Johanna Lumme

VITAMIN D STATUS IN NORTHERN FINLAND BIRTH COHORT 1966 AND IN WOMEN WITH REPRODUCTIVE DISORDERS
JOHANNA LUMME

VITAMIN D STATUS IN NORTHERN FINLAND BIRTH COHORT 1966 AND IN WOMEN WITH REPRODUCTIVE DISORDERS

Academic dissertation to be presented with the assent of the Doctoral Programme Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 4 of Oulu University Hospital, on 4 November 2022, at 12 noon
Abstract

Vitamin D is essential for bone health, but additional positive health effects of vitamin D have been observed in recent decades. Due to the lack of solar light in northern latitudes, the risk of vitamin D deficiency is elevated, which may predispose people to several adverse health consequences. In Finland, a nationwide systematic vitamin D fortification of dairy products and fat spreads has been launched, but evidence of the effects of this public health action is scarce, especially among the population in northern Finland. Vitamin D has been suggested to be linked to reproductive disorders in women such as infertility, polycystic ovary syndrome (PCOS) and early-onset menopausal transition, but previous research results have been conflicting.

The aim of the present study was to evaluate vitamin D status in the population living in northern latitudes with an increased risk for vitamin D deficiency and in women with reproductive disorders. The study population included 31- and 46-year-old participants from the prospectively collected large Northern Finland Birth Cohort 1966. The impacts of the vitamin D fortification program were investigated with vitamin D measurements before and after the start of vitamin D fortification in dairy products and fat spreads. Use of vitamin D supplementation was also observed. The aim was to assess whether a history of fertility problems, PCOS or early-onset climacteric phase in women were associated with vitamin D status.

The study results showed that vitamin D status improved, and that the prevalence of vitamin D deficiency decreased after the initiation of the vitamin D fortification program. Seasonal variation in vitamin D also diminished. In women, a history of infertility and decreased fecundability associated with lower vitamin D status. However, based on the study results, PCOS did not seem to increase the risk for vitamin D inadequacy. While no association was observed between climacteric status and vitamin D, hormone replacement therapy (HRT) use had an independent positive effect on vitamin D status.

In conclusion, the study results support the use of vitamin D fortification and vitamin D supplementation. Sufficient vitamin D intake should be ensured in women with reproductive issues, especially those with infertility problems.

Keywords: 25(OH)D, early menopause, hormone replacement therapy, infertility, polycystic ovary syndrome, population-based study, vitamin D, vitamin D fortification, vitamin D supplementation
Lumme, Johanna, D-vitamiinipitoisuudet Pohjois-Suomen syntymäkohortin 1966 väestössä ja D-vitamiini yhteys naisten lisääntymisterveyteen.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala

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Tiivistelmä


Asiasanat: 25(OH)D, D-vitamiini, D-vitamiinisäisyys, hormonikorvaus, lapsenmuutos, munasarjojen monirakkulaoirehyttymä, varhaiset vaihdevuodot, väestöpohjainen tutkimus
To Aino, Väinö and Jesse
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Oulu, 2022

Johanna Emilia Lumme
### Abbreviations

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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>1,25(OH)(_2)D</td>
<td>1,25-dihydroxyvitamin D, calcitriol</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D, calcidiol</td>
</tr>
<tr>
<td>25(OH)D(_2)</td>
<td>25-hydroxyvitamin D(_2)</td>
</tr>
<tr>
<td>25(OH)D(_3)</td>
<td>25-hydroxyvitamin D(_3)</td>
</tr>
<tr>
<td>AMH</td>
<td>Anti-Müllerian hormone</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CMIA</td>
<td>Chemiluminescence microparticle immunoassay</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D binding protein</td>
</tr>
<tr>
<td>DEQAS</td>
<td>Vitamin D external quality assessment scheme</td>
</tr>
<tr>
<td>EM</td>
<td>Early-onset menopause, early menopause</td>
</tr>
<tr>
<td>ES</td>
<td>Endocrine Society</td>
</tr>
<tr>
<td>ESHRE</td>
<td>European Society of Human Reproduction and Embryology</td>
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<tr>
<td>fP-Gluc</td>
<td>Fasting plasma glucose</td>
</tr>
<tr>
<td>fS-Ins</td>
<td>Fasting serum insulin</td>
</tr>
<tr>
<td>FAI</td>
<td>Free androgen index</td>
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<tr>
<td>FINAS</td>
<td>Finnish Accreditation Service</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>H2000–2011</td>
<td>Health 2000 and 2011 studies</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostatic model assessment of insulin resistance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>High-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectrometry</td>
</tr>
<tr>
<td>MET</td>
<td>Metabolic equivalent of task</td>
</tr>
<tr>
<td>µg/d</td>
<td>µg/day</td>
</tr>
<tr>
<td>NFBC1966</td>
<td>Northern Finland Birth Cohort 1966</td>
</tr>
<tr>
<td>NNC</td>
<td>National Nutrition Council</td>
</tr>
<tr>
<td>OCP</td>
<td>Oral contraceptive pill</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
</tr>
<tr>
<td>POI</td>
<td>Premature ovarian insufficiency</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>RXR</td>
<td>Retinoid X receptor</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>STRAW</td>
<td>Stages of Reproductive Aging Workshop</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to pregnancy</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VDSP</td>
<td>Vitamin D Standardization Certification Program</td>
</tr>
<tr>
<td>VitD</td>
<td>Vitamin D</td>
</tr>
</tbody>
</table>
Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


*The authors contributed equally to this work

††The authors contributed equally to this work

Publication IV is included in the doctoral thesis of Susanna Savukoski.
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1 Introduction

Vitamin D is essential in the regulation of calcium and phosphorus metabolism in the human body (Charoenngam et al., 2019). Vitamin D enhances the absorption of calcium in the intestine and bone mineralization. Prolonged vitamin D deficiency causes rickets in children and osteomalacia in adults. In recent years, vitamin D has also been suggested to be involved in non-skeletal diseases including autoimmune (Antico et al., 2012) and cardiovascular diseases (CVDs) (Pilz et al., 2016; L. Wang et al., 2012), type 2 diabetes (T2D) (Lips et al., 2017), cancers (Mondul et al., 2017), and mental illnesses (Anglin et al., 2013). Vitamin D might play a role in the regulation of immunity and inflammatory functions (Adams & Hewison, 2008; Di Rosa et al., 2011; Prietl et al., 2013; Zhou & Hyppönen, 2022). Inadequate vitamin D status may even be a risk factor for all-cause mortality (Gaksch et al., 2017). The active form of vitamin D signals in cells via vitamin D receptors (VDRs), which have been found in multiple tissues including reproductive organs (pituitary gland, uterus, ovaries, and placenta), indicating a possible role in sex hormone regulation, reproductive functions, and women’s reproductive health (Lerchbaum & Obermayer-Pietsch, 2012).

Vitamin D is produced naturally from solar ultraviolet B (UVB) radiation, which induces vitamin D production in the skin (Wacker & Holick, 2013). During the long winters in northern latitudes, the amount of sunlight is decreased, and vitamin D must be obtained from the diet and/or vitamin D supplements. Since it is found naturally in only a few foods (e.g., fish, eggs, and mushrooms), the Nordic countries, including Finland, have implemented systematic national food fortification programs to improve vitamin D intake and prevent vitamin D deficiency. Vitamin D supplementation is also recommended (Raulio et al., 2017). However, evidence of the impact of these public health actions is scarce, especially among the population of northern Finland.

Infertility and decreased fecundability are prevalent problems in Western countries. Studies have suggested that vitamin D might be associated with fecundability, and establishing a link between reproduction and vitamin D might provide an easy way to enhance fertility. Infertility can be caused by multiple conditions, including polycystic ovary syndrome (PCOS) and early-onset menopausal transition, both of which might also be a risk factor for vitamin D insufficiency (Anagnostis et al., 2013).

PCOS is one of the most common endocrinologic diseases in fertile-aged women, affecting approximately 10% of those women (March et al., 2010). Typical
features of PCOS are menstrual disturbance, hyperandrogenism, and polycystic ovaries. Metabolic derangements and metabolic syndrome, including obesity, impaired glucose tolerance, and insulin resistance (IR), are also associated with PCOS (Bozdag et al., 2016). Obesity and PCOS are common causes of fertility problems. Since vitamin D deficiency has been noted to be involved in the metabolic pathways affected in PCOS and might be linked to decreased fecundability, it is essential to evaluate vitamin D status in women with PCOS (Várbiró et al., 2022). Although previous studies of vitamin D in fertility problems and women with PCOS have shown inconsistent results, they have generally involved heterogenous, small, and selected patient groups.

Early menopause (EM; the occurrence of menopause before age 45) and premature ovarian insufficiency (POI; the occurrence of menopause before age 40) predispose women to several morbidities and health problems. POI in particular has been shown to be associated with risks for CVD, diabetes, and osteoporosis (Mishra et al., 2017; Muka et al., 2016). Since vitamin D has been linked to all these diseases and is important for maintaining bone health, it is essential to acknowledge vitamin D status in this patient group. In women with POI, systematic hormone replacement therapy (HRT) and sufficient vitamin D intake are recommended (Webber et al., 2016). HRT has been observed to alleviate the health derangements connected with EM, but studies of the link between vitamin D and HRT use are lacking.
2 Review of literature

In this thesis, vitamin D is referred to according to International System (IS) terminology. The circulating form of vitamin D is referred to as 25-hydroxyvitamin D (25(OH)D) and measured from serum. Vitamin D status is used to refer to 25(OH)D concentrations in the serum. The active form of vitamin D is referred to as 1,25-dihydroxyvitamin D or calcitriol (1,25(OH)₂D). Units of 25(OH)D and 1,25(OH)₂D measurements are in nmol/L. Intake of vitamin D from dietary sources and supplementation is reported in µg (micrograms) per day (µg/d).

2.1 Vitamin D

2.1.1 Background of vitamin D

Vitamin D is a fat-soluble hydrophobic steroid vitamin that acts as a hormone in the human body (DeLuca, 2004). Prolonged severe vitamin D deficiency can lead to rickets among children and osteomalacia among adults (Holick, 2007). Rickets was found in England in the 17th century (Wolf, 2004), but the precise molecular structure of vitamin D was only identified in the 1930s. In Finland, rickets was described in 1839 by Elias Lönnrot, who treated it with cod liver oil and sun baths (Ala-Houhala et al., 1995). Archiater Ylppö reported that approximately 35% of three- to six-month-old babies and 50%–70% of one- to two-year old children had sign of rickets in 1925 (Ylppö, 1925).

The recommendation to use vitamin D supplements, especially for children, was introduced in Finland in the 1940s (Hallman et al., 1964). At that time, the recommended dose was 100 µg/d. After reports of vitamin D intoxication and a decline in the incidence of the rickets from 7% to 0.6%, the recommended dosage was decreased to 25 µg/d. In 1992, the recommended daily vitamin D supplementation dose was set at 10 µg (Sosiaali- ja terveyshallitus, 1992). Still, vitamin D deficiency has raised concerns of becoming a major global public health problem (Charoenngam et al., 2019).

2.1.2 Sources of vitamin D

The main source of vitamin D in the human body is the skin, where UVB radiation from the sun causes 7-dehydrocholesterol conversion to previtamin D₂. Previtamin
D$_3$ is further transformed into vitamin D$_3$ (cholecalciferol) in the skin (Figure 1). In addition to solar synthesis, vitamin D is obtained from the diet as vitamin D$_2$ and vitamin D$_3$. Vitamin D$_2$ is the other isoform (ergocalciferol) and is obtained only from dietary sources. Plant-based products contain vitamin D$_2$, and animal-based products contain vitamin D$_3$ (National Institute for Health and Welfare, 2019). The bioavailability of vitamin D$_3$ has been suggested to be higher than the vitamin D$_2$ isoform in a few studies (Armas et al., 2004; Lehmann et al., 2013).

In northern latitudes, the amount of sunlight and thus vitamin D synthesis in the skin decreases during the winter season, when darkness is frequent (Huotari & Herzig, 2008; Leary et al., 2017). In addition, awareness of the importance of sun protection for reducing skin cancer has grown, decreasing dermal vitamin D synthesis in the summer months (Matsuoka et al., 1987). In the human diet, only a few food products naturally contain vitamin D in significant amounts (National Institute for Health and Welfare, 2019). Dietary sources of vitamin D are shown in Table 1 (National Institute for Health and Welfare, 2019; Roth et al., 2018).

Table 1. Food products naturally containing vitamin D, modified according to the Finnish National Food Composition Database (2019).

<table>
<thead>
<tr>
<th>Food product</th>
<th>Vitamin D form</th>
<th>Dose in µg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant-based</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mushrooms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Funnel chantarelle</td>
<td>D$_2$</td>
<td>15.4</td>
</tr>
<tr>
<td>Chantarelle</td>
<td>D$_2$</td>
<td>5.8</td>
</tr>
<tr>
<td>Porcini</td>
<td>D$_2$</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Animal-based</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>D$_3$</td>
<td>2.2</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>D$_3$</td>
<td>6.5</td>
</tr>
<tr>
<td>Fish$^1$</td>
<td>D$_3$</td>
<td>13.4</td>
</tr>
<tr>
<td>Liver$^2$</td>
<td>D$_3$</td>
<td>0.6</td>
</tr>
<tr>
<td>Chicken</td>
<td>D$_3$</td>
<td>0.7</td>
</tr>
<tr>
<td>Pork</td>
<td>D$_3$</td>
<td>0.4</td>
</tr>
<tr>
<td>Beef</td>
<td>D$_3$</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$^1$The average of 10 commonly consumed fish in Finland: pike, perch, vendace, Baltic herring, salmon, tuna, rainbow trout, pollock, whitefish, and zander per 150g portion. $^2$The average of chicken, pork, beef, and reindeer liver.

Thus, it is difficult to obtain the necessary amount of vitamin D in northern latitudes, which increases the risk of vitamin D deficiency. Some Nordic countries (mainly Finland, Sweden, and Norway) have launched fortification programs to ensure
sufficient vitamin D intake and prevent low vitamin D levels (Table 2) (Itkonen et al., 2018, 2020). Finnish nutrition recommendations have been modified based on the Nordic Nutrition Recommendations.

2.1.3 Recommendations for vitamin D intake

In Finland, the first fortifications of margarine with vitamin D began in 1941 (Virtanen, 2012). The first National Nutrition Council (NNC) recommendations appeared in 1987 and included a recommended daily dietary and supplement intake of vitamin D. Still, inadequate vitamin D status was observed in multiple studies in the first decade of the present century, and intake of vitamin D was shown to be below recommended levels in not only Finland but also in other Nordic countries (Lamberg-Allardt et al., 2013; Ministry of Social Affairs and Health, 2006; Raulio et al., 2017). Since there was a need to increase vitamin D intake, the Finnish NNC in December 2002 instituted the systematic fortification of all fluid dairy products (except organic products) and plant-based alternatives with 0.5 µg/100g of vitamin D₃. Since then, all fat spreads except butter have also been systematically fortified with 10 µg/100g of vitamin D₃ (Raulio et al., 2017).

After the start of the systematic fortification program, low vitamin D status was still found in a few age groups. Vitamin D status at or below the level set as insufficient was found in 21.3% of the population in 2002–2004 (Ministry of Social Affairs and Health, 2006). Thus, recommendation for vitamin D fortification was doubled in 2010 (dairy products, 1.0 µg/100g; fat spreads, 20 µg/100g) (Raulio et al., 2017). The fortification of these products was not mandatory, but most of the dairy products and fat spreads sold and served in Finland are fortified.

To increase vitamin D intake, the use of oral vitamin D supplementation is recommended (Table 3). The NNC recommends 10 µg/d of vitamin D supplementation during the darkest times of the year (October–March) for adults who do not consume fish and vitamin D-fortified food products (i.e., dairy products or fat spreads) daily. The recommendations for vitamin D supplements for different age groups and recommended total daily intake of vitamin D are shown in Table 3. The recommended total vitamin D intake is 10 µg/d except for those 75 years or older, for whom it is 20 µg/d (Finnish Food Authority, 2014).
Table 2. Vitamin D fortification of fluid dairy products and fat spreads in the Nordic countries.

<table>
<thead>
<tr>
<th>Product</th>
<th>Finland (Itkonen et al., 2018, 2020)</th>
<th>Sweden (Itkonen et al., 2020; Livsmedelsverket, 2018; Summerhays et al., 2019)</th>
<th>Norway (Nasjonalt råd for ernæring, 2018)</th>
<th>Denmark (Grønborg et al., 2019, 2020)</th>
<th>Iceland (Itkonen et al., 2020)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy products</td>
<td>2002: 0.5 µg/100g</td>
<td>2007: extra low-fat milk (&lt; 1.5% fat) 0.38–0.5 µg/100g</td>
<td>2006: Extra low-fat and lactose-free milk, 0.4 µg/100g</td>
<td>No systematic fortification</td>
<td>No systematic fortification</td>
</tr>
<tr>
<td></td>
<td>2010: 1 µg/100g fluid milk products, lactose-free and vegetable-based alternatives, yogurt, sour milk</td>
<td>2018: milk, fermented milk (≤ 3% fat), including lactose-free and vegetable-based alternatives 0.75–1.1 µg/100g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat spreads</td>
<td>2002: 10 µg/100g</td>
<td>2007: margarine and cooking fats 7.5–10 µg/100 g</td>
<td>2006: 10 µg/100g</td>
<td>No systematic fortification</td>
<td>No systematic fortification</td>
</tr>
<tr>
<td></td>
<td>2010: 20 µg/100g</td>
<td>2018: 19.5–21.0 µg/100g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>Fortification allowed since 2005 in fat spreads, sports drinks, and lactose-free milk products</td>
<td>Some milk products, some domestic foods (mostly fat spreads), and some imported foods (vegetable oils, cereals) are fortified.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Recommended dosage of vitamin D supplements for target groups and recommendations for total daily intake of vitamin D. Modified after Finnish Food Authority (2014).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Daily vitamin D supplement throughout the year (µg)</th>
<th>Recommended total vitamin D daily intake (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks–1 year</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2–17 years</td>
<td>7.5</td>
<td>10</td>
</tr>
<tr>
<td>18–60 years</td>
<td>10²</td>
<td>10</td>
</tr>
<tr>
<td>61–74 years</td>
<td>10²</td>
<td>10</td>
</tr>
<tr>
<td>≥ 75 years</td>
<td>20 (or 10)²</td>
<td>20</td>
</tr>
<tr>
<td>Pregnant or lactating women</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

¹ Total amount of vitamin D from dietary sources and any supplements. ² No daily use of fortified dairy products, fat spreads, and/or fish at the darkest times of the year (October–March). ³ Smaller doses may be sufficient for those who consume large amounts of fortified milk products, fat spreads, and/or fish.

Upper limits for safe vitamin D intake have been also set by the European Food Safety Authority and the NNC (Nordic Council of Ministers, 2014; Turck et al., 2018). The upper tolerable intake limit for infants 0–6 months old is 25 µg/d, for infants 6–12 months old 35 µg/d, for children 1–17 years old 40 µg/d, and for adolescents and adults 100 µg/d. The risk of vitamin D toxicity, hypercalcemia, arises with long-lasting high intake doses and overly elevated 25(OH)D concentrations (Roth et al., 2018).

2.1.4 Vitamin D metabolism and physiological effects

Vitamin D synthetized in the skin (vitamin D₃) or absorbed in the intestine from the diet and supplements (vitamin D₂ and D₃) is mainly bound to vitamin D binding protein (DBP) in the circulation and transported to the liver (Figure 1) (Holick, 2007). In the liver, both vitamin D isoforms are hydroxylated into 25-hydroxyvitamin D (25(OH)D, calcidiol) by vitamin D-25-hydroxylases. The concentration of 25(OH)D is proportional to the vitamin D intake from the skin and intestine and is the main form of vitamin D in the circulation with a long half-life. Thus, serum 25(OH)D concentration is considered the main reflector and best indicator of an individual’s vitamin D status (Holick, 2007; Hosslein-Nezhad & Holick, 2013). Then, 25(OH)D is mainly bound to DBP in the serum, and the 25(OH)D-DBP complex is transported to the kidneys (DeLuca, 2004), where the proximal tubules epithelial cells convert 25(OH)D into an active form of vitamin
D (1,25(OH)₂D, cholecalciferol) by 25(OH)D-1α-hydroxylase (Holick, 2007), and 1,25(OH)₂D is bound again to DBP in circulation. Parathyroid hormone (PTH), circulating calcium, phosphate, calcitonin, fibroblast growth factor, and 1,25(OH)₂D itself regulate the activation of 25(OH)D to 1,25(OH)₂D (Lehmann & Meurer, 2010). The most bioactive form of vitamin D is 1,25(OH)₂D, which together with 25(OH)D regulates the metabolism of calcium, phosphorus, and bone. Both 25(OH)D and 1,25(OH)₂D are catabolized by 25(OH)D-24-hydroxylase into a biologically inactive water-soluble form that is excreted in the bile (Lehmann & Meurer, 2010).

![Vitamin D metabolism](image)

**Fig. 1. Vitamin D metabolism.** DBP: Vitamin D binding protein, UVB: Ultraviolet B radiation, VDR: Vitamin D receptor.

The main role of vitamin D is to maintain calcium and phosphorus at target levels in the circulation by increasing the calcium and phosphorus absorption in the intestine and enhancing the activation of osteoclasts and osteoblasts in the bone; this is known as the classical function of vitamin D (Holick, 2007; Hossein-Nezhad & Holick, 2013). In the target cells, 1,25(OH)₂D acts via VDRs (Norman, 2008). 1,25(OH)₂D binds to VDR; this complex is further bound to a retinoid X receptor (RXR). This complex down- or up-regulates the target gene’s activity by binding to a specific nucleotide sequence in DNA (vitamin D response element, VDRE).
Figure 2 demonstrates the binding of 1,25(OH)\(_2\)D to VDR, RXR, and VDRE in the cell, nucleus, and target gene, respectively. VDRs are more commonly found in the intestine and bone, but also in most tissues in the human body. About 3% of the human genome may be regulated by the 1,25(OH)\(_2\)D-VDR complex. Since several genes in the body are regulated by vitamin D, additional physiological effects have been suggested; these are the non-classical functions of vitamin D (Holick, 2007; Norman, 2008). VDRs have been also expressed from the organs of the reproductive system such as the pituitary gland, uterus, ovaries, and placenta, suggesting possible effects of vitamin D on reproductive health (Lerchbaum & Obermayer-Pietsch, 2012).

![Figure 2](image)

**Fig. 2.** Binding of 1,25(OH)\(_2\)D to VDR, RXR, and VDRE in the cell. 1,25(OH)\(_2\)D: 1,25-dihydroxyvitamin D, RXR: Retinoid X receptor, VDR: Vitamin D receptor, VDRE: Vitamin D response element.

Vitamin D may play a role in inhibiting cell proliferation and regulating innate and adaptive immunity, which influence fertilization and the progression of pregnancy. These functions could have an impact on overall endocrine health, especially in the presence of vitamin D insufficiency (Anagnostis et al., 2013; Holick, 2007; Lerchbaum & Obermayer-Pietsch, 2012). Figure 3 shows the target organs of 1,25(OH)\(_2\)D in terms of both classical and non-classical functions.
2.1.5 Assessment and definition of vitamin D status

Several laboratory methods have been developed to assess vitamin D status (e.g., serum 25(OH)D concentration): chromatographic methods, protein-binding methods, and immunochemical methods (Carter, 2011; Janssen et al., 2012). Each methodology has its own advantages and disadvantages. The gold standard of methods is liquid chromatography tandem mass spectrometry (LC-MS/MS); it can detect both the vitamin D$_2$ and D$_3$ isoforms, but it is expensive and requires expertise. However, the main challenge of the different methods is that 25(OH)D concentration results can vary based on the method used, which makes it hard to compare and interpret the results between vitamin D studies. Thus, different international collaborative procedures have been launched. The International Vitamin D External Quality Assessment Scheme (DEQAS) was established in 1989 to ensure the reliability of 25(OH)D assays (Carter et al., 2010). In 2010, the Vitamin D Standardization Certification Program (VDSP) was launched by the U.S. National Institutes of Health (NIH) to enable the standardization of 25(OH)D measurements over time and across different locations and laboratory procedures (Durazo-Arvizu et al., 2017; Sempos et al., 2012, 2016). Since then, the VDSP has coordinated the standardization of 25(OH)D measurements.
The definitions of vitamin D deficiency, insufficiency, and sufficiency according to the Institute of Medicine (IOM) and Endocrine Society (ES) guidelines are shown in Table 4 (Holick et al., 2011; Ross et al., 2011). The ES has proposed that the 25(OH)D concentration cutoff for vitamin D sufficiency should be raised to 75–125 nmol/L, based on a decrease in non-skeletal outcomes related to vitamin D and maintaining PTH at normal levels (Holick et al., 2011; Płudowski et al., 2013). In clinical practice in Finland, 25(OH)D concentrations below 50 nmol/L are considered to indicate insufficient vitamin D status; those individuals should be treated with higher intake of vitamin D. For patients with osteoporosis and those at high risk for developing it (e.g., anorexia patients), 25(OH)D concentrations above 75 nmol/L are recommended.

Table 4. Definition of 25(OH)D concentrations based on IOM$^1$ and ES$^2$ guidelines.

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>IOM (Ross et al., 2011)</th>
<th>ES (Holick et al., 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>&lt; 30</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>30–50</td>
<td>50–75</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>&gt; 50</td>
<td>&gt; 75</td>
</tr>
</tbody>
</table>

$^1$IOM: Institute of Medicine, $^2$ES: Endocrine society, 25(OH)D: 25-hydroxyvitamin D.

2.1.6 Possible predisposing factors for insufficient vitamin D status

Environmental factors and lifestyle

The season of the year and latitude are important influencers of serum 25(OH)D concentration. UVB radiation decreases during the dark winters in northern latitudes, which predisposes people to have low 25(OH)D concentrations, since total solar radiation is diminished (Wacker & Holick, 2013). In addition, use of sunscreen inhibits the absorption of UVB radiation into the skin and thus blocks vitamin D synthesis (Gilchrest, 2008; Matsuoka et al., 1987). Melanin pigmentation serves as natural protection against solar UVB radiation (Wacker & Holick, 2013). Less vitamin D is formed on skin with darker melanin pigmentation, which increases the risk for vitamin D inadequacy compared to people whose skin has lighter pigmentation (Holick & Chen, 2008). Thus, ethnicity might be a risk factor for vitamin D insufficiency (Scragg et al., 2007). Cultural factors such as a requirement for much or nearly all of the body to be covered in public might be an additional risk for vitamin D inadequacy (Gannagé-Yared et al., 2000).
The results of the relationship between smoking and vitamin D have been contradictory. While an inverse association between smoking and vitamin D status has been detected, the opposite finding has also been observed. Contradictory findings have also been reported regarding alcohol consumption and vitamin D status. In a few studies, vitamin D status has been suggested to be higher in individuals who consume alcohol moderately than in both abstainers and those with high levels of alcohol consumption (Jääskeläinen et al., 2013; Larose et al., 2014; Palaniswamy et al., 2017; Touvier et al., 2015). Relatively consistent results have been observed as to a positive association between physical activity and vitamin D (Daly et al., 2012; Larose et al., 2014; Rabenberg et al., 2015; Touvier et al., 2015). These findings have been explained by the assumption that physical activity relates to time spent outdoors, which entails solar exposure that enhances vitamin D status.

**BMI and obesity**

Several epidemiological studies have reported an inverse association between body mass index (BMI) and vitamin D (Pereira-Santos et al., 2015; Saneei et al., 2013), and obesity (BMI ≥ 30 kg/m²) might carry a risk for vitamin D deficiency (Pereira-Santos et al., 2015). Multiple explanations of the background mechanism have been presented. Intake of vitamin D from diet and supplements might be lower in individuals with higher BMIs (Johnson et al., 2012; Kamycheva et al., 2003). Physical activity is also presumably lower in obese individuals (Pourshahidi, 2015). Vitamin D is a fat-soluble hydrophobic molecule that might be sequestered in adipose tissues. Based on the sequestration hypothesis, this might lead to vitamin D insufficiency, since 25(OH)D is not able to revert to serum when it is stored in such tissues (Blum et al., 2008; Didriksen et al., 2015; Wortsman et al., 2000). Volumetric dilution has also been suggested as an explanation for the connection between lower vitamin D status and obesity (Drincic et al., 2012).

**Genetic variations**

Individual variation in the molecular response of vitamin D has been suggested; some people may require higher vitamin D intake to obtain the same 25(OH)D status. The hypothesis has been posited that genetic variations and single nucleotide polymorphisms (SNP) related to vitamin D metabolism might be a background factor affecting vitamin D status. Thus, genetic differences should also be considered when evaluating vitamin D status. For example, SNPs related to VDR
gene polymorphism have been proposed to be connected to PCOS (Liang et al., 2019).

**Socioeconomic factors**

Socioeconomic status (SES) has been found to be linked with vitamin D status, especially a positive association between vitamin D and higher SES (Jääskeläinen et al., 2013; Lips et al., 2021; Sowah et al., 2017). A higher risk for vitamin D deficiency has been found in indoor workers, shift workers, and healthcare workers (Sowah et al., 2017). Positive associations between both higher levels of educational attainment and high income and vitamin D status have also been observed (Tønnesen et al., 2016). These findings might be explained by poorer lifestyle choices, quality of diet, higher BMIs, smoking, and less physical activity in lower-SES groups.

**Age, sex, and marital status**

Inconsistent results have been observed regarding the association between vitamin D and age, sex, and marital status. Vitamin D production in the skin declines with age, so older age has been suggested to be a risk for vitamin D insufficiency. However, these findings have been contradictory, and positive and non-significant associations have also been reported (Daly et al., 2012; Freedman et al., 2013; Hilger et al., 2014; Hintzpeter et al., 2008; Pasco et al., 2009; Touvier et al., 2015). Although differences between vitamin D status in women and men have been noted, similar 25(OH)D concentrations have generally been found (Freedman et al., 2013; Hilger et al., 2014; Larose et al., 2014). A few studies have suggested that being married or cohabiting has beneficial effects on vitamin D status (Hintzpeter et al., 2008; Pasco et al., 2009).

**Chronic diseases**

Studies have reported inverse associations between 25(OH)D concentration and multiple diseases, including T2D (Lips et al., 2017), CVD (Pilz et al., 2016; Wang et al., 2012), autoimmune diseases (Antico et al., 2012), certain cancers (Mondul et al., 2017), depression (Anglin et al., 2013), and all-cause mortality (Gaksch et al., 2017; Hossein-Nezhad & Holick, 2013; Wacker & Holick, 2013). In middle age, the prevalence of these chronic non-communicable diseases increases, and
menopause might add an additional risk for metabolic derangements in women. Vitamin D status at or above the sufficient level in this age group might be especially important for identifying individuals at risk for low 25(OH)D concentrations.

2.1.7 Longitudinal vitamin D status

To reduce the risk of vitamin D insufficiency, nutritional campaigns have been launched in most Nordic countries. Despite these nationwide nutritional interventions based on vitamin D food fortification and supplementation, the positive effects on 25(OH)D concentrations have been limited. The Finnish national health survey Health 2000 and 2011 studies (H2000–2011) showed a mean increase of 17 nmol/L in serum 25(OH)D concentrations (Jääskeläinen et al., 2017). The study samples were gathered before and after the fortification waves, but most of the participants were from southern Finland (Jääskeläinen et al., 2017). However, study samples from Norway and Sweden have demonstrated relatively stable vitamin D status in recent decades, even though those countries also initiated vitamin D fortification programs (Jorde et al., 2010; Summerhays et al., 2019). Similar findings have been observed in three studies from the United States (McKibben et al., 2016; Mirfakhraee et al., 2017; Schleicher et al., 2016) and in one from the Netherlands (Van Schoor et al., 2014). Table 5 presents studies that longitudinally assess vitamin D status.

2.1.8 Summary

Vitamin D is essential for bones, but in recent years several other health benefits have been acknowledged. Vitamin D deficiency is a public health concern in Finland, and fortification of dairy products and fat spreads has been initiated, with the use of vitamin D supplements also recommended. Despite the vast amount of vitamin D-related research, the effects of nationwide fortification and supplementation programs and the longitudinal evaluation of vitamin D status have been modest at best, especially in northern Finland.
Table 5. Previously published studies assessing vitamin D status longitudinally.

<table>
<thead>
<tr>
<th>Name</th>
<th>Vitamin D status</th>
<th>Study population</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tromsø study (Jorde et al., 2010)</td>
<td>↔</td>
<td>General population from northern Norway 50–74 years, n = 2,668.</td>
<td>1994–2008 (plus intervention study one year)</td>
</tr>
<tr>
<td>NHANES study (Schleicher et al., 2016)</td>
<td>↔</td>
<td>Non-institutionalized population from United States, n = 75,280, aged ≥ 12 years.</td>
<td>1988–2010 (excluding the year 2000)</td>
</tr>
<tr>
<td>Health 2000 and 2011 studies (Jääskeläinen et al., 2017)</td>
<td>↑</td>
<td>Nationwide population register from Finland ≥ 30 years, n = 3,328 at both timepoints.</td>
<td>2000–2011</td>
</tr>
<tr>
<td>Dallas Heart study (Mirfakhraee et al., 2017)</td>
<td>↔</td>
<td>Dallas, Texas, United States, 18–65 years, n = 2045.</td>
<td>Seven years, 2000–2002 and 2007–2009</td>
</tr>
</tbody>
</table>

↑ Vitamin D status increased over time, ↔ Vitamin D status stayed stable over time
2.2 Infertility

2.2.1 Definition, epidemiology, and risk factors

The classic definition of infertility is a failure to achieve clinical pregnancy within 12 months of unprotected and regular intercourse (Vander Borght & Wyns, 2018; Zegers-Hochschild et al., 2017). Infertility is defined as primary if a woman has never had a clinical pregnancy. Secondary infertility is observed if a woman has previously had a clinical pregnancy but is now unsuccessful at achieving one (Zegers-Hochschild et al., 2017). Fecundability can be defined as decreased if time to pregnancy (TTP) has exceeded 12 months with active exposure to pregnancy (Koivunen et al., 2008).

Depending on the population in question, the prevalence of infertility has been estimated to be 8%–15%. The number of infertile patients has been increasing in recent decades, and one of six couples in Western countries now suffer from infertility problems (Farquhar et al., 2019; Vander Borght & Wyns, 2018). Approximately 35% of infertility cases are due to female-related factors and 30% to male-related factors; 15% of infertility can be attributed to both female- and male-related reasons. The remaining ~15% of infertility cases remain unexplained (Farquhar et al., 2019; Skoracka et al., 2021; Vander Borght & Wyns, 2018).

In women, disorders that may have a role in infertility are PCOS, POI, endometriosis, uterine fibroids, and endometrial polyps. Infections, systemic diseases, and iatrogenic causes can also interfere with fecundability and induce infertility. Based on the affected organ, the causes can be divided into ovarian, oviductal, and uterine (National Collaborating Centre for Women’s and Children’s Health, 2004; Vander Borght & Wyns, 2018).

However, multiple additional factors have been associated with fertility problems and thus may lead to infertility. Age has a major influence on fecundability. In healthy women, fertility begins to decline from 25–30 years of age, and age is one of the main reasons for the increasing prevalence of infertility in Western countries, where the mean age for a woman’s first delivery is rising to 30 years. Another important reason for the increase in reproductive problems is worldwide obesity (World Health Organization, 2019). Overweight (BMI 25.0–29.9 kg/m²) or obesity (BMI ≥ 30 kg/m²) can impair fecundability, and elevated BMI is a risk factor for infertility (Nelson & Fleming, 2007; Pasquali et al., 2007;
Zain & Norman, 2008). In addition, women with high BMIs have an elevated risk for PCOS-related anovulation (Brewer & Balen, 2010; Legro, 2012), which can further attenuate fertility. Smoking and alcohol consumption are also noted as lifestyle issues that may adversely affect reproductive success (Homan et al., 2007). Additionally, the impacts of nutrition and an unhealthy diet on infertility have been investigated. An unhealthy diet may have a detrimental effect on fertility (Hart, 2016). Potential ways to improve fertility through nutrition have also been proposed (Skoracka et al., 2021). Figure 4 presents the factors that may be associated with infertility and reproductive health.

![Figure 4](image.png)

**Fig. 4. Factors that may be associated with reproductive health, including infertility.**

PCOS: Polycystic ovary syndrome.

### 2.2.2 Vitamin D and infertility

Vitamin D has been suggested as essential for endocrinological and reproductive functions (Anagnostis et al., 2013; Lerchbaum & Obermayer-Pietsch, 2012). The VDRs and enzymes required for vitamin D metabolism are expressed in the pituitary gland, endometrium, fallopian epithelial cells, ovaries, decidua, and placenta (Anagnostis et al., 2013). Ensuring sufficient vitamin D intake from the diet and vitamin D supplements could be a simple way to increase fertility, especially in areas at risk for vitamin D insufficiency (Vanni et al., 2014).
Multiple animal studies have reported a link between vitamin D and fecundability (Skoracka et al., 2021). Diet-induced vitamin D deficiency reduced mating success and fertility and increased the risk of pregnancy complications in rats (Luk et al., 2012). Several detrimental effects on fertility have been observed in VDR and 1α-hydroxylase knockout mice. For example, hypergonadotropic hypogonadism and reduction of aromatase activity and gene expression have been detected. Aromatase is also an important molecule in the biosynthesis of estradiol from androgen precursors in humans, indicating that vitamin D may have an impact on sex hormone synthesis. In mice, uterine hypoplasia, impaired folliculogenesis, anovulation, and a decreased number of fetuses with lower weight have also been found in VDR and 1α-hydroxylase knockout mice (Lerchbaum & Obermayer-Pietsch, 2012; Shahrokhi et al., 2016).

Despite animal studies that have observed an inverse association between vitamin D status and fertility, the results in human studies have been inconclusive (Chiu et al., 2018; Gaskins & Chavarro, 2018; Lerchbaum & Rabe, 2014). Inadequate 25(OH)D concentrations have been observed in women undergoing infertility treatments (Ozkan et al., 2010). Studies investigating the impact of 25(OH)D concentrations on success rates of infertility treatments have reported positive results (J. Chu et al., 2018), but inconsistencies between studies have also reported regarding pregnancy success and vitamin D status (Vanni et al., 2014).

A link of vitamin D with fertility has been sought from the endocrine system of reproduction. Active vitamin D has been proposed to alter the steroidogenesis of sex hormones. It may directly stimulate the synthesis of progesterone and estradiol in human ovarian cells (Parikh et al., 2010). In addition, several enzymes, such as 17β-hydroxysteroid dehydrogenase and aromatase, regulate the steroidogenesis of sex hormones. Vitamin D may act favorably on fertility by affecting the synthesis of these enzymes (Krishnan et al., 2010; Shahrokhi et al., 2016; Wang & Tuohimaa, 2007). Anti-Müllerian hormone (AMH) is the best marker of ovarian reserve in women and decreases with age as menopause approaches (Broer et al., 2014). Vitamin D may positively influence AMH levels (Merhi et al., 2012), but discrepancies in the association between vitamin D status and AMH levels have been observed (Moridi et al., 2020). In PCOS, meanwhile, AMH levels have been proposed to be abnormally high due to the characteristics of the syndrome (Garg & Tal, 2016). PCOS is one of the main causes of fertility problems in women and may explain the discrepancies between studies, since several were conducted among women with PCOS (Moridi et al., 2020). Conflicting results have also been reported in studies evaluating the association between pregnancy rates and vitamin
D status in healthy women (Fung et al., 2017; Møller et al., 2012; Somigliana et al., 2016). However, small sample sizes, variations in age group and ethnic background, and selected patient groups from outpatient clinics in those studies may partly explain the discrepancies.

During pregnancy, several changes in vitamin D metabolism may occur. The concentration of active 1,25(OH)₂D and DBP increases significantly (Pilz et al., 2018). Insufficient 25(OH)D concentrations have been observed in pregnant women despite the frequent use of prenatal vitamins. Low vitamin D status may be associated with miscarriage risk and other pregnancy complications like pre-eclampsia and gestational diabetes (Aghajafari et al., 2013; Gonçalves et al., 2018; Palacios et al., 2016; Zhang et al., 2017). The mechanism might be explained by the immunomodulatory effects of vitamin D (Cyprian et al., 2019). Table 6 presents previous studies linking reproductive functions and vitamin D.

### 2.2.3 Seasonal variation of fecundability

Previous research assessing seasonal variation and fecundability has suggested a seasonal pattern of births (Dahlberg & Andersson, 2019). Several confounding cultural and behavioral factors, comfort, and personal choice vary across seasons and may explain this finding. However, variations in TTP have also been reported between seasons (Stolwijk et al., 1996; Wesselink et al., 2020). TTP might be extended in spring (February–April) and decrease in late summer and fall (August–October) (Stolwijk et al., 1996). A study suggested a parallel seasonal variation in fertilization and embryo quality rates in women with in vitro fertilization treatments (Rojansky et al., 2000). This may encourage a hypothesis regarding nutritional background and the possibility that seasonal variation in vitamin D status may explain the observations, since vitamin D status tends to be at its lowest levels at the end of winter (Klingberg et al., 2015).

### 2.2.4 Summary

The prevalence of low fecundability and infertility is increasing. Vitamin D may be associated with fertility, reproductive functions, and reproductive outcomes, but previous observations are contradictory, and studies have been conducted with selected and small populations. The mechanisms by which vitamin D may act on reproductive functions, and the causes behind those mechanisms, are unclear.
Table 6. Studies assessing the link between reproductive functions and vitamin D.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Result</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroidogenesis of sex hormones</td>
<td>Vitamin D directly stimulated the synthesis of progesterone and estradiol in ovarian cells (Parikh et al., 2010).</td>
<td>Molecular study</td>
</tr>
<tr>
<td>Synthesis of the enzymes related to sex hormone synthesis</td>
<td>Vitamin D favorably affected aromatase and 17β-hydroxysteroid dehydrogenase synthesis (Krishnan et al., 2010; Wang &amp; Tuohimaa, 2007).</td>
<td>Molecular study</td>
</tr>
<tr>
<td>AMH levels(^1)</td>
<td>Vitamin D had a positive impact on AMH levels (Chu et al., 2021; Merhi et al., 2012).(^1)</td>
<td>Cross-sectional study from prospectively collected cohort. Review and meta-analysis of 18 observational studies and six interventional studies.</td>
</tr>
<tr>
<td>Discrepant findings have also been observed</td>
<td>(Moridi et al., 2020).</td>
<td></td>
</tr>
<tr>
<td>Infertility treatments</td>
<td>Lower vitamin D status in women undergoing infertility treatments (Ozkan et al., 2010). Beneficial impact of vitamin D on success rates of infertility treatments (Chu et al., 2018).</td>
<td>Prospective cohort study</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>Conflicting results on association between pregnancy rates and vitamin D status in healthy women (Fung et al., 2017; Møller et al., 2012; Somigliana et al., 2016).</td>
<td>Systematic review and meta-analysis of 11 published cohort studies</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>Association of miscarriages and low vitamin D status (&lt; 50 nmol/L) (Zhang et al., 2017).</td>
<td>Systematic review and meta-analysis of five case-control and cohort studies</td>
</tr>
</tbody>
</table>

\(^1\) AMH: Anti-Müllerian hormone

2.3 Polycystic ovary syndrome (PCOS)

2.3.1 Definition, epidemiology, and clinical features

PCOS is the most common endocrinologic disease in fertile-aged women (Bozdag et al., 2016). There are three criteria for standardizing the diagnosis of PCOS: the National Institutes of Health (NIH), Rotterdam, and Androgen Excess and PCOS
(AE-PCOS) society criteria (Table 7). The first guideline to appear was from the NIH and consisted of both clinical and/or biochemical hyperandrogenism and chronic oligo-anovulation (Zawadzki & Dunaif, 1992). After the NIH criteria were promulgated, variations in the definition of PCOS were noticed, and debates over correct PCOS diagnosis persisted. Thus, the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (Rotterdam criteria) suggested that a PCOS diagnosis requires at least two of the following findings: oligo-anovulation, clinical and/or biochemical hyperandrogenism, or polycystic ovaries in ultrasonography (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004).

Under the Rotterdam criteria, polycystic ovaries are indicated if at least one ovary has 12 or more follicles 2–9 mm in diameter and/or elevated ovarian volume (i.e., > 10 mm). The most recent effort was proposed by the AE-PCOS, which highlighted the hyperandrogenism of PCOS and suggested hyperandrogenism, ovarian dysfunction (including oligo-/anovulation and/or polycystic ovaries, and the exclusion of other endocrinologic conditions (Azziz et al., 2006).

**Table 7. Different diagnostic criteria for polycystic ovary syndrome.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NIH</th>
<th>Rotterdam¹</th>
<th>AE-PCOS society</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical and/or biochemical</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>hyperandrogenism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligo-/anovulation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polycystic ovaries in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ultrasonography</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹At least two of three symptoms are required. AE-PCOS: Androgen Excess and Polycystic Ovary Syndrome, NIH: National Institutes of Health.

The prevalence of PCOS depends on which diagnostic criteria are used; it varies between 4%–8% under the NIH criteria, 2%–20% under the Rotterdam criteria, and 2%–15% under the AE-PCOS criteria. In addition, study populations and designs and persistent difficulties with the phenotypic characterization of PCOS may explain discrepancies in prevalence between studies. However, the approximate prevalence of PCOS is 5%–15% in fertile-aged women (March et al., 2010).

Clinical hyperandrogenism is observed by assessing the degree of hirsutism and biochemical hyperandrogenism by the concentration of total or free testosterone or by calculating the free androgen index (FAI) based on the serum concentrations of testosterone and sex hormone binding globulin (SHBG). The degree of hirsutism has been shown to perform well in identifying women with
PCOS characteristics (Kazemi et al., 2015; Taponen, 2004; Taponen et al., 2003). Additional hyperandrogenic features are acne and androgenic alopecia (male-pattern hair loss) (Franks, 1995).

Menstrual irregularities like oligomenorrhea (irregular cycles) and anovulation are another clinical characteristic of PCOS. They are usually classified together as oligo-amenorrhea, which is indicated if menstrual cycle length is \( \geq 35 \) days more than twice a year or there are < 10 menstruations per year; meanwhile, amenorrhea is detected if menses are absent for three or more months (Azziz et al., 2009).

### 2.3.2 Health risks of PCOS

PCOS is a heterogenous disease affecting the endocrine system that also causes several clinical and physiological disturbances (Xu & Qiao, 2022). The precise etiology of PCOS is unclear, but multiple genetic and environmental causes have previously been connected to PCOS. Elevated androgen levels may be linked with multiple health and metabolic derangements, including obesity, impaired glucose metabolism, and IR. The most important and commonly demonstrated association is between obesity and PCOS. Depending on the study population, it has been shown that a high number of women with PCOS are overweight (BMI 25.0–29.9 kg/m\(^2\)) or obese (BMI 30 kg/m\(^2\) or more) (Azziz et al., 2009). In addition, abdominal obesity has been noted to be associated with more severe metabolic derangements than female-type obesity. Thus, the distribution of adipose tissue is crucial.

Previous studies have shown that elevated BMI and especially elevated abdominal obesity could play a role in the development and pathophysiology of PCOS (Legro, 2012). In any case, obesity appears to exacerbate the features and metabolic derangements of PCOS. In obese women with PCOS, risk factors for CVD are elevated, including abnormal glucose metabolism, IR, dyslipidemia, and metabolic syndrome. Studies have observed that possible ways to alleviate the metabolic disturbances associated with PCOS are lifestyle changes, including weight loss and regular physical activity, and the insulin sensitizer metformin (Garzia et al., 2022; Moran et al., 2011), which may also mitigate the fertility derangements associated with PCOS (Collée et al., 2021).
2.3.3 PCOS and fertility

Lower fertility has been found in women with PCOS. Menstrual dysregulation caused by oligo-ovulation and anovulation is one of the main effects of PCOS. Thus, PCOS is the primary reason for anovulatory infertility in women, and infertility is more likely in women with PCOS than in healthy women (El Hayek et al., 2016). In addition to fecundability problems, the risk of pregnancy complications such as gestational diabetes, hypertension, pre-eclampsia, and miscarriage have been reported as elevated (Hart, 2007; Hart & Doherty, 2015). These reproductive problems are presumably connected to gynecologic and endocrine disturbances related to PCOS. Studies have suggested that the risk of pregnancy outcome impairments is independent of a woman’s BMI (Rees et al., 2016; Roos et al., 2011). The adverse metabolic and reproductive health consequences related to PCOS are shown in Figure 5.

![Adverse health consequences associated with polycystic ovary syndrome](image)

Fig. 5. Adverse metabolic and reproductive health consequences related to polycystic ovary syndrome.

2.3.4 Vitamin D and PCOS

Previous studies have suggested that vitamin D could play a positive role in metabolic and reproductive health in women with PCOS (He et al., 2015; Irani & Merhi, 2014; Maidana et al., 2019; Muscogiuri et al., 2017; Shahrokhi et al., 2016; Thomson et al., 2012). Since VDRs have been found in several tissues and in the ovaries, the main organs affected by PCOS, there may be a regulatory function. Insufficient vitamin D status has been suggested to be common in women with PCOS, and vitamin D status has been linked with metabolic alterations and reproductive functions (Várbiró et al., 2022). However, the results of observational studies are contradictory, and some studies have reported similar and higher 25(OH)D concentrations compared to healthy women (Bacopoulou et al., 2017; He
et al., 2015; Thomson et al., 2012). Studies on vitamin D status in women with PCOS have primarily been conducted with small clinical samples without assessing the important confounders influencing vitamin D and PCOS. Low 25(OH)D concentration has been associated with obesity, and since obesity is common among the population with PCOS, it may be difficult to reliably evaluate the impact of obesity on vitamin D status in this population using small samples (Joham et al., 2016; Pereira-Santos et al., 2015).

Previous clinical trials demonstrating the effect of vitamin D on hormonal and metabolic derangements have shown that vitamin D may reduce testosterone levels and inflammatory biomarkers and improve IR and lipid metabolism in women with PCOS (Jin et al., 2020; Łagowska et al., 2018; Luo et al., 2021; Zhao et al., 2021). However, some clinical trials have reported conflicting or weak results regarding hormonal and metabolic health in such women (Krul-Poel et al., 2013; Pergialiotis et al., 2017). Ethnicity is a confounding factor in vitamin D status; for example, a review conducted by Łagowska et al. focused on studies from Iran, decreasing the generalizability of the observations and interfering with their interpretation (Łagowska et al., 2018; Menichini et al., 2022).

Still, the precise mechanism by which vitamin D may act in relation to PCOS remains unidentified (Colonese et al., 2015). High androgen levels in women with PCOS have been shown to be a key cause in derangements of metabolic health and to cause aberrations in adipose tissue and glucose metabolism (Maidana et al., 2019). The link between vitamin D and PCOS may arise from deranged endocrine pathways such as insulin secretion and sex hormone synthesis. Vitamin D may have a beneficial impact by increasing insulin responsiveness with stimulation of expression of insulin receptors. VDRE is located in the insulin gene, and active vitamin D (1,25(OH)2D) might be involved in transcription of the insulin gene. In addition, insulin secretion is a calcium-related process and, since vitamin D regulates intra- and extracellular calcium, hypovitaminosis may have adverse effects (Pittas et al., 2007). Vitamin D has also been suggested to have anti-inflammatory properties that could modulate and reduce the low-grade inflammation related to obesity, metabolic syndrome, and IR (Palaniswamy et al., 2020). Recently, polymorphism in VDR genes has been suggested to be associated with lower vitamin D status in women with PCOS and to endocrine and metabolic disturbances related to PCOS and the risk for PCOS (Reis et al., 2017; Vulcan et al., 2021). Figure 6 presents the proposed mechanisms by which vitamin D deficiency might interfere with metabolic derangements and fertility in women with PCOS.
2.3.5 Summary

Infertility and decreased fertility are common in women with PCOS. Several metabolic and reproductive health derangements are also related to PCOS, including obesity, impaired glucose metabolism, and IR. A link between vitamin D and PCOS has been suggested, but the precise role of vitamin D in PCOS remains unknown. Large observational studies with unselected populations with important confounders (BMI, lifestyle, and seasonal and latitudinal effects) are lacking.

2.4 Early-onset menopausal transition

2.4.1 Definition, epidemiology, and natural course of menopause

In women in Western countries, the median age for natural menopause is 51 (Mishra et al., 2017). Several factors combine to impact the age at which
menopause occurs, with the strongest influence observed to be genetic (Mishra et al., 2017). Environmental and lifestyle exposures have additional effects. Thus, final menopausal age varies widely between individuals. Lower BMI, smoking, and lower SES have been suggested to be associated with menopausal transition at younger ages (Costanian et al., 2018; Gold, 2001).

A woman who experiences her final menstrual period between ages 40 and 44 is defined as having EM (Mishra et al., 2017), while menopause before age 40 is classified as POI. The prevalence of EM and POI have been reported as 7.6%–12.2% and 1.1%–3.7%, respectively (Golezar et al., 2019). The ESHRE criteria for POI require amenorrhea for ≥ 4 months and follicle-stimulating hormone (FSH) values > 25 IU/L in two measurements four weeks apart (Webber et al., 2016). In most POI patients, the etiology is idiopathic, but autoimmune diseases have been reported to be associated with POI. In addition, genetic disorders such as Turner syndrome predispose women to POI (Nelson, 2009; Webber et al., 2016).

In the menopausal transition (perimenopause, climacteric phase), multiple natural hormonal changes occur in women’s bodies. A decline in the ovarian follicle pool causes a decrease in inhibin B levels, and FSH concentrations begin to elevate. Estradiol levels begin to rise as a result of these higher FSH concentrations but fall after the final menstrual period. SHBG levels decline, which leads to an increase in free testosterone levels and causes a change in the ratio of total testosterone to estradiol, although significant changes in absolute levels of testosterone do not occur during menopause (Burger et al., 2007). The hormonal changes associated with menopause induce not only uncomfortable symptoms such as hot flashes and night sweats (Brinton et al., 2015) but also adverse metabolic and bone alterations during and after menopause (Joon Cho et al., 2008; Väänänen & Härkönen, 1996). After menopause, women may be at elevated risk for metabolic syndrome, its related features, and diabetes (Gruppo di Studio Progetto Menopausa, 2005; Joon Cho et al., 2008; Razmjou et al., 2018; Wu et al., 2001). Derangements of inflammatory biomarkers have also been noted during menopause (Lee et al., 2009). Estrogen maintains bone mineral density (BMD); after estradiol levels fall during the menopausal transition, bone resorption start to accelerate and BMD decreases (Väänänen & Härkönen, 1996).
2.4.2 Health risks with early-onset menopausal transition and hormone replacement therapy (HRT)

EM and POI are risks for women’s metabolic and overall health (Mishra et al., 2017; Savukoski et al., 2019). Compared to premenopausal women at same age, in women with early-onset menopausal transition, adverse changes in fat distribution and a higher prevalence of metabolic syndrome have been found (Daan et al., 2016; Muka et al., 2016). Risk of CVD, cardiovascular mortality, and all-cause mortality may subsequently be elevated (Daan et al., 2016). Associations between early-onset menopausal transition and decreased glucose tolerance, lower insulin sensitivity, and diabetes have been suggested (Lee et al., 2013; Muka et al., 2017; Savukoski et al., 2021). In addition, EM and POI are risk factors for bone health; the risk of osteoporosis and fragility fractures are higher in women with EM than in those who experience menopause later in life (Amarante et al., 2011; Svejme et al., 2012).

Systemic HRT with estrogen (with or without progesterone) is used to relieve menopausal symptoms, but HRT has also been found to have several cardiovascular and bone health-related benefits (MacLennan et al., 2004). HRT may enhance lipid profile, prevent cardiovascular morbidity, improve BMD, and lower the risk of osteoporosis (Arabi, 2003; Godsland, 2001; Savolainen-Peltonen et al., 2016; Žegura et al., 2006). Thus, use of systemic HRT is recommended for women with POI (Webber et al., 2016). There are two different administration routes for systemic HRT: transdermal and oral (Pan et al., 2022). Transdermal HRT bypasses the gut and first-pass hepatic metabolism, whereas the use of oral HRT may alter estrogen levels. Oral HRT also induces hepatic protein synthesis, which causes the main adverse side effects of oral HRT (Kopper et al., 2008). Transdermal HRT may be a safer option and more effective in preventing CVD than oral HRT (Kopper et al., 2008).

2.4.3 Vitamin D and early-onset menopausal transition

Only a few studies have assessed the association between early-onset menopausal transition and vitamin D status, and no clear associations have been observed (Purdue-Smithe et al., 2018). However, low 25(OH)D concentrations and EM may impair bone health, elevate cardiovascular health risks, and impair glucose metabolism (Lips et al., 2017; Lips & Van Schoor, 2011; Pilz et al., 2016; L. Wang et al., 2012). Vitamin D has also been observed to have anti-inflammatory properties, which could alleviate the CVD risks of EM (Mutt et al., 2012; Nadir et
al., 2010; Sassi et al., 2018). A lower risk for EM due to higher calcium and vitamin D intake in the diet has been suggested (Kebapcilar et al., 2013). To prevent osteoporosis and other adverse health changes, both adequate vitamin D intake and HRT are recommended for women with POI (Webber et al., 2016). However, studies have reported that the prevalence of low vitamin D status is higher in postmenopausal women with osteoporosis and in women with POI (Kebapcilar et al., 2013; Kuchuk et al., 2009; Purdue-Smithe et al., 2017). Earlier observations have found that the use of oral contraceptive pills (OCPs) containing estrogen may be associated with higher serum 25(OH)D concentrations (Møller et al., 2013; Palaniswamy et al., 2017). At the same, no or only weak associations have been observed with the use of postmenopausal HRT and 25(OH)D concentrations (Bikle & Schwartz, 2019; Rejnmark et al., 2006; Shirazi et al., 2013; Touvier et al., 2015).

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D status in women with POI (Kebapcilar et al., 2013)</td>
<td>Cross-sectional case-control study, POI n = 35, controls n = 28.</td>
<td>Women with POI had significantly lower vitamin D status than women with normal menstrual cycles.</td>
</tr>
<tr>
<td>Vitamin D and calcium intake and risk for EM (Purdue-Smithe et al., 2017)</td>
<td>Cohort study with food frequency questionnaire, women with EM n = 2,041.</td>
<td>Higher intake of dietary vitamin D and calcium associated with lower risk of EM.</td>
</tr>
<tr>
<td>Association of vitamin D status and risk for EM (Purdue-Smithe et al., 2018)</td>
<td>Nested case-control study, EM n = 328, controls n = 328.</td>
<td>No association between vitamin D and risk for EM, modest association between DBP and risk of EM.</td>
</tr>
<tr>
<td>Vitamin D and HRT use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Association of HRT treatment of 5 years with vitamin D status (Rejnmark et al., 2006)</td>
<td>Clinical trial, postmenopausal women, 89 with and 98 without HRT.</td>
<td>DBP increased with HRT, but 25(OH)D concentration was not affected by HRT use.</td>
</tr>
<tr>
<td>Association of HRT use with vitamin D status (Shirazi et al., 2013)</td>
<td>Cohort study, n = 727 women.</td>
<td>HRT use was not associated with 25(OH)D concentration.</td>
</tr>
<tr>
<td>Association of HRT use with vitamin D status (Touvier et al., 2015)</td>
<td>Cross-sectional study, n = 995 women, n = 394 postmenopausal, (n = 361 with HRT)</td>
<td>HRT use was not associated with 25(OH)D concentration.</td>
</tr>
</tbody>
</table>

Previous studies evaluating the association of early-onset menopausal transition or HRT with vitamin D status are shown in Table 8.

### 2.4.4 Summary

Women with EM and POI are at elevated risk for several adverse health consequences and morbidities. Vitamin D might be one way to help mitigate these issues. Thus, women with early-onset menopausal transition may benefit in particular from adequate vitamin D status. However, only a few studies have assessed vitamin D status in this patient group.
3  Aims of the present study

The purpose of this study was to explore the vitamin D status in the general population and in women with reproductive disorders living in a risk area for vitamin D deficiency and insufficiency. The study participants were gathered from the Northern Finland Birth Cohort 1966 (NFBC1966), which provided a large, population-based study sample.

The specific aims of the study were as follows:

1. To assess changes and seasonal variations in vitamin D status in a longitudinal setup before and after Finland’s implementation of a national food fortification program. The use of vitamin D supplementation and the association of vitamin D supplementation with 25(OH)D concentrations were evaluated.

2. To determine whether a history of infertility or decreased fecundability is associated with 25(OH)D concentration in women by age 31 and to investigate the association of previous reproductive outcomes with vitamin D status at that age.

3. To evaluate whether women with PCOS have lower 25(OH)D concentrations than healthy controls at age 31. In women with PCOS, factors associated with vitamin D status were assessed. The association between a history of fertility problems and vitamin D status in women with PCOS was observed.

4. To investigate vitamin D concentrations in relation to menopausal status in women by age 46 and to determine the association between HRT use and 25(OH)D concentrations in women with early onset of climacterium.
4 Materials and methods

4.1 Study population

Study participants were drawn from the large, population-based Northern Finland Birth cohort 1966 (NFBC1966), which includes 89.9% of all women who were pregnant with an estimated due date in 1966 from the most northern parts of Finland, Oulu and Lapland (n = 12,055). The cohort study began in the 24th gestational week; cross-sectional follow-ups were conducted at birth, one, 14, 31, and 46 years. The present study used the follow-ups at ages 31 and 46; both consisted of comprehensive questionnaires and clinical examinations (Nordström et al., 2022).

At 31 years, in 1997, cohort participants who were alive and whose contact information was available were sent a postal questionnaire (n = 11,543, women n = 5,688). Responses were available from 8,690 (75.3%, women n = 4,523). Participants living in northern Finland or in the Helsinki metropolitan area (n = 8,503, women n = 4,093) were asked to undergo a thorough clinical examination; 6,007 (70.6%, women n = 3,127) NFBC1966 members attended such an examination.

At 46 years, in 2012, 10,331 NFBC1966 members (women n = 5,123) received an updated postal questionnaire, of whom 7,146 (69.2%, women n = 3,848) responded. Clinical examination data were received from 5,832 (56.5%, women n = 3,263) participants at age 46.

Both the 31- and 46-year follow-ups included comprehensive questionnaires that asked about participants’ social background, lifestyle, behavior, work status, reproductive and medical history, previously diagnosed diseases, and use of medication and vitamin supplements. Clinical examinations at both timepoints consisted of blood samples with a wide range of measurements.

Study I included both men and women at two timepoints: ages 31 and 46. Studies II–IV included only women. Studies II–III used data from the follow-up at age 31, while Study IV used age 46. Covariates and laboratory samples were used at these timepoints unless otherwise noted. Figure 7 presents a flowchart of the study population.
Fig. 7. Flowchart of the study population. 25(OH)D: 25-hydroxyvitamin D.

Register data used in the study

The NFBC1966 data has been linked to various nationwide registers to be used with the cohort data. Information from Finland’s Population Register Center (now called the Digital and Population Data Service Agency) was used to categorize latitude in all four studies. In Study IV, data on prescriptions, purchases of
medicines, medicine expenses and reimbursement for medicine expenses were obtained from the Social Insurance Institution of Finland’s statistics on reimbursements for prescription medicines. A license is required to access those data, which are available from 1995 onward (The Social Insurance Institution of Finland, 2020). The Care Register for Health Care of Finland’s National Institute of Health and Welfare was used to identify women with POI.

4.2 Study groups

4.2.1 Vitamin D in Northern Finland Birth Cohort 1966 population (Study I)

In Study I, both 31-year and 46-year questionnaires and clinical examination data from all men and women were used; 25(OH)D concentrations in the NFBC1966 participants were measured at ages 31 and 46 (Figure 7). At 31 years, there were 5,600 measurements available, while there were 5,791 at 46 years. To be able to assess longitudinal changes in vitamin D status in Study I, only those participants with vitamin D measurements at both timepoints were retained (n = 3,650).

4.2.2 Infertility and decreased fecundability population (Study II)

Questionnaire and clinical examination data from women at age 31 were used in Study II. The 31-year follow-up questionnaire inquired, “Have you or has your partner been examined for infertility?” and “Have you been treated for infertility?” Women who answered yes to either question were assigned to the infertility group (n = 375). Fecundability was determined as time until the first pregnancy in months from the questionnaire information. Active exposure to pregnancy without using contraception for over one year was categorized as decreased fecundability (n = 338). Women without infertility examinations or treatments and fecundability times of less than a year were included in the control group (n = 2,051). Those participants who had never tried to become pregnant (n = 754) and those with partner infertility were excluded from the data (n = 38). The number of previous pregnancies, ectopic pregnancies, miscarriages, and deliveries were classified as zero, one, two, or more based on questionnaire information.
4.2.3 Women with self-reported PCOS (Study III)

To determine women with and without self-reported PCOS symptoms, questionnaire data from ages 31 and 46 were used in Study III. At age 31, the questionnaire asked, “Is your menstrual cycle often (more than twice a year) over 35 days?” and “Do you have excessive body hair?” Of 31-year-old women who answered these questions, 125 (4.2%) answered yes to both. Since oligo-amenorrhea and hirsutism are typical hormonal and metabolic features of the syndrome, the two questions had previously been validated as accurate in identifying PCOS (Taponen, 2004; Taponen et al., 2003). At age 46, the questionnaire asked, “Have you ever been diagnosed as having polycystic ovaries and/or polycystic ovary syndrome?”; 181 (5.0%) women answered yes. A population of women with self-reported PCOS (n = 280) was formed from the combination of answers at the two ages. All women without PCOS symptoms at age 31 who also answered no to the PCOS question at age 46 formed the control population (n = 1,573). As pregnancy and the use of hormonal contraceptives might improve irregular cycles and cause oligo-amenorrhea, those participants were excluded from the data at 31 years (n = 1,488), as were participants who did not give permission to use their data (31 years, n = 44; 46 years, n = 20).

4.2.4 Climacteric and preclimacteric women (Study IV)

The climacteric and preclimacteric populations in Study IV were formed based on the questionnaire and clinical examination at age 46 and on medicine reimbursement data from the register information of the Social Insurance Institution of Finland’s statistics on reimbursements for prescription medicines. FSH concentrations were measured in NFBC1966 at age 46.

At age 46, the NFBC1966 questionnaire included a question about menstrual history. Women without menstruation ≥ 4 months (i.e., amenorrhea) and FSH ≥ 25 IU/L were categorized as climacteric. Women with FSH < 25 IU/L and regular or irregular menstrual cycles were classified as preclimacteric. Climacteric women were considered perimenopausal or postmenopausal, while preclimacteric women were classified as premenopausal or early perimenopausal. This classification was based on ESHRE guidelines for POI and the Stages of Reproductive Aging Workshop (STRAW) +10 for reproductive stages, since there are no general recommendations for determining climacterium. Women who had made HRT purchases in the year prior to the 46-year follow-up in the medicine reimbursement
register and who reported using HRT in the 46-year questionnaire were all included in the climacteric group. Only FSH concentrations were used if women had been hysterectomized or currently used a progestin-only treatment (pill, capsule or, intrauterine device). Women using combined estrogen-progestin OCPs (n = 201) or tamoxifen (n = 14) were excluded. Ultimately, the climacteric group included 375 women and the preclimacteric group 2,244 women. In the climacteric group, 94 women (25.1%) used HRT.

4.3 Methods

4.3.1 Laboratory methods

At ages 31 and 46, blood samples in NFBC1966 were drawn after overnight fast between 8 and 10 a.m. by trained laboratory nurses. After being centrifuged, the serum samples at age 31 were placed in long-term freezing at –70°C before being defrosted and analyzed. The serum samples at age 46 were stored at –20°C and then –80°C before analyses.

25-hydroxyvitamin D measurements (Studies I–IV)

The serum samples drawn at age 31 were defrosted and analyzed in four batches in 2008 and 2009 for 25(OH)D measurements. Serum 25-hydroxyvitamin D$_2$ (25(OH)D$_2$) and 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) concentrations were analyzed with the LC-MS/MS (Elstree, Hertfordshire, United Kingdom), a procedure validated by the DEQAS. Total serum 25(OH)D concentrations were calculated as the sum of 25(OH)D$_2$ and 25(OH)D$_3$ concentrations. The coefficient of variation (CV) was less than 16% for the assay. The 25(OH)D concentrations measured with LC-MS/MS were verified with Diasorin radioimmunoassay (RIA, Stillwater, MN, United States), including a $^{125}$I-labeled tracer. In addition, the Centers for Disease Control and Prevention’s VDSP was used to calibrate 25(OH)D concentrations. First a chemiluminescence microparticle immunoassay (CMIA) Architect i2000SR automatic analyzer (Abbott Diagnostics, IL, United States) was used to analyze a subset of 25(OH)D samples. Then, based on those subset measurements, an equation was used to calculate VDSP-calibrated 25(OH)D concentrations at age 31. Calibrated 25(OH)D concentrations were comparable with laboratory methods in
NFBC1966 follow-up studies at different timepoints and with other published vitamin D studies. The calibrated 25(OH)D concentrations were used in Studies I and II.

The serum samples drawn at age 46 were analyzed with CMIA Architect i2000SR automatic analyzer (Abbott Diagnostics, Illinois, United States). Repeated quality control samples were included in the assay with the study samples to calculate CVs: in internal control samples with serum 25(OH)D > 100 nmol/L, the CV was 3.2%; in samples with medium serum 25(OH)D levels ~80 nmol/L, the CV was 3.1%; and with serum 25(OH)D < 40 nmol/L, the CV was 3.6%. In blinded quality control pairs in which 25(OH)D levels were not known, the CVs were 1.1%. The CMIA method was compared with high-performance liquid chromatography (HPLC); the results showed a high correlation of 0.922, and the reproducibility of CMIA was also highly reliable (R = 0.98).

**Outliers and categorization of 25(OH)D**

Outliers were calculated and excluded to add reliability to 25(OH)D measurements. An observation located at an abnormal distance from other measurements was defined as an outlier. Using interquartile ranges (IQRs) of 25(OH)D concentrations, outliers were calculated with two equations: the first quartile cutoff − 1.5 × IQR for the lower limit, and the third quartile cutoff + 1.5 × IQR for the upper limit.

Study I contained 112 outliers, Study II 23, Study III 6, and Study IV 59. The number of participants with 25(OH)D measurements after excluding outliers in Studies I–IV is presented in Table 9. In addition, in Study I, only those participants with measurements at both follow-ups (i.e., 31 and 46 years) were included to assess longitudinal changes in 25(OH)D concentrations. No significant difference was detected in vitamin D status, background, or clinical characteristics between the full sample and those with repeated measures at the two timepoints.

In Studies I and II, vitamin D status groups < 30, 30–50, 50–75, and > 75 nmol/L were used, based on both IOM and ES guidelines to describe vitamin D status as accurately as possible. Vitamin D status groups based only on IOM guidelines were used in Study III (< 30, 30–50, > 50 nmol/L) and only on ES guidelines in Study IV (< 50, 50–75, > 75 nmol/L), due to the higher mean distribution and quartiles of 25(OH)D concentrations in the 46-year follow-up than in the 31-year follow-up. In all four studies, quartiles of 25(OH)D concentrations were examined to compare distributions of the study populations in question.
Table 9. Number of 25(OH)D measurements in Studies I to IV.

<table>
<thead>
<tr>
<th>Study participants and study groups</th>
<th>Number of participants with 25(OH)D measurements¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td></td>
</tr>
<tr>
<td>31-year follow-up</td>
<td>5,564</td>
</tr>
<tr>
<td>46-year follow-up</td>
<td>5,715</td>
</tr>
<tr>
<td>Measurement at both follow-up points</td>
<td>3,650</td>
</tr>
<tr>
<td>Study II (women at 31 years)</td>
<td></td>
</tr>
<tr>
<td>Infertility</td>
<td>239</td>
</tr>
<tr>
<td>Decreased fecundability</td>
<td>203</td>
</tr>
<tr>
<td>Controls</td>
<td>1,324</td>
</tr>
<tr>
<td>Study III (women at 31 years)</td>
<td></td>
</tr>
<tr>
<td>Self-reported PCOS²</td>
<td>194</td>
</tr>
<tr>
<td>Controls</td>
<td>1,052</td>
</tr>
<tr>
<td>Study IV (women at 46 years)</td>
<td></td>
</tr>
<tr>
<td>Preclimacteric</td>
<td>2,193</td>
</tr>
<tr>
<td>Climacteric women with HRT³</td>
<td>76</td>
</tr>
<tr>
<td>Climacteric women without HRT³</td>
<td>275</td>
</tr>
</tbody>
</table>

¹25(OH)D: 25-hydroxyvitamin D, ²PCOS: Polycystic ovary syndrome, ³HRT: Hormone replacement therapy.

**Hormonal, inflammatory, and metabolic laboratory biomarkers (Studies III and IV)**

Samples were analyzed in NordLab Oulu (formerly called the Oulu University Hospital Laboratory, Oulu, Finland), a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO 15189). In Study III, concentrations of serum testosterone, high-sensitivity C-reactive protein (hs-CRP), fasting serum insulin (fS-Ins), and fasting plasma glucose (fP-Gluc) were used at age 31.

Testosterone concentrations were determined using Agilent triple quadrupole 6410 LC/MS equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Inc., Wilmington, DE, United States). The intra- and interassay CVs were 4.0% and 5.6%, respectively.

Inflammatory biomarker hs-CRP concentrations were measured with immunoenzymometric assay (Medix Biochemica, Espoo, Finland). The intra- and interassay coefficients of variation for the method were 4.2% and 5.2%, respectively.
After being stored at 4°C, fP-Gluc samples were analyzed the same day they were drawn. The fS-Ins samples were stored at −20°C and analyzed within seven days of being drawn; they were measured using RIA (Pharmacia Diagnostics, Uppsala, Sweden), while fP-Gluc was measured using a glucose dehydrogenase method (Granuprist 250, Diagnostica Merck, Darmstadt, Germany). For fS-Ins, the intra- and interassay coefficients of variation were 5.3% and 7.6%, respectively; for fP-Gluc, they were 1.5% and 2.3%, respectively. The concentrations of fP-Gluc and fS-Ins were used to calculate homeostatic model assessment of insulin resistance (HOMA-IR) values to estimate IR (Tang et al., 2015; Wallace et al., 2004):

\[ \text{HOMA-IR} = \frac{(\text{fP-Gluc} \times \text{fS-Ins})}{22.5} \]

In Study IV, serum FSH at age 46 was used to divide participants into groups; FSH was analyzed using an immunochemiluminometric method (Advia Centaur, Siemens Healthcare Diagnostics, Tarrytown, NY, United States).

4.3.2 Covariates

**BMI**

During the 31- and 46-year follow-up examinations, weight and height were measured by trained nurses. BMI was calculated by weight in kg divided by the square of height in m. If that BMI measurement was missing, information on weight and height from the postal questionnaires was used. Clinically measured and self-reported BMI have been shown to provide equivalent results (Ollila et al., 2016).

**Vitamin D from diet and supplementation**

Postal questionnaires at 31 and 46 years included questions about medication and vitamin supplements. At age 31, the question was “How often do you use the following medication? Vitamins or trace elements 1. Not at all, 2. Sometimes, 3. Regularly or continually”; strength and dose were also queried. Participants reporting any vitamin D-containing multivitamin or dietary supplementation use were classified as vitamin D supplementation users. A participant not reporting vitamin D supplementation use or vitamin D-containing multivitamins use were classified as no vitamin D supplementation use. An internet search of vitamins and mineral supplements was performed to determine whether they contained vitamin
D$_2$ or vitamin D$_3$. Emails were sent to manufacturers if the vitamin D content was not clear from the distributor’s or manufacturer’s website (Berry et al., 2017).

At age 46, the open-ended question was as follows: “List here all the medications you are taking with dose and amount (over-the-counter drugs, prescription drugs, vitamins, and dietary supplements). Do you use them regularly or as needed, and what do you take the medication for?” The frequency of vitamin D supplementation was classified as regular use if the participant answered daily or regular use. The answers every other day, twice a week, during dark times, in the winter, and so on were considered irregular. Participants who did not answer the question were classified as non-users. All vitamin D-containing supplements, multivitamins, calcium, and omega-3 supplements were included. If the vitamin D content of such products was missing, it was estimated through an internet search for vitamin D content information provided by the manufacturer. The frequency of vitamin D supplementation was used even when the dose of the vitamin D supplement remained unresolved ($n = 76$).

Studies I and IV used a food frequency questionnaire that assessed the frequency of food consumption during the six months prior to the 46-year follow-up study. Vitamin D consumption from dairy products, spreadable fats, and fish was calculated using the National Food Composition Database in Finland, which is maintained by the National Institute for Health and Welfare. The consumption of dairy products was estimated with the question “How many glasses (0.2 L) do you usually drink/eat per day of: 1. Milk, 2. Sour milk, 3. Other dairy products (e.g., yogurt, other fermented milk products, ice cream)?” All dairy products except cheese were evaluated to contain 1 $\mu$g/100 g of vitamin D. The consumption of fat spreads was estimated from the answers to three questions: “What type of bread spread do you usually use?” “How many times do you eat bread per day?” and “How much spread do you put on a slice of bread?” The vitamin D content in fat spreads varies by spread type: butter and organic butter contain 0 $\mu$g/g of vitamin D, plant-based sterol and stanol margarines and vegetable oil spreads 0.2 $\mu$g/g, and vegetable oil mixtures 0.1 $\mu$g/g. One serving (150 g) of fish was estimated to contain 13.4 $\mu$g of vitamin D based on the 10 most commonly consumed fish, according to the Finnish National Food Composition Database. If a participant ate fish almost daily, the vitamin D dose was evaluated to be 13.4 $\mu$g/d; if twice a week, 3.8 $\mu$g/d, and if once a week, 1.9 $\mu$g/d. Less frequent consumption was assessed to be 0 $\mu$g of vitamin D from fish.
Background information

In Study I, marital status was categorized as married if the participant was married or cohabiting and unmarried if divorced, widowed, or not cohabiting or married. In Study II, relationships were classified into two groups: those who had been in a relationship if ever married, cohabiting, separated, or widowed, and those who had no previous relationships of that nature.

To assess the SES of the study participants, either occupation or education was used. In Studies I and II, occupational status was categorized as higher-level employee, lower-level employee or entrepreneur, manual worker or farmer, and not working. In Study III, SES was categorized as professional, skilled worker, unskilled worker, farmer, and other. In Study IV, educational status was divided into basic, secondary, and higher education.

Environmental factors

To estimate participants’ solar exposure, latitude of residence, season of blood sampling, and frequency of sunny holidays were used. Information on participants’ residences during the follow-up studies in 1997 and 2012–2013 was collected from the Finnish population register center. Residence information was used to categorize latitude as 60°N (Helsinki and other provinces in middle and southern Finland), 65°N (the city of Oulu), and ≥ 65°N (Finland’s northernmost provinces of Oulu and Lapland).

Blood sampling season was defined as low-vitamin D season (November–May) and high-vitamin D season (June–October), based on the dates of each participant’s clinical examinations at ages 31 and 46. At age 46, clinical examinations were conducted throughout the year, but at age 31 February and March were not included due to the holiday season.

In Study IV, the frequency of sunny holidays abroad within the previous 10 years was collected from the questionnaire at age 46 and categorized as sunny holidays at least once a year, every two to three years, and never.

Lifestyle

Smoking status was categorized in Studies I and III as non-smoker, occasional or former smoker, and active smoker, and in Studies II and IV as non-smoker (including occasional/former smoker) or active smoker.
Alcohol consumption was estimated in g/d based on consumption of wine, beer, and spirits in the six months prior to the questionnaires. Alcohol intake was grouped into abstainers (0 g/d), low-risk drinking (≤ 40 g/d for men and ≤ 20 g/d for women), and high-risk drinking (> 40 g/d for men and > 20 g/d for women).

Physical activity was estimated based on the frequency and duration of leisure time activities as metabolic equivalent of task (MET) scores in hours per week (3 METs = light physical activity, 5 METs = brisk physical activity). The covariates in Studies I–IV are shown in Table 10.

### 4.3.3 Statistical methods

The distribution of the continuous variables was assessed visually with histogram normality curves. Normally distributed continuous variables were analyzed with independent sample *t*-tests and one-way analysis of variance when appropriate. Kruskal-Wallis and Mann–Whitney *U* tests were used for non-parametric continuous variables physical activity (Studies I–IV) and vitamin D supplementation dose (Study I). Pearson’s chi-square tests and Fisher’s exact tests were used for categorical variables. In these analyses, results were presented as mean (standard deviation [SD]), median (IQR), and prevalence (%), as appropriate.

Multivariable linear regression analysis was used to assess the association of different factors with 25(OH)D in all four studies. The results of the multivariable linear regression analyses are presented with beta coefficients with 95% confidence intervals (CIs). In all studies, variables in the models were selected based on results from the tests listed above and on knowledge from previous research (Jääskeläinen et al., 2013; Palaniswamy et al., 2017). After the variables in the models were tested one by one, they were all added to the regression models simultaneously. Two-way interactions between the main explanatory variables were also explored in all models. On the grounds of goodness-of-fit tests, only significant interaction terms of the independent variables were included in the final models, with *p*-values below 0.05 considered statistically significant. The analyses were performed with IBM SPSS Statistics for Windows, Version 25.0.0.0 (IBM Corp. Armonk, NY, United States). In addition, RStudio Version 1.1.456 was used in Study II.
<table>
<thead>
<tr>
<th>Covariate</th>
<th>Classified</th>
<th>Information based on</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season of blood sampling</td>
<td>Low vitamin D season: November–May, High vitamin D season: June–October.</td>
<td>Clinical examination at 31 and 46 years.</td>
<td>All</td>
</tr>
<tr>
<td>Smoking</td>
<td>Non-smoker, (occasional/former smoker), and active smoker.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>All</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Abstainers: 0 g/d, Low risk drinking: ≤ 40 g/d for men and ≤ 20 g/d for women, High-risk drinking: &gt; 40 g/d for men and &gt; 20 g/d for women.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>All</td>
</tr>
<tr>
<td>BMI</td>
<td>kg/m².</td>
<td>Clinical examination at 31 and 46 years, questionnaire information if no measured value.</td>
<td>All</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Metabolic equivalent of task scores in hours per week.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>All</td>
</tr>
<tr>
<td>Laboratory batch</td>
<td>Batches I to IV.</td>
<td>Laboratory information at 31 years.</td>
<td>I–III</td>
</tr>
<tr>
<td>Occupational status</td>
<td>Higher-level employee, lower-level employee or entrepreneur, manual worker or farmer, not working.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>I, II</td>
</tr>
<tr>
<td>Educational status</td>
<td>Basic, secondary, and higher education.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>I and IV</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married: Married or cohabiting, Unmarried: Not cohabiting or married, divorced, or widowed.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>I</td>
</tr>
<tr>
<td>Relationship status</td>
<td>Ever been in a relationship (ever married, cohabiting, separated, or widowed), No previous relationships.</td>
<td>Questionnaire information at 31 years.</td>
<td>II</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>Professional, skilled worker, unskilled worker, farmer, and other.</td>
<td>Questionnaire information at 31 and 46 years.</td>
<td>III</td>
</tr>
<tr>
<td>Sunny holidays</td>
<td>At least once a year, every two to three years, and never.</td>
<td>Questionnaire information at 46 years.</td>
<td>IV</td>
</tr>
</tbody>
</table>

g/d: grams per day, MET: Metabolic equivalent of task.
Specific statistical methods in Study I

Pearson’s chi-square tests for categorical variables, one-way analysis of variance for normally distributed continuous variables, and Kruskal–Wallis tests for non-parametric continuous variables were conducted to assess differences by vitamin D status and time. To study the differences in vitamin D intake characteristics between sexes, an independent samples t-test, Pearson’s chi-squared test, and the Mann–Whitney U test were performed.

In the multivariable linear regression model, serum 25(OH)D measurements were used as z-scores to control for the effects of different covariates. Standardization in the form of z-scores was performed with sex, season of blood sampling, latitude of residence, and technical laboratory variation (batch corrections 1–4 only for the 31-year measurements). The z-scores were calculated separately with different combinations of categories of these variables and then combined into a single variable. The 31- and 46-year z-score variables for 25(OH)D concentrations were used in the linear regression analysis.

A multivariable linear regression model was used to assess the association of different factors with 25(OH)D. In the model, the 46-year 25(OH)D z-score was the dependent variable, and vitamin D intake from fluid dairy products, fat spreads, and fish, vitamin D supplement use, BMI, and 31-year 25(OH)D z-score were independent variables. Marital status, physical activity, smoking, alcohol consumption, occupation, and education and their relevant two-way interactions were tested in the model, but no interaction was found, and their effects did not change the estimates of the other variables in the model.

Two sensitivity analyses were performed in Study I. In the first, a similar multivariable linear regression analysis that included the outlier data was conducted; the other excluded women using OCP and HRT, since that might affect serum 25(OH)D concentrations (Palaniswamy et al., 2017). The results of the multivariable regression models remained unchanged after the changes and exclusions described above.

In Study I, statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0.0.0 (IBM Corp. Armonk, NY, United States). Figure 1 was prepared using CorelDRAW Graphics Suite 2019, Version 21.0.0.593 (Corel Corporation, Ottawa, ON, Canada) and Figures 2 and 3 using GraphPad Prism Version 8.0.1.244 (GraphPad Software, San Diego, CA, United States).
Specific statistical methods in Study II

To analyze differences between women with a history of infertility, decreased fecundability, and controls, independent sample t-tests and one-way analysis of variance, as appropriate, were used for normally distributed continuous variables BMI and 25(OH)D. The Mann–Whitney U test was used for the non-parametric continuous variable physical activity, and Pearson’s chi-square test was used to compare the distributions of categorical variables across the infertility, decreased fecundability, and control groups.

Two multivariable linear regression models were conducted to assess the independent association between a history of infertility and 25(OH)D concentrations (model one) and between a history of decreased fecundability and 25(OH)D concentrations (model two). Model one included 25(OH)D concentration as the dependent variable and infertility, relationship status, alcohol consumption, season of blood sampling, latitude of residence, technical laboratory variation (batch correction), BMI, and physical activity as independent variables. Model two included 25(OH)D concentration as the dependent variable and decreased fecundability, relationship status, season of blood sampling, latitude of residence, laboratory batch effect, BMI, and physical activity as independent variables.

Sensitivity analyses were performed in Study II. The first such analysis was a similar multivariable linear regression including use of vitamin D supplementation, and the second excluded women who were pregnant at the 31-year follow-up, since hemodilution might change 25(OH)D concentrations (Takaoka et al., 2020). Weeks of gestation for the pregnant women were not known.

The statistical analyses were executed with IBM SPSS Statistics for Windows, Version 26.0.0.0 (IBM Corp, Armonk, NY, United States) and RStudio Version 1.1.456. Figure 1 was prepared using CorelDRAW Graphics Suite 2019, Version 21.0.0.593 (Corel Corporation, Ottawa, ON, Canada). Figures 2 and 3 were conducted using IBM SPSS Statistics for Windows, Version 28.0.0.0 (IBM Corp, Armonk, NY, United States).

Specific statistical methods in Study III

Independent samples t-tests or nonparametric Mann–Whitney U tests were carried out, as appropriate, to compare continuous variables (25(OH)D, BMI, physical activity, testosterone, HOMA-IR, hs-CRP) between women with and without self-reported PCOS. Pearson’s chi-square tests or Fisher’s exact tests were used to
compare the distributions of categorical variables (vitamin D status groups and quartiles, season of blood sampling, latitude of residence, alcohol consumption, smoking, SES) across women with self-reported PCOS and control women.

A multivariable linear regression model was carried out to find independent associations between different exposures with 25(OH)D concentrations. The final model included 25(OH)D concentration at 31 years as the dependent variable and self-reported PCOS, BMI, season of blood sampling, latitude of residence, and technical laboratory variation (batch correction for vitamin D) as independent variables.

A sub-analysis with self-reported participants with PCOS was conducted to assess mean testosterone concentrations between vitamin D quartiles. An additional sub-analysis including only women with PCOS was performed to compare serum 25(OH)D concentrations in women with and without fecundability problems. Infertility, decreased fecundability, and control study groups were defined as in Study II. Multivariable linear regression models were conducted with only women with PCOS to find independent associations with 25(OH)D concentrations, including infertility or decreased fecundability, season of blood sampling, latitude of residence, laboratory batch effect, BMI, and physical activity.

The statistical analyses were executed using IBM SPSS Statistics for Windows, Version 25 (IBM Corp., Armonk, NY, United States). The forest plot was created using RStudio Version 1.1.453.

Specific statistical methods in Study IV

To compare climacteric with preclimacteric women and climacteric women with and without HRT, independent samples t-tests for normally distributed continuous variables and Mann–Whitney U tests for non-parametric variables were conducted. Pearson’s chi-square tests were used to compare categorical variables between these study groups.

A multivariable linear regression model was developed to evaluate the independent association between climacteric status and 25(OH)D concentration in the study population. The confounding factors in the model were latitude of residence, season of blood sampling, smoking, BMI, physical activity, dietary vitamin D, and dose of vitamin D supplementation.

Another multivariable linear regression model was performed as a sub-analysis including only climacteric women to investigate factors associated with 25(OH)D concentrations, including use of HRT, latitude of residence, season of blood
sampling, smoking, BMI, physical activity, dietary vitamin D, and dose of vitamin D supplementation.

The statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0.0 (IBM Corp, Armonk, NY, United States). Figure 1 was prepared using CorelDRAW Graphics Suite 2019, Version 21.0.0.593 (Corel Corporation, Ottawa, ON, Canada), and Figures 2 and 3 were executed using GraphPad Prism Version 8.0.1.244 (GraphPad Software, San Diego, CA, United States).

4.3.4 Ethical issues

The ethical committee of the Northern Ostrobothnia Hospital District and University of Oulu originally approved the study setting of the NFBC1966 (94/2011, 12/2003). Study procedures followed the 1964 Helsinki Declarations and its later amendments. Permissions for nationwide register data to be used in the study were requested and obtained from the registers’ administrations. Participants provided written informed consent to use data from the NFBC1966 follow-ups. Separate consent was gathered from the participants to use register data for scientific purposes. Identification of individual study participants is not possible.
5 Results and Discussion

Figure 8 presents a summary of each of the four studies' characteristics, populations, main outcomes, and results.

![Characteristics and results of Studies I–IV](image)

**Fig. 8. Characteristics and results of Studies I–IV. 25(OH)D: 25-hydroxyvitamin D, HRT: Hormone replacement therapy, NFBC1966: Northern Finland Birth Cohort 1966, PCOS: Polycystic ovary syndrome.**

5.1 Determinants and longitudinal changes in vitamin D status (Study I)

The characteristics of the Study I population at ages 31 (in 1997) and 46 (in 2012–2013) between vitamin D status groups are shown in Table 11. Vitamin D status groups were classified into four categories based on 25(OH)D concentration: < 30, 30–50, 50–75, and > 75 nmol/L.
### Table 11. Background characteristics of the study population in different vitamin D status groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitamin D status groups at 31-year follow-up</th>
<th>Vitamin D status groups at 46-year follow-up</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 30</td>
<td>30–50</td>
<td>50–75</td>
</tr>
<tr>
<td>Marital status¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>259 (75.4)</td>
<td>903 (75.1)</td>
<td>1,176 (75.0)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>84 (24.6)</td>
<td>300 (24.9)</td>
<td>391 (25.0)</td>
</tr>
<tr>
<td>Occupational status²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Higher level employee</td>
<td>59 (18.1)</td>
<td>258 (21.8)</td>
<td>261 (17.0)</td>
</tr>
<tr>
<td>Lower-level employee/entrepreneur</td>
<td>134 (41.2)</td>
<td>475 (40.2)</td>
<td>640 (41.6)</td>
</tr>
<tr>
<td>Manual worker/farmer</td>
<td>98 (30.2)</td>
<td>321 (27.1)</td>
<td>421 (27.4)</td>
</tr>
<tr>
<td>Not working</td>
<td>34 (10.5)</td>
<td>129 (11.0)</td>
<td>215 (14.0)</td>
</tr>
<tr>
<td>BMI²</td>
<td>24.6 (4.2)</td>
<td>24.5 (4.0)</td>
<td>24.6 (4.2)</td>
</tr>
<tr>
<td>Smoking¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>168 (49.1)</td>
<td>601 (50.3)</td>
<td>740 (47.4)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>90 (26.3)</td>
<td>302 (25.3)</td>
<td>430 (27.5)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>84 (24.6)</td>
<td>293 (24.6)</td>
<td>392 (25.1)</td>
</tr>
<tr>
<td>Alcohol consumption¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abstainer</td>
<td>40 (11.9)</td>
<td>134 (11.5)</td>
<td>140 (9.2)</td>
</tr>
<tr>
<td>Low-risk drinker</td>
<td>280 (83.8)</td>
<td>996 (84.5)</td>
<td>1,306 (85.6)</td>
</tr>
<tr>
<td>High-risk drinker</td>
<td>14 (4.3)</td>
<td>49 (4.2)</td>
<td>80 (5.2)</td>
</tr>
<tr>
<td>Season of blood sampling¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-vitamin D season</td>
<td>86 (25.0)</td>
<td>475 (39.5)</td>
<td>1,216 (77.5)</td>
</tr>
<tr>
<td>Low-vitamin D season</td>
<td>260 (75.0)</td>
<td>728 (60.5)</td>
<td>354 (22.5)</td>
</tr>
<tr>
<td>Latitude¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60°N</td>
<td>57 (16.5)</td>
<td>249 (20.5)</td>
<td>201 (12.7)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Vitamin D status groups at 31-year follow-up</td>
<td>Vitamin D status groups at 46-year follow-up</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 30</td>
<td>30–50</td>
<td>50–75</td>
</tr>
<tr>
<td>65°N</td>
<td>57 (16.5)</td>
<td>244 (20.2)</td>
<td>332 (21.0)</td>
</tr>
<tr>
<td>≥ 65°N</td>
<td>232 (67.0)</td>
<td>719 (59.3)</td>
<td>1,048 (66.3)</td>
</tr>
</tbody>
</table>

1 n (%); 2 kg/m², mean (SD); 3 MET, metabolic equivalent of task, median [IQR].
Occupational status differed between the vitamin D status groups at age 31, but the difference disappeared at the age-46 follow-up. This might be explained by differences in occupation groups between the follow-up points. The not working category might have been more pronounced at the 31-year follow-up due to maternity leaves and a recession in Finland.

BMI was lower and amount of physical activity was elevated in the higher vitamin D status groups. There was a larger difference between the lowest and highest vitamin D status groups at 46 years than at 31 years. The association between vitamin D and BMI has been shown in multiple earlier studies (Pereira-Santos et al., 2015; Wortsman et al., 2000), as has the connection between vitamin D and physical activity (Jääskeläinen et al., 2013; Jorde et al., 2010). These findings are also in line with previously published research and longitudinal observations about smoking, alcohol consumption, BMI, and physical activity, which differed between vitamin D status groups (Jääskeläinen et al., 2013; Mutt et al., 2019; Palaniswamy et al., 2017; Pereira-Santos et al., 2015).

At 31 years, 25(OH)D measurements were drawn more often during the high-vitamin D season, whereas at 46 years more measurements were drawn during the low-vitamin D season. At both timepoints, the study population was mainly located in Northern Finland.

5.1.1 Vitamin D status, temporal change, and seasonal differences

Between the two follow-up points of Study I (1997–2012), Finland launched a national fortification program for dairy products and fat spreads (Raulio et al., 2017). Figure 9 presents the timeline of NFBC1966 31- and 46-year follow-up studies and vitamin D fortification waves I (2002) and II (2010).

![Fig. 9. Timeline of the NFBC1966 31- and 46-year follow-up studies and vitamin D fortification waves I (2002) and II (2010). NFBC1966: Northern Finland Birth Cohort 1966.](image)

In addition, a systematic vitamin D supplementation recommendation was instituted between the follow-up timepoints (Finnish Food Authority, 2014). The mean serum 25(OH)D concentration increased by 10.6 nmol/L (SD 24.4) in the
At 31 years, 25(OH)D concentration was 54.2 nmol/L (SD 18.5); at 46 years, it was 64.8 (SD 19.4) nmol/L. The prevalence of the lowest vitamin D groups (25(OH)D < 50 nmol/L) decreased almost 20% from 42.7% at 31 years to 23.5% at age 46 years. The highest vitamin D group (25(OH)D > 75 nmol/L) increased from 14.0% at 31 years to 28.8% at 46 years. The participants who had the lowest levels of 25(OH)D experienced a greater increase than the other vitamin D status groups. In the vitamin D status groups at 31 years (< 30, 30–50, 50–75, and > 75 nmol/L), the changes in serum 25(OH)D were 34.9, 21.9, 4.5, and −13.8 nmol/L, respectively. These observations show the importance and effectiveness of vitamin D fortification and regular use of vitamin D supplementation. Similar observations were reported in a study by Jääskeläinen et al. (Jääskeläinen et al., 2017).

During the 15-year period between follow-ups, seasonal variations in 25(OH)D concentrations decreased between the summer and winter months from 17.2 nmol/L at age 31 to 8.3 nmol/L at age 46. At age 31, the mean concentration of 25(OH)D in the winter months (November–May) was 43.6 (SD 15.4) nmol/L; in the summer months, it was 60.8 (SD 17.2) nmol/L. At age 46, the difference in 25(OH)D concentrations was less pronounced: 61.7 (SD 19.7) nmol/L in winter and 69.0 (SD 18.0) nmol/L in summer. In addition, between 31 and 46 years, the mean increase in 25(OH)D concentration was more pronounced in the winter months (18.1 nmol/L) than in the summer months (8.2 nmol/L).

The improvement of vitamin D status in this study appears to be attributable to the implementation of a food fortification policy in Finland and shows the effect of a national health policy among a middle-aged general population. The participants with the lowest vitamin D status at baseline appeared to benefit the most from fortification, indicating the importance and effectiveness of that program. In addition, seasonal variation in vitamin D status declined, with most participants having a year-round vitamin D status ≥ 50 nmol/L. These observations are consistent with the results of a previous Finnish study from H2000–2011, in which the population was mainly located in the southern parts of Finland. However, studies from Northern Sweden (Summerhays et al., 2019), Norway (Jorde et al., 2010), the Netherlands (Van Schoor et al., 2014), and from the United States (McKibben et al., 2016; Mirfakhraee et al., 2017; Schleicher et al., 2016) found no clear time trend or showed relatively stable serum 25(OH)D concentrations in longitudinal screening, although seasonal variations were observed. The fortification policies in these countries were not as systematic as in Finland, which might help explain the differences. In addition, all the studies were conducted with
different ethnic populations and age groups, which might have an impact on vitamin D status and thus affect the results.

### 5.1.2 Vitamin D intake

Dietary intake of vitamin D, the use of vitamin D supplements, and the distribution of vitamin D intake between the serum 25(OH)D status groups at age 46 are presented in Table 12. Total dietary vitamin D intake was calculated from the consumption of fortified dairy products, fortified fat spreads, and fish; on average, it was 11.0 μg/d. On average, 6.0 μg/d of vitamin D were obtained from fortified dairy products, 4.3 μg/d from fortified fat spreads, and 2.0 μg/d from fish.

At age 46, the intake of dietary vitamin D varied significantly between vitamin D status groups (Table 12). The total vitamin D intake and intake of dairy products and fish were higher in the highest vitamin D status groups (50–75 and > 75 nmol/L). However, the intake of vitamin D from fortified fat spreads did not differ significantly between vitamin D status groups. Vitamin D intake from fortified dairy products and fat spreads was also investigated as quartiles. There was a dose–response relationship between fortified dairy intake quartiles and serum 25(OH)D concentrations (60.4, 63.2, 65.0, and 68.8 nmol/L from lowest to highest quartile, \( p < 0.001 \)). However, no significant difference in vitamin D status was observed between the fortified fat spread intake quartiles (66.1, 63.8, 64.0, and 64.9 nmol/L, \( p = 0.14 \)).

At age 46, vitamin D supplements were used by 25.8% of study participants (Table 12). Among those users, 71.5% took vitamin D supplements regularly and 28.5% irregularly. As expected, regular supplementation use was more likely in the > 75 nmol/L and 50–75 nmol/L groups (29.4% and 16.6%, respectively) than in vitamin D status groups with 25(OH)D < 50 nmol/L (12.8%, \( p < 0.001 \)). The median of vitamin D supplementation dose was 10.0 μg (IQR 12.5). The dose of vitamin D supplementation increased toward the highest vitamin D status group (Table 12).

Both the general Nordic and specific Finnish nutrition recommendations for vitamin D intake are 10 μg/d (Itkonen et al., 2020; Raulio et al., 2017). This was exceeded by study participants, with an average intake of 11.0 μg/d. This result indicates the adequacy of the present fortification policy. The amount of vitamin D fortification in Sweden and Norway is lower, and intake has not increased similarly in either country (Itkonen et al., 2020).
Table 12. Vitamin D status in the study population at 31 and 46 years and vitamin D intake at 46 years from diet and supplements, compared to vitamin D status groups at 46 years.

<table>
<thead>
<tr>
<th>Vitamin D characteristic</th>
<th>n</th>
<th>Mean ± SD</th>
<th>&lt; 30</th>
<th>30–50</th>
<th>50–75</th>
<th>&gt; 75</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D at 31 years 1</td>
<td>3,650</td>
<td>54.2 (18.5)</td>
<td>346 (9.5)</td>
<td>1,213 (33.2)</td>
<td>1,581 (43.3)</td>
<td>510 (14.0)</td>
<td>NA</td>
</tr>
<tr>
<td>25(OH)D at 46 years 1</td>
<td>3,650</td>
<td>64.8 (19.4)</td>
<td>92 (2.5)</td>
<td>765 (21.0)</td>
<td>1,740 (47.7)</td>
<td>1,053 (28.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Change in 25(OH)D 1</td>
<td>3,650</td>
<td>10.6 (24.4)</td>
<td>34.9 (19.1)</td>
<td>21.9 (19.9)</td>
<td>4.5 (20.0)</td>
<td>-13.8 (20.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin D intake at 46 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dietary intake 2</td>
<td>3,638</td>
<td>11.0 (5.8)</td>
<td>8.9 (18.7)</td>
<td>9.6 (5.1)</td>
<td>11.4 (6.1)</td>
<td>11.6 (5.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fortified dairy products 2</td>
<td>3,555</td>
<td>6.0 (4.1)</td>
<td>4.3 (3.9)</td>
<td>5.0 (3.4)</td>
<td>6.3 (4.4)</td>
<td>6.6 (4.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fortified fat spreads 2</td>
<td>2,766</td>
<td>4.3 (2.6)</td>
<td>4.4 (2.7)</td>
<td>4.2 (2.5)</td>
<td>4.4 (2.8)</td>
<td>4.1 (2.5)</td>
<td>0.054</td>
</tr>
<tr>
<td>Fish 2</td>
<td>3,384</td>
<td>2.0 (2.4)</td>
<td>1.4 (2.0)</td>
<td>1.8 (2.2)</td>
<td>2.0 (2.4)</td>
<td>2.2 (2.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Use of supplements 3</td>
<td>3,650</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>674</td>
<td>3 (3.3)</td>
<td>73 (9.5)</td>
<td>288 (16.5)</td>
<td>310 (29.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irregular</td>
<td>268</td>
<td>4 (4.3)</td>
<td>40 (5.3)</td>
<td>135 (7.8)</td>
<td>89 (8.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2,708</td>
<td>85 (92.4)</td>
<td>652 (85.2)</td>
<td>1,317 (75.7)</td>
<td>654 (62.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplementation dose 4</td>
<td>866</td>
<td>10.0 [12.5]</td>
<td>4.4 [17.5]</td>
<td>10.0 [10.0]</td>
<td>10.0 [12.5]</td>
<td>16.3 [20.0]</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

1 nmol/L, mean (SD), 2 µg/d, mean (SD), 3 n (%), 4 µg/d, median (IQR). 25(OH)D: 25-hydroxyvitamin D.
In line with previous research, vitamin D intake from diet came mostly from dairy products, and dose–response was observed in serum 25(OH)D concentrations in increasing milk intake quartiles. Unlike vitamin D from dairy products, there was no association between vitamin D from fortified fat spreads and serum 25(OH)D concentrations. This finding was also observed in the H2000–2011 study (Jääskeläinen et al., 2017). This difference might be explained by health and lifestyle factors associated with the intake of dairy and fat products. Consumption of dairy products is suggested to be related to an overall healthier diet and even a decreased risk for diseases, especially T2D, metabolic syndrome, and CVD (Crichton et al., 2019; Theodoratou et al., 2014). In contrast, higher consumption of fat might reflect an unhealthier lifestyle and obesity (Raatz et al., 2017). Additionally, some differences might be connected to the bioavailability of vitamin D in fortified fat spreads and fortified milk (Grossmann & Tangpricha, 2010; Yang et al., 2013). Based on the findings reported here and in previous research, it may be worth asking whether the current policy of vitamin D fortification of fat spreads actually benefits the general population in terms of improved vitamin D status. To the best of our knowledge, there is no evidence to demonstrate such benefits in the literature.

At 46 years, vitamin D supplementation was used by an average 26% of the study participants, in contrast to the H2000–2011 study, which reported that supplements were used by over 40% of the population (Jääskeläinen et al., 2017). The questionnaire used in the present study asked about all medications and supplementation with an open-ended question, which may have led to an underestimation of supplementation consumption. Still, a significant positive effect on 25(OH)D concentrations was observed with regular supplementation use. The mean age of the H2000-2011 population was 10 years higher, which might also explain the difference between the studies, since supplementation use is generally more common in older age groups (Jääskeläinen et al., 2017). In addition, possible differences in behavior and habits by region might be involved, since the study participants of the present study were mainly located in Northern Finland.

**Vitamin D between genders**

Table 13 shows the differences between vitamin D status and intake between female and male participants. The mean 25(OH)D concentration was significantly higher in both women and men between the timepoints at 46 and 31 years ($p < 0.001$). However, there was no significant difference when comparing the 25(OH)D
concentrations or vitamin D status groups between genders at either timepoint. There were significant differences between genders in vitamin D intake. Men obtained more vitamin D from dietary sources than women (12.3 µg vs. 10.1 µg, \( p < 0.001 \)). Intake of vitamin D from fortified dairy products (\( p < 0.001 \)) and fat spreads (\( p < 0.001 \)) was higher among men than among women, but no difference was observed in the intake of vitamin D from fish. Rather, the frequency of vitamin D supplementation use and median vitamin D supplementation dose were both higher among women than among men (24.5% vs. 10.8%, \( p < 0.001 \), and 11.3 µg/d vs. 10.0 µg/d, \( p = 0.009 \), respectively).

Table 13. Vitamin D between female and male study participants.

<table>
<thead>
<tr>
<th>Vitamin D characteristic</th>
<th>Women</th>
<th>Men</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D concentration(^1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At age 31</td>
<td>2,051</td>
<td>1,599</td>
<td>0.39</td>
</tr>
<tr>
<td>At age 46</td>
<td>2,051</td>
<td>1,599</td>
<td>0.083</td>
</tr>
<tr>
<td>Vitamin D status group at 31(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>210 (10.2)</td>
<td>136 (8.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>30–50</td>
<td>671 (32.7)</td>
<td>542 (33.9)</td>
<td></td>
</tr>
<tr>
<td>50–75</td>
<td>895 (43.6)</td>
<td>686 (42.9)</td>
<td></td>
</tr>
<tr>
<td>&gt; 75</td>
<td>275 (13.4)</td>
<td>235 (14.7)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D status group at 46(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>47 (2.3)</td>
<td>45 (2.8)</td>
<td>0.44</td>
</tr>
<tr>
<td>30–50</td>
<td>422 (20.6)</td>
<td>343 (21.5)</td>
<td></td>
</tr>
<tr>
<td>50–75</td>
<td>972 (47.4)</td>
<td>768 (48.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 75</td>
<td>610 (29.7)</td>
<td>443 (27.7)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake at age 46(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dietary intake</td>
<td>2,046</td>
<td>1,592</td>
<td>0.001</td>
</tr>
<tr>
<td>Fortified dairy products</td>
<td>2,006</td>
<td>1,549</td>
<td>0.001</td>
</tr>
<tr>
<td>Fortified fat spreads</td>
<td>1,550</td>
<td>1,216</td>
<td>0.001</td>
</tr>
<tr>
<td>Fish</td>
<td>1,922</td>
<td>1,462</td>
<td>0.077</td>
</tr>
<tr>
<td>Use of supplements(^2)</td>
<td>2,051</td>
<td>1,599</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Regular</td>
<td>502 (24.5)</td>
<td>172 (10.8)</td>
<td></td>
</tr>
<tr>
<td>Irregular</td>
<td>204 (9.9)</td>
<td>64 (4.0)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1,345 (65.6)</td>
<td>1,363 (85.2)</td>
<td></td>
</tr>
<tr>
<td>Supplementation dose(^4)</td>
<td>643</td>
<td>223</td>
<td>0.009</td>
</tr>
</tbody>
</table>

\(^1\text{nmol/L, mean (SD), } ^2\text{\%}, \ ^3\text{µg/day, mean (SD), } ^4\text{µg/day, median [IQR], 25(OH)D: 25-hydroxyvitamin D.}\)
Sex differences in vitamin D status were not found, which is in line with an earlier systematic review of observational studies worldwide (Hilger et al., 2014). In men, higher intake of energy and vitamin D-containing food products in the diet might explain the difference in sources of vitamin D between the sexes and is important to acknowledge. Parallel findings have previously been published (Jääskeläinen et al., 2017; Pietinen et al., 2010; Raulio et al., 2017). Women might benefit from being reminded about the regular use of vitamin D supplementation.

5.1.3 Predictors of vitamin D status

A multivariable linear regression model was developed to examine the independent association of multiple predictors of vitamin D status at 46 years. The 25(OH)D concentration was used as the z-score, which was standardized for sex, season of blood sampling, and latitude of residence. At 46 years, the 25(OH)D z-score was positively associated with the vitamin D intake from fortified dairy products and fish and with regular use of vitamin D supplements (Figure 10). However, the estimated intake of vitamin D from fortified fat spreads was not associated with the 46-year 25(OH)D z-score. A negative association was observed between the 46-year 25(OH)D z-score and BMI. Interestingly, the 25(OH)D at 46 years was positively predicted by the 31-year 25(OH)D z-score.

In the multivariable linear regression model, several confounders were adjusted, including dietary vitamin D intake and supplementation. To our knowledge, only one published study using an adult population has a similar finding of an association between present 25(OH)D concentration and previous vitamin D status. This result may suggest that individual factors (e.g. lifestyle, sociodemographic, or genetic) play an important role in vitamin D status, along with dietary intake, supplementation, and other vitamin D sources (Wang et al., 2010). As a hypothesis, individual biological pathways or genetic factors might be connected to vitamin D status. Vitamin D absorption from the intestine, liver and kidney function, chronic diseases, and medications could each affect overall vitamin D metabolism and thus 25(OH)D concentrations. In the future, more detailed research into the role of these pathways and the impacts of individual factors on vitamin D status is required.
Fig. 10. Results of the multivariable linear regression analyses of the 25(OH)D z-score with different exposures at age 46 (2012-2013) in the Northern Finland Birth Cohort 1966. 25(OH)D: 25-hydroxyvitamin D, BMI: Body mass index. VitD: Vitamin D.
5.1.4 Summary

After Finland’s implementation of fortification of dairy products and fat spreads, improvement in vitamin D status was observed, and the prevalence of vitamin D insufficiency declined. The regular use of vitamin D supplements also appeared to be an important factor in achieving adequate vitamin D intake. Seasonal variations in 25(OH)D concentrations diminished between the two follow-up timepoints. Despite the positive effect of public health actions on vitamin D status, the 31-year 25(OH)D concentrations independently associated with serum 25(OH)D concentrations at 46 years, indicating a possible individual behavioral or genetic factor that at least partly explains vitamin D status.

5.2 Vitamin D status in women with infertility and decreased fecundability (Study II)

There were 375 women with a history of infertility, 338 women with decreased fecundability, and 2,051 controls without infertility problems and fecundability below 12 months in NFBC1966. Women with a history of infertility and decreased fecundability had higher BMIs than controls \((p = 0.04\) and \(p = 0.03\), respectively). Alcohol consumption in women with infertility were more at the high-risk level than in controls \((p = 0.04)\). Women with decreased fecundability were more likely to have been in a relationship \((p = 0.02)\) and were more physically active \((p = 0.02)\) than controls.

5.2.1 Vitamin D status between study groups and reproductive outcomes

Concentrations of 25(OH)D were observed as means (SDs), vitamin D status groups (< 30, 30–50, 50–75, > 75 nmol/L), and quartiles (< 39.1, 39.1–53.1, 53.1–66.5, > 66.5) between study groups. In women with previous infertility examinations or treatments, the mean 25(OH)D concentration was lower (51.2 nmol/L, SD 18.9, \(p = 0.019\)), but there was no significant difference in the decreased fecundability group (53.1 nmol/L, SD 17.9, \(p = 0.39\)) when compared to the controls (54.2 nmol/L, SD 18.2; Table 14). Vitamin D status group < 30 nmol/L was also more frequent in women with previous infertility problems than among controls, whereas vitamin D status > 75 nmol/L was more frequent among controls.
(\(p = 0.019\)). No significant differences were observed between vitamin D quartiles or in women with decreased fecundability. The lowest vitamin D quartile was more pronounced in the infertility group compared to controls, but that difference did not reach a significant level.

Table 14. 25(OH)D concentrations and distributions between women with previous infertility or decreased fecundability and controls.

<table>
<thead>
<tr>
<th>25(OH)D status</th>
<th>Infertility(^3) (n = 239)</th>
<th>Decreased fecundability(^4) (n = 203)</th>
<th>Controls (n = 1,324)</th>
<th>(p)-value(^3)</th>
<th>(p)-value(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D(^1)</td>
<td>51.2 (18.9)</td>
<td>53.1 (17.9)</td>
<td>54.2 (18.2)</td>
<td>0.019</td>
<td>0.39</td>
</tr>
<tr>
<td>25(OH)D groups(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>38 (15.9)</td>
<td>20 (9.9)</td>
<td>123 (9.3)</td>
<td>0.019</td>
<td>0.86</td>
</tr>
<tr>
<td>30–50</td>
<td>77 (32.2)</td>
<td>72 (35.5)</td>
<td>439 (33.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–75</td>
<td>97 (40.6)</td>
<td>83 (40.9)</td>
<td>584 (44.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 75</td>
<td>27 (11.3)</td>
<td>28 (13.8)</td>
<td>178 (13.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D quartiles(^2)</td>
<td></td>
<td></td>
<td></td>
<td>0.063</td>
<td>0.46</td>
</tr>
<tr>
<td>&lt; 39.1</td>
<td>71 (29.7)</td>
<td>54 (26.6)</td>
<td>317 (23.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39.1–53.1</td>
<td>59 (24.7)</td>
<td>44 (21.7)</td>
<td>327 (24.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53.1–66.5</td>
<td>63 (26.4)</td>
<td>57 (28.1)</td>
<td>327 (24.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 66.5</td>
<td>46 (19.2)</td>
<td>48 (23.6)</td>
<td>353 (26.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) nmol/L, mean (SD), \(^2\) n (%), \(^3\) \(p\)-value comparing women with previous infertility examination or infertility treatment versus controls. \(^4\) \(p\)-value comparing women with decreased fecundability versus controls. 25(OH)D: 25-hydroxyvitamin D.

Serum 25(OH)D concentrations were also investigated as means (SDs) and vitamin D status groups (< 30, 30-50, 50-75, > 75 nmol/L) in terms of reproductive outcomes: pregnancies, miscarriages, ectopic pregnancies, and deliveries. There was no significant difference between mean 25(OH)D concentrations and rates of previous pregnancies (\(p = 0.38\)), ectopic pregnancies (\(p = 0.28\)), or deliveries (\(p = 0.07\)). The vitamin D status groups also did not differ between these reproductive outcomes.

However, in women with two or more miscarriages, the mean 25(OH)D concentrations were significantly lower (51.0 nmol/L, SD 19.5) than in women without previous miscarriages (54.2 nmol/L, SD 18.1, \(p = 0.04\)). Multiple miscarriages appeared to be more frequent in women with vitamin D status < 30 nmol/L and less common in the group with vitamin D > 75 nmol/L, but the difference was not significant (\(p = 0.079\)).
5.2.2 Association of infertility and decreased fecundability with vitamin D status

Multivariable adjusted linear regression models 1 and 2 were used to assess the independent association of a history of infertility and decreased fecundability with 25(OH)D concentrations, with adjustments made for relevant confounding factors. Multivariable adjusted linear regression model 1 demonstrated that a history of infertility had a negative association with 25(OH)D concentration ($\beta = -2.7$, 95% CI -4.6, -0.7, $p = 0.008$; Table 15). In addition, previous decreased fecundability had an association with lower 25(OH)D concentration ($\beta = -4.1$, 95% CI -7.4, -0.8, $p = 0.014$; Table 15) in the multivariable adjusted linear regression model 2.

Table 15. Association of 25(OH)D and relevant exposures in multivariable linear regression models 1 and 2.

<table>
<thead>
<tr>
<th>Multivariable linear regression models</th>
<th>$\beta$ Coefficient</th>
<th>95% CI of $\beta$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infertility$^2$</td>
<td>-2.7</td>
<td>-4.6, -0.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Low-vitamin D season$^3$</td>
<td>-9.3</td>
<td>-16.3, -2.3</td>
<td>0.009</td>
</tr>
<tr>
<td>$^{4}$BMI</td>
<td>-0.6</td>
<td>-1.1, -0.05</td>
<td>0.031</td>
</tr>
<tr>
<td>$^{5}$Physical activity$^{4,5}$</td>
<td>0.2</td>
<td>0.003, 0.3</td>
<td>0.045</td>
</tr>
<tr>
<td>Model 2$^6$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased fecundability$^2$</td>
<td>-4.1</td>
<td>-7.4, -0.8</td>
<td>0.014</td>
</tr>
<tr>
<td>Low-vitamin D season$^3$</td>
<td>-9.2</td>
<td>-16.2, -2.2</td>
<td>0.010</td>
</tr>
<tr>
<td>$^{4}$BMI</td>
<td>-0.5</td>
<td>-1.1, -0.004</td>
<td>0.048</td>
</tr>
<tr>
<td>$^{5}$Physical activity$^{4,5}$</td>
<td>0.2</td>
<td>0.04, 0.4</td>
<td>0.013</td>
</tr>
</tbody>
</table>

$^1$Model 1 also included relationship status, latitude, alcohol consumption, and laboratory effect, none of which had a significant association with 25(OH)D concentrations. $^2$No fertility problems as reference category. $^3$High-vitamin D season as reference category. $^4$Continuous variable. $^5$Metabolic equivalent of task scores: physical activity in hours per week. $^6$Model 2 also included relationship status, latitude, and laboratory effect, none of which had a significant association with 25(OH)D concentrations. 25(OH)D: 25-hydroxyvitamin D, CI: Confidence interval.

Higher BMI and low-vitamin D season were associated with lower 25(OH)D concentrations and physical activity with higher 25(OH)D concentrations in both
models. Other adjusted factors were not associated with 25(OH)D concentrations in the models (e.g., alcohol consumption in model 1 and latitude of residence and relationship status in both models). An inverse association between BMI and 25(OH)D concentrations has been demonstrated in multiple studies and was also found in the present study’s population (Palaniswamy et al., 2017; Pereira-Santos et al., 2015). Bioavailability of 25(OH)D might be decreased with higher BMI, so these individuals might need to maintain an even higher vitamin D status (Wortsman et al., 2000). In women with decreased fecundability, the frequency of physical activity was higher than in controls. Consistent with previous studies, physical activity was positively associated with 25(OH)D concentrations in the linear regression models (Jääskeläinen et al., 2013; Palaniswamy et al., 2017).

Previously, the association of infertility problems or fecundability and 25(OH)D concentrations has only been investigated in a general population in a cohort study setting in a few instances. A greater possibility of pregnancy was observed in a study by Fung et al. in women with mean 25(OH)D concentrations over 50 nmol/L, compared to women with 25(OH)D below 50 nmol/L (Fung et al., 2017). These findings were also reported in a population from the United States, where the probability of pregnancy was associated with 25(OH)D concentrations (Jukic et al., 2019; Mumford et al., 2018). Studies with Danish or Italian populations reported opposite results (Møller et al., 2012; Somigliana et al., 2016). However, the participant rate in both of those studies were low, and the follow-up time was short. Møller et al. did not find an association between fecundability or pregnancy outcomes and serum 25(OH)D concentrations. However, that result may have been affected by the fact that their study participants were healthy women who used OCPs, in addition to the low sample size (Møller et al., 2012). In the Italian study by Somigliana et al., 25(OH)D concentrations were measured in women who were already pregnant, which might have caused changes in 25(OH)D concentrations due to hemodilution (Somigliana et al., 2016; Takaoka et al., 2020). The studies excluded women with previous infertility and focused on healthy women; they thus only partially reflected the situation of the general population (Fung et al., 2017; Jukic et al., 2019; Møller et al., 2012). In one study, vitamin D supplementation use was not examined (Mumford et al., 2018).

Vitamin D has been suggested to have an immunomodulatory impact (Sassi et al., 2018; Tamblyn et al., 2015) that influences embryonic implantation, placentation, and pregnancy success (Gonçalves et al., 2018; Tamblyn et al., 2015). In the present study’s population, 25(OH)D concentrations were lower in women who miscarried than among women without miscarriages, suggesting a possible
link with vitamin D. Other studies have reported inconsistent results as to an association between miscarriages and 25(OH)D (Amegah et al., 2017; Andersen et al., 2015; Zhang et al., 2017). However, those results should be interpreted with caution, since the participation rates in those studies were small.

Multiple enzymes influence the steroidogenesis of sex hormones (Shahrokhi et al., 2016). The positive effect of vitamin D on fertility has been proposed to arise from its impact on the synthesis of enzymes like 17β-hydroxysteroid dehydrogenase (Wang & Tuohimaa, 2007) and aromatase (Krishnan et al., 2010). Furthermore, ovarian reserve marker AMH levels and 25(OH)D concentrations have been suggested to be positively correlated, indicating that vitamin D may regulate folliculogenesis (Chu et al., 2021; Irani & Merhi, 2014). The relevance of these observations to fertility needs further research.

In a few studies, vitamin D has been postulated to play a positive role in the success of infertility treatments (Chu et al., 2018; Vanni et al., 2014). Probability of pregnancy and live birth rates may be elevated in women with adequate vitamin D status during infertility treatments (Chu et al., 2018). However, some studies have reported the opposite results (Aleyasin et al., 2011; Neville et al., 2016). In a study by Abadia et al., a positive association was observed between 25(OH)D concentrations and fertilization rates, but no association was found with clinical pregnancy or live birth rates in women who underwent in vitro fertilization or intracytoplasmic sperm injection treatments (Abadia et al., 2016).

In the NFBC1966 study population, 25(OH)D concentrations did not differ by number of previous pregnancies, ectopic pregnancies, or deliveries. Although the control population had higher vitamin D status, the mean concentration was still within the 50 nmol/L limit of insufficiency (Holick et al., 2011); 25(OH)D concentrations over 50 nmol/L are considered sufficient and have been observed to prevent increase of PTH concentration and lower the risk of rickets (Holick et al., 2011). Even higher concentrations of 25(OH)D might be required for favorable health effects and improvement in fertility to be observed with confidence (Bischoff-Ferrari et al., 2006; Cozzolino et al., 2020). Hence, observational studies with large sample sizes are needed to determine the optimal 25(OH)D concentrations that might increase reproductive outcome rates and enhance fecundability.
5.2.3 Summary

In this study population, previous infertility problems and decreased fecundability were associated with an increased rate of lower 25(OH)D concentrations. Sufficient vitamin D status might benefit women with fertility problems, especially obese women who may be at increased risk for vitamin D deficiency. Measurement of 25(OH)D concentration might be advisable, and appropriate intake of vitamin D should be considered for women of reproductive age who are planning pregnancy.

5.3 Vitamin D levels in women with PCOS (Study III)

5.3.1 Clinical and biochemical features of the PCOS study population

As to PCOS, 25(OH)D measurements were available from 192 women with self-reported PCOS and 1,048 controls. Compared to the controls, women with self-reported PCOS were more likely to live in Finland’s northernmost latitudes ($p = 0.002$). In clinical and other biochemical features, women with self-reported PCOS had higher mean BMIs (26.23 kg/m$^2$, SD 6.05 vs. 23.61 kg/m$^2$, SD 4.19, $p < 0.001$; Table 16), testosterone (1.40 nmol/L, SD 0.65 vs. 1.03 nmol/L, SD 0.43, $p < 0.001$), and HOMA-IR (1.23, SD 0.77 vs. 1.00, SD 0.43, $p < 0.001$) concentrations than in controls. Hs-CRP concentrations were almost twice as high in women with self-reported PCOS than in controls (2.62 mg/L SD 4.01 vs. 1.63 mg/L, SD 3.41, $p < 0.001$).

Table 16. Clinical and other biochemical features in women with self-reported PCOS and non-PCOS controls at age 31.

<table>
<thead>
<tr>
<th>Feature</th>
<th>PCOS (n = 192–268)</th>
<th>Controls (n = 1,048–1,560)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m$^2$ $^1$</td>
<td>26.23 (6.05)</td>
<td>23.61 (4.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Testosterone levels, nmol/L $^1$</td>
<td>1.40 (0.65)</td>
<td>1.03 (0.43)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HOMA-IR $^1$</td>
<td>1.23 (0.77)</td>
<td>1.00 (0.43)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hs-CRP, mg/L $^1$</td>
<td>2.62 (4.01)</td>
<td>1.63 (3.41)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$^1$mean (SD), HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, hs-CRP: high-sensitivity C-reactive protein, PCOS: Polycystic ovary syndrome.
5.3.2 Comparison of vitamin D status between women with PCOS and controls

Serum 25(OH)D concentrations were compared between women with self-reported PCOS and non-symptomatic controls as means (SDs), vitamin D status groups (< 30, 30–50, 50–75, > 75 nmol/L), and vitamin D quartiles (< 39.0, 39.0–49.5, 49.5–59.7, > 59.7 nmol/L). As a sub-analysis, testosterone concentrations were compared in 25(OH)D quartiles only among women with PCOS.

There was no difference in mean 25(OH)D concentrations between women with self-reported PCOS and controls (50.35 nmol/L, SD 13.51 vs. 48.30 nmol/L, SD 13.37, \( p = 0.051 \); Table 17). In addition, the distribution across vitamin D status groups (\( p = 0.24 \)) and 25(OH)D quartiles (\( p = 0.058 \)) did not differ between women with PCOS and controls.

Table 17. Serum 25(OH)D concentrations and distributions in women with self-reported PCOS and in non-PCOS controls at age 31.

<table>
<thead>
<tr>
<th>25(OH)D status</th>
<th>PCOS (n = 192)</th>
<th>Controls (n = 1,048)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (^1)</td>
<td>50.35 (13.51)</td>
<td>48.30 (13.37)</td>
<td>0.051</td>
</tr>
<tr>
<td>25(OH)D status groups (^2)</td>
<td></td>
<td></td>
<td>0.241</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>12 (6.3)</td>
<td>93 (8.9)</td>
<td></td>
</tr>
<tr>
<td>30–50</td>
<td>87 (45.3)</td>
<td>473 (45.1)</td>
<td></td>
</tr>
<tr>
<td>50–75</td>
<td>85 (44.3)</td>
<td>460 (43.9)</td>
<td></td>
</tr>
<tr>
<td>&gt; 75</td>
<td>8 (4.2)</td>
<td>22 (2.1)</td>
<td></td>
</tr>
<tr>
<td>25(OH)D quartiles (^2)</td>
<td></td>
<td></td>
<td>0.058</td>
</tr>
<tr>
<td>&lt; 39.0</td>
<td>38 (19.8)</td>
<td>299 (28.5)</td>
<td></td>
</tr>
<tr>
<td>39.0–49.5</td>
<td>57 (29.7)</td>
<td>251 (24.0)</td>
<td></td>
</tr>
<tr>
<td>49.5–59.7</td>
<td>51 (26.6)</td>
<td>280 (26.7)</td>
<td></td>
</tr>
<tr>
<td>&gt; 59.7</td>
<td>46 (23.9)</td>
<td>218 (20.8)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)nmol/L (mean, SD), \(^2\) (%), 25(OH)D: 25-hydroxyvitamin D, PCOS: Polycystic ovary syndrome.

In a sub-analysis with only self-reported women with PCOS, there was no difference in testosterone concentrations between the different vitamin D quartiles. Testosterone was 1.20 nmol/L in the lowest 25(OH)D quartile (\( n = 37 \)), 1.40 nmol/L in the second quartile (\( n = 56 \)), 1.44 nmol/L in the third quartile (\( n = 51 \)), and 1.48 nmol/L in the highest 25(OH)D quartile (\( n = 42 \)) \( p < 0.239 \).
This study assessed serum 25(OH)D concentrations in women with self-reported PCOS versus those in non-PCOS controls. A multivariable linear regression model was developed to investigate the independent association between PCOS and 25(OH)D concentration when adjusting with several essential confounding factors. Figure 11 demonstrates the positive association between self-reported PCOS and 25(OH)D concentrations ($\beta = 2.46$, 95% CI 0.84, 4.08, $p = 0.003$). A negative association was found between BMI and 25(OH)D in the same model, while positive associations were observed between 25(OH)D concentrations and living at 65°N latitude and high-sunlight months. When adding HOMA-IR and hs-CRP to the linear regression model, the results remained essentially the same: self-reported PCOS had a positive association with 25(OH)D ($\beta = 2.39$, 95% CI 0.65, 4.13, $p = 0.007$).

Despite 25(OH)D concentrations being positively associated with self-reported PCOS, after adjusting for several essential confounding factors, the mean 25(OH)D concentrations in the women with self-reported PCOS were only slightly above the 50nmol/L limit, while controls were below the limit. Over 50% of women in both study groups had serum 25(OH)D concentrations below 50 nmol/L, which is near the level reported in previous research (He et al., 2015).
Women with PCOS were expected to have lower vitamin D status than non-PCOS controls, since the onset of chronic diseases has previously been linked to low concentrations of vitamin D, and the human body’s normal physiology might be interfered with by hypovitaminosis D (Holick, 2007). In addition, multiple metabolic risk factors were observed in women with PCOS: high BMI, IR, and low-grade inflammation (elevated HOMA-IR and hs-CRP concentrations). Higher rates of T2D, hypertension, and dyslipidemia in women with PCOS have also been reported (Ollila et al., 2016, 2017, 2019). Studies have previously indicated that PCOS might be a risk factor for vitamin D insufficiency, in addition to obesity, metabolic disturbances, and IR (Couto Alves et al., 2017; Joham et al., 2016; Thomson et al., 2012; Wehr et al., 2009). Those with PCOS and higher BMIs have been suggested to have more insufficient vitamin D status than women with PCOS who are in the normal weight range (Joham et al., 2016; Thomson et al., 2012). Despite metabolic derangements and high BMIs in women with PCOS noted in the present study’s population and in previous studies, women with PCOS had 25(OH)D concentrations that were comparable with controls, although vitamin D status was low in both study groups.

The results of previous observational studies have been inconsistent as to vitamin D status in women with PCOS. Most of the findings have been the opposite of the results presented here (Davis et al., 2019; Krul-Poel et al., 2018; Maidana et al., 2019), but similar results have also been observed (He et al., 2015; Mahmoudi et al., 2010). Studies presenting lower 25(OH)D concentrations in women with PCOS have been carried out with relatively small sample sizes. Participants were also recruited from infertility clinics and not from the general population, as is the case with the NFBC1966 sample, although the Rotterdam criteria were also followed (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004). In most previous research, analyses were performed without essential confounding factors (Davis et al., 2019; He et al., 2015; Maidana et al., 2019), and that might help explain contradictory and inconsistent results.

A study by Krul-Poel et al. included 639 women with PCOS and 449 controls; it is one of the largest previously published vitamin D studies. It observed lower vitamin D status in anovulatory PCOS women than in healthy controls (Krul-Poel et al., 2018). In that study, women with PCOS showed anovulatory infertility and were recruited from infertility clinics, so their PCOS phenotypes might have been more severe. As with the present study, women with PCOS had higher BMIs than controls. However, in contrast to the NFBC1966 control population, the controls in the study by Krul-Poel were fertile, with normal delivery less than 18 months
before the study. Thus, their health status might have been better than would be found among controls drawn from the general population. 25(OH)D samples were taken soon after recent pregnancies, so many control participants were likely nursing. The use of vitamin D supplementation was probably more regular in that population, which might help explain the discrepancies (Krul-Poel et al., 2018).

Vitamin D has been suggested to have anti-inflammatory actions and beneficial effects on glucose metabolism (Akbari et al., 2018; He et al., 2015; Jamilian et al., 2017; Mutt et al., 2012; Sassi et al., 2018). However, higher vitamin D status in the PCOS group did not result in lower hs-CRP concentrations than in the NFBC1966 control group. The favorable health actions in the PCOS group might require even higher vitamin D status.

Hyperandrogenism and IR are key causes of the metabolic derangements in women with PCOS (Colonese et al., 2015; Reyes-Muñoz et al., 2018). Beneficial effects of vitamin D have also been found regarding androgen levels and insulin responsiveness in PCOS women (Colonese et al., 2015; Reis et al., 2017; Thomson et al., 2012; Yildizhan et al., 2009). However, according to a study by Pittas et al., vitamin D supplementation might not protect prediabetic patients from T2D (Pittas et al., 2019), which could explain why vitamin D did not decrease HOMA-IR levels in the NFBC1966 population with PCOS. In addition, testosterone concentrations were higher in women with PCOS than in controls. In a sub-analysis, testosterone concentrations did not differ between vitamin D quartiles, which might have been anticipated based on earlier studies (Thomson et al., 2012; Yildizhan et al., 2009).

The results of the present study cannot be explained by background characteristics since they did not differ substantially. A higher proportion of women with PCOS lived in the northernmost parts of Finland, increasing their risk of having inadequate vitamin D status (Gagnon et al., 2010; Huotari & Herzig, 2008), but the mean 25(OH)D concentrations were still sufficient compared to controls.

Vitamin D is a fat-soluble molecule that can be sequestered in adipose tissue (Mutt et al., 2014), decreasing the bioavailability of vitamin D and possibly accounting for the negative association between BMI and 25(OH)D concentrations and the differences between the two groups (Joham et al., 2016). In addition, the actions of active vitamin D (calcitriol, 1,25(OH)2D) are generated in cells via VDRs (Reis et al., 2017). Multiple genes related to glucose and lipid metabolism are modulated by VDRs (Bouillon et al., 2008). Although PCOS is closely related with these metabolic derangements, it could be considered a multigenic disease (Colonese et al., 2015). Studies suggesting an increased risk for PCOS in women with VDR polymorphism have also been published, and the association may related
to effects on testosterone and insulin concentrations (Dasgupta et al., 2015; Liang et al., 2019; Reis et al., 2017; Santos et al., 2017; Szafarowska et al., 2019). The findings of the present study may suggest 25(OH)D resistance in women with PCOS, which could result from VDR gene polymorphism (Ranjzad et al., 2011; Reis et al., 2017). Further investigation is required to determine the genetic relationship between PCOS and vitamin D.

5.3.3 Vitamin D status in women with PCOS and fecundability problems (Studies II and III)

A subgroup analysis was performed to evaluate 25(OH)D concentrations in the PCOS population with and without previous fecundability problems. A history of infertility and decreased fecundability were defined as in Study II and PCOS as in Study III. History of infertility (p < 0.001) and decreased fecundability (p < 0.001) were more frequent in women with self-reported PCOS than in non-symptomatic controls (Table 18).

<table>
<thead>
<tr>
<th>Fecundability characteristic</th>
<th>PCOS (n = 146–165)</th>
<th>Controls (n = 872–874)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility ^1</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>77 (46.7)</td>
<td>123 (14.1)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>88 (53.3)</td>
<td>749 (85.9)</td>
<td></td>
</tr>
<tr>
<td>Decreased fecundability ^1</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>58 (39.7)</td>
<td>125 (14.3)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>88 (60.3)</td>
<td>749 (85.7)</td>
<td></td>
</tr>
</tbody>
</table>

^1 n (%), ^2 mean (SD), PCOS: Polycystic ovary syndrome.

In terms of group size, 25(OH)D concentrations were available from 56 women with PCOS and infertility, 40 women with PCOS and decreased fecundability, and 62 women with PCOS and no fecundability problems. Unadjusted mean serum 25(OH)D concentrations were lower in women with a history of infertility (51.8 nmol/L, SD 19.1, p = 0.046; Table 19), but not among those with a history of decreased fecundability (53.4 nmol/L, SD 19.06, p = 0.16) when compared with women with PCOS who did not have fecundability problems (58.6 nmol/L, SD 17.2).
Multivariable linear regression models were also developed in the PCOS population to assess the independent association of fecundability problems with 25(OH)D concentrations. In the first model, there was no association between a history of infertility and 25(OH)D concentrations in women with PCOS. In addition, no significant association was found in the second model assessing association between decreased fecundability and 25(OH)D concentrations in women with PCOS.

Table 19. 25(OH)D concentrations and distributions of 25(OH)D status groups in women with PCOS, with and without fecundability problems.

<table>
<thead>
<tr>
<th>25(OH)D status</th>
<th>PCOS + infertility</th>
<th>PCOS + decreased fecundability</th>
<th>PCOS + normal fecundability</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>8 (14.3)</td>
<td>4 (10.0)</td>
<td>1 (1.6)</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>30–50</td>
<td>18 (32.1)</td>
<td>13 (32.5)</td>
<td>22 (35.5)</td>
<td>0.076</td>
<td>0.30</td>
</tr>
<tr>
<td>50–75</td>
<td>22 (39.3)</td>
<td>16 (40.0)</td>
<td>27 (43.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 75</td>
<td>8 (14.3)</td>
<td>7 (17.5)</td>
<td>12 (19.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1nmol/L, mean (SD), 2n (%), 3p-value comparing women with PCOS and previous infertility examination or infertility treatment versus women with PCOS and normal fecundability. 4p-value comparing women with PCOS and decreased fecundability versus women with PCOS and normal fecundability. 25(OH)D: 25-hydroxyvitamin D, PCOS: Polycystic ovary syndrome.

PCOS is a primary cause of anovulatory infertility in women (El Hayek et al., 2016). In healthy women, associations between low 25(OH)D concentrations and prolonged menstrual cycles have been suggested (Jukic et al., 2018). Previously, vitamin D supplementation has been suggested to promote follicle development and restore menstrual irregularities in women with PCOS (Fang et al., 2017; Fung et al., 2017; Thomson et al., 2012). Although unadjusted 25(OH)D concentrations were lower in women with a history of infertility and PCOS than among women with PCOS but without a history of infertility problems, there was no association between infertility or decreased fecundability and 25(OH)D concentrations in women with PCOS after adjusting for season of blood sampling, latitude of residence, relationship status, BMI, and physical activity. Still, women with severe PCOS, who could have high BMIs and more troubling metabolic derangements, might be at the greatest risk for fertility problems and thus could benefit most from having at least adequate vitamin D status. Despite the present study’s representative
overall sample size, the numbers of women with PCOS symptoms in the fertility subgroups were low.

5.3.4 Summary

In the NFBC1966 study population, women with PCOS seemed not to be at increased risk for inadequate vitamin D status. In addition, PCOS was associated with higher vitamin D status after adjustments in a linear model. Women with PCOS had more infertility problems and decreased fecundability. Vitamin D status was lower in women with PCOS who had previous infertility problems, but this difference was not significant after adjustments. However, based on previous observations and the present study’s results, maintaining sufficient vitamin D concentration in women with PCOS is recommended, especially in overweight women suffering from fertility problems.

5.4 Early-onset climacterium and vitamin D status (Study IV)

Measurements of 25(OH)D were available from 351 climacteric and 2,193 preclimacteric women at age 46. Concentrations of 25(OH)D were compared between climacteric and preclimacteric women and between climacteric women with and without HRT use. Of the climacteric women, eight were diagnosed with POI according to the Care Register for Health Care, and two thirds of the women whose menstrual anamnesis was available had a duration below 24 months. HRT was used by 76 (21.7%) of the climacteric women; a higher proportion used oral (n = 46, 60.5%) than transdermal HRT (n = 30, 39.5%).

Compared with preclimacteric women, climacteric women were more likely to live in the northern parts of Finland (49.6% vs. 41.3%, p < 0.001) and to be smokers (21.8% vs. 17.3%, p = 0.047). Other background characteristics, including season of blood sampling, vitamin D intake from diet and supplementation, alcohol consumption, level of education, frequency of sunny holidays abroad, BMI, and physical activity, did not differ between study groups.

No difference in background and clinical characteristics or vitamin D intake was observed in climacteric women using HRT (n = 76) compared with climacteric women not using HRT (n = 275), but climacteric women using HRT (n = 76) were more physically active (p = 0.033).
5.4.1 Vitamin D status in climacteric and preclimacteric women

Mean serum 25(OH)D concentrations were higher in climacteric women (68.1, SD 19.8 nmol/L) than in preclimacteric women (65.2, SD 19.3 nmol/L, \( p = 0.01 \); Table 20). When investigating distributions in vitamin D status groups (< 50, 50–75, > 75 nmol/L), < 50 nmol/L was more frequent in preclimacteric women and > 75 nmol/L more frequent in climacteric women. A similar observation was made as to vitamin D quartiles.

Table 20. Serum 25-hydroxyvitamin D concentrations and distributions between study groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preclimacteric women (n = 2,193)</th>
<th>Climacteric women (n = 351)</th>
<th>Climacteric women with HRT (n = 76)</th>
<th>Climacteric women without HRT (n = 275)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D, nmol/L, mean (SD)</td>
<td>65.2 (19.3)</td>
<td>68.1 (19.8)</td>
<td>72.6 (19.4)</td>
<td>66.8 (19.8)</td>
</tr>
<tr>
<td>( p )-value</td>
<td>0.01(^1)</td>
<td>0.025(^2)</td>
<td>0.004(^1)</td>
<td>0.048(^2)</td>
</tr>
</tbody>
</table>

Vitamin D status groups, n (%)

| < 50 | 501 (22.8) | 61 (17.4) | 9 (11.8) | 52 (18.9) |
| 50–75 | 1,056 (48.2) | 160 (45.6) | 30 (39.5) | 130 (47.3) |
| > 75 | 636 (29.0) | 130 (37.9) | 37 (48.7) | 93 (33.8) |
| \( p \)-value | 0.004\(^1\) | 0.048\(^2\) | 0.013\(^2\) | 0.048\(^2\) |

Vitamin D quartiles, n (%)

| < 51.67 | 554 (25.3) | 74 (21.1) | 11 (14.5) | 63 (22.9) |
| 51.7–64.4 | 553 (25.2) | 79 (22.5) | 17 (22.4) | 62 (22.5) |
| 64.4–78.1 | 555 (25.3) | 87 (24.8) | 16 (21.1) | 71 (25.8) |
| > 78.1 | 531 (24.2) | 111 (31.6) | 32 (42.1) | 79 (28.7) |
| \( p \)-value | 0.022\(^1\) | 0.013\(^2\) | 0.013\(^2\) | 0.013\(^2\) |

\(^1\) Preclimacteric vs. climacteric women, \(^2\) Climacteric women with HRT vs. climacteric women without HRT.

25(OH)D: 25-hydroxyvitamin D, HRT: Hormone replacement therapy.

However, when adjusting for relevant confounders (season of blood sampling, latitude, smoking, vitamin D from diet and supplementation, BMI, and physical activity) in the multivariable linear regression model, climacteric status was not associated with differences in 25(OH)D concentrations (\( \beta = 4.5, 95\% \text{ CI} \sim 1.4, 10.4, p = 0.137 \); Figure 12) between preclimacteric and climacteric women.

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Fig. 12. Results of multivariable regression model showing associations with 25(OH)D with different exposures. 25(OH)D: 25-hydroxyvitamin D, CI: Confidence interval, VitD: Vitamin D.
5.4.2 HRT use in climacteric women and vitamin D

In climacteric women, mean serum 25(OH)D concentrations were higher among women using HRT (72.6, SD 19.4 nmol/L) than among women not using HRT (66.8, SD 19.8 nmol/L, \( p = 0.025 \), Table 20). Frequency of vitamin D status < 50 nmol/L was more pronounced and vitamin D status > 75 nmol/L less frequent in women not using HRT than among HRT users (\( p = 0.048 \)). Similar results were found for vitamin D quartiles. In addition to the results shown in Table 20, climacteric women using HRT had higher mean serum 25(OH)D concentrations than preclimacteric women (\( p = 0.001 \)). However, no difference was observed between climacteric women not using HRT and preclimacteric women (\( p = 0.196 \)).

There was also no difference in mean 25(OH)D concentrations between oral (70.7, SD 21.4 nmol/L) and transdermal administration routes of HRT (75.5, SD 15.7 nmol/L, \( p = 0.29 \)).

Figure 13 presents the results of a linear regression model in only climacteric women examining associations between 25(OH)D concentrations and HRT use, BMI, vitamin D from diet and supplementation, smoking, physical activity, season of blood sampling, and latitude. HRT use had an association with higher 25(OH)D concentrations (\( \beta = 5.9 \text{ nmol/L}, 95\% \text{ CI 1.3, 10.5}, p = 0.013 \)).

Prior studies of vitamin D in women with an early-onset climacteric phase are lacking. Kebapcilar et al. found that vitamin D deficiency was more frequent in women younger than 40 with POI compared to healthy controls (Kebapcilar et al., 2013). However, their study included only 35 POI women and 28 controls. Purdue-Smithe et al. found no association between premenopausal vitamin D concentrations and the risk of EM in a larger follow-up study (Purdue-Smithe et al., 2017). No studies observing vitamin D status in women with EM have been published.

Oral HRT use was more frequent than transdermal administration, but there was no significant difference in 25(OH)D concentrations between the two administration routes. The fluctuation in 25(OH)D concentrations was wider with oral HRT users. In oral HRT use, gut and first-pass hepatic metabolism might cause alterations in estradiol levels (Kopper et al., 2008) which could help explain the more stable 25(OH)D concentrations found with transdermal HRT administration.
Fig. 13. Forest plot showing beta coefficient of predictive factors’ associations with 25(OH)D concentrations in climacteric women at age 46. 25(OH)D: 25-hydroxyvitamin D, CI: Confidence interval, HT: Hormone replacement therapy, VitD: Vitamin D.
Previous findings suggest a positive association between OCP use and 25(OH)D concentrations (Møller et al., 2013; Shirazi et al., 2013). This finding was confirmed in the present study’s population at the 31-year follow-up (Palaniswamy et al., 2017). However, a positive association between systematic HRT use and 25(OH)D concentration has not been demonstrated. Three studies have shown results that are vary from or are the opposite of ours, but the age ranges of the women were wider, and those studies had smaller numbers of participants (Rejnmark et al., 2006; Shirazi et al., 2013; Touvier et al., 2015). Shirazi et al. investigated 727 Swedish women with mean age 56.9 years with and without HRT use and found no significant positive association between 25(OH)D concentrations and HRT use (Shirazi et al., 2013). Rejnmark et al. studied 25(OH)D concentrations in peri- and postmenopausal women with (n = 89) and without (n = 98) oral HRT use in a five-year longitudinal study (Rejnmark et al., 2006). In that study, although HRT use was not associated with 25(OH)D concentrations, HRT use did have a positive association with DBP. The analyses in that study were conducted without adjustments for essential confounding factors using repeated measures analysis of variance. Touvier et al. reported on a cross-sectional study of 995 French women (394 postmenopausal) and found no association between HRT use and 25(OH)D concentrations. (Touvier et al., 2015). In addition, a randomized placebo-controlled study by Heikkinen et al. did not show any effect of HRT alone on 25(OH)D concentrations in 69 postmenopausal women with a mean age of 53. However, vitamin D supplementation alone and combined with HRT did increase 25(OH)D concentrations (Heikkinen et al., 1998).

A few mechanisms as to how estrogen elevates 25(OH)D concentrations have been postulated. Studies have hypothesized that estradiol might increase the induction of 25-hydroxylase activity in the liver, which in turn could increase 25-hydroxylation (Bouillon et al., 2020; Nelson et al., 2009; Palaniswamy et al., 2017). However, only two animal studies have investigated the connection of estradiol with 25-hydroxylase activity (Castillo et al., 1977; Saarem & Pedersen, 1987), and no human studies have been conducted. One study suggested that estrogen might enhance 1-alpha-hydroxylase activity (Gallagher et al., 1980). In addition, some research has proposed that estrogen might increase the concentration of the main carrier of 25(OH)D in blood DBPs (Bouillon et al., 2020). DBP and 25(OH)D concentrations have a positive correlation, and most of the 25(OH)D in the serum is bound to DBPs. Thus, increased DBP rates could also elevate 25(OH)D concentrations (Fraser & Milan, 2013; Hutchinson et al., 2017).
Overall, in both preclimacteric and climacteric women, vitamin D status was at sufficient levels, which can be considered a fairly good outcome, since the risk of vitamin D inadequacy is elevated in northern latitudes (Huotari & Herzig, 2008). In addition, 25(OH)D concentrations were in line with previous observations in Finland (Jääskeläinen et al., 2017; Rautilo et al., 2017). The NFBC1966 46-year follow-up study was conducted in 2012–2013, after the start of national food fortification of fluid dairy products and fat spreads, accompanied by a recommendation for vitamin D supplementation (Itkonen et al., 2020; Jääskeläinen et al., 2013). These are the essential reasons for the improvement in vitamin D status among preclimacteric and climacteric women. Women with EM may still benefit from ensuring adequate vitamin D intake, especially those without regular HRT use (Webber et al., 2016).

Previous studies from this same population found that early onset of the climacteric phase is associated with CVD risk factors such as higher cholesterol levels and impaired insulin sensitivity (Savukoski et al., 2019, 2021). HRT use in postmenopausal women has been suggested to have a protective effect against CVD (Grodstein et al., 2006; Savolainen-Peltonen et al., 2016), but vitamin D could also act as an atheroprotective factor (Krishna, 2019; Mutt et al., 2019; Rai & Agrawal, 2017). Vitamin D is an important molecule in bone health (Lips & Van Schoor, 2011), but positive impacts of HRT use on BMD have also been observed (Cauley et al., 2003; The Writing Group for the PEPI, 1996). As a hypothesis, vitamin D-related pathways might be linked to the positive health consequences of HRT use.

The amount of physical activity was higher in HRT users, but the other background characteristics did not differ between climacteric women with and without HRT use. The intake of vitamin D from diet and supplements also did not differ. After adjustments in the multivariable linear model, the association between HRT use and 25(OH)D concentration remained significant, but the possibility that lifestyle and/or socioeconomic factors could at least partly explain the difference in 25(OH)D concentrations cannot be completely eliminated.

5.4.3 Summary

As Study IV shows, early-onset menopausal transition was not associated with lower vitamin D status. However, the use of systematic HRT was associated with increased 25(OH)D concentrations in a linear regression model of climacteric women at age 46. Further studies assessing this association are required to better elucidate the reasons for this finding.
6 Strengths and limitations

These studies have multiple strengths. The participants were from a large, systematically collected birth cohort and thus represent symptoms and diseases at the general population level. The participation rate of the cohort was high and included participants from homogenous ethnic, cultural, and genetic backgrounds. The participants were born in the same area within one year of one another, which enabled a focus on a specific age group at each study timepoint (31 and 46 years) and examining the possibility that vitamin D status might be affected by age (Hilger et al., 2014). Comprehensive cohort study questionnaires and clinical examinations were used to obtain and control for several potential confounders that might be associated with vitamin D status, infertility, PCOS, and EM. Due to the study questionnaire, dietary data and information on vitamin D supplementation use were also available.

25(OH)D concentrations were measured from a large number of participants. At 31 years, serum concentrations of 25(OH)D were determined using the LC-MS/MS method, which is the gold standard of 25(OH)D measurements (Galior et al., 2018). CMIA VDSP-calibrated 25(OH)D concentrations at age 31 provided the opportunity to assess vitamin D in a longitudinal setting, which makes the present study’s observations comparable with other vitamin D studies (Durazo-Arvizu et al., 2017; Sempos et al., 2016).

The observations in Study I offer essential insights for the future about the importance of vitamin D food fortification and vitamin D supplementation in public health policy. In Study II, fecundability was studied by using multiple variables (previous infertility examinations and treatments, along with reproductive outcomes) with validated methods (Koivunen et al., 2008; Laru et al., 2021). The association of vitamin D and PCOS was defined in an unselected population, whereas most previous PCOS studies have used populations selected from hospitals or private fertility clinics; general population studies have been lacking. Menopause status in Study IV was examined using both menstrual history and FSH values, which increases the reliability of the study group division. The use of medication was not based on self-reporting; instead, Finland’s national registry data were obtained to verify the use of HRT.

Of course, the four studies have certain limitations. Only one 25(OH)D measurement was available per study timepoint. Causality cannot be assessed due to the cross-sectional observational setting of all four studies. In Study I, it would have been more informative to assess the efficacy of fortification if the baseline
25(OH)D concentrations had been available before each fortification wave. The use of vitamin D supplementation was queried with an open-ended question and was not concurrent with a previously published nationwide study (Jääskeläinen et al., 2017). However, vitamin D supplementation was positively associated with 25(OH)D concentrations, which implies that the results are reliable. In Studies I and III, we did not have the opportunity to use information about vitamin D supplementation at age 31. However, the use of vitamin D supplementation was uncommon in 1997, the study timepoint (Jääskeläinen et al., 2013), so this missing information is an acceptable limitation. Since the fortification of dairy products and fat spreads started in 2002 and dietary intake of vitamin D is modest, the lack of precise dietary data from the 31-year follow-up is also acceptable in Studies I–III (Raulio et al., 2017).

In Study II, 31 was an appropriate age to evaluate early fecundability problems since the mean age of first delivery in Finland in 1997 was 27.7 for nulliparous women and 29.8 for all deliveries (Gissler et al., 2014; Official Statistics of Finland (OSF), 2021). Concentrations of 25(OH)D were also measured after the onset of fecundability difficulties. In Study III, PCOS diagnoses were based on self-reported symptoms and diagnoses, but this population has previously been shown to successfully identify women with PCOS (Ollila et al., 2016, 2019; Taponen, 2004; Taponen et al., 2003). Since participants using hormonal contraceptives were excluded, the number of women with PCOS may be even higher, as hormonal contraceptives are a common treatment for PCOS symptoms (Teede et al., 2019). The use of OCPs might elevate 25(OH)D concentrations (Møller et al., 2013; Palaniswamy et al., 2017), which could have affected the results of Study III. Although there was a large overall cohort, the number of women with both PCOS and previous fecundability problems were small and needs further evaluation.

In the 46-year follow-up in Study IV, only one FSH measurement was available from study participants, although the ESHRE criteria for POI recommend two measurements, preferably obtained two weeks apart (Webber et al., 2016). However, the menstrual history of the participants was also used to increase the reliability of the study group division. The national register data of medication reimbursements was used, but the number of participants using HRT was too low to assess any effects of administration route or HRT dose on vitamin D status.
7 Conclusion

1. In this study population, mean serum 25(OH)D concentrations increased over 10 nmol/L during the 15-year period after the start of a national intervention of food fortification. The prevalence of serum 25(OH)D concentrations < 50 nmol/L was almost halved. Study detected a substantial reduction in seasonal variations in vitamin D status. Vitamin D intake from diet exceeded the recommended level. The regular use of vitamin D supplements might be an important factor especially in women in achieving adequate vitamin D intake. Vitamin D status at 31 years was observed to be independently associated with vitamin D status at 46 years, which might indicate genetic and/or behavioral factors. Vitamin D intake from diet and supplements still need further improvement at the general population level.

2. In women with a history of infertility, the mean serum 25(OH)D concentrations were lower than among women without previous fertility problems. A history of infertility and decreased fecundability were associated with lower 25(OH)D concentrations in women at age 31. Women with a history of fertility problems should be advised about the value of sufficient vitamin D intake. Enhancing vitamin D status in women with fertility derangements, especially those at risk of deficiency (e.g., obese women and women not regularly consuming vitamin D-rich foods), might be beneficial for fecundability.

3. In this study population, vitamin D status was similar in women with PCOS and in controls. After adjustments for confounding factors, 25(OH)D concentrations were actually higher in the women with PCOS than in the controls. In women with PCOS with or without a history of infertility vitamin D status did not differ in the adjusted model. No greater propensity for vitamin D insufficiency was observed in the sample of women with PCOS than in controls.

4. In this study, the early onset of climacterium by age 46 was not associated with inadequate 25(OH)D concentrations when adjusted for essential confounding factors. Use of systematic HRT was associated with higher 25(OH)D concentrations in women with early onset of the climacteric phase, but the number of HRT users was low. The study observations support the advantages of systematic HRT use. Vitamin D may be involved with the positive health effects of HRT in women with early-onset climacterium. Adequate vitamin D intake is recommended for women facing menopause earlier than average to minimize long-term adverse health effects.

In the future, research is warranted to clarify individual factors that may affect vitamin D status. In addition, studies are required to identify the optimal vitamin D
status in terms of reproductive health. Studies should address whether women benefit from 25(OH)D concentrations over 50 nmol/L. Further evaluation is also needed of the mechanisms of HRT use on vitamin D status.
List of references


should I eat? **Fertility and Sterility**, 110(4), 560–569. https://doi.org/10.1016/j.fertnstert.2018.05.027


of rickets]. Duodecim, 80, 185–189.


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Reis, G. V. O. P. dos, Gontijo, N. A., Rodrigues, K. F., Alves, M. T., Ferreira, C. N., &


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Theodoratou, E., Tzoulaki, I., Zgaga, L., & Ioannidis, J. P. A. (2014). Vitamin D and multiple health outcomes: Umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ (Online), 348*, g2035. https://doi.org/10.1136/bmj.g2035


Zhao, J.-F., Li, B.-X., & Zhang, Q. (2021). Vitamin D improves levels of hormonal,
oxidative stress and inflammatory parameters in polycystic ovary syndrome: a meta-analysis study. *Annals of Palliative Medicine, 10*(1), 169–183. https://doi.org/10.21037/apm-20-2201

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Publication IV is included in the doctoral thesis of Susanna Savukoski.

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