

## Smoking during pregnancy reduces vitamin D levels in a Finnish birth register cohort

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### Conflict of Interest

None.

### Authors' contributions

AIL performed the analyses and wrote the first draft of the manuscript with JHE and SH. SH coined the study question. JHE analyzed the patient data and HÖ compiled the clinical data sheet. HSN is an expert in metabolism during pregnancy and HMS is the PI of the birth registry cohort study for pregnancy complications and oversaw the laboratory assays for OH(25)D. All co-authors contributed to and approved the final manuscript.

### Ethics approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the Hospital District of Helsinki and Uusimaa (HUS/2186/2016). The use of health registers was approved by the National Institute for Health and Welfare (THL/444/5.05.00/2013).

### Short title

Vitamin D levels in Finnish smoking mothers

## **Abstract**

**Objective:** Maternal vitamin D levels in pregnancy may have implications for both the mother and foetus. Deficiency of vitamin D has been linked to several pregnancy complications and foetal skeletal health. Smoking has been associated with reduced serum levels of vitamin D metabolite 25(OH)D.

**Design:** A nested case-control study within the Finish Maternity Cohort, a population based cohort which includes first trimester sera from 98% of pregnancies in Finland since 1987. The selection consisted of women with uncomplicated pregnancies. We studied sera concentration of 25(OH)D in 313 non-smoking and 46 self-reported smoking pregnant women.

**Setting:** We hypothesize that pregnant smokers may have an increased risk of low 25(OH)D levels especially during winter months.

**Subjects:** A control group consisting of uncomplicated pregnancies from a non-published pregnancy complication study included 359 uncomplicated pregnancies. Individuals, who reported that they do not smoke were considered to be 'non-smokers' (N=313) and those, who reported continued smoking after the first trimester of pregnancy were considered to be 'smokers' (N=46).

**Results:** Smokers had significantly lower levels of 25(OH)D irrespective of sampling time ( $p < 0.0001$ ). Furthermore, during the low sun-exposure season, only 14% of smokers met the guideline levels of 40 nmol/L for serum 25(OH)D in comparison with 31% of non-smokers.

**Conclusions:** Expectant mothers who smoke have an increased risk of vitamin D deficiency during low sun exposure months in the Northern regions. Further studies are needed to assess the associated risks for maternal and foetal health as well as possible long-term implications for the infant.

## **Key words**

Pregnancy, Smoking, 25(OH)D, vitamin D

## Introduction

Smoking is known to be associated with low bone mass and increased risks of fractures, one of the plausible mechanisms being depressed 25-Hydroxyvitamin D (25(OH)D) levels among smokers.

Vitamin D is also a versatile modulator of the immune system with anti-inflammatory and anti-oxidant properties <sup>(1)</sup>. Pregnant women in Finland were studied from 1997-2004 and during this time, a significant increase of daily vitamin D intake from mean 6.2 to 8.9 µg was recorded <sup>(2)</sup>. The higher level is below the recommended 10 µg daily intake.

The adverse effects of tobacco on reproductive health and pregnancy is well established. Tobacco increase the risk of infertility both in women and men. Increased risk of pregnancy loss is also observed among smokers compared with non-smokers <sup>(3)</sup>. Tobacco contains hundreds of harmful substances and well-known carcinogens. Nicotine itself is vasoconstrictor, neuroteratogen impacting cytotrophoblast proliferation and differentiation <sup>(4)</sup>. Nicotine and carbon monoxide cross the placenta rapidly and with chronic exposure the levels in the foetal compartment exceeds those on the maternal side. Although, the exact mechanisms are unknown tobacco results in adverse pregnancy outcomes, the association between tobacco and increased risk of foetal mortality has been recognized for decades <sup>(3)</sup>. Smoking is associated with low birth weight, preterm birth, placental abruption, still birth, sudden infant death syndrome and neurobehavioral effects such as attention deficit hyperactivity disorder. Maternal smoking has profound effects on pregnancy and the future health of the offspring throughout life. Low birth weight and intrauterine growth retardation is often followed by childhood obesity and metabolic changes leading to elevated blood pressure and even elevated risk of type II diabetes <sup>(4)</sup>.

In Denmark, Brot et al reported a 10% decrease of circulating levels of 25(OH)D in smoking perimenopausal women <sup>(5)</sup>. Levels of 25(OH)D have been shown to decline in a dose-dependent manner, in correlation with increased pack years of smoking <sup>(6)</sup>. During pregnancy, smoking appears to have a similar effect, the decline of maternal 25(OH)D being in the order of 10-15%. Maternal 25OHD levels in turn correlate with foetal concentrations and 25(OH)D is known to cross placentas in *in vitro* placental perfusion studies, whereas calcitriol does not cross the placenta <sup>(7)</sup>. However, the transfer of 25(OH)D from maternal to the foetal compartment has very little effect on maternal 25(OH)D.

Vitamin D is involved in several bone homeostasis and immunoregulatory pathways where it has complex roles. Some studies suggest that prenatal exposure to vitamin D deficiency or lower than non-pregnant parathyroid hormone (PTH) levels affects neonatal outcomes, such as skeletal health, caries, and growth <sup>(8,9)</sup>. In addition, vitamin D plays a role in the human immune system and its deficiency is suggested to be associated with a number of pregnancy complications such as pre-eclampsia, small for gestational age infants, gestational diabetes mellitus, and recurrent pregnancy loss <sup>(10-14)</sup>. However, in pregnant women with severe vitamin D deficiency and vitamin D-dependent rickets type I or type II, in the absence of vitamin D supplementation, no change is observed in their own health or for their infant's skeletal health <sup>(15)</sup>.

This study sets out to evaluate the effect of smoking and season on maternal 25(OH)D levels in early pregnancies prior to pregnancy related haemodilution and profound metabolic changes. The cohort with non-smoking and smoking women was selected from uncomplicated pregnancies to rule out the effects of pregnancy complications on 25(OH)D levels. The cohort represents a population with low sun exposure.

## **Methods**

We used a national population-based Finnish Medical Birth Register (FMBR) to identify smoking and non-smoking subjects and to investigate the effect of smoking during pregnancy on vitamin D levels <sup>(16)</sup>.

FMBR contains data on demographics, pregnancy, and delivery characteristics as well as diagnoses on all live births or still births since 1987 delivered after pregnancy week 22 or weighing >500 g during the first post-natal week. When data from multiple pregnancies was available, the most recent was used as an index pregnancy. A control group consisting of women with uncomplicated pregnancies from a non-published pregnancy complication study included 359 uncomplicated pregnancies. Of these women, 46 were smokers and 313 non-smokers. Serum samples from first trimester were identified within the Finnish Maternity Cohort (FMC) by a unique personal identification code, which has been assigned to each resident of Finland. Selected serum samples were then analysed for vitamin D levels.

The FMC of the Northern Finland Biobank Borealis was established in 1983 as a nationwide effort of National Institute for Health and Welfare, Finland and is comprised of 2 million serum samples collected during the first and early second trimester of pregnancy (5th to 95th percentile: months 2–

4 of pregnancy) from over 950,000 women. The FMC covers more than 98% of pregnancies in Finland with archived prenatal serum specimens drawn for routine screening for congenital infections (HIV, Hepatitis B and syphilis). Following informed consent, the remaining serum samples (one sample of 1-3 mls for each pregnancy) are stored at -25°C in a protected biorepository at the Northern Finland Biobank Borealis in Oulu and are available for scientific research ([www.esis.fi](http://www.esis.fi)).

25(OH)D was measured in the first-trimester serum sample available in the FMC using a chemiluminescence microparticle immunoassay (CMIA) using an Architect i2000SR automatic analyser (Abbott Diagnostics) according to the manufacturer's instructions. The CMIA test of Abbott Diagnostics has previously been found to succeed best at assay of serum 25(OH)D compared to other available methods<sup>(17)</sup>. Furthermore, storage time have been found to have no effect on 25(OH)D levels<sup>(18)</sup>. Coefficients of variation derived from repeated quality control samples included in the assay with the study samples were calculated. In internal control samples with "high" 25(OH)D (>100 nmol/L) the CV=3.5%, "medium" 25(OH)D levels (~80 nmol/L) 1.8%, and "low" 25(OH)D levels (<40 nmol/L), 3.0%. In blinded QC pairs where 25(OH)D levels were not known, the CV was 1.1%.

The smoking status in this study is self-reported. Individuals, who reported that they do not smoke were considered to be 'non-smokers' (N=313) and those, who reported continued smoking after the first trimester of pregnancy were considered to be 'smokers' (N=46).

### Statistics

Independent-samples t-test was used to assess the statistical departure of vitamin D levels in smokers' sera from levels observed in controls, because BMI did not differ between groups (Table 1) and no other data was available to be included as covariants. Vitamin D data was normally distributed according to Kolmogorov-Smirnov test in both smokers and non-smokers. According to the Shapiro-Wilk test, the non-smoker's data was not normally distributed ( $p=0.003$ ), but by observation of the data histograms and box plots, this was deemed to be due to two outliers (less than 1% of data) with extremely high levels of vitamin D, while the bulk of the data conformed well to the normal distribution. We are aware, that the sample sizes differ between smokers and non-smokers, but homogeneity of these groups was verified by Levene's statistical test and no heterogeneity of variance was discovered. In the subanalysis by season, the non-equal variances probability value was reported when the Levene's test of homogeneity of variance indicated departure from equal variances. Statistical analyses were carried out in SPSS vs. 24 (IBM).

## Results

We have analysed the levels of vitamin D during first trimester of healthy pregnancies in a Finnish population. The clinical characteristics of the study population are described in Table 1.

The mean vitamin D levels in first trimester are shown in Figure 1. The levels of vitamin D are significantly lower (p-value < 0.001) in smokers (mean = 32.1, SD = 14.6) than in non-smokers (mean = 41.6, SD = 19.5). Figure 1 is in corroboration of previously published data indicating the decreasing effect of smoking on vitamin D levels <sup>(6)</sup>.

The main findings were that the 25(OH)D levels of smokers in winter are 74% of the 25(OH)D levels of non-smokers and 77% in summer. Figure 2 shows that the relationship between smoking and vitamin D is more pronounced in the winter months when exposure to sun is extremely low. This association is observed during summer months (P-value = 0.028) and winter months (P-value < 0.001). Within the groups, the 25(OH)D difference between summer and winter seasons were 41% among smoking and 38% among non-smoking pregnant women.

## Discussion

We have studied 25(OH)D levels in first trimester of pregnancy in a Finnish population according to the season of sampling. Among the smokers, only four women (14%) in winter and nine women (53%) in summer had 25(OH)D of 40 nmol/L or more in comparison to 55 women (31%) in winter and 106 women (78%) in summer among non-smokers. The recommended reference level for 25(OH)D in Finland is 40-80 nmol/L. Vitamin D deficiency is common in Finland particularly during the winter months <sup>(19)</sup>. In contrast, the US National Health and Nutrition Examination Survey (NHANES) reports that 7% are deficient at <30 nmol/L and 72% of American pregnant and lactating women have 25(OH)D levels of 50 nmol/L or more <sup>(20)</sup>.

25(OH)D with a half-life of 14-20 d is a long-lived plasma metabolite and has been considered a stable and reliable marker of vitamin D status <sup>(15)</sup>. Although measurement is technically challenging, the evidence on the relative difference between non-smoking and smoking pregnant women is in the order of at least 25% independently of seasonal variability (p-value <0.001). The selection of normal pregnancies to this cohort excludes the bias brought about by pregnancy complications that may be associated with alterations in 25(OH)D concentrations. On the other hand, the proportion of individuals that regularly uses vitamin D supplements as part of their diet is

not known in the registry data used in this study <sup>(21)</sup>. While ethnicity of the study population is not recorded, the study population is considered homogenous as a representation of the fair Finnish general population.

Although, the exact mechanisms are not known how tobacco results in the adverse effects on pregnancy, the association between tobacco and increased risk of foetal mortality has been recognized for decades <sup>(3)</sup>. We do not know the exact mechanisms behind smoking and vitamin D deficiency, and even less is known how the exacerbated effect of smoking on 25(OH)D levels is mediated during pregnancy. In this registry based study, detailed and verified information on possible confounding factors were not available. However, our data supports, that in a susceptible woman, smoking during pregnancy may be a particular risk for pregnancy complications during winter months, because this group has the lowest vitamin D levels and therefore a highest risk of pregnancy complications. Further studies are needed to decipher the interaction between PTH, vitamin D, and maternal smoking in relation to neonatal outcomes. There is no evidence of smoking affecting individual PTH levels <sup>(22)</sup>. Furthermore, unlike 25(OH)D, PTH does not cross the placenta <sup>(8)</sup>. Therefore, the possible adverse consequences of smoking during pregnancy in relation to calcium homeostasis are likely related to the exaggerated deficiency of vitamin D among the smoking mothers as observed in our data. Furthermore, these effects may be interrelated and may offer new options for early preventive measures.

### Conclusion

The present study shows that smoking during pregnancy is associated with a 27 % decline in 25(OH)D levels in first trimester. Our results also indicate that seasonal variation in 25(OH)D levels is similar among smoking and non-smoking pregnant women in their first trimester of pregnancy. Taken together, these changes result in very low 25(OH)D levels among pregnant smokers especially in wintertime, since most of the smoking women are below the recommended reference limits. To what extent the compound effect of smoking and low sun exposure directs maternal and foetal outcome and later development and immunology of the child are still open questions. In addition, our study does not address whether being below the target area is more dangerous in early versus late pregnancy. This cohort needs to be expanded to address these issues in the future.



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Table 1. Clinical characteristics of smoking and non-smoking pregnancies.

	non-smokers (N=313)	smokers (N=46)	p-value *
	mean (SD)		
Maternal age (y)	27.56 (3.80)	23.63 (3.74)	<0.001
BMI (N=111 vs N=8)	24.08	24.42	NS
Primiparity (%)	2.24	34.78	<0.001 †
Gestational age at delivery (days)	280.80 (7.86)	279.47 (9.31)	NS
Birth weight (kg)	3677.76 (484.01)	3471.85 (406.84)	0.001
Birth height (cm)	50.70 (1.88)	50.06 (1.66)	0.001
Male gender (%)	50.80	36.96	NS
Month of sampling ‡	6.48 (3.48)	5.20 (3.44)	NS
Year of sampling	2000 (5.83)	2000 (6.14)	NS

NS not significant, SD standard deviation, BMI body mass index

\*  $p < 0.05$  is considered statistically significant

† Mann-Whitney U test, t-test if not indicated

‡ 1=January – 12=December

## Figure legends

Figure 1. Women who smoke (N=46) during pregnancy have significantly lower vitamin D levels in serum than non-smoking (N=313) women.

Figure 2. Differences between smokers and non-smokers in vitamin D levels are particularly pronounced during the winter months (November to May), when sun light is limited in Finland. Smokers, winter N=29; non-smokers, winter N=175; smokers, summer N=17; non-smokers, summer N=136.

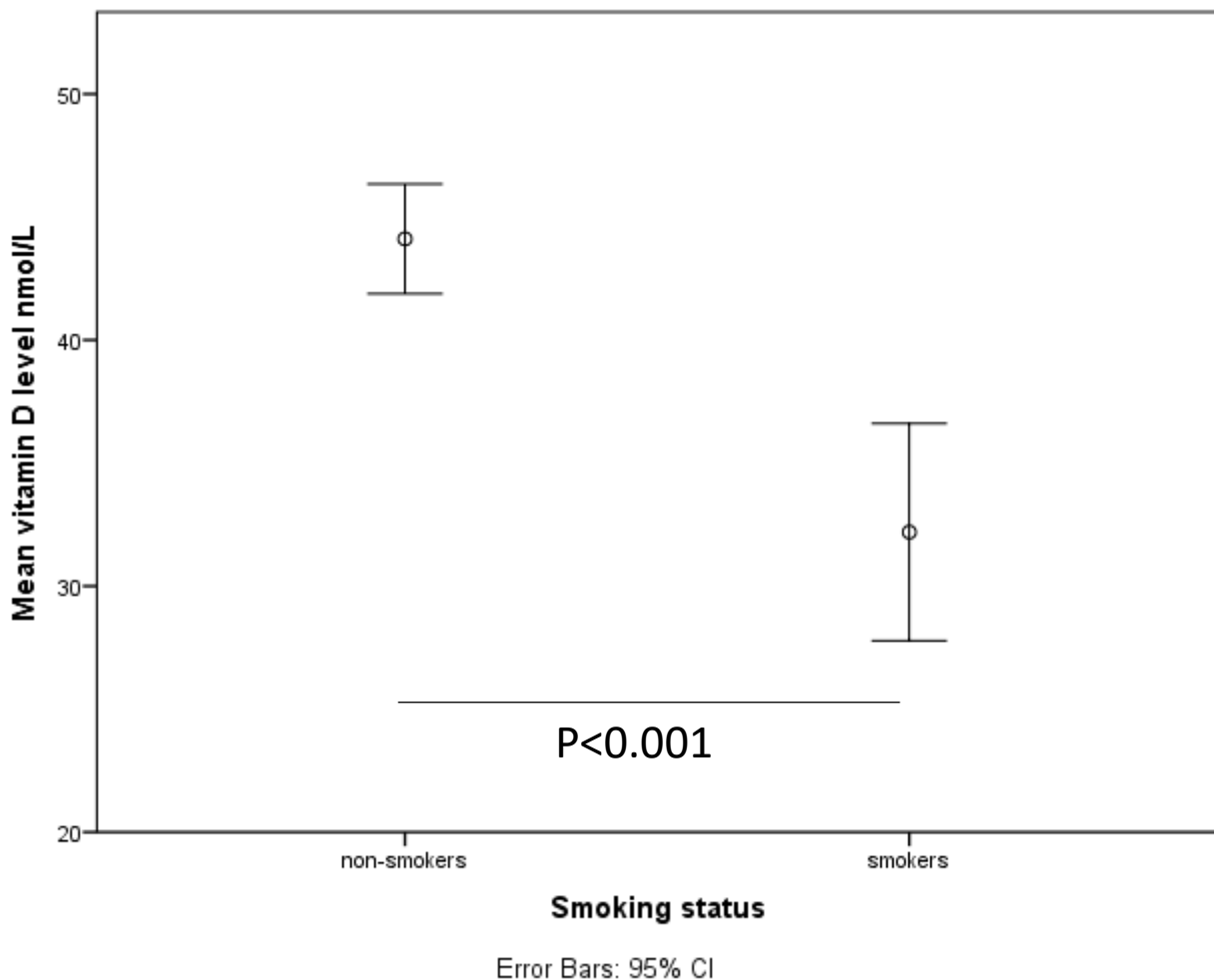


Figure 1.

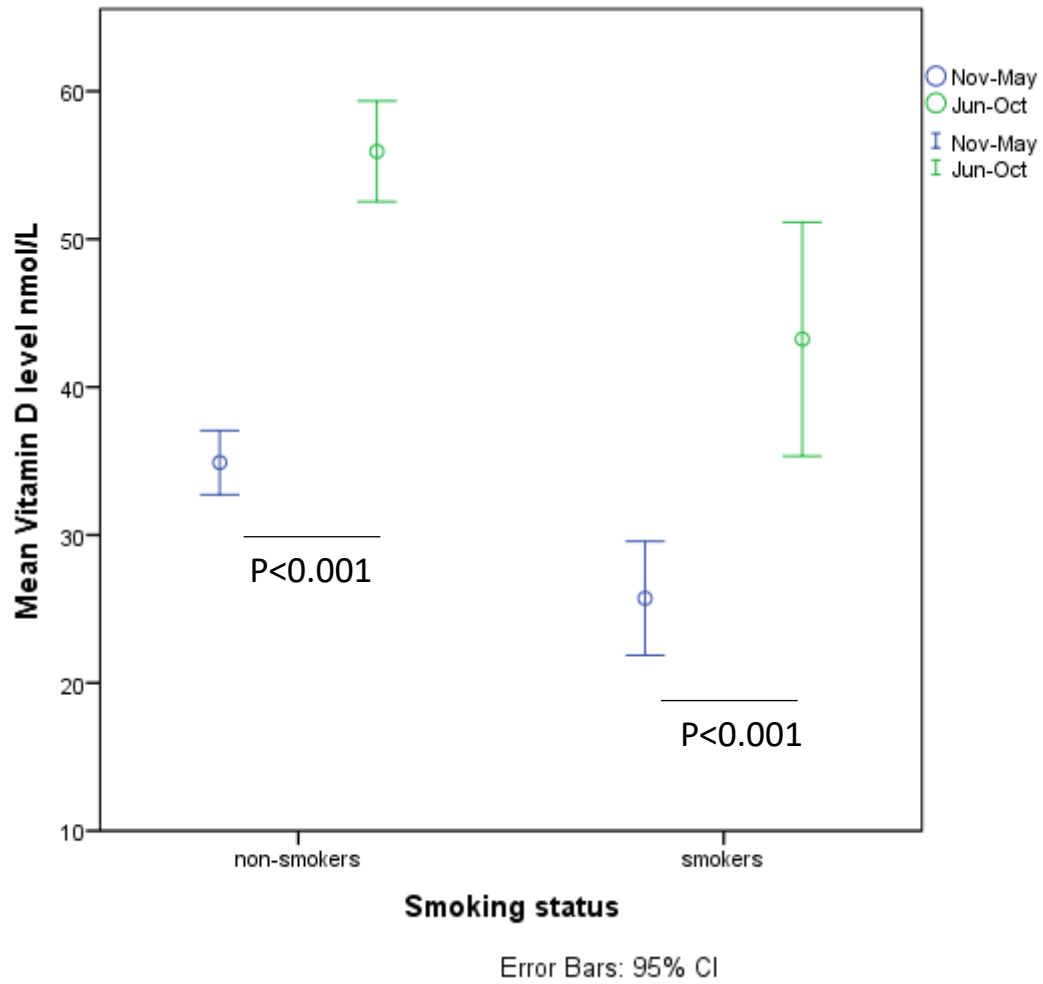


Figure 2.