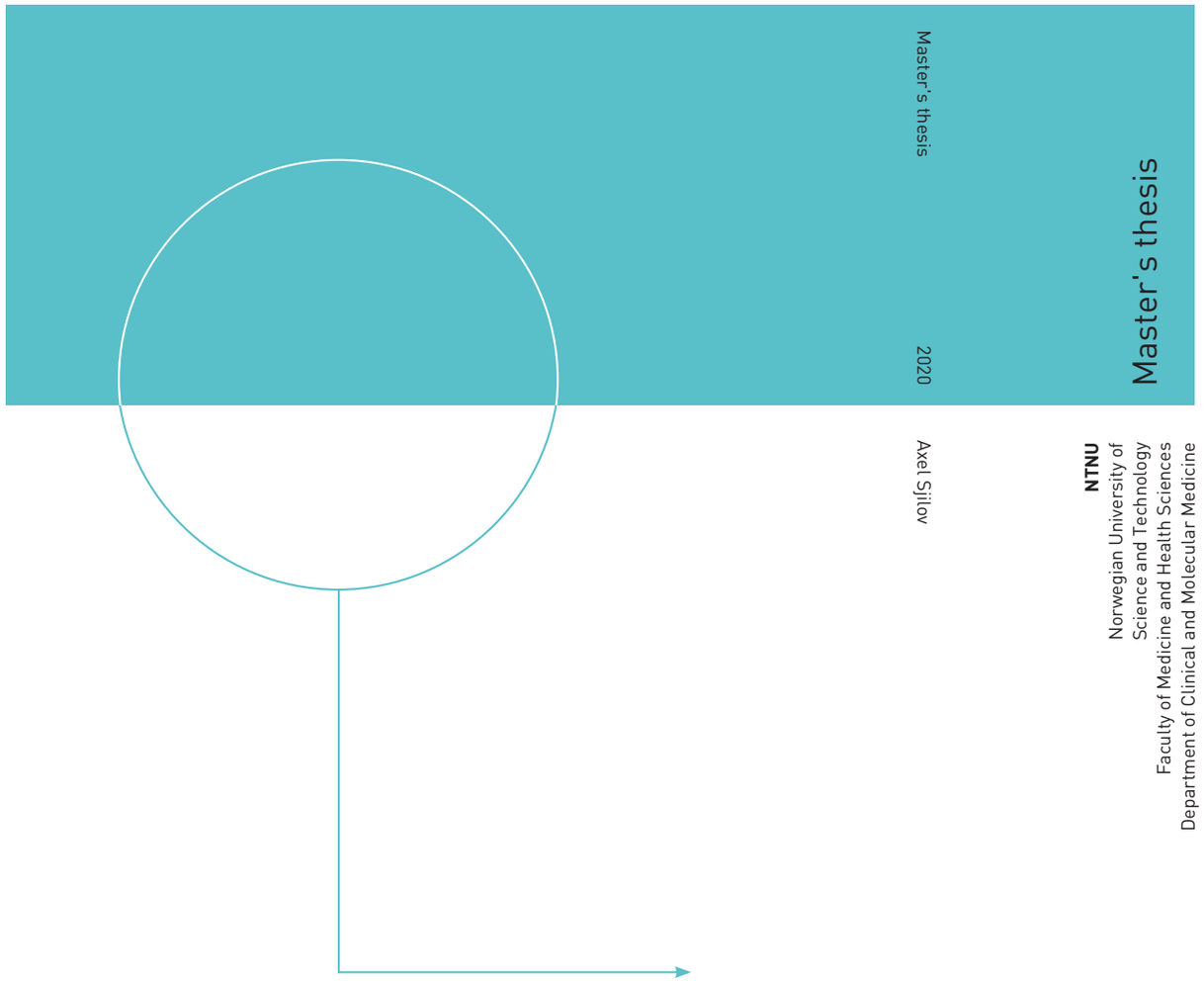


Axel Sjilov

Should we be concerned about the vitamin A status among Norwegian pregnant women?

August 2020

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of
Science and Technology

Should we be concerned about the vitamin A status among Norwegian pregnant women?

Axel Sjilov

Medisin - Profesjonsstudium
Submission date: August 2020
Supervisor: Unni Syversen
Co-supervisor: Miriam K. Gustafsson

Norwegian University of Science and Technology
Department of Clinical and Molecular Medicine

Should we be concerned about the vitamin A status among Norwegian pregnant women?

Supervisor: Professor, Unni Syversen (IKOM, NTNU and Department of Endocrinology, Trondheim University Hospital (St. Olavs hospital)

Co-supervisor: Associate professor, Miriam K. Gustafsson (ISM, NTNU and Division of Mental Health Care, Trondheim University Hospital (St. Olavs hospital)

Medical student: Axel Sjilov (NTNU)

Abstract

Background

Vitamin A deficiency affects approximately 19 million pregnant women worldwide, especially in developing countries. The vitamin is obtained from the diet and supplements. It is important for a wide range of functions such as immunity, growth and vision. Moreover, it is crucial for skeletal development during fetal life. Notably, both low and high levels may be harmful for the skeleton.

The aim of this study was to assess vitamin A status among Norwegian, pregnant women.

Methods

This is a secondary analysis of a randomized controlled trial which examined the impact of antenatal exercise on gestational diabetes. Altogether 855 healthy, fair-skinned Norwegian women from the cities of Trondheim and Stavanger participated. Blood samples were collected during second and third trimester (pregnancy week 18-22 and 32-36), as well as data on pregnancy outcomes and BMI. Relevant background information was obtained from questionnaires, and data concerning labour from the birth registry. We retrieved data on

vitamin D levels that were analyzed previously. Vitamin A (retinoic acid) was measured by high performance liquid chromatography (HPLC) and 25(OH)D by electrochemiluminescence immunoassay (ECLIA), at Trondheim University Hospital.

Findings

Mean vitamin A levels were 1.35 and 1.09 µmol in second and third trimester, respectively. Participants from Trondheim had on average 0.10 µmol/L higher serum retinol levels than those from Stavanger. Vitamin A inadequacy (serum retinol ≤ 1.05 µmol/L) was observed in 9.3 % (n=79) and 44.9 % (n=325) of the women in second and third trimester, respectively. In third trimester, 2.0 % (n=14) had vitamin A deficiency (< 0.70 µmol/L). Vitamin A and D was negatively correlated with birthweight.

Conclusions

A large proportion of the pregnant women had inadequate vitamin A status by the third trimester. Vitamin A levels correlated negatively with birth weight. Given the important effects of vitamin A for fetal development and future health of the child, the high occurrence of inadequacy is of concern.

Background

Maternal vitamin A deficiency – A public health problem

Vitamin A deficiency is a public health problem causing high morbidity and mortality in many countries, especially in children and pregnant women in low and middle income countries. During pregnancy there is a high nutritional demand, particularly in the third trimester due to accelerated fetal development. According to the WHO, vitamin A deficiency affects approximately 19 million pregnant women worldwide and is a public health issue at a population level [1-2].

Effects of vitamin A

Vitamin A is important for a wide range of functions, such as vision, reproduction, growth, immunity, maintenance of epithelial tissue, and regulation of cell proliferation and differentiation [3-11]. Both vitamin A deficiency and excessive intake can cause embryonic malformations [12]. Thus, it plays a role in the pathogenesis of a variety of diseases such as cardiovascular disease, type 2 diabetes, anemia, obesity, and osteoporosis. Vitamin A

deficiency during pregnancy disrupt proper stem cell differentiation and slows fetal growth and development [10, 13-14], and may increase the risk for future disease. In concordance, our research group observed a significant positive association between prenatal maternal retinol levels and offspring peak bone mass [15]. This may indicate that low maternal retinol levels increase the risk for future osteoporosis in the offspring [16-17].

Dietary sources

We obtain vitamin A from the diet either as preformed vitamin A (retinol) from animal sources or as provitamin A carotenoids, e.g. beta-carotene, from plants. Beta-carotene is cleaved in the intestinal mucosa by carotene dioxygenase, yielding retinaldehyde, which can be transferred to retinol [10]. Examples of food from animal sources containing vitamin A include liver, eggs, fish and milk (including breast milk). Plant-based sources include spinach, carrots, sweet potatoes, and yellow maize among others [18]. Vitamin A from animal sources can be toxic at a high dose, whereas beta-carotene does not cause vitamin A toxicity even at large doses since the body converts only what is needed. Retinol is stored in the liver. Some of the retinol that is absorbed in the intestine is delivered to tissues other than the liver by chylomicrons remnants. The second most important organ for clearance of chylomicron remnants is bone [10].

WHO recommendations

Vitamin A supplementation is only recommended for pregnant women in areas where vitamin A deficiency is a severe public health problem*, to prevent night blindness**.

* Vitamin A deficiency is a severe public health problem if ≥ 5% of women in a population have a history of night blindness in their most recent pregnancy in the previous 3–5 years that ended in a live birth, or if ≥ 20% of pregnant women have a serum retinol level <0.70 µmol/L. Determination of vitamin A deficiency as a public health problem involves estimating the prevalence of deficiency in a population by using specific biochemical and clinical indicators of vitamin A status [1].

In this study we aimed to examine vitamin A status in pregnant Norwegian fair-skinned women, associations of vitamin A levels and pregnancy outcomes and birth weight. We also

had access to previously collected data on vitamin D status in these women [19], which enabled us to study the association between vitamin A and D levels.

Materials and methods

Study design and population

This is a secondary analysis of data from a randomized controlled trial (RCT), where 855 pregnant Norwegian women from the cities of Trondheim (n=660) and Stavanger (n=195) participated between 2007 and 2009. The objective was to investigate the antenatal health effects of an exercise program, and the primary outcome was gestational diabetes mellitus Healthy fair-skinned women, 18 years and older, with a singleton live fetus were included. Exclusion criteria were high-risk pregnancies and diseases that could hinder participation in the exercise program. The two groups were homogenous at inclusion and after the intervention, and were merged in the current study [20].

Data collection

The participants were recruited consecutively, and clinical data and blood samples were collected in second and third trimester (pregnancy week 18-22 and 32-36). Body weight and height were measured at inclusion. Questionnaires regarding sociodemographic variables, diet and supplements, childbirths, medical history, smoking behavior and physical activity were completed. A self-administered optical mark readable Food Frequency Questionnaire (FFQ) containing around 180 food items was used to collect information [19].

Serum analyses

Blood samples were collected after fasting and sera were stored at - 80 °C. Analyses of all-trans retinoic acid (vitamin A) and 25-hydroxyvitamin *D* (25(OH)D) were conducted in 2015 at Department of Laboratory Medicine, St. Olavs hospital, Trondheim University Hospital. Vitamin A was analyzed by high performance liquid chromatography (HPLC) and 25(OH)D by electrochemiluminescence immunoassay (ECLIA).

Definition of vitamin A and D deficiency

Vitamin A inadequacy is defined as ≤ 1.05 µmol/L serum retinol. Vitamin A deficiency is defined as < 0.70 µmol/L serum retinol [21].

Vitamin D (serum 25(OH)D levels) <50 nmol/L are classified as insufficiency and 25(OH)D levels <30 nmol/L as deficiency (VDD) according to the US Institute of Medicine (IOM) and Nordic Nutrition recommendations [22-24].

Ethics

The study was conducted in accordance with the ethical principles in the declaration of Helsinki, approved by the Regional Committee for Medical and Health Research Ethics (REK 4.2007.81) and registered in the ClinicalTrials.gov (NCT 00476567)

Statistical analysis

Data were analyzed using SPSS statistics version 26.0 (Armonk, NY: IBM Corp). In general data are presented as the arithmetic mean ± SD or 95 % confidence intervals (CI). For comparison of serum parameters between second and third trimester and between the two cities, paired t-test was used as the data were normally distributed. When comparing serum retinol levels between Trondheim and Stavanger, an independent sample t-test in SPSS. Pearson's correlation was used to measure the strength of association between vitamin A and other variables. Significance was set at $P \leq 0.05$.

Results

Maternal characteristics

Table 1 shows the baseline demographic and clinical characteristics of the 855 pregnant women attending the study at inclusion during the second trimester (mean inclusion point was week 20.0 ± 1.7). Mean age at delivery was 30.5 ± 4.3 years. The women were well-educated. About 57 % (n=486) had no previous children, 30 % (n=254) had one former child, and 13 % (n=115) had two or more children. 1.1 % (n=9) reported smoking. Mean BMI (kg/m^2) at study inclusion (week 20.0 ± 1.7) was 24.8 ± 3.2. 32.9 % (n=281) of the participants were classified as overweight (BMI 25-29.99), and 7.4 % (n=63) were classified as obese (BMI ≥ 30). Only 0.2 % (n=2) were classified as underweight (BMI ≤ 18.5). 3.5 % (n=30) of the offspring had a birth weight ≤ 2.50 kg, classified as low birth weight, according to WHO criteria [25]. 17 % (n=149) had a birth weight ≥ 4.00 kg, which is categorized as fetal macrosomia [26].

Mean vitamin D intake was 10.4 ± 7.0 µg, of these 5.5 ± 6.5 µg came from supplements. More than half of the women 59.3 % (n=507) had a lower intake than the recommended 10 µg

per day, whereas 18.4 % (n=157) got more than 10 µg vitamin D from supplements. Daily fish intake was 54.8 ± 38.3 g, which is below the recommended intake of minimum 300 g weekly; 45.0 % (n=383) had a fish intake below [27-28]. Mean calcium intake was 974.8 ± 374.1 mg daily. About half of the women 47.1 % (n=401) had a lower daily intake than the recommended 900 mg. Data on vitamin A supplements were not available

Table 1. Baseline demographic and clinical characteristics of the study population†.

Maternal characteristics	Total (*n* = 855)	Trondheim 63° N (*n* = 660)	Stavanger 58° N (*n* = 195)
Age (years)	30.5 ± 4.3	30.4 ± 4.3	30.6 ± 4.5
Gestational length at inclusion (weeks)*	20.0 ± 1.7	20.0 ± 1.7	20.7 ± 1.5
Education level *n* (%)			
Elementary school	5 (0.6)	3 (0.5)	2 (1.0)
High School	90 (10.5)	64 (9.7)	26 (13.3)
University	760 (88.9)	593 (89.8)	167 (85.6)
Paid work or self-employed *n* (%)**	793 (92.9)	614 (93.2)	184 (94.4)
Parity *n* (%)			
0	486 (56.8)	374 (56.7)	112 (57.4)
1	254 (29.7)	199 (30.2)	55 (28.2)
2	90 (10.5)	68 (10.3)	22 (11.3)
3+	25 (2.9)	19 (2.9)	6 (3.1)
Smoking *n* (%)**	9 (1.1)	5 (0.8)	4 (2.1)
Inclusion body mass index (kg/m^2)**	24.8 ± 3.2	24.9 ± 3.3	24.7 ± 3.0
Blood pressure (mm Hg)			
Systolic	108.9 ± 8.6	108.9 ± 8.6	108.9 ± 8.5
Diastolic	68.7 ± 7.8	69.4 ± 7.7	66.5 ± 7.7
Gestational hypertension *n* (%)††	9 (1.1)	8 (1.2)	1 (0.5)
Gestational diabetes *n* (%)*** †††	5 (0.6)	5 (0.8)	0
Daily total vitD intake (µg)	10.4 ± 7.0	10.6 ± 7.1	9.8 ± 6.7
Daily total vitD intake < 10 µg *n* (%)	507 (59.3)	383 (58.0)	124 (63.6)
Daily vitD from supplements (µg)****	5.5 ± 6.5	5.7 ± 6.6	5.0 ± 6.4
Daily intake of ≥ 10 µg vitD from supplements *n* (%)****	157 (18.4)	124 (18.8)	33 (17.0)
Daily intake of fish (g)****	54.8 ± 38.3	54.4 ± 38.5	56.0 ± 37.4
Intake of fish < 300 g/week *n* (%)****	383 (45.0)	299 (45.4)	84 (43.3)
Daily intake of calcium (mg)****	974.8 ± 374.1	976.9 ± 373.3	967.7 ± 377.8
Daily calcium intake < 900 mg *n* (%)****	401 (47.1)	304 (46.2)	97 (50.0)
Exercised regularly pre-pregnancy *n* (%)	610 (71.3)	476 (72.1)	134 (68.7)

Continuous variables are given as means ± standard deviation (SD), and categorical variables are given as (*n*) with percentages (%).
The Norwegian authorities' recommendations for pregnant women are a daily vitD supplement intake of 10 µg, a weekly intake of 300-450 g fish and additionally 900 mg calcium per day.
†The inclusion appointment was between 18-22 weeks of pregnancy.
††Gestational hypertension is defined as systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, or both in women with no pregestational hypertension.
†††The criteria for gestational diabetes were fasting glucose level in whole blood ≥ 6.1 mmol/L, or plasma glucose ≥ 7.0 mmol/L, or 2-hour glucose level ≥ 7.8 mmol/L after oral glucose tolerance test in women with no pregestational diabetes.
*Ten women from Trondheim and two from Stavanger are missing.
**One woman from Trondheim is missing.
***14 women from Trondheim and five women from Stavanger are missing.
****One woman from Stavanger and two women from Trondheim are missing.
Abbreviation: vitD, vitamin D.
The data in table 1 is taken from https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0195041.t001.

See table 2 for vitamin D, calcium and fish intake during the third trimester. Daily total vitamin D intake was reduced by 0.1 µg from second to third trimester. 60.8 % (n=463) women had a lower daily intake than the recommended minimum of 10 µg. 5.6 ± 6.8 µg came one average from supplements, while 18.3 % (n=139) obtained recommended dosage of at least 10 µg vitamin D from supplements. During the third trimester daily intake of fish had declined from a mean of 54.8 ± 38.3 during second trimester to 49.1 ± 32.3 g. 51.5 % (n=390) women got less fish per week than the minimum recommended of at least 300 g. Daily intake of calcium was 960.6 ± 344.5 mg/day, and 45.9 % (n=348) participants were below the daily recommended of 900 mg.

Table 2. Vitamin D, calcium and fish intake in third trimester*.

Variables	Total population (*n* = 761)	Trondheim 63° N (*n* = 602)	Stavanger 58° N (*n* = 159)
Daily total vitD intake (µg)	10.3 ± 7.3	10.3 ± 7.4	10.4 ± 6.9
Daily total vitD intake < 10 µg *n* (%)	463 (60.8)	366 (60.8)	97 (61.0)
Daily vitD from supplements (µg)**	5.6 ± 6.8	5.6 ± 6.8	5.9 ± 6.7
Daily intake of ≥ 10 µg vitD from supplements *n* (%)**	139 (18.3)	108 (18.0)	31 (19.5)
Daily intake of fish (g)**	49.1 ± 32.3	49.5 ± 32.6	47.5 ± 30.9
Intake of fish < 300 g/week *n* (%)**	390 (51.5)	306 (51.1)	84 (52.8)
Daily intake of calcium (mg)**	960.6 ± 344.5	962.7 ± 352.1	952.9 ± 315.1
Daily intake of calcium < 900 mg *n* (%)**	348 (45.9)	276 (46.1)	72 (45.3)

Continuous variables are given as means ± standard deviation (SD) and categorical variables are given as numbers (*n*) with percentages (%). The Norwegian authorities' recommendations for pregnant women are a daily vitD supplement intake of 10 µg, a weekly intake of 300-450 g fish and additionally 900 mg calcium per day.
*The appointment was between 32-36 weeks of pregnancy.
**Three from Trondheim are missing.
Abbreviation: vitD, vitamin D.

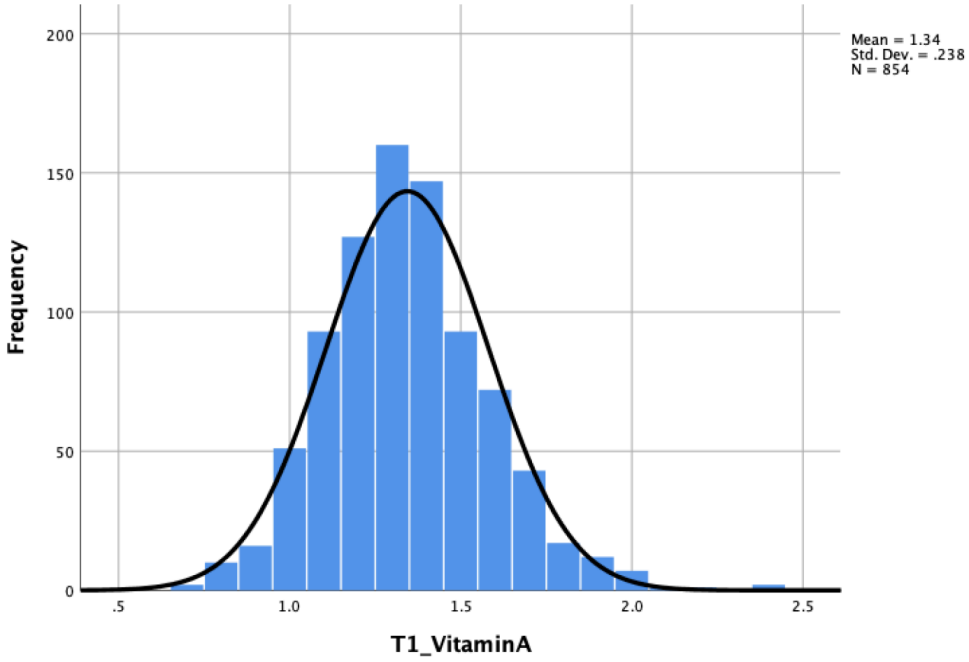
Vitamin A (serum retinol) measurements

Table 3 shows the prevalence of vitamin A inadequacy and proportion of women in different categories of vitamin A levels. Graph 1, 1.1, 2, 2.1 gives an overview of the normal distribution as well as q-q plots both trimesters, see also boxplots 1 and 2.
Mean serum retinol was 1.35 µmol/L± 0.238 in second trimester and 1.09 ± 0.245 µmol/L by the third trimester. No women were deficient at second trimester measurements, whereas 9.3 % (n=79) had vitamin A inadequacy. By the third trimester 2.0 % (n=14) of the women were deficient, and the prevalence of vitamin A inadequacy had risen to 44.9 % (n=325).

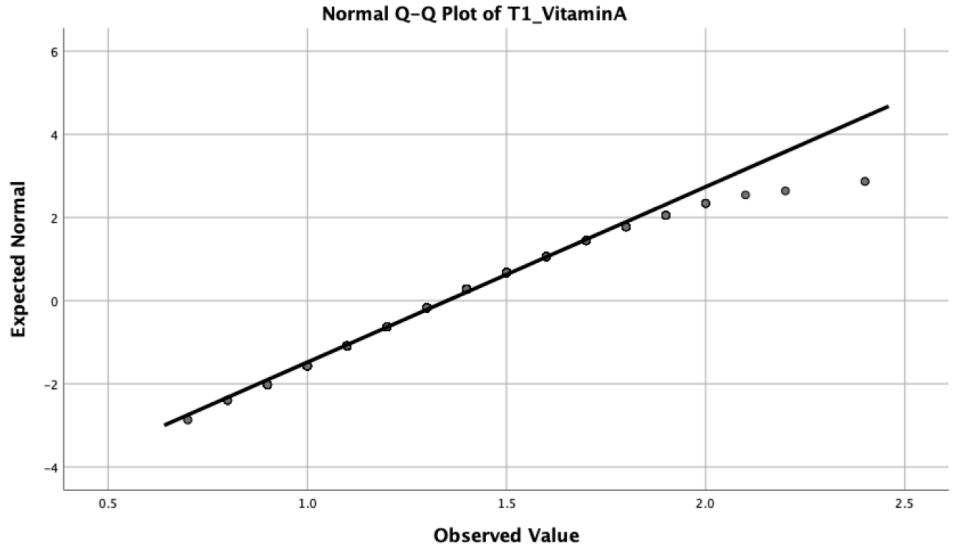
Table 3. Prevalence of vitamin A inadequacy and proportion of women in different categories of vitamin A levels.

Serum retinol (μmol/L)	**Second trimester** (n = **854**)	**Third trimester** (n = **723**)
Total mean serum retinol	1.35 μmol/L	1.09 μmol/L
< 0.7 n (%)	0 (0.0)	14 (0.02)
≤ 1.05 n (%)	79 (9.3)	325 (44.9)
1.05-1.49 n (%)	527 (61.7)	345 (47.7)
1.50-1.99 n (%)	237 (27.8)	50 (6.9)
≥ 2.00 n (%)	11 (1.3)	3 (0.4)

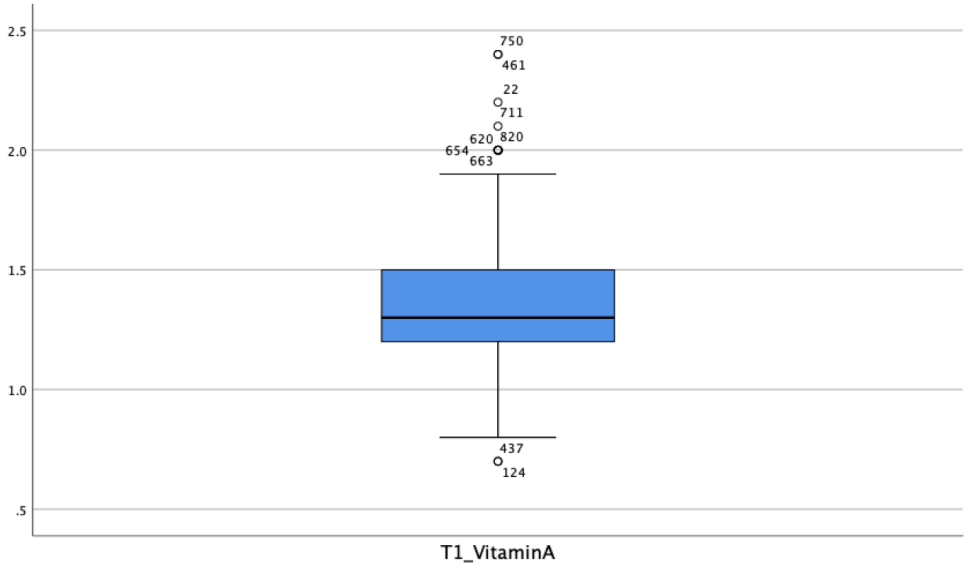
Graph 1: Vitamin A measurements second trimester



Graph 1.1: vitamin A meaurements second trimester

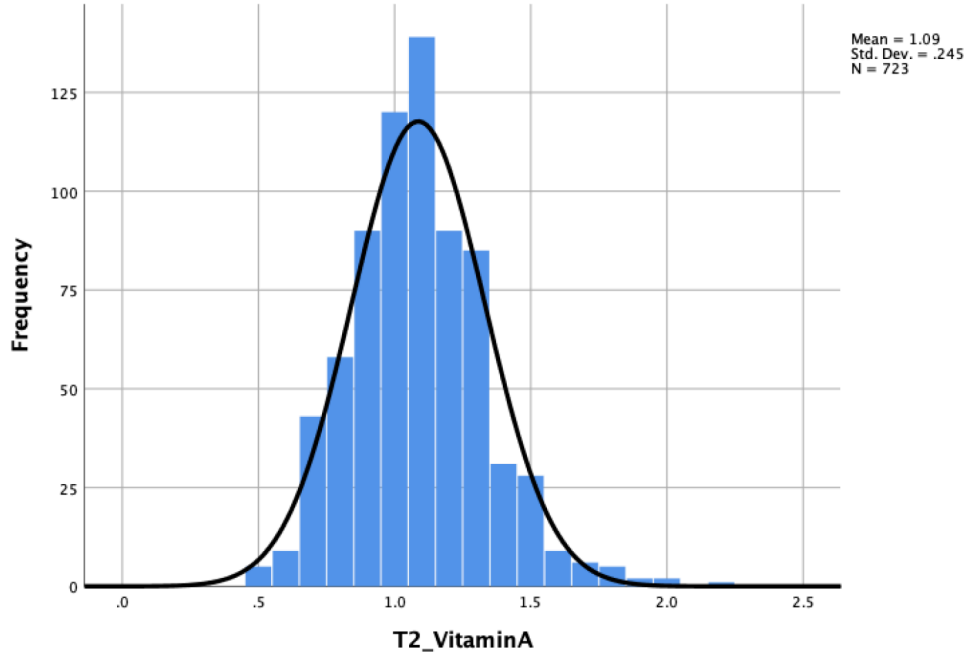


Box-plot 1: vitamin A measurements second trimester

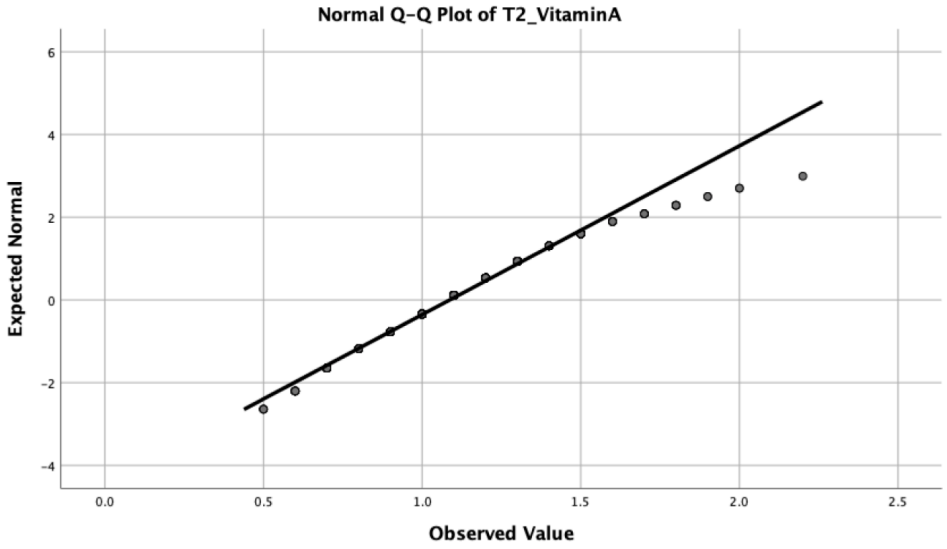


Box plot of vitamin A levels in all participants during second trimester. The median and 25th to 75th percentile are represented with the box and the entire data range between the whiskers.

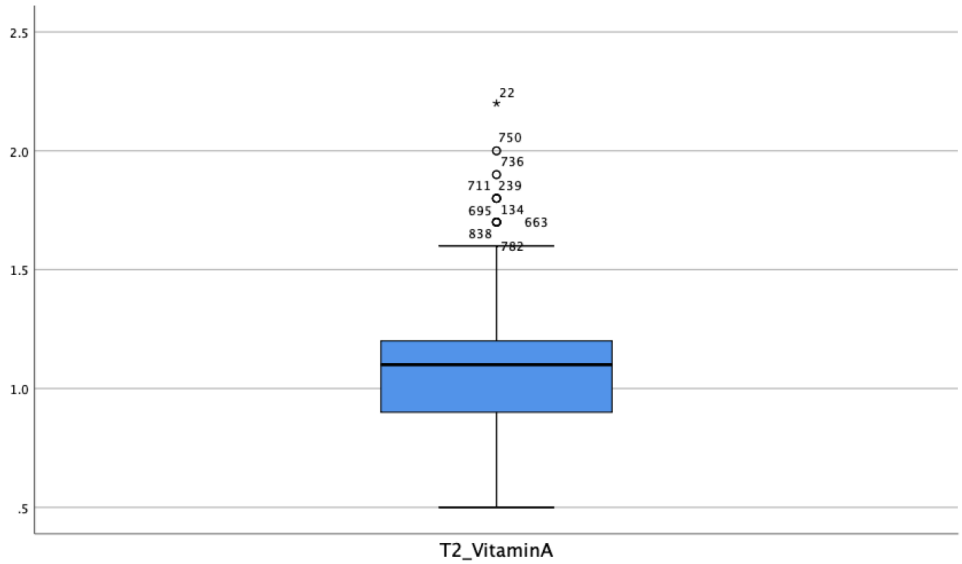
Graph 2: Vitamin A measurements third trimester



Graph 2.1: vitamin A meaurements third trimester



Box-plot 2: vitamin A measurements third trimester



Box plot of vitamin A levels in all participants during third trimester. The median and 25th to 75th percentile are represented with the box and the entire data range between the whiskers.

Differences in serum levels of vitamin A between participants from Trondheim and Stavanger

Mean serum retinol was significantly higher for women from Trondheim during second and third trimester (mean 1.24 vs 1.14 µmol/L, P=0.000014) See table 4.

Table 4. Mean serum retinol for second and third trimester comparing the study population of Trondheim and Stavanger.

Trimester	Trondheim	Stavanger	All*
Second	1.37 µmol/L	1.26 µmol/L	1.35 µmol/L
Third	1.11 µmol/L	1.01 µmol/L	1.09 µmol/L
Both trimesters	1.24 µmol/L	1.14 µmol/L	1.22 µmol/L

*Mean for all study participants

Regular exercise pre- and during pregnancy and vitamin A levels

During the second trimester, there was no difference in mean vitamin A levels in the women who had exercised regularly pre-pregnancy and the ones who did not. The same applied to women who exercised regularly during pregnancy and woman not exercising regularly during pregnancy.

Vitamin A and BMI

Table 5 shows mean serum retinol levels during second and third trimester for different categories of BMI. BMI correlated positively with vitamin A levels in second trimester (r=0.117, P= 0.001), but not with vitamin A levels in third trimester (r=0.026, P=0.488).

Table 5. Mean serum level of retinol according to categories of BMI

BMI second trimester	Second trimester (µmol/L)	Third trimester (µmol/L)
≤ 18.5 (*n* = 2 (0.2 %))	1.3	1.0
18.5 - 24.99 (*n* = 508 (59.4 %))	1.33	1.09
25.0 - 29.99 (*n* = 281 (32.9 %))	1.37	1.07
≥ 30.0 (*n* = 63 (7.4 %))	1.37	1.12

*Mean serum retinol value among all BMI-categories

Relation between levels of vitamin A (retinol) and D (25(OH)D)

Table 6 shows number of women in different ranges of vitamin A status and their mean levels of vitamin D. When excluding participants with vitamin A levels ≥ 2.00 µmol/L, we observed no correlation between vitamin A and D neither in second (r=0.067, P=0.53) nor third trimester (r=0.062, P=0.095).

Table 6. Relation between levels of vitamin A (retinol) and D (25(OH)D)

Vitamin A (serum retinol in µmol/L) second trimester (n=854)	**25 (OH)D (nmol/L) second trimester (n=854)**
≤ 1.05 (*n* = 79)	63.4
1.05-1.49 (*n* = 527)	65.7
1.50-1.99 (*n* = 237)	68.0
≥ 2.00 (*n* = 11)	60.5
Vitamin A (serum retinol in µmol/L) third trimester (*n* = 723)	**25 (OH)D (nmol/L) third trimester (*n* = 723)**
≤ 1.05 (*n* = 325)	63.1
1.05-1.49 (*n* = 345)	63.8
1.50-1.99 (*n* = 50)	76.4
≥ 2.00 (*n* = 3)	46.0

Birth weight and corresponding vitamin A and D levels.

Birth weight was negatively correlated with both vitamin A and D levels in third trimester (r=-0.176, P ≤ 0.001 and r=-0.109, P= 0.003 respectively). Table 7.1-2 shows birth weight and corresponding vitamin A and D levels.

Table 7.1. Birth weight and corresponding vitamin A levels

Birth weight	**Serum retinol second trimester**	**Serum retinol third trimester**	**Mean serum retinol both trimesters**
< 2.50 kg (*n* = 30)	1.34 µmol/L	1.21 µmol/L	1.28 µmol/L
2.50-2.99 kg (*n* = 79)	1.37 µmol/L	1.14 µmol/L	1.26 µmol/L
3.00-3.49 kg (*n* = 290)	1.35 µmol/L	1.13 µmol/L	1.24 µmol/L
3.50-3.99 kg (*n* = 304)	1.34 µmol/L	1.05 µmol/L	1.20 µmol/L
≥ 4.00 kg (*n* = 149)	1.32 µmol/L	1.03 µmol/L	1.18 µmol/L

Table 7.2. Birth weight and corresponding vitamin D levels

Birth weight	**Vitamin D second trimester**	**Vitamin D third trimester**	**Mean vitamin D both trimesters**
< 2.50 kg (*n* = 30)	71.2 nmol/L	79.9 nmol/L	75.6 nmol/L
2.50-2.99 kg (*n* = 79)	73.2 nmol/L	73.4 nmol/L	73.3 nmol/L
3.00-3.49 kg (*n* = 290)	65.7 nmol/L	64.0 nmol/L	64.9 nmol/L
3.50-3.99 kg (*n* = 304)	64.9 nmol/L	62.3 nmol/L	63.6 nmol/L
≥ 4.00 kg (*n* = 149)	63.8 nmol/L	61.8 nmol/L	62.8 nmol/L

Discussion

This is one of the largest longitudinal studies to address vitamin A status in pregnant women. We have previously investigated antenatal vitamin D status in the same study population, and found that a high proportion of these well-educated, healthy women were vitamin D insufficient 34 % (n=246) and deficient 7 % (n=50) by the third trimester [19]. Now we show that 45% (n=325) of the women also exhibited vitamin A inadequacy during the third trimester, and 2.0 % (n=14) deficiency. Average serum retinol level throughout second and third trimester was 1.22 µmol/L Mean serum retinol dropped by 0.26 µmol/L from second to third trimester, respectively from 1.35 µmol/L to 1.09 µmol/L. Moreover, we found that 15.5% (n=112) of the women displayed both vitamin A inadequacy/deficiency and vitamin D insufficiency in the third trimester. Finally, both vitamin A and D levels were negatively correlated with birth weight.

Our findings are in line with a study among Turkish women (n=427) where 45.5% had vitamin A inadequacy during pregnancy. A higher percentage of the women exhibited deficiency, 16.9% versus 1.9% in our study [29]. The prevalence of vitamin A deficiency seems to be highest in developing countries. In an Iranian study, 24.6% of a sample of 3270 pregnant women exhibited vitamin A deficiency; 18.5% of 200 in Bangladesh; 15.8% of 101 in Nigeria; 13.8% of 738 in Guinea-Bissau; and 10.6% of 160 pregnant adolescents in Venezuela [30]. Vitamin A deficiency is also a serious public health issue in the Republic of the Congo and Brazil [30]. Similarly, a review concluded that there was a substantial number of pregnant women with vitamin A deficiency in developing areas of South Asia [30]. A review including studies conducted in Ethiopia, Kenya, Nigeria, and South Africa showed a prevalence of vitamin A deficiency by 21% to 48% among pregnant women [30].

We observed a substantial decline in retinol levels from second to third trimester. This concords with previous studies from both industrialized and developing countries. In a large study from 1954, a 9% decline in serum retinol values occurred between the first and third trimester, whereas others have reported a reduction of about 14% in the serum retinol levels [31]. Among the participants in the present study mean levels dropped by 19% (0.26 µmol/L). Hemodilution and nutritional status are the two main factors contributing to this pattern of declining serum retinol during pregnancy: [32].

The findings of the present study are in contrast to a previous study by our research group including 41 pregnant women, where mean retinol levels through second and third trimester were substantially higher (1.66 μmol/L) [15]. This discrepancy may be attributed to alteration in the dietary intake of vitamin A between the time periods the studies were conducted. Notably, the vitamin A content in tran (cod liver oil) was reduced by 75% in 2001. Before 2002 a teaspoon (5 ml) of cod liver oil contained 1000 μg retinol. This is higher than the recommended dosage for women (600 – 700 μg), both among pregnant and non-pregnant [33-34]. After the reduction of retinol content, 5 ml tran now contains only 250 μg of vitamin A. According to The Norwegian Directorate of Health, the mean procurement of fish per day per person in 1986-88 was 41 g, and in 2007-09, 37 g per day [35]. These factors could partly explain the lower vitamin A levels in the present study.

Interestingly, women from Trondheim exhibited a significantly higher retinol level than those from Stavanger (0.10 μmol/L). We have previously reported that 51.5 % (n=390) of the pregnant women ate less fish than recommended during the third trimester ref. Fish is a source of both vitamin A and D, and low intake will contribute to hypovitaminosis. On average the study participants from the two cities had a similar daily intake of fish. The study participants from Trondheim had a slightly higher intake of vitamin D through supplementation, which could indicate that they might also get more vitamin A through supplementation as well. Unfortunately, we did not have data specifically on vitamin A supplementation.

The low fish intake during pregnancy could be attributed to that certain fish species, such as swordfish, king mackerel and bigeye tuna (found in sushi) have been reported to contain mercury [36]. Ingestion of mercury has been associated with developmental delays and brain damage in the fetus [37]. It is likely that this caution towards certain fish species contribute to that some pregnant women avoid eating fish all together.

We observed that vitamin A levels seemed to be positively related with vitamin D levels, possibly reflecting that many dietary sources contain both vitamin A and D. However, women who had vitamin A levels $\geq$ 2.00 μmol/L had the lowest mean vitamin D level. Only 11 women in the second and three women in third trimester exhibited these levels. Since both vitamin A and D use the same retinoid X receptor (RXR), higher levels of vitamin A can potentially interfere with the action of vitamin D [38].

There is yet no consensus on the optimal serum level of retinol, neither in the pregnant nor non-pregnant state. In a meta-analysis addressing the association between vitamin A and fracture risk, serum retinol levels between 1.99 and 2.31 μmol/L seemed to be the optimal range in the non-pregnant [39]. In the present study, mean serum retinol was 1.22 μmol/L throughout pregnancy, and only nine women had levels in the suggested optimal range during second trimester, and three women during the third trimester.

We observed a negative correlation between birth weight and both vitamin A and D levels in third trimester. The mothers whose offspring had low birth weight, had on average 0.20 μmol/L higher retinol level than those giving birth to babies ≥ 4.00 kg. This could reflect that larger fetuses are demanding more vitamin A, thus depleting the maternal storages to a greater extent than smaller fetuses. This concords with observations from a Chinese study, showing that mothers with larger offspring had lower levels of vitamin A in late pregnancy [40]. In contrast, a study from Israel reported that low maternal vitamin A levels were associated with lower birth weight [41]. Likewise, mean vitamin D level for women giving birth to offspring ≤ 2.50 kg was significantly higher than for those with offspring ≥ 4.00 kg.
A study of 596 pregnant fair-skinned women performed in the UK did not find any association between maternal vitamin D level and offspring's size [42]. On the other hand, a study from Iran showed that maternal vitamin D deficiency may increase the risk for lower birth weight. In the Iranian study almost half of the women were vitamin D deficient [43], versus only about one fifth in the UK study [42]. Maternal obesity is a known risk factor for macrosomia in the offspring [44]. This was not the case in our study population, as mean birth weight in offspring of obese women (BMI ≥ 30, n=63) was 3.7 kg, whereas mean BMI in women having offspring ≥ 4.00 kg was 25.6.
Maternal BMI at inclusion correlated positively with serum retinol levels in second trimester. A positive correlation between BMI (pre-pregnancy and at inclusion) and serum retinol has also been reported in pregnant adolescents [45]. These findings may reflect that retinoids are stored in adipose tissue and are converted to the active metabolite retinoic acid when needed. [46].

There is increasing evidence for developmental origins of disease. Our research group has previously shown a positive association between prenatal maternal retinol levels and offspring peak bone mass suggesting that low maternal levels may increase risk for future osteoporosis

[15]. Maternal vitamin A deficiency is also known to affect pulmonary development and promote airway hyper responsiveness. Accordingly, vitamin A deficiency in fetal life is associated with increased risk for asthma [47].

We observed a high prevalence of vitamin A inadequacy among these well-educated, healthy women during pregnancy. A substantial proportion of the women also exhibited vitamin D insufficiency. Given the many potential health consequences of hypovitaminosis A and D for mother and offspring, our findings are of concern. There is a need for increased attention with respect to vitamin A and D supplementation during pregnancy and also in the non-pregnant state. Recommendations concerning vitamin A and D intake should be updated.

The major strengths of the present study are the large number of participants, high follow-up rate, and blood sampling both in second and third trimester during pregnancy. Moreover, analyses were performed concurrently, applying the same instruments and procedures. The study sites were located in two cities, which enabled comparison of different geographical regions of Norway.

The participants were well-educated Caucasian women with low-risk pregnancies, which may affect the generalizability. [48]. Measurement of retinol levels in plasma is useful for assessing vitamin A inadequacy. However, assessment of marginal vitamin A status may be difficult because [14]. Moreover, data on intake of vitamin A were lacking.
Finally, several comparisons were made, thus increasing the probability for false positive findings. Hence, the results need to be interpreted with care.

Conclusion

In this large study we show that vitamin A inadequacy is prevalent among healthy Norwegian women in third trimester of pregnancy. 15.5 % (n=112) displayed both vitamin A inadequacy and vitamin D insufficiency. Birth weight was negatively correlated with maternal vitamin A and D levels. Our findings are of concern as they may affect the child's future health. The recommendations for vitamin A intake should be revised.

References

1. WHO. *Vitamin A supplementation during pregnancy. WHO e-Library of Evidence for Nutrition Actions (eLENA)*. (Last update 11. February 2019)
2. WHO. *Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency*. Geneva, World Health Organization, 2009: p. VII
3. Yang C, Chen J, Liu Z, Yun C, Piao J, Yang X. Prevalence and influence factors of vitamin A deficiency of Chinese pregnant women. Nutr J. 2016;15:12.
4. Chen H, Qian N, Yan L, Jiang H. Role of serum vitamin A and E in pregnancy. Exp Ther Med. 2018;16(6):5185-9.
5. Bastos Maia S, Costa Caminha MdF, Lins da Silva S, Rolland Souza AS, Carvalho Dos Santos C, Batista Filho M. The Prevalence of Vitamin A Deficiency and Associated Factors in Pregnant Women Receiving Prenatal Care at a Reference Maternity Hospital in Northeastern Brazil. Nutrients. 2018;10(9):1271.
6. Pozniakov SP. [Mechanism of action of vitamin A on cell differentiation and function]. Ontogenez. 1986;17(6):578-86.
7. Debreceni B, Debreceni L. Role of vitamins in cardiovascular health and disease. *Research Reports in Clinical Cardiology*. 2014;5:283-295
8. Valdés-Ramos R, Guadarrama-López AL, Martínez-Carrillo BE, Benítez-Arciniega AD. Vitamins and type 2 diabetes mellitus. Endocr Metab Immune Disord Drug Targets. 2015;15(1):54-63.
9. Jeyakumar SM, Vajreswari A. Vitamin A as a key regulator of obesity & its associated disorders: Evidences from an obese rat model. Indian J Med Res. 2015;141(3):275-84.
10. Conaway HH, Henning P, Lerner UH. Vitamin a metabolism, action, and role in skeletal homeostasis. Endocr Rev. 2013;34(6):766-97.
11. Thorne-Lyman AL, Fawzi WW. Vitamin A and carotenoids during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis. Paediatr Perinat Epidemiol. 2012;26 Suppl 1(0 1):36-54.
12. Nordic Council of Ministers, Nordic Council of Ministers *Nordic Nutrition Recommendations 2012*(2014),5(11):1 http://dx.doi.org/10.6027/Nord2014-002

13. See AW, Kaiser ME, White JC, Clagett-Dame M. A nutritional model of late embryonic vitamin A deficiency produces defects in organogenesis at a high penetrance and reveals new roles for the vitamin in skeletal development. Dev Biol. 2008;316(2):171-90.
14. https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/ [hentet 29. juli 2020].
15. Balasuriya CND, Larose TL, Mosti MP, Evensen KAI, Jacobsen GW, Thorsby PM, et al. Maternal serum retinol, 25(OH)D and 1,25(OH)2D concentrations during pregnancy and peak bone mass and trabecular bone score in adult offspring at 26-year follow-up. PLOS ONE. 2019;14(9):e0222712.
16. Feskanich, D., et al., Vitamin A intake and hip fractures among postmenopausal women. Jama, 2002. 287(1): p. 47-54.
17. Michaelsson, K., et al., Serum retinol levels and the risk of fracture. N Engl J Med, 2003. 348(4): p. 287- 94.
18. Gilbert C. What is vitamin A and why do we need it?. *Community Eye Health*. 2013;26(84):65.
19. Gustafsson MK, Romundstad PR, Stafne SN, Helvik AS, Stunes AK, et al. (2018) Alterations in the vitamin D endocrine system during pregnancy: A longitudinal study of 855 healthy Norwegian women. PLOS ONE 13(4): e0195041
20. Stafne SN, Salvesen K, Romundstad PR, Eggebø TM, Carlsen SM, Mørkved S. Regular exercise during pregnancy to prevent gestational diabetes: a randomized controlled trial. Obstet Gynecol. 2012;119(1):29-36.
21. LIMA ABMd, GARCÊZ LS, OLIVEIRA IKF, SANTOS MMd, DA PAZ SMRS, PAIVA AdA. Vitamin A deficiency and factors associated with retinol levels in public school students. Revista de Nutrição. 2017;30:605-14.
22. Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. Nutrition Bulletin / Bnf. 2014;39(4):322–50. PMC4288313. pmid:25635171
23. Nordic Nutrition Recommendations 2012: Integrating Nutrition and Physical Activity. 5th ed: Nordic Council of Ministers; 2014. p. 627.
24. Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D, Calcium. The National Academies Collection: Reports funded by National Institutes of Health. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Dietary Reference

Intakes for Calcium and Vitamin D. Washington (DC): National Academies Press (US) National Academy of Sciences.; 2011.

25. WHO. Global nutrition targets 2025: low birth weight policy brief (WHO/NMH/NHD/14.5). Geneva: World Health Organization; 2014.

26. Lu Y, Zhang J, Lu X, Xi W, Li Z. Secular trends of macrosomia in southeast China, 1994-2005. BMC Public Health. 2011;11:818.

27. https://nifes.hi.no/forskningstema/sjomat-og-helse/helseeffekter-ved-sjomatinntak/sjomat-og-graviditet/

28. https://www.matportalen.no/matvaregrupper/tema/fisk_og_skalldyr/

29. Meram I, Bozkurt AI, Kilincer S, Ozcirpici B, Ozgur S. Vitamin A and beta-carotene levels during pregnancy in Gaziantep, Turkey. Acta Medica (Hradec Kralove). 2004;47(3):189-93.

30. Bastos Maia S, Rolland Souza AS, Costa Caminha MF, Lins da Silva S, Callou Cruz R, Carvalho Dos Santos C, et al. Vitamin A and Pregnancy: A Narrative Review. Nutrients. 2019;11(3).

31. Food and Nutrition Bulletin, vol. 22, no. 3 © 2001, The United Nations University

32. https://journals.sagepub.com/doi/pdf/10.1177/156482650102200305

33. Forsmo S, Fjeldbo SK, Langhammer A. Childhood cod liver oil consumption and bone mineral density in a population-based cohort of peri- and postmeno- pausal women. The Nord-Trøndelag Health Study. Am J Epidemiol 2007; doi: 10.1093/aje/kwm320.

34. https://www.legemiddelhandboka.no/T15.1.10/Kosttilskudd_under_svangerskap [hentet 14. juli 2020].

35. Helsedirektoratet (The Norwegian Directorate of Health) «UTVIKLINGEN I NORSK KOSTHOLD 2019» Matforsyningsstatistikk og forbruksundersøkelser. Released 02/2020. Publication number IS-2880.

36. https://americanpregnancy.org/is-it-safe/mercury-levels-in-sushi/

37. McKean SJ, Bartell SM, Hansen RL, Barfod GH, Green PG, Hertz-Picciotto I. Prenatal mercury exposure, autism, and developmental delay, using pharmacokinetic combination of newborn blood concentrations and questionnaire data: a case control study. Environ Health. 2015;14:62.

38. Rohde CM, Manatt M, Clagett-Dame M, DeLuca HF. Vitamin A Antagonizes the Action of Vitamin D in Rats. The Journal of Nutrition. 1999;129(12):2246-50.

39. Wu AM, Huang CQ, Lin ZK, Tian NF, Ni WF, Wang XY, et al. The relationship between vitamin A and risk of fracture: meta-analysis of prospective studies. J Bone Miner Res. 2014;29(9):2032-9.

40. Chen H, Qian N, Yan L, Jiang H. Role of serum vitamin A and E in pregnancy. Exp Ther Med. 2018;16(6):5185-9.

41. Gazala E, Sarov B, Hershkovitz E, Edvardson S, Sklan D, Katz M, et al. Retinol concentration in maternal and cord serum: Its relation to birth weight in healthy mother-infant pairs. Early human development. 2003;71:19-28.

42. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, et al. Maternal vitamin D status during pregnancy and child outcomes. European Journal of Clinical Nutrition. 2008;62(1):68-77.

43. Khalessi N, Kalani M, Araghi M, Farahani Z. The Relationship between Maternal Vitamin D Deficiency and Low Birth Weight Neonates. J Family Reprod Health. 2015;9(3):113-7.

44. Zhang C, Hediger ML, Albert PS, Grewal J, Sciscione A, Grobman WA, et al. Association of Maternal Obesity With Longitudinal Ultrasonographic Measures of Fetal Growth: Findings From the NICHD Fetal Growth Studies–Singletons. JAMA Pediatrics. 2018;172(1):24-31.

45. Spíndola Garcêz L, de Sousa Paz Lima G, de Azevedo Paiva A, Maria Rebêlo Sampaio da Paz S, Lázaro Gomes EI, Nunes VS, et al. Serum Retinol Levels in Pregnant Adolescents and Their Relationship with Habitual Food Intake, Infection and Obstetric, Nutritional and Socioeconomic Variables. Nutrients. 2016;8(11):669.

46. Frey SK, Vogel S. Vitamin A metabolism and adipose tissue biology. Nutrients. 2011;3(1):27-39.

47. Checkley W, West KP, Wise RA, Wu L, LeClerq SC, Khatry S, et al. Supplementation with vitamin A early in life and subsequent risk of asthma. European Respiratory Journal. 2011;38(6):1310-9.

48. Andersen LF, Solvoll K, Johansson LR, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. American journal of epidemiology. 1999;150(1):75–87. Epub 1999/07/10. pmid:10400557.