Aspects of Vitamin D Deficiency in Elderly People in Nursing Homes and in Patients with Type 2 Diabetes

with Emphasis on Mortality, Cardiovascular
Morbidity and Mental Health

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Cover picture: 70-year-old man in sunlight.

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To Fredrik,
Filip and Klara

Whatever you are, try to be a good one
William Makepeace Thackeray

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ABSTRACT

Background

Institutionalised elderly people living in northern latitudes may be at elevated risk for vitamin D deficiency. They are recommended to take oral vitamin D supplements, but the main source of vitamin D is sunlight. Previous studies have shown an association between low levels of vitamin D and several diseases, but important knowledge about vitamin D in elderly people in nursing homes and in patients with type 2 diabetes is lacking. The aims of this thesis were to study aspects of vitamin D deficiency in these two populations and to explore whether low vitamin D levels were associated with mortality, cardiovascular morbidity and mental health. Also, we aimed to examine whether an intervention with encouragement to spend time outdoors during summer could increase vitamin D levels in the elderly in nursing homes.

Methods

The present thesis is based on four papers. Papers I and IV are confined to nursing home residents >65 years. Paper I is based on data from SHADES (The Study of Health and Drugs in the Elderly). Paper IV is based on the study Sunlight and Vitamin D in Older People in Nursing Homes. Papers II and III are based on data from CARDIPP (Cardiovascular Risk Factors in Patients with Diabetes—a Prospective Study in Primary Care), with patients with type 2 diabetes aged 55–66 years. Papers I–III were prospective observational cohort studies and Paper IV was a cluster randomised intervention trial.

In **Paper I**, serum 25-hydroxyvitamin D₃ (25(OH)D₃) was analysed on three occasions. The vital status of the subjects was ascertained and hazard ratios (HRs) for mortality according to baseline 25(OH)D₃ quartiles (Q) were calculated.

In **Paper II**, serum 25(OH)D₃ was analysed at baseline. HRs for the first myocardial infarction, stroke or cardiovascular mortality according to 25(OH)D₃ were calculated.

In **Paper III**, serum 25(OH)D₃ was analysed at baseline. The SF-36 questionnaires measuring vitality and mental health were administered at baseline and after four years.

In **Paper IV**, the intervention group was encouraged to go outside for 20–30 minutes every day for two months during the summer of 2018. Before and after the summer, serum 25(OH)D was analysed and SF-36 questionnaires measuring vitality and mental health were administered.

Results

In **Paper I**, 80% of the participants had 25(OH)D₃ < 50 nmol/l. Vitamin D deficiency was associated with an increased mortality risk. Compared with Q4 (25(OH)D₃ >48 nmol/l), the HR (with a 95% confidence interval (CI)) for mortality was 2.02 (1.31–3.12) in Q1 (25(OH)D₃ <29 nmol/l) ($p < 0.05$), 2.03 (1.32–3.14) in Q2 (25(OH)D₃ 30–37 nmol/l) ($p < 0.05$) and 1.6 (1.03–2.48) in Q3 (25(OH)D₃ 38–47 nmol/l) ($p < 0.05$).

In **Paper II**, serum 25(OH)D₃ was inversely associated with the risk of cardiovascular morbidity and mortality. The HR per nmol/l was 0.98 (95% CI: 0.96-0.99) (p=0.001), when adjusted for age, sex and season.

In **Paper III**, serum 25(OH)D₃ was inversely associated with poor mental health at baseline. The odds ratio (OR) for 10 nmol/l increase in 25(OH)D₃ was 0.90 (95% CI: 0.83-0.96) (p=0.003), but not at follow-up (p>0.05). Serum 25(OH)D₃ was not associated with vitality at baseline (p>0.05), nor at follow-up after adjustments.

In **Paper IV**, the 25(OH)D levels increased significantly in the intervention group during the summer: from a median (interquartile range (IQR)) of serum 25(OH)D of 42.5 (23.0) nmol/l to 53.5 (33.0) nmol/l (p=0.011). The 25(OH)D levels increased in the control group as well, but the increase was not significant. The intervention group reported better mental health after the summer compared to before the summer (p=0.015), unlike the control group.

Conclusions

Low vitamin D levels were associated with increased mortality in elderly people in nursing homes, and with cardiovascular morbidity/ mortality and poor mental health in patients with type 2 diabetes. From our studies, we cannot draw conclusions about causality. The results indicate that the vitamin D levels give prognostic information. Active encouragement to spend time outdoors during summer improved the vitamin D levels and mental health in elderly people in nursing homes, and such activity could be considered as a complement to oral vitamin D supplementation in the summer.

POPULÄRVETENSKAPLIG SAMMANFATTNING

D-vitamin bildas i huden när vi utsätts för solljus och i mindre mängder så kan vi även få D-vitamin via mat såsom fet fisk och berikade mejeriprodukter. På våra breddgrader i Sverige så tillverkar inte huden D-vitamin under vinterhalvåret (mellan oktober och mars), vilket medför att nivåerna av D-vitamin i kroppen sjunker. Äldre personer på särskilt boende löper särskilt stor risk för D-vitaminbrist och alla äldre rekommenderas att ta ett dagligt tillskott med D-vitamin. Tidigare studier har visat ett samband mellan låga D-vitaminnivåer och en rad olika sjukdomar, men det finns fortfarande kunskapsluckor bl. a. gällande äldre personer som bor på särskilt boende och patienter med typ 2-diabetes.

Syftet med denna avhandling var att studera olika aspekter på D-vitaminbrist hos dessa två grupper och att undersöka om D-vitaminnivåerna var kopplade till risken för död, hjärtkärlsjukdom och nedsatt mental hälsa. Vi ville också undersöka om man genom att aktivt uppmuntra äldre personer på särskilt boende att vara utomhus sommartid kunde öka deras D-vitaminnivåer.

I SHADES-studien (delarbete 1) visades att det fanns ett samband mellan D-vitaminbrist och en ökad risk för tidigare död hos äldre (>65 år) på särskilt boende i södra Sverige (Eslöv, Jönköping och Linköping). Risken att dö var dubbelt så stor hos de två fjärdedelar av studiedeltagarna som hade lägst och näst lägst D-vitaminvärden jämfört med den fjärdedel som hade högst värden. D-vitaminbrist var mycket vanligt, då 80% av studiedeltagarna hade för lågt D-vitaminvärde.

I CARDIPP-studien visades att det fanns ett samband mellan låga D-vitaminnivåer och risken för hjärtkärlsjukdom (hjärtinfarkt och stroke) och död pga hjärtkärlsjukdom hos medelålders (55-66 år) patienter med typ 2-diabetes (delarbete 2).

I CARDIPP-studien sågs även ett samband mellan låga D-vitaminnivåer och nedsatt mental hälsa utifrån svar på en enkät som heter SF-36 (delarbete 3). Patienterna fick svara på samma enkät 4 år senare, men det sågs inget samband mellan D-vitaminvärdet när de gick in i studien och den

mentala hälsan vid uppföljningen. Ett annat område som enkäten undersökte var vitalitet, men något tydligt samband mellan D-vitaminnivåerna och patienternas vitalitet kunde inte visas.

I studien Solljus och D-vitamin hos äldre på särskilt boende (delarbete 4) visades att den grupp av de äldre på särskilt boende som blev aktivt uppmuntrade till att vara utomhus 20-30 minuter mitt på dagen varje dag under 2 månader sommaren 2018 fick ökade D-vitaminnivåer och bättre mental hälsa enligt enkäten SF-36, medan vi inte såg lika tydlig skillnad i D-vitaminnivå eller mental hälsa hos den grupp som inte fick denna uppmuntran.

Sammanfattningsvis så fann vi ett samband mellan låga D-vitaminnivåer och ökad risk för tidigare död hos äldre på särskilt boende samt mellan låga D-vitaminnivåer och hjärtkärlsjukdom och nedsatt mental hälsa hos patienter med typ 2-diabetes. Detta behöver dock inte betyda att det är de låga D-vitaminnivåerna som är den bakomliggande orsaken, men dessa studier antyder att D-vitaminvärdet ändå kan ge information om prognosen. Eftersom aktivt uppmuntrande till att spendera tid utomhus på sommaren ledde till ökade D-vitaminnivåer och förbättrad mental hälsa hos äldre på särskilt boende, så kan man överväga detta som ett komplement till D-vitamintabletter hos de äldre på sommaren.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. **Samefors M**, Östgren CJ, Mölsted S, Lannering C, Midlöv P, Tengblad A. Vitamin D deficiency in elderly people in Swedish nursing homes is associated with increased mortality. *Eur J Endocrinol* 2014;170(5):667-75.
- II. **Samefors M**, Scragg R, Lanne T, Nystrom FH, Ostgren CJ. Association between serum 25(OH)D₃ and cardiovascular morbidity and mortality in people with type 2 diabetes: a community-based cohort study. *Diabet Med* 2017;34(3):372-9.
- III. **Samefors M**, Scragg R, Nystrom FH, Östgren CJ. Is there an association between serum 25(OH)D₃ and mental well-being in patients with type 2 diabetes? Results from a cohort study in primary care. *Hormones* 2020;19(3):361-7.
- IV. **Samefors M**, Tengblad A, Östgren CJ. Sunlight exposure and vitamin D levels in older people- an intervention study in Swedish nursing homes. *J Nutr Health Aging* 2020. DOI 10.1007/s12603-020-1435-z (Online ahead of print).

ABBREVIATIONS

AIC	Akaike Information Criteria
BMI	Body Mass Index
CARDIPP	Cardiovascular Risk Factors in Patients with Diabetes—a Prospective Study in Primary Care
CI	Confidence Interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DBP	Vitamin D-Binding Protein
DFRI	The Downton Fall Risk Index
D2d trial	The Vitamin D and Type 2 Diabetes Trial
FGF-23	Fibroblast Growth Factor 23
GFR	Glomerular Filtration Rate
GP	General Practitioner
HbA1c	Haemoglobin A1c
HDL	High-Density Lipoprotein
HPLC	High Performance Liquid Chromatography
HR	Hazard Ratio
ICD-10	International Classification of Diseases, 10th Version
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IOM	Institute of Medicine
IQR	Interquartile Range
IU	International Unit
LC–MS	Liquid Chromatography with Mass Spectrometry
LDL	Low-Density Lipoprotein
MACE	Major Adverse Cardiovascular Events
MD	Medical Doctor
MNS	Modified Norton Scale
N	Number of People

NS	Non-Significant
OR	Odds Ratio
PhD	Doctor of Philosophy
PTH	Parathyroid Hormone
Q	Quartile
RCT	Randomised Clinical Trial
RR	Relative Risk
SD	Standard Deviation
SHADES	The Study of Health and Drugs in the Elderly
SMHI	The Swedish Meteorological and Hydrological Institute
SNP	Single Nucleotide Polymorphism
UV	Ultraviolet
UVB	Ultraviolet B
VDR	Vitamin D Receptor
ViDA	Vitamin D Assessment Study
VIDAL	Vitamin D and Longevity trial
VITAL	The VITamin D and OmegA-3 Trial
VLDL	Very-Low-Density Lipoprotein
WHO	World Health Organization
1-OHase	25-Hydroxyvitamin D-1 α -Hydroxylase
1.25(OH) ₂ D	1.25-Dihydroxyvitamin D
24-OHase	25-Hydroxyvitamin D-24-Hydroxylase
25(OH)D	25-Hydroxyvitamin D

PROLOGUE

Eight years ago, when I was a GP registrar, I became interested in vitamin D and thought it was exciting with its possible links to various health problems. Was it possible that vitamin D deficiency, which is common at our northern latitudes due to lack of sunlight, could explain some of the health problems that I met every day as a doctor in general practice?

When it was time for me to do the mandatory individual written scientific work that was included in the specialist medical training programme, my wish was to write about vitamin D. I asked Professor Sigvard Mölstad, at that time research leader of the Primary Care Unit of Research and Development at Futurum, Jönköping County Region, about it and he introduced me to SHADES. I wrote my individual scientific work based on data from SHADES and found it interesting. Eventually, this work developed to my first paper and is included in this thesis. Anders Tengblad, MD, PhD, and at the time GP at the same primary health care centre as me, was my supervisor in this work and he inspired and guided me. At a research meeting, I met Professor Carl Johan Östgren, who encouraged me to start my research. I was enrolled as a PhD student at Linköping University in May 2013.

Doctoral studies and research are a journey and I am glad that I began that journey. One great inspiration in my research was the opportunity to be part of the National Research School in General Practice, and from this I had the opportunity to go on a pre-doc stay at the School of Population Health in Auckland, New Zealand in 2015 with Professor Robert Scragg as my supervisor. This was very instructive and inspiring for my research, and a memory for life. Another highlight during my time as a PhD student has been to complete Paper IV. To carry out all the different parts of the study from the beginning to the end, of course with good guidance from my supervisors, has taught me a lot.

I believe that clinical work as a doctor is good for research, and that the research is good for the clinical work and patient care. Primary care is complex. I believe that it is important that medical research is performed in the complex context of general practice, as well as in all specialist categories, as this results in greater benefits for our patients.

INTRODUCTION

Vitamin D

Vitamin D (calciferol) is a category of steroid-like substances, including the main forms vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) and their hydroxylated derivatives. Despite the name, vitamin D is not a true vitamin. Instead, it is more accurate to consider it a steroid hormone precursor, because of the human body's capacity for endogenous synthesis of vitamin D₃ through UV exposure of the skin (1). The major role of vitamin D is to increase the intestinal absorption of calcium for the mineralisation of the skeleton. Also, vitamin D is essential for the regulation of calcium levels in the blood. In the case of severe vitamin D deficiency, the newly formed bone is not mineralised, causing rickets in children and osteomalacia in adults. Less severe vitamin D deficiency leads to increased serum PTH, bone resorption, osteoporosis and increased risk of fractures (2). In recent years, vitamin D has been found to have extra-skeletal effects, and possible associations between vitamin D and several other diseases have been found.

History

Vitamin D is one of the oldest hormones. For over 500 million years, it has been produced in ocean-dwelling phytoplankton and zooplankton when exposed to sunlight (3, 4).

Rickets was clearly described for the first time by Whistler, Boot, Glisson and Mayow in the 17th century, as they had observed that children in cities in Europe developed skeletal deformities and growth retardations (4, 5). The incidence of rickets increased during the industrial revolution, when people settled down in cities close to each other. Also, as a result of the air pollution due to coal and wood combustion, children in the industrialised cities had little direct exposure to sunlight. By the end of the 19th century, manifestations of rickets were highly prevalent both in Europe and North America. The association between rickets and lack of sunlight was first recognised at the beginning of the 20th century and treatment of rickets with sunshine was reported (3, 6). A few years later, food was subjected to UV radiation for the treatment and prevention of rickets, followed by fortification of food with synthetically produced vitamin D (3). Many researchers contributed to the discovery of vitamin D, among them the German steroid chemist Adolf Windaus who made a decisive contribution and won the

1928 Nobel prize in chemistry “for his studies on the constitution of the sterols and their connection with vitamins”(7).

In Sweden, rickets, also known as *engelska sjukan* (the English disease), has been known since the middle of the 18th century (8). For the last 80 years, small children have been recommended vitamin D as protection against rickets, first in cod liver oil and later in concentrated oil (9). The recommendation of drops containing vitamin D to all small children was clearly given by the Swedish authority the National Board of Health and Welfare in an announcement 1978 (10) and is still current.

Sources of Vitamin D

The main source of vitamin D is exposure to sunlight, which accounts for more than 90% of the body’s vitamin D supply. In lesser amounts, vitamin D can be obtained from the diet (4, 11).

Dermal Synthesis of Vitamin D through UV Radiation

When UVB radiation (wavelengths of 290-315 nm) from sunlight penetrates the skin, 7-dehydrocholesterol is converted to pre-vitamin D₃, which is then converted to vitamin D₃ (cholecalciferol). The cutaneous vitamin D synthesis is impacted by several environmental and individual factors. Examples of environmental factors impacting the available UV radiation are latitude, season, time of the day, the amount of ozone, clouds and aerosols in the atmosphere and the reflectivity of the surface. Examples of individual factors are skin type, age, clothing and sunscreen use (12). At our northern latitudes, dermal vitamin D production does not occur during October to March, as during this period the sun is not high enough in the sky for its UVB rays to penetrate the atmosphere (13, 14). A systematic review and meta-analysis of factors of importance for the cutaneous synthesis of 25(OH)D showed that the UV dose and the baseline 25(OH)D concentration had a significant impact on the achieved 25(OH)D level (15). Another study reported that the size of the exposed skin area, UVB dose, skin erythema and BMI were the major determinants of dermal production of vitamin D after UVB exposure (16). Exposure of face and hands induced smaller 25(OH)D₃ production in comparison with exposure of larger skin areas, contrary to another study showing a more effective dermal production in the face and the hands than in other body areas, suggesting an evolutionary adaptation for preventing vitamin D deficiency (17).

For people with fair pigmentation, it is believed that direct sunlight exposure of the head, neck and bare arms for 6-8 minutes 2-3 times a week in June and July in Sweden is enough for a vitamin D₃ production

corresponding to 5-10 micrograms/day of vitamin D₃. For people with dark pigmentation up to 15 minutes would be needed (18, 19).

Vitamin D in Food

Vitamin D₃ is found in fatty fishes such as salmon, herring, mackerel and tuna, cod liver oil and other fish oils, egg yolks and meat products such as liver. Vitamin D₂ is found in small quantities in yeast and wild mushrooms (11, 20).

In several countries, including Sweden, some foods, especially dairy products, are fortified with vitamin D and are therefore important vitamin D sources. In Sweden, all milk, sour milk products and lactose-free products with fat <3%, vegetable-based alternatives and solid/ fluid margarine are mandatorily fortified with vitamin D (21). The Nordic countries share common nutritional recommendations (19), but there are large differences between the countries in how the recommendations are implemented and which policies (including fortification and recommendations of supplements) are used to achieve the recommended vitamin D intake and an adequate vitamin D status (22). Based on the latest version of the Nordic Nutrition Recommendations 2012 (19), the Swedish Food Agency recommends all people have a daily vitamin D intake of 10 micrograms, except adults >75 years and people with little/ no sun exposure who are recommended to have 20 micrograms. To meet this need, a daily oral vitamin D supplement is recommended for children <2 years, people >75 years, vegetarians, people not eating fish or fortified food and people with little/ no sun exposure (18). The recommendations are given on the basis of evidence of a protective effect of vitamin D on bone health, total mortality and fall risk (19). One IU corresponds to 0.025 micrograms of vitamin D, for example: 400 IU are equal to 10 micrograms.

The effect of vitamin D supplementation on serum 25(OH)D concentration was explored in a systematic review with individuals >50 years, given daily doses of 5-250 micrograms (median 20 micrograms) for 1 to 60 months (median 8.5 months). Among community-dwelling people, the average increase in serum 25(OH)D was 1.95 nmol/l per one microgram given vitamin D₃ without calcium per day. Supplementation with vitamin D₂ was associated with lower increases in serum 25(OH)D concentrations than vitamin D₃ (23).

Vitamin D Metabolism

Vitamin D₂ and Vitamin D₃ from the diet are incorporated into chylomicrons which are further processed into lipoproteins (e.g. VLDL and LDL)

and transported by the lymphatic system to the venous circulation (1). Vitamin D (representing both D₂ and D₃) is fat soluble and can be stored in adipose tissue (11), but the depot is not large enough to prevent seasonal changes in vitamin D concentrations (14). In the blood circulation, vitamin D is bound to the DBP and transported to the liver, where it is metabolised into calcidiol: 25(OH)D, by the enzyme vitamin D-25-hydroxylase (Figure 1). Serum 25(OH)D is the major circulating form of vitamin D. As 25(OH)D is biologically inactive, it must be converted into the biologically active form, namely the hormone calcitriol: 1,25(OH)₂D by the enzyme 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1, 1-OHase) in the kidneys. The production of 1,25(OH)₂D is stimulated by PTH from the parathyroid glands and is carefully regulated also by its own negative feedback, by levels of calcium and phosphorus in the blood and by levels of FGF-23 secreted from the bone. The 1,25(OH)₂D increases the enzyme 25-hydroxyvitamin D-24-hydroxylase (24-OHase), which catabolises 25(OH)D and 1,25(OH)₂D into biologically inactive calcitroic acid, which is excreted in the bile.

The main target organs of 1,25(OH)₂D are the kidneys, the intestine and the bone. In the small intestine, 1,25(OH)₂D increases the absorption of calcium and phosphorus. In the bone, 1,25(OH)₂D interacts with the osteoblasts, which induces the pre-osteoclasts to become mature osteoclasts. The osteoclasts resorb calcium and phosphorus from the bone and release calcium and phosphorus into the blood. Adequate levels of calcium and phosphorus are important for mineralisation of the bone (11).

Serum 25(OH)D has a half-life of approximately two weeks (24), compared to 1,25(OH)₂D with a half-life of approximately four hours (25). Another study showed a longer half-life of serum 25(OH)D of about two months (15).

Figure 1. Synthesis and metabolism of vitamin D in the regulation of calcium, phosphorus and bone metabolism. Figure from Holick 2007: Vitamin D deficiency. Reproduced with permission from the Massachusetts Medical Society, publisher of the New England Journal of Medicine.

Vitamin D Receptor

The $1.25(\text{OH})_2\text{D}$ exerts its effects through the VDR, which is located in the nucleus of the vitamin D target cells where it regulates expressions of genes whose products control different biological functions including calcium and phosphate homeostasis, bone remodelling, immunomodulation and cell differentiation. Initially, the VDR was discovered in the intestine, bone, kidney, and parathyroid glands (26). In recent years, the VDR has been detected in several other tissues and organs with non-calcium-regulating cell types (27, 28). Also, the expression of 25-hydroxyvitamin D-1 α -hydroxylase has been found in a large number of tissues, which enables active vitamin D synthesis in a paracrine way; and a large number of genes have been found to be under the control of $1.25(\text{OH})_2\text{D}$. Together, this enables a wide variety of additional extra-skeletal vitamin D hormone functions (11, 29, 30). Apart from the classical slow pathway of $1.25(\text{OH})_2\text{D}$ activating this nuclear receptor which alters gene transcription and subsequently regulates cellular activity, there is also evidence of rapid non-genomic membrane signalling through a receptor localised in plasma membrane caveolae in some cells, for example regulating ion channel activity (31, 32). The physiological consequences of the non-genomic pathway are not fully understood (30).

Vitamin D Deficiency

The optimal level of serum 25(OH)D is controversial. However, most experts including the IOM in the United States define vitamin D deficiency as a serum 25(OH)D level below 50 nmol/l (11, 33, 34). Others consider serum levels below 75 nmol/l to be insufficient (25). The reason for this is that the serum 25(OH)D levels are inversely associated with the PTH levels until the serum 25(OH)D reaches a point of approximately 75 nmol/l, where the PTH levels begin to level off (35, 36). However, studies have shown a large variation in the plateau level of PTH (33, 37). In different individuals and in different clinical settings, PTH can be at slightly different levels at comparable 25(OH)D status. Not only the 25(OH)D levels are of importance for the regulation of PTH, but also for example the renal function and the calcium levels (38). The dissociation sometimes seen between 25(OH)D and PTH levels may also be due to the not fully understood interaction between the endocrine and the paracrine vitamin D systems (39).

As vitamin D deficiency causes secondary hyperparathyroidism, it leads to increased bone turnover, bone mineral loss and increased risk of fractures. Serum 25(OH)D levels of 25-50 nmol/l are associated with slightly elevated serum PTH levels, often within the normal reference ranges. Serum 25(OH)D levels of <25 nmol/l are associated with moderately increased PTH levels and increased bone turnover. In the case of severe vitamin D

deficiency, the serum PTH is greatly elevated, and the bone mineralisation is incomplete leading to osteomalacia in adults or to rickets in children (40).

Vitamin D deficiency may have many causes, including behavioural factors such as vegan diet, clothing and sun avoidance. Vitamin D deficiency can also have specific causes such as reduced skin synthesis, for example because of ageing or skin pigmentation, decreased bioavailability because of obesity or malabsorption e.g. due to intestinal diseases, increased catabolism because of drugs such as anticonvulsants or glucocorticoids, decreased production of 25(OH)D because of liver failure, increased urinary loss of 25(OH)D because of nephrotic syndrome, decreased production of 1,25(OH)₂D because of chronic kidney disease, heritable forms of rickets or acquired disorders such as tumour-induced osteomalacia, primary hyperparathyroidism, granulomatous disorders or hyperthyroidism (11).

Prevalence of Vitamin D Deficiency

Vitamin D deficiency is highly prevalent in people worldwide (41). Population-based studies have estimated that 20% of the adults in Australia (42) and 29% of the adults in the USA (43) are vitamin D deficient (25(OH)D <50 nmol/l), as well as 40% of the young and adult individuals in Europe (44). However, there are large differences within Europe. Studies have shown that people in the Nordic countries often have better vitamin D status than people in the countries around the Mediterranean. This is despite the higher latitudes and lower UV radiation from the sun in the Nordic countries and it is speculated that this may be due to increased sun seeking behaviour and nutritional factors (45). Studies have shown that the mean 25(OH)D concentrations among adults in the Nordic countries were 60–65 nmol/l, except for lower concentrations in Iceland. Seasonal variations were seen. However, despite the adequate mean concentrations, a notable proportion had 25(OH)D <50 nmol/l, especially in risk groups, for example immigrant groups (22).

Vitamin D Toxicity

Vitamin D intoxication has been observed for serum 25(OH)D concentrations above 374 nmol/l (11), but concerns about risks for potential adverse effects have been raised at serum 25(OH)D levels above 125 nmol/l (34). Vitamin D intoxication is very rare, but might lead to pronounced hypercalcaemia, hyperphosphatemia, renal failure and in the worst case death. Vitamin D intoxication is caused by ingestion of very high oral doses of vitamin D for a prolonged period of time (46, 47). Sunlight itself cannot cause vitamin D intoxication. In the case of excessive sunlight exposure, the UVB

radiation degrades the overbalance of previtamin D₃ and vitamin D₃ into inactive photoproducts (11). However, excessive sunlight exposure may damage the skin and increase the risk of skin cancer.

Measuring Vitamin D Status

Serum 25(OH)D is considered the best biomarker of the vitamin D status and is measured in clinical praxis (11). It is believed that serum 1.25(OH)₂D does not reflect the vitamin D status, so measurement of serum 1.25(OH)₂D is only indicated in special situations such as VDR defects, vitamin D intoxication and severe vitamin D deficiency (48). Serum 25(OH)D was also the most reliable and robust marker of the vitamin D status, when compared to other biomarkers such as PTH, bone turnover markers, bone mineral density and calcium absorption (49).

There are two main types of routinely used methods to measure 25(OH)D: immunoassays and methods based on chromatographic separation followed by non-immunological direct detection (HPLC, LC-MS/MS) (50, 51). Large differences in the results of the 25(OH)D assays have been reported and the reliability of the 25(OH)D measurements has been questioned. An international standardisation project (Vitamin D Standardisation Program) aims to standardise the serum 25(OH)D concentration measurements both in clinical and research laboratories, in order to facilitate pooling of data in systematic reviews (52).

The 25(OH)D concentration is reported in units of nmol/l, except in the United States where it is reported in ng/ml. The values in ng/ml can be converted (approximately) into nmol/l, using the formula: ng/ml $\times$ 2.5 $\approx$ nmol/l.

Specific Aspects of Vitamin D

Vitamin D and Older People

Vitamin D deficiency is highly prevalent in older people (40), especially in people living in nursing homes (53-58). However, there may be differences between different groups. One study showed that vitamin D deficiency was common in elderly (>75 years old) Swedish people in four high-risk groups (patients with hip fracture/ stroke patients/ frequent emergency ward visitors/ nursing home residents), especially in the nursing home subgroup, but healthy participants in an age-matched control group had adequate vitamin D levels (59).

Possible explanations for the high prevalence of vitamin D deficiency in elderly might be that elderly people often stay indoors, and that they have dietary deficiency and decreased cutaneous production of vitamin D with increased age, due to a reduction of the amount of 7-dehydrocholesterol in the skin (6). The intestinal absorption of dietary vitamin D is preserved even at advanced age. The metabolism remains relatively normal, but might be affected by chronic diseases, and the formation of $1.25(\text{OH})_2\text{D}$ in the kidneys can be restricted by renal function impairment (40). Despite this, sunlight is still an important vitamin D source for older people. A Dutch study with community-dwelling older people showed that sun exposure, vitamin D intake and vitamin D-related genes substantially contributed to the variation in serum $25(\text{OH})\text{D}$, and of those three factors, sun exposure was the most important (60).

Vitamin D and Mortality

Several systematic reviews/ meta-analyses of observational studies have shown an association between low levels of vitamin D and an increased risk of all-cause mortality (61-65). In a German study, low vitamin D levels were associated with increased all-cause mortality also among people who reported their health status to be good to excellent (66). Several studies that found an inverse association between the vitamin D levels and mortality also controlled for prior diseases or excluded individuals with certain chronic diseases at baseline (61). A small number of studies have shown an increased risk of all-cause mortality not only at low, but also at high $25(\text{OH})\text{D}$ concentrations, and they refer to it as a U-shaped association (67) or a reverse J-shaped association (68). However, most of the studies show a decreasing risk of all-cause mortality for increasing $25(\text{OH})\text{D}$ below a threshold concentration, above which the risk remains stable (61, 62).

A Cochrane review from 2014 showed that vitamin D supplementation decreased mortality in adults (RR 0.97 (95% CI: 0.94-0.99), $p = 0.02$). When analysing vitamin D_2 and vitamin D_3 separately, only vitamin D_3 decreased mortality (69). Another systematic review from 2014 also suggested a small effect of vitamin D supplementation on all-cause mortality, but with remaining uncertainty in the analysis (70). A recent systematic review and meta-analysis showed no association between vitamin D supplementation and all-cause mortality in adults compared with placebo or no treatment, but supplementation with vitamin D_3 reduced the risk of cancer related death (71). Major studies are underway. A double-blind, randomised, placebo-controlled trial called the D-Health trial is ongoing, studying the effect of monthly 60 000 IU vitamin D_3 vs. placebo for five years, in 21 315 participants in Australia. The primary outcome is all-cause mortality, and

secondary outcomes are total cancer incidence and specifically colorectal cancer incidence (72). The VIDAL trial will analyse the effect of intermittent high dose vitamin D supplementation for five years on all-cause mortality in 20 000 adults (65-84 years old) in the United Kingdom (73).

Vitamin D and Cardiovascular Disease

Since the hypothesis that vitamin D may prevent cardiovascular disease was presented in 1981 (74), several studies have explored the relationship between 25(OH)D and cardiovascular disease in general populations. A meta-analysis of prospective studies has shown an inverse association between 25(OH)D and the risk of cardiovascular disease (75). A Swedish study with rural men with a follow-up time of 20 years showed that the relative risk of ischaemic heart disease was halved in the quartile with the highest vitamin D level compared with the participants in the lowest quartile (76).

The fact that the VDR is present in vascular smooth muscle, cardiomyocytes and immune cells enables the vitamin D to affect the cardiovascular system. Potential mechanisms behind the relationship between vitamin D deficiency and vascular disease include chronic inflammation, endothelial dysfunction, vascular calcification and regulation of the renin-angiotensin system (77). The exact vascular role of vitamin D in the development of atherosclerosis, myocardial infarction and stroke is not fully understood. Associations between vitamin D deficiency and other risk factors for cardiovascular disease such as obesity, diabetes mellitus, dyslipidaemia and hypertension have been found, which is why other mechanisms are also plausible (78). On the other hand, a possible acceleration of atherosclerosis and vascular calcification because of accumulation of an excess of diet-derived 25(OH)D₃ along with the transporting lipoproteins in the artery walls and atherosclerotic plaques has also been suggested (1).

The randomised controlled trials to date have not shown cardiovascular benefits of vitamin D supplementation in the general population. The ViDA study investigated the effect of monthly 100 000 IU vitamin D₃ vs. placebo in 5110 participants aged 50-84 years in New Zealand, with a median follow-up time of 3.3 years. They found no effect of vitamin D supplementation on the main outcomes: cardiovascular disease, acute respiratory infections, non-vertebral fractures, falls and all cancer. However, some beneficial effects were seen regarding bone mineral density, lung function and arterial function, mainly in participants with 25(OH)D concentrations < 50 nmol/l (79). The VITAL study studied the effect of vitamin D₃ (2000 IU/day) and omega-3 fatty acids vs. placebo on cancer and cardiovascular disease in 25 871 individuals in the United States, with a median follow-up

time of 5.3 years. They found no reduction in invasive cancer incidence or cardiovascular events in mostly non-deficient people (80). In a meta-analysis, no association between vitamin D supplementation and cardiovascular disease measured as MACE, myocardial infarction, stroke, or cardiovascular or all-cause mortality was seen (81). However, the effects of vitamin D supplementation may differ among individuals and groups due to differences in body composition, environmental factors, and genetic variations in the DBP and the VDR, which is why a personalised medicine approach is important (1).

Vitamin D and Type 2 Diabetes

Several prospective observational studies have demonstrated an association between low levels of vitamin D and increased incidence of type 2 diabetes (82, 83) and increased insulin resistance (84-86).

The effect of vitamin D on the glucose homeostasis is plausible, as the VDR is present in the insulin-producing β -cells in the pancreas. $1.25(\text{OH})_2\text{D}$ may regulate the β -cell function by different mechanisms, e.g. by influencing insulin secretion by regulating the intracellular calcium ion level and increasing the resistance of the β -cells to apoptosis (87). The association between vitamin D deficiency and insulin resistance might be mediated through inflammation, since studies show an immunomodulating role of vitamin D (88) and since a chronic, low-grade inflammation in the pancreatic islets is believed to be a part of the etiopathology of type 2 diabetes (89). Genetic variations in the DBP and the VDR may also predispose for type 2 diabetes (90), but a Mendelian randomisation study found no significant association between SNPs within or near $25(\text{OH})\text{D}$ -related genes and the risk of type 2 diabetes, leading to questioning of whether the association between $25(\text{OH})\text{D}$ deficiency and type 2 diabetes is causal (91).

The D2d trial tested whether vitamin D supplementation at a dose of 4000 IU/day reduced the risk of type 2 diabetes among adults with prediabetes compared with placebo. They included 2423 participants with a median follow-up of 2.5 years. The vitamin D supplementation did not result in a significantly lower risk of diabetes compared to placebo. However, a high percentage of the participants had adequate vitamin D concentrations at baseline, which may have prevented detection of a significant effect in the entire cohort. A post hoc analysis implied a lower risk of diabetes in the vitamin D supplementation group among the participants with a baseline serum $25(\text{OH})\text{D}$ level below 30 nmol/l (92). Another randomised clinical trial as part of the Tromsø Study with 511 individuals with prediabetes and mostly with an adequate vitamin D status at inclusion, did not show any

positive effect, for the five years, of vitamin D 20 000 IU/week on the risk of developing type 2 diabetes or measures of glucose metabolism, serum lipids, or blood pressure compared with placebo (93).

Vitamin D and Mental Health

The idea of vitamin D deficiency being a cause of depression is appealing, as the seasonality in some forms of depression, usually with mood changes during autumn and winter, often coincides with the annual decline in serum 25(OH)D levels. Systematic reviews and meta-analyses have shown an association between low vitamin D levels and risk of depression (94, 95). A recent meta-analysis of prospective cohort studies that examined the association between serum 25(OH)D levels and the risk of depression in older adults has shown similar results, with an inverse association between serum 25(OH)D and the risk of depression. Every 25 nmol/l increase in serum 25(OH)D was associated with a 12% decrease in the depression risk (96).

A meta-analysis of randomised controlled trials about the effect of vitamin D supplementation on depression or depressive symptoms in adults did not show any significant reduction in depression after vitamin D supplementation, but most of the studies included people with low levels of depression and sufficient vitamin D levels. The results were also complicated by the heterogeneity in vitamin D doses and in times of intervention (97).

Possible autocrine/ paracrine effects of vitamin D in the brain are suggested, as the VDR (98) and the enzyme 25-hydroxyvitamin D₃-1 α -hydroxylase required for production of 1.25(OH)₂D₃ (99), are distributed throughout the human brain. The active hormone can also be enzymatically eliminated in the brain. Vitamin D may influence brain functions through various mechanisms. There is preclinical evidence that vitamin D can alter neurotransmitter expressions, for example regulating catecholamine levels through the rate-limiting enzyme for dopamine, tyrosine hydroxylase (100), which might be of importance for the development of depression. In depression, the glucocorticoid signalling may be dysregulated and an interaction between vitamin D and glucocorticoids in the hippocampus has been found (101). Another possible link between vitamin D deficiency and depression is a low-grade inflammation, as vitamin D may have a modulatory effect on pro-inflammatory cytokines and down-regulates inflammatory mediators in the brain (102). Vitamin D has also been shown to regulate neurotrophic factors such as nerve growth factor, to affect neuroplasticity processes such as axogenesis, and to have a neuro-protective effect, for example by inducing the synthesis of Ca²⁺ binding proteins, and it may inhibit

the synthesis of inducible nitric oxide synthase involved in ischaemia or in neurodegenerative conditions (103). A direct neuroregulatory effect is also possible, as genetic variance in the VDR gene has been shown to influence cognitive functions and the prevalence of depressive symptoms in older individuals (104). However, a Mendelian randomisation study found no significant association between the studied SNPs associated with 25(OH)D concentrations and depression/ depressive symptoms, indicating no causal relationship between genetically determined 25(OH) D concentrations and the onset of depression/ depressive symptoms. However, a limitation was that the analysed SNPs only explained 2.8% of the variance of vitamin D concentrations (105). Another plausible explanation is that depressive disorders lead to a poor diet and less time outdoors, resulting in less sunlight exposure and lower 25(OH)D concentrations i.e., it is possible that low vitamin D levels are not the cause but the effect of the depression.

Older People in Nursing Homes

The structure of the Swedish population has changed greatly during the last hundred years. The average life expectancy has increased gradually; in 2019 it was 84.7 years for women and 81.3 years for men (106).

In Sweden, 6% of all women and 4% of all men >65 years lived in a nursing home (*särskilt boende*) in 2019. In the oldest age group (>90 years), 36 % of all women and 25 % of all men lived in a nursing home in 2019. Overall, 108 500 people lived in nursing homes and two-thirds of them were female (107). The proportion of elderly that get social assistance, for example nursing home stays, is decreasing and there is a shift towards care at home. One year after moving to a nursing home, 41% of the men and 29% of the women have passed away, which indicates that people living in nursing homes in Sweden are seriously ill and/or have a great need for nursing (108). This is an important, but difficult patient category to study, not least due to the high co-morbidity in this population.

Since the new millennium, approximately 90 000 people in Sweden have died every year. In 2019, the most common cause of death in Sweden was cardiovascular disease, which caused over 31% of all deaths in both men and women. The second most common cause of death was tumour diseases, which caused 25% of the deaths in women and 28% of the deaths in men in 2019. Other common causes of death were mental illnesses and behavioural disorders such as dementia, respiratory diseases, neurological disorders and external causes of death (109).

Type 2 Diabetes

The disease diabetes mellitus has been known since ancient times. The name diabetes comes from a Latin and Greek word meaning *to pass through* (110), referring to the excessive discharge of urine, and the name mellitus comes from a Latin word meaning *sweetened with honey* (111), together referring to the sweetness of the urine in people with diabetes.

Diabetes mellitus is a major and growing health issue. In 2019, it was estimated that 463 million people aged 20-79 years (9.3% of all adults in this age group) in the world had diabetes, with the majority of the people living in low- and middle-income countries. The number of adults in the same age group living with diabetes in 2030 is projected to be 578 million and in 2045 to 700 million. Undiagnosed diabetes is very common: it is estimated that 50% of the people with diabetes are unaware of the disease. The vast majority of all diabetes mellitus worldwide is type 2 diabetes, accounting for around 90%.

Diabetes mellitus occurs when the blood glucose levels are elevated, because of an inability of the pancreas to produce any or enough of the hormone insulin or because the body cannot effectively use the produced insulin. In type 2 diabetes, the blood glucose is initially elevated because of insulin resistance, which means that the body's cells are unable to respond fully to the insulin. With time, this situation prompts an increase in insulin production, which might lead to failure of the pancreatic beta cells to keep up with the increasing demand, eventually leading to insufficient insulin production. Type 2 diabetes is strongly linked with obesity, as well as with increased age, ethnicity and family history, even if the causes are not fully understood.

The threshold levels for the diagnosis of diabetes is shown in Figure 2, together with threshold levels for IGT and IFG (intermediate hyperglycaemia), which are conditions with blood glucose levels above the normal range but below the threshold levels for diabetes (112).

Figure 2. Threshold levels for the diagnosis of diabetes. Figure from the International Diabetes Federation, IDF Diabetes Atlas, 9th edition. Reproduced with the permission of IDF. The values given for the oral glucose tolerance test apply to venous blood tests.

The diagnostic criteria for diabetes and intermediate hyperglycaemia are based on the recommendations from WHO (113), and the recommendations have been reviewed and updated by WHO and IDF (114). There are small, but important differences between the recommendations from ADA and WHO, for example with a lower limit for the fasting glucose level in the diagnosis of IFG (5,6–6,9 mmol/l) in the ADA recommendations (115, 116). The role of HbA1c in diagnosing diabetes has been discussed (117). HbA1c as a diagnostic test for type 2 diabetes is included in the Swedish guidelines: at least two elevated HbA1c values, or one elevated HbA1c value together with one elevated plasma glucose value as above, is diagnostic for diabetes.

Diabetes mellitus can lead to serious complications, especially if the disease is left unchecked or poorly controlled over a long time. The complications

might be macrovascular due to damage in the larger blood vessels, i.e. cardiovascular diseases such as coronary heart disease, ischaemic stroke, hypertension, diabetes cardiomyopathy and peripheral arterial disease. Other complications may be microvascular due to damage in the small blood vessels, i.e. neuropathy, nephropathy and retinopathy, and the latter might lead to blindness. With good management of type 2 diabetes, commonly referred to as an intensive multifactorial risk strategy, serious complications can be delayed or even prevented. The basis of such treatment is promoting a healthy lifestyle including regular physical activity, weight loss in case of overweight, and smoking cessation in case of smoking. The next treatment step is oral antidiabetic medication with metformin as the first treatment, followed by several different available therapy options. If this is not sufficient to control the blood glucose levels, insulin injection therapy may be needed. It is also crucial to carefully manage blood pressure and lipid levels (112).

AIMS

General Aim

To study aspects of vitamin D deficiency in elderly people in nursing homes and in patients with type 2 diabetes, and to explore whether low vitamin D levels are associated with mortality, cardiovascular morbidity and mental health.

Specific Aims

- To investigate whether low vitamin D levels are associated with an increased mortality risk in elderly people in Swedish nursing homes, to investigate the extent to which elderly people in nursing homes have vitamin D deficiency and how the levels of vitamin D change over time.
- To explore the association between vitamin D levels and cardiovascular morbidity and mortality in patients with type 2 diabetes.
- To explore the association between vitamin D levels and mental well-being in terms of vitality and mental health in patients with type 2 diabetes.
- To determine whether an intervention with encouragement to spend time outdoors during summertime can increase the levels of vitamin D in elderly people living in nursing homes.

MATERIAL AND METHODS

The present thesis is based on four papers. An overview is presented in Table 1.

Table 1. Overview of Studies included in the Thesis.

Paper	Study design	Study population and sample size	Data analyses
Paper I: Vitamin D deficiency in elderly people in Swedish nursing homes is associated with increased mortality.	A prospective observational cohort study.	333 people >65 years living in 11 nursing homes in Eslöv, Jönköping and Linköping in southern Sweden.	Descriptive and comparative parametric statistical analyses (t-test, ANOVA). Chi-square test. Spearman's rank correlation. Cox regression analyses. Logistic regression analyses.
Paper II: Association between serum 25(OH)D ₃ and cardiovascular morbidity and mortality in people with type 2 diabetes: a community-based cohort study.	A prospective observational community-based cohort study.	698 people with type 2 diabetes aged 55-66 years from 22 primary health care centres in Östergötland and Jönköping in southern Sweden.	Descriptive and comparative parametric statistical analyses (t-test, ANOVA). Chi-square test. Linear regression analyses. Cox regression analyses. AIC.
Paper III: Is there an association between serum 25(OH)D ₃ and mental well-being in patients with type 2 diabetes? Results from a cohort study in primary care.	A prospective observational community-based cohort study.	698 people with type 2 diabetes aged 55-66 years from 22 primary health care centres in Östergötland and Jönköping in southern Sweden.	Descriptive and comparative non-parametric statistical analyses (The Mann-Whitney U test and Kruskal-Wallis H test). Parametric statistics (one sample t-test). Chi-square test. The Fischer's exact test. Spearman's rank correlation. Binary logistic regression analyses.
Paper IV: Sunlight exposure and vitamin D levels in older people: an intervention study in Swedish nursing homes.	A cluster randomised intervention trial.	In total, 40 people >65 years living in three nursing homes in Jönköping in southern Sweden.	Descriptive and comparative non-parametric (The Mann-Whitney U test and Wilcoxon signed-rank test). Parametric statistical analyses (t-test). Chi-square test. Fischer's exact test.

The first and the fourth publications are confined to elderly people living in nursing homes. The first paper is based on data from SHADES. The fourth paper is based on the study Sunlight and Vitamin D in Older People in Nursing Homes. The second and the third publications are confined to patients with type 2 diabetes and these papers are based on data from CARDIPP. An overview of the papers according to the study populations is presented in Figure 3.

Figure 3. The four papers in this thesis according to the different study populations.

Study Populations

SHADES

SHADES was a prospective observational cohort study with the aim of describing and analysing mortality, morbidity, health status and use of drugs among elderly people living in nursing homes in Sweden. The study was conducted in 11 nursing homes in three Swedish cities: Eslöv (latitude 55°N), Jönköping (latitude 57°N) and Linköping (latitude 58°N) between 2007 and 2011. All people who lived in or moved into the selected nursing homes were invited to participate. The last inclusion was made in March 2011. The analysis of the deceased was conducted in March 2012. Exclusion criteria were: planned short-term stay, for example due to rehabilitation or palliative care, severe illnesses, language problems, and age under 65 years. SHADES included a total of 429 people. Ninety-six individuals were excluded in the dataset of this study: 55 for being on vitamin D medication in

combination with calcium, and 41 due to lack of data (Figure 4). Thus, 333 individuals remained for analysis in this study, of whom 226 (68%) were women and 107 (32%) men. The mean age for the women was 86 years and for the men 83 years.

Figure 4. Flow chart of SHADES. (Paper I)

CARDIPP

CARDIPP was a prospective observational community-based cohort study. The aim of the study was to explore the impact from the different cardiovascular risk factors on early cardiovascular organ damage to facilitate earlier and individually tailored intervention in patients with type 2 diabetes. The participants were consecutively recruited from 22 primary health care centres in Östergötland and Jönköping in Sweden between 2005 and 2008. All participants had type 2 diabetes and were aged 55–65 years. Exclusion criteria were difficulties understanding the Swedish language and severe physical or mental disease. The primary health care centres were of different sizes and had different demographic locations, but the model of treatment and care of type 2 diabetes were similar and followed the national guidelines for diabetes care. In total, CARDIPP enrolled 761 patients. Sixty-three were excluded from the dataset of our studies: 20 due to vitamin D

supplementation and 44 due to missing data on vitamin D status. One person with missing data on vitamin D status also reported taking vitamin D supplements. Thus, 698 individuals remained for analysis in these studies. Of the participants, 463 (66%) were male and 235 (34%) female. The mean age for men was 60.7 years and for women 60.5 years. All participants were invited to a reinvestigation after four years.

Sunlight and Vitamin D in Older People in Nursing Homes

This study was a cluster randomised intervention trial with the aim of investigating whether an intervention with encouragement to spend time outdoors during summertime could increase the levels of vitamin D and the mental well-being of people living in Swedish nursing homes. The study participants were consecutively recruited between April and May, 2018. Inclusion criteria for participants were: living in the selected nursing homes in Jönköping in southern Sweden, to be expected to stay in the nursing homes over the summer, and age > 65 years. Exclusion criteria included short-term stay in the nursing home, palliative care/ short expected survival, other reasons to believe that the resident was not expected to go outdoors, and inability to give informed consent to the study. Residents with severe dementia were not asked to participate. In total, 42 people were included in the study. The intervention group consisted of people living in two selected small nursing homes (n=20), and the control group were people living in another selected large nursing home (n=22). In the intervention group, 18 people remained for analysis and in the control group, all the participants remained for analysis, as shown in Figure 5.

Figure 5. Flow chart of the study Sunlight and Vitamin D in Older People in Nursing Homes. (Paper IV)

Methods

SHADES

Data on age, gender, the date of moving into the nursing home, the date of inclusion in the study and the date of leaving the nursing home or death were recorded. Every six months during the study, the participants were examined by a nurse and data were collected from the medical records about diagnoses, chronic diseases and medication as well as visits to a GP, visits to an emergency ward and hospitalisation. The diagnoses were classified according to the ICD-10. The causes of death were ascertained from the Swedish Cause of Death Register (The National Board of Health and Welfare, Stockholm, Sweden) with high validity (118). The functional level of the participants was estimated with the second subscale Physical Activity in the MNS (119) at inclusion. The DFRI (120) was used to investigate earlier falls. The height was measured to the nearest centimetre and the weight was measured to the nearest kilogram. BMI was calculated as weight (kg) divided by height (m) squared. The blood pressure was measured three times while sitting and the mean value was used for analysis.

Laboratory Measurements

Venous blood samples were collected in the fasting state. The levels of serum 25(OH)D₃ were analysed three times: at inclusion and after one and two years with HPLC technology. After extraction, separation of 25(OH)D₃ was performed using the HPLC-system (reagent kit and HPLC column from Chromsystems Instruments & Chemicals GmbH, Munich, Germany) with UV detection at a wavelength of 265 nm (UV detector from Dionex ThermoFisher Scientific Inc, Sunnyvale, California, USA). At baseline, haemoglobin, creatinine and cystatin C were analysed. GFR was estimated by cystatin C (eGFR) in ml/min/1.73m² and was calculated as: eGFR= 84.69 x (1/cystatin C^{1.680}) (121). The blood samples were analysed at Ryhov county hospital, Jönköping, Sweden.

CARDIPP

The nurses measured the body weight to the nearest 0.1 kilogram and the height to the nearest centimetre at inclusion. From this, BMI was calculated. The blood pressure was measured manually in a sitting position and the average of three measurements was used for analysis (122). The examination included a standardised anamnesis including previous diseases, diabetes duration (time from diagnosis of type 2 diabetes until the baseline examination) and ongoing medication inclusive of vitamin D and other supplements. The study participants filled out a general questionnaire

about their marital status and occupation and reported their exercise, smoking and drinking habits.

SF-36

In Paper III, we use the results of the short form health survey SF-36, which the participants filled out for measurement of their mental well-being in terms of vitality and mental health both at baseline and at follow-up after four years. SF-36 is a standardised questionnaire measuring self-reported health and is derived from the Medical Outcomes study in the 1980s (123). The participants answered the nine questions depicted in Table 2. All items were assessed with a range from 1 to 6: 1= all of the time, 2= most of the time, 3= a good bit of the time, 4= some of the time, 5= a little of the time and 6= none of the time. The responses to each item were scored and summed according to a standardised scoring protocol, resulting in a maximum score of 100 (124). SF-36 has been evaluated in Sweden regarding data quality, scaling assumptions, reliability and construct validity and found to be reliable (125).

Table 2 Questions on Vitality and Mental Health from the Short Form Health Survey SF-36

How much of the time during the past 4 weeks:

1. did you feel full of pep?
 2. have you been a very nervous person?
 3. have you felt so down in the dumps that nothing could cheer you up?
 4. have you felt calm and peaceful?
 5. did you have a lot of energy?
 6. have you felt downhearted and blue?
 7. did you feel worn out?
 8. have you been a happy person?
 9. did you feel tired?
-

The health concept of vitality was measured using questions 1, 5, 7 and 9, and that of mental health using questions 2, 3, 4, 6 and 8.

Laboratory Measurements

At baseline, a fasting venous blood sample was drawn and was used for immediate routine laboratory analyses and frozen for later analyses. From the frozen samples, serum 25(OH)D₃ and PTH were analysed using chemiluminescence on a Cobas e602 unit (Roche Diagnostics Scandinavia AB, Bromma, Sweden). GFR was estimated using the CKD-EPI equation (126).

In Paper II, the study participants were followed from the index date to the first event of an endpoint, emigration or to 31 December 2014. The primary endpoint was a composite endpoint of the first non-fatal or fatal event of hospitalisation for acute myocardial infarction or stroke or cardiovascular mortality, i.e. MACE. This endpoint was determined with high validity (118, 127) by linkage to the Swedish Cause of Death and In-Patient Care Diagnosis registries (The National Board of Health and Welfare, Stockholm, Sweden). MACE is a well-established composite endpoint, frequently used in cardiovascular research (128-130).

Sunlight and Vitamin D in Older People in Nursing Homes

We registered data on age, gender and smoking habits at inclusion. A nurse measured the height and the waist circumference to the nearest centimetre and the weight to the nearest kilogram. The blood pressure was recorded in a sitting position after five minutes of rest. The second subscale Physical Activity, in the MNS, was used to estimate the functional level at baseline (119). We used the questionnaire SF-36 to measure the study participant's mental well-being in terms of vitality and mental health before and after the intervention (Table 2).

Laboratory Measurements

Venous non-fasting blood samples were drawn from all participants both before the start of the intervention (in April/May 2018) and after the intervention (in September/October 2018). Serum 25(OH)D was analysed with a 1-step delayed chemiluminescent microparticle immunoassay technique with an Architect i2000SR immunoassay analyser, Abbott Laboratories, Abbott Park, Illinois, USA at Ryhov county hospital, Jönköping, Sweden.

The Intervention

The intervention consisted of active encouragement orally and in writing to the study participants in the intervention group to go outside for 20-30 minutes between 11 a.m. and 3 p.m. every day for two consecutive months, starting on any day between May 15 and July 1, 2018. The staff in the nursing homes were asked to continuously remind the participants in the intervention group to go outside every day. Written advice from the Swedish Radiation Safety Authority about sun protection was given, including information about protection with clothes, hat, sunglasses and sunscreen, to stay in the shade when the sun was at its highest in the sky and to avoid sunlight if the skin was burned. The control group lived as usual. All participants were instructed to record the days with time spent outdoors in a diary.

Food

We recorded how many times fish/ seafood was on the menu in the nursing homes during the intervention period: 56 times in one of the nursing homes for the intervention group, 52 times in the other nursing home for the intervention group, and 58 times in the nursing home for the control group.

Weather

Data on sunlight time was collected from SMHI, measured in Växjö, Sweden, located approximately 106 km from the nursing homes. The summer of 2018 was sunny in southern Sweden. The number of sunlight hours was 352 in May, 308 in June, 357 in July and 188 in August measured as time with direct solar radiation above 120 W/m² determined using a contrast sensor (131).

Statistical Analyses

In Paper I, data were analysed with Statistica 10 (StatSoft Inc., Tulsa, Oklahoma, USA). In Papers II–IV, data were analysed with IBM SPSS statistics 20-25 (International Business Machines Corporation, New York, USA).

In Papers I and II, parametric statistical analyses were used. Mean and SD were used to present the average value and the measure of statistical dispersion. A student's t-test and ANOVA were used to compare mean levels of continuous variables between groups.

In Papers III and IV, non-parametric tests were used. Median and IQR were used to present the average value and the measure of statistical dispersion. The Mann-Whitney U test (Papers III and IV), a Kruskal-Wallis H test (Paper III) and a related samples Wilcoxon signed-rank test (Paper IV) were used to compare median levels of continuous variables between groups. Regarding the results of SF-36, we presented mean and SD. The one sample t-test was used to compare our results of SF-36 with the mean values of the Swedish population, as the norms for the general populations are given in mean values (Papers III and IV). Student's t-tests were used to compare mean levels of the SF-36 results between the groups (Paper IV).

In all four papers, the Chi-square test was used to investigate associations between categorical data. In Papers III and IV, Fischer's exact test was used when more than 20% of the cells had an expected frequency below 5.

In Paper I, the period May–October was categorised as summer and November–April as winter to adjust for the season when the blood sample for vitamin D was collected. In Papers II and III, the period April–September was categorised as summer, and January–March and October–December as winter.

Statistical significance was defined as a p-value <0.05. Statisticians were consulted in all four papers.

Specifically in Paper I

Correlation between two variables was calculated with Spearman's rank correlation. Cox regression analyses were used to calculate HRs for mortality in the different 25(OH)D₃ quartiles, with the fourth quartile as a reference. Logistic regression analyses were used to calculate the OR for cardiovascular events, falls and fractures in the different 25(OH)D₃ quartiles, with the fourth quartile as a reference.

Specifically in Paper II

Linear regression analyses were used to assess associations between serum 25(OH)D₃ and variables with differences between serum 25(OH)D₃ quartiles. Cox regression analyses were used to calculate HRs for the MACE with serum 25(OH)D₃ both as a continuous variable and in serum 25(OH)D₃ quartiles, with the fourth quartile as a reference. Since several parallel Cox regression analyses were performed, the AIC (132) was used to evaluate the model with the best fit.

Specifically in Paper III

Spearman's correlation coefficients were analysed to assess associations between serum 25(OH)D₃ and key continuous variables, vitality and mental health. Binary logistic regression analyses were used to calculate OR for vitality and mental health associated with serum 25(OH)D₃. The median scores were used to dichotomise the concepts of vitality and mental health from the SF-36.

Ethical Considerations

All studies were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each individual prior to participating in the studies. The studies resulting in the four papers were approved by the Regional Ethical Review Board, Linköping, Sweden, Dnr M150-07 (SHADES), Dnr M26-05 (CARDIPP) and Dnr 2017/105-31 (Sunlight and Vitamin D in Older People in Nursing Homes). All participants could withdraw at any point if they chose to. All data were pseudonymised and presented on a group level, and specific individuals could not be singled out or identified.

RESULTS

Paper I

The study participants were categorised into four quartiles according to their serum 25(OH)D₃ levels at baseline. Baseline characteristics are presented in Table 3. The mean (SD) serum 25(OH)D₃ concentration for the whole study population at baseline was 40.2 (16.0) nmol/l, with no significant difference between men and women. Eighty percent of the participants had 25(OH)D₃ <50 nmol/l. The vitamin D levels decreased from the first to the second and the third measurements. Higher 25(OH)D₃ concentrations were seen from May to October compared to November to April: 39.2 vs. 35.7 nmol/l ($p < 0.01$).

The mean follow-up time of the study was three years. During the follow-up period, 147 (44%) of the 333 people in the study had died of any cause. In Q1 (25(OH)D₃ <29 nmol/l), 59% of the participants died compared to 28% in Q4 (25(OH)D₃ >48 nmol/l). The most common causes of death according to the death certificates were: cardiovascular disease (48%), infections (14%), dementia (10%), general organ failure (10%), neoplastic disease (3%), kidney failure (3%) and other causes (12%).

Figure 6 shows the cumulative survival for the people in the different vitamin D quartiles. HR (with 95% CI) for mortality was 2.02 (1.31-3.12) in Q1 compared with Q4 ($p < 0.05$), 2.03 (1.32-3.14) in Q2 compared with Q4 ($p < 0.05$) and 1.6 (1.03-2.48) in Q3 compared with Q4 ($p < 0.05$). The Cox regression analysis is adjusted for differences in age, gender, BMI, systolic and diastolic blood pressure, GFR estimated from cystatin C, the season when the blood sample for vitamin D was collected, the length of time living in the nursing home, previous cardiovascular diseases, neoplastic diseases, dementia and the level of physical activity. Of these variables, only increased age and a limited level of physical activity were associated with an increased mortality risk.

Table 3 Baseline Characteristics according to 25(OH)D₃ Quartiles

	Serum 25(OH)D ₃ quartile				p-value
	1	2	3	4	
Number	86	82	85	80	
25(OH)D ₃ range (nmol/l)	11-29	30-37	38-47	48-120	
25(OH)D ₃ mean (nmol/l)	24.1 +/-4.1	33.4 +/-2.2	42.1 +/-2.8	62.3 +/-14.7	
Male	38% (n=33)	23% (n=19)	42% (n=36)	24% (n=19)	ns
Age (years)	86.3 +/- 6.2	84.7 +/- 6.8	84.5 +/- 7.0	84.3 +/- 7.2	ns
male	85.8 +/- 6.4	83.2 +/- 5.6	81.9 +/- 7.4	79.4 +/- 6.7	p<0.01 Q1-Q4
female	86.6 +/- 6.1	85.2 +/- 7.1	86.3 +/- 6.2	85.8 +/- 6.8	ns
Time in nursing home					
<1 year	48% (n=41)	54% (n=44)	55% (n=47)	68% (n=54)	p=0.05 Q1-Q4
1-3 years	30% (n=26)	22% (n=18)	26% (n=22)	16% (n=13)	ns
>3 years	22% (n=19)	24% (n=20)	19% (n=16)	16% (n=13)	ns
Body Mass Index (kg/m ²)	24.6 +/- 4.5	24.7 +/- 5.6	25.9 +/- 5.1	24.9 +/- 3.9	ns
male	24.6 +/- 4.0	25.4 +/- 3.8	24.8 +/- 4.8	24.9 +/- 3.8	ns
female	24.6 +/- 4.8	24.5 +/- 6.0	26.7 +/- 5.2	24.9 +/- 3.9	ns
Haemoglobin (g/l)	126.3 +/- 14.5	125.0 +/- 14.8	126.4 +/- 12.5	125.4 +/- 14.2	ns
male	127.8 +/- 15.0	126.4 +/- 11.8	128.6 +/- 13.5	126.8 +/- 16.7	ns
female	125.4 +/-14.3	124.5 +/- 15.6	124.9 +/- 11.5	125.0 +/- 13.6	ns
Creatinine (mikromol/l)	79.0 +/- 30.0	86.3 +/- 34.4	98.9 +/- 64.5	93.9 +/- 37.0	p<0.05 Q1-Q3
male	92.2 +/- 39.4	101.0 +/- 43.4	117.8 +/- 91.3	106.5 +/- 55.0	ns
female	70.8 +/- 18.2	81.9 +/- 30.3	84.8 +/- 25.8	89.9 +/- 28.8	p<0.05 Q1-Q3,4
GFR (estimated by cystatin C) (ml/min/1.73m ²)	62.4 +/- 25.4	62.8 +/- 27.2	56.0 +/- 24.6	56.4 +/- 25.8	ns
male	63.1 +/- 25.9	60.8 +/- 26.4	55.4 +/- 27.2	56.3 +/- 20.8	ns
female	62.0 +/- 25.3	63.4 +/- 27.7	56.4 +/- 22.8	56.4 +/- 27.3	ns
Systolic Blood Pressure (mm Hg)	130.5 +/- 26.3	130.3 +/- 23.6	135.4 +/- 21.6	135.9 +/- 23.1	ns
male	128.2 +/- 26.9	121.4 +/- 19.4	126.2 +/- 18.2	132.4 +/- 27.7	ns
female	132.0 +/- 26.0	132.7 +/- 24.2	143.0 +/- 21.4	137.0 +/- 21.6	ns
Diastolic Blood Pressure (mm Hg)	70.5 +/- 11.2	71.7 +/- 11.4	72.6 +/- 10.5	73.4 +/- 11.6	ns
male	71.5 +/- 9.7	68.5 +/- 9.7	71.5 +/- 9.2	70.7 +/- 10.8	ns
female	69.8 +/- 12.2	72.6 +/- 11.7	73.5 +/- 11.4	74.2 +/- 11.8	ns
Frequency of patients with hyper- tension diagnosis (%)	24% (n=21)	28% (n=23)	27% (n=23)	40% (n=32)	ns
male	36% (n=12)	32% (n=6)	22% (n=8)	47% (n=9)	ns
female	17% (n=9)	27% (n=17)	31% (n=15)	38% (n=23)	ns
Frequency of patients with cardio- vascular diagnosis other than hy- pertension (%)	55% (n=47)	60% (n=49)	60% (n=51)	54% (n=43)	ns
male	52% (n=17)	68% (n=13)	64% (n=23)	53% (n=10)	ns
female	57% (n=30)	57% (n=36)	57% (n=28)	54% (n=33)	ns
Level of physical activity in the modified Norton scale					
1. bedridden	-	-	-	-	ns
2. chair-bound	35% (n=30)	25% (n=20)	24% (n=20)	23% (n=18)	ns
3. walks with help	24% (n=21)	17% (n=14)	14% (n=12)	15% (n=12)	ns
4. ambulant	41% (n=35)	58% (n=47)	61% (n=51)	63% (n=50)	ns

Data are presented as means and SD or as percentages and (n). Differences between groups were calculated with ANOVA and differences between proportions were calculated with χ^2 test. Significant differences are presented with p-values. (Paper I)

Figure 6. Cumulative survival according to 25(OH)D₃ quartiles. Cox regression analyses were used to calculate HRs for mortality in the different 25(OH)D₃ quartiles, with the fourth quartile as a reference. (Paper I)

Paper II

The mean (SD) serum 25(OH)D₃ concentration was 50.7 (21.9) nmol/l for all participants, with no significant difference between men and women. Fifty-five % of the participants had 25(OH)D₃ <50 nmol/l. The study participants were categorised into four quartiles according to their 25(OH)D₃ levels.

The study had a mean follow-up time of 7.3 years. During this period, 57 (8%) of the 698 study participants died of any cause, 33 (5%) were hospitalised for a non-fatal or fatal acute myocardial infarction, and 31 (4%) were hospitalised for a non-fatal or fatal stroke (three had both an acute myocardial infarction and a stroke). The number who coded positive for our primary endpoint (i.e. the first non-fatal or fatal event of hospitalisation for acute myocardial infarction or stroke or cardiovascular mortality) was 66 (9%). Serum 25(OH)D₃ was inversely associated with the risk of cardiovascular morbidity and mortality. The HRs of cardiovascular morbidity and mortality for the three lowest vitamin D quartiles compared with the highest quartile are shown in Table 4, with adjustment made in three models (Model 1 adjusts for age, gender and season for the blood sample collection; Model 2 includes adjustment for smoking, physical activity and BMI; and Model 3 also includes systolic blood pressure, PTH, albumin-corrected calcium, CRP, GFR, LDL/HDL ratio, HbA_{1c}, duration of diabetes and previous cardiovascular disease). The analyses showed strong evidence of a dose-response association, with the HR being highest in the lowest vitamin D quartile. The proportions of people without cardiovascular morbidity and mortality in the different 25(OH)D₃ quartiles (in Model 1) are shown in Figure 7.

When serum 25(OH)D₃ was analysed as a continuous variable, the HR (95% CI) per nmol/l was 0.98 (95% CI: 0.96-0.99) (p=0.001) in Model 1. The association remained significant in all models i.e. after adjusting for all previous mentioned covariates. We found significant improvements in AIC when adding vitamin D to the models. No association between PTH and cardiovascular morbidity and mortality was seen.

Table 4 Association between 25(OH)D₃, Covariates and CVD Morbidity and Mortality

Variables	Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)
25(OH)D ₃ quartile (nmol/l)	p=0.008	p=0.053	p=0.012
Q1 (7.5-35.5)	3.46 (1.60-7.47)	2.77 (1.26-6.13)	3.09 (1.30-7.35)
Q2 (35.5-47.5)	2.26 (1.01-5.06)	1.72 (0.74-4.00)	0.87 (0.32-2.36)
Q3 (47.5-61.8)	1.62 (0.70-3.76)	1.38 (0.58-3.30)	1.51 (0.60-3.83)
Q4 (61.8-164.9)	1.00	1.00	1.00
Age (years)	1.07 (0.98-1.16)	1.08 (0.99-1.18)	1.11 (0.99-1.24)
Men (vs. women)	1.88 (1.06-3.35)	1.82 (0.98-3.40)	1.31 (0.64-2.69)
Summer (vs. winter)	2.09 (1.28-3.42)	1.67 (0.98-2.83)	1.43 (0.77-2.64)
Smoking		p=0.14	p=0.10
Current		1.89 (0.84-4.26)	1.46 (0.55-3.88)
Previous		1.90 (0.98-3.69)	2.35 (1.05-5.26)
Non-		1.00	1.00
Exercise		p=0.39	p=0.81
Sedentary		2.62 (0.72-9.46)	2.17 (0.45-10.60)
Mild		1.70 (0.52-5.62)	1.75 (0.40-7.64)
Moderate		1.47 (0.38-5.62)	1.83 (0.36-9.39)
Regular		1.00	1.00
BMI (kg/m ²)		1.07 (1.01-1.12)	1.02 (0.95-1.09)
Systolic Blood Pressure (mmHg)			1.01 (0.99-1.03)
PTH (pg/ml)			1.00 (0.98-1.02)
Calcium, alb-corr (mmol/l)			8.22 (0.20-338.49)
CRP (mg/l)			1.05 (1.00-1.10)
GFR (ml/min/1.73m ²)			1.00 (0.97-1.02)
LDL/HDL ratio			0.91 (0.61-1.35)
HbA1c (mmol/mol and %)			1.04 (1.02-1.06)
Duration of diabetes (years)			1.00 (0.96-1.05)
Previous CVD (yes vs. no)			2.47 (1.27-4.79)

(Paper II)

Figure 7. Proportion of people without cardiovascular morbidity and mortality according to 25(OH)D₃ quartiles. Cox regression analyses were used to calculate HRs for cardiovascular morbidity and mortality in the different 25(OH)D₃ quartiles with the fourth quartile as a reference, when adjusting for age, gender and season. (Paper II)

Paper III

We had data on vitality and mental health from the SF-36 questionnaire for 98% of the study participants at baseline and for 67% at the follow-up after four years. Table 5 show the results of SF-36. Compared to the Swedish general population (mean score for vitality 68.8, mean score for mental health 80.9) (125), our study participants had a significantly lower mean score for vitality ($p < 0.001$), but no difference ($p > 0.05$) compared to the Swedish general population was seen regarding mental health.

Table 5 Results of SF-36

	At baseline		At 4-year follow-up	
	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)
		n=687		n=469
Vitality Score	70 (30)	63.7 (23.2)	70 (35)	64.8 (22.5)
		n=686		n=467
Mental Health Score	84 (20)	80.4 (17.5)	84 (20)	80.4 (17.1)

The logistic regression analyses (Table 6) showed that baseline serum 25(OH)D₃ was inversely associated with poor mental health at baseline ($p < 0.05$), but not at follow-up after four years. For vitality, no association with serum 25(OH)D₃ was found at baseline ($p > 0.05$). At follow-up, an inverse association between 25(OH)D₃ and low vitality was seen at first, but it disappeared after adjustment for diabetes-related data and lifestyle factors.

There were several differences between the study participants who attended the reinvestigation and those who did not. The non-attendees (n=229) more often were unmarried/living alone, sedentary, smokers, obese, had longer diabetes duration plus had lower levels of 25(OH)D₃, PTH and creatinine and lower diastolic blood pressure than the people who attended the reinvestigation ($p < 0.05$).

Table 6 Odds Ratios of Mental Well-being at Baseline and at 4-Year Follow-up Associated with a 10 nmol/l Increase in Baseline 25(OH)D₃

	At baseline OR (95% CI), p-value	At 4-year follow-up OR (95% CI), p-value
Vitality (score below median)	n=687	n=469
Unadjusted	0.94 (0.87-1.00), p=0.065	0.89 (0.82-0.97), p=0.009*
Adjusted for age & sex	0.95 (0.89-1.02), p=0.19	0.91 (0.83-1.00), p=0.039*
Adjusted for age, sex, season, PTH & albumin-corrected calcium	0.96 (0.89-1.03), p=0.26	0.89 (0.81-0.98), p=0.014*
Adjusted for age, sex, BMI, HbA1c & duration of diabetes	0.97 (0.90-1.05), p=0.42	0.94 (0.86-1.04), p=0.21
Adjusted for age, sex, BMI, marital status, physical activity, smoking & alcohol habits.	0.96 (0.89-1.04), p=0.33	0.94 (0.86-1.04), p=0.24
Mental Health (score below median)	n=686	n=467
Unadjusted	0.90 (0.83-0.96), p=0.003*	0.94 (0.87-1.03), p=0.18
Adjusted for age & sex	0.91 (0.84-0.98), p=0.010*	0.96 (0.88-1.04), p=0.32
Adjusted for age, sex, season, PTH & albumin-corrected calcium	0.91 (0.84-0.98), p=0.017*	0.94 (0.86-1.03), p=0.21
Adjusted for age, sex, BMI, HbA1c & duration of diabetes	0.92 (0.85-1.00), p=0.046*	0.98 (0.90-1.08), p=0.71
Adjusted for age, sex, BMI, marital status, physical activity, smoking & alcohol habits	0.91 (0.84-0.99), p=0.021*	0.98 (0.89-1.07), p=0.65

A p-value of <0.05 was considered significant (*). (Paper III)

Paper IV

Before the summer, the median (IQR) of serum 25(OH)D was 42.5 (23.0) nmol/l in the intervention group and 52.0 (36.0) nmol/l in the control group. In total, 55% had 25(OH)D <50 nmol/l. After the summer, the 25(OH)D levels had increased significantly in the intervention group ($p=0.011$) to a median (IQR) of serum 25(OH)D of 53.5 (33.0) nmol/l. The control group had a median (IQR) of serum 25(OH)D of 65.0 (35.0) nmol/l after the summer, but the increase was not significant. Figure 8 shows the changes in serum 25(OH)D during the summer.

Figure 8. Box and whisker plot (median, first and third quartile, range) of 25(OH)D. The related samples Wilcoxon signed-rank test was used to compare median levels of 25(OH)D before and after the summer. A p-value of <0.05 was considered significant (*). (Paper IV)

The intervention group reported better mental health in SF-36 after the summer compared to before the summer ($p=0.015$) as shown in Table 7. In the control group, no difference in the scores for vitality or mental health was seen during the summer. Compared to the Swedish population >65 years (mean score for vitality 66.4, mean score for mental health 80.3 (124)), our study participants had significantly lower mental health scores before ($p=0.007$), but not after the summer ($p>0.05$). Regarding vitality, our study participants had significantly lower scores both before ($p<0.001$) and after the summer ($p=0.015$) than the older Swedish population.

Table 7 Results of SF-36

	Intervention group	Control group	p-value
Vitality score			
Before summer	51.9 (22.7)	46.6 (30.6)	$p=0.54$
After summer	58.8 (17.9)	50.8 (33.0)	$p=0.40$
p-value	$p=0.15$	$p=0.17$	
Mental health score			
Before summer	72.2 (20.3)	67.1 (27.3)	$p=0.51$
After summer	82.4 (16.4)	71.8 (29.1)	$p=0.21$
p-value	$p=0.015^*$	$p=0.09$	

Data are presented as means and SD. The independent samples t-test was used to compare mean levels in scores between the intervention group and the control group. The paired samples t-test was used to compare mean levels in scores before and after the summer. A p-value of <0.05 was considered significant (*). (Paper IV)

DISCUSSION

Elderly People in Nursing Homes (Papers I and IV)

Our studies showed that vitamin D deficiency was highly prevalent in elderly people living in nursing homes in Sweden, as a majority of our study participants had vitamin D levels below 50 nmol/l. This finding corresponds with the findings of others (53-58). Elderly people in Swedish nursing homes are generally frail and often seriously ill, which was evident in our studies as the co-morbidity and the mortality were high. For example, in Paper IV many of the residents in the selected nursing homes were not eligible for participation because of severe dementia, and in Paper I, 44% of the study participants died during the study period (with a mean follow-up time of three years). In Paper I, those with the highest 25(OH)D₃ values had lived in the nursing homes for the shortest time and vice versa. Also, we found that the vitamin D levels decreased over time, which could be a consequence of deterioration of chronic diseases, general physical impairment and reduced sun exposure. The results indicate that the vitamin D status can be a marker of deteriorating health.

In Paper I, Vitamin D deficiency was associated with an increased risk of mortality. The risk of death was doubled in Q1 and Q2 compared with Q4. Our results are consistent with the results of a previous study on female nursing home residents in Austria (56). From our study, we cannot identify the mechanisms behind the increased mortality risk associated with vitamin D deficiency. For example, we found no association between the vitamin D levels and the risk of cardiovascular events. The variation in documentation in the medical journals and the fact that the number of registered events in the different categories according to diagnoses was relatively small might have contributed to the lack of an univocal explanation behind the association between low vitamin D levels and the increased mortality risk.

In Paper I, the vitamin D levels changed during the year, with higher concentrations in the summer months, which indicates the importance of sunlight on the vitamin D level even in this group of elderly. Paper IV showed a significant increase in serum 25(OH)D after active encouragement to spend time outdoors during summertime i.e. through increased natural sunlight exposure. Even if the increase was modest and not quite equivalent to the expected result of the recommended daily dose of vitamin D (an

increase in serum 25(OH)D of 11-13 nmol/l corresponds to vitamin D supplementation of approximately 5.5-7 micrograms, compared to the recommended daily dose of 20 micrograms), we showed that it was possible to achieve higher vitamin D concentrations in elderly in nursing homes through active encouragement to spend time outdoors. This corresponds with the results of a small study from New Zealand (latitude 37°S) (133). Our results are in contrast to a 12-month intervention study from Australia (latitude 34°S) (134), but a sub study showed that the UV dose had a small, but positive effect on the vitamin D levels after six months for those who started their sunlight sessions in spring or autumn (135).

In Paper IV, the intervention also improved self-perceived mental health, but this study could not identify the reason for the improved mental health after the summer in the intervention group. Apart from the improved 25(OH)D level, possible explanations for the improved mental health may include increased social interaction and more physical activities when spending time outdoors.

Patients with Type 2 Diabetes (Papers II and III)

In Paper II, we found that low baseline 25(OH)D₃ was associated with an increased risk of cardiovascular morbidity and mortality in a population of middle-aged patients with type 2 diabetes. Our results correspond with some (136, 137), but not all (138) previous studies of patients with type 2 diabetes. The association found in our study was consistent when adjusting for cardiovascular risk factors such as limited physical activity, physiological variables such as PTH, and previous cardiovascular disease. Our results indicate that the association between 25(OH)D₃ and cardiovascular disease does not involve PTH. Physical activity is another important variable, as inactivity is a risk factor for cardiovascular disease (139) and also likely to be associated with less time spent outdoors leading to reduced sunlight exposure and lower vitamin D values.

In Paper III, we found an inverse association between serum 25(OH)D₃ and poor mental health at baseline, but not at follow-up on mental well-being after four years. The finding that vitamin D was associated with mental health at baseline is in line with one (140) of the two previous studies confined to populations with diabetes (140, 141). We found no association between serum 25(OH)D₃ and vitality at baseline, and the association first seen at follow-up disappeared after controlling for data regarding diabetes status and lifestyle factors. This suggests that other factors are of greater importance for vitality than vitamin D in patients with type 2 diabetes.

Strengths and Limitations

This thesis is based on four papers with both strengths and limitations. Strengths of SHADES (Paper I) included the prospective study design and that older people in nursing homes were included, as this is a population category not particularly well-studied previously. Another strength was that the vitamin D levels were measured repeatedly, making it possible to compare the levels over time. A limitation was that the blood samples were collected with a one-year interval, which might have involved a risk of skewed distribution of the blood sample collection during the different seasons. Due to the observational study design, we could not draw conclusions about causality.

A major strength of CARDIPP (Papers II and III) was that it was community-based, as type 2 diabetes in Sweden is managed by a GP at a primary health care centre, which means that the results could be generalised to the people with type 2 diabetes in the community. Other strengths were the prospective study design and that data could be analysed with respect to several confounding factors. CARDIPP also had some limitations. The observational study design precluded conclusions about causality and we could not rule out residual confounding factors.

- In Paper II, no participant was lost to follow-up due to the registry data on mortality, which was a strength. A limitation was that the number of events of the primary endpoint (MACE) was relatively low.
- In Paper III, the limitations included that we had no data on the medical history of depression/affective disorders, nor any data on antidepressant medication. The follow-up analyses had several weaknesses: we had a single baseline 25(OH)D₃ for calculation of the association both at baseline and follow-up. One-third of the study population was lost to follow-up. There were several differences between those who did not attend the reinvestigation and those who did, including lower 25(OH)D₃ values for those who did not participate in the reinvestigation, which might mean a potential selection bias. The size of the associations among serum 25(OH)D₃ and vitality and mental health in Spearman's correlations differed from the size of the results of the logistic regression analyses, which is why we could not rule out moderation of an unrecognised factor or a non-linear association.

Strengths of Sunlight and Vitamin D in Older People in Nursing Homes (Paper IV) were its randomised design and that it included older people

living in nursing homes. Another strength was that the intervention was feasible, inexpensive and considered to be clinically relevant. Limitations of the study included the small study size and the open label design i.e. the participants and staff were not blinded to the intervention.

Clinical Implications and Future Research

Multiple observational epidemiological studies have found an association between low vitamin D levels and an increased risk of several diseases, but there is a concern that confounding and reverse causation may explain the results (66, 142). Also in our studies, an association between low vitamin D levels and increased risk of mortality in elderly people in nursing homes, and an association between low vitamin D levels and cardiovascular morbidity and mortality and poor mental health in patients with type 2 diabetes have been found. The clear associations immediately raise the question of whether premature death, cardiovascular disease and poor mental health could be prevented by increasing vitamin D levels. However, our studies do not answer that question.

Among researchers in the field, many hoped that RCTs would show whether increasing vitamin D levels prevented non-skeletal diseases, but to date, data showing convincing benefits of vitamin D supplementation on several outcomes are missing. Regarding mortality risk, some previous studies of vitamin D supplementation have shown decreased mortality with vitamin D supplementation (69, 70), while others have not (71). The results of the D-Health Trial on all-cause mortality are still expected (72). Regarding cardiovascular disease, RCTs of vitamin D supplementation have not shown any reduction in the risk of cardiovascular disease in the general population (79, 80). No reduction in depression has been seen after vitamin D supplementation, but there is heterogeneity in the performed studies (97).

This discrepancy between the results of the observational studies and the RCTs has led to an ongoing discussion about the role of vitamin D in health, and how to proceed. Some researchers stress that the difficulty showing positive effects of vitamin D supplementation in RCTs points to small effects of vitamin D supplementation, if any. They argue for new and better-designed RCTs as the only way to determine the role of vitamin D in health (143). Others argue that observational studies will continue to contribute to the evidence which is the basis for the role of vitamin D in health, as RCTs of vitamin D supplementation also have a range of limitations. The limitations of RCTs about vitamin D include: a low response rate, resulting

in a selected group of worried but healthy study participants, which affects the generalisability; and a number of limitations that decrease the differences in outcome between the vitamin D supplementation group and the placebo group, leading to underestimation of the effects of supplementation. Examples of the latter are: inclusion of vitamin D-sufficient people, a need for participation in the study for many years to give the ability to detect outcomes that are rare or long-term, which in turn decreases compliance and retention, and the possibility for participants in both arms to take vitamin D supplements on their own and to have blood testing, which increase the risk of contamination and unblinding. Researchers in the field also highlight Mendelian randomisation studies as one important observational design for future research (144).

In Sweden, oral vitamin D supplementation is recommended to all people over 75 years to ensure an adequate intake of vitamin D, but the main source of vitamin D is sunlight. The strategy of active encouragement to go outdoors explored in Paper IV could be used as a complement to oral vitamin D supplementation during the summer, which is appealing as polypharmacy is a common problem for many older people (145). If so, it is important to give balanced information about the benefits and risks of sunlight exposure as UV radiation increases the risk of skin cancer. However, it is doubtful whether the level of the achieved 25(OH)D values in our study was high enough to maintain adequate levels throughout the year without vitamin D supplements.

There are still uncertainties about the role of vitamin D in health. Vitamin D may be used as a marker of ill health and may provide prognostic information, which possibly can be used as a clinical tool. My hope is that our research will contribute one of many pieces to help solve the ongoing puzzle about vitamin D.

CONCLUSIONS

General Conclusions

Low vitamin D levels are associated with increased mortality in elderly people in nursing homes, and with cardiovascular morbidity/ mortality and poor mental health in patients with type 2 diabetes. From our studies, we cannot draw conclusions about causality. Serum 25(OH)D could be considered as a prognostic factor. Active encouragement to spend time outdoors during summertime improved the vitamin D levels and mental health in elderly people in nursing homes and could be considered as a complement to oral vitamin D supplementation in the summer.

Specific Conclusions

- Vitamin D deficiency was associated with increased mortality in elderly people living in Swedish nursing homes. Vitamin D deficiency was very prevalent in this study population and the vitamin D levels changed during the year.
- Serum 25(OH)D₃ was inversely associated with the risk of cardiovascular morbidity and mortality in middle-aged people with type 2 diabetes.
- Serum 25(OH)D₃ was inversely associated with poor mental health at baseline, but not at follow-up, in middle-aged people with type 2 diabetes. Serum 25(OH)D₃ was not associated with vitality after adjustment.
- An intervention with active encouragement to spend time outdoors during summertime improved the levels of serum 25(OH)D and self-perceived mental health in older nursing home residents.

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