive/dark olive	129	606	46	162	Ref	38	151	1.08	0.62-1.91	0.78	45	286	0.80	0.48-1.36	0.41	0.32

Footnote:

1. VDscore4 [calculated using annual D-UVB, supplement use, oily fish consumption and estimated hourly sunlight exposure] and Tertiles of these were used to explore the relationship
2. Adjusted model has been adjusted for: smoking status, alcohol intake, BMI, oesophageal-gastric reflux, qualifications, and gastric ulcers. Cut offs used were tertiles of VDscore4
3. Analysis included those who received a primary gastric or oesophageal cancer diagnosis
4. VDscore4 IQR
5. Those with brown/black skin were excluded from the analysis due to very low numbers (n=7 cases)

6.4.4 Survival Cohort Characteristics

When examining the survival cohort (n=235), 43 participants (18%) died from disease and 15 (6%) died from other causes. Median follow up was 10.3 years (IQR: 7.8-13.3 years). Similar baseline characteristics of the overall UK Biobank cohort were found for the survival cohort, for example vitamin D supplementation use was 4%, vitamin D supplement use 24 hrs prior to interview was <1% and similar levels of fair or very fair skin types were also found in (79% vs 76% in entire cohort) (**Table 6.5**, **Table 6.11**). However, there were also some differences, 31% of oesophageal and gastric cancer participants were female, compared to 54% of the total UK Biobank cohort. Unsurprisingly, there was a higher percentage of smokers or past smokers in the survival cohort (67% vs 45%) (**Table 6.11**). The distribution of characteristics were mostly similar when stratified by annual D-UVB quartile, however, a large percentage of those with osteoporosis were found to have lower D-UVB doses and were part of quartile 1 .

6.4.5 Survival Analysis in Survival Cohort

There was no strong evidence of a relationship between annual D-UVB or VDscore4 and survival of cancer when comparing highest to lowest tertiles. No association was found for cancer-specific death using annual D-UVB (HR=1.24, 95%CI: 0.61-2.52) or VDscore4 (HR=1.65, 95%CI: 0.71-3.82) (**Table 6.12, 6.13**). Similar results were found for adjusted models (annual D-UVB: HR=1.76, 95%CI: 0.80-3.87, VDscore4: HR=1.66, 95%CI: 0.66-4.14). These trends were also noted for all-cause mortality (**Table 6.12, 6.13**). Correspondingly, in Kaplan-Meier graphs no significant difference between tertiles of annual D-UVB or VDscore4 were observed (**Figure 6.4, 6.5**). A large difference in beta-coefficient between adjusted and unadjusted VDscore4 tertiles was observed in tertile 2 (unadjusted: HR=1.60, adjusted: HR=1.05). The main drivers of this difference in hazard ratios was found to be a combination of adjustments for skin colour, gender and gastric reflux. These variables had a large impact on the beta-coefficient, demonstrating that these variables are important risk factors for cancer-specific mortality in this group. Such a dramatic difference between adjusted and unadjusted models was not observed in Tertile 3. When restricted to primary gastric cancer a significant decrease in all-cause and cancer-specific mortality when comparing tertile two with tertile one was observed for VDscore4 (cancer-specific: HR= 0.22, 95%CI: 0.05-0.93, all-cause: HR= 0.26, 95%CI: 0.07-0.99) (**Table 6.13**). The relationship between annual D-UVB, VDscore4 and non-cancer related mortality was also investigated, however no associations were found, although the number of events was low (**Appendix 7**).

Table 6.11: Baseline characteristics of survival cohort.

All participants in survival cohort (N=235).

Characteristics	All	Annual D-UVB			
		Quartile 1 (27650-33170)	Quartile 2 (33190-34400)	Quartile 3 (34401-37550)	Quartile 4 (37580-41060)
	N (%)	N (%)	N (%)	N (%)	N (%)
Annual D-UVB	34400 (33190-37580)	31580 (2959-32330)	33870 (33670-34080)	35250 (34780-35560)	38800 (38660-39640)
Sex					
Female	72 (31)	25 (35)	14 (19)	18 (25)	15 (21)
Male	163 (69)	34 (21)	44 (27)	41 (25)	44 (27)
Age	63 (58-66)	63 (59-67)	62 (56-66)	63 (59-66)	62 (56-66)
BMI (NA=1)[1]					
Underweight	8 (3)	2 (25)	0 (0)	3 (38)	3 (38)
Normal weight	116 (50)	21 (18)	30 (26)	38 (33)	27 (23)
Overweight	72 (31)	23 (32)	19 (26)	12 (17)	18 (25)
Obese	37 (16)	11 (30)	9 (24)	6 (16)	11 (30)
Extremely Obese	1 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Weight loss (NA=2)					
Yes	72 (31)	19 (26)	20 (28)	16 (22)	17 (24)
No	161 (69)	40 (25)	37 (23)	43 (27)	41 (25)
Supplement Users					
Yes	10 (4)	6 (60)	0 (0)	2 (20)	2 (20)
No	225 (96)	53 (24)	58 (26)	57 (25)	57 (25)
Supplement use 24hrs prior to interview					
Yes	1 (<1)	1 (100)	0 (0)	0 (0)	0 (0)
No	234 (100)	58 (25)	59 (25)	59 (25)	59 (25)
Oily Fish Consumption (NA=2)					
Low	108 (46)	28 (26)	24 (22)	30 (28)	26 (24)
Medium	125 (53)	31 (25)	34 (27)	28 (22)	32 (26)
High	2 (1)	0 (0)	0 (0)	1 (50)	1 (50)
Skin colour (NA=7)					
Very fair	18 (8)	3 (17)	6 (33)	5 (28)	4 (22)
Fair	161 (71)	39 (24)	42 (26)	36 (22)	44 (27)
Olive	29 (13)	9 (31)	3 (10)	14 (48)	3 (10)
Dark olive	7 (3)	3 (43)	2 (29)	0 (0)	2 (29)
Brown	8 (4)	0 (0)	2 (25)	1 (13)	5 (63)
Black	5 (2)	2 (40)	0 (0)	2 (40)	1 (20)

Ease of skin tanning (NA=7)					
Never tan	47 (21)	12 (26)	12 (26)	12 (26)	11 (23)
Get mildly tanned	34 (15)	8 (24)	7 (21)	5 (15)	14 (41)
Moderate tan	91 (41)	22 (24)	29 (32)	20 (22)	20 (22)
Get very tanned	52 (23)	13 (25)	9 (17)	19 (37)	11 (21)
Smoking Status (NA=2)					
Current smoker	29 (12)	8 (28)	6 (21)	10 (34)	5 (17)
Past smoker	128 (55)	32 (25)	29 (23)	32 (25)	35 (27)
Never smoker	76 (33)	19 (25)	22 (29)	16 (21)	19 (25)
Alcohol Consumption					
Current drinker	200 (85)	54 (27)	47 (24)	50 (25)	49 (25)
Past drinker	18 (8)	4 (22)	6 (33)	2 (11)	6 (33)
Never	17 (7)	1 (6)	5 (29)	7 (41)	4 (24)
Time spent outdoors					
Low	62 (26)	17 (27)	9 (15)	16 (26)	20 (32)
Medium	125 (53)	31 (25)	34 (27)	31 (25)	29 (23)
High	48 (20)	11 (23)	15 (31)	12 (25)	10 (21)
Sun Protection use (NA=1)					
Always	35 (15)	12 (34)	8 (23)	4 (11)	11 (31)
Mostly	71 (30)	14 (20)	21 (30)	18 (25)	18 (25)
Sometimes	81 (35)	20 (25)	17 (21)	22 (27)	22 (27)
Rarely/Never	44 (19)	12 (27)	12 (27)	14 (32)	6 (14)
Do not go in sun	3 (1)	1 (33)	0 (0)	1 (33)	1 (33)
Upper gastrointestinal cancer					
Oesophageal	118 (50)	27 (23)	32 (27)	26 (22)	33 (28)
Gastric cancer	117 (50)	32 (27)	26 (22)	33 (28)	26 (22)
Barrett's oesophagus					
Yes	11 (5)	2 (18)	5 (45)	1 (9)	3 (27)
No	224 (95)	57 (25)	53 (24)	58 (26)	56 (25)
Osteoporosis					
Yes	7 (3)	4 (57)	1 (14)	1 (14)	1 (14)
No	228 (97)	55 (24)	57 (25)	58 (25)	58 (25)

Footnote:

[1] BMI categories: Underweight: <18.5, Normal weight: 18.6-24.9, Overweight: 25-29.9, Obese: 30-39.9, Extremely Obese: ≥40

Table 6.12: Association between annual D-UVB tertiles and the survival of primary oesophageal and gastric cancer

Cox proportional hazard analysis looking at the effect of annual D-UVB in the survival of **primary** oesophageal and gastric cancer stratified by cancer type [1, 2]

Outcome			Tertile 1 (3110-33270)			Tertile 2 (34070-34990)					Tertile 3 (38100-39260)					P-trend
		N	N	% died	HR	N	% died	HR	95% CL	p-val	N	% died	HR	95% CL	p-val	
	CS mortality															
All	*Unadjusted*	235	78	18%	Ref	78	15%	0.86	0.40-1.86	0.70	79	22%	1.24	0.61-2.52	0.55	0.27
	Adjusted	235	78	18%	Ref	78	15%	0.74	0.32-1.76	0.49	79	22%	1.76	0.80-3.87	0.16	*0.08*
Cancer location	*Oesophageal*	118	38	21%	Ref	37	14%	0.44	0.10-1.93	0.28	43	28%	1.53	0.51-4.57	0.45	0.19
	Gastric	117	40	15%	Ref	41	17%	0.60	0.17-2.11	0.42	36	14%	1.43	0.37-5.51	0.61	0.39
Histology	*OEAC*	74	23	22%	Ref	25	16%	0.59	0.10-3.47	0.56	26	31%	2.10	0.49-8.96	0.32	0.12
	AC mortality															
All	*Unadjusted*	235	78	26%	Ref	78	22%	0.91	0.48-1.74	0.78	79	22%	1.15	0.62-2.12	0.66	0.35
	Adjusted	235	78	26%	Ref	78	22%	0.80	0.39-1.67	0.56	79	22%	1.51	0.76-3.00	0.24	0.14
Cancer location	*Oesophageal*	118	38	16%	Ref	37	13%	0.87	0.31-2.41	0.79	43	18%	1.52	0.61-3.78	0.37	0.25
	Gastric	117	40	18%	Ref	41	17%	0.60	0.18-2.06	0.42	36	19%	1.37	0.40-4.73	0.62	0.39
Histology	*OEAC*	74	23	43%	Ref	25	28%	0.65	0.18-2.39	0.52	26	31%	1.12	0.35-3.58	0.85	0.39

Footnote:

[1] Adjusted model has been adjusted for age, sex, skin colour, weight loss, smoking status, alcohol intake, BMI, cancer type and oesophageal or gastric reflux, weight loss, any cardiovascular condition. No information about cancer stage was available to adjust for this confounder.

[2] AC: all cause; CS: cancer specific; up/mid third Oes: upper/middle oesophageal cancer (classed by ICD-10-CM Diagnosis Code C15.5); lower third Oes: lower oesophageal cancer (classed by ICD-10-CM Diagnosis Code C15.3/15.4), OEAC; oesophageal adenocarcinoma, OESCC ; oesophageal squamous cell carcinoma

[3] All hazards were found to be proportional.

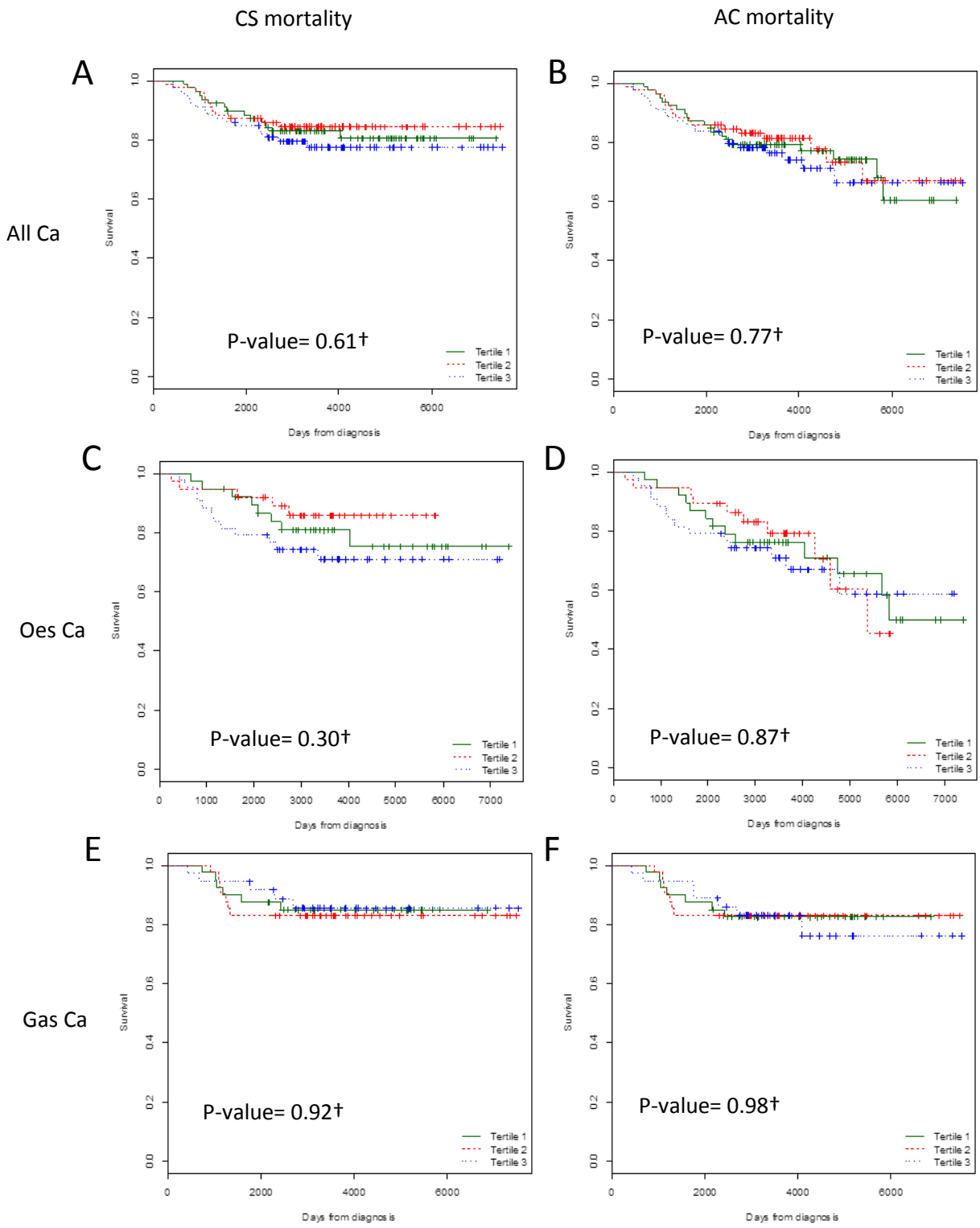

Figure 6.4: Kaplan-Meier analysis for mortality for Primary oesophageal and gastric cancers split by annual D-UVB tertiles

A) cancer-specific Mortality**, B)** All-cause mortality **C)** Oesophageal Cancer specific mortality **D)** Oesophageal cancer all-cause mortality, **E)** Gastric Cancer specific mortality **F)** Gastric all-cause mortality † log rank test. Tertile 1=Green, Tertile 2=Red and Tertile 3=Blue.

Table 6.13: Association between VDscore4 tertiles and the survival of primary oesophageal and gastric cancer

Cox proportional hazard analysis looking at the effect of VDscore4 in the survival of **primary** oesophageal and gastric cancer stratified by cancer type [1,2,3,4].

Outcome		N	Tertile 1 (3-6)[5]			Tertile 2 (10-16)					Tertile 3 (20-23)					P-trend
			N	% died	HR	N	% died	HR	95% CL	p-val	N	% died	HR	95% CL	p-val	
	CS mortality															
All	*Unadjusted*	235	62	13%	Ref	91	20%	1.60	0.70-3.69	0.27	82	21%	1.65	0.71-3.82	0.25	0.42
	Adjusted	235	62	13%	Ref	91	20%	1.05	0.42-2.60	0.92	82	21%	1.66	0.66-4.14	0.28	0.40
Cancer location	*Oesophageal*	118	26	8%	Ref	48	23%	1.87	0.37-9.45	0.44	44	27%	2.83	0.56-14.20	0.22	0.24
	Lower third Oes	51	10	20%	Ref	16	19%	0.33	0.01-8.80	0.50	25	32%	0.73	0.04-12.6	0.83	0.83
	Gastric	117	36	16%	Ref	43	16%	0.22	0.05-0.93	**0.04**	38	13%	0.49	0.12-2.01	0.32	0.15
	AC mortality															
All	*Unadjusted*	235	62	23%	Ref	91	23%	1.14	0.58-2.25	0.70	82	28%	1.35	0.69-2.62	0.38	0.43
	Adjusted	235	62	23%	Ref	91	23%	0.82	0.39-1.81	0.62	82	28%	1.29	0.59-2.70	0.50	0.37
Cancer location	*Oesophageal*	118	26	27%	Ref	48	29%	1.25	0.41-3.80	0.70	44	36%	1.95	0.64-5.93	0.24	0.23
	Lower third Oes	51	10	50%	Ref	16	19%	0.29	0.02-3.90	0.35	25	44%	0.86	0.10-7.65	0.89	0.96
	Gastric	117	36	19%	Ref	43	16%	0.26	0.07-0.99	**0.05**	38	18%	0.51	0.15-1.81	0.30	0.52
Histology	*OEAC*	74	14	29%	Ref	31	32%	1.25	0.21-7.55	0.81	29	34%	1.70	0.33-8.88	0.53	0.23

Footnote:

1 VDscore4 calculated using annual D-UVB, supplement use, oily fish consumption and estimated hourly sunlight exposure

2 Adjusted model has been adjusted for age, sex, Skin colour, weight loss, smoking status, alcohol intake, BMI, cancer type and oesophageal or gastric reflux, weight loss, any cardiovascular condition. All hazards were found to be proportional

3 AC: all cause; CS: cancer specific; up/mid third oes: upper/middle oesophageal cancer (classed by ICD-10-CM Diagnosis Code C15.5); lower third oes: lower oesophageal cancer (classed by ICD-10-CM Diagnosis Code C15.3/15.4). OEAC; oesophageal adenocarcinoma, OESCC ; oesophageal squamous cell carcinoma

4 Due to small numbers analysis for some subtypes was unable to be carried out.

5 VDscore4 IQR

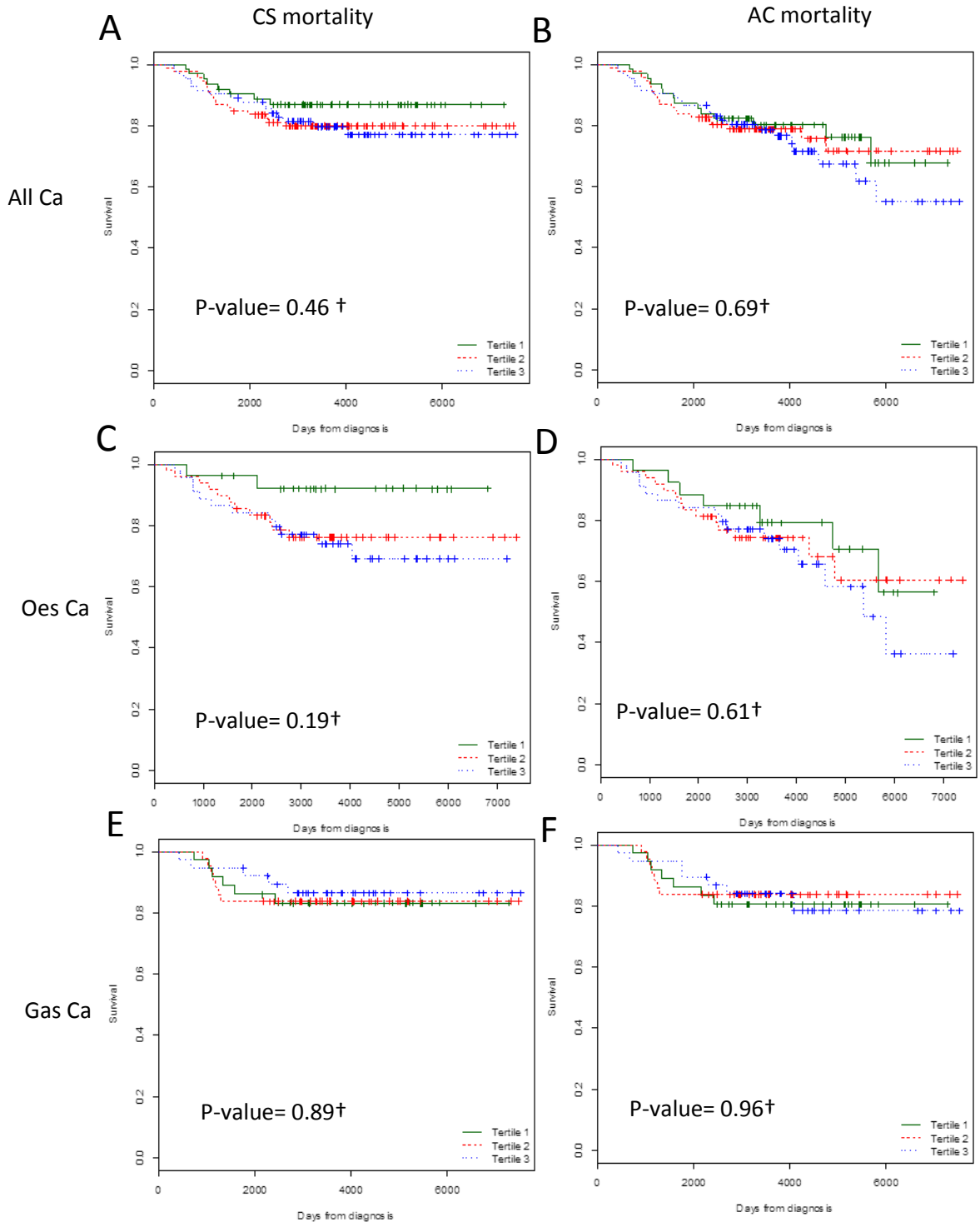

Figure 6.5: Kaplan-Meier analysis for mortality for Primary oesophageal and gastric cancers split by VDscore4 tertiles

A) cancer-specific Mortality**, B)** All-cause mortality **C)** Oesophageal Cancer specific mortality **D)** Oesophageal cancer all-cause mortality, **E)** Gastric Cancer specific mortality **F)** Gastric all-cause mortality † log rank test. Tertile 1=Green, Tertile 2=Red and Tertile 3=Blue

6.5 Discussion

The main aim of this chapter was to examine the association between vitamin D and the *risk* and *survival* of oesophageal and gastric cancer using annual D-UVB and VDscore4.

6.5.1 Oesophageal and Gastric Cancer Occurrence in Risk Cohort

The association between vitamin D and odds of developing oesophageal and gastric cancer was examined within the risk cohort. 25(OH)D measurements were not available for this cohort and therefore the relationship between 25(OH)D status and odds of developing upper gastrointestinal cancer was not explored.

The relationship between vitamin D and risk of upper gastrointestinal cancer was explored using annual D-UVB and VDscore as vitamin D estimates. Overall, a strong inverse relationship for higher annual D-UVB dose and VDscore4 and a reduced odds of developing any gastrointestinal cancer was found, when comparing the highest tertile to the lowest tertile (a 21% reduction for annual D-UVB and a 31% reduction for VDscore4), oesophageal cancer (24% reduction for VDscore4 was found when comparing tertile three to tertile one), and oesophageal adenocarcinoma (37% reduction for VDscore4). No consistent associations were found for squamous cell carcinoma cases or gastric cancer.

In accordance with this study, other studies which measured UVB dose have found a reduction in cancer incidence [126, 306]. One such study was carried out by Tran *et al.* who found that higher lifetime UV radiation was associated with a reduced odds of adenocarcinoma [6] and the results are in agreement with the work carried out in this chapter [301]. Additionally, Tran *et al.* [6] also found no association with oesophageal squamous cell carcinoma, a result which is echoed in this chapter [301].

It is interesting to note the differences in results between histological subtypes of oesophageal cancer, seen here and reported by Tran *et al*. There could be many reasons why this could be the case, (as both cancer types have different aetiologies). A study by Trowbridge *et al*. found high expression of VDR located in the mucosa of Barrett's oesophagus suffers, but not in normal mucosa [307]. This increase in VDR expression might indicate an increased sensitivity to 25(OH)D. As Barrett's oesophagus is highly associated with adenocarcinoma, but not squamous cell carcinoma, these results could indicate why no protective effect of annual D-UVB or VDscore4 is observed with squamous cell carcinoma cases [308]. However, due to the small sample size for squamous cell carcinoma cases, perhaps no significant associations were found for this group due to the lack of statistical power.

Interestingly, the study by Tran *et al.* was carried out in Australia, where UV radiation is dramatically higher than in the UK [309]. The fact that the relationship is still persistent in this study, even in a location with much lower UV doses compared to Australia, suggests that UVB may be an important factor in reducing the odds of developing upper gastrointestinal cancer even at high latitudes.

Many ecological studies are also in agreement with these findings and have found a strong relationship between UV radiation and oesophageal and gastric cancer risk [7, 104, 106]. For example, Boscoe *et al.* found a decrease in cancer risk rates in the USA, including gastric and oesophageal cancers, in areas with higher UVB [7]. Chen *et al*. found a similar relationship in China when satellite measurements of cloud-adjusted ambient UVB were used: incidence rates of oesophageal and gastric cancer were found to be reduced in areas with higher UVB [104]. A comparable relationship has also been shown in France for oesophageal cancers [106]. This has also shown to be true for other cancer types with a recent monograph by the W.H.O outlining evidence of an inverse relationship between UV radiation and breast, colorectal, prostate, ovary cancers and Non-Hodgkin's lymphoma [310].

This inverse relationship is in contrast to some previous literature as some studies examining the relationship between vitamin D and the risk of oesophageal or gastric cancer have reported a no significant overall effect [8, 9, 221, 311]. These studies reported harmful associations in stratified analysis when 25(OH)D concentration was used as the vitamin D estimate. 25(OH)D concentration is considered the best vitamin D status estimate at a specific point in time, however, it is unknown if this is the best measurement when examining odds of cancer development, as it only provides a one-time estimate of vitamin D, which is heavily seasonally biased. Additionally, some of the 25(OH)D concentration used in these studies were taken many years before cancer diagnosis. Chen *et al.*, used a one-time pre-trial 25(OH)D measurement taken one year prior to the start of the study, after which the participants were followed for a period of five years. Therefore, there could be in some cases, up to six years between blood draw and cancer diagnosis. As 25(OH)D can differ dramatically between seasons, health, and changes in diet or sun exposure practices, the differences in concentration of 25(OH)D between pre-trial and time of diagnosis could have existed. By using an annual D-UVB estimate, rather than a one-time measurement, this study gives a broader portrayal of vitamin D status as it is not subject to seasonal bias. Furthermore, VDscore4 also captures information on supplement use, utilisation of UVB and some dietary aspects of vitamin D. However, there are merits and disadvantages of each estimate used.

Two recent systematic reviews and meta-analysis, which have been published on the topic failed to find any association between dietary intake and upper gastrointestinal cancer risk and no association was found for gastric cancer and 25(OH)D concentration [189, 226]. A significant positive relationship reported for oesophageal cancer risk and 25(OH)D concentration was reported [189]. However, the studies included in this meta-analysis were reported to contain a large number of Chinese participants from the Linxian region [8, 9, 202], which is known for its high level of oesophageal cancer cases and it is unknown if local environmental factors have played a role in this association. Additionally, all of these studies failed to take into account supplementation (by far the more important dietary contributor) when dietary sources were examined. This thesis on the other hand, incorporated supplement use, oily fish consumption, annual D-UVB dose and average time spent outdoors to give an overall estimate of vitamin D status.

When examining interactions between confounding variables and risk of upper gastrointestinal cancer, no significant associations were found. This suggests that these confounders are not affecting this relationship and a relationship between vitamin D estimates (annual D-UVB and VDscore4) and oesophageal and gastric cancer risk is present. However, this should be examined in a larger cohort designed with this research question in mind before conclusions can be drawn.

6.5.2 Oesophageal and Gastric Cancer Survival Analysis in Survival Cohort

As discussed in the literature review, there has been little research into the association between vitamin D and survival of oesophageal and gastric cancer. No study has examined the association in oesophageal cancer and vitamin D, and only one study has examined the relationship between vitamin D and gastric cancer survival [190].

The relationship between vitamin D and survival of oesophageal and gastric cancer was first assessed separately. Next, both cancers were examined together as is frequently done due to their anatomical proximity and some shared aetiology. However, all findings should be interpreted with care because adjustment for stage was possible as this information was not available.

No association between annual D-UVB or VDscore4 and upper gastrointestinal cancer survival was found. Similar results were found when stratified by cancer type and cancer subtype. Lack of adjustment for stage [a key determinant of survival] may have affected the statistical models and ability to detect associations. No significant association was found when examining the relationship between annual D-UVB, VDscore4 and cancer-specific mortality. This was also true

for all-cause mortality. As no study has exclusively examined vitamin D and oesophageal cancer survival or its subtypes, even this null result is novel.

A study by Ren *et al.* found increased survival of gastric cancer in those with higher 25(OH)D concentrations. The study conducted here found a significant association between tertile two of VDscore4 and decreased all-cause and cancer specific mortality when compared to lower tertiles, however, this association was not present in highest tertile of VDscore4. Unfortunately, it is difficult to compare these two studies as the comparators were different, as Ren *et al.*, examined the differences in survival between sufficient and insufficient individuals, while this study compared VDscore4 tertiles. Additionally, no defined threshold of deficiency can be established yet using VDscores, and further research is needed to establish this. Therefore there may be some evidence that gastric cancer mortality is increased in those with lower VDscores and deficiency in 25(OH)D but further research is needed before conclusions can be drawn.

While published studies are scarce and current analysis limited, overall, these results are in agreement with previous results: no overall association was found for primary upper gastrointestinal cancers or oesophageal cancers, and an increase in gastric cancer mortality was found for those with low VDscores. However, further studies are needed which can examine this relationship using both 25(OH)D concentration and VDscores which are adjusted for cancer stage. These results highlight the need for cancer specific and subtype-specific analysis when carrying out observational studies.

6.5.3 Implications of this Research

This research demonstrated that annual D-UVB and VDscore4 are associated with a reduced risk of oesophageal and gastric cancer. This finding adds to sparse literature on the topic of vitamin D and oesophageal or gastric cancer risk. Furthermore, most of the studies to date which have found an inverse association between vitamin D and upper gastrointestinal cancers have been ecological studies, which are flawed due to their ecological nature. This study also found a strong relationship in a high latitude country which suggests that D-UVB is important even when it is not present at high doses. However, this study was carried out using vitamin D estimates (annual D-UVB and VDscore4). Therefore, it is imperative that further research in a larger cohort is carried out. This future study should examine the relationship between vitamin D and the *risk* and *survival* of all subtypes of upper gastrointestinal cancer e.g.: oesophageal adenocarcinoma, oesophageal squamous cell carcinoma, cardia and non-cardia gastric cancer. Furthermore, this

relationship should be examined using multiple measures of vitamin D in order to compare results from each, in order to increase the strength of the study, as all current measures of vitamin D have flaws.

The results of this study also suggest the potential benefit of using annual D-VB and VDscore4 as estimates for vitamin D status, for example, when 25(OH)D concentration is unavailable. Furthermore, using annual D-UVB, gives an estimation of longer term vitamin D status and provides a non-seasonally biased estimation of vitamin D. This estimate also has the potential to be used alongside 25(OH)D concentrations when available, in order to ensure that the seasonality aspect of 25(OH)D is correctly accounted for. If similar results are found for both estimates separately, this would give further strength to a study.

Moreover, this VDscore4 is simple and offers a method of estimation of vitamin D status which is inexpensive, incorporates the most important sources of vitamin D over a longer time period and has the potential to be used, in a number of different populations. This is unlike previously predicted vitamin D estimates which often use coefficients from regression analysis which could change form one cohort to another, or 25(OH)D concentrations which can be expensive, seasonally biased and only estimates vitamin D status at one particular point in time. VDscore4 could be further improved with more detailed dietary and lifestyle information.

The results from this study are important for sun exposure guidelines. As mentioned in previous chapters, there are mixed messages with regards to sun exposure being communicated to the public, with some, including the HSE suggesting that seeking UVB exposure to obtain vitamin D is unnecessary [246]. However, this chapter has demonstrated that higher annual UVB might reduce the odds of developing oesophageal and gastric cancer. High exposure to UVB is detrimental to health, however, this study offers evidence that some UVB exposure might be beneficial for some aspects of health. Perhaps strict sun avoidance practices which are often publicized in order to reduce skin cancer rates, are not as helpful as publicising the need for more controlled UVB exposure. It is hoped that this research will be able to demonstrate to policy makers that controlled exposure of D-UVB, rather than strict avoidance, is what is needed. This research can also help illustrate the importance of vitamin D supplementation during some parts of the year, especially in countries where D-UVB exposure is low.

6.5.4 Strengths and Limitations

The strengths in this study lie with the use of a very large cohort with extensive data on many aspects of health and comprehensive information of many factors which impact vitamin D

status. 25(OH)D concentration was not available in this cohort, however, using an accurate method of estimating individual ambient D-UVB dose, along with other important vitamin D related variables, simple vitamin D estimates which take into account UVB dose, time spent outdoors, supplementation use and oily fish consumption were created.

Furthermore, this study was able to individually examine the risk and survival of different upper gastrointestinal cancers and subtypes. This is important due to the differing aetiologies between cancer types and subtypes. A further strength of this chapter is the novel research into vitamin D and the survival of oesophageal cancer which has not been examined in detail previously.

This study was strengthened by assigning controls to cases in a 1:5 ratio, which increases the precision and statistical power of the study. This is also the largest study to date examining both the impact of vitamin D in the survival of oesophageal and gastric cancer, although no adjustments for cancer stage could be made in this analysis.

In this study, all cases and controls who had received a diagnosis of skin cancer, including non-melanoma skin cancer were excluded. As an association between UV exposure and skin cancer is well established [44, 45], by excluding all skin cancer cases, individuals with greater exposure to D-UVB would also be selected out. As these individuals were not included in this analysis, this may have reduced the statistical power of the study; as those with the highest D-UVB exposure were excluded and inclusion of this group could have strengthened the effect sizes of a protective effect.

Supplementation has been shown to be one of the most important sources of vitamin D in high latitude countries [255]. Using this knowledge, along with results from the TUDA cohort which suggested supplementation was the most important determinant of vitamin D, this study placed the most emphasis on this variable when calculating the Vitamin D scores. However, due to the limited number of people taking supplements in this cohort (4%), all of them were allocated to the “tertile 3” group. Due to this, the differences in quartiles of cw-D-UVB group, sun enjoyment and oily fish status were effectively lost in this group and supplementation was driving the results, this could be considered a limitation of this study. In addition, these individuals might represent a particular sub-population in terms of diet and lifestyle and it could be these factors which are in fact reducing their odds of cancer development, rather than vitamin D.

A further limitation of this study is the lack of 25(OH)D concentration for this cohort. However, unless adequately adjusted for season, this measurement may not be the best measurement to use when examining cancer. Cancer is a slowly developing disease and a one-time 25(OH)D measurement or other seasonally biased proxy of vitamin D status might not give a

representative portrayal of vitamin D status over a longer period of time, especially when examining a disease which can take years to develop. However, if accurately adjusted this measurement would have been useful to test associations with upper gastrointestinal cancer risk and survival. Furthermore, this measurement could have been used to examine the relationship between 25(OH)D concentration and Vitamin D scores in a very large cohort.

As mentioned previously, the assessment of vitamin D status is quite complex. There are many types of estimates, and very few of these give a comprehensive picture of vitamin D. Due to the seasonal nature of vitamin D, and the fact it can be obtained from multiple sources, along with the differences in its synthesis between individuals, an accurate assessment of vitamin D status is almost impossible, especially for large heterogeneous cohorts.

Estimates attempting to incorporate multiple sources over a year long period to negate the temporal aspect of vitamin D measurement are a viable option [145]. These type of measurements could include multiple measures of 25(OH)D too, however this is rarely carried out. Annual D-UVB and VDscore4 are examples of estimates which attempt to fulfil the needs of a "non-seasonally biased vitamin D estimate" which can be used in a large cohort. These measurements are not without their own flaws.

As with all estimates, there is a degree of uncertainty involved. These are proxy measurements which rely on certain assumptions to estimate vitamin D. For example, annual D-UVB makes the assumption of constant utilisation of the ambient UVB dose available and that the dose available is correlated with the dose which is absorbed by individuals. This is not often the case as time spent outdoors and clothing coverage have a dramatic impact on absorption of UVB and may differ throughout the year. This estimate also assumes that the same dose of UVB results in similar changes in vitamin D concentration in all individuals. However, this is not the case as older or darker skinned individuals may need higher levels of UVB in order to synthesis similar levels of vitamin D.

VDscore4 also relies on some assumptions; for example, this estimate accounts for oily fish intake but ignores other dietary source of vitamin D. Additionally, differing levels of consumption of this variable are treated as negligible as the same score was given to those who eat oily fish daily and those who only eat it only once weekly. This is also true for the supplementation aspect, as no information on supplementation dose was available in this cohort. Due to this, those taking any dose of vitamin D supplements are treated equally in this score, and presumed to have similar levels of vitamin D. However, this would not be the case as those taking higher doses, such as 1000 IU/d would most likely have a higher vitamin D status than those only taking the recommended daily dose of 400 IU/d. Therefore, this assumption is

a limitation of this study. In addition, supplement use, oily fish consumption and time spent outdoors were all self-reported in this study and this score assumes that this information was accurately reported by participants, however this may not have been the case.

The implications of these assumptions are important as they could be over- or under-estimating the vitamin D status. For example, assuming similar utilisation of UVB for all individuals means that overestimation is likely on those who spend a below-average amount of time outdoors, or those who are covered up with little skin exposed. This assumption was one reason why VDscore4 was developed as it can adjusted, albeit crudely, for utilisation of UVB.

Although these estimates can over and under estimate levels of vitamin D, there is currently no adequate alternative to deal with these issues. Accurate vitamin D status assessment without the use of monthly blood measurements is impossible, and as such, proxy estimations which account for UVB, dietary sources, and supplementation use are currently the most feasible approach, particularly for research in large cohorts. In order to get the best estimation of vitamin D, what is needed is a specifically designed large cohort with detailed information on supplementation use, supplement dose and dietary vitamin D. Not all of this information was available in this cohort and this is a limitation of this study. More detailed information about vitamin D should be used in future studies.

There is a further issue with using vitamin D proxy measures which also needs to be addressed and this is the assumption that variables which impact upon the relationship between vitamin D and cancer risk or survival, will also impact upon this relationship when a vitamin D proxy measurement is used. This might not always be the case. It has been shown in previous chapters that VDscores and cw-D-UVB are highly associated with 25(OH)D and these proxy measurements are also related to personal characteristics of individuals, in a somewhat similar manner as 25(OH)D. However, it is not known if this is also the case with VDscore4 or annual D-UVB. For example gastric-ulcers were found to be associated with VDscore4 but these are also risk factors for cancer occurrence. It is unknown however, if this variable would be a confounder if 25(OH)D was measured. Assuming that variables which impact 25(OH)D also effect VDscore4 or annual D-UVB in the same manner, or vice versa, is a limitation of the use of proxy measurements and therefore of this chapter. This adjustment could therefore lead to under adjustment or unnecessarily adjustment of this model. Unnecessary adjustment can lead to a decrease in precision of an estimate, while under-adjustment can lead to the presence of confounding variables as these are not adjusted for. Both of these issues can have an impact upon the accuracy of the model.

Annual D-UVB was calculated based on the date of attendance of participants to their biobank interview. As this was an annual estimation, the temporal aspect of UVB exposure did not impact upon the UVB-dose assigned to each individual; this estimate was solely based upon their place of residence. It was presumed that the majority of individuals would reside at that location for the duration of the study. This assumption is another limitation of this study.

The main advantage of calculating annual D-UVB based on time of attendance was that VDscore4 could be calculated for participants in this cohort. For the risk analysis this was a beneficial approach as this thesis was examining UVB and lifestyle factors of individuals not long before they developed cancer (median time from recruitment to cancer diagnosis was 3.09 years). As D-UVB does not change dramatically from year to year at any given location, this estimate would have also been predictive of UVB directly before their diagnosis (assuming they resided at the same location). However, as information about location of residence is only available for the date of biobank attendance, and it is not known if participants may have changed location in the time between attendance and cancer diagnosis. This is a limitation of this approach, as any change in location would have results in an altered D-UVB dose prior to diagnosis and could lead to misclassification of some individuals.

For the survival analysis, this approach was appropriate as any dietary or lifestyle changes which may have occurred after diagnosis, such as taking more of an active interest in their heath, would have been captured accurately in VDscore4 as these individuals were recruited after their diagnosis.

As the overall biobank cohort was substantial, a modest number of people who had been diagnosed with oesophageal and gastric cancer were able to be selected, either prior to or after their biobank interview. As these are considered relatively rare conditions, sample sizes in studies using these cancer types are often small or are made up of a number of combined cohorts.

Due to the large sample size and variability of recruitment location for this cohort, it should be representative of the UK as a whole. However, this may not have been the case; there may have been some selection bias with this cohort as individuals who sign up to be a part of such a cohort may have a greater interest in their own health and this may have impacted on the representativeness of the cohort. For example, the incidence rate of oesophageal cancer in the UK in 2015 was found to be 0.6% of the overall population. However, the incidence rate of oesophageal cancer in this cohort was found to be 0.02% of the population. Therefore, this cohort may not have captured accurate prevalence of oesophageal or gastric cancer cases which would be representative of the UK as a whole. This impacts upon the generalisability of this

cohort and the reproducibility of the results in this chapter. Additional studies should be carried out to confirm the results shown here.

It has been mentioned that sample sizes for this analysis are larger or comparable to sample sizes in other published studies on this topic, and in fact this study is the second largest study to date examining the relationship between vitamin D and upper gastrointestinal cancer risk, however, the number of cancer cases included in this study are still fairly small. Due to this, the power of some subtype analysis may have been too small to determine if there is a statistical relationship present. This is a large limitation of this study and for future studies examining oesophageal and gastric cancer risk and survival, a large cohort is needed before any firm conclusion about an association between vitamin D and subtypes of these cancers can be made. The small numbers of cancer cases in this cohort should be considered when interpreting results.

Follow-up time for the survival cohort was sufficient: median time from cancer diagnosis to death/censoring date was 10.3 years. However, these participants had already been diagnosed with cancer at time of biobank recruitment and attendance for interview (median time from cancer diagnosis to biobank attendance was 3.4 years). Due to this, those who had severer cancers may have been selected out (either due to death or being unwell) and not recruited to the UK biobank. A consequence there was a smaller number of events within this group than expected (18% mortality overall) which may have been due to a survivorship bias. This may have, in part, been a reason why no significant results were found for the survival cohorts, as the number of events were too small. This should be taken into account when interpreting results from this chapter.

This chapter aimed to examine multiple things, the relationship between annual D-UVB, VDscore and cancer risk and survival. It also aimed to examine this relationship in upper gastrointestinal cancer overall and subtypes of cancer. This led to multiple analysis being undertaken. Due to this, issues with multiple testing need to be taken into consideration. Multiple testing increases the probability of the occurrence of type 1 error in the analysis. If the Bonferroni correction method was added to this analysis, then a p-value of less than 0.004 would be needed to prove significance for survival analysis and a p-value of 0.003 would be needed to prove significance in the risk cohort. None of the results reached this level of significance when examining the relationship between cancer survival and VDscore4 or annual D-UVB. However, this level of significance was observed in some instances when examining risk of upper gastrointestinal cancers. This suggests that multiple testing in this case was a limitation to some aspects of this chapter.

Another weakness to acknowledge is that the data used in this study was pre-collected data. Although this was fairly comprehensive, there are some variables which would have been useful. For example information on cancer stage was not available and as this is such an important determinant of cancer mortality, the lack of this adjustment is quite consequential. Cancer stage is heavily associated with cancer survival, those with cancer stages of 3 or 4 would have a worse baseline prognosis than those who have stage 1 or 2 cancer. At the same time there could be a relationship between UVB and cancer stage; those who have more advanced stages in cancer may have spent less time outdoors due to their illness and because of this they would have scored lower when their VDscore4 was generated. This lack of adjustment could lead to confounding. The strength of the relationship between cancer stage and mortality is likely to nullify any relationship which may be present between annual D-UVB or VDscore4 and mortality. Additionally, information on Helicobacter Pylori infection was not available, which is a risk factor for gastric cancer, and could have been useful when determining a relationship between VDscore4 and upper gastrointestinal cancers.

6.6 Conclusion

Higher tertiles of annual D-UVB and VDscore4 were strongly associated with reduced odds of developing upper gastrointestinal cancer, especially oesophageal cancers, even in a high latitude country. These results support the hypothesis that vitamin D may be beneficial in the prevention of these cancers. This study also highlights the potential beneficial effect of sunshine on health, if exposure was modest and moderate. Small amounts of exposure to low levels of D-UVB, along with supplementation use could be beneficial in reducing the development of upper gastrointestinal cancer. No overall association between annual D-UVB, VDscore4s, and survival of oesophageal or gastric cancer was found. However, further research needs to be conducted using multiple assessments of vitamin D in a large cohort before conclusions can be made.

7 UVB, 25(OH)D and Upper Gastrointestinal Cancer in an Irish Cohort

7.1. Aim

The aim of this chapter was three-fold. Firstly, as 25(OH)D was not available in the UK biobank, examining the relationship between 25(OH)D and upper gastrointestinal survival was not possible. This information was available in the Irish cohort of oesophageal and gastric cancer and investigating this relationship was the first aim of this chapter. Secondly, as this thesis previously examined the relationship between annual D-UVB and oesophageal and gastric survival in the UK biobank cohort, this relationship was also investigated in an Irish cohort in order to compare and contrast results. Finally, this chapter wanted to investigate the relationship between our vitamin D estimates and cancer survival, while looking at the impact weight loss has on this relationship.

7.2. Introduction

The beneficial effect of vitamin D on cancer risk and survival has been suggested in number of studies [5, 7]. While strong inverse associations for both annual D-UVB and VDscore4 were found when examining the odds of developing upper gastrointestinal cancer in a cohort of participants from the UK, no association between annual D-UVB or VDscore4 and the survival of upper gastrointestinal cancer were found. This lack of association may have been due to the lack of adjustment for important cancer related variables, most notably cancer stage.

Using an Irish cohort of oesophageal and gastric cancer cases, the association between 25(OH)D concentration and annual D-UVB, and cancer survival will be investigated. The key difference between the analyses carried out in this chapter compared to chapter six is that 25(OH)D concentrations and cancer stage were available for this Irish cohort.

Furthermore, as vitamin D is a fat soluble vitamin, which can be stored in the tissue, body size can affect the relationship between circulating 25(OH)D and the total vitamin D stored in the body [312]. Subjects who are overweight or obese have been consistently shown to have lower circulating 25(OH)D than their normal weight counterparts, while they may have the same total amount of vitamin D [313]. This is an important aspect of vitamin D that needs to be taken into account when examining health outcomes which are related with obesity, such as upper gastrointestinal cancers [314], but has not been previously done. Furthermore, 25(OH)D has been shown to be released from adipose tissue into circulation following weight loss [315-319]. This is a further consideration which should be taken into account when examining 25(OH)D in

upper gastrointestinal cancers as weight loss within this cancer is common [320, 321] and associated with a poorer prognosis [322, 323]. Therefore, there could be an interesting relationship between weight loss, vitamin D and cancer survival within an upper gastrointestinal cancer cohort and this chapter also aimed to investigate this complex relationship in an Irish cohort.

7.3. Methods

7.3.1. Study Population

Data from an upper gastrointestinal cancer database at the Oesophageal and Gastric Centre in St James's Hospital, Dublin was used for this study (2008-2014). All samples from this biobank which had serum samples available in 2014 were included in this study and had their 25(OH)D concentration measured. This is biobank is continuously collecting data, and new participants are being added. At the time when this study was initiated, 610 patients would have been registered in the database but 265 patients were included in this analysis; 210 with oesophageal adenocarcinoma or squamous cell carcinoma, and 55 with gastric adenocarcinoma. Serum sample, necessary for 25(OH)D assessment was not available for the remainder. Exclusion criteria included being pregnant, HIV or Hepatitis C positive and a previous history of upper gastrointestinal cancers. Ethical approval was obtained from the joint St James's Hospital/AMNCH ethics committee. All participants gave written informed consent. Blood and tissue samples were taken from participants on the morning of surgery (which was typically 1-9 months post diagnosis). Hospital records were first examined for mortality data by the biobank's data manager. Mortality was coded as follows: died of disease, died of other causes, post-operative death, died cause unknown, no-evidence of disease, and alive. If hospital records did not have information on death or follow-up, recent attendance at outpatient departments and an online directory of death notices in Ireland (RIP.ie) were examined to determine the survival status of patients. Finally, if this was unsuccessful, the patient's GP was contacted for further follow-up information. Follow up time was calculated from date of diagnosis to date of death or last follow up, if the patient was not known to have died (or date of death due to non-cancer-related deaths in cancer-specific mortality analysis). Censoring date was the 27th July 2015. Cancer specific death was coded as those who had "died of disease" while all-cause mortality was coded as all those who had "died of disease", "died of other causes", "died cause unknown" and those who had a post-operative death.

The majority of information was collected using hospital documentation (patients' medical charts, hospital databases and multidisciplinary team meetings) prior to surgery. Self-reported symptoms (weight loss [yes/no], dysphagia [ability to eat anything, partial solids, or liquids only], epigastric pain, nausea etc.) and BMI (calculated using height and weight measurements) were recorded by a physician at first presentation shortly after cancer diagnosis which was typically 1-9 months prior to surgery and blood collection. Information regarding lifestyle, including smoking (past smoker, current smoker, and non-smoker) and alcohol use (non-drinker, heavy drinker, social drinker, and ex-drinker) was also collected. Overall cancer stage was determined

by a physician following numerous staging investigations, including but not exclusively by oesophago-gastro duodenoscopy, endoscopic ultrasound, PET scan, laparoscopy and cytology, and fine needle aspiration of lymph nodes.

7.3.2. 25(OH)D Measurement

Serum was prepared from peripheral blood. Total 25(OH)D ($25(OH)D_2$ and $25(OH)D_3$) was measured from serum samples by LC-MS/MS. All samples were assayed at the Biochemistry Department of St James's Hospital, Dublin, Ireland, which is verified by the Vitamin D External Quality Assessment Scheme and National Institute of Standards and Technology from initial 25(OH)D measurements taken randomly throughout the year. While 25(OH)D is considered to be the best estimation of vitamin D status as a specific point in time, using a point estimate without adjustment for season is not an accurate measure of year-round vitamin D status, due to the seasonal fluctuations of this vitamin [145, 324, 325]. This is especially important when examining the effect of vitamin D on slowly developing health outcomes such as cancer. Adjustment for month of blood draw has been shown to be an effective method of accounting for seasonality. Wang *et al.* found that accounting for month of blood draw when determining categories of 25(OH)D is the best method as it reduces bias towards the null and also does not introduce bias away from the null [145]. This approach was used in this thesis: May-adjusted 25(OH)D was calculated. This was carried out to give a longer-term average of vitamin D in individuals which is not seasonally biased. This adjustment was carried out by using regression to find the mean differences between 25(OH)D values in May, and all other months of the year (while adjusting for age and sex); this difference (the beta coefficient) was then used to adjust the 25(OH)D concentration, given the month of blood draw, as has been done previously [255] (**Figure 7.1**). This scaled the 25(OH)D concentrations so that they were all similar to concentrations taken in May.

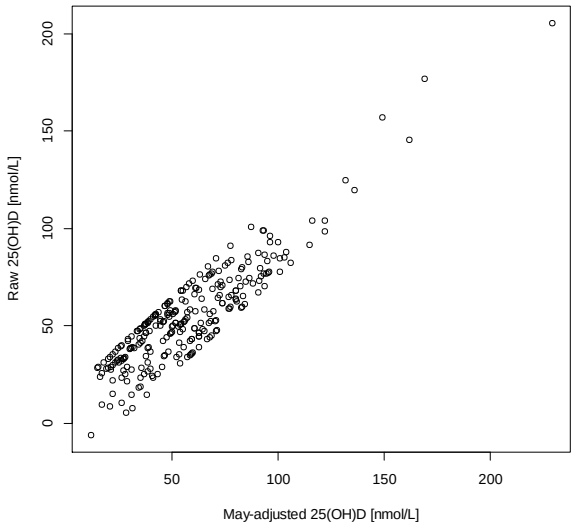

Figure 7.1: Raw 25(OH)D vs May-adjusted 25(OH)D

7.3.3. Annual D-UVB Dose

The TEMIS database which records daily D-UVB doses on the island of Ireland from 2005-2015 was used in this study. Annual D-UVB was calculated in the period from six months prior to diagnosis to six months after diagnosis. From the original 265 patients, 7 were excluded as their residential address was unknown. A further ten participants were excluded from this analysis as they did not survive for six months post-diagnosis. Therefore in total 248 patients were examined when investigating annual D-UVB. When examining cancer stages 1-3 the total sample size was 229. Annual D-UVB was determined in a similar manner as previously described, whereby daily D-UVB doses were extracted for each individual based on their residential location for 365 days, from six months prior to diagnosis. Annual D-UVB was chosen as it covers a 1-year cycle and anything greater or less than one year would bias the estimate. This time period was chosen for a number of reasons. Firstly, when examining cancer survival it is important to estimate vitamin D or UVB around the time of diagnosis, rather than years prior to diagnosis as UVB dose or vitamin D concentration may change following diagnosis [326]. Secondly, as 25(OH)D was taken shortly after diagnosis, it was necessary to calculate UVB dose around a similar time frame so that direct comparisons could be made between the estimates. As some individuals did not live past one year following 25(OH)D blood draw, these individuals would have had to be left out if the exact same date was chosen for D-UVB measurement and 25(OH)D concentration, as D-UVB is estimated based on past UVB exposure. Due to small sample size it was important to try include the highest number individuals possible. Additionally,

as this thesis is examining *annual* D-UVB in this cohort, location is what is determining D-UVB dose. As most individuals are unlikely to move location within a 6 month period of getting diagnosed with cancer, even if D-UVB was calculated on day of diagnosis, 6-months prior or 6-months after, the difference in D-UVB dose would be negligible.

Annual D-UVB doses were then split into tertiles. As annual doses of D-UVB do not change dramatically from year to year, annual D-UVB would be correlated with annual D-UVB doses a year later. Information on vitamin D supplement use, dietary vitamin D sources or time spent outdoors was not available for this cohort and as such VDscore4 could not be calculated for this cohort.

7.3.4. Statistical Analysis

All analyses were performed in R (R Development Core Team, 2011) and using the R-package 'Survival' (Thomas Lumley, 2015). Mean (SD), median (IQR) and proportions are given to describe the data. 25(OH)D tertiles were established based on raw and May-adjusted 25(OH)D concentration (lowest tertile being the reference). Tertiles were also calculated for annual D-UVB dose. Kaplan-Meier survival curves were used to illustrate the effect of vitamin D status (and other variables) on upper gastrointestinal cancer-specific and all-cause mortality. Cox proportional hazards models were used to estimate hazard ratios after adjustment for important covariates. All hazard ratios were checked for proportionality through examination of Schoenfeld residuals. If this was not the case, an interaction of tertiles with a time component was added to these models. Covariates considered were: age, sex, alcohol intake, smoking status, dysphagia score, cancer stage, cancer type (oesophageal or gastric), cancer subtype (oesophageal adenocarcinoma or oesophageal squamous cell carcinoma), if they had chemotherapy, dysplasia, co-morbid disease, number of days spent in the ICU and weight loss. The final model was chosen using backwards stepwise regression based off of AIC, BIC, R^2 value and the maximum number of participants (**Table 7.1**). The final model contained age, sex, alcohol intake, smoking status, dysphagia score, cancer stage, cancer type and cancer subtype. Primary analysis was carried out on all cancer stages 1-3. Subtype analysis was excluded if there were less than 10 participants in any tertile. The cancer stages were restricted as stage 0 is not a true cancer stage but high grade dysplasia, cancer-specific mortality is very low among these cases. On the other hand, in stage four cancer cases the cancer had spread to distant sites and consequentially this group of patients is very heterogeneous. Furthermore, this group may also have had different treatments or have made significant lifestyle changes and therefore

participants in this group would have been considerably different from each other. However, we did include this group in some secondary analysis which is shown in the appendices.

As before, due to the similar risk factors for upper gastrointestinal cancers, both combined and individual analysis was conducted in this chapter, as has been done previously in numerous studies [8, 302].

Stratified analysis by cancer type, cancer subtype and weight loss was undertaken. $P<0.05$ was considered statistically significant.

Table 7.1: Model selection for survival analysis

Model	R^2	AIC	BIC	Missing data
1[1]	0.29	212.70	245.52	205
2[2]	0.38	535.10	585.80	128
3[3]	0.33	764.72	812.86	63
4[4]	0.30	898.55	946.87	41
5[5]	0.30	896.57	942.36	41
6[6]	0.30	903.42	946.84	40
7[7]	0.28	903.20	944.10	40

Footnote:

1 *Model 1* contains: Sex + age at diagnosis+ Alcohol Intake+ dysphagia+ Weight loss +BMI + Cancer stage+ cancer type + cancer subtype+ Number of Days in ICU + Co morbid disease + Smoking Status + Chemotherapy + Dysplasia + annual UVB/25(OH)D

2 *Model 2* contains: Model 1 minus Dysplasia

3 *Model 3* contains: Model 2minus BMI

4 *Model 4* contains: Model 3 minus days spent in the ICU.

5 *Model 5* contains: Model 4 minus any co-morbid disease.

6 *Model 6* contains: Model 5 minus chemotherapy.

7 *Model 7* contains: Model 6 minus weight loss.

Model 8 was chosen as there was very little difference in the R^2 between model 7 and 8, but the AIC and BIC were lower in model 8. Model 8 contained sex, age at diagnosis, alcohol intake, dysphagia, cancer stage, cancer type, cancer subtype, smoking status and annual UVB/25(OH)D

7.4.Results

7.4.1. Baseline Characteristics

Baseline characteristics of the cohort are shown in **Table 7.2**. In total, 265 patients (68 female, 26%) were included in this study; median age of participants was 66y (range: 29-87y). Blood measurements were spread throughout the year, although 57% were taken in summer and autumn. Median 25(OH)D concentration was 54.2 nmol/L (IQR: 37.6-72.8) and is comparable to other Irish studies [276, 327]. A large proportion of the cohort had stage two or three cancer (37% and 35%), and a considerable percentage of the cohort was overweight or obese (N=136; 51%). Additionally, 136 (51%) reported weight loss as a symptom. In total 109 (41%) died from the disease and 20 (7%) died from other causes. Median follow up was 2.34 years (IQR: 1.3-4.5 years) (**Table 7.2**). As expected, cancer stage was a major determinant of cancer mortality (**Figure 7.2**).

Table 7.2: Baseline characteristics of cohort[1]

Characteristics	**All**	**25(OH)D**			**Weight loss**	
		Tertile 1	**Tertile 2**	**Tertile 3**	**Yes**	**No**
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
25(OH)D (nmol/L; median & IQR)	54.2 (38-73)	31.1 (25-42)	51.3 (43-63)	81.5 (69-95)	55.5 (37-74)	52 (43-63)
			P<2e-16 [2]		*P=0.79* [2]	
Annual D-UVB (mJ/cm^2; median & IQR)	31928 (31245-32622)	30990 (30430-31260)	31960 (31790-32180)	32910 (32660-33310)	31970 (31340-32600)	31880 (31180-32650)
			P<2e-16 [2]		*P=0.19* [2]	
Age (years; median & IQR)	66 (59-72)	66 (59-74)	66 (59-72)	66 (59-73)	65 (59-72)	68 (60-73)
			P= 0.89 [2]		*P= 0.29* [2]	
Sex						
Female	68 (26)	27 (37)	23 (36)	18 (27)	34 (51)	33 (49)
Male	197 (74)	64 (32)	65 (32)	68 (36)	102 (53)	92 (47)
			P= 0.41		*P=0.80*	
Subtype						
OEAC[3]	172 (64)	52 (30)	60 (35)	60 (35)	90 (53)	79 (48)
OESCC[3]	38 (15)	12 (31)	12 (31)	14 (38)	20 (50)	18 (50)
Gastric	55 (21)	27 (48)	16 (30)	12 (21)	26 (49)	28 (51)
			P= 0.12		*P= 0.81*	
Cancer Grade (NA=2, <1%) [4,5]						
Stage 0	7 (3)	1 (14)	2 (29)	4 (57)	1 (14)	6 (86)
Stage 1	51 (21)	17 (31)	19 (35)	18 (35)	10 (19)	43 (81)
Stage 2	95 (37)	34 (33)	37 (37)	28 (29)	51 (51)	47 (48)
Stage 3	83 (35)	36 (38)	26 (28)	31 (34)	65 (72)	26 (28)
Stage 4	10 (4)	2 (20)	3 (30)	5 (50)	8 (80)	2 (20)
			P= 0.57		***P= 5.8x10^{-9}***	
Barrett's Oesophagus (NA=79, 30%)						
Yes	103 (39)	31 (30)	35 (35)	37 (35)	44 (44)	57 (56)
No	83 (31)	25 (29)	30 (37)	28 (34)	54 (65)	29 (35)
			P= 0.94		***P= 0.004***	

Characteristics	All	25(OH)D			Weight loss	
		Tertile 1	Tertile 2	Tertile 3	Yes	No
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Dysplasia (NA=150, 57%)						
Yes	63 (24)	15 (23)	26 (41)	23 (36)	24 (39)	39 (61)
No	52 (19)	15(29)	22 (44)	14 (27)	32 (64)	18 (36)
			P=0.59		***P= 0.01***	
Alcohol Consumption (NA=16, 6%) [5]						
Non-drinker	72 (27)	26 (34)	19 (29)	27 (37)	32 (46)	39 (54)
Ex-drinker	12 (4)	7 (58)	2 (17)	3 (25)	5 (42)	7 (58)
Social drinker	133 (50)	39 (29)	46 (34)	48 (37)	70 (53)	62 (47)
Heavy Drinker	32 (13)	14 (44)	14 (41)	4 (16)	21 (66)	11 (34)
			P= 0.06		*P= 0.23*	
Smoking Status (NA=10, 4%)						
Current smoker	61 (23)	26 (43)	22 (34)	13 (23)	43 (70)	18 (30)
Never smoker	87 (32)	28 (31)	27 (33)	32 (36)	38 (45)	48 (55)
Past smoker	107 (41)	35 (32)	35 (32)	37 (35)	50 (47)	56 (53)
			P= 0.32		***P= 0.003***	
Season of Blood Draw [6]						
Winter	62 (23)	21 (34)	24 (39)	17 (27)	31 (52)	29 (48)
Spring	52 (20)	16 (28)	18 (34)	18 (38)	27 (53)	25 (47)
Summer	69 (26)	27 (39)	19 (27)	23 (34)	38 (51)	30 (49)
Autumn	82 (31)	27 (33)	27 (33)	28 (33)	40 (49)	41 (51)
			P= 0.85		*P= 0.89*	
Weight loss (NA=4, 1%)						
Yes	136 (51)	50 (37)	39 (29)	47 (34)	-	-
No	125 (47)	40 (31)	49 (40)	36 (29)	-	-
			P= 0.20		-	
BMI (NA=53, 20%)						
Under/Normal (<18.5-24.9)	76 (36)	25 (32)	22 (29)	29 (39)	50 (65)	26 (35)
Overweight (>25)	77 (36)	24 (31)	26 (34)	27 (35)	40 (52)	37 (48)
Obese (30-39)	59 (28)	21 (35)	24 (42)	14 (23)	23 (40)	36 (60)
			P= 0.43		***P=0.008***	
Dysphagia score (NA=33, 13%)						
Able to eat anything	111 (42)	41 (37)	39 (36)	31 (27)	41 (37)	70 (63)
Eat partial solids/only liquids	121 (45)	38 (31)	40 (33)	43 (36)	81 (67)	40 (33)
			P= 0.44		***P= 4.8x10^{-6}***	
Follow-up (days; median & IQR)	854 (468-1627)	815 (477-1506)	1107 (657-1767)	685 (374-1332)	877 (367-1353)	1006 (664-1707)
			P= 0.004 [2]		***P= 0.0002*** [2]	
Survival						
Alive	136 (51)	40 (29)	54 (41)	42 (29)	50 (37)	84 (63)
Dead	129 (49)	51 (38)	34 (25)	44 (38)	86 (68)	41 (32)
			P= 0.073		*P=6.0x10^{-7}*	
Cancer	109 (85)	41 (38)	31 (28)	37 (34)	73 (67)	*35 (33)*
Other	20 (15)	10 (50)	3 (15)	7 (35)	13 (65)	*6 (35)*
			P= 0.37		***P= 2.6x10^{-5}***	

Footnote:

1 Chi square tests were done on the majority of the characteristics

2 Anova tests carried out to examine differences between tertiles of 25(OH)D and weight loss

3 OEAC: oesophageal adenocarcinoma, OESCC: oesophageal squamous cell carcinoma

[4] Cancer grade: TMN clinical grade;
[5] Stages 0 and 1 and 3 and 4 collapsed together.
[6] drinking status: social drinker (<14 UPW - female <21 UPW - male); heavy drinker (>14 UPW - female >21 UPW - male);
[7] Seasons: winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug), autumn (Sep-Nov).

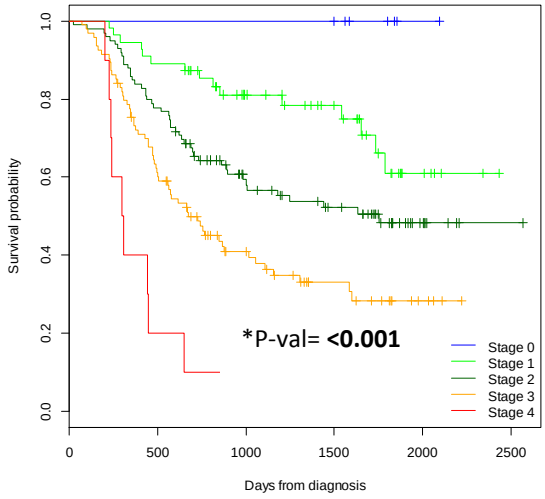

Figure 7.2: Kaplan-Meier curve for cancer-specific mortality by stage.

[1] *log rank test

7.4.2. 25(OH)D and Survival

In brief, no evidence of the relationship was found between 25(OH)D and either all-cause or cancer-specific mortality in adjusted analysis (**Table 7.3**). When comparing highest *vs.* lowest May-adjusted 25(OH)D tertile, no significant associations were found for cancer specific or all-cause mortality, both when stages 1-3 were examined and when all cancer cases were included (**Table 7.3**, **Appendix 8: Table 1**). Contrastingly, when examining Kaplan-Meier curves a statistically significant trend towards better all-cause survival for those in May-adjusted tertile two was noted (**Figure 7.3**), however these are not adjusted for important cancer related variables.

In a Cox-proportional hazard model a significant increased risk of oesophageal adenocarcinoma cancer-specific mortality was observed for those in tertile three (n=162, HR=2.25, 95% CI: 1.06-4.76). A 125% increased rate of cancer death was found for this group when compared to tertile 1, however significance was lost when all-cause mortality was investigated (**Table 7.3, Figure 7.4**). Conversely, a suggestive protective association between 25(OH)D and cancer-specific oesophageal squamous cell carcinoma death was found for higher tertiles of 25(OH)D, when restricted to stages one to three (HR=0.22, 95% CI: 0.04-1.27), however, this association was also lost when all-cause mortality was examined (**Table 7.3**)**.**

When examining gastric cancer, a significant protective association was observed for cancer-specific mortality when comparing tertile two to tertile one (HR=0.04, 95% CI: 0.002-0.72) (**Table 7.3**). However, sample sizes within this group were small (n=14 cases in Tertile 2).

Table 7.3: Association between 25(OH)D and upper gastrointestinal cancer mortality (stages 1-3)

Cox proportional hazard analysis examining the effect of 25(OH)D in the survival of upper gastrointestinal cancer[1,2].

Vitamin D variable	Outcome	N	Tertile 1			Tertile 2					Tertile 3					P-trend
			N	% who died	HR	N	% who died	HR	95% CL	p-val	N	% who died	HR	95% CL	p-val	
	Cancer specific mortality															
All	*Raw 25(OH)D*[3]	246	81	41%	Ref	85	42%	1.17	0.68-2.02	0.57	80	40%	1.50	0.83-2.73	0.18	0.15
	May-adjusted 25(OH)D[4,5]	246	87	45%	Ref	82	34%	0.96	0.55-1.66	0.88	77	44%	1.62	0.94-2.80	*0.08*	0.19
Oesophageal	*Raw 25(OH)D*	197	55	44%	Ref	73	45%	1.21	0.67-2.20	0.53	69	41%	1.35	0.70-2.61	0.37	0.39
	May-adjusted 25(OH)D[4]	197	63	46%	Ref	68	38%	1.20	0.67-2.16	0.54	66	45%	1.63	0.88-3.00	0.12	0.41
OEAC	*Raw 25(OH)D*	162	47	45%	Ref	59	39%	0.97	0.48-1.96	0.93	56	43%	1.47	0.69-3.14	0.32	0.20
	May-adjusted 25(OH)D[4]	162	52	42%	Ref	56	36%	1.24	0.61-2.51	0.55	54	48%	2.25	1.06-4.76	**0.03**	0.19
OESCC	*May-adjusted 25(OH)D*[4]	35	11	63%	Ref	12	50%	0.74	0.19-2.85	0.66	12	30%	0.22	0.04-1.27	*0.09*	*0.06*
Gastric	*Raw 25(OH)D*	49	26	35%	Ref	12	25%	0.10	0.006-1.58	0.10	11	36%	1.25	0.19-8.05	0.81	0.56
	May-adjusted 25(OH)D[4]	49	24	38%	Ref	14	16%	0.04	0.002-0.72	**0.02**	11	36%	1.29	0.10-17.32	0.85	0.78
	All-cause mortality															
All	*Raw 25(OH)D*	246	81	52%	Ref	85	47%	0.98	0.60-1.60	0.93	80	48%	1.45	0.85-2.47	0.25	0.06
	May-adjusted 25(OH)D[4]	246	87	56%	Ref	82	38%	0.82	0.50-1.37	0.45	77	52%	1.66	0.99-2.79	*0.05*	*0.05*
Oesophageal	*Raw 25(OH)D*	197	55	49%	Ref	73	67%	1.20	0.68-2.10	0.52	69	48%	1.46	0.78-2.70	0.23	0.14
	May-adjusted 25(OH)D[4]	197	63	54%	Ref	68	43%	1.13	0.65-1.97	0.65	66	53%	1.72	0.97-3.06	*0.07*	0.12
OEAC	*Raw 25(OH)D*	162	47	51%	Ref	59	46%	0.94	0.49-1.80	0.85	56	46%	1.40	0.68-2.87	0.36	0.25
	May-adjusted 25(OH)D[4]	162	52	52%	Ref	56	39%	1.13	0.59-2.16	0.72	54	52%	2.01	0.99-4.05	*0.05*	0.19
OESCC	*May-adjusted 25(OH)D*[4]	35	11	63%	Ref	12	50%	0.71	0.20-2.62	0.61	12	58%	0.56	0.14-2.21	0.41	0.74
Gastric	*Raw 25(OH)D*	49	26	58%	Ref	12	25%	0.05	0.005-0.47	**0.009**	11	45%	0.53	0.10-3.00	0.48	0.79
	May-adjusted 25(OH)D[4]	49	24	63%	Ref	14	21%	0.05	0.006-0.41	**0.005**	11	45%	0.29	0.04-2.05	0.21	0.70

Footnote:

1 OEAC: oesophageal adenocarcinoma; OESCC: oesophageal squamous cell carcinoma.

2 Adjusted for age, sex, smoking status, alcohol intake, cancer stage, cancer type, cancer subtype and dysphagia score. (When raw 25(OH)D is used, this model is also adjusted for season of blood draw)

3 Raw 25(OH)D, IQR range per tertile: all: Tertile 1: 24.8-37 nmol/L Tertile 2: 48.6-59.3 nmol/L Tertile 3: 72.6-93.4 nmol/L; OES: Tertile 1: 26-38 nmol/L Tertile 2: 48.6-59.3 nmol/L Tertile 3: 72.7-93.1 nmol/L; OEAC: Tertile 1: 26.3-38.15 nmol/L Tertile 2: 49.3-59.6 nmol/L Tertile 3: 71.5-93.0 nmol/L;

OESCC: Tertile 1: 25.3-35.5 nmol/L Tertile 2: 47.9-58.9 nmol/L Tertile 3: 76.7-93.8 nmol/L; Gastric: Tertile 1: 21.0-35.4 nmol/L Tertile 2: 48.1-54.2 nmol/L Tertile 3: 71.2-95.6 nmol/L

[4] May-Adjusted 25(OH) D is adjusted as if all 25(OH)D was sampled in May. all: Tertile 1: 27.0-38.6 nmol/L Tertile 2: 48.4-56.6 nmol/L Tertile 3: 68.7-84.7 nmol/L; OES: Tertile 1: 28.2-39.3 nmol/L Tertile 2: 48.3-57.4 nmol/L Tertile 3: 68.2-84.5 nmol/L; OEAC: Tertile 1: 27.7-39.3 nmol/L Tertile 2: 48.3-60.4 nmol/L Tertile 3: 68.2-84.8 nmol/L; OESCC: Tertile 1: 32.2-38.9 nmol/L Tertile 2: 49.4-61.1 nmol/L Tertile 3: 69.2-80.0 nmol/L; Gastric: Tertile 1: 17.6-34.1 nmol/L Tertile 2: 51.0-54.5 nmol/L Tertile 3: 75.4-84.5 nmol/L

[5] All cox hazards were found to be proportional.

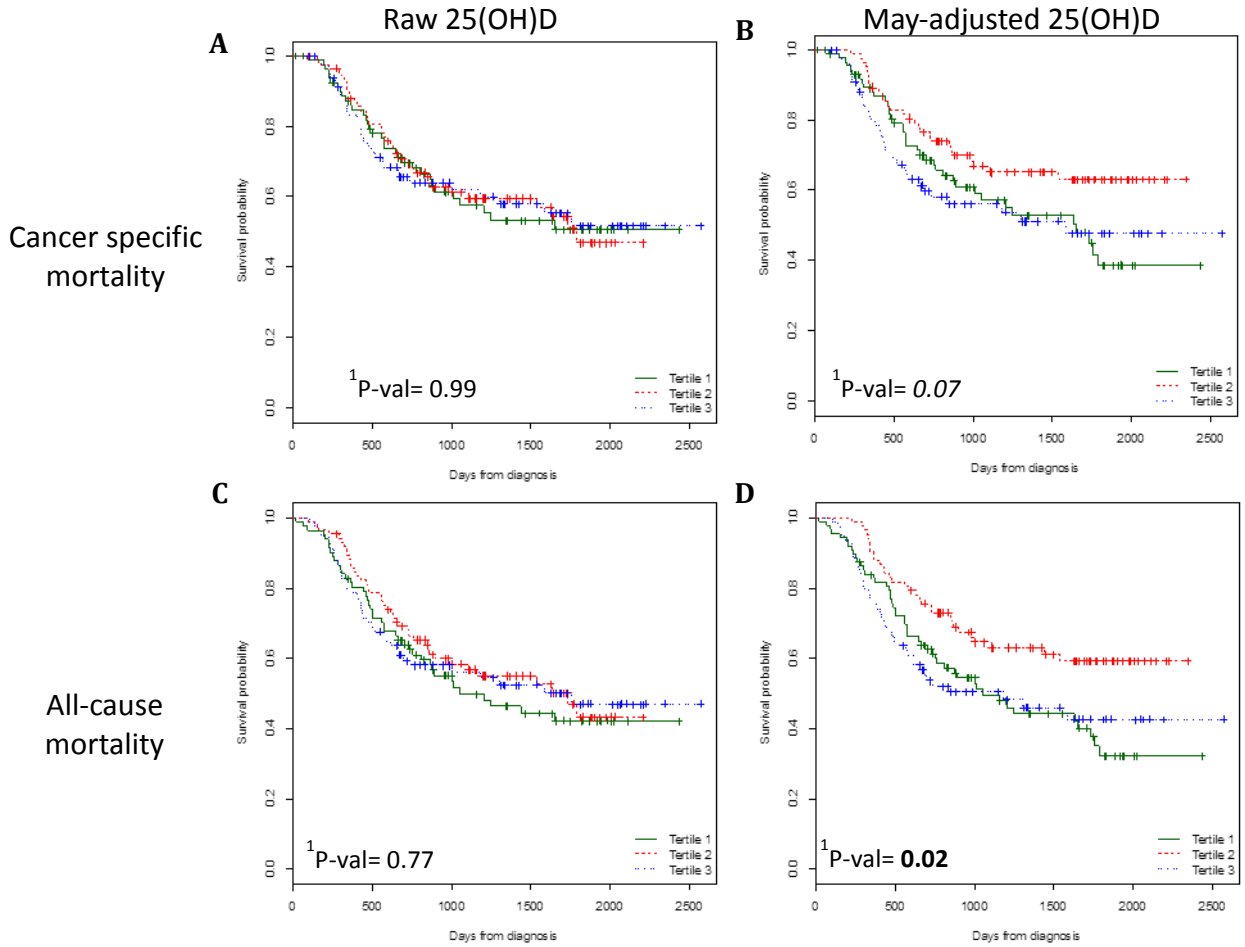

Figure 7.3: Kaplan-Meier analysis for survival using raw 25(OH)D and may-adjusted 25(OH)D (cancer stages 1-3)

Kaplan-Meier analysis per 25(OH)D tertile for **A)** Raw 25(OH)D cancer-specific mortality, **B)** May-adjusted 25(OH)D Cancer-specific mortality, **C)** raw 25(OH)D all-cause mortality, **D)** May-adjusted 25(OH)D all-cause mortality [1] log rank test. Tertile 1=green, Tertile 2= red, Tertile 3= blue. Raw 25(OH)D, IQR range per tertile: all: Tertile 1: 24.8-37 nmol/L Tertile 2: 48.6-59.3 nmol/L Tertile 3: 72.6-93.4 nmol/L; May adjusted 25(OH)D IQR range: Tertile 1: 27.0-38.6 nmol/L Tertile 2: 48.4-56.6 nmol/L Tertile 3: 68.7-84.7 nmol/L

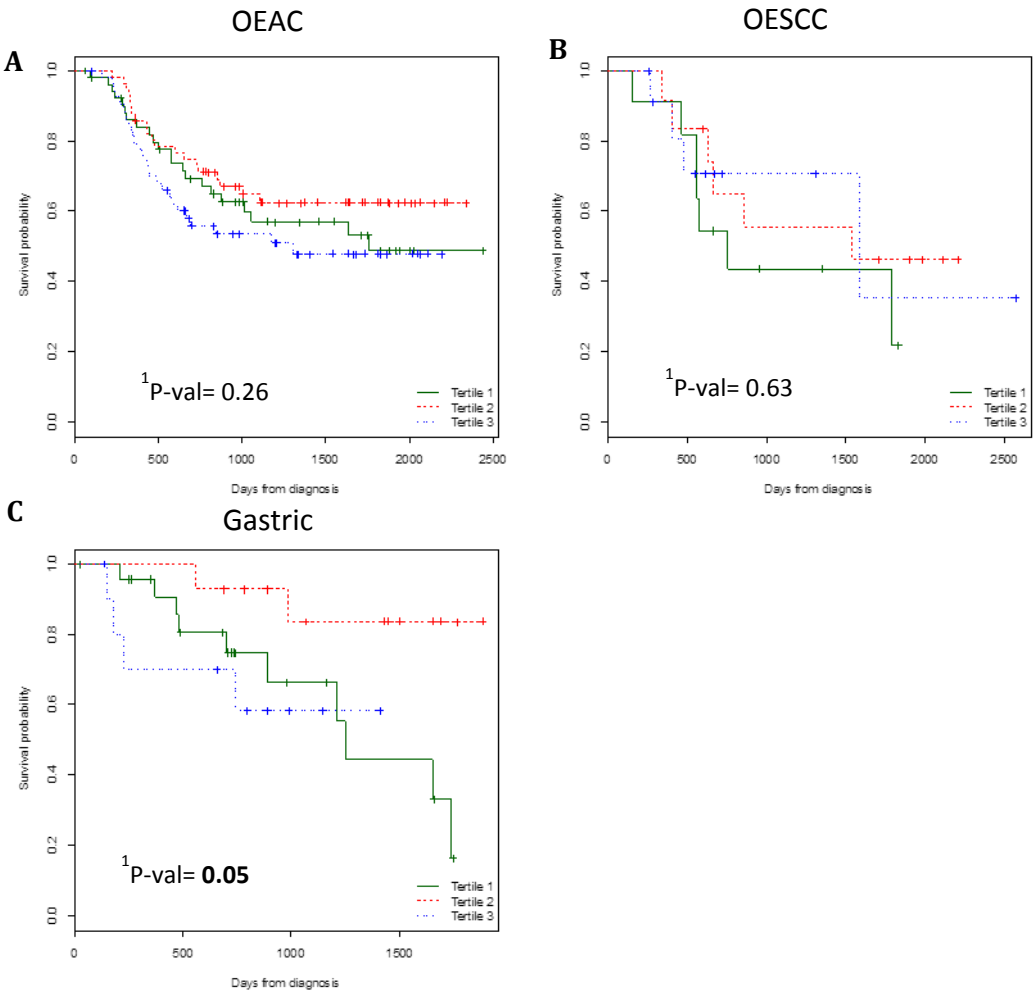

Figure 7.4: Kaplan-Meier analysis for survival using may-adjusted 25(OH)D and cancer-specific mortality stratified by cancer type (cancer stages 1-3)

Kaplan-Meier analysis for cancer specific mortality May-adjusted 25(OH)D stratified by **A)** Oesophageal Adenocarcinoma **B)** Oesophageal Squamous cell carcinoma**, C)** Gastric Cancer. [1] Log rank test. Tertile 1=green, Tertile 2= red, Tertile 3= blue. IQR range: OEAC: Tertile 1: 27.7-39.3 nmol/L Tertile 2: 48.3-60.4 nmol/L Tertile 3: 68.2-84.8 nmol/L; OESCC: Tertile 1: 32.2-38.9 nmol/L Tertile 2: 49.4-61.1 nmol/L Tertile 3: 69.2-80.0 nmol/L; Gastric: Tertile 1: 17.6-34.1 nmol/L Tertile 2: 51.0-54.5 nmol/L Tertile 3: 75.4-84.5 nmol/L

7.4.3. Annual D-UVB and Survival

When investigating the relationship between cancer mortality and annual D-UVB, no significant overall association was found using cox proportional hazard ratios or Kaplan Meier curves (**Table 7.4, Figure 7.5, appendix 8: Table 2**). In contrast to what was observed for 25(OH)D a suggestively reduced all-cause mortality was found comparing tertile 2 to tertile 1 in all gastrointestinal cases (HR=0.62, 95% CI:0.36-1.06) but this risk was increased for subtype analysis except gastric when examining tertile 3 (**Table 7.4**). This reduced risk in tertile 2 was found to be significant for both cancer-specific and all-cause mortality when all cancer cases were included (cancer specific: HR=0.54, 95% CI:0.31-0.96), but not in tertile 3 (**Appendix 8: Table 2**). When examining the Kaplan Meier curves no significant association was found in any subtype of cancer.

Table 7.4: Association between annual D-UVB and survival of upper gastrointestinal cancers (stages 1-3)

Cox proportional hazard analysis examining the effect ambient annual D-UVB in the survival of upper gastrointestinal cancer[1, 2,3,4.]

Annual D-UVB variable		Tertile 1			Tertile 2					Tertile 3					p-trend
Outcome	N	N	% who died	HR	N	% who died	HR	95% CL	p-val	N	% who died	HR	95% CL	p-val	
Cancer specific mortality															
All	229	81	48%	Ref	75	32%	0.62	0.35-1.10	0.10	73	44%	1.10	0.64-1.90	0.74	0.83
Oesophageal	183	63	51%	Ref	60	33%	0.68	0.36-1.27	0.22	61	48%	1.30	0.73-2.33	0.27	0.37
OEAC	149	53	74%	Ref	47	34%	0.83	0.41-1.69	0.61	51	47%	1.40	0.70-2.78	0.34	0.30
OESCC	34	10	70%	Ref	13	31%	0.39	0.08-2.02	0.27	10	50%	1.66	0.38-7.07	0.50	0.32
Gastric	46	18	38%	Ref	15	26%	0.38	0.05-2.74	0.34	12	25%	0.30	0.01-8.47	0.48	0.98
All-cause mortality															
All	229	81	54%	Ref	75	37%	0.62	0.36-1.06	*0.08*	73	49%	1.18	0.70-1.10	0.54	0.69
Oesophageal	183	63	50%	Ref	60	35%	0.65	0.35-1.19	0.16	61	54%	1.46	0.84-2.54	0.17	0.18
OEAC	149	53	51%	Ref	47	34%	0.74	0.37-1.48	0.40	51	53%	1.55	0.80-2.99	0.19	0.14
OESCC	34	10	80%	Ref	13	38%	0.64	0.15-2.80	0.55	10	60%	2.02	0.49-8.28	0.33	0.33
Gastric	46	18	50%	Ref	15	46%	0.41	0.09-1.84	0.25	12	25%	0.12	0.008-1.81	0.12	0.31

Footnote:

1 OEAC: oesophageal adenocarcinoma; OESCC: oesophageal squamous cell carcinoma

2 Adjusted for age, sex, dysphagia score, smoking status, alcohol intake, cancer stage, weight loss, cancer type and cancer subtype.

3 All hazards were found to be proportional.

4 Annual D-UVB IQR range: all: Tertile 1: 30400-31260 mJ/cm^2, Tertile 2: 31780-32170 mJ/cm^2, Tertile 3: 32660-33310 mJ/cm^2; OES: Tertile 1: 30330-31260 mJ/cm^2, Tertile 2: 31800-32170 mJ/cm^2, Tertile 3: 32660-33380 mJ/cm^2; OEAC: Tertile 1: 30310-31250 mJ/cm^2, Tertile 2: 31790-32110 mJ/cm^2, Tertile 3: 32660-33390 mJ/cm^2; OESCC: Tertile 1: 30500-31330 mJ/cm^2, Tertile 2: 31800-32340 mJ/cm^2, Tertile 3: 32600-33120 mJ/cm^2; Gastric: Tertile 1: 30770-31200 mJ/cm^2, Tertile 2: 31720-32160 mJ/cm^2, Tertile 3: 32760-33030 mJ/cm^2.

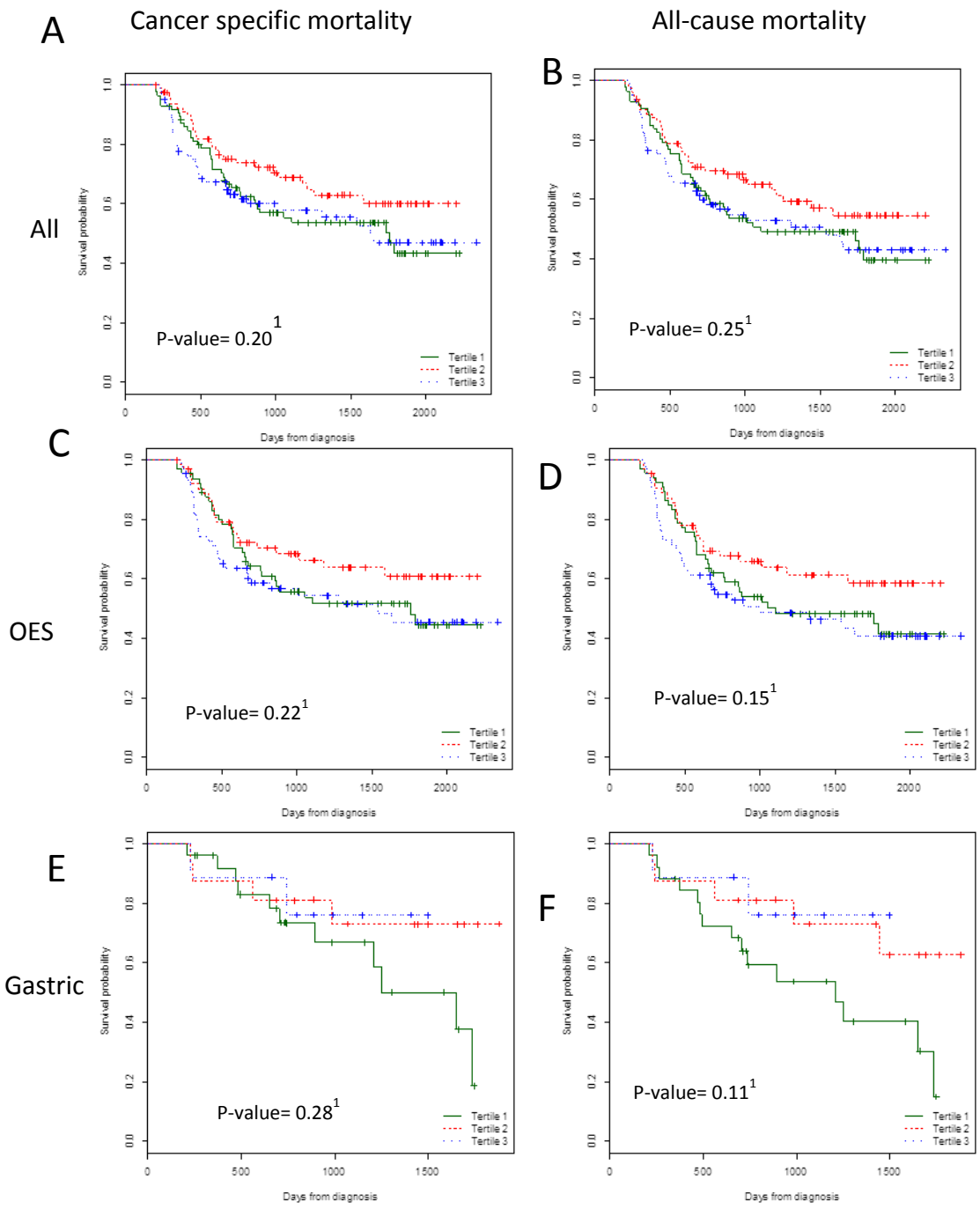

Figure 7.5: Kaplan-Meier analysis for annual D-UVB and upper gastrointestinal cancer mortality (stages 1-3)

Kaplan-Meier analysis per annual D-UVB tertile for **A)** annual D-UVB tertiles cancer-specific mortality, **B)** annual D-UVB tertiles all-cause mortality, **C)** Oesophageal only annual D-UVB tertiles cancer-specific mortality, **D)** Oesophageal only annual D-UVB tertiles all-cause mortality, **E)** Gastric only annual D-UVB tertiles cancer-specific mortality, **F)** Gastric only annual D-UVB tertiles all-cause mortality. [1] log rank test. Annual D-UVB IQR range: all: Tertile 1: 30400-31260 mJ/cm2, Tertile 2: 31780-32170 mJ/cm2, Tertile 3: 32660-33310 mJ/cm2; OES: Tertile 1: 30330-31260 mJ/cm2, Tertile 2: 31800-32170 mJ/cm2, Tertile 3: 32660-33380 mJ/cm2; Gastric: Tertile 1: 30770-31200 mJ/cm2, Tertile 2: 31720-32160 mJ/cm2, Tertile 3: 32760-33030 mJ/cm2.

7.4.4. Weight Loss, 25(OH)D, Annual D-UVB, and Survival

Patients who experienced weight loss had a significantly greater mortality than those who did not (**Figure 7.6**)**.** This was also found to be the case when restricted by cancer stage, except stage 2 (**Figure 7.6**) Weight loss was also associated with cancer stage, as those with earlier stages of cancer also had less weight loss. Additionally, those suffering from dysphagia and only able to eat partial solids or liquids experienced more weight loss (**Table 7.5**)**.** No associations between 25(OH)D and cancer specific mortality were observed for all upper gastrointestinal cancers however, a 120% increase in cancer-specific oesophageal mortality was observed when comparing tertile three to tertile one in those who lost weight (HR=2.20, 95% CI: 1.07-4.49) (**Table 7.6**). Similar significant results were found when all cancer cases were included (**Appendix 8: Table 3**). These trends were also noted for all upper gastrointestinal cancers and oesophageal cancers in all-cause mortality (**Table 7.7, Appendix 8: Table 4**). Contrastingly however, no significant associations for cancer-specific mortality or oesophageal mortality were observed in those who did not lose weight (**Table 7.7, 7.8**). However, a significant association was found for all-cause mortality in those with oesophageal cancer when P for trend was examined.

When examining Kaplan-Meier curves differences between the weight loss group and the non-weight loss group were also noted. A significant difference between tertiles in any gastrointestinal cancer were observed, with tertile three showing the worst survival and tertile 2 showing the best. This trend was also noted when restricted to oesophageal cases. No association was found for gastric cancer, although sample sizes were small for this group. In contrast to the weight loss group; tertile three in the no weight loss group had the best survival in all analysis undertaken, while tertile one had the worst, although no significant differences were found (**Figure 7.7**).

This association was further investigated using interaction analysis and non-significant results were observed (**Figure 7.8**). However when the distribution of mortality within in these two groups is widely different, for example mortality decreases as you increase 25(OH)D concentration in those who did not lose weight (Tertile 1: 34% , Tertile 2: 28% and Tertile 3: 22%), while the opposite is true in those who did lose weight (Tertile 1: 54% , Tertile 2: 42% and Tertile 3: 62%). This relationship was found despite a similar distribution of cancer stage in both groups; for example, the percentage of each tertile with stage 3 cancers are reasonably consistent across all tertiles (No weight loss: Tertile 1: 35%, Tertile 2: 38% and Tertile 3: 27%; weight loss Tertile 1: 42% , Tertile 2: 25% and Tertile 3: 34%), so this relationship unlikely to be explained by a larger percentage of stage 3 cancers in tertile 3.

No overall association was found for cancer specific or all-cause mortality when examining weight loss and annual D-UVB dose in this cohort (**Table 7.9, 7.10, Appendix 8: Table 5 and 6**).

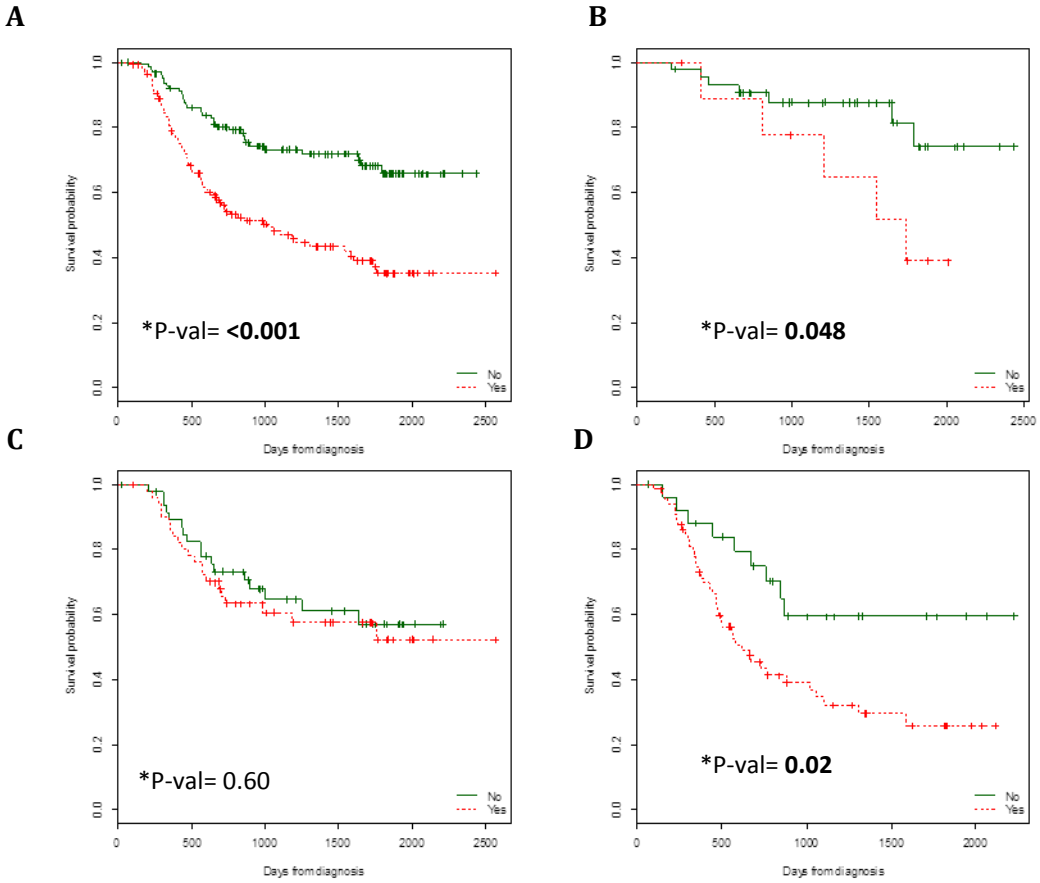

Figure 7.6: Kaplan-Meier analysis cancer-specific survival and weight loss (stages 1-3)
Kaplan-Meier analysis for cancer-specific mortality in those who did or did not have had weight loss symptoms. **A)** Overall, **B)** stage 1, **C)** stage 2, **D)** stage 3 *Log rank test

Table 7.5: Association between weight loss and selected variables [1].

Variable		Beta	SE	p-value
Age		-0.0002	0.003	0.94
Sex	Female	Ref	Ref	Ref
	Male	0.06	0.01	0.53
BMI	Underweight	0.18	0.18	0.30
	Normal	Ref	Ref	Ref
	Overweight	-0.15	0.09	0.10
	Obese	-0.22	0.09	**0.02**
Cancer Subtype	Gastric	Ref	Ref	Ref
	OEAC	-0.02	0.11	0.84
	OESCC	-0.11	0.14	0.43
Cancer stage	Stage 0	-0.54	0.20	**0.007**
	Stage 1	-0.25	0.11	**0.02**
	Stage 2	Ref	Ref	Ref
	Stage 3	0.13	0.08	0.11
	Stage 4	0.16	0.16	0.31
Alcohol intake	Non drinker	Ref	Ref	Ref
	Ex drinker	-0.22	0.19	0.25
	Social drinker	0.05	0.09	0.58
	Heavy drinker	0.06	0.12	0.61
Dysphagia	Anything	Ref	Ref	Ref
	Partial solids/liquids	0.18	0.08	**0.04**
Smoking Status	Never	Ref	Ref	Ref
	Past	-0.003	0.08	0.97
	Current	0.09	0.10	0.34
Season	Autumn	Ref	Ref	Ref
	Spring	-0.08	0.10	0.43
	Summer	0.03	0.09	0.75
	Winter	0.02	0.10	0.82
25(OH)D status		-0.0008	0.002	0.61

Footnote:
[1] OEAC: oesophageal adenocarcinoma, OESCC: oesophageal squamous cell carcinoma

Table 7.6: Association between 25(OH)D and cancer-specific mortality stratified by weight loss (stages 1-3)

Cox proportional hazard analysis looking at the effect of 25(OH)D in the cancer specific mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms [1,2].

	Model		N	Tertile 1			Tertile 2					Tertile 3					p-trend
				N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	All	*Raw 25(OH)D*	126	41	51%	ref	42	55%	1.40	0.69-2.81	0.36	43	53%	2.20	1.06-4.59	**0.03**	**0.05**
		May-adjusted 25(OH)D[3]	126	48	54%	ref	36	42%	1.05	0.50-2.23	0.88	42	62%	2.20	1.16-4.20	**0.02**	0.12
	OES	*Raw 25(OH)D*	104	31	52%	ref	37	57%	1.54	0.73-3.26	0.26	36	50%	1.99	0.87-4.54	0.10	0.14
		May-adjusted 25(OH)D	104	38	71%	ref	31	45%	1.21	0.56-2.62	0.62	35	66%	2.20	1.07-4.49	**0.03**	0.23
No weight loss	All	*Raw 25(OH)D*	117	39	31%	ref	43	30%	1.52	0.52-4.48	0.45	34	24%	1.58	0.43-5.78	0.49	0.28
		May-adjusted 25(OH)D	117	38	34%	ref	46	28%	1.39	0.52-3.76	0.51	32	25%	1.60	0.41-6.20	0.50	*0.08*
	OES	*Raw 25(OH)D*	74	23	35%	ref	36	33%	1.48	0.35-6.27	0.60	31	26%	1.81	0.37-8.81	0.46	0.29
		May-adjusted 25(OH)D	74	24	38%	ref	37	32%	1.74	0.53-5.73	0.36	29	24%	2.09	0.45-9.72	0.35	0.18

Footnote:

1 May-Adj; May adjusted 25(OH)D and model adjusted for age, sex, dysphagia, smoking status, alcohol intake, cancer stage, cancer type, cancer subtype. (Raw 25(OH)D was also adjusted for season)

2 Gastric cancer or subtypes of oes cancer could not be investigated due to small sample sizes in these groups

3 All cox hazards were found to be proportional

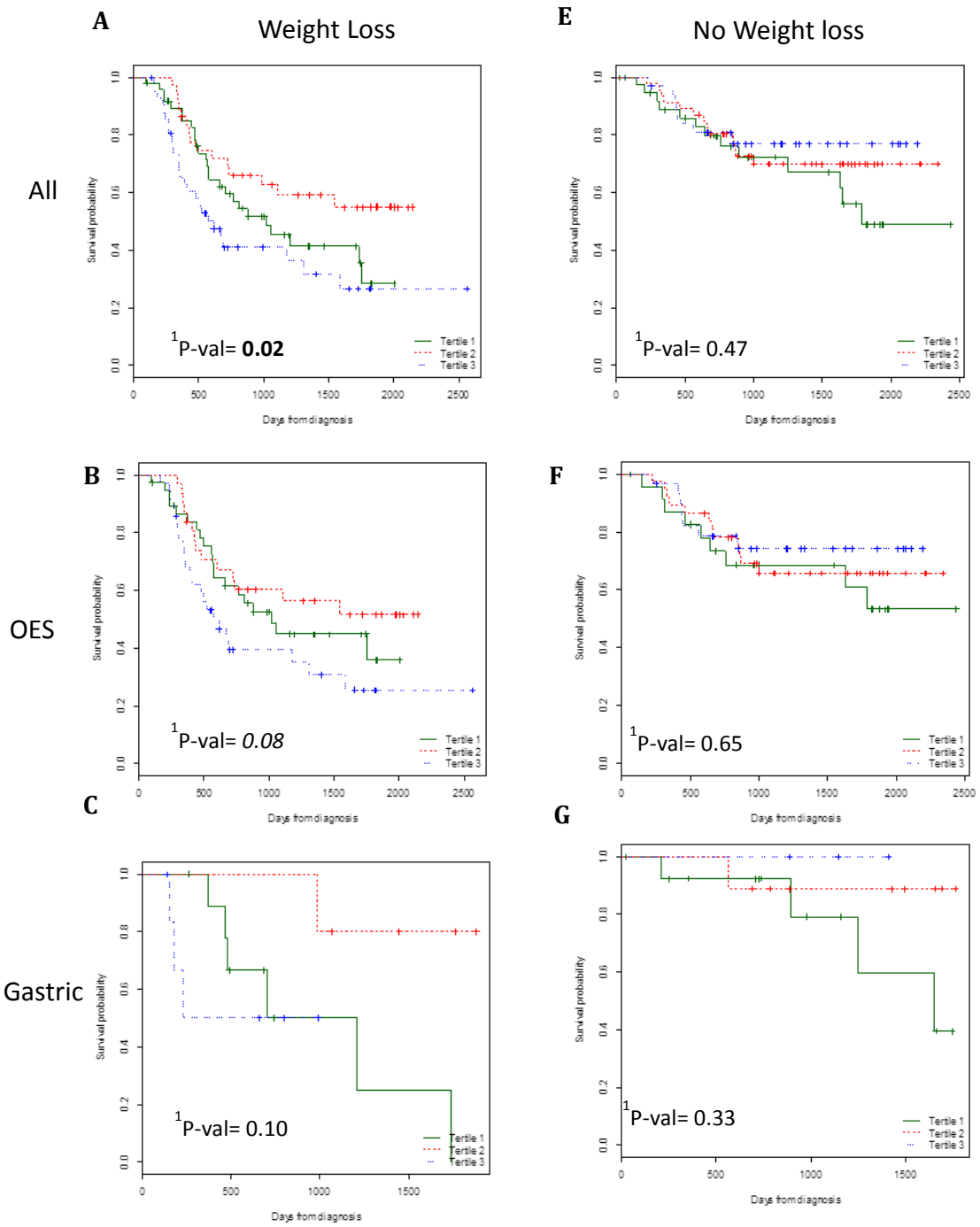

Figure 7.7: Kaplan-Meier analysis, 25(OH)D, cancer specific mortality and weight loss

Kaplan-Meier analysis for cancer specific mortality stages 1-3 using May-adjusted 25(OH)D stratified by **A)** those who lost weight**, B)** Oesophageal Cancer-with weight loss **C)** Gastric Cancer-with weight loss**, D)** Those who did not lose weight **E)** Oesophageal Cancer-with no weight loss **F)** Gastric-with no weight loss, [1]log rank test

Table 7.7: Association between 25(OH)D and all-cause mortality stratified by weight loss (stage 1-3)

Cox proportional hazard analysis looking at the effect of 25(OH)D in the all-cause mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms [1,2].

		Model	N	Tertile 1			Tertile 2					Tertile 3					p-trend
				N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	**All**	*Raw 25(OH)D*	127	41	61%	ref	42	64%	1.36	0.71-2.60	0.35	43	63%	2.26	1.15-4.45	**0.02**	**0.02**
		May-adjusted 25(OH)D	127	48	65%	ref	36	50%	1.09	0.55-2.15	0.81	42	71%	2.29	1.24-4.18	**0.007**	**0.05**
	OES	*Raw 25(OH)D*	85	31	55%	ref	37	68%	1.78	0.87-3.65	0.12	36	64%	2.37	1.07-5.24	**0.03**	0.07
		May-adjusted 25(OH)D	85	38	61%	ref	31	52%	1.24	0.60-2.53	0.56	35	75%	2.50	1.25-4.96	**0.009**	0.10
No weight loss	**All**	*Raw 25(OH)D*	117	39	44%	ref	43	30%	0.91	0.35-2.39	0.85	34	26%	1.18	0.38-3.72	0.78	0.18
		May-adjusted 25(OH)D	117	38	47%	ref	46	28%	0.89	0.35-2.19	0.79	32	25%	1.28	0.39-4.22	0.69	**0.04**
	OES	*Raw 25(OH)D*	74	23	43%	ref	36	33%	0.97	0.26-3.68	0.96	31	29%	1.43	0.26-3.68	0.62	*0.08*
		May-adjusted 25(OH)D	74	24	46%	ref	37	32%	1.17	0.38-3.60	0.79	29	28%	1.87	0.46-7.63	0.38	**0.04**

Footnote:

[1] Adjusted for age, sex, dysphagia score, smoking status, alcohol intake, cancer stage, cancer type, and cancer subtype.

[2] All cox hazards were found to be proportional

Table 7.8: Survival analysing examining 25(OH)D tertiles with interaction with weight loss

Cox proportional hazard analysis examining the effect of 25(OH)D in the survival of upper gastrointestinal cancer[1].

Outcome	N	Tertile 1			Tertile 2					Tertile 3					p-trend
		N	% who died	HR	N	% who died	HR	95% CL	p-val	N	% who died	HR	95% CL	p-val	
Cancer specific mortality															
All	242	86	45%	Ref	82	34%	1.08	0.35-3.32	0.89	74	44%	2.38	0.71-7.98	0.16	0.82
All oesophageal	194	62	47%	Ref	68	38%	0.97	0.28-3.34	0.95	64	47%	2.34	0.63-8.72	0.20	0.70
All-cause mortality															
All	242	86	57%	Ref	82	38%	1.52	0.53-4.31	0.43	74	51%	2.32	0.77-7.0	0.13	0.90
All oesophageal	194	62	55%	Ref	68	41%	1.30	0.41-4.19	0.66	64	53%	2.32	0.69-7.78	0.17	0.92

Footnote:

1 Adjusted for age, sex, smoking status, alcohol intake, cancer stage, cancer type, cancer subtype and dysphagia score.

2 May-Adjusted 25(OH)D is adjusted as if all 25(OH)D was sampled in May.

3 All cox hazards were found to be proportional.

Table 7.9: Cox proportional hazard analysis, annual D-UVB, cancer-specific mortality and weight loss (stages 1-3)

Cox proportional hazard analysis looking at the effect of annual D-UVB in the cancer specific mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms[1, 2].

	Model		N	Tertile 1			Tertile 2					Tertile 3					p-trend
				N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	**All**	*Annual D-UVB*	118	37	59%	Ref	44	43%	0.73	0.35-1.52	0.40	37	59%	1.49	0.72-3.10	0.29	0.26
	OES	*Annual D-UVB*	99	29	62%	Ref	37	43%	0.78	0.36-1.68	0.53	33	63%	1.74	0.82-3.68	0.15	0.17
No weight loss	**All**	*Annual D-UVB*	108	44	39%	Ref	29	17%	0.34	0.09-1.25	0.10	35	26%	0.77	0.29-2.06	0.61	0.67
	OES	*Annual D-UVB*	83	34	41%	Ref	21	19%	0.46	0.10-2.20	0.33	28	29%	0.65	0.19-2.21	0.49	0.87

Footnote:

[1] Model adjusted for age, sex, dysphagia, smoking status, alcohol intake, cancer stage, cancer type and cancer subtype

[2] All hazard ratios were found to be proportional

Table 7.10: Cox proportional hazard analysis, annual D-UVB, all-cause mortality and weight loss (stages 1-3)

Cox proportional hazard analysis looking at the effect of annual D-UVB in the all-cause mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms [1, 2].

	Model		N	Tertile 1			Tertile 2					Tertile 3					p-trend
				N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	**All**	*Annual D-UVB*	118	37	70%	Ref	44	50%	0.76	0.38-1.49	0.42	37	65%	1.57	0.80-3.11	0.19	0.16
	OES	*Annual D-UVB*	99	29	72%	Ref	37	46%	0.71	0.35-1.46	0.35	33	69%	1.78	0.88-3.60	0.11	0.11
No weight loss	**All**	*Annual D-UVB*	108	44	41%	Ref	29	21%	0.33	0.09-1.17	*0.09*	35	31%	1.02	0.42-2.47	0.97	0.87
	OES	*Annual D-UVB*	83	34	41%	Ref	21	19%	0.50	0.11-2.35	0.38	28	36%	1.06	0.36-3.15	0.91	0.58

Footnote:

[1] Model adjusted for age, sex, dysphagia, smoking status, alcohol intake, cancer stage, cancer type and cancer subtype.

[2] All hazard ratios were found to be proportional

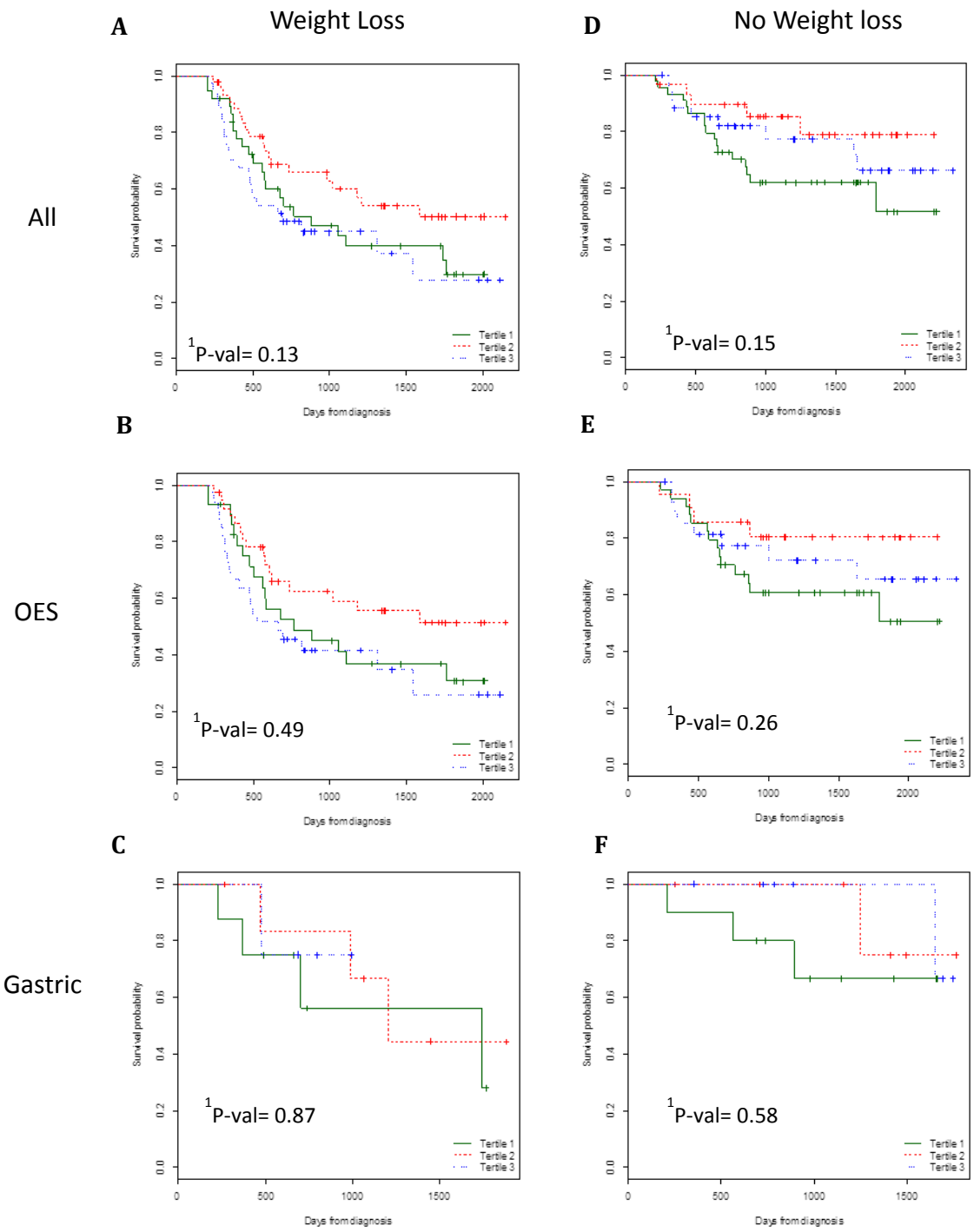

Figure 7.8: Kaplan-Meier analysis, annual D-UVB, cancer specific mortality and weight loss (stages 1-3)

Kaplan-Meier analysis for cancer specific mortality annual D-UVB stratified by **A)** those with weight loss **B)** Oesophageal Cancer-with weight loss**, C)** Gastric Cancer-with weight loss, **D)** those with no weight loss **E)** Oesophageal cancer-with no weight loss**, F)** Gastric Cancer-with no weight loss.

7.5. Discussion

Overall, no clear and consistent association was found between 25(OH)D concentration or UVB dose and cancer-specific or all-cause mortality. A robust positive association was found between 25(OH)D concentration and oesophageal adenocarcinoma mortality in stages 1-3, but some evidence suggesting a negative relationship was observed for oesophageal squamous cell carcinoma. Likewise, a protective association was observed in gastric cancer when comparing 25(OH)D concentration from those in tertile 2 to those in tertile 1. However, sample sizes were small, particularly in gastric cancer and squamous cell carcinoma groups.

7.1.1. 25(OH)D, Annual D-UVB and Upper Gastrointestinal Cancer

In this study no convincing overall association between upper gastrointestinal cancer survival and 25(OH)D or annual D-UVB was found. However, significant positive associations between tertile three vs tertile one of 25(OH)D concentration and oesophageal adenocarcinoma mortality were observed when restricted to stages one to three of cancer, particularly among those who lost weight. However, a suggestively significant inverse association was observed for tertile three vs tertile one of oesophageal squamous cell carcinoma mortality.

Studies which have examined the relationship between upper gastrointestinal cancer mortality and vitamin D are scarce. In fact there are no studies which have examined this association in oesophageal cancer patients, and only one has examined it in gastric cancer patients.

However, there have been a few studies which have examined the relationship between vitamin D and survival in multiple digestive cancers jointly. For example, Freeman *et al.*, examined the association in gastric, oesophageal, pancreatic and liver cancers and found no significant associations [328]. Giovannucci and colleagues on the other hand found an inverse association between vitamin D and mortality, where every 25 nmol/L increment in *predicted levels* of 25(OH)D resulted in a 45% reduction in cancer mortality for “all digestive” cancers. However, this study also failed to examine cancer types in detail and does not specify which digestive cancers were included in the analysis. As these studies investigated the relationship in all digestive cancers without cancer-specific or site-specific analysis, it is not known if this approach may have masked any relationship which exists between vitamin D and individual cancer types.

Further studies which investigated this relationship and have found both harmful and protective effects, however these are mostly ecological in design and other causes underlying the observed differences cannot be dismissed [7, 71, 103, 106, 329-331]. This debate has been problematic to resolve due to the limited number of studies, different measures of vitamin D looking at this

relationship [189] and the different mix of cancer types which are included in the analysis. Therefore, this positive association between May-adjusted 25(OH)D and oesophageal adenocarcinoma mortality and similarly the suggestive protective association between May-adjusted 25(OH)D and oesophageal squamous cell carcinoma mortality are novel results.

Similarities can also been drawn from this thesis and that of Ren *et al.* who noted gastric cancer patients with higher 25(OH)D concentration had overall improved five year survival when compared to patients with deficient levels of 25(OH)D (<50 nmol/L) [45]. This was also seen when 25(OH)D tertiles were examined as those in tertile 2 (range: 44-62 nmol/L) had reduced mortality levels compared to those with lower 25(OH)D concentration, however, the number of cases was small. Similar results were also noted for gastric cancer in the UK biobank cohort in Chapter 6.

There were some differences between the results observed using annual D-UVB and 25(OH)D concentration. There are many reasons why these differences might have arose; for instance 25(OH)D concentrations were adjusted so that they are less seasonally biased, however, even these May-adjusted 25(OH)D concentrations may not have captured a long term "average" vitamin D status, accurately. Furthermore, annual D-UVB only take into account one source of vitamin D while 25(OH)D can account for numerous sources, most notably, supplementation. Unfortunately, this information was not available for this cohort.

There was mostly agreement between the results found in the UK biobank cohort in chapter six and the Irish biobank cohort used in this chapter, although null-findings dominated. Both VDscore4 in the UK cohort and 25(OH)D concentrations in the Irish cohort noted a decreased risk of gastric cancer survival when comparing tertile two with tertile one. A positive association was found examining 25(OH)D concentration in the Irish cohort and oesophageal adenocarcinoma mortality, but this association was not observed using the other estimates. The differences which arise between the two cohorts and different estimates may have been due to many factors, such as differing adjustments being made (e.g. Biobank cohort was lacking in cancer stage information). Additionally, there was different follow up times for each of the cohorts and these cases were recruited at different times during the disease process which may have also contributed to the differences observed.

7.5.1. 25(OH)D, Annual D-UVB, Weight Loss and Survival

Vitamin D is a fat-soluble vitamin D and can be absorbed into adipose tissue before being released into circulation following weight loss [315-319]. This is an important aspect of vitamin

D which is rarely taken into account and is of importance when examining the association between vitamin D and some cancers. This is because being overweight or obese is a risk factor for upper-gastrointestinal cancers, but at the same time, once these cancers are diagnosed, weight loss can be common and is associated with a poorer diagnosis [320, 321]. Such weight loss could cause subsequent release of 25(OH)D from fat storage, and have an impact on the relationship between survival and vitamin D, as high 25(OH)D may act as a marker for poorer prognosis, but it is weight loss which is driving this relationship. This adds complication to the relationship between 25(OH)D and cancer survival, and this thesis tried to examine this in more detail, hypothesising that weight loss may affect this relationship. However, this cohort was not designed for this purpose and as such only some basic analysis could be conducted. Further research using a specifically designed cohort is needed before conclusions can be drawn.

In accordance with other studies, it was found in this study that the presence of weight loss increased mortality at most stages of cancer [322, 323], however, significant differences were not found for those with stage 2 cancer and it is unknown why this is the case.

When restricted to those who did not have weight loss symptoms, those with higher 25(OH)D concentrations were observed to have improved cancer specific-survival, although this was not found when all-cause mortality was examined, as those with higher tertiles were found to have an increased mortality when continuous models were employed. Contrastingly, when examining those who had weight loss symptoms those with the highest 25(OH)D concentrations were found to have an increased risk of mortality. Furthermore, the distribution of mortality between those who lost weight and those who did not were found to be widely different. However, no significant results were observed when interaction analysis was carried out. Therefore, no conclusions on the modificatory effect of weight loss on the relationship between vitamin D and oesophageal and gastric cancer survival can be made from these results. However, this is a topic which has not previously been examined in the literature and further studies should be conducted in order to examine this in detail using an adequately designed study as it is possible that weight loss could have an impact on this relationship in some cancer types.

7.5.2. Study Implications

This study has important implications for further vitamin D research. The importance of cancer site specific and subtype specific analysis when examining the association between vitamin D and cancer has been shown, as different results between subtypes were found. Oesophageal

cancer was not found to have a significant association with 25(OH)D tertiles, but oesophageal adenocarcinoma was. Often, similar cancers and cancer subtypes are grouped together to increase sample sizes and statistical power in studies but if the direction of the effect is different, this may bias the findings. Statistical relationships that were observed support the notion that a relationship between vitamin D and oesophageal cancer is site and subtype specific.

Furthermore, this research may help inform the study designs of future vitamin D research. This study considered and discussed the limitations of using 25(OH)D as an estimate for vitamin D, when examining the relationship between vitamin D and health outcomes. Primarily, due to the strong seasonality bias. Despite best efforts adjusting for season with only a single measurement, this measurement is going to be imperfect.

This study investigated the modificatory role of weight loss in the relationship between vitamin D status and survival. While no definite results were found, suggestive results reported here, together with a strong prior expectation due to the well-established link between weight loss and 25(OH)D, warrant further investigation and consideration when examining health outcomes associated with weight loss.

7.5.3. Strengths and Limitations

One of the strengths in this study lies with its design. 25(OH)D concentration was used in this study, which is considered the most accurate point measure of vitamin D status. Additionally, annual D-UVB was also investigated, which gives a longer-term estimation of vitamin D status.

25(OH)D concentrations were measured using the "gold-standard" method (LC/MS). In the current study 25(OH)D was measured post-diagnosis rather than years prior to diagnosis. Previous studies examining vitamin D and cancer survival often used historic concentrations of 25(OH)D [332]. As this section is examining survival and not risk, 25(OH)D concentrations after diagnosis is expected to be more relevant for survival, as a number of factors could potentially change after a diagnosis of cancer e.g.: diet or lifestyle (time spent outdoors). Therefore, using 25(OH)D which was taken years prior to diagnosis may not be indicative of 25(OH)D at diagnosis and lead to misclassification of vitamin D status. However, 25(OH)D dose taken post-diagnosis may also be a limitation of this study as 25(OH)D could also be altered due to medications or treatments an individual is prescribed. This is one of the reasons why having another measure of vitamin D may be useful. Additionally, the 25(OH)D concentrations in this analysis was May-adjusted, in order to account for the seasonality of vitamin D. However, this estimate gives an

average vitamin D dose based on only one measurement of 25(OH)D concentration. An actual "average" estimate would need take into account multiple estimates of vitamin D and would give a more accurate estimation of an individual's vitamin D status and reduce the impact of season of blood draw. The lack of longitudinal 25(OH)D measurements in this study is a limitation. Additionally, multiple measures of 25(OH)D could have highlighted a change in 25(OH)D pre- or post- weight loss. Additionally, no measurements of how much weight loss a patient had under gone was available for this cohort and therefore the power to investigate a link between weight loss, 25(OH)D and mortality was impaired.

Vitamin D mortality was examined in individual sites and subtypes separately, which is critical because of the differing aetiologies these may have. However, in doing so sample size was affected: when stratified by cancer site and type the sample sizes, particularly within the gastric cancer group, were very small. This meant that there might not have been enough power in these groups to detect a relationship even if one was present. Additionally, the same issue arose when examining vitamin D, weight loss and survival: as the cohort was split, sample sizes in these groups were reduced. Due to this, there might not have been enough power to detect a significant association. Therefore it is important that these relationships be examined using larger sample sizes and an appropriate study design.

As mentioned in chapter 6, multiple testing considerations also needs to be addressed. If the Bonferroni correction method was added to this analysis then a p-value of less than 0.006 would be needed to prove significance for the weight loss analysis, a p-value of less than 0.01 would be needed to prove significance in the annual D-UVB analysis and a p-value of 0.002 would be needed when examining 25(OH)D concentration analysis. As none of our results reached this level of significance, consideration needs to be made as to whether the results which were observed were true findings or if they were spurious results resulting from type 1 error. As this study aimed to examine the impact of vitamin D in upper gastrointestinal cancer survival overall and in specific cancer types and subtypes, multiple testing was a necessary aspect of this chapter, and is a limitation of this study.

There are also some limitations with the cohort itself. Although this is chapter uses data from the only oesophageal and gastric Biobank in the country, the majority of participants recruited were from the Dublin area, and therefore they might not be representative of the country as a whole. Furthermore, selection of participants was limited to those who had sufficient serum samples for 25(OH)D measurement. Therefore the generalisability of this study is a further limitation and further studies should be carried out using a nationally representative cohort with a large sample size.

Furthermore, no information on vitamin D related variables such as supplement use or sun exposure was available and as such these could not incorporated into the analysis and VDscore4 could not be calculated for these participants which is a further limitation of this study.

7.5. Conclusion

This study investigated the relationship between 25(OH)D and annual D-UVB and mortality in oesophageal and gastric cancer in an Irish cohort. No consistent results were found. This may have been a false negative result due to a lack of power of this study, as sample sizes were small. However, a detrimental relationship between 25(OH)D concentration and mortality when restricted to cancer stage one to three, and in those with oesophageal adenocarcinoma was observed.

Additionally, this chapter investigated whether weight loss in cancer patients might be confounding the association between 25(OH)D status and mortality, while some suggestive findings were reported no conclusions can be made due to the number of limitations of this cohort such as small sample size and inadequate data on weight loss. A larger study measuring weight loss at different times after a diagnosis and additional vitamin D related variables, should be conducted before any conclusions on the subject can be drawn.

8 Conclusion

8.1 Key Findings

8.1.1 Summary

This thesis described UVB over Ireland and the UK, examined the relationship between vitamin D-related variables to 25(OH)D (including an accurate ambient UVB dose, supplements and other important variables), developed a simple vitamin D scoring system and examined its use for the prediction of vitamin D sufficiency and deficiency. Finally, it investigated the role of 25(OH)D concentration, ambient D-UVB and a vitamin D score in oesophageal and gastric cancer occurrence and survival.

D-UVB doses over Ireland and the UK which were adjusted for important variables (ozone, cloud cover, altitude, and wavelengths of UV) using the detailed TEMIS database were examined. Comparisons were made between and within the two countries. Large differences in D-UVB dose were observed despite a small latitude and longitude differential. This was the first time UVB was explored in such detail in these countries.

Cw-D-UVB dose for two cohorts were calculated and association analysis was undertaken. A strong relationship between 25(OH)D concentration and cw-D-UVB was observed, including among individuals who are taking high dose vitamin D supplements and those aged over 60y. The relationship between UVB and 25(OH)D within these groups had not been reported previously, most likely due to poor power due to small sample sizes or a crude estimate of UVB doses. A strong relationship between 25(OH)D and other vitamin D related variables such as supplementation use, sun enjoyment or time spent outdoors and oily fish consumption was also confirmed. This demonstrates that other variables, along with D-UVB have an important relationship with 25(OH)D concentrations.

Using this information, a simple vitamin D scoring system was developed, and vitamin D scores obtained in this way were found to be associated with 25(OH)D in two Irish cohorts where 25(OH)D concentrations were available. Furthermore, it was determined that vitamin D scores were important variables for classifying individuals into deficient and sufficient, based on their 25(OH)D concentration.

Vitamin D score, annual D-UVB, and May-adjusted 25(OH)D, were then used to investigate a relationship between vitamin D and the risk and survival of oesophageal or gastric cancer in British and Irish cohorts.

Overall, a strong association between both higher annual D-UVB and vitamin D score and a reduced risk of oesophageal and gastric cancer was found in a large British cohort. Those with higher tertiles of vitamin D score and annual UVB were found to have a reduced odds of developing upper gastrointestinal cancer compared to the lowest tertile. This was also observed when oesophageal adenocarcinoma was examined. Unfortunately 25(OH)D was not available in this cohort to examine this relationship

Contrastingly, an increase in oesophageal adenocarcinoma mortality with higher tertiles of 25(OH)D concentration was found in an Irish cohort. These results were however dissimilar to those relating to gastric cancer, as an indication of decreased mortality in those with higher concentrations of 25(OH)D concentrations was found, however sample sizes were particularly small within this group.

Additionally, throughout this thesis it has been discussed that the use of multiple measures of vitamin D could improve vitamin D research, as there are multiple flaws associated with most vitamin D assessment measures. This thesis discusses the importance of acknowledging the strengths and weaknesses of each one. Furthermore, this thesis demonstrates that using a vitamin D score may be a simple and useful approach in future research, in conjunction with other measures, as it can incorporate many sources of vitamin D into a single estimate. Once this method is searched further, it has the possibility of being used in a clinical setting to screen those who may be in need to 25(OH)D assessment. Vitamin D score could also have the potential to be used as a longer-term average estimate of vitamin D status (in conjunction with 25(OH)D or alone), when assessing associations vitamin D might have with health outcomes.

This thesis had many important outcomes, however, further research needs to be carried out in a large cohort where ideally both 25(OH)D concentration and other vitamin D related factors are captured, before definitive conclusions can be drawn about the relationship between vitamin D and the risk or survival of oesophageal or gastric cancer.

8.1.2 Overall conclusion of thesis

One of the major difficulties with vitamin D research is that there is no “gold standard” measurement of vitamin D status. The lack of a standardised method of estimating vitamin D has so far hampered previous work on the topic. Vitamin D has been implicated in having a role in the development of numerous diseases and conditions [333]. However, the majority of these studies have used 25(OH)D measurements to examine their hypothesis. 25(OH)D is an acceptable measurement when estimating deficiency or sufficiency of vitamin D at a specific

point in time, however, unless properly adjusted, this measurement is not an accurate estimation of "average long term vitamin D". This is due to the multiple factors which can alter 25(OH)D status, most notably, the temporal aspect, as 25(OH)D changes substantially from season to season. Without proper adjustment for month of blood draw or season, 25(OH)D measurements can lead to non-differential misclassification which can in turn affect the results of association's studies [144, 145]. What is needed however, is multiple measures of 25(OH)D taken for each participant over a year long period. This approach would give a more accurate estimate as it would reduce the bias from seasonal variation and give an average estimate of vitamin D. This method would be the "ideal" approach to vitamin D research if it was achievable, however this is rarely a viable option for large scale epidemiology studies due to the need for ethical approval for multiple blood measurements and the financial costs involved. In conjunction with multiple 25(OH)D concentrations, other measures of assessing vitamin D could be used. For example, one approach could be to use an accurate dosimeter to estimate how much UVB the individual was exposed to. During the period of time the participant is wearing a dosimeter, they could also keep a food and supplement diary which could then be used to estimate dietary vitamin D. Once these two measurements are taken they could be combined together using regression methods to develop a vitamin D estimation which takes into account all sources of vitamin D. This however would rely on a daily self-reported food diary and compliance rates for this might not be 100%. Additionally, high compliance would also be required with the wearing of the dosimeter which would need to be placed on clothes by the individual every day. Furthermore, the financial cost of dosimeters can be quite high which would reduce a functionality of this method on a large scale.

The above scenarios outline how a "gold standard" approach to vitamin D estimation is possible, however, this approach rarely plausible for research studies. Due to the logistic, timing and financial considerations most studies opt for a one time measurement of 25(OH)D or an estimation of only one source of vitamin D (e.g. UVB estimate or dietary estimate only). For example, Mulholland *et al*. estimated dietary vitamin D, and examined its association to the risk of oesophageal adenocarcinoma, however supplementation or UVB was not taken into account in this study and this may have caused misclassification of some individual's vitamin D status [136]. Other studies have attempted to predict 25(OH)D dose using regression models in order to incorporate multiple sources of vitamin D [121, 288]. However, these studies do not always incorporate information about the most important sources of vitamin D. For example, Deschasaux *et al.* did not incorporate dietary sources of supplementation in their estimate [288]. Furthermore, some of these studies have also failed to use an accurate measure of

important sources in their estimate, for example, Giovannucci *et al.* estimates UVB exposure through "leisure-time physical activity". This may be an inaccurate measure of UVB exposure as no information is given about whether this leisure time activity was carried out outdoors or indoors. Deschasaux *et al.* also failed to use an accurate measure of UVB and instead opted for "season" as a proxy UVB measure. This thesis has demonstrated that the use of season is not as predicable and as accurate as an ambient UVB dose. Season fails to take into account altitude and microclimate. Additionally, UVB doses at the beginning and end of a season could be different, which has also been demonstrated in this study. Therefore, it is of limited use in vitamin D research, and more accurate individual measures should be used when creating a vitamin D estimate. The variation in UVB observed here demonstrates that previously created estimates of vitamin D may not be the most accurate estimate possible. Additionally, these studies fail to acknowledge that 25(OH)D concentration is not an "average vitamin D" estimate and without an adequate adjustment, the temporal aspect of 25(OH)D status can have a considerable impact when examining associations between vitamin D and health outcomes.

This lack of consistency was one of the main motivators for this thesis. This thesis aimed to investigate the relationship between vitamin D and oesophageal gastric cancer risk and survival using multiple estimates of vitamin D. In doing this, this thesis wanted to create a simple vitamin D estimate using detailed D-UVB doses which could give an "average vitamin D" assessment.

In order to develop a simple "average vitamin D" estimate which could be used in large studies there were multiple important considerations which needed to be addressed. Primarily, this estimate needed created using mostly "freely available" measurements. Secondly, this estimate needed to incorporate the most important sources of vitamin D into one estimate, as has been suggested by previous articles [137].

This thesis did not have access to multiple 25(OH)D measurements and vitamin D related variables in the same cohort. Therefore, in order to develop an average vitamin D proxy measure which included information on multiple sources of vitamin D, it was necessary to investigate the relationship between vitamin D related variables and 25(OH)D first, before any average vitamin D measure could be constructed.

Two of the most important sources of vitamin D are supplementation and UVB exposure. These were important sources which needed to be captured adequately in this research. This thesis chose to use the TEMIS database when estimating UVB dose. This database was chosen as it provides a detailed and comprehensively adjusted D-UVB estimate. This UVB dose estimate has been adjusted for altitude, cloud cover, ozone layer as well as restricting the UVB to wavelengths which can synthesize vitamin D.

A detailed investigation into this variable was conducted prior to its use in the creation of a vitamin D estimate. It was important to determine two things when investigating UVB. Firstly, what was the relationship between UVB dose estimate and 25(OH)D and secondly, was such a detailed estimate of UVB necessary for a simple vitamin D estimate.

In order to determine the answer to the first question, cw-D-UVB was created and investigated. This was a comprehensively adjusted D-UVB estimate which also accounts for the accumulation and diminution of vitamin D in the body. This estimate was determined for each individual calculated in a number of cohorts. Cw-D-UVB dose was shown to be associated with 25(OH)D concentrations in two Irish cohorts. This thesis has also demonstrated that, when categorised into quartiles, those in lower quartiles of cw-D-UVB had lower 25(OH)D concentrations than those in higher quartiles. These individuals were more likely to be in the insufficient vitamin D range when compared to those in the higher quartiles. These findings suggest the importance of D-UVB in preventing vitamin D deficiency. Furthermore, cw-D-UVB was found to be still associated with 25(OH)D concentrations in those who were taking high dose supplements, and in older individuals, who are known to have a decreased potential for cutaneous vitamin D synthesis. cw-D-UVB was also found to be an important variable in Boruta analysis when classifying individuals into vitamin D deficient and sufficient groups and when prediction of deficiency was examined using random forest modelling. Cw-D-doses were also been found to have seasonal fluctuations similar to 25(OH)D concentrations, in that they peak about a month after daily D-UVB doses do. This “lag” effect which is observed in 25(OH)D concentrations has not been accounted for previously in UVB dose estimates. This demonstrates that UVB is a strong predictor of vitamin D status even in vulnerable cohorts, at high latitudes. These results suggest that UVB should be an important component of any vitamin D proxy which is subsequently created.

A strong association was found between 25(OH)D and cw-D-UVB doses, however, it was also important to determine if such a detailed UVB estimate was necessary. When investigating UVB doses over Ireland and the UK using detailed UVB grids, it was found that despite the small changes in latitude and longitude, D-UVB doses over Ireland and the UK varied dramatically. A strong north to south D-UVB gradient was noted, as well as some evidence of an east to west D-UVB gradient. Higher D-UVB doses were consistently found as one moves further south or further east in the two countries.

However, even though large variations were observed between areas in Ireland and the UK, it was difficult to determine if these variations would have meaningful impact upon 25(OH)D dose. The direct relationship between vitamin D and UVB is very difficult to measure. As there are so

many other factors, which can hinder UVB production of vitamin D in the skin, the direct impact of certain levels of UVB on vitamin D is unknown. To add further complication to the matter, 25(OH)D measurements incorporate all sources of vitamin D and as UVB is only one source, the levels of 25(OH)D which can be attributed solely to UVB are unknown. Furthermore, as UVB can be measured in a number of different ways and can be adjusted for a number of different important factors, even comparing UVB does from two different sources is difficult. As such, the relationship between UVB and vitamin D can and will change slightly depending on the UVB measurement used. Due to these issues outlined above there is no standard amount of UVB which is needed to increase 25(OH)D by 1 nmol/L.

In order to determine if these variations in UVB are meaningful and need to be taken into account when creating a vitamin D estimate multiple 25(OH)D measurements for a large number of individuals who live in different latitudes would be required. As this information was not available in this thesis it is impossible to determine what would be a meaningful level of variation in UVB. However, information on 25(OH)D and cw-D-UVB dose for four time points was available in a small cohort, although, this cohort was located in similar latitudes.

Overall it was found that on average using regression methods, a 1000 mJ/cm^2 increase in cw-D-UVB dose resulted in a 2.4 nmol/L increase in 25(OH)D in the placebo group. On a case by case basis however, the results were slightly different. For example in Patient 25 from the Crohns disease cohort, an increase of just over 7000 mJ/cm^2 resulted in a change in 25(OH)D by 20 nmol/L, rather than the 17.2 nmol/L which would have been expected using beta coefficient from the regression model. This change of 20 nmol/L in Patient 25 resulted in deficiency over the time period (25(OH)D concentration dropped from 41 nmol/L to 21 nmol/L over the four month time period). This result demonstrates that in the case of this thesis, a change of 7000 mJ/cm^2 cw-D-UVB dose can have a meaningful effect on vitamin D status. It has also previously been shown in this thesis that there was an average daily difference of 15.75 mJ/cm^2 between the most northerly and most southerly locations in Ireland. Over a year long period the difference between these locations could be 5,748.8 mJ/cm^2. This difference could hypothetically result in a difference in 25(OH)D concentration of approximately 15 nmol/L solely based on location. This 15 nmol/L could be the difference between deficiency and sufficiency in an individual. Furthermore, this level of variation was seen with only a small differential of latitude (difference of 3.9°). If this was to be examined in countries which have a much larger latitude differential, such as Chile or the USA, then the variation would be even more relevant.

After a thorough investigation into UVB, this thesis demonstrated that it was possible to create a UVB estimate which was associated with 25(OH)D concentration in two cohorts and that the

variation in UVB which was observed between locations in England and Ireland could potentially have a direct impact upon 25(OH)D dose. These are important findings, as previous studies which have used UVB as a proxy for vitamin D often measure UVB only at a single location or estimate it over a large geographic area with different climates and altitudes [102, 105, 243]. This broad-brush approach is likely to affect the accuracy of the UVB estimate and thus bias association analysis findings towards the null due to imprecision in the estimate. All of the above results suggest that a detailed cw-D-UVB dose is a valuable estimate of 25(OH)D status.

25(OH)D status incorporates all sources of vitamin D, while cw-D-UVB only takes one source into account, therefore in order to build create a vitamin D estimate, other sources of vitamin D should be incorporated. An important source of vitamin D at a high latitude location is supplementation [157]. This thesis created a basic VDscore which contained information about supplementation and cw-D-UVB. Supplementation has been shown to be the most important source of vitamin D in high latitudes and was therefore determined to be the most important variable when creating VDscore1. This initial VDscore was found to be highly associated with 25(OH)D status in two Irish cohorts.

However, this VDscore1 did not adjust for two important factors, a dietary vitamin D source and utilisation of UVB. Although dietary sources of vitamin D are scarce and levels of vitamin D within sources low, they are still an important sources of vitamin D and therefore should be considered when creating a vitamin D estimate. Additionally, although UVB dose was incorporated into the model, this variable could not capture utilisation of UVB. In order increase the precision of the estimate a second and third vitamin D estimate was created which incorporated both of these important vitamin D related factors, (VDscore2 and VDscore3). These vitamin D scores were also found to be highly associated with 25(OH)D status in an Irish cohort. Furthermore, a linear relationship between vitamin D scores and 25(OH)D concentration was found, and mean and median 25(OH)D concentration was shown to increase with every increase in vitamin D score. It was also observed using random forest modelling that the prediction of vitamin D deficiency and sufficiency could be improved by the addition of cw-D-UVB + supplementation and VDscores (VDscore1, VDscore2 and VDscore3), compared to the baseline model.

The creation of cw-D-UVB, and VDscores demonstrated the possibility that a simple vitamin D estimate could be created which was strongly associated with 25(OH)D. However, in doing this, these estimates were also seasonally biased and did not give an “average” vitamin D status estimate. These estimates do have the potential to be very useful in research however. These estimates could be useful as a simple identification tool or pre-assessment of vitamin D status to prioritise those who need to have 25(OH)D assessment carried out. This system could flag

those at risk of deficiency, subsequent 25(OH)D measurements can be taken to determine if these individuals are deficient. After further research and replication, these VDscores could be developed into a simple cost-effective clinical tool as the variables which make up this score are easily and freely available. This would be a much more personalised system than is currently in use, as only those who are considered "at risk", such as those over 65, routinely have their vitamin D tested. Furthermore, this VDscore has the potential to be used in some studies where permissions to take blood samples are difficult to obtain e.g.: in children or institutionalised community-based individuals.

The uses and outcomes from these VDscores are important, however these scores did not provide an "average" vitamin D estimate. This is due to the temporal nature of cw-D-UVB and by consequence, VDscore2 and VDscore3. Therefore, using these estimates to examine the relationship between UVB and a health outcome would be inappropriate as the levels of cw-D-UVB and VDscore2 and VDscore3 could change significantly depending on *when* during the year these estimates were created. This could lead to misclassification of individuals and problems when determining associations.

Therefore, annual D-UVB was calculated in order to estimate D-UVB doses "year-round" without seasonal bias. VDscore4 was also created. This incorporated all elements of VDscore2 except it used a non-seasonally biased estimate of UVB. These estimates contained information on some of the most important sources of vitamin D (UVB, supplementation and dietary sources), with the most weight given to those variables which were shown to be most important at predicting 25(OH)D status in previous chapters. However, as these estimates contain "annual D-UVB" they provided an "average vitamin D" estimate unlike what has been carried out by previous studies [121]. Therefore, these estimates could be used to examine the association between vitamin D and a health outcomes without the prospect of a seasonal-bias hampering any associations found. The association between these vitamin D estimates and the risk and survival of oesophageal and gastric cancer was then investigated, this was one of the main aims of this thesis.

The risk of upper gastrointestinal cancer was first investigated. Previous studies examining vitamin D and cancer risk and survival have demonstrated a strong inverse relationship between vitamin D and various other cancers [101]. Similarly, a recent systematic review and meta-analysis found a significant reduction in overall cancer mortality and progression with increasing circulating vitamin D [134]. However no consistent relationship has been observed between vitamin D and upper gastrointestinal cancers [189, 220, 226, 334]. The current literature on the association between vitamin D and upper gastrointestinal cancers is strikingly limited. Higher

25(OH)D concentrations have been associated with an increased risk of oesophageal cancer in a recent meta-analysis of three studies [189]. All available studies that used 25(OH)D concentrations as their vitamin D estimate, contained large numbers of the Han Chinese population [8, 9, 202]. These were mainly from the Linxian region of China, where oesophageal cancer rates are very high and environmental factors may be influencing this relationship. Previous literature on gastric cancer have found no significant associations with vitamin D dose [226]. However, one study which examined life-time UVB found a decreased risk for oesophageal adenocarcinoma in those with higher UVB levels, but no consistent association for squamous cell carcinoma [6].

An inverse relationship was found between annual D-UVB and VDscores and oesophageal and gastric cancer occurrence. A reduced odds of any oesophageal or gastric cancer was found in all analysis. This relationship was further strengthened when restricted to oesophageal adenocarcinoma cases. However, 25(OH)D concentrations were not available to assess this relationship using this vitamin D measurement. This thesis strongly encourages further studies to be carried out using both 25(OH)D concentrations and vitamin D scores to determine the association between vitamin D and oesophageal and gastric cancer risk.

The relationship between vitamin D status and survival of oesophageal or gastric cancer was next examined in two different cohorts, an Irish cohort and a UK cohort. Although it has been previously mentioned that using 25(OH)D can be seasonally biased, this estimate could be used to examine associations with health outcomes, if it is accurately adjusted for month of blood draw [145]. This method would bring all individuals to similar levels of vitamin D and as such the seasonal-bias in the estimate is reduced. This has been carried out in this thesis as 25(OH)D was May-adjusted.

25(OH)D concentration was not available in the UK cohort and VDscore4 could not be calculated in the Irish cohort as vitamin D variables such as supplementation use and time spent outdoors were not available. Hence, only comparisons can be made between annual D-UVB in both cohorts. No overall significant associations between vitamin D and upper gastrointestinal cancers were found in either cohort using any of the three estimates of vitamin D (**Table 8.1**).

Previous studies on the topic have also found no associations [188], however no previous study has examined in detail each cancer type and cancer subtype making the results shown in this thesis novel. Sample sizes between the two cohorts were comparable, n= 265 (172 oesophageal cases and 93 gastric cancer cases) in the Irish cohort and n=235 (118 oesophageal cases and 117 gastric cancer cases) in the UK cohort.

It was also noted that those who were in Tertile 2 had less gastric cancer mortality when compared to tertile 1, when examining both VDscores and 25(OH)D dose. This result however was not observed using annual D-UVB in either cohort.

There were also some differences between estimates. An increased risk of oesophageal adenocarcinoma mortality was noted in those with higher 25(OH)D concentrations in the Irish cohort. However, this association was not supported using vitamin D scores in the UK cohort or using annual D-UVB in both the Irish and the UK cohort.

The differences between the results of both estimates may have been due to the lack of adjustment for cancer stage in the UK Biobank cohort. Alternatively, the relationship between 25(OH)D and oesophageal adenocarcinoma may have been confounded, for example by weight loss, as results in this thesis suggested that different associations for 25(OH)D concentration tertiles may exist in those who did or did not lose weight, however further research is needed to explore this issue more thoroughly.

These results suggest that associations between vitamin D and oesophageal and gastric cancer might be different depending on what cancer type or subtype is in question. Biologically this is possible as different cancers have different aetiologies. However, further studies need to be carried out using both seasonally adjusted 25(OH)D concentrations and other estimates of vitamin D such as VDscore4 before conclusions on this topic can be made.

All estimates used in this study have their own individual flaws and ideally in future research, all estimates (annual D-UVB, VDscores and 25(OH)D) should be used to estimate the relationship between vitamin D and health outcomes.

The main aim of this thesis was to examine the association between vitamin D and oesophageal and gastric cancer risk and survival using multiple estimates of vitamin D. However, in examining UVB in detail and its relationship with cancer, it became apparent sun exposure guidelines are not as clear or as accurate as perhaps they should be, with some guidelines from different societies even being contradictory [245, 246]. This leads to confusion among the general population about the best practice for obtaining vitamin D without increasing their risk of skin cancer. Total avoidance of UVB may not be necessary but rather controlled exposure should be employed.

The majority of societies and health authorities around the world suggest that 5-15 minutes of sun exposure without sun protection is what is needed to become sufficient in vitamin D without increasing your risk of skin cancer in the future [49]. However, these guidelines fail to take into account any variation of UVB throughout the year or in different parts of the world. This thesis

has demonstrated in detail that UVB can vary considerably depending on location. This could have a large impact on cutaneous synthesis of vitamin D and therefore this "one-size-fits-all" sun exposure guidelines may not be appropriate for all individuals for all times during the year.

Such variation in UVB exposure has also been shown in other studies, for example a study by O'Neill *et al.* observed almost a 2.5 fold increase in yearly UVB in Greece when compared to Ireland or the UK. In countries with high levels of UVB, sun exposure at certain times of the day should be limited or avoided e.g. at mid-day when the UVB dose is the strongest. However, time of day would not be such a large factor in countries such as the UK and Ireland where UVB doses do not reach such high levels. Furthermore, 5-15 minutes of sun exposure may be sufficient in countries such as Greece for adequate vitamin D synthesis, but on a cloudy day in Ireland and the UK this level of exposure may not be enough to ensure sufficiency.

Moreover, these guidelines fail to account for differences in vitamin D deficiency in different populations. For example, it was noted that D-UVB was an important predictor in those over 60y in this thesis, however it was also noticed that role of D-UVB was reduced in those over 75y, this may be due to the reduced ability for older individuals for cutaneous synthesises of vitamin D. Previous studies have also demonstrated that older individuals have lower vitamin D levels than younger counterparts and as this groups is also more at risk of osteoporosis [22, 54], it may be advisable for older individuals to be considered separately to the population as a whole. Therefore population group specific guidelines may also be advisable.

However, the impact of time-of-year specific and person-specific guidelines are acknowledged in some areas. Australia for example has different recommendations for different times of the year, and for those who are more at risk of vitamin D deficiency [335]. These recommendations would allow the public to make an information decision about their exposure to UVB and tailor it depending on the time of year. These recommendations also fall short as they fail to recognise the difference between UVB doses at different latitudes and also the impact of time of the day and cloud cover.

Melanoma has been linked to high sun exposure [336], however, it has also been observed that occupational exposure to sunlight can reduce melanoma risk [67, 68]. Most melanomas occur in areas of the body not exposed to sun [70], and lower 25(OH)D has been associated with thicker tumours and a poorer prognosis [69]. Along with evidence demonstrating high prevalence of vitamin D deficiency, even within countries with high UVB level [290, 337, 338], these results lead to greater confusion about vitamin D, skin cancer and sun practices.

Accurate understanding of UVB radiation and its relationship to 25(OH)D is paramount when disentangling this relationship. This thesis noted UVB to be an important predictor of vitamin D deficiency in an older cohort even in those taking supplements, and utilisation of UVB is important for preventing deficiency, especially in those who were not supplemented. Additionally, higher annual D-UVB were found to be associated with a reduced odds of upper gastrointestinal cancer in the UK. These results are important as they demonstrate that UVB is not negligible even at this high northern latitude. This study recommends more specific regional sun behaviour guidelines and acknowledgment of the importance of controlled UVB exposure, rather than avoidance. Furthermore, the general public should be educated on these topics along with information about the potential benefits of vitamin D.

Overall this thesis added important information to the field of vitamin D research and cancer. This was the first study to examine the association between vitamin D and oesophageal cancer survival and the second to examine the association between vitamin D and gastric cancer survival. Additionally, this thesis investigated the relationship between vitamin D and risk of oesophageal and gastric cancer in one of the largest cohorts to date. However, further research is needed using multiple measures of vitamin D in larger cohorts before firm conclusions can be made. Additionally, this thesis investigated the creation and use of different vitamin D estimates. These freely available and simple estimates have the potential to be used in future vitamin D research in conjunction with more well-known estimates of vitamin D status, such as 25(OH)D or as a screening tool to identify those at risk of deficiency. However, further research on these vitamin D estimates in larger studies is required before specific conclusions on their use can be made.

Table 8.1: Comparison between survival in oesophageal and gastric cancer in an Irish and the UK cohort

Overall results when examining the relationship between vitamin D estimates (VDscore4, D-UVB and 25(OH)D) in primary oesophageal and gastric cancer survival in two different cohorts.

Mortality		UK biobank cohort [1]		Irish biobank cohort [2]	
	Cancer type	**VDscore4**	**Annual D-UVB**	**25(OH)D**	**Annual D-UVB**
Cancer specific	Any uGI	No significant association	No significant association	No significant association	No significant association
	Oes	No significant association	No significant association	No significant association	No significant association
	OEAC	No significant association	No significant association	Increased mortality in Tertile 3	No significant association
	OESCC	No significant association	No significant association	No significant association	No significant association
	Gastric	Decreased mortality in Tertile 2	No significant association	Decreased mortality in Tertile 2	No significant association
All-cause	Any uGI	No significant association	No significant association	No significant association	No significant association
	Oes	No significant association	No significant association	No significant association	No significant association
	OEAC	No significant association	No significant association	No significant association	No significant association
	OESCC	No significant association	No significant association	No significant association	No significant association
	Gastric	Decreased mortality in Tertile 2	No significant association	Decreased mortality in Tertile 2	No significant association

Footnote:

[1] model adjusted for age, sex, smoking status, alcohol intake, cancer type, BMI, oesophageal or gastric reflux, weight loss, skin colour and any cardiovascular condition.

[2] model adjusted for age, sex, smoking status, alcohol intake, cancer type, cancer subtype, cancer stage and dysphagia. This cohort could not be adjusted for BMI , Barrett's oesophagus or dysplasia due to the large number of missing information from this variable (NA=53, 80 and 151 respectively).

3 uGI: upper gastrointestinal cancer, Oes: oesophageal cancer, OEAC: oesophageal adenocarcinoma, OESCC: oesophageal squamous cell carcinoma.

8.2 Strengths and Limitations

8.2.1 Study Strengths

Study strengths and weakness have already been mentioned in individual chapters however this section briefly details the overall strengths and weaknesses of this thesis.

One of the main strengths of this study is that D-UVB doses has been calculated for almost 500,000 people in total (n= 471,460) using UVB measurements with the best temporal and special resolution to date. This TEMIS database, as has been highlighted previously, restricted wavelengths to only those which are important for vitamin D synthesis, adjusted for ozone column, cloud cover, surface elevation and surface UV reflectivity within a relatively small geographic area. This offered improvements on other UVB measurements previously used [102, 242, 243].

This thesis also developed a number of simple VDscores which was found to be associated with 25(OH)D concentration. Both a simple hierarchal method and a simple regression method were used to develop these scores. These scores only included vitamin D related variables, rather than personal variables, such as age and BMI. This is an important strength as it allows versatility for this vitamin D scoring system. As it has been shown that using personal characteristics to develop a vitamin D proxy can then bias any relationship between the vitamin D proxy and a health outcome. By excluding personal variables in this method, this estimate could be utilised when classifying sufficiency and deficiency in a large Irish cohort but also in the examination of vitamin D on the risk and survival of upper gastrointestinal cancers.

Other strengths of this study include that this was the first to investigate the relationship between vitamin D and oesophageal cancer survival, as previous studies on the topic have combined this with other digestive cancer types. Additionally, this study adds information to sparse evidence regarding vitamin D and gastric cancer survival as only one other study has investigated this topic in detail. This thesis was also the first to examine UVB doses across Ireland and the UK in detail and quantify the variation which exists based on latitude, longitude and time of year.

8.2.2 Study Limitations

There were a number of limitations to this study, primarily, as this thesis used previously collected data from different cohorts, variables differ between cohort groups and some desirable pieces of information were not available in all cohorts. For example, supplementation dose, amount of weight loss and pre-post diagnosis 25(OH)D concentrations were not available in some cohorts, such as the

TUDA and Biobank cohort. One major piece of information which was missing from this thesis is cancer stage from the UK biobank cohort. Without the adjustment for cancer stage the results examining survival in this cohort should be interpreted with caution.

Additionally, there was no information with regards to sun enjoyment, sun exposure, dietary sources of vitamin D, and supplement use in the Irish oesophageal and gastric cancer cohort and as such VDscores could not the be created for this cohort.

Serum measurements of 25(OH)D for the large UK biobank cohort were not released in time for completion of this thesis, which are due to be released in 2018. Using 25(OH)D concentrations it would have been possible to explore the relationship between 25(OH)D concentration and the risk and survival of oesophageal and gastric cancer in the UK biobank cohort. This would have allowed direct comparison with the results from the Irish cohort and also comparisons with the results which were obtained using VDscore4. Additionally, these 25(OH)D concentrations would have been useful to develop VDscore2 further. Using 25(OH)D concentrations from such a large cohort of people may have permitted a threshold at which individuals become deficient using VDscore2 to be determined. Another potential limitation is that a number of variables in the cohorts used were generated from self-reported questionnaires given to participants and this could be subject to recall bias.

The annual D-UVB estimates which were examined in both the UK biobank and the Irish biobank cohorts were calculated at different times after diagnosis. In the UK biobank annual D-UVB was calculated after diagnosis, but the time between diagnosis and annual D-UVB estimation could have varied from a few months to a number of years. On the other hand the annual D-UVB doses was calculated quite soon after diagnosis in the Irish cohort (from 1-9 months). This difference in timing should not have a large impact upon the annual-D-UVB dose calculated as UVB does not vary much from year to year, however this should still be noted when interpreting results of this study.

Utilisation of the TEMIS database is a strength of this thesis, however, there is also a limitation in its use. The UV data from the database was calculated using a peak action spectrum of 295 nm which was derived from the final draft version of ref. [239], however the published report of ref. [239] had a peak of 298nm. This leads to a difference in daily UV dose values of a factor of about 2.2. The use of a different action spectrum however does not affect the statistical relationships presented throughout this thesis, just the absolute value of the presented cw-D-UVB dose. Furthermore, the results in this thesis have been reproduced using a new version of the TEMIS database which uses a peak of 298 nm.

One of the main challenges of this thesis was the use of multiple cohort's in order to describe, develop and examine the role of vitamin D and UVB estimates in the risk and survival of oesophageal and gastric cancer. This approach was necessary for a number of reasons. Firstly, different cohorts were

required to examine different aspects of this thesis. For example, the Crohns disease cohort was used as it contained longitudinal measures of 25(OH)D under highly controlled circumstances of an RCT. Similarly the use of the TUDA cohort was necessary for this thesis as it was a large cohort which had information on vitamin D related variables and 25(OH)D concentration, this allowed an investigation into variables which impact vitamin D concentrations and the development of a detailed VDscore. Furthermore, both the UK and Irish Biobank cohorts were necessary as they allowed the examination of the risk and survival of oesophageal and gastric cancer using multiple vitamin estimates. The use of many different cohorts in this this was required in order to investigate the hypothesis being tested. Furthermore, the use of these cohorts could be considered a strength of this thesis, as it allowed the examination of VDscores and cw-D-UVB in different individuals, with similar results being found, increasing the reproducibility of this work. However, the use of multiple cohorts in this thesis was also a limitation of this study. As different cohorts were used, these had different variables and as such the same associations could not be examined in all cohorts. For example, the Crohn's cohort had no information on utilisation of UVB nor dietary sources of vitamin D and as such these could not be included into the VDscore created. Similarly, variations in "utilisation of UVB variable" was observed between the TUDA cohort and the UK biobank cohort. The TUDA cohort examined "sun enjoyment" as a proxy for utilisation of UVB while the UK biobank used "time spent outdoors" to describe this variable. The answers for these variables were treated in a similar manner when creating VDscores, i.e.: it was assumed that time spent outdoors was related to average vitamin D in the same manner as "enjoyment of the sun" was related to 25(OH)D concentration. This may not have been the case and the use of different cohorts with different variables is a limitation of this thesis.

Another limitation of this thesis is the use of cohorts which may not be representative of the general population. This thesis used cohorts which consisted of individuals with Crohn's disease and older individuals in the early development phase of chronic conditions such as osteoporosis, dementia and cardiovascular diseases. The relationship between vitamin D related variables and 25(OH)D was examined in these cohorts, however the relationships which were found may not be representative of the relationship which could be found using healthy members of the general population. This limitation of this study could impact upon the generalisability and reproducibility of the results shown in this thesis, as differences in the relationship between certain variables and 25(OH)D could exist between those with certain conditions and healthy adults. Therefore, associations which were found between variables and 25(OH)D concentration should be examined in a large cohort of healthy individuals to fully investigate this relationship, unfortunately this was not possible in this thesis. Furthermore, this thesis was conducted using participants who were Irish and English, therefore the study results can only be inferred for people living at similar northern latitudes who have similar

dietary or sun exposure patterns. This limits the generalisability of these results as it is unknown if these results would also be found in different populations. Further research in other cohorts at different locations with different dietary, sun exposure and supplementation habits (i.e. widespread fortification of foods) should be explored in the future to ensure reproducibility and generalisability of these results.

Finally, sample sizes in a number of cohorts was small. The longitudinal Crohn's disease cohort had less than 100 participants and the risk and survival analysis of oesophageal and gastric cancer in the UK and Irish cohort's cancer also contained a small number of primary cancer cases. Further studies with substantial study sizes are needed in the future to examine the association between vitamin D estimates and the risk and survival of oesophageal and gastric cancer.

Another limitation of this study which has been described in detail in chapter 5 and 6 is the assumptions made by the vitamin D estimates used in this thesis. The use of vitamin estimates assumes that the ambient UVB dose measured is in fact received by individuals and utilised for vitamin D synthesis, this might not be the case as skin cover by clothing could prevent synthesis of vitamin D. Furthermore, these estimates assume similar increases in vitamin D regardless of supplementation dose taken or quantity of oily fish consumption. This is also not the case, as higher supplementation dose and oily fish consumption would lead to higher levels of vitamin D. However, the addition of supplementation dose and oily fish consumption dose would add complication to this estimate which has the potential to reduce the ease at which this estimate could be calculated. Furthermore, this thesis assumes that this VDscore would be appropriate at all times of the year and all locations, this might not be the case and this thesis only investigated this relationship in an Irish and a UK cohort. Therefore, this study should be examined in a large cohort of individuals from a different location to determine if this estimate could be used for vitamin D research in other locations.

Furthermore, this thesis aimed to develop and examine the use of vitamin D estimates which gave a "long-term average" of vitamin D, rather than a point estimate. These variables contained some of the most important sources of vitamin D (supplementation, UVB and dietary sources). However, these estimates assumed that the relationship between these variables and a point estimate such as 25(OH)D concentration is the same as the relationship between these variables and a "long-term average" vitamin D dose. This may not be the case as the relationship between variables and 25(OH)D dose might not be the same as the relationship for a longer term estimate. For example, oily fish consumption may contribute to 25(OH)D concentration by a certain degree, but it is unknown if oily fish consumption would contribute to a longer term estimate of vitamin D to the same degree or if the contribution of other variables become more important over a prolonged time, which would render the contribution of oily fish less important. This could not be examined in this thesis as multiple

measurements of 25(OH)D along with vitamin D related variables would be needed in one cohort to examine this and this information was not available. This is an important limitation of this study and the relationship between vitamin D scores and multiple measures of 25(OH)D averaged over time should be investigated further.

The aim of this thesis was to examining the relationship between vitamin D and oesophageal and gastric cancer risk and survival using multiple vitamin D estimates. This thesis also wanted to examine this relationship in cancer subtypes. However, with the inclusion of a number of different hypotheses in this thesis, there could be some issues with multiple testing. As described in chapter 6 and 7, multiple testing increases the likelihood of type one error in results. This can reduce the reliability of the results as some of the significant results which were observed may be due to type one error. This is a limitation of this study and the hypothesis selected. However, it was necessary to examine the relationship between vitamin D and upper gastrointestinal cancers together and separately examine the relationship between vitamin D and the different subtypes of cancer. As these arise in different cell types and may have different risk factors it is plausible to suggest they may have a different relationship with vitamin D. Additionally, when the Bonferroni correction was employed some of the results which were observed were still found to be significant. This suggests that although multiple testing may be an issue with some of the results and limitation of this study, the strong relationships which were found between vitamin D estimates and upper gastrointestinal cancer occurrence did not occur due to type one error.

8.3 Implications of this Research

Vitamin D research is topical, there has been a resurgence into its research in recent years as vitamin D has been implicated in numerous disease and conditions. There has been a large push towards improving the vitamin D status of the general public. Whether this is through broad fortification of consumer products, educating people about the importance of vitamin D and how to find sources in the diet, or proposing appropriate sun exposure guidelines. The results from this thesis add important information to the field of vitamin D research.

This thesis has carried out valuable in-depth research into UVB doses in Ireland and the UK. This research highlights the differences which exist despite small latitude differential in both countries. This research could have important implications on future vitamin D research as this study highlights the need for detailed UVB measurements in future studies.

Cw-D-UVB has been individually calculated using the greatest spatial and temporal resolution to date. It has been shown that UVB and sun exposure are still of strong importance in those who are aged

over 60 years old and those taking high dose supplements. Additionally, the importance of supplementation in an older cohort has been demonstrated as it was noted that supplementation was an important factor in maintaining healthy vitamin D status for all levels of ambient radiation at this northerly location, irrespective of sun enjoyment. Any dose of vitamin D supplement was found to approximately increase 25(OH)D concentration by 35-40 nmol/L in this elderly cohort. These results are relevant in advancing health guidelines in relation to supplementation use and sun exposure for this vulnerable cohort.

A simple vitamin D score was also developed. This score was found to be useful as it could incorporate numerous sources of vitamin D and could rank a population based on their vitamin D status. This scoring system could be useful for estimating if an individual is at risk of vitamin D deficiency and if 25(OH)D assessment necessary, however further research is needed before this can be achieved.

Additionally, this vitamin D scoring system could be used as a vitamin D estimate when investigating the relationship between vitamin D and various health outcomes, as has been done in this study in relation to the risk and survival of gastric cancer. As this vitamin D score was not determined by health related determinants of vitamin D such as age, BMI and physical activity, as has been done previously [266, 290, 339], it minimises bias when investigating future health outcomes. Furthermore, as it uses a detailed annual D-UVB dose in its calculation, it is not seasonally biased unlike other vitamin D measurements.

8.4 Recommendations for Further Research

Outputs from this thesis are a helpful addition to vitamin D research, however, further research on this topic would be beneficial to the field. There are a number of recommendations for further research arising on foot of this thesis.

8.4.1 Development of a more Sophisticated Vitamin D Scoring System

The simple method of vitamin D has been shown to be useful estimating average vitamin D status based on a number of variables, however this scoring system could be enhanced in the future with the addition of more variables such as dose of vitamin D supplementation and tailored as needed for different populations. Investigating this in the UK biobank cohort, once 25(OH)D data becomes available would be useful as it is a well characterised and large cohort.

25(OH)D concentrations in such a large cohort would also be useful to determine thresholds of vitamin D score at individuals at risk of deficiency. When this information becomes available, future research can look in more detail about how to develop this vitamin D scoring system into a useful clinical tool to help determine vitamin D deficiency in individuals.

8.4.2 Exploration into 25(OH)D and Risk of Oesophageal and Gastric Cancer

Data on 25(OH)D in the UK biobank cohort was not available during the time period this thesis was undertaken. 25(OH)D information would allow direct comparison to risk analysis carried out from a number of previous studies [8, 9, 202]. Additionally, as this would be the first to investigate the relationship in a European population, differences between Chinese and European cohorts could be investigated.

8.4.3 Exploration into 25(OH)D and Survival of Oesophageal and Gastric Cancer

Once 25(OH)D becomes available in the UK biobank cohort it will be possible to directly compare the association between vitamin D and upper gastrointestinal cancer survival in both the Irish and UK cohort. Additionally, the cohorts could be combined and survival analysis could be repeated with an increased sample size and statistical power. This would allow researchers to carry out the largest study to date examining the relationship between 25(OH)D and survival of oesophageal and gastric cancer. Furthermore, with this larger sample size, stratified analysis on subtypes of oesophageal cancer with adequate sample sizes could be achieved.

Moreover, having 25(OH)D concentration for this cohort would allow comparisons to be made between analysis using 25(OH)D concentration and analysis using vitamin D scores in the same large cohort.

8.4.4 Further Exploration into the Effect of Weight Loss on Vitamin D and Survival

This thesis investigated the relationship between vitamin D, survival of oesophageal and gastric cancer and the modificatory role of weight loss. However, a small sample size and inadequate information on weight loss and 25(OH)D concentration pre/post weight loss meant that this relationship could not be assessed thoroughly. Future research on this topic should be carried out using detailed information about how much weight was lost before cancer diagnosis and multiple measurements of 25(OH)D.

8.4.5 Further Exploration Vitamin D and Other Cancer Types

Using the UK biobank cohort analysis can be carried out to explore the relationship between vitamin D (using 25(OH)D, annual D-UVB and vitamin D scores) and the risk and survival of many different cancers and other conditions. Samples sizes for more prevalent cancers such as breast cancer and colorectal cancer would be larger than the sample size of the oesophageal and gastric cancer cases and therefore stronger associations between vitamin D estimates and the risk and survival of these cancers may be found.

8.4.6 Further Exploration into the Differences in UVB dose Between Countries

The TEMIS database has detailed information regarding vitamin D UVB dose every day from 2005 for all of Europe. This is a valuable resource which is underutilised. Further research could explore the differences in UVB doses over Europe and this information could inform government health bodies about the best sun exposure practices to advice their citizens on with regional specificity. Additionally, this exploration could determine if widespread vitamin D supplementation should be advised in certain countries for certain times of the year.

8.5 Final Remarks

Overall, this thesis developed a number of vitamin D estimates. These estimates were found to be strongly associated with 25(OH)D status. Furthermore, this thesis developed a simple “average” vitamin D estimate which incorporated information on three of the main sources of vitamin D (supplementation, diet and UVB).

Using the vitamin D measures developed, this study found a strong inverse relationship between annual D-UVB, vitamin D scores and the risk of oesophageal and gastric cancer. This inverse relationship was also observed when examining the relationship between annual D-UVB, VDscore4, 25(OH)D and mortality in gastric cancer. However, no consistent associations were found for oesophageal cancer mortality. Further research is needed in a larger cohort using 25(OH)D concentrations, vitamin D score and annual D-UVB in order to fully explore the relationship between vitamin D and upper gastrointestinal cancers.

8.6 Dissemination and Other Achievements during the Study

From the start of this PhD in 2014, I have developed numerous skills, completed several training courses and presented at three conferences. I have published three peer reviewed articles in international journals based on my literature review and findings from this thesis. I have also published a systematic review and meta-analysis and a commentary piece related to the field of study during my time carrying out this PhD. Four additional articles will hopefully be published from the research outlined in this thesis. Each of these outputs are outlined in further detail below.

8.6.1 Funding Received During This Project

I applied for and received funding from the "Trinity Trust Travel grant" in 2016, in order to fund travel to the 4th International Vitamin Conference in Copenhagen.

8.6.2 Training Courses Completed

I have completed the following training courses throughout my PhD:

- Online course in R programming, *Coursera (Oct 2014)*
- Online course in Epidemiology, *Coursera (Oct 2014)*
- Techniques and Strategies in Molecular Medicine*, Molecular Medicine Ireland (Dec 2015)*
- CAPSL Postgraduate Teaching Assistants: Introduction to Teaching, *Trinity College Dublin (Sep 2016)*.

8.6.3 Publications

Stemming directly from my PhD work, I have published three articles, one systematic review and meta-analysis (joint first author) and two original articles (first author).

- Zgaga L.*, O'Sullivan F.*, Cantwell M.M, Murray L.J., Thota P.N., and Coleman H.G. "Markers of Vitamin D Exposure and Esophageal Cancer Risk: A Systematic Review and Meta-analysis." Cancer Epidemiology and Prevention Biomarkers 25.6 (2016): 877-886 [189]. *Joint first author

- O'Sullivan F., Laird E., Kelly D., van Geffen J., van Weele M., McNulty H., Hoey L., Healy M., McCarroll K., Cunningham C., Casey M., Ward M., Strain J.J., Molloy A.M., Zgaga L. "Ambient

UVB dose and Sun Enjoyment Are Important Predicators of Vitamin D Status in an Older Population" Journal of Nutrition 147 (2017): 1-12.

- O'Sullivan F., van Geffen J., van Weele M., Zgaga L. "Annual Ambient UVB at Wavelengths that Induce Vitamin D Synthesis is Associated with Reduced Oesophageal and Gastric Cancer Risk: a Nested Case-Control Study" Photochemistry and Photobiology (2018)

I have also published two additional papers from a related field of study:

- Vaughan-Shaw P., O' Sullivan F., Farrington S.M., Theodoratou E., Campbell H., G Dunlop M.G., Zgaga L. "The Impact of Vitamin D Pathway Genetic Variation and Circulating 25-Hydroxyvitamin D on Cancer Outcome: Systematic Review and Meta-Analysis" British Journal of Cancer, (2017) 1-19
    - I was lead analyst on this study and carried out all forest plots and meta-analysis for this study, as well as being involved in the article selection, information extraction, scoring and writing of the article.

- Zgaga, L., Vaughan-Shaw P., O' Sullivan F., *et al.* "Reply to 'Comment on 'The impact of vitamin D pathway genetic variation and circulating 25-hydroxyvitamin D on cancer outcome: systematic review and meta-analysis''." British Journal of Cancer (2017).

8.6.4 Presentations

I have attended six conferences and presented at three of these.

- O' Sullivan F., Healy M., King S., O' Sullivan J., Reynolds J., Zgaga L. "The Effect of Circulating 25-Hydroxyvitamin D Level on Survival in Oesophageal and Gastric Cancer Patients", 10th International Cancer Conference, Trinity College Dublin, October 2016 (poster presentation)

- O' Sullivan F., Healy M., King S., O' Sullivan J., Reynolds J., Zgaga L. "The Association Between 25-Hydroxyvitamin D and Upper Gastrointestinal Cancer Survival, and the Modificatory Role of Weight Loss" Irish Society of Gastroenterology Conference, Galway, June 2016 (poster presentation)

- O' Sullivan F., Raftery T., Kelly D., O' Sullivan M., Zgaga L. "Using Cumulative and Weighted UVB at Wavelengths that Induce Vitamin D Synthesis (cw-vitD-UVB) and Vitamin D Supplementation for Enhancing Prediction of 25-Hydroxyvitamin D", 4th International Vitamin Conference, Copenhagen, May 2016 (Poster and oral presentation)

- Attended three consecutive multidisciplinary Bone Study Conferences, Trinity College Dublin *(March 2014-2016)*

8.6.5 Papers Drafted

- O' Sullivan F., Raftery T., van Geffen J., van Weele M., McNamara D., O'Morain C., Mahmud N., Healy M., Kelly D., O' Sullivan M., Zgaga L. "Sunshine is an important determinant of vitamin D status even among high-dose supplement users: secondary analysis of a randomised controlled trial" *(submitted environmental health perspectives, March 2018)*

- O' Sullivan F., Healy M., King S., O' Sullivan J., Reynolds J., Zgaga L. "The Effect of Circulating 25(OH)D Concentration on the Survival of Upper Gastrointestinal Cancer" *(2018 submission expected)*

- O' Sullivan F., Kelly D., Laird E., Zgaga L. "The use of a simple vitamin D scoring system to determine an effect of Vitamin D in the Risk and Survival of Upper Gastrointestinal Cancers" (*2018 submission expected*)

8.6.6 Skills Developed during the PhD

- Teaching skills (taught, prepared and assessed epidemiology module for 200 4th year medical students consisting of one 3-hour and one 1.5-hour seminar, each given eight times throughout the year)
- Communication and leadership skills: Managed small research projects with 2nd year medical students
- Presentation skills through presenting at conferences
- Statistical programming in R
- Experience handling large data sets
- Regression models (stepwise multivariable linear regression, conditional logistic regression)

- Systematic review and Meta-analysis techniques (Forest plots, Heterogeneity analysis, Funnel plots, quality assessments, Newcastle-Ottawa (NO) scoring, Meta-regression)
- Risk and survival analysis techniques (Kaplan-Meier (KM) graphs, Cox proportional hazards model)
- Classification and prediction techniques (Receiver operator curves and Area under the curve)

8.6.7 Other projects I was Involved in during this PhD

I have also been involved in some other projects during my PhD

- Applied for and received data on colorectal cancer from the National cancer registry of Ireland

- Wrote and sent out a press release for the paper entitled “Ambient UVB dose and Sun Enjoyment Are Important Predicators of Vitamin D Status in an Older Population”, which was subsequently published as an article in; The Irish times, The Irish independent, The Leitrim observer, the Journal.ie and a segment on Q102 radio station and many others.

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363. Daly, R.M., D.J. Magliano, C. Gagnon, Z.X. Lu, D.M.E.W. Dunstan, K.A. Sikaris, P.Z. Zimmet, P.R. Ebeling, and J.E. Shaw, *Lower serum 25-hydroxyvitamin d levels are associated with greater all-cause and cancer-related mortality among Australian adults: Findings from the Australian diabetes, obesity and lifestyle study (AUSDIAB).* Osteoporosis International, 2011. **22**: p. S551.
364. Giovannucci, E. and A.T. Chan, *Role of vitamin and mineral supplementation and aspirin use in cancer survivors.* Journal of Clinical Oncology, 2010. **28**(26): p. 4081-4085.
365. Gorham, E.D., S.B. Mohr, F.C. Garland, and C.F. Garland, *Vitamin D for Cancer Prevention and Survival*. Vitamin D: Physiology, Molecular Biology, and Clinical Applications, Second Edition, ed. M.F. Holick. 2010. 813-840.
366. Grant, W.B., *Does solar ultraviolet irradiation affect cancer mortality rates in China?* Asian Pac J Cancer Prev, 2007. **8**(2): p. 236-42.
367. Kelly, D., E. Theodoratou, S.M. Farrington, R. Fraser, H. Campbell, M.G. Dunlop, and L. Zgaga, *The contributions of adjusted ambient ultraviolet B radiation at place of residence and other determinants to serum 25-hydroxyvitamin D concentrations.* Br J Dermatol, 2016. **174**(5): p. 1068-78.

10 Appendices

10.1 Appendix 1: Search strategy for oesophageal and gastric cancer meta-analysis

Oesophageal cancer risk

- **WEB OF SCIENCE**
  - You searched for: TOPIC:((vitamin D cholecalciferol) OR(ergocalciferol) OR (25-hydroxyvitamin D) OR (vitamin D receptor(s)) OR (calcitriol receptor(s)) OR (cholecalciferol)) *AND* TOPIC:((Barrett's (o)esophagus) OR (neoplasm(s)) OR ((o)esophageal cancer) OR (adenocarcinoma) OR (squamous cell carcinoma) OR (tumour(s))) *AND* TOPIC: ((single nucleotide polymorphism(s)) OR (UVB) OR (ultraviolet) OR (genetic polymorphism(s)) OR (sun exposure) OR (solar radiation) OR (latitude) OR (sunlight) OR (geographic variation))
  - 240 papers found including relevant ones
    - Gu
    - Van winkle X2
    - Abnet 2010
- **EMBASE**
  - #1= 'vitamin d'/exp OR 'vitamin d' OR 'cholecalciferol'/exp OR 'cholecalciferol' OR 'ergocalciferol'/exp OR 'ergocalciferol' OR '25-hydroxyvitamin d'/exp OR '25-hydroxyvitamin d' OR 'vitamin d receptor'/exp OR 'vitamin d receptor' OR 'calcitriol receptor'/exp OR 'calcitriol receptor'
  - #2= 'single nucleotide polymorphism' OR 'uvb' OR 'ultraviolet' OR 'genetic polymorphism' OR 'sun exposure' OR 'solar radiation' OR 'latitude' OR 'sunlight' OR 'geographic variation'
  - #3= 'Barrett's esophagus' OR 'neoplasm' OR 'esophageal cancer' OR 'adenocarcinoma' OR 'squamous cell carcinoma' OR 'tumour'
  - Main search: 4= #1 OR #2 AND #3
  - 742 papers found including relevant ones:
    - Janmaat
    - Wang
    - Chang

- **PUB MED/MEDLINE**
  - Search ((((((((((vitamin D) OR ergocalciferol) OR 25-hydroxyvitamin D) OR vitamin D receptor(s)) OR calcitriol receptor(s)) OR cholecalciferol)))) AND ((((((Barrett's (o)esophagus) OR neoplasm(s)) OR (o)esophageal cancer) OR adenocarcinoma) OR squamous cell carcinoma) OR tumour(s))) AND (((((((single nucleotide polymorphism(s)) OR ultraviolet) OR genetic polymorphism(s)) OR sun exposure) OR UVB, solar radiation) OR sunlight) OR latitude) OR geographic variation)
  - 604 papers found including relevant ones:

- Gu
- Tran
- Li

As I was not involved in the original search, I do not have information about exactly which studies were excluded and why.

Restricted to 2014-2015

- **WEB OF SCIENCE**
  - You searched for: TOPIC:((vitamin D cholecalciferol) OR(ergocalciferol) OR (25-hydroxyvitamin D) OR (vitamin D receptor(s)) OR (calcitriol receptor(s)) OR (cholecalciferol)) *AND* TOPIC:((Barrett's (o)esophagus) OR (neoplasm(s)) OR ((o)esophageal cancer) OR (adenocarcinoma) OR (squamous cell carcinoma) OR (tumour(s))) *AND* TOPIC: ((single nucleotide polymorphism(s)) OR (UVB) OR (ultraviolet) OR (genetic polymorphism(s)) OR (sun exposure) OR (solar radiation) OR (latitude) OR (sunlight) OR (geographic variation))
  - 24 papers found including relevant ones (n=3)
    - Sunlight, ultraviolet radiation, vitamin D and skin cancer: how much sunlight do we need? By: Holick, Michael F (review)
    - Solar ultraviolet irradiance and cancer incidence and mortality. By: Grant (review)
    - Vitamin D receptor polymorphisms and cancer. By: Gandini (review)

- **EMBASE**
  - #1= 'vitamin d'/exp OR 'vitamin d' OR 'cholecalciferol'/exp OR 'cholecalciferol' OR 'ergocalciferol'/exp OR 'ergocalciferol' OR '25-hydroxyvitamin d'/exp OR '25-hydroxyvitamin d' OR 'vitamin d receptor'/exp OR 'vitamin d receptor' OR 'calcitriol receptor'/exp OR 'calcitriol receptor'
  - #2= 'single nucleotide polymorphism' OR 'uvb' OR 'ultraviolet' OR 'genetic polymorphism' OR 'sun exposure' OR 'solar radiation' OR 'latitude' OR 'sunlight' OR 'geographic variation'
  - #3= 'Barrett's esophagus' OR 'neoplasm' OR 'esophageal cancer' OR 'adenocarcinoma' OR 'squamous cell carcinoma' OR 'tumour'
  - Main search: 4= #1 OR #2 AND #3
  - 89 papers found including relevant ones: (n=5)
    - Mechanistic effects of calcitriol in cancer biology Díaz L. (review)
    - Vitamin D receptor polymorphisms are associated with reduced esophageal vitamin D receptor expression and reduced esophageal adenocarcinoma risk Janmaat V.T (already have)
    - Vitamin D receptor gene polymorphisms and esophageal cancer risk in a Chinese population: A negative study Gu H (already have)
    - Vitamin D receptor polymorphisms and cancer. Gandini S (duplicate)

- Common genetic variants related to vitamin D status are not associated with esophageal squamous cell carcinoma risk in China Wang J.-B (included)

- **PUB MED/MEDLINE**
  - Search ((((((((((vitamin D) OR ergocalciferol) OR 25-hydroxyvitamin D) OR vitamin D receptor(s)) OR calcitriol receptor(s)) OR cholecalciferol)))) AND ((((((Barrett's (o)esophagus) OR neoplasm(s)) OR (o)esophageal cancer) OR adenocarcinoma) OR squamous cell carcinoma) OR tumour(s))) AND ((((((((single nucleotide polymorphism(s)) OR ultraviolet) OR genetic polymorphism(s)) OR sun exposure) OR UVB, solar radiation) OR sunlight) OR latitude) OR geographic variation)
  - 70 papers found including relevant ones (n=7):

After duplication removal

Holick, M.F., *Sunlight, UV-Radiation, Vitamin D and Skin Cancer: How Much Sunlight Do We Need?*, in *Sunlight, Vitamin D and Skin Cancer*. [340]
Grant, W.B., *Solar ultraviolet irradiance and cancer incidence and mortality*. [341]
Gandini, S., et al., *Vitamin D receptor polymorphisms and cancer.* [342]
Díaz, L., et al., *Mechanistic effects of calcitriol in cancer biology.* [343]
Janmaat, V.T., et al., *Vitamin D receptor polymorphisms are associated with reduced esophageal vitamin D receptor expression and reduced esophageal adenocarcinoma risk.* [206]
Gu, H., et al., *Vitamin D receptor gene polymorphisms and esophageal cancer risk in a Chinese population: a negative study. [205]*
Wang, T.J., et al., *Common genetic determinants of vitamin D insufficiency: a genome-wide association study.* [210]
Bikle, D.D., *Vitamin D and cancer: The promise not yet fulfilled.* [344]

Excluded studies

- Studies by Gu *et al.* and Janmaat *et al.*, were already included in the original study list so these were excluded from the updated list.
- Studies by Holick *et al.,* Grant *et al.,* Gandini *et al.,* Diaz *et al.,* and Bikle *et al.,* were excluded as they were review articles.
- This left 1 extra study by Wang *et al.,* which was included in the meta-analysis.

Oesophageal cancer survival

- **WEB OF SCIENCE**
  - You searched for: TOPIC:((vitamin D cholecalciferol) OR(ergocalciferol) OR (25-hydroxyvitamin D) OR (vitamin D receptor(s)) OR (calcitriol receptor(s)) OR (cholecalciferol)) *AND* TOPIC:((Barrett's (o)esophagus) OR (neoplasm(s)) OR ((o)esophageal cancer) OR (adenocarcinoma) OR (squamous cell carcinoma) OR (tumour(s))) *AND* TOPIC: ((single nucleotide polymorphism(s)) OR (UVB) OR (ultraviolet) OR (genetic polymorphism(s)) OR (sun exposure) OR (solar radiation) OR (latitude) OR (sunlight) OR (geographic variation)) *AND* TOPIC: :((Death) OR (mortality) OR (survival))
  - 128 papers found

- **EMBASE**
    - #1= 'vitamin d'/exp OR 'vitamin d' OR 'cholecalciferol'/exp OR 'cholecalciferol' OR 'ergocalciferol'/exp OR 'ergocalciferol' OR '25-hydroxyvitamin d'/exp OR '25-hydroxyvitamin d' OR 'vitamin d receptor'/exp OR 'vitamin d receptor' OR 'calcitriol receptor'/exp OR 'calcitriol receptor'
    - #2= 'single nucleotide polymorphism' OR 'uvb' OR 'ultraviolet' OR 'genetic polymorphism' OR 'sun exposure' OR 'solar radiation' OR 'latitude' OR 'sunlight' OR 'geographic variation'
    - #3= 'Barrett's esophagus' OR 'neoplasm' OR 'esophageal cancer' OR 'adenocarcinoma' OR 'squamous cell carcinoma' OR 'tumour'
    - #4= 'Death' OR 'Mortality' OR 'Survival'
    - Main search: 5= #1 OR #2 AND #3 AND #4
    - 121 papers found

- **PUB MED/MEDLINE**
    - Search ((((((((((vitamin D) OR ergocalciferol) OR 25-hydroxyvitamin D) OR vitamin D receptor(s)) OR calcitriol receptor(s)) OR cholecalciferol)))) AND ((((((Barrett's (o)esophagus) OR neoplasm(s)) OR (o)esophageal cancer) OR adenocarcinoma) OR squamous cell carcinoma) OR tumour(s))) AND ((((((((single nucleotide polymorphism(s)) OR ultraviolet) OR genetic polymorphism(s)) OR sun exposure) OR UVB, solar radiation) OR sunlight) OR latitude) OR geographic variation) AND ((((((Death) OR (mortality) OR (survival)
    - 85 papers found

After duplication removal

- 15 full articles selected

Grant, (2010), An ecological study of cancer incidence and mortality rates in France with respect to latitude, an index for vitamin D production [106]
Trowbridge, Vitamin D and the Epidemiology of Upper Gastrointestinal Cancers: A Critical Analysis of the Current Evidence [345]
Grant, (2007) An ecologic study of cancer mortality rates in Spain with respect to indices of solar UVB irradiance and smoking [105]
Boscoe, Solar ultraviolet-B exposure and cancer incidence and mortality in the United States, 1993-2002 [7]
Chen, (2013) Relationship between cancer survival and ambient ultraviolet B irradiance in China [329]
Chen, (2010)Relationship between cancer mortality/incidence and ambient ultraviolet B irradiance in China [104]
Giovannucci, The epidemiology of vitamin D and cancer incidence and mortality: A review (United States) [346]
Fleischer, (2016), Solar radiation and the incidence and mortality of leading invasive cancers in the United States. [347]
Grant, (2016) Roles of Solar UVB and Vitamin D in Reducing Cancer Risk and Increasing Survival [126]
Jorde, Polymorphisms related to the serum 25-hydroxyvitamin D level and risk of myocardial infarction, diabetes, cancer and mortality. The Tromso Study [348]

Levin, Genetic variants and associations of 25-hydroxyvitamin D concentrations with major clinical outcomes [349]
Skeie, Cod liver oil, other dietary supplements and survival among cancer patients with solid tumours [350]
Köstner, Vitamin D receptor (VDR) polymorphisms in basal cell carcinomas (BCC) and cutaneous squamous cell carcinomas (SCC) [351]
Berlanga-taylor, An integrated approach to defining genetic and environmental determinants for major clinical outcomes involving vitamin D [352]
Freedman, Prospective study of serum vitamin D and cancer mortality in the United States. [188]

Excluded studies

- Studies by Fleischer *et al.*, Chen *et al.* (2010), Chen *et al.* (2013), Grant *et al.* (2010), and Grant *et al.* (2007), were excluded as they were ecological studies.
- Studies by Berlanga-taylor *et al.*, Giovannucci et al., Grant *et al.* (2016), and Trowbridge *et al.* were excluded as they were review articles.
- The study by Köstner *et al.* was excluded as it used vitamin D analogues.
- Studies by Jorde *et al.*, Boscoe *et al.*, Skeie *et al*., Freedman *et al*. and Levin *et al.* were excluded as they were deemed not relevant after reading the full article.

Gastric cancer risk

- **WEB OF SCIENCE**
  - You searched for: TOPIC:((vitamin D cholecalciferol) OR(ergocalciferol) OR (25-hydroxyvitamin D) OR (vitamin D receptor(s)) OR (calcitriol receptor(s)) OR (cholecalciferol)) *AND* TOPIC:((Gastric cancer) OR (neoplasm(s)) OR (stomach cancer) OR (cardia adenocarcinoma) OR (non-cardia adenocarcinoma) OR (tumour(s))) *AND* TOPIC: ((single nucleotide polymorphism(s)) OR (UVB) OR (ultraviolet) OR (genetic polymorphism(s)) OR (sun exposure) OR (solar radiation) OR (latitude) OR (sunlight) OR (geographic variation))
  - 412 papers found.

- **EMBASE**
  - #1= 'vitamin d'/exp OR 'vitamin d' OR 'cholecalciferol'/exp OR 'cholecalciferol' OR 'ergocalciferol'/exp OR 'ergocalciferol' OR '25-hydroxyvitamin d'/exp OR '25-hydroxyvitamin d' OR 'vitamin d receptor'/exp OR 'vitamin d receptor' OR 'calcitriol receptor'/exp OR 'calcitriol receptor'
  - #2= 'single nucleotide polymorphism' OR 'uvb' OR 'ultraviolet' OR 'genetic polymorphism' OR 'sun exposure' OR 'solar radiation' OR 'latitude' OR 'sunlight' OR 'geographic variation'
  - #3= ‘Gastric cancer' OR 'neoplasm' OR ‘stomach cancer' OR ' cardia adenocarcinoma' OR ‘non-cardia adenocarcinoma' OR 'tumour'
  - Main search: 4= #1 OR #2 AND #3
  - 849 papers found.

- **PUB MED/MEDLINE**
    - Search ((((((((((vitamin D) OR ergocalciferol) OR 25-hydroxyvitamin D) OR vitamin D receptor(s)) OR calcitriol receptor(s)) OR cholecalciferol)))) AND ((((((Gastric cancer) OR neoplasm(s)) OR stomach cancer) OR cardia adenocarcinoma) OR non-cardia adenocarcinoma) OR tumour(s))) OR (((((((single nucleotide polymorphism(s)) OR ultraviolet) OR genetic polymorphism(s)) OR sun exposure) OR UVB, solar radiation) OR sunlight) OR latitude) OR geographic variation)
    - 703 papers found.

After duplication removal

- 25 full articles selected

Cong, L., et al. (2015). "FokI Polymorphism of the Vitamin D Receptor Gene Is Associated with Susceptibility to Gastric Cancer: A Case-Control Study." [224]
Fleischer, A. B. and S. E. Fleischer (2016). "Solar radiation and the incidence and mortality of leading invasive cancers in the United States." [347]
Freedman, D. M., et al. (2010). "Serum 25-hydroxyvitamin D and cancer mortality in the NHANES III study (1988-2006)." [188]
Fukuda, Y., et al. (2008). "Multilevel analysis of solar radiation and cancer mortality using ecological data in Japan." [330]
Giovannucci, E., et al. (2006). "Prospective study of predictors of vitamin D status and cancer incidence and mortality in men." [121]
Grant, W. B. (2010). "Re: Overview of the cohort consortium vitamin d pooling project of rarer cancers." [353]
Grant, W. B. (2010). "Relation between prediagnostic serum 25-hydroxyvitamin D level and incidence of breast, colorectal, and other cancers." [354]
Grant, W. B. (2015). "The roles of solar UVB and vitamin D in reducing the risk of cancer." [355]
Grant, W. B. and S. B. Mohr (2009). "Ecological Studies Of Ultraviolet B, Vitamin D And Cancer Since 2000." [356]
Helzlsouer, K. J. and V. S. Comm (2010). "Overview of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers." [124]
Holick, M. F. (2013). "Vitamin D, sunlight and cancer connection." [357]
Moon, S. J., et al. (2005). "Ultraviolet radiation: effects on risks of prostate cancer and other internal cancers." [358]
Shen, X. B., et al. (2014). "Screening of susceptibility genes and multi-gene risk analysis in gastric cancer." [225]
Shui, I. and E. Giovannucci (2014). "Vitamin D status and cancer incidence and mortality." [359]
Takahashi, E. (1974). "Stomach cancer and ecologic factors in Japan." [331]
Trowbridge, R., et al. (2013). "Vitamin D and the Epidemiology of Upper Gastrointestinal Cancers: A Critical Analysis of the Current Evidence." [360]
Unal, D., et al. (2014). "Lack of any Association between Season of Diagnosis and Survival of Gastric Cancer Cases in Kayseri, Turkey." [361]
Chen W, Dawsey SM, Qiao YL, Mark SD, Dong ZW, Taylor PR, et al. Prospective study of serum 25(OH)-vitamin D concentration and risk of oesophageal and gastric cancers. [9]
Mayne, Nutrient Intake and Risk of Subtypes of Esophageal and Gastric Cancer [200]

Abnet CC, Chen Y, Chow WH, Gao YT, Helzlsouer KJ, LeMarchand L, et al. Circulating 25-hydroxyvitamin D and risk of esophageal and gastric cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. [202]
Ren, Prognostic effects of 25-hydroxyvitamin D levels in gastric cancer [190]
Cornée J, Pobel D, Riboli E, Guyader M, Hémon B. A case-control study of gastric cancer and nutritional factors in Marseille, France. [222]
La Vecchia C, Ferraroni M, D'Avanzo B, Decarli A, Franceschi S. Selected micronutrient intake and the risk of gastric cancer. [223]
Pelucchi C, Tramacere I, Bertuccio P, Tavani A, Negri E, La Vecchia C. Dietary intake of selected micronutrients and gastric cancer risk: An Italian case-control study. [221]
Chen W, Clements M, Rahman B, Zhang S, Qiao Y, Armstrong BK. Relationship between cancer mortality/incidence and ambient ultraviolet B irradiance in China. Cancer Causes Control. [104]

Excluded studies

- Studies by Chen *et al.* (2010), Ren *et al.*, Fleischer *et al.*, Freedman *et al.* and Fukuda *et al.* all contained information on mortality and were excluded due to this.
- The study by Unal *et al.* was considered not relevant as it was an ecological study design
- The study by Giovannucci *et al.* was excluded as it used predicted 25(OH)D concentrations.
- Studies by Trowbridge *et al.*, Shui *et al.*, Grant *et al.* (2009), Grant *et al.* (2010), Grant *et al.* (2015) and Holick *et al.* were review articles and were excluded.
- Studies by Helzlsouer *et al.* and Grant *et al.* (2010) were found to be commentary pieces and so were excluded.
- Studies by Moon *et al.* and Takahashi et al. were excluded as they did not have information on gastric cancer.
- This left 8 studies which were included in the meta-analysis and are outlined in detail in Chapter 2.

Gastric cancer survival

WEB OF SCIENCE

- You searched for: TOPIC:((vitamin D cholecalciferol) OR (ergocalciferol) OR (25-hydroxyvitamin D) OR (vitamin D receptor(s)) OR (calcitriol receptor(s)) OR (cholecalciferol)) *AND* TOPIC:((Gastric cancer) OR (neoplasm(s)) OR (stomach cancer) OR (cardia adenocarcinoma) OR (non-cardia adenocarcinoma) OR (tumour(s))) *AND* TOPIC: ((single nucleotide polymorphism(s)) OR (UVB) OR (ultraviolet) OR (genetic polymorphism(s)) OR (sun exposure) OR (solar radiation) OR (latitude) OR (sunlight) OR (geographic variation)) *AND* TOPIC: :((Death) OR (mortality) OR (survival))
- 126 papers found.

- **EMBASE**
    - #1= 'vitamin d'/exp OR 'vitamin d' OR 'cholecalciferol'/exp OR 'cholecalciferol' OR 'ergocalciferol'/exp OR 'ergocalciferol' OR '25-hydroxyvitamin d'/exp OR '25-hydroxyvitamin d' OR 'vitamin d receptor'/exp OR 'vitamin d receptor' OR 'calcitriol receptor'/exp OR 'calcitriol receptor'
    - #2= 'single nucleotide polymorphism' OR 'uvb' OR 'ultraviolet' OR 'genetic polymorphism' OR 'sun exposure' OR 'solar radiation' OR 'latitude' OR 'sunlight' OR 'geographic variation'
    - #3= 'Gastric cancer' OR 'neoplasm' OR 'stomach cancer' OR ' cardia adenocarcinoma' OR 'non-cardia adenocarcinoma' OR 'tumour'
    - #4= 'Death' OR 'Mortality' OR 'Survival'
    - Main search: 5= #1 OR #2 AND #3 AND #4
    - 129 papers found.

- **PUB MED/MEDLINE**
    - Search ((((((((((vitamin D) OR ergocalciferol) OR 25-hydroxyvitamin D) OR vitamin D receptor(s)) OR calcitriol receptor(s)) OR cholecalciferol)))) AND ((((((Gastric cancer) OR neoplasm(s)) OR stomach cancer) OR cardia adenocarcinoma) OR non-cardia adenocarcinoma) OR tumour(s))) OR (((((((single nucleotide polymorphism(s)) OR ultraviolet) OR genetic polymorphism(s)) OR sun exposure) OR UVB, solar radiation) OR sunlight) OR latitude) OR geographic variation) AND ((((((Death) OR (mortality) OR (survival)
    - 80 papers found.

After duplication removal

- 19 full articles selected

Ren, Prognostic effects of 25-hydroxyvitamin D levels in gastric cancer [334]
Fleischer, A. B. and S. E. Fleischer (2016). "Solar radiation and the incidence and mortality of leading invasive cancers in the United States." [347]
Freedman, D. M., et al. (2010). "Serum 25-hydroxyvitamin D and cancer mortality in the NHANES III study (1988-2006)." [188]
Fukuda, Y., et al. (2008). "Multilevel analysis of solar radiation and cancer mortality using ecological data in Japan." [330]
Bayer, vitamin D and cancer [362]
Berlanga-taylor, An integrated approach to defining genetic and environmental determinants for major clinical outcomes involving vitamin D [352]
Chen, Relationship between cancer survival and ambient ultraviolet B irradiance in China (2013) [329]
Chen, Relationship between cancer mortality/incidence and ambient ultraviolet B irradiance in China (2010) [104]
Daly, Lower serum 25-hydroxyvitamin d levels are associated with greater all-cause and cancer-related mortality among Australian adults: Findings from the Australian diabetes, obesity and lifestyle study (AUSDIAB) [363]
Giovannucci, Role of vitamin and mineral supplementation and aspirin use in cancer survivors [364]
Gorham, Vitamin D for Cancer Prevention and Survival [365]
Grant (2002), An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation [5]
Grant (2007), Does solar ultraviolet irradiation affect cancer mortality rates in China? [366]
Grant (2008), Solar ultraviolet irradiance and cancer incidence and mortality [341]

Grant (2016), Roles of Solar UVB and Vitamin D in Reducing Cancer Risk and Increasing Survival [126]
Jorde, Polymorphisms related to the serum 25-hydroxyvitamin D level and risk of myocardial infarction, diabetes, cancer and mortality. The Tromso Study [348]
Levin, Genetic variants and associations of 25-hydroxyvitamin D concentrations with major clinical outcomes [349]
Trowbridge, Vitamin D and the Epidemiology of Upper Gastrointestinal Cancers: A Critical Analysis of the Current Evidence [360]
Unal, Lack of any Association between Season of Diagnosis and Survival of Gastric Cancer Cases in Kayseri, Turkey [361]

Excluded studies

- Studies by Fleischer *et al.*, Fukuda *et al.*, Chen *et al.* (2010), Chen *et al.* (2013), Grant *et al.* (2002), and Grant *et al.* (2007) were excluded as they were ecological studies.
- Studies by Berlanga-taylor *et al.*, Giovannucci et al., Gorham *et al.*, Grant *et al.* (2008), Grant *et al.* (2016), and Trowbridge *et al.* were excluded as they were review articles.
- The study by Bayer *et al.* was excluded as it was not in English.
- Studies by Daly *et al.*, Jorde *et al.*, Unal *et al.*, Freedman *et al*. and Levin *et al.* were excluded as they were deemed not relevant after reading the full article.
- This left 1 study which was included in the review and is outlined in detail in Chapter 2.

Updated search in Cochrane library 2017:

Oesophageal cancer Risk

- #1= vitamin d OR cholecalciferol OR ergocalciferol OR 25-hydroxyvitamin d OR vitamin d receptor OR calcitriol receptor
- #2= single nucleotide polymorphism OR uvb OR ultraviolet OR genetic polymorphism OR sun exposure OR solar radiation OR latitude OR sunlight OR geographic variation
- #3= Barrett's esophagus OR neoplasm OR esophageal cancer OR adenocarcinoma OR squamous cell carcinoma OR tumour
- Main search: 4= #1 OR #2 AND #3

- 24 articles found
  - 21 reviews
  - 3 protocols
- 1 relevant review found (Bjelakovic [Vitamin D supplementation for prevention of cancer in adults]
  - References checked and no additional suitable studies were found

Oesophageal cancer survival

- #1= vitamin d OR cholecalciferol OR ergocalciferol OR 25-hydroxyvitamin d OR vitamin d receptor OR calcitriol receptor
- #2= single nucleotide polymorphism OR uvb OR ultraviolet OR genetic polymorphism OR sun exposure OR solar radiation OR latitude OR sunlight OR geographic variation
- #3= Barrett's esophagus OR neoplasm OR esophageal cancer OR adenocarcinoma OR squamous cell carcinoma OR tumour
- #4= Death OR Mortality OR Survival
- Main search: 5= #1 OR #2 AND #3 AND #4

- 21 articles found
  - 18 reviews
  - 3 protocols
- 1 relevant review found (Bjelakovic [Vitamin D supplementation for prevention of mortality in adults]
  - References checked and no additional suitable studies were found

Gastric cancer Risk

- #1= vitamin d OR cholecalciferol OR ergocalciferol OR 25-hydroxyvitamin d OR vitamin d receptor OR calcitriol receptor
- #2= single nucleotide polymorphism OR uvb OR ultraviolet OR genetic polymorphism OR sun exposure OR solar radiation OR latitude OR sunlight OR geographic variation
- #3= Gastric cancer OR neoplasm OR stomach cancer OR cardia adenocarcinoma OR non-cardia adenocarcinoma OR tumour
- Main search: 4= #1 OR #2 AND #3

- 31 articles found
  - 30 reviews
  - 1 protocols
- 1 relevant review found (Bjelakovic [Vitamin D supplementation for prevention of cancer in adults]
  - References checked and no additional suitable studies were found

Gastric cancer survival

- #1= vitamin d OR cholecalciferol OR ergocalciferol OR 25-hydroxyvitamin d OR vitamin d receptor OR calcitriol receptor
- #2= single nucleotide polymorphism OR uvb OR ultraviolet OR genetic polymorphism OR sun exposure OR solar radiation OR latitude OR sunlight OR geographic variation
- #3= Gastric cancer OR neoplasm OR stomach cancer OR cardia adenocarcinoma OR non-cardia adenocarcinoma OR tumour
- #4= Death OR Mortality OR Survival

    - Main search: 5= #1 OR #2 AND #3 AND #4

- 27 articles found
    - 26 reviews
    - 1 protocols
- 1 relevant review found (Bjelakovic [Vitamin D supplementation for prevention of mortality in adults]
    - References checked and no additional suitable studies were found

10.2 Appendix 2: Scoring of meta-analysis studies

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation ✸
   b) yes, eg record linkage or based on self reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases ✸
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls ✸
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint) ✸
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for (Supplementation for dietary vitamin D intake and month of blood draw for 25(OH)D) (Select the most important factor.) ✸
   b) study controls for any additional factor ✸ (smoking)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) ✸
   b) structured interview where blind to case/control status ✸
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes ✸
   b) no

3) Non-Response rate
   a) same rate for both groups ✸
   b) non respondents described
   c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community ✵
   b) somewhat representative of the average _____ in the community ✵
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort ✵
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
   a) secure record (eg surgical records) ✵
   b) structured interview ✵
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes ✵
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for (Supplementation for dietary vitamin D intake and month of blood draw for 25(OH)D) (select the most important factor) ✵
   b) study controls for any additional factor ✵ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment ✵
   b) record linkage ✵
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) ✵
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for ✵
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) ✵
   c) follow up rate < ____% (select an adequate %) and no description of those lost
   d) no statement

10.3 Appendix 3: Work divide of the thesis

Systematic review and meta-analysis.

- *Oesophageal cancer risk and vitamin D*
    - The initial search process was carried out by Dr. Helen Coleman and Dr. Lina Zgaga relevant articles were isolated prior to initiation of my PhD
    - Updated search process was carried out by myself and Dr. Helen Coleman
    - Titles and abstracts were independently examined by at least two researchers (myself, Dr. Helen Coleman and Dr. Lina Zgaga)
    - The quality Scoring processes and information extraction was carried out by myself and Dr. Helen Coleman.
    - All quantitative analysis was carried out by myself, such as forest plots, i^2 calculations and funnel plots.
- *Oesophageal cancer survival and vitamin D*
    - All search processes, assessment of studies, extraction of information was carried out by myself.
    - All quantitative analysis was carried out myself
- *Gastric cancer risk and vitamin D*
    - All search processes, assessment of studies, extraction of information was carried out by myself.
    - All quantitative analysis was carried out myself
- *Gastric cancer survival and vitamin D*
    - All search processes, assessment of studies, extraction of information was carried out by myself.
    - All quantitative analysis was carried out myself

TEMIS database

- This database was constructed by the Royal Netherlands Meteorological Institute and is freely available for use. This database has been adjusted for cloud cover, ozone layer and altitude by Dr. Jos van Geffen and Dr. Michiel van Weele.
- Data cleaning and amalgamation was carried out by myself. All files for all grids were in separate files and in an unusable format. This needed to be cleaned and amalgamated to the correct format. Additionally, there were some blackout days where D-UVB could not be calculated as there was no information on cloud cover or ozone layer. Average values of D-UVB from other years for that day in that location were used as substitutes for those blackout days and these needed to be incorporated into the main dataset.
- Calculation of Annual D-UVB, cw-D-UVB and all other measures using this database was carried out myself.

135 cw-D-UVB development

- This 135 cw-D-UVB was developed by Dr Dervla Kelly and Dr. Lina Zgaga, as is outlined in [367]. 135 days was deemed optimal in the analysis from this article after numerous days had been investigated. This why was 135 days was chosen to be used in this thesis. However, sensitivity analysis was carried out by myself (data not shown) for 60-day and 120 day cw-D-UVB and similar results therefore for continuality purposes 135 days was included.

- 135 cw-D-UVB or annual D-UVB dose for all participants included this thesis was calculated by myself.

Cohorts

- Data from all cohorts were collected by previous individuals. This thesis was a secondary analysis of those databases.

10.4 Appendix 4: UVB Grids

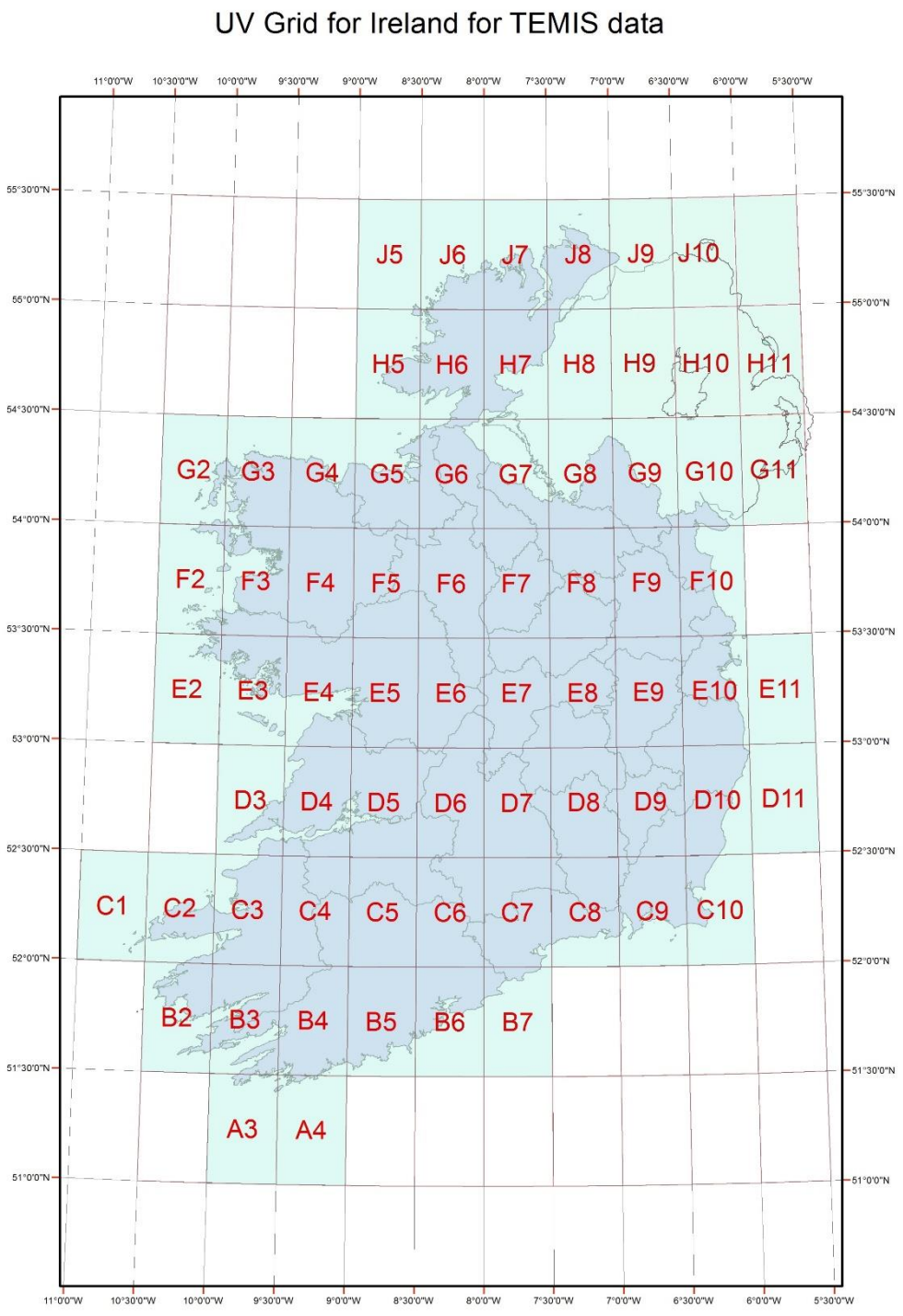

Figure 10.1: TEMIS Grid covering Ireland

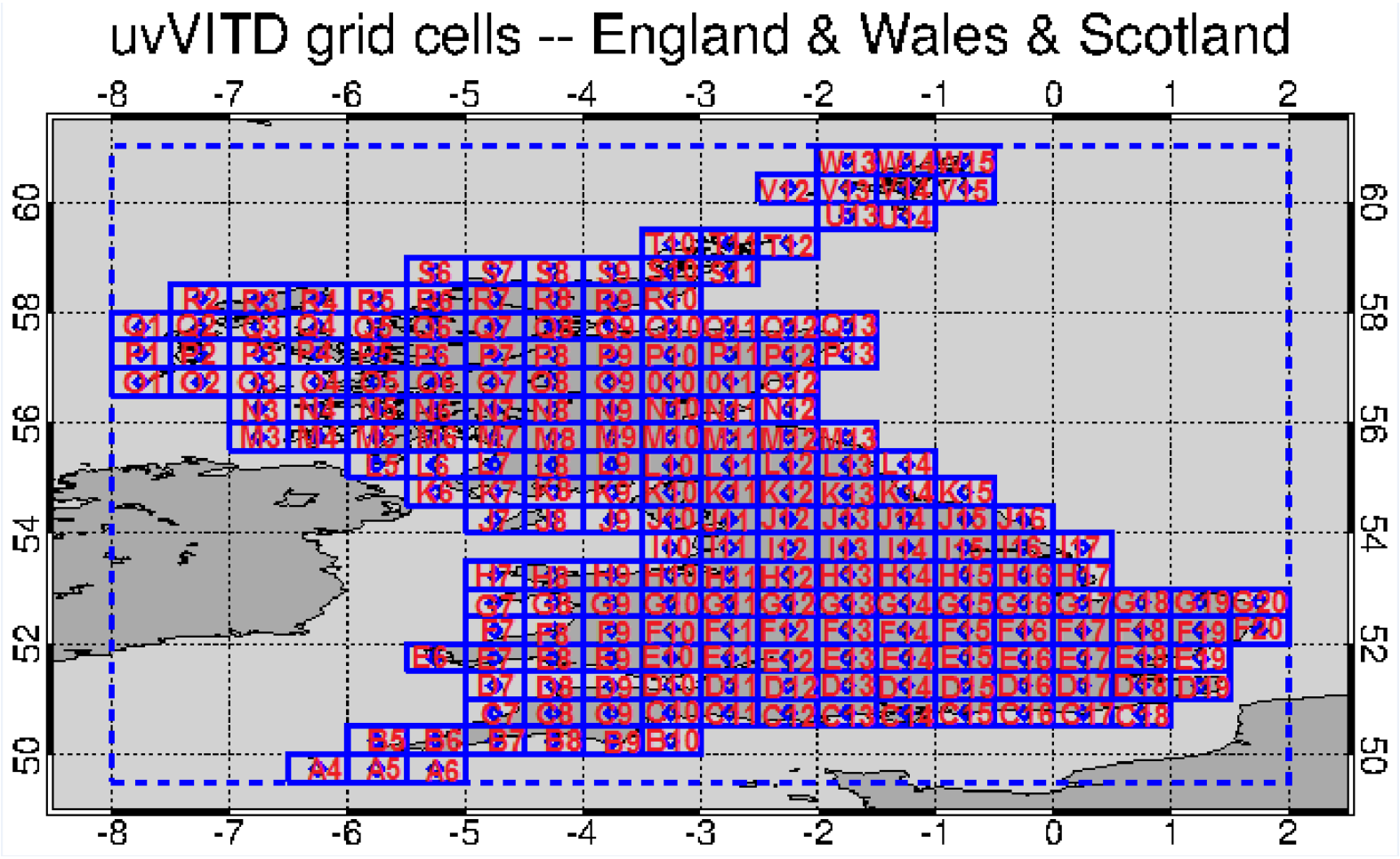

Figure 10.2: TEMIS Grid covering UK

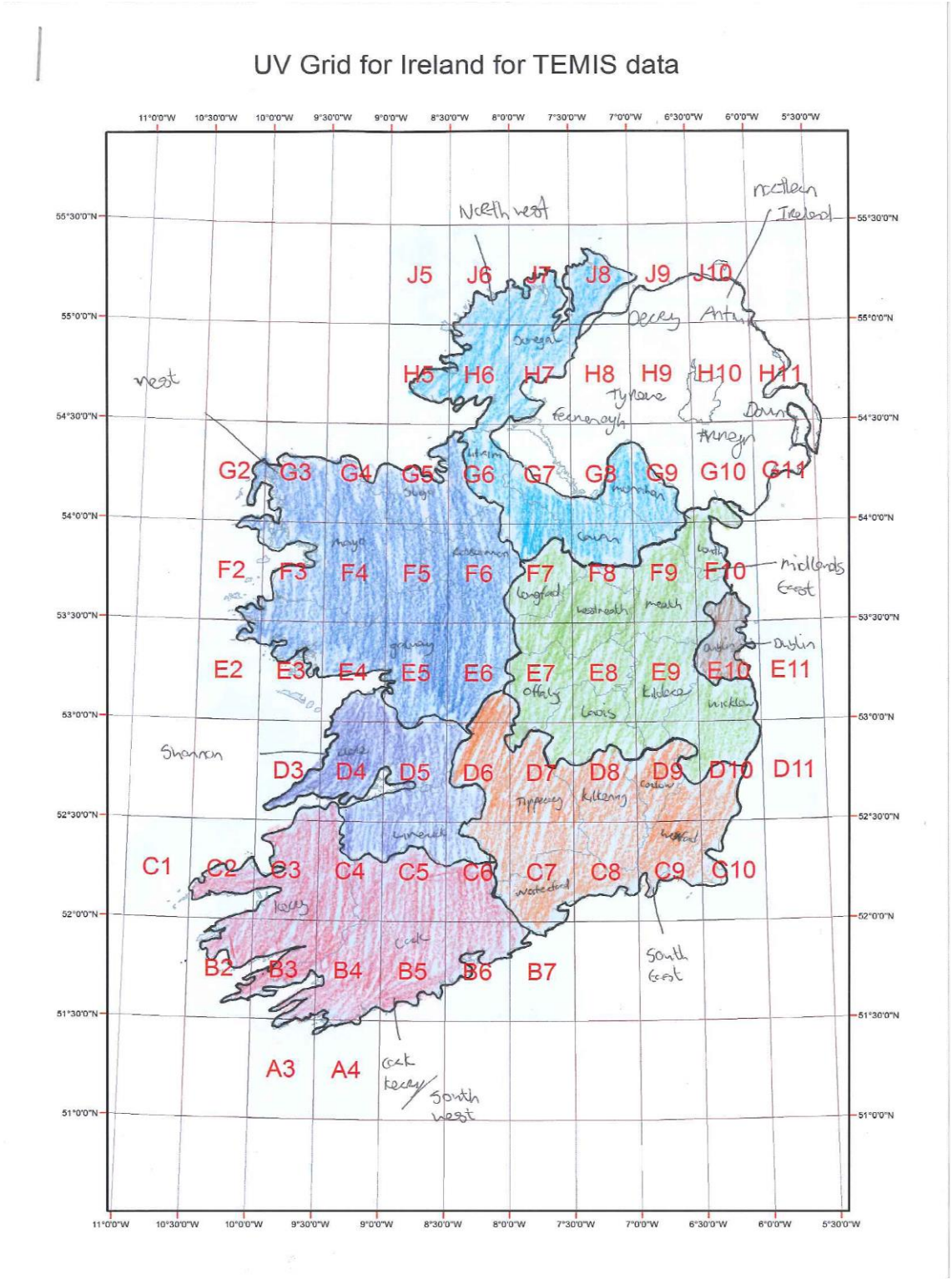

Figure 10.3: Eight regions covering Ireland

Table 10.1: Counties and TEMIS grid references in each of the regional areas

Northern Ireland	North West	West	Midlands East	Dublin	South East	Shannon	South West
Counties							
Down	Donegal	Roscommon	Louth	Dublin	Tipperary	Clare	Cork
Antrim	Leitrim	Sligo	Meath		Kilkenny	Galway	Kerry
Derry	Cavan	Mayo	Kildare		Carlow		
Tyrone	Monaghan	Galway	Longford		Wexford		
Armagh			Westmeath		Waterford		
Fermanagh			Offaly				
			Laois				
			Wicklow				
Grids							
J9	J6	G2	F7	E10	D6	D3	A3
J10	J7	G3	F8		D7	D4	A4
H8	J8	G4	F9		D8	D5	C2
H9	H6	G5	F10		D9		C3
H10	H7	F3	E7		C7		C4
H11	G6	F4	E8		C8		C5
G10	G7	F5	E9		C9		C6
G11	G8	F6	D10		C10		B2
	G9	E2					B3
		E3					B4
		E4					B5
		E5					B6
		E6					

10.5 Appendix 5: Diagnostic plots chapter 4

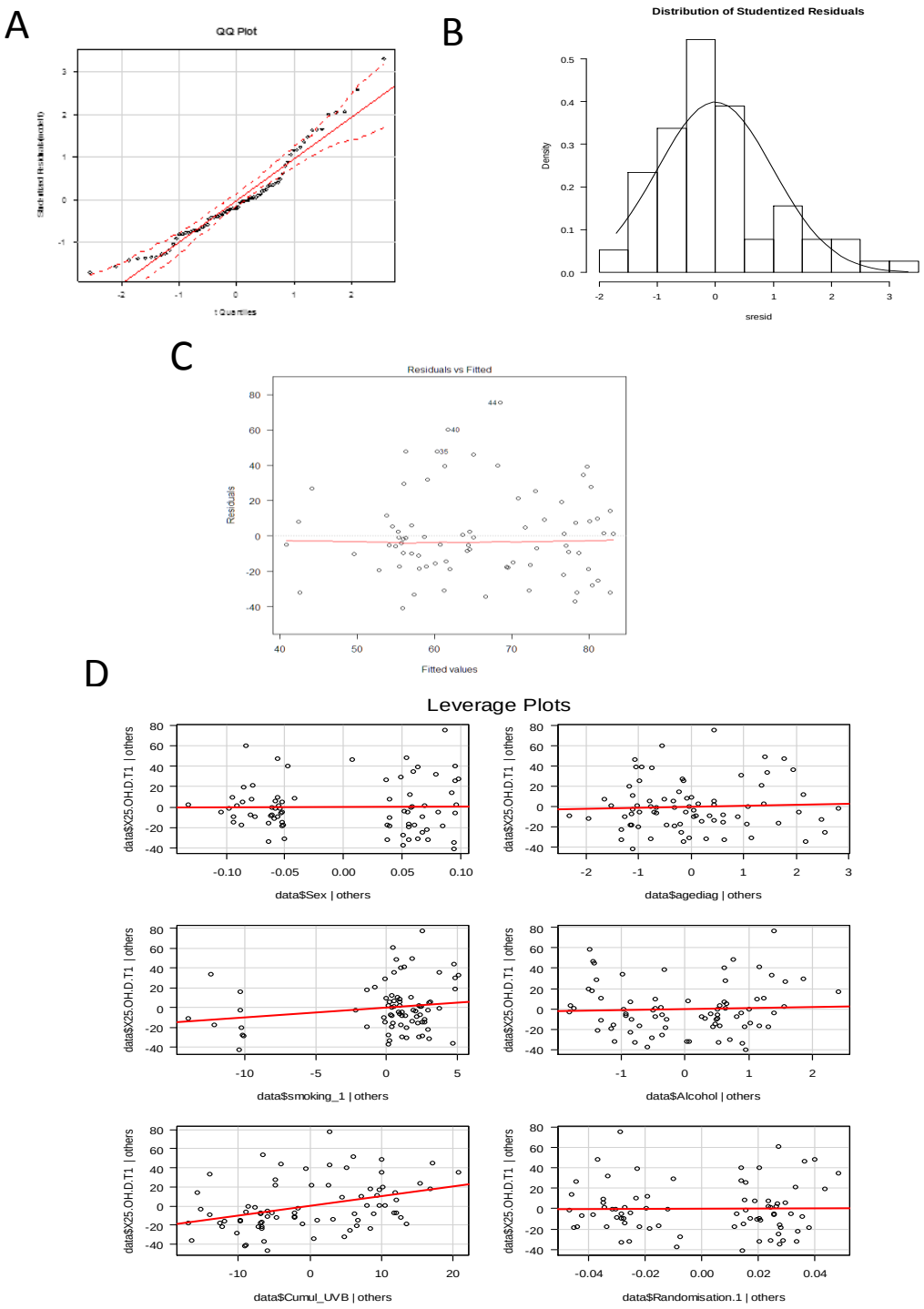

Figure 10.4: Diagnostic plots for regression model examining association between T1 cw-D-UVB and T1 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status.

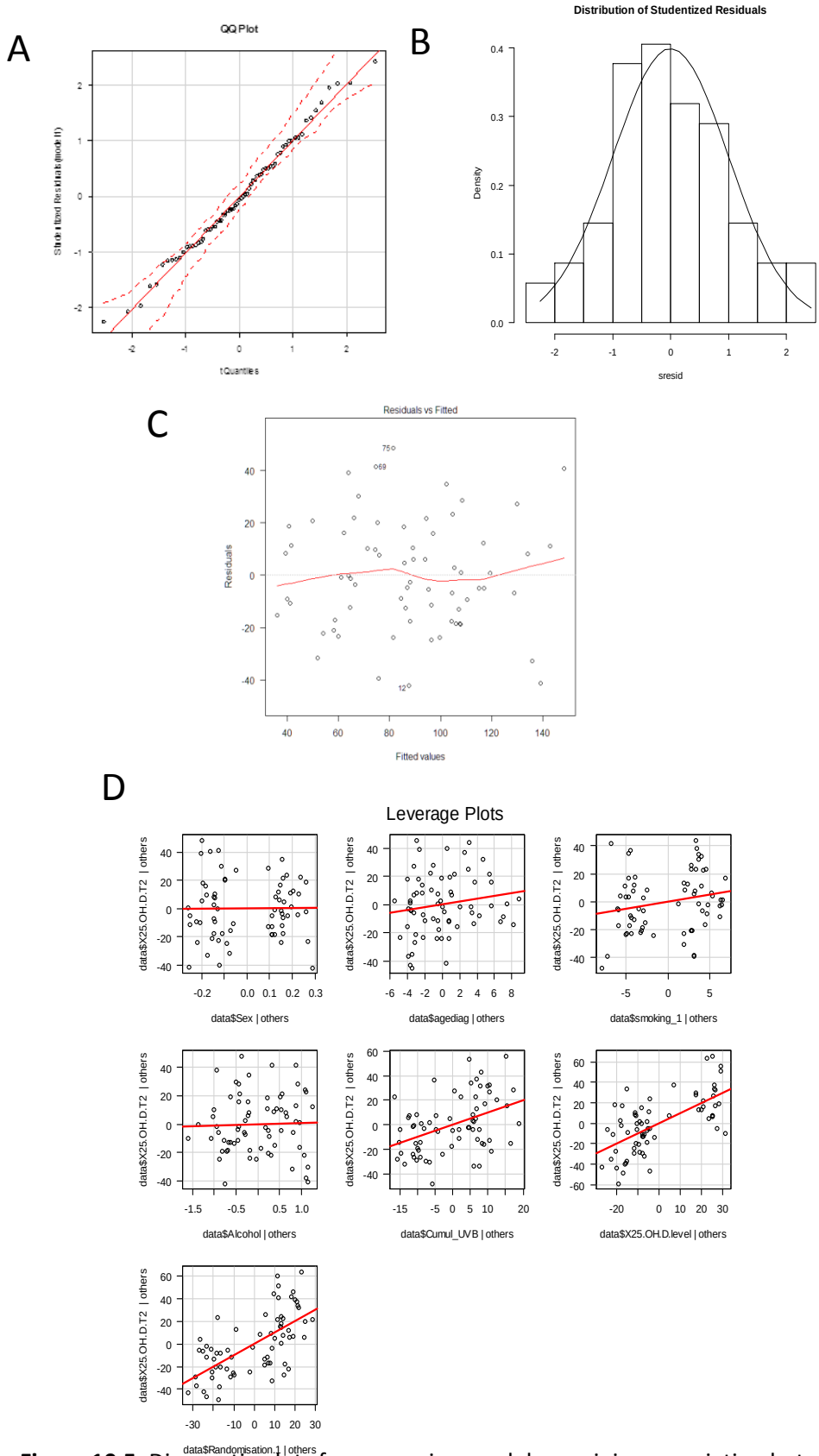

Figure 10.5: Diagnostic plots for regression model examining association between T1 VDscore1 and T1 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status.

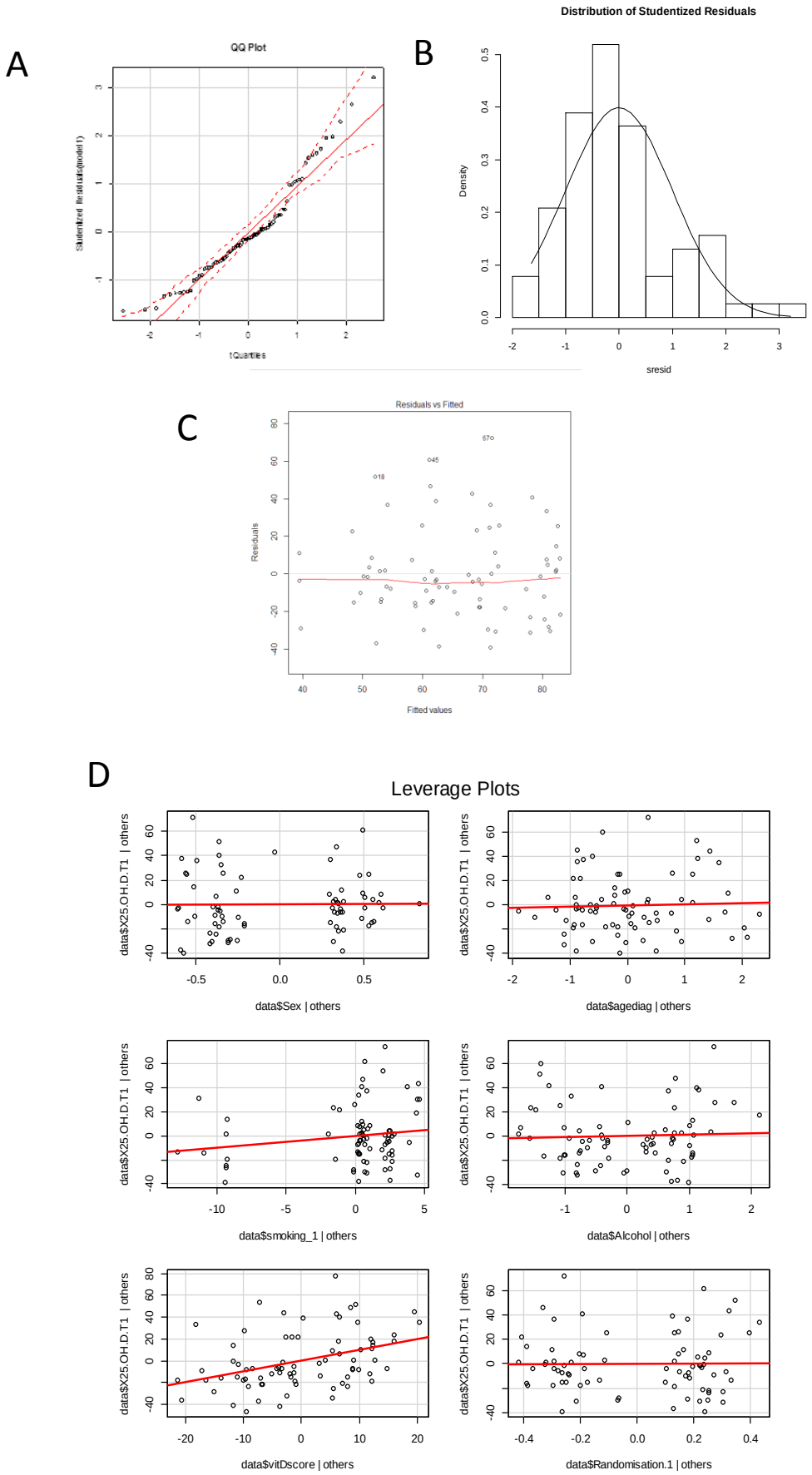

Figure 10.6: Diagnostic plots for regression model examining association between T2 cw-D-UVB and T2 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status, baseline 25(OH)D concentration level and randomisation.

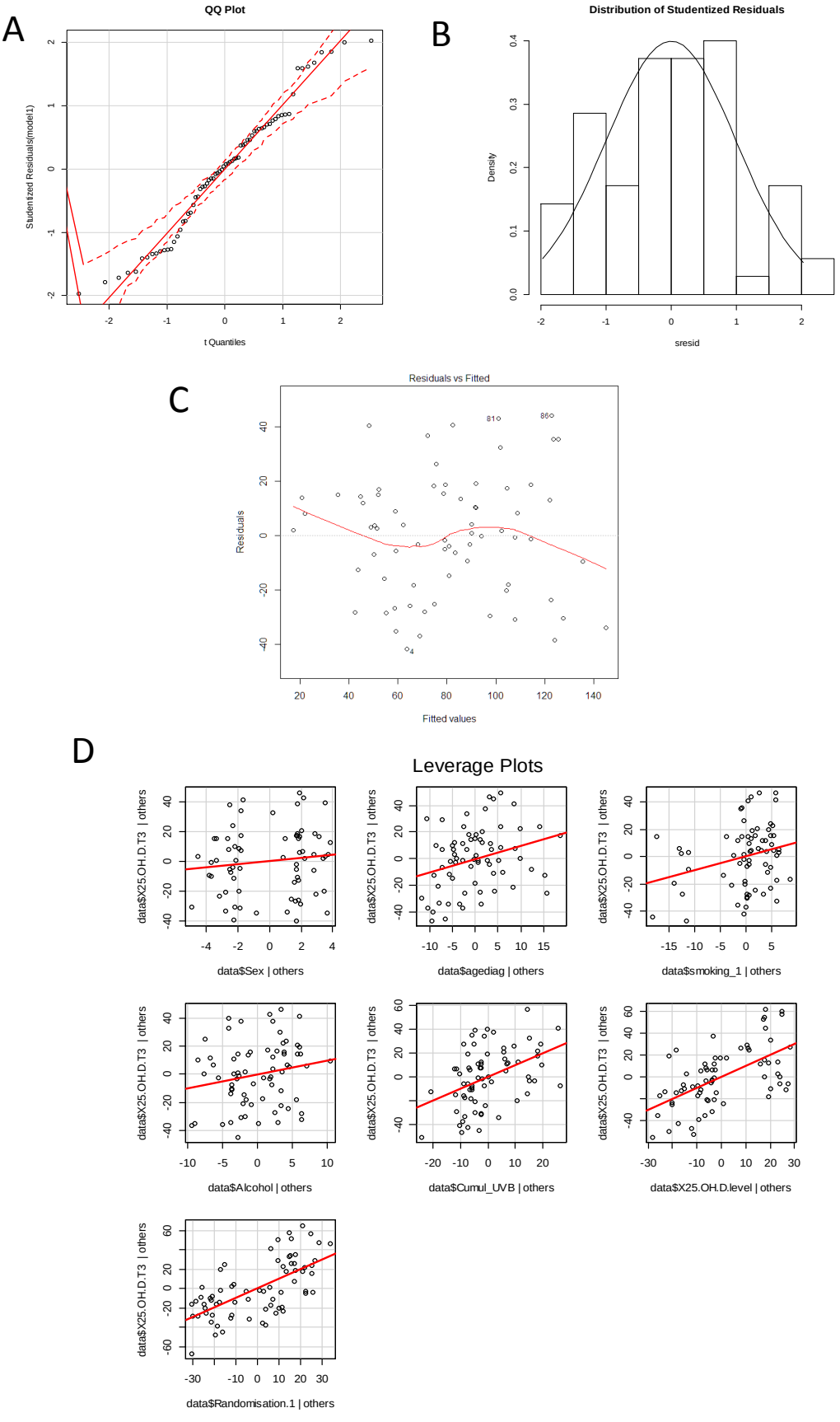

Figure 10.7: Diagnostic plots for regression model examining association between T2 VDscore1 and T2 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status, Baseline 25(OH)D concentration level.

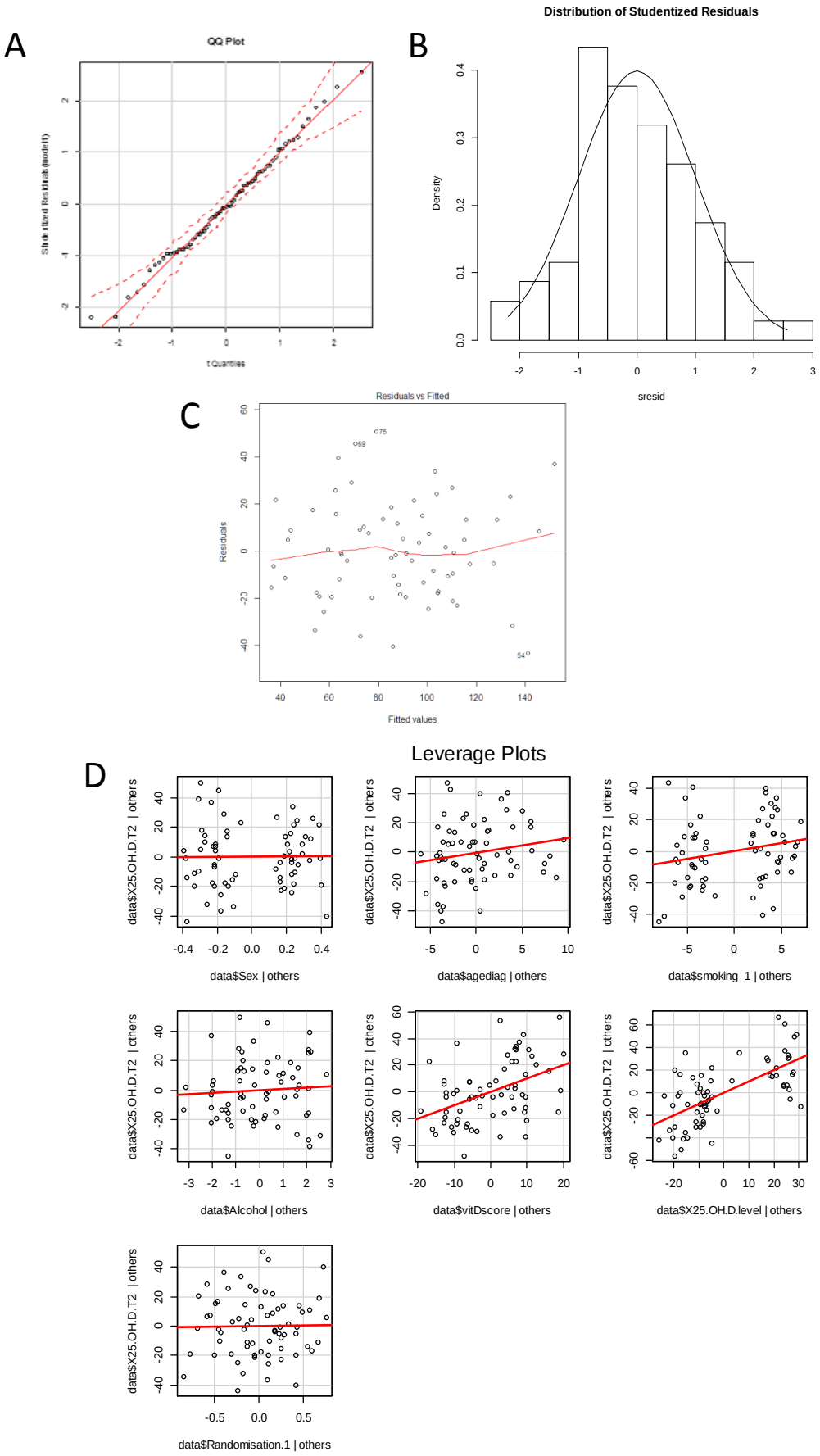

Figure 10.8: Diagnostic plots for regression model examining association between T3 cw-D-UVB and T3 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status, baseline 25(OH)D concentration level and randomisation.

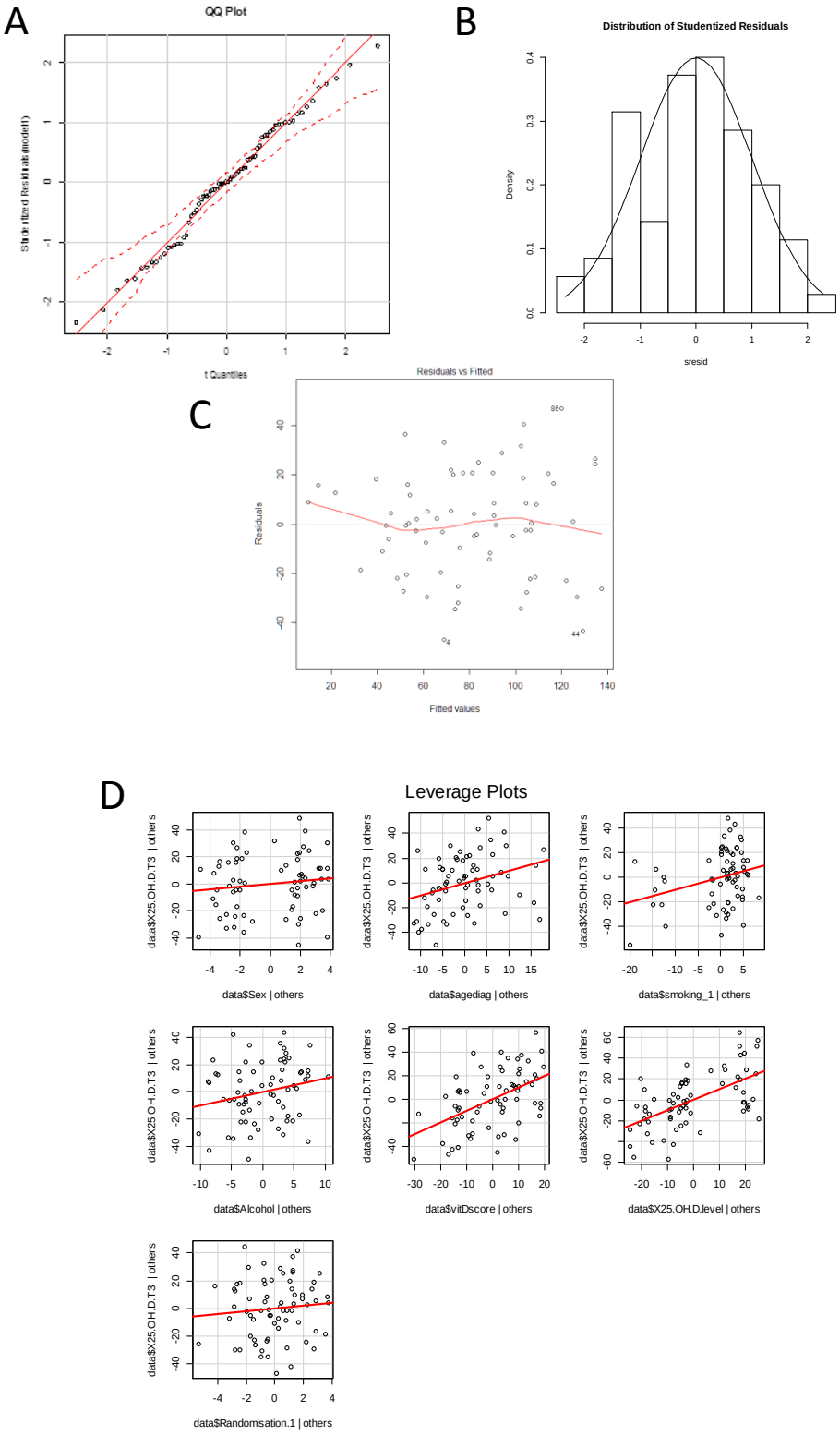

Figure 10.9: Diagnostic plots for regression model examining association between T3 VDscore1 and T3 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status, Baseline 25(OH)D concentration level.

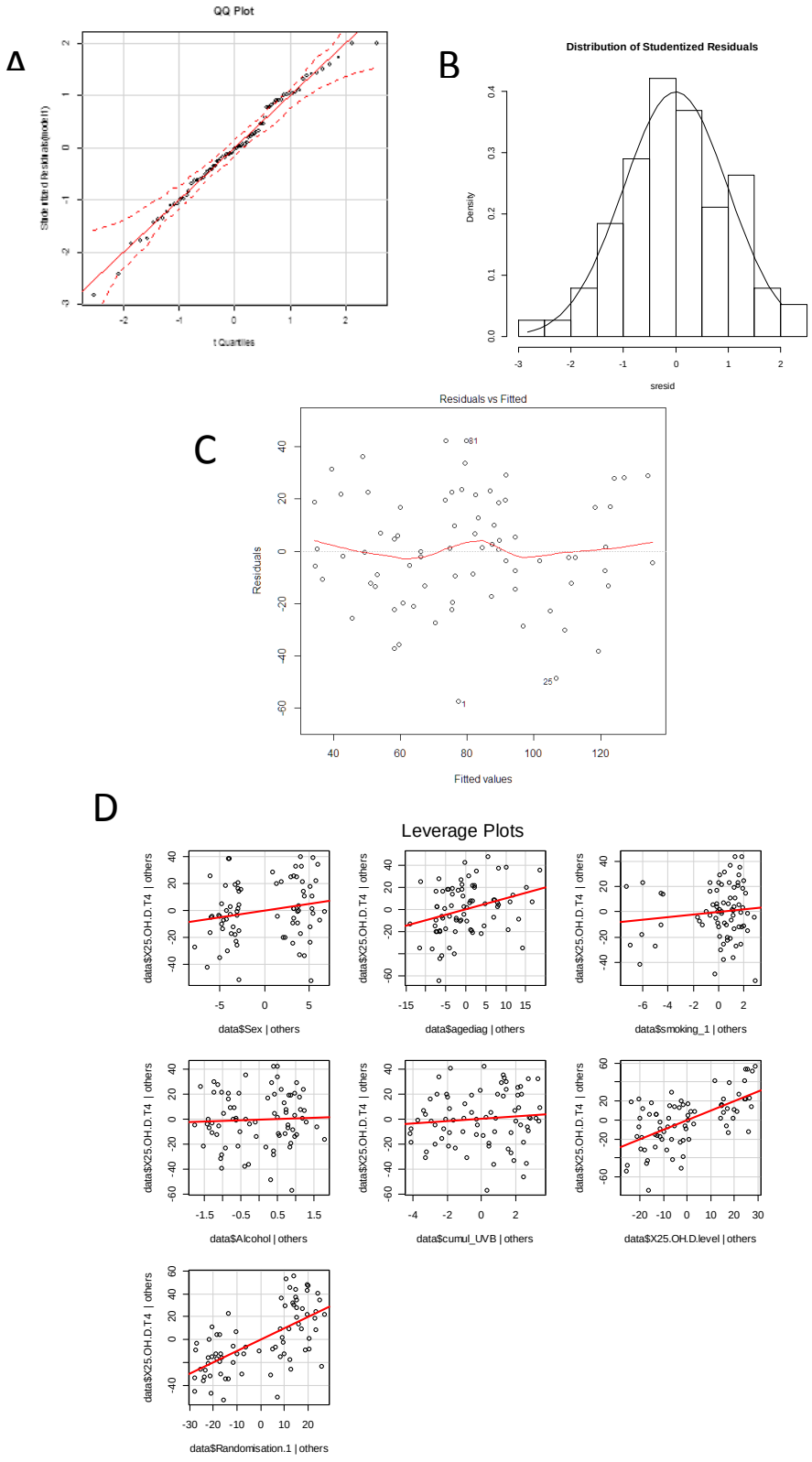

Figure 10.10: Diagnostic plots for regression model examining association between T4 cw-D-UVB and T4 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status, baseline 25(OH)D concentration level and randomisation.

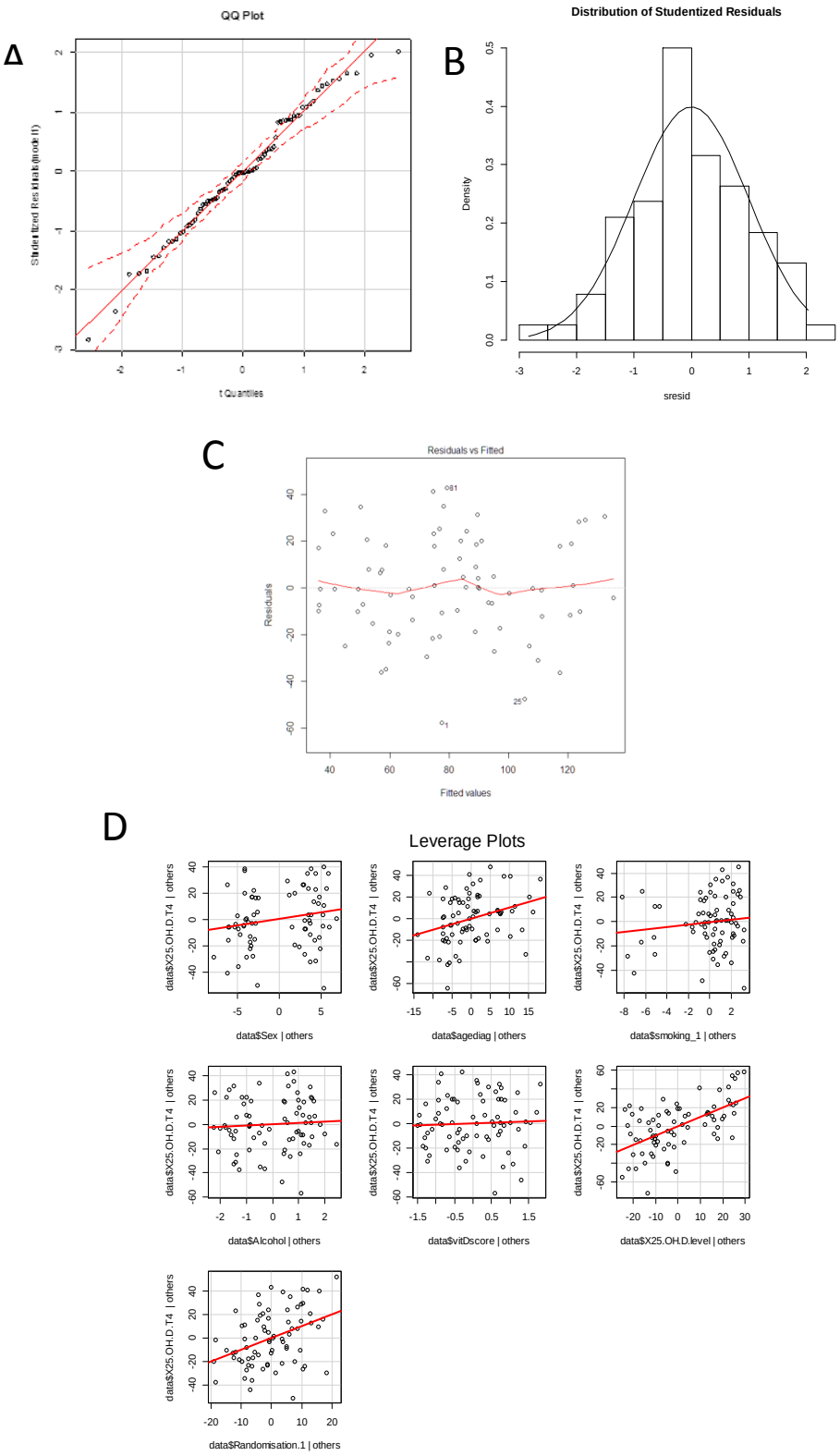

Figure 10.11: Diagnostic plots for regression model examining association between T4 VDscore1 and T4 25(OH)D dose. Adjusted also for gender, age, alcohol consumption, smoking status, Baseline 25(OH)D concentration level.

10.6 Appendix 6: Diagnostic plots Chapter 5

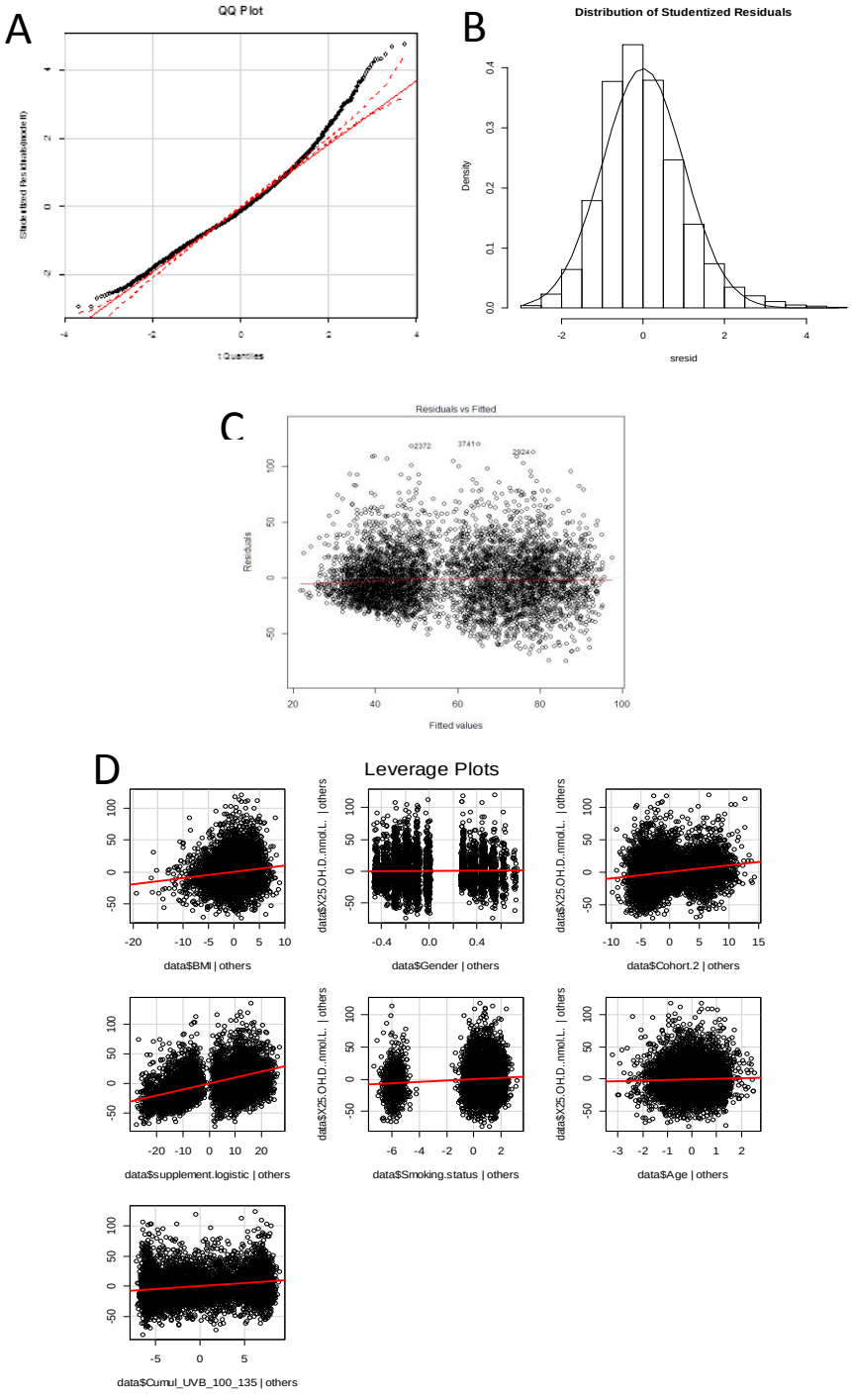

Figure 10.12: Diagnostic plots for regression model examining association between cw-D-UVB and 25(OH)D dose. Adjusted also for supplement use, gender, age, cohort type, BMI, and smoking status.

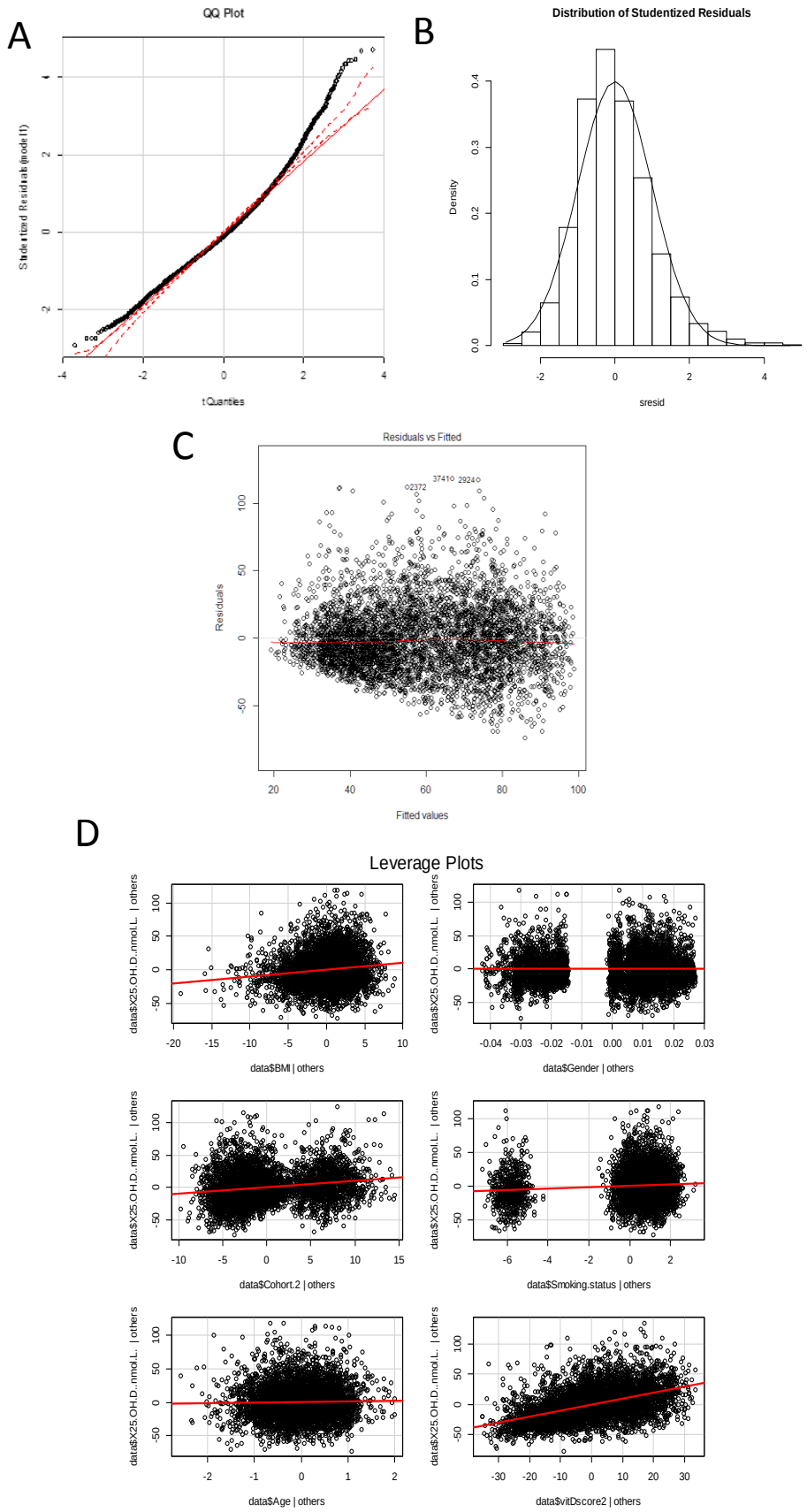

Figure 10.13: Diagnostic plots for regression model examining association between VDscore2 and 25(OH)D dose. Adjusted also for gender, age, cohort type, BMI, and smoking status. VDscore1 and 3 were also tested but data is not shown.

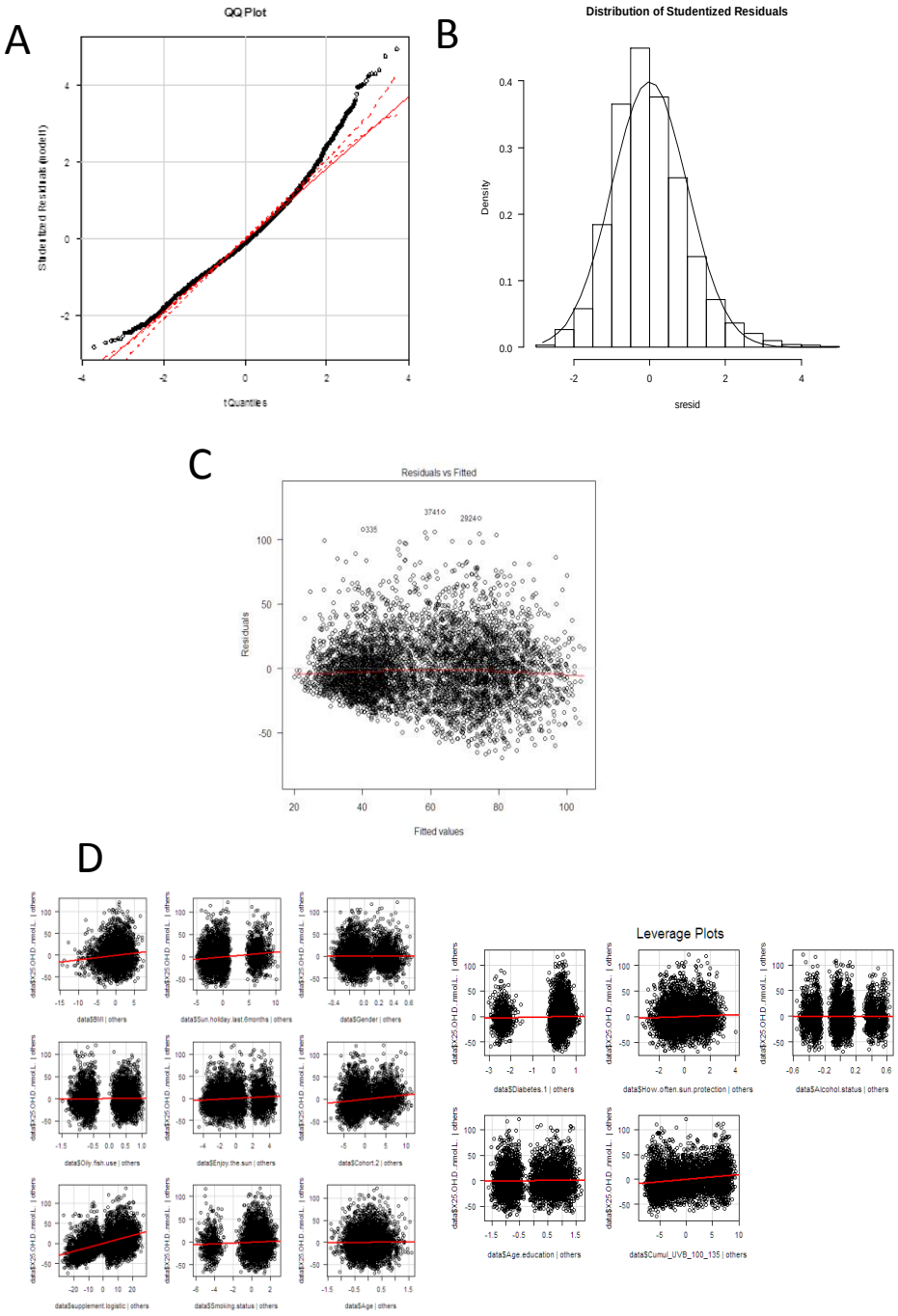

Figure 10.14: Diagnostic plots for regression model examining association between cw-DUVB and Personal characteristics. Models examining 25(OH)D, VDscore,1 VDscore2 and 3 were also tested but data is not shown.

10.7 Appendix 7: Examination of the association between annuals D-UVB and VDscore4 and other mortality-excluding cancer.

Table 10.2: Association between VDscore4 tertiles and the survival of primary oesophageal and gastric cancer
Cox proportional hazard analysis looking at the effect of annual D-UVB in the survival of **primary** oesophageal and gastric cancer stratified by cancer type[1, 2]

Outcome			**Tertile 1** (3-6)			**Tertile 2** (10-16)					**Tertile 3** (20-23)					**P-trend**
		N	**N**	**% died**	**HR**	**N**	**% died**	**HR**	**95% CL**	**p-val**	**N**	**% died**	**HR**	**95% CL**	**p-val**	
	other mortality															
All	*Unadjusted*	235	62	10%	Ref	91	3%	0.43	0.11-1.72	0.23	82	7%	0.90	0.29-2.82	0.86	0.90
	Adjusted	235	62	10%	Ref	91	3%	0.26	0.05-1.39	0.11	82	7%	0.51	0.11-2.35	0.39	0.96

Footnote:

[6] VDscore4 calculated using annual D-UVB, supplement use, oily fish consumption and estimated hourly sunlight exposur2

[7] Adjusted model has been adjusted for age, sex, Skin colour, weight loss, smoking status, alcohol intake, BMI, cancer type and oesophageal or gastric reflux, weight loss, any cardiovascular condition. All hazards were found to be proportional

Table 10.3: Association between Annual D-UVB tertiles and the survival of primary oesophageal and gastric cancer
Cox proportional hazard analysis looking at the effect of annual D-UVB in the survival of **primary** oesophageal and gastric cancer stratified by cancer type [1]

Outcome			**Tertile 1** (3110-33270)			**Tertile 2** (34070-34990)					**Tertile 3** (38100-39260)					**P-trend**
		N	**N**	**% died**	**HR**	**N**	**% died**	**HR**	**95% CL**	**p-val**	**N**	**% died**	**HR**	**95% CL**	**p-val**	
	other mortality															
All	*Unadjusted*	235	78	8%	Ref	78	6%	1.04	0.32-3.42	0.95	79	5%	0.85	0.24-3.04	0.81	0.89
	Adjusted	235	78	8%	Ref	78	6%	1.04	0.23-4.56	0.96	79	5%	1.00	0.21-4.72	0.99	0.67

Footnote:

Adjusted model has been adjusted for age, sex, skin colour, weight loss, smoking status, alcohol intake, BMI, cancer type and oesophageal or gastric reflux, weight loss, any cardiovascular condition. No information about cancer stage was available to adjust for this confounder.

10.8 Appendix 8: Associations with all cancer stages

Table 10.4: Association between may-adjusted 25(OH)D and upper gastrointestinal cancer mortality (all stages)

Cox proportional hazard analysis examining the effect of 25(OH)D in the survival of upper gastrointestinal cancer[1].

Outcome	N	Tertile 1			Tertile 2					Tertile 3					p-trend
		N	% who died	HR	N	% who died	HR	95% CL	p-val	N	% who died	HR	95% CL	p-val	
Cancer specific mortality															
All[4]	265	91	45%	Ref	88	35%	1.00	0.98-1.00	0.34	86	43%	1.00	0.99-1.00	0.76	0.54
All oesophageal	210	64	47%	Ref	72	38%	1.20	0.67-2.12	0.53	74	45%	1.49	0.81-2.71	0.20	0.54
OEAC	172	52	42%	Ref	60	35%	1.27	0.64-2.56	0.50	60	46%	2.11	0.99-4.49	**0.05**	0.32
OESCC	38	12	60%	Ref	12	50%	0.96	0.25-3.77	0.96	14	36%	0.41	0.09-1.90	0.25	**0.05**
Gastric	55	27	41%	Ref	16	25%	0.14	0.02-1.23	*0.08*	12	33%	3.24	0.24-43.50	0.37	0.97
All-cause mortality															
All	265	91	56%	Ref	88	39%	0.84	0.52-1.38	0.50	86	51%	1.53	0.92-2.53	0.10	0.34
All oesophageal	210	64	55%	Ref	72	40%	1.14	0.66-1.97	0.63	74	53%	1.58	0.90-2.79	0.11	0.17
OEAC	172	52	52%	Ref	60	38%	1.17	0.61-2.23	0.64	60	52%	1.88	0.93-3.79	*0.08*	0.39
OESCC	38	12	60%	Ref	12	50%	0.87	0.24-3.22	0.84	14	57%	0.81	0.23-2.86	0.74	0.96
Gastric	55	27	59%	Ref	16	31%	0.08	0.01-0.45	**0.005**	12	42%	0.49	0.08- 2.96	0.43	0.12

Footnote:

[4] OEAC: oesophageal adenocarcinoma; OESCC: oesophageal squamous cell carcinoma.

[5] Adjusted for age, sex, smoking status, alcohol intake, cancer stage, cancer type, cancer subtype and dysphagia score.

[6] May-Adjusted 25(OH)D is adjusted as if all 25(OH)D was sampled in May.

[7] All cox hazards were found to be proportional except when examining the cancer specific mortality in the entire data-set using 25(OH)D tertiles. In order to compensate for this, this model was additionally adjusted for the interaction between tertiles of 25(OH)D and follow up time, when this was carried out hazards were found to be proportional.

Table 10.5: Association between annual D-UVB and survival of upper gastrointestinal cancers (all cancer stages)

Cox proportional hazard analysis examining the effect ambient annual D-UVB in the survival of upper gastrointestinal cancer[1, 2.]

Annual D-UVB variable	N	Tertile 1			Tertile 2					Tertile 3					p-trend
		N	% who died	HR	N	% who died	HR	95% CL	p-val	N	% who died	HR	95% CL	p-val	
Cancer specific mortality															
All	248	86	47%	Ref	81	33%	0.54	0.31-0.96	**0.03**	81	40%	1.11	0.66-1.87	0.96	0.75
Oesophageal	196	66	48%	Ref	64	34%	0.61	0.32-1.14	0.12	67	48%	1.35	0.76-2.36	0.30	0.30
OEAC	159	55	45%	Ref	51	35%	0.78	0.38-1.60	0.50	55	45%	1.41	0.72-2.78	0.32	0.34
OESCC[3]	37	11	63%	Ref	13	31%	0.96	$1x10^{-4}$-$8x10^{3}$	0.99	12	58%	0.91	$7x10^{-5}$-$2x10^{4}$	0.99	0.31
Gastric[3]	52	20	40%	Ref	17	29%	1.03	0.80-1.32	0.84	14	29%	1.03	0.57-1.86	0.91	0.45
All-cause mortality															
All	248	86	50%	Ref	81	40%	0.57	0.33-0.97	**0.04**	81	49%	1.15	0.70-1.88	0.58	0.63
Oesophageal	196	66	53%	Ref	64	38%	0.60	0.33-1.10	0.10	67	54%	1.47	0.86-2.51	0.16	0.14
OEAC	159	55	49%	Ref	51	37%	0.69	0.34-1.40	0.31	55	51%	1.51	0.79-2.89	0.21	0.16
OESCC	37	11	72%	Ref	13	38%	0.66	0.15-2.94	0.59	12	60%	2.18	0.52-9.10	0.28	0.31
Gastric[3]	52	20	50%	Ref	17	47%	1.00	0.15-6.6	0.98	14	29%	1.03	0.21-4.90	0.97	0.14

Footnote:

1 OEAC: oesophageal adenocarcinoma; OESCC: oesophageal squamous cell carcinoma

2 Adjusted for age, sex, dysphagia score, smoking status, alcohol intake, cancer stage, weight loss, cancer type, cancer subtype.

3 The majority of cox hazards were found to be proportional except when examining the tertiles of D-UVB in cancer-specific mortality in Gastric cancer and ESCC and all-cause mortality in Gastric cancer. In order to compensate for this, these models were additionally adjusted for the interaction between tertiles of 25(OH)D and follow up time, when this was carried out hazards were found to be proportional.

Table: Association between 25(OH)D and cancer-specific mortality stratified by weight loss (All stages)

Cox proportional hazard analysis looking at the effect of 25(OH)D in the cancer specific mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms [1].

Model	Stratified by	N	Tertile 1			Tertile 2					Tertile 3					P-trend
			N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	All[2]	136	50	54%	ref	39	44%	0.99	0.96-1.02	0.67	47	62%	1.00	0.98-1.03	0.52	0.15
	OES	90	32	63%	ref	28	50%	1.19	0.55-2.55	0.65	30	86%	2.00	0.99-4.06	**0.05**	0.39
	Gastric	26	12	58%	ref	7	43%	0.005	$9x10^{-6}$-2.5	*0.09*	7	43%	8.15	0.73-1.06	*0.09*	0.12
No weight loss	All	125	40	35%	ref	49	29%	1.50	0.58-3.85	0.40	36	19%	1.69	0.44-6.50	0.44	*0.07*
	OES	79	25	40%	ref	40	33%	1.91	0.64-5.67	0.24	32	22%	2.24	0.49-10.23	0.30	0.15
	Gastric[2]	28	15	26%	ref	9	10%	$4x10^{+9}$	0-inf	0.99	4	0%	$3x10^{-10}$	0-inf	0.99	0.34

Footnote:

1 May-Adj; May adjusted 25(OH)D and model adjusted for age, sex, dysphagia, smoking status, alcohol intake, cancer stage, cancer type, and cancer subtype.

2 The majority of cox hazards were found to be proportional except when examining the weight loss tertiles of 25(OH)D in cancer-specific mortality overall. In order to compensate for this, this model was additionally adjusted for the interaction between tertiles of 25(OH)D and follow up time, when this was carried out hazards were found to be proportional.

Table 10.6: Association between may-adjusted 25(OH)D and all-cause mortality stratified by weight loss (all-stages)

Cox proportional hazard analysis looking at the effect of 25(OH)D in the all-cause mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms.

Model	Stratified by	N	Tertile 1			Tertile 2					Tertile 3					P-trend
			N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	**All**[2]	137	50	64%	ref	39	51%	0.99	0.96-1.03	0.64	47	72%	1.00	0.99-1.02	0.56	**0.05**
	OES	90	38	61%	ref	32	50%	1.21	0.59-2.46	0.61	40	75%	2.26	1.15-4.46	**0.02**	0.21
	Gastric	27	12	75%	ref	7	57%	$2x10^{-13}$	0-inf	0.99	7	57%	$1x10^{-31}$	0-inf	0.99	0.10
No weight loss	**All**	126	40	48%	ref	49	29%	0.97	0.40-2.32	0.94	36	20%	1.36	0.41-4.49	0.61	**0.03**
	OES	79	25	48%	ref	39	33%	1.31	0.46-3.66	0.61	33	24%	2.01	0.49-8.22	0.33	**0.03**
	Gastric	28	15	46%	ref	9	10%	$7x10^{9}$	0-inf	0.99	4	0%	$5x10^{-10}$	0-inf	0.99	0.30

Footnote:

[1] Adjusted for age, sex, dysphagia score, smoking status, alcohol intake, cancer stage, cancer type, and cancer subtype.

[2] The majority of cox hazards were found to be proportional except when examining the weight loss tertiles of 25(OH)D in all-cause mortality overall. In order to compensate for this, this model was additionally adjusted for the interaction between tertiles of 25(OH)D and follow up time, when this was carried out hazards were found to be proportional.

Table 10.7: Cox proportional hazard analysis, annual D-UVB, cancer-specific mortality and weight loss (all-stages)

Cox proportional hazard analysis looking at the effect of annual D-UVB in the cancer specific mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms[1, 2].

		Model	N	Tertile 1			Tertile 2					Tertile 3					p-trend
				N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	**All**	*Annual D-UVB*	128	39	59%	Ref	48	44%	0.61	0.29-1.26	0.18	41	61%	1.64	0.82-3.29	0.16	0.14
	OES	*Annual D-UVB*	105	29	62%	Ref	40	43%	0.69	0.32-1.50	0.35	36	64%	1.88	0.91-3.91	*0.09*	0.12
No weight loss	**All**	*Annual D-UVB*	117	47	36%	Ref	31	19%	0.41	0.13-1.36	0.15	39	26%	0.67	0.25-1.78	0.42	0.56
	OES	*Annual D-UVB*	90	37	38%	Ref	22	23%	0.60	0.14-2.56	0.49	31	29%	0.55	0.16-1.90	0.35	0.65

Footnote:

[3] Model adjusted for age, sex, dysphagia, smoking status, alcohol intake, cancer stage, cancer type and cancer subtype

[4] All hazard ratios were found to be proportional

Table 10.8: Cox proportional hazard analysis, annual D-UVB, all-cause mortality and weight loss (all-stages)

Cox proportional hazard analysis looking at the effect of annual D-UVB in the all-cause mortality of oesophageal and gastric cancer in those who have or do not have weight loss symptoms [1, 2].

		Model	N	Tertile 1			Tertile 2					Tertile 3					p-trend
				N	% who died	HR	N	% who died	HR	95% CI	p-value	N	% who died	HR	95% CI	p-value	
Weight loss	All	*Annual D-UVB*	128	39	69%	Ref	48	52%	0.65	0.33-1.28	0.21	41	66%	1.62	0.84-3.11	0.15	0.10
	OES	*Annual D-UVB*	105	29	72%	Ref	40	48%	0.63	0.30-1.30	0.21	36	69%	1.82	0.91-3.63	0.09	*0.08*
No weight loss	All	*Annual D-UVB*	117	47	38%	Ref	31	23%	0.42	0.13-1.31	0.14	39	31%	0.89	0.37-2.17	0.80	0.73
	OES	*Annual D-UVB*	90	37	38%	Ref	22	23%	1.00	0.85-1.18	0.99	31	35%	1.00	0.85-1.18	0.97	0.80

Footnote:

[3] Model adjusted for age, sex, dysphagia, smoking status, alcohol intake, cancer stage, cancer type and cancer subtype.

[4] All hazard ratios were found to be proportional except in oesophageal cancer in the non-weight loss group. This model was adjusted for time in order to compensate for this.