# ASSOCIATION BETWEEN VITAMIN D INTAKE AND CORONARY HEART DISEASE MORTALITY AMONG MEN AND WOMEN WITH CORONARY HEART DISEASE IN KUOPIO, FINLAND.

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# ASSOCIATION BETWEEN VITAMIN D INTAKE AND CORONARY HEART DISEASE MORTALITY AMONG MEN AND WOMEN WITH CORONARY HEART DISEASE IN KUOPIO, FINLAND

Vitamin D can be derived from quite limited amount of foods, from exposing the skin to the sun or by supplementing with tablets. CHD is a common disease in the world with increased mortality from the early 20$^{th}$ century. Previous studies have investigated the association between vitamin D intake and CHD mortality. However, the results are inconclusive, indicating the need for continued research.

Vitamin D intake of 415 patients with CHD were analyzed to predict CHD mortality. During the 5 year follow up, there were 16 CHD deaths. After adjusting for age and sex there was no association between vitamin D intake and CHD death with RR 1.10 (95% confidence interval (CI) 1.04-1.17,p= 0.677), CVD death, 1.07 ( 95% CI 1.02-1.12, p=0.578), and total death RR 1.05( 95% CI 1.00-1.10, p=0.266.

The association remained insignificant after further adjustment for known confounders. There was no association between different groups of vitamin D intake and the three endpoints: total, CVD and CHD mortality (p for trend across groups: 0.30, 0.74 and 0.89, respectively).

After adjustment for age, gender and other confounders, those in the vitamin D group of 5-7 µg/day had RR of 1.27 (95% CI: 0.52,3.12) of total death, compared to those in the lowest group of < 5 µg/day (p for trend across groups: 0.30).

Results from this study suggest that there is no association between vitamin D intake and CHD mortality in subjects with existing CHD . The clinical implication of this study is that vitamin D intake among men and women with CHD could benefit from further research.

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I dedicate this work to the two people that I love most in the world; Victor Nyange and my little baby Jocelyn Taya Nyange.

# ABBREVIATIONS

| | |
|---|---|
| AMI | Acute myocardial infarction. |
| AMIS | Acute myocardial ischemia. |
| BP | Blood pressure. |
| CVD | Cardiovascular disease. |
| CHD | Coronary heart disease. |
| CAD | Coronary artery disease. |
| CABG | Coronary artery bypass grafting. |
| CI | Confidence interval. |
| EUROASPIRE | European action on secondary prevention through intervention to reduce events. |
| HF | Heart failure. |
| HDL | High density lipoprotein. |
| 25(OH)D | 25 hydroxyvitamin D. |
| IHD | Ischemic heart disease. |
| IOM | Institute of medicine. |
| LDL | Low density lipoprotein. |
| MI | Myocardial infarction. |
| NHANES | National Health and Nutritional Examination. |
| PTH | Parathyroid hormone. |
| PTCA | Percutaneous transluminal coronary angioplasty. |
| RDA | Recommended dietary allowance. |
| RR | Risk ratio. |

| | |
|---|---|
| SBP | Systolic blood pressure. |
| WHO | World health organization. |

# LIST OF TABLES

# LIST OF FIGURES

# Table of Contents

# 1. INTRODUCTION

According to the World Health Organization (WHO), almost 23.6 million deaths will be attributed to cardiovascular diseases (CVD), mainly stroke and heart diseases by the year 2030. CVDs include coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart diseases, deep vein thrombosis and pulmonary embolism. The behavioral risk factors of CVD are smoking, physical inactivity and harmful use of alcohol. The metabolic risk factors are high blood pressure, raised blood glucose, raised blood lipids, overweight and obesity. Underlying factors include social, economic and cultural change manifested by globalization, urbanization, and population ageing. Other determinants include poverty, stress and hereditary factors (WHO 2011).

CHD is among the leading causes of death in industrialized countries, with higher rates in men than in women, although as women age, CHD related mortality increases. Nutritional factors have been shown to play a major role in the etiology and management of CHD. In recent years, scientists have continued to discover sources, roles and purpose of vitamin D in the body (Ross et al. 2011).

The earliest evidence on the importance of vitamin D to human health began with the industrial revolution in Europe. This was a period characterized with massive migration to the cities, leading to congestion and environmental pollution. Consequently, children had inadequate exposure to sunlight as the structures were squeezed and without adequate sun exposure. This was associated with increased prevalence of rickets in children (Holick 2004). Similar observations were made in the United States. Towards the end of the $20^{th}$ century, reported cases of rickets had spread all over the world.

Vitamin D deficiency and rickets continued to be of interest, with evidence showing reduced prevalence of rickets in 1930s, when fortification of foods with the vitamin was introduced.(Holick 2004).

Studies investigating the association between vitamin D and CHD began in the 1970s. One of the earliest studies conducted in Denmark in 1978 where circulating 25 hydroxyvitamin D (25(OH) D) concentration was measured in 128 patients admitted with ischemic symptoms and 409 controls.

Findings showed that 25(OH)D concentrations were lower in ischemic patients at 58.75 nmol/L, compared to controls who had 72 nmol/L (Leu & Giovannucci 2011). A nested case-control study of patients with myocardial infarction (MI) using data from the Tromso Heart Study in northern Norway using 30 cases and 60 matched controls, reported a slightly lower 25(OH)D level in cases 59 nmol/L compared to controls 63.5 nmol/L. These two studies suggested that there were effects of low vitamin D intake on patients with heart complications. Despite these findings, few human studies with regard to vitamin D and its relation to CHD have been reported (Leu & Giovannucci 2011).

There is continued interest in vitamin D's broad spectrum of health benefits and outcomes. The relationship between vitamin D and bone health has been well established (Ross et al. 2011). This paved the way for research on investigating relationship between vitamin D and other health outcomes. However, evidence concerning the relationship between vitamin D intake and CHD is still inconclusive with scientist recommending more studies and clinical trials.

In this study we seek to investigate the existence of an association between vitamin D and CHD mortality in patients who have existing CHD.

# 2. LITERATURE REVIEW

In the 1980s and 1990s several observations on the relation between vitamin D and CHD suggested that vitamin D could explain differences in mortality from ischemic heart disease (IHD). The National Health and Nutritional Examination Surveys (NHANES) conducted between 1988 and 2004 showed that individuals with vitamin D deficiency had a higher risk of reporting incidences of heart failure (HF) and MI than those individuals with higher intake of vitamin D (Judd & Tangpricha 2009).

Randomized controlled trials done on German elderly women concluded that little amounts of vitamin D 400IU per day could reduce systolic blood pressure (Pfeifer et al. 2001). On the contrary, the Women's Health Initiative (WHI) showed no significant difference with regard to diastolic blood pressure in women randomized to consume either calcium or vitamin D supplements (400 IU per day) at the end of 7 years of follow up (Scragg et al. 1995). A review concluded that vitamin D insufficiency is common in the world and that the current evidence does not strongly support screening for vitamin D deficiency in patients with CHD or those who are at risk of CHD (Judd & Tangpricha 2009).

A meta analysis done on vitamin D and cardiovascular outcomes found that trial data available are unable to demonstrate a statistical significant reduction in mortality and CHD risk associated with vitamin D intake (Elamin et al. 2011). Similar results were observed in another meta analysis by Grandi et al. (2010) which concluded that data from prospective investigations suggest an inverse association between vitamin D intake and CHD and recommended that more research is needed.

## 2.1. Vitamin D

Vitamin D is a fat-soluble vitamin obtained endogenously through the skin and is induced by ultra violet radiation (Figure 1).

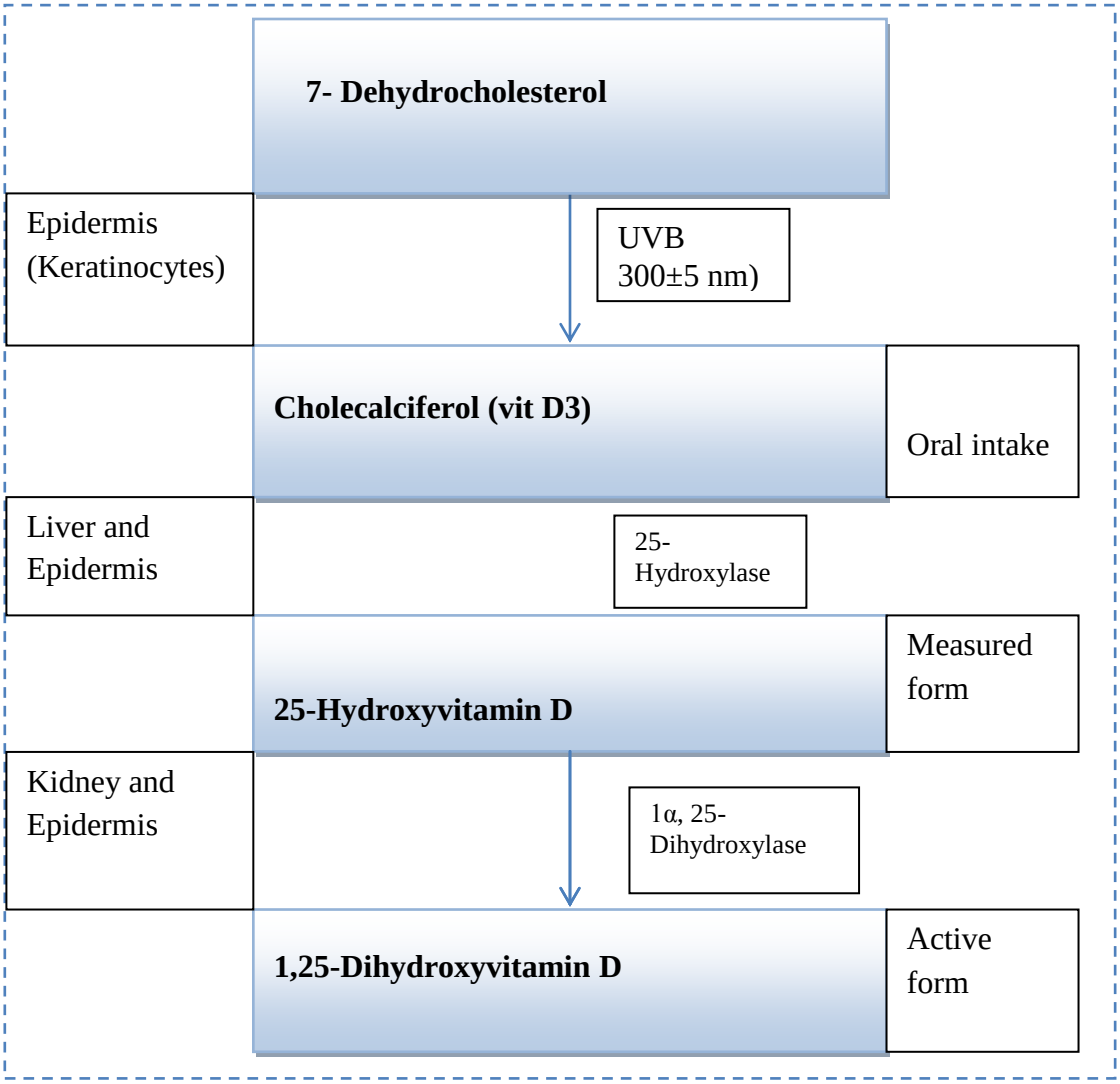


Figure 1:Pathway of vitamin D synthesis in the body (LoPiccolo & Lim 2010).

Ultraviolet B at a wavelength of 300±5 nm converts 7-dehydrocholesterol (pro-vitamin $D_3$) into pre-vitamin $D_3$ in the basal and suprabasal layers of the epidermis. Pre-vitamin $D_3$ then undergoes

non-enzymatic isomerization to form cholecalciferol, known as vitamin $D_3$. Cholecalciferol enters the circulation and is transported by vitamin D binding protein to the liver, where hydroxylation by 25-hydroxylase to 25(OH)D occurs. In the end 25(OH)D is transported by vitamin D binding protein to the kidney where hydroxylation by 1α-hydroxylase to 1, 25-dihydroxyvitamin D [1α, $25(OH)_2D$] occurs, yielding the hormonally active form of vitamin D (LoPiccolo & Lim 2010).

Vitamins $D_2$ (ergocalciferol) and $D_3$ are also widely available in the form of over-the-counter dietary supplements. Vitamin D reduces CHD risk by inhibiting vascular smooth muscle proliferation, regulating blood pressure and glucose metabolism and reducing inflammation (Manson 2010). Studies have suggested that vitamin $D_2$ is inferior to vitamin $D_3$ in raising serum 25(OH) D, and may even suppress the endogenous formation of 25(OH )D and $1\alpha,25(OH)_2D$ (LoPiccolo & Lim 2010).

According to the Institute of Medicine (IOM) in the United States the estimated average requirement per day for vitamin D in 2010 was 400 IU/day for all ages. The recommended dietary allowance and upper level intake in children ranges from 600-2500 IU/day (1 to 3 years) and 600–3000 IU/day (4 to 8 years), respectively (Institute of Medicine 2010).

The rest of the population aged from 9 to 70 have a recommendation of 600 IU/day as the recommended dietary allowance (RDA) and an upper tolerable level intake of 4000 IU/ day (Institute of Medicine 2010). There has been no consensus on the cut points for vitamin D deficiency and its definition has not been well defined. The current International Osteoporosis Foundation (IOF) guidelines define it as having serum 25(OH)D of less than 25 nmol/L (McGreevy & Williams 2011).

A vitamin D concentration of 50 nmol/L is considered a normal/ sufficient level of circulating 25(OH)D (Holick 2005)**.** Wallis et al. (2008) mentions that a 25(OH)D level of >75 nmol/L is sufficient and that the measurement of parathyroid hormone (PTH) alone is not a reliable measure of vitamin D adequacy especially in patients with chronic renal failure or calcium insufficiency.

Dietary sources of vitamin D include oily fish such as (salmon, mackerel, and herring ), egg yolk and some mushrooms. Cod liver oil that is extracted from fish is also a good source of the vitamin. In the United States milk, some juices, some breads, yoghurts, cereals, chocolate mixes and cheese

are fortified with vitamin D. There are also multivitamins available in various amounts 400 to 50000 IU (LoPiccolo & Lim 2010, Holick & Chen 2008).

In Finland vitamin D sources are mainly from fish, fortified dairy products and margarine (Pietinen et al. 2010). The fortification of fat spreads with vitamin D in Finland started in the 1950s and fortification of milk products began in 2003. This has greatly increased vitamin D intake but surveys show that the intake are still inadequate in both men and women (Pietinen et al. 2010).

### 2.1.1. Risks of vitamin D deficiency

Vitamin D deficiency is common in the northern latitude. This could be attributed to the low amounts of UVB light during winter. A British study with 7437 participants, found that half of the participants had 25(OH)D concentration of below 40 nmol/L during winter and spring (McGreevy & Williams 2011). A sunscreen with a sun protection of 15 absorbs incident UVB radiation and could reduce vitamin D3 synthesis by 99% (McGreevy & Williams 2011)**.** Aging is also associated with decreased vitamin D3 level due to the reduced cutaneous production of vitamin D (McGreevy & Williams 2011). Members of some religions that cover their body, and also women and children in geographical areas with long winters, institutionalized persons, and HIV infection drugs that increase the catabolism of 25(OH)D through induction of CYP450 system also increase the risk of vitamin D deficiency (McGreevy & Williams 2011).

Vitamin D deficiency could lead to deranged metabolism in the body, causing conditions such as high blood pressure and adult onset diabetes mellitus (Holick & Chen 2008), as shown in Figure 2.

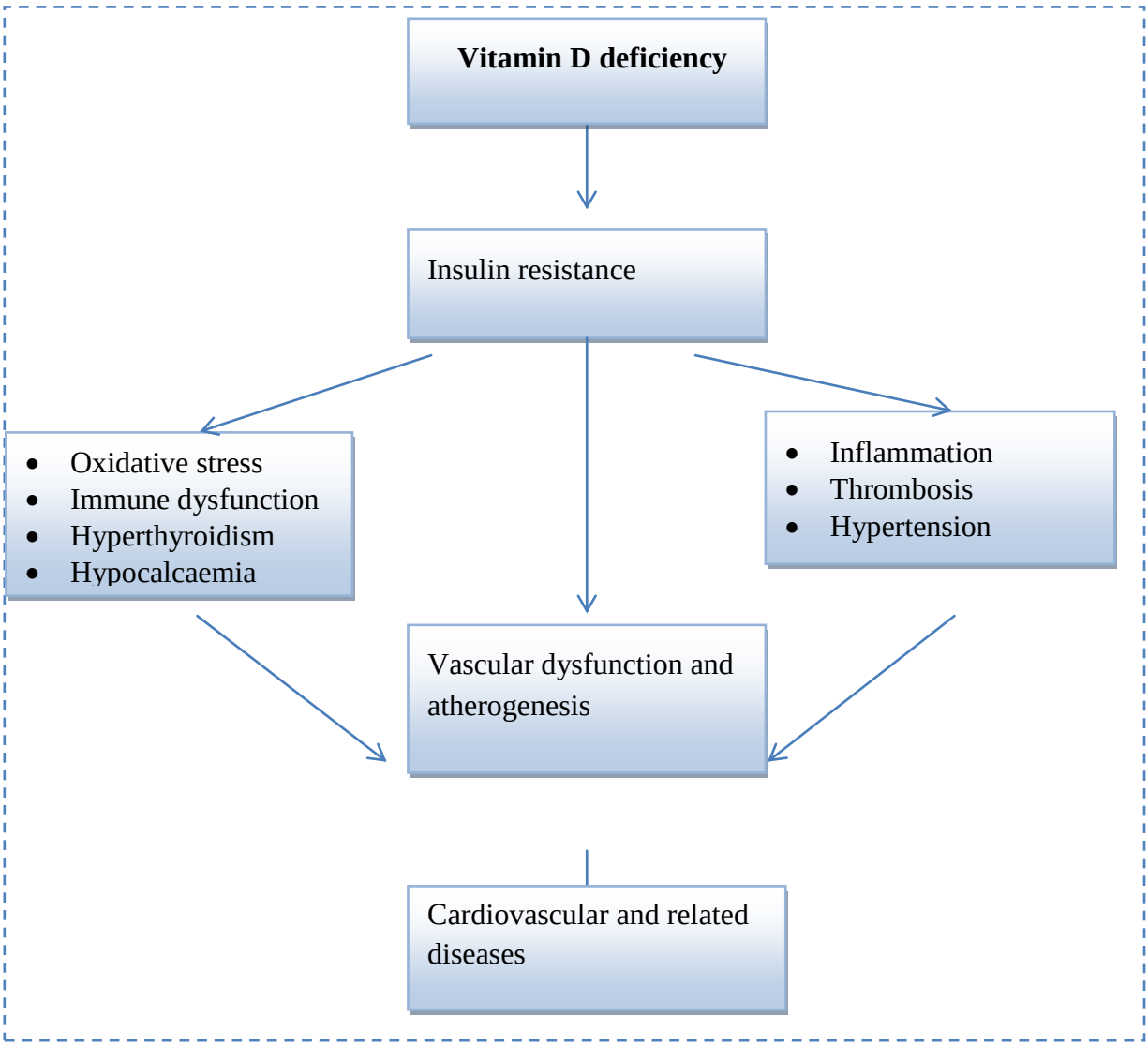


Figure 2: Pathways through which vitamin D leads to CHD risk (Artaza et al. 2009).

Vitamin D deficiency could lead to oxidative stress, immune dysfunction, hyperthyroidism, hypocalcaemia, inflammation, thrombosis and hypertension. These complications could lead to vascular dysfunction and atherogenesis and consequently CHD (Artaza et al. 2009).

An analysis to investigate prevalence of vitamin D deficiency and the relation of Vitamin D intake to prevalent and incident CHD risk factors and disease showed that vitamin D deficiency was associated with CHD (Anderson et al. 2010).

The continued worsening trend of nutritional insufficiency and the improving knowledge of the non hormonal actions of vitamin D and its metabolites have increased the interest in the synthesis, metabolism, and action of vitamin D. Vitamin D deficiency has continued to be linked to HBP, MI, stroke, diabetes, congestive heart failure, peripheral vascular disease, atherosclerosis and endothelial dysfunction (McGreevy & Williams 2011, Gouni-Berthold et al. 2009, Michos & Melamed 2008). Similarly, a population study conducted in Israel supported the fact that low 25(OH)D intake is associated with CHD (Steinvil et al. 2011).

Table 1: Serum vitamin D and coronary heart disease risk, and dietary vitamin D and CHD mortality.

| **Name of study, author,** | **Type of study** | **Population type** | **Population size, n** | **Results** |
|---|---|---|---|---|
| Parathyroid hormone and vitamin D markers for cardiovascular and all cause mortality in heart failure by Schierbeck 2011 | Prospective follow cohort | Male and female HF outpatients were followed-up for 3½ years. | 148 | Vitamin D deficiency was prevalent in 43% of the population and vitamin D was independently associated HF. |
| Vitamin D status and the risk of cardiovascular disease death by Kilkkinen 2009 | Prospective cohort study. | Finnish adults | 6219 participants free of CHD at baseline with measured serum 25(OH)D intake, 27.1 years of follow up | There were 640 CHD deaths, with low vitamin D intake being associated with CHD events. |
| Vitamin D intake and risk of cardiovascular disease in US men and women by Sun Q 2011. | Prospective cohort study | American adults | 118,864 men free of CVD at baseline | 9886 cases of CHD was reported. A higher intake of vitamin D was associated with a lower incidence of CHD in men but not in women. |
| 25 hydroxyvitamin D and risk of myocardial infarction in men by Giovannucci 2008 | Prospective study. | Aged 40 to 75 free of CVD at baseline | 18225 men | After 10 year follow up 454 men developed CHD with low serum vitamin D concentration being associated with CHD. |
| Relationship with 25 hydroxyvitamin D with all cause and cardiovascular disease mortality in older community dwelling adults by Semba 2010 | Prospective cohort study. | men aged >= 65years | 1006 followed up for 6.5 years. | Individuals with low serum 25(OH)D were at a higher risk of all-cause and CHD mortality. |

| | | | | |
|---|---|---|---|---|
| Low serum hydroxyvitamin D levels are associated with increased mortality risk in a general population: the Tromso study by Hutchinson 2010 | Prospective cohort study. | Male and female | The study had 7161 participants from the Tromso study. | During a follow-up period of 11.7 years, 1359 (19.0%) participants died. Low serum 25(OH)D intake were associated with increased CHD mortality. |
| Association of serum 25 hydroxyvitavitmin D with the risk of death in a general older population in Finland by Virtanen 2011 | Prospective cohort study. | Men and women aged between 53 to 73 without CHD at the beginning of the study. | 552 men and 584 women | Low concentration of serum 25(OH)D was associated with CHD deaths. |
| Serum and dDietary vitamin D and cardiovascular disease risk in elderly men by Messenger 2011 | Prospective cohort study | Evaluating between serum and dietary vitamin D intake and incidence of CHD | 3094 men | There were 371 CHD cases, there was no association between vitamin D concentration deficiency compared to sufficiency with CHD incidence. |

### 2.2 Vitamin D and coronary heart disease risk

In recent years, there has been an increase in interest to evaluate other potential functions of vitamin D and particularly with its relation to CHDs. Few studies have demonstrated an increased risk for CHD death in individuals with vitamin D deficiency (Virtanen et al. 2011). Some experimental studies have suggested several CHD protective mechanisms such as anti-atherosclerotic, anti-inflammatory and direct cardio protective actions (Gouni-Berthold et al. 2009, Borges et al. 2011, Pilz et al. 2009).

On the contrary, other epidemiologic studies have found an inverse association between vitamin D deficiency and prevalence of CHDs as well as individual cardio-metabolic risk factors (Anagnostis et al. 2010). There has also been contrasting findings with other studies documenting that there exists no relationship between serum 25(OH)D and the incidence of CHD (Messenger et al. 2011).

In a study that prospectively analyzed large electronic medical records database to determine the prevalence of vitamin D deficiency and its intake to prevalent and incidence of CHD risk factors, the findings demonstrated that vitamin D intake were highly associated with CHD at ($p< 0.0001$) (Anderson et al. 2010). Conclusively vitamin D status can lead to CHD incidence whether CVD status of the participants is established at the beginning or at the end of the study. The database contained 41,504 patient records with at least one measurement of vitamin D intake.

Using data from the Health Professionals Follow up Study, Giovannucci (2008) concluded that low 25(OH)D concentrations were associated with an increased risk of CHD (Table 1). Similarly, previous cross-sectional studies have reported that vitamin D deficiency is associated with increased risk of CVD including hypertension, HF and IHD (Judd & Tangpricha 2009).

#### 2.2.1 Vitamin D and blood pressure

Hypertension is a growing health concern and is postulated to affect 1.6 billion people worldwide by the year 2025. A review concluded that there is evidence that supports the association of vitamin D and blood pressure (Martini & Wood 2008). Another review also showed that vitamin D helps in the treatment of hypertension (Wallis et al. 2008). The result showed that there was a fall in diastolic and systolic blood pressure after exposure to sunlight 3 times a week daily for 6 weeks (Wallis et al. 2008).

Wang et al. (2008) investigated the association between intake of dairy products, calcium and vitamin D and the occurrence of hypertension in 28,886 US women aged 45 years and above. The results of the study showed that there is an association between dietary vitamin D and hypertension.

A similar study of two prospective cohort studies with 613 men from Health Professionals Follow up Study and 1198 women from the Nurses' Health Study investigating the association between plasma 25(OH) D and risk of incident hypertension concluded that there was an inverse relation between 25(OH) D and risk of incident hypertension (Forman et al. 2007).

### 2.2.2 Vitamin D and parathyroid hormone

Individuals with excessively high levels of PTH that accompany primary or secondary hyperparathyroidism, and patients with end stage renal disease, have increased the risk of CHD mortality(Hedback & Oden 1998, Nilsson et al. 2002).

There are few reports about PTH levels and risk of CHD mortality in older adults without kidney disease. One study reported an association between higher PTH levels and increased risk of CHD mortality among institutionalized older adults who did not have primary hyperparathyroidism. The study had 842 subjects (82 men and 660 women) and after a follow up period of 11months 345 died (Sambrook et al. 2004). Another study demonstrated an increased risk of all-cause and CHD mortality with higher PTH levels among 978 men in the community (Hagstrom et al. 2009).

## 2.3 Vitamin D supplements and coronary heart disease

Randomized controlled trials with vitamin D supplementation have found some effect on CHD risk reduction. A study that randomized British men with vitamin D3 100,000 IU/day given every four months for over 5 years, showed that there was a reduction in CHD events and mortality (Manson 2010). Women's Health Initiative study with 36,282 post-menopausal women participants aged 50 to 79 were tested with vitamin D3 (400 IU/day) for a seven year period. The study revealed that there was no increase or decrease in CHD mortality with vitamin D supplementation (Hsia et al. 2007).

## 2.4 Serum Vitamin D level and coronary heart disease mortality

Currently there are ongoing investigations as to whether there exists a relation between serum vitamin D level and CHD mortality in patients with heart conditions (Judd & Tangpricha 2009). However, a little evidence demonstrating the association is available (Zittermann et al. 2009). Accumulating

evidence from several non-randomized studies also indicate that deficient 25(OH)D concentrations are associated with excess CHD mortality in the general population (Zittermann et al. 2009).

A study investigating whether low serum 25(OH)D concentrations are associated with an increased CHD mortality concluded that a positive relationship exists. Hutchinson and colleagues (2010) similarly found an association between serum 25(OH)D concentrations and CHD mortality. The study included both male and female participants hence it is possible to generalize the findings to a wider population (Hutchinson et al. 2010). Another study showed that, vitamin D deficiency was prevalent in 43% of the population and concluded that vitamin D was independently associated with CHD mortality (Schierbeck et al. 2011) ( Table 1).

Contrary to the above findings, other studies have found that both low and high serum 25(OH)D should not be encouraged. This was evident in the Uppsala Longitudinal Study investigating the association between plasma 25(OH)D and mortality with a sample of 1194 adults. Approximately 50% higher total mortality rate was observed among men in the lowest 10% and the highest 5% of plasma 25(OH)D concentrations compared with intermediate concentrations. The conclusion was that both high and low concentrations of plasma 25(OH)D are associated with elevated risks of CHD mortality (Michaelsson et al. 2010).

# 3 AIM OF THE STUDY

The main aim of this study was to evaluate the association between dietary vitamin D intake and risk of CHD mortality in men and women with established CHD.
This study also investigates the association between vitamin D intake and CVD and total mortality.

HYPOTHESIS

There exists an association between sufficient vitamin D intake with reduced CHD, CVD and total mortality.

# 4. SUBJECTS

Our study evaluates participants from the European Action on Secondary Prevention through Intervention to Reduce Events (EUROASPIRE) study. This is a prospective cohort study with subjects having clinically established CHD, and admitted at the Kuopio University Hospital. The follow up period was 5 years (Erkkilä et al. 2003).

The Finnish cohort of the EUROASPIRE study comprised 415 patients (285 men, 130 women) with clinically established CHD, between 1991–1994 and who were aged <71 years at the time of hospital admission. Discharge lists, cardiac surgery and coronary angiography registers were used to retrospectively categorize patients into four categories (Erkkilä et al. 2003).

1. Patients going through their first elective or emergency coronary artery bypass grafting (CABG).
2. Those having their first elective or emergency percutaneous transluminal coronary angioplasty (PTCA) but with no previous CABG.
3. Those having their first or recurrent acute myocardial infarction (AMI) but with no CABG or PTCA.
4. Those admitted with known symptoms of acute myocardial ischemia (AMIS) but with no confirmation of AMI and with no record of CABG, PTCA, or AMI.

The researchers identified 125 patients with CABG, PTCA, and AMIS and 156 with AMI. The baseline examination was conducted in 1995.

The following number of patients from each category participated in the study: CABG, 109; PTCA, 106; AMI, 101; and AMIS, 99; with an overall participation rate of 82%, a total of 415 patients were included in the study. The Finnish center was the only one of the EUROASPIRE centers to carry out detailed dietary studies and measurements. All patients gave their approved consent to be part of this study and the study was approved by the Ethics Committee of the University of Kuopio (Erkkilä et al. 2003).

# 5. METHODS

## 5.1 Interview and examination

Structured questionnaires were used to conduct patient interviews with information on demographics, including years of schooling; smoking habits; and use of drugs. Measurements taken included, weight, height, waist circumference, hip circumference and blood pressure (BP). Weight was measured while the subjects wore light clothing with no shoes. Body mass index (BMI) was calculated as weight (kg)/height ($m^2$) and waist circumference was measured midway between the lower rib margin and the iliac crest. Hip circumference was measured at the point yielding the maximum circumference over the buttocks (Erkkilä et al. 2003).

An automatic digital sphygmomanometer (Takeda UA 731; A&D Co.Ltd, Abingdon, United Kingdom) was used to measure BP while the subjects were in a sitting position and after they had rested for 5 min. Non smoking status was validated by performing a breath carbon monoxide measurement (≤ 10 parts per million) (Bedfont Scientific EC 50,Sittingbourne, United Kingdom). The diagnosis of diabetes was based on previous diagnosis by a physician or if their fasting plasma glucose concentration was ≥ 7 mmol/l (Erkkilä et al. 2003).

## 5.2 Food records

A 4-day food record (3 weekdays and 1weekend day) was completed at home by the patients and they estimated the amounts of foods consumed using portion sizes listed in a booklet. The food records were returned during the interview, and all records were checked by a clinical nutritionist and missing information was completed if necessary. MICRO-NUTRICA dietary analysis program (version 2.0; Finnish Social Insurance Institution, Turku, Finland), was used to calculate nutrient intakes (Erkkilä et al. 2003).

## 5.3 Laboratory measurements

Collection of blood samples was done between 0800 and 1000 after patients had fasted for 12 hours. Serum lipids, lipoproteins, low density lipoprotein (LDL) and high density lipoprotein (HDL) were then analyzed. The serum samples were stored at -70 degrees until they were ready for analysis while the fresh serum was used to measure the serum total and lipoprotein lipids. Ultracentrifugation for 18h at 4 degrees, 144 000 by g, and density of 1.006 kg/L to remove VLDL, then dextran sulfate-magnesium chloride was used to precipitate LDL from the fluid (Penttila et al. 1981). Finally, HDL

was analyzed from the remaining fluid. Calculation of LDL cholesterol was done by subtracting the amount of HDL from the fluid containing HDL and LDL cholesterol (Erkkilä et al. 2003).

Commercial kits (kits 237574 and 701904; Boehringer GmbH, Mannheim, Germany) and a Kone Specific Clinical Analyzer (Kone Ltd, Espoo, Finland) were used to analyze cholesterol in the whole serum and in separated lipoproteins and serum triacylglycerols. Serum lipids were analyzed using standardized enzymatic methods. Amperometric enzymatic method (GlucoseAuto & Stat GA 110 analyzer; Daiichi Co, Kyoto, Japan) was used to analyze plasma glucose (Erkkilä et al. 2003).

### 5.4 Endpoint ascertainment

The date of the earliest event or the end of the follow-up period (30 April 2001 for deaths and 31 December 2000 for hospitalizations) was the censoring date. The endpoints included deaths from all causes, CVD and CHD.

CHD deaths included codes 120-125 from the International Classification of Diseases, $10^{th}$ version, and CVD deaths included codes 120-28, 160-69, G45 and G46. Computer linkage of Finnish social security numbers to the national death register (Statistics Finland, Helsinki) was used to ascertain deaths and to retrieve copies of death certificates. The national hospital discharge registers of the National Research and Development Centre for Welfare and Health on the basis of social security numbers was used to obtain data on AMIs, strokes, and revascularization procedures, medical records were also obtained (Erkkilä et al. 2003).

# 6. STATISTICAL ANALYSIS

Statistical analysis was done using SPSS for WINDOWS version 17. Test for normality was done using the Kolmogorov – Smirnov test. Frequencies, percentages, means and standard deviations of the various variables were analyzed to describe the data.

Chi- square test was used to explore the relationship between categorical variables. The difference in baseline characteristics was analyzed using non- parametric tests. One way analysis of variance (ANOVA) was used to compare means among groups.

Vitamin D intake was divided into three categories of less than 5 µg/day, 5 µg/day to 7 µg/day and greater than 7 µg/day. Education was divided in to those who studied for less than 8 years, 9–11 years and more than 11 years. Elevated BP was defined as systolic blood pressure (SBP) of over 140mmHg and diastolic blood pressure more than 90mmHg. Current smokers, diabetes, the use of beta blockers, lipid lowering drugs, aspirin and other anti-platelet drugs, ACE inhibitors and family history of CHD were all categorized as yes or no.

The Cox proportional hazard model was used to assess relative risks of different endpoints. Vitamin D intake was entered in the model as a categorical variable after it was classified in three groups.

The model was first adjusted for age and sex only. The second model was then adjusted for sex, age, diagnostic category (CABG or PTCA compared with AMI or AMIS), total serum cholesterol concentration, triglycerides concentration, BMI, diabetes mellitus, education and energy intake. P value of less than 0.05 was considered statistically significant.

# 7. RESULTS

## 7.1 Baseline characteristics

The participants had a mean age of 60 years, with more than half being men (66.9%). Most of the participants at baseline were overweight, with a mean waist hip ratio of 0.93. The mean BMI was 28.2 $kg/m^2$, 13.2% of the participants had diabetes and 81.9% had a family history of CHD at baseline (Table 2). During the follow up period, there were a total of 34 deaths with of which 16 were due to CHD. The mean energy intake was 1731kcal/day. The mean vitamin D intake was 5.3µg/day and a maximum of 24µg/day.

Other baseline characteristics of the study participants are shown in table 2.

Table 2. Baseline characteristics of the CHD patients (n = 415)

| Variables | | Mean or n (%) | ±SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Age(years) | | 60.9 | 8.41 | 25.0 | 73.9 |
| Sex | Male | 66.9% | | | |
| | Female | 33.1% | | | |
| Body mass index (kg/m²) | | 28.2 | 4.0 | 16.7 | 45.5 |
| Waist to hip ratio | | 0.93 | 0.07 | 0.72 | 1.09 |
| Years of formal education | <8 years | 270 ( 65.1 ) | | | |
| | 9- 11 years | 92 ( 22.2 ) | | | |
| | ≥ 12 years | 53 ( 12.8 ) | | | |
| Current smoker | | 51 (20.1%) | | | |
| Smoking ( years) | | 23.4 | 11.8 | 0 | 60.00 |
| Systolic blood pressure ( mmHg ) | | 141 | 22 | 82 | 204 |
| Diastolic blood pressure ( mmHg) | | 82 | 12 | 44 | 140 |
| Vitamin D intake (mg/day) | | 5.3 | 3.9 | 0.4 | 24.3 |
| Energy(kcal/day ) | | 1731 | 511 | 635 | 3410 |
| | | LIPIDS | | | |
| Serum total cholesterol (mmol/l) | | 6.12 | 1.18 | 2.13 | 11.96 |
| LDL cholesterol (mmol/l) | | 4.25 | 1.04 | 1.28 | 10.83 |
| HDL cholesterol(mmol/l) | | 1.22 | 0.29 | 0.47 | 2.77 |
| Triglycerides(mmol/l) | | 1.94 | 1.50 | 0.60 | 23.12 |
| | | DIAGNOSES AT BASELINE | | | |
| CABG | | 109 ( 26.2% ) | | | |
| PTCA | | 106 ( 25.5% ) | | | |
| AMI | | 101( 24.3% ) | | | |
| AMIS | | 99 ( 23.8% ) | | | |
| Diabetes diagnosed or plasma glucose ≥ 7mmol/l | | 70 (13.2%) | | | |
| Family history of CHD | | 339 (81.9%) | | | |
| | | USE OF MEDICATION | | | |
| Lipid lowering drugs | | 59 (11.1%) | | | |
| ACE inhibitors | | 72 (17.3%) | | | |
| Beta blockers | | 323 (77.8%) | | | |
| Aspirin and other anti-platelet | | 341 (82.2%) | | | |
| | | EVENTS DURING FOLLOW UP | | | |
| CHD deaths | | 16 (47.05%) | | | |
| CVD deaths | | 18 (52.94%) | | | |

LDL (low density lipoprotein), HDL (high density lipoprotein ), CABG ( coronary artery bypass grafting ),PTCA (percutaneous transluminal coronary angioplasty ),AMI (acute myocardial infarction), AMIS (acute myocardial ischemia), CHD (coronary heart disease).

### 7.2 Characteristics of CHD patients in vitamin D groups

After categorizing CHD patients according to amount of vitamin D intake per day, it was observed that a majority consumed less than 5µg/day in both genders. Similarly, it was noted that regardless of the years of education, most of the participants consumed less than 5µg/day. Participants who had chronic illnesses such as diabetes and hypertension were also noted to consume insufficient amount of vitamin D. It was also noted that 69.4% of the patients who were current smokers had a low vitamin D intake of less than 5µg/day and 55.1% had a family history of CHD.

Details of other CHD patients' characteristics are shown in table 3.

Table 3. Characteristics of the CHD patients in vitamin D groups.

| **Categorical variables** | **< 5 µg/day n= 232** | **5- 7 µg/day n = 83** | **> 7 µg/day n = 86** |
|---|---|---|---|
| Sex | | | |
| Men | 51.6% | 25.1% | 23.3% |
| Women | 72.4% | 10.6% | 17.1% |
| Education | | | |
| <8yrs | 57.6% | 19.5% | 22.9% |
| 9- 11 | 65.9% | 14.8% | 19.3% |
| >12 | 45.1% | 37.3% | 17.6% |
| Current Smoking | 69.4% | 18.4% | 12.2% |
| Diabetes | 65.7% | 20.9% | 13.4% |
| High blood pressure | 56.4% | 23.3% | 20.3% |
| Beta blockers | 56.4% | 22% | 21.7% |
| Lipid lowering drugs | 54.7% | 24.8% | 20.5% |
| Aspirin and platelet drugs | 57.2% | 20.7% | 22.2% |
| ACE inhibitors | 49.3% | 24.6% | 26.1% |
| Family History of CHD | 55.1% | 24.5% | 20.4% |
| Age (years) | 60.6 ± 8.7 | 60.5 ± 8 | 61.7 ± 8.2 |
| Body mass index (kg/m²) | 28.3 ± 4.3 | 27.8 ± 3.5 | 28.2 ± 3.7 |
| Waist to hip ratio | 0.93 ± 0.08 | 0.95 ± 0.06 | 0.93 ± 0.07 |
| Waist measured (cms) | 92 ± 10 | 93 ± 9 | 93 ± 9 |
| Total cholesterol (mmol/l) | 6.16 ± 1.18 | 6.01 ± 1.2 | 6 .06 ± 1.09 |
| LDL cholesterol (mmol/l) | 4.25 ± 1.08 | 4.20 ±1.03 | 4.30 ± 0.98 |
| HDL cholesterol (mmol/l) | 1.23 ± 0.29 | 1.20 ±0.30 | 1.25 ± 0.28 |
| Triglycerides (mmol/l) | 2.0 5 ± 1.78 | 1.20 ±1.14 | 1.67 ± 0.85 |
| Systolic blood pressure(mmHg) | 141.8 ± 22.7 | 138.9 ± 20.0 | 140.1 ± 23.5 |
| Diastolic blood pressure(mmHg) | 82.8 ± 12.8 | 80.8 ±11.1 | 81.9 ± 11.9 |

All values are mean SD or percentages

CHD (coronary heart disease), CVD (cardiovascular diseases).

LDL (low density lipoprotein), HDL (high density lipoprotein)

### 7.3 Vitamin D intake and risk of death

The first cox model was adjusted for age and sex for the all endpoints (total death CVD death and CHD death) with continuous vitamin D intake as exposure. The results show that there is no association between vitamin D intake and CHD death with RR 1.10 (95% confidence interval (CI) 1.04-1.17,p= 0.677) , CVD death 1.07( 95% CI 1.02-1.12, p=0.578), and total death RR 1.05( 95% 1.00-1.10, p=0.266 (Table 4). The association remained insignificant after further adjustment for known confounders such as total serum cholesterol concentration, triglycerides concentration, BMI, diabetes mellitus, education and energy intake and diagnostic category (CABG or PTCA compared with AMI or AMIS).

Table 4. Risk of death (95% confidence interval) with vitamin D intake adjusted for age and sex.

| Death | Risk ratio( 95% Confidence Interval) | P value |
|---|---|---|
| CHD death | 1.10(1.04, 1.17) [16] | 0.677 |
| CVD death | 1.07(1.02,1.12) [19] | 0.578 |
| Total death | 1.05(1.00, 1.10) [34] | 0.266 |

[ ] Number of deaths.

There was no trend in hazard ratios across different groups of vitamin D intake for CHD mortality, CVD death and total deaths (p for trend across groups: 0.30, 0.74 and 0.89 respectively). After adjustment for age, gender and other confounders, those in the dietary vitamin D group of 5-7 µg/day had RR of 1.27 [95% (CI): 0.52,3.12) of total death, compared to those in the lowest group (< 5 µg/day ) (p for trend across groups 0.30). After re-analyzing vitamin D intake in two groups (≤ 7 µg/day versus >7 µg/day) and all the endpoints, the results remained non significant. Detailed results are shown in table 5.

Table 5. Risk of death (95% confidence interval) with vitamin D intake in two and three categories.

| **Death** | **< 5 µg/day** | **5 - 7 µg/day** | **>7 µg/day** | **P for trend** | **Vitamin D ≤ 7ug/day vs >7 ug/day** |
|---|---|---|---|---|---|
| CHD death | 1 [8] | 0.97 ( 0.41, 2.29 ) [2] | 0.67 (0.23, 1.98 ) [6] | 0.89 | 1.46 (0.70, 3.06 ) |
| CVD death | 1 [11] | 1.06( 0.50, 2.25 ) [2] | 0.59(0.21, 1.63 ) [6] | 0.74 | 1.51 ( 0.79, 2.89 ) |
| Total death | 1 [21] | 1.27(0.52,3.12 ) [6] | 0.98(0.32,2.94 ) [7] | 0.30 | 1.37( 0.67, 2.79 ) |

[N] Number of deaths in each tertile.

# 8. DISCUSSION

## 8.1Vitamin D intake and mortality

In contrast to most previous studies, the findings from this study demonstrate that there is no association between vitamin D intake and CHD mortality, and all cause mortality among men and women with CHD in Kuopio Finland after adjustment for age and sex. There results remained non significant after further adjustment for previously known CHD risk factors.

These findings are consistent with some, but not all previous studies, providing evidence that there is no association between vitamin D and CHD mortality. A previous study by Messenger and colleagues (2011) had similar findings (Messenger et al. 2011) but had more CHD events observed. This could be attributed to the larger number of participants. The study had 3094 male participants, with a mean age of 76.4 years. Our study participants had a mean age of 60 years. A meta analysis found no association in two studies between vitamin D intake at baseline, and increased risk for cardiovascular and all-cause mortality (Grandi et al. 2010). Another study concluded that a low vitamin D intake is not associated with CHD mortality (Cawthon et al. 2010). The study had male participants aged at least 65 years, while our study had 415 male and female participants. There are also studies that have shown an association between vitamin D intake and CHD death. An example is a cohort study that provided evidence that inadequate amounts of vitamin D in the body may predict a higher risk of CHD death (Melamed et al. 2008**).** The relation between vitamin D and CHD is complex, and most studies including our study show no association between vitamin D intake and CHD mortality.

Low vitamin D intake was noted in women. Similar findings were demonstrated in a study by Hirani et al. (2010), where 25(OH)D were significantly higher in men than in women. It was suggested that men consumed vitamin D from diet, or had more exposure to the sun than women. This makes women more susceptible to low 25(OH)D level. No association between 25(OH)D and all-cause mortality was observed, although men with lower 25(OH)D tended to have worse health status than men with higher 25(OH)D levels; however, these differences tended to be small in magnitude (Cawthon et al. 2010). For example, a higher percentage of women consumed lower amounts of vitamin D, while a higher percentage of men ate the recommended amounts of vitamin D.

A low vitamin D intake has continued to be associated with non communicable conditions such as CHD especially among the older population (Hirani et al. 2010). In our study, however, additional adjustment for age did not affect the predictive value of vitamin D intake with regard to mortality. Generally vitamin D intake in Europe is low at 2-3 µg/day, especially amongst the elderly (Ovesen et al. 2003). The FINDIET study reported that the average vitamin D intake levels still fall below the recommended amounts of 400 IU/day according to IOM (Institute of Medicine 2010, Pietinen et al. 2010). There has been no consensus on the RDA of vitamin D in the European region, mainly due to its dual nature in supply to the body. The amount obtained endogenously varies, making it difficult to have accurate recommendations. The RDA in most European countries is 5 to 10 µg/day with higher amounts recommended in the elderly and infants. Other studies suggest vitamin D intake of 50 to 100 µg/day (Vieth et al. 2001). In this study the mean vitamin D intake was 5.2 µg/day, which is slightly above the minimum recommended. The current Finnish recommendations for adults are 7.5µg/day and for subjects over 60 years, a supplement providing 20µg/day is recommended (Finnish National Nutritional Council 2012).

As individuals age the concentration of 25(OH)D decreases due to the declining efficacy of the skin to produce vitamin D (Hirani et al. 2010, MacLaughlin & Holick 1985). This has been associated with immobility, low use of supplements and other age related diseases. The elderly spend less time exposed to sunlight especially those in institutions (Ovesen et al. 2003). The population in our study had a mean age of 60, making them susceptible to insufficient vitamin D intake. Increased BMI had increased odds of having a lower vitamin D status when compared to those with a lower BMI (Hirani et al. 2010). Low vitamin D intake could be a precursor to high BMI (Forman et al. 2007). A large population of the subjects in our study was overweight with a mean BMI of 28.2 kg/ $m^2$. Other studies have continued to suggest that individuals with increased BMIs have a low vitamin D status due to body fat (Hirani et al. 2010).

A study conducted in Finland revealed that vitamin D intake was low in 20% of hospitalized elderly men and 26% of hospitalized elderly women in Finland. The prevalence was lower in outpatients with 6% and 2% of men and women, respectively (Kauppinen-Makelin et al. 2001). Interestingly, low vitamin D levels are exhibited in healthy individuals during winter, with

25(OH)D concentrations reduced to <25 nmol/l in 26.2% of women and 28.6% of the men in Finland (Lamberg-Allardt et al. 2001).

In summary, and in contrast to many but not all previously reported studies, vitamin D intake was not associated with an increased risk of all-cause or cause-specific CHD mortality. Dietary vitamin D does not protect an individual with CHD from death. Randomized trials are needed to determine the causal relation between vitamin D and mortality.

# 9. STRENGTHS AND LIMITATIONS

The strengths of this study is that we had a representative sample of both men and women with a high participation rate, prospectively collected data and few losses during the follow up period. This then makes it possible to generalize the findings to the larger population in Kuopio, Finland, owing to the fact that CHD are common in this region of the country.

The use of nationwide mortality register to obtain information on CHD using death certificates has been shown to have a reasonably good validity. Information on CHD and its risk factors at baseline was from the physicians examination. However the population in the study was rather small compared to other studies which could have affected the results of this study.

The low number of deaths also reduces the power to find several associations. Data on several CHD risk factors facilitated adjustment for potential confounders but at the same time it can be speculated that individuals with chronic illnesses may have had reduced 25(OH)D concentrations because of limited exposure to the sun and inadequate dietary intake. This could suggest that vitamin D status is a non specific indicator of chronic illness rather than a direct contributor to the pathogenesis of disease. Vitamin D intake measurement was not repeated during follow up that might have led to different results. These findings can easily be generalized to the Finnish population with CHD and not to the wider population.

# 10. SUMMARY AND CONCLUSION

In this study, there is no association between vitamin D intake and CHD. However, other studies have shown a positive correlation. Therefore, gaps still exist in identifying the relationship between vitamin D intake and CHD.

The association between vitamin D intake and CHD mortality was non significant, after adjustment for age, sex and other confounders. Although there could be a possible causal link between vitamin D and CHD mortality, further investigations and studies from different populations is necessary for further clarification. The existence of a causal link between vitamin D intake and CHD mortality could be demonstrated in a randomized controlled clinical trial.

In conclusion, the intake of vitamin D, may not have significant CHD related public health implications. However, more research on the health benefits of vitamin D intake and CHD would be recommended. Due to the role of CHD as a leading cause of death in developed countries, identification of new CHD risk factors is fundamental.

# 11.REFERENCES

Anagnostis P, Athyros VG, Adamidou F, Florentin M & Karagiannis A. Vitamin D and cardiovascular disease: a novel agent for reducing cardiovascular risk? Current vascular pharmacology 2010;8:720-730.

Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, Lappe DL, Muhlestein JB & Intermountain Heart Collaborative (IHC) Study Group. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. The American Journal of Cardiology 2010;106:963-968.

Artaza JN, Mehrotra R & Norris KC. Vitamin D and the cardiovascular system. Clinical journal of the American Society of Nephrology : CJASN 2009;4:1515-1522.

Borges MC, Martini LA & Rogero MM. Current perspectives on vitamin D, immune system, and chronic diseases. Nutrition (Burbank, Los Angeles County, Calif.) 2011;27:399-404.

Cawthon PM, Parimi N, Barrett-Connor E, Laughlin GA, Ensrud KE, Hoffman AR, Shikany JM, Cauley JA, Lane NE, Bauer DC, Orwoll ES, Cummings SR & Osteoporotic Fractures in Men (MrOS) Research Group. Serum 25-hydroxyvitamin D, parathyroid hormone, and mortality in older men. The Journal of clinical endocrinology and metabolism 2010;95:4625-4634.

Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A, Erwin PJ, Hensrud DD, Murad MH & Montori VM. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. The Journal of clinical endocrinology and metabolism 2011;96:1931-1942.

Erkkilä AT, Lehto S, Pyorala K & Uusitupa MI. n-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. The American Journal of Clinical Nutrition 2003;78:65-71.

Finnish National Nutritional Council 2012, , *Nutrition is an important part of health*. Available: http://www.ravitsemusneuvottelukunta.fi/portal/en/ [2012, 10/26] .

Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC & Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 2007;49:1063-1069.

Gouni-Berthold I, Krone W & Berthold HK. Vitamin D and cardiovascular disease. Current vascular pharmacology 2009;7:414-422.

Grandi NC, Breitling LP & Brenner H. Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. Preventive medicine 2010;51:228-233.

Hagstrom E, Hellman P, Larsson TE, Ingelsson E, Berglund L, Sundstrom J, Melhus H, Held C, Lind L, Michaelsson K & Arnlov J. Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. Circulation 2009;119:2765-2771.

Hedback G & Oden A. Increased risk of death from primary hyperparathyroidism--an update. European journal of clinical investigation 1998;28:271-276.

Hirani V, Tull K, Ali A & Mindell J. Urgent action needed to improve vitamin D status among older people in England! Age and Ageing 2010;39:62-68.

Holick MF. Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease, type 1 diabetes, autoimmune diseases, and some cancers. Southern medical journal 2005;98:1024-1027.

Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American Journal of Clinical Nutrition 2004;80:1678S-88S.

Holick MF & Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. The American Journal of Clinical Nutrition 2008;87:1080S-6S.

Hsia J, Heiss G, Ren H, Allison M, Dolan NC, Greenland P, Heckbert SR, Johnson KC, Manson JE, Sidney S, Trevisan M & Women's Health Initiative Investigators. Calcium/vitamin D supplementation and cardiovascular events. Circulation 2007;115:846-854.

Hutchinson MS, Grimnes G, Joakimsen RM, Figenschau Y & Jorde R. Low serum 25-hydroxyvitamin D levels are associated with increased all-cause mortality risk in a general population: the Tromso study. European journal of endocrinology / European Federation of Endocrine Societies 2010;162:935-942.

Institute of Medicine 2010, 12/1-last update, *DRIs for Calcium and Vitamin D*. Available: http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/DRI-Values.aspx [2012, 4/20] .

Judd SE & Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. The American Journal of the Medical Sciences 2009;338:40-44.

Kauppinen-Makelin R, Tahtela R, Loyttyniemi E, Karkkainen J & Valimaki MJ. A high prevalence of hypovitaminosis D in Finnish medical in- and outpatients. Journal of internal medicine 2001;249:559-563.

Lamberg-Allardt CJ, Outila TA, Karkkainen MU, Rita HJ & Valsta LM. Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2001;16:2066-2073.

Leu M & Giovannucci E. Vitamin D: epidemiology of cardiovascular risks and events. Best practice & research.Clinical endocrinology & metabolism 2011;25:633-646.

LoPiccolo MC & Lim HW. Vitamin D in health and disease. Photodermatology, photoimmunology & photomedicine 2010;26:224-229.

MacLaughlin J & Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. The Journal of clinical investigation 1985;76:1536-1538.

Manson JE. Vitamin D and the heart: why we need large-scale clinical trials. Cleveland Clinic journal of medicine 2010;77:903-910.

Martini LA & Wood RJ. Vitamin D and blood pressure connection: update on epidemiologic, clinical, and mechanistic evidence. Nutrition reviews 2008;66:291-297.

McGreevy C & Williams D. New insights about vitamin D and cardiovascular disease: a narrative review. Annals of Internal Medicine 2011;155:820-826.

Melamed ML, Michos ED, Post W & Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Archives of Internal Medicine 2008;168:1629-1637.

Messenger W, Nielson CM, Li H, Beer T, Barrett-Connor E, Stone K & Shannon J. Serum and dietary vitamin D and cardiovascular disease risk in elderly men: A prospective cohort study. Nutrition, metabolism, and cardiovascular diseases : NMCD 2011;.

Michaelsson K, Baron JA, Snellman G, Gedeborg R, Byberg L, Sundstrom J, Berglund L, Arnlov J, Hellman P, Blomhoff R, Wolk A, Garmo H, Holmberg L & Melhus H. Plasma vitamin D and mortality in older men: a community-based prospective cohort study. The American Journal of Clinical Nutrition 2010;92:841-848.

Michos ED & Melamed ML. Vitamin D and cardiovascular disease risk. Current opinion in clinical nutrition and metabolic care 2008;11:7-12.

Nilsson IL, Yin L, Lundgren E, Rastad J & Ekbom A. Clinical presentation of primary hyperparathyroidism in Europe--nationwide cohort analysis on mortality from nonmalignant causes. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2002;17 Suppl 2:N68-74.

Ovesen L, Andersen R & Jakobsen J. Geographical differences in vitamin D status, with particular reference to European countries. The Proceedings of the Nutrition Society 2003;62:813-821.

Penttila IM, Voutilainen E, Laitinen P & Juutilainen P. Comparison of different analytical and precipitation methods for direct estimation of serum high-density lipoprotein cholesterol. Scandinavian Journal of Clinical and Laboratory Investigation 1981;41:353-360.

Pfeifer M, Begerow B, Minne HW, Nachtigall D & Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. The Journal of clinical endocrinology and metabolism 2001;86:1633-1637.

Pietinen P, Paturi M, Reinivuo H, Tapanainen H & Valsta LM. FINDIET 2007 Survey: energy and nutrient intakes. Public health nutrition 2010;13:920-924.

Pilz S, Dobnig H, Nijpels G, Heine RJ, Stehouwer CD, Snijder MB, van Dam RM & Dekker JM. Vitamin D and mortality in older men and women. Clinical endocrinology 2009;71:666-672.

Ross AC, Taylor CL, Yaktine AL & Del Valle HB. Dietary reference intakes for calcium and vitamin D. National Academy Press 2011.

Sambrook PN, Chen JS, March LM, Cameron ID, Cumming RG, Lord SR, Schwarz J & Seibel MJ. Serum parathyroid hormone is associated with increased mortality independent of 25-hydroxy vitamin d status, bone mass, and renal function in the frail and very old: a cohort study. The Journal of clinical endocrinology and metabolism 2004;89:5477-5481.

Schierbeck LL, Jensen TS, Bang U, Jensen G, Kober L & Jensen JE. Parathyroid hormone and vitamin D--markers for cardiovascular and all cause mortality in heart failure. European journal of heart failure 2011;13:626-632.

Scragg R, Khaw KT & Murphy S. Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults. European journal of clinical nutrition 1995;49:640-646.

Steinvil A, Leshem-Rubinow E, Berliner S, Justo D, Finn T, Ish-shalom M, Birati EY, Shalev V, Sheinberg B & Rogowski O. Vitamin D deficiency prevalence and cardiovascular risk in Israel. European journal of clinical investigation 2011;41:263-268.

Vieth R, Chan PC & MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. The American Journal of Clinical Nutrition 2001;73:288-294.

Virtanen JK, Nurmi T, Voutilainen S, Mursu J & Tuomainen TP. Association of serum 25-hydroxyvitamin D with the risk of death in a general older population in Finland. European journal of nutrition 2011;50:305-312.

Wallis DE, Penckofer S & Sizemore GW. The "sunshine deficit" and cardiovascular disease. Circulation 2008;118:1476-1485.

WHO 2011, September, 2011-last update, *Cardiovascular diseases Fact sheet N°317*. Available: http://www.who.int/mediacentre/factsheets/fs317/en/index.html [2011, September, 2011/29] .

Zittermann A, Gummert JF & Borgermann J. Vitamin D deficiency and mortality. Current opinion in clinical nutrition and metabolic care 2009;12:634-639.