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PUBLIC HEALTH IMPORTANCE OF VITAMIN D

RESULTS FROM THE POPULATION-BASED HEALTH 2000/2011 SURVEY

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ACADEMIC DISSERTATION

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ABSTRACT

Vitamin D is essential for skeletal health. In recent decades, vitamin D deficiency has also been suggested to be an independent risk factor for other harmful health outcomes. In the early 2000s, vitamin D status of the Finnish population was insufficient due to low dietary intake and limited exposure to sunlight in Finland especially during winter. Thus, during 2000s nutritional policy acts, including voluntary, systematic fortification of fluid milk products and fat spreads, were executed to improve the vitamin D status of the Finnish population.

The aims of the present study were to examine the vitamin D status of Finnish adults, its sociodemographic, lifestyle and metabolic health related determinants and the temporal change between the years 2000-2011. The study also aimed to examine whether low vitamin D status is associated with depression prevalence or predicts weight gain or an increase in waist circumference during the 11-year follow-up.

The study population consisted of participants aged 30 years and over from the nationally representative Health 2000 Survey (n=8028) and its follow-up, the Health 2011 Survey (n=7964). The Health 2000/2011 Survey included questionnaires, interviews and a comprehensive health examination including laboratory measurements. Serum 25-hydroxyvitamin D concentration (S-25(OH)D), a measure of vitamin D status, was analysed by radioimmunoassay in 2000 for 6134 (76%) participants and in 2011 by chemiluminescent immunoassay for 4051 (51%) participants. To improve the comparability of the methods, measurements were standardized according to the Vitamin D Standardization Program (VDSP) using liquid chromatography-tandem mass spectrometry.

Information on diet was assessed by a validated food frequency questionnaire (FFQ). Diagnosis of depression was based on a Composite International Diagnostic Interview (CIDI) and current depressive symptoms on the Beck Depression Inventory (BDI). Height, weight and waist circumference were measured in a health examination by trained nurses. Sociodemographic and lifestyle factors were determined by questionnaires and interviews.

Statistical analyses were based on linear and logistic regression models adjusted for potential confounding factors.

In 2000, more than half of the Finnish adult population had a vitamin D status under 50 nmol/L, which is generally considered to be insufficient. Remarkable improvement was seen during the 2000s leading to over 90% of adults having sufficient vitamin D status in 2011. The increase was mainly explained by the systematic vitamin D fortification policy and increased use of vitamin D supplements, but other factors, such as higher amount of ultraviolet B radiation in 2011, may have also contributed to improvement. In

2011, a sufficient vitamin D status was possible to reach without the use of supplements if vitamin D fortified milk products and fat spreads were consumed daily and fish at least twice a week. Further, the results showed that vitamin D status was positively associated with a healthy lifestyle measured with 5-item lifestyle index (components: body mass index, physical activity, smoking, alcohol consumption and diet).

In men, low vitamin D status was cross-sectionally associated with a higher prevalence of depressive disorder and predicted an increase in waist circumference during the 11-year follow-up but not weight gain. In women, vitamin D status was not associated with either depressive disorder and symptoms or increase in waist circumference and weight gain.

In conclusion, low vitamin D status as a potential public health concern in Finland has improved during the 2000s, indicating the success of nutritional policy acts. The results of the present study do not overall consistently support the hypothesis that low vitamin D status is an independent risk factor for depression or obesity. However, it is possible that low vitamin D status may be associated with a higher risk of abdominal obesity especially in men, but the association may also be due to residual confounding.

Keywords: Population Study, Vitamin D, Serum 25-hydroxyvitamin D, Depression, Obesity

TIIVISTELMÄ

D-vitamiini on välttämätön luuston terveydelle. Viime vuosikymmeninä on esitetty, että D-vitamiinilla saattaa olla myös muita terveyttä edistäviä vaikutuksia. Suomalaisväestön D-vitamiinitasot olivat matalia vielä 2000-luvun alussa, sillä D-vitamiinin saanti ravinnosta oli niukkaa ja altistuminen auringon valolle on Suomessa vähäistä erityisesti talvisin. Tämän vuoksi suomalaisväestön D-vitamiinitasoja pyrittiin parantamaan 2000-luvulla ravitsemuspoliittisin päätöksin, kuten suosittelemalla nestemäisten maitotuotteiden ja ravintorasvojen täydentämistä D-vitamiinilla.

Tämän tutkimuksen tavoitteena oli tutkia suomalaisen aikuisväestön D-vitamiinitasoja ja niissä tapahtuneita muutoksia vuosien 2000 ja 2011 välillä. Tutkimuksessa selvitettiin myös D-vitamiinitasoihin yhteydessä olevia sosiodemografisia tekijöitä sekä elintapoihin ja metaboliseen terveyteen liittyviä tekijöitä. Lisäksi tutkittiin, ovatko elimistön matalat D-vitamiinitasot yhteydessä masennuksen esiintyvyyteen poikkileikkausasetelmassa ja ennustavatko ne painonnousua tai vyötärönympäryksen kasvua 11 vuoden seurannan aikana.

Tutkimus perustui kansallisesti edustavien Terveys 2000 –tutkimuksen (n=8028) ja sen seurantatutkimuksen, Terveys 2011 –tutkimuksen (n=7964), 30 vuotta täyttäneiden otoksiin, joissa kerättiin terveystarkastusten, haastatteluiden ja kyselyiden avulla monipuolisesti tietoa terveydestä ja siihen yhteydessä olevista tekijöistä. Elimistön D-vitamiinitason mittarina käytettiin seerumin 25-hydroksi-D-vitamiinipitoisuutta (S-25(OH)D), joka määritettiin vuonna 2000 radioimmunomenetelmällä 6134 (76%) tutkittavalta ja vuonna 2011 kemiluminesenssi-immunomenetelmällä 4051 (51%) tutkittavalta. Vertailukelpoisuuden varmistamiseksi vuosien 2000 ja 2011 mittaustulokset standardoitiin käyttäen nestekromatografia-tandem-massaspektrometriaa.

Ruoankäyttöä ja ravintoaineiden saantia arvioitiin validoidun frekvenssityyppisen ruoankäyttökyselyn avulla (FFQ, food frequency questionnaire). Masennushäiriö määritettiin mielenterveyshaastattelun (CIDI, Composite International Diagnostic Interview) perusteella ja masennusoireet BDI-kyselyn (Beck Depression Inventory) avulla. Koulutetut tutkimushoitajat mittasivat pituuden, painon ja vyötärönympäryksen. Elintapoja ja sosiodemografisia tekijöitä selvitettiin kyselyjen ja haastatteluiden avulla.

Tilastolliset analyysit perustuivat lineaarisiin ja logistisiin regressiomalleihin, joissa huomioitiin mahdolliset sekoittavat tekijät.

Vuonna 2000 yli puolella suomalaisista D-vitamiinitaso oli alle 50 nmol/l, jota pidetään yleisesti riittämättömänä. Suomalaisten D-vitamiinitasot nousivat huomattavasti 2000-luvun aikana, ja vuonna 2011 D-vitamiinitaso oli riittävä yli 90 prosentilla väestöstä. D-vitamiinitasojen

nousu selittyy systemaattisella elintarvikkeiden D-vitamiinitäydentämisellä sekä lisääntyneellä D-vitamiinilisien käytöllä, mutta myös muut tekijät, kuten runsaampi ultravioletti-B-säteilyn määrä vuonna 2011, ovat saattaneet myötävaikuttaa nousuun. Vuonna 2011 riittävä D-vitamiinitaso oli mahdollista saavuttaa ilman D-vitamiinilisien käyttöä, mikäli ruokavalio sisälsi nestemäisiä maitotuotteita ja levitettäviä ravintorasvoja päivittäin sekä kalaa vähintään kaksi kertaa viikossa. Lisäksi elintapaindeksiin (osatekijät: painoindeksi, fyysinen aktiivisuus, tupakointi, alkoholin kulutus ja ruokavalio) perustuvat tulokset osoittivat, että terveellisiä elintapoja noudattavilla D-vitamiinitasot olivat keskimäärin suuremmat kuin niitä noudattamattomilla.

Miehillä matalat D-vitamiinitasot olivat yhteydessä suurempaan masennushäiriön esiintyvyyteen poikkileikkausasetelmassa ja ennustivat vyötärönympäryksen kasvua, mutta eivät painonnousua, 11 vuoden seurannan aikana. Naisilla D-vitamiinitasojen ei havaittu olevan yhteydessä masennushäirön tai masennusoireiden esiintyvyyteen eikä painonnousuun tai vyötärönympäryksen kasvuun 11 vuoden seurannan aikana.

Yhteenvetona voidaan todeta, että suomalaisten keskimääräiset D-vitamiinitasot ovat nousseet huomattavasti 2000-luvun aikana osoittaen ravitsemuspoliittisten päätösten olleen kansanterveyden näkökulmasta onnistuneita. Tutkimuksen tulokset eivät kuitenkaan yksiselitteisesti vahvista hypoteeseja siitä, että matala D-vitamiinitaso on yhteydessä masennuksen tai lihavuuden riskiin. On kuitenkin mahdollista, että matala D-vitamiinitaso saattaa lisätä vyötärölihavuuden riskiä erityisesti miehillä, mutta toisaalta yhteyden taustalla saattavat olla myös muut tekijät.

Avainsanat: Väestötutkimus, D-vitamiini, Seerumin 25-hydroksi-D-vitamiini, Masennus, Lihavuus

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications and some unpublished results from further analyses:

I. Jääskeläinen T, Knekt P, Marniemi J, Sares-Jäske L, Männistö S, Heliövaara M, Järvinen R. Vitamin D status is associated with sociodemographic factors, lifestyle and metabolic health. (2013) *Eur J Nutr;*52(2):513-525.

II. Jääskeläinen T*, Itkonen ST*, Lundqvist A, Erkkola M, Koskela T, Lakkala K, Dowling KG, Hull GL, Kröger H, Karppinen J, Kyllönen E, Härkänen T, Cashman KD, Männistö S, Lamberg-Allardt C. The positive impact of general vitamin D food fortification policy on vitamin D status in a representative adult Finnish population: evidence from an 11-y follow-up based on standardized 25-hydroxyvitamin D data. (2017) *Am J Clin Nutr*;105(6):1512-1520. (*equally contributed).

III. Jääskeläinen T, Knekt P, Suvisaari J, Männistö S, Partonen T, Sääksjärvi K, Kaartinen NE, Kanerva N, Lindfors O. Higher serum 25-hydroxyvitamin D concentrations are related to a reduced risk of depression. (2015) *Br J Nutr*;113(9):1418-1426.

IV. Jääskeläinen T, Männistö S, Härkänen T, Sääksjärvi K, Koskinen S, Lundqvist A. Does vitamin D status predict weight gain or increase in waist circumference? – Results from the Health 2000/2011 Survey (submitted).

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ABBREVIATIONS

$1,25(OH)_2D$	1α-25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AHEI	Alternate Healthy Eating Index
AusDiab	Australian Diabetes, Obesity and Lifestyle Study
BDI	Beck Depression Inventory
BIA	Bioimpedance Analysis
BMI	Body Mass Index
CaMOs	Canadian Multicentre Osteoporosis Study
CDC	Center for Disease Control and Prevention
CES-D	Centre for Epidemiologic Studies Depression scale
CI	Confidence Interval
CIDI	Composite International Diagnostic Interview
CLIA	Chemiluminescence-immunoassay
CYP27B1	25-hydroxyvitamin D-1α hydroxylase
DEGS	German Health Interview and Examination Survey for Adults
DSM-IV	the Diagnostic and Statistical Manual of Mental Disorders, 4th revised edition
ECLIA	Electrochemiluminescence-immunoassay
EFSA	The European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food Frequency Questionnaire
FHS	The Framingham Heart Study
GNHIES	German National Health Interview and Examination Survey
HDL	High Density Lipoprotein
HEALTH 2000	The Health 2000 Survey
HEALTH 2011	The Health 2011 Survey
HPFS	Health Professionals Follow-up Study

HPLC	High Performance Liquid Chromatography
HR	Hazard ratio
HUNT	The Nord-Trøndelag Health Study
InCHIANTI	Invecchiare Chianti, Aging in the Chianti Area
IOM	Institute of Medicine
IRAS	Insulin Resistance Atherosclerosis Family Study
IU	International Unit
LC–MS/MS	Liquid Chromatography Tandem Mass Spectrometry
mAHEI	modified Alternate Healthy Eating Index
NDNS	National Diet and Nutrition Survey
NFBC	Northern Finland Birth Cohort
NHANES	National Health and Nutrition Examination Survey
NHS	New Hoorn Study
NIST	National Institute of Standards and Technology
NORDEN	Nordic Council of Ministers
OR	Odds Ratio
PA	Physical Activity
P450	P450 enzymes
PHQ-9	Patient Health Questionnaire depression module
PTH	Parathormone
RCT	Randomized Controlled Trial
RIA	Radioimmunoassay
S-25(OH)D	Serum 25-hydroxyvitamin D
SACN	Scientific Advisory Committee on Nutrition
SCAN	the Schedules for Clinical Assessment in Neuropsychiatry
SCL	Symptom Check List
SMD	Standardised Mean Difference
SNPs	Single Nucleotide Polymorphisms
SOF	Study of Osteoporotic Fractures

SU.VI.MAX	SUpplémentation en VItamines et Minéraux AntioXydants
THL	National Institute for Health and Welfare
UK	United Kingdom
US	United States
USRT	US Radiologic Technologists Study
UVB	Ultraviolet B
VDR	Vitamin D Receptor
VDSP	Vitamin D Standardization Program
WC	Waist Circumference
WHO	World Health Organization

1 INTRODUCTION

Vitamin D is a fat-soluble nutrient which acts like a steroid hormone in the human body (DeLuca 2004). Vitamin D is essential for maintaining normal blood levels of calcium and phosphorus required for skeletal health. Globally, the most important source of vitamin D is its formation in the skin after exposure to sunlight (Holick 2003). Natural food sources of vitamin D are rare, mainly including fish, egg yolk and some mushrooms (Lamberg-Allardt 2006).

Vitamin D status is usually measured by analysing serum 25-hydroxyvitamin D concentration, which reflects both dietary intake and exposure to sunlight. Sufficient vitamin D status is still slightly controversial, but serum concentrations under 50 nmol/L are generally considered to be insufficient for bone health (Institute of Medicine Food and Nutrition Board 2011). In Finland, insufficient vitamin D status has been common in the past decades due to low dietary intake and the Finnish location at Northern latitudes leading to limited exposure to sunlight, especially in winter. In the beginning of the 2000s, insufficient vitamin D status was considered a potential public health concern in Finland (Lamberg-Allardt et al. 2001). Thus, to improve vitamin D status at the population level, the voluntary systematic fortification of fluid milk products and fat spreads was started in the beginning of 2003 (Ministry of Trade and Industry of Finland 2002). In 2010 the recommendations for fortification levels were doubled (National Nutrition Council 2010). The vitamin D status of the Finnish adult population and the effects of the food fortification policy has previously been studied based on small subpopulations (Lamberg-Allardt and Viljakainen 2006) or independent cross-sectional samples (Raulio et al. 2017). Thus, there is still a need for large-scale, prospective population-based surveys to examine the vitamin D status of Finnish adults and the effects of nutritional policy acts on it.

During the recent decades it has been proposed that vitamin D may also have non-skeletal health effects. Vitamin D deficiency has been suggested to be associated with an increased risk of cancers, autoimmune diseases, cardiovascular diseases and mental health problems, for example (Holick 2007). Low vitamin D status has also been linked to an increased risk of overweight and obesity, but the cause-effect directions are still unclear (Pourshahidi 2015). Despite the large number of studies focusing on possible non-skeletal health effects of vitamin D during recent decades, the epidemiological evidence is still mainly controversial (Lamberg-Allardt et al. 2013). For example, it is still unclear whether the associations between vitamin D status and health outcomes may be due to confounding. Although some of the factors associated with vitamin D status, for example physical activity (Hintzpeter et al. 2008; Miettinen et al. 2014; Larose et al. 2014), are

well-known, the whole picture is still unclear. It is important to reveal the determinants of vitamin D status based on large population-based samples so that potential confounding factors can be examined and carefully taken into account when studying the potential health effects of vitamin D.

The present study extends previous research by examining the vitamin D status of Finnish adults, its sociodemographic, lifestyle and metabolic determinants and the change in vitamin D status between the years 2000 and 2011 based on a large population-based sample. Further, the association of vitamin D status with two major public concerns in Finland, depression and obesity, will be examined.

2 REVIEW OF THE LITERATURE

2.1 PRODUCTION, METABOLISM AND PHYSIOLOGICAL EFFECTS OF VITAMIN D

2.1.1 PRODUCTION AND METABOLISM

Vitamin D is a fat-soluble, hormone-like vitamin which is either synthesised in the skin or obtained from diet or supplements (DeLuca 2004) (Figure 1). Vitamin D3, a natural form of vitamin D, is produced in the skin after exposure to UVB (ultraviolet B) radiation (Lehmann and Meurer 2010). In this photolytic process in the skin, 7-dehydrocholesterol, also called provitamin D3, in the epidermal and dermal cells in the skin absorbs UVB radiation, thus converting to previtamin D3. Previtamin D3 is transformed into vitamin D3 (cholecalciferol) upon nonenzymatic isomerisation induced by heat. Production of vitamin D in the skin is dependent on the amount and duration of exposure to UVB radiation, protection from sunlight, and the angle of the sunlight (zenith angle) which is dependent on the time of day, season and latitude. In Finland, the zenith angle in winter (November to February) is so oblique that the amount of UVB radiation reaching the Earth's surface is very limited and cutaneous production of vitamin D is very low.

Vitamin D_3 synthesised in the skin or obtained from diet and supplements is biologically inert (Lehmann and Meurer 2010) and requires two hydroxylations, first in the liver, converting to 25(OH)D (25-hydroxyvitamin D) and second in the kidneys to transform into to biologically active 1,25(OH)2D (1α-25-dihydroxyvitamin D) (Figure 1). The first reaction is catalysed by P450 enzymes. The second reaction is catalysed by CYP27B1 (25-hydroxyvitamin D-1α hydroxylase) and regulated by parathormone, calcium, phosphate, calcitonin, fibroblast growth factor and $1,25(OH)_2D$ itself.

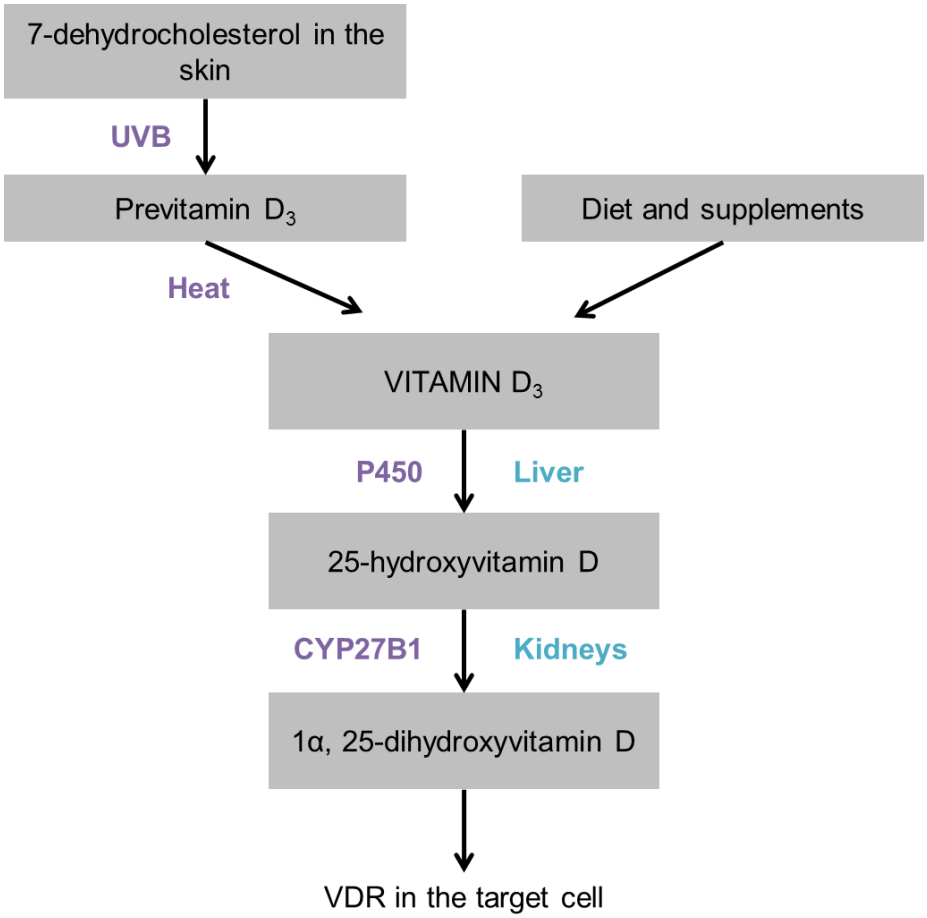

Figure 1. Metabolism of vitamin D3. Abbreviations: UVB Ultaviolet B radiation; P450 P450 enzymes; CYP27B1 25-hydroxyvitamin D-1α hydroxylase; VDR Vitamin D receptor

2.1.2 PHYSIOLOGICAL EFFECTS

When biologically active $1,25(OH)_2D$ enters the cell, it binds to the VDR (vitamin D receptor) (Figure 1) (Lehmann and Meurer 2010). The complex formed by $1,25(OH)_2D$ and VDR forms a heterodimer with a retinoid receptor. The heterodimer binds to a responsive gene followed by transcription, translation and protein synthesis, for example formation of calcium-binding proteins. The classic function of vitamin D is to maintain normal blood levels of calcium and phosphorus required for normal bone formation and mineralisation and the proper functioning of muscles and the nervous system as well as general cellular function in all cells of the body (DeLuca 2004). The active form of vitamin D ($1,25(OH)_2D$) stimulates 1) the

active transport of calcium and phosphate in the intestinal cell, 2) together with PTH (parathormone) bone resorption and calcium and phosphate mobilisation and, 3) together with PTH reabsorption of calcium in the distal renal tubule. Severe vitamin D deficiency leads to rickets and osteomalacia (Holick 2007). Vitamin D deficiency may also contribute the pathogenesis of osteoporosis by inducing PTH secretion resulting in higher bone turnover and increased resorption. In the elderly, vitamin D has been shown to reduce the risk of falling (Bischoff-Ferrari et al. 2009a) and non-vertebral fractures (Bischoff-Ferrari et al. 2009b). In high, long-term doses, however, vitamin D is toxic and may lead to hypercalcemia and hyperphosphataemia (Holick 2007).

2.2 DIETARY INTAKE OF VITAMIN D

2.2.1 FOOD SOURCES

Dietary vitamin D is obtained from natural dietary sources, from fortified food items and from vitamin-D-containing supplements (Lamberg-Allardt 2006). There are only a few good naturally rich dietary sources of vitamin D3 ie. fish and fish products as well as egg yolk (Table 1). Also meat contains a small amount of vitamin D3. In addition, wild mushrooms include vitamin D2.

Table 1. The examples of the natural dietary sources of vitamin D according to Fineli, National Food Composition Database in Finland (Reinivuo et al. 2010)

Food item	Vitamin D, µg/100 g
Fish	
Eel	25.6
Powan	22.1
Herring	20.5
Perch	15.2
Salmon, fillet	8.0
Tuna	7.2
Pike-perch	6.9
Salmon, whole	5.2
Rainbow trout, whole	5.1
Pike	2.1
Egg yolk	6.5
Egg	2.2
Meat and offal	
Liver, pork	1.1
Liver, beef	0.6
Chicken	0.7
Pork	0.4
Beef	0.2
Mushrooms	
Funnel chantarelle	15.4
Chantarelle	5.8
Porcini	2.6

The main dietary sources of vitamin D vary between countries, according to national dietary habits and food fortification policies. In the Nordic countries, fish and fortified fat spreads are important contributors to vitamin D intake (Lamberg-Allardt et al. 2013). In addition, in Norway and Iceland cod liver oil is a traditional source of vitamin D. In Finland and Sweden, fortified milk products are also an important source (see chapter 2.4.1), with fluid milk products contributing about 40% to vitamin D intake among younger adults and about 30% among older adults in Finland (Lamberg-Allardt et al. 2013; Helldan et al. 2013). The situation seems quite similar in other European countries, fat spreads being the main source in Holland, and fish in France as well as in Spain, for example (Spiro and Buttriss 2014). Among the UK (United Kingdom) adult population, however, despite the fact

that meat contains only a small amount of vitamin D, meat and meat products are the main sources of vitamin D followed by fat spreads and fish (Spiro and Buttriss 2014). In the US (United States) and Canada, fortified foods, mainly milk products, contribute over half of the dietary vitamin D intake (Calvo and Whiting 2013).

2.2.2 RECOMMENDATIONS

Recommendations for vitamin D intake given by selected authorities are shown in Table 2. In Finland, the recommended intake for working-aged adults was raised from 5.0 µg/day to 7.5 µg/day in 2005 and further to 10.0 µg/day in 2014, in line with the Nordic Nutrition Recommendations (Nordic Council of Ministers 2014; National Nutrition Council 2014). The recommendation for older adults (≥75 years) is higher, 20.0 µg/day. Within Europe, the recommended levels vary between 10.0 and 20.0 µg/day, and in North America the IOM (The North American Institute of Medicine) recommends 15.0 µg/day (Table 2). The IOM, EFSA (The European Food Safety Authority) and Nordic Council of Ministers have consistently indicated that the upper intake level for adults is 100 µg/day (Institute of Medicine Food and Nutrition Board 2011; European Food Safety Authority 2016; Nordic Council of Ministers 2014).

Table 2. Recommendations for vitamin D intake and upper intake for adult populations given by selected authorities.

Authority	Countries	Recommended intake, µg/day	Upper intake levels, µg/day
Nordic Council of Ministers (NORDEN) 2012	Denmark Finland Iceland Norway Sweden	10	100
Dutch Health Ministry 2012	Netherlands	10	
The UK Scientific Advisory Committee on Nutrition (SACN) 2016	UK	10	
The North American Institute of Medicine (IOM) 2011	Canada US	15[1]	100
The European Food Safety Authority (EFSA) 2016		15[2]	100
German Nutrition Society 2012	Austria German Switzerland	20[3]	

Abbreviations: US United States; UK United Kingdom

[1] Based on conditions of minimal sun exposure

[2] Dietary Reference Value

[3] While endogenous synthesis is lacking

2.2.3 INTAKE

In the large epidemiological studies, 24h recalls and food frequency questionnaires are widely used to estimate food intake (Biro et al. 2002). 24h recall has been suggested to be a good method to estimate population means and distributions whereas food frequency questionnaires are especially suitable for large population studies to estimate foods and drinks usually consumed and to rank individuals according to their food and nutrient intake. One major limitation for both, however, is that they are dependent on the respondent's memory.

Vitamin D intake in Europe has been examined by using standardized 24-h dietary recall in the EPIC (European Prospective Investigation into Cancer and Nutrition) study among adults aged 35-74 years from 10 countries (Freisling et al. 2010). Furthermore, vitamin D intake in Europe has been summarised in a systematic review (Spiro and Buttriss 2014). Both ended up with parallel conclusions: within Europe, vitamin D intake tends to be highest in the Nordic countries and lowest in Southern Europe. Based on the results from EPIC, the mean intakes were relatively low, 4.8 and 3.3 μg/day for men and women, respectively, but considerable variation between the countries was seen (Freisling et al. 2010). According to the systematic review (Spiro and Buttriss 2014), vitamin D intake varied in most Nordic countries, (e.g., Finland, Sweden and Norway) from 6-10 μg/day and in the other European countries (Denmark, Germany, Ireland, Italy, Portugal, the Netherlands) the mean intakes were between 3-5 μg/day, with very low (1.6 μg/day in men and 1.7 μg/day in women) intakes were reported in Spain. According to the FINDIET 2012 Survey (Helldan et al. 2013), the mean intake in Finland was 11 μg/day in men and 9 μg/day in women. As a comparison, based on data from the NHANES 2005-2006 (National Health and Nutrition Examination Survey), in the US mean vitamin intakes were about 5 and 4 μg/day in men and women, respectively (Bailey et al. 2010).

2.3 VITAMIN D STATUS

2.3.1 MEASUREMENT METHODS

Circulating 25(OH)D concentration is a widely accepted metabolite to determine an individual's vitamin D status because its serum concentration is high and half-life is relatively long, 2 to 3 weeks (Holick 2009). The $1,25(OH)_2D$ metabolite is not suitable due to the fact that its half-life is only 4-6 hours and circulating concentrations are thousands of times lower than the concentrations of 25(OH)D.

The laboratory measurements used to analyse S-25(OH)D (serum 25-hydroxyvitamin D) concentration can be categorised to immunoassays and physical detection methods (Holick 2009). The limitation of immunoassays, including radioimmunoassay, enzyme immunoassay and chemiluminescent immunoassay, is that they react also interfering vitamin D metabolites (e.g. 24,25-dihydroxyvitamin D) and typically overestimate serum concentrations of 25(OH)D by 10-20 %. The main physical detection methods, i.e. HPLC (high performance liquid chromatography) and LC–MS/MS (liquid chromatography tandem mass spectrometry), are more accurate but they require more resources such as expensive equipments and expert staff.

Due to the variation in results analysed with different assays when measuring vitamin D status, an international collaborative – VDSP (the Vitamin D Standardization Program) – was founded in 2010 (Sempos et al.

2012; Binkley et al. 2017). Its major objective was to develop methodology to standardise S-25(OH)D measurement to the gold standard reference measurement procedures of the NIST (National Institute of Standards and Technology), Ghent University and the CDC (Centers for Disease Control and Prevention). Since 2010, VDSP has coordinated the standardisation of S-25(OH)D measurements of past, current and future studies (Binkley et al. 2017). Briefly, the protocol includes five steps: 1) Estimation of the number of samples to be re-analysed (approximately 150 samples), 2) Selection of the specific stored samples, 3) re-measuring the samples using certified assay, 4) developing the mathematical model to calibrate original values to standardised values and, 5) applying the model to the entire data to standardise all values (Binkley et al. 2017).

2.3.2 THRESHOLDS FOR SUFFICIENT VITAMIN D STATUS

The IOM has defined the following thresholds for vitamin D status: <30 nmol/L (deficiency), 30-49 nmol/L (insufficiency) and ≥50 nmol/L (sufficient) (Institute of Medicine Food and Nutrition Board 2011). Finnish national nutrition recommendations are in line with this, considering serum concentration of at least 50 nmol/L sufficient for health (National Nutrition Council 2014). However, higher thresholds have also been suggested. The American Endocrine Society defines vitamin D deficiency as a vitamin D status <50 nmol/L and insufficiency <75 nmol/L (Holick et al. 2011).

2.3.3 VITAMIN D STATUS IN POPULATIONS

Concerning global vitamin D status, the systematic review including 194 studies from 44 countries showed that the prevalence of vitamin D insufficiency (S-25(OH)D <50 nmol/L) was 37.3% (Hilger et al. 2014). The mean S-25(OH)D values ranged widely, from 4.9 to 136.2 nmol/L. Comparing the results between the countries or within countries between the different studies is, however, challenging. A majority of the studies were carried out in small subpopulations instead of large samples of general populations. Further, there are also differences in the season of blood sampling and laboratory methodology used, which make comparison more difficult.

Table 3 shows S-25(OH)D concentrations among adult populations, which are calibrated according to the VDSP to improve the comparability between the studies (see chapters 2.3.1 and 4.3.1) in selected European countries (Cashman et al. 2015; Cashman et al. 2016). It seems that among the Nordic populations, in Norway and Denmark, the prevalence of vitamin D insufficiency is lower compared with the UK, Germany and Netherlands.

Table 3. The mean S-25(OH)D concentrations standardised according to the VDSP in selected European adult populations (Adapted from Cashman et al. 2015 and Cashman et al. 2016)

Country, Study	n Age range	Mean (95% CI)	< 50 nmol/l % (95 % CI)
Norway Tromsø Study (2008)	12 817 30-87	65.0 (55.2, 74.7)	18.6 (7.3, 39.6)
Denmark Health 2006 (2006-2008)	3409 19-72	65.0 (60.9, 69.1)	23.6 (18.3, 28.7)
UK NDNS (2008-2012)	911 19-91	46.6 (43.6,49.6)	57.9 (55.4, 60.7)
Germany DEGS (2008-2011)	6995 18-79	50.1 (49.6, 50.5)	54.5 (53.4, 55.6)
Netherlands, NHS (2006–2007)	2625 40-66	59.5	33.6

Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D; VDSP the Vitamin D Standardization Program; CI Confidence Interval; UK united Kingdom; NDNS National Diet and Nutrition Survey; DEGS German Health Interview and Examination Survey for Adults; NHS New Hoorn Study

In Finland, the mean concentration of S-25(OH)D has been shown to be 63.3 and 66.5 nmol/L in men and women, respectively (Raulio et al. 2017), and in Northern Sweden 65.2 and 71.0 nmol/L in men and women, respectively (Ramnemark et al. 2015). Finally, among the US adult population, based on data from the NHANES 2006-2008, vitamin D status varied in men between 57.3 and 58.9 and in women between 56.5 and 62.7 nmol/L depending on the age group (Institute of Medicine Food and Nutrition Board 2011).

2.3.4 FACTORS ASSOCIATED WITH VITAMIN D STATUS

Due to the fact that exposure to sunlight is an important source of vitamin D, vitamin D status is usually lower in the winter months compared with the summer months (Rabenberg et al. 2015; Touvier et al. 2015; Larose et al. 2014). Further, because melanin in the skin absorbs UVB-radiation, skin pigmentation is also associated with vitamin D status (Chen et al. 2007). For example, the results based on the NHANES have shown that non-Hispanic

blacks had remarkably lower (30 nmol/L) vitamin D status compared to non-Hispanic whites living in the same area (Scragg et al. 2007). Potential associations of other sociodemographic, lifestyle and metabolic health-related factors with vitamin D status are reviewed in the next paragraphs and in Tables 4-6 with the main focus on original cross-sectional studies focusing on determinants of vitamin D status in general adult populations published over the past ten years.

The association of vitamin D status with sex or age is controversial (Table 4). Two studies have suggested that vitamin D status is lower in women compared with men, while no sex difference was found in two other studies. Because the capacity of the skin to produce vitamin D decreases with increasing age, it has been proposed that older age is associated with lower vitamin D status (MacLaughlin and Holick 1985). However, although five studies of ten have found an inverse association, positive and non-significant associations have also been reported. Only a few studies have focused on the association between vitamin D status and marital status, suggesting that vitamin D status may be higher among those who are married or cohabiting compared with others. The evidence on the association between vitamin D status and education level is controversial with inverse, positive and null associations all having been reported.

Table 4. *The association between vitamin D status and selected sociodemographic factors in general adult populations.*

Name of the study, country n, sex, age range (Reference)	**Female sex**	**Age**	**Being married or cohabiting**	**Education**
NFBC 1966, Finland n=4758, F+M, 31 y (Palaniswamy et al. 2017)				
DEGS1, Germany n=6995, F+M, 18-79 y (Rabenberg et al. 2015)		F M		F[1] M[1]
SU.VI.MAX, France n=1828, F+M, ≤ 65y (Touvier et al. 2015)				
The HUNT, Norway n=2584, F+M, 19-55y (Larose et al. 2014)				
FIN-D2D, Finland n=2822, F+M, 45-74 y (Miettinen et al. 2014)				
The USRT, US n=1500, F+M, 48-93 y (Freedman et al. 2013)				
The AusDiab, Australia n=11,218, F+M, ≥25 y (Daly et al. 2012)				
CaMos, Canada n=1912, F+M, ≥35 y (Greene-Finestone et al. 2011)				
Geelong Osteoporosis Study Australia, n=1494, F, 20-94 y (Pasco et al. 2009)			[2] [3]	
GNHIES, Germany n=4030, F+M, 18-79 y (Hintzpeter et al. 2008)		F M		

Green=positive Red=inverse Gray=no significant association, White=not available;

Abbreviations: NFBC Northern Finland Birth Cohort; DEGS1 the German Health Interview and Examination Survey for Adults; SU.VI.MAX SUpplémentation en VItamines et Minéraux AntioXydants; HUNT The Nord-Trøndelag Health Study; USRT US Radiologic Technologists study; AusDiab Australian Diabetes, Obesity and Lifestyle Study; CaMOs Canadian Multicentre Osteoporosis Study; GNHIES German National Health Interview and Examination Survey; F Female; M male

[1]Socio-economic status including education, occupation and household income; 2 20-54y; [3] ≥55 y

Regarding BMI (body mass index), an inverse association for vitamin D status has been reported in almost all studies in Table 5. Further, almost all of the studies in Table 5 have relatively consistently shown that vitamin D status is typically higher among physically active compared to non-active subjects. One explanation for this is that physical activity is related to the time spent outdoors. However, it has been shown that both, indoor and outdoor activities were associated with higher vitamin D status compared to inactivity, but the association with outdoor activities was stronger (Scragg and Camargo 2008). Further, some studies have reported lower vitamin D status among smokers compared with non-smokers, whereas others have not found significant association, leaving this issue controversial. Regarding alcohol consumption, there are some suggestions that higher alcohol consumption may be associated with higher vitamin D status. Also, a high intake of vitamin D from diet or the use of vitamin D supplements typically increases vitamin D status (Larose et al. 2014; Greene-Finestone et al. 2011). The association between vitamin D status and quality of diet is poorly known, but one study has found a positive association between vitamin D status and healthy diet measured with a 5-item (ie. red meat, rye or crisp bread, berries or fruit, salads and vegetables) diet score (Palaniswamy et al. 2017).

Table 5. *The association between vitamin D status and body mass index and selected lifestyle factors in general adult populations.*

Name of the study, country n, sex, age range (Reference)	Body mass index	Physical activity[1]	Current Smoking	Alcohol consump-tion	Quality of diet[2]
NFBC 1966, Finland n=4758, F+M, 31 y (Palaniswamy et al. 2017)					
DEGS1, Germany n=6995, F+M, 18-79 y (Rabenberg et al. 2015)					
SU.VI.MAX, France n=1828, F+M, ≤ 65y (Touvier et al. 2015)					
The HUNT, Norway n=2584, F+M, 19-55y (Larose et al. 2014)					
FIN-D2D, Finland n=2822, F+M, 45-74 y (Miettinen et al. 2014)	F / M				
The USRT, US n=1500, F+M, 48-93 y (Freedman et al. 2013)			F / M		
The AusDiab, Australia n=11,218, F+M, ≥25 y (Daly et al. 2012)					
CaMos, Canada n=1912, F+M, ≥35 y (Greene-Finestone et al. 2011)					
Geelong Osteoporosis Study Australia, n=1494, F, 20-94 y (Pasco et al. 2009)			[3] / [4]		
GNHIES, Germany n=4030, F+M, 18-79 y (Hintzpeter et al. 2008)					

Green=positive Red=inverse Gray=no significant association, White=not available;

Abbreviations: NFBC Northern Finland Birth Cohort; DEGS1 the German Health Interview and Examination Survey for Adults; SU.VI.MAX SUpplémentation en VItamines et Minéraux AntioXydants; HUNT The Nord-Trøndelag Health Study; USRT US Radiologic Technologists study; AusDiab Australian Diabetes, Obesity and Lifestyle study; CaMOs Canadian Multicentre Osteoporosis Study GNHIES German National Health Interview and Examination Survey; F Female; M male

[1] Including both indoor and outdoor activity; [2] Measured with diet score [3] 20-54 years, [4] ≥55 years

Table 6 shows that an inverse association between vitamin D status and blood pressure has been reported. Further, low vitamin D status may be associated with an unfavourable serum lipid profile with increased serum triglyceride and decreased HDL (high density lipoprotein) cholesterol concentrations. The association of vitamin D status with serum fasting glucose is quite poorly known and controversial.

Table 6. *The association between vitamin D status and blood pressure, serum lipids and fasting glucose in general adult populations.*

Name of the study, country n, sex, age range (Reference)	**Blood pressure**		**Serum HDL-C**	**Serum Triglyce-rides**	**Serum Fasting glucose**
FIN-D2D, Finland n=2822, F+M, 45-74 y (Miettinen et al. 2014)	F	M			
NHANES, USA n=3529, F+M, ≥20 y (Maki et al. 2012)					
FHS, US n=3890, F+M (Cheng et al. 2010)					
Tromso Study, Norway n=4125, F+M, ≥30 y (Jorde et al. 2010a)					
Tromso Study, Norway n=10105, F+M, ≥30 y (Jorde et al. 2010b)					
HPFS, US n=900, M, 40-75 y (Giovannucci et al. 2008)					
1958 British Birth Cohort, UK n=6810, F+M, 45 y (Hypponen et al. 2008)					

Green=positive Red=inverse Gray=no significant association, White=not available

Abbreviations: HDL-C High density lipoprotein concentration; NHANES National Health and Nutrition Examination Survey; FHS The Framingham Heart Study; HPFS Health Professionals follow-up study; F Female, M male; US United States; UK United Kingdom

2.4 STRATEGIES TO IMPROVE VITAMIN D STATUS

2.4.1 FOOD FORTIFICATION WITH VITAMIN D

Food fortification with vitamin D has been suggested to be an effective strategy to improve vitamin D status at the population level (Cashman 2015). The positive effect of food fortification on vitamin D status has been shown in quite small randomised controlled trials (O'Donnell et al. 2008; Black et al. 2012) but the evidence based on population-based samples is limited.

Within Europe, the vitamin D fortification of low-fat milk products and fat spreads is mandatory in Sweden. Also, in Norway low-fat milk is fortified at the level of 0.4 μg/100 g and fat spreads at the level of 10 μg/100 g (Norwegian Scientific Committee for Food Safety 2013). Further, in the UK most fat spreads, and some other foods such as breakfast cereals, are fortified with vitamin D on a voluntary basis (Scientific Advisory Commitee on Nutrition 2016). It has been estimated that in the US and Canada a remarkable part of individuals' intake of vitamin D from food is from fortified foods (Calvo et al. 2004; Calvo and Whiting 2013). In the US almost all milk is fortified with vitamin D on a voluntary basis (0.96 μg/100 g) and in Canada, the fortification of milk (0.8-1.0 μg/100 g) and margarine (13.0 μg/100 g) is mandatory.

In Finland, fat spreads have been fortified on a voluntary basis since the 1950s at the level of 5.0-10.0 μg/100 g (Suojanen 2003). In December 2002, the Finnish National Nutrition Council aimed to increase vitamin D intake at the population level and launched the new recommendations for systematic, voluntary fortification. The recommendation was to fortify fat spreads with vitamin D at a concentration of 10.0 μg/100 g and all fluid milk products (with the exception of organic products) and respective lactose-free milk-, soy-, and cereal-based drinks at a concentration of 0.5 μg/100 g (Figure 2) (Ministry of Trade and Industry of Finland 2002). The aim was that most of the adult population would reach the Nordic vitamin D intake recommendation at that time 5.0 μg/day (National Nutrition Council 1998), which was further increased to 7.5 μg/day in 2005 (National Nutrition Council 2005). According to the FINDIET 2007 Survey, vitamin D intakes were 7 and 5 μg/day in men and women, respectively (Paturi et al. 2008). Further, a study of different population subsets showed that although vitamin D status improved after the implementation of the fortification policy in 2003, insufficient vitamin D status was still common especially among supplement non-users in winter (Lamberg-Allardt and Viljakainen 2006). Thus, in 2010 the recommendations for the fortification levels were doubled (National Nutrition Council 2010). The improvement of vitamin D intake and vitamin D status has recently been demonstrated based on independent cross-sectional samples (study years 2002, 2007 and 2012) of Finnish adults (Raulio et al. 2017).

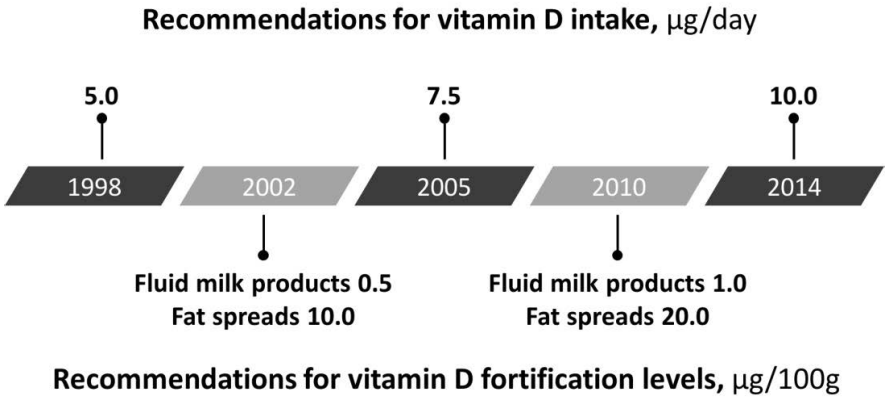

Figure 2. Recommendations for vitamin D intake for adult population and fortification levels in Finland since 1998.

2.4.2 SUPPLEMENTATION

Recommendations for vitamin D supplementation vary between countries (Spiro and Buttriss 2014). However, in general, vitamin D supplementation is recommended in many countries, including Finland, for vulnerable groups such as children, pregnant and breastfeeding women and older people. In Finland, vitamin D supplementation is recommended for adults at a level of 10 µg/day between October to March if fortified milk products and fat spreads are not consumed daily and fish at least 2-3 times per week (National Nutrition Council 2014).

2.5 HEALTH EFFECTS OF VITAMIN D

2.5.1 OVERVIEW

Vitamin D is essential for skeletal health. In recent decades, vitamin D deficiency has been suggested to also be associated with increased risk of many other chronic diseases such as cardiovascular diseases, cancers and autoimmune diseases (Theodoratou et al. 2014). Potential biological mechanisms behind these associations mainly include the finding that vitamin D receptors are expressed not only in cells related to calcium and phosphate metabolism but also in many other cells and tissues in the human body, suggesting that vitamin D may also have non-skeletal health effects (DeLuca 2008). In addition, the vitamin D activating enzyme (CYP27B1) has been found in extra-renal tissues, indicating that the active form of vitamin D, $1,25(OH)_2D$, is also formed locally in other tissues of the human body

(Lehmann and Meurer 2010). However, evidence from epidemiological studies is still limited and controversial.

In the next chapters, the epidemiologic evidence on the association between vitamin D status and two major public health concerns in Finland, depression and obesity, will be reviewed.

2.5.2 DEPRESSION

Depression is a major increasing public health concern throughout the world with the effects on work dysfunction and use of health care resources (Ustun et al. 2004). In Finland, the prevalence of any depressive disorder has increased from 7.3% to 9.6% between the years 2000 and 2011 (Markkula et al. 2015). It has been estimated that in Finland financial costs due to depression are almost a billion euros annually (Sillanpää et al. 2008). In addition to psychological and psychosocial factors, the pathogenesis of depression is proposed to include biological factors (Belmaker and Agam 2008). The potential pathophysiological mechanims have been linked to functional and structural brain abnormalities, such as genetic vulnerability and reduced activity in noradrenergic and serotonergic neurotransmission (Palazidou 2012).

A systematic review including ten **cross-sectional** studies (n=69 to 7970) examining the association between vitamin D status and depression showed that low vitamin D status was associated with a higher prevalence of depression (OR [odds ratio]=1.31, 95% CI [confidence interval] 1.0-1.71) (Anglin et al. 2013). Further, a recently published Norwegian population-based study indicated that low serum 25(OH)D concentrations are associated with higher prevalence of depressive symptoms estimated by the SCL (Symptom Check List) (Kjaergaard et al. 2011). On the other hand, among 6331 German participants aged 18–79 years the association between vitamin D status and current depressive symptoms measured by the Patient Health Questionnaire depression module (PHQ-9) was significant only in the summer but not in winter (Rabenberg et al. 2016).

A total of 5 **cohort studies,** most of them carried out among the elderly, with at least 500 participants have examined whether vitamin D status predicts the incidence of depression (Table 7). Two of them have found no association. However, in two studies low vitamin D status predicted incident depression during the follow-up (May et al. 2010; Williams et al. 2015) and in Italian study it predicted incident depressive mood among women (Milaneschi et al. 2010). In two cohort studies, in addition to incident depression the association between vitamin D status and change in depressive symptoms during the follow-up has also been examined with no significant associations (Husemoen et al. 2016; Jovanova et al. 2017).

Table 7. Summary of the results from cohort studies including at least 500 participants with vitamin D status at baseline as a predictor of depression. The statistical significant results are printed in bold.

Name of the study Baseline years Location (reference)	Study population n, age range, gender	Length of follow-up (years)	S-25(OH)D laboratory method	S-25(OH)D classification	Measurement of depression	Main results
Rotterdam study 1997-1999 Rotterdam, the Netherlands (Jovanova et al. 2017)	n=3251 ≥ 55 years at baseline Men and women	Average 10 (SD 3.5)	Elecsys vitamin D total assay	Continuosly (SD of the square root of S-25(OH)D)	Dutch version of SCAN, classification according to DSM-IV	HR (95% CI): 0.95 (0.86, 1.05)[1]
Health2006 2006-2008 Denmark (Husemoen et al. 2016)	n=1936 18-69 years at baseline Men and women	5	CLIA	Estimates are presented per 10 nmol/L S-25(OH)D	SCL-90-R Self-reported doctor-diagnosed depression	OR (95% CI): 1.03 (0.93, 1.14); p=0.59[2]
Health ABC 1998-1999 USA (Williams et al. 2014)	n=2325 Men and women 70-79 years at baseline	4	RIA	<20 vs. ≥30 ng/mL	CES-D short score ≥10 or antidepressant medication use	HR (95% CI): **1.65 (1.23, 2.22)** p for trend <0.01[3]

Table 7. continues

Name of the study Baseline years Location (ref.)	Study population n, age range, gender	Length of follow-up (years)	S-25(OH)D laboratory method	S-25(OH)D classification	Measurement of depression	Main results
2000-2009 USA (May et al. 2010)[4]	n=7358 Men and Women ≥ 50 years at baseline	Average 1.07 years, maximum 6.64 years	CLIA	≤15 vs. >50 ng/mL	A clinical diagnosis of depression [ICD-9]	HR (95% CI): **2.70 (1.35, 5.40)** **p 0.05[5]**
InCHIANTI 1998-1999 Italy (Milaneschi et al. 2010)	n=640 Men and Women ≥ 65 years at baseline	6	RIA	<50 vs. ≥50 nmol/L	CES-D (Depressive mood)	HR (95% CI): **Women: 2.09 (1.25, 3.49)** **p 0.005[6]** Men: 1.46 (0.81, 2.65) p 0.21[6]

Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D concentration; SD standard deviation; SCAN the Schedules for Clinical Assessment in Neuropsychiatry; DSM-IV the Diagnostic and Statistical Manual of Mental Disorders, 4th revised edition; HR Hazard ratio; CI Confidence Interval; CLIA chemiluminescence-immunoassay; SCL Symptom Check List; OR Odds Ratio; Health ABC the Health, Aging and body composition study; RIA Radioimmunoassay; CES-D Centre for Epidemiologic Studies Depression scale; ICD-9 International Classification of Diseases, Ninth Edition; InCHIANTI Invecchiare Chianti, aging in the Chianti area; BMI body mass index;

[1]Model: gender, age, baseline depressive symptoms, BMI, alcohol consumption, smoking status, marital status, Activity of Daily living score

[2]Model: sex, age, seasonality, history of chronic disease, BMI, physical activity, educational level, smoking status, alcohol consumption, fruit and vegetable intake, supplement use, ethnicity

[3]Model: age, sex, race, season, education, diabetes, cardiovascular diseases, BMI, Modified Mini-Mental State score, kidney disease, smoking status, alcohol consumption, marital status, physical activity, history of depression

[4] Cardiovascular patients

[5] Model: Not reported

[6] Model: age, baseline CES-D, Activity of daily living disabilities, use of antidepressants, number of chronic diseases, Short Physical Performance Battery, high parathormone, season of data collection

Randomised controlled trials (RCTs) on the treatment effect of vitamin D supplementation on depression have given contradictory results (Gowda et al. 2015). In a meta-analysis including 9 RCTs (total n=4923), vitamin D supplementation did not, overall, reduce depressive symptoms among adults with diagnosed depression or depressive symptoms (standardised mean difference 0.28; 95% CI -0.14, 0.69; p=0.19) (Gowda et al. 2015). However, there was variability in the duration of the interventions and vitamin D doses between the studies included in the meta-analysis. In 4 of them, the duration of the intervention was a maximum of 8 weeks, in 3 of them 6 to 24 months and in 2 of them at least 36 months. Further, the dose of vitamin D exceeded 4000 IU (international units)/day (100 μg/day) in 5 of the studies. In the rest of them, it ranged from 400 to 1500 IU/day (10 to 37.5 μg/day). Also the number of participants (from 42 to 2252) and outcome measurements ranged widely between the studies. Three of the studies included in the meta-analysis used the Beck Depression Inventory (BDI), finding no significant association. Finally, a recently published RCT ended in similar conclusions (Yalamanchili and Gallagher 2018). One year of treatment with vitamin D (three groups: 400-800 IU/day [10-20 μg/day], 1600-3200 IU/day [40-80 μg/day] and 4000-4800 IU/day [100-120 μg/day]) did not influence the Geriatric depression score among older women (n=270).

2.5.3 OBESITY

Obesity, shortly defined as an abnormal or excessive accumulation of fat in adipose tissue with potential harmful health effects, is a growing public health concern worldwide (World Health Organization 2000). Obesity has many health consequences including premature death and increased risk of many chronic diseases such as diabetes, coronary heart disease and sleep apnea. The most useful tool to measure obesity at the population level is BMI, calculated as weight divided by height squared (kg/m^2) (World Health Organization 2000). In Finland, every fifth adult is obese (BMI≥30 kg/m^2) (Männistö et al. 2015). In high-income countries the highest prevalence of obesity has been shown in the US, about 30% (Finucane et al. 2011).

Epidemiological evidence on the association between vitamin D status and obesity, measured by different indicators, is mainly based on **cross-sectional studies**. A meta-analysis including 23 cross-sectional studies has shown that the prevalence of vitamin D deficiency was 35% higher in obese compared to normal-weight subjects (prevalence ratio 1.35; 95% CI 1.21, 1.50) (Pereira-Santos et al. 2015). Further, another meta-analysis including 34 cross-sectional studies reported the inverse association between vitamin D status and BMI (combined Fisher's Z of correlation coefficients =-0.15; 95% CI -0.19, -0.11) (Saneei et al. 2013).

The evidence based on **cohort studies** with vitamin D status as a predictor of obesity, however, is limited and controversial (Table 8). Low vitamin D status at baseline predicted incident obesity in the Norwegian population during an 11-year follow-up (Mai et al. 2012) as well as among Spanish men and women during a 4-year follow-up (Gonzalez-Molero et al. 2013). Le Blanc et al. (2012) found no overall association between vitamin D status at baseline and change in weight during the follow-up, but they found a significant interaction between weight change categories (loss, stable and gain). In the weight gain category, they found an inverse association between vitamin D status at baseline and and weight change. However, the remaining three studies have reported no significant association between vitamin D status at baseline and incident overweight, weight gain, body fat gain or increase in BMI during follow-up (Table 8).

Finally, **RCTs** do not support the hypothesis that vitamin D supplementation has an effect on adiposity measures (Pathak et al. 2014; Chandler et al. 2015). A meta-analysis including 18 RCTs with outcome measures of body weight, BMI, fat mass, percentage of fat mass or lean body mass concluded that vitamin D supplementation did not decrease the measures of obesity (Pathak et al. 2014). Concerning BMI small but non-significant decrease was found (standardised mean difference [SMD]=-0.097; 95% CI -0.21, 0.02; p=0.09). The duration of the interventions ranged from four weeks to three years, being at least one year in six of the studies. Also, the doses of vitamin D ranged widely, being at least 4000 IU/day (100 µg/day) in four studies. Another meta-analysis including a total of 26 RCTs (partly overlapping with Pathak et al. 2014) with a minimum duration of 3 months and 50 participants and using change in BMI, fat mass or weight as an outcome also concluded that vitamin D supplementation had no effect on adiposity measures (Chandler et al. 2015). When comparing vitamin D supplementation with placebo, weighted mean differences (95% CI) were -0.06 kg/m^2 (-0.14, 0.03), -0.05 kg (-0.32, 0.23) and -0.43 kg (-1.69, 0.84) for BMI, weight and fat mass, respectively. The median duration of the intervention was 12 months ranging from 3 to 84 months. A great variety was also seen in the doses of vitamin D (0.75 µg/day to over 300 µg/day).

Table 8. Summary of the results from cohort studies with vitamin D status at baseline as a predictor of obesity measured by different indicators. The statistical significant results are printed in bold.

Name of the study Baseline years Location (reference)	**Study population n, age range, gender**	**Length of follow-up (years)**	**S-25(OH)D laboratory method**	**S-25(OH)D classifi-cation**	**Anthropo-metric measure-ments**	**Classification**	**Main results**
The Population Study of Women in Gothenburg 1968-1969 Sweden (Lehtinen-Jacks and Agelii et al. 2016)	n=759 Women 38-60 years at baseline BMI <25 kg/m² at baseline	6 to 35	25(OH)D total assay and a competitive ECLIA protein-binding assay	Lowest quartile (51.45 nmol/l) vs. upper three quartiles combined	Weight and height: measured	Incident overweight: BMI≥25 kg/m²	RR[1] (95% CI) BMI≥25 1.00 (0.83–1.20)
KORA-Age Study 2009 Southern Germany (Vogt et al. 2016)	n=735, 65-90 years at baseline Men and women	3	ECLIA	For every 1 SD higher baseline 25(OH)D	Weight: measured Body fat gain: BIA	> 3% weight/body fat gain vs. stable	OR[2] (95% CI) weight gain: M 0.93 (0.65, 1.31) F 0.93 (0.65, 1.33) OR[2] (95% CI) body fat gain: M 1.04 (0.76, 1.42) F 0.72 (0.52, 1.00)

Table 8. continues

Name of the study Baseline years Location (reference)	Study population n, age range, gender	Length of follow-up (years)	S-25(OH)D laboratory method	S-25(OH)D classifi-cation	Anthropo-metric measure-ments	Classification	Main results
The Pizarra Study 2002-2004 Southern Spain (Gonzalez-Molero et al. 2013)	n=961 23-72 at baseline Men and women	4	ECLIA	≤ 42,5 vs. >42,5 nmol/L	Weight and height: measured	Incident obesity: BMI≥30 kg/m²	OR[3] (95% CI) BMI≥30: **2.35 (1.03, 5.4)**
						Weight gain >3.7 kg	OR[3] (95% CI) Weight gain >3.7 kg: **2.37 (1.23, 4.58)**
HUNT 1995-1997 Norway (Mai et al. 2012)	n=2165, < 65 years at follow-up Men and women	11	CLIA	< 50 vs. ≥75 nmol/L	Weight, height and WC: measured	Incident obesity: BMI≥30 or WC ≥88/102 cm	OR[4] (95% CI) BMI≥30: **1.73, (1.24, 2,41)** OR[4] (95% CI) WC ≥88/102: **1.56 (1.22, 1.99)**
SOF 1986-1988 US (LeBlanc et al. 2012)	n = 1054 ≥ 65 years at baseline Women	4.5	LC-MS/MS	≥ 30 vs. < 30 ng/mL	Weight and height: measured	Weight change	Mean change in weight[5]: -3.3 vs. -2.8 pounds; P 0.24
IRAS 1999-2002 US (Young et al. 2009)	n=1081 Men and women Hispanic and African Americans	5.3	RIA[6]	Continuous	Weight and height: measured	Change in BMI	Regression coefficient ± SE[7] Hispanics: 0.01±0.01; P 0.38 African Americans: -0.04±0.03;P 0.15

Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D; ECLIA Electrochemiluminescence-immunoassay; RR relati ve risk; SD standard deviation; BMI body mass index; BIA bioimpedance analysis; OR odds ratio; CI confidence interval; M male; F Female; HUNT The Nord-Trøndelag Health Study; WC waist circumference; SOF Study of Osteoporotic Fractures; CLIA chemiluminescence-immunoassay; LC-MS/MS Liquid Chromatography Tandem Mass Spectrometry; IRAS The Insulin Resistance Atherosclerosis Family Study; SE standard error; RIA radioimmunoassay

[1]Model: Age, season, menopause and education

[2]Model: Age, sex, season of serum collection, disability, physical activity, smoking status, diabetes, diabetes medication, stroke, kidney disease, follow-up time

[3]Model: Age, sex, season, intact parathyroid hormone and the presence of diabetes

[4]Model: Sex, age, smoking, education, physical activity, social benefits,economical difficulties at baseline

[5] Not reported

[6] Plasma 25(OH)D

[7] Model: Age, sex, BMI at baseline, clinic site

2.5.4 POTENTIAL BIOLOGICAL MECHANISMS

The presence of vitamin D activating enzyme CYB27B1 and vitamin D receptors in adipose tissue, as well as in the brain and central nervous system, indicates that vitamin D may have a role in the regulation of them (Eyles et al. 2005; Earthman et al. 2012). Regarding depression, it has been proposed that vitamin D deficiency may contribute to disruptions in the neuroendocrine and central nervous systems, and these disruptions may lead to further dysregulation of neurotransmission and neurotransmitter metabolism and signalling, neuroprotection, neuroimmunomodulation, and the release of glucocorticoids, potentially leading to depression (Obradovic et al. 2006; Garcion et al. 2002). Regarding obesity, it has been suggested that vitamin D may have anti-obesity effects by affecting the gene expression related to adipocyte differentiation, lipolysis and lipogenesis (Earthman et al. 2012). Further, it is possible that the factors associated with both low vitamin D status and increased risk of depression, such as elevated PTH concentration, explain the association (Earthman et al. 2012; Watson and Marx 2002).

There are also some hypotheses of reverse causative mechanisms, for example decreased bioavailability (Wortsman et al. 2000) or volumetric dilution (Drincic et al. 2012) of vitamin D in obesity, which indicates that obesity may induce vitamin D deficiency. In addition to biological mechanisms, it is also possible that depression or obesity leads to decreased outdoor activities or lower vitamin D intake from diet (Stumpf and Privette 1989; Pourshahidi 2015).

It should be taken into account that the molecular response to vitamin D supplementation and intake varies between individuals (Carlberg and Haq 2016). It has been suggested that those having a low molecular response to vitamin D may require higher S-25(OH)D concentration to achieve the same potential health effects of vitamin D as those having a high response. The molecular basis of these differences is not yet fully understood but they may, at least partly, be explained by genetic variation and SNPs (single nucleotide polymorphisms). Thus, in addition to measuring S-25(OH)D genetic differences should also be taken into account, if possible, when estimating an individuals vitamin D status and its changes.

3 AIMS

The aim of this thesis was to evaluate the vitamin D status of Finnish adults, its temporal changes and factors related to it, as well as the role of vitamin D status in the prevalence of depression and in the development of obesity. The specific objectives were:

1. To study the vitamin D status of Finnish adults in the year 2000 and to determine sociodemographic, lifestyle and metabolic health factors related to it (I)
2. To study the change in vitamin D status in the Finnish adult population between the years 2000 and 2011 and to evaluate the effect of the systematic vitamin D fortification policy (II)
3. To study a cross-sectional association between vitamin D status and prevalence of depressive disorder and depressive symptoms in the year 2000 (III)
4. To study whether vitamin D status predicts weight gain or an increase in waist circumference during the 11-year follow-up between the years 2000 and 2011 (IV)

4 METHODS

4.1 STUDY POPULATION

The study is based on the population-based Health 2000/2011 Survey conducted in Finland by the National Institute for Health and Welfare (THL) and its co-operators. The Health 2000 Survey (Health 2000) was carried out from 2000 to 2001 (Heistaro 2008) and its follow-up, the Health 2011 Survey (Health 2011), from 2011 to 2012 (Lundqvist and Mäki-Opas 2016). The main aim of the Health 2000/2011 Survey was to provide a comprehensive overview on health, functional capacity and welfare, as well as their determinants and changes in the Finnish adult population.

The original Health 2000 sample comprised 9922 persons aged 18 or over of whom 8028 were aged 30 or over. The sample frame was regionally stratified according to five university hospital regions (strata): Helsinki, Turku, Tampere, Kuopio and Oulu (Figure 3). In the first stage of sampling, 80 health centre districts, 16 from each university hospital regions, were sampled as a cluster. The health centre districts of 15 largest towns of Finland were selected with probability 1 and the sample size for each health centre district was proportional to its proportion of population. The rest 65 health centre districts were selected using a systematic sampling with probabilities proportional to size (PPS-SYS design). In the second stage, systematic random sampling was used to draw the sample from each health centre districts using the nationwide population register. This two-stage stratified cluster sample represents the adult population living in mainland Finland.

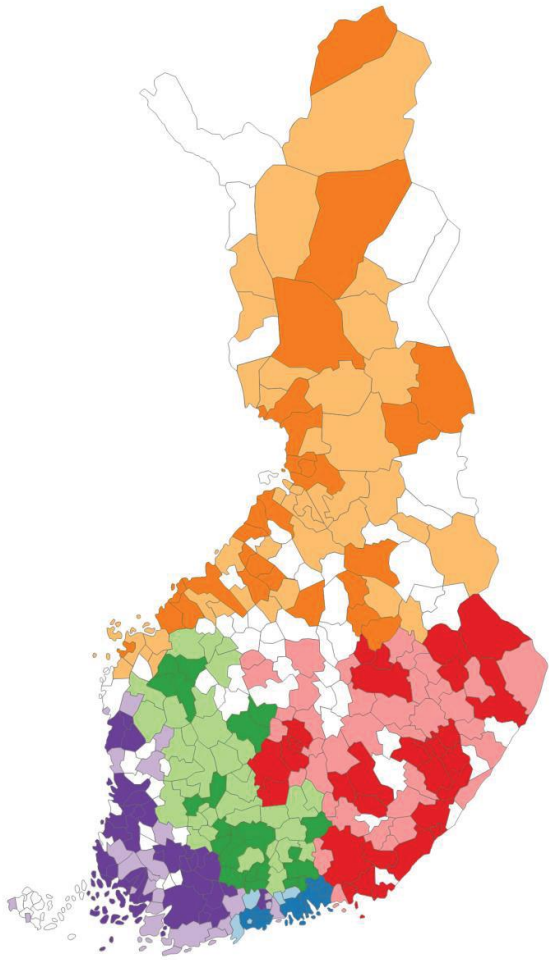

Figure 3. The study areas of the Health 2000/2011 Survey. Differerent colors: the Univesity hospital regions, Darker shades: actual Health 2000 health centre districts, Lighter shades: the areas, where some of the members in the sample migrated between 2000 and 2011(The source of the figure: Lundqvist A, Mäki-Opas T (eds.) (2016) Health 2011 – Methods)

A total of 84% of the sample aged 30 or over participated in a health examination in Health 2000 (Figure 4). All members of the original Health 2000 sample (n=9922) who were not dead, living abroad or refused further studies were invited 11 years later to the follow-up examination, Health 2011 (n=8135). A total of 59% of them participated in a health examination. In this thesis, the samples of participants aged ≥30 years were utilised. S-25(OH)D concentrations were analysed from 6134 and 4051 participants aged ≥30 years in Health 2000 and Health 2011, respectively. In both years S-25(OH)D concentrations were available for 3328 participants.

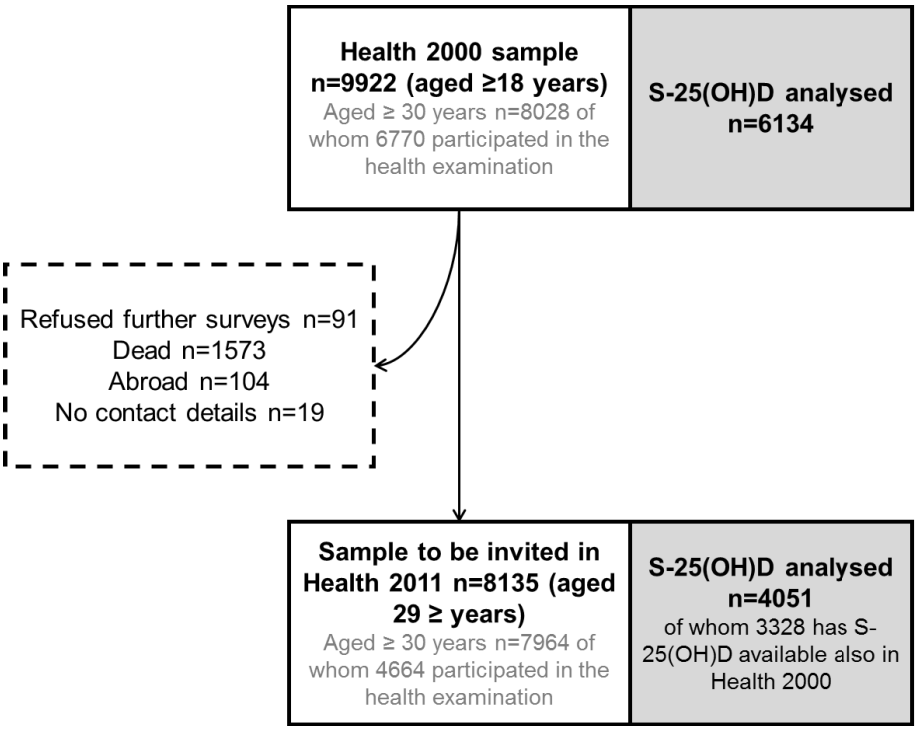

Figure 4. Flowchart of the research data. Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D concentration

The inclusion criteria in different substudies of the thesis are summarised in Table 9.

Table 9. The inclusion criteria in different substudies of the thesis.

Substudy	Data	n	Inclusion criteria
I	Health 2000	5714	Men and non-pregnant women Aged 30-79 years S-25(OH)D concentration available
II	Health 2000/2011	6134/ 4051	Men and women Aged 30 years and over S-25(OH)D concentration available
II[1]	Health 2000/2011	3328	Men and women Aged 30 years and over in 2000 S-25(OH)D available in both years
III	Health 2000	5371	Men and non-pregnant women Aged 30-79 years S-25(OH)D available Current depressive symptoms and diagnosis of depressive disorder assessed
IV	Health 2000/2011	2924	Men and non-pregnant women Aged 30-64 at baseline S-25(OH)D available in 2000 Weight and height were measured in both years

Abbreviations: s-25(OH)D serum 25-hydroxyvitamin D

[1]When analysing the changes in individual-level between the years 2000 and 2011

Descriptive statistics including selected variables of those Health 2000/2011 participants whose S-25(OH)D concentrations were analysed is shown in Table 10.

Table 10. Descriptive statistics of the study populations including participants aged ≥30 years and whose S-25(OH)D is available.

	Health 2000	Health 2011
Study years	2000-2001	2011-2012
n[1]	6134	4051
Women, %	54.4	55.3
Age, years, mean (SD)	52.8 (14.9)	56.1 (13.5)
Married or cohabiting, %	69.7	74.1
High education[2], %	28.3	41.3
BMI< 30 kg/m², %	77.6	76.3
Physically active at leisure time, %	72.6	73.0
Non-smoker, %	73.7	81.8

Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D; SD standard deviation; BMI body mass index

[1] Number of those participants whose S-25(OH)D concentration is available

[2] At least Bachelor's level education

4.2 ETHICAL CONSIDERATION

Health 2000 was approved by the Ethical Committee for Research in Epidemiology and Public Health on May 2000, whereas Health 2011 by the Coordinating Ethics Committee on March 2011 at the Hospital District of Helsinki and Uusimaa. The participants were informed about the content of the survey and written informed consent was obtained from all participants in both years. Data protection was taken carefully into account in all stages of the survey. Personal data were replaced by examination codes in the examination files.

4.3 MEASUREMENTS

The Health 2000/2011 Survey included self-administered questionnaires, interviews and a comprehensive health examination, including laboratory measurements (Heistaro 2008; Lundqvist and Mäki-Opas 2016). The main measurements utilised in this thesis are summarised in the next chapters. The data of the substudy III was re-analysed for this summary and measurements utilised in these re-analyses are presented in the next chapters.

4.3.1 LABORATORY MEASUREMENTS

Vitamin D status

In the present study, S-25(OH)D concentration was considered as a measure of vitamin D status. The fasting blood samples were drawn as a part of a health examination in both surveys and stored at −70°C. Health 2000 samples, utilised in all substudies of the thesis, were collected between September 2000 and March 2001 and Health 2011 samples, utilised in the substudies II and IV, from August to December in 2011. In Health 2000, 65% of the blood samples were collected during winter (i.e., November to February) whereas in Health 2011, the proportion was 37%. From Health 2000 samples the S-25(OH)D concentration was analysed with the use of radioimmunoassay (DiaSorin, Minnesota) between January 2001 and November 2002 whereas from Health 2011 samples with the use of chemiluminescent immunoassay (Architect ci8200; Abbot Laboratories, Abbot Park, IL) from February to March in 2014. The interassay coefficients of variation were 7.8% and 3.6% for Health 2000 and Health 2011, respectively.

Due to the method-related differences in S-25(OH)D concentrations (Sempos et al. 2012), the results were standardised according to the Vitamin D Standardization Program (VDSP) protocol to improve the comparability between the Health 2000 and 2011 measurements (Cashman et al. 2015). Briefly, a statistical algorithm-defined sub-sample of participants (n=238 in 2000 and n=101 in 2011) were re-analysed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) by the Vitamin D Research Group at University College Cork. The calibration equations were developed between original Health 2000 or Health 2011 S-25(OH)D data and new LC-MS/MS-measured S-25(OH)D data; this equation was applied to the entire S-25(OH)D data from Health 2000 or Health 2011 (Cashman et al. 2015).

Thresholds for S-25(OH)D concentrations in substudy II were based on IOM 2011 definitions: <30 nmol/L (deficiency), 30-49 nmol/L (insufficiency) and ≥50 nmol/L (sufficient) (Institute of Medicine Food and Nutrition Board 2011). In substudy III, participants were divided into gender-specific quartiles according to their S-25(OH)D concentration (men: 8-33, 34-43, 44-55, 56-132 nmol/L; women: 7-34, 35-43, 44-54, 55-134 nmol/L) and in substudy IV, into gender-specific quintiles according to S-25(OH)D concentration at baseline (men: 8-32, 33-40, 41-48, 49-58, 59-121; women: 8-32, 33-39, 40-47, 48-57, 58-123 nmol/L). The month of blood sampling was utilised in the analysis of substudies I and II as a categorised variable (September to March in Health 2000 and August to December in Health 2011; each month as a separate class). In substudy II, when comparing the change in S-25(OH)D concentration between the years 2000 and 2011 in individual-level, the change in blood sampling season was

defined as follows: 1) same season (blood sampling in Health 2011 within the same month or ≤ 1 month earlier or later than in H2000; n = 1762) or 2) different season (blood sampling in H2011 ≥2 month earlier or later than in H2000; n = 1566).

Fasting glucose and serum lipids

In Health 2000, concentrations of serum fasting glucose and triglycerides were analysed using enzymatic methods (Glucose, Hexokinase, Olympus System Reagent, Germany and Triglycerides, GPO PAP, Olympus System Reagent, Germany) (Heistaro 2008). Serum HDL cholesterol concentration was analysed using direct method (HDL-C Plus, Roche Diagnostics GmbH, Germany). The analyses were performed within 6 months after the blood samples were taken. The interassay coefficients of variation for serum fasting glucose concentration were 2.1% and 2.3% at the mean level of 9.3 and 5.2 mmol/l, respectively, and for triglyceride concentration 2.1% and 3.2% at the mean level of 1.4 and 1.5 mmol/l, respectively. For serum HDL cholesterol concentration the interassay coefficients of variation were 4.8% and 5.3% at the mean levels of 1.3 and 1.4 mmol/l, respectively.

4.3.2 ANTHROPOMETRIC AND CLINICAL MEASUREMENTS AND METABOLIC SYNDROME

Anthropometric and clinical measurements

Height was measured with a stadiometer (Person-Check, Medizintechnic, KaWe, Kirchner & Wilhelm, Germany in Health 2000 and Seca 213 in Health 2011) (Heistaro 2008; Lundqvist and Mäki-Opas 2016). In Health 2000, height was recorded to an accuracy of 0.5 cm, whereas in Health 2011 to an accuracy of 0.1 cm.

Weight was primarily measured as a part of bioimpedance body composition analysis (InBody 3.0 in Health 2000 and Seca 514 in Health 2011) in light clothing without shoes, and recorded to an accuracy of 0.1 cm. If bioimpedance analysis was not possible, a floor scale was used. BMI was calculated as weight divided by height squared. BMI was categorised according to WHO classification as follows: <18.5 kg/m² (underweight), 18.5-24.9 kg/m² (normal weight), 25.0-29.9 kg/m² (overweight), ≥30 kg/m² (obese) (World Health Organization 2000). In substudy III, BMI was further dichotomised using the cut-off ≥30 kg/m² and in substudy IV, using the cut-off 25 kg/m².

Waist circumference was measured on bare skin in a standing position from the mid-point between the lowest rib bones and the high point of the iliac crest during light expiration with a non-elastic tape. In Health 2000, waist circumference was recorded to an accuracy of 0.5 cm, whereas in

Health 2011 to an accuracy of 0.1 cm. In substudy I, elevated waist circumference was defined as determined in the harmonisation definition of metabolic syndrome, ≥94 cm in men and ≥80 cm in women (Alberti et al. 2009). In substudy IV, elevated waist circumference was defined according to WHO (World Health Organization 2000) classification, ≥102 cm and ≥88 cm in men and women, respectively.

Blood pressure was measured twice from the right arm after five minutes of quiet seating using a standard mercury manometer (Mercuro300; Speidel & Keller, Jungingen, Germany) with a two minute interval. The mean value was used in the analyses.

Metabolic syndrome

In substudy I, metabolic syndrome was determined according to the harmonisation definition (Alberti et al. 2009) as a presence of any three of the following five risk factors: elevated waist circumference (≥94 cm in men and ≥80 cm in women), elevated serum fasting glucose concentration (≥5.6 mmol/L), elevated serum triglyceride concentration (≥1.7 mmol/L), reduced serum HDL cholesterol concentration (<1.0 mmol/L in men and <1.3 mmol/L in women) and elevated blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic ≥85 mmHg or use of anti-hypertensive medication). In substudy I, the components of metabolic syndrome were included in Model 2 as classified above.

4.3.3 QUESTIONNAIRES AND INTERVIEWS

Sociodemographic factors

Information on age and sex was obtained from the Population Register Centre of Finland (Heistaro 2008; Lundqvist and Mäki-Opas 2016). In different substudies, the participants were categorised by age as follows: I and II 30-44, 45-54, 55-64, 65-74, ≥75 and IV 30-39, 40-49, 50-59, 60-64 years. In substudies I and III, age was included in the models as a continuous variable. Marital status was assessed in the interview by a question with five catogories: 1) married, 2) living with your partner, 3) divorced or living apart, 4) widowed or 5) single. Marital status was utilised in substudy I as a variable with four categories by combining the first two categories, and in substudies II-IV was dichotomised into being married or living with your partner vs. divorced, widow/er and single. Education was also assessed as a part of the interview with the questions related to basic education, highest completed education level and the years studied full-time. In substudy I, education was utilised based on full-time study years and the participants were categorised according to study years as follows: 1) <7 years, 2) 7-12 years and 3)>12 years. In substudies III and IV, education was utilised based on highest

completed education level and the participants were dichotomised into high education (tertiary education; at least Bachelor's level education) vs. middle (secondary education) or low (primary education).

Lifestyle factors

Data on diet and use of vitamin D supplements were collected by a validated self-administered food frequency questionnaire (FFQ) whose purpose was to estimate the average food intake of the participants over the preceding year with the question "How often have you eaten the following foods over the past 12 months" (Männistö et al. 1996; Paalanen et al. 2006). The FFQ included 128 (Health 2000) or 131 (Health 2011) commonly used food items or dishes with specified serving sizes (e.g., glass, portion, slices, dL), which were presented in the following subgroups: "dairy products", "cereals", "spreads", "vegetables", "potatoes, rice and pasta", "meat", "fish", "chicken, turkey and eggs", "fruits and berries", "desserts", "sweets and snacks" and "beverages". The participants recorded their average consumption of food items and prepared dishes in 9 frequency categories: never/rarely, 1-3 times per month, once per week, 2-4 times per week, 5-6 times per week, once per day, 2-3 times per day, 4-5 times per day and 6+ times per day.

In both study years, the average food consumption and intakes of nutrients per day were calculated based on the continuously updated National Finnish Food Composition Database (Fineli) using Finessi, software developed at THL (Reinivuo et al. 2010). Vitamin D intake from diet (µg/day) was utilised either divided into quintiles (substudy I) or dichotomised using the cut-off point ≥10 µg/day (substudy II) according to the Finnish nutrition recommendations (National Nutrition Council 2014). In substudy I, the participants were categorised according to their intakes of fish and shellfish (g/day) into quintiles and according to their intake of margarine (g/day) into quartiles. In substudy II, the participants were categorised according to their consumption of main dietary sources of vitamin D – fish, fluid milk products and fat spreads – based on frequency categories from the FFQ form as follows: 1) daily consumption of fluid milk products as one glass of milk or sour milk or one pot of yoghurt at least once a day, 2) daily consumption of fat spreads as one teaspoon of margarines or low-fat spreads (butter and butter-vegetable oil mixture excluded) at least once a day and 3) fish consumption at least twice a week as one portion (e.g., plateful of fish soup, one portion of frozen fish or salmon) of fish at least twice a week. All of the above-mentioned three components were scored 0 (no) to 1 (yes) and summed to a total score, which ranged from 0 (no dietary vitamin D sources) to 3 (all of the main dietary vitamin D sources).

The use of supplements was asked, with minor differences between the years. In Health 2000, the participants were asked to fill in the name and dose of the product by themselves, whereas in Health 2011 the product type was provided in the FFQ form (multivitamin and mineral, vitamin B, vitamin

C, vitamin D, calcium, magnesium, fatty acid, other). Further, the use of supplements was categorised in Health 2000 into "irregular" or "regular", whereas in Health 2011 there were three categories: "never use", "occasionally or intermittenly" and "daily or almost daily". In substudy II, those who were not using supplements or used them irregularly in either Health 2000 or Health 2011 were classified as supplement non-users.

In substudy I, diet was evaluated by a modification of the 9-item Alternate Healthy Eating Index (AHEI) (McCullough et al. 2002). Compared to the original 9-item AHEI, alcohol consumption and use of multivitamins were excluded because alcohol consumption was analysed as an independent lifestyle factor and there is no recommendation for habitual multivitamin use in Finland. Thus, the modified Alternate Healthy Eating Index (mAHEI) included seven components: intake of vegetables (g/day), intake of fresh fruits and berries (g/day), intake of legumes, nuts, seeds and soya beans (g/day), ratio of white-to-red meat, intake of rye (g/day), ratio of polyunsaturated-to-saturated fat and intake of trans fat (g/day). The components were categorised into quintiles and, with the exception of trans fat intake, received scores in ascendant order such that the lowest quintile received 1 point and the highest quintile 5 points. Trans fat intake was scored in the opposite order. A total score of the mAHEI was calculated by summing up all the component scores and ranged from 7 (lowest) to 35 (highest). A higher score was considered to represent a healthier diet compared to lower scores. In substudy I, total mAHEI score was divided into gender-specific quintiles (men: 7–16, 17–19, 20–22, 23–25, 26–34; women: 8–16, 17–19, 20–22, 23–25, 26–35 points).

The frequency and type of leisure-time physical activity were assessed from a self-administred questionnaire by a question "How much do you exercise and strain yourself physically in your leisure time?". The question included four categories: "1) In my leisure time I read, watch TV and do other activities in which I do not move much and which do not strain me physically, 2) In my leisure time I walk, cycle and move in other ways at least 4 hours per week, 3) In my leisure time I exercise at least 3 hours per week and 4) In my leisure time I practice regularly several times per week for competition". For the analyses of substudy I, the participants were categorised according to their physical activity during leisure-time into three classes by combining the last two categories as follows: 1) No leisure-time physical activity, 2) Moderate ≥4h/week and 3) Vigorous ≥3h/week. In substudies II-IV, the participants were dichotomized into active (categories 2-4) vs. inactive (category 1).

The information on consumption of alcohol was also based on questionnaire. A total consumption of alcohol (g/day) measure was calculated based on questions related to the frequency of alcohol use and typical doses. For the analysis, participants were categorised according to their total consumption of alcohol into three classes: 1) 0 g/week, 2) male: 1-199, female: 1-99 g/week, and 3) male: ≥200, female: ≥100 g/week. For

substudies III-IV, alcohol consumption was further dichotomised into moderate consumption (male: 1-199, female: 1-99 g/week) vs. non-consumption or high consumption. The cut-off values are modified from the Finnish Current Care Guidelines (Working group appointed by the Finnish Medical Society Duodecim and the Finnish Society of Addiction Medicine 2015).

Smoking habits were assessed in the interview. The participants were asked: "Have you ever smoked during your life-time?", "Have you smoked at least 100 times during your life-time", "Have you ever smoked daily for at least one year?" and "When did you smoke last?". Based on these four questions, participants were categorised according to their smoking habits as follows: 1) smokes daily, 2) smokes occasionally, 3) has stopped smoking, and 4) not smoking at all or never smoked daily for at least one year. For the analysis, smoking habits were further categorised into a variable with three classes ("current" including classes 1 and 2, "former" including class 3 and "never" including class 4) by combining the two first categories (substudy I) or dichotomised into current smoker (class 1) vs. other (classes 2-3) (substudies II-IV).

The lifestyle index, utilised in substudy I, was modified based on criteria presented by Hu et al. (2001). Healthy lifestyle was defined based on five components as follows: 1) BMI <25 kg/m^2, 2) regular moderate physical activity for at least 4h per week or vigorous activity for at least 3h per week, 3) no current smoking, 4) moderate alcohol consumption defined as 1-199 g/week in men or 1-99 g/week in women and 5) an above-median total score on the mAHEI (21 points). For each component, participants who met the criteria for a healthy lifestyle received 1 point, while those who did not meet the criteria were scored 0. Thus, the total score ranged from 0 to 5, with higher scores suggesting a healthier lifestyle.

Depression

In Health 2000, diagnosis of depressive disorder was assessed using the computerised version of the Munich-Composite International Diagnostic Interview (CIDI), developed by the WHO for purposes of epidemiological research (World Health Organization 1990). The CIDI allows the estimation of diagnoses for major mental disorders, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) based on questions concerning symptoms experienced during the past 12 months (American Psychiatric Association 1994; Wittchen et al. 1998).

Current depressive symptoms were measured using the 21-item Beck Depression Inventory (BDI) (Beck et al. 1961), which was a part of the questionnaire in Health 2000. The cut-off point of ≥10 was used to assess the current depressive symptoms (Beck et al. 1988).

4.4 STUDY DESIGNS

Substudies I and III of the thesis had a cross-sectional study design based on the Health 2000 dataset only whereas substudies II and IV were conducted as longitudinal design based on both Health 2000 and Health 2011 datasets.

4.5 STATISTICAL ANALYSES

The statistical analyses of this thesis were based on linear and logistic regression models. They were carried out taking into account the sampling design using SAS 9.2 (SAS Institute 2009) (substudy I) or SAS 9.3 (SAS Institute 2011) (substudies II-IV) and Sudaan 10.0.1 (Research Triangle Institute 2015) (substudies I-IV). Inverse probability weights were used to correct the effects of non-participation (Härkänen et al. 2016). In substudies I and II, the results are presented as weighted, model-adjusted means or prevalences, which were based on predictive margins (Graubard and Korn 1999) and their standard deviations (SD) (unadjusted; substudy I) or 95% confidence intervals (CI) (substudy II). In substudies III and IV, the results are presented as weighted ORs and their 95% CIs based on logistic regression models. Tests for heterogeneity were performed with the use of the Wald test and tests for trend using the Wald test by including the exposure variable (III: S-25(OH)D in quartiles; IV S-25(OH)D at baseline in quintiles) as a continuous variable in the model. P-values <0.05 were considered statistically significant. The statistical analyses of this thesis including confounding variables included in the models are summarised in Table 11.

In substudy I, two different linear regression models (Table 11) were used to estimate the model-adjusted means of S-25(OH)D concentration in the categories of potential determinants.

In substudy II, the joint dataset of Health 2000 and Health 2011 was used as a standard population in the analyses so that changes in the distribution of potential confounding factors did not confound the interpretation of the results. The analyses included both, comparing population averages in 2000 and 2011 (n=6134 in 2000 and 4051 in 2011) and analyzing the changes in individual-level between 2000 and 2011 among those whose S-25(OH)D concentrations were available for both years (n=3328).When comparing the population averages, the mean vitamin D intake and the prevalence of intake ≥10 μg/day were analysed using linear and logistic regression, respectively, adjusted for categorical age and stratified by sex. The prevalences of vitamin D supplement users were studied using logistic regression models adjusted for sex and categorical age. The mean VDSP-standardised S-25(OH)D concentrations and prevalences according to selected cut-offs were analysed using linear and logistic regression, respectively, adjusted for categorical age, sex and the month of the blood sampling. The analyses were carried out for both, total population and subpopulation of supplement non-users. Further,

substudy II included the following analyses focusing on changes in individual-level (Table 11):

a) The mean change in S-25(OH)D in the categories of S-25(OH)D at baseline was analysed using linear regression models for both, total population and subpopulation of supplement non-users (Table 11, IIa)
b) The effect of vitamin D fortification of fluid milk products was examined among those vitamin D supplement non-users who had not changed their consumption of fluid milk products (i.e., started or stopped) during the follow-up. The means of S-25(OH)D concentration in 2000 and 2011, as well as the mean change between the years, in categories of fluid milk consuming (consumer in both years, n=1017 vs. non-consumer in both years, n=229) was analysed using linear regression (Table 11, IIb).
c) The effect of vitamin D fortification of fat spreads (margarines or low-fat spreads; butter and butter–vegetable oil mixtures were excluded) was examined among those vitamin D supplement non-users who had not changed their consumption of fat spreads (i.e., started or stopped) during the follow-up. The means of S-25(OH)D concentration in 2000 and 2011, and the mean change between the years, in categories of fat spread consuming (consumer in both years, n=558 vs. non-consumer in both years, n=436) was analysed using linear regression (Table 11, IIc).

In substudy III, the participants were divided into quartiles according to their S-25(OH)D concentration and ORs and their 95% CIs for depressive disorder and depressive symptoms were estimated using logistic regression. The original analyses of substudy III were presented for the total population including both men and women adjusted for sex, age (continuous) and month of blood sampling (September to March). Due to the fact that sex modified the association between vitamin D status and depressive disorder (p for interaction <0.01) the further analyses of this summary were stratified by sex. The potential confounding factors for these further analyses of this summary, originally listed based on the results of substudy I and literature, which were independently associated (p <0.20) with the exposure (S-25(OH)D) and outcome (depressive disorder) were included in Model 2 (Table 11) (Rothman 1986).

In substudy IV, logistic regression was used to analyse the ORs and their 95% CIs for ≥10% weight gain and ≥10% increase in waist circumference according to quintile of S-25(OH)D concentration at baseline (Table 10, IV). The possible effect modification by sex, blood sampling season (November to March vs. other) and either BMI at baseline (weight gain; normal weight defined as BMI <25 kg/m^2 vs. other) or waist circumference at baseline (<102cm in men or <88 cm in women vs. other) were estimated by including the interaction term between S-25(OH)D concentration at baseline and possible effect modifier in age- and sex-adjusted models. The association

between S-25(OH)D at baseline and a ≥10% increase in waist circumference was modified by sex (p for interaction <0.01; all other p-values for interactions studied >0.30). Thus, the analyses were stratified by sex. The potential confounding factors which were associated (p<0.20) with exposure (S-25(OH)D at baseline) and outcomes (≥10% weight gain and ≥10% increase in waist circumference) were included in Model 2 (Table 11) (Rothman 1986).

Table 11. Summary of the statistical analyses of the thesis.

	Substudy I	Substudy II[1]	Substudy III[2]	Substudy IV[2]
Design	Cross-sectional	Longitudinal	Cross-sectional	Longitudinal
Data	Health 2000	Health 2000/2011	Health 2000	Health 2000/2011
Main exposures	Sociodemographic, lifestyle and metabolic health related factors	S-25(OH)D at baseline (IIa), fluid milk consumption (IIb), fat spread consumption (IIc)	S-25(OH)D (quartiles)	S-25(OH)D at baseline (quintiles)
Main outcomes	S-25(OH)D	Δ S-25(OH)D	Depressive disorder Depressive symptoms	≥10% weight gain ≥10% increase in WC
Confounding factors included in the models	**Model 1**: age, sex and month of the blood sampling **Model 2**: model 1+ marital status, education, BMI, leisure-time PA, smoking, alcohol consumption, the total score on mAHEI, blood pressure, serum HDL cholesterol, serum triglycerides and serum fasting glucose	**IIa Model**: age at baseline, sex, change in blood sampling season **IIb Model**: age (or age at baseline when analyzing the mean change), sex, blood sampling season or change in blood sampling season (when analyzing the mean change), fish consumption in 2000 and 2011 and fat spread consumption in 2000 and 2011	**Model 1**: age **Model 2**: model1+ marital status, BMI, leisure-time PA, smoking and alcohol consumption	**Model 1**: age **Model 2**: model 1+ marital status, education, leisure-time PA, smoking status and alcohol consumption

Table 11. continues

Substudy I	Substudy II[1]	Substudy III[2]	Substudy IV[2]
	IIc Model: age (or age at baseline when analyzing the mean change), sex, blood sampling season (or change in blood sampling season when analyzing the mean change), fish consumption in 2000 and 2011 and fluid milk consumption in 2000 and 2011		

Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D; WC Waist circumference; BMI Body Mass Index; PA physical activity; mAHEI modified Alternate Healthy Eating Index; HDL High Density Lipoprotein

[1]Based on Vitamin D Standardization Program (VDSP) standardised S-25(OH)D concentrations

[2] The analyses are stratified by sex.

5 RESULTS

5.1 ASSOCIATION BETWEEN VITAMIN D STATUS AND SOCIODEMOGRAPHIC, LIFESTYLE AND METABOLIC HEALTH FACTORS (I)

In 2000, the mean concentration of S-25(OH)D after adjustment for age and month of blood sampling was 45.5 (SD 16.9) and 45.2 (SD 16.6) nmol/L in men and women, respectively, with no difference between sexes (p for the difference 0.47). The participants with higher vitamin D status were more likely older, married or cohabiting and had high education compared to others (data not shown). Further adjustment for sociodemographic, lifestyle and metabolic health factors did not notably change the interpretations of the results.

Vitamin D status was positively associated with a healthy lifestyle estimated by the lifestyle index (Figure 5). Those having the highest score on the lifestyle index, indicating the healthiest lifestyle, had 15.8 nmol/L higher S-25(OH)D concentrations compared with participants with the unhealthiest lifestyle (53.0 nmol/L [SD 17.9] vs. 37.2 nmol/L [SD 13.9]) after adjustment for sex, age and month of blood sampling.

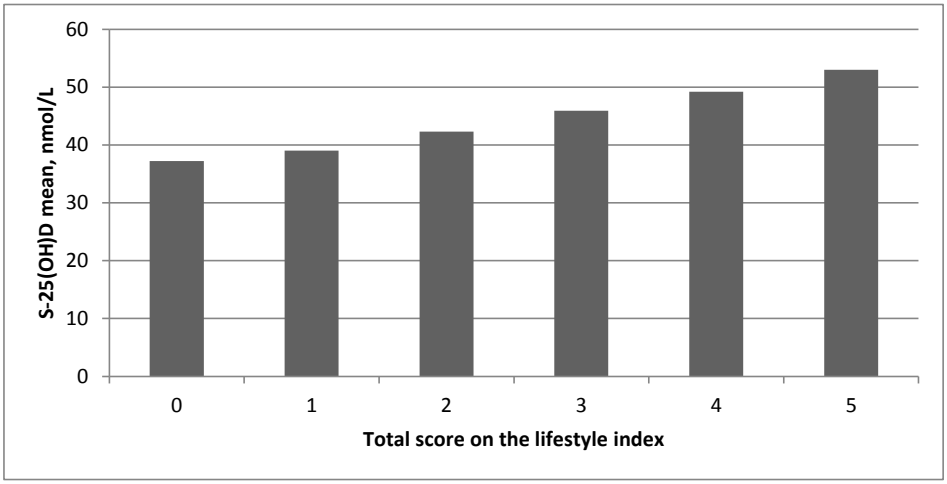

Figure 5. The mean S-25(OH)D concentration adjusted for sex, age and month of blood sampling according to the total score on the lifestyle index. 0 points indicates unhealthiest lifestyle and 5 points healthiest lifestyle. P for trend <0.01. The components of the lifestyle index: body mass index, leisure-time physical activity, smoking, alcohol consumption and diet. Number of participants: 0 n=91, 1 n=444, 2 n=1169, 3 n=1628, 4 n=1385, 5 n=430. Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D.

Further, each single component of the lifestyle index was independently associated with S-25(OH)D concentration i.e., S-25(OH) concentration was higher among those with BMI 18.5-24.9 kg/m^2 (normal-weight), who were physically active during leisure-time (including both indoor and outdoor activity), non-smokers, moderate alcohol consumers or had healthy diet compared to others (Table 12). In Model 2, adjustment for sociodemographic, lifestyle and metabolic health factors slightly attenuated the results but did not notably change the interpretation of them.

Table 12. The mean S-25(OH)D concentration according to BMI and lifestyle (model-adjusted means and their unadjusted SDs).

	S-25(OH)D concentration			
	Model 1[1]		Model 2[2]	
	Mean	(SD)	Mean	(SD)
BMI[3] kg/m²				
<18.5	45.5	(26.2)	50.5	(27.2)
18.5–24.9	47.2	(17.6)	47.2	(17.6)
25.0–29.9	45.4	(16.3)	45.6	(16.2)
≥30.0	42.3	(15.3)	43.8	(15.4)
Leisure time PA[3,4]				
Vigorous ≥3h/week	48.4	(16.7)	47.6	(16.7)
Moderate ≥4 h/week	46.4	(16.7)	46.3	(16.8)
No leisure time PA	41.2	(15.9)	43.4	(15.8)
Smoking[3]				
Never smoked	46.4	(16.8)	46.6	(16.8)
Former smoker	47.4	(17.1)	47.1	(17.0)
Current smoker	41.5	(15.5)	43.0	(15.5)
Alcohol consumption[3,5]				
None	42.4	(16.0)	43.9	(16.0)
Moderate	47.1	(16.6)	46.7	(16.5)
High	45.0	(18.1)	46.2	(18.3)
Quintiles of mAHEI[6,7]				
5 (highest)	48.7	(17.0)	48.0	(17.0)
4	48.1	(17.2)	47.6	(17.3)
3	46.3	(17.0)	46.4	(17.0)
2	43.9	(15.9)	44.2	(15.8)
1 (lowest)	41.8	(15.4)	42.8	(15.4)

Abbreviations: SD standard deviation; S-25(OH)D serum 25-hydroxyvitamin D; BMI body mass index; PA Physical activity; mAHEI modified Alternate Healthy eating index; HDL High-density lipoprotein

[1] Model 1: sex, age (continuos), month of blood sampling (September to March) and factor studied

[2] Model 2 further included: marital status (married or cohabiting, divorced, widow/er, unmarried), education (<7, 7–12, >12 years), body mass index (<18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m2), leisure-time physical activity (no, moderate ≥4 h/week, vigorous ≥3h/week), smoking (never, former, current), alcohol consumption (M 0, 1-199, ≥200, F 0, 1-99, ≥100 g/week), total score on mAHEI index (quintiles), blood pressure (normal, elevated), serum HDL cholesterol (M ≤1.0, >1.0, F ≤1.3, >1.3 mmol/l), serum triglycerides(<1.7, ≥1.7 mmol/l), serum fasting glucose (<5.6, ≥5.6 mmol/l)

[3] P-values for heterogeneity <0.01 for both model 1 and model 2

[4] Including both indoor and outdoor activity

[5] None=0g/w, moderate male 1–199, female 1–99 g/w, high= male ≥200, female ≥100

[6] P-values for trend < 0.01 for both model 1 and model 2

[7] Quintiles: M 7–16, 17–19, 20–22, 23–25, 26–34, F 8–16, 17–19, 20–22, 23–25, 26–35

Those who met the criteria of metabolic syndrome had lower mean (43.0 nmol/L [SD 15.7]) S-25(OH)D concentration compared to those who did not meet the criteria (47.1 nmol/L [SD 17.4]). In Model 1, each component of metabolic syndrome was independently associated with S-25(OH)D concentration (Table 13). In Model 2, the inverse association between S-25(OH)D and waist circumference and positive association between S-25(OH)D and serum HDL cholesterol remained statistically significant.

Table 13. The mean S-25(OH)D concentration according to metabolic health-related factors (model-adjusted means and their unadjusted SDs).

	S-25(OH)D concentration					
	Model 1[1]			Model 2[2]		
	Mean	(SD)	P[3]	Mean	(SD)	P[3]
Waist circumference, cm			<0.01			<0.01
M <94, F <80	47.7	(17.9)		47.4[4]	(18.0)	
M ≥94, F ≥80	44.2	(16.1)		45.0	(16.0)	
Blood pressure			<0.01			0.41
Normal	46.2	(16.6)		46.1	(16.7)	
Elevated[5]	44.8	(16.8)		45.7	(16.7)	
HDL cholesterol, mmol/L			<0.01			<0.01
M >1.0, F >1.3	47.1	(17.1)		46.8	(17.1)	
M ≤1.0, F ≤1.3	42.0	(15.4)		43.9	(15.4)	
Triglycerides, mmol/L			<0.01			0.11
<1.7	46.6	(17.1)		46.1	(17.0)	
≥1.7	42.9	(15.8)		45.2	(15.8)	
Fasting glucose, mmol/L			<0.01			0.10
<5.6	45.9	(16.8)		46.1	(16.8)	
≥5.6	44.4	(16.6)		45.3	(16.6)	

Abbreviations: SD standard deviation; S-25(OH)D serum 25-hydroxyvitamin D; HDL High-density lipoprotein; M=male, F=female

[1] Model 1: sex, age (continuos), month of blood sampling (September to March) and factor studied

[2] Model 2 further included: marital status (married or cohabiting, divorced, widow/er, unmarried), education (<7, 7–12, >12 years), body mass index (<18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m2), leisure-time physical activity (no, moderate ≥4 h/week, vigorous ≥3h/week), smoking (never, former, current), alcohol consumption (M 0, 1-199, ≥200, F 0, 1-99, ≥100 g/week), total score on mAHEI index (quintiles), blood pressure (normal, elevated), serum HDL cholesterol (M ≤1.0, >1.0, F ≤1.3, >1.3 mmol/l), serum triglycerides(<1.7, ≥1.7 mmol/l), serum fasting glucose (<5.6, ≥5.6 mmol/l)

[3] P for heterogeneity

[4] Body mass index was excluded and waist circumference (M <94, ≥94, F <80, ≥80 cm) included in the model

[5] Elevated: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or antihypertensive medication. Normal: Not elevated nor medication

5.2 THE CHANGE IN VITAMIN D INTAKE AND VITAMIN D STATUS BETWEEN 2000 AND 2011 (II)

5.2.1 VITAMIN D INTAKE AND USE OF SUPPLEMENTS

In Health 2011, vitamin D intake from diet was 14.1 µg/day (95% CI 13.8, 14.5) in men and 11.7 µg/day (95% CI 11.4, 11.9) in women, which was almost twice as high compared to Health 2000 (men: 7.2 µg/day, 95% CI 7.1, 7.4; women 6.8 µg/day, 95% CI 6.7, 7.0). In Health 2011, 72.3% (95% CI 69.8, 74.7) and 56.4% (95% CI 54.2, 58.6) of men and women, respectively, reached the recommended vitamin D intake ≥10 µg/day. The major dietary contributors for vitamin D intake in Health 2011 were fish (38%), fluid milk products (34%) and fat spreads (10%) whereas in Health 2000 fish contributed 57%, fluid milk products 4% and fat spreads 9%. Further, the prevalence of vitamin D supplement users was four times higher in Health 2011 (41.1 %; 95% CI 39.4, 42.8) compared to Health 2000 (11.4%; 95%CI 10.6, 12.3).

5.2.2 VITAMIN D STATUS

When analysing the change in S-25(OH)D concentration between the years 2000 and 2011, the VDSP-standardised S-25(OH)D concentrations were used (see Methods chapter 4.3.1). The mean S-25(OH)D concentration in 2011 was 65.4 nmol/L, which was 17.8 nmol/L higher compared to year 2000 (Table 14). The prevalences of insufficient S-25(OH)D concentration (< 50 nmol/l) were 55.7% and 9.1% in 2000 and 2011, respectively. Further, in 2000, 13.0% of the population were vitamin D deficient (<30 nmol/L) whereas in 2011 the prevalence was 0.6%.

Among the subpopulation of vitamin D supplement non-users, the mean S-25(OH)D concentration in 2000 was 46.9 nmol/L and in 2011 14.8 nmol/L higher, 61.7 nmol/L (Table 14). The prevalence of insufficient (<50 nmol/L) S-25(OH)D concentration in 2000 was 58.5 nmol/L, whereas in 2011 it was 13.7 nmol/L. In Health 2011, if the main dietary vitamin D sources (fish at least twice a week, fluid milk products and fat spreads daily) were consumed, 90.8% of the supplement non-users reached the serum 25(OH)D concentration of 50 nmol/L.

Table 14. The VDSP-standardised mean concentration of S-25(OH)D and prevalences according to selected cut-offs <30, <50 and ≥75 nmol/L.

	Health 2000		Health 2011	
Total population				
n	6134		4051	
Mean, nmol/L (95%CI)	47.6	(47.2, 48.0)	65.4	(65.0, 65.9)
Prevalences according to selected cut-offs:				
< 30 nmol/L, % (95% CI)	13.0	(12.8, 14.7)	0.6	(0.3, 0.9)
< 50 nmol/L, %, (95% CI)	55.7	(54.3, 57.1)	9.1	(8.1, 10.3)
≥ 75 nmol/L, % (95% CI)	4.1	(3.6, 4.8)	19.9	(18.5, 21.3)
Supplement non-users				
n	4956		2110	
Mean, nmol/L (95%CI)	46.9	(46.5, 47.4)	61.7	(61.1, 62.3)
Prevalences according to selected cut-offs:				
< 30 nmol/L, % (95% CI)	13.7	(12.8, 14.7)	0.9	(0.5, 1.6)
< 50 nmol/L, %, (95% CI)	58.5	(57.1, 60.0)	13.7	(11.9, 15.7)
≥ 75 nmol/L, % (95% CI)	3.8	(3.3, 4.5)	12.6	(11.2, 14.1)

Abbrevitations: VDSP Vitamin D Standardization Program; S-25(OH)D serum 25-hydroxyvitamin D; CI confidence interval

The values are model-adjusted means (nmol/L) or prevalences (%) based on predictive margins adjusted for sex, age (30-44, 45-54, 55-64, 65-74, ≥75 years) and month of blood sampling (September to March in 2000 and August to December in 2011). All p-values for the difference between the Health 2000 and Health 2011 <0.01.

Seasonal variation in S-25(OH)D concentrations was seen in both years, but the difference in the mean concentrations was similar over the months studied (Figure 6).

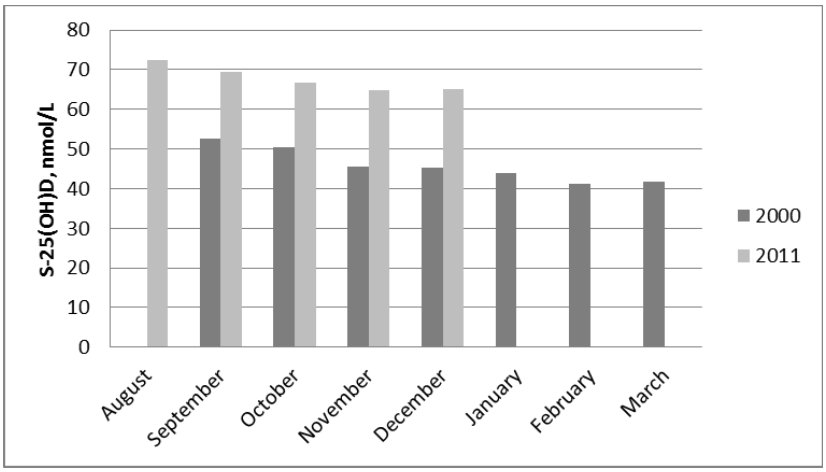

Figure 6. The mean VDSP-standardized S-25(OH)D concentrations adjusted for age and sex according to the month of blood sampling in Health 2000 (2000) and Health 2011 (2011).Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D; VDSP Vitamin D Standardization Program

When analysing the changes in individual-level among those participants whose VDSP-standardised S-25(OH)D concentrations were available in both years (n=3328) S-25(OH)D concentration improved most (34.3 nmol/L) in participants with vitamin D deficiency at baseline and least (11.2 nmol/L) among those whose S-25(OH)D concentration was sufficient (≥ 50 nmol/L) at baseline (Table 15). Parallel results were seen when analysing the subpopulation of supplement non-users.

Table 15. The mean change in VDSP-standardised S-25(OH)D concentration between 2000 and 2011 according to baseline concentration of S-25(OH)D

	S-25(OH)D at baseline, nmol/L		
	<30.0	**30-49.9**	**≥50**
Total population			
n	381	1507	1440
Δ S-25(OH)D	34.3	24.2	11.2
(95% CI)	(32.9, 35.6)	(23.5, 24.8)	(10.6, 11.9)
Supplement non-users			
n	215	723	640
Δ S-25(OH)D	32.2	22.0	9.6
(95% CI)	(30.5, 33.9)	(21.1, 22.8)	(8.7, 10.5)

Abbreviations: VDSP Vitamin D Standardization Program; S-25(OH)D serum 25-hydroxyvitamin D; CI confidence interval

The values are model-adjusted, weighted mean changes in S-25(OH)D adjusted for sex, categorical age at baseline and change in blood sampling season. P-value for heterogeneity <0.01 for both total population and supplement non-users.

5.2.3 THE EFFECT OF VITAMIN D FORTIFICATION TO VITAMIN D STATUS

The effect of vitamin D fortification of fluid milk products was analysed in the subpopulation of supplement non-users who were either fluid milk consumers (n=1017) or non-consumers (n=229) in both study years. Similarly, the effect of vitamin D fortification of fat spread was analysed among supplement non-users who were either fat spread consumers (n=558) or non-consumers (n=436) in both study years.

In 2000, there was no difference in S-25(OH)D concentrations between fluid milk consumers (46.3 nmol/L; 95% CI 45.4, 47.2) and non-consumers (47.8 nmol/L; 95% CI 46.1, 49.4). In 2011, the mean concentration of S-25(OH)D in fluid milk consumers (66.2 nmol/L; 95% CI 65.5, 66.9) was 5 nmol/L higher compared to non-consumers (61.7 nmol/L; 95% CI 59.9, 63.4) (p for the difference <0.01). Thus, the mean change in S-25(OH)D between 2000 and 2011 in daily fluid milk consumers was 6.0 nmol/L higher compared to non-consumers.

In 2000, daily fat spread consumers had higher mean S-25(OH)D concentrations (49.4 nmol/L; 95% CI 48.2, 50.5) compared with non-consumers (43.9 nmol/L; 95% CI 42.5, 45.2). Among non-consumers, the mean change in S-25(OH)D concentrations between 2000 and 2011 was 5 nmol/L higher compared with consumers, and in Health 2011 there was no difference in the mean S-25(OH)D concentration between the groups (data not shown).

5.3 CROSS-SECTIONAL ASSOCIATION BETWEEN VITAMIN D STATUS AND DEPRESSION PREVALENCE (III)

In Health 2000, a total of 115 (4.6%) men and 239 (8.4%) women had diagnosed depressive disorder. Further, 547 (21.7%) men and 857 (30.1%) women had current depressive symptoms (BDI ≥10 points).

In the original analyses of substudy III where the data for men and women were analysed together, vitamin D status was associated with a higher prevalence of depressive disorder when adjusted for sex, age and month of blood sampling (OR for highest vs. lowest quintile 0.56, 95% CI 0.40, 0.78; p for trend <0.01).

Due to the fact that sex modified the association between vitamin D status and depressive disorder (p for interaction <0.01) further analyses of this summary were stratified by sex. The results of the stratified analyses showed that in men, low vitamin D status was associated with a higher prevalence of depressive disorder when adjusted for age (Model 1) (OR for the highest vs. lowest quintile 0.34, 95% CI 0.19, 0.60; p for trend <0.01) (Table 16). After further adjustment for sociodemographic and lifestyle factors (Model 2), this association was slightly attenuated but remained significant (OR for the highest vs. lowest quintile 0.51, 95% CI 0.29, 0.91; p for trend 0.01). Further, low vitamin D status was associated with a higher prevalence of current depressive symptoms among men in Model 1 (OR for the highest vs. lowest quintile 0.59, 95% CI 0.45, 0.77; p for trend <0.01) but in Model 2 this association attenuated to non-significant (OR for the highest vs. lowest quintile 0.79, 95% CI 0.59, 1.05; p for trend 0.13).

The results of the further stratified analyses showed that in women, there were no significant associations between vitamin D status and depressive disorder (OR for the highest vs. lowest quintile 0.75, 95% CI 0.51, 1.10; p for trend 0.12) or current depressive symptoms (OR for the highest vs. lowest quintile 0.82, 95% CI 0.65, 1.04; p for trend 0.10) in Model 1 and these associations were further attenuated after adjustment for sociodemographic and lifestyle factors.

Table 16. Diagnoses of depressive disorder and depressive symptoms by quartiles of S-25(OH)D concentration (Odds ratios and 95% confidence intervals)

	S-25(OH)D quartiles[1]							
	1	2		3		4		p for trend
	ref.	OR	95% CI	OR	95% CI	OR	95% CI	
Men								
Depressive disorder, *n*	*47*	*31*		*20*		*17*		
Model 1[2]	1	0.62	0.39, 0.99	0.41	0.24, 0.70	0.34	0.19, 0.60	<0.01
Model 2[3]	1	0.73	0.45, 1.18	0.55	0.32, 0.95	0.51	0.29, 0.91	0.01
Depressive symptoms, BDI ≥ 10, *n*	*165*	*123*		*124*		*135*		
Model 1	1	0.62	0.47, 0.81	0.59	0.45, 0.79	0.59	0.45, 0.77	<0.01
Model 2	1	0.69	0.52, 0.91	0.71	0.54, 0.94	0.79	0.59, 1.05	0.13
Women								
Depressive disorder, *n*	*70*	*65*		*56*		*48*		
Model 1[2]	1	1.02	0.71, 1.45	0.91	0.63, 1.31	0.75	0.51, 1.10	0.12
Model 2[3]	1	1.14	0.79, 1.65	1.02	0.69, 1.50	0.88	0.59, 1.33	0.50
Depressive symptoms, BDI ≥ 10, *n*	*226*	*213*		212		*206*		
Model 1	1	0.98	0.78, 1.23	0.94	0.74, 1.18	0.82	0.65, 1.04	0.10
Model 2	1	1.08	0.85, 1.36	1.07	0.84, 1.36	0.98	0.77, 1.25	0.89

Abbreviations: OR odds ratio; CI confidence interval; S-25(OH)D serum 25-hydroxyvitamin D; BDI Beck Depression Inventory

Men total n=2524, Women total n=2847

[1] Men: Q1 8-33, Q2 34-43, Q3 44-55, Q4 56-132 nmol/L; Women: Q1 7-34, Q2 35-43, Q3 44-54, Q4 55-134 nmol/L

[2]Model 1: sex, age (continuous)

[3]Model 2: further included marital status (being married vs. divorced, widow/er, unmarried), education (high education vs. middle or low), body mass index (< 30, ≥ 30 kg/m²), leisure-time physical activity (yes, no), smoking (yes, no), moderate (in men 1-199 g/week, in women 1-99 g/week) alcohol consumption

5.4 LONGITUDINAL ASSOCIATIONS BETWEEN VITAMIN D STATUS AND WEIGHT GAIN AND INCREASE IN WAIST CIRCUMFERENCE BETWEEN 2000 AND 2011 (IV)

In men, there was a borderline significant inverse association between low vitamin D status at baseline and ≥10% weight gain during the follow-up, when adjusted for age (OR for the highest vs. lowest quintile 0.67, 95% CI 0.40, 1.11; p for trend 0.03) (Table 17). After further adjustment for potential confounders, this association was slightly attenuated (OR for the highest vs. lowest quintile 0.77, 95% CI 0.45, 1.32; p for trend 0.09). In men, low vitamin D status at baseline predicted a ≥10% increase in waist circumference when adjusted for age (OR for the highest vs. lowest quintile 0.43, 95% CI 0.25, 0.73; p for trend <0.01). After further adjustment for potential confounders the association remained statistically significant (OR for the highest vs. lowest quintile 0.46, 95% CI 0.26, 0.81; p for trend <0.01).

In women, vitamin D status at baseline was not associated with either a ≥10% weight gain (OR for highest vs. lowest quintile 0.81, 95% CI 0.55, 1.18; p for trend 0.32) or a ≥10% increase in waist circumference (0.82, 95% CI 0.56, 1.20; p for trend 0.23) when adjusted for age. Further adjustment for sociodemographic and lifestyle factors did not notably change the results.

Table 17. *Weight gain and increase in waist circumference during the follow-up according to serum 25-hydroxyvitamin D concentration at baseline (Odds ratios and 95% confidence intervals)*

	S-25(OH)D quintiles at baseline[1]									
	1	**2**		**3**		**4**		**5**		**p for trend**
	ref.	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Men										
Weight gain ≥10% , *n*	*49*	*39*		*24*		*22*		*29*		
Model 1[2]	1.00	0.81	0.50, 1.32	0.49	0.28, 0.83	0.48	0.28, 0.84	0.67	0.40, 1.11	0.03
Model 2[3]	1.00	0.91	0.56, 1.49	0.56	0.32, 0.97	0.55	0.31, 0.97	0.77	0.45, 1.32	0.09
WC increasing ≥ 10%, *n*	*53*	*48*		*29*		*18*		*24*		
Model 1	1.00	0.91	0.58, 1.43	0.51	0.31, 0.84	0.33	0.18, 0.58	0.43	0.25, 0.73	<0.01
Model 2	1.00	0.96	0.60, 1.53	0.57	0.34, 0.95	0.36	0.20, 0.65	0.46	0.26, 0.81	<0.01
Women										
Weight gain ≥10% , *n*	*99*	*84*		*63*		*76*		*63*		
Model 1	1.00	0.92	0.65, 1.31	0.73	0.50, 1.07	0.94	0.65, 1.35	0.81	0.55, 1.18	0.32
Model 2	1.00	0.89	0.62, 1.29	0.81	0.55, 1.19	1.01	0.70, 1.47	0.92	0.62, 1.36	0.89
WC increasing ≥ 10%, *n*	*87*	*88*		*59*		*74*		*62*		
Model 1	1.00	1.09	0.77, 1.56	0.73	0.50, 1.07	0.96	0.67, 1.39	0.82	0.56, 1.20	0.23
Model 2	1.00	1.07	0.75, 1.55	0.77	0.52, 1.15	1.00	0.69, 1.46	0.89	0.60, 1.32	0.50

Abbreviations: S-25(OH)D serum 25-hydroxyvitamin D; OR odds ratio; CI confidence interval; WC waist circumference
Men total n=1342, Women total n=1582
P value for interaction between sex and S-25(OH)D at baseline <0.001 for ≥10% increase in waist circumference.
[1]Men: 8-32, 33-40, 41-48, 49-58, 59-121; Women: 8-32, 33-39, 40-47, 48-57, 58-123
[2]Model 1: age (30-39, 40-49, 50-59, 60-69, 70-79) at baseline
[3] Model 2: further included marital status (being married vs. other), education (high education vs. other), leisure-time physical activity (yes, no), smoking (yes, no), moderate (in men 1-199 g/week, in women 1-99 g/week) alcohol consumption (yes, no) at baseline

6 DISCUSSION

6.1 VITAMIN D INTAKE AND STATUS IN FINLAND IN THE 2000s

In the beginning of the 2000s, vitamin D intake in Finland was quite low, 5.8 and 3.8 μg/day in men and women, respectively (Männistö et al. 2003). Further, a study carried out in a subpopulation of young adults from Southern Finland showed that vitamin D status, measured as S-25(OH)D concentration, was low (Lamberg-Allardt et al. 2001). The results of the present study based on nationally-representative, VDSP-standardised S-25(OH)D data from the Finnish population confirm this finding by showing that in 2000 only 44% the population reached sufficient vitamin D status, 50 nmol/L.

During the 2000s, nutritional policy acts to improve vitamin D intake and status at the population level were implemented in Finland. First, a systematic fortification of fat spreads and fluid milk products with vitamin D was initiated in the beginning of 2003. Fat spreads were already widely fortified on a voluntary basis before systematic fortification policy at the level of 5.0-10.0 μg/100 g (Suojanen 2003), but in 2003 the fortification level was established at 10.0 μg/100 g (Ministry of Trade and Industry of Finland 2002). Fluid milk products were an entirely novel product to fortify with vitamin D at the level of 0.5 μg/100 g. Second, the nutrition recommendations for vitamin D intake were increased in 2005 from 5.0 μg/day to 7.5 μg/day (National Nutrition Council 2005). Finally, in 2010 the recommendations for the fortification levels were doubled so that the fortification levels for fat spreads and fluid milk products were 10.0 μg/100 g and 1.0 μg/100 g, respectively (National Nutrition Council 2010). This was due the finding that the new recommendation for vitamin D intake was not reached at the population level (Paturi et al. 2008). Further, a study including different subsets of the Finnish population showed that about one-third of vitamin D supplement non-users still had insufficient vitamin D status (Lamberg-Allardt and Viljakainen 2006).

The present study showed that the vitamin D status of Finnish adults has improved remarkably between the years 2000 and 2011. In 2011, only 9% of the population had insufficient vitamin D status. These findings were in line with another Finnish population-based study, which was based on independent cross-sectional samples (Raulio et al. 2017). Due to the prospective design of the present study, there was a unique opportunity to compare the vitamin D status of participants before and after nutritional policy acts. The important finding was that vitamin D status had improved especially among those having vitamin D deficiency or insufficiency in 2000. Further, slight differences between sociodemographic groups observed in

2000 were attenuated, and most importantly, almost all of the adult population reached sufficient vitamin D status, 50 nmol/L, in 2011.

The results showed that, in 2011, if fat spreads and fluid milk products were consumed daily and fish at least twice a week (the main dietary sources of vitamin D) over 90% of the population was able to reach sufficient vitamin D status without the use of vitamin D supplements. Further, vitamin D intake from diet was doubled between 2000 and 2011, being 14 and 12 µg/d in men and women, respectively, in 2011. Thus, the fortification level in Finland seems to be sufficient and the use of vitamin D supplements is not routinely needed among the general adult population. Unless vitamin D sources are not consumed as mentioned above, supplementation (10 µg/day) is recommended for the working-aged population from October to March (National Nutrition Council 2014).

The contribution of fat spreads to vitamin D intake remained at the same level (about 10%) during the study period. This is probably due to the fact that fat spreads were already fortified at the level of 5.0-10.0 µg/100 g on a voluntary basis before systematic fortification policy started (Suojanen 2003). The contribution of fluid milk products as a novel vitamin D source for vitamin D intake increased from 4% to 34% between 2000 and 2011. Further, the increase in vitamin D status between 2000 and 2011 was 6 nmol/L higher in fluid milk consumers compared to non-consumers.

It has generally been suspected that fortification of single staples does not remarkably increase vitamin D intake at the population level (Hayes and Cashman 2017). For example in Canada, where the fortification of milk products and fat spreads is mandatory and at approximately at the same level as is recommended in Finland, both vitamin D intake and vitamin D status are still insufficient (Vatanparast et al. 2010). One explanation for the successful fortification policy in Finland might be that milk products are also widely consumed in adult population (Helldan et al. 2013) and thus they were a good choice to fortify with vitamin D. Nowadays, both vitamin D intake and status seem to be quite high in Finland compared to many countries in Europe, especially compared to Southern Europe (Spiro and Buttriss 2014). In addition to Finland, also in other Nordic countries, vitamin D status has been reported to be at quite sufficient levels (Cashman et al. 2015; Ramnemark et al. 2015). By way of exception to other Nordic countries, however, in Denmark food fortification with vitamin D has not yet been widely implemented (Grønborg et al. 2018). It should also be noted that comparing results between countries is challenging due to different time-periods and seasons of data collection and differences in food intake and laboratory methods.

It is obvious that systematic vitamin D fortification policy has improved the vitamin D intake and status of Finnish adults between 2000 and 2011, but other factors have also had a role on the observed improvement. The present study showed that the proportion of vitamin D supplement users had increased from 11% to 41% during the 2000s. Similar results have been found

in another Finnish study comparing cross-sectional samples of adults in 2002, 2007 and 2012, which showed that the proportion of supplement users had increased especially between the years 2007 and 2012 (Raulio et al. 2017). Further, some other factors such as increased travelling and higher amounts of UVB -radiation in 2011 compared to 2000 (Lakkala et al. 2008; Mäkelä et al. 2016) may somewhat explain the results. Further, the non-systematic fortification of some single foods other than fluid milks and fat spreads may have slightly increased vitamin D intake and status. Finally, some season-related methodological limitations should also be taken into account (see chapter 6.4.2).

6.2 DETERMINANTS OF VITAMIN D STATUS

In the present study, no association between vitamin D status and sex was found. This finding is in line with two studies conducted in the US and Norway (Freedman et al. 2013; Larose et al. 2014), but two other studies carried out in Finland and France have shown that vitamin D status is lower among women compared to men (Palaniswamy et al. 2017; Touvier et al. 2015). The potential relationship between vitamin D status and age is also still controversial. In line with the results of this study, two studies conducted in Finland (Miettinen et al. 2014) and the US (Freedman et al. 2013) have reported positive association, but an inverse association (Touvier et al. 2015) and a non-significant (Larose et al. 2014) association have also been shown. Further, in the present study, vitamin D status was higher among participants who were married or cohabiting compared to those in other marital status groups. The previous evidence on the association between vitamin D status and marital status is limited to one German study showing similar results (Hintzpeter et al. 2008). However, in contrast to findings of the present study that high education is positively associated vitamin D status, the majority of previous studies have not found association between vitamin D status and education (Touvier et al. 2015; Larose et al. 2014; Miettinen et al. 2014). One explanation for the conflicting results concerning the associations between vitamin D status and sociodemographic factors may be differences in study populations, for example different age groups studied, or variety in sample sizes.

The present study showed a positive association between vitamin D status and healthy lifestyle. All of the five components (BMI, physical activity, smoking status, alcohol consumption and diet) of the lifestyle index were also independently associated with vitamin D status, but the difference between those with the healthiest and unhealthiest lifestyles, as determined by the lifestyle index, was remarkably higher than observed when examining the single components. This finding suggests that higher vitamin D status indicates a generally healthy lifestyle. The inverse association between vitamin D status and obesity as well as positive association between vitamin

D status and physical activity has also been consistently shown in other studies (Palaniswamy et al. 2017; Rabenberg et al. 2015; Larose et al. 2014). In the present study current smokers had a lower vitamin D status compared to non-smokers. This finding is in line with some other studies (Miettinen et al. 2014; Larose et al. 2014), but non-significant associations have also been reported (Palaniswamy et al. 2017; Touvier et al. 2015), leaving this issue slightly controversial. Regarding alcohol consumption, in the present study, vitamin D status was lowest among abstainers and highest among moderate consumers. However, the difference in vitamin D status between moderate and high consumers was small after adjustment for potential confounders. In three other studies (Palaniswamy et al. 2017; Touvier et al. 2015; Larose et al. 2014), vitamin D status was lowest among abstainers and increased with increasing consumption of alcohol.

The present study is one of a few studies which have investigated the association between vitamin D status and the quality of diet, estimated with the mAHEI–index. A clear trend was found: the higher the score on the mAHEI–index, indicating a healthier diet, the higher the vitamin D status. Similar results have been found in one other study conducted in Finland where the quality of diet was estimated with a 5-item (i.e., red meat, rye or crisp bread, berries or fruit, salads and vegetables) dietary index (Palaniswamy et al. 2017). These findings support the hypothesis that not only high dietary intake of vitamin D sources but also a generally healthy diet including for example high consumption of vegetables and fruits, is positively associated with vitamin D status.

Regarding metabolic health, both present and previous studies have quite consistently indicated that S-25(OH)D concentration is positively associated with serum HDL-cholesterol (Maki et al. 2012; Cheng et al. 2010; Jorde et al. 2010b; Jorde et al. 2010a). Some earlier studies have also shown an inverse association between vitamin D status and blood pressure, serum triglycerides and fasting glucose (Cheng et al. 2010; Jorde et al. 2010b; Jorde et al. 2010a). In the present study, however, these associations were not statistically significant after controlling for potential confounders.

The potential mechanisms behind the associations summarised above are mainly unclear. Concerning obesity, there are many hypotheses explaining the inverse association, such as decreased bioavailability of vitamin D in obese subjects (Wortsman et al. 2000). Further, it is probable that physical activity is related to time spent outdoors. It is also probable that potential determinants are associated with each other, for example it has been proposed that the association between vitamin D status and BMI is actually due to differences in physical activity and dietary habits between normal weight and obese subjects (Pourshahidi 2015). Furthermore, the associations are complex. For example, despite the biological fact that aging decreases the skin's capacity to synthesise vitamin D (MacLaughlin and Holick 1985), in Health 2000, fish consumption (Montonen et al. 2008) and health-promoting physical activity (Aromaa and Koskinen 2004) were more

frequent among older age groups compared to younger groups, probably explaining the observed positive association between vitamin D status and age. Sociodemographic differences in vitamin D status may also reflect general population group differences in health, which have been observed for example between marital status groups (Joutsenniemi et al. 2006).

Regarding the components of metabolic health, some plausible biological mechanisms explaining the associations with vitamin D status have been presented. For example, it has been suggested that vitamin D regulates the renin–angiotensin system which has a role in the regulation of blood pressure (Li et al. 2002). However, as found in the present study, adjustment for potential confounding factors weakened the observed associations between vitamin D status and components of metabolic health, suggesting that they may also be due to confounding. Finally, there are also differences in vitamin D-related gene expression and molecular response to vitamin D intake. A 5-month RCT with high-dose vitamin D supplementation among 71 pre-diabetic participants showed that about half of the study population were considered conventional responders to vitamin D, whereas the other half were categorised as lower responders (Carlberg et al. 2013).

In conclusion, it seems that vitamin D status is positively associated with a generally healthy lifestyle and may also indicate favourable sociodemographic status and good metabolic health. These factors are also well-known to be protective factors for many chronic diseases and public health concerns. For example, this study is one of the first to show that a generally healthy diet is associated with higher vitamin D status. Both high quality of diet (Fung et al. 2007) and high vitamin D status (Mattila et al. 2007) have been shown to be associated with lower risks of type 2 diabetes, for instance. Thus, it is possible that sociodemographic, lifestyle and metabolic health-related determinants of vitamin D status may, at least partly, explain the hypothesised associations between vitamin D status and health outcomes. For this reason, potential confounding factors should be carefully taken into account when studying the health effects of vitamin D.

6.3 HEALTH EFFECTS OF VITAMIN D

Vitamin D has a key role in regulating calcium and phosporus metabolism and is essential for musculoskeletal health (Holick 2007). During recent decades, low vitamin D status has also been proposed to be associated with many non-skeletal health outcomes (Holick 2007), but the evidence is still mainly controversial (Lamberg-Allardt et al. 2013).

6.3.1 VITAMIN D STATUS AND DEPRESSION

In the present study, low vitamin D status was associated with a higher prevalence of depressive disorder and current depressive symptoms in men

when adjusted for age only. The association with depressive disorder was slightly attenuated after adjustment for sociodemographic and lifestyle factors, but remained statistically significant. The association between vitamin D status and current depressive symptoms, however, attenuated to non-significant after further adjustments. In women, low vitamin D status was not associated with either depressive disorder or current depressive symptoms.

Parallel to the present findings, two population-based cross-sectional studies conducted in China and the US have reported a lack of association between vitamin D status and depressive symptoms after controlling for potential confounders (Pan et al. 2009; Zhao et al. 2010). In contrast to these findings, however, a large population-based cross-sectional study carried out in Norway has shown that low vitamin D status was also associated with higher prevalence of depressive symptoms after adjustment for potential confounders (e.g., chronic diseases, BMI, physical activity, smoking and alcohol consumption) (Kjaergaard et al. 2011). Further, contrary to the findings of the present study, the association in the Norwegian study was stronger among women compared with men. Also, a meta-analysis of ten cross-sectional studies concluded that there is an inverse association between vitamin D status and depression (Anglin et al. 2013). However, it should be taken into account that the majority of the studies included in the meta-analysis were carried out among older adults. The major limitation of the cross-sectional studies is that any conclusions about temporal aspects or causality cannot be drawn. For example, in the recent German population-based cross-sectional study, the inverse association between vitamin D status and depressive symptoms was significant only in the summer, not in the winter, thus, the authors proposed that vitamin D may be a consequence of depression rather than a cause of it (Rabenberg et al. 2016). They suggested that depressed persons spent less time outdoors leading to less exposure to UVB -radiation and a lower vitamin D status.

The evidence based on cohort studies regarding vitamin D and depression is also inconsistent. Three cohort studies (Milaneschi et al. 2010; May et al. 2010; Williams et al. 2015), all carried out among the elderly, have found an inverse association between vitamin D status and incident depression, whereas three other studies (Chan et al. 2011; Husemoen et al. 2016; Jovanova et al. 2017) have reported no association. It should be noted, however, that the results of May et al. (2010) were based on a subpopulation of cardiovascular patients and Milaneschi et al. (2010) reported a significant association only for women. Further, the results of RCTs are somewhat mixed and the recent meta-analysis (Gowda et al. 2015) does not overall support the hypothesis that vitamin D supplementation has a significant effect on depressive disorder or depressive symptoms. Although there are some hypotheses about biological mechanisms behind the potential association, for example the presence of vitamin D-activating enzyme and

vitamin D receptors in the brain and central nervous system (Eyles et al. 2005), the evidence on the potential presence of exact mechanisms is limited.

In conclusion, the present evidence does not consistently support the hypothesis that low vitamin D status is a risk factor for depression. The findings of the present study demonstrated the importance to comprehensively take into account potential effect modifying and confounding factors when studying the association between vitamin D status and depression.

6.3.2 VITAMIN D STATUS AND OBESITY

The present study showed that low vitamin D status at baseline predicted the increase in waist circumference during an 11-year follow-up among men. The association with weight gain was weaker and was further attenuated after adjustment for potential confounders. In women, vitamin D status was not associated with an increase in waist circumference or weight gain.

The evidence based on cohort studies is inconsistent. In line with the present findings, in a Norwegian population-based cohort study, low vitamin D status at baseline predicted incident obesity, measured as an increase in waist circumference, during an 11-year follow-up (Mai et al. 2012). However, contrary to the present findings, in the Norwegian study, the results were presented for both sexes and were also significant when using the increase in BMI as an outcome. Further, low vitamin D status at baseline has been shown to predict weight gain and increases in BMI in a cohort study carried out in Southern Spain (Gonzalez-Molero et al. 2013). In accordance with the findings of the present study, however, a lack of association between vitamin D status and weight gain or increase in BMI has been reported (Young et al. 2009; LeBlanc et al. 2012; Lehtinen-Jacks and Agelii et al. 2016; Vogt et al. 2016). Furthermore, the results from RCTs are inconsistent and overall have not shown that vitamin D supplementation affects BMI, body weigth or fat mass (Chandler et al. 2015; Pathak et al. 2014). It should also be noted that the duration of the interventions and doses of vitamin D supplementation varied widely between the RCTs. In two meta-analyses including 18 and 26 RCTs, the duration of the interventions ranged from 4 weeks to 3 years (Pathak et al. 2014) and from 3 months to 7 years (median duration: 12 months) (Chandler et al. 2015). Daily doses of vitamin D supplements ranged from under 10 μg/day to over 100 μg/day.

Cross-sectional studies have quite consistently shown the inverse association between vitamin D status and obesity (Saneei et al. 2013; Pereira-Santos et al. 2015), but it is still unclear whether low vitamin D status induces obesity or vice versa. Some cohort studies have examined the association in reverse order (i.e., whether obesity predicts vitamin D deficiency). Interestingly, in the Spanish study, which concluded that vitamin D deficiency is a risk factor for developing obesity, obesity at baseline did not predict vitamin D deficiency during the 4-year follow-up (Gonzalez-Molero et

al. 2013). However, contrary to this finding, BMI at baseline was inversely associated with change in vitamin D status during a 2-year follow-up among 866 Puerto Rican adults living in Boston (Jamal-Allial et al. 2014). Furthermore, results based on the Norwegian Tromsø study have shown that the increase in BMI was associated with a decrease in vitamin D status and vice versa (Jorde et al. 2010c). Therefore, based on results of the studies summarised in the two previous paragraphs, one possibility is that the association between vitamin D status and obesity may work both ways, vitamin D status as a predictor of obesity and vice versa. Growing evidence of genetic markers related to vitamin D may clarify the controversial cause–effect directionality between vitamin D status and obesity. A recently published bi-directional Mendelian randomisation analysis, for instance, suggested that high BMI leads to vitamin D deficiency rather than vice versa (Vimaleswaran et al. 2013).

The mixed results of the cohort studies may be partly explained by the fact that the comparison of them is challenging. First, instead of a general adult population, some of them were carried out among older adults (Vogt et al. 2016; LeBlanc et al. 2012) or ethnic subgroups (Young et al. 2009; Jamal-Allial et al. 2014). Second, measurement and classification of both vitamin D status and obesity vary widely between the studies. In the present study 5% and 10% weight gain and increase in waist circumference were used as cut-off -points because it has been proposed that 5-10% weight loss promotes health in obese subjects (World Health Organization 2000). Third, there is also variability in the length of follow-up times and sample sizes of the studies. Finally, although sex and age are usually taken into account in the analyses, the potential confounding factors incuded in the models vary between the studies.

The present study suggested that if there is an association between vitamin D status and obesity, it is particularly related to abdominal obesity, which has many harmful health effects (Huxley et al. 2010). This is supported by the cross-sectional findings from the Framingham Heart Study, which concluded that low vitamin D status was especially associated with visceral fat (Cheng et al. 2010). However, there is no explanation for the present finding that the association between low vitamin D status and increase in waist circumference was significant only for men, pointing out the possibility that there is some residual confounding which could not be controlled for, especially in men.

In conclusion, the association between vitamin D status and obesity seems to be complex. The inverse association based on cross-sectional studies is quite consistent, but the temporal aspects and possible cause–effect directionality are unclear. Further, if low vitamin D status predicts obesity, it is probable that the etiology behind the association is multifactorial, including the genetic, metabolic and environmental factors summarised by Pourshahidi et al. (2015) and Earthman et al. (2012). The present finding that vitamin D status may be especially related to an

increased risk of abdominal obesity might support the presence of some biological mechanisms related to the regulation of adipose tissue. However, as the present study has indicated, vitamin D status is positively associated with a healthy lifestyle and it is likely that this at least partly explains the association.

6.4 PUBLIC HEALTH IMPORTANCE OF VITAMIN D

In the beginning of the 2000s, the vitamin D status of Finnish adults was insuffucient due to low dietary intake and limited exposure to sunlight especially in the winter time. At the same time, there were an increasing number of observational studies suggesting that vitamin D insufficiency may have many non-skeletal harmful health effects. Thus, vitamin D insufficiency was considered as a potential public health concern in Finland. As a result, during the 2000s, nutritional policy acts, including the fortification of food with vitamin D and increased recommendation for vitamin D intake, were made to improve the vitamin D status of the Finnish population.

The results of the present study showed that both vitamin D intake from food and supplements and the vitamin D status of Finnish adults has increased remarkably between 2000 and 2011. Thus, it seems that in the general adult population vitamin D status is now at a sufficient level. However, it should be taken into account that there are still specific risk groups for vitamin D deficiency, such as certain immigrant groups whose vitamin D status has been shown to be insufficient (Castaneda et al. 2012).

In the present study, the association between vitamin D status and two major public health concerns in Finland, depression and obesity, was examined based on a large population-based sample. The results tend to attenuate after controlling for potential confounders, such as lifestyle factors, and they do not consistently indicate that low vitamin D status is a risk factor for depression or obesity. Further, despite the large number of studies focusing on possible health effects of vitamin D during recent decades, a recent systematic literature review concluded that the evidence for the health–promoting effects of vitamin D is only consistent for bone health, total mortality and the risk of falling (Lamberg-Allardt et al. 2013). The results from the recently published meta-analyses of RCTs are parallel: they do not overall support the hypothesis that supplementation with vitamin D will have large-scale non-skeletal health effects (Rejnmark et al. 2017; Autier et al. 2017). However, the authors pointed out some methodological limitations, such as small sample sizes and the fact that most RCTs are conducted in populations with sufficient vitamin D status suggesting the need for large-scale RCTs (Rejnmark et al. 2017). Overall, it could be concluded, but with caution, that the non-skeletal health effects of vitamin D may be more limited than it has been hypothesised during recent decades. From the public health point of view, it is important to maintain sufficient

vitamin D status at the population level in Finland, which is essential for bone health. However, evidence that vitamin D contributes in the prevention of other public health concerns is still limited.

6.5 METHODOLOGICAL ISSUES

6.5.1 STUDY POPULATION AND DESIGNS

The major strength of the present study was that it was based on a large, nationally representatative sample of Finnish adults (Heistaro 2008; Lundqvist and Mäki-Opas 2016) allowing the generalisability of the results to the entire Finnish adult population. However, it should be taken into account that substudies I-IV of the thesis are based on different subpopulations of those participants whose S-25(OH)D concentrations were determined and non-participation may slightly decrease representativeness to entire Finnish population. High participation rates are important to reduce the potential biases caused by non-participation. In Health 2000, the participation rate in the health examination was high, 84%, and S-25(OH)D concentrations were available for 76% of the members of the sample aged 30 years of age and older. In Health 2011, the corresponding figures were lower, 59% and 50%. In 2011, non-participation was especially common among men, in younger age groups and in lower education groups (Härkänen et al. 2016). In the present study, inverse probability weights were used to handle this missing data and to reduce potential bias caused by the increased non-participation from 2000 to 2011 (Härkänen et al. 2016). However, this method corrects only the distribution of known background variables (eg. earlier mentioned sex, age and education) among participants to match the distribution in the population. Thus, it is possible that increased non-participation may have potentially caused bias to the results.

Substudies I and III were conducted using a cross-sectional design, and therefore, the temporal order of exposure and outcome remains unclear. The longitudinal design of substudies II and IV provided the opportunity to evaluate temporal aspects.

6.5.2 ANALYSES OF SERUM 25(OH)D

In the present study, S-25(OH)D concentration was used as a biomarker of vitamin D status, reflecting both vitamin D produced in the skin and obtained from the diet and supplements. S-25(OH)D is a widely used and accepted metabolite to indicate vitamin D status due to its relatively long half-life and high serum concentration (Holick 2009). However, there are still some methodological challenges related to seasonal variation, laboratory analyses and storing of the serum samples.

First, because cutaneous synthesis of vitamin D is a remarkable source of vitamin D under ideal conditions (Holick 2003), notable seasonal variation in vitamin status was seen. The serum samples used in the present study were taken between September 2000 and March 2001 and between August and December 2011. In Health 2011, a higher proportion of blood samples were drawn closer to summer (i.e., Aug-Oct) compared to Health 2000, and among those who had serum samples available for both years, the samples were taken within the same season (same month or ± one month) for 53% of the participants. In substudy II, we compared the vitamin D status of the study population between 2000 and 2011. Thus, the ideal would have been that serum samples had been taken within the same season in both surveys. When comparing population averages in 2000 and 2011, we controlled for this by using a joint dataset of Health 2000 and Health 2011 as a standard population and by including the sampling season in the model. The individual-level analyses among those participants who had S-25(OH)D concentrations available in both study years were adjusted for the change in blood sampling season. However, despite these adjustments, this methodological limitation may explain a minor part of the observed increase in S-25(OH)D between the study years.

S-25(OH)D was measured only once in both surveys and single measurements do not provide any information on intra-individual seasonal variation during a year. However, in substudy I, the month of blood sampling season was included in the model, and showed that it had no remarkable effect on the results. In the analyses of substudies III-IV in this summary, the month of blood sampling was not included in the model because it did not meet the the criteria of confounding. It is still possible that intra-individual seasonal variation may have some minor confounding effect on our results. In the ideal situation, S-25(OH)D concentration would have been measured repeatedly.

Second, it is well-known that S-25(OH)D concentrations may vary according to the laboratory method used (Sempos et al. 2012). Thus, the major advantage of the present study was that although different laboratory methods were used in 2000 and 2011, the VDSP-standardised S-25(OH)D concentrations were used when the results from 2000 were compared with the results from 2011 (Cashman et al. 2015). Standardised S-25(OH)D concentrations are more comparable to each other and also internationally to VDSP-standardised samples from other countries.

Third, blood samples were collected at the health examination, processed to serum and stored at -70°C before analysing S-25(OH)D concentrations in both surveys. S-25(OH)D is shown to be stable during the storage at -20°C (Ocke et al. 1995) as well as during multiple freeze–thaw cycles (Antoniucci et al. 2005), but it is possible that S-25(OH)D concentrations have slightly declined during storage.

Finally, it should be taken into account that as presented earlier, the vitamin D status of the study population has improved remarkably during

the follow-up. This may slightly have affected the results when the association between vitamin D status at baseline and weight gain and increase in waist circumference were examined (substudy IV). However, vitamin D status in Finland has been low decades before this improvement (Kilkkinen et al. 2009). Further, recommended levels for the fortification of fat spreads and fluid milk products were doubled in 2010, just before the follow-up time ended.

6.5.3 OTHER MEASUREMENTS

The strength of this study was that the Health 2000/2011 Survey included a large amount of measurements conducted according to standardised methods at both baseline and follow-up (Heistaro 2008; Lundqvist and Mäki-Opas 2016). There was the possibility to first analyse a large variety of potential determinants of S-25(OH)D and then widely take into account potential confounding and effect modifying factors when examining the health effects of vitamin D. However, it is still possible that all potential confounding factors could not be revealed and some residual confounding remains. This is one explanation for the findings of the present study that the associations between vitamin D status and depressive disorder and increase in waist circumference were significant only for men.

In Health 2000, the quality maintenance and control was carefully taken into account (Heistaro 2008). The designs used in the quality assessment included repeated measurements, parallel measurements, reference measurements and standard measurements. In addition to continuous quality control specific quality days were also organised. Although the quality assurance in 2011 was not as systematic as in 2000 due to limited resources, quality assessment of the key measurements has been shown to be satisfactory (Lundqvist and Mäki-Opas 2016).

Food and nutrient intake was estimated with the FFQ, which is considered a standard method for large epidemiological studies (Willett 2012). The FFQ in the present study was modified for the Health 2000/2011 Survey from the questionnaires used in two other surveys in Finland (Pietinen et al. 1988; Männistö et al. 1996), with the main aim to rank participants according to their food consumption and nutrient intake (Paalanen et al. 2006). The main limitations of the FFQ method are related to the facts that the method is based on the respondents' memory and the list of food items is pre-defined, possibly excluding some food items. In Health 2000/2011, the list of food items, however, was quite comprehensive including 128 items in 2000 and 131 items in 2011. In Health 2000, the validity of the FFQ was estimated by using a 3-day food record as the reference method in a subpopulation of 294 participants (Paalanen et al. 2006). The results showed that overall the mean nutrient intakes were higher when measuring with the FFQ compared to the food record, especially in women. Concerning nutrient intake, the energy-adjusted Pearson correlation

coefficients varied from 0.14 to 0.66 in men and from 0.20 to 0.70 in women. Concerning food consumption, the equivalent values were 0.09 to 0.89 and 0.01 to 0.85 in men and women, respectively. The validation study concluded that despite the tendency of the FFQ to overestimate nutrient intake, the correlation coefficients were at the same level as measured in other studies. Further, in 2000, the repeatability of the FFQ was estimated by repeating the questionnaire 5-9 months after the first questionnaire in a subpopulation of 180 participants (Montonen et al. 2008). The results showed that reliability coefficients ranged between 0.32-0.68 for food groups and 0.40-0.69 for nutrient intake. The study concluded that the repeatability is at the same level as in other studies and could be considered sufficient for the purposes of epidemiologic research. Further, there are some limitations related to dietary variables used in substudy II. Sour whole milk was included as a fluid milk product in the analyses, although it is not usually fortified with vitamin D. Organic products are not fortified with vitamin D, but they could not be excluded from the analysis. Finally, there are some limitations regarding the mAHEI-index used. The mAHEI -index was modified from the AHEI -index (McCullough et al. 2002), but its validity in the Finnish population has not been estimated. For example, the inclusion of trans fat intake, which is a minor problem in the Finnish diet, could be criticised.

The strength of the study was that the main health outcomes, depression and indicators of obesity, were measured with reliable methods. The diagnosis of depressive disorder was based on CIDI, a structured mental health interview developed by the WHO especially for epidemiologic studies (World Health Organization 1990). The quality control measures of CIDI showed that in Health 2000 the inter-interview reliability was very good (Pirkola et al. 2005). Further, weight, height and waist circumference were measured by trained nurses with standardised methods (Heistaro 2008; Lundqvist and Mäki-Opas 2016).

There are also limitations related to self-reported variables. In the present study, only leisure-time physical activity was estimated, underestimating total physical activity among those who are physically active at work or on their way to work. Further, alcohol consumption, for instance, is typically underestimated in surveys (Mäkelä et al. 2010).

6.6 IMPLICATIONS FOR FUTURE RESEARCH

The vitamin D status of Finnish adults has improved remarkably during the 2000s. Although the vitamin D status of Finnish adult population seems now to be sufficient, it is important to monitor the situation in the future based on large population-based samples.

It is also important to reveal determinants of vitamin D status, which are still partly unknown. Uncovering the potential genetic markers behind low

vitamin D status would increase the knowledge of vitamin D on a more individualised level.

The association between low vitamin D status and increased risk of chronic diseases and public health concerns, including depression and obesity, is still quite inconsistent and should be clarified in further studies. More prospective epidemiological studies, based on large samples and accurate methodology, are still needed. It is especially important to take into account possible confounders and effect modifiers. Also, the knowledge of biological mechanisms behind these potential associations is limited and should be further examined in experimental studies.

If convincing evidence of non-skeletal health effects of vitamin D is obtained, large-scale randomised trials designed to examine the effects of vitamin D supplementation on specific, carefully-selected health effects are required to confirm the findings. Further, thresholds of optimal vitamin D status for potential non-skeletal health effects in different populations as well as in different population subgroups should be confirmed based on standardised methodology.

7 CONCLUSIONS

In general, vitamin D status is positively associated with a healthy lifestyle, but the role of low vitamin D status as an independent risk factor for depression or obesity is inconsistent. The following specific main conclusions of the thesis can be drawn:

1. In 2000, less than half of the Finnish adult population had sufficient (S-25(OH)D ≥ 50 nmol/L) vitamin D status. There was a strong, positive association between vitamin D status and a healthy lifestyle (i.e., being of normal-weight, physically active at leisure-time, non-smoker, moderate alcohol consumer and adhering to a healthy diet). This should be carefully taken into account when studying the potential health effects of vitamin D because lifestyle factors may confound the associations between vitamin D status and health outcomes.

2. The vitamin D status of the Finnish adult population improved since 2000, and over 90% of the population had sufficient vitamin D status in 2011. This suggests that the systematic vitamin D fortification policy has been successful. Those subjects who consume vitamin D fortified milk products and fat spreads daily and fish at least twice a week reach sufficient vitamin D status without vitamin D supplements.

3. Low vitamin D status was associated with a higher prevalence of depressive disorder among men but not among women. An independent association was not found between vitamin D status and depressive symptoms among both men and women.

4. Low vitamin D status at baseline predicted an increase in waist circumference during an 11-year follow-up among men but not among women. Vitamin D status at baseline did not predict weight gain. This suggests that if there is a link between vitamin D status and development of obesity, it is related especially to abdominal obesity.

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