Determinants of vitamin D status

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Preface

This master thesis was based on the data obtained from the Western Norway Coronary Angiography Cohort. I would like to thank those who participated or contributed to the data collection.

I would like to thank my main supervisor, Professor Ottar Nygård for sharing some of his knowledge and expertise on the topic of this thesis. I would also like to thank my co-supervisor, Vegard Lysne PhD for providing valuable feedback and helpful suggestions for improvements. A massive thank you, to my co-supervisor, Johnny Laupsa-Borge PhD student, for guidance and valuable feedbacks throughout the working process, and for answering questions that have emerged.

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Abstract

Background: Vitamin D is a fat-soluble vitamin with important functions in several different tissues, and poor vitamin D status has been found to be associated with chronic diseases like type 1 diabetes, metabolic syndrome, cardiovascular disease, infectious diseases, and several forms of cancer. Different modifiable and non-modifiable factors have been suggested to be associated with serum 25(OH)D levels. The prevalence of insufficient serum 25(OH)D level is high, and determinants of vitamin D status are not fully elucidated. The main purpose of this thesis was to explore factors associated with circulating vitamin D levels which could contribute to a better understanding of potential causes of vitamin D deficiency.

Methods: This was a cross-sectional study of 4118 patients who underwent coronary angiography at Haukeland University Hospital or Stavanger University Hospital with suspected or verified stable angina pectoris (SAP) and available data on 25(OH)D serum concentrations. To assess the relationship between serum 25(OH)D and dietary, clinical, and biochemical variables measured in the two cohort studies, linear regression was used, while quantile regression analyses were conducted to assess the relationships between 25(OH)D status and the same variables in selected quantiles of 25(OH)D levels. To estimate the strength of the linear relationships between serum 25(OH)D levels and the measured predictors on a standardized scale, Spearman`s rank correlation coefficients, rhos, were calculated.

Results: The results showed that serum 25(OH)D levels were positively associated with blood sampling during the summer months and vitamin D intake through diet, fish, and egg consumption, and with circulating concentrations of HDL cholesterol (HDL-C), riboflavin, pyridoxal phosphate (PLP), pyridoxic acid (PA), folate, cobalamin, vitamin A, vitamin E, choline, and sarcosine. Negative associations were found for blood sampling during the winter season and body mass index (BMI), with circulating concentrations of triglycerides (TGs), blood glucose, and hemoglobin A1c (HbA1C), and with total

homocysteine (tHcy), dimethylglycine (DMG), and C-reactive protein (CRP) levels at low serum 25(OH)D concentrations.

Conclusions: In this cross-sectional study, we found that serum 25(OH)D levels were associated with seasonality, vitamin D intake, and BMI, and with circulating levels of HDL-C, TGs, PLP, vitamin A, and vitamin E, total homocysteine, as well as markers of glucose metabolism. These results may motivate future experimental studies further investigating determinants of vitamin D status and their mechanistic relationships, leading to better prevention of vitamin D deficiency.

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Abbreviations

25(OH)D	25-hydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
7-DHC	7-dehydrocholesterol
ApoA-I	Apolipoprotein A-I
ApoB	Apolipoprotein B
BECAC	Bergen Coronary Angiography Cohort
BMI	Body mass index (kg/m^2)
CAD	Coronary artery disease
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Vitamin D binding protein
DMG	Dimetylglycine
eGFR	Estimated glomerular filtration rate
FFQ	Food frequency questionnaire
HBA1c	Glycosylated haemoglobin
HDL	High density lipoprotein
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LDL	Low density lipoprotein
MMA	Methylmalonic acid
PA	4-pyridoxic acid
PL	Pyridoxine levels
PLP	Pyridoxal 5-phosphate
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RXR	Retinoid X-receptor

SAP	Stable angina pectoris
tCys	Total cysteine
TG	Triglycerides
tHcy	Total homocysteine
VDR	Vitamin D receptor
VDRE	Vitamin D responsive elements
WECAC	Western Norway Coronary Angiography Cohort
WENBIT	Western Norway B-Vitamin Intervention Trial

1 Introduction

Vitamin D, calciferol, is a fat soluble vitamin essential for calcium and phosphorus metabolism, and insufficient levels of vitamin D are known to be detrimental to bone health (1,2). Several functions besides calcium and phosphorus metabolism have been discovered, and poor vitamin D status has been found to be associated with chronic diseases like type 1 diabetes, metabolic syndrome, cardiovascular disease (CVD), infectious diseases, and several forms of cancer (1,3–5). A high global prevalence of insufficient vitamin D levels increases the risk of health consequences in a large group of people (3,4). Different factors have been suggested to be associated with circulating levels of vitamin D (6–8), but potential determinants of vitamin D status have not been fully elucidated. The main aim of this thesis was to investigate relationships between serum levels of vitamin D and dietary, clinical, and biochemical variables measured in a large cohort.

1.1 Vitamin D

1.1.1 The "sunshine" vitamin

Lack of sunlight has historically been strongly associated with the skeletal disease rickets (1,2). Early in the 1900s, it was suggested that rickets was caused by a dietary deficiency, and animal studies showed that the supply of cod liver oil prevented and cured the disease, and this resulted in the discovery of vitamin D as an essential nutrient (1,2).

The observed connection between sunlight exposure, vitamin D, and the development of rickets led to the identification of the two precursors of vitamin D, named 7-dehydrocholesterol (7-DHC) and ergosterol (1,2,9). 7-DHC is a sterol produced by animals, while ergosterol is found in plants, and both of these precursors change structure when exposed to ultraviolet irradiation from sunlight (1,2,9). When exposed to ultraviolet irradiation, 7-DHC is transformed into the provitamin D called vitamin D_3 or cholecalciferol, while ergosterol is transformed into ergocalciferol, also called vitamin D_2

(1,2,9). Because of the importance of sunlight exposure in the synthesis of vitamin D, the vitamin is also called the “sunshine vitamin” (1,2,9).

1.1.2 Vitamin D metabolism

7-DHC in the epidermis of the skin absorbs UV-radiation between 290 nm and 315 nm, causing an isomerization that involves photolytic ring opening to produce the 9,10-seco-sterol previtamin D_3 (1,9,10). Previtamin D_3 has the thermodynamically unstable s-cis, s-cis conformation and is easily transformed to vitamin D_3 through a non-enzymatic heat-induced isomerization, which cause the hydrophilic and hydrophobic interactions between the previtamin D_3 and the membrane fatty acids to break, and vitamin D_3 is released from the skin cell membrane into the blood (1,2,10). If the production of vitamin D_3 is high and the serum levels are above required, some of the vitamin D_3 can be stored in the fat tissue and be released in periods with insufficient synthesis (1,11,12).

Vitamin D_2 and D_3 have to be further activated before the vitamin can perform its functions in the target tissues (2,9). The vitamin D_3 produced in the skin or ingested through the diet is transported in the blood bound to vitamin D-binding protein (DBP) or incorporated into chylomicrons, and delivered to the liver where vitamin D_3 is converted to 25-hydroxyvitamin D_3 (25(OH)D) by the vitamin D 25-hydroxylase CYP2R1 (1,2). 25(OH)D_3 is released in the blood and transported to the kidneys, where a 25-hydroxyvitamin D-1α-hydroxylase called CYP27B1 converts 25(OH)D_3 to the active form 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), also called calcitriol (1,2,9). 1,25$(OH)_2D_3$ is transported in the blood to different tissues in the body and function like a steroid hormone that bind to cell membrane and nuclear vitamin D receptors (VDR) to regulate gene expression (1,9).

Vitamin D metabolism is tightly regulated based on calcium and phosphorus levels (1,2). Low serum calcium levels activate the calcium receptors on the parathyroid glands. This cause an increased release of parathyroid hormone (PTH), which in turn stimulates the

kidneys to increase the activity of 25(OH)D-1α-hydroxylase, which result in increased serum levels of calcitriol (1). Low phosphorus levels also lead to an increased activity of 25(OH)D-1α-hydroxylase to increase the circulating levels of calcitriol. Low levels of both calcium and phosphorus substantially increase the activity of 25(OH)D-1α-hydroxylase and thereby lead to high levels of calcitriol as a protective mechanism to normalize calcium and phosphorus levels (1).

The breakdown of calcitriol also occurs in the kidneys, and this catabolism is also tightly regulated based on calcium and phosphorus levels. 25(OH)D-24-hydroxylase (CYP24A1) is the catabolic enzyme responsible for the degradation of $1,25(OH)_2D_3$ to calcitroic acid, which is then excreted in the bile (1,2). High levels of PTH and calcitriol increases the activity of the 25(OH)D-24-hydroxylase, while low serum phosphorus levels down-regulate the activity of the 25(OH)D-24-hydroxylase and thereby the degradation of calcitriol to maintain sufficient serum levels (1,2).

1.1.3 Vitamin D functions

The vitamin D receptor

The actions of vitamin D are mediated through the vitamin D receptor (VDR), a ligand-activated transcription factor located in the target tissues (1,2,13). Binding of $1,25(OH)_2D_3$ to the VDR leads to the formation of two independent protein interaction surfaces on the VDR, one that allows binding of the heterodimer retinoid X-receptor (RXR), and one that is necessary for recruitment of large coregulatory complexes required for regulation of gene expression (1). The VDR recognizes a specific DNA sequence called vitamin D response element (VDRE), and the VDR-RXR complex binds to the VDRE. The regulation of gene expression is not mediated directly by the VDR, but indirectly through the recruitment of coregulatory complexes with the ability to make the desired changes of gene expression (1). These coregulatory complexes contain one VDR-interacting component, as well as many subunits with different enzymatic functions that acts by enhancing or suppressing the expression of the targeted genes (1). This way, through binding to the VDR and regulating gene expression, vitamin D has the ability to regulate the activity and different functions in a number of cells and tissues.

Effects of vitamin D

The primary function of vitamin D is to maintain calcium and phosphorus homeostasis (1,2). Serum calcium levels should be maintained at a very constant level, at 2.1 to 2.6 mmol/L, to attain and maintain a healthy bone mineral density (12). Vitamin D has three primary functions with the intent of increasing calcium levels if they fall below normal levels, which include stimulating the absorption of calcium and phosphorus in the intestine, mobilization of calcium and phosphorus from the bone tissue, and also stimulating reabsorption of calcium in the renal tube (2,12).

The VDR has also been found in tissues besides the ones involved in calcium and phosphorus metabolism, including the islet cells of the pancreas, the parathyroid glands, B cells and T cells of the immune system, in the macrophages, in epithelial cells of the intima of blood vessels, in cells of the stomach, in keratinocytes of skin, in epithelial cells of the colon, and in cells of the placenta (1,2,12). Thus, vitamin D has several non-skeletal functions (1,2,12). Among the observed functions of vitamin D, it has been found to affect the keratinocytes (1,13), maintaining parathyroid status (1,12), and have an impact on the immune system (1,12,14). However, it is still a work in progress to investigate the wide range of non-skeletal effects of vitamin D.

1.1.4 Sources of vitamin D

Dietary sources of vitamin D are quite limited and include fatty fish, cod liver oil, egg yolk, and foods fortified with vitamin D, such as dairy products. Vitamin D supplements are also an important source of vitamin D in some parts of the population (1,15). However, exposure of sunlight on the skin is considered to be the most important source of the vitamin (1,2). Vitamin D occurs in two different structural forms, named vitamin D_2, or ergocalciferol, and vitamin D_3, or cholecalciferol. Vitamin D_2 is found in plant sources like mushrooms and yeast, while vitamin D_3 is found in animal and fortified foods and is synthesized in the skin (1).

1.1.5 Recommended vitamin D levels

25(OH)D is the circulating form measured to determine vitamin D status (1,9). The Nordic Nutrition Recommendations 2012 (16), the US Institute of Medicine (17), and the recommendations from Germany, Austria, and Switzerland (18) consider a serum level of above 50 nmol/L 25(OH)D (20 ng/mL) as sufficient, while a level of under 30 nmol/L (12 ng/mL) is regarded as deficient (19). However, the levels of serum 25(OH)D considered as adequate and the definition of vitamin D deficiency has been widely discussed. In parts of the literature, a serum level of above 30 ng/mL (75 nmol/L) 25(OH)D is considered to be sufficient to optimize health, while levels under 20 ng/mL (50 nmol/L) is considered as a vitamin D deficiency (1,9,20). There have also been uncertainties regarding the daily intake of vitamin D needed to achieve the optimal serum levels of 25(OH)D, and the recommendations vary across different countries and health authorities. The US recommendation for vitamin D intake to achieve the recommended level of 50 nmol/L to support bone health is set to 15 µg for children and adults (17), while the dietary guidelines from Germany, Austria, and Switzerland have estimated adequate vitamin D intake to be 20 µg/d for children, adolescents, and adults (18). However, the Nordic recommendations to maintain sufficient serum levels is set to 10 µg/d for children, adults, pregnant women, and lactating women, and 20 µg/d for adults over 75 years old (19).

Highly increased serum levels of 25(OH)D are toxic and can lead to consequences like hypercalcaemia, hyperphosphatemia, nephrocalcinosis, and kidney failure (1,19). Serum 25(OH)D concentrations above 375 nmol/L indicates vitamin D toxicity (21). This usually occurs with excessive oral intake, most often associated with supplementation above the recommended doses (1,21). The tolerable upper intake level (UL) is set to 100 µg/d for adults and adolescents, 50 µg/d for children 1–10 years of age, and 25 µg/d for infants (19). Excessive sunlight exposure will not cause vitamin D intoxication due to photodegradation of previtamin D_3 to inactive sterols in the skin, in addition to the protective effect of melanin production against further irradiation (22).

1.1.6 Vitamin D deficiency

Prevalence

The prevalence of vitamin D deficiency varies across different parts of the world and between different population groups. Reviews aimed at providing an overview of the global vitamin D status have found that vitamin D deficiency is a global problem affecting all age groups, but the prevalence is particularly high in girls and women from the middle east (3,8).

Data from "The Tromsø study" and "The North Trøndelag health study" show that a large proportion of the Norwegian adult population has suboptimal serum 25(OH)D levels (below 50 nmol/L), while a relatively small proportion have levels below 25 nmol/L, considered as deficient (23). The vitamin D status varies across different groups in the Norwegian population, and it has been found that among the adult ethnic Norwegian population aged 45–75, the majority have sufficient vitamin D levels, while among the elderly at nursing homes and the non-western immigrants, it is estimated that more than 70% have insufficient serum 25(OH)D levels (24).

Consequences of vitamin D deficiency

Vitamin D deficiency has primarily been associated with detrimental effects on bone health. Lack of vitamin D affects the calcium homeostasis, causing decreased calcium absorption in the intestine and reabsorption of calcium in the kidneys, decreased levels of calcium in the blood, and thereby impaired bone mineralization (1,2,9). A consequence of severely deficient vitamin D and calcium levels in children is poor bone development and the condition rickets, characterized by bowed legs, knock knees, and growth retardation (1,9,11). In adults, vitamin D deficiency could cause impaired bone mineralization leading to the bone disease osteomalacia and increased risk of osteoporosis (1,2).

The vitamin D receptor (VDR) is also found in muscle tissue, and vitamin D deficiency has been shown to impair muscle function and cause muscle weakness, which in turn

increases the risk of falling (11,25). A proposed explanation of the effect of vitamin D on muscle function and the risk of falling is that $1,25(OH)_2D$ binds to a vitamin D receptor in muscle tissue stimulating *de novo* protein synthesis, muscle cell growth, and improved muscle function (1,25). Closely related to the effects on muscle function and risk of falling is the connection between vitamin D status and fracture risk. A dose-dependent associations between vitamin D status and the risk of fractures have been observed (1,25). A meta-analysis found that supplementing with 10 μg/d or below did not reduce the risk of fractures, while vitamin D supplementation at 12.5 to 17.5 μg/d and higher achieved serum 25(OH)D levels that seemed to reduce the risk of nonvertebral fractures by 20% and hip fractures by 18% (26).

In addition to the detrimental effects on bone health and muscle function, vitamin D deficiency is associated with increased risk of several chronic diseases, including CVD, autoimmune diseases, infectious diseases, multiple sclerosis, type 1 diabetes, and different forms of cancer (1,2,11).

Both excessive and insufficient vitamin D status have been suggested to be associated with an increased risk of CVD (1,2). In observational studies from the 1980s, it was observed a seasonal variation in cases of cardiovascular events in accordance with varying sunlight exposure, and it was hypothesized that vitamin D is associated with the risk of CVD (1). Insufficient vitamin D status is associated with several different risk factors of CVD, including hypertension, peripheral vascular disease, diabetes mellitus, and abnormal lipid profiles (2,27–32). Furthermore, several meta-analyses found that low levels of serum 25(OH)D were associated with an increased risk of CVD (33,34). Potential mechanisms explaining the protective effects of vitamin D against CVD include beneficial effects on cardiac function, blood pressure, insulin resistance, lipid metabolism, and inflammatory processes (1). Studies investigating the effects of vitamin D supplementation on the risk of CVD are, however, inconsistent, and it is yet premature to draw firm conclusions about the effects of vitamin D supplements (35–37).

The abilities of vitamin D to regulate gene expression in several different tissues have led to the hypothesis that vitamin D could have anti-cancer effects and that vitamin D status affects cancer risk (1). Activation of VDR by binding of calcitriol elicit a wide variety of responses, which could influence cellular growth, proliferation, apoptosis, and immune function, and thereby affecting the risk of cancer development (1,38). Vitamin D also seems to have angiogenesis inhibitory effects causing tumor growth retardation and tumor regression (1,38).

Associations between serum 25(OH)D levels and different types of cancers, including colorectal cancer, breast cancer, and prostate cancer, have been suggested (1,11,16). A meta-analysis indicated that circulating 25(OH)D was inversely associated with cancer incidence and cancer mortality (39), and that serum 25(OH)D levels were inversely related to the risk of colorectal cancer, but no association was found for breast and prostate cancer (40). When looking at the effects of vitamin D supplementation on cancer incidence and mortality, the results are inconclusive (41,42).

Immunomodulatory and anti-inflammatory effects of vitamin D have also been hypothesized, and it has been suggested that vitamin D may thereby affect the risk of developing autoimmune diseases (1,43). A meta-analysis looking at the effects of vitamin D on systemic inflammation and autoimmune disease concluded that the data was insufficient to indicate a relation between vitamin D and reduced risk of autoimmune disease (14). However, several meta-analyses investigating the relationship between rheumatoid arthritis and vitamin D status have found that patients with rheumatoid arthritis have lower serum 25(OH)D compared to healthy controls, and that there is a negative association between serum 25(OH)D and rheumatoid arthritis disease activity (44,45). Similar results have been observed when looking at associations between vitamin D status and type 1 diabetes, where subjects with type 1 diabetes had 6.3 nmol/ lower serum 25(OH)D levels compared to the control group (46).

Vitamin D deficiency has also been suggested to increase the risk of infectious diseases (47), and vitamin D has been proposed to have a protective effect on diseases like

respiratory tract infections and tuberculosis (1,48,49). Some meta-analyses found that vitamin D supplementation reduced the risk of acute respiratory tract infection (odds ratio (OR) 0.64 and odds ratio 0.88), where the protective effects were strongest in those with profound vitamin D deficiency at baseline (49,50), while another meta-analysis observed a weaker protective effect of vitamin D supplementation on the risk of respiratory tract infections in previously healthy individuals (relative risk (RR) 0.94) (48). When looking at the associations between vitamin D and tuberculosis, some meta-analyses found that vitamin D deficiency is associated with an increased risk of tuberculosis (51,52).

Vitamin D also seems to influence brain function, and it has been found associations between insufficient vitamin D status and several neurological diseases, including schizophrenia, Parkinson´s disease, Alzheimer´s disease, and reduced cognitive function (53,54). Data from experimental trials indicate that vitamin D is a neuroactive steroid, and that vitamin D signaling is involved in brain development and function in adults (53,54). Meta-analyses found that lower 25(OH)D levels were associated with poorer cognitive function (55) and that individuals with Alzheimer's disease had lower 25(OH)D concentrations compared to healthy controls (55,56).

In addition to the range of chronic diseases, several studies have also found an inverse relationship between 25(OH)D levels and all-cause mortality (57,58). However, the effect of vitamin D supplementation on all-cause mortality is unclear. Some meta-analyses found that intake of vitamin D supplements were associated with decreased total mortality rates (59,60), while another analysis observed no association between vitamin D supplementation and all-cause mortality (61).

1.1.7 Factors associated with vitamin D status

Vitamin D status is affected by several different factors, both modifiable and non-modifiable. Among the modifiable factors are sunlight-exposure, vitamin D content in the diet, bodyweight, smoking, and lifestyle factors such as physical activity (6,7,62–64).

The non-modifiable factors include gender, age, and skin-color (6,7,62,63,65). However, there is still uncertainty regarding determining factors of vitamin D status and potential risk factors of vitamin D deficiency. This thesis is based on a cohort of patients with stable angina pectoris (SAP). A previous study based on these data found that serum 25(OH)D concentrations were inversely associated with cardiovascular mortality (66), highlighting the need to provide more information about potential determinants of vitamin D.

2 Objectives

Vitamin D has a wide variety of functions in the human body, and insufficient vitamin D levels may have major health consequences. Importantly, the prevalence of insufficient serum 25(OH)D levels is high, and potential determinants of vitamin D status are not fully elucidated. This thesis aimed to explore a wide variety of factors, both demographic characteristics, anthropometric measures, biochemical variables, and dietary data, to investigate which factors were associated with serum 25(OH)D levels, measured at baseline in a large cohort of patients with stable angina pectoris.

Specific objectives

- Assess cross-sectional associations between 25(OH)D serum levels and a variety of dietary, clinical, and biochemical variables by linear regression modeling.
- Explore associations between serum 25(OH)D and dietary, clinical and biochemical variables at different levels of 25(OH)D by quantile regression analysis.
- Assess the strengths of linear relationships between serum 25(OH)D levels and the measured variables of interest on a standardized scale by correlation analysis.

3 Methods

3.1 Cohorts

This was a cross-sectional study based on data obtained from the *Western Norway Coronary Angiography Cohort* (WECAC), investigating factors associated with serum 25(OH)D levels at baseline in a large clinical cohort. WECAC included the participants from both *Bergen Coronary Angiography Cohort* (BECAC) and *Western Norway B Vitamin Intervention Trial* (WENBIT). BECAC was a prospective cohort study that followed patients who underwent elective coronary angiography at Haukeland University Hospital between January 2000 and April 2004 (67). The overall aim of BECAC was to study various prognostic markers of cardiovascular endpoints and cause-specific mortality in patients with suspected heart diseases (67). WENBIT (ClinicalTrials.gov Identifier: NCT00354081) was a randomized, controlled, double blind study investigating effects of homocysteine-lowering therapy on mortality and cardiac events in patients undergoing coronary angiography, hypothesizing that a daily supplement with B vitamins would reduce the risk of cardiovascular mortality and serious cardiovascular events among patients with coronary artery disease (68).

The inclusion criteria for the cohort was age over 18 years, patients able to give informed consent, with and without significant coronary artery disease (CAD) who had undergone coronary angiography just before inclusion, and was prepared to undergo long-term follow-up (67,68). Patients with known alcohol abuse or serious mental illness, or with known active malignant disease were not eligible to participate in the study. The study was conducted according to the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Data Protection Authority, and written informed consent was obtained from all participants (68).

In total 5210 men and women who underwent coronary angiography at Haukeland University Hospital or Stavanger University Hospital between April 1999 and April 2004

were included in BECAC and WENBIT (4241 patients were included in BECAC and an additional 969 patients were included in WENBIT). From this cohort, a total of 4166 patients with suspected or verified stable angina pectoris (SAP) were eligible for inclusion. Of these patients, 4118 had available measures of 25(OH)D concentrations and were included in the analyses in the current thesis (**Figure 1**).

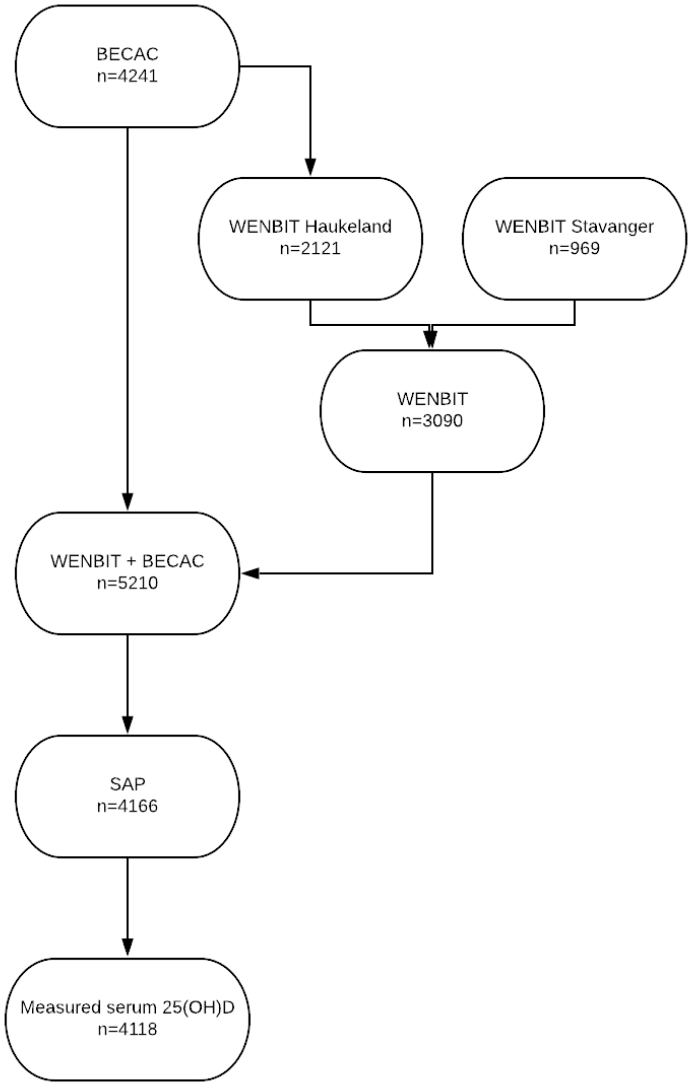

Figure 1. Flow of subjects from BECAC and WENBIT with stable angina pectoris (SAP) and available measurements of serum 25(OH)D included in the analyses.

3.2 Baseline characteristics

Information about the participants was obtained by clinical examinations, anthropometric measurements, blood sampling, and questionnaires on lifestyle, medical history, and dietary habits. Self-administered questionnaires were used to obtain information on participants lifestyle, and medical history gave information about health status and was verified by comparing with hospital records. Smoking status was classified based on self-reported smoking habits and plasma cotinine levels. Current smoking was defined as a self-reported smoker, having stopped smoking less than 90 days ago, or plasma cotinine levels above 85 nmol/L. Estimated glomerular filtration rates (eGFR) were calculated by using the formula suggested by the Chronic Kidney Disease Epidemiology Collaboration (69).

3.2.1 Laboratory data

Clinical examinations and blood sampling at baseline and during follow-up were conducted by trained study personnel. At Haukeland University Hospital, the blood samples were taken from non-fasting patients before the angiography, while at Stavanger University Hospital, fasting blood samples were taken in conjunction with the angiography. Routine blood analyses were performed at the hospital laboratories, while blood sample for biobanking were immediately prepared to serum and plasma and stored at −80°C until analyzed (68).

Analyses of plasma 25(OH)D_2 and 25(OH)D_3 concentrations were performed by using liquid chromatography tandem mass spectrometry (LC-MS/MS) in the period between 2011 and 2012 at Bevital AS, Bergen, Norway (66,68).

3.2.2 Dietary data

Information on dietary habits was obtained from a semiquantitative food frequency questionnaire (FFQ) the participants completed at enrollment (68,70). The FFQ was developed at the Department of Nutrition, University of Oslo, and included 169 food items that were grouped according to traditional Norwegian meal patterns. It was

designed to obtain information on usual food intake during the past year, including the frequency of consumption of different food groups and amounts given as household measures or units, such as slices or pieces. Questions on supplement use were also included in the FFQ. Based on the information from the FFQ, nutrient intake was calculated by using a database and software system developed at the Department of Nutrition, University of Oslo (Kostberegningssystem, version 3.2; University of Oslo, Norway).

3.3 Statistical analysis

Baseline characteristics of the study population are presented as arithmetic means and standard deviation (SD) for demographic characteristics, as number and percentages for categorical variables, and as geometric means and ranges (1SD ranges) for biochemical variables. The geometric SD ranges were calculated by dividing and multiplying the geometric means with the geometric SD factors to obtain the lower and upper limits, respectively. The descriptive statistics are categorized into quartiles of 25(OH)D levels. P-values for the linear trend across quartiles were derived from unadjusted linear regression models for the continuous variables, while unadjusted logistic regression models were used to derive p-values for trend across quartiles of 25(OH)D levels for the categorical variables. All statistical analyses were conducted with R, version 3.6.1 (https://www.R-project.org) (71), and data transformation and exploration were done by using *tidyverse* packages (72).

To assess the relationship between serum 25(OH)D levels and dietary, clinical, and biochemical variables linear regression analysis were used. Linear regression is a method to investigate associations between variables, and allows prediction of the values of the dependent variable based on the values of the independent variable (73). A simple linear regression model was used to assess the association between serum 25(OH)D and the different parameters. In addition, multiple linear regression models were used to adjust for potential confounding factors, i.e., to isolate the relationship between serum 25(OH)D levels and the variable of interest from the effects of the confounding variables. Multiple regression allows inclusion of more than one

independent variable, and is used to reveal associations between multiple predictor variables and a single outcome variable (74). The first multiple regression model was adjusted for sex and age, and the second was adjusted for age, sex, BMI, smoking habits, and GFR. We adjusted for age, sex, BMI, smoking habits, and GFR as these covariates have been found to be associated with serum 25(OH)D levels (6,7,62,75), and also several of the variables of interest. Among observed relationships between potential confounders and the variables we investigated, are associations between BMI and biochemical variables, such as standard lipids and parameters glycemic control (76), the relationships of sex and age with amino acid profile (77) and lipid profile (78), and the associations between GFR and biochemical variables, such as vitamin status (79). The linear regression analyses were performed with the *lm* function, while logistic regression analyses were conducted with the *glm* function in the *stats* package, version 3.6.2 (https://www.rdocumentation.org/packages/stats) (80).

Quantile regression analysis was used to assess the relationship between 25(OH)D concentrations and the different parameters in selected quantiles of 25(OH)D. This type of regression analysis makes it possible to assess the relationship between the independent variables and serum 25(OH)D at different levels of 25(OH)D (81). This may disclose varying degrees of association between the independent variables and 25(OH)D in different categories of vitamin D status. Quantile regression was conducted both for the unadjusted model, the model adjusted for age and sex, and the model adjusted for age, sex, BMI, smoking habits, and GFR. The quantile regression analyses were performed with the *lqm* function in the *quantreg* package, version 5.51 (https://cran.r-project.org/web/packages/quantreg/) (82).

To estimate the strengths of linear relationships between serum 25(OH)D levels and the measured variables of interest on a standardized scale, Spearman`s rank correlation coefficients, rhos, with bootstrapped confidence intervals and p-values, were calculated. Spearman`s correlation measures the strength and direction of the monotonic relationship between two variables. A positive relationship, which implies that as the value of one of the variables increases so does the other variable, will give a positive

correlation coefficient, while a negative relationship, which implies that as the value of one variable increases the value of the other variable decreases, is shown as a negative correlation coefficient (83). The Spearman`s correlation coefficient can take values from −1 to +1, where a correlation coefficient of +1 indicates a perfect positive association, while a correlation coefficient of −1 indicates a perfect negative association, and a coefficient of 0 indicates that there is no association between the two variables (84). To control for the effects of potential confounding factors, partial correlation analyses were also conducted by adjusting for age and sex in one model, and for age, sex, BMI, smoking habits, and GFR in another model. The Spearman`s rank correlation coefficients were calculated with the *cor.test* function in the *RVAideMemoire* package and the partial correlations were estimated with the *pcor.test* function in the *RVAideMemoire* package, version 0.9-73 (https://cran.r-project.org/web/packages/RVAideMemoire) (85).

3.3.1 Model validation

Linearity, normality, and homoscedasticity are assumptions that should be met to justify the use of linear regression (73,86). In a linear regression model, it is assumed that the relationship between the dependent variable and the independent variables is linear. A linear regression model is also limited by the fact that it models the mean of the conditional probability distribution, and the results are more accurate if this distribution is normally distributed. The assumption of homoscedasticity includes that the variance of the residuals should be the same for all values of the independent variables. Through model validation, these assumptions can be checked to determine whether the chosen model is suitable for its purposes (87,88).

To check for the assumptions of linearity, normality, and homoscedasticity, and examine the validity of the regression models in this thesis, graphical analyses of residuals were conducted. To assess the linearity, the residual values were plotted against predicted values of the independent variable in a scatter plot. The residuals were presented on the y-axis, while the predicted values were presented on the x-axis, and a horizontal line was drawn where the residuals equal zero. Residual plots showing no systematic pattern and a line approximately horizontal at zero, indicated linearity (86,89). In

addition, partial residual plots were used to assess linearity in the multiple regression models. In partial residual plots the dependent variable is adjusted for the linear effects of the independent variables except the one of interest. The partial residuals were plotted against predictive values for each of the independent variables in component-plus-residual-plots. Plots with residuals randomly scattered close to the zero-line, indicated that the assumption of linearity was held (86). The assumption of homoscedasticity was evaluated by plotting square root standardized residuals against the predicted values of the independent variable. Residuals randomly spread along a horizontal line, indicated that the assumption of homoscedasticity was valid (86,89). To assess the assumption of normality of the residuals, a normal quantile-quantile (QQ) plot was used. In this plot, the quantiles of the observed residuals were plotted against the quantiles of the standard normal distribution, and where the points in the scatter plot followed along the 45 degree line, we assumed normality (86,89). The diagnostic plots and model validation in this thesis were conducted with the *plot* function in the *ggfortify* package, version 0.4.10 (https://cran.r-project.org/package=ggfortify) (90), and partial residual plots were made with the *crPlots* function in the *car* package, version 3.0-7 (91) (https://cran.r-project.org/package=car).

Potential nonlinear associations between serum 25(OH)D and different variables was visualized by plotting the relationships using splined functions in the regression models, and the function *geom_smooth* in the package *ggplot2*, version 3.2.0 (https://cran.r-project.org/web/packages/ggplot2).

To visualize the results from the statistical analysis different plots and figures were made. All plots were made with the *ggplot2* package, version 3.2.0 (https://cran.r-project.org/web/packages/ggplot2).

4 Results

4.1 Baseline characteristics

Of the 4166 participants eligible for inclusion in this study, 48 were excluded due to missing data on serum 25(OH)D levels, and the remaining 4118 (2961 (72%) males and 1157 (28%) females) were included in the analyses. The mean age (SD) in the study population was 61.8 (10.4) years.

Geometric mean serum 25(OH)D level (1SD range) in the study group was 55.4 (38.3–80.1) nmol/L, with a range from 3.37 nmol/L to 205 nmol/L. A total of 1423 (35%) of the 4118 participants, had serum 25(OH)D concentrations below the recommended level of 50 nmol/L, while the remaining 2695 had serum 25(OH)D levels considered as sufficient. The baseline table is divided into quartiles of 25(OH)D levels, showing the linear trend of the different variables across the quartiles. **Tables 1-4** present baseline data on demographic characteristics, anthropometric measurements, levels of standard lipids, blood glucose, inflammatory markers, vitamin status, dietary data, amino acids, and amino acid metabolites, both in the total cohort and across quartiles of 25(OH)D levels.

Table 1. Demographic characteristics, anthropometric measurements, smoking habits, GFR and vitamin D intake of the study population at baseline across quartiles of 25OH vitamin D levels.[1]

		Quartiles of vitamin D levels				
Variable	**Total cohort (n = 4118)**	**Q1 (n = 1030)**	**Q2 (n = 1029)**	**Q3 (n = 1030)**	**Q4 (n = 1029)**	**P-value[2]**
25(OH)D (nmol/L)	55.4 (38.3, 80.1)	34.0 (26.0, 44.4)	51.3 (47.9, 55.0)	64.1 (60.0, 68.4)	84.3 (73.1, 97.1)	
Age (years)	61.8 (10.4)	60.1 (11.0)	61.4 (10.3)	62.9 (9.85)	62.6 (10.0)	<0.001
Male sex (n)	2961 (71.9)	716 (69.5)	733 (71.2)	762 (74.0)	750 (73.0)	0.075
BMI (kg/m^2)	26.3 (4.00)	26.9 (4.48)	26.6 (4.07)	26.1 (3.71)	25.5 (3.54)	<0.001
Current smoker (n)	1063 (26)	340 (33.0)	248 (24.1)	220 (21.4)	255 (24.8)	<0.001
eGFR (mL/min/ 1,73^2)	85.5 (66.3, 110)	88.4 (69.1, 113)	86.9 (69.2, 109)	84.2 (65.7, 108)	82.7 (62.2, 110)	<0.001
Waist circumference (cm)	95.8 (11.6)	97.3 (12.6)	96.4 (11.6)	95.6 (11.3)	94.1 (10.6)	<0.001
Vitamin D intake (µg/d)	10.8 (8.48)	7.95 (5.48)	9.88 (7.60)	11.8 (9.40)	12.9 (9.51)	<0.001

[1]Values are arithmetic means (SDs), geometric means (1SD range), and numbers (%) across quartiles of serum 25OH vitamin D levels. BMI, body mass index (kg/m^2); eGFR, estimated glomerular filtration ratio. [2]P-values are derived from unadjusted linear regression models for the continuous variables and logistic regression models for the categorical variables.

Table 2. Standard lipids, blood glucose, and inflammatory markers in serum at baseline across quartiles of 25OH vitamin D levels.[1]

		Quartiles of vitamin D levels				
Variable	**Total cohort (n = 4118)**	**Q1 (n = 1030)**	**Q2 (n = 1029)**	**Q3 (n = 1030)**	**Q4 (n = 1029)**	**P-value[2]**
TG (mmol/L)	1.54 (0.92, 2.58)	1.67 (0.96, 2.91)	1.54 (0.94, 2.52)	1.49 (0.91, 2.46)	1.46 (0.89, 2.40)	<0.001
Total cholesterol (mmol/L)	4.95 (3.97, 6.17)	4.98 (3.95, 6.27)	4.94 (3.98, 6.13)	4.92 (3.96, 6.11)	4.96 (4.01, 6.13)	0.673
LDL-C (mmol/L)	2.94 (2.11, 4.09)	2.95 (2.11, 4.12)	2.94 (2.13, 4.07)	2.92 (2.10, 4.08)	2.92 (2.10, 4.08)	0.794
HDL-C (mmol/L)	1.24 (0.78, 1.95)	1.18 (0.90, 1.56)	1.23 (0.93, 1.62)	1.23 (0.57, 2.68)	1.31 (1.00, 1.71)	<0.001
Non-HDL-C (mmol/L)	3.62 (2.69, 4.86)	3.71 (2.74, 5.02)	3.62 (2.71, 4.85)	3.57 (2.67, 4.78)	3.56 (2.65, 4.78)	0.002
ApoA-I (g/L)	1.29 (1.05, 1.59)	1.24 (1.00, 1.54)	1.28 (1.03, 1.58)	1.30 (1.07, 1.59)	1.35 (1.11, 1.63)	<0.001
ApoB (g/L)	0.87 (0.67, 1.14)	0.88 (0.67, 1.16)	0.87 (0.67, 1.14)	0.87 (0.67, 1.12)	0.87 (0.67, 1.13)	0.259
Type 2 diabetes (n)	455 (11.0)	140 (13.6)	112 (10.9)	110 (10.7)	93 (9.04)	<0.001
HbA1c (%)	6.08 (4.91, 7.54)	6.22 (4.98, 7.78)	6.06 (4.90, 7.48)	6.09 (4.95, 7.49)	5.96 (4.81, 7.38)	<0.001
Serum glucose (mmol/L)	6.00 (3.69, 9.77)	6.21 (4.54, 8.49)	6.11 (4.62, 8.07)	5.85 (2.55, 13.39)	5.85 (4.39, 7.81)	<0.001
CRP (mg/L)	1.86 (0.59, 5.84)	2.25 (0.59, 8.59)	1.79 (0.63, 5.12)	1.68 (0.57, 4.96)	1.76 (0.61, 5.10)	0.003
Neopterin (nmol/L)	8.57 (5.85, 12.5)	8.59 (5.75, 12.8)	8.37 (5.72, 12.2)	8.59 (6.11, 12.1)	8.72 (5.85, 13.0)	0.006

[1]Values are geometric means (1SD range), and numbers (%) across quartiles of serum 25OH vitamin D levels. TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; LDL-C, serum low density lipoprotein cholesterol; ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HBA1c, glycosylated haemoglobin; CRP, C-reactive protein. [2]P-values are derived from unadjusted linear regression models for the continuous variables and logistic regression models for the categorical variables.

Table 3. Dietary data at baseline across quartiles of 25OH vitamin D levels.[1]

		Quartiles of vitamin D levels				
Variable	**Total cohort (n = 4118)**	**Q1 (n = 1030)**	**Q2 (n = 1029)**	**Q3 (n = 1030)**	**Q4 (n = 1029)**	**P-value[2]**
Riboflavin (µg/dL)	12.4 (5.90, 26.2)	11.3 (5.62, 22.7)	12.4 (5.97, 26.0)	12.9 (6.20, 26.8)	13.2 (5.91, 29.4)	<0.001
PL (nmol/L)	10.6 (5.43, 20.9)	9.48 (5.12, 17.5)	10.2 (5.42, 19.2)	11.1 (5.74, 21.4)	12.0 (5.64, 25.5)	0.072
PLP (nmol/L)	43.7 (24.0, 79.6)	38.3 (20.8, 70.4)	42.9 (24.3, 75.9)	46.5 (26.0, 83.2)	47.7 (25.8, 88.2)	<0.001
PA (nmol/L)	28.0 (14.3, 54.8)	24.9 (13.5, 45.8)	26.8 (14.5, 49.7)	29.1 (14.9, 56.7)	31.8 (15.0, 67.6)	0.005
Folate (nmol/L)	11.0 (5.99, 20.3)	10.0 (5.55, 18.0)	11.03 (6.06, 20.0)	11.3 (6.21, 20.5)	11.8 (6.22, 22.5)	<0.001
Cobalamin (pq/mL)	361 (228, 570)	343 (214, 549)	358 (231, 554)	368 (238, 569)	375 (232, 606)	0.001
MMA (nmol/L)	0.17 (0.12, 0.25)	0.17 (0.11, 0.26)	0.17 (0.12, 0.24)	0.17 (0.12, 0.24)	0.17 (0.12, 0.25)	0.736
Vitamin A (µmol/L)	2.84 (2.25, 3.59)	2.71 (2.11, 3.48)	2.79 (2.22, 3.50)	2.88 (2.31, 3.60)	2.99 (2.38, 3.74)	<0.001
Vitamin E (µmol/L)	30.2 (24.1, 38.0)	29.2 (22.8, 37.4)	29.9 (24.0, 37.1)	30.6 (24.6, 38.2)	31.3 (25.2, 38.9)	<0.001
Low fat milk consumption (g/day)	131 (188)	137 (203)	122 (179)	134 (187)	129 (183)	0.598
Fish consumption (g/day)	110 (70.7)	96.1 (62.5)	112 (69.9)	112 (76.8)	119 (70.0)	<0.001
Egg consumption (g/day)	16.6 (11.9)	15.6 (11.9)	17.1 (11.8)	16.3 (11.7)	17.1 (11.9)	0.090

[1]Values are arithmetic means (SDs) and geometric means (1SD range) across quartiles of serum 25OH vitamin D levels. PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; MMA, methylmalonic acid. [2]P-values are derived from unadjusted linear regression models.

Table 4. Serum levels of amino acids and amino acid metabolites at baseline across quartiles of 25OH vitamin D levels.[1]

		Quartiles of vitamin D levels				
Variable	**Total cohort (n = 4118)**	**Q1 (n = 1030)**	**Q2 (n = 1029)**	**Q3 (n = 1030)**	**Q4 (n = 1029)**	**P-value[2]**
Serine (µmol/L)	97.2 (26.1, 361)	99.7 (41.0, 243)	98.5 (28.5, 340)	95.1 (21.0, 432)	95.5 (21.0, 433)	0.728
Glycine (µmol/L)	211 (163, 272)	206 (158, 268)	209 (163, 269)	211 (162, 274)	216 (168, 278)	<0.001
DMG (µmol/L)	4.20 (2.99, 5.90)	4.23 (2.92, 6.13)	4.13 (2.98, 5.73)	4.22 (3.05, 5.83)	4.21 (3.00, 5.92)	0.339
Sarcosine (µmol/L)	1.50 (1.06, 2.12)	1.43 (0.99, 2.06)	1.50 (1.08, 2.10)	1.51 (1.09, 2.10)	1.56 (1.11, 2.20)	<0.001
Choline (µmol/L)	9.71 (7.48, 12.6)	9.36 (7.01, 12.5)	9.68 (7.58, 12.4)	9.74 (7.57, 12.5)	10.1 (7.83, 12.9)	<0.001
Betaine (µmol/L)	38.9 (28.3, 53.5)	37.8 (27.0, 52.8)	38.5 (28.1, 52.8)	39.3 (28.8, 53.6)	40.2 (29.5, 54.7)	<0.001
Methionine (µmol/L)	27.0 (20.8, 35.1)	26.5 (20.3, 34.5)	27.1 (20.9, 35.2)	27.0 (21.1, 34.5)	27.4 (20.8, 36.0)	0.023
tHcy (µmol/L)	10.7 (7.75, 14.8)	10.9 (7.65, 15.5)	10.5 (7.70, 14.2)	10.6 (7.87, 14.4)	10.8 (7.79, 15.0)	0.537
tCys (µmol/L)	290 (254, 331)	288 (252, 329)	288 (253, 328)	293 (257, 334)	292 (256, 332)	<0.001

[1]Values are geometric means (1SD range) across quartiles of serum 25OH vitamin D levels. DMG, plasma dimetylglycine; tHcy, total homocysteine; tCys, total cysteine.
[2]P-values are derived from unadjusted linear regression models.

4.2 Factors associated with vitamin D status

The following sections present a summary of the results from the regression and Spearman`s correlation analyses investigating the associations between serum 25(OH)D levels and measured variables of interest.

Vitamin D intake

Data on estimated daily vitamin D intake was available for 2068 of the study participants. The mean vitamin D intake in the total population was estimated to 10.8 (8.48) µg/d. 779 of the 2068 with available data on vitamin D intake had an estimated daily intake above the recommendation of 10 µg/d. Among the participants with serum 25(OH)D levels above 50 nmol/L, the mean vitamin D intake was estimated to 11.9 (9.2) µg/d, while the mean daily intake among the subjects with serum levels below 50 nmol/L was estimated to 8.3 (5.9) µg. Both the unadjusted and the adjusted regression and Spearman`s correlation analyses showed that vitamin D intake was positively associated with the serum 25(OH)D level (**Tables 5 and 6**). The fully adjusted regression model showed that each additional 1 µg higher daily vitamin D intake was associated with an increase in serum 25(OH)D of 0.47 nmol/L.

Season

Great variation was seen when analyzing the associations between month or season of the study visit and the measured serum 25(OH)D levels. The lowest vitamin D levels were measured in the subjects included in March, with a geometric mean (1SD range) serum 25(OH)D concentration of 49.5 (35.3–69.5) nmol/L, while the highest levels were observed in the blood samples taken in August, with a geometric mean (1SD range) of 69.0 (53.4–89.0) nmol/L. This implies a difference of 19.5 nmol/L between the geometric means of the months with highest and lowest observed vitamin D levels.

When categorizing date of study visit in quarters of the year, the results revealed a similar trend with lower serum 25(OH)D levels during the winter months compared to the summer months (**Table 6**). Blood samples in the period from January through March had the lowest serum 25(OH)D levels with a mean of 50.0 (34.8–71.8) nmol/L, while the

quarter with the highest levels of serum 25(OH)D was July through September with a mean of 66.5 (50.1–88.3) nmol/L. This relationship was also shown in the regression and correlation analyses, where the summer months were positively associated with serum 25(OH)D levels, while the winter months were negatively associated with vitamin D status in both the unadjusted and the adjusted models. The proportion of subjects included in the period from January to March with serum 25(OH)D levels below 50 nmol/L, was estimated to 48%, while among subjects included in the period from July through August, the proportion with insufficient serum 25(OH)D levels was 14%. The variation of serum 25(OH)D levels according to months of the year are shown in **Figure 2**.

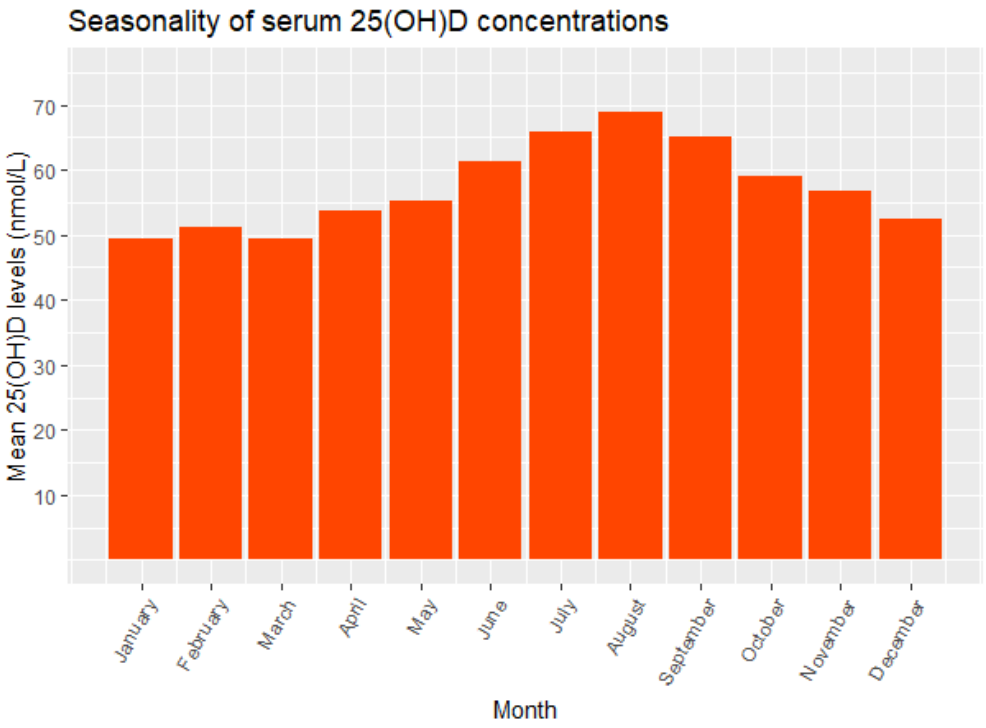

Figure 2. Mean 25(OH)D levels measured at each month of study visit.

Anthropometric measurements

Both the unadjusted linear regression and correlation analyses and the models adjusted for age and sex showed that both BMI and waist circumference were inversely related to serum 25(OH)D levels (**Tables 5 and 6**). When classified according to WHOs body mass

index classification (92), it was observed that subjects classified as “normal weight” (BMI 18.5–24.9) had a mean serum 25(OH)D concentration of 58.1 (40.5–83.3) nmol/L, the subjects in the “overweight” category (BMI 25.0–29.9) had a mean serum 25(OH)D level of 55.7 (38.5–80.5) nmol/L, while the subjects classified as “obese class 1” (BMI 30.0–34.9), “obese class 2” (BMI 35.0–39.9), and “obese class 3” (BMI above 40) had mean serum 25(OH)D concentrations of 50.9 (35.8–72.4), 46.3 (31.0–69.0), and 43.6 (29.5–64.4) nmol/L, respectively. 30% of the normal weight subjects had insufficient serum 25(OH)D levels, while 34 and 46% of the subjects categorized as overweight or obese had an insufficient vitamin D status.

Table 5. Associations between serum 25(OH)D levels and demographic characteristics, anthropometric measurements, smoking habits, GFR, and vitamin D intake.[1]

	Regression model					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Age (years)	0.22 (0.16, 0.28)	<0.001				
Sex (male = 1)	1.24 (−0.13, 2.61)	0.075				
BMI (kg/m^2)	−0.73 (−0.88, −0.57)	<0.001	−0.67 (−0.82, −0.52)			
Current smoker (n)	−2.92 (−4.33, −1.52)	<0.001	−1.75 (−3.19, −0.31)	0.018		
eGFR (mL/min/ 1,73^2)	−0.17 (−0.21, −0.14)	<0.001	−0.16 (−0.20, −0.11)	<0.001		
Waist circumference (cm)	−0.19 (−0.25, −0.13)	<0.001	−0.23 (−0.29, −0.17)	<0.001	−0.04 (−0.15, 0.07)	0.473
Vitamin D intake (µg/d)	0.47 (0.38, 0.57)	<0.001	0.49 (0.39, 0.58)	<0.001	0.47 (0.38, 0.57)	<0.001

[1] Values are regression coefficients (95% CI) and p-values. Regression coefficients represent the mean difference of 25(OH)D (nmol/L) per one unit increase of the predictor variable. BMI, body mass index; eGFR, estimated glomerular filtration rate. [2] Model 1: unadjusted; [3] Model 2: adjusted for sex and age; [4] Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

Table 6. Correlation between serum 25(OH)D levels and demographic characteristics, anthropometric measurements, smoking habits, GFR, and vitamin D intake.[1]

	Correlation coefficients for unadjusted and adjusted models					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**
Age (years)	0.10 (0.07, 0.14)	<0.001				
Sex (1 = male)	0.03 (−0.00, 0.06)	0.041				
BMI (kg/m^2)	−0.14 (−0.17, −0.11)	<0.001	−0.13 (−0.16, −0.10)	<0.001		
Current smoker (n)	−0.07 (−0.11, −0.04)	<0.001	−0.05 (−0.08, −0.02)	<0.001		
eGFR (mL/min/ 1,73^2)	−0.15 (−0.18, −0.12)	<0.001	−0.11 (−0.14, −0.08)	<0.001		
Waist circumference (cm)	−0.11 (−0.14, −0.08)	<0.001	−0.13 (−0.16, −0.09)	<0.001	−0.01 (−0.05, 0.02)	0.358
Vitamin D intake (µg/d)	0.21 (0.17, 0.26)	<0.001	0.21 (0.17, 0.26)	<0.001	0.21 (0.17, 0.25)	<0.001
Season	0.18 (0.14, 0.22)	<0.001	0.17 (0.13, 0.21)	<0.001	0.17 (0.13, 0.21)	<0.001
Season 1[5]	−0.22 (−0.26, −0.19)	<0.001	−0.17 (−0.22, −0.15)	<0.001	−0.16 (−0.22, −0.14)	<0.001
Season 2[6]	−0.00 (−0.04, 0.04)	0.933	0.00 (−0.04, 0.03)	0.900	−0.01 (−0.04, 0.03)	0.291
Season 3[7]	0.25 (0.22, 0.29)	<0.001	0.16 (0.15, 0.22)	<0.001	0.15 (0.14, 0.22)	<0.001
Season 4[8]	−0.01 (−0.05, 0.03)	0.680	−0.02 (−0.05, 0.02)	0.199	−0.02 (−0.05, 0.02)	0.262

[1]Values are Spearman`s *rhos* (bootstrapped 95% CIs) and p-values. BMI, body mass index (kg/m^2); eGFR, estimated glomerular filtration ratio. [2]Model 1: unadjusted; [3]Model 2: adjusted for sex and age; [4]Model 3: adjusted for sex, age, BMI, smoking habits, and GFR. [5] Season 1, January−March; [6] Season 2, April−June; [7] Season 3, July−September; [8] Season 4, October−December.

Standard lipids

The results showed that serum levels of 25(OH)D was positively associated with HDL-cholesterol (HDL-C) and apolipoprotein (Apo) A-I, and inversely with triglycerides (TGs) in both the unadjusted and the adjusted linear regression models and Spearman`s correlation analyses (**Tables 7 and 8, Figure 3)**. The fully adjusted linear regression model showed that each additional 0.1 mmol/L higher baseline HDL-C was associated with 0.6 nmol/L higher 25(OH)D. The geometric mean (1SD range) HDL-C level in subjects with sufficient 25(OH)D levels was 1.26 (0.75–2.13) mmol/L, while the geometric mean (1 SD range) HDL-C level in subjects with insufficient vitamin D status was 1.19 (0.90–1.58) mmol/L.

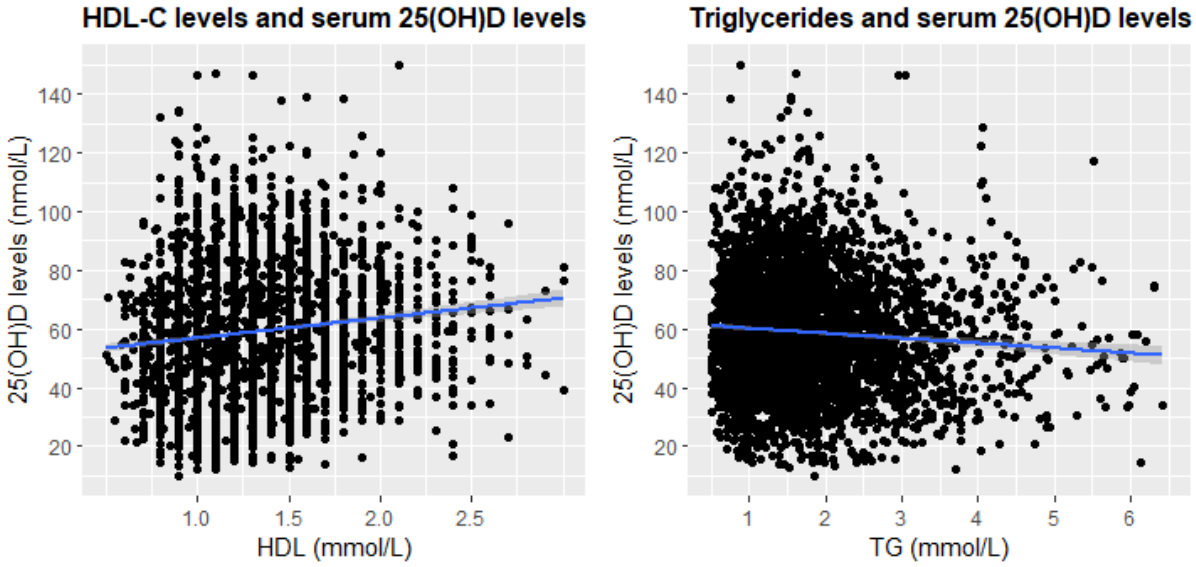

Figure 3. Scatter plot and regression line from the unadjusted linear regression model. The left graph shows the relationship between HDL-C level and serum 25(OH)D level, while the right graph shows the association between TGs and serum 25(OH)D level. HDL-C, High density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D, TG; triglycerides.

Glucose metabolism

Serum levels of 25(OH)D associated negatively with blood glucose, HbA1C, and being diagnosed with type 2 diabetes in both the unadjusted linear regression model and Spearman`s correlation analyses and the models adjusted for confounding variables (**Tables 7 and 8**). The number of participants diagnosed with type 2 diabetes in the study population was 455. The geometric mean (1SD range) serum 25(OH)D level in

these subjects were estimated to 51.9 (34.2–78.8) nmol/L, compared to 55.9 (39.0–80.0) nmol/L in the participants without the diagnosis.

Inflammation

A negative relationship was observed between CRP and 25(OH)D levels in serum in both the unadjusted and adjusted regression models (**Table 7**). However, the fully adjusted quantile regression model showed that the negative association was stronger at lower levels of serum 25(OH)D, and the association was weaker at higher 25(OH)D levels. This relationship is presented in **Figure 4**. A similar trend was observed for the association between serum 25(OH)D and neopterin in the fully adjusted quantile regression model, where the negative association was stronger at lower levels of serum 25(OH)D, and the relationship disappeared at the upper quantiles of serum 25(OH)D (**Supplemental Table 9**). Thus, at lower levels of 25(OH)D, higher CRP and neopterin was associated with lower 25(OH)D, while at higher levels of 25(OH)D, this relationship tended to disappear.

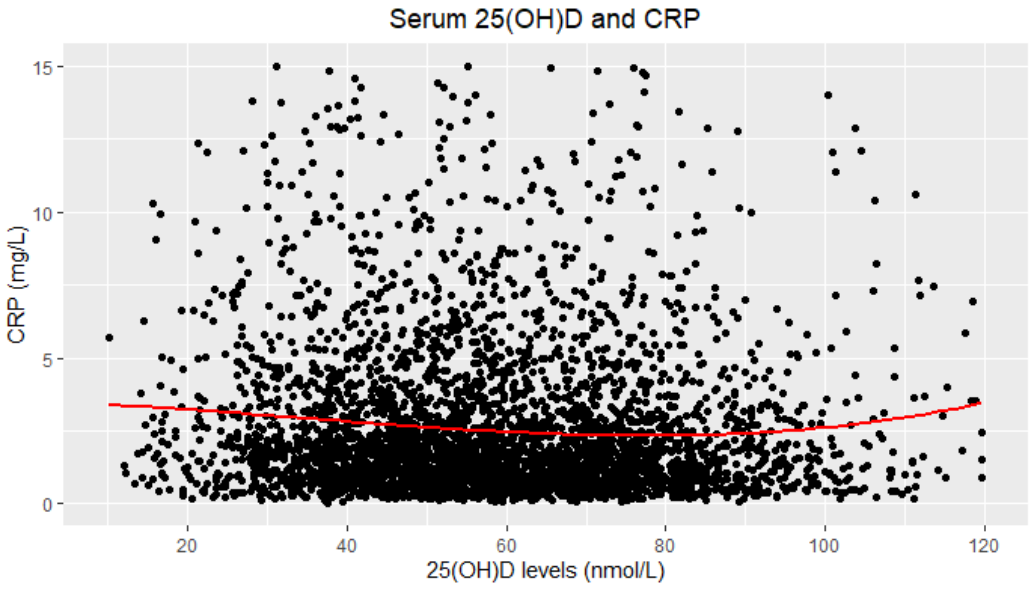

Figure 4 Plot showing the association between serum 25(OH)D and CRP using a splined function in the unadjusted regression model. 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein

Table 7. Associations between 25(OH)D and standard lipids, blood glucose, and inflammatory markers in serum.[1]

	Regression model					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
TG (mmol/L)	−1.69 (−2.20, −1.20)	<0.001	−1.48 (−1.99, −0.98)	<0.001	−1.11 (−1.62, −0.60)	<0.001
Total cholesterol (mmol/L)	−0.11 (−0.64, 0.41)	0.673	0.06 (−0.46, 0.59)	0.814	0.15 (−0.37, 0.67)	0.582
LDL-C (mmol/L)	−0.08 (−0.68, 0.52)	0.794	0.07 (−0.53, 0.67)	0.822	0.16 (−0.43, 0.75)	0.602
HDL-C (mmol/L)	6.68 (5.07, 8.29)	<0.001	7.09 (5.40, 8.78)	<0.001	6.01 (4.28, 7.74)	<0.001
Non-HDL-C (mmol/L)	−0.82 (−1.34, −0.29)	0.002	−0.61 (−1.14, −0.09)	0.023	−0.39 (−0.91, 0.13)	0.142
ApoA-I (g/L)	10.7 (8.45, 13.0)	<0.001	12.1 (9.69, 14.5)	<0.001	10.8 (8.41, 13.2)	<0.001
ApoB (g/L)	−1.43 (−3.91, 1.05)	0.259	−0.64 (−3.11, 1.84)	0.615	0.13 (−2.33, 2.59)	0.917
Type 2 diabetes (n)	−3.32 (−5.28, −1.36)	<0.001	−4.10 (-6.05, -2.14)	<0.001	−2.29 (−4.26, −0.31)	0.023
HbA1C (%)	−0.97 (−1.42, −0.53)	<0.001	−1.02 (−1.46, −0.57)	<0.001	−0.75 (−1.19, −0.31)	0.001
Serum glucose (mmol/L)	−0.59 (−0.85, −0.34)	<0.001	−0.67 (−0.92, −0.41)	<0.001	−0.44 (−0.70, −0.19)	0.001
CRP (mg/L)	−0.13 (−0.22, −0.05)	0.003	−0.14 (−0.22, −0.05)	0.001	−0.12 (−0.20, −0.04)	0.005
Neopterin (nmol/L)	0.11 (0.03, 0.20)	0.006	0.07 (−0.02, 0.15)	0.109	−0.09 (−0.18, 0.01)	0.065

[1] Values are regression coefficients (95% CI) and p-values. Regression coefficients represent the mean difference of 25(OH)D (nmol/L) per one unit increase of the predictor variable. TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; LDL-C, serum low density lipoprotein cholesterol; ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HBA1c, glycosylated haemoglobin; CRP, C-reactive protein. [2] Model 1: unadjusted; [3] Model 2: adjusted for sex and age; [4] Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

Table 8. Correlation between 25(OH)D and standard lipids, blood glucose and inflammatory markers in serum.[1]

	Correlation coefficients for unadjusted and adjusted models					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**
TG (mmol/L)	−0.09 (−0.12, −0.06)	<0.001	−0.08 (−0.11, −0.05)	<0.001	−0.05 (−0.08, −0.02)	<0.001
Total cholesterol (mmol/L)	−0.00 (−0.03, 0.03)	0.990	0.01 (−0.02, 0.04)	0.523	0.01 (−0.02, 0.05)	0.466
LDL-C (mmol/L)	−0.00 (−0.03, 0.03)	0.889	0.01 (−0.02, 0.04)	0.735	0.01 (−0.02, 0.04)	0.712
HDL-C (mmol/L)	0.14 (0.11, 0.17)	<0.001	0.15 (0.12, 0.18)	<0.001	0.13 (0.10, 0.16)	<0.001
Non-HDL-C (mmol/L)	−0.04 (−0.08, −0.01)	0.006	−0.03 (−0.06, −0.00)	0.030	−0.02 (−0.05, 0.01)	0.110
ApoA-I (g/L)	0.15 (0.12, 0.18)	<0.001	0.17 (0.14, 0.20)	<0.001	0.15 (0.12, 0.19)	<0.001
ApoB (g/L)	−0.02 (−0.05, 0.01)	0.217	−0.01 (−0.04, 0.02)	0.477	−0.00 (−0.03, 0.03)	0.703
Type 2 diabetes (n)	−0.05 (−0.08, −0.02)	0.001	−0.02 (−0.05, −0.00)	0.123	−0.02 (−0.04, 0.01)	0.143
HbA1C (%)	−0.08 (−0.11, −0.05)	<0.001	−0.08 (−0.11, −0.05)	<0.001	−0.06 (−0.09, −0.03)	<0.001
Serum glucose (mmol/L)	−0.08 (−0.11, −0.05)	<0.001	−0.09 (−0.13, −0.06)	<0.001	−0.07 (−0.10, −0.04)	<0.001
CRP (mg/L)	−0.09 (−0.12, −0.06)	<0.001	−0.09 (−0.12, −0.06)	<0.001	−0.06 (−0.09, −0.03)	<0.001
Neopterin (nmol/L)	0.05 (0.02, 0.08)	0.003	0.00 (−0.03, 0.03)	1.000	−0.06 (−0.09. −0.03)	<0.001

[1]Values are Spearman`s *rhos* (bootstrapped 95% CIs) and p-values. TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; LDL-C, serum low density lipoprotein cholesterol; ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HBA1c, glycosylated haemoglobin; CRP, C-reactive protein. [2] Model 1: unadjusted; [3] Model 2: adjusted for sex and age; [4] Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

Amino acids

Serum 25(OH)D levels associated positively with sarcosine and choline levels in both the unadjusted and adjusted regression models and Spearman`s correlation analyses. An inverse association was found between serum 25(OH)D level and total homocysteine and DMG in the fully adjusted regression model (**Tables 9 and 11**). The fully adjusted quantile regression analysis showed that the negative association between total homocysteine and vitamin D status was stronger at lower levels of serum 25(OH)D, and that the relationship disappeared at the upper quantiles of serum 25(OH)D (**Supplemental Table 11**). The association between serum 25(OH)D and total homocysteine is presented in **Figure 5**.

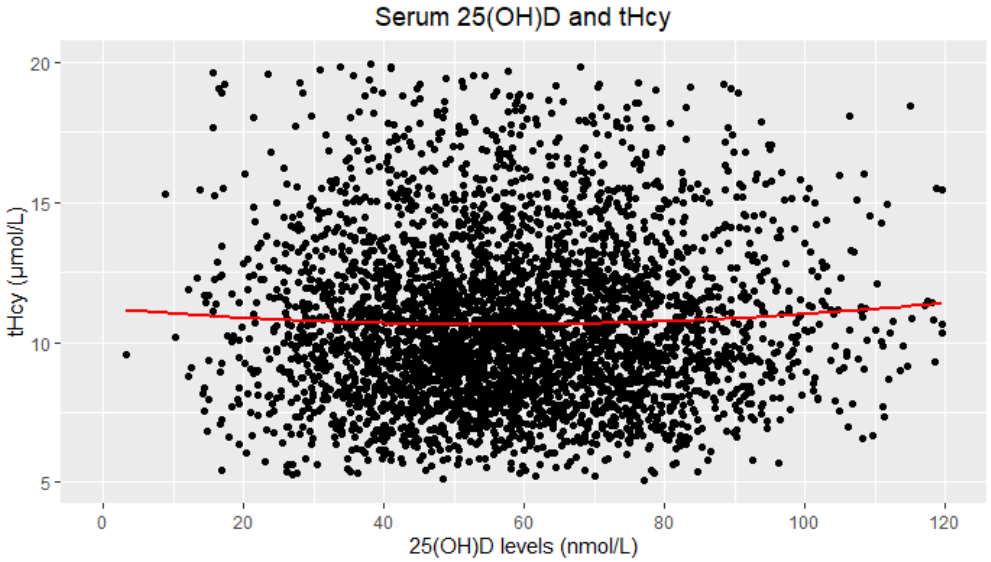

Figure 5 Plot showing the association between serum 25(OH)D and tHcy using a splined function in the unadjusted regression model. 25(OH)D, 25-hydroxyvitamin D; tHcy, total homocysteine.

Vitamin status and dietary variables

Serum 25(OH)D levels was positively related to fish and egg consumption. Positive associations were also found for serum levels of vitamin A, vitamin E, riboflavin, pyridoxal phosphate (PLP), 4-pyridoxic acid (PA), folate, and cobalamin in both the unadjusted and the adjusted linear regression models and Spearman`s correlation analyses (**Tables 10 and 12**).

Figure 6 presents Spearman`s correlation coefficients and bootstrapped confidence intervals for the key observations of associations between serum 25(OH)D and the different variables, showing the direction and strength of the relationships on a standardized scale. Results from the quantile regression analyses investigating the associations between different quantiles of 25(OH)D and the independent variables are presented in the **Supplemental Tables 1-4** for the unadjusted model, **Supplemental Tables 5-8** for the model adjusted for age and sex, and **Supplemental Table 9-12** for the model adjusted for age, sex, BMI, smoking habits, and GFR.

Table 9. Associations between serum 25(OH)D and amino acid levels.[1]

	Regression model					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Serine (µmol/L)	−0.01 (−0.03, 0.02)	0.728	0.00 (−0.03, 0.03)	0.867	−0.01 (−0.04, 0.02)	0.492
Glycine (µmol/L)	0.02 (0.01, 0.03)	<0.001	0.02 (0.01, 0.03)	<0.001	0.01 (−0.01, 0.02)	0.301
DMG (µmol/L)	−0.07 (−0.21, 0.07)	0.339	−0.11 (−0.25, 0.02)	0.109	−0.17 (−0.31, −0.03)	0.016
Sarcosine (µmol/L)	2.62 (1.62, 3.61)	<0.001	2.50 (1.51, 3.49)	<0.001	1.79 (0.80, 2.78)	<0.001
Choline (µmol/L)	0.81 (0.58, 1.04)	<0.001	0.62 (0.38, 0.86)	<0.001	0.46 (0.20, 0.71)	<0.001
Betaine (µmol/L)	0.09 (0.04, 0.13)	<0.001	0.06 (0.02, 0.11)	0.006	0.03 (−0.02, 0.08)	0.199
Methionine (µmol/L)	0.09 (0.01, 0.17)	0.023	0.09 (0.02, 0.17)	0.020	0.07 (−0.01, 0.15)	0.088
tHcy (µmol/L)	0.04 (−0.09, 0.16)	0.537	−0.07 (−0.19, 0.06)	0.302	−0.26 (−0.39, −0.12)	<0.001
tCys (µmol/L)	0.03 (0.02, 0.05)	<0.001	0.01 (−0.01, 0.03)	0.255	0.00 (−0.02, 0.02)	0.967

[1] Values are regression coefficients (95% CIs) and p-values. Regression coefficients represent the mean difference of 25(OH)D (nmol/L) per one unit increase of the predictor variable. DMG, plasma dimetylglycine; tHcy, total homocysteine; tCys, total cysteine. [2] Model 1: unadjusted; [3] Model 2: adjusted for sex and age; [4] Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

Table 10. Associations between serum 25(OH)D levels, vitamin status, and dietary variables.[1]

	Regression model					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Riboflavin (µg/dL)	0.05 (0.03, 0.07)	<0.001	0.04 (0.02, 0.06)	<0.001	0.04 (0.02, 0.06)	<0.001
PL (nmol/L)	0.01 (0.00, 0.01)	0.072	0.01 (0.00, 0.01)	0.062	0.01 (0.00, 0.01)	0.075
PLP (nmol/L)	0.03 (0.02, 0.04)	<0.001	0.03 (0.02, 0.04)	<0.001	0.03 (0.01, 0.04)	<0.001
PA (nmol/L)	0.01 (0.00, 0.01)	0.005	0.01 (0.00, 0.01)	0.006	0.01 (0.00, 0.01)	0.030
Folate (nmol/L)	0.11 (0.06, 0.12)	<0.001	0.10 (0.05, 0.15)	<0.001	0.09 (0.05, 0.14)	<0.001
Cobalamin (pq/mL)	0.00 (0.00, 0.00)	0.001	0.00 (0.00, 0.01)	0.001	0.00 (0.00, 0.01)	0.004
MMA (nmol/L)	−0.50 (−3.38, 2.39)	0.736	−1.73 (−4.61, 1.15)	0.238	−2.73 (−5.58, 0.12)	0.061
Vitamin A (µmol/L)	4.51 (3.65, 5.37)	<0.001	4.59 (3.74, 5.45)	<0.001	4.25 (3.31, 5.18)	<0.001
Vitamin E (µmol/L)	0.26 (0.18, 0.34)	<0.001	0.27 (0.19, 0.35)	<0.001	0.27 (0.19, 0.34)	<0.001
Low fat milk consumption (g/day)	0.00 (−0.00, 0.01)	0.598	0.00 (−0.00, 0.01)	0.356	0.00 (−0.00, 0.01)	0.384
Fish consumption (g/day)	0.03 (0.02, 0.04)	<0.001	0.03 (0.02, 0.04)	<0.001	0.03 (0.02, 0.04)	<0.001
Egg consumption (g/day)	0.06 (−0.01, 0.13)	0.090	0.07 (−0.00, 0.14)	0.056	0.11 (0.04, 0.18)	0.003

[1] Values are regression coefficients (95% CIs) and p-values. Regression coefficients represent the mean difference of 25(OH)D (nmol/L) per one unit increase of the predictor variable. PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; MMA, methylmalonic acid. [2] Model 1: unadjusted; [3] Model 2: adjusted for sex and age; [4] Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

Table 11. Correlations between serum 25(OH)D and amino acid levels.[1]

	Correlation coefficients for unadjusted and adjusted models					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**
Serine (µmol/L)	0.00 (−0.03, 0.03)	0.888	0.01 (−0.02, 0.04)	0.607	−0.00 (−0.03, 0.03)	0.022
Glycine (µmol/L)	0.07 (0.04, 0.10)	<0.001	0.08 (0.05, 0.11)	<0.001	0.02 (−0.01, 0.05)	0.314
DMG (µmol/L)	0.01 (−0.03, 0.04)	0.662	−0.02 (−0.05, 0.01)	0.202	−0.04 (−0.07, −0.01)	0.005
Sarcosine (µmol/L)	0.10 (0.07, 0.13)	<0.001	0.10 (0.07, 0.13)	<0.001	0.07 (0.04, 0.11)	<0.001
Choline (µmol/L)	0.09 (0.06, 0.12)	<0.001	0.07 (0.03, 0.10)	<0.001	0.05 (0.02, 0.08)	0.003
Betaine (µmol/L)	0.09 (0.06, 0.12)	<0.001	0.07 (0.04, 0.10)	<0.001	0.04 (0.01, 0.07)	0.012
Methionine (µmol/L)	0.04 (0.01, 0.07)	0.020	0.04 (0.01, 0.07)	0.026	0.02 (−0.01, 0.06)	0.193
tHcy (µmol/L)	0.01 (−0.02, 0.04)	0.468	−0.03 (−0.06, 0.00)	0.058	−0.08 (−0.11, −0.05)	<0.001
tCys (µmol/L)	0.06 (0.02, 0.09)	<0.001	0.01 (−0.02, 0.05)	0.395	−0.00 (−0.03, 0.03)	0.737

[1] Values are spearman`s *rhos* (bootstrapped 95% CIs) and p-values. DMG, plasma dimetylglycine; tHcy, total homocysteine; tCys, total cysteine. [2] Model 1: unadjusted; [3] Model 2: adjusted for sex and age; [4] Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

Table 12. Correlations between serum 25(OH) D levels, vitamin status, and dietary variables.[1]

	Correlation coefficients for unadjusted and adjusted models					
	Model 1[2]		Model 2[3]		Model 3[4]	
Variable	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**	**Spearman's rho (95% CI)**	**P-value**
Riboflavin (µg/dL)	0.06 (0.04, 0.09)	<0.001	0.05 (0.02, 0.08)	0.003	0.04 (0.01, 0.07)	0.022
PL (nmol/L)	0.17 (0.14, 0.20)	<0.001	0.16 (0.10, 0.18)	<0.001	0.11 (0.06, 0.14)	<0.001
PLP (nmol/L)	0.15 (0.12, 0.18)	<0.001	0.16 (0.13, 0.19)	<0.001	0.14 (0.11, 0.17)	<0.001
PA (nmol/L)	0.17 (0.14, 0.20)	<0.001	0.15 (0.10, 0.17)	<0.001	0.07 (0.04, 0.11)	<0.001
Folate (nmol/L)	0.11 (0.08, 0.14)	<0.001	0.11 (0.08, 0.14)	<0.001	0.11 (0.08, 0.14)	<0.001
Cobalamin (pq/mL)	0.07 (0.04, 0.10)	<0.001	0.08 (0.05, 0.12)	<0.001	0.06 (0.03, 0.10)	<0.001
MMA (nmol/L)	0.05 (0.02, 0.08)	0.003	0.01 (−0.02, 0.04)	0.669	−0.03 (−0.06, −0.00)	0.018
Vitamin A (µmol/L)	0.16 (0.13, 0.19)	<0.001	0.16 (0.13, 0.19)	<0.001	0.14 (0.11, 0.17)	<0.001
Vitamin E (µmol/L)	0.13 (0.10, 0.16)	<0.001	0.14 (0.10, 0.17)	<0.001	0.14 (0.10, 0.17)	<0.001
Low fat milk consumption (g/day)	0.01 (−0.03, 0.05)	0.637	0.01 (−0.03, 0.05)	0.605	0.02 (−0.03, 0.06)	0.681
Fish consumption (g/day)	0.12 (0.08, 0.16)	<0.001	0.12 (0.08, 0.16)	<0.001	0.13 (0.09, 0.17)	<0.001
Egg consumption (g/day)	0.04 (0.00, 0.09)	0.043	0.05 (0.00, 0.09)	0.054	0.07 (0.02, 0.11)	0.007

[1]Values are spearman`s *rhos* (bootstrapped 95% CIs) and p-values. Regression coefficients represent the mean difference of 25(OH)D (nmol/L) per one unit increase of the predictor variable. PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; MMA, methylmalonic acid. [2]Model 1: unadjusted; [3]Model 2: adjusted for sex and age; [4]Model 3: adjusted for sex, age, BMI, smoking habits, and GFR.

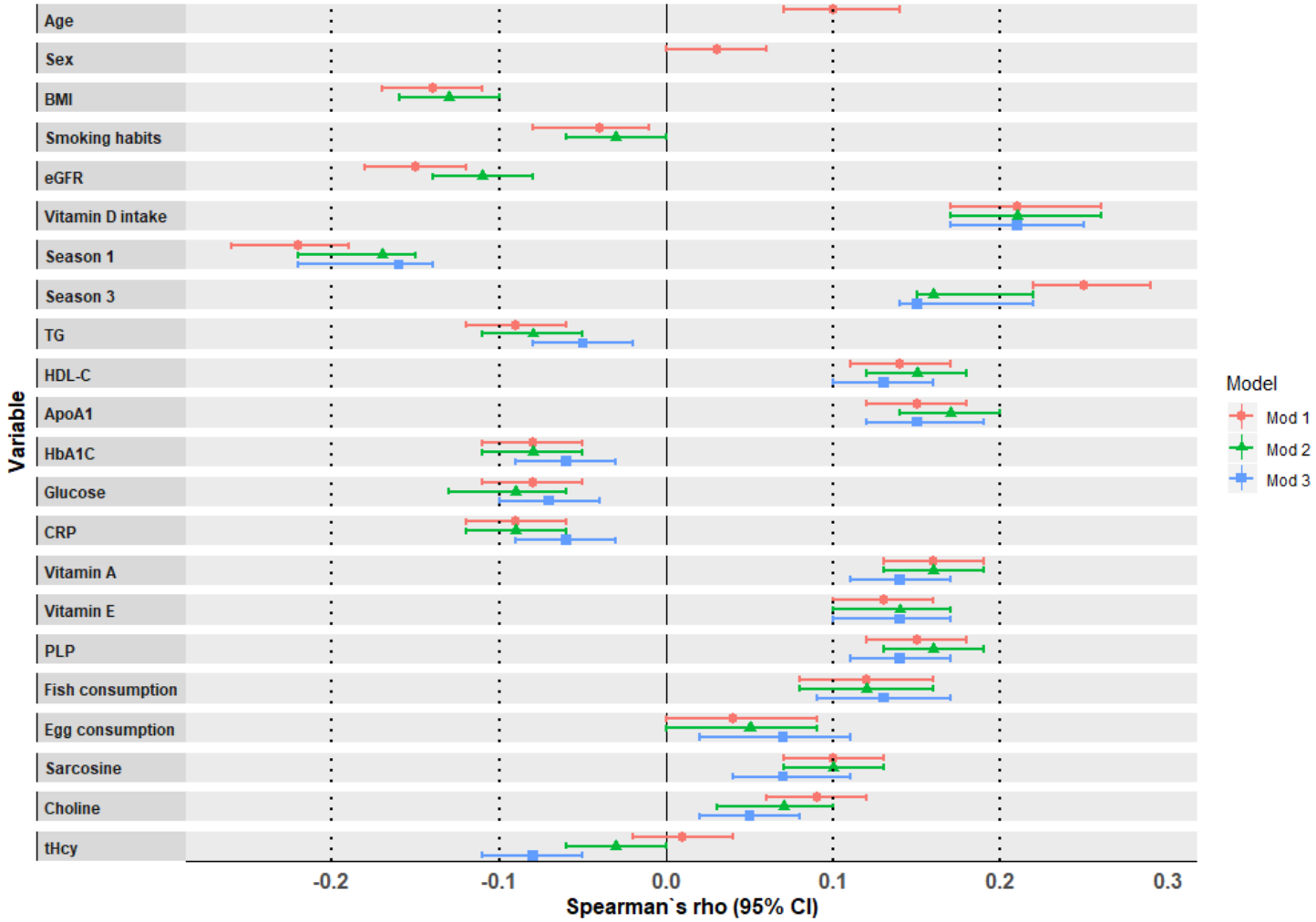

Figure 6. Forest plot presenting Spearman`s rho and bootstrapped 95% CI for key findings of associations between serum 25(OH)D and variables of interest. Model 1: unadjusted; Model 2: adjusted for sex and age; Model 3: adjusted for sex, age, BMI, smoking habits, and GFR. BMI, body mass index (kg/m²); eGFR, estimated glomerular filtration ratio; Season 1, January-March; Season 3, July-September; TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; ApoA-I, Apolipoprotein A-I; HBA1c, glycosylated haemoglobin; CRP, C-reactive protein; PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; tHcy, total homocysteine; tCys, total cysteine.

5 Discussion

5.1 Main results

In this study, we explored a wide variety of variables to assess which were associated with serum 25(OH)D levels among 4118 patients with stable angina pectoris included in a large cohort. The results showed a seasonal variation of serum 25(OH)D concentration, with the highest concentrations measured in the blood samples taken in August and the lowest concentrations in the subjects included in March. Further, we found that serum levels of 25(OH)D were positively associated with dietary vitamin D, fish intake, and egg consumption, and with serum levels of HDL-C, riboflavin, PLP, PA, folate, cobalamin, vitamin A, vitamin E, choline, and sarcosine. Factors that showed an inverse relationship with serum 25(OH)D concentrations were BMI, and serum levels of TGs, blood glucose, HbA1C, and total homocysteine, DMG, and CRP at lower 25(OH)D serum levels.

5.2 Discussion of methods

5.2.1 Study design

This was a cross-sectional study, a type of observational study design where the variables of interest are measured at the same time point (93). Cross-sectional studies can be used to investigate associations between risk factors and outcomes or relationships between different variables, as was the case in the present thesis (94). This study design gives the opportunity to investigate many variables at the same time that can be more rigorously assessed in future studies (94)(95).

Cross-sectional studies are, however, limited by the fact that the outcome and the exposure are measured at the same time, and it is not possible to determine whether the exposure preceded the outcome, decide the direction of an association, and derive causal relationships (93)(94)(95). This principle of temporality is important to take into consideration when interpreting the results from cross-sectional studies.

Sample size

A strength in the present thesis is the relatively large study sample, with 4118 subjects included. The size of the study sample is important for the confidence in the estimates, because a larger sample size will be less affected by variability in a heterogeneous population. We have more available information, and so the uncertainty reduces (96–98). On the other hand, very large sample sizes tend to show even small differences or associations as statistically significant results, and it is important to be aware of this when evaluating the results and distinguish between what is statistically and what is clinically significant associations (97).

Study population

A study sample is a finite part of participants included from the target population, while the target population is the entire set of subjects the researchers want to obtain information on (99). The results from analyses of the study population may be generalized to the target population to a certain degree, referred to as external validity. The source population in this study was patients in Western-Norway referred to coronary angiography for suspected coronary artery disease, and the target population was all patients with stable angina pectoris. The cohort in this thesis was characterized by a large proportion of older men, and was found to be quite similar to other populations who had verified coronary artery disease in other European hospitals (68). Hence, the results should be generalizable to other populations with stable angina pectoris. However, the results may not be generalized to the general population because the subjects with stable angina pectoris included may have several characteristics associated with CVD differentiating them from the general population. Further longitudinal studies of the general population are needed to draw conclusion regarding variables associated with vitamin D status in the general population.

Data collection

The data collection methods are prone to random and systematic errors, that could affect the precision and validity of the study (100). The sampling method is a potential source of both systematic and random errors, due to the fact that the sample is a

selection from the target population and large parts of the target population are for different reasons not included in the study (100,101). Selection bias occurs when a population is selected by some factors that create association between two variables when they are not associated in the target population (102). Collider stratification bias, a type of selection bias, occurs when we condition on a common effect of otherwise unrelated variables, and could lead to a spurious association between the variables (103). In the case of this thesis, if vitamin D status affects the risk of SAP, and thereby the chance of inclusion in the cohort, it could lead to spurious association between vitamin D status and other variables affecting the risk of SAP and chance of inclusion, due to selection bias. This kind of selection bias may introduce associations in the included cohort that do not exist in the general population.

Random errors due to the study sample can be minimized by increasing the sample size (101). In WECAC, all available patients who underwent coronary angiography and fulfilled the inclusion criteria were recruited to the study, which lead to inclusion of a large study sample over a broad time period, and thereby reduced risk of random errors due to the sampling method. Non-response bias, that occur when subject who refuse to participate in the study are significantly different from the included subjects, may also bias the results (101). The observations in this study are probably not much affected by non-response bias, because of the design of the study with just one case of data collection, conducted in conjunction with elective coronary angiography, and the fact that participation did not require much effort from the participants.

The measurements of key variables are also potential sources of random errors and may affect the precision of the study. The data on serum 25(OH)D concentrations included in our study were measured by LC-MS/MS at Bevital AS, a laboratory certified by the Vitamin D External Quality Assessment Scheme, with a between-day coefficient of variation (CV) of 7–8% (104), indicating a small degree of random errors in the included measurements of 25(OH)D concentrations.

A potential source of systematic error in this study is the fact that blood sampling was conducted differently at Haukeland University Hospital and Stavanger University Hospital. At Haukeland University Hospital, blood sampling was done non-fasting prior to coronary angiography, while at Stavanger University Hospital, blood samples were taken after angiography and at least 8 hours of fasting. The non-standardized blood sampling could be a potential source of systematic error and reduce the validity in our study. This could have been handled by adjusting for site of study visit or non-fasting state of blood sampling, which could have reduced the risk of systematic error in the results.

Parts of the data collection were done by self-administrated questionnaires to obtain information on diet, dietary supplements, smoking habits, medical conditions, and use of drugs. Questionnaires based on self-report may be subject to self-reporting bias, and are a potential source of systematic and random error in the data collection (105). Self-reported data are prone to social desirability bias, where the reported data may be affected by underestimation or overestimation due to social desirability or approval. Another usual challenge with self-reported data is the ability to recall information. This type of bias is referred to as recall bias, and could potentially cause an underestimation or overestimation of the true associations (105). When conducting the data collection for WECAC, several measures were used to minimize the potential biases. Firstly, reported medical history was controlled against hospital records by trained personnel, and reported smoking status was complemented with measurement of cotinine concentration to provide an objective measurement. In addition, measurement of weight and anthropometry were conducted by trained personnel to minimize bias.

Dietary data collection is prone to both social desirability bias and recall bias, and could be affected by overreporting of socially desirable behaviors and difficulties with recalling dietary habits. A semiquantitative food frequency questionnaire was designed in a way to obtain best possible information on usual food intake during the past year and minimize bias. However, the dietary data may still be a source of uncertainty in the data collection.

5.2.2 Statistical analysis

In this cross-sectional study, we chose to conduct linear regression and Spearman`s correlation analyses to investigate associations and estimate effect sizes. Linear regression is used to measure the relationship between a dependent variable (outcome) and one or more independent variables (predictors). The Spearman`s correlation coefficient describes the strength and direction of the monotonic relationship between two variables, and linear regression also allows us to estimate how much the dependent variable either increases or decreases as the independent variables are changed (73,106).

Importantly, linear regression is limited to linear and curvilinear relationships, and nonlinear associations may be missed (107). Linear regression is also limited by the fact that it investigates the relationship between the mean of the dependent variable and the independent variables, and in some cases, it is more valuable to look at the different levels of the dependent variable. We therefore also included quantile regression analysis to investigate the associations between serum 25(OH)D and the variables of interest at different serum levels of 25(OH)D.

Quantile regression allows us to estimate the effect of an independent variable on a specified quantile of the dependent variable (81,108). Unlike linear regression, quantile regression makes no assumption about the distribution of the residuals, and can be used when the conditions of linear regression are not met (108,109). If the data are multimodal or highly skewed, quantile regression will be able to capture this relationship. Quantile regression is also more robust against outliers compared to the ordinary linear regression (108). Categorization of continuous variables and using them in analysis is also associated with some limitations. Such categorization lead to an assumption that the effect or association is homogeneous within each category (110). This assumption may reduce the power of the statistical test and the ability to detect associations. Categorization also makes it more difficult to compare results across different studies, because the categories used in the analyses depend on the study population and may differ between the compared studies (110). However, as an addition

to linear regression models, quantile regression may contribute to a more complete view of the associations of interest, especially in cases of nonlinear associations. The quantile regression analyses allowed us to assess the associations between serum 25(OH)D and the independent variables at quartiles of serum 25(OH)D levels and explore potential nonlinear relationships.

Another challenge in observational studies is the effect of confounding variables. A confounding variable is causally associated with both the dependent variable and the independent variable, and is not on the causal pathway between the exposure and outcome (111). Confounding variables lead to results that don`t reflect the actual relationship between the variables we want to study (112). To account for factors known to be associated with vitamin D status and several of the variables we wanted to investigate, we conducted multiple linear regression analyses in addition to the simple linear regression analyses. This way we adjust for the effect of known confounding variables to isolate the relationship of interest.

P-value can be defined as "the probability that the chosen test statistic would have been at least as large as its observed value if every model assumption were correct, including the test hypothesis" (113). If the p-value is lower than the alpha level chosen, often 0.05, the association is stated to be statistically significant (113,114). However, statistical significance, or a small p-value, is not equivalent to scientific or clinical significance, and larger p-values do not imply a lack of importance (115). As mentioned, with a large sample, like in the current study, an inferential test may show statistically significant results even at very weak associations, while the effect sizes are not affected by the sample size (116,117). Although the significance level does not say anything about the size, direction, or clinical relevance of an association, the p-value is influenced by the variability in responses and may say something about the consistency in our findings (113). Notably, the interpretation of effect sizes estimated by linear regression and correlation analyses should be done with caution, and the clinical significance of an effect size also depends on theoretical background and comparison with previous work on the topic (116,118).

5.3 Discussion of results

5.3.1 Sunlight exposure and season

The results from this study showed a great variation of serum 25(OH)D in different months and quarters of the year. In line with several previous studies (6,117,118), we observed that serum 25(OH)D levels were higher during the quarter from July through September compared to the winter season. The seasonal variation observed in this and several previous studies is mainly explained by the fact that the sunlight exposure is insufficient for adequate vitamin D synthesis in the skin to maintain the vitamin D status throughout the winter months at the latitude of the Nordic countries (121). The UV radiation intensity is highest during the summer months and decreases during the winter. Besides, there is a greater degree of outdoor activities during the summer compared to the winter season, causing a considerable difference in serum 25(OH)D levels between summer and winter months (6,119).

Sunlight exposure is reckoned as the major source of vitamin D for most people worldwide (1,120,121). A sufficient amount of sunlight exposure to stimulate the production of vitamin D_3 in the skin can be enough to produce adequate levels of serum 25(OH)D (1,19). It is estimated that during June and July in the Nordic countries exposure of sunlight to 25% of the body surface for about 6-8 minutes 2 to 3 times a week provides vitamin D equivalent to the recommended daily intake of 10 µg (19). The amount of vitamin D_3 produced in the skin from sunlight exposure is also affected by exposed skin surface, use of sunscreen, and skin pigmentation, where skin type 4 (black skin) requires a higher degree of sunlight exposure compared to skin type 1 (white skin) to attain the same amount of vitamin D_3 production (19,65). It has also been found that the production of vitamin D_3 in the skin as a response to sunlight exposure is decreasing with age (65).

Based on the observed seasonal variation of 25(OH)D concentrations, it has been discussed if single 25(OH)D measurements should be adjusted for seasonal variation to better assess vitamin D status (122–124). Seasonal variation in 25(OH)D concentrations implies that a person could have adequate 25(OH)D levels during the summer and

autumn months, but due to the reduced sunlight exposure, insufficient 25(OH)D levels in the winter and spring (123). It has been suggested that serum 25(OH)D concentration regarded as sufficient should vary according to season of blood sampling. In a study of healthy, postmenopausal women, and middle-aged and elderly men, the predicted sine curves indicated that serum 25(OH)D concentrations of 70-90 nmol/L in men and 60-70 nmol/L in women during summer and fall were required to ensure 25(OH)D concentrations above 50 nmol/L throughout the year (123). Similarly, a study of the subjects in WECAC, using a cosinor model, estimated that a concentration of 65.8 nmol/L would be required in August to remain vitamin D sufficient in February (122). These findings indicate that taking seasonal variation into account may increase the accuracy of the assessment of vitamin D status and improve the identification of patients at risk of developing insufficient vitamin D levels throughout the year.

The results from this and previous studies indicate that sunlight exposure and seasonal variation are important determining factors of vitamin D status. However, vitamin D status is not explained in its entirety as a result of seasonal variation, and other variables also influence serum levels of 25(OH)D.

5.3.2 Diet

The positive association between serum 25(OH)D and daily vitamin D intake observed in this thesis, is in line with the results from several previous studies showing that estimated vitamin D intake is positively associated with serum 25(OH)D, and is one of the major determinants of vitamin D status (125–127).

Some of the previous studies investigating determinants of vitamin D status found that estimated vitamin D intake did not affect the risk of insufficient or deficient vitamin D status (6,62). In these studies, it was observed that a higher estimated vitamin D intake did not decrease the risk of insufficient or deficient serum 25(OH)D concentrations, and the major determinants of vitamin D status was, however, season, latitude, age, obesity, and physical activity. In a study of a population of Danish adults, it was found that there

was no difference in association between vitamin D intake and insufficient or deficient serum 25(OH)D levels in the group at the lower quartile of vitamin D intake compared to the group in the upper quartile of vitamin D intake. However, use of supplements was not included in the analysis, which may affected the results (6). In addition, a large study of a middle-aged Caucasian population also observed no difference in association between serum 25(OH)D levels and vitamin D intake when comparing quartiles of vitamin D intake (62).

Other studies have, however, found that dietary intake of vitamin D is positively associated with serum 25(OH)D level. Several studies conducted in Norway have shown that intake of fatty fish and cod liver oil are positively associated with serum 25(OH)D levels and inversely associated with prevalence of vitamin D deficiency (63,126). A cross-sectional study of a Norwegian adult population found that mean serum 25(OH)D level in the participants with daily intake of cod liver oil was 65.5 nmol/L, compared to 57.9 nmol/L in the participants with no cod liver oil intake (63). In a study of Icelandic children, it was also found that current vitamin D intake was positively associated with vitamin D status (128). Among the children taking the recommended daily dose of vitamin D supplement, 83% had a sufficient level of serum 25(OH)D, while only 51% of the children not taking any vitamin D supplement had a sufficient serum 25(OH)D level (128).

Optimal 25(OH)D levels

As sunlight exposure and factors that affect sunlight exposure are important determining factors of vitamin D status, it complicates the estimation of daily needed vitamin D intake through diet to achieve specific serum 25(OH)D concentrations, and there is still lack of consensus as to the daily recommended dose to attain the desired level of serum 25(OH)D (16,129). The dietary recommendations for daily vitamin D intake are, therefore, estimated based on conditions of minimal sunlight exposure (17). In addition, due to the nonlinear dose-response of vitamin D intake on serum 25(OH)D levels, it makes it more complicated to estimate the needed daily vitamin D intake (16,130). Studies of the dose-response relation between vitamin D intake and serum

25(OH)D have shown that the association is dependent on the basal concentration, where the response is greater when the basal concentration is low (16,129,131). It has been estimated that at low starting serum 25(OH)D levels, the average increase in serum 25(OH)D is 1.2 nmol/L for every μg of vitamin D_3 given as a daily oral dose, while at higher starting 25(OH)D levels, the increase is only 0.7 nmol/L or less per μg vitamin D_3 (131).

Other studies looking at the dose-response relationship have found a nonlinear relationship between vitamin D intake and serum 25(OH)D levels, with a great increase in serum 25(OH)D with dosages up to 25 μg/d, and a flattened response as daily intake exceeds 25 μg/d (17). This indicates that increasing a daily vitamin D intake from a very low intake to a recommended or slightly higher intake has a greater effect on serum 25(OH)D levels than increasing a daily vitamin D intake that is already above the recommended dose. In addition, several studies observed a higher response of serum 25(OH)D to total vitamin D intake at lower compared to higher latitudes (>49,5 °N) (17,132). Taken together, these findings regarding the dose-response relationship between dietary intakes of vitamin D and serum 25(OH)D concentrations have made it difficult to estimate a daily vitamin D intake needed to achieve an optimal serum 25(OH)D level. The suggested daily vitamin D intake needed to achieve optimal serum 25(OH)D concentrations varies. While some suggest that at least 15 μg/d is needed to attain a mean serum 25(OH)D level of 50 nmol/L and at least 20–25 μg/d is needed to reach a serum level of 75 nmol/L (131), others suggest that the daily intake should be 25–50 μg or even higher to maintain serum 25(OH)D levels that are optimal for health (133,134).

In summary, the results from this and previous studies indicate that dietary intake of vitamin D is one of the significant determinants of serum 25(OH)D level, and that a habitual diet containing vitamin D-rich food items contributes to attain a sufficient level of serum 25(OH)D.

5.3.3 Anthropometric measurements

We found that serum 25(OH)D levels were negatively related to BMI and waist circumference. These findings are in line with several previous studies showing that overweight was associated with lower 25(OH)D concentrations (135–137). A meta-analysis of observational studies found that the prevalence of vitamin D deficiency was 35% higher in obese subjects compared to the normal weight group (135). In addition, a meta-analysis of 21 cohorts observed an inverse association between serum 25(OH)D and BMI, where each unit increase in BMI was associated with 1.15% lower serum 25(OH)D concentrations (136). Similar results was found in an observational study of 250 adults, where BMI was inversely associated with serum 25(OH)D, and each unit increase in BMI was associated with a decrease of 0.74 nmol/L serum 25(OH)D (137). These associations between serum 25(OH)D and anthropometric measurements have also been observed in several observational studies from the Nordic countries, showing that overweight and obesity is associated with increased risk of insufficient serum 25(OH)D levels (6,7,138).

Several hypotheses explaining the observed association between overweight and vitamin D status have been suggested. One potential explanation is that overweight and obesity are associated with other factors, such as inactivity and limited outdoor activity, causing insufficient sunlight exposure and thereby a low production of vitamin D_3 in the skin (139,140). However, previous studies have shown that serum 25(OH)D levels are lower in obese individuals compared to normal weight individuals despite similar sunlight exposure, and that the increase of 25(OH)D levels in obese subjects are significantly lower than in normal weight subjects when exposed to identical amount of UV-B radiation (141). A suggested explanation to the differences in vitamin D status between obese and normal weight individuals is that vitamin D_3 is sequestered in subcutaneous fat, and because obese subjects have more fat available for this process, the serum levels of 25(OH)D are therefore lower (139–141).

Another hypothesized explanation is that volumetric dilution makes up the observed differences of vitamin D status in obese compared to normal weight individuals

(139,140,142). According to this hypothesis, the low serum 25(OH)D levels in obese individuals are explained by the larger body size, meaning that putting the same amount into a larger pool, will result in a lower concentration (139,140,142). It has therefore been suggested that vitamin D dosage to treat deficiency should be adjusted according to body weight to supply a sufficient amount (142).

Taken together, the results from the present thesis, are in line with several previous findings, indicating that increasing body weight is inversely associated with serum 25(OH)D levels, and may potentially be one of the determining factors of vitamin D status.

5.3.4 Lifestyle

Closely related to sunlight exposure, diet, and bodyweight, it has been suggested that the lifestyle of an individual is one of the determining factors of vitamin D status. Previous studies have found vitamin D status to be associated with lifestyle factors such as degree of outdoor activity, exercise, and clothing style (6,7,62,65). A lifestyle characterized by limited physical activity, minimal time outdoors, and a clothing style that includes covering most of the skin, has been shown to be associated with significantly lower serum 25(OH)D levels (6,7,62,65). Data from *The Tromsø Study* looking at vitamin D status in Norwegian adolescents, showed that some of the lifestyle factors related to serum 25(OH)D levels were use of snuff, physical activity, sunbathing holiday, and use of solarium (143). The effects of lifestyle on vitamin D status is largely explained by the associations between lifestyle and sunlight exposure, healthy diet, and bodyweight, which are important determinants of vitamin D status.

5.3.5 Standard lipids

In this study, HDL-C was found to be positively associated with serum 25(OH)D level, while an inverse relationship was observed between 25(OH)D and TGs. Several previous studies also found that 25(OH)D associated positively with HDL-C (144,145) and inversely with TGs (144–146). In a study of patients referred for the diagnosis and

treatment of hyperlipidemia and CVD, serum 25(OH)D was positively associated with HDL-C, with a Spearman`s rho of 0.19, and inversely associated with TGs, with a Spearman`s rho of −0.25 (144). This shows similar, but stronger associations between serum 25(OH)D and the lipids, as in the current thesis. In a cross-sectional study from the University of Tromsø including 10105 subjects, it was found an increase in total cholesterol, HDL-C, and LDL-C, and a decrease in LDL-C/HDL-C ratio and TGs across increasing serum 25(OH)D concentrations (145). The strongest associations were observed for HDL-C and TGs with a difference of 6.0 and 18.5% between the lowest and highest quartile of serum 25(OH)D. The results in the current thesis are similar to these previous findings, where HDL-C was positively and TGs inversely associated with serum 25(OH)D.

Based on the observed associations between vitamin D status and several of the standard lipids, it has been suggested a relationship between serum 25(OH)D levels and cardiovascular health, which has also been observed in several previous studies (33–35). In several trials, it was observed that changes in serum lipids were small and the effect of vitamin D supplementation on lipid concentrations did not differ from the placebo group (147,148). A meta-analysis found that vitamin D supplementation was related to an increase in LDL-C and a small increase in total cholesterol, while HDL-C and TGs were slightly reduced (149). Another systematic review and meta-analysis showed that vitamin D supplementation reduced serum levels of total cholesterol, LDL-C, and TGs (150). Thus, the long-term effects of vitamin D supplementation on the lipid profile and risk of CVD are still uncertain.

Taken together, the results from this thesis, and previous studies, indicate a relationship between vitamin D status and the standard lipid profile. However, these associations are still non fully elucidated, and further work is needed to investigate the connection between vitamin D status, possible causal pathways, effects of vitamin D supplementation, and the clinical relevance regarding CVD risk.

5.3.6 Glycemic control

We observed a negative relationship between vitamin D status and serum glucose, HbA1c, and type 2 diabetes diagnosis. The observed association between serum 25(OH)D level and glycemic control have also been found in several previous studies (151–154). A cross-sectional study of postmenopausal women showed that fasting serum glucose was inversely associated with serum 25(OH)D, with a Pearson`s correlation coefficient of −0.15 (151). In addition, a study of young men and women found an inverse association between fasting plasma glucose and serum 25(OH)D (152). In a cohort of 7198 Caucasian subjects, it was found an inverse relationship between serum 25(OH)D and HbA1c (153). Similar results were found in a large cohort of adults over the age of 20 years, showing that lower levels of serum 25(OH)D were associated with higher HbA1c (154). Previous studies have also investigated the effects of vitamin D supplementation on glycemic control (155,156). A meta-analysis of 47 randomized controlled trials (RCTs) including nondiabetic adults found a weak positive effect, where vitamin D supplementation reduced fasting glucose by 0.11 mmol/L (155). Another meta-analysis of RCTs showed a summary mean difference in fasting glucose between the intervention and placebo group of −0.12 mmol/L (156). These findings indicate a potential beneficial effect of vitamin D supplementation of serum glucose. However, the effects of vitamin D supplementation on glycemic control and risk of diabetes is still not fully elucidated.

Based on observed associations between vitamin D status and glucose homeostasis, it has been suggested that vitamin D status could be associated with diabetes risk (1,157). A meta-analysis of 13 cross-sectional studies found that patients with type 1 diabetes had lower serum 25(OH)D levels than the control group, and that there was an association between vitamin D deficiency and type 1 diabetes (157). Similar findings have been observed when looking at type 2 diabetes, showing that 25(OH)D was inversely related to risk of type 2 diabetes (158,159). A meta-analysis of 21 prospective studies found an inverse association between serum 25(OH)D and risk of type 2 diabetes, where the relative risk of type 2 diabetes when comparing the highest to lowest category of 25(OH)D levels was 0.62 (158). In addition, a meta-analysis of 8 observational studies observed a 43% lower risk of developing type 2 diabetes among

the individuals with the highest vitamin D status (above 62.5 nmol/L) compared to the subjects in the group with the lowest vitamin D status (under 35 nmol/L)(159). The results from the current thesis are in line with these findings, indicating an association between serum 25(OH)D and type 2 diabetes.

The observed associations between serum 25(OH)D levels and circulating markers of glycemic control in this thesis, and the relationship between vitamin D and beta-cell function and insulin sensitivity observed in previous studies (152,160,161), indicate that this relationship may be of clinical significance, and should be further investigated in future studies.

5.3.7 Inflammation

A weak inverse association was observed between serum 25(OH)D level and the inflammatory markers CRP and neopterin at lower serum levels of 25(OH)D, and these associations seemed to be reduced at higher 25(OH)D levels. Similar results have been found in a large cross-sectional study of an adult population, where it was observed an inverse relation between serum 25(OH)D and CRP at 25(OH)D levels below the median (52.5 nmol/L), while a positive association was observed at serum 25(OH)D levels above the median, equivalent to a geometric mean CRP change of 0.06 mg/L for each 1 nmol/L change in serum 25(OH)D (162). Similarly, a cross-sectional study of a general adult population observed a U-shaped relationship between vitamin D status and high sensitivity-CRP (hs-CRP) (163). It was found that hs-CRP concentrations decreased up to a 25(OH)D concentration of about 52.5 nmol/L and increased when the 25(OH)D concentrations exceeded 62.5 nmol/L (163). In addition, an inverse relationship between serum 25(OH)D and CRP was observed in a cross-sectional study of an elderly population, where the lowest quartile of 25(OH)D was associated with higher CRP compared to the upper quartile (OR = 1.23) (164). When looking at the effects of vitamin D supplementation, pooled data from four RCTs investigating the effects of vitamin D supplementation in subjects without vitamin D deficiency found a slight increase in hs-CRP in the subjects given vitamin D supplement (delta value = 0.02 mg/L) whereas in the placebo group there was a slight decrease (165). However, a meta-analysis of RCTs

evaluating the impact of vitamin D supplementation on CRP found a weighted mean difference in CRP of −0.26 mg/L following administration of vitamin D (166). The association between serum 25(OH)D and CRP observed in this thesis and previous studies, indicate a potential nonlinear relationship. However, this relation, and the effects of vitamin D supplementation on inflammatory markers, are still not fully elucidated.

Based on observed associations between vitamin D status and inflammatory markers, it has been suggested that vitamin D status may be related to risk of infectious and autoimmune diseases like tuberculosis, rheumatoid arthritis, and type 1 diabetes (1,167,168). A high prevalence of insufficient serum 25(OH)D levels has been found in subjects with these infectious and autoimmune diseases (14). Future studies should further investigate the mechanisms of the association between serum 25(OH)D and immune function, and the effect of vitamin D on risk of infectious and autoimmune diseases.

5.3.8 Vitamins

Serum 25(OH)D concentration was found to be positively associated with riboflavin, pyridoxal phosphate, 4-pyridoxic acid, folate, cobalamin, vitamin A, and vitamin E in the present study. These associations may be explained by interacting effects of the vitamins, that other vitamins may have functions affecting the metabolism or actions of vitamin D (169,170), or the fact that the fat-soluble vitamins have similar absorption, transport, and storage mechanisms (9).

B vitamins

The positive association between serum levels of 25(OH)D and pyridoxal phosphate, the active form of vitamin B6, was the strongest observed relationship among the B vitamins. There is not much previous data on the association between serum 25(OH)D and PLP, although a cross-sectional study on community-dwelling middle-aged and older people showed that vitamin B6 was positively associated with serum 25(OH)D

concentration, with a partial Pearson`s *r* of 0.35 for women and 0.21 for men (171). It has been suggested that PLP may have modulatory effects on the action of steroid hormones, including calcitriol, which may partly explain the observed association between serum 25(OH)D and PLP (169,172). The associations between serum levels of 25(OH)D and the B vitamins included in our analyses were weak, and existing evidence does not provide any conclusive data supporting a clinically significant association between vitamin D status and circulating levels of B vitamins. Thus, the connection is still highly uncertain.

Fat-soluble vitamins

In a large cohort of adults above 35 years of age examining vitamin D levels by quartiles, serum levels of vitamin A and vitamin E were positively associated with serum 25(OH)D (64). The common mechanisms involved in the metabolism of the fat-soluble vitamins may partly explain why 25(OH)D was associated with vitamin A and E. Another potential connection, is the observed interactions between vitamin D and vitamin A, where high concentrations of 9-cis retinoic acid, a vitamin A metabolite, may prevent calcitriol to perform its gene regulatory functions (170,173). Our observations are in line with existing knowledge and previously observed connections between serum 25(OH)D and other fat-soluble vitamins.

5.3.9 Amino acids

The plasma concentration of several amino acids appeared to correlate with serum 25(OH)D. Sarcosine and choline associated positively with 25(OH)D, while an inverse relationship was found for total homocysteine at lower levels of 25(OH)D, but this association appeared to be weaker at higher serum levels of 25(OH)D. The relationship between 25(OH)D and homocysteine was the strongest observed association among amino acids in our data. A similar relationship was also observed in a large community-based cohort of asymptomatic adults, where they found an inverse association between homocysteine and serum 25(OH)D at 25(OH)D levels below median (52.5 nmol/L), while a weak positive association was observed at 25(OH)D levels above median level (174). A longitudinal study of 4475 participants showed that baseline homocysteine

concentrations were inversely associated with serum 25(OH)D, where homocysteine concentrations were 0.182 μmol/L lower for each additional 25 nmol/L of serum 25(OH)D concentration (175). However, when the analyses were repeated separately by subgroup of baseline 25(OH)D, it was observed that each additional 25 nmol/L 25(OH)D was associated with 1.056 μmol/L lower homocysteine in the subjects with 25(OH)D below 50 nmol/L and 0.150 μmol/L in those with serum 25(OH)D above 50 nmol/L (175). A quite similar trend was found in the current thesis, where the inverse association between serum 25(OH)D and homocysteine was weaker at higher levels of serum 25(OH)D.

The observed associations between serum levels of 25(OH)D and different amino acids were weak in our population, and the results from previous studies are inconclusive. However, a relationship between vitamin D and homocysteine, similar to the observed association in the current thesis, has been found in previous studies (174,175), indicating that this relationship may be of clinical significance, and should be further investigated in future studies.

6 Future perspectives

The prevalence of suboptimal vitamin D status is still high, both in Norway and worldwide (8,23). Insufficient vitamin D status is associated with health risks, and prevention of vitamin D deficiency is a public health priority (23,176). Thus, research into the determinants of vitamin D status is vital to get a better understanding of the causes of insufficient vitamin D status, and thereby improving strategies to prevent vitamin D deficiency.

Due to the nature of the study design in the present study, we are not able to draw any conclusions on causal relationships. However, the results from this and previous studies indicate associations between serum 25(OH)D level and season, vitamin D intake, lipid profile, PLP, vitamin A, vitamin E, BMI, and glucose metabolism. These observations may be the basis of future studies further investigating the associations between serum 25(OH)D and the different variables. Longitudinal studies allows us to measure the characteristics of the same individuals on several occasions over time, and may be used to evaluate the relationship between the potential determinants and serum levels of 25(OH)D over time (177,178). These types of studies have the ability to demonstrate temporality, and cause-and-effect relationships can be examined for the associations between serum 25(OH)D levels and different variables already observed (177,179). As mentioned, vitamin D status is affected by seasonal variation, which makes the assessment of associations between serum 25(OH)D and potential determinants more complicated. A suggested method to deal with seasonality of 25(OH)D is cosinor modelling (122), which could provide a more precise estimate of the associations between serum 25(OH)D and different determinants. Further, interventional studies, where a potential determinant of vitamin D status is changed and the effects on serum levels of 25(OH)D are measured, can be conducted to assess determinants of vitamin D status and measure the effect of preventive measures of vitamin D deficiency.

7 Conclusions

In this study, we explored a wide variety of factors to investigate associations with serum 25(OH)D level, aiming to get a deeper understanding of potential determinants of vitamin D status. In conclusion, we observed a seasonal variation of serum 25(OH)D, where the highest concentrations were measured in August, and the lowest concentrations were measured in March. Serum levels of 25(OH)D were positively associated with dietary vitamin D, fish and egg consumption, and with serum levels of HDL-C, PLP, vitamin A, and vitamin E. Inverse associations were observed for BMI, and serum levels of TGs, total homocysteine, blood glucose, HbA1C and CRP.

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Supplementary table 1. Associations between 25(OH)D levels and demographic characteristics in selected quantiles of 25(OH)D levels from the unadjusted regression model.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Age (years)	−0.16 (−0.24, −0.09)	<0.001	−0.00 (−0.07, 0.07)	0.947	0.19 (0.12, 0.26)	<0.001	0.41 (0.43, 0.48)	<0.001	0.62 (0.54. 0.69)	<0.001
Sex (1 = male)	1.09 (−1.39, 3.57)	0.383	1.62 (−0.44, 3.68)	0.120	1.47 (−0.31, 3.25)	0.103	0.68 (−1.29, 2.64)	0.493	−1.50 (−4.25, 1.25)	0.279
BMI (kg/m2)	−1.55 (−1.70, −1.42)	<0.001	−1.20 (−1.33, −1.07)	<0.001	−0.77 (−0.90, −0.65)	<0.001	−0.27 (−0.40, −0.13)	<0.001	0.20 (0.01, 0.32)	0.003
Current smoker (n)	−6.12 (−8.02, −4.21)	<0.001	−5.65 (−7.05, −4.24)	<0.001	−3.22 (−5.04, −1.41)	<0.001	−0.83 (−2.48, 0.82)	0.317	−1.53 (−4.63, 1.57)	0.325
eGFR (mL/min per 1,73^2)	−0.44 (−0.48, −0.40)	<0.001	−0.32 (−0.36, −0.28)	<0.001	−0.19 (−0.23, −0.15)	<0.001	−0.03 (−0.07, 0.01)	0.090	0.10 (0.06, 0.14)	<0.001
Waist circumference (cm)	−0.44 (−0.50, −0.38)	<0.001	−0.34 (−0.40, −0.27)	<0.001	−0.20 (−0.27, −0.14	<0.001	−0.06 (−0.12, 0.00)	0.050	0.00 (0.00, 0.13)	0.050
Vitamin D intake (µg/d)	0.39 (0.19, 0.59)	<0.001	0.29 (0.10, 0.47)	0.003	0.42 (0.30, 0.55)	<0.001	0.45 (0.25, 0.65)	<0.001	0.51 (0.18, 0.85)	0.003
Season 1	−6.65 (−9.41, −3.89)	<0.001	−9.30 (−11.2, −7.39)	<0.001	−10.3 (−11.9, −8.66)	<0.001	−10.3 (−13.3, −7.20)	<0.001	−8.99 (−13.1, −4.85)	<0.001
Season 2	0.10 (−2.58, 2.79)	0.939	1.25 (−1.22, 3.71)	0.314	0.06 (−1.45, 1.57)	0.936	−1.10 (−3.79, 1.59)	0.414	−0.79 (−4.73, 3.14)	0.688
Season 3	10.9 (8.25, 13.6)	<0.001	12.4 (9.81, 14.9)	<0.001	12.6 (9.76, 15.4)	<0.001	11.0 (8.41, 13.5)	<0.001	9.31 (6.36, 12.3)	<0.001
Season 4	1.76 (−0.64, 4.15)	0.146	1.05 (1.34, 3.45)	0.381	−0.40 (−2.06, 1.25)	0.628	−2.13 (−4.38, 0.12)	0.063	0.59 (−3.31, 4.48)	0.764

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the unadjusted regression model. BMI, body mass index (kg/m^2); eGFR, estimated glomerular filtration ratio; season 1, January-March; season 2, April-June; season 3, July-September; season 4, October-December.

Supplementary table 2. Associations between 25(OH)D levels and lipid profile, blood glucose and inflammatory markers in selected quantiles of 25(OH)D levels from the unadjusted regression model.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
TG (mmol/L)	−1.66 (−2.17, −1.14)	<0.001	−2.08 (−2.79, −1.37)	<0.001	−1.96 (−2.60, −1.32)	<0.001	−1.24 (−2.27, −0.21)	0.019	−1.19 (−2.10, 0.28)	0.011
Total cholesterol (mmol/L)	−1.16 (−1.89, −0.43)	0.003	−1.04 (−1.99, −0.09)	0.032	−0.38 (−0.89, 0.13)	0.139	0.40 (−0.47, 1.26)	0.361	1.32 (0.01, 2.63)	0.049
LDL-C (mmol/L)	−0.66 (−1.53, 0.20)	0.128	−1.21 (−2.18, −0.24)	0.016	−0.48 (−1.27, 0.31)	0.228	0.93 (0.06, 1.80)	0.037	0.68 (−0.95, 2.31)	0.406
HDL-C (mmol/L)	5.44 (2.91, 7,97)	<0.001	6.31 (3.87, 8.76)	<0.001	6.50 (4.96, 8.05)	<0.001	7.03 (5.09, 8.96)	<0.001	7.30 (4.98, 9.61)	<0.001
Non-HDL-C (mmol/L)	−1.04 (−1.73, −0.34)	0.004	−1.35 (−2.35, −0.35)	0.009	−1.20 (−1.67, −0.73)	<0.001	−0.57 (−1.27, 0.14)	0.111	−0.58 (−2.01, 0.85)	0.419
ApoA1 (g/L)	7.78 (4.12, 11.45)	<0.001	9.62 (6.20, 13.03)	<0.001	10.6 (7.66, 13.5)	<0.001	11.9 (8.12, 15.77)	<0.001	11.7 (6.89, 16.5)	<0.001
ApoB (g/L)	−3.44 (−6.68, −0.19)	0.039	−3.66 (−7.02, −0.30)	0.033	−3.02 (−6.61, 0.56)	0.096	−0.67 (−3.26, 1.93)	0.608	0.37 (−5.59, 6.34)	0.901
Type 2 diabetes (n)	−5.33 (−8.11, −2.54)	<0.001	−3.58 (−6.12, −1.05)	0.007	−2.66 (−4.41, −0.91)	0.004	−3.45 (−6.66, −0.24)	0.036	−0.28 (−4.19, 3.64)	0.887
HbA1c (%)	−1.81 (−3.13, −0.50)	0.008	−1.90 (−2.73, −1.07)	<0.001	−1.14 (−1.70, −0.57)	<0.001	−0.56 (−1.38, 0.25)	0.170	−0.01 (−1.52, 1.51)	0.999
Serum glucose (mmol/L)	−1.26 (−1.64, −0.88)	<0.001	−1.07 (−1.65, −0.49)	<0.001	−0.75 (−1.06, −0.44)	0.001	−0.36 (−0.76, 0.05)	0.083	−0.05 (−0.65, 0.56)	0.882
CRP (mg/L)	−0.24 (−0.37, −0.11)	<0.001	−0.23 (−0.38, −0.08)	0.003	−0.22 (−0.37, −0.08)	0.003	−0.04 (−0.23, 0.14)	0.652	−0.01 (−0.10, 0.09)	0.887
Neopterin (nmol/L)	−0.17 (−0.59, 0.24)	0.407	−0.10 (−0.39, 0.19)	0.481	0.05 (−0.09, 0.20)	0.451	0.29 (−0.12, 0.70)	0.167	0.52 (−0.28, 1.32)	0.199

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the unadjusted regression model.
TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; LDL-C, serum low density lipoprotein cholesterol; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; HbA1c, glycosylated haemoglobin; CRP, C-reactive protein.

Supplementary table 3. Associations between 25(OH)D levels and dietary data in selected quantiles of 25(OH)D levels from the unadjusted regression model.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Riboflavin (µg/dL)	−0.02 (−0.07, 0.02)	0.329	0.01 (−0.06, 0.09)	0.721	0.04 (0.02, 0.05)	<0.001	0.17 (0.05, 0.29)	0.005	0.12 (−0.05, 0.30)	0.157
PL (nmol/L)	−0.02 (−0.03, 0.00)	0.055	0.00 (−0.00, 0.01)	0.147	0.01 (−0.00, 0.01)	0.095	0.01 (−0.04, 0.07)	0.629	0.08 (0.00, 0.15)	0.04
PLP (nmol/L)	−0.16 (−0.22, −0.10)	<0.001	−0.08 (−0.11, −0.04)	<0.001	0.02 (0.00, 0.03)	0.025	0.16 (0.12, 0.20)	<0.001	0.35 (0.26, 0.45)	<0.001
PA (nmol/L)	−0.01 (−0.07, 0.05)	0.663	−0.02 (−0.06, 0.03)	0.424	0.00 (0.00, 0.01)	0.009	0.05 (−0.01, 0.11)	0.101	0.12 (−0.10, 0.33)	0.272
Folate (nmol/L)	0.04 (−0.12, 0.20)	0.615	0.02 (−0.06, 0.09)	0.618	0.07 (0.01, 0.13)	0.035	0.16 (0.06, 0.25)	0.002	0.21 (−0.13, 0.55)	0.225
Cobalamin (pq/mL)	−0.06 (−0.06, −0.05)	<0.001	−0.03 (−0.03, −0.02)	<0.001	0.00 (−0.00, 0.00)	0.473	0.03 (0.02, 0.03)	<0.001	0.06 (0.06, 0.07)	<0.001
MMA (nmol/L)	−6.21 (−18.1, 5.67)	0.298	−0.62 (−8.16, 6.92)	0.870	−1.75 (−8.49, 4.99)	0.604	1.78 (−6.20, 9.77)	0.656	4.36 (−16.6, 25.4)	0.678
Vitamin A (µmol/L)	2.20 (0.40, 5.08)	0.018	3.67 (2.25, 5.08)	<0.001	4.25 (3.09, 5.41)	<0.001	5.14 (3.56, 6.71)	<0.001	7.95 (6.23, 9.68)	<0.001
Vitamin E (µmol/L)	−0.45 (−0.55, −0.36)	<0.001	−0.16 (−0.25, −0.07)	<0.001	0.21 (0.11, 0.31)	<0.001	0.65 (0.55, 0.75)	<0.001	1.06 (0.95, 1.17)	<0.001
Low fat milk consumption (g/day)	−0.07 (−0.08, −0.06)	<0.001	−0.04 (−0.04, −0.03)	<0.001	−0.00 (−0.01, 0.00)	0.173	0.03 (0.03, 0.04)	<0.001	0.08 (0.06, 0.10)	<0.001
Egg consumption (g/day)	−0.07 (−0.21, 0.07)	0.317	−0.01 (−0.19, 0.17)	0.884	−0.01 (−0.11, 0.09)	0.789	0.29 (0.09, 0.50)	0.006	0.37 (0.04, 0.70)	0.030
Fish consumption (g/day)	−0.17 (−0.19, −0.14)	<0.001	−0.07 (−0.08, −0.05)	<0.001	0.02 (0.00, 0.03)	0.020	0.12 (0.10, 0.13)	<0.001	0.21 (0.19, 0.24)	<0.001

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the unadjusted regression model.
PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; MMA, methylmalonic acid.

Supplementary table 4. Associations between 25(OH)D levels and amino acids and amino acid metabolites in selected quantiles of 25(OH)D levels from the unadjusted regression model.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Serine (µmol/L)	−0.23 (−0.26, −0.20)	<0.001	−0.13 (−0.16, −0.11)	<0.001	−0.02 (−0.05, 0.01)	0.187	0.11 (0.09, 0.14)	<0.001	0.23 (0.20, 0.26)	<0.001
Glycine (µmol/L)	−0.09 (−0.10, −0.08)	<0.001	−0.04 (−0.05, −0.03)	<0.001	0.01 (0.00, 0.02)	0.003	0.07 (0.07, 0.08)	<0.001	0.13 (0.12, 0.14)	<0.001
Dmg (µmol/L)	−0.13 (−0.73, 0.47)	0.657	−0.11 (−0.55, 0.33)	0.627	−0.11 (−0.41, 0.19)	0.453	0.12 (−0.35, 0.60)	0.607	0.08 (−0.10, 1.16)	0.883
Sarcosine (µmol/L)	2.12 (0.68, 3.56)	0.005	2.47 (1.08, 3.85)	<0.001	2.50 (1.26, 3.74)	<0.001	3.60 (1.88, 5.33)	<0.001	3.82 (1.76, 5.88)	<0.001
Choline (µmol/L)	−0.45 (−1.10, 0.19)	0.162	0.24 (−0.25, 0.73)	0.332	0.68 (0.44, 0.92)	<0.001	1.37 (0.91, 1.84)	<0.001	2.23 (1.42, 3.04)	<0.001
Betaine (µmol/L)	−0.47 (−0.56, −0.38)	<0.001	−0.21 (−0.26, −0.15)	<0.001	0.05 (0.01, 0.10)	0.025	0.37 (0.32, 0.43)	<0.001	0.70 (0.61, 0.78)	<0.001
Methionine (µmol/L)	−0.61 (−0.75, −0.46)	<0.001	−0.27 (−0.38, −0.15)	<0.001	0.04 (−0.05, 0.13)	0.337	0.49 (0.39, 0.59)	<0.001	0.78 (0.61, 0.94)	<0.001
tHcy (µmol/L)	−0.35 (−0.92, 0.22)	0.225	−0.37 (−0.61, −0.12)	0.004	−0.07 (−0.21, 0.08)	0.364	0.32 (−0.12, 0.76)	0.146	0.88 (0.32, 1.44)	0.003
tCys (µmol/L)	−0.05 (−0.06, −0.03)	<0.001	−0.02 (−0.03, 0.00)	0.070	0.03 (0.01, 0.04)	0.001	0.08 (0.06, 0.09)	<0.001	0.12 (0.10, 0.14)	<0.001

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the unadjusted regression model.
DMG, plasma dimetylglycine; tHcy, total homocysteine; tCys, total cysteine.

Supplementary table 5. Associations between 25(OH)D levels and demographic characteristics in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age and sex.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
BMI (kg/m2)	−1.01 (−1.17, −0.85)	<0.001	−0.86 (−1.00, −0.71)	<0.001	−0.68 (−0.82, −0.54)	<0.001	−0.58 (−0.71, −0.44)	<0.001	−0.36 (−0.58, −0.14)	0.002
Current smoker (n)	−1.88 (−3.26, −0.50)	0.009	−1.88 (−3.21, −0.54)	0.007	−1.75 (−3.10, −0.40)	0.012	−1.72 (−3.08, −0.36)	0.014	−1.47 (−2.84, −0.10)	0.036
eGFR (mL/min per 1,73^2)	−0.27 (−0.34, −0.20)	<0.001	−0.23 (−0.28, −0.17)	<0.001	−0.16 (−0.22, −0.10)	<0.001	−0.07 (−0.13, −0.02)	0.013	−0.05 (−0.11, 0.02)	0.137
Waist circ. (cm)	−0.36 (−0.42, −0.30)	<0.001	−0.33 (−0.39, −0.26)	<0.001	−0.24 (−0.30, −0.18)	<0.001	−0.14 (−0.21, −0.07)	<0.001	−0.07 (−0.16, 0.01)	0.087
Season 1	−9.20 (−10.9, −7.49)	<0.001	−9.24 (−11.0, −7.53)	<0.001	−9.24 (−11.0, −7.53)	<0.001	−9.24 (−11.0, −7.52)	<0.001	−9.24 (−10.9, −7.52)	<0.001
Season 2	−0.18 (−1.66, 1.29)	0.805	−0.18 (−1.65, 1.29)	0.807	−0.18 (−1.65, 1.29)	0.808	−0.18 (−1.66, 1.30)	0.809	−0.18 (−1.64, 1.29)	0.808
Season 3	11.7 (9.78, 13.7)	<0.001	11.7 (9.78, 13.7)	<0.001	11.7 (9.79, 13.7)	<0.001	11.7 (9.79, 13.7)	<0.001	11.7 (9.78, 13.7)	<0.001
Season 4	−0.61 (−2.13, 0.91)	0.425	−0.61 (−2.13, 0.90)	0.419	−0.61 (−2.13, 0.90)	0.420	−0.61 (−2.13, 0.91)	0.421	−0.62 (−2.13, 0.90)	0.418
Vitamin D intake (µg/d)	0.42 (0.29, 0.56)	<0.001	0.45 (0.35, 0.56)	<0.001	0.48 (0.38, 0.59)	<0.001	0.49 (0.35, 0.63)	<0.001	0.50 (0.35, 0.64)	<0.001

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age and sex. BMI, body mass index (kg/m^2); eGFR, estimated glomerular filtration ratio; waist circ., waist circumference; season 1, January-March; season 2, April-June; season 3, July-September; season 4, October-December.

Supplementary table 6. Associations between 25(OH)D levels and lipid profile, blood glucose and inflammatory markers in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age and sex.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
TG (mmol/L)	−1.89 (−2.45, −1.32)	<0.001	−1.69 (−2.19, −1.19)	<0.001	−1.49 (−1.97, −1.00)	<0.001	−1.46 (−1.98, −0.95)	<0.001	−0.85 (−1.50, −0.21)	0.011
Total cholesterol (mmol/L)	−0.23 (−1.07, 0.61)	0.589	−0.61 (−1.22, 0.00)	0.052	0.06 (−0.46, 0.58)	0.817	0.16 (−0.41, 0.73)	0.584	0.90 (0.01, 1.78)	0.047
LDL-C (mmol/L)	−0.34 (−1.11, 0.43)	0.374	−0.11 (−0.79, 0.56)	0.738	0.07 (−0.60, 0.73)	0.841	0.12 (−0.55, 0.79)	0.720	0.72 (−0.02, 1.47)	0.057
HDL-C (mmol/L)	7.00 (5.41, 8.59)	<0.001	7.08 (5.48, 8.68)	<0.001	7.09 (5.50, 8.68)	<0.001	7.10 (5.49, 8.70)	<0.001	7.19 (5.49, 8.78)	<0.001
Non-HDL-C (mmol/L)	−1.84 (−2.60, −1.08)	<0.001	−0.63 (−1.25, −0.02)	0.042	−0.62 (−1.12, −0.12)	0.016	−0.45 (−0.95, 0.06)	0.082	0.31 (−0.45, 1.08)	0.416
ApoA-I (g/L)	12.06 (9.67, 14.45)	<0.001	12.07 (9.65, 14.48)	<0.001	12.07 (9.67, 14.47)	<0.001	12.13 (9.72, 14.54)	<0.001	12.08 (9.68, 14.49)	<0.001
ApoB (g/L)	−0.84 (−3.40, 1.72)	0.513	−0.64 (−3.16, 1.88)	0.612	−0.64 (−3.15, 1.88)	0.614	−0.63 (−3.15, 1.89)	0.617	−0.60 (−3.16, 1.95)	0.638
Type 2 diabetes	−4.10 (−6.10, −2.09)	<0.001	−4.11 (−6.11, −2.10)	<0.001	−4.09 (−6.10, −2.09)	<0.001	−4.10 (−6.11, −2.09)	<0.001	−4.09 (−6.10, −2.08)	<0.001
HbA1c (%)	−2.34 (−3.00, −1.68)	0.008	−1.39 (−2.02, −0.76)	<0.001	−1.02 (−1.53, −0.50)	<0.001	−0.91 (−1.52, −0.31)	0.004	0.20 (−0.70, 1.11)	0.652
Serum glucose (mmol/L)	−1.28 (−1.67, −0.88)	<0.001	−0.91 (−1.30, −0.53)	<0.001	−0.67 (−0.96, −0.38)	<0.001	−0.44 (−0.78, −0.10)	0.012	−0.03 (−0.49, 0.43)	0.902
CRP (mg/L)	−0.28 (−0.41, −0.16)	<0.001	−0.25 (−0.35, −0.14)	<0.001	−0.19 (−0.31, −0.06)	0.004	−0.05 (−0.22, 0.12)	0.559	−0.02 (−0.11, 0.07)	0.601
Neopterin (nmol/L)	−0.19 (−0.43, 0.05)	0.123	−0.11 (−0.31, 0.09)	0.275	0.06 (−0.08, 0.20)	0.394	0.10 (−0.06, 0.25)	0.210	0.23 (0.10, 0.36)	0.001

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age and sex. TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; LDL-C, serum low density lipoprotein cholesterol; ApoA-I, Apolipoprotein A1; ApoB, Apolipoprotein B; HBA1c, glycosylated haemoglobin; CRP, C-reactive protein.

Supplementary table 7. Associations between 25(OH)D levels and vitamin status and dietary data in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age and sex.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Riboflavin (µg/dL)	−0.04 (0.00, 0.07)	0.046	0.05 (0.03, 0.08)	<0.001	0.04 (0.03, 0.06)	<0.001	0.03 (−0.00, 0.06)	0.056	0.07 (0.01, 0.13)	0.029
PL (nmol/L)	0.00 (−0.01, 0.02)	0.715	0.01 (−0.00, 0.02)	0.175	0.01 (0.00, 0.02)	0.002	0.01 (0.00, 0.01)	0.021	−0.00 (−0.03, 0.03)	0.981
PLP (nmol/L)	0.01 (−0.02, 0.03)	0.578	0.02 (0.01, 0.04)	0.010	0.04 (0.03, 0.05)	<0.001	0.03 (0.01, 0.06)	0.003	0.07 (0.04, 0.10)	<0.001
PA (nmol/L)	0.00 (−0.01, 0.01)	0.662	0.01 (0.00, 0.01)	0.005	0.01 (0.00, 0.01)	0.001	0.01 (0.00, 0.01)	0.030	0.00 (−0.02, 0.03)	0.912
Folate (nmol/L)	0.06 (0.00, 0.12)	0.046	0.07 (0.00, 0.13)	0.042	0.11 (0.04, 0.18)	0.002	0.14 (0.06, 0.21)	<0.001	0.16 (0.06, 0.27)	0.004
Cobalamin (pq/mL)	−0.00 (−0.01, 0.00)	0.395	0.00 (−0.00, 0.01)	0.437	0.00 (−0.00, 0.00)	0.501	0.01 (0.00, 0.02)	0.030	0.02 (0.01, 0.02)	0.001
MMA (nmol/L)	−1.74 (−7.51, 4.04)	0.549	−1.73 (−7.51, 4.05)	0.550	−1.73 (−7.51, 4.05)	0.550	−1.73 (−7.51, 4.05)	0.550	−1.73 (−7.51, 4.05)	0.550
Vitamin A (µmol/L)	3.03 (1.66, 4.39)	<0.001	4.53 (3.64, 5.43)	<0.001	4.59 (3.71, 5.46)	<0.001	4.60 (3.73, 5.48)	<0.001	4.78 (3.72, 5.85)	<0.001
Vitamin E (µmol/L)	−0.09 (−0.20, 0.01)	0.080	0.11 (−0.01, 0.24)	0.073	0.26 (0.15, 0.37)	<0.001	0.45 (0.31, 0.59)	<0.001	0.63 (0.45, 0.82)	<0.001
Low fat milk consumption (g/day)	−0.00 (−0.01, −0.00)	0.018	−0.01 (−0.01, 0.00)	0.114	0.00 (−0.00, 0.01)	0.630	0.01 (−0.00, 0.01)	0.087	0.01 (0.00, 0.02)	0.019
Egg consumption (g/day)	−0.03 (−0.11, 0.04)	0.377	0.02 (−0.08, 0.12)	0.671	0.02 (−0.08, 0.13)	0.666	0.14 (0.03, 0.25)	0.017	0.22 (0.04, 0.40)	0.017
Fish consumption (g/day)	0.01 (−0.00, 0.03)	0.102	0.03 (0.01, 0.04)	0.003	0.02 (0.01, 0.04)	0.004	0.04 (0.02, 0.06)	<0.001	0.03 (0.01, 0.05)	0.018

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age and sex. PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; MMA, methylmalonic acid.

Supplementary table 8. Associations between 25(OH)D levels and amino acids and amino acid metabolites in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age and sex.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Serine (µmol/L)	−0.12 (−0.16, −0.08)	<0.001	−0.05 (−0.08, −0.02)	0.004	−0.01 (−0.03, 0.02)	0.647	0.06 (0.03, 0.09)	<0.001	0.14 (0.10, 0.19)	<0.001
Glycine (µmol/L)	−0.02 (−0.03, −0.01)	<0.001	0.00 (−0.01, 0.01)	0.454	0.02 (0.01, 0.03)	<0.001	0.06 (0.04, 0.07)	<0.001	0.07 (0.05, 0.09)	<0.001
DMG (µmol/L)	−0.45 (−0.86, −0.04)	0.033	−0.27 (−0.58, 0.04)	0.086	−0.12 (−0.34, 0.11)	0.304	0.01 (−0.23, 0.25)	0.945	0.23 (−0.20, 0.65)	0.285
Sarcosine (µmol/L)	2.23 (1.20, 3.26)	<0.001	2.45 (1.47, 3.42)	<0.001	2.50 (1.51, 3.48)	<0.001	2.51 (1.55, 3.47)	<0.001	2.54 (1.54, 3.54)	<0.001
Choline (µmol/L)	−0.22 (−0.65, 0.21)	0.312	0.36 (−0.01, 0.74)	0.058	0.55 (0.28, 0.82)	<0.001	0.73 (0.42, 1.04)	<0.001	1.31 (0.76, 1.86)	<0.001
Betaine (µmol/L)	−0.07 (−0.14, −0.01)	0.029	−0.01 (−0.06, 0.04)	0.731	0.06 (0.01, 0.10)	0.010	0.13 (0.07, 0.19)	<0.001	0.18 (0.09, 0.27)	<0.001
Methionine (µmol/L)	−0.22 (−0.35, −0.10)	<0.001	−0.03 (−0.12, 0.06)	0.489	0.06 (−0.03, 0.15)	0.172	0.24 (0.12, 0.35)	<0.001	0.34 (0.18, 0.49)	<0.001
tHcy (µmol/L)	−0.38 (−0.57, −0.19)	<0.001	−0.33 (−0.46, −0.20)	<0.001	−0.10 (−0.27, 0.06)	0.217	0.14 (−0.11, 0.39)	0.252	0.36 (0.13, 0.60)	0.003
tCys (µmol/L)	−0.07 (−0.08, −0.06)	<0.001	−0.03 (−0.05, −0.02)	<0.001	0.00 (−0.01, 0.02)	0.492	0.05 (0.03, 0.06)	<0.001	0.09 (0.08, 0.11)	<0.001

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age and sex. DMG, plasma dimetylglycine; tHcy, total homocysteine; tCys, total cysteine.

Supplementary table 9. Associations between 25(OH)D levels and waist circumference, lipid profile, blood glucose and inflammatory markers in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age, sex, BMI, smoking habits and GFR. [1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Waist circumference (cm)	−0.10 (−0.23, −0.02)	0.097	−0.08 (−0.21, 0.04)	0.187	−0.03 (−0.15, 0.09)	0.564	0.02 (−0.10, 0.14)	0.736	0.07 (−0.05, 0.20)	0.230
TG (mmol/L)	−1.10 (−1.55, −0.66)	<0.001	−1.13 (−1.58, −0.68)	<0.001	−1.10 (−1.55, −0.65)	<0.001	−1.10 (−1.55, −0.65)	<0.001	−1.07 (−1.53, −0.62)	<0.001
Total cholesterol (mmol/L)	0.09 (−0.50, 0.67)	0.771	0.09 (−0.50, 0.68)	0.755	0.11 (−0.47, 0.70)	0.698	0.12 (−0.46, 0.71)	0.674	0.14 (−0.44, 0.73)	0.626
LDL-C (mmol/L)	0.06 (−0.52, 0.65)	0.826	0.09 (−0.50, 0.68)	0.767	0.10 (−0.49, 0.69)	0.735	0.10 (−0.49, 0.69)	0.725	0.12 (−0.48, 0.71)	0.693
HDL-C (mmol/L)	6.12 (4.12, 8.13)	<0.001	6.13 (4.12, 8.13)	<0.001	6.13 (4.12, 8.13)	<0.001	6.13 (4.13, 8.14)	<0.001	6.14 (4.13, 8.14)	<0.001
Non-HDL-C (mmol/L)	−0.46 (−1.01, 0.09)	0.101	−0.45 (−1.00, 0.10)	0.104	−0.43 (−0.99, 0.12)	0.124	−0.43 (−0.98, 0.13)	0.127	−0.41 (−0.97, 0.14)	0.143
ApoA1 (g/L)	10.96 (8.32, 13.60)	<0.001	10.97 (8.33, 13.60)	<0.001	10.97 (8.33, 13.61)	<0.001	10.97 (8.34, 13.61)	<0.001	10.98 (8.34, 13.61)	<0.001
ApoB (g/L)	−0.13 (−2.73, 2.48)	0.922	−0.11 (−2.72, 2.49)	0.930	−0.11 (−2.71, 2.49)	0.932	−0.11 (−2.71, 2.49)	0.933	−0.11 (−2.71, 2.50)	0.934
Type 2 diabetes (n)	−2.29 (−4.27, −0.30)	0.025	−2.28 (−4.27, −0.30)	0.025	−2.28 (−4.27, −0.30)	0.025	−2.28 (−4.27, −0.30)	0.025	−2.28 (−4.26, −0.30)	0.025
HbA1c (%)	−0.79 (−1.25, −0.33)	0.001	−0.78 (−1.26, −0.31)	0.002	−0.76 (−1.24, −0.29)	0.002	−0.75 (−1.23, −0.28)	0.003	−0.68 (−1.15, −0.21)	0.006
Serum glucose (mmol/L)	−0.52 (−0.76, −0.28)	<0.001	−0.46 (−0.70, −0.22)	<0.001	−0.45 (−0.69, −0.21)	<0.001	−0.43 (−0.68, −0.19)	<0.001	−0.40 (−0.64, −0.16)	0.002
CRP (mg/L)	−0.18 (−0.26, −0.10)	<0.001	−0.26 (−0.37, −0.14)	<0.001	−0.18 (−0.27, −0.10)	<0.001	−0.06 (−0.16, 0.05)	0.280	−0.04 (−0.13, 0.05)	0.390
Neopterin (nmol/L)	−0.59 (−0.80, −0.38)	<0.001	−0.45 (−0.70, −0.20)	<0.001	−0.10 (−0.29, 0.09)	0.306	−0.00 (−0.22, 0.21)	0.976	0.11 (−0.14, 0.37)	0.375

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age, sex, BMI, smoking-habits and GFR. TG, serum triglycerides; HDL-C, serum high density lipoprotein cholesterol; LDL-C, serum low density lipoprotein cholesterol; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; HBA1c, glycosylated haemoglobin; CRP, C-reactive protein.

Supplementary table 10. Associations between 25(OH)D levels and vitamin status and dietary data in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age, sex, BMI, smoking habits and GFR.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Vitamin D intake (µg/d)	0.47 (0.36, 0.57)	<0.001	0.50 (0.38, 0.61)	<0.001	0.49 (0.40, 0.59)	<0.001	0.42 (0.29, 0.55)	<0.001	0.42 (0.27, 0.57)	<0.001
Riboflavin (µg/dL)	0.03 (−0.00, 0.07)	0.117	0.04 (0.02, 0.07)	0.002	0.03 (0.02, 0.05)	<0.001	0.02 (−0.00, 0.05)	0.099	0.06 (0.01, 0.11)	0.030
PL (nmol/L)	0.00 (−0.01, 0.01)	0.780	0.01 (−0.00, 0.02)	0.209	0.01 (0.00, 0.02)	0.018	0.01 (0.00, 0.01)	0.026	−0.00 (−0.01, 0.00)	0.830
PLP (nmol/L)	0.01 (−0.01, 0.02)	0.354	0.03 (0.01, 0.05)	0.005	0.03 (0.02, 0.05)	<0.001	0.02 (0.00, 0.04)	0.013	0.03 (0.02, 0.05)	<0.001
PA (nmol/L)	−0.00 (−0.01, 0.01)	0.695	0.00 (−0.00, 0.01)	0.105	0.00 (0.00, 0.01)	0.033	0.00 (−0.00, 0.01)	0.073	0.00 (−0.02, 0.02)	0.858
Folate (nmol/L)	0.06 (−0.00, 0.11)	0.055	0.06 (−0.00, 0.12)	0.066	0.09 (0.02, 0.16)	0.009	0.12 (0.07, 0.17)	<0.001	0.10 (0.02, 0.18)	0.013
Cobalamin (pq/mL)	−0.00 (−0.01, 0.00)	0.484	0.00 (−0.00, 0.00)	0.090	0.00 (−0.00, 0.01)	0.563	0.00 (−0.00, 0.01)	0.230	0.01 (−0.00, 0.02)	0.106
MMA (nmol/L)	−2.86 (−8.81, 3.10)	0.340	−2.85 (−8.81, 3.10)	0.340	−2.85 (−8.80, 3.10)	0.341	−2.85 (−8.80, 3.10)	0.341	−2.85 (−8.80, 3.10)	0.341
Vitamin A (µmol/L)	4.27 (3.12, 5.41)	<0.001	4.28 (3.11, 5.44)	<0.001	4.30 (3.14, 5.46)	<0.001	4.31 (3.15, 5.47)	<0.001	4.34 (3.18, 5.50)	<0.001
Vitamin E (µmol/L)	0.07 (−0.01, 0.16)	0.097	0.20 (0.10, 0.29)	<0.001	0.27 (0.19, 0.34)	<0.001	0.33 (0.23, 0.43)	<0.001	0.39 (0.26, 0.51)	<0.001
Low fat milk consumption (g/day)	−0.00 (−0.01, 0.00)	0.328	−0.00 (−0.01, 0.01)	0.869	0.00 (−0.00, 0.01)	0.575	0.00 (−0.00, 0.01)	0.834	0.00 (−0.00, 0.01)	0.538
Egg consumption (g/day)	0.01 (−0.00, 0.15)	0.245	0.08 (0.00, 0.16)	0.039	0.10 (0.03, 0.18)	0.008	0.12 (0.03, 0.22)	0.009	0.17 (0.02, 0.33)	0.025
Fish consumption (g/day)	0.03 (0.02, 0.04)	<0.001	0.03 (0.01, 0.04)	<0.001	0.03 (0.02, 0.05)	<0.001	0.03 (0.01, 0.05)	0.001	0.02 (0.00, 0.05)	0.047

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age, sex, BMI, smoking-habits and GFR. PL, Pyridoxine levels; PLP, Pyridoxal 5-phosphate; PA, 4-pyridoxic acid; MMA, methylmalonic acid.

Supplementary table 11. Associations between 25(OH)D levels and amino acids and amino acid metabolites in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age, sex, BMI, smoking habits and GFR.[1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Serine (µmol/L)	−0.07 (−0.11, −0.02)	0.003	−0.04 (−0.07, −0.01)	0.022	−0.01 (−0.04, 0.02)	0.469	0.02 (−0.02, 0.05)	0.372	0.05 (−0.01, 0.11)	0.086
Glycine (µmol/L)	−0.01 (−0.03, 0.01)	0.156	−0.01 (−0.02, 0.00)	0.143	0.00 (−0.01, 0.01)	0.922	0.02 (0.00, 0.04)	0.013	0.03 (0.02, 0.05)	<0.001
Dmg (µmol/L)	−0.21 (−0.56, 0.14)	0.239	−0.20 (−0.55, 0.16)	0.267	−0.17 (−0.53, 0.20)	0.361	−0.16 (−0.51, 0.19)	0.351	−0.15 (−0.48, 0.19)	0.381
Sarcosine (µmol/L)	1.79 (0.61, 2.98)	0.004	1.79 (0.61, 2.98)	0.004	1.80 (0.62, 2.99)	0.004	1.82 (0.63, 3.00)	0.003	1.82 (0.64, 3.00)	0.003
Choline (µmol/L)	0.16 (−0.18, 0.50)	0.344	0.34 (0.06, 0.62)	0.020	0.46 (0.17, 0.76)	0.003	0.51 (0.18, 0.83)	0.003	0.60 (0.22, 0.98)	0.003
Betaine (µmol/L)	−0.02 (−0.09, 0.05)	0.623	−0.03 (−0.09, 0.03)	0.331	0.03 (−0.04, 0.09)	0.423	0.06 (0.00, 0.11)	0.039	0.06 (−0.02, 0.14)	0.162
Methionine (µmol/L)	−0.02 (−0.13, 0.09)	0.698	0.01 (−0.08, 0.11)	0.774	0.06 (−0.04, 0.16)	0.224	0.15 (0.04, 0.26)	0.007	0.16 (0.03, 0.29)	0.018
tHcy (µmol/L)	−0.56 (−0.90 −0.22)	0.002	−0.43 (−0.62, −0.25)	<0.001	−0.38 (−0.55, −0.20)	<0.001	−0.21 (−0.38, −0.03)	0.021	−0.01 (−0.32, 0.30)	0.962
tCys (µmol/L)	−0.06 (−0.08, −0.04)	<0.001	−0.03 (−0.05, −0.01)	0.004	−0.00 (−0.02, 0.01)	0.651	0.02 (0.00, 0.04)	0.019	0.06 (0.04, 0.08)	<0.001

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age, sex, BMI, smoking-habits and GFR. DMG, plasma dimetylglycine; tHcy, total homocysteine; tCys, total cysteine.

Supplementary table 12. Associations between 25(OH)D levels and season of study visit in selected quantiles of 25(OH)D levels derived from a regression model adjusted for age, sex, BMI, smoking habits and GFR. [1]

	Quantile									
	0.10		0.25		0.50		0.75		0.90	
Variable	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Season 1	−8.96 (−10.6, −7.30)	<0.001	−8.96 (−10.6, −7.31)	<0.001	−8.96 (−10.6, −7.31)	<0.001	−8.96 (−10.6, −7.30)	<0.001	−8.96 (−10.6, −7.30)	<0.001
Season 2	−0.53 (−2.11, 1.06)	0.507	−0.53 (−2.11, 1.06)	0.509	−0.53 (−2.11, 1.06)	0.508	−0.53 (−2.11, 1.06)	0.507	−0.53 (−2.11, 1.06)	0.507
Season 3	11.6 (9.70, 13.5)	<0.001	11.6 (9.71, 13.5)	<0.001	11.6 (9.71, 13.5)	<0.001	11.6 (9.71, 13.5)	<0.001	11.6 (9.71, 13.5)	<0.001
Season 4	−0.44 (−1.94, 1.07)	0.563	−0.44 (−1.94, 1.07)	0.563	−0.44 (−1.95, 1.07)	0.561	−0.44 (−1.95, 1.07)	0.561	−0.44 (−1.95, 1.07)	0.562

[1]Values are regression coefficients (β) at each quantile of serum 25(OH)D with bootstrapped 95% confidence intervals and p-values from the regression model adjusted for age, sex, BMI, smoking-habits and GFR. Season 1, January-March; season 2, April-June; season 3, July-September; season 4, October-December.