VITAMIN D LEVELS DURING FIRST TRIMESTER OF PREGNANCY IN FINNISH WOMEN

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Vitamin D is a secosteroid and the most significant source is direct exposure of the skin to sunlight. It can also be obtained from dietary sources, nutritional supplementation and food fortification. Vitamin D deficiency is known for a long time, but only relatively recently researchers were able to identify its chemical structures.

Vitamin D deficiency during pregnancy may have a negative impact on both mother and child. There are studies which investigated maternal vitamin D status worldwide, but the results are inconclusive and further research is warranted, especially in the Nordic area where there are long dark winters.

The aim of the present study is to determine 25(OH)D concentrations during first trimester of pregnancy in Finnish women and to study whether 25(OH)D concentrations were associated with different maternal or neonatal factors. Serum vitamin D_3 and vitamin D_2 concentrations of 405 Finnish pregnant women were analyzed during the first trimester of pregnancy.

In total, only 10.9% of pregnant women had sufficient vitamin D_3 levels (>80nmol/l or >32ng/ml), the rest of pregnant women were 25(OH)D insufficient and deficient. Maternal vitamin D_3 concentrations were associated with season of sampling, age of the mother and maternal smoking status before pregnancy. Vitamin D_2 levels were detectable only in 5.2% of women.

Low vitamin D_3 levels might be a public health issue concerning both future mother and offspring.

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1 Abbreviations

µl: microlitre

25(OH)D: 25 hydroxy-cholecalciferol = 25- hydroxyvitamin D = calcidiol

BMI: Body Mass Index

EFSA: The European Food Safety Authority

GDM: Gestational Diabetes Mellitus

HPLC: High performance liquid chromatography

IU (International Unit): measurement based on biological activity or effect: 1 IU of vitamin D is defined as the activity of 0.025 µg of cholecalciferol or ergocalciferol

ml: millilitre

MS: Multiple Sclerosis

ng/ml: nanogram per milliliter

nmol/l: nanomole per liter

NNR 5: Nordic Nutrition Recommendations, fifth edition

PE: Preeclampsia

rpm: Revolutions per minute

SZA: Solar Zenith Angle

UVB: Ultraviolet B

v/v: Volume to volume

VDD: Vitamin D Deficiency

Vitamin D: calciferol (including both vitamin D_2 and vitamin D_3)

Vitamin D_2: Ergocalciferol

Vitamin D_3: Cholecalciferol

Conversions:

1 ng/ml= 2.496 nmol/l≈ 2.5 nmol/l

40 IU = 1 µg

1 nmol/l= 0.4 ng/ml

2   Introduction

2.1   History of vitamin D

The main condition of vitamin D deficiency, rickets, was known since the mid 17th century. At that time, Doctor Daniel Whistler published his only work "The Rickets", "De morbo puerili Anglorum, quem patrio idiomate indigenae vocant", written in Latin (Anonymous1968). But even before this publication, there is a painting by Hans Burgkmair dating since 1509 depicting a rachitic child (Cone 1980).

It took three centuries before vitamin D was discovered and rightfully recognize its health benefits. Both structures of vitamin D_2 and vitamin D_3 were described and differentiated by Adolf Windaus and his colleagues in the 1930's (Wolf 2004).

 Last century was marked with strong, but not continuous scientific activity, in order to comprehend the mysteries of the elusive vitamin D. Nowadays there is a growing body of scientific papers whose main targets are to asses and determine the health benefits, different clinical and patho-physiological relations of vitamin D and health.

Between January 1922 and March 2014, there have been 58.298 published articles, listed in PubMed, which included the term "vitamin D" in the title, and there has been a steady increase from the 1980' forwards.

2.2   Vitamin D metabolism

In general, the majority of the population meets its essential vitamin D amount by exposure of the skin to sunlight (ultraviolet radiation; UVB = 290-315 nm) and secondary from nutritional sources. The most significant source of vitamin D for humans worldwide is the endogenous pathway. This translates into the ability of the skin to synthesize the steroid pro-hormone (7-dehydrocholesterol) from the sunshine exposure (Bodnar, Simhan 2010). The UVB radiation transforms 7-dehydrocholesterol in the epidermis to previtamin D_3, which is converted to vitamin D_3 by a thermal process (Webb, Kline & Holick 1988).

Following the dermal synthesis or dietary intake, vitamin D reaches the liver where the first hydroxylation takes place to form 25-hydroxy-cholecalciferol or 25(OH)D_3, which is the major circulating form of the vitamin in the body. Additionally, 25(OH)D_3 is metabolized

again in the kidney in order to become an active form 1α,25-dihydroxycholecalciferol (other abbreviations and names for the vitamin D active form are the following: 1α,25-dihydroxyvitamin D_3 or 1,25-(OH)$_2$D or 1α,25-dihydroxyvitamin D or calcitriol) (Chen et al. 2007).
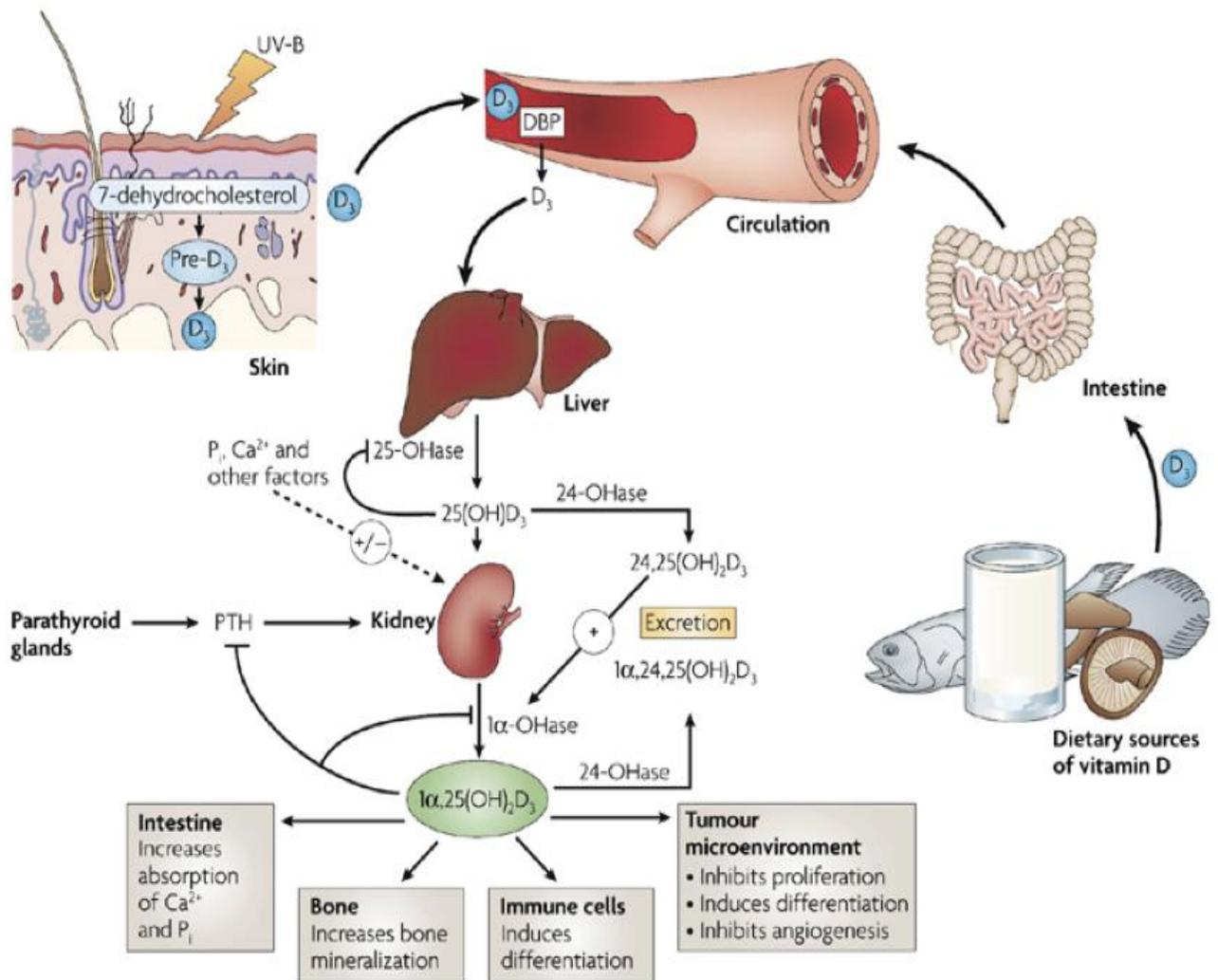

Figure 1. The metabolism and possible effects of vitamin D, image from Nature Reviews Cancer (Deeb, Trump & Johnson 2007).

25(OH)D stimulates the small intestine to enhance its dietary calcium and phosphorus absorption. It sustains normal values of calcium and phosphorus in the systemic circulation. If the exogenous calcium sources are insufficient, vitamin D together with the parathyroid hormone (PTH) will direct part of the bone calcium towards the general circulation in order to have balanced calcium serum values (Chen et al. 2007).
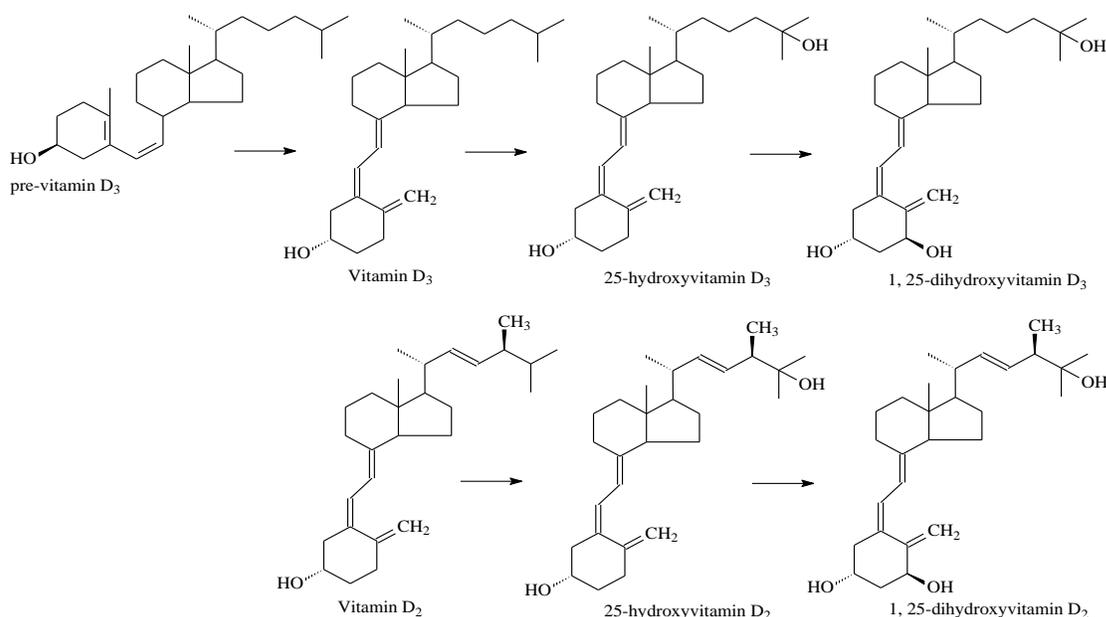
Figure 2. Molecular structures of vitamin D metabolism.

According to geographic coordinates above 35-37 $^{\circ}$ North and South latitude, vitamin D is not being produced in the dermis during the cold season, or winter, and the values of 25(OH)D reach their lowest levels between February and March. One of the significant natural factors that influence the pre-hormone production in the human skin is the solar zenith angle (SZA). The SZA together with the local atmospheric features establish the available amount of UVB radiation. The SZA could be amplified or shortened either by the daily global rotation or by incrementing in the north-south diameter. The concentration of the SZA is determined by season and geographical latitude (Chen et al. 2007, Webb 2006, Webb, Holick 1988). In the cold season at latitudes higher than 35° in both northern and southern hemispheres, the 25(OH)D dermal productions is deficient (Holick 2002).

The vitamin D status in the human body is assessed by measuring the concentrations of a specific marker, which is 25(OH)D. As shown in Table1, it was concluded that there is reliable scientific evidence that serum values of 25(OH)D above 50 nmol/l(>20ng/ml) are recommended for a favorable health status for humans (Grant, Holick 2005). However, there is no agreed medical consensus regarding the limits of vitamin D intake, deficiency, insufficiency, and sufficiency, thus the values below are considered an estimate for these limits.

Table 1. Concentrations of vitamin D and health status (Grant, Holick 2005).

25(OH)D concentration (nmol/l)	25(OH)D concentration (ng/ml)	Health (status)
<50	</≤ 20	Deficiency
50-80	20-32	Insufficiency
80-250	32-100	Sufficiency
135-225	54-90	Normal (sunny countries)
>/≥250	>/≥100	Excess (Surplus)
>/325	>150	Intoxication

2.3 Vitamin D deficiency (VDD)

It has been generally agreed that the vitamin D_3 value below 50 nmol/l(<20 ng/ml) of 25(OH)D is a marker for vitamin D deficiency (VDD) (Holick, Chen 2008). However, Bhatt and coworkers suggested that urban Asian Indians who were participating in their study were vitamin D deficient with values <25 nmol/l (<10ng/ml), vitamin D insufficient as <75 nmol/l (<30ng/ml), and vitamin D sufficient >75 nmol/l (>30ng/ml) (Bhatt et al. 2014).

Similarly, in the American Endocrine Society Clinical Practice Guidelines, the authors defined vitamin D deficiency as <50nmol/l(<20ng/ml), and concentrations ranging between 52.5-72.5 nmol/l(21-29 ng/ml) were considered moderate vitamin D deficiency (Holick et al. 2011).

Some of the causes of VDD are factors that decrease the transmission of UVB such as season and altitude. It is known that position of the sun affects the UVB photons reaching the earth surface (Webb, Kline & Holick 1988, Holick 2003). Dressing code, sunscreen protection usage and dark skin pigmentation prevent the penetration of the solar UVB into the dermis,

which lead to reduced vitamin D synthesis in the skin (Chen et al. 2007, Holick 2003, Matsuoka et al. 1987).

Other factors which might determine VDD are the ageing process, due to diminishing values of dermal 7-dehydrocholesterol, and obesity, due to the large adipose tissue which may seize the circulating vitamin D and its deposition (Holick, Chen 2008, Wortsman et al. 2000).

2.4   Vitamin D research in Finland – brief history

Schultzer and Christensen studied vitamin D in Finland in the late 1940's, reporting vitamin D_2 treatment of parathyroid tetany (Schultzer, Christensen 1946).

Next, in the 1950's, some articles were published about vitamin D during childhood. Hultin described "Principles of vitamin D administration for children", and Koponen published "Vitamin D requirements in infant feeding" (Hultin 1956, Koponen 1957). In the 1980's there were several studies published regarding the seasonal difference of vitamin D concentrations in Finnish population (Savolainen et al. 1980, Elomaa et al. 1982, Kokkonen, Koivisto & Kirkinen 1983), and similarly in pregnant women and their offspring (Ala-Houhala 1985, Ala-Houhala et al. 1988).

In northern Finland there is a seasonal variation of vitamin D in pregnant women and their neonates, in winter the concentrations being lower than in summer (Kokkonen, Koivisto & Kirkinen 1983), and women who supplemented during pregnancy had higher 25(OH)D concentrations than the ones who did not take any supplements (Lamberg-Allardt, Larjosto & Schultz 1984). Kuoppala found that vitamin D concentrations were significantly lower in pregnant women with insulin-dependent diabetes, than in women with gestational diabetes (GDM) and in the control subset (Kuoppala 1988).

Several other Finnish studies investigated vitamin D status during and after pregnancy, for example: vitamin D supplementation during pregnancy and lactation (Ala-Houhala 1985), association between maternal supplementation and breast-milk concentrations (Ala-Houhala et al. 1988), and association between vitamin D levels in mothers and children. In this last study, the highest vitamin D values were recorded after delivery during summer and the lowest during spring (Kuoppala et al. 1986). In recent Finnish studies, possible effects of maternal

insufficient 25(OH)D levels were assumed on fetal bone development (Viljakainen et al. 2010), and higher risk to develop cancer (Toriola et al. 2010).

Finland was part of The Environmental Determinants of Diabetes in the Young study (TEDDY), which studied the use of dietary supplements (mostly vitamin D and fatty acids) during pregnancy in four different countries (Germany, Finland, Sweden and USA). Results showed that 87% of the Finnish pregnant women were taking some type of supplements beside diet. Maternal vitamin D containing supplement intake increased in Finland from 63% to 79% during the study period, but not in the other study countries (Aronsson et al. 2013). However, results from several studies carried out in Finland have shown that vitamin D supplementation during pregnancy is not reaching the optimal intake recommended by the national dietary guidelines (Erkkola et al. 1998, Arkkola et al. 2006).

Despite the Finnish vitamin D food fortification policy since 2003 and the present nutrition guidelines, which recommend vitamin D dietary supplements all year round, vitamin D status in pregnant women has seasonal variations and is still rather poor (Prasad et al. 2010, Pietinen et al. 2010).

2.5 Vitamin D and pregnancy

2.5.1 General

Developing fetus acquires its vitamin D across the placenta and thus maternal vitamin D status may influence the intrauterine fetal development and infant health later. During pregnancy, vitamin D values tend to decrease from their optimal requirements for the body for several reasons. This phenomenon may lead to developing either vitamin D insufficiency or deficiency, which may affect the mother directly and later the offspring.

Nevertheless, due to insufficient high quality human data, especially randomized controlled trials, the vitamin D concentration range for which both mother and offspring get the most health benefits is not exactly known (Christesen et al. 2012a).

However, the same authors, in a different review conclude that maternal vitamin D concentrations are inversely associated with specific pregnancy outcomes such as,

preeclampsia (PE), gestational diabetes (GDM), and infertility parameters (Christesen et al. 2012b).

There are specific risk factors that have the main influence to predispose VDD among pregnant women. These factors, either exogenous or endogenous, are for example: season, obesity and body mass index (BMI), skin pigmentation or ethnicity, dressing code, maternal parity, diet and supplementation.

2.5.2 Season

Season is considered to be one of the important factors that affect vitamin D status in humans, and in particularly pregnant women. Season is related to the sunlight exposure which affects the formation of vitamin D in the body.

There have been many studies showing associations between sunlight exposure or different seasons and vitamin D concentrations among pregnant women.

In an American study, Bodnar and associates showed that during cold seasons, vitamin D levels reached their lowest concentrations, and in the summer months values peaked (Bodnar et al. 2009). Even in Turkey, where the sunshine is plentiful, most of the pregnant women had hypovitaminosis and half of the study population was severely deficient throughout winter seasons (Ustuner et al. 2011). Two Indian studies established that over 80% of the pregnant females were vitamin D deficient, having the lowest values during winter (Jain et al. 2011, Marwaha et al. 2011). Corresponding to previous research, the authors considered the season to be a risk factor for VDD (Perampalam et al. 2011, Bowyer et al. 2009, Perez-Lopez et al. 2011).

However, Darling and coworkers found that Asian child-bearing age women who were living in Great Britain had very low vitamin D concentrations throughout the year, while seasonal variability was detected only in Caucasian women (Darling et al. 2012).

2.5.3 Obesity and body mass index (BMI)

Among non-pregnant obese women, the concentrations of serum 25(OH)D are lower than those in normal weighted women (Arunabh et al. 2003, Ortega et al. 2009, McKinney, Breitkopf & Berenson 2008). McGill and associates showed that the higher the BMI and waist circumference, the lower 25(OH)D concentrations in a New Zealand obese population (McGill

et al. 2008). An American study found similar results in the obese participants; the 25(OH)D values were systematically lower in obese than in their normal weight counterparts (Parikh et al. 2004).

Similarly among pregnant women, maternal vitamin D values during first trimester of pregnancy have been negatively associated with maternal BMI (Perez-Lopez et al. 2011, Zhang et al. 2008, Yu et al. 2011). In a Danish study, vitamin D insufficiency during early pregnancy was positively correlated with higher prepregnancy BMI (Andersen et al. 2013).

Josefson and colleagues found that obese mothers had lower cord blood vitamin D concentrations at birth than normal weighted women (Josefson et al. 2013). Similarly, Bodnar and associates showed that vitamin D values in mothers and their infants were significantly lower after delivery in those women with pre-pregnant obesity than their leaner counterparts (Bodnar et al. 2007a). Josefson and collaborators showed how newborns of obese mothers had lower vitamin D concentrations even if these values were at similar level in mothers (Josefson et al. 2013). Thus it is possible that obese mothers might transmit less vitamin D to fetuses during pregnancy. As a conclusion of these studies, a significant inverse association between vitamin D status during pregnancy and the maternal BMI is evident; the higher maternal BMI, the lower 25(OH)D concentrations.

The association between obesity and VDD might lie in the decline of its bioavailability from fat tissue (Wortsman et al. 2000). In higher BMI, the production of the dermal 7-dehydrocholesterol is not altered, but fat tissue might modify its discharge from the skin into the systemic circulation. So, even though it is formed in the skin, vitamin D will remain as a deposit and will not reach the next passages to be further metabolized, but only to a lesser degree than in normal weighted individuals (Wortsman et al. 2000).

As a summary, vitamin D insufficiency in obese individuals might be due to lower bioavailability of vitamin D_3 from endogenous and exogenous sources because of its accumulation in the fatty tissues of the body (Wortsman et al. 2000).

2.5.4   Skin pigmentation (or ethnicity)

The different human ethnicity is associated with various skin color shadings with a specific permeability to the body. The skin pigmentation differences in humans are adaptive and

correlate to the adjustment of ultraviolet radiation to the skin. Melanin plays a filtering and photo protective role. Nevertheless, the solar radiation requirement for medium to very dark skinned people is from two to six times higher than in lightly pigmented individuals in order to synthesize the same amount of 7-dehydrocholesterol. There are also differences in gender; usually females are less melanized as the males from the same ethnic group (Webb, Kline & Holick 1988, Jablonski, Chaplin 2000). This phenomenon features skin pigmentation to be a risk factor for VDD.

Among non-pregnant women the vitamin D values are highest usually in the fair pigmented Caucasians and lowest in deeply pigmented African-American (McKinney, Breitkopf & Berenson 2008, Harris, Dawson-Hughes 1998, Scragg et al. 2004, Ginde et al. 2010). Darling and coworkers recently showed that Asian females living in the UK were lacking vitamin D throughout the year, while the white residents were less deficient (Darling et al. 2012). Canadian cross-sectional study found that 76% of the aboriginal females had low serum 25(OH)D values (Lebrun et al. 1993).

During pregnancy similar results have been obtained; Afro-American, African, Indian, Asian, and Arab or Middle-Eastern women had significantly lower vitamin D concentrations than white Caucasians (Bodnar et al. 2009, Perez-Lopez et al. 2011, Feleke et al. 1999, Clifton-Bligh, McElduff & McElduff 2008). This might be due to deeply pigmented skin, dressing code and the lack of vitamin D supplementation in the food chain (Feleke et al. 1999). Further, Andersen and colleagues showed that pregnant Danish women of non-European origin were associated with lower vitamin D concentrations (Andersen et al. 2013).

In a systematic review on maternal vitamin D status, the researchers concluded that VDD was common in different regions of the world even in the levels with excess sun-light. Exposure of sun differs greatly from Northern to Southern Europe and from Europe to Asia and Africa. Regardless of ethnic background and country of origin, VDD was highly prevalent among pregnant women all over the world (Schroth, Lavelle & Moffatt 2005).

2.5.5 Dressing code

There is great evidence how vitamin D concentrations are significantly lower among pregnant women wearing concealing clothing. The practice of (wearing) purdah or concealing clothes

and veiling is prevalent in Muslim countries, and in the Asian subcontinent being part of the Hindu tradition. Most of these countries have a tropical climate and enjoy plenty of sunshine and long daylight hours. However, some individuals, most of them are females, are rarely or almost never exposed straightly to UVB radiation. Due to these dressing habits there is a chronic lack of direct sun exposure of the skin, which leads to a reduction of vitamin D synthesis in the body that might result in VDD (Sachan et al. 2005). Most of the studies evaluating significance of concealing dressing have been conducted in countries in which the majority of the female population follows a specific (religious) dress code.

In two Turkish studies, maternal dressing code was directly associated with VDD in third trimester pregnant women (Pehlivan, I et al. 2002), and nursing mothers (Andiran, Yordam & Ozon 2002). Nearly half of the nursing mothers wearing covered attire had VDD (Andiran, Yordam & Ozon 2002).

In addition, in two Iranian studies, the authors concluded that almost two of three expecting mothers had VDD (Maghbooli et al. 2007), and the majority of the parturients had very low 25(OH)D concentrations mostly due to a veiled dress code (Bassir et al. 2001).

Other evidence sustaining the high prevalence of maternal VDD in tropical countries comes from different studies conducted in the Gulf region (Saudi Arabia, Kuwait and United Arab Emirates). The hypovitaminosis was mainly due to the lack of sun light exposure by adopting a strict dressing behavior covering the arms, hands and even face (Serenius, Elidrissy & Dandona 1984, Taha, Dost & Sedrani 1984, el-Sonbaty, Abdul-Ghaffar 1996).

A Dutch study showed that 91% of newborns born to immigrant pregnant women wearing concealing clothes had VDD. Further, they showed that maternal and umbilical cord 25(OH)D concentrations significantly correlated (Dijkstra et al. 2007). Bowyer and collaborators found that more than 70% of veiled pregnant women in Australia were suffering from hypovitaminosis D (Bowyer et al. 2009).

Only in a small British study of 160 pregnant women the authors could not relate the dressing codes to the hypovitaminosis; these women were selected from a non-European ethnic minority population. However, half of the participants were severely vitamin D deficient (50% had <20nmol/l vitamin D concentrations) in the beginning of pregnancy, and half of women

who had lived in Britain for longer than 3 years had still subnormal vitamin D levels even after supplementation (Datta et al. 2002).

2.5.6   Maternal parity

Multiparty is estimated to be a risk factor for VDD due to depletion of the vitamin D reserves in the body especially if there is a lack of vitamin D supplementation or faulted dietary behaviors within the pregnancy spacing periods (Gharaibeh, Stoecker 2009, Jensen et al. 2012). A Jordanian study suggests that women who delivered five or more children had much lower 25(OH)D concentrations compared with primiparous women (Gharaibeh, Stoecker 2009). Multiparity has also been a potential risk factor of decreased vitamin D supplementation to newborns after birth. Dratva and coworkers showed that being pregnant second time almost halves the chances to supplementation in Swiss nursing mothers (Dratva, Merten & Ackermann-Liebrich 2006).

However, not all studies have shown association between parity and vitamin D levels. Both Tao and collaborators and Narchi and coworkers did not find significant differences between parity and vitamin D concentrations (Narchi et al. 2010, Tao et al. 2012). Moreover, the pregnancy spacing itself could be a determining factor for VDD; i.e. the shorter the spacing period the higher the risk for lower 25(OH)D concentrations in the next pregnancy. There are also studies which observed that nulliparity itself may a risk factor for vitamin D deficiency (Perez-Lopez et al. 2011).

2.5.7   Diet and supplementation

Deficient diet could be considered a primary risk factor for VDD in pregnancy, especially with certain dietary behavior of specific groups, for instance in Asia, Middle East and other parts of the world whose populations do not consume enough vitamin D rich foods (different types of fatty fish, fish products, liver and mushrooms) or vitamin D fortified foods.

Halicioglu and colleagues found that the 25(OH)D concentrations of the expectant women who did not consume regularly milk and dairy products were significantly lower compared with pregnant women who had an adequate dairy intake (Halicioglu et al. 2012). Similarly a Jordanian research reported that over 75% of the women of reproductive age participating in the study consumed infrequently or never fish; only 7.5% confirmed prenatal supplements

usage; 70% of the mothers consume insufficiently milk and dairy products, which are unpasteurized and non-fortified for vitamin D (Gharaibeh, Stoecker 2009).

Nevertheless, data collected from the Norwegian Mother and Child Cohort Study (MoBA) discovered that over 80% from the expecting women were using dietary supplements; most frequently fish liver and oil. While 63% did not meet the daily recommended vitamin D intake in this study, 99% of the non-users of any supplement were vitamin D insufficient (Haugen et al. 2008). A Swiss study found that multiparous and young or old (<25 and over 35 years) women took more rarely supplements during pregnancy, and gave less often supplements to newborns (Dratva, Merten & Ackermann-Liebrich 2006).

Many studies have shown that regardless of vitamin D supplementation during pregnancy, maternal concentrations are suboptimal especially during cold season (Holmes et al. 2009). More than half of the Danish pregnant women were vitamin D insufficient even though almost 70% of them were reported to use supplements (Jensen et al. 2012). Similarly, regardless 60% of the expecting Belgian women were using vitamin D supplementation, 45% of them were 25(OH)D deficient (Vandevijvere et al. 2012).

2.5.8 Dietary Recommendations for vitamin D

We gathered information from three different sources: the Nordic Nutrition Recommendations (NNR 5) from the Nordic countries (Denmark, Finland, Iceland, Norway, Sweden, the Faroe Islands, Greenland, and Åland), the European Food Safety Authority (EFSA), and the Finnish Nutrition Recommendations 2014.

Table 2. NNR 5 recommended daily intake of vitamin D*.

Age	Vitamin D (μg)
<6 months (from 4 weeks of age)	10
6-23 months	10
2-18 years	10
Adults (females and males, years)	
18-74	10
≥75	20
Pregnant women	10
Lactating	10

*From 4 weeks of age, infants should receive 10 μg vitamin D per day as a supplement. Elderly people with little or no sun exposure should receive a supplement of 20 μg vitamin D_3 per day in addition to the dietary intake.

Table 2 shows the present Nordic Nutrition Recommendations 2012 guideline issued in October 2013 (the fifth edition, NNR 5). The Recommended Intake for vitamin D was increased from 7.5 µg to 10 µg per day for children above 2 years and for adults, while for the elderly ($\geq$ 75 years of age) the recommendation is 20 µg per day.

Table 3. EFSA recommendations tolerable upper intake levels (ULs) for vitamin D.

Summary of Tolerable Upper Intake Levels for vitamin D Age (years)	Tolerable Upper Intake Level (UL) for vitamin D (µg/day)
Children	
0-1	25
1-10	50
11-17	100
Adults $\geq$ 18	100
Pregnant women	100
Lactating women	100

Further, in July 2012, EFSA has reviewed the Tolerable Upper Intake Levels for vitamin D intake (Table 3). The UL for adults and adolescents has been raised from 50µg to 100µg daily; the UL for children aged 1-10 years has been increased from 25µg to 50µg daily.

Table 4. The recommended daily intake of vitamin D[1] (translated from Finnish).

	Age	Vitamin D (µg)
Children	<6 months[2]	10
	6-23 months	10
	2-17 years	10
Adults (females and males, years)	17-60	10
	61-74	10[2]
	$\geq$75	20[2]
Pregnancy		10[2]
Breastfeeding		10[2]

[1]The recommended daily intake is from the diet. Nutrient losses due to food preparation techniques and treatment-related methods have been taken into account in the design of the diets.

[2]In Finland, vitamin D supplementation recommendations apply all year-round as follows: for children from 2-weeks to 2 years of age (10 µg/day), for pregnant women and nursing mothers (10 µg/day), and for people above 60 years of age (20 µg/day).

Table 4 comprises the vitamin D recommendations from the most recent national Finnish Nutrition Recommendations 2014 (Terveyttä ruoasta - Suomalaiset ravitsemussuositukset 2014).

2.5.9 Other factors

Maternal smoking is jeopardizing the health of both the future mother and the offspring. Smoking during pregnancy negatively affected the fetal growth (Jaddoe et al. 2007). Older pregnant women (≥40 years) who smoked during pregnancy were more likely to give birth to preterm and small for gestational age (SGA) newborns (Salihu et al. 2005).

Other adverse effect of smoking on pregnancy is the susceptibility of developing VDD (Andersen et al. 2013, Jensen et al. 2012, Vandevijvere et al. 2012, Diaz-Gomez et al. 2007). However, the mechanisms by which active- or second-hand smoking have an impact on vitamin D status of the mother and the child, are not clear and need to be investigated further more.

Some studies have shown negative correlations between maternal age and vitamin D levels during pregnancy; thus older mothers were having lower vitamin D concentrations (Gharaibeh, Stoecker 2009, Tao et al. 2012). However, a Danish study which concentrated more on supplementation intake, found that older mothers were having higher vitamin D concentrations than the younger counterparts (Josefson et al. 2013).

Low socio-economic status is shown to be one of the most important risk factors for lower vitamin D concentrations in pregnant women as well as in their newborn infants in some studies (Andiran, Yordam & Ozon 2002, Vandevijvere et al. 2012, Pehlivan et al. 2003). Nevertheless Narchi and coworkers, found no important differences between maternal education and vitamin D levels during pregnancy (Narchi et al. 2010). A Pakistani study revealed that the vitamin D levels of pregnant women who had no educational background were marginally higher than those belonging to a higher socio-economical group. According to this study the difference was not due to diet, which in both groups was deficient in vitamin D rich foods, but due to a different outdoor behavior. Women from disadvantaged communities tend to spend more time outside than the educated ones (Atiq et al. 1998).

2.6 Pregnancy complications and Vitamin D deficiency

2.6.1 Vitamin D deficiency and preeclampsia (PE)

Preeclampsia (PE) can be defined as new onset gestational hypertension and proteinuria for the first time after 20 weeks gestation (Bodnar et al. 2007b). This disorder impacts both the mother and the child, and delivery ends the clinical features of PE. It is multifactorial of nature and this aspect makes it more difficult to model.

There are several (epidemiologically proven) risk factors associated with incidence of PE. Some of these risk factors are maternal hypertension, gestational diabetes (GDM), diabetes mellitus, obesity (BMI above 29 kg/m^2), and twin pregnancy, but also living in a Nordic country (Ros, Cnattingius & Lipworth 1998). It is suggested also that vitamin D deficiency could be associated with the development of PE (Bodnar et al. 2007b, Baker et al. 2010, Rylander, Lindqvist 2011). Nevertheless, the mechanism that clarifies the pathway of VDD towards PE is not clear.

The association between development of preeclampsia during pregnancy and VDD has been evaluated in many different studies. The association between midgestation VDD and the risk of severe preeclampsia later during pregnancy was found in one nested case-control study (Baker et al. 2010). Bodnar and collaborators concluded that maternal VDD is a risk factor for PE and further, newborns born to preeclamptic mothers had lower vitamin D status than those of control mothers (Bodnar et al. 2007b). However, not all studies had found correlation between maternal vitamin D status from the first half of pregnancy and the risk of developing PE or adverse pregnancy outcomes later (Powe et al. 2010, Shand et al. 2010, Yu et al. 2012).

There are several factors which may impact (adversely or positively) the association between VDD and PE for example: season, vitamin D supplementation, altitude and latitude, obesity, ethnicity, and dress code. Haugen and coworkers found a protective role of vitamin D supplementation on pregnant Norwegian women from the risk of PE, while vitamin D intake from the diet alone did not have the same effect. The possible reasons for the difference were not explained (Haugen et al. 2009). A Finnish cohort study concluded that regular vitamin D supplementation in infancy was associated with reduced risk of PE during future pregnancy,

even though the dose of supplementation did not show any association with the risk of PE (Hyppönen et al. 2007).

In the Nordic countries, the differences of sunlight exposure are considerable between seasons, causing less production of vitamin D by the skin. A recent Swedish cross-sectional study found that deficiency of sunlight was associated with increased risk of eclampsia in pregnant women possibly due to vitamin D insufficiency (Rylander, Lindqvist 2011). A systematic review found that PE rates were also higher during winter months in non-tropical regions or when delivery occurred during the maximal humidity season in tropical areas (TePoel, Saftlas & Wallis 2011). Further, an Indian study discovered a positive association between the incidence of eclampsia and the monsoon season. Nevertheless, in this study the climatic influence, whether it is dry or humid, did not play an important role in the development of preeclampsia or hypertension during pregnancy (Subramaniam 2007).

Fowles and coworkers investigated whether there is an association between maternal nutrient intake and placental biomarkers and possible preeclampsia. The outcome was inconclusive in terms of isolating a single nutrient potentially responsible for inhibiting early placental growth and PE development. Nevertheless, the 25(OH)D concentrations were below the range in 71.2% of the subjects (Fowles et al. 2012).

2.6.2 Vitamin D deficiency and Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus (GDM) is glucose intolerance with the commencement or first detection during pregnancy (Buchanan et al. 2007, American Diabetes Association 2000). It is associated with several risk factors such as: high maternal age, obesity or overweight, prior history of GDM, family history of type 2 diabetes mellitus (T2DM), and ethnicity (Buchanan, Page 2011, Gibson, Waters & Catalano 2012, Rajab et al. 2012). There is a growing body of scientific evidence between low vitamin D values and impaired glucose metabolism during pregnancy (Zhang et al. 2008, Clifton-Bligh, McElduff & McElduff 2008, Maghbooli et al. 2008, Azar et al. 2011). The results from a Canadian study pointed that pregnant women who developed GDM had lower 25(OH) D serum concentrations than the control group. The lower vitamin D concentrations skewed towards the ethnic groups rather than the white Caucasian majority, and there was also a seasonal difference in vitamin D values (Parlea et al. 2012). Rudnicki and Mølsted-Pedersen included in their study Danish pregnant women who already

had developed GDM and were given intravenously vitamin D. The authors showed that the therapy decreased the fasting glucose values in GDM participants on a short term (Rudnicki, Molsted-Pedersen 1997).

Even as VDD is quite common among Indian pregnant women, no correlation was discovered between low vitamin D concentrations and risk of GDM and/or glucose intolerance (Farrant et al. 2009). Moreover, in a London case-control study, which included first trimester pregnant women, the maternal values of 25(OH)D were not predictive for development of GDM (Savvidou et al. 2011).

The above mentioned studies have demonstrated some evidence of the association between VDD and GDM. Nonetheless, this association and its mechanism are still obscure.

2.6.3 Vitamin D deficiency and possible consequences on newborns

Maternal vitamin D status is the fetal source of vitamin D during pregnancy and supplementation during breastfeeding after birth. The direct consequences of VDD could appear during gestation, affecting the pregnant females themselves with short term impact, while the long term consequences of maternal VDD can be seen on their newborns later. The children of VDD mothers might start to suffer from hypovitaminosis D in their early days (Thomas et al. 2011). This status of deficiency can be improved by adequate supplementation (Crocker et al. 2011). Viljakainen and colleagues showed that low maternal vitamin D status might decrease the bone density of the child, but it was improved with adequate supplementation (Viljakainen et al. 2011). And the second prominent outcome in the offspring is the risk of developing rickets (Prentice 2011). It is obvious that VDD is a risk factor for rickets in children (Kaludjerovic, Vieth 2010). The newborns to mothers who are vitamin D sufficient have a normal fetal development and mineral incrementation. While the neonates to vitamin D insufficient mothers might experience fetal growth retardation, decreased mineral accretion, reduced postnatal development and weight gain (Pawley, Bishop 2004).

Nabulsi and collaborators concluded that VDD, caused by maternal veiling, was associated with decreased musculoskeletal parameters in boys, but not in girls (Nabulsi et al. 2008). Consequently, the birth size of the neonates might be associated with vitamin D values of the mothers. Thus, offspring born to VDD mothers are susceptible to be smaller in size (small for

gestational age or SGA) (Morley et al. 2009). However, there are several factors that may influence the association of maternal VDD and fetal growth, including racial background and genotyping (Bodnar et al. 2010). Similarly, Shibata and coworkers found that the overall prevalence of VDD in their gravid Japanese population was considerably high. Lower vitamin D results were positively associated with the danger of bone mass impairment and delivering prematurely (Shibata et al. 2011).

A Dutch prospective birth cohort study named Child, Parent and Health: Lifestyle and Genetic Constitution (KOALA [in Dutch]), which included first trimester healthy pregnant women, found no correlations between maternal vitamin D supplementation and child lung function. However, when referred to child supplementation after birth, they found a significant association between vitamin D intake and higher 25(OH)D concentrations at age 2 years (Cremers et al. 2011). From the recent Finnish DIPP study (Diabetes Prevention and Prediction study), no association was found between the risks of developing severe beta cell autoimmunity in the offspring and daily maternal intake of vitamin D from external sources during pregnancy, such as food and supplements (Marjamäki et al. 2010).

Multiple sclerosis (MS) is a central nervous system disorder of unknown etiology. Both environmental and genetic factors contribute to the development of the disease (Burrell et al. 2011). One of the environmental factors that might have a role in the development of MS is gestational VDD. A Swedish study supported the emerging evidence that there might be an association between the risk of MS and the season of birth. Low maternal vitamin D levels could be possible intermediators between season and MS (Salzer, Svenningsson & Sundstrom 2010). According to Mirzaei and colleagues the maternal 25(OH)D intake from nutrient sources during pregnancy was oppositely associated with the MS development in the female offspring (Mirzaei et al. 2011)

3   Study aims

The aim of the study was to determine serum concentrations of 25-hydroxyvitamin D during first trimester of pregnancy in normal uncomplicated Finnish pregnant women who were living in the eastern part of Finland (Kuopio area), and to study whether 25(OH)D concentrations were associated with different maternal and neonatal clinical determinants.

4   Materials and method

4.1   Study population: maternal and perinatal data

Blood samples of Caucasian Finnish women from the Eastern Finland region were collected routinely for screening for Down's syndrome during first trimester of pregnancy (9th – 11th gestational weeks). All women who delivered in Kuopio University Hospital (Kuopion Yliopistollinen Sairaala, KYS) during April 2008-March 2009 with adequate serum samples for vitamin D analysis were selected for further study.

Data on season and gestational age at sampling, maternal age, parity, numbers of earlier gestations and spontaneous abortions, maternal BMI, and smoking was obtained from Kuopio University Hospital Birth register database. Variables were recoded as following: season of sampling (March-May, June-August, September-November vs. December-February), gestational week at sampling ($\leq$10, 10-11 vs. $\geq$11 gestational weeks), maternal age at delivery ($\leq$26, 27-32 vs. >32 years), maternal BMI at first trimester ($\leq$21, 21-23, $\geq$23 Kg/m^2), maternal parity (0, 1 vs. $\geq$2), maternal earlier smoking before pregnancy (earlier >5 cigarettes/daily, but not during pregnancy vs. never smoking), gestational age at delivery (259-278, 279-285, $\geq$286 days), the mode of birth (vaginal, non-elective vs. elective Caesarean section), fetal gender (female/male), birth weight ($\leq$3320, 3321-3733 vs. >3733 grams) and duration of hospital stay after birth ($\leq$2, 3 vs. >3 days)

A new recheck was done with study population with exclusions of all women with other than Finnish nationality and pregnancy complications (such as high blood pressure and gestational diabetes) including 405 women in final analysis. Serum $25(OH)D_3$ and $25(OH)D_2$ levels were analyzed from all women

Table 5. Inclusion criteria of the study population.

Inclusion criteria
Only singletons
Delivery after term >36 gestational weeks
No GDM during pregnancy and no earlier diabetes mellitus
BMI $\leq$30 Kg/m^2
No small gestational ages (<5th percentile)
No smoking during pregnancy
Blood pressure <140/90 mmHg or no preeclampsia during ongoing pregnancy
Newborn does not need intensive care treatment

4.2 Laboratory analysis

The serum concentrations of vitamin D_3 and vitamin D_2 were analysed by the High Performance Liquid Chromatography using coulometric electrode array detector (HPLC-CEAD) method (Nurmi et al. 2013), in the biochemistry laboratory from University of Eastern Finland, Institute of Public Health and Clinical Nutrition, Kuopio Campus.

The pre-treatment of the frozen samples prior to the analysis is as follows:

A precise volume (500 µl) of the sample was thawed and transferred into disposable tubes. It was added 350 µl methanol-isopropanol 80:20 (v/v) and shaken vigorously for 30s (1350 rpm). Afterwards, both vitamins D [25(OH)D_2 and 25(OH)D_3] were extracted three times using 2 ml of hexane and shaking for 2 minutes. Then the sample was being centrifuged for 5-10 minutes at 3450 rpm at room temperature. The hexane phase was collected and transferred into a durable glass tube. All three combined hexane phases were evaporated under nitrogen gas (N_2) flow at 25°C. The remaining dry residue was dissolved in 150 µl of 60 mM sodium per chlorate buffer: methanol 20:80 (v/v) and shaken for 30s. Then the sample was centrifuged for 10 minutes at 3450 rpm and 100 µl transferred into 0.2 ml conical HPLC vials. The injection volume in the HPLC-CEAD device was 30 µl (Nurmi et al. 2013).

The general formula used for the calculation of both vitamin D_3 and D_2 is $Fx = E \times \frac{D}{C}$

Where F_x represents the function; E represents the measured value/concentration of $25(OH)D_3$ or $25(OH)D_2$ nmol/l by HPLC, so it is the raw data; D represents the dissolving volume (150 μl) of sodium per chlorate buffer: methanol added to the dry residue; C represents the taken volume from the serum sample, which is usually 500 μl, but could vary according to the sample.

4.3   Statistical analysis

SPSS version 17.0 was used to perform the statistical analysis. Associations between different maternal and prenatal factors and vitamin D_3 levels in univariate analysis were performed with the Chi-square test and continuous variables by Kruskal-Wallis test. In multivariate test, vitamin D_3, values were ln-transformed, and approximate normal distribution was confirmed. Covariance analysis was used to investigate the relationship between the vitamin D_3 and the variety of significant variables found in univariate tests. All variables that were significant in the univariate analysis at $P<0.11$ were included simultaneously in the covariance analysis. If the proportion of missing values exceeded 5% for any case, the remaining data were included in the analysis as a separate group. The cut-off level for statistical significance was two-sided $P<0.05$.

5   Results

Table 6. Maternal and neonatal characteristics of the study population.

Characteristics	x (SD)	*N*(%)
Maternal age, years	29	5.2
≤26	139	34.3
27–31	136	33.6
≥32	130	32.1
Married	227	56.0
Maternal parity		
Nulliparous	182	44.9
Primiparous	140	34.6
Multiparous	83	20.5
Number of earlier gestations	1.3	1.4
Number of earlier spontaneous abortions	0.3	0.7
Smoking before pregnancy	82	20.2
Maternal BMI in first trimester, kg/m^2	21.7	1.8
≤21	147	36.3
21–23	150	37.0
≥23	108	26.7
Duration of gestation when sampling, weeks	10.2	0.9
Season of sampling		
Spring	57	14.1
Summer	128	31.6
Autumn	126	31.1
Winter	94	23.2
Duration of gestation at birth, days	281	8
Mode of delivery		
Vaginal, spontaneous	330	81.5
Vaginal, vacuum traction	37	9.1
Elective cesarean	20	4.9
Nonelective cesarean	18	4.4
Fetal sex, male	213	52.6
Birth weight, grams	3546	421
Duration of hospital stay after birth, days	2.9	1.4

Table 6 shows basic demographic and clinical characteristics of the study population. All women in the study were Finnish Caucasian who were not reported smoking during pregnancy with BMI equal to or less than 29 kg/m^2 in the first trimester. All women had normal blood pressure values followed during pregnancy in outpatient health centers with regular free visits and no one developed pre-eclampsia or gestational diabetes during ongoing pregnancy. In average, highest systolic and diastolic blood pressure values during ongoing pregnancy were

118 (SD 10.1) mmHg and 72 (7.8) mmHg. All women delivered after term pregnancy (≥37 weeks) with normal weighted (gestational adjusted) newborn, who did not need neonatal intensive care treatment immediately after birth.

Table 7. Vitamin D_3 and D_2 levels in first trimester of 405 normal Finnish nonsmoking women.

Variable	Mean (SD)	Min. – Max.	Median	N (%)
Vitamin D_3 (nmol/l)	54.9 (18.7)	10.3 – 117.8	53.6	
Deficiency (<25 nmol/l)				19 (4.7)
Deficiency (<50 nmol/l)				164 (40.5)
Insufficiency (50-80 nmol/l)				197 (48.6)
Sufficiency (>80 nmol/l)				44 (10.9)
Vitamin D_2 (nmol/l)	0.48 (2.8)	0 – 39.3	0	
Not detectable				384 (94.8)
If detectable < 6 nmol/l				10 (2.5)
If detectable ≥ 6 nmol/l				11 (2.7)

Table 7 shows vitamin D_3 and D_2 concentrations sampled during first trimester of pregnancy. Most samples were obtained at 10th (range 8-12th) gestational week mostly during summer and autumn; less samples were collected during spring and winter (Table 6). In 94.8% of women vitamin D_2 was not detectable and only 21 (5.2%) women had detectable amounts of vitamin D_2. Due to low numbers of women with detectable amounts of vitamin D_2, only vitamin D_3 concentrations were further analyzed.

In 40.5% of the study population (N=164), vitamin D_3 concentrations were below 50 nmol/l, which is attributed to a deficient status. Almost half of the population (48.6%) had vitamin D_3 concentrations between 50-80 nmol/l(N=197), and only 10.9 % had values greater than 80 nmol/llimit, which represents sufficiency. Among total population, the mean vitamin D_3 level was 54.9 nmol/lwith minimum from 10 nmol/l to maximum 118 nmol/l. Sampling season was most significantly associated with vitamin D_3 levels. Deficient levels of vitamin D_3, were almost 3-times more frequently obtained from samples during winter (61.7 % of women) compared to samples during summer (23.4%) (p<0.0001).

Table 8. Maternal and neonatal variables and first trimester vitamin D₃ levels and deficiency in serum samples of 405 Finnish pregnant normal women.

Variable	N (%)	Vitamin D_3 nmol/l	P value	Deficiency N (%)	P value
Maternal age, years			0.0001		0.002
Lowest tertile (<27)	139 (34.3)	49.8		74 (53.2)	
Middle tertile (27–32)	136 (33.6)	57.8		51 (37.5)	
Highest tertile (>32)	130 (32.1)	57.5		39 (30.0)	
Marital status			0.069		0.050
Married	227 (56.0)	56.5		80 (35.2)	
Not married	178 (44.0)	52.9		84 (47.2)	
Maternal parity			0.122		0.104
Nulliparous	182 (44.9)	55.0		74 (40.7)	
Primiparous	140 (34.6)	53.1		64 (45.7)	
Multiparous	83 (20.5)	57.7		26 (31.3)	
Number of earlier gestations			0.170		0.134
None	146 (36.0)	55.0		59 (40.4)	
One	127 (31.4)	52.9		61 (48.0)	
Two or more	132 (32.6)	56.8		44 (33.3)	
Number of earlier spontaneous abortions			0.512		0.335
None	332 (82.0)	54.7		138 (41.6)	
One	45 (11.1)	54.6		18 (40.0)	
Two or more	28 (6.9)	58.7		8 (28.6)	
Maternal BMI at 1st trimester kg/m2			0.032		0.013
Lowest tertile (<21)	147 (36.3)	52.7		67 (45.6)	
Middle tertile (21–23)	150 (37.0)	54.3		61 (40.7)	
Highest tertile (>23)	108 (26.7)	58.7		36 (33.3)	
Maternal smoking before pregnancy			0.0001		0.0001
No	323 (79.8)	57.0		116 (35.9)	
Yes	82 (20.2)	46.8		48 (58.5)	
Duration of gestation at the time of sampling (weeks)			0.862		0.579
– 10	190 (46.9)	54.9		76 (40.0)	
10–11	155 (38.3)	55.2		62 (40.0)	

11-	60 (14.8)	54.1		26 (43.3)	
Season at the time of sampling			0.0001		0.0001
Winter	94 (23.2)	45.3		58 (61.7)	
Spring	57 (14.1)	50.3		25 (43.9)	
Summer	128 (31.6)	61.8		30 (23.4)	
Autumn	126 (31.1)	57.2		51 (40.5)	

Vitamin D_3 values are mean values. P values are determined by Kruskal-Wallis or Chi-square or test. Vitamin D_3 deficiency is determined serum values <50 nmol/l.

In univariate analysis, vitamin D_3 levels were significantly higher in older women and those with higher BMI in first trimester compared to those women who were younger and had lower BMI (Table 8). Levels also tended to be higher (p<0.069) in married women. In contrast, women who reported to quit smoking during pregnancy but had smoked earlier before pregnancy had significantly lower levels of vitamin D_3 and also more significantly had deficient levels (up to 58.5% of women who had smoked before pregnancy compared to 36.7% of non-smoking women, p<0.001). Similarly, 47.2% of cohabitated women had deficient levels of vitamin D_3 in first trimester compared to 35.2% of married women (p<0.050). No other maternal prenatal factor was associated with vitamin D_3 levels or deficiency.

Table 9. Estimated selected coefficients from the covariance analysis to predict vitamin D$_3$ levels in first trimester serum samples of 405 Finnish women.

	Coefficients (SD)	95% CI	*P*- value
Intercept	3.86 (0.06)	3.8 - 4.0	0.001
Season of sampling			
spring *vs.* winter	0.16 (0.06)	0.05 - 0.27	0.005
summer *vs.* winter	0.34 (0.05)	0.25 - 0.43	0.0001
autumn vs. winter	0.24 (0.05)	0.15 - 0.33	0.0001
Maternal age			
≤ 27 *vs.* >32 years	-0.12 (0.05)	-0.22 - -0.03	0.009
28–32 *vs.* >32 years	-0.002 (0.04)	-0.09 - 0.08	0.957
Maternal parity			
0 *vs.* ≥2	0.02 (0.05)	-0.08 - 0.11	0.726
1 *vs.* ≥2	-0.06 (0.05)	-0.16 - 0.03	0.192
Maternal BMI, kg/m2			
≤ 21 vs. >23	-0.06 (0.04)	-0.15 - 0.025	0.165
21–23 *vs.* >23	-0.02 (0.04)	-0.10 - 0.07	0.660
Maternal smoking			
Smoking before but not during pregnancy *vs.* non-smoking	-0.14 (0.04)	-0.23 - -0.06	0.001
Married			
No *vs.* yes	-0.029 (0.037)	-0.10 - 0.044	0.440

R2= 0.20. All these variables were included in multivariate model.

In multivariate analysis, vitamin D$_3$ levels were associated significantly with season of sampling, maternal age and maternal earlier smoking before pregnancy. Levels were lowest in women who were sampled during winter, who were young and who were quitters that had smoked more than 5 cigarettes daily before pregnancy (Table 9). Maternal parity or BMI values and marital status were not associated in the vitamin D$_3$ concentrations in multivariate analysis.

First trimester vitamin D$_3$ levels were not associated with the gestational age at birth in women who delivered after term pregnancy, with the mode of delivery, fetal sex, birth weight, or duration of hospital stay after birth (data not shown).

6 Discussion

This study showed that most of the Finnish pregnant women with uncomplicated pregnancy were 25(OH)D deficient or insufficient in the first trimester, with only a few (10.9%) having sufficient (>80 nmol/l or >32 ng/ml) vitamin D concentrations although Finland has national policies which are health oriented with guidelines and nutritional recommendations for vitamin D supplementation, and vitamin D fortified food products are well available.

The factors associated with maternal 25(OH)D concentrations were sampling season, smoking before pregnancy and maternal age.

The sampling season in first trimester of pregnancy was significantly associated with maternal vitamin D_3 concentrations as shown also in earlier studies (Ustuner et al. 2011, Bowyer et al. 2009, Perez-Lopez et al. 2011). This result is similar and could be integrated in the larger body of literature, which found that the lowest concentration is during winter and highest during summer (Bodnar et al. 2009, Perampalam et al. 2011) . This is partly due to the fact that Kuopio and surrounding Eastern Finland areas are situated around 62°N latitude, having long dark winters, and relatively short summers.

We found significant evidence about the association between maternal smoking before pregnancy (even though women reported to had quit smoking during pregnancy) and decreased 25(OH)D levels, which is in line with other international studies (Perez-Lopez et al. 2011, Andersen et al. 2013).

The accuracy of self-reported smoking is to a certain extent unreliable, because participants tend to underreport smoking (Webb et al. 2003, Shipton et al. 2009). Smoking status can be assessed more objectively by using plasma cotinine levels, which is an active metabolite of nicotine, considered to be the gold standard biomarker for smoking exposure (Benowitz 1996). Unfortunately we did not measure cotinine in this study.

It is already documented that cigarette smoking increases the risk of developing fractures later in life, due to a decrease in bone mineral density (BMD) in both women and men (Ward, Klesges 2001). Among other several factors, the loss of bone mass in elderly smokers may be originated in impaired intestinal calcium absorption (Need et al. 2002, Krall, Dawson-Hughes

1999) and also due to lower estrogen concentrations in female smokers (Jensen, Christiansen & Rodbro 1985). Brot at al. found in their study that middle-aged women had lower concentrations of vitamin D, 1,25(OH)D and parathyroid hormone (PTH) and also recorded bone loss compared to non-smokers (Brot, Jorgensen & Sorensen 1999).

Nevertheless, the underlying mechanism of VDD in smokers is yet to be elucidated. We can speculate that once a person, in this case a pregnant woman quits smoking, the daily routine and even eating habits change. Since in Finland smoking indoors is banned, the person is not getting out as often as before, thus the exposure to direct sunlight might also decrease.

In our study, older maternal age was associated with higher vitamin D_3 concentrations, which is similar to what previous research has found (Ginde et al. 2010). Potential reasons may be that older women are more health concerned, might have healthier eating habits than the younger ones, taking more regularly supplements, fortified foods or sunbathing more. Another possibility is their internal vitamin D pool, mostly the adipose reserve of 25(OH)D, might be larger. However, including maternal BMI into the multivariate analysis, significant association was still noted between maternal age and vitamin D levels thus higher BMI did not fully explain association between older maternal age and higher vitamin D levels. In contrast, we did not find any association between maternal BMI and vitamin D levels in multivariate analysis; but we had excluded all obese women and evaluated only normal-weighted pregnant women.

However, in univariate analysis, women with lower BMI had significantly more often lower vitamin D levels compared to women with higher BMI.

When women, regardless of origin, either Finnish or immigrants, receive nutritional and general pregnancy counseling, we should pay more attention to vitamin D supplementation. All the factors should be taken into account by advising and encouraging, according to the individual food tolerance, to increase the intake of natural occurring vitamin D rich foods (fatty fish, mushrooms, eggs) or vitamin D fortified foods, regular supplementation and sun bathing whenever is possible.

The entire issue becomes more complicated due to the limitations of various analytical methods, a lack of consensus and a clear cut agreed measurement method for 25(OH)D

concentrations. Not even the entire gold standard methodological concern is not definitely settled, and many laboratories use different methods to assess vitamin D concentrations. While there is relevant research and some authors conclude that the high performance liquid chromatography (HPLC) is the gold standard method of assessing 25(OH)D (Zerwekh 2008), in recent years liquid chromatography-tandem mass spectroscopy (LC-MS/MS) is considered to be the gold standard analytical method of assessing vitamin D concentrations (El-Khoury, Reineks & Wang 2011).

The present diversity of assays, not being a unified system, raises some important issues.

In the external quality control scheme for 25(OH)D organized by Labquality participated 33 laboratories across Finland. The laboratories analyzed two serum samples for which reference values were obtained from AS Vitas Norway. Both serum samples had 25(OH)D concentration on an upper level of normal range (67.4 and 76.9 nmol/l). The laboratories applied either immunometric or chromatographic methods. Coefficients of variation between the laboratories were 16 and 18%, which are similar to those obtained in the Vitamin D External Quality Assessment Scheme (DEQAS) (Wallace et al. 2010).

The most problematic concern arose from those laboratories in the Labquality quality control scheme, which had random variation in their results so that they obtained higher values from the lower sample and vice versa. The bias and variability revealed regarding the respective Finnish labs is similar to what Lai and coworkers concluded in their study, which found a disconcerting rate of bias and variability inter-laboratories, between different assays, and also inter-assays (Lai et al. 2012).

This type of variation was observed among those laboratories using immunometric methods, but also among those using HPLC or LC-MS. Although it is not possible to see directly from the DEQAS reports, it is very likely that similar type of variation occurs also in those 1200 participating laboratories (from 54 countries, January 2013) in DEQAS rounds (DEQAS, 2014), from Europe and the USA.

Nevertheless, our HPLC-CEAD method has been validated by participating in the DEQAS rounds four times per year. According to our results for 25(OH)D$_3$, the HPLC-CEAD method performance is ±10% of the target values provided by the National Institute of Standards and Technology (NIST). Our chromatography is able to separate 3-C-epimer, and our results have been compared to the NIST values for pure 25(OH)D$_3$.

6.1   Strengths and limitations of the study

This study included 405 serum samples from normal, uncomplicated, pregnant Finnish women who were selected only from the eastern part of Finland. We can conclude such sample might be representative for the pregnant women in this specific region of the country.

The information about vitamin D_3 and D_2 concentrations were collected by analyzing all serum samples in the same laboratory and using the same analytical method, HPLC, which is considered to be a reliable analytical method. Information about vitamin D from biomarkers is more valid than other methods. The information about the background of the participants (socioeconomic, sociodemographic) used in the study were collected from an accurate and comprehensive database, the Birth Registry.

However, one limitation is the lack of data on maternal vitamin D supplementation and dietary habits during pregnancy, in addition to sun exposure and/or sun protection screen used. Another limitation could be the lack of genetic information of vitamin D polymorphisms. Nonetheless, the Finnish population from the Eastern part of the country is homogenous, so less variation could affect the study. A larger sample is needed, comprising of participants from different areas of the country, in order to form a better picture about the vitamin D concentrations during pregnancy in Finland.

6.2   Conclusion and implications

According to the existing literature and present results, vitamin D deficiency and insufficiency are prevalent, particularly in pregnant women, worldwide and also in Finland, which is considered an important public health concern.

The human genome contains 2776 vitamin D receptor binding sites (Ramagopalan et al. 2010), suggesting that vitamin D implications are beyond bone health and calcium balance. But despite all the research carried out in this field, there is no scientific consensus over the vitamin D health implications beyond bone and calcium homeostasis roles.

More large multicentre clinical trials are necessary to establish the vitamin D clinical involvement in human health and disease.

The present study adds to current scientific information regarding vitamin D values in apparently healthy Finnish pregnant women.

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