

Dissertation

**A parametric Weibull individual participant data,
one-step meta-analysis of standardized
25-hydroxyvitamin D and mortality in 26916
participants from a European consortium**

submitted by
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Statutory Declaration

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of “Good Scientific Practice and Ombuds Committee at the Medical University of Graz”.

July 9th, 2017, Martin Gaksch

1. Foreword

This doctoral thesis is the result of my time working as a research assistant at the Division of Endocrinology and Metabolism, Department of Internal Medicine, Medical University of Graz under the supervision of Prof. Stefan Pilz. Initially working on the Styrian Vitamin D Hypertension Trial, I switched my thesis' topic as the opportunity arose to take part in a European Union (EU) project and to focus on the implementation of an individual participant meta-analysis of standardized vitamin D concentrations across several European cohort studies. The results of my efforts are published in the article "Vitamin D and mortality: Individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium" in PLoS ONE (1). The thesis is an extended piece of work including additional material and unpublished computations of the topic.

I am indebted to the participants in different countries for their willingness to participate in the study.

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5. Abbreviations

25(OH)D	25-Hydroxyvitamin D
AD	Aggregate data
AFT	Accelerated failure time
AGES	Age, Gene/Environment Susceptibility-Reykjavik Study
BMI	Body mass index
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEGS	German Health Interview and Examination Survey for Adults
e.g.	example given
etc.	et cetera
EU	European Union
GFR	Glomerular filtration rate
HR	Hazard ratio
HTN	Arterial hypertension
i.e.	id est
ICC	Intra-class correlation coefficient
IOM	Institute of Medicine
IPD	Individual patient or participant data
IU	International units
LASA	Longitudinal Aging Study Amsterdam
LDL	Low-density lipoprotein cholesterol
LURIC	Ludwigshafen Risk and Cardiovascular Health Study

MET	Metabolic equivalent of task
NHS	New Hoorn Study
NIST	U.S. National Institute of Standards and Technology
ODIN	EU-project ‘Food-based solutions for eradication of vitamin D deficiency and health promotion throughout the life cycle’
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RMP	Reference measurement procedure
SAS	SAS software of SAS Institute for advanced analytics
SBP	Systolic blood pressure
UVB	Ultraviolet B light
VDSP	Vitamin D Standardization Program

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8. Abstract [German]

Hintergrund: In der Literatur finden sich Hinweise, dass ein Mangel an Vitamin D ein Risikofaktor für Mortalität ist. Frühere Metaanalysen verwendeten jedoch keine standardisierten Labormethoden für die Messung von 25-Hydroxyvitamin D (25[OH]D) Konzentrationen und waren limitiert durch nicht standardisierte, klinische Endpunkte oder aggregierte Daten anstatt von Individualdaten (IPD). Wir haben daher eine IPD-Metaanalyse über den Zusammenhang von standardisierten Serum 25(OH)D-Konzentrationen und Mortalität durchgeführt.

Methoden: In einem europäischen Konsortium aus acht prospektiven Studien, darunter sieben Kohorten der Allgemeinbevölkerung, verwendeten wir Protokolle aus dem Vitamin D Standardization Program (VDSP) zur Standardisierung von 25(OH)D-Konzentrationen. Wir führten one-step Metaanalysen durch, um Assoziationen von 25(OH)D und Gesamtmortalität als primären Endpunkt und Herz-Kreislauf- und Krebs-Mortalität als sekundäre Endpunkte zu identifizieren. Diese Metaanalyse ist auf ClinicalTrials.gov, unter der Nummer NCT02438488 registriert.

Ergebnisse: Wir analysierten 26916 StudienteilnehmerInnen (medianes Alter 61,6 Jahre, 58% Frauen) mit einer medianen 25(OH)D-Konzentration von 53,8 nmol/L. Während einem medianen Beobachtungszeitraum von 10,5 Jahren starben 6802 Personen. Das Risiko der Gesamtmortalität unterscheidet sich nicht signifikant für 25(OH)D-Konzentrationen von 50 bis 125 nmol/L oder höher. Im Vergleich zu TeilnehmerInnen mit 25(OH)D-Konzentrationen von 75 bis 99,99 nmol/L zeigten die TeilnehmerInnen mit 25(OH)D-Konzentrationen von 40 bis 49,99, von 30 bis 39,99 und unter 30 nmol/L Hazard Ratios (mit 95% Konfidenzintervall) für die Gesamtmortalität von 1,15 (1,00-1,29), 1,33 (1,16-1,51) bzw. 1,67 (1,44-1,89). Ähnliche Ergebnisse waren für die kardiovaskuläre Mortalität, jedoch nicht für die Krebs-Mortalität ersichtlich.

Interpretation: In der ersten IPD-Metaanalyse mit standardisierter Messung von 25(OH)D-Konzentrationen beobachteten wir eine Assoziation zwischen niedrigem 25(OH)D und erhöhtem Sterblichkeitsrisiko. Das Ergebnis ist wichtig für das öffentliche Gesundheitswesen um eine adäquate Grundlage für die Behandlung und Prävention von Vitamin-D-Mangel bedingten Todesfällen zu schaffen.

9. Abstract

Background: Vitamin D deficiency may be a risk factor for mortality but previous meta-analyses lacked standardization of laboratory methods for 25-hydroxyvitamin D (25[OH]D) concentrations and were limited by use of unstandardized clinical outcomes and by use of aggregate data instead of individual participant data (IPD). We therefore performed an IPD meta-analysis on the association between standardized serum 25(OH)D and mortality.

Methods: In a European consortium of eight prospective studies, including seven general population cohorts, we used Vitamin D Standardization Program (VDSP) protocols to standardize 25(OH)D data. Meta-analyses using a one-step procedure on IPD were performed to study associations of 25(OH)D with all-cause mortality as the primary outcome and with cardiovascular and cancer mortality as secondary outcomes. This meta-analysis is registered at ClinicalTrials.gov, number NCT02438488.

Findings: We analysed 26916 study participants (median age 61.6 years, 58 % females) with a median 25(OH)D concentration of 53.8 nmol/l. During a median follow-up time of 10.5 years, 6802 persons died. All-cause mortality risk did not significantly differ by 25(OH)D concentration for concentrations ranging from 50 to 125 nmol/l and higher. Compared to participants with 25(OH)D concentrations of 75 to 99.99 nmol/l, the adjusted hazard ratios (with 95% confidence interval) for mortality in the 25(OH)D groups with 40 to 49.99, 30 to 39.99, and <30 nmol/l were 1.15 (1.00-1.29), 1.33 (1.16-1.51), and 1.67 (1.44-1.89), respectively. We observed similar results for cardiovascular mortality but there was no significant association between level of 25(OH)D and cancer deaths.

Interpretation: In the first IPD meta-analysis using standardized measurement of 25(OH)D we observed an association between low 25(OH)D and increased risk of mortality. It is of public health interest to evaluate whether vitamin D deficiency is associated with increased mortality to establish a basis for treatment and prevention of vitamin D deficiency related deaths.

10. Introduction

Vitamin D has received increased attention due to the relatively high prevalence of vitamin D deficiency and new insights on the physiological role of vitamin D in classical target tissues as well as evidence of extra-skeletal effects. These extra-skeletal vitamin D actions in humans include, for instance, inhibition of cancer progression, effects on the cardiovascular system, and immunomodulatory properties in certain autoimmune diseases.(2) Vitamin D deficiency is evident in many parts of the globe and shows prevalence rates between 13.0% and 40.4% according to the threshold of vitamin D deficiency being used as <30nmol/l (12ng/ml) and <50nmol/l (20ng/ml).(3) Taken together the diversity of clinical implications, the high prevalence of vitamin D deficiency and the low barrier of oral vitamin D substitution makes vitamin D deficiency a highly important global health issue.

10.1 Vitamin D

Vitamin D refers to vitamin D₂ (ergocalciferol) and D₃ (cholecalciferol), which are secosteroids, *id sunt* steroids with a broken ring molecule structure. Both molecules differ structurally in the side chain, where vitamin D₂ has a C₂₂-C₂₃ double bond and an additional methyl group at C₂₄.(4) Both vitamin D₂ and D₃ are based on lanosterol, which is the starting point of biochemical steroid synthesis into cholesterol (or the equivalent ergosterol in fungi and sitosterol in plants). While vitamin D₂ is mainly found in many but not all algae as well as yeasts, vitamin D₂ in humans can only be derived from nutritional resources. Vitamin D₃ may come both from dietary sources or may be endogenously produced from 7-dehydrocholesterol when human skin is exposed to ultraviolet light B (UVB).(5)

The vitamin D synthesis pathway is a very early phenomenon in the evolution of all eukaryotes, and its synthetic pathway is highly conserved during evolution. The photochemical conversion of 7-dehydrocholesterol, also called pro-vitamin D₃, by UVB produces pre-vitamin D₃. The conversion occurs at wavelength of 290 to 315 nm and would also happen at lower wavelengths, but solar radiation below 290 nm is usually absorbed by the ozone layer.(6) The photochemical reaction is considered to be a highly efficient protection of early eukaryotes against DNA damage induced by UVB when there was a low atmospheric O₂ concentration and thus no protecting ozone layer a few billion years ago. (5,7)

The vitamin D synthesis in the human skin is effective, if skin exposure to UVB is sufficient. Toxic effects are most probably impossible, since daily conversion of 7-dehydrocholesterol into pre-vitamin D reaches a plateau after 15% of total conversion and then biologically inactive derivatives such as lumisterol and tachysterol are produced. Lumisterol and tachysterol may form back to pre-

vitamin D when pre-vitamin D concentrations are low and thus serve as additional storages for vitamin D. (8)

Pre-vitamin D₃ is biologically inactive, thermodynamically unstable and undergoes a temperature-sensitive rearrangement of the three double bonds to form vitamin D₃. Vitamin D₃ diffuses into the blood, where it must be bound to vitamin D binding protein (DBP) due to its fat-soluble properties to be transported to the liver. Vitamin D₃ from dietary sources may be either transported by DBP or by chylomicrons to the liver.(2)

In the liver, vitamin D is hydroxylated at C-25 to 25-hydroxyvitamin D [25(OH)D] by a 25-hydroxylase. 25(OH)D is the major circulating and storing form of vitamin D. Its serum concentration is commonly used to estimate the overall vitamin D status of an individual because 25(OH)D concentrations best reflect vitamin D input from all sources. (9)

10.2 Synthesis in the Skin

The synthesis of vitamin D in the skin is the most important source of 25(OH)D in humans. The efficiency of vitamin D synthesis depends on the intensity of UVB and is therefore determined by external factors, such as season and latitude. In regions with significant seasonal variation of UVB intensity, synthesis of vitamin D varies during the year with peak concentrations of plasma 25(OH)D measured in the months following summer. (10) In Europe, there is a clear trend of decreasing UVB availability when moving from South to North ranging from ~35° N, where there are essentially no periods of insufficient UVB intensity during the year, to ~69° N, where there are at least eight months where UVB intensity is insufficient for large parts of the population to maintain a sufficient vitamin D status.(10,11)

Factors for individual variability of 25(OH)D synthesis include behavioural reasons, like in- or outdoor-related work, frequency of sun-bathing, habitual application of sunscreen, and clothing habits. Cutaneous factors of individual 25(OH)D variability include the capacity of dermal vitamin D synthesis, which may decrease with advanced age and is less efficient in older than in younger adults. Also, melanin absorbs UVB and thus contributes to lower capacity of vitamin D synthesis in the skin of certain ethnicities with a darker skin type.(12)

10.3 Factors Influencing Vitamin D Status

Beside synthesis, factors for inter-individual variability of 25(OH)D include body mass index (BMI) or body fat percentage, vitamin D and calcium intake, and individual genetic background. Higher BMI has been associated with smaller increases in 25(OH)D concentrations in response to vitamin D supplementation (9,13-15) as vitamin D is fat soluble and suggested to be trapped in adipose tissue.(14) Combined intake of calcium and vitamin D is associated with a little lower

surge of serum 25(OH)D concentrations than vitamin D supplementation alone (16,17) although the underlying reasons are still not fully understood.(14) Anyway, a combined intake of vitamin D and calcium may be necessary for vitamin D to exert its full range of clinical actions and was at least shown to be superior in reducing fracture risk compared to vitamin D supplementation alone (see below).

Genome-wide association studies have identified genetic loci that are associated with circulating 25(OH)D concentrations.(18,19) The loci included genes involved in synthesis of cholesterol and vitamin D precursors [e.g. 7-dehydrocholesterol reductase (DHCR7) and cytochrome P450 2R1 (CYP2R1)], in vitamin D transport [i.e. for vitamin D transport by DBP, encoded by group-specific component (GC)], and in hydroxylation of vitamin D [i.e. 24-hydroxylation (CYP24A1)] and thus in degradation of vitamin D.(20)

10.4 Food Sources of Vitamin D

Dietary intake is a second vitamin D source and generally of less importance. If vitamin D synthesis is, however, limited or absent, oral vitamin D supply becomes essential for humans. Food sources of vitamin D include meat and meat products, fish, eggs, milk and milk products, and cereals. The distribution and diversification of intake patterns vary from country to country and rely on the extent of voluntary fortification of manufacturers, fortification policies as well as the individual use of supplements, which can be either vitamin D₂ or vitamin D₃.(10) Natural food sources of vitamin D may be limited or may not be consumed on a regular basis. Average intakes in populations within the EU range between 120 to 300 international units (IU; 3-7.5 µg) per day, depending on the country. (21) Anyway, the range does not meet most of dietary recommendations in individual European countries which frequently vary between 400 to 800 IU (10 to 20 µg; divide by 40 to convert from IU into µg) per day.(10)

10.5 Population Subgroups at Risk for Inadequate Vitamin D Status

According to the United States (U.S.) National Institute for Health and Clinical Excellence (NICE) evidence-based recommendations on the prevention of vitamin D deficiency, especially following population subgroups are at increased risk of vitamin D deficiency (22):

- Infants and children aged under 5,
- Pregnant and breastfeeding women, particularly teenagers and young women,
- People over 65,
- People who have low or no exposure to the sun, for example, those who cover their skin for cultural reasons, who are housebound or confined indoors for long periods

- People with darker skin, for example, people of African, African-Caribbean or South Asian family origin.

According to *Holick* and the Endocrine Society Task Force (23), following population subgroups are further at increased risk of vitamin D deficiency:

- Children as well as young and middle-aged adults are at equally high risk worldwide,
- General population of Australia, the Middle East, India, Africa, and South America,
- Obese adults (BMI > 30 kg/m²),
- Patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism,

10.6 Definitions of Deficiency, Inadequate and Adequate Vitamin D Status

In the literature, there have been large controversies and inconsistencies regarding standard definition of vitamin D deficiency, adequate and optimal vitamin D status. According to U.S. Institute of Medicine (IOM) recommendations for vitamin D (24,25), a serum 25(OH)D concentration <30 nmol/l (<12 ng/ml) is defined as 'deficient', serum 25(OH)D concentrations ranging from 30 to 50 nmol/l (from 12 to 20 ng/ml) may be 'inadequate' in some people, and serum 25(OH)D >50 nmol/l (>20 ng/ml) concentrations are considered 'sufficient' for almost the whole population. The IOM recommendations further state that serum 25OHD concentrations above 75 nmol/l (30 ng/ml) are not consistently associated with increased benefit and that serum 25OHD concentrations above 125 nmol/l (50 ng/ml) may be a reason for concern. To convert serum 25OHD concentrations from nmol/l to ng/ml, a divisor of 2.496 was used.(24) Although not in the range of sufficient, the IOM recommendations consider a concentration between 40 and 50 nmol/l not necessarily a range for intervention with supplementation.(10)

The Endocrine Society Task Force (23) advocates higher thresholds for deficiency and insufficiency, which are defined as 25(OH)D concentrations below 50 nmol/l (20 ng/ml) and 25(OH)D concentrations of 52.5 to 72.5 nmol/l (21 to 29 ng/ml), respectively. The Endocrine Society recommendations further suggest a population screening for vitamin D deficiency in individuals at risk for deficiency.(23)

10.7 Recommendations Regarding Oral Supplementation

The IOM recommends beyond infancy and up to an age of 70 years a daily vitamin D intake of 600 IU (15µg) per day to maintain adequate vitamin D supply and for people >70 years 800 IU (20µg) daily. In line with the IOM, the Endocrine Society Task Force recommends an intake of 600 IU

(15µg) per day of vitamin D for adults aged 19 to 50 years, 600 to 800 IU (15 to 20 µg) per day for adults aged 50 to 70 years, and 800 IU (20 µg) per day for adults aged 70+ years. To regain an adequate vitamin D status from deficiency, higher daily oral doses of vitamin D are usually needed. The IOM and the Endocrine Society Task Force both advise not to exceed daily doses of vitamin D supplementation of 4,000 IU (100µg) without medical supervision for children aged over 8 years and adults.(23,24)

Either vitamin D2 or vitamin D3 is suggested for the treatment and prevention of vitamin D deficiency. There is considerable evidence that the two forms can be assumed to have the same potency, as most of their features in metabolism and actions are identical. Some evidence points towards the notion that at high doses, D2 is less effective than D3, but further evidence from experimental animal data suggests a lower risk of toxicity for D2 than for D3.(24)

The U.K. government's Scientific Advisory Committee on Nutrition (SACN) since July 2016 recommends a reference nutrient intake for vitamin D of 10 µg/day (400 IU/day) for everyone in the general UK population aged 4 years and above as well as for infants from age 1 up to 4 years. Children aged under 1 year are being recommended 8.5-10 µg/day (340-400 IU).(26)

The European Food Safety Authority (EFSA) issued intake recommendations for vitamin D in June 2016. EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA) advocates a daily intake of 15 µg/day (600 IU/day) for adults as well as for children aged 1-17 years and pregnant and lactating women. For infants aged 7-11 months, an adequate intake was set at 10 µg/day.(27)

10.8 Risk of Intoxication

In the 1930s and 1940s of the last century food fortification with vitamin D was started to defy the incidence rate of rickets. In the subsequent post-war decades food fortification programs were then terminated due to fear of vitamin D overdosing and intoxication.(8,12,28) Oral vitamin D excess was shown to be responsible for soft tissue calcification in experimental studies of animals and infants. Vitamin D intoxication has been associated with infantile hypercalcemia with supra-valvular aortic stenosis, mental retardation and craniofacial malformation. More recent studies have shown that elevated 1,25(OH)₂D concentrations with a phosphate deficient diet had no promotional effects on vascular calcification but a combination of elevated 1,25(OH)₂D and phosphate overload seemed to facilitate a process of tissue calcification. Meanwhile, several RTCs have demonstrated a safety of administration of daily vitamin D doses up to 5000 IU or single oral dose of up to 300000 IU.(8)

10.9 Further 25(OH)D Metabolism

After the hydroxylation of vitamin D and subsequent formation of 25OHD in the liver, 25OHD is transported to the kidneys bound to DBP. In the kidneys, 25OHD is hydroxylated at the α -position of C-1 by 1 α -hydroxylase (mitochondrial cytochrome CYP27B1) to form 1 α ,25-dihydroxyvitamin D [1,25(OH)₂D], which is biologically active, and therefore often termed the “active vitamin D hormone”.(29,30) The bioconversion of 25OHD to 1,25(OH)₂D is strictly regulated by serum calcium and serum phosphorus concentrations, 1,25(OH)₂D and parathyroid hormone (PTH) blood concentrations. (30) Due to its tight, homeostatic regulation 1,25(OH)₂D is therefore considered less useful in assessing human overall vitamin D status.(8) 1,25(OH)₂D is responsible for most, if not all, of the biologic actions of vitamin D.(2)

In the kidney, 25(OH)D may be also converted into 24,25(OH)₂D by hydroxylation at C-24 by 24-hydroxylase (mitochondrial cytochrome CYP24A1). CYP24A1 limits the amount of 1,25(OH)₂D by producing 24,25(OH)₂D, which is mainly biologically inactive, and therefore decreases the pool of 25(OH)D available for 1-hydroxylation and primarily fosters the elimination of vitamin D.(2)

10.10 Regulation Of 1,25(OH)₂D₃

CYP27B1 and CYP24A1 are tightly regulated. Elevated parathyroid hormone (PTH), because of hypocalcaemia, induces CYP27B1 and 1,25(OH)₂D synthesis in the kidney.(31-34) 1,25(OH)₂D itself suppresses PTH production in the parathyroid gland in a feedback loop by directly inhibiting translation of the PTH gene and indirectly by enhancing transcription of the calcium sensing receptor (CaSR) and by elevating serum calcium concentration.(35-39) CYP24A1 is inversely regulated and stimulated by 1,25(OH)₂D₃ and inhibited by low calcium and PTH. (31,32,34)

Other regulators of 1,25(OH)₂D include fibroblast growth factor-23 (FGF-23), calcitonin, insulin, phosphorus, growth hormone, and prolactin.(40-42)

10.11 Vitamin D Actions

The biological actions of 1,25(OH)₂D are mediated by the vitamin D receptor (VDR) which belongs to the steroid receptor family. VDR forms a complex with retinoid X receptor (RXR), a type of nuclear receptor, for activation of vitamin D target genes.(43) The activated VDR/RXR complex binds to specific DNA sequences, called vitamin D response elements (VDREs), and leads to either activation or repression in the transcription of related target genes.(2) It is estimated that VDR may be involved in the expression of 100 to 1250 genes directly and/or indirectly, which would present 0.5 to 5% of the total human genome.(44) An analysis of oral vitamin D₃ supplementation revealed that of the 291 genes in human white blood cells affected there was at

least a 1.5 fold induced expression of genes related to 81 pathways and at least a 1.5 fold inhibition of genes affecting 88 pathways. Affected pathways included but were not limited to zinc and other metal iron transporters, taste transduction, EGF, PDGF and IGF-1 signalling pathways, DNA damage bypass, glucose uptake, various RNA transcription functions, and various enzyme metabolisms.(44)

10.11.1 Classical Role of Vitamin D

10.11.1.1 *Bone*

The classical role of vitamin D is to ensure adequate calcium supply for serum calcium concentration in the first place and, then, for bone mineralization thereby preventing rickets and osteomalacia.(5,45) The bone structural integrity relies on sufficient calcium supply from the blood, which itself depends on intestinal calcium absorption and renal calcium reabsorption, and both processes are enhanced by vitamin D. Vitamin D actions in the bone during a negative calcium balance are mainly directed to preserve serum calcium concentrations, so it then promotes increased bone resorption and impairs bone mineralization at the expenses of skeletal integrity.(2) Still not entirely clear, there is some evidence that if calcium supply is sufficient, vitamin D not only stops promoting bone resorption but may also encourage terminal differentiation of osteoblasts and may directly facilitate re-mineralization.(46) Meta-analyses of randomized controlled trials (RCTs) have documented a significant reduction of fracture rates by using vitamin D supplementation in combination with calcium.(47,48) Supplementation of vitamin D in combination with calcium is thus a standard treatment for patients suffering from osteoporosis.(20,49,50)

10.11.1.2 *Intestine and Kidney*

The major action of 1,25(OH)₂D and the VDR in the intestine is calcium absorption and increasing calcium transport through the enterocyte. Activation of VDR augments the calcium influx through the apical membrane calcium channel ‘transient potential vanilloid type 6’ (TRPV6) of the enterocyte, enhances binding to the calcium binding protein calbindin-D9k, and regulates unloading of calcium through the basolateral ‘plasma membrane calcium ATPase 1b’ (PMCA1b) into the blood.(2) Without vitamin D, only 10 to 15% of dietary calcium is absorbed and, after interaction of 1,25(OH)₂D with VDR binding sites in enterocytes, efficiency of intestinal calcium absorption may be increased up to 30 to 40%.(9)

In the kidney, most of the filtered calcium is passively reabsorbed at the proximal tubules independent of 1,25(OH)₂D. 1,25(OH)₂D-dependent absorption does not take place until the urine reaches the distal tubule where calcium uptake requires an active transcellular mechanism. The

active molecular reabsorption model resembles intestinal calcium absorption, but with calcium entry through TRPV type 5, and calcium extrusion by the sodium/calcium exchanger (NCX1) and PMCA1b.(2)

10.11.2 Vitamin D as Risk Marker

For risk assessment in human studies, although 1,25(OH)₂D and VDR are used for elicitation of molecular vitamin D pathways in experimental research, generally 25(OH)D concentrations are used in epidemiological studies for estimating vitamin D status prevalence. This is because 25(OH)D has a half-life of three weeks and is not under strict control of calcitropic hormones, therefore it is not prone to sudden endogenous changes in concentration and considered a more reliable marker for vitamin D status than 1,25(OH)₂D.(2)

For observational studies on vitamin D status and diseases, rather pre-diagnostic vitamin D measurement designs are used, because post-diagnostic vitamin D measurements may be susceptible to reverse causality and post-diagnostic 25(OH)D concentrations may not present the cause but rather the consequence of a given disease. In addition, post-diagnostic 25(OH)D concentrations may be influenced by disease-related factors such as lower sun exposure, physical activity, inflammation, and lower food intake of an individual affected.(2)

10.11.3 Pleiotropic Actions of Vitamin D

VDR is found in a broad range of tissues other than stated above, including brain, heart, skeletal muscle, liver, pancreas, and immune tissues (38), and vitamin D has been shown to influence a variety of physiological functions. Evidence suggests that during evolution vitamin D first had a role of inducing P450 enzymes for xenobiotic detoxification in several organs and catabolic purposes. In theory, with development of first vertebrates to live on land, which usually presents a calcium poor environment, vitamin D became functional with terrestrial calcium homeostasis.(51-53)

10.11.3.1 Cardiovascular System

Observational evidence supports an association between low 25(OH)D concentrations and cardiovascular disease (CVD) as there is a seasonal oscillation of cardiovascular events with a peak incidence in winter and at increasing distance from the equator.(54-56) VDR has been found in cells throughout the cardiovascular system, including cardiomyocytes, arterial wall cells, and immune cells. Observational studies suggest an involvement of the vitamin D system in cardiovascular function and progression of diseases, including inflammation, thrombosis, and aberrations of the renin-angiotensin aldosterone system. Furthermore, VDR may be important in

controlling cardiac hypertrophy and fibrosis, and delaying the development of atherosclerosis.(8,28,57,58) Vitamin D deficiency also may be associated with congestive heart failure.(59) Animal studies have demonstrated that vitamin D deficiency in mice may lead to hypertension.(60) A clinical study on UVB exposition of patients with hypertension revealed a subsequent normalization in blood pressure (both systolic and diastolic blood pressure were reduced by 6 mmHg).(61) Investigating the effect of vitamin D supplementation on blood pressure, a study could show a trend towards normalization of blood pressure in patients with arterial hypertension.(62) Recent studies on vitamin D or UVB exposure and blood pressure could, however, not document clear effects.(63,64)

10.11.3.2 Vitamin D as a Risk Factor for CVD

In observational studies low 25(OH)D concentrations have been associated with increased risk of mortality due to CVD endpoints including heart failure, sudden cardiac death (SCD), heart attack or stroke.(28,65-69) Vitamin D deficiency is associated with inflammation and cardiovascular risk markers, including higher coronary artery calcium scores, impaired endothelial function and increased vascular stiffness (70). Furthermore, vitamin D deficiency is suggested a promoter for atherosclerosis (71) and is linked to other vascular risk factors such as hyperlipidaemia and diabetes mellitus.(28) In contrast to these findings, results from RCTs testing a risk lowering effect of vitamin D supplementation on CVD outcomes have remained unclear (69,72) and most RCTs have not shown any clear effect of vitamin D supplementation on CVD outcomes.(20,50,69,72)

10.11.3.3 Malignant Diseases

1,25(OH)₂D was shown to slow down cancer cell growth of certain types of cancer by arresting cells in the G₀/G₁ phase of the cell cycle, by inducing their differentiation or by inducing apoptotic cell death, including HL60 melanoma, leukaemia. Furthermore, 1,25(OH)₂D may influence angiogenesis, alter cell adhesion and migration, and reduce the invasive properties of cancer cells. VDR expression is mostly retained in cancer growth and only about 5% of tumour cells lack the VDR gene or exhibit VDR gene alterations.(2)

In animal models, vitamin D deficiency was coupled with increased the risk for colonic tumour formation (73) and enhances tumour growth of various cancer types in mice.(74-76) Preclinical cancer models further suggest that both intake of vitamin D₃ and UVB are able to inhibit malignant progression of intestinal tumours (77) and that treatment with 1,25(OH)₂D₃ or its analogues ameliorates the response to chemotherapy.(78,79)

10.11.3.4 Vitamin D as a Risk Factor for Malignant Diseases

Observational studies in humans have shown partly inconsistent results for associations between 25(OH)D concentrations and cancer risk. There is an exception regarding colorectal cancer where compelling evidence has revealed an inverse association between 25(OH)D concentrations and cancer risk.(80,81) A meta-analysis documented an association between low 25(OH)D concentrations and overall survival of patients with breast cancer (82), but another suggests no clear overall relationship between circulating 25(OH)D concentrations and breast cancer risk.(83) Limitations regarding an association have been found for European study participants as well as pre-menopausal women.(82,83) Higher 25(OH)D concentrations have been significantly associated with reduced cancer-specific mortality for patients with lymphoma and cancers of the upper digestive tract.(84,85) For other cancer types, including melanoma, non-melanoma skin cancer, prostate, and bladder cancer, no clear association between 25(OH)D concentrations and cancer risk could be shown.(86-88) In line with the last argument, a meta-analysis could not or not consistently show a significant association between 25(OH)D concentrations and cancer-specific mortality for a combined set of cancer-specific endpoints. (89)

10.11.3.5 Inflammation

Several studies have shown that low 25(OH)D levels are associated with parameters of inflammation, but the direction of the cause and effect relationship still has to be elucidated as several scientists argue that a low 25(OH)D level may simply reflect inflammatory processes. This hypothesis is supported by data showing that inflammation modulates vitamin D metabolism and vitamin D binding protein levels, but there is also evidence that vitamin D itself exerts a variety of effects on immune cells and inflammatory processes.(2)

10.12 Vitamin D as a Risk Factor for All-Cause Mortality

A compelling body of evidence has shown a significant association between low 25(OH)D concentrations and overall mortality for various patient cohorts as well as for the general population.(65-68,84,89-93) Moreover, vitamin D supplementation has been shown to reduce overall mortality in elderly and about 150 patients need to be treated over five years for one additional life to be saved.(69) In a meta-analysis of RCTs covering a broader but nevertheless older population, vitamin D supplementation alone did not reduce the risk of all-cause mortality. (84) However in subgroup analyses, vitamin D3 supplementation reduced all-cause mortality significantly by 11% while vitamin D2 supplementation had no effect on mortality.

While the major body of epidemiological studies have consistently confirmed that individuals with low 25(OH)D concentrations are at increased risk of mortality, clinical evidence from RCTs

documenting a reduction in mortality in other population groups than the elderly as well as in the general population is sparse so far. Moreover, the precise shape of the 25(OH)D to mortality association curve is still not entirely clear. Many studies were underpowered and limited by analyses constrained to stratification of the study population according to 25(OH)D groups, resulting in a non-continuous evaluation of the association or in no definition of a curve at all. There have been reports on U- or reverse J-shaped curves of the association between 25(OH)D and mortality, which raised questions on the optimal range for vitamin D status, on the ultimate definition of vitamin D deficiency as well as the possible hazard of high-normal vitamin D concentrations.(20)

10.13 Limitations in Further Defining the Optimal Range for Vitamin D Status

There are several limitations when comparing observational studies and further defining the optimal range for vitamin D status and cut-off points for vitamin D deficiency. Major limitations when comparing studies relate to differences in study design, endpoint definitions, choice of vitamin D assay methodology as well as the season or months in which blood samples were taken, geographic latitude of the population under study, as well as the age range, ethnicity and sex of the study populations. Because of these limitations, the optimal range for vitamin D status and the ultimate definition of vitamin D insufficiency are still being discussed. Beside the IOM (24) and Endocrine Society Task Force (23) estimations and recommendations, there still exist a variety of different vitamin D deficiency prevalence estimates and related cut-off points being used across population studies thus further hampering comparisons of prevalence of vitamin D deficiency and optimal range for vitamin D status.(10)

10.14 Standardization of Vitamin D

As mentioned earlier, differences in analytic method for serum 25(OH)D are likely to contribute to deficiency prevalence estimates between populations. As shown in several reports, available 25(OH)D assays can yield markedly differing results. (3,94-96) To make vitamin D deficiency prevalence estimates more reliable and a comparison between studies more feasible, a U.S. National Institutes of Health-Office of Dietary Supplements (NIH-ODS) guided international collaborative initiative, namely the Vitamin D Standardization Program (VDSP), developed protocols for standardizing 25(OH)D data from current and previous surveys to the standardized concentrations as measured by the U.S. National Institute of Standards and Technology (NIST) and Ghent University reference measurement procedure (RMP).(97-99) The protocols are intended to be used to calibrate the reference measurement procedures to assigned values for concentrations of total 25(OH)D, 25(OH)D₂, 25(OH)D₃, and 3-epimer of 25(OH)D₃ for single studies.(99)

10.15 Aims of The Present Work

In the present analysis, the knowledge gap between standardized 25(OH)D concentrations and mortality is addressed.⁽¹⁾ Therefore, original and VDSP-standardized 25(OH)D concentrations as well as clinical outcomes from a collaboration of eight prospective studies across Europe within the EU-project ‘Food-based solutions for eradication of vitamin D deficiency and health promotion throughout the life cycle’ (ODIN) are being used. We employ an individual participant data (IPD), collaborative meta-analysis to address all-cause mortality as the primary outcome, and cardiovascular and cancer mortality as secondary outcomes in association with original and VDSP-standardized 25(OH)D concentrations.⁽¹⁾

11. Material and Methods

11.1 Study Identification and Selection

We established a collaboration to undertake this meta-analysis of IPD. Potential participants in a work-package (work package 8 [WP8]) of the European Commission-funded ODIN project (www.odin-vitd.eu/) were invited to attend a one-day workshop in Amsterdam in November 2012 to discuss aims, implementation and development of the task. (100) Invited European-based participants were mainly identified based on having recently published data from large prospective cohorts on vitamin D and mortality. There were a few prerequisites for inclusion of individual cohort studies in ODIN such as availability of bio-banked samples and validated prospective data on clinical outcomes. We subsequently invited representatives of the InCHIANTI (Invecchiare in Chianti, aging in the Chianti area; n=1453) study and the SU.VI.MAX (Supplementation en Vitamines et Mineraux Antioxydants; n=13017) study to express interest but they did not respond (SU.VI.MAX) or declined (InCHIANTI). The Danish Osteoporosis Prevention Study (DOPS; n=1006) failed the criteria due to inadequate samples. (1)

Included studies were the 4th survey of the Tromsø study (n= 26956), the Ludwigshafen Risk and Cardiovascular Health Study (LURIC; n= 3316), the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES; n= 5764), the New Hoorn Study (NHS; n=2807), the Aarhus Mammography Cohort Study (n=2555), the German Health Interview and Examination Survey for Adults (DEGS; n=4030) and the first (n=1 509) and second (n=919) cohort of the Longitudinal Aging Study Amsterdam (LASA). (1)

Within each cohort, we standardized cardiovascular and cancer end points on an individual basis, using definitions based on clinical and research expertise, availability of data, published guidelines and definitions, and the Standardized Data Collection for Cardiovascular Trials Initiative. (100) Definitions were sought to fit as closely as possible by each participating institution. (1)

All cohort studies were conducted in accordance with the declaration of Helsinki and written informed consent was obtained from all participants. This meta-analysis is registered at ClinicalTrials.gov, number NCT02438488 and adheres to recommendations for the rationale, conduct, and reporting of IPD meta-analysis. (101)

11.2 Participant Selection

The present analysis was based on individuals with complete data on age, sex, BMI, season of blood sampling, 25(OH)D concentration, vital status at follow-up and follow-up-time (which was at least 1 day). Participants with missing data were excluded from the analysis and we performed no data imputation. (1)

11.3 Participant Involvement

No participants were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design, or implementation of the study. No participants were asked to advise on interpretation or writing up of results. Easily comprehensible parts of the results will be disseminated to the public at <http://www.odin-vitd.eu/>.(1)

11.4 Study Details

11.4.1 Tromsø Study

The Tromsø Study, conducted by the University of Tromsø in cooperation with the National Health Screening Service, is a repeated population-based study in the municipality of Tromsø, Norway, situated at 69°N.(102,103) The baseline visit for the present analysis is the fourth survey, which was performed in 1994-1995, with repeated follow-up surveys conducted at six to seven years intervals. Of the 26956 participants, there were missing values for 25-hydroxyvitamin D (25[OH]D) (n=19796), and BMI (n=15), so that the final sample for the present study compromised 7145 individuals.(1)

11.4.2 Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

The LURIC Study is a prospective, hospital-based cohort study among 3316 study participants who were routinely referred to a tertiary care medical centre in south-west Germany, situated at 49°N, between 1997 and 2000.(104) Inclusion criteria were the availability of a coronary angiogram, German ancestry, and clinical stability except for acute coronary syndromes (ACS). Exclusion criteria were any acute illness other than ACS, any chronic disease where non-cardiac disease predominated and a history of malignancy within the past five years. Participants were continuously followed up with respect to fatal events. As there were missing values for 25(OH)D (n=17), the final sample compromised 3299 individuals.(1)

11.4.3 Age, Gene/Environment Susceptibility (Ages) Reykjavik Study

The AGES Reykjavik Study is conducted by a collaboration of the National Institute on Aging, National Institutes of Health, USA and, the Icelandic Heart Association. The AGES-Reykjavik sample is drawn from an established population-based cohort, the Reykjavik Study, and presents a repeated population-based study conducted in Iceland, situated at latitude of about 64°N. AGES-Reykjavik examinations began in 2002. At that time, there were 11549 previously examined Reykjavik Study cohort members still alive. At the end of AGES-Reykjavik examinations in February 2006, the study compromised 5764 survivors of the Reykjavik Study cohort. The AGES-Reykjavik examination is a single wave of examination, completed in three clinic visits, with a participant's full examination completed within a four to six-week time window.(105) Of the 5764 individuals, there were missing values for 25(OH)D (n=245), BMI (n=8), mortality follow-up (n=1), so that the final sample compromised 5510 individuals.(1)

11.4.4 The New Hoorn Study

From July 2006 until November 2007, the population-based New Hoorn Study (NHS) on glucose tolerance was performed in the city of Hoorn, the Netherlands. A random sample of 6180 men and women aged 40-65 years was drawn from the municipal population registry of Hoorn, situated at 53°N.(106) Of the 6 180 people who were invited, 2807 agreed to participate (45.4%). Of the non-attendees, 47% provided a reason for not participating, of which the most common were no time to participate (43%) and already having regular health checks (24.5%). For the present analysis, 216 individuals of 2807 were excluded, as there were missing frozen samples for measurement of 25(OH)D (n=182), and missing data on mortality follow-up (n=30) and BMI (n=4), so that the final sample compromised 2591 individuals.(1)

11.4.5 Aarhus Mammography Cohort Study

Between May 1st, 2003 and July 1st, 2007, 2555 women referred to a diagnostic mammography examination at Aarhus University Hospital (Aarhus, Denmark) were included in the Aarhus Mammography Cohort Study. The primary focus of this study was the association between pre-diagnostic plasma 25(OH)D concentrations and risk of breast cancer. Included women have been followed prospectively with assessment of mortality and incident diseases using The Danish National Hospital Discharge Register and the Danish Cancer Register.(107) There were missing values for BMI (n=63) and mortality follow-up (n=19), so that the final sample compromised 2473 individuals. The mainland of Denmark compromises latitude from 54°N to 57°N.(1)

11.4.6 German Health Interview and Examination Survey for Adults (DEGS)

DEGS is primarily designed as a periodically repeated cross-sectional national health interview and examination survey of adults in Germany. The target population comprises adults 18-79 years of age with permanent residence in Germany according to local population registries. To lay the ground for longitudinal studies, persons who had participated in the 1997-1999 national health interview and examination survey (GNHIES98) were invited to take part in DEGS1 in 2008-2011, provided they had agreed to be re-contacted and were still contactable. Of the 7124 persons who participated in GNHIES98, a subsample of 4030 randomly selected men and women also participated in the affiliated German Nutrition Survey (GeNuS) and had data on serum 25(OH)D concentrations. For the current analysis, this random subsample of 4030 participants was selected, and GNHIES98 and GeNuS were set as baseline visit and DEGS1 as follow-up visit. For the current analysis, 168 individuals were excluded due to missing values for age, sex, 25(OH)D (n=113), month of blood sampling, BMI (n=16), and mortality follow-up (n=39), so that the final sample compromised 3862 individuals.(108,109) The mainland of Germany compromises latitude from 47°N to 54°N.(1)

11.4.7 Longitudinal Aging Study Amsterdam (LASA) - First Cohort

LASA is an ongoing multidisciplinary cohort study on predictors and consequences of changes in older persons. In 1992/1993, a random sample of men and women aged 55 years old and over, stratified by age, sex, urbanization grade, and expected five-year mortality rate, was drawn from the population registers of eleven municipalities, in three regions of the Netherlands (n=3107). Measurement cycles were repeated every three years and included a main interview and medical interview. Blood samples were obtained in the first follow-up cycle 1995/1996, which for the present analysis, was defined as baseline visit. The blood samples were centrifuged and stored at -20 °C. Of the 1509 individuals that completed the baseline examination in 1995/96, there were missing values for 25(OH)D (n=189), month of blood sampling (n=3), and mortality follow-up (n=18), so that the final sample comprised 1302 individuals.(110,111) The study comprises latitude from 51°N to 53°N.(1)

11.4.8 Longitudinal Aging Study Amsterdam (LASA) - Second Cohort

An additional cohort was recruited from the same sampling frame in 2002/2003, exactly ten years after the first LASA cycle of the original cohort. This new cohort consisted of 1002 men and women who were born between 1938 and 1947. Of the 919 individuals with complete demographic data of the baseline examination, there were missing values for 25(OH)D (n=181), and missing data on mortality follow-up (n=4), so that the final sample comprised 734 individuals.(1)

11.5 Construction of An IPD Database

Investigators from each study cohort provided individual participant data on a MS Excel template (Microsoft Excel, Redmond, Washington, USA) or translated comparable data into a SPSS (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, NY) or SAS (SAS Institute Inc., 100 SAS Campus Drive, Cary, USA) data file. The template was harmonized prior to the exchange by all investigators to alleviate co-operation and to ensure clear assignment of the data. Data was transmitted to the analysis coordination centre (Medical University of Graz, Austria) by email or by password protected file transfer exchange server. Datasets contained no personally identifiable information, e.g. names or absolute dates. For transmitting, missing data was indicated as “9999” for missing values and “8888” for general missing covariates in the datasets. Database management was processed by SPSS version 20 and higher.(1)

11.6 Endpoint Definitions

The purpose of this chapter is to provide a framework of definitions for cardiovascular and cancer end points within the current analysis. These definitions are based on clinical and research expertise, availability of data, published guidelines, and definitions, and refer to the Standardized Data Collection for Cardiovascular Trials Initiative.(100) Definitions were sought to fit as closely as possible by each participating institution.(1)

According to the International Classification of Diseases (ICD) system, cardiovascular death was pre-specified as acute myocardial infarction (ICD-9: 410; ICD-10: I21, I22)(112), sudden cardiac death (ICD-9: 427; ICD-10: I46-I49, R00)(113), death due to heart failure (ICD-9: 428; ICD-10: I50)(114), death due to stroke (ICD-9: 362.3, 430, 431, 433.01, 433.11, 433.21, 433.31, 433.81, 433.91, 434.01, 434.11, 434.91, 436.x; ICD-10: H34.1, I60, I61, I63, I64)(115), and other causes of cardiovascular death. Other causes of death refer to a cardiovascular death not included in the above categories [e.g. pulmonary embolism, cardiovascular intervention (other than one related to an AMI), aortic aneurysm rupture, or peripheral arterial disease].(1,100)

Cancer death was defined as death from cancer sites as follows(116): Lung, trachea, and bronchus (ICD-7: 162; ICD-9: 162; ICD-10: C33, C34), colorectal cancer death (ICD-7: 153, 154; ICD-9: 153, 154; ICD-10: C18-C21), other cancer of the digestive system, i.e., oesophagus, stomach, liver, pancreatic cancer (ICD-7: 150, 151, 155, 157; ICD-9: 150, 151, 155, 157; ICD-10: C15, C16, C22, C25), breast cancer death (ICD-7: 170; ICD-9: 174, 175, 233; ICD-10: C50), prostate cancer death (ICD-7: 177; ICD-9: 185; ICD-10: C61), skin cancer death (ICD-7: 190, 191; ICD-9: 172, 173, 232; ICD-10: C43-C44), and other cancers, including buccal, larynx, melanoma, gynaecological sites, kidney, bladder, brain, multiple myeloma (ICD-7: 140-148, 152, 155.1, 160, 161, 162.2, 172-176, 178-181, 191-197 ;ICD-9: 140-149, 152, 156, 160, 161, 163-165, 170-171, 179, 181-184, 186-189, 190-199, 203, 209; ICD-10: C00 - C14, C17, C23 - C24, C26 - C32, C37 - C41, C45 - C49, C51 - C52, C54 - C60, C62 - C65, C67 - C80, C88, C90, C97).(1)

11.6.1 Tromsø Study

Information on endpoints of all-cause and cause-specific mortality in the Tromsø Study was derived from a National registry. Each participant was linked to data from the Norwegian Causes of Death Registry using a personal identification number to identify vital status. Cause of death is in Norway based on the ICD system and the underlying cause of death was used. ICD-9 was applied for deaths occurring up to 1996 and ICD-10 was applied for deaths occurring in 1996 and thereafter.(1,117)

Cardiovascular death was specified as acute myocardial infarction (ICD-9: 410; ICD-10: I21.9, I22.9), sudden cardiac death (ICD-9: 427.3; ICD-10: I44.1, I45.6, I46.1, I46.9, I49.1, I49.9, I51.4,

I51.6, I51.9), death due to heart failure (ICD-9: 428.9; ICD-10: I50.0, I50.1, I50.9), death due to stroke (ICD-9: 430, 431, 433.1, 434.9, 436; ICD-10: H34.0, I60.0, I60.1, I60.2, I60.8, I60.9, I61.2, I61.3, I61.4, I61.9, I62.0, I63.0, I63.1, I63.2, I63.3, I63.4, I63.9, I64, I69.1, I69.3, I69.4), and other causes of cardiovascular death.(1)

Other causes of cardiovascular death contained coronary/peripheral atherosclerosis (ICD-9: 414.0, 414.3, 414.8, 414.9; ICD-10: I70.2, I70.9), unspecified cerebrovascular/peripheral vascular disease (ICD-9: 437.9; ICD-10: I73.9, I67.2, I67.8, I67.9), generalized and unspecified atherosclerosis (ICD-9: 440.9), aneurysm (ICD-9: 441.0, 441.1, 441.4, 441.5, 441.6; ICD-10: I71.0-I71.5, I71.8, I71.9), endocarditis (ICD-10: I38, I33.0), atrial fibrillation, (rheumatic) valve disease (ICD-9: 394.9, 424.1; ICD-10: I05.8, I06.0, I06.9, I08.0, I35.0, I35.1, I35.2, I35.9), hypertensive (heart) disease (ICD-10: I10, I11.0), hypertensive kidney disease (ICD-10: I12.0, I12.9, I13.2), angina pectoris (ICD-10: I20.9), and ischemic heart disease (ICD-10: I24.1, I25.1, I25.2, I25.5), thrombosis and embolism (ICD-10: I26.9, I74.2, I74.3, I80.2, I80.3), other pulmonary heart diseases (ICD-10: I27.0, I27.9), chronic constrictive pericarditis (ICD-10: I31.1), cardiomyopathy (ICD-10: I42.0, I42.2, I42.6, I42.9), myocarditis (ICD-10: I45.6), arteritis (ICD-10: I77.6).(1)

Cancer death was defined as death from cancer sites as follows: Lung, trachea, and bronchus (ICD-9: 162.9; ICD-10: C33, C34.0-C34.3, C34.9), colorectal cancer death (ICD-9: 153; ICD-10: C19, C20, C18.0-C18.7, C18.9, C21.0, C21.1), other cancer of the digestive system, i.e., oesophagus, stomach, liver, pancreatic cancer (ICD-9: 150, 151, 155.0, 155.1, 157; ICD-10: C15.4, C15.5, C15.8, C15.9, C16.0, C16.2-C16.6, C16.8, C16.9, C22.0, C22.1, C23, C25.0, C25.1, C25.8, C25.9), breast cancer death (ICD-10: C50.2- C50.5, C50.8, C50.9), prostate cancer death (ICD-9: 185; ICD-10: C61), skin cancer death (ICD-9: 172; ICD-10: C43.3, C43.5-C43.7, C43.9), and other cancers, including buccal, larynx, melanoma, gynaecological sites, kidney, bladder, brain, multiple myeloma (ICD-9: 161, 171, 183.0, 184.9, 188, 190, 199.1, 200.1, 202.1, 204.1; ICD-10: C01, C02.1, C02.9, C04.9, C07, C08, C09.9, C13.9, C14, C17.9, C24.0, C24.1, C24.9, C26.9, C31, C32.0, C32.2, C32.9, C38.0, C38.4, C41.0, C45.0, C45.7, C45.9, C48.0-C48.2, C49.0, C49.2, C49.9, C51.9, C52, C53.9, C55, C56, C54.9, C57.4, C57.9, C64, C65, C73, C67.9, C68.9, C69.3, C71.0, C71.2, C71.9, C74, C75.9, C76.2, C80, C81.9, C82.1, C83.0, C83.1, C83.3, C83.7, C85.1, C85.7, C85.9, C88.0, C90.0, C91.0, C91.1, C91.3, C91.9, C92.0, C92.1, C92.5, C95.9, C96.1).(1)

11.6.2 Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

Information about vital status was obtained from local person registries. Medical records of local hospitals, death certificates, and autopsy data were used to classify the causes of death into cardiovascular and non-cardiovascular mortality. Classification of the causes of death was independently done by two experienced physicians who were blinded to any data of the study subjects except of those that were necessary for the coding of the causes of death. In the event of a

disagreement regarding a specific case, the final classification was done by one of the principle investigators of LURIC (W. M.).(1,118)

11.6.3 Age, Gene/Environment Susceptibility (Ages) Reykjavik Study

Information on vital status and the causes of death was based on data from a complete adjudicated registry of deaths available from the Icelandic National Roster.(1,119)

Cardiovascular death was pre-specified as acute myocardial infarction (ICD-10: I21, I22), sudden cardiac death (ICD-10: I47-I49, R00), death due to heart failure (ICD-10: I50), death due to stroke (ICD-10: H34.1, I60, I61, I63, I64, G45). Other causes refer to a cardiovascular death not included in the above categories [e.g. pulmonary embolism, cardiovascular intervention (other than one related to an AMI), aortic aneurysm rupture, or peripheral arterial disease].(1)

Cancer death was defined as death from cancer sites as follows: Lung, and bronchus (ICD-10: C34), colorectal cancer death (ICD-10: C18-C21), other cancer of the digestive system, i.e., oesophagus, stomach, pancreatic cancer (ICD-10: C15, C16, C25), breast cancer death (ICD-10: C50), prostate cancer death (ICD-10: C61), skin cancer death (ICD-10: C43-C44), and other cancers, including buccal, larynx, melanoma, gynaecological sites, kidney, bladder, brain, multiple myeloma (ICD-10: C00-D49, other than listed above).(1)

11.6.4 The New Hoorn Study

Information on vital status was obtained from the Dutch Municipal Population Register. No specific causes of death have been yet coded.(1)

11.6.5 Aarhus Mammography Cohort Study

Information on endpoints of all-cause and cause-specific mortality was derived from The Danish National Hospital Discharge Register and the Danish Cancer Register.(1,120)

Data from death certificates were used to decide the fundamental cause of death. Coding was based on the ICD-10 classification, but the coding has been performed by the physician filling in the death certificate. ICD-10 codes I00-I99 have resulted in a death being considered as a cardiovascular death, and C00-D49 have resulted in a death being classified as due to cancer.(1)

11.6.6 German Health Interview and Examination Survey for Adults (DEGS)

Information on endpoints of all-cause and cause-specific mortality in the German Health Interview and Examination Survey for Adults was derived from local population registries and death

certificates.(121) Cardiovascular death was specified in ICD-10 codes as I00 to I99. Cancer death was specified in ICD 10 codes as C00 to C97.(1)

11.6.7 Longitudinal Aging Study Amsterdam, First and Second Cohort

Occurrence of deaths among the study participants was retrieved up to 1st November 2013 through linkage with population register data. Primary causes of death were obtained from the Dutch Central Bureau of Statistics. The cause of death was coded according to the ICD system, 10th Revision (ICD-10).(1,122)

Cardiovascular death was pre-specified as acute myocardial infarction (ICD-9: 410; ICD-10: I21, I22), sudden cardiac death (ICD-9: 427; ICD-10: I46-I49, R00), death due to heart failure (ICD-9: 428; ICD-10: I50), death due to stroke (ICD-9: 362.3, 430, 431, 433.01, 433.11, 433.21, 433.31, 433.81, 433.91, 434.01, 434.11, 434.91, 436.x; ICD-10: H34.1, I60, I61, I63, I64), and other causes of cardiovascular death. Other causes included pulmonary embolism, aortic aneurysm rupture, or peripheral arterial disease (ICD-9: 415.1, 441.3, 441.6, 440.2; ICD-10: I26.9, I71.0, I71.3, I71.4, I71.9, I73.9).(1)

Cancer death was defined as death from cancer sites as follows: Lung, trachea, and bronchus (ICD-9: 162; ICD-10: C33, C34), colorectal cancer death (ICD-9: 153, 154; ICD-10: C18-C21), other cancer of the digestive system, i.e., oesophagus, stomach, liver, pancreatic cancer (ICD-9: 150, 151, 155, 157; ICD-10: C15, C16, C22, C25), breast cancer death (ICD-9: 174, 175, 233; ICD-10: C50), prostate cancer death (ICD-9: 185; ICD-10: C61), skin cancer death (ICD-9: 172, 173, 232; ICD-10: C43-C44), and other cancers, including gynaecological sites, and malignant neoplasms of lymphoid, hematopoietic and related tissue (ICD-10: C53.9, C81.9, C85.9, C91.0, C91.1, C92.0, C92.1).(1)

11.7 Covariate Definitions

Age was defined as age in years at baseline visit and inserted as linear term in regression models. Sex was inserted as a binary variable coding as “0” for women and “1” for men. BMI was defined as weight in kilograms divided by height in meters squared and inserted as linear term. Season of blood sampling at baseline was coded as dummy variable for each of the four Seasons, with spring defined as March to May (with coding “0” for baseline blood sampling not during spring, and “1” for during spring), summer as June to August (with coding “0” for baseline blood sampling not during summer, and “1” for during summer), autumn as September to November (with coding “0” for baseline blood sampling not during autumn, and “1” for during autumn), and winter as December to February (with coding “0” for baseline blood sampling not during winter, and “1” for during winter). Autumn was defined as reference season and left out in the regression equation.(89)

Supplemental intakes of calcium and vitamin D were defined as intake by supplements at baseline. Supplemental calcium intake was coded as a binary variable coding as “0” for no supplemental intake and “1” for positive supplemental intake of calcium at the baseline visit. Supplemental vitamin D intake was coded as a binary variable coding as “0” for no supplemental intake and “1” for positive supplemental intake of vitamin D at the baseline visit. Present arterial hypertension was defined as (definitions listed according to priority - if more than one point was available in a study the higher ranked definition was given priority): Participants already on antihypertensive drug treatment, physician-reported arterial hypertension, self-reported arterial hypertension, office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg according to 2003 guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC).(123) Present arterial hypertension was inserted as a binary variable with “0” coding for no arterial hypertension present and “1” for arterial hypertension present at baseline. Present diabetes mellitus was specified (definitions listed according to priority - if more than one point was available in a study the higher ranked definition was given priority) as: Those participants on glucose lowering drugs, physician-reported, self-reported or according to American Diabetes Association (ADA) criteria fasting glucose ≥ 7.0 mmol/l, two hours post-load glucose ≥ 11.1 mmol/l or HbA1c $\geq 6.5\%$.(124) Present diabetes mellitus was inserted as a binary variable with “0” coding for no diabetes mellitus present and “1” for diabetes mellitus present at baseline. Current smoking habit was dichotomized as no active smoking (“0”) versus active smoking (“1”) at baseline. Physical activity was coded in three levels according to the frequency of leisure activity in hours per week from low to high frequency.(125) Each level was transformed into a dummy variable and inserted into a regression model as a binary variable coding for “0” level not present versus “1” level present and the third (highest frequency) group was defined as reference and left out in the regression equation. Basically, physical activity was defined as leisure activity, with a degree of medium or vigorous intensity. If calculated, medium or vigorous intensity were determined as metabolic equivalent of task (MET) of three and higher, equivalent to 600-1499 kcal per week. The lowest level of physical activity includes participants on moderate and vigorous activity with a frequency of less than one hour per week. Additionally, participants without participating in any regular leisure activity (e.g. bedridden participants) or participating only in light activities (intensity MET-score ≤ 2.9 , e.g. light household, billiards, walking slowly) were included into the lowest level of physical activity, regardless of their frequency. Participants assigned to light activities are defined as participants who are not participating in any moderate or vigorous activities or any regular activity with MET-score of three and higher. The medium level of physical activity comprises participants with moderate and vigorous activity (intensity MET-score of three and higher) with a frequency of one to three hours per week and the highest level of physical activity comprises participants with moderate and vigorous activity (intensity MET-score of three and higher) with a frequency of more than three hours per week. History of CVD

was coded as binary variable “0” for negative and “1” for positive history. History of CVD included previous acute myocardial infarction and previous stroke/ transient ischemic attack at baseline visit. History of cancer was a binary variable coding as “0” for negative and “1” for positive history of cancer at baseline. History of cancer included cancer of lung, trachea, and bronchus, colorectal cancer, oesophagus-, stomach-, liver-, and pancreatic cancer, breast cancer, prostate cancer, skin cancer, and other cancer sites including buccal, larynx, melanoma, gynaecological sites, kidney, bladder, brain, and multiple myeloma. Estimated glomerular filtration rate in mL/min/1.73m² was calculated from creatinine at baseline visit according to the four-variable Modification of Diet in Renal Disease (MDRD) Study equation and inserted as a linear term in the regression equations. C-reactive protein (CRP) at baseline was inserted as linear term in mg/l. Parathyroid hormone (PTH) was inserted as linear term in pmol/l, low density lipoprotein (LDL) was inserted as linear term in mmol/l, and systolic blood pressure (SBP) as linear term in mmHg in the regression equation.(1)

11.8 Covariate Assessment

Table 1: List of Additional Baseline Confounders Available for Model Building of Parametric Survival Model in The Eight Observational Cohort Studies Included in The Meta-Analysis.

Characteristic	Tromsø	LURIC	AGES	NHS	Aarhus	DEGS	LASA, first cohort	LASA, second cohort
Age	X	X	X	X	X	X	X	X
Sex	X	X	X	X	X	X	X	X
Season	X	X	X	X	X	X	X	X
BMI	X	X	X	X	X	X	X	X
Current smoking	X	X	X	X	X	X	X	X
Present Diabetes	X	X	X	X	X	X	X	X
Present HTN	X	X	X	X	X	X	X	X
History of CVD	X	X	X	N.A.	X	X	X	X
History of cancer	X	X	X	N.A.	X	X	X	X
Physical activity	X	X	X	X	N.A.	X	X	X
eGFR	X	X	X	N.A.	X	X	X	X
CRP	N.A.	X	X	N.A.	N.A.	X	X	N.A.
Calcium Supplements	X	X	X	N.A.	X	N.A.	X	N.A.
Vitamin D Supplements	X	X	X	N.A.	X	N.A.	N.A.	N.A.
PTH	X	X	X	N.A.	X	X	X	X
SBP	X	X	X	X	N.A.	X	X	X
LDL-C	X	X	X	X	N.A.	X	X	X
Glucose	N.A.	X	X	X	N.A.	X	X	X

Abbreviations: Tromsø = Tromsø Study; LURIC = Ludwigshafen Risk and Cardiovascular Health Study; AGES = Age, Gene/Environment Susceptibility Reykjavik Study; NHS = New Hoorn Study; Aarhus = Aarhus Mammography Cohort Study; DEGS = German Health Interview and Examination Survey for Adults; LASA = Longitudinal Aging Study Amsterdam; X = Confounder available; N.A. = Confounder not available; BMI = Body mass index; HTN = Arterial hypertension; CVD = Cardiovascular disease; eGFR = estimated glomerular filtration rate according to the four-variable Modification of Diet in Renal Disease (MDRD) Study equation; CRP = C-reactive protein; PTH = Parathyroid hormone; SBP = Systolic blood pressure; LDL-C = Low density lipoprotein cholesterol. Basic adjustment variables age, sex, BMI, season of blood sampling, vital status at follow up and follow-up/failure time were minimum requirements and were available in all cohorts.(1)

11.8.1 Tromsø Study

Present diabetes: The diagnosis of diabetes mellitus was derived from questionnaires (“Do you have, or have you had diabetes?”), through elevated HbA1c (6.5%), by linkage of the participant list to the University Hospital of North Norway digital discharge diagnosis registry (ICD-9 codes 250, 357.2, 362.0, 583.8, 648.0, 648.8, 790.2, ICD-10 codes E10.0-E14, O24 and R73) and hospital records.(1)

Present arterial hypertension: Present diagnosis of arterial hypertension was established as intake of antihypertensives or office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg.(1)

Physical activity: Information on physical activity was obtained from a questionnaire on light and hard leisure activity. Light activity was asked for as “*Light activity (not sweating or out of breath): How has your physical activity in leisure time been during this last year? Think of your weekly average for the year. Time spent going to work count as leisure time (hours per week).*” Hard leisure activity was obtained as followed: “*Hard physical activity (sweating/out of breath): How has your physical activity in leisure time been during this last year? Think of your weekly average for the year. Time spent going to work count as leisure time (hours per week).*” For recoding, only the Tromsø variable of hard leisure activity was used, and the weekly frequency was divided from a four-levelled scale, which compromised the answers, “no activity”, “less than one hour per week”, “one to two hours per week”, and “three hours or more per week”, into the three-levelled co-variable according to the current definition. For re-coding, the lowest level of hard leisure activity was kept as it is, answer two and three were combined to the medium level of physical activity and answer four was re-coded into the highest level of physical activity.(1)

Active smoker status: Active smoking status was obtained from questionnaire (“*Do you smoke cigarettes daily?*”).(1)

History of cardiovascular disease: Positive history of cardiovascular disease was defined as positive history of myocardial infarction or positive history of stroke. History of myocardial infarction and stroke were self-reported (“*Do you have, or have you had a heart attack?*”, and “*Do you have, or have you had a cerebral stroke/brain haemorrhage?*”) and obtained from medical records.(1)

History of cancer: Information on cancer incidence and cancer location was retrieved from the Cancer Registry.(1)

Creatinine: Plasma creatinine in $\mu\text{mol/l}$ was analysed by a modified Jaffe reaction on a Roche Hitachi 911 automated analyser (Boehringer Mannheim/Hitachi, Indianapolis, USA).(1)

Intake of calcium by supplements: Supplemental intake of calcium was obtained from questionnaires (“Have you used calcium tablets or bone meal during the last 14 days?”) and hospital records.(1)

Intake of vitamin D by supplements: Supplemental intake of vitamin D was obtained from questionnaires (“Have you used vitamin D supplement during the last 14 days?”) and hospital records.(1)

Measurement of serum 25(OH)D: Sera from the second visit of the study were stored at -70°C , and after a median storage time of 13 years, they were thawed in March 2008 and analysed for 25(OH)D₃ by electro chemiluminescence immunoassay (ECLIA) from Roche (Roche Diagnostics, Mannheim, Germany) using an automated clinical chemistry analyser (Modular E170, Roche Diagnostics, Mannheim, Germany). According to the manufacturer, the assay has, for total analytical precision, a coefficient of variation (CV) $\leq 7.8\%$ as judged in any of three different concentrations (48.6, 73.8, and 177.0 nmol/l). The cross-reactivity with 25(OH)D₂ was $<10\%$, and the analytical sensitivity was 10 nmol/l.(102) Since this assay overestimated 25(OH)D in smokers (126), standardization was performed separately for smokers and non-smokers.(1)

Body mass index: Height and weight were measured while the subjects wore light clothing and no shoes and BMI was calculated as kg/m^2 .(1,103)

Blood pressure: Blood pressure was measured with an automatic device (Dinamap Vital Signs Monitor 1846; Critikon Inc, Tampa, FL). The subjects were seated for two minutes. Three recordings were made at two minutes intervals, and the mean of the last two measurements was used.(1,127)

Low-density lipoprotein: In the fourth survey of the Tromsø Study, serum total cholesterol (TC) and triacylglycerol (TAG) concentrations were analysed by enzymatic colorimetric methods with commercial kits (CHOD-PAP for TC and GPO-PAP for TAG; Boehringer-Mannheim, Mannheim, Germany). Serum high-density lipoprotein cholesterol (HDL-C) was measured after the precipitation of lower-density lipoproteins with heparin and manganese chloride. The serum LDL was calculated using the formula $\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TAG} \times 0.46)$, provided the serum TAG value was $<4.0\text{mmol}/\text{l}$.(1,128)

Parathyroid hormone: Intact PTH was measured on Immulite (Diagnostic Products Corporation, Los Angeles, CA, USA) based on a two-site chemiluminescent immunometric assay. The reference range in our laboratory is 1.1-6.8 pmol/l for those below the age of 50, and 1.1-7.5 pmol/l for those 50 years or above, the between assay CV being 6-8% in the actual range.(1,129)

11.8.2 Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

Present diabetes: Diabetes mellitus was diagnosed according to the criteria published by the American Diabetes Association (ADA) 1997 (124) and by the World Health Organization (WHO) (130). These criteria for the definition of diabetes mellitus either demanded a pathological fasting (plasma glucose ≥ 126 mg/dl) or a two-hour plasma glucose concentration ≥ 200 mg/dl after an oral glucose tolerance test. In addition, an individual with a documented history of diabetes was defined as diabetic, regardless whether the laboratory criteria for diabetes were met or not. Requirement for an antidiabetic medication, i.e., oral antidiabetic and/or insulin use for the control of glycaemia, was accepted as a documentation of diabetes, as were medical documents (i.e., hospital discharge letters or other documentation) with a diagnosis of diabetes (including diabetics treated by diet only).(1)

Present arterial hypertension: Present diagnosis of arterial hypertension was established as intake of antihypertensive medication or office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg.(1)

Physical activity: Daily physical activity was recorded using a non-validated 11-point scale ranging from bedridden to extremely active. Key points on the scale were “1” meant bed rest, “2” meant mostly supine, “3” meant not very active, “6” meant usual office work, “9” meant heavy work or sports, and “11” meant extremely sportive. Recoding was performed according to WHO recommendations into (1 thru 7=1), (8=2) and (9 thru 11=3).(1,131)

Active smoker status: Active smoking status was self-reported (“Do you smoke cigarettes regularly?”).(1)

History of cardiovascular disease: Positive history of cardiovascular disease was defined as positive history of myocardial infarction or positive history of stroke. Previous event of stroke was obtained from medical records. A previous myocardial infarction (MI) was diagnosed if a MI had been survived for more than one month before enrolment into LURIC. The diagnosis could either be based on ECG (see Winkelmann BR et al.(103) for detailed information), or it was based on a report of a diagnosis of MI in a medical document (discharge letter, catheterization report). Additionally, previously unrecognized MI (often called silent MI) was included as history of CVD. Silent MI was defined as a subtype of previous MI, where the individual had not been hospitalized during the acute phase of the MI and the diagnosis of MI was established in retrospect. Previously unrecognized MI was diagnosed in the presence of new pathologic Q-waves in at least two adjacent leads with or without wall motion abnormalities. Isolated documentation of wall motion abnormality, vessel occlusion without further clinical evidence or evidence of scarring by imaging was not sufficient for the diagnosis of a previously unrecognized MI.(1)

History of cancer: Participants who had malignant cancer in the previous five years were excluded from the LURIC Study. Information on history of cancer prior to the previous five years was obtained from questionnaire.(1)

Creatinine: Serum creatinine was measured by Jaffé method (twin mode) on a CREA/Hitachi 717 automated analyser (Roche Mannheim, Germany).(1)

C-reactive protein: CRP was analysed by an immunoturbidimetric assay on a Hitachi 911 analyser (Roche Mannheim, Germany).(1)

Intake of calcium by supplements: Supplemental intake of calcium was obtained from medication history questionnaire with explicit field for vitamin intake. Retrospective search tags were Ossofortin, Ortho core PLUS, Ca BT, Calcium BT, Phosetamin, Calcium Dura, Solugastril, Calcium Sandoz, Calcium forte, Calcium, Calcimagon, Frubase Ca forte, Ca-Acetat, also multivitamin preparations were considered.(1)

Intake of vitamin D by supplements: Supplemental intake of vitamin D was obtained from medication history questionnaire with explicit field for vitamin intake. Retrospective search tags were Vigatoletten, Ossofortin, Rocaltrol, Vitamin D3, D-Tracetten, Frubase Ca forte, and Calcimagon. Also, multivitamin preparations were considered.(1)

Body mass index: Body weight was measured without shoes and in light clothing by a trained nurse. Body height was recorded to the nearest centimetre with the subject barefoot and in the upright position.(1)

Blood pressure: Blood pressure was measured with an automated oscillometric device (Omron MX4, Omron Healthcare GmbH, Hamburg, Germany) while supine for at least 10 minutes. At least three consecutive measurements of systolic and diastolic blood pressures were taken 30 seconds apart and mean values of these measurements are reported. Measurements were considered invalid and repeated if they varied > 10 mmHg systolic, > 5 mmHg diastolic, or heart rate of more than five beats per minute from each other (except for atrial fibrillation).(1,104)

Low-density lipoprotein cholesterol: Low-density lipoprotein cholesterol was measured after separating lipoproteins with a combined ultracentrifugation-precipitation method.

Parathyroid hormone: Intact PTH was determined in serum by Electro Chemiluminescence Immunoassay (ECLIA) on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany), with a normal range of 15-65 pg/ml and an inter-assay CV of 5.7-6.3%.(1)

Parathyroid hormone: Intact PTH was determined in serum by Electro Chemiluminescence Immunoassay (ECLIA) on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany), with a normal range of 15-65 pg/ml and an inter-assay CV of 5.7-6.3%.(1)

11.8.3 Age, Gene/Environment Susceptibility (Ages) Reykjavik Study

Present diabetes: Diabetes was defined using glucose lowering drugs, or fasting glucose ≥ 7.0 mmol/l and self-report according to questionnaire (“*Has some doctor or other health personnel ever told you that you had diabetes?*”).(1)

Present arterial hypertension: Information on present arterial hypertension was obtained either from questionnaire (“*Has a doctor or other health provider ever told you that you had hypertension or high blood pressure?*”), from physiological measurements (office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg), or usage of antihypertensive medication (specifically Anatomical Therapeutic Chemical (ATC) classification code beginning with C02).(1)

Physical activity: Participants answered questions about frequency of current moderate or vigorous physical activity (“*How often did you participate in moderate or vigorous physical activities in the past 12 months?*”). Answers were categorized into never, rarely, occasionally, moderate or high frequency of participation. Options one to three were combined to basic level of physical activity, option four was assigned the second level of physical activity and options five and six were set level three. Participants with only light activities were assigned to basic level of physical activity.(1)

Active smoker status: Active smoking status was self-reported (“*Do you smoke cigarettes now?*” and “*Do you smoke cigar or pipe now?*”).(1)

History of cardiovascular disease: History of cardiovascular disease was defined as history of myocardial infarction or history of stroke. History of myocardial infarction and stroke were either self-reported obtained from the Directorate of Health or from hospital records.(1)

History of cancer: History of cancer was obtained from hospital records.(1)

Creatinine: Serum creatinine was measured using the Roche-Hitachi 912 instrument with Roche Creatinine Jaffé compensated method; Roche Diagnostics, Mannheim, Germany. The CV for the creatinine assay was 2.5%.(1,132)

C-reactive protein: High sensitivity CRP was measured on a Hitachi 912, using reagents from Roche Diagnostics and following the manufacturer’s instructions. Both within- and between-assay quality control procedures were used and the CV of the method was 1.3% to 3.4%, respectively, through the period of data collection. The assay could detect a minimal CRP concentration of 0.1 mg/l and values below this level were classified as undetectable. All participants in this study had detectable CRP levels.(1,133)

Intake of calcium by supplements: Calcium intake by supplements was defined as use of any calcium supplements according to questionnaire or list of „Over the counter drug use “. Question asked was „Do you take calcium tablets? “. (1)

Intake of vitamin D by supplements: Intake of vitamin D supplements was defined as regular use of any vitamin D supplements according to questionnaire or list of „Over the counter drug use “. In the definition all occurrences of products with information on vitamin D supplements, multivitamins, and fish liver oil were used. People were also asked to bring all medications and supplements to the AGES-visit. Questions asked were „Do you take multi-vitamins? “ and „On average how often do you take cod or Saithe liver oil or liver oil pills (not halibut liver oil pills)?“(1)

Measurement of serum 25(OH)D: Blood was collected during the first clinic visit to AGES-study, from September 2002 to January 2006, and fasting serum samples were kept frozen at -80°C on-site in the IHA biorepository. Quantitative determination of total 25(OH) D (D2 and D3) was conducted by means of a direct, competitive chemiluminescence immunoassay (CLIA), using the LIAISON 25 OH Vitamin D Total assay (DiaSorin, Inc., Stillwater, Minnesota). The inter-assay CV was <6.5%, using a previously frozen serum pool as the control sample and <12.7% when the calculated data were from measurements using Liaison quality controls.(1)

Body mass index: Body mass index was calculated as weight in kilogram divided by height in meters squared. Baseline weight was measured using a digital scale and height was measured using a stadiometer.(1)

Blood pressure: Blood pressure: Blood pressure was measured with a mercury sphygmomanometer with a large cuff, and the mean value of two consecutive blood pressure measurements was used in the analysis.(1)

Low-density lipoprotein: Total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and triglycerides were analysed on a Hitachi 912 chemistry automated analyser, using reagents from Roche Diagnostics and following the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). LDL was calculated using the Friedewald equation.(1)

Parathyroid hormone: Intact PTH was measured on Roche Elecsys 2010 analyser from Roche Diagnostics GmbH, Mannheim using electro chemiluminescence technology on a two-site immunoassay. PTH values were converted from pg/ml to pmol/l by multiplication with 0.106. The inter-assay CV was less than 3.1% when using a frozen serum pool as the control sample and was less than 2.8% when using Roche quality controls.(1,134)

Glucose: Glucose was analysed on a Hitachi 912 chemistry automated analyser, using reagents from Roche Diagnostics and following the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Inter-assay CV was 2.0%.(1)

11.8.4 The New Hoorn Study

Present diabetes: Based on the results of the OGTT, participants were categorized into 3 groups using the WHO '06 criteria (135): normal glucose metabolism (NGM), impaired glucose regulation, i.e. impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) based on fasting glucose greater of equal 6.1 mmol/l and/or 2h post OGTT glucose greater of equal 7.8 mmol/l or newly detected diabetes mellitus (NDM). In addition, known diabetes mellitus (KDM) was defined using insulin or oral hypoglycaemic agents, and self-reported known diabetes (“*Have you ever been told by a medical doctor (or another health professional) that you have diabetes?*”). For the present analysis participants with known, as well as newly diagnosed and participants with IFG were considered positive for this variable.(1,106)

Present arterial hypertension: Present arterial hypertension was defined in participants already on antihypertensive drug treatment or according to WHO 2003 statement (127) and in participants with office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg.(1)

Physical activity: Physical activity was obtained from questionnaires on leisure activity, with focus on frequency and degree of exertion. Information on four kinds of regularly performed sports plus biking was obtained. After recoding each type of activity from minutes per day into hours per week, the overall time for no activity, light and moderate to vigorous degree of exertion was calculated for every type of sports and biking. Then, the overall weekly time for each level of intensity was calculated as the sum of different kinds of sports performed at each level of intensity. The time variables were then stratified into three levels of frequency separately for light and moderate to vigorous intensity. For moderate to vigorous intensity, frequency of less than one hour per week was re-coded to the lowest level of physical activity, one to three hours per week were set as the medium level of physical activity and three hours or more per week were set as the highest level of physical activity. For light intensity, frequency of less than two hour per week was re-coded to the lowest level of physical activity, two to six hours per week were set as the medium level of physical activity and six hours or more per week were set as the highest level of physical activity. Finally, out of the stratified frequency variables of light and moderate to vigorous intensity, the participant was signed the higher value out of both variables.(1)

Active smoker status: Information on active smoking status was obtained from questionnaire (“*Do you smoke?*”).(1)

Measurement of serum 25(OH)D: The NHS was the only cohort study with no original 25(OH)D measurements; so that the NHS was not calibrated, but measured in full a liquid chromatography-mass spectrometry method at University College Cork.(1)

Body mass index: Height and weight were measured without shoes and heavy clothes. Body mass index was calculated as weight (in kg) divided by height (in meters) square.(1)

Blood pressure: Blood pressure was measured three times on the right arm after a 10-minute rest period, using a Colin Press BP 8800p Non-Invasive Blood Pressure Monitor (Colin Medical Technology Corporation, USA). Final blood pressure was calculated as the mean of the last two measurements.(1)

Low-density lipoprotein: Triglycerides, total and high-density lipoprotein cholesterol were determined from fasting plasma samples by enzymatic techniques (Boehringer-Mannheim, Mannheim, Germany). Low-density lipoprotein cholesterol was estimated with the Friedewald formula, except in individuals with triglycerides >4.5 mmol/l.(1)

Glucose: Glucose was measured in venous plasma by the glucose-oxidase method (Glucoquant/hexokinase/G6P-DH; Boehringer-Mannheim, Mannheim, Germany). HbA1c was assessed using a DCCT standardized reversed-phase cation exchange chromatography (HA8160 analyser, Menarini, Florence, Italy). The intra-assay CV was 0.65% at a mean of 4.89% and the inter-assay CV 1.55% at a mean of 5.52%.(1)

11.8.5 Aarhus Mammography Cohort Study

Present diabetes: The diagnosis is based on a hospitalization discharge (ICD) code, self-reported treatment with anti-diabetic drugs, or both.(1)

Present arterial hypertension: The diagnosis is based on a hospitalization discharge (ICD) code, self-reported treatment with anti-hypertensive drugs, or both.(1)

Active smoker status: Smoking status was self-reported by the participants in a self-administered questionnaire (“Do you smoke?”).(1)

History of cardiovascular disease: Positive history of cardiovascular disease was defined as positive history of myocardial infarction or positive history of stroke. History of stroke and history of myocardial infarction were retrieved from The Danish National Hospital Discharge Register.(1)

History of cancer: History of cancer was retrieved from The Danish National Hospital Discharge Register and the Danish Cancer Register.(1)

Creatinine: Creatinine was measured by standard laboratory methods at the Aarhus University Hospital laboratory.(1)

Intake of calcium by supplements: The use of calcium supplements was self-reported in a self-administered questionnaire (“Do you daily use supplements of calcium? If yes, please state the name of the supplement”).(1)

Intake of vitamin D by supplements: The use of vitamin D supplements, including use of multivitamin pills, was self-reported in a self-administered questionnaire (“*Do you daily use supplements of vitamin D? If yes, please state the name of the supplement*” and “*Do you use multivitamin tablets?*”).(1)

Measurement of serum 25(OH)D: Prior to the mammography, a blood sample from each participant was collected. Samples were divided in aliquots and stored immediately at -80°C until analysis. At the end of the study, plasma 25(OH)D concentrations were analysed by isotope dilution liquid chromatography-tandem mass spectrometry by a method adapted from Maunsell et al.(136) Mean coefficients of variation for 25(OH)D3 were 6.4% and 9.1% at concentrations of 66.5 and 21.1 nmol/l.(1,120)

Body mass index: Height and weight were self-reported by the participants in a self-administered questionnaire, which was filled in prior to the mammography examination.(1)

Parathyroid hormone: Intact parathyroid hormone (PTH) was measured in plasma using a second-generation electro chemiluminescent immunoassay (ECLIA) on an automated instrument (Cobas e601; Roche Diagnostics, GmbH, Mannheim, Germany). According to the manufacturer, the reference interval for PTH concentrations is 1.6-6.9 pmol/l. The lower limit of detection was 0.127 pmol/l, and total imprecision (CV%) was 3.3% at 3.69 pmol/l and 2.7% at 26.6 pmol/l.(1)

11.8.6 German Health Interview and Examination Survey for Adults (DEGS)

Present diabetes: Diabetes mellitus was defined as either a self-reported history of physician-diagnosed diabetes in a standardized physician-administered computer-assisted personal interview (CAPI), or current intake of glucose lowering drugs (Anatomical Therapeutic Chemical [ATC] classification code A10) within the last seven days prior to baseline examination, or a baseline hbA1c value of $\geq 6.5\%$ according to ADA 2010 criteria.(137) Participants were asked “*Have you ever been diagnosed with diabetes by a doctor?*”.(1)

Present arterial hypertension: Present diagnosis of arterial hypertension was defined as either a self-reported history of physician-diagnosed hypertension or office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg. Participants were asked “*Have you ever been diagnosed with high or elevated blood pressure by a doctor?*”.(1)

Physical activity: Participants were asked 1) for how many hours per week they regularly engage in sports (no sports, less than 1 hour, 1-2 hours, 2-4 hours or more than 4 hours per week) and 2) on how many occasions per week and for how many minutes per occasion they engage in sports or other physical activities in a way that they start to sweat or get out of breath.(1)

From their answers on the first question, participants were categorized into <1 hour, 1-2 hours or >2 hours of sport per week. From the answers to the second question, the time spent on moderate intensity physical activity per week was calculated, and participants were categorized into <1 hour, 1-3 hours or >3 hours of moderate intensity physical activity per week. Eventually, level of physical activity per week was classified as low, medium, and high and participants were allocated to these categories based on the higher categories of both classifications derived from the first and second question.(1,138)

Active smoker status: Active smoking status was assessed by self-administered questionnaire. Participants were asked “Did you use to smoke or do you smoke now?”, if “yes, I smoke now” then the participants were also asked “daily or occasionally?”.(1)

History of cardiovascular disease: Positive history of cardiovascular disease was defined as positive lifetime history of physician-diagnosed myocardial infarction or positive lifetime history of physician-diagnosed stroke based on self-reports in the CAPI. Participants were asked „Has a doctor ever diagnosed you as having a myocardial infarction?” and „Has a doctor ever diagnosed you as having a stroke? “. (1)

History of cancer: Lifetime history of physician-diagnosed cancer was assessed by self-reports of participants in the CAPI. Participants were asked „Has a doctor ever diagnosed you as having cancer? “. (1)

Creatinine: Serum creatinine was measured by a kinetic alkaline picrate method on an ARCHITECT ci8200 analyser (Abbott Diagnostics, Wiesbaden, Germany). Maximum intra- and inter-assay CV were 1.27% and 2.02%, respectively.(1)

C-reactive protein: CRP was measured by Immunoturbidimetry on an ARCHITECT ci8200 analyser (Abbott Diagnostics, Wiesbaden, Germany). Maximum intra- and inter-assay CV were 3.95% and 4.5%, respectively.(1)

Measurement of serum 25(OH)D: The participants were asked to fast for at least 3 h, whereupon venous blood samples were drawn for biochemical analyses. Extra serum was aliquoted and stored at -40°C. Serum 25(OH)D concentrations were measured from June to September 2005 in the Epidemiological Research Laboratory of the Robert Koch-Institute, using LIAISON chemiluminescence immunoassay (CLIA; DiaSorin Inc., Stillwater, MN, USA). Inter- and intra-assay coefficients of variation for serum 25(OH)D were 11.7% and 9.9%, respectively. The lower detection limit of the assay was 5 nmol/l.(1,139)

Body mass index: Body weight was measured with a calibrated electronic scale (type: SECA) to the nearest 0.1 kg and body height was measured with a levelling board on the electronic scale to the nearest 0.1 cm. Height and weight were measured by trained personnel while the participants wore

light clothing and no shoes and body mass index was calculated as weight in kg divided by height in meters squared.(1,140)

Blood pressure: Blood pressure was measured by a physician three times after at least three minutes rest, using a mercury sphygmomanometer (Erkameter 3000, Erka, Bad Jözl, Germany). Mean systolic and diastolic blood pressure was calculated from the second and the third measurements.(1,141)

Low-density lipoprotein cholesterol: LDL cholesterol was estimated using the Friedewald equation.(1,142)

Parathyroid hormone: Serum intact parathyroid hormone concentrations (iPTH) were measured from June to September 2005 in the Epidemiological Research Laboratory of the Robert Koch-Institute, using LIAISON chemiluminescence immunoassay (CLIA) (DiaSorin Inc., Stillwater, MN, USA). Inter- and intra-assay coefficients of variation were 7.2% and 3.7%, respectively. The lower detection limit of the assay was 0.106 pmol/l.(1,139)

Glucose: Glucose was measured by the glucose oxidase technique using Mega Merck Kits (Merck, Darmstadt, Germany). Inter-assay coefficients of variation ranged between 1.11% and 2.62%, respectively.(1)

11.8.7 Longitudinal Aging Study Amsterdam (LASA), First Cohort

Present diabetes: Present diabetes mellitus was defined in those participants that were on glucose lowering drugs, and in those with self-reported diabetes mellitus (“*Do you have diabetes?*”).(1)

Present arterial hypertension: Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg and/or use of anti-hypertensive medication. To obtain information on medication use, the participants had to show all the prescribed drugs he/she used at the moment of the medical interview at home. The names, types and doses were noted by the interviewer. All medication was classified according to the internationally accepted ATC-classification. Medications with ATC-code starting with C02, C07, C08 or C09 were classified as anti-hypertensive medication.(1)

Physical activity: Information on physical activity was obtained during each main interview of LASA. The following activities were addressed: walking outdoors, bicycling, light household, heavy household, and two sports activities. In general, the respondents were asked how often and for how long in the previous two weeks they had engaged in each activity. The LASA Physical Activity Questionnaire (LAPAQ) has been validated against seven-day physical activity diaries and seven-day pedometer counts in a subsample of 439 LASA participants. For LASA first and second cohort, a constructed variable was created according to the time per week spent on activities with

MET score higher than three: The first level of physical activity encompassed individuals that spent less than one hours per week on activities with MET score higher than three, individuals within the second group spent one to three hours per week and the third level involved individuals with more than three hours. Patients with activities at MET score below three are automatically assigned to the lowest group of physical activity.(1,143)

Active smoker status: In LASA, both current smoking status (never, former, current smoker) and smoking history (age when started smoking, age when stopped smoking) were assessed at each examination during the medical interview (“*Do you smoke?*”).(1)

History of cardiovascular disease: Positive history of cardiovascular disease was defined as positive history of myocardial infarction or positive history of stroke. History of myocardial infarction and stroke were self-reported. Questions were “Have you had a myocardial infarction?”, “Did you ever have a stroke or a transient ischemic attack?”, “When did this happen?” and a detailed questionnaire on symptoms left after the event.(1)

History of cancer: History of cancer was self-reported. Questions were “*Do you have a tumour or cancer or have you ever had it?*” and “*From which age do you have this disease?*”(1)

Creatinine: Serum creatinine in $\mu\text{mol/l}$ was measured using the Jaffe alkaline picrate reaction with a Hitachi 747 analyser.(1)

C-reactive protein: The serum concentrations of CRP were determined using a sensitive enzyme-linked immunosorbent assay (ELISA) at Sanquin Research, Amsterdam. CRP concentrations were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and a biotinylated monoclonal antibody (mAb) against CRP (CLB anti-CRP-2) as the detecting antibody. CRP was measured in duplicate, and averages were used. The detection limit was 0.8 ng/ml, the inter-assay CV was < 4.2% CRP.(1,144)

Intake of calcium by supplements: Calcium intake by supplements was defined as ATC-code A12AA from prescribed medication.(1)

Measurement of serum 25(OH)D: Serum 25(OH)D measurements took place in 1997/1998 by a radioimmunoassay (Nichols Diagnostics Capistrano, CA, USA). The inter-assay CV was 11 % on average concentrations of 27 and 141 nmol/l. All measurements were performed at the Endocrine Laboratory of the VU University Medical Centre.(1,111)

Body mass index: Body weight was measured without clothes and shoes using a calibrated bathroom balance scale; body height was measured using a stadiometer. BMI was calculated as body weight in kilograms divided by height in meters squared.(1,145)

Blood pressure: Blood pressure (in mmHg) was measured after 5 min of rest at the upper left arm with subjects in a seated position, using an oscillometric blood pressure monitor (Omron Corporation, Tokyo, Japan).(1)

Low-density lipoprotein: HDL cholesterol and triglycerides were determined by an enzymatic colorimetric test (Roche diagnostics, Mannheim, Germany). The inter-assay CV was <2.8% for triglycerides, and <6.4% for HDL cholesterol. All laboratory analyses were performed in EDTA plasma samples stored at -80° C, at the Department of Clinical Chemistry of the VUmc in 2005. LDL-cholesterol was calculated as total cholesterol - HDL-cholesterol - VLDL-cholesterol; VLDL-cholesterol was calculated as 0.456 x total triglyceride concentration expressed in mmol/l (Friedewald). This was done only for triglyceride concentrations of < 5.0 mmol/l.(1)

The concentration of LDL-cholesterol is usually calculated by the formula of Friedewald et al., because isolation of the LDL fraction requires ultracentrifugation, a technique not generally available in service laboratories. The Friedewald formula provides an adequate estimate of LDL-cholesterol for most fasting specimens but is known to be less reliable as triglyceride concentration increases. Therefore, the formula was only used if triglycerides were < 5.0 mmol/l. LDL cholesterol could not be calculated in 9 subjects, because triglyceride concentrations were 5.0 mmol/l in these subjects.(1)

Parathyroid hormone: Parathyroid hormone (pmol/l) was measured by means of immunoradiometric assay (Incstar Corp., Stillwater, MN, USA), with an inter-assay CV of 12%. The analyses were carried out at the Endocrine Laboratory of the VU University Medical Centre.(1,146)

Glucose: The cut-off of 0.247 mmol/l for fructosamine corresponds to the cut-off of 6.1 mmol/l for fasting plasma glucose in terms of sensitivity and specificity in discriminating subjects with glucose intolerance from subjects with normal glucose tolerance.(147) Because the instructions before blood sampling allowed respondents to take tea and dry toast but no dairy products, we could not guarantee fasting blood samples. Fructosamine is little affected by eating, unlike the plasma glucose concentration. Therefore, we used serum fructosamine as a proxy for plasma glucose. Fructosamine was determined by a colorimetric test (Roche Diagnostics, Mannheim, Germany).(1)

11.8.8 Longitudinal Aging Study Amsterdam (LASA), Second Cohort

The methods of LASA, second cohort, were the same as the methods of the first cohort. Differences between cohorts in covariate assessment are given below.(1)

Measurement of serum 25(OH)D: For all serum 25(OH)D measurements, a radioimmunoassay was used (DiaSorin, Stillwater, MN, USA). Blood was collected at the baseline visit, in 2002/2003, and

25(OH)D measurements were performed in 2010/2011. The inter-assay CV was 10 % at average concentrations of 30 and 65 nmol/l. All measurements were performed at the Endocrine Laboratory of the VU University Medical Centre.(1,111)

Parathyroid hormone: Parathyroid hormone (pmol L⁻¹) was measured by means of immunometric assay (Luminescence (Architect, Abbott, Laboratories, Diagnostics Division, Abbott park, Chicago, Illinois USA), with an inter-assay CV of 5%. The analyses were carried out at the Endocrine Laboratory of the VU University Medical Centre.(1)

11.9 Re-Analysis of Bio-Banked Serum/Plasma for Total 25-Hydroxyvitamin D by Liquid Chromatography-Tandem Mass Spectrometry

Serum or plasma samples (referred to as serum hereafter) of every participating institution of WP8 were shipped to University College Cork where the Vitamin D Research Group re-measured total 25(OH)D concentrations by a certified LC-MS/MS method. The Vitamin D Research Group at University College Cork is a participant in the VDSP (97) and is certified by Centres for Disease Control and Prevention's Vitamin D Standardization Certification Program (148). Both the VDSP and the certification program reports total 25(OH)D using the higher order reference laboratories. The quality and accuracy of serum total 25(OH)D analysis by the LC-MS/MS in the laboratory at University College Cork is monitored on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, London, UK).(3,149)

The methods have been described in detail elsewhere.(98,150) In brief, the established LC-MS/MS method measures 25(OH)D₂ and 25(OH)D₃ independently of concentrations of the 3-epimer of 25(OH)D₃ [3-epi-25(OH)D₃], which is not chromatographically resolved from 25(OH)D₃ by most routine LC-MS/MS methods. The presence of 3-epi-25(OH)D₃ can pose problems for LC-MS/MS methods because the precursor ion and fragmentation patterns are the same as 25(OH)D, thus failure to account for these metabolites can result in overestimation of 25(OH)D₃ as the quantitatively more abundant metabolite. (3,149)

The intra-assay CV of the method was <5% for all 25-hydroxyvitamin D metabolites, while the inter-assay CV was <6%. The inter-assay CV for total 25(OH)D was 3.6%.(3,149)

11.9.1 Applying the VDSP Protocol for Standardization of Serum 25(OH)D Data from Past Surveys to The ODIN WP8 Study Populations

The VDSP protocol for standardization of serum 25(OH)D data from past surveys was employed for the current work which generally entails three steps as outlined in detail elsewhere (97,98):

After the development of a master equation to convert values based on the LC-MS/MS measurement procedure to the reference measurement procedures at Ghent University and NIST (Protocol 1), which was already established by certification.(1)

A statistically defined sub-sample of the stored (bio-banked) sera from a study population is re-analysed and an equation to convert all past serum 25(OH)D values to the current measurement procedure (Protocol 2) is developed.(1)

The equation is used to convert the previous serum 25(OH)D values to the Vitamin D Research Group certified procedure.(1)

The statistically defined sub-sample is facilitated through an algorithm was developed within the VDSP, and published.(148)

“The maximum projected sample size of stored serum samples required for this protocol and with this collection of population studies was calculated using procedures for the estimation of the predicted LC-MS/MS-based 25(OH)D value for a given serum 25(OH)D value from the original method of analysis (e.g., immunoassay or LC-MS/MS) with a pre-defined precision of a 95% confidence interval, which have been described elsewhere.(99,148)

Serum samples within each study population separately were selected by first dividing the range of the previous method-based serum 25(OH)D measurements into quartiles, with each quartile being sampled according to a uniform distribution.(148) This method has been shown, via computer simulations, to be statistically more efficient than uniform random sampling in the entire range.(148) The selected serum samples for each population study were retrieved from the respective bio-banks and each set shipped to the Vitamin D Research Group, where they were re-analysed for serum total 25(OH)D using LC-MS/MS (as outlined above). Serum total 25(OH)D concentration within each sample were calculated as the sum of respective 25(OH)D₂ and 25(OH)D₃ concentrations.

The relationship between serum 25(OH)D in the statistical algorithm-defined subset of the sera for each of the WP8 studies separately, as measured by the original method and re-analysed by our traceable LC-MS/MS method, was evaluated using regression analysis, as described elsewhere.(99) Several best fit lines were evaluated for each data set (as defined by R² as well as consideration of the residuals plots), and the resulting regression equation which provided the best fit was applied to the entire data set for that population study, as per the VDSP Protocol 2.”(3,149)

11.10 Statistical Analyses

11.10.1 Rationale for a One-Step Meta-Analysis

The current work displays an IPD meta-analysis which was implemented in a one-step approach. In a one-step approach one statistical model is used, while accounting for the clustering between cohort studies, to estimate an overall effect.(151) Rather than the two-step approach, where aggregated data (AD) in the form of summary statistics is calculated for individual studies first and then effect estimates are combined to a final model, the one-step approach is considered the gold standard of meta-analyses, because the risk for ecological bias is lower than in AD meta-analysis, allows better control of confounding by participant- and study-level covariates, and improves power for detecting interactions and subgroup analyses.(101) Although it is widely believed that in meta-analysis one- and two-step approaches deliver comparable results, for binary outcomes, e.g. time-to-death and time-to-follow-up in survival analysis, the one-step approach produces more reliable results than the two-step method when few studies or few subjects per study are available.(1,152)

11.10.2 Primary Outcome

We studied associations of 25(OH)D with all-cause mortality as the primary outcome.(1)

11.10.3 Parametric Regression Model

In survival analysis, there are various types of regression models, including the Cox proportional hazards model being the most popularly used. The Cox model is semi-parametric because it does not require strong assumptions on the distribution of survival time. The form of the baseline hazard function is therefore not specified, only how the hazard changes (relative hazard) with the inclusion of a covariate vector is of interest. It assumes that the predictors of a given model have a multiplicative (positive or negative) effect on the hazard and that their effect is constant over time. As a result, hazard ratio β , which is interpreted as changing rate of hazard, is calculated. Hazard ratios do not reflect a time unit of the study and may be difficult to interpret intuitively.(153)

Unlike the Cox model, in parametric regression models all parts of the model are specified, both the distribution of survival time and the hazard changing effect of any covariates. Parametric regression models postulate a direct relationship between the predictors and the survival time, resulting in change of survival time and making its clinical interpretation easier.(153) Advantages of parametric models in survival analysis further include employment of accelerated failure time (AFT) models, use of full maximum likelihood to estimate effect estimates more efficiently (with

smaller standard errors) and the possibility to perform more sophisticated analyses, such as including random effects.(154,155)

AFT models provide an alternative to the commonly used proportional hazards models. They can include accelerative or decelerative effects of a covariate on survival time while proportional hazards models assume that the effect of a covariate on the hazard scale is multiplicative.(154)

11.10.3.1 Exploring the Parametric Data Distribution

A first idea of parametric distribution of the ODIN dataset can be obtained by nonparametrically methods including plotting estimated probability density function (pdf) over a range of survival times T .(156)

SAS Code 1: Printing the Probability Density Function (See Appendix, Page 151)

11.10.3.2 Assessing the Suitability of a Parametric Model

Prior to fitting a model based on an assumed parametric form for the hazard function, a preliminary study of the validity of this assumption should be carried out. If the hazard function is reasonably constant over time, this would indicate that the exponential distribution might be a suitable model for the data. On the other hand, if the hazard function increased or decreased monotonically with increasing survival time, a model based on the Weibull distribution would be indicated.(155)

Cumulative hazard plots are used for visually examining distributional model assumptions for reliability data.(157)

11.10.3.2.1 The Hazard Function, $h(t)$, and Cumulative Hazard Function, $H(t)$

The hazard function describes the relative likelihood of the event occurring at time t ($f(t)$), conditional on the subject's survival up to that time t ($S(t)$):

$$h(t) = \frac{f(t)}{S(t)}$$

In the cumulative hazard function is the integral of the hazards function over time. It is therefore calculated by integrating the hazard function over an interval of time.

SAS Code 2: Printing the Hazard Function and Cumulative Hazard Function with Nelson-Aalen Estimator (See Appendix, Page 151)

11.10.3.2.2 Log-Log Transformation

Furthermore, a log-log transformation of the survival function against $\log(t)$ for the ODIN dataset indicates a nearly straight-line relationship and confirms that the Weibull distribution is an appropriate model for the underlying data.(155)

SAS Code 3: Printing A Weibull Probability Plot Using a Log-Log Scale (See Appendix, Page 151)

11.10.3.2.3 Quantile-Quantile Plot

A central Weibull assumption is that treatments have a multiplicative effect on survival time that is consistent over time.(158)

To evaluate the validity of this assumption, a quantile-quantile (Q-Q) plot of empirical against theoretical quantiles of failure time can be plotted to further assess if the Weibull distribution might fit the underlying ODIN data set.(155)

SAS Code 4: Printing A Quantile-Quantile Plot (See Appendix, Page 151)

11.10.3.3 Comparing Parametric Distributions

In practice, the selection of a certain parametric distribution is done by comparing the model fit for a variety of different distributions. Parametric distributions with multiple parameters defining their shape, like the generalized gamma distribution being defined by three parameters (three degrees of freedom), may have a better fit. For the sake of parsimony, a distribution with the fewest parameters among candidate distributions may be chosen that gives the best fit. Comparisons may be done graphically by using cumulative hazard plots or probability plots and by numerical model selection. Model selection indices such as the Akaike information criterion (AIC) and log-likelihood allow for numeric comparison and may be less subjective than comparing graphs.(153,155)

Some commonly assumed parametric distributions in survival models include exponential, Weibull, log-normal, and gamma distributions.(155,159)

SAS Code 5: Printing A Histogram of The Pdf and Incorporating Different Parametric Distributions (See Appendix, Page 151)

SAS Code 6: Estimation and Inference for Weibull, Exponential, Log-Normal, Gamma and Log-Logistic Regression Model (See Appendix, Page 152)

11.10.3.3.1 Likelihood Ratio Test

After attaining model fit parameters, a likelihood ratio test, which is essentially a chi-squared test (χ^2), can be calculated from log-likelihood parameters. The likelihood ratio test is a test used for comparing the sufficiency of a smaller model versus a more complex model. As a result, it displays likelihood ratios and p-values that are used to decide whether to reject the null model in favour of the alternative model. The likelihood ratio is small if the alternative model is a better fit than the null model.(153)

SAS Code 7: Likelihood Ratio Test (See Appendix, Page 153)

11.10.3.4 Parameter Estimation

The Weibull model itself has formerly been used to derive the Framingham coronary heart disease (CHD) risk equation (160) and a coronary heart disease risk score for type II diabetes from Tayside data.(161)

PROC LIFEREG can be used to obtain maximum likelihood estimate of parameters. By default, the LIFEREG procedure computes initial values for the parameters by using ordinary least squares and the log-likelihood function is maximized thereafter by means of a ridge-stabilized Newton-Raphson algorithm. The parameter estimates obtained from PROC LIFEREG can be later used as starting values for the PROC NLMIXED procedure where the final model is employed in.(155)

SAS Code 8: Estimating Weibull Parameters (See Appendix, Page 153)

11.10.3.5 Motivation for Inclusion of Random Effects

IPD meta-analyses combine data from different trials to increase the power of statistical analyses, regardless whether they follow a one-step or two-step approach. Frequently in the analysis of survival data, survival times within the same group are correlated due to unobserved co-variates. Single studies may differ in their design, clinical and patients' characteristics, methods, and setting. As a result, unobserved co-variates may cause the overall effect of interest to vary across studies and result in increased imprecision of overall effect estimates. Clustered data can be handled by a random effects approach, also called the frailty model, where a random covariate is introduced into the model induces dependence among clusters. In SAS, the estimation in frailty model can be carried out in PROC NLMIXED.(1,162)

11.10.3.5.1 Heterogeneity

To assess the size of the random effect and the heterogeneity across studies, we calculated an intra-class correlation coefficient (ICC) and its 95% CI.(151) The ICC was defined as the ratio of between-cluster variance to total variance, while the total variance is the sum of between-cluster and within-cluster variance. The between-cluster variance was estimated by the NL MIXED procedure and the within-cluster variance was set $\pi^2/6$ to take account of the asymmetric Weibull distribution with its long tail to the right.(163) The coefficient can be interpreted as the I² measure of inconsistency proposed by Higgins and colleagues, whereby 25% represents small heterogeneity, 50% represents medium heterogeneity, and 75% represents large heterogeneity.(1,164)

SAS Code 9: Estimating Intra-Class Correlation Coefficient (ICC) Of A Model (See Appendix, Page 153)

11.10.3.6 The Weibull AFT Model

In AFT models with Weibull, exponential, lognormal, or loglogistic distribution, the logarithm of failure time $Y = \log(T)$ is treated as the response variable and includes an error term ϵ that is assumed to follow a particular distribution. In general, the AFT model takes the form of:

$$\text{Prediction} = \text{intercept} + \text{coefficient}(\text{predictor}) + \text{scale (quantile function)} \quad (\text{Equation 1})$$

or,

$$\log(T_i) = \beta_0 + \beta_1 x_{1i} + \dots + \beta_p x_{pi} + \sigma \epsilon_i \quad (\text{Equation 2})$$

where $\log(T_i)$ is the log-transformed failure time for the i th individual, $x_1 \dots x_p$ are explanatory variables with coefficients $\beta_1 \dots \beta_p$, the intercept β_0 is the log-transformed failure time when the transformed accelerating variables and the percentile of the quantile function are 0, σ is the scale parameter, and ϵ_i is the error term, representing residual or unexplained variation in the log-transformed failure times.(155,158)

Under the AFT model parameterization, the distribution chosen for T_i dictates the distribution of the error term ϵ_i . For the Weibull model, the survival time T follows an accelerated failure time distribution and $\log(T)$ has an extreme value distribution.(158,165)

A Weibull regression model can be written in both accelerated and proportional forms, allowing for simultaneous description of treatment effect in terms of HR and relative change in survival time [event time ratio (ETR)].(165)

The Weibull hazard model parameters can be expressed as transforms of the Weibull AFT parameters, and can be parameterized by the shape parameter $\lambda = 1/\sigma$, and the event rate parameter $\alpha_i = e^{-(\beta_0 + \beta_1 x_{1i} + \dots + \beta_p x_{pi})/\sigma}$. Please note, that parameterization notation differs between authors, and the current work adheres to the notation used in the SAS implementation of Liu X.(166)

Then, the Weibull hazard function can be formulated as (166,167):

$$h(t, \boldsymbol{\beta}) = \alpha \lambda (\alpha t)^{\lambda-1} \quad (\text{Equation 3})$$

the corresponding survival function as (166):

$$G(t, \boldsymbol{\beta}) = e^{-(\alpha t)^\lambda} \quad (\text{Equation 4})$$

and the corresponding probability density function as (166,167):

$$g(t, \boldsymbol{\beta}) = \alpha \lambda (\alpha t)^{\lambda-1} e^{-(\alpha t)^\lambda} \quad (\text{Equation 5})$$

Adding random effects ω , the model becomes a frailty model (166-168):

$$g(t, \boldsymbol{\beta}) = \alpha \lambda (\alpha t)^{\lambda-1} e^{-(\alpha t)^\lambda} e^\omega \quad (\text{Equation 6})$$

The value λ determines the shape of the Weibull hazard function. If $\lambda=1$, the Weibull AFT model reduces to the exponential AFT regression model where the hazard is constant over time. When $\lambda > 1$, the hazard rate decreases over time. When $0.5 < \lambda < 1$, the hazard increases at a decreasing rate. When $0 < \lambda < 0.5$, the hazard increases at an increasing rate and when $\lambda = 0.5$, the hazard rate is constantly increasing. (166,167)

If the density of failure, hazard function, and survival distribution function at time t are given as $g(t, \boldsymbol{\beta})$, $h(t, \boldsymbol{\beta})$, and $G(t, \boldsymbol{\beta})$, the log-likelihood can be written as (Equation 7):

$$\begin{aligned} ll(\boldsymbol{\beta}; t) &= \sum_{i \in U_u} \log g(t_i, \boldsymbol{\beta}) + \sum_{i \in U_c} \log G(t_i, \boldsymbol{\beta}) = \\ &= \sum_{i \in U_u} \log h(t_i, \boldsymbol{\beta}) + \sum_{i=1}^n \log G(t_i, \boldsymbol{\beta}) \end{aligned}$$

where U_u is the set of uncensored observations and U_c is the set of censored observations, and n denotes the total sample size. (167)

11.10.3.7 SAS Implementation

For the analysis in ODIN, a Weibull model was implemented in SAS PROC NLMIXED procedure (SAS Institute Inc., 100 SAS Campus Drive, Cary, USA) with random effects to account for clustering across cohort studies. PROC NLMIXED fits nonlinear mixed models by maximizing an approximation to the likelihood integrated over the random effects.(169)

We used PROC LIFEREG in SAS to suggest suitable starting values (parms) of the model's parameters implemented in NLMIXED, but assuming only fixed effects.(170)

Parameter estimation was based on maximum likelihood estimation using a Quasi-Newton optimization and Broyden-Fletcher-Goldfarb-Shanno algorithm.(171) Zero-valued times cannot be evaluated for the log likelihood contribution (168), therefore follow-up-time had to be > 0 .

To account for heterogeneity across study centres, random effects were employed. To numerically integrate over the distribution of random effects, Gauss-Hermite quadrature was used in the likelihood equations and in calculation of conditional intercepts. The inclusion of random effects in the model was preferred regardless of results of formal testing for statistical heterogeneity.(169)

The present analysis was based on individuals with complete data on age, sex, BMI, season of blood sampling, 25(OH)D concentration, vital status at follow-up and follow-up-time (which was at least 1 day). Participants with missing data were excluded from the analysis and we performed no data imputation.(1)

Survival function and probability density function in our code of the NLMIXED model are termed G_t and g . The *PARMS* statement suggested by PROC LIFEREG sets the initial values to the fixed-effect-only estimates $b_0 \dots b_{44}$ and improves convergence over the default starting values in the linear predictor *linp*. The random effect is entered as u at the end of *linp*. Its distribution is specified as a normal distribution on the RANDOM statement which is currently the only distribution available in PROC NLMIXED.(168)

SAS Code 10: Basic NLMIXED Weibull Model Implementation and Categorical Variable Approach and Adjustment for Age, Sex and Month of Blood Sampling (See Appendix, Page 153)

11.10.3.8 25(OH)D Groups

For mortality analyses, 25(OH)D was modelled two ways, using a traditional categorical variable approach with seven groups and a restricted cubic splines approach.(91) In the present work, also fractional polynomials will be used for model 2.

The IPD population was divided into seven ordinal risk groups according to their baseline 25(OH)D measurement. Thresholds of 25(OH)D incorporating both Institute of Medicine (25) and Endocrine Society (23) as well as other published values were defined as follows: Severely vitamin D deficient (<30.00 nmol/l); two groups of patients at risk for inadequacy (from 30 to

39.99 nmol/l and 40 to 49.99 nmol/l); vitamin D sufficient (from 50 to 74.99 nmol/l); two groups of 25(OH)D concentrations which are sufficient although not consistently associated with increased benefit (from 75 to 99.99 nmol/l and 100 to 124.99 nmol/l); and high vitamin D concentrations (≥ 125 nmol/l) with possible reason for concern. To convert nmol/l to ng/ml divide by 2.496).(1,25)

Hazard ratios (HR) can be estimated by including the estimate statement into the model adjusted for the model scale parameter, after the reference group is set 0. The log hazard ratio is then given by $-\beta/\sigma$.(166)

SAS Code 11: Estimating Vitamin D Group 1 as Example Given: (See Appendix, Page 154)

11.10.3.9 Cubic Splines

While grouping of a continuous risk factor and its analysis within a risk-step function is popular and in most cases robust, it is unreasonable to postulate that risk suddenly increases as a category cut point is crossed. From a statistical viewpoint, cut point selection itself may influence results and precision of estimates may be reduced when continuous variables are pooled to groups.(172) When using splines for regression, the range of values of the predictor is subdivided using a set of knots and separate regression curves are fit between the knots.

Cubic splines tend to be poorly behaved at the two tails (before the first knot and after the last knot). To avoid this, restricted cubic splines are used. When using a restricted cubic spline (i.e. natural splines), one obtains a continuous smooth function that is linear before the first knot, a piecewise cubic polynomial between adjacent knots, and linear again after the last knot.(173)

In the present work, the restricted cubic-splines approach was chosen to retain the continuous nature of 25(OH)D values and to calculate HRs with 95% confidence intervals (CI) at the median value of each group. We chose the 25(OH)D group with the lowest mortality risk as the reference.(89,91) The highest category is generally open-ended and too heterogeneous for being used as a reference group.(174)

The number of knots is usually more important than their location. *Stone* (1986) showed that five knots are enough to provide a good fit to any patterns that are likely to arise in practice. According to *Harrell*, the cubic-splines were determined with five knots at the fifth, 27.5th, 50th, 72.5th, and 95th percentile of 25(OH)D.(175)

For the inclusion of restricted cubic splines in regression models, the SAS macro %DASPLINE was carried out provided by *Harrell F.E.* at the Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, USA.(176)

SAS Code 12: Estimating Additional Variables for Restricted Cubic Splines (See Appendix, Page 154)

For n knots, the macro creates set of $(n - 2)$ new dummy variables for 25(OH)D in the dataset (additional variables for *variable* are automatically named *variable1*, *variable2*, *variable3*, etc.) in addition to the original parameter.(176)

The new variables X_i for number n of knots are defined as (Equation 8):

$$X_{i+1} = \frac{(X_1 - t_i)_+^3 - (t_n - t_{n-1})^{-1} \{ (X_1 - t_{n-1})_+^3 (t_n - t_i) - (X_1 - t_n)_+^3 (t_{k-1} - t_i) \}}{(t_n - t_1)^2}, i$$

$$= 1, \dots, n - 2$$

where

$$(u)_+ = \begin{cases} u, & \text{if } u > 0 \\ 0, & \text{if } u \leq 0 \end{cases} \quad \text{(Equation 9)}$$

and where $t_i, i = 1, \dots, n$ are the knot values, $X_i, i = 1, \dots, n - 1$ are the variables to be created, X_1 is the original variable, and u is the result of a term between $(\dots)_+$.

The new set of variables must be included into the linear term in addition to the original variable and including their coefficients in the linear term of the NLMIXED model.

Since the new set of variables is simply a restatement of the predictor, restricted cubic splines can be used in any type of regression (ordinary least squares, logistic, survival).(173)

The knots must be transferred into the final model, as well a given set of 25(OH)D values for HR computation (e.g. at group medians). To compute HRs at the given set of 25(OH)D values, again the estimate statement can be included into the model. The log hazard ratio for a 25(OH)D values of interest is the difference in hazard rates between the 25(OH)D value and a given reference value, adjusted for the model scale parameter $-(\beta_{G1} - \beta_{ref})/\sigma$.

SAS Code 13: Basic NLMIXED Weibull Model Implementation and Restricted Cubic Spline Approach, Adjustment for Age, Sex and Month of Blood Sampling, And Incorporating Random Effects (See Appendix, Page 154)

11.10.3.10 Fractional Polynomials

Besides employing cubic splines to retain the continuous nature of 25(OH)D values, the Multivariable fractional polynomial (MFP) method can be used to preserve continuous nature of a covariate when a non-linear relationship is suspected. For each covariate other than 25(OH)D, we tested non-linear trends and estimated the two trend coefficients (β_{p1} and β_{p2}) of the following second-order fractional polynomial log-linear model: $\log(\text{HR}) = \beta_{p1}p_1 + \beta_{p2}p_2$, where p_1 and p_2 were chosen from a predefined set, $P = (-2, -1, -0.5, 0, 0.5, 1, 2, 3)$. Briefly, fractional polynomials models are especially useful when one wishes to preserve the continuous nature of the covariates in a regression model, but suspects that some or all of the relationships may be non-linear.(172)

Consideration of the family of second-order fractional polynomials specifically is worthwhile,

because the first-order models can model only monotonic curves and because fractional polynomials with an order >2 are rarely required in practice.(177)

In the present work, MFP were only used to validate the use of cubic splines. Displayed will be a comparison results for categorical variable, cubic splines and fractional polynomials approach of regression model 2 of Cork standardized 25OHD concentrations and all-cause-mortality. Further use of fractional polynomials was restricted in the current work as fractional polynomials, like unrestricted cubic splines, tend to behave poorly at both extremes of the curve.

For employment of MFP, the SAS macro %mfp8 was carried out provided by *Meier-Hirmer C.* and *Carina Ortseifen O.* at the Freiburg Centre for Data Analysis and Modelling, University of Freiburg, Germany.(178)

SAS Code 14: Estimating A Fractional Polynomial Term For 25(OH)D (See Appendix, Page 158)

For 25(OH)D, the macro revealed a set of powers of 0, 0.5 for 25(OH)D:

$$FP(0, 0.5) = \beta_1 \ln(X_1) + \beta_2 \sqrt{X_1} \quad (\text{Equation 10})$$

11.10.3.11 Nadir of Cubic Splines

Cubic splines were used to estimate the nadir of the mortality curve, i.e. the concentration of 25(OH)D with the lowest mortality risk. While for the restricted cubic spline function the last two β s are redundant as for $X_1 > t_5$ linearity is forced and the last two β s are just combinations of the other β s, for nadir computation they must be included in the linear term again for unrestricted cubic splines.(91)

β_5 and β_6 are defined as (91):

$$\beta_5 = \frac{1}{(t_5 - t_4)} [\beta_2(t_1 - t_5) + \beta_3(t_2 - t_5) + \beta_4(t_3 - t_5)] \quad (\text{Equation 11})$$

$$\beta_6 = \frac{1}{(t_4 - t_5)} [\beta_2(t_1 - t_4) + \beta_3(t_2 - t_4) + \beta_4(t_3 - t_4)] \quad (\text{Equation 12})$$

The linear term can be rewritten(91) as (Equation 13):

$$\begin{aligned} \text{logit}[25(OH)D] = & \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - t_1)^3 I_{(t_1, \infty)}(X_1) + \beta_3 (X_1 - t_2)^3 I_{(t_2, \infty)}(X_1) \\ & + \beta_4 (X_1 - t_3)^3 I_{(t_3, \infty)}(X_1) + \beta_5 (X_1 - t_4)^3 I_{(t_4, \infty)}(X_1) \\ & + \beta_6 (X_1 - t_5)^3 I_{(t_5, \infty)}(X_1) + \Omega' \mathbf{Z}_i \end{aligned}$$

To obtain the nadir, the first order derivative of the spline function may be taken (91).

$$\begin{aligned} \frac{d\text{logit}}{dx_1} = & \beta_1 + 3\beta_2(X_1 - t_1)^2 I_{(t_1, \infty)}(X_1) + 3\beta_3(X_1 - t_2)^2 I_{(t_2, \infty)}(X_1) \\ & + 3\beta_4(X_1 - t_3)^2 I_{(t_3, \infty)}(X_1) + 3\beta_5(X_1 - t_4)^2 I_{(t_4, \infty)}(X_1) \\ & + 3\beta_6(X_1 - t_5)^2 I_{(t_5, \infty)}(X_1) \end{aligned} \tag{Equation 14}$$

For 5 knots, 2 extrema may be found.

SAS Code 15: Nadir Computation for Cubic Splines (See Appendix, Page 158)

The zero points (extrema) can be obtained by a general quadratic formula (91):

$$\frac{d\text{logit}}{dx_1} = A_j X_1^2 + B_j X_1 + C_j, \quad X_1 \in (t_j, t_{j+1}], j = 0, \dots, 5 \tag{Equation 15}$$

$$A_j = 3 \sum_1^j \beta_{i+1} \tag{Equation 16}$$

$$B_j = -6 \sum_1^j t_i \beta_{i+1} \tag{Equation 17}$$

$$C_j = \beta_1 + 3 \sum_1^j t_i^2 \beta_{i+1} \tag{Equation 18}$$

$$X(\beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6) = \frac{-B_j \pm \sqrt{B_j^2 - 4A_j C_j}}{2A_j} \tag{Equation 19}$$

SAS Code 16: Implementing Quadratic Formula and Estimating Nadir for Cubic Splines (See Appendix, Page 159)

11.10.3.12 *Nadir of Fractional Polynomials*

While FP1 functions are always monotonic, whereas FP2(p_1, p_2) functions could be either monotonic or unimodal (i.e. have only a maximum or a minimum). An FP2 function is monotonic when

$$\text{sign}(\beta_1 \times \beta_2) \text{sign}(p_2) = \text{sign}(p_1), \text{ with } \text{sign}(0) \equiv 1 \quad (\text{Equation 20})$$

and unimodal (that is β_1, β_2 having opposite signs) otherwise. (91)

To obtain the nadir, of the given spline function (Equation 21):

$$\text{logit} = \beta_1 \ln(X_1) + \beta_2 \sqrt{X_1}$$

the first order derivative (Equation 22):

$$\frac{d\text{logit}}{dX_1} = \frac{2\beta_1 - \beta_2 \sqrt{X_1}}{2X_1}$$

is set zero as (Equation 23):

$$X(\beta_1, \beta_2) = \frac{4\beta_1^2}{\beta_2^2}$$

SAS Code 17: Estimating Nadir for Fractional Polynomials (See Appendix, Page 159)

11.10.4 Descriptive Statistics

Continuously distributed data are provided as mean and standard deviation when normally distributed and as median with interquartile ranges (IQR) when skewed. Original and standardized 25(OH)D measurements as well as other covariates at baseline were provided both for each study centre and the whole IPD cohort. Differences between original and standardized 25(OH)D concentrations were assessed by Student's paired t-test. For comparisons of characteristics across vitamin D status groups, we used ANOVA for continuous and χ^2 test for categorical data, as appropriate.(1)

11.10.5 Parametric Model Adjustment

Our outcome analyses were adjusted for risk factors of mortality and determinants of vitamin D status. In model 1 we adjusted for age (in years), sex (male/female), and season of blood collection (Spring, Summer, Autumn, and Winter). In model 2, our main statistical model, we additionally adjusted for BMI (in kg/m²). In model 3, we additionally adjusted for diabetes mellitus (yes/no) and arterial hypertension (yes/no), and in model 4 we added history of cancer (yes/no), history of cardiovascular disease (yes/no) and current smoking status (yes/no) as covariates.(1)

11.10.6 Additional Adjustments

Additional adjustments had model 2 as reference model and included adjustments for supplemental intake of calcium, supplemental intake of vitamin D, physical activity, estimated glomerular filtration rate (eGFR), parathyroid hormone, C-reactive protein, systolic blood pressure, low density lipoprotein cholesterol, and glucose. Additional adjusting covariates were not available in every cohort study, so additional adjustments were only performed in the studies that could provide those covariates (1):

First, supplemental intake of calcium (yes/no) was added to model two. The first additional adjustment analysis was performed in all studies, but DEGS and LASA, second cohort, as no information on supplemental usage was available.(1)

Second, supplemental intake of vitamin D (yes/no) was added to model two in all studies but LASA, first and second cohort, and DEGS, as no information on supplemental usage of vitamin D was available in these studies.(1)

Third, additional adjustment of model two for physical activity (three dummy variables for low, medium and high frequency of physical activity) was processed in all studies but the Aarhus mammography cohort. For sensitivity analysis, we also left out DEGS, as the participants in DEGS revealed a high proportion of younger, physically active individuals.(1)

Fourth, adjustment for eGFR (in mL/min/1.73m²) was added to model two. The eGFR was calculated from creatinine at baseline visit according to the four-variable Modification of Diet in Renal Disease (MDRD) Study equation and was added to model two in all studies but NHS, as up to the current analysis, no creatinine measurements were available in NHS.(1)

In a fifth analysis, adjustment for parathyroid hormone (in pmol/l) was added to model two in all studies but NHS, Aarhus mammography cohort and LASA, second cohort.(1)

In a sixth analysis, adjustment for C-reactive protein (in mg/l) was added to model two in all studies but NHS, Aarhus mammography cohort and LASA, second cohort.(1)

In a seventh analysis, adjustment for systolic blood pressure (in mmHg) was added to model two in all studies but the Aarhus mammography cohort, and DEGS.(1)

In an eighth analysis, adjustment for low density lipoprotein cholesterol (in mmol/l) was added to model two in all studies but the Aarhus mammography cohort, and DEGS.(1)

In a ninth analysis, adjustment for glucose (in mmol/l) was added to model two in all studies but the Tromsø Study, Aarhus mammography cohort, and DEGS.(1)

All models on original 25(OH)D were performed without NHS, as NHS had no original 25(OH)D measurements. For standardized 25(OH)D, we computed all models 1) with data of NHS and 2) without data from NHS to provide comparable results between models of original and standardized 25(OH)D measurements.(1)

11.10.7 Sensitivity Analyses

Pre-specified subgroup analyses were performed to stratify for risk factors for vitamin D deficiency and mortality. Specifically, we stratified for sex (females/males), age groups (<60 yrs.; 60 to <70 yrs.; ≥70 yrs.), BMI groups (<25 kg/m²; 25 to <30 kg/m²; ≥30 kg/m²), calcium supplementation (yes/no), vitamin D supplementation (yes/no), history of CVD (yes/no), and history of cancer (yes/no). Further sensitivity analyses were restricted to general population cohorts (i.e. all cohorts except LURIC) and to individuals that died > 1 year and > 3 years after baseline examination.(1)

11.10.8 Proportional Hazards Assumption and Residuals

For continuous variables, we tested the proportionality of hazards by plotting scaled Schoenfeld residuals as a function of time. For categorical covariates, the proportional hazards assumption was visually tested by plotting $-\log[-\log(S(t))]$ vs. time for strata of each covariate.(156,179)

SAS Code 18: Testing the Proportional Hazards Assumption for Continuous and Categorical Covariates. (See Appendix, Page 159)

To detect model specification errors, we employed cumulative residual plots of Cox-Snell residuals against covariates.(179,180)

SAS Code 19: Printing Plots of Cox-Snell Residuals. (See Appendix, Page 159)

11.10.9 Secondary Outcomes

Secondary outcomes were cardiovascular mortality and cancer mortality, and were available in all cohort studies except NHS. We utilized traditional Cox proportional hazards and the modified risks regression according to the method of *Fine* and *Gray* (181) to account for competing risks. In brief, proportional hazards may not be satisfied in multi-centre settings, so baseline hazards were allowed to vary across single cohort studies.(182) The analysis was carried out in one step and clusters of the Tromsø Study, LURIC, the AGES Reykjavik Study, the Aarhus mammography cohort, DEGS and the first LASA cohort. For NHS, no cause-specific mortality was available at the time-point of the analysis.(1)

The competing risk analyses were performed using R Version 3.1.1 and ‘crrSC’ package, Version 1.1, which is an extension of the ‘cmprsk’ package, Version 2.2-7 (2014-06-17; Bob Gray) to Stratified and Clustered data.(181,183)

11.10.10 Risk of Bias

The current analysis is based on a collaborative meta-analysis of observational studies and selection criteria were based on availability of serum samples and mortality follow-up as well as agreement to participate in the project. The participating institutions were not selected by a systematic literature review or by any outcome. We consider the data at low risk for study selection bias, publication bias and data availability bias as the work includes studies that were selected based on pre-specified criteria that are mainly related to data availability.(184,185) As participants included and those with missing values did not significantly differ in study parameters, we consider the risk for participant selection bias and attrition bias also to be relatively low. Due to

computational issues one participant with follow-up time of zero days had to be excluded from all analyses as the maximum likelihood estimation technically cannot handle zero values. It is unlikely that this exclusion had significant impact on the results.(1)

Performance and detection bias as well as outcome or availability bias should be negligible due to the implementation of random effects, the anonymized nature of the data transfer and the clear endpoint definitions of the work.(1)

11.10.11 Statistical Software

All statistical tests were two sided using an α level of 0.05 if not otherwise specified. All meta-analyses were conducted with SAS Version 9.2 (SAS Institute Inc., 100 SAS Campus Drive, Cary, USA) or R Version 3.1.1 (2014-07-10; Copyright © The R Foundation for Statistical Computing). Data management was performed with SPSS version 20 or higher (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, NY) or MS Excel 2013 (Microsoft. Redmond, Washington, USA).(1)

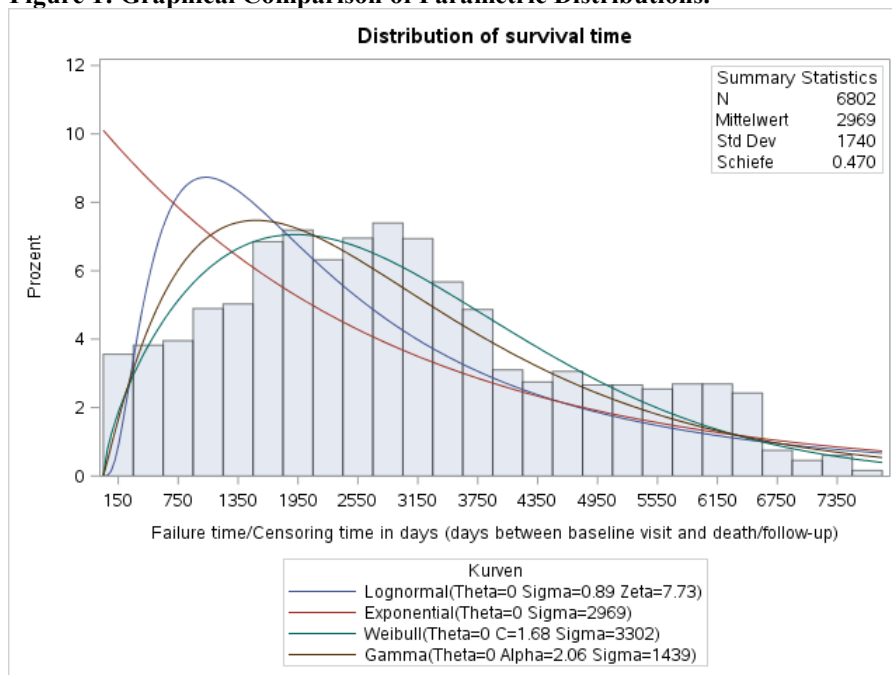
12. Results

In this section, results of parametric model fitting, baseline characteristics of the ODIN database, and results for primary and secondary outcomes are presented.

12.1 Parametric Model Characteristics

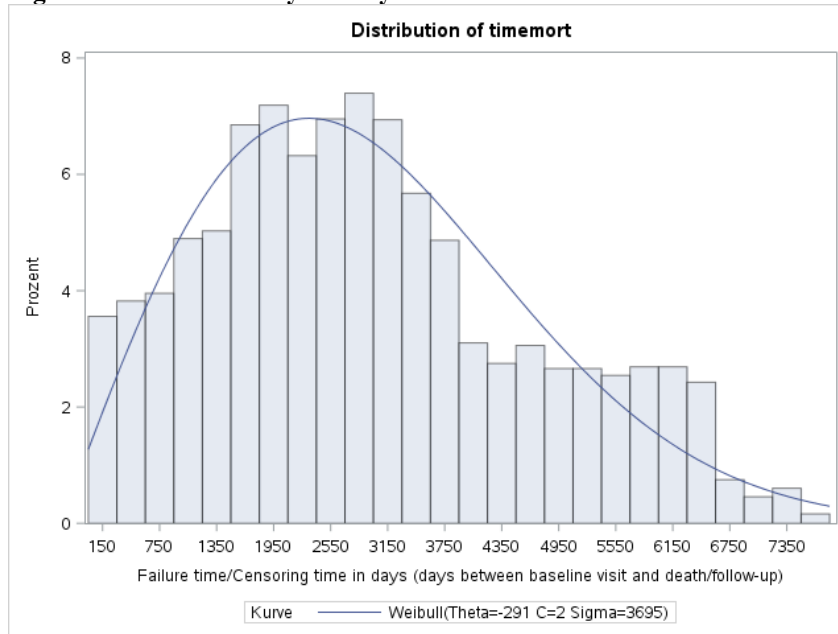
A primary analysis of the distribution of survival data of the ODIN dataset can be obtained by graphical analysis of the probability density function to the underlying data (Figure 1).

Figure 1: Graphical Comparison of Parametric Distributions.



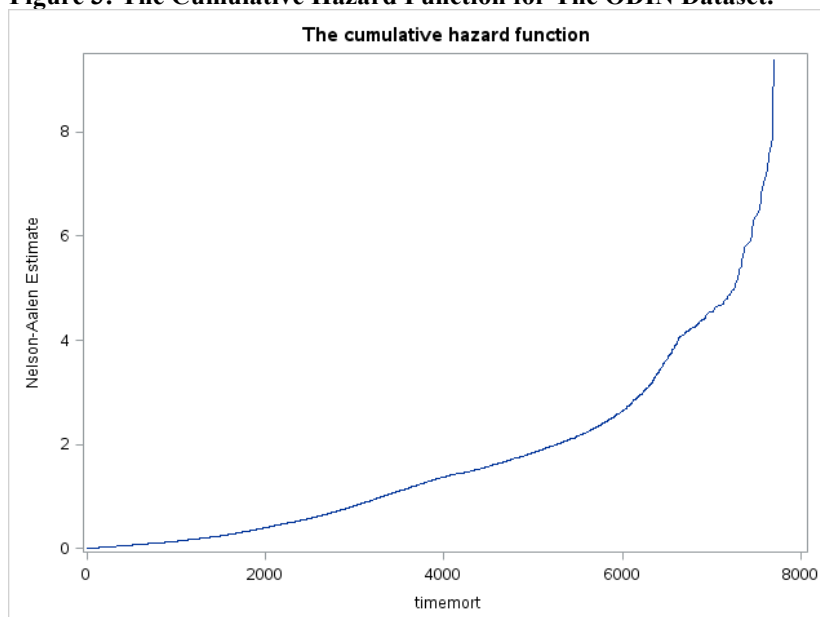
Parametric distributions including exponential, Weibull, log-normal, and gamma distributions applied to the ODIN dataset. The log-logistic distribution is not part of the proc univariate procedure in SAS.

Comparing the exponential, Weibull, log-normal, and gamma distributions graphically, the Weibull distribution seems as a good fit for the underlying ODIN dataset (Figure 2).

Figure 2: The Probability Density Function.

Probability density function for the ODIN dataset

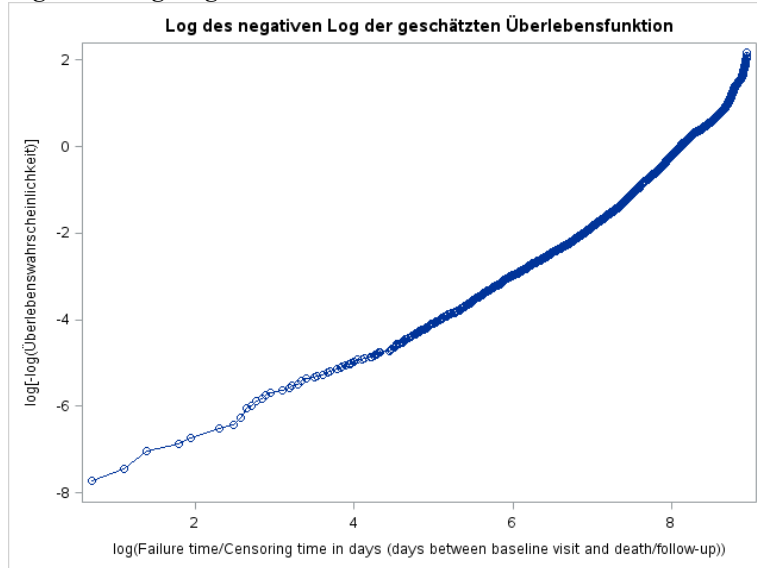
The cumulative hazard function (Figure 3) is not constant increasing over survival time, but increasing at accelerated rate over increasing survival time as failure rate itself increases with time. Thus, a model based on the Weibull rather than the exponential distribution is indicated. In theory, the exponential distribution is a special case of Weibull distributions where the hazard is constant over time.(155)

Figure 3: The Cumulative Hazard Function for The ODIN Dataset.

The cumulative hazard function for the ODIN dataset.

In the log-log plot (Figure 4) of $\log[-\log(\text{survival rate})]$ against $\log(\text{time})$ of a Weibull distribution assembles a nearly straight line.

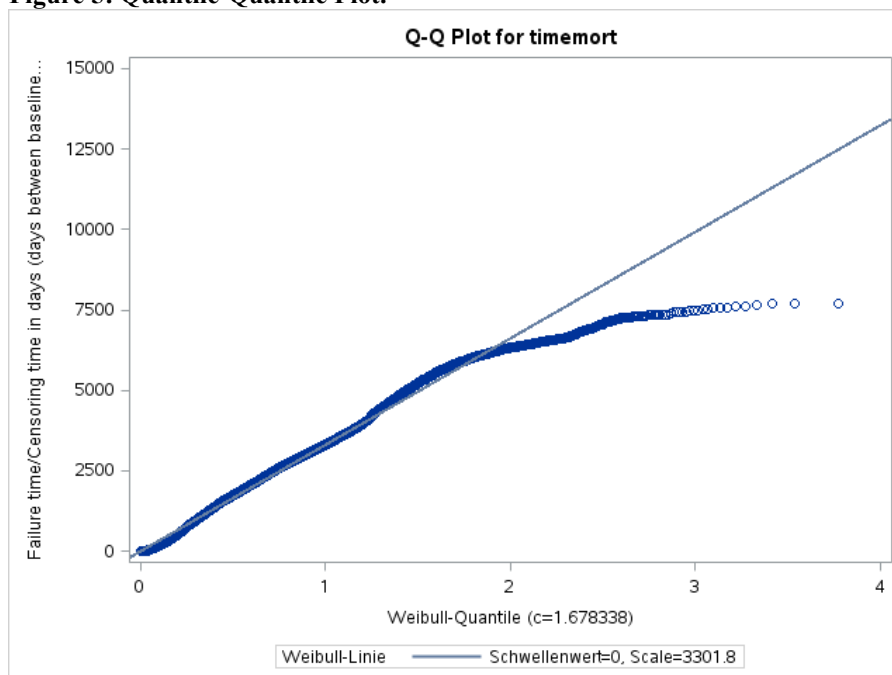
Figure 4: Log-Log Plot.



Log-log transformation of the survival function against $\log(\text{time})$ for the ODIN dataset

The quantile-quantile plot (Figure 5) suggests that a Weibull distribution may fit the ODIN data set well for the first 5000 days (i.e. about 13 years) of follow-up.

Figure 5: Quantile-Quantile Plot.



Q-Q plot of quantiles of failure times against estimates of the quantiles.

In numerical comparison by PROC LIFEREG, between exponential, Weibull, log-logistic, log-normal, and gamma distributions, the Weibull and gamma distributions reveal the smallest information criterion (Table 2).

Table 2: Numerical Comparison of Parametric Distributions.

Distribution	Degrees of freedom	Log-Likelihood	AIC
Exponential	1	-20054.55231	145329.1
Weibull	2	-19638.55188	144499.1
Log-logistic	2	-19701.03791	144624.1
Log-normal	2	-20100.77556	145423.6
Gamma	3	-19602.34903	144428.7

Model fit parameters including log-likelihoods and AICs for different distributions applied to the ODIN dataset.

According to the results of the ODIN dataset, the smallest AIC is calculated for the gamma model which is referred to as the null model. The likelihood ratio test compares the Weibull distribution (2 degrees of freedom) model with the gamma distribution model (3 degrees of freedom).

Table 3: Likelihood Ratio Test Between Weibull And Gamma Distribution.

LRT	Difference in df	p-value
72.4057	1	p<0.001

Likelihood Ratio Test (LRT) compares the Weibull distribution (2 degrees of freedom) model with the gamma distribution model (3 degrees of freedom). df=degrees of freedom.

The likelihood ratio test shows that the model is a significantly better fit for the underlying data than the null model and rejects the null hypothesis. The gamma model may be a better fit according to the AIC but for the sake of parsimony (fewer degrees of freedom) the Weibull model may be chosen.(154)

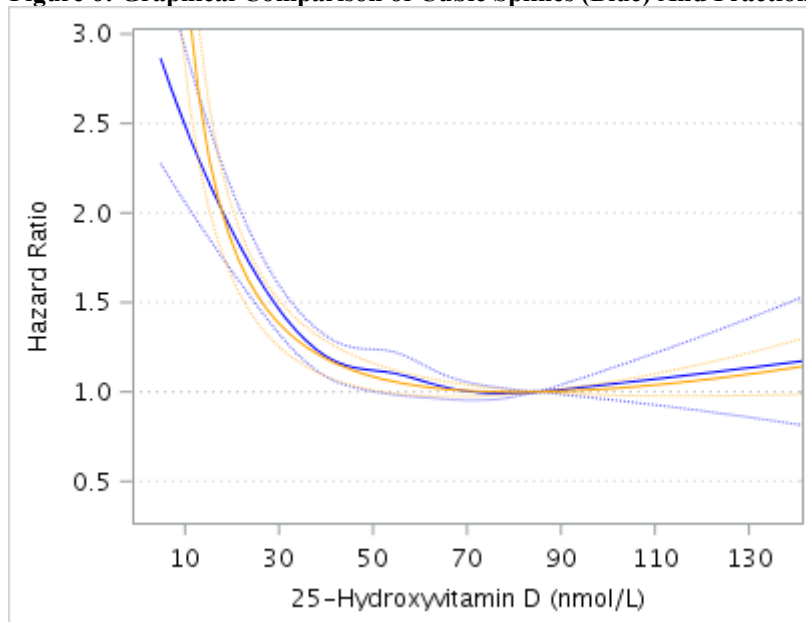
Comparing hazard ratios for mean values of 25(OH)D groups between a categorical, restricted cubic splines and fractional polynomial, the results are similar (Table 4). For the highest and lowest 25(OH)D concentrations, fractional polynomials tend to overestimate hazard ratios (Figure 6).

Table 4: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized 25-Hydroxyvitamin D Concentrations in nmol/l and Statistical Approach for Full Database.

Category	<30	30-39.99	40-49.99	50-74.99	75-99.99	100-124.99	≥125
Mean*, nmol/l	22.0	35.4	45.2	60.9	84.7	108.7	142.8
Sample size, n	2903	3099	5015	11892	3150	684	173
Deaths, n	999	892	1386	2935	522	57	11
Categorical HR (95% CI)	1.67 (1.44-1.89)	1.33 (1.16-1.51)	1.15 (1.00-.29)	1.05 (0.93-1.17)	1.00	1.00 (0.66-1.33)	0.98 (0.27-1.68)
Fractional polynomials HR (95% CI)	1.71 (1.53-1.88)	1.26 (1.15-1.37)	1.13 (1.04-1.21)	1.04 (0.98-1.08)	1.00	1.03 (0.98-1.09)	1.15 (0.99-1.31)
Nadir MFP ^a , nmol/l (95% CI)	80.7 (62.0-99.4)						
Cubic-splines HR (95% CI)	1.80 (1.60-2.00)	1.30 (1.17-1.42)	1.14 (1.03-1.25)	1.09 (0.97-1.15)	1.00	1.07 (0.93-1.20)	1.18 (0.81-1.55)
Nadir CS ^b , nmol/l (95% CI)	78.1 (67.9-88.3)						

Statistical approach was based on a 1) categorical, 2) restricted cubic splines and 3) fractional polynomial model approach. Hazard rates for all approaches were calculated for the (*) mean 25-hydroxyvitamin D value of each category with reference to the nadir of cubic splines. Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI). Models were adjusted for age, sex, season of blood drawing, and body mass index (BMI) at baseline visit. ^aNadir MFP was calculated using fractional polynomials. ^bNadir CS was calculated using cubic splines.

Figure 6: Graphical Comparison of Cubic Splines (Blue) And Fractional Polynomials (Orange).



Mortality rates adjusted for adjusted for age, sex, body mass index and season of blood drawing by standardized 25-hydroxyvitamin D concentrations using a restricted cubic splines (blue) and fractional polynomials approach (orange). Hazard ratios are referring to the 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

12.2 Baseline Characteristics

Baseline characteristics of the entire study population and of all eight individual cohort studies comprising 26 916 participants are shown in Table 5. Table 6 shows study specific details on 25(OH)D assays and data availability. A comparison of original and standardized 25-hydroxyvitamin-D values with mean difference and percentages of participants below 30, 40 and 50 nmol/l 25(OH)D for each cohort study is provided in Table 7. Associations between standardized 25(OH)D concentrations and baseline variables are shown in Table 8. Several risk factors for mortality outcomes such as e.g. BMI, smoking, arterial hypertension, diabetes mellitus, C-reactive protein and low physical activity were associated with low 25(OH)D concentrations.(1)

General mortality follow-up data are shown for the entire study population, as well as for each individual study in Table 9. Characteristics of survivors (n=20 114) compared to deceased study participants (n=6 802) are shown in Supplementary Table 10.(1)

Table 5: Baseline Characteristics of The Entire Study Population and The Individual Cohort Studies.

Characteristic	Unit	Total cohort (N=26 916)	Tromsø (N=7 145)	LURIC (N=3 299)	AGES (N=5 510)	NHS (N=2 591)	Aarhus (N=2 473)	DEGS (N=3 862)	LASA, first cohort (N=1 302)	LASA, second cohort (N=734)
Age	(years)	61.6 (51.9-71.8)	60.1 (53.7-67.2)	64.5 (56.3-70.5)	76 (72-81)	54 (48-59)	50.5 (42.2-58.4)	43 (32-57)	75.1 (69.9-81.1)	60.2 (57.3-62.6)
Sex	(% women)	58	61	30	57	54	100	56	51	54
Season ^a										
Winter	(%)	27	32	23	26	26	26	16	29	51
Spring	(%)	27	35	20	26	15	21	28	22	38
Summer	(%)	16	4	25	15	26	24	20	23	10
Autumn	(%)	30	29	32	33	33	29	36	26	<1
BMI	(kg/m ²)	25.9 (23.4-28.8)	25.5 (23.2-28.3)	27.1 (24.7-29.7)	26.7 (24.1-29.6)	25.6 (23.4-28.3)	23.1 (21.3-26)	25.9 (23.1-29.2)	26.5 (24.1-29.2)	26.8 (24.3-29.6)
Current smoking	(%)	24	33	20	12	21	23	32	18	27
Physical activity ^b	(% missing)	13	2	3	3	19	N.A.	<1	3	1
Low frequency	(%)	59	64	80	65	9	N.A.	55	61	43
Medium frequency	(%)	28	29	12	23	50	N.A.	31	34	47
High frequency	(%)	13	7	8	12	41	N.A.	14	5	10
Present Diabetes ^c	(%)	11	3	32	12	23	2	8	8	7
Glucose	(mmol/l)	5.4 (4.9-5.9)	N.A.	5.1 (4.6-5.9)	5.5 (5.2-6.0)	5.4 (4.5-6.6)	N.A.	5.2 (4.9-5.6)	5.6 (5.0-6.7)	4.8 (4.1-5.5)
Present HTN ^d	(%)	60	56	93	81	40	13	45	78	61

SBP	(mmHg)	138 (125-154)	140 (127-157)	123 (123-157)	140 (128-155)	131 (121-144)	N.A.	131 (129-145)	151 (134-169)	139 (126-154)
LDL-C	(mmol/l)	3.6 (2.9-4.4)	4.4 (3.6-5.2)	3.0 (2.4-3.8)	3.5 (2.8-4.2)	3.3 (2.7-3.9)	N.A.	3.6 (3.0-4.4)	3.6 (3.1-4.3)	3.3 (2.8-4.0)
History of CVD ^c	(%)	14	7	46	19	N.A.	2	3	10	7
History of cancer	(%)	8	5	7	16	N.A.	7	3	12	8
eGFR	(mL/min/1.73m ²)	83 (69-97)	97 (86-109)	81 (70-92)	67 (57-78)	N.A.	88 (78-98)	88 (78-98)	65 (57-73)	68 (61-76)
CRP	(mg/dl)	2.0 (0.9-4.4)	N.A.	2.7 (1.2-7.4)	1.9 (1.0-3.8)	N.A.	N.A.	1.4 (0.6-3.1)	3.2 (1.5-6.5)	N.A.
Calcium Supplements	(%)	7	7	1	17	N.A.	26	N.A.	11	N.A.
Vitamin D Supplements	(%)	21	40	1	79	N.A.	54	N.A.	N.A.	N.A.
PTH	(pmol/l)	3.4 (2.4-4.7)	2.5 (1.9-3.5)	3.1 (2.3-4.1)	4.5 (3.6-5.6)	N.A.	4.1 (3.2-5.3)	3.2 (1.8-4.6)	3.2 (2.5-4.3)	5.6 (4.3-7.0)

Baseline characteristics are presented as median with interquartile range or percentage where appropriate. Abbreviations: Tromsø = 4th Tromsø Study; LURIC = Ludwigshafen Risk and Cardiovascular Health Study; AGES = Age, Gene/Environment Susceptibility Reykjavik Study; NHS = New Hoorn Study; Aarhus = Aarhus Mammography Cohort Study; DEGS = German Health Interview and Examination Survey for Adults; LASA = The Longitudinal Aging Study Amsterdam; BMI = Body mass index; HTN = Arterial hypertension; SBP = Systolic blood pressure; LDL-C = Low density lipoprotein cholesterol; CVD = Cardiovascular disease; eGFR = Estimated glomerular filtration rate according to the four-variable Modification of Diet in Renal Disease (MDRD) formula; CRP = C-reactive protein; PTH = Parathyroid hormone; N.A. = Not available. ^aSeason of baseline blood sampling was defined as spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). ^bPhysical activity was defined as frequency of medium- or vigorous leisure activity and was stratified in low (<1 hour per week), medium (1-3 hours) and high frequency (>3 hours per week). ^cPresent diabetes mellitus at baseline was defined as (listed according to priority - highest priority first): Those participants on glucose lowering drugs, physician-reported, self-reported or according to ADA: fasting glucose \geq 7.0 mmol/l, 2h postload glucose \geq 11.1 mmol/l or HbA1c \geq 6.5% (ICD-9: 250; ICD-10: E10-E14). ^dPresent arterial hypertension at baseline was defined as (listed according to priority - highest priority first): Participants already on antihypertensive drug treatment, physician-reported, self-reported HTN, office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg (ICD-9: 401,405; ICD-10: I10, I15). ^eHistory of CVD at baseline was defined as positive history of myocardial infarction and/or stroke.(1)

Table 6: Description of Cohort Studies Included in Meta-Analyses of The Associations Between Serum 25-Hydroxyvitamin D Concentrations and Mortality Outcomes.

Study name	Place and years of baseline examination	Study sample description	25(OH)D assay	Study population total, N	With measured Total 25(OH)D [†] , N	Included in Analysis [‡] , N	Excluded [‡] , %
Overall	—	—	—	47856	27133	26916	43.8
Tromsø	Tromsø, north Norway, 1994-95 [§]	Population-based	Modular E170 Roche ^a	26956	7160	7145	73.5
LURIC	Ludwigshafen, Germany, 1997-2000	Hospital-based [¶]	RIA Diasorin ^b	3316	3299	3299	0.5
AGES	Reykjavík, Iceland, 2002-06	Population-based	CLIA Diasorin ^c	5764	5519	5510	4.4
NHS	City of Hoorn, the Netherlands, 2006/07	Population-based	LC-MS [#]	2807	2625	2591	7.7
Aarhus	County of Aarhus, Denmark, 2003-07	Population-based	LC-MS ^d	2555	2555	2473	3.2
DEGS^l	Nation-wide, Germany, 1997-99	Population-based	CLIA Diasorin ^c	4030	3917	3862	4.2
LASA, first cohort	Regions Amsterdam, Zwolle and Oss, the Netherlands, 1995/96	Population-based, older cohort	RIA ^e	1509	1320	1302	13.7
LASA, second cohort	Similar to LASA 1, 2002/03	Population-based, younger cohort	CLIA Diasorin ^c	919	738	734	20.1

Characteristics are presented as total number (N) or percentage (%) where appropriate. Abbreviations: Tromsø = Tromsø Study; LURIC = Ludwigshafen Risk and Cardiovascular Health Study; AGES = Age, Gene/Environment Susceptibility Reykjavik Study; NHS = New Hoorn Study; Aarhus = Aarhus Mammography Cohort Study; DEGS = German Health Interview and Examination Survey for Adults; LASA = Longitudinal Aging Study Amsterdam; 25(OH)D=25-hydroxyvitamin D; CLIA = Chemiluminescent Immunoassay; LC-MS = Liquid chromatography-mass spectrometry; RIA = radioimmunoassay; ^aManufacturers of 25(OH)D assays originally used in the single cohort studies: ^aECLIA on a Modular E170 Roche (Roche Diagnostics, Rotkreuz, Switzerland), ^bRIA (DiaSorin Inc, Antony, France, and Stillwater, MN), ^cCLIA Diasorin-Liaison (Diasorin, Stillwater, USA), ^dAarhus hospital in-house isotope dilution liquid chromatography-tandem mass spectrometry (not commercially available); ^eRIA (Nichols Diagnostics Capistrano, CA, USA). [†]Number of participants with available measurements of standardized 25(OH)D. [‡]Inclusion criterion for the present analysis were available participant data for original and standardized 25(OH)D measurements, data on vital status at follow-up and follow-up time/censoring time, data on age, sex, body mass index and season of blood sampling. [§]Baseline visit for the Tromsø Study was the 4th cycle. [¶]Study participants were referred to diagnostic angiography. [#]NHS had no original 25(OH)D and was measured in full by the same LC-MS method used for the standardization of the other studies. ^l For the present analysis, the German National Health Interview and Examination Survey 1998 (GNHIES98) with the integrated German Nutrition Survey 1998 (GeNuS98) was the baseline visit.(1)

Table 7: Comparison of Original and Standardized 25-Hydroxyvitamin D Values, Both in nmol/l.

Study	Original 25(OH)D ^a , nmol/l	Percentage <30nmol/l ^b , %	Percentage <40nmol/l ^b , %	Percentage <50nmol/l ^b , %	Standardized 25(OH)D, nmol/l	Percentage <30nmol/l ^c , %	Percentage <40nmol/l ^c , %	Percentage <50nmol/l ^c , %	Mean difference ±SE ^d , nmol/l
Overall (N=26916)^e	52.5 (37.1-69.2)	13.9	26.6	41.4	53.8 ^f (41.4-66.1)	11.0 ^e	22.3 ^e	42.0 ^e	0.1838 ±0.0542 ^{*,e}
Tromsø (N=7145)	57.0 (44.8-70.4)	4.9	16.5	35.4	53.5 (46.5-61.2)	0.7	8.7	36.7	-4.4704 ±0.1394 [*]
LURIC (N=3299)	39.0 (25.3-57.5)	33.6	51.7	65.9	38.6 (25.0-56.8)	34.0	52.5	66.5	-0.5333 ±0.0070 [*]
AGES (N=5510)	51.8 (35.8-67.6)	17.3	31.3	47.0	59.1 (44.0-68.8)	8.4	19.6	33.6	3.7098 ±0.0998 [*]
NHS (N=2591)	N.A.	N.A.	N.A.	N.A.	59.1 (44.7-73.1)	9.1	18.8	33.5	N.A.
Aarhus (N=2473)	61.9 (45.6-78.3)	8.5	18.2	31.0	58.0 (43.0-72.0)	10.1	21.5	36.7	-4.5252 ±0.0588 [*]
DEGS (N=3862)	46.0 (32.2-71.0)	21.7	40.0	56.1	55.9 (39.0-86.4)	14.3	26.3	42.1	6.8579 ±0.1331 [*]
LASA, first cohort (N=1302)	51.0 (36.2-68.2)	17.5	31.1	48.2	48.5 (35.6-61.9)	17.2	33.0	52.9	-4.1741 ±0.1557 [*]
LASA, second cohort (N=734)	55.0 (42.0-68.1)	7.6	20.7	40.5	54.3 (41.7-66.9)	7.6	21.4	41.1	-1.4601 ±0.0882 [*]

Comparison of original and standardized 25-hydroxyvitamin-D values with mean difference and standard errors (SE) were calculated by Student’s paired t-test. Characteristics are presented as median with interquartile range (IQR), mean difference with SE or percentage (%) where appropriate. Abbreviations: 25(OH)D = 25-hydroxyvitamin D; Tromsø = Tromsø Study; LURIC = Ludwigshafen Risk and Cardiovascular Health Study; AGES = Age, Gene/Environment Susceptibility Reykjavik Study; NHS = New Hoorn Study; Aarhus = Aarhus Mammography Cohort Study; DEGS = German Health Interview and Examination Survey for Adults; LASA = Longitudinal Aging Study Amsterdam; N.A. = Not available; *Statistically significant (P<0.05). ^aOriginal 25(OH)D values were measured by study-specific assays. ^bPercentage of individuals with original 25(OH)D concentration below given threshold. ^cPercentage of individuals with standardized 25(OH)D concentration below given threshold. ^dMean absolute difference was defined as standardized 25(OH)D measurements minus original measurements. ^eThe overall number, percentages and mean difference ±SE consisted of individual participants from Tromsø, LURIC, AGES, Aarhus, LASA, DEGS, first and second cohort, as NHS had no original 25(OH)D measurements. ^fThe overall median 25(OH)D value for all studies (including NHS; n=26 916) was 55.4 nmol/l (41.7-66.8). For all studies, the percentage of individuals with standardized 25(OH)D <30, <40, and <50 nmol/l was 10.96, 22.5, and 41.15 %, respectively. For all studies from the general population (i.e. all studies except LURIC; n=23 617), the percentage of individuals with original measurements of 25(OH)D <30, <40, and <50 nmol/l was 7.7, 18.3, and 37.6%, respectively.(1)

Table 8: Standardized 25-Hydroxyvitamin D Concentrations by Baseline Characteristics of The Study Populations, In nmol/l.

Characteristic	Total cohort	Tromsø	LURIC	AGES	NHS	Aarhus Mammography	DEGS	LASA, first cohort	LASA, second cohort
Age, in years									
<60	55.0 (42.7-69.1)*	54.8 (47.7-62.7)*	42.5 (28.0-60.0)*	N.A.	58.1 (43.5-72.2)*	57.0 (42.0-73.0)*	56.9 (39.5-88.2)*	N.A.	53.9 (42.7-68.0)
60-69.9	53.4 (42.4-65.0)*	52.8 (46.0-60.3)*	41.0 (26.5-58.0)*	58.3 (43.0-67.7)	62.7 (48.3-75.9)*	61.0 (48.0-73.0)*	56.3 (40.8-84.4)*	58.5 (43.1-68.5)*	54.7 (41.1-66.9)
≥70	53.7 (39.0-65.5)*	51.4 (44.7-58.7)*	32.4 (20.9-46.4)*	59.2 (44.0-68.9)	N.A.	59.0 (48.0-66.8)*	45.0 (30.0-67.4)*	45.5 (32.4-58.9)*	N.A.
Sex									
Women	54.4 (42.0-67.0)*	53.5 (46.1-61.2)	32.4 (20.9-52.8)*	57.6 (42.2-67.2)*	60.0 (46.0-75.6)*	58.0 (43.0-72.0)	55.8 (39.4-89.2)*	44.3 (32.3-58.3)*	53.9 (42.3-69.1)
Men	53.9 (41.0-66.6)*	53.5 (47.1-61.1)	41.0 (27.7-58.8)*	61.1 (46.9-71.1)*	57.7 (43.1-70.2)*	N.A.	56.0 (38.5-82.6)*	53.4 (40.0-65.3)*	54.8 (41.4-65.5)
Season^a									
Winter	50.4 (38.9-61.9)*	51.5 (44.3-59.0)*	31.6 (20.9-45.2)*	57.8 (41.7-67.5)*	49.1 (36.5-65.3)*	51.0 (39.0-67.0)*	47.0 (36.6-69.9)*	44.6 (30.1-58.7)*	54.0 (42.4-67.3)*
Spring	53.9 (40.0-65.7)*	56.9 (49.7-66.6)*	28.2 (19.4-40.3)*	60.5 (44.3-71.1)*	67.1 (55.1-81.7)*	53.0 (39.0-68.0)*	46.0 (30.0-71.0)*	43.7 (33.9-59.0)*	53.4 (40.8-65.5)*
Summer	61.2 (47.0-75.6)*	62.3 (54.0-69.9)*	51.1 (35.6-66.2)*	61.2 (47.8-71.0)*	63.5 (49.8-76.3)*	67.5 (55.0-83.0)*	63.1 (43.8-92.2)*	53.9 (40.9-66.2)*	62.0 (48.2-72.7)*
Autumn	54.7 (43.4-67.3)*	51.2 (45.1-58.2)*	44.5 (30.2-60.2)*	58.4 (43.8-67.2)*	50.3 (36.2-62.6)*	60.0 (47.0-74.0)*	47.0 (36.6-69.9)*	53.0 (40.1-62.8)*	46.5 (38.7-52.6)*
BMI^b									
Normal or Underweight	56.9 (44.1-69.5)*	54.5 (47.5-62.8)*	37.0 (23.8-57.0)*	61.4 (45.9-70.5)*	62.8 (48.7-76.7)*	60.0 (46.0-74.0)*	59.9 (40.8-91.8)*	51.2 (36.8-65.4)*	56.1 (42.4-66.9)
Overweight	54.2 (41.8-66.3)*	53.7 (46.8-60.9)*	41.0 (26.8-58.2)*	59.5 (45.7-69.0)*	58.2 (43.4-71.9)*	57.0 (41.0-70.0)*	56.0 (39.0-87.6)*	48.7 (36.6-61.3)*	55.6 (42.9-69.0)
Obesity	48.6 (36.7-61.2)*	49.6 (43.4-57.2)*	36.1 (23.8-52.1)*	53.0 (39.2-64.8)*	51.9 (37.4-64.5)*	47.0 (35.0-61.0)*	49.1 (36.0-69.8)*	43.4 (32.1-55.6)*	50.9 (40.0-64.5)
Smoking									
No current smoking	55.0 (42.1-67.1)*	53.9 (46.8-61.6)*	39.3 (25.8-56.5)	59.7 (45.3-69.1)*	59.9 (46.1-74.0)*	58.0 (45.0-73.0)*	55.8 (39.2-84.5)	48.7 (35.9-62.1)	56.5 (44.0-69.6)*
Current	52.0 (40.4-65.0)*	52.6 (45.6-60.5)*	36.0 (22.8-57.0)	52.9 (36.4-66.0)*	55.2 (40.1-71.3)*	55.0 (38.0-72.0)*	55.8 (38.1-89.6)	45.7 (33.9-61.5)	47.7 (38.1-61.5)*
Characteristic	Total cohort	Tromsø	LURIC	AGES	NHS	Aarhus Mammography	DEGS	LASA, first cohort	LASA, second cohort
Physical activity^c									
Low frequency	51.3 (38.8-63.4)*	52.6 (45.7-60.3)*	35.9 (23.3-53.1)*	58.0 (41.7-67.9)*	56.3 (39.6-69.0)*	N.A.	53.6 (36.6-80.6)*	44.2 (32.2-59.1)*	51.6 (40.7-66.9)

Moderate frequency	57.0 (45.1-68.9)*	55.3 (48.1-62.8)*	47.9 (33.2-63.8)*	61.0 (47.6-70.0)*	59.3 (44.8-74.1)*	N.A.	59.4 (42.1-91.2)*	53.8 (41.2-65.7)*	55.2 (42.4-67.5)
High frequency	60.2 (47.8-73.1)*	55.0 (48.3-63.0)*	57.0 (40.4-74.2)*	62.7 (52.6-70.9)*	62.7 (50.1-75.7)*	N.A.	60.5 (42.0-90.8)*	56.9 (48.1-65.0)*	58.4 (47.6-68.3)
Present diabetes^d									
No	55.0 (42.8-67.2)*	53.6 (46.7-61.4)*	41.3 (27.0-59.5)*	59.5 (44.5-69.0)*	60.4 (46.2-73.8)*	58.0 (44.0-72.3)*	56.6 (39.9-87.8)*	48.9 (36.1-62.3)*	54.9 (42.6-68.3)*
Yes	46.2 (31.2-61.9)*	49.9 (43.4-56.9)*	34.4 (21.7-49.4)*	55.7 (40.3-66.9)*	54.8 (40.4-71.1)*	49.0 (33.0-67.0)*	47.2 (31.3-68.7)*	41.2 (30.5-54.8)*	44.0 (35.0-57.6)*
Glucose, in mmol/l									
<7.0	55.7 (38.6-70.5)*	N.A.	39.5 (26.0-57.7)*	59.3 (44.2-68.9)*	60.5 (46.2-73.9)*	N.A.	56.3 (39.7-87.2)*	51.1 (39.2-67.4)	59.4 (46.1-70.9)
≥7.0	48.2 (32.2-64.5)*	N.A.	33.9 (20.9-48.1)*	57.1 (41.3-67.2)*	53.2 (39.4-69.8)*	N.A.	47.1 (32.4-70.5)*	52.5 (35.8-63.8)	47.0 (40.3-61.5)
Present HTN^e									
No	56.9 (45.0-69.6)*	54.6 (47.5-62.3)*	46.8 (28.3-63.9)*	60.2 (47.1-69.0)*	59.9 (45.8-74.2)*	58.0 (44.0-72.0)	59.0 (41.5-90.9)*	48.5 (36.7-61.9)	56.6 (42.5-70.3)*
Yes	52.3 (39.4-65.0)*	52.7 (45.8-60.4)*	38.1 (24.8-56.0)*	59.0 (43.3-68.7)*	57.5 (43.1-71.8)*	57.0 (43.0-74.0)	52.3 (36.4-78.3)*	48.6 (35.4-62.0)	52.4 (41.5-66.2)*
SBP, in mmHg									
<140	55.2 (42.3-67.9)*	54.5 (47.5-62.2)*	39.5 (25.3-57.5)*	59.3 (44.4-68.9)	59.5 (45.7-74.0)*	N.A.	57.8 (40.5-89.7)*	48.7 (36.3-62.2)	54.3 (42.1-68.2)
≥140	52.3 (40.6-64.4)*	52.5 (45.7-60.1)*	37.8 (24.8-56.0)*	59.0 (43.7-68.6)	57.4 (42.5-71.8)*	N.A.	52.3 (35.6-78.2)*	48.7 (35.6-62.1)	54.5 (41.7-66.7)
LDL, (in mmol/l)									
<2.6	53.6 (37.1-68.7)	54.8 (46.4-63.6)	37.3 (24.6-55.6)	60.5 (46.1-72.1)*	57.0 (43.6-74.1)	N.A.	61.5 (42.4-92.5)*	42.0 (27.9-55.3)*	55.9 (45.4-67.2)
2.6-4.0	53.9 (40.5-67.2)	53.4 (46.2-61.3)	39.5 (25.5-57.0)	59.1 (43.9-68.9)*	59.4 (45.5-72.4)	N.A.	56.9 (39.8-88.4)*	48.2 (35.7-61.7)*	59.9 (45.1-72.4)
≥4.1	54.1 (44.2-64.4)	53.7 (46.9-61.2)	37.8 (24.2-56.7)	58.4 (42.7-67.4)*	59.3 (44.2-73.2)	N.A.	53.9 (37.8-81.6)*	51.3 (38.6-64.5)*	55.8 (46.1-68.4)
History of CVD^f									
No	54.4 (42.5-66.6)*	53.5 (46.6-61.2)	39.5 (26.0-57.0)*	59.3 (44.5-68.7)	N.A.	58.0 (43.0-72.0)*	56.0 (39.2-86.4)	48.6 (36.1-62.3)*	54.5 (41.7-66.9)
Yes	48.6 (32.9-63.2)*	53.2 (46.1-60.3)	37.3 (24.2-56.0)*	58.3 (42.2-69.0)	N.A.	64.0 (42.0-81.5)*	48.1 (34.0-74.7)	44.8 (30.0-58.5)*	52.5 (43.3-68.6)
History of cancer									
No	53.8 (41.6-66.2)*	53.5 (46.6-61.2)*	39.1 (25.3-57.0)	59.4 (44.4-69.1)*	N.A.	58.0 (44.0-73.0)	56.0 (39.0-86.8)	48.7 (36.1-62.3)	54.3 (42.3-67.3)
Yes	53.4 (39.3-64.6)*	52.8 (45.6-60.9)*	35.9 (21.7-52.4)	58.1 (42.4-67.1)*	N.A.	58.0 (41.0-71.0)	53.9 (35.0-82.7)	46.1 (33.9-60.4)	52.2 (38.6-66.7)
eGFR, in mL/min/1.73m²									
<60	53.2 (37.3-66.8)*	55.2 (47.1-63.5)	33.4 (20.4-50.3)*	58.3 (42.2-68.8)*	N.A.	62.0 (45.5-76.0)	50.5 (36.4-73.1)*	47.3 (35.0-60.7)	58.4 (45.3-70.3)*
≥60	53.9 (42.0-66.0)*	53.5 (46.5-61.2)	39.5 (25.8-57.3)*	59.4 (44.8-68.8)*	N.A.	58.0 (43.0-72.0)	56.1 (38.9-87.1)*	49.2 (36.0-62.7)	53.4 (41.3-66.7)*
CRP, in mg/l									

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<2	55.0 (38.3-69.7)*	N.A.	41.0 (27.7-58.5)*	59.6 (45.5-68.8)*	N.A.	N.A.	55.0 (38.7-83.4)*	51.4 (38.8-64.6)*	N.A.
≥2	51.0 (34.4-67.1)*	N.A.	37.1 (23.6-55.0)*	58.6 (42.2-68.8)*	N.A.	N.A.	57.3 (39.7-90.2)*	47.7 (34.9-60.5)*	N.A.
Intake of calcium supplements									
No	52.6 (39.7-64.1)*	53.9 (46.8-61.5)*	38.6 (25.0-56.8)*	58.5 (42.3-68.3)*	N.A.	55.0 (40.0-70.0)*	N.A.	48.1 (34.7-61.3)*	N.A.
Yes	61.4 (50.5-71.0)*	57.3 (48.5-63.9)*	39.7 (30.7-63.3)*	62.5 (51.9-70.7)*	N.A.	65.0 (55.0-77.0)*	N.A.	55.7 (40.4-66.0)*	N.A.
Intake of vitamin D supplements									
No	48.0 (35.3-59.5)*	52.2 (45.3-59.8)*	38.6 (25.0-56.5)*	41.1 (30.9-56.8)	N.A.	52.0 (37.0-68.0)*	N.A.	N.A.	N.A.
Yes	60.1 (49.6-69.5)*	57.3 (49.8-64.7)*	51.6 (34.6-78.1)*	61.6 (49.7-70.6)*	N.A.	61.0 (49.0-74.0)*	N.A.	N.A.	N.A.
PTH, in pmol/l									
<6.8	52.8 (39.8-66.6)*	52.3 (45.4-59.6)*	39.5 (25.8-57.3)*	60.2 (45.2-69.4)*	N.A.	58.0 (45.0-74.0)*	57.6 (40.4-89.4)*	49.6 (36.5-62.5)*	55.9 (43.8-69.6)*
≥6.8	41.0 (27.8-56.7)*	47.3 (40.6-52.5)*	25.3 (18.1-38.0)*	39.9 (29.4-55.9)*	N.A.	47.5 (32.0-62.8)*	37.9 (24.4-58.8)*	35.7 (22.4-46.2)*	48.1 (36.6-62.5)*

Characteristics are presented as median 25-Hydroxy-vitamin D [25(OH)D] values with interquartile range. Abbreviations: Tromsø = Tromsø Study; LURIC = Ludwigshafen Risk and Cardiovascular Health Study; AGES = Age, Gene/Environment Susceptibility Reykjavik Study; NHS = New Hoorn Study; Aarhus = Aarhus Mammography Cohort Study; DEGS = German Health Interview and Examination Survey for Adults; LASA = Longitudinal Aging Study Amsterdam; BMI = Body mass index; HTN = Arterial hypertension; SBP = Systolic blood pressure; LDL = Low density lipoprotein; CVD = Cardiovascular disease; eGFR = Estimated glomerular filtration rate according to the four-variable Modification of Diet in Renal Disease (MDRD) formula; CRP = C-reactive protein; PTH = Parathyroid hormone; N.A. = Not available; *Statistically significant (P<0.05) by analysis of variance. ^aSeason of baseline blood sampling was defined as spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). ^bDefinition of categories of BMI by World Health Organization BMI categories: normal or underweight (<25 kg/m²), overweight (25 to <30 kg/m²); obesity (≥30 kg/m²). ^cPhysical activity was defined as frequency of medium- or vigorous leisure activity and was stratified in low (<1 hour per week), medium (1-3 hours) and high frequency (>3 hours per week). ^dDiabetes mellitus at baseline was defined as (listed according to priority - highest priority first): Those participants on glucose lowering drugs, physician-reported, self-reported or according to ADA: fasting glucose ≥ 7.0 mmol/l, 2h postload glucose ≥ 11.1 mmol/l or HbA1c ≥ 6.5% (ICD-9: 250; ICD-10: E10-E14). ^eArterial hypertension at baseline was defined as (listed according to priority - highest priority first): Participants already on antihypertensive drug treatment, physician-reported, self-reported HTN, office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg (ICD-9: 401,405; ICD-10: I10, I15). ^fHistory of CVD at baseline was defined as positive history of myocardial infarction and/or stroke.(1)

Table 9: Description of Follow-Up Time in Cohort Studies and Mortality Outcomes.

Study Name	End of follow-up, Year	Median follow-up time, years	Participants at risk, N	Total Deaths, N	CVD Deaths, N	Cancer Deaths, N	Other Deaths, N	Unspecified Deaths, N
Overall	-	10.5	26916	6802 [†]	1810	1414	1450	958
Tromsø	2013	17.8	7145	2203	665	661	N.A.	877
LURIC	2010	9.9	3299	985	613	141	210	21
AGES	2013 [†]	8.9	5510	2146 [†]	252	307	487	2
NHS	2014	7.5	2591	69	N.A. [‡]	N.A. [‡]	N.A. [‡]	N.A. [‡]
Aarhus	2014	8.7	2473	78	11	56	10	1
DEGS*	2010	12.1	3862	282	89	85	55	53
LASA, first cohort	2013	14.4	1302	973	175	153	638	6
LASA, second cohort	2013	10.8	734	66	5	11	50	0

Characteristics are presented as median follow-up time and total number (N) of death where appropriate. Abbreviations: Tromsø = Tromsø Study; LURIC = Ludwigshafen Risk and Cardiovascular Health Study; AGES = Age, Gene/Environment Susceptibility Reykjavik Study; NHS = New Hoorn Study; Aarhus = Aarhus Mammography Cohort Study; DEGS = German Health Interview and Examination Survey for Adults; LASA = Longitudinal Aging Study Amsterdam; 25(OH)D=25-hydroxyvitamin D; CVD death = Cardiovascular death; CLIA = Chemiluminescent Immunoassay; LC-MS = Liquid chromatography-mass spectrometry; RIA = radioimmunoassay; N.A. = not available; *For the present analysis, the German National Health Interview and Examination Survey 1998 (GNHIES98) with the integrated German Nutrition Survey 1998 (GeNuS98) was the baseline visit. [†]For AGES, follow-up for death of all causes extends through end of year 2013, but cause-specific death was coded through the year 2009. Due to the difference in follow-up time the number of total deaths and cause-specific deaths differ. [‡]For NHS only data on all-cause mortality was available.(1)

Table 10: Baseline Characteristics of Individuals Alive and Deceased.

Characteristic	Alive (n = 20114)	Deceased (n = 6802)
Age, years	57.9 (48.3-67.4)*	72.6 (66.4-79.0)*
Sex, % women	62*	46*
Season		
Winter, %	26.6*	27.0*
Spring, %	26.5*	14.0*
Summer, %	16.6*	30.3*
Autumn, %	30.3*	30.3*
BMI, kg/m ²	25.8 (23.7-28.7)*	26.2 (23.7-29.1)*
Current smoking, %	24	25
Physical activity		
Low frequency, %	53.7*	73.7*
Medium frequency, %	31.5*	19.5*
High frequency, %	14.8*	6.8*
Present Diabetes, %	9*	17*
Glucose, mmHg	5.4 (4.8-6.0)*	5.5 (5.1-6.2)*
Present HTN, %	53*	80*
SBP, mmHg	137.0 (124.0-151.5)*	146.0 (131.0-164.0)*
LDL, mmol/l	3.6 (2.9-4.4)*	3.7 (2.9-4.5)*
History of CVD, %	10*	24*
History of cancer, %	5*	14*
eGFR, mL/min/1.73m ²	85.9 (72.5-98.9)*	75.7 (61.8-92.2)*
CRP, mg/dl	1.7 (0.8-3.8)*	2.7 (2.2-6.1)*
Calcium Supplements, %	12*	12*
Vitamin D Supplements, %	46*	50*
PTH, pmol/l	3.5 (2.5-4.7)*	3.5 (2.5-4.8)*
Original 25(OH)D, nmol/l	53.6 (38.3-70.5)*	49.7 (33.9-66.0)*
Standardized 25(OH)D, nmol/l	55.4 (42.8-68.1)*	50.9 (38.2-62.7)*

Characteristics are presented as median with interquartile range or percentage where appropriate.

Abbreviations: BMI = Body mass index; HTN = Arterial hypertension; SBP = Systolic blood pressure; LDL = Low density lipoprotein; CVD = Cardiovascular disease; eGFR = Estimated glomerular filtration rate according to the four-variable Modification of Diet in Renal Disease (MDRD) formula; CRP = C-reactive protein; PTH = Parathyroid hormone; 25(OH)D = 25-Hydroxy-vitamin D; N.A. = Not available;

*Statistically significant (P<0.05) by analysis of variance or chi square test. ^aSeason of baseline blood sampling was defined as spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). ^bPhysical activity was defined as frequency of medium- or vigorous leisure activity and was stratified in low (<1 hour per week), medium (1-3 hours) and high frequency (>3 hours per week). ^cDiabetes mellitus at baseline was defined as (listed according to priority - highest priority first): Those participants on glucose lowering drugs, physician-reported, self-reported or according to ADA: fasting glucose \geq 7.0 mmol/l, 2h postload glucose \geq 11.1 mmol/l or HbA1c \geq 6.5% (ICD-9: 250; ICD-10: E10-E14). ^dArterial hypertension at baseline was defined as (listed according to priority - highest priority first): Participants already on antihypertensive drug treatment, physician-reported, self-reported HTN, office systolic and/or diastolic blood pressure of equal to or higher than 140 and/or 90 mmHg (ICD-9: 401,405; ICD-10: I10, I15). History of CVD at baseline was defined as positive history of myocardial infarction and/or stroke.(1)

12.3 25(OH)D And All-Cause Mortality

Results for the IPD meta-analysis on standardized 25(OH)D and total mortality (correspondent model 1 to 4) are presented in Table 11, and the respective cubic splines regression curves are shown in Figure 7 to Figure 10.(1)

Table 11: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized 25-Hydroxyvitamin D Concentrations in nmol/l and Statistical Approach for Full Database.

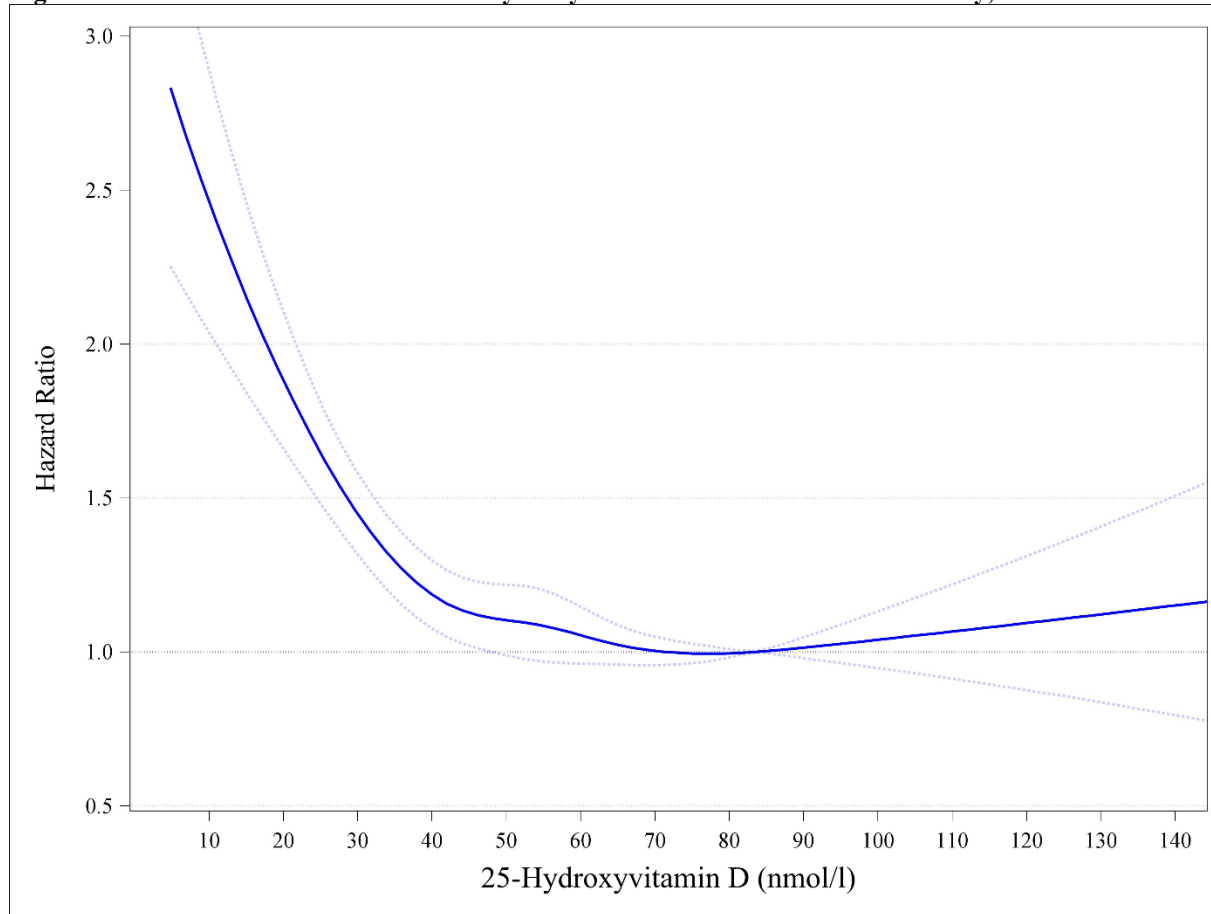
Category	<30	30-39.99	40-49.99	50-74.99	75-99.99	100-124.99	≥125
Median*, nmol/l	23.0	35.9	45.3	60.4	83.6	107.2	135
Sample size, n	2903	3099	5015	11892	3150	684	173
Deaths, n	999	892	1386	2935	522	57	11
Model 1^a							
Categorical HR (95% CI)	1.65 (1.43-1.87)	1.32 (1.14-1.49)	1.13 (0.99-1.28)	1.05 (0.93-1.16)	1.00	1.00 (0.66-1.33)	0.97 (0.27-1.67)
Cubic-splines HR (95% CI)	1.75 (1.56-1.93)	1.28 (1.16-1.39)	1.13 (1.02-1.23)	1.05 (0.96-1.14)	1.00	1.06 (0.92-1.19)	1.14 (0.82-1.46)
Nadir, nmol/l (95% CI)	77.6 (68.2-87.0)						
Model 2^b							
Categorical HR (95% CI)	1.67 (1.44-1.89)	1.33 (1.16-1.51)	1.15 (1.00-1.29)	1.05 (0.93-1.17)	1.00	1.00 (0.66-1.33)	0.98 (0.27-1.68)
Cubic-splines HR (95% CI)	1.76 (1.58-1.95)	1.29 (1.17-1.41)	1.14 (1.03-1.24)	1.06 (0.96-1.15)	1.00	1.06 (0.92-1.19)	1.13 (0.81-1.45)
Nadir, nmol/l (95% CI)	78.1 (67.9-88.3)						
Model 3^c							
Categorical HR (95% CI)	1.61 (1.39-1.83)	1.32 (1.14-1.50)	1.14 (1.00-1.29)	1.06 (0.94-1.18)	1.00	1.03 (0.68-1.37)	0.97 (0.26-1.69)
Cubic-splines HR (95% CI)	1.72 (1.53-1.90)	1.27 (1.15-1.38)	1.12 (1.02-1.23)	1.05 (0.96-1.14)	1.00	1.06 (0.91-1.20)	1.15 (0.77-1.53)
Nadir, nmol/l (95% CI)	77.7 (68.7-86.7)						
Model 4^d							
Categorical HR	1.50	1.24	1.12	1.05	1.00	1.07	0.87

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(95% CI)	(1.28-1.71)	(1.07-1.42)	(0.97-1.27)	(0.92-1.18)	1.00	(0.69-1.45)	(0.21-1.53)
Cubic-splines HR (95% CI)	1.56 (1.38-1.74)	1.19 (1.08-1.31)	1.08 (0.97-1.19)	1.04 (0.95-1.12)	1.00	1.04 (0.89-1.19)	1.10 (0.72-1.49)
Nadir, nmol/l (95% CI)	78.6 (69.3-88.0)						

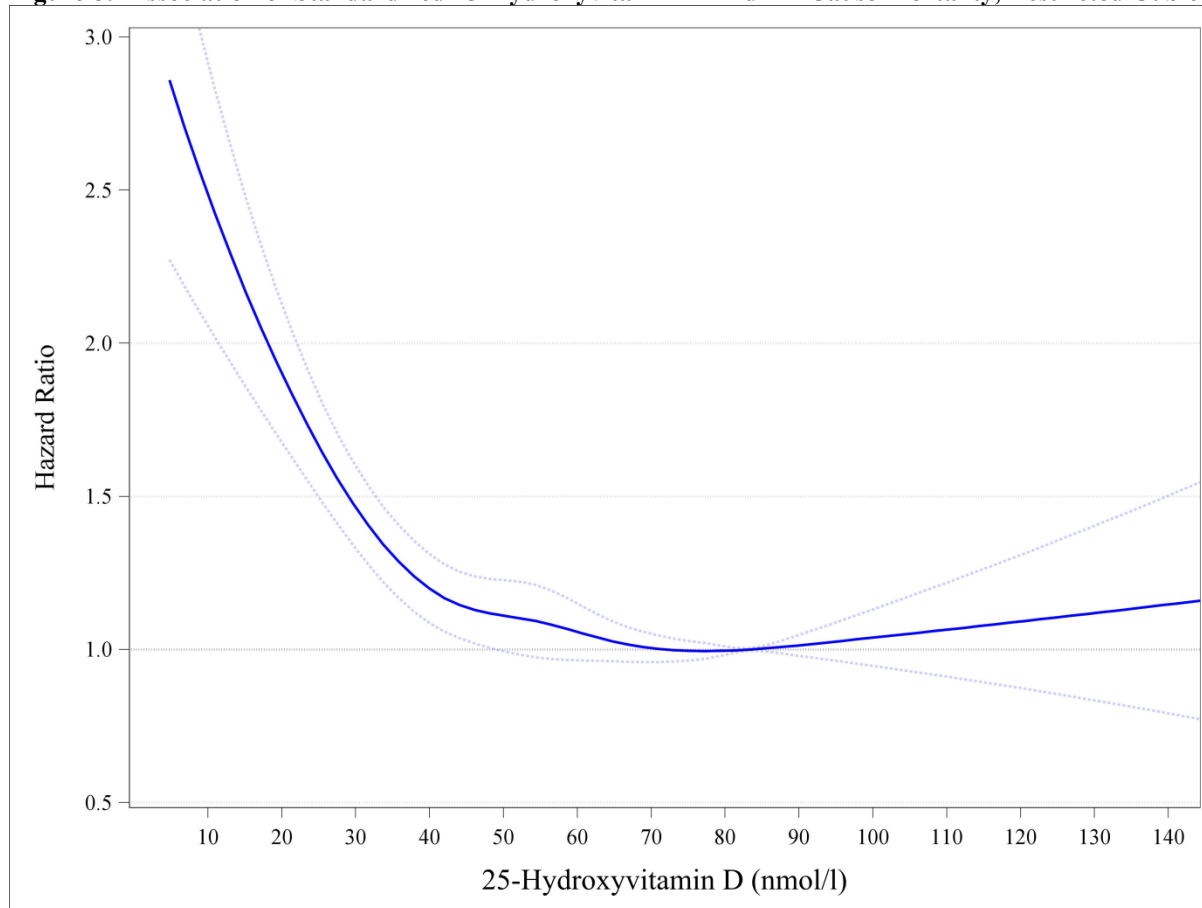
Statistical approach was based on 1) categorical models and 2) cubic splines models. Estimates of the cubic splines approach were calculated for the (*) median 25-hydroxyvitamin D value of each category. Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI). The nadir is the concentration of 25-hydroxyvitamin D with the lowest predicted risk. ^aHazard rates adjusted for age, sex, and season of blood drawing at baseline visit. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI) at baseline visit. ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension at baseline visit. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer at baseline visit. History of CVD was defined as history of myocardial infarction and history of stroke. History of CVD and history of cancer were not available in the New Hoorn Study (NHS) therefore Model 4 was conducted without NHS. One participant was excluded from all analyses due to having a follow-up time of t=0 days.(1)

Figure 7: Association of Standardized 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 1.



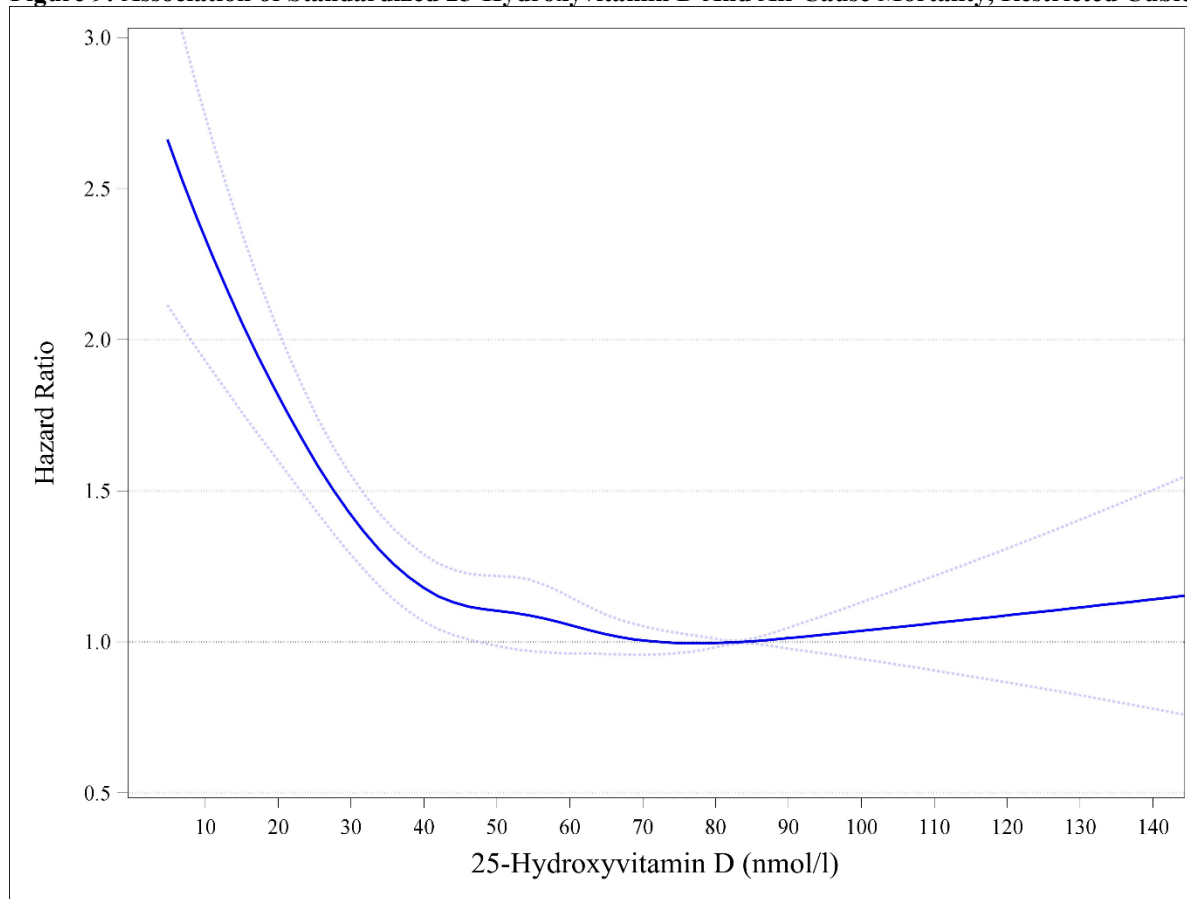
Hazard rate adjusted for age, sex, and season of blood drawing at baseline visit. Hazard ratios [blue line with 95% confidence interval as the dotted blue lines] are referring to the 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

Figure 8: Association of Standardized 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 2.



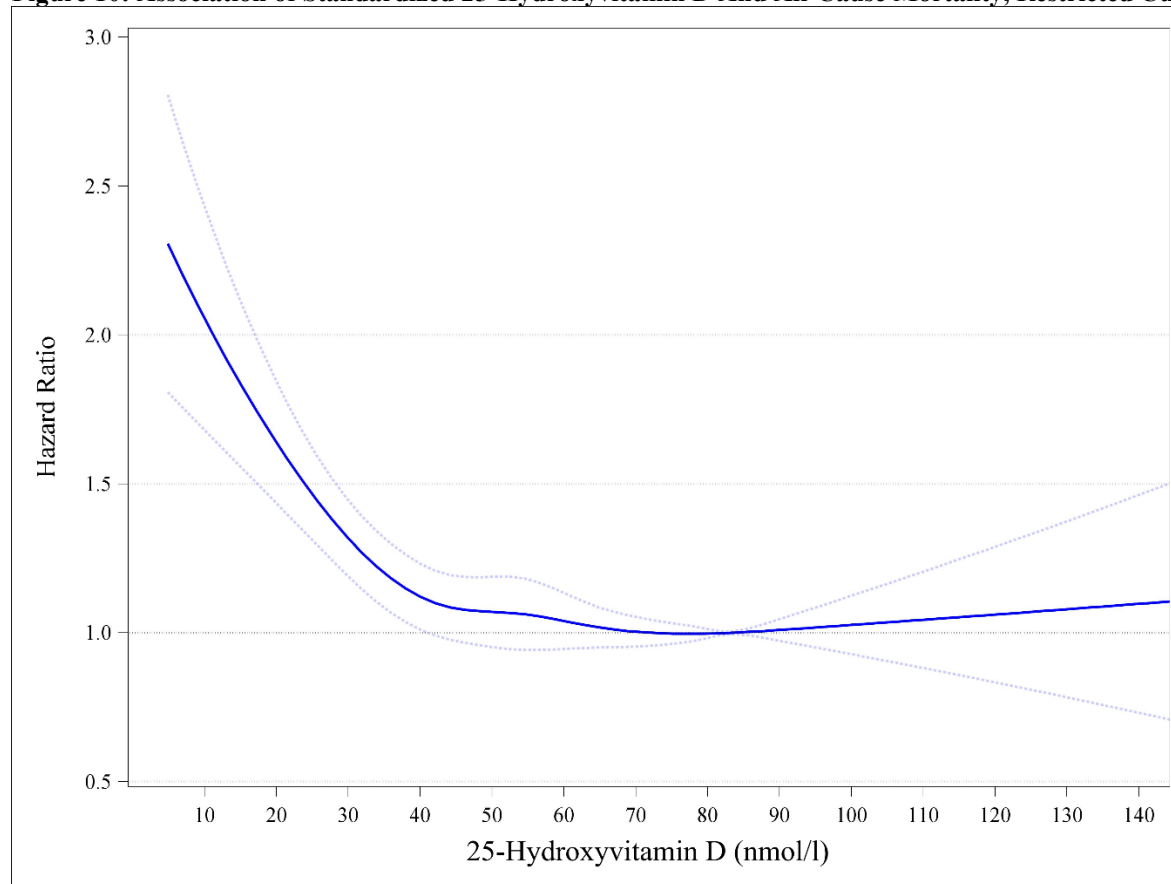
Hazard rate adjusted for adjusted for age, sex, body mass index and season of blood drawing by standardized 25-hydroxyvitamin D concentrations. Hazard ratios [blue line with 95% confidence interval as the dotted blue lines] are referring to the 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Figure 9: Association of Standardized 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 3.



Hazard rate adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension at baseline visit. Hazard ratios [blue line with 95% confidence interval as the dotted blue lines] are referring to the 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

Figure 10: Association of Standardized 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 4.



Hazard rate adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer at baseline visit. History of CVD was defined as history of myocardial infarction and history of stroke. History of CVD and history of cancer were not available in the New Hoorn Study (NHS) therefore Model 4 was conducted without NHS. Hazard ratios [blue line with 95% confidence interval as the dotted blue lines] are referring to the 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

Mortality risk was not significantly different for participants with 25(OH)D concentrations ranging from 50 to 125 nmol/l and higher. Mortality was marginally increased in participants with 25(OH)D concentrations from 40 to 49.99 nmol/l and was significantly increased in the group with 25(OH)D concentrations from 30 to 39.99 nmol/l, and even more pronounced for 25(OH)D concentrations below 30 nmol/l. Results for mortality risk according to standardized 25(OH)D using additional adjustments are shown in Table 12.(1)

Table 12: Additional Adjustments for Hazard Ratios of Death from All Causes (95% CI) By Standardized Total 25-Hydroxy-Vitamin D Concentrations in nmol/l and Statistical Approach.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	100-124.99	≥125
Additional model 1 ^a	Plus adjustment for intake of calcium ^a	1.78 (1.47-2.09)	1.36 (1.13-1.60)	1.19 (0.99-1.39)	1.09 (0.93-1.25)	1.00	1.03 (0.53-1.54)	1.18 (0.00-2.70)
Additional model 2 ^b	Plus adjustment for intake of vitamin D ^b	1.82 (1.42-2.22)	1.37 (1.08-1.67)	1.17 (0.94-1.41)	1.06 (0.87-1.25)	1.00	0.96 (0.38-1.55)	1.02 (0.00-2.58)
Additional model 3 ^c	Plus adjustment for adjustment for PA ^c	1.64 (1.42-1.86)	1.32 (1.14-1.49)	1.12 (0.98-1.26)	1.05 (0.93-1.17)	1.00	1.00 (0.66-1.33)	0.98 (0.27-1.69)
Additional model 4 ^d	Plus adjustment for eGFR ^d	1.70 (1.46-1.94)	1.35 (1.17-1.54)	1.17 (1.01-1.32)	1.06 (0.93-1.18)	1.00	1.03 (0.67-1.40)	1.01 (0.25-1.77)
Additional model 5 ^e	Plus adjustment for PTH ^e	1.75 (1.43-2.08)	1.41 (1.15-1.67)	1.22 (1.00-1.44)	1.14 (0.95-1.33)	1.00	1.04 (0.57-1.52)	1.03 (0.22-1.84)
Additional model 6 ^f	Plus adjustment for CRP ^f	1.67 (1.34-2.00)	1.31 (1.04-1.57)	1.14 (0.91-1.37)	1.06 (0.87-1.25)	1.00	1.00 (0.52-1.48)	0.99 (0.00-2.01)
Additional model 7 ^g	Plus adjustment for SBP ^g	1.66 (1.42-1.89)	1.31 (1.13-1.49)	1.14 (0.99-1.28)	1.05 (0.92-1.17)	1.00	1.00 (0.65-1.35)	0.98 (0.25-1.71)
Additional model 8 ^h	Plus adjustment for LDL-C ^h	1.67 (1.44-1.91)	1.30 (1.12-1.49)	1.14 (1.00-1.29)	1.05 (0.92-1.17)	1.00	0.99 (0.64-1.34)	0.99 (0.25-1.73)
Additional model 9 ⁱ	Plus adjustment for glucose ⁱ	1.73 (1.44-2.02)	1.31 (1.09-1.54)	1.11 (0.92-1.30)	1.08 (0.92-1.24)	1.00	0.98 (0.60-1.37)	1.02 (0.18-1.85)

Statistical approach was based on categorical models. Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: DEGS = German Health Interview and Examination Survey for Adults; NHS = The New Hoorn Study; HR = Hazard ratio with 95% confidence interval (CI). Hazard rates adjustment as:

^aAdditional cumulative adjustment for supplemental intake of calcium (yes/no) after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on supplemental intake of calcium, the analysis was performed without the New Hoorn Study (NHS), and the Longitudinal Aging Study Amsterdam (LASA), second cohort.

^bAdditional cumulative adjustment for supplemental intake of vitamin D (yes/no) after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on supplemental intake of vitamin D, the analysis was performed without NHS, and LASA, first and second cohort.

^cAdditional cumulative adjustment for physical activity (PA), either low frequency (yes/no), medium (yes/no) or high frequency (yes/no), after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on physical activity, the analysis was performed without the Aarhus Mammography Cohort Study.

^dAdditional cumulative adjustment for estimated glomerular filtration rate (eGFR) in mL/min/1.73m² according to the four-variable Modification of Diet in Renal Disease formula, after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on eGFR, the analysis was performed without NHS.

^eAdditional cumulative adjustment for parathyroid hormone (PTH) in pmol/l, after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on PTH, the analysis was performed without NHS.

^fAdditional cumulative adjustment for C-reactive protein (CRP) in mg/l, after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on CRP, the analysis was performed without the New Hoorn Study, Aarhus Mammography Cohort Study and LASA, second cohort.

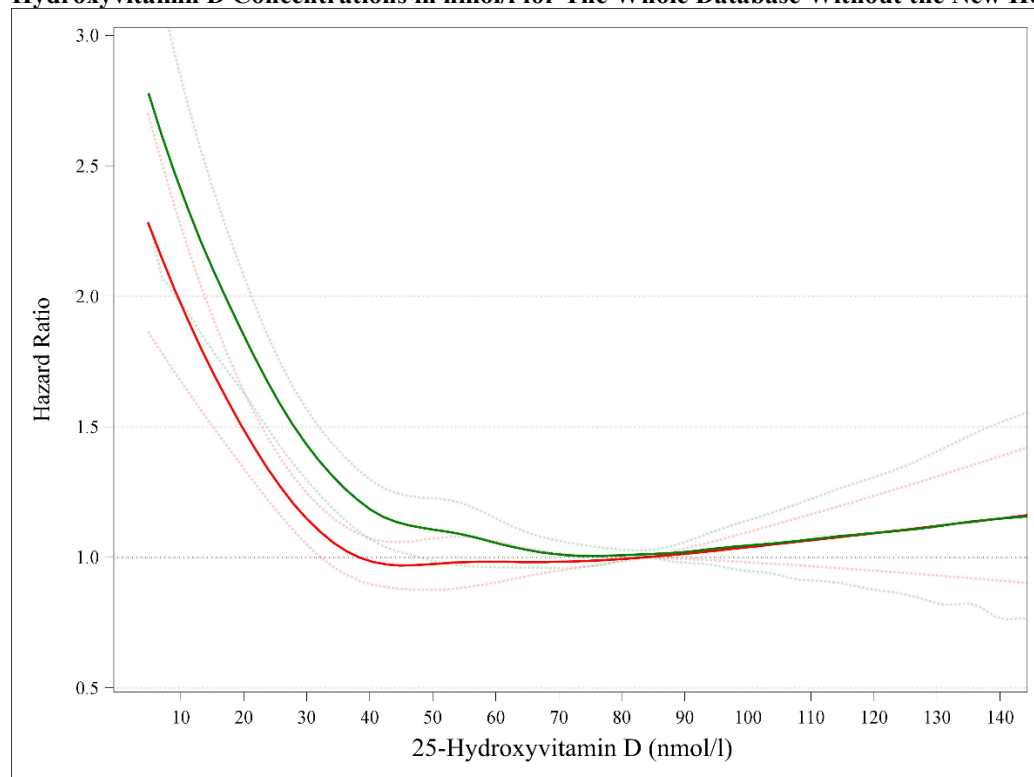
^gAdditional cumulative adjustment for systolic blood pressure (SBP) in mmHg, after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on SBP, the analysis was performed without the Aarhus Mammography Cohort Study.

^hAdditional cumulative adjustment for low-density lipoprotein cholesterol (LDL-C) in mmol/l, after adjustment for age, sex, season of blood drawing and BMI. Due to missing data on LDL-C, the analysis was performed without the Aarhus Mammography Cohort Study.

ⁱAdditional cumulative adjustment for glucose in mmol/l, after adjustment for age, sex, season of blood drawing and BMI.(1)

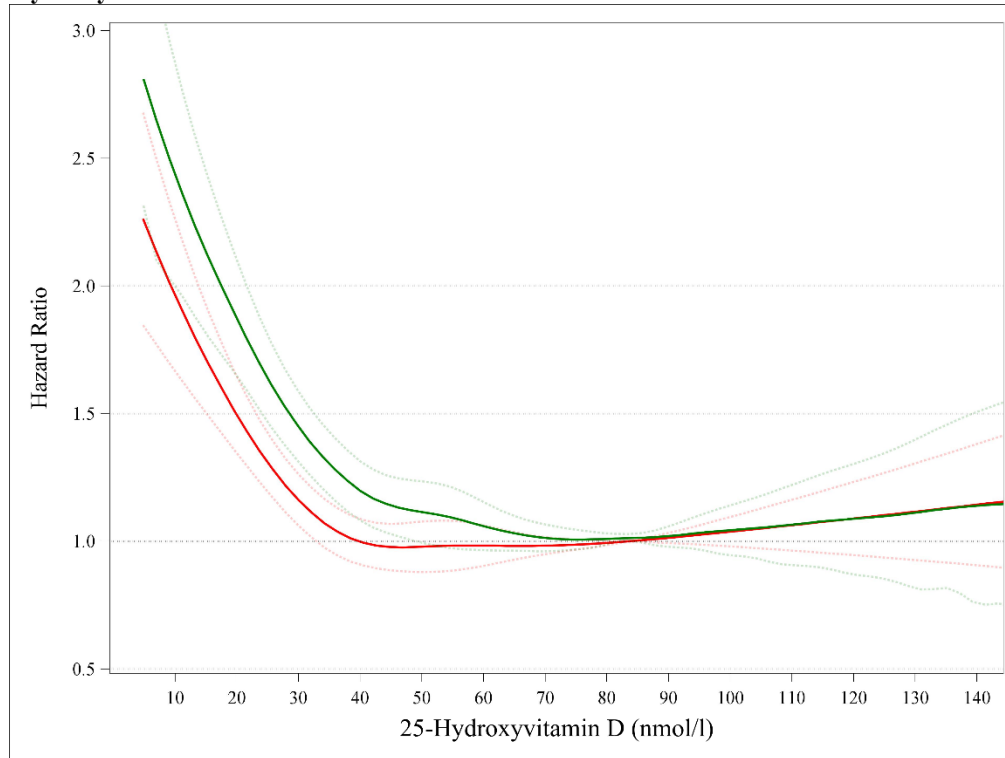
For direct comparison between original and standardized 25(OH)D concentrations in relation to all-cause mortality, we present results for model 1 to 4 in Figure 11 to Figure 14 and in corresponding Table 13 and Table 14 for the seven studies with available standardized and original 25(OH)D concentrations. The New Hoorn Study had no original 25(OH)D concentrations and was left out for direct comparison between original and standardized 25(OH)D concentrations in relation to all-cause mortality.(1)

Figure 11: Association Of 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 1, Comparison of Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Whole Database Without the New Hoorn Study.



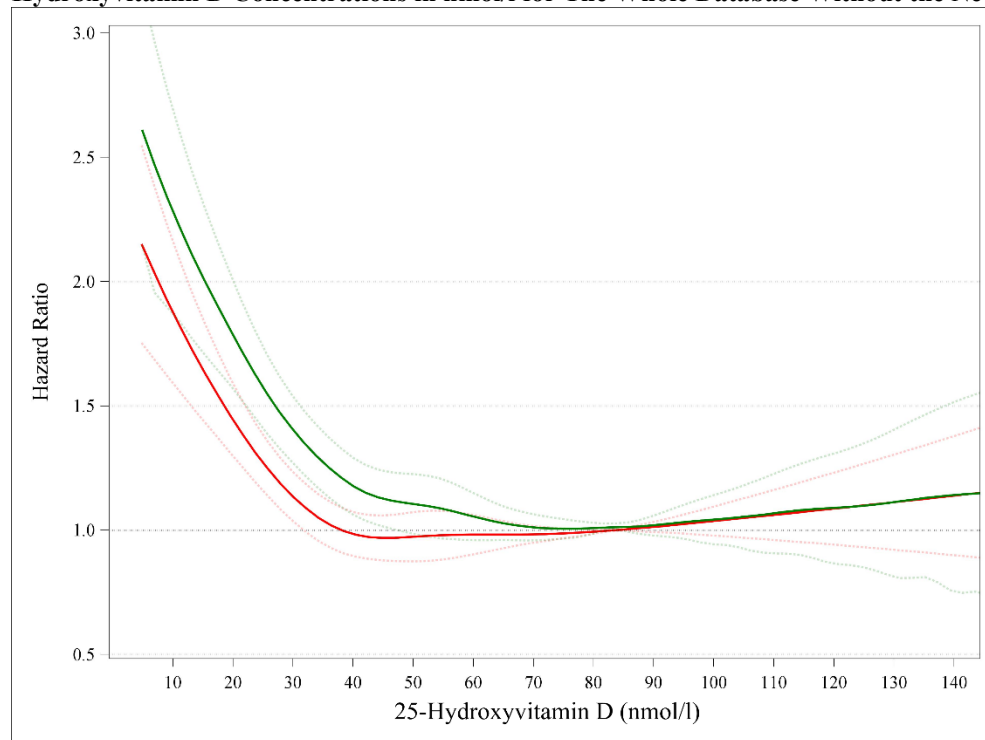
Hazard rates adjusted for age, sex, and season of blood drawing. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (green line with 95% CI as the dotted green lines) excluding the New Hoorn study as the New Hoorn study had no original 25-hydroxyvitamin D values. Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

Figure 12: Association Of 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 1, Comparison of Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Whole Database Without the New Hoorn Study.



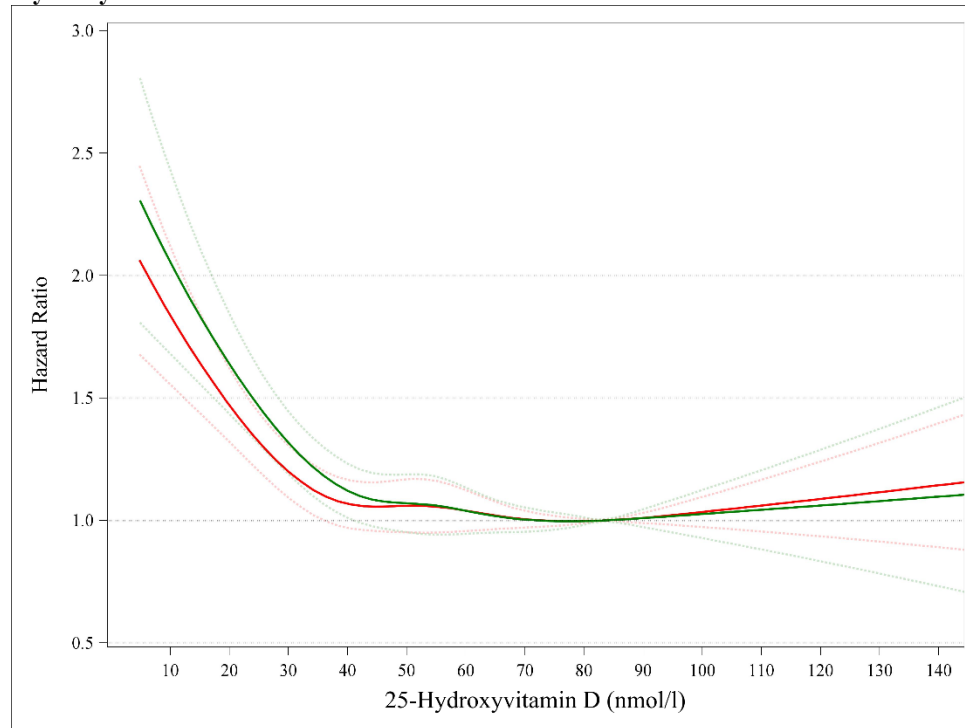
Hazard rates adjusted for age, sex, season of blood drawing, and body mass index. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (green line with 95% CI as the dotted green lines) excluding the New Hoorn study as the New Hoorn study had no original 25-hydroxyvitamin D values. Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Figure 13: Association Of 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 3, Comparison of Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Whole Database Without the New Hoorn Study.



Hazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension at baseline visit. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (green line with 95% CI as the dotted green lines) excluding the New Hoorn study as the New Hoorn study had no original 25-hydroxyvitamin D values. Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

Figure 14: Association Of 25-Hydroxyvitamin D And All-Cause Mortality, Restricted Cubic Splines Model 4, Comparison of Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Whole Database Without the New Hoorn Study.



Hazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer at baseline visit. History of CVD was defined as history of myocardial infarction and history of stroke. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (green line with 95% CI as the dotted green lines) excluding the New Hoorn study as the New Hoorn study had no original 25-hydroxyvitamin D values. Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).

Table 13: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized 25-Hydroxyvitamin D Concentrations in nmol/l and Statistical Approach for Full Database Without the New Hoorn Study.

Category	<30	30-39.99	40-49.99	50-74.99	75-99.99	100-124.99	≥125	
Median*, nmol/l	23.1	35.8	45.3	60.4	82.2	107.5	134.2	
Sample size, n	2359	2631	4410	10923	2372	315	44	
Deaths, n	932	847	1342	2886	470	39	4	
Model 1 ^a	Categorical HR (95% CI)	1.65 (1.42-1.88)	1.33 (1.14-1.51)	1.14 (0.99-1.29)	1.05 (0.92-1.17)	1.00	1.00 (0.65-1.36)	0.98 (0.25-1.72)
	Cubic-splines HR (95% CI)	1.74 (1.56-1.93)	1.28 (1.16-1.40)	1.13 (1.02-1.24)	1.05 (0.96-1.14)	1.00	1.06 (0.92-1.20)	1.13 (0.80-1.46)
	Nadir, nmol/l (95% CI)	78.5 (67.6-89.4)						
Model 2 ^b	Categorical HR (95% CI)	1.67 (1.44-1.91)	1.34 (1.16-1.53)	1.15 (1.00-1.30)	1.05 (0.93-1.18)	1.00	1.01 (0.65-1.36)	0.99 (0.25-1.73)
	Cubic-splines HR (95% CI)	1.76 (1.57-1.96)	1.29 (1.17-1.42)	1.14 (1.03-1.25)	1.05 (0.96-1.15)	1.00	1.05 (0.91-1.19)	1.12 (0.79-1.45)
	Nadir, nmol/l (95% CI)	78.9 (67.0-90.9)						
Model 3 ^c	Categorical HR (95% CI)	1.62 (1.39-1.84)	1.33 (1.14-1.51)	1.15 (1.00-1.30)	1.06 (0.94-1.19)	1.00	1.03 (0.67-1.39)	0.98 (0.23-1.73)
	Cubic-splines HR (95% CI)	1.71 (1.52-1.91)	1.27 (1.15-1.39)	1.13 (1.01-1.24)	1.05 (0.96-1.13)	1.00	1.06 (0.91-1.20)	1.14 (0.75-1.54)
	Nadir, nmol/l (95% CI)	78.7 (67.6-89.8)						
Model 4 ^d	Categorical HR (95% CI)	1.50 (1.28-1.71)	1.24 (1.07-1.42)	1.12 (0.97-1.27)	1.05 (0.92-1.18)	1.00	1.07 (0.69-1.45)	0.87 (0.21-1.53)
	Cubic-splines HR (95% CI)	1.56 (1.38-1.74)	1.19 (1.08-1.31)	1.08 (0.97-1.19)	1.04 (0.95-1.12)	1.00	1.04 (0.89-1.18)	1.10 (0.72-1.49)
	Nadir, nmol/l (95% CI)	78.6 (69.3-88.0)						

Statistical approach was based on 1) categorical models and 2) cubic splines models. Estimates of the cubic splines approach were calculated for the median 25-hydroxyvitamin D value (*) of each category. Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with

95% confidence interval (CI). The nadir is the concentration of 25-hydroxyvitamin D with the lowest predicted risk. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 14: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Original 25-Hydroxyvitamin D Concentrations in nmol/l and Statistical Approach for Full Database Without the New Hoorn Study.

Category	<30	30-39.99	40-49.99	50-74.99	75-99.99	100-124.99	≥125	
Median*, nmol/l	22.2	35.4	45.1	60.9	83.4	108	143	
Sample size, n	3705	3404	4004	8638	3385	870	319	
Deaths, n	1342	985	1067	2325	788	177	49	
Model 1 ^a	Categorical HR (95% CI)	1.45 (1.29-1.61)	1.11 (0.98-1.24)	0.98 (0.87-1.09)	1.01 (0.91-1.11)	1.00	1.12 (0.90-1.34)	1.26 (0.82-1.71)
	Cubic-splines HR (95% CI)	1.40 (1.27-1.53)	1.04 (0.95-1.13)	0.97 (0.88-1.06)	0.98 (0.91-1.06)	1.00	1.06 (0.97-1.15)	1.16 (0.91-1.41)
	Nadir, nmol/l (95% CI)	43.4 (37.2-49.5)						
Model 2 ^b	Categorical HR (95% CI)	1.46 (1.30-1.63)	1.12 (0.99-1.25)	0.99 (0.88-1.10)	1.01 (0.92-1.11)	1.00	1.12 (0.90-1.34)	1.26 (0.82-1.70)
	Cubic-splines HR (95% CI)	1.41 (1.28-1.54)	1.05 (0.96-1.15)	0.98 (0.89-1.07)	0.98 (0.91-1.06)	1.00	1.06 (0.97-1.15)	1.15 (0.90-1.40)
	Nadir, nmol/l (95% CI)	44.1 (35.8-52.4)						
Model 3 ^c	Categorical HR (95% CI)	1.41 (1.25-1.57)	1.10 (0.97-1.23)	0.97 (0.86-1.08)	1.01 (0.91-1.11)	1.00	1.10 (0.88-1.32)	1.28 (0.83-1.74)
	Cubic-splines HR (95% CI)	1.36 (1.24-1.49)	1.03 (0.94-1.13)	0.97 (0.88-1.06)	0.98 (0.91-1.06)	1.00	1.06 (0.96-1.15)	1.15 (0.89-1.40)
	Nadir, nmol/l (95% CI)	43.6 (36.4-50.7)						
Model 4 ^d	Categorical HR (95% CI)	1.47 (1.29-1.64)	1.20 (1.06-1.35)	1.08 (0.95-1.20)	1.08 (0.97-1.19)	1.00	1.10 (0.87-1.33)	1.31 (0.83-1.80)
	Cubic-splines HR (95% CI)	1.40 (1.27-1.53)	1.11 (1.01-1.21)	1.06 (0.96-1.16)	1.04 (0.96-1.12)	1.00	1.06 (0.96-1.15)	1.15 (0.88-1.42)
	Nadir, nmol/l	83.2						

(95% CI)

(75.9-90.5)

Statistical approach was based on 1) categorical models and 2) cubic splines models. Estimates of the cubic splines approach were calculated for the median 25-hydroxyvitamin D value (*) of each category. Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI). The nadir is the concentration of 25-hydroxyvitamin D with the lowest predicted risk. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

For each individual study cohort, we present all-cause mortality data according to standardized and original 25(OH)D concentrations in Table 15 to Table 22 and in Figure 15 to Figure 19.(1)

Table 15: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Tromsø Study.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		27.8	37.2	45.8	58.5	79.7	112.0
Sample size, n		49	572	2019	4173	317	15
Deaths, n		19	211	700	1193	77	3
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	1.24 (0.60-1.88)	1.19 (0.88-1.50)	0.98 (0.75-1.21)	0.89 (0.68-1.09)	1.00	1.07 (0.00-2.29)
Model 2 ^b	Categorical HR (95% CI)	1.26 (0.61-1.91)	1.21 (0.89-1.53)	0.99 (0.76-1.22)	0.89 (0.69-1.09)	1.00	1.08 (0.00-2.30)
Model 3 ^c	Categorical HR (95% CI)	1.20 (0.58-1.82)	1.23 (0.90-1.56)	1.02 (0.78-1.27)	0.93 (0.72-1.15)	1.00	1.12 (0.00-2.37)
Model 4 ^d	Categorical HR (95% CI)	1.12 (0.54-1.70)	1.11 (0.82-1.41)	0.96 (0.73-1.19)	0.89 (0.69-1.10)	1.00	1.14 (0.00-2.48)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	0.96 (0.76-1.16)	0.86 (0.72-1.00)	0.73 (0.62-0.84)	0.85 (0.74-0.95)	1.00	1.28 (0.96-1.61)
Model 2 ^b	Categorical HR (95% CI)	0.97 (0.77-1.18)	0.87 (0.72-1.01)	0.73 (0.62-0.85)	0.85 (0.74-0.95)	1.00	1.28 (0.95-1.61)
Model 3 ^c	Categorical HR (95% CI)	0.91 (0.72-1.11)	0.85 (0.71-0.99)	0.71 (0.60-0.82)	0.84 (0.73-0.95)	1.00	1.26 (0.93-1.58)
Model 4 ^d	Categorical HR (95% CI)	1.44 (1.12-1.76)	1.35 (1.11-1.59)	1.07 (0.90-1.25)	1.05 (0.92-1.19)	1.00	1.12 (0.83-1.41)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates

adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 16: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Ludwigshafen Risk and Cardiovascular Health Study.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		20.9	35.1	45	59.5	84.3	108.4
Sample size, n		1123	609	463	791	253	60
Deaths, n		477	173	116	175	37	7
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	2.92 (1.98-3.85)	1.73 (1.14-2.31)	1.46 (0.95-1.97)	1.27 (0.85-1.70)	1.00	0.63 (0.15-1.11)
Model 2 ^b	Categorical HR (95% CI)	2.94 (1.99-3.89)	1.74 (1.15-2.34)	1.48 (0.96-2.00)	1.28 (0.85-1.71)	1.00	0.67 (0.18-1.17)
Model 3 ^c	Categorical HR (95% CI)	2.69 (1.82-3.56)	1.61 (1.06-2.15)	1.37 (0.89-1.85)	1.25 (0.83-1.67)	1.00	0.70 (0.18-1.22)
Model 4 ^d	Categorical HR (95% CI)	2.63 (1.76-3.48)	1.57 (1.04-2.10)	1.41 (0.91-1.90)	1.25 (0.83-1.68)	1.00	0.71 (0.19-1.24)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	2.92 (1.98-3.85)	1.73 (1.14-2.31)	1.46 (0.95-1.97)	1.27 (0.85-1.70)	1.00	0.63 (0.15-1.11)
Model 2 ^b	Categorical HR (95% CI)	2.94 (1.99-3.89)	1.74 (1.15-2.33)	1.47 (0.96-1.99)	1.28 (0.85-1.70)	1.00	0.63 (0.16-1.11)
Model 3 ^c	Categorical HR (95% CI)	2.70 (1.83-3.58)	1.62 (1.07-2.16)	1.38 (0.89-1.86)	1.25 (0.83-1.67)	1.00	0.67 (0.16-1.78)
Model 4 ^d	Categorical HR (95% CI)	2.62 (1.77-3.46)	1.57 (1.04-2.10)	1.40 (0.91-1.90)	1.25 (0.83-1.66)	1.00	0.67 (0.16-1.17)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI,

active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 17: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Age, Gene/Environment Susceptibility Reykjavik Study.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		24.9	35.5	45	62.7	81.3	110.1
Sample size, n		460	622	767	2881	722	58
Deaths, n		218	266	289	1075	276	22
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	1.48 (1.21-1.74)	1.32 (1.10-1.55)	1.05 (0.88-1.23)	1.04 (0.90-1.18)	1.00	1.29 (0.73-1.85)
Model 2 ^b	Categorical HR (95% CI)	1.51 (1.28-1.75)	1.22 (1.02-1.43)	1.09 (0.91-1.26)	1.11 (0.96-1.27)	1.00	1.35 (1.02-1.68)
Model 3 ^c	Categorical HR (95% CI)	1.49 (1.26-1.72)	1.22 (1.01-1.42)	1.09 (0.91-1.27)	1.12 (0.96-1.27)	1.00	1.33 (1.00-1.67)
Model 4 ^d	Categorical HR (95% CI)	1.38 (1.16-1.60)	1.18 (0.98-1.39)	1.04 (0.87-1.22)	1.13 (0.97-1.29)	1.00	1.42 (1.07-1.78)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	1.50 (1.27-1.73)	1.21 (1.01-1.41)	1.08 (0.90-1.25)	1.11 (0.96-1.27)	1.00	1.37 (1.03-1.70)
Model 2 ^b	Categorical HR (95% CI)	1.53 (1.29-1.77)	1.23 (1.03-1.44)	1.09 (0.91-1.27)	1.12 (0.96-1.28)	1.00	1.36 (1.03-1.70)
Model 3 ^c	Categorical HR (95% CI)	1.53 (1.29-1.76)	1.22 (1.02-1.43)	1.10 (0.92-1.28)	1.12 (0.97-1.28)	1.00	1.36 (1.02-1.69)
Model 4 ^d	Categorical HR (95% CI)	1.39 (1.17-1.61)	1.18 (0.98-1.38)	1.05 (0.87-1.23)	1.13 (0.97-1.29)	1.00	1.40 (1.05-1.76)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for

age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 18: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Newly Measured 25-Hydroxyvitamin D Concentrations in nmol/l for The New Hoorn Study.

Category		<30	30-39•99	40-49•99	50-74•99	75-99•99	≥100
Median*, nmol/l		24.8	35.5	45.4	62.2	83.1	108.4
Sample size, n		235	253	380	1148	477	98
Deaths, n		9	5	9	30	14	2
Model 1 ^a	Categorical HR (95% CI)	1.85 (0.20-3.49)	0.88 (0.00-1.81)	0.91 (0.13-1.69)	0.97 (0.35-1.59)	1.00	0.76 (0.00-1.88)
Model 2 ^b	Categorical HR (95% CI)	1.44 (0.00-4.34)	0.74 (0.69-0.79)	0.76 (0.72-0.81)	0.89 (0.87-0.92)	1.00	0.77 (0.73-0.82)
Model 3 ^c	Categorical HR (95% CI)	1.41 (0.00-4.21)	0.73 (0.68-0.78)	0.74 (0.69-0.79)	0.90 (0.88-0.92)	1.00	0.78 (0.74-0.83)
Model 4 ^d	Categorical HR (95% CI)	1.16 (0.00-3.61)	0.65 (0.59-0.72)	0.65 (0.59-0.72)	0.81 (0.77-0.85)	1.00	0.82 (0.78-0.86)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median newly measured 25-hydroxyvitamin D value of each category. ^aHazard rate adjusted for age, sex, and season of blood drawing. ^bHazard rate adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rate adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rate adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 19: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Aarhus Mammography Cohort Study.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		25.0	36.0	46.0	62.0	84.0	110.0
Sample size, n		251	280	377	1048	412	105
Deaths, n		7	16	12	35	6	2
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	2.39 (0.00-4.90)	3.81 (0.47-7.15)	2.28 (0.21-4.36)	2.08 (0.55-3.61)	1.00	1.28 (0.00-2.94)
Model 2 ^b	Categorical HR (95% CI)	2.16 (0.00-4.45)	3.33 (0.36-6.31)	2.10 (0.18-4.02)	2.03 (0.54-3.52)	1.00	1.29 (0.00-2.95)
Model 3 ^c	Categorical HR (95% CI)	2.17 (0.00-4.46)	3.14 (0.33-5.96)	2.13 (0.18-4.07)	2.06 (0.55-3.58)	1.00	1.32 (0.00-3.02)
Model 4 ^d	Categorical HR (95% CI)	1.78 (0.00-3.77)	3.00 (0.31-5.68)	1.97 (0.16-3.77)	2.07 (0.54-3.60)	1.00	1.09 (0.00-2.53)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	2.41 (0.00-4.91)	3.77 (0.48-7.06)	2.23 (0.21-4.26)	2.03 (0.55-3.51)	1.00	1.23 (0.00-2.83)
Model 2 ^b	Categorical HR (95% CI)	2.20 (0.00-4.51)	3.33 (0.36-6.30)	2.07 (0.18-3.96)	2.02 (0.54-3.50)	1.00	1.23 (0.00-2.84)
Model 3 ^c	Categorical HR (95% CI)	2.24 (0.00-4.58)	3.16 (0.33-5.99)	2.13 (0.19-4.07)	2.04 (0.55-3.54)	1.00	1.29 (0.00-2.97)
Model 4 ^d	Categorical HR (95% CI)	1.83 (0.00-3.88)	3.03 (0.32-5.75)	1.97 (0.16-3.78)	2.09 (0.55-3.63)	1.00	1.07 (0.00-2.49)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI,

active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 20: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The German Health Interview and Examination Survey for Adults.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		22.2	34.9	44.7	59.7	89.9	110.9
Sample size, n		553	464	607	993	747	498
Deaths, n		59	43	44	59	51	26
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	1.44 (0.88-2.01)	1.28 (0.75-1.81)	0.98 (0.58-1.38)	0.84 (0.52-1.15)	1.00	0.94 (0.49-1.39)
Model 2 ^b	Categorical HR (95% CI)	1.42 (0.86-1.98)	1.26 (0.74-1.79)	0.96 (0.56-1.35)	0.83 (0.52-1.14)	1.00	0.95 (0.49-1.40)
Model 3 ^c	Categorical HR (95% CI)	1.35 (0.82-1.89)	1.24 (0.72-1.75)	0.99 (0.58-1.41)	0.83 (0.52-1.15)	1.00	0.96 (0.50-1.42)
Model 4 ^d	Categorical HR (95% CI)	1.31 (0.79-1.83)	1.29 (0.74-1.84)	1.00 (0.58-1.41)	0.87 (0.53-1.20)	1.00	0.97 (0.50-1.44)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	1.62 (0.90-2.33)	1.05 (0.55-1.55)	0.80 (0.39-1.20)	1.10 (0.59-1.60)	1.00	1.04 (0.44-1.64)
Model 2 ^b	Categorical HR (95% CI)	1.59 (0.88-2.30)	1.03 (0.54-1.52)	0.78 (0.28-1.17)	1.09 (0.59-1.59)	1.00	1.05 (0.44-1.65)
Model 3 ^c	Categorical HR (95% CI)	1.51 (0.84-2.18)	1.03 (0.54-1.52)	0.77 (0.38-1.17)	1.07 (0.58-1.56)	1.00	1.06 (0.45-1.67)
Model 4 ^d	Categorical HR (95% CI)	1.53 (0.85-2.22)	1.10 (0.57-1.64)	0.81 (0.39-1.22)	1.18 (0.63-1.74)	1.00	1.08 (0.44-1.72)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI,

active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Table 21: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Longitudinal Aging Study Amsterdam, First Cohort.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		23.8	35.6	44.5	60.5	82.5	104.1
Sample size, n		224	205	260	502	104	7
Deaths, n		204	165	201	341	57	5
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	1.63 (1.22-2.04)	1.33 (0.98-1.68)	1.30 (0.98-1.62)	1.09 (0.84-1.33)	1.00	1.06 (0.66-1.47)
Model 2 ^b	Categorical HR (95% CI)	1.63 (1.21-2.04)	1.34 (0.99-1.69)	1.30 (0.98-1.62)	1.09 (0.85-1.33)	1.00	1.07 (0.66-1.47)
Model 3 ^c	Categorical HR (95% CI)	1.51 (1.13-1.90)	1.28 (0.94-1.62)	1.21 (0.91-1.51)	1.06 (0.82-1.30)	1.00	1.03 (0.64-1.42)
Model 4 ^d	Categorical HR (95% CI)	1.37 (1.02-1.72)	1.21 (0.90-1.53)	1.17 (0.88-1.46)	1.05 (0.81-1.28)	1.00	1.03 (0.64-1.43)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	1.63 (1.22-2.04)	1.33 (0.98-1.68)	1.30 (0.98-1.62)	1.09 (0.84-1.33)	1.00	1.06 (0.66-1.47)
Model 2 ^b	Categorical HR (95% CI)	1.64 (1.22-2.06)	1.34 (0.99-1.69)	1.30 (0.98-1.62)	1.09 (0.85-1.33)	1.00	1.06 (0.66-1.47)
Model 3 ^c	Categorical HR (95% CI)	1.52 (1.13-1.90)	1.28 (0.95-1.62)	1.21 (0.91-1.51)	1.06 (0.82-1.29)	1.00	1.03 (0.64-1.42)
Model 4 ^d	Categorical HR (95% CI)	1.37 (1.02-1.72)	1.22 (0.90-1.54)	1.17 (0.88-1.46)	1.05 (0.81-1.28)	1.00	1.03 (0.64-1.43)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI,

active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

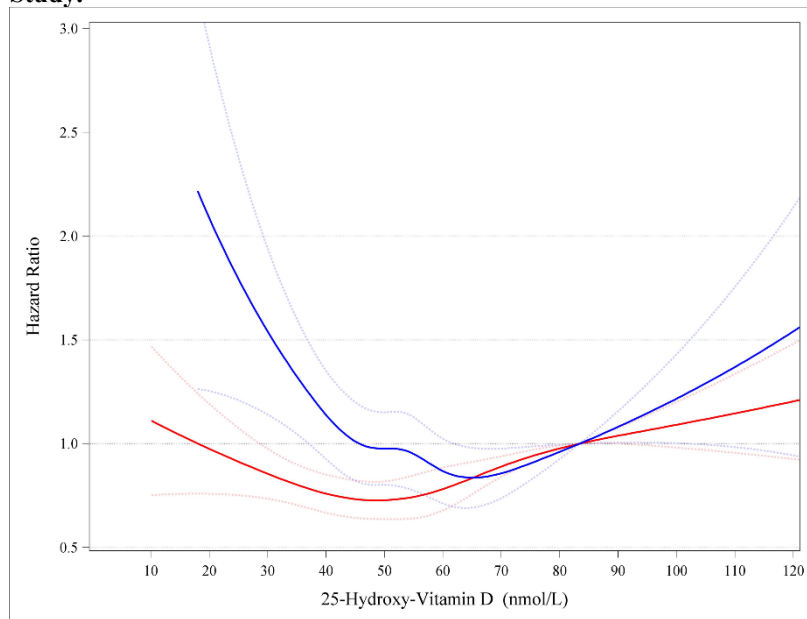
Table 22: Adjusted Hazard Ratio of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Longitudinal Aging Study Amsterdam, Second Cohort.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		25.6	36.2	45.0	60.7	82.1	106.1
Sample size, n		56	101	145	329	93	10
Deaths, n		6	13	15	27	4	1
Standardized measurements							
Model 1 ^a	Categorical HR (95% CI)	3.00 (0.00-6.82)	3.96 (0.00-8.44)	2.68 (0.00-5.68)	2.27 (0.00-4.67)	1.00	2.58 (0.00-6.98)
Model 2 ^b	Categorical HR (95% CI)	3.11 (0.00-7.08)	4.11 (0.00-8.77)	2.80 (0.00-5.92)	2.32 (0.00-4.76)	1.00	2.73 (0.00-7.40)
Model 3 ^c	Categorical HR (95% CI)	2.99 (0.00-6.82)	3.90 (0.00-8.35)	2.73 (0.00-5.78)	2.27 (0.00-4.66)	1.00	2.65 (0.00-7.19)
Model 4 ^d	Categorical HR (95% CI)	2.46 (0.00-5.63)	3.23 (0.00-6.90)	2.27 (0.00-4.82)	2.25 (0.00-4.62)	1.00	2.49 (0.00-6.74)
Original measurements							
Model 1 ^a	Categorical HR (95% CI)	3.00 (0.00-6.83)	3.97 (0.00-8.46)	2.69 (0.00-5.69)	2.27 (0.00-4.67)	1.00	2.58 (0.00-6.99)
Model 2 ^b	Categorical HR (95% CI)	3.11 (0.00-7.09)	4.12 (0.00-8.79)	2.80 (0.00-5.94)	2.32 (0.00-4.77)	1.00	2.74 (0.00-7.42)
Model 3 ^c	Categorical HR (95% CI)	2.99 (0.00-6.83)	3.92 (0.00-8.39)	2.74 (0.00-5.81)	2.28 (0.00-4.68)	1.00	2.67 (0.00-7.23)
Model 4 ^d	Categorical HR (95% CI)	2.48 (0.00-5.68)	3.26 (0.00-6.96)	2.29 (0.00-4.86)	2.26 (0.00-4.66)	1.00	2.50 (0.00-6.78)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Category characteristics refer to standardized 25-hydroxyvitamin D concentrations. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median standardized 25-hydroxyvitamin D value of each category. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI,

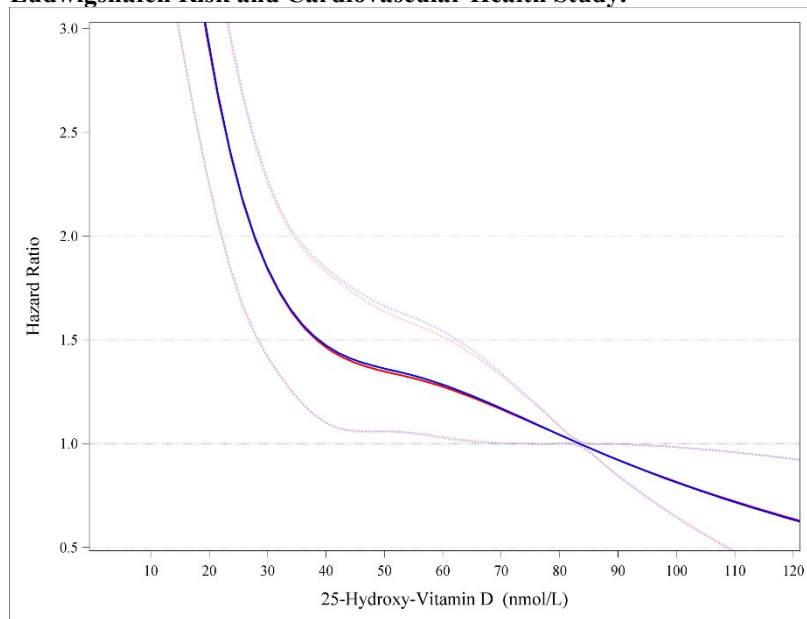
active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

Figure 15: Hazard Ratios of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Tromsø Study.



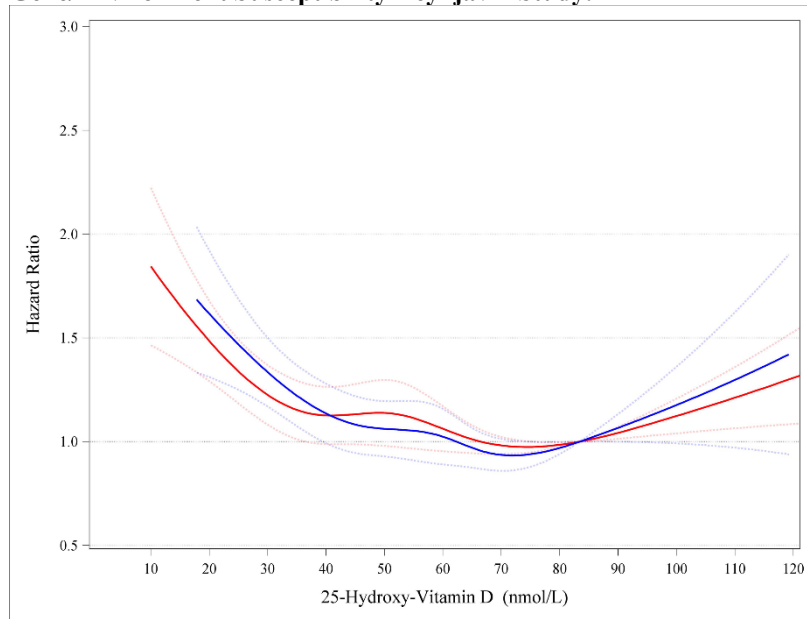
Hazard rates for all-cause mortality adjusted for age, sex, season of blood drawing, and body mass index. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (blue line with 95% CI as the dotted blue lines). Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Figure 16: Hazard Ratios of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Ludwigshafen Risk and Cardiovascular Health Study.



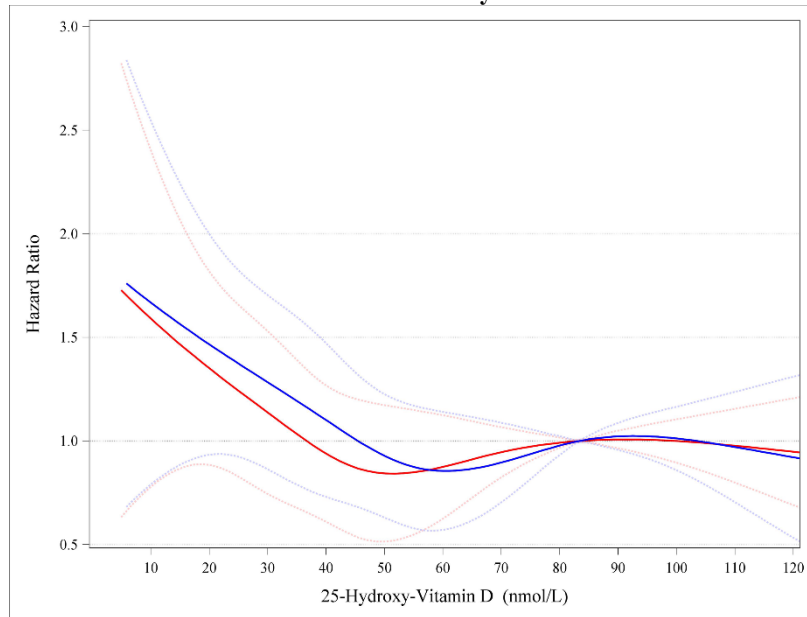
Hazard rates for all-cause mortality adjusted for age, sex, season of blood drawing, and body mass index. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (blue line with 95% CI as the dotted blue lines). Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Figure 17: Hazard Ratios of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Age, Gene/Environment Susceptibility Reykjavik Study.



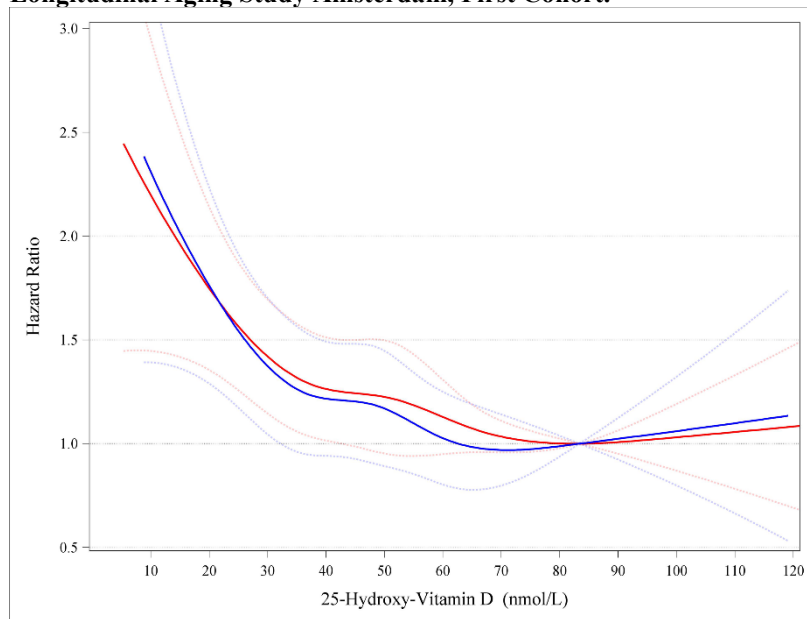
Hazard rates for all-cause mortality adjusted for age, sex, season of blood drawing, and body mass index. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (blue line with 95% CI as the dotted blue lines). Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Figure 18: Hazard Ratios of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The German Health Interview and Examination Survey for Adults.



Hazard rates for all-cause mortality adjusted for age, sex, season of blood drawing, and body mass index. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (blue line with 95% CI as the dotted blue lines). Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Figure 19: Hazard Ratios of Death from All Causes (95% CI) By Standardized Versus Original 25-Hydroxyvitamin D Concentrations in nmol/l for The Longitudinal Aging Study Amsterdam, First Cohort.



Hazard rates for all-cause mortality adjusted for age, sex, season of blood drawing, and body mass index. Hazard ratios are shown for original 25-hydroxyvitamin D (red line with 95% CI as the dotted red lines) and for standardized 25-hydroxyvitamin D (blue line with 95% CI as the dotted blue lines). Hazard ratios are referring to the standardized 25-hydroxyvitamin D concentration of 83.4 nmol/l (i.e. the median 25-hydroxyvitamin D concentration for the group with 25-hydroxyvitamin D concentrations from 75 to 99.99 nmol/l).(1)

Sensitivity analyses for standardized 25(OH)D concentrations are shown in Table 23.(1)

Table 23: Adjusted Hazard Ratio of Death from All Causes (95% CI) According to Standardized 25-Hydroxyvitamin D Concentrations in nmol/l in Subgroup Analysis.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	100-124.99	≥125	
Median*, nmol/l		23.0	35.9	45.3	60.4	83.6	107.2	135	
Sample size, n		2903	3099	5015	11892	3150	684	173	
Deaths, n		999	892	1386	2935	522	57	11	
Model 2 ^a	Subgroups of sex ^a	Female (n=15 616)	1.55 (1.23-1.87)	1.27 (1.01-1.53)	1.11 (0.89-1.32)	1.02 (0.83-1.20)	1.00	0.94 (0.42-1.46)	0.89 (0.00-2.12)
		Male (n=11 300)	1.78 (1.45-2.11)	1.38 (1.13-1.63)	1.18 (0.98-1.38)	1.08 (0.91-1.24)	1.00	1.11 (0.64-1.58)	1.00 (0.11-1.88)
Model 2 ^b	Subgroups of age ^b	<60 yrs. (n=12 214)	2.29 (1.27-3.31)	1.38 (0.76-2.01)	1.26 (0.74-1.77)	1.18 (0.73-1.64)	1.00	0.76 (0.02-1.50)	0.94 (0.00-2.49)
		60 to <70 yrs. (n=6 483)	2.05 (1.45-2.64)	1.34 (0.97-1.72)	1.17 (0.88-1.47)	1.02 (0.78-1.26)	1.00	1.02 (0.40-1.63)	0.70 (0.00-1.57)
		≥70 yrs. (n=8 219)	1.89 (1.56-2.22)	1.41 (1.16-1.66)	1.17 (0.97-1.37)	1.06 (0.90-1.22)	1.00	1.04 (0.54-1.54)	1.08 (0.00-2.58)
Model 2 ^c	Subgroups of BMI ^c	<25 kg/m ² (n=11 008)	1.84 (1.45-2.24)	1.38 (1.08-1.68)	1.12 (0.90-1.34)	1.03 (0.85-1.21)	1.00	1.19 (0.61-1.76)	0.51 (0.00-1.37)
		25 to <30 kg/m ² (n=11 026)	1.54 (1.22-1.87)	1.37 (1.09-1.65)	1.25 (1.01-1.49)	1.11 (0.92-1.31)	1.00	0.73 (0.30-1.17)	1.24 (0.02-2.46)
		≥30 kg/m ² (n=4 882)	1.55 (1.01-2.04)	1.16 (0.80-1.52)	0.95 (0.66-1.24)	0.94 (0.67-1.22)	1.00	1.23 (0.30-2.16)	1.18 (0.00-2.81)
Model 2 ^d	Subgroups of intake of calcium ^d	No intake (n=15 254)	1.87 (1.52-2.22)	1.43 (1.16-1.70)	1.24 (1.02-1.47)	1.14 (0.95-1.32)	1.00	1.09 (0.50-1.68)	1.35 (0.00-3.08)
		Intake (n=2 071)	1.20 (0.47-1.92)	1.06 (0.53-1.59)	0.97 (0.56-1.38)	0.92 (0.61-1.23)	1.00	0.87 (0.00-1.81)	N.A.
Model 2 ^e	Subgroups of intake of vitamin D ^e	No intake (n=8 810)	2.32 (1.39-3.24)	1.59 (0.95-2.24)	1.38 (0.83-1.93)	1.26 (0.77-1.75)	1.00	0.81 (0.00-1.79)	1.09 (0.00-3.12)

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		Intake (n=7 890)	1.46 (0.92-2.00)	1.42 (1.00-1.84)	1.14 (0.85-1.43)	1.01 (0.81-1.22)	1.00	1.12 (0.33-1.91)	0.99 (0.00-4.05)
Model 2 ^f	Subgroups of history of CVD ^f	No history (n=20 934)	1.54 (1.29-1.79)	1.28 (1.08-1.48)	1.09 (0.93-1.25)	1.01 (0.87-1.15)	1.00	0.95 (0.56-1.34)	1.02 (1.12-1.93)
		History (n=3 376)	2.12 (1.53-2.72)	1.62 (1.15-2.08)	1.52 (1.09-1.95)	1.29 (0.95-1.62)	1.00	1.34 (0.42-2.26)	0.90 (0.00-2.20)
Model 2 ^g	Subgroups of history of cancer ^g	No history (n=22 260)	1.70 (1.44-1.96)	1.33 (1.13-1.53)	1.15 (0.98-1.31)	1.06 (0.92-1.20)	1.00	1.03 (0.64-1.42)	1.05 (0.22-1.88)
		History (n=1 992)	1.53 (0.98-2.08)	1.36 (0.88-1.85)	1.16 (0.77-1.56)	0.98 (0.68-1.28)	1.00	0.92 (0.01-1.82)	0.54 (0.00-1.90)
Model 2 ^h	Sensitivity analysis ^h	>365 ds (n=26 604)	1.65 (1.42-1.87)	1.33 (1.15-1.51)	1.13 (0.99-1.28)	1.04 (0.92-1.16)	1.00	1.04 (0.69-1.39)	1.04 (0.29-1.79)
Model 2 ⁱ	Sensitivity analysis ⁱ	>1095 ds (n=25 917)	1.61 (1.37-1.85)	1.32 (1.13-1.51)	1.15 (0.99-1.30)	1.04 (0.91-1.17)	1.00	1.10 (0.70-1.50)	1.24 (0.35-2.13)
Model 2 ^j	Sensitivity analysis ^j	General population (n=23 617)	1.43 (1.21-1.66)	1.33 (1.13-1.52)	1.11 (0.96-1.26)	1.03 (0.90-1.15)	1.00	1.03 (0.90-1.15)	1.13 (0.72-1.54)

Statistical approach was based on categorical models adjusted for age, sex, season of blood drawing, and BMI (i.e. Model 2). Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); yrs. = Years; kg/m² = Kilogram per meter squared; ds = Days; BMI = Body mass index; CVD = Cardiovascular disease. ^aSubgroup comparison of female versus male sex. ^bSubgroup comparison of three categories of age: <60 yrs., 60 to <70 yrs. and ≥70 yrs. ^cSubgroup comparison of three categories of BMI defined by World Health Organization BMI categories: normal or underweight (<25 kg/m²), overweight (25 to <30 kg/m²); obesity (≥30 kg/m²). ^dSubgroup comparison of categories of no intake of calcium supplementation versus positive intake of calcium supplementation. ^eComparison of categories of no intake of vitamin D supplementation versus positive intake of vitamin D supplementation. ^fSubgroup comparison of categories of no history of CVD versus positive history of CVD. History of CVD was defined as history of myocardial infarction and history of stroke. ^gSubgroup comparison of categories of no history of cancer versus positive history of cancer. ^hSensitivity analysis restricted to participants who died >1 year after baseline examination. ⁱSensitivity analysis restricted to participants who died >3 yrs. after baseline examination. ^jSensitivity analysis restricted to general population cohorts (i.e. all cohorts except LURIC).(1)

For all our meta-analyses, the intra-class correlation coefficients (ICC) as a measure of inconsistency was below 25%, thus indicating small heterogeneity (Table 24).(1)

Table 24: Intra-Class Correlation Coefficients for Models 1 To 4 Presented by Standardized and Original 25-Hydroxyvitamin D Concentrations and Statistical Approach for Full Database.

	Statistical Approach	Standardized 25(OH)D	ICC (%)	Original 25(OH)D	ICC (%)
Model 1 ^a	Categorical model		1.1 (0.0-2.4)		1.7 (0.0-3.3)
	Cubic-splines model		1.6 (0.0-4.8)		1.5 (0.0-3.8)
Model 2 ^b	Categorical model		1.3 (0-3.0)		2.1 (0.0-4.4)
	Cubic-splines model		1.8 (0.0-6.6)		0.6 (0.1-1.2)
Model 3 ^c	Categorical model		0.9 (0-2.3)		2.0 (0.0-4.4)
	Cubic-splines model		1.3 (0.0-4.8)		0.9 (0.0-2.1)
Model 4 ^d	Categorical model		0.7 (0.0-2.1)		0.6 (0.0-1.5)
	Cubic-splines model		0.5 (0.0-1.3)		0.5 (0.0-1.3)

Intra-class correlation coefficients (ICC) for models 1 to 4 are presented as percentages with 95% confidence interval and are based on 1) categorical models and 2) restricted cubic splines models. ^aHazard rates adjusted for age, sex, and season of blood drawing. ^bHazard rates adjusted for age, sex, season of blood drawing, and body mass index (BMI). ^cHazard rates adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension. ^dHazard rates adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer. History of CVD was defined as history of myocardial infarction and history of stroke.(1)

12.4 25(OH)D and Cause-Specific Mortality

There was a significant inverse association between standardized 25(OH)D concentrations and cardiovascular mortality (Table 25) while no association was found between standardized 25(OH)D and cancer deaths (Table 26).(1)

Table 25: Adjusted Hazard Ratio of Death from Cardiovascular Causes (95% CI) By Standardized 25-Hydroxyvitamin D Concentrations in nmol/l in Competing Risk Analysis for Full Database Without the New Hoorn Study.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		22.2	35.4	45.1	60.9	83.4	112
Sample size, n		2668	2847	4634	10775	2642	759
Deaths, n		391	259	379	665	100	16
Model 1 ^a	Categorical HR (95% CI)	3.18 (1.82-5.53)	1.99 (1.64-2.40)	1.72 (1.47-2.02)	1.35 (1.12-1.63)	1.00	0.95 (0.69-1.31)
Model 2 ^b	Categorical HR (95% CI)	3.10 (1.78-5.41)	1.93 (1.60-2.33)	1.69 (1.47-1.95)	1.34 (1.12-1.61)	1.00	0.95 (0.69-1.31)
Model 3 ^c	Categorical HR (95% CI)	2.54 (1.74-3.71)	1.74 (1.61-1.89)	1.68 (1.41-2.00)	1.38 (1.11-1.70)	1.00	0.91 (0.65-1.29)
Model 4 ^d	Categorical HR (95% CI)	2.21 (1.50-3.26)	1.61 (1.46-1.77)	1.65 (1.39-1.97)	1.37 (1.12-1.67)	1.00	0.92 (0.62-1.36)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median 25-hydroxyvitamin D value of each category. ^aHazard rate adjusted for age, sex, and season of blood drawing at baseline visit. ^bHazard rate adjusted for age, sex, season of blood drawing, and body mass index (BMI) at baseline visit. ^cHazard rate adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension at baseline visit. ^dHazard rate adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer at baseline visit. History of CVD was defined as history of myocardial infarction and history of stroke. History of CVD and history of cancer were not available in the New Hoorn Study (NHS) therefore Model 4 was conducted without NHS.(1)

Table 26: Adjusted Hazard Ratio of Death from Cancer Causes (95% CI) By Standardized 25-Hydroxyvitamin D Concentrations in nmol/l in Competing Risk Analysis for Full Database Without the New Hoorn Study.

Category		<30	30-39.99	40-49.99	50-74.99	75-99.99	≥100
Median*, nmol/l		22.2	35.4	45.1	60.9	83.4	112
Sample size, n		2668	2847	4634	10775	2642	759
Deaths, n		152	156	312	673	104	17
Model 1 ^a	Categorical HR (95% CI)	1.08 (0.74-1.60)	1.11 (0.91-1.35)	1.29 (1.12-1.49)	1.25 (1.07-1.47)	1.00	0.80 (0.60-1.06)
Model 2 ^b	Categorical HR (95% CI)	1.10 (0.75-1.61)	1.13 (0.93-1.36)	1.30 (1.13-1.50)	1.26 (1.07-1.48)	1.00	0.80 (0.60-1.07)
Model 3 ^c	Categorical HR (95% CI)	1.15 (0.78-1.69)	1.16 (0.94-1.42)	1.33 (1.14-1.55)	1.26 (1.07-1.49)	1.00	0.82 (0.61-1.10)
Model 4 ^d	Categorical HR (95% CI)	1.08 (0.73-1.60)	1.07 (0.87-1.32)	1.25 (1.07-1.46)	1.24 (1.06-1.45)	1.00	0.79 (0.60-1.04)

Categories are based on the Institute of Medicine report 2011 used cut-off values. Abbreviations: HR = Hazard ratio with 95% confidence interval (CI); *The median is the median 25-hydroxyvitamin D value of each category. ^aHazard rate adjusted for age, sex, and season of blood drawing at baseline visit. ^bHazard rate adjusted for age, sex, season of blood drawing, and body mass index (BMI) at baseline visit. ^cHazard rate adjusted for age, sex, season of blood drawing, BMI, present diabetes mellitus, and present arterial hypertension at baseline visit. ^dHazard rate adjusted for age, sex, season of blood drawing, BMI, active smoker status, history of cardiovascular disease (CVD), and history of cancer at baseline visit. History of CVD was defined as history of myocardial infarction and history of stroke. History of CVD and history of cancer were not available in the New Hoorn Study (NHS) therefore Model 4 was conducted without NHS.(1)

13. Discussion

The current work and the respective paper (1) established the first one-step IPD meta-analyses of standardized 25(OH)D concentrations in association with mortality. We (1) could show that low 25(OH)D concentrations are associated with an increased risk of mortality. Our study replicates and extends the findings from previous meta-analyses.(84,89,92,174) The main finding of this work is that individuals with 25(OH)D concentrations below approximately 30 to 40 nmol/l (~12 ng/ml to 20 ng/ml) are at increased risk for mortality and that the relationship between vitamin D status and mortality steeply increases below this range of 25(OH)D concentrations.

While implementing only categorical and restricted cubic splines models in the corresponding paper (1), the present doctoral thesis further employs a fractional polynomials implementation for the modelling of the association between 25(OH)D concentrations and mortality. In the thesis, individuals with 25(OH)D concentrations below approximately 30 to 40 nmol/l revealed increased hazard ratios for all-cause mortality regardless whether a 25(OH)D concentrations were modelled as categorical, restricted cubic splines or fractional polynomials term. Moreover, the nadir of approximately 80 nmol/l (~32 ng/ml) for the lowest mortality rate could be shown for the fractional polynomial implementation and thus approves the nadir computation of the cubic splines model.(1)

The shape of the 25(OH)D and mortality curve was comparable for fractional polynomial- and restricted cubic splines implementation and has important implications for the clinical significance of 25(OH)D levels in terms of their relationship with mortality. While there is an ongoing debate on whether 50 and/or 75 nmol/l should be used as a threshold for 25(OH)D classification of deficiency, we have clearly shown in our IPD meta-analysis that there is no significant and consistent difference in risk of mortality for 25(OH)D levels ranging from approximately 40 to 125 nmol/l.(1,9,25)

For the present analyses, we used a one-step meta-analysis approach, which synthesises the IPD from all studies in a single step whilst accounting for clustering of patients within studies, rather than a commonly used two-step meta-analysis approach, where each study is summarized by its factor-outcome association estimate and variance in the first stage, and these AD are then appropriately combined across studies in the second stage.(152) Although it is widely accepted that both approaches deliver comparable results (151), there are concerns that for mortality analyses the one-step approach may produce more robust results with regard to estimates for pooled effects, standard errors, between-study heterogeneity and correlation between random effects.(152) Especially in our case, where there are few studies included in the meta-analyses, a one-stage method may produce more reliable results than the two-stage method, as the method increases total sample size. This reduces the risk of incidental findings and increases the precision of study

results.(152) Moreover, the one-step approach offers more flexibility regarding implementation of non-linear associations (e.g. cubic splines, fractional polynomials, etc.), and the extended ability to explore heterogeneity and subgroup analyses.(152)

The standardization of 25(OH)D concentrations had a nonsignificant impact on the mortality curve which was similar in behaviour to a cumulative model adjustment for potential confounders, so that the steepness of the association between 40 and 80 nmol/l presented a small (but not significant) increase. While there were thus no dramatic changes in 25(OH)D concentrations and their association with mortality in our meta-analysis, it should be underlined that previous studies reported on significant assay differences in 25(OH)D concentrations underlining the importance of using standardized 25(OH)D levels.(1,94,98,99,186)

The present study had limited statistical power to evaluate the association between 25(OH)D and mortality for individuals with 25(OH)D concentrations higher than ~110 nmol/l (~40 ng/ml) as mortality data was sparse (<5% of whole dataset) in the range of >112 nmol/l (44.8 ng/ml) of 25(OH)D concentrations. From our analyses, we cannot draw a final conclusion whether mortality risk increases again or further decreases beyond 100 nmol/l, but our results point towards a statistically nonsignificant risk increase.

Statistical heterogeneity was low between studies according to the ICC across different models so that incorporation of random effects into our models would not have been mandatory. After careful consideration, random effects were nonetheless included in the analyses, as theoretically, for 25(OH)D, unmeasured confounding was conceivable in the form of e.g. different latitudes and sun exposure, distributions of genetic variants of vitamin D metabolism, or varying intake patterns of vitamin D of participants across Europe, which we could not adjust for. Ignoring heterogeneity would lead to an overly precise summary result.(101) Anyway, results were not significantly different with and without random effects (data not shown). Using an estimated within-cluster variance of $\pi^2/6$ when estimating ICC as proposed by *Rodríguez* (163) had no significant impact on the measurement of heterogeneity in comparison to a classical Pearson's correlation coefficient (data not shown). Because of the Weibull distribution of our model, our findings may only be valid for a follow-up time of 13 years or less and may therefore differ for a longer follow-up period.

Regarding secondary endpoints, we could show a significant association of 25(OH)D concentrations with CVD mortality, but not with cancer mortality. The underlying model for cause-specific mortality was based on a competing-risk method described by *Fine* and *Gray* (181) which was implemented in the '*cmprsk*' package in R as weighted estimating equation (WEE). A WEE is a marginal modelling approach and thus per definition not a conditional model, leaving out clustering. To overcome limitations, we employed a competing risks regression for clustered data ('*crrSC*' package) which was implemented by *Zhou B.* and *Latouche A.* (183). The '*crrSC*'

package is an extension of the competing-risk method where baseline hazard could vary across levels of the stratification covariate. While there may already be more elaborated implementations of stratified competing risks regressions, they are still not yet widely applied.(183) While the choice of regression modelling and implementation potentially had an impact on the magnitude of the summary result regarding CVD mortality, we doubt that it had any influence on the conclusiveness of cancer mortality outcomes. We further analysed cancer-specific outcomes within different regression model implementations which showed a similar trend (data not shown). In this context, published data from RCTs and meta-analyses of RCTs did, by the majority, not show a risk lowering effect of vitamin D supplementation on cardiovascular or cancer endpoints.(23,25,69,72,187)

We are well aware that thresholds for 25(OH)D vary according to the outcomes studied and that the clinical significance of vitamin D is currently based on its effects on bone and mineral metabolism, but all-cause mortality is a clearly defined highly relevant endpoint that might be causally related to vitamin D.(92) As mentioned in the introduction, some albeit not all, meta-analyses of RCTs have suggested that vitamin D supplementation may reduce mortality.(92,187) Our findings support this hypothesis although we'd like to emphasise that individuals might probably benefit from vitamin D treatment with regard to mortality only if their initial 25(OH)D concentrations were lower than approximately 30 to 40 nmol/l. For individuals with 25(OH)D concentrations higher than 40 nmol/l no consistent conclusion can be based on our results regarding a risk lowering effect for mortality. Considering that most previous and ongoing vitamin D RCTs were not restricted to individuals with 25(OH)D concentrations lower than approximately 30 to 40 nmol/l, it still remains an important research question to elucidate whether individuals at or below this range benefit from vitamin D supplementation with regard to mortality, cardiovascular diseases or cancer outcomes. Thus, for design, analysis, and interpretation of clinical trials on vitamin D supplementation as well as for the care of patients, a threshold of approximately 30 to 40 nmol/l should be considered regarding mortality. Our results indicate that individuals with 25(OH)D below 30 to 40 nmol/L or even lower may benefit from vitamin D supplementation with regard to mortality.(1,188,189)

A limitation of our work is the focus on European cohort studies, which may limit the generalisability of our findings to other populations. Our data stems from studies ranging from Germany to Iceland, thus Eastern as well as Southern Europe countries are not represented in our analyses. We could not adjust for any form of osteoporosis or fractures as there were no data available.

Our findings remained stable throughout adjustments for a panel of potential confounders and were materially unchanged in several sensitivity analyses. Our cohort consists of general population studies as well as hospital-based surveys and results remained materially unchanged when excluding hospital-based surveys. Although groups of participants who died and survived differed

significantly regarding age with the deceased group clearly containing more elderly, the association between 25(OH)D concentrations and mortality remained stable in sensitivity analyses. Moreover, we could observe a trend towards a pronounced association of 25(OH)D and mortality for participants of younger age.

Due to the observational character of our analyses, we cannot draw definite conclusions about causality. Moreover, the association between 25(OH)D and mortality may be subject to reverse causation, i.e. that 25(OH)D concentrations are a result of underlying diseases or causal risk factors such as e.g. inflammation, obesity, poor nutrition or low physical activity. In the present meta-analysis, the association between 25(OH)D and mortality remained significant despite adjustments for several potential confounders and despite inclusion of random effects. Nevertheless, we cannot fully rule out residual confounding, especially if confounders were not subject to clustering, i.e. present across Europe. In this context, it should be acknowledged that most findings from observational studies on the associations between vitamin D deficiency and increased risk of various chronic diseases could not be replicated in RCTs, suggesting the existence of unmeasured or unconsidered confounding factors or reverse causation.(1,187,190)

Main strengths of our meta-analysis are the use of standardized 25(OH)D concentrations, the one-step IPD meta-analysis approach and implementation of a parametric survival model, and the inclusion of previously unpublished data on vitamin D status and mortality (i.e. first 25(OH)D data of the NHS and 10-year follow-up data of the LURIC study).(1) Main strengths of the thesis are the computation of nadir and modelling of the continuous nature of 25(OH)D through restricted cubic splines and fractional polynomials.

In conclusion, we could show that individuals with 25(OH)D concentrations below approximately 30 to 40 nmol/L are at increased risk of mortality. There was no significant association between vitamin D status and mortality for 25(OH)D concentrations above that range, therefore arguing that RCTs addressing this issue should be restricted to severely vitamin D deficient individuals because across most parts of the 25(OH)D distribution there was no meaningful association with mortality.(1)

14. References

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15. Appendix

15.1 SAS Codes

SAS Code 1: Printing the Probability Density Function

```
ods graphics on;
proc univariate data=ODIN(where=(death=1));
title ' The Weibull probability density function';
var timemort;
histogram timemort / weibull (sigma=est c=2 theta=est);
run;
ods graphics off;
title;
```

SAS Code 2: Printing the Hazard Function and Cumulative Hazard Function with Nelson-Aalen Estimator

```
ods output ProductLimitEstimates = ple;
proc lifetest data=ODIN(where=(death=1)) nelson plots=hazard(bw=365);
time timemort*death(0);
run;
proc sgplot data = ple;
title "The cumulative hazard function";
series x = timemort y = CumHaz;
run;
```

SAS Code 3: Printing A Weibull Probability Plot Using a Log-Log Scale

```
ods output ProductLimitEstimates = ple;
proc lifetest data=ODIN(where=(death=1)) nelson plots=(s,ls,lls,h,p);
time timemort*death(0);
run;
```

SAS Code 4: Printing A Quantile-Quantile Plot

```
proc univariate data= ODIN (where=(death=1));
var timemort;
qqplot timemort / weibull (theta=0 shape=est scale=est);
title2 "Weibull Quantile-Quantile Plot";
run;
```

SAS Code 5: Printing A Histogram of The Pdf and Incorporating Different Parametric Distributions

```
ods graphics on;
ods select Histogram ParameterEstimates GoodnessOfFit FitQuantiles;
proc univariate data= ODIN (where=(death=1));
var timemort;
histogram /
                                weibull (theta = 0)
                                gamma (theta = 0)
                                lognormal (theta = 0)
                                exponential (theta = 0)
                                odstitle = title;
inset n mean(5.3) std='Std Dev'(5.3) skewness(5.3)
      / pos = ne header = 'Summary Statistics';
title "Distribution of survival time";
title2 "Tests of Fit";

run;
```

SAS Code 6: Estimation and Inference for Weibull, Exponential, Log-Normal, Gamma and Log-Logistic Regression Model

```
ods output ParameterEstimates (match_all=bydist persist=proc) =
table12_6;

#####EXPONENTIAL#####;
proc lifereg data = work.import;
  model timemort*death(0)= / distribution=exponential;

run;

#####WEIBULL#####;
proc lifereg data = work.import;
  model timemort*death(0)= / distribution=weibull;

run;

#####LNORMAL#####;
proc lifereg data = work.import;
  model timemort*death(0)= / distribution=lnormal;

run;

#####LLOGISTIC#####;
proc lifereg data = work.import;
  model timemort*death(0)= / distribution=llogistic;

run;

#####GAMMA#####;
proc lifereg data = work.import;
  model timemort*death(0)= / distribution=gamma;
run;
ods output close;

data table12_5;
  set &bydist;
  retain group 0;
  keep Parameter estimate stderr group;
  if Parameter = "Intercept" then group= group+1;
run;
proc format;
  value group 1 = "Exponential"
             2 = "Weibull"
             3 = "Log Logistic"
             4 = "Log Normal"
             5 = "Gamma";

run;
options nocenter FORMCHAR='|----|+|---+=|-\<>*';
proc tabulate data=table12_5;
  format group group.;
  class group Parameter;
  var estimate stderr;
  table parameter=' ', group=' '* (estimate='Est' stderr='SE') *sum='
'*f=5.3/RTS=13;
run;
```

SAS Code 7: Likelihood Ratio Test

```
DATA lrt_pval;
* Log likelihood results for each distribution from the results above;
  Weib = -19638.55188;
  Gamma = -19602.34903;

  df_1 = 2;
  df_2 = 3;

* Likelihood Ratio Tests;
  LRT = -2* (Weib - Gamma);
  ddf = abs(df_1- df_2);
* p-values;
  P_Value = 1 - probchi(LRT,ddf);
RUN;

PROC PRINT DATA = lrt_pval;

  TITLE 'LR test statistic and p-value';
RUN;
```

SAS Code 8: Estimating Weibull Parameters

```
ods graphics on;
title 'Lifereg with WEIBULL distribution';
proc lifereg data= ODIN;
model timemort*death(0)= / covb dist=weibull;
  probplot
  ppout
  npintervals=simul;
  inset;
run;
ods graphics off;
```

SAS Code 9: Estimating Intra-Class Correlation Coefficient (ICC) Of A Model

```
ESTIMATE 'ICC' s2u / ( s2u + ((Const_pi**2)/6));
```

SAS Code 10: Basic NLMIXED Weibull Model Implementation and Categorical Variable Approach and Adjustment for Age, Sex and Month of Blood Sampling

```
proc nlmixed data=ODIN cov ecov tech=quanew update=bfgs;

*Optimization Algorithm = Quasi-Newton optimization and Broyden-Fletcher-
Goldfarb-Shanno algorithm (Stedman MR, Lew RA, Losina E, Gagnon DR,
Solomon DH, Brookhart MA. A comparison of statistical approaches for
physician-randomized trials with survival outcomes. Contemp Clin Trials.
2012 Jan 33(1):104-15.);

*Following statements represent starting values for estimation process,
absolute values are derived from Proc LIFEREG;
parms b0=12.3208 b1=-0.0564 b5=-0.0578 b6=-0.3377 b71=0.0055 b41=0.0043
b42=-0.0043 b44=0.0445 lamda=1.6118; * s2u=0.0;
bounds lamda > 0; *Lamda is set positive;

linp = b0 + b1* Cork25ohd + b5*age + b6*sex + b71*bmi +
b41*monthbaseline11 + b42*monthbaseline12 + b44*monthbaseline14; * + u;

* Cork25ohd is the variables indicating standardized 25(OH)D. age is the
age at baseline visit. sex is the individual participants sex. bmi is the
```

individuals's body mass index at baseline visit. monthbaseline11 ... 14 are the variables indicating month of baseline blood sampling. ;

```
alpha = exp(-linp);
G_t = exp(-(alpha*timemort)**lamda); *The survival distribution function
with death incidacting the status variable and timemort indicating the
censoring/failure time;
g = lamda*alpha*((alpha*timemort)**(lamda-1))*G_t; *The probability
density function;
ll = (death=1)*log(g) + (death=0)*log(G_t); *The log-likelihood function;
model timemort ~ general(ll); *Computes the conditional estimates;
* random u ~ normal(0,s2u) subject=centerid out=EB_corkspline2;

run;
```

SAS Code 11: Estimating Vitamin D Group 1 as Example Given:

```
estimate ' Group 1' exp(-(g1)*lamda);
```

SAS Code 12: Estimating Additional Variables for Restricted Cubic Splines

```
%include "survrisk.sas"; *%DASPLINE macro location;

* SAS macro „%DASPLINE“ was provided by Harrell FE at the Department of
Biostatistics, Vanderbilt University School of Medicine, Nashville, TN,
USA (Harrell FE., Jr DASPLINE Macro. [January 16th, 2015];
http://biostat.mc.vanderbilt.edu/twiki/pub/Main/SasMacros/survrisk.txt).;

%daspline (Cork25ohd, nk=5, data=ODIN); *5 knots at the fifth, 27.5th,
50th, 72.5th, and 95th percentiles;
data ODIN2; set ODIN; *New output data set;
&_ Cork25ohd;

run;
```

SAS Code 13: Basic NLMIXED Weibull Model Implementation and Restricted Cubic Spline Approach, Adjustment for Age, Sex and Month of Blood Sampling, And Incorporating Random Effects

```
proc nlmixed data=ODIN2 cov ecov tech=quanew update=bfgs;

parms b0=12.3208 b01=0.0189 b1=-0.0564 b2=0.2547 b3=-0.3368 b5=-0.0578
b6=-0.3377 b71=0.0055 b41=0.0043 b42=-0.0043 b44=0.0445 lamda=1.6118
s2u=0.0;
bounds lamda > 0;

*Knots for Cork25ohd at (computed as the fifth, 27.5th, 50th, 72.5th, and
95th percentile): 22.10000038 43.02 54.2 65.3 93.30000305;
K1=22.10000038; * Knot 1;
K2=43.02; * Knot 2;
K3=54.2; * Knot 3;
K4=65.3; * Knot 4;
K5=93.30000305; * Knot 5;
*Reference point and group medians;
Ref1=83.4; * Reference point = Median group 5;
G1=22.8; * Estimate point 1: Median group 1;
G2=35.6528300; * Estimate point 2: Median group 2;
```

```

G3=45.2000008; * Estimate point 3: Median group 3;
G4=60.3; * Estimate point 4: Median group 4;
G5=83.4; * Estimate point 5: Median group 5;
G6=107.0999985; * Estimate point 6: Median group 6;
G7=135.0; * Estimate point 7: Median group 7;

Const_pi = CONSTANT('PI'); *pi is a constant;

linp = b0 + b01*Cork25ohd + b1*Cork25ohd1 + b2*Cork25ohd2 + b3*Cork25ohd3
+ b5*age + b6*sex + b71*bmi + b41*monthbaseline11 + b42*monthbaseline12 +
b44*monthbaseline14 + u; *u is the random effect term;

alpha = exp(-linp);
G_t = exp(-(alpha*timemort)**lamda);
g = lamda*alpha*((alpha*timemort)**(lamda-1))*G_t;
ll = (death=1)*log(g) + (death=0)*log(G_t);
model timemort ~ general(ll);
random u ~ normal(0,s2u) subject=centerid out=EB_corkspline2; *The random
effect is normally distributed (s2u is the estimated between-cluster
variance);

ESTIMATE 'ICC' s2u / ( s2u + ((Const_pi**2)/6)); *The ICC is defined
according to Rodriguez, G. and Elo, I. (2003). Intra-class correlation in
random-effects models for binary data. The Stata Journal, 3(1):32-46.;
predict 1-G_t out=cdf_corkspline2; *The cumulative distribution function;

predict alpha out=alpha_corkspline2;

*Predictions for plot of hazard ratios (the curve has to be further
implemented in graphics procedures as PROC GPLOT or PROC SGRENDER);

DROP _kd_;
_kd_ = (K5 - K1)**.6666666666666666 ;
predict exp(-(b01*Cork25ohd
+b1*(max((Cork25ohd-K1)/_kd_,0))**3+(K4-K1)*max((Cork25ohd-
K5)/_kd_,0))**3-(K5-K1)*max((Cork25ohd-K4)/_kd_,0))**3/(K5-K4))
+b2*(max((Cork25ohd-K2)/_kd_,0))**3+(K4-K2)*max((Cork25ohd-
K5)/_kd_,0))**3-(K5-K2)*max((Cork25ohd-K4)/_kd_,0))**3/(K5-K4))
+b3*(max((Cork25ohd-K3)/_kd_,0))**3+(K4-K3)*max((Cork25ohd-
K5)/_kd_,0))**3-(K5-K3)*max((Cork25ohd-K4)/_kd_,0))**3/(K5-K4))
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0))**3+(K4-K1)*max((Ref1-K5)/_kd_,0))**3-(K5-
K1)*max((Ref1-K4)/_kd_,0))**3/(K5-K4))
+b2*(max((Ref1-K2)/_kd_,0))**3+(K4-K2)*max((Ref1-K5)/_kd_,0))**3-(K5-
K2)*max((Ref1-K4)/_kd_,0))**3/(K5-K4))
+b3*(max((Ref1-K3)/_kd_,0))**3+(K4-K3)*max((Ref1-K5)/_kd_,0))**3-(K5-
K3)*max((Ref1-K4)/_kd_,0))**3/(K5-K4))
)
)*lamda)
out=alpha2_corkspline2;

*Estimates for group medians (hazard ratios);
DROP _kd_;
_kd_ = (K5 - K1)**.6666666666666666;
estimate "G1" exp(-(b01*G1
+b1*(max((G1-K1)/_kd_,0))**3+(K4-K1)*max((G1-K5)/_kd_,0))**3-(K5-
K1)*max((G1-K4)/_kd_,0))**3/(K5-K4))

```

```

+b2*(max((G1-K2)/_kd_,0)**3+((K4-K2)*max((G1-K5)/_kd_,0)**3-(K5-
K2)*max((G1-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((G1-K3)/_kd_,0)**3+((K4-K3)*max((G1-K5)/_kd_,0)**3-(K5-
K3)*max((G1-K4)/_kd_,0)**3)/(K5-K4))
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
))*lamda);

estimate "G2" exp(-(b01*G2
+b1*(max((G2-K1)/_kd_,0)**3+((K4-K1)*max((G2-K5)/_kd_,0)**3-(K5-
K1)*max((G2-K4)/_kd_,0)**3)/(K5-K4))
+b2*(max((G2-K2)/_kd_,0)**3+((K4-K2)*max((G2-K5)/_kd_,0)**3-(K5-
K2)*max((G2-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((G2-K3)/_kd_,0)**3+((K4-K3)*max((G2-K5)/_kd_,0)**3-(K5-
K3)*max((G2-K4)/_kd_,0)**3)/(K5-K4))
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
))*lamda);

estimate "G3" exp(-(b01*G3
+b1*(max((G3-K1)/_kd_,0)**3+((K4-K1)*max((G3-K5)/_kd_,0)**3-(K5-
K1)*max((G3-K4)/_kd_,0)**3)/(K5-K4))
+b2*(max((G3-K2)/_kd_,0)**3+((K4-K2)*max((G3-K5)/_kd_,0)**3-(K5-
K2)*max((G3-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((G3-K3)/_kd_,0)**3+((K4-K3)*max((G3-K5)/_kd_,0)**3-(K5-
K3)*max((G3-K4)/_kd_,0)**3)/(K5-K4))
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))
))*lamda);

estimate "G4" exp(-(b01*G4
+b1*(max((G4-K1)/_kd_,0)**3+((K4-K1)*max((G4-K5)/_kd_,0)**3-(K5-
K1)*max((G4-K4)/_kd_,0)**3)/(K5-K4))
+b2*(max((G4-K2)/_kd_,0)**3+((K4-K2)*max((G4-K5)/_kd_,0)**3-(K5-
K2)*max((G4-K4)/_kd_,0)**3)/(K5-K4))
+b3*(max((G4-K3)/_kd_,0)**3+((K4-K3)*max((G4-K5)/_kd_,0)**3-(K5-
K3)*max((G4-K4)/_kd_,0)**3)/(K5-K4))
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4))

```

```
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
))*lamda;
```

```
estimate "G5" exp(-(b01*G5
+b1*(max((G5-K1)/_kd_,0)**3+((K4-K1)*max((G5-K5)/_kd_,0)**3-(K5-
K1)*max((G5-K4)/_kd_,0)**3)/(K5-K4)
+b2*(max((G5-K2)/_kd_,0)**3+((K4-K2)*max((G5-K5)/_kd_,0)**3-(K5-
K2)*max((G5-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((G5-K3)/_kd_,0)**3+((K4-K3)*max((G5-K5)/_kd_,0)**3-(K5-
K3)*max((G5-K4)/_kd_,0)**3)/(K5-K4)
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
))*lamda);
```

```
estimate "G6" exp(-(b01*G6
+b1*(max((G6-K1)/_kd_,0)**3+((K4-K1)*max((G6-K5)/_kd_,0)**3-(K5-
K1)*max((G6-K4)/_kd_,0)**3)/(K5-K4)
+b2*(max((G6-K2)/_kd_,0)**3+((K4-K2)*max((G6-K5)/_kd_,0)**3-(K5-
K2)*max((G6-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((G6-K3)/_kd_,0)**3+((K4-K3)*max((G6-K5)/_kd_,0)**3-(K5-
K3)*max((G6-K4)/_kd_,0)**3)/(K5-K4)
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
))*lamda);
```

```
estimate "G7" exp(-(b01*G7
+b1*(max((G7-K1)/_kd_,0)**3+((K4-K1)*max((G7-K5)/_kd_,0)**3-(K5-
K1)*max((G7-K4)/_kd_,0)**3)/(K5-K4)
+b2*(max((G7-K2)/_kd_,0)**3+((K4-K2)*max((G7-K5)/_kd_,0)**3-(K5-
K2)*max((G7-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((G7-K3)/_kd_,0)**3+((K4-K3)*max((G7-K5)/_kd_,0)**3-(K5-
K3)*max((G7-K4)/_kd_,0)**3)/(K5-K4)
-
(b01*Ref1
+b1*(max((Ref1-K1)/_kd_,0)**3+((K4-K1)*max((Ref1-K5)/_kd_,0)**3-(K5-
K1)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b2*(max((Ref1-K2)/_kd_,0)**3+((K4-K2)*max((Ref1-K5)/_kd_,0)**3-(K5-
K2)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
+b3*(max((Ref1-K3)/_kd_,0)**3+((K4-K3)*max((Ref1-K5)/_kd_,0)**3-(K5-
K3)*max((Ref1-K4)/_kd_,0)**3)/(K5-K4)
))*lamda);
```

run;

SAS Code 14: Estimating A Fractional Polynomial Term For 25(OH)D

```

libname ODINdata "/folders/myfolders/ODIN/";
DATA ODINdata.ODIN2;
SET ODINdata.ODIN;

RUN;

%mp8 (DSNAME=ODINdata.ODIN2,
YNAME=timemort,
XNAME=oldvitd,
xbin= sex monthbaseline11 monthbaseline12 monthbaseline14,
xincl= timeagebaseline bmi,
MODEL=S,
PW=-2 -1 -0.5 0 0.5 1 2 3,
M=2,
CENSVAR=death,
CENSVAL=0,
TIES=BRESLOW,
ALPHA=0.05,
MSELECT=RA2,
MACPATH=/folders/myfolders/ODIN,
DSOUT=FPOUT,
SHOWRES=d /* n */
);

DATA NFFP;
SET FPOUT;
OUT=1;
RUN;

```

SAS Code 15: Nadir Computation for Cubic Splines

```

b1_cs=3*(((K5-K1)/_kd_)+(3*((K4-K5)/_kd_)-(K5-K1)*(3*(K5-K4)/_kd_))/(K5-K4));
b2_cs=3*(((K5-K2)/_kd_)+(3*((K4-K5)/_kd_)-(K5-K2)*(3*(K5-K4)/_kd_))/(K5-K4));
b3_cs=3*(((K5-K3)/_kd_)+(3*((K4-K5)/_kd_)-(K5-K3)*(3*(K5-K4)/_kd_))/(K5-K4));

b1_cs2=3*(((K5-K1)/_kd_)**2+(3*((K4-K5)/_kd_)**2-(K5-K1)*(3*(K5-K4)/_kd_)**2)/(K5-K4));
b2_cs2=3*(((K5-K2)/_kd_)**2+(3*((K4-K5)/_kd_)**2-(K5-K2)*(3*(K5-K4)/_kd_)**2)/(K5-K4));
b3_cs2=3*(((K5-K3)/_kd_)**2+(3*((K4-K5)/_kd_)**2-(K5-K3)*(3*(K5-K4)/_kd_)**2)/(K5-K4));

b1_cs3=3*(((K1-K5)/_kd_)+(3*((K4-K5)/_kd_)-(K5-K1)*(3*(K5-K4)/_kd_))/(K5-K4));
b2_cs3=3*(((K2-K5)/_kd_)+(3*((K4-K5)/_kd_)-(K5-K2)*(3*(K5-K4)/_kd_))/(K5-K4));
b3_cs3=3*(((K3-K5)/_kd_)+(3*((K4-K5)/_kd_)-(K5-K3)*(3*(K5-K4)/_kd_))/(K5-K4));

b1_cs32=3*(((K1-K5)/_kd_)**2+(3*((K4-K5)/_kd_)**2-(K5-K1)*(3*(K5-K4)/_kd_)**2)/(K5-K4));
b2_cs32=3*(((K2-K5)/_kd_)**2+(3*((K4-K5)/_kd_)**2-(K5-K2)*(3*(K5-K4)/_kd_)**2)/(K5-K4));
b3_cs32=3*(((K3-K5)/_kd_)**2+(3*((K4-K5)/_kd_)**2-(K5-K3)*(3*(K5-K4)/_kd_)**2)/(K5-K4));

```

SAS Code 16: Implementing Quadratic Formula and Estimating Nadir for Cubic Splines

```
A = (3*(b1+b2+b3));
B = (-6*(b1*b1_cs+b2*b2_cs+b3*b3_cs));
C = (b01 + 3*(b1*b1_cs2+b2*b2_cs2+b3*b3_cs2));

A_2 = (3*(b1+b2+b3));
B_2 = (-6*(b1*b1_cs3+b2*b2_cs3+b3*b3_cs3));
C_2 = (b01 + 3*(b1*b1_cs32+b2*b2_cs32+b3*b3_cs32));

estimate "Nadir cubic splines" ((-B+sqrt((B**2)-(4*A*C)))/(2*A))*lamda*(-1);
estimate "Nadir2 cubic splines " ((-B_2+sqrt((B_2**2)-(4*A_2*C_2)))/(2*A_2))*lamda*(-1);
```

SAS Code 17: Estimating Nadir for Fractional Polynomials

```
estimate "Nadir MFP" ((2*b1)/b2)**2
```

SAS Code 18: Testing the Proportional Hazards Assumption for Continuous and Categorical Covariates.

```
proc phreg data=ODINdata.hazardCork2 noprint;
model timemort*death(0)= age;
output out = out_res XBETA=XB RESMART=Mart RESDEV=Dev RESSCO=SCO_age
RESSCH=Sch_age;
run;
```

```
Title "Schoenfeld residuals against age";
proc sgplot data = out_res;
yaxis grid;
refline 0 / axis = y;
loess y=SCH_age x=timemort/ clm;
run;
```

```
Title "Proportional hazards assumption for categorial covariates";
ods output ProductLimitEstimates = ple;
proc lifetest data=ODINdata.hazardCork2(where=(death=1)) nelson
plots=(s,ls,lls,h,p);
time timemort*death(0);
strata varCork25ohdgroups;
run;
```

SAS Code 19: Printing Plots of Cox-Snell Residuals.

```
proc lifereg data=ODINdata.hazardCork2;
model timemort*death(0)= varCork25ohdgroups1 varCork25ohdgroups2
varCork25ohdgroups3
varCork25ohdgroups4 varCork25ohdgroups6 varCork25ohdgroups7 age sex bmi
monthbaseline11
monthbaseline12 monthbaseline14/ distribution=weibull;
output out=weibull cresidual=CREs sresidual=SRES cdf=f;
run;

data weibull1;
set weibull;
cox = -log( 1-f );
run;
```

```
proc lifetest data=weibull1 outsurv=surv_wei noprint;
  time cox*death(0);
run;
data surv_wei;
  set surv_wei;
  ls = -log(survival);
run;

Title "Martingale residuals";
proc sgplot data =surv_wei;
  yaxis grid;
  scatter y=cox x=ls;
  scatter y=cox x=cox;
run;
```