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Vitamin D, muscle strength, prolonged labour, Caesarean sections and lifestyle

*Clinical and intervention studies in pregnant Somali
and Swedish women and new mothers*

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Abstract

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Long-term severe vitamin D deficiency may cause osteomalacia with muscle weakness, pain, soft bones, cramps and eventually death. In a pilot study, I found many Somali women to be vitamin D deficient and very weak. This raised my interest and resulted in the research questions regarding if and how this weakness was linked to vitamin D deficiency, if it could be treated with simple lifestyle advice and supplementation, and if pronounced deficiency could cause serious birth outcomes due to prolonged labour?

Study I showed that 90% of pregnant Somali women (n = 52) and new mothers from primary antenatal care suffered from vitamin D deficiency with pronounced muscular weakness and signs of skeletal degradation, compared with 10% of the Swedish women (n = 71). Handgrip strength was predicted by vitamin D levels.

Study II showed that vitamin D supplementation among those with insufficient levels at baseline reversed deficiency and skeletal degradation. Furthermore, increased strength in hands and legs was predicted by the amount of supplement intake.

Study III aimed to investigate the vitamin D levels and the birth outcomes of cesarean sections and assisted birth for prolonged labour. A directed acyclic graph was established to adjust for covariates. A causal effect of critically low (unmeasurable) vitamin D levels on the outcomes of caesarean sections, emergency caesarean sections and assisted birth for prolonged labour was found with the increased odds of four, nine and six times to one for the birth outcomes, respectively.

Lifestyle associated with vitamin D may concern many due to risk of low sun exposure, for example, when working long office hours, engaging in excessive computer gaming, using sunscreen, wearing veiled clothes, or when having special diets like vegan food.

It is important to monitor individuals in primary- and antenatal care with muscle weakness and risk factors for vitamin D deficiency, especially in the high-risk group of Somali pregnant women and new mothers.

Keywords: : caesarean section, dystocia, immigrant, muscle strength, obstetric labour complications, osteomalacia, physical performance, Somalia, vitamin D, vitamin D deficiency

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*To my wonderful family
for their support with this project*

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Kalliokoski P, Bergqvist Y, Löfvander M: Physical performance and 25-hydroxyvitamin D: a cross-sectional study of pregnant Swedish and Somali immigrant women and new mothers. *BMC Pregnancy Childbirth* 2013; 13:237.
- II Kalliokoski P, Rodhe N, Bergqvist Y, Löfvander M: Long-term adherence and effects on grip strength and upper leg performance of prescribed supplemental vitamin D in pregnant and recently pregnant women of Somali and Swedish birth with 25-hydroxyvitamin D deficiency: a before-and-after treatment study. *BMC Pregnancy Childbirth* 2016; 16:353.
- III Kalliokoski P, Nohlert E, Rodhe N, Löfvander M: Critically low vitamin D levels may cause emergency caesarean sections: a cohort study of Somali and Swedish women in antenatal primary care. (*Submitted*)
- IV Kalliokoski P, Widarsson M, Rodhe N, Löfvander M: Impact on Vitamin D related lifestyle in Somali women with severe deficiency following medical advice by doctor: A mixed method study of before and after treatment (*Manuscript*)

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Abbreviations

AB	Assisted Birth
AB-PL	Assisted Birth for Prolonged Labour
ALP	Alkaline Phosphatase
Ca	Calcium
CS	Caesarean Section
DAG	Directed Acyclic Graph
ECS	Emergency Caesarean Section
ECS-PL	Emergency Caesarean for Prolonged Labour
nmol/L	nanomol per litre
PL	Prolonged Labour
S-25(OH)D	Serum levels of 25-HydroxyVitamin D (25(OH)D or 25OHD)
VDR	Vitamin D Receptor
pmol/L	pikomol per Litre
PTH	ParaThyroid Hormone
UV-light	UltraViolet light

The beginning

The winter took a dark, cold grip on our town Borlänge in Dalecarlia in December 2009. At that time, Hamdi Sheeck, an assistant nurse from Somalia working at our primary health care centre, came to my office. “Paul, why am I so dizzy?”, she asked. She felt tired, weak and unwell. When she first started working at the primary health care centre the previous summer, she was a happy and healthy person.

I examined her blood pressure and took blood samples, including levels of haemoglobin, blood glucose and thyroid hormone, all of which were normal. I found no other obvious explanation for her symptoms. After a couple of days, it suddenly struck me: What if she had a vitamin D deficiency? It seemed unlikely, and the tentative association between vitamin D and dizziness was very vague; however, I had read that balance could be affected by vitamin D deficiency, and she had risk factors for vitamin D deficiency such as wearing veiled clothing and having strong skin pigmentation.

Her blood test showed a serum level of 25-hydroxyvitamin D (S-25(OH)D) of 21 nmol/L. Deficiency is considered when the level of S-25(OH)D is less than 25 nmol/L according to Swedish and British references compared to the reference level less than 50 nmol/L in the US.

When we talked about the symptoms and complaints with vitamin D deficiency, another Somali assistant nurse at our primary healthcare centre, Fawsiya Bisharo said, “then, I surely have a deficiency as well”. Sure enough, she had... Both assistant nurses were seemingly healthy, working for the primary health care centre as translators as well, and were now found to have vitamin D deficiency. When we talked about the possible consequences of vitamin D deficiency, such as weakness, pain and depression, Fawsiya said; “I know many Somali women that experience this”.

Now, when I had two young fellow staff members with vitamin D deficiency, there may be more? Not uncommon that immigrant women of southern origin in our catchment area complained about pain and looked very tired. They often walked in a certain way, where the upper body sank onto the hip while leaning on the stepping leg; moreover, they looked as though they were suffering and unhappy. It made me realise that vitamin D deficiency could be a tentative explanation for the complaints of many individuals.

I was advised to do a pilot study, and my manager gave me permission to take in ten patients with pain, weakness and depression. I had read that women were more prone to developing vitamin D deficiency. Since the Somali

women were very keen about dressing with veiled clothes, perhaps many of them were deficient?

Our two assistant nurses helped me from the outset to find Somali women with the above-mentioned complaints, and they were invited to come for blood tests. After ten tubes of blood tests, I had nine with S-25(OH)D deficiency. I now realised that the problem could be much larger than I ever expected!

I read the Swedish National Food Administration's recommendations on vitamin D in pregnancy and lactation and it struck me that it could be a serious problem. If the deficiency was widespread here in Borlänge, it may be common in Sweden and Scandinavia and perhaps in other parts of the world with little sun as well?

I was especially touched by the passages in the text on possible effects of vitamin D deficiency on the foetus. I decided I wanted to study if pregnant women had such low 25(OH)D levels, as one could suspect from the results in our pilot study, which incorporated a couple of women who were pregnant and who also had very low S-25(OH)D levels. If they had low levels, perhaps we would be able to help both mother and child by treating the mother?

Again, I received another good advice from my wise senior colleague Dr Lars Jerdén to contact Assistant Professor Monica Löfvander at Centre for Clinical Research in Falun. She had extensive experience in working with immigrants in Stockholm, before moving to my neighbour town Falun at that time. I presented my findings of S-25(OH)D deficiency and my interest in studying the levels of S-25(OH)D among pregnant women from Somalia and Sweden as a comparison. Monica Löfvander immediately became interested since she had thought about similar things herself. She helped me immediately to write an application for funding for blood sampling in our first vitamin D study. Since then, she has been my scientific mentor.

Women from our pilot study kept dropping in since many Somali women seemed to be plagued by pain and tiredness. Soon I had results from 30 women, and 90% still had vitamin D deficiency.

With the help of my scientific mentor, we made pilot study protocols. With good help from the assistant nurses, Fawsiya and Hamdi, I could examine the thirty Somali women. While doing the pilot study, I asked the women to do one single squat, and to my consternation many women seemed to have very weak muscles. Many of them had to hold on to tables and chairs while arising and some even fell on the floor. They laughed and received help getting up from the floor. Several had a positive Trendelenburg's test.

One of the physiotherapists at the healthcare centre told me about the hand grip dynamometer named Grippit, which is a validated instrument for measuring grip strength. We suspected that doing the maximum repeated squats in a time interval, as in sit-to-stand test, would be problematic for pregnant women. We therefore had to adapt the leg tests for pregnancy. Measuring grip strength could become a good test of strength in pregnant women even if they were heavily pregnant.

With the results from the pilot study, we turned to the statistician Jan Ifver who helped us to do a power calculation for our first study. Thereafter, I applied to the ethical board in Uppsala to get approval. We wanted to study the vitamin D levels among pregnant and lactating mothers and to study possible associations with muscle weakness and pain, which I had observed in my clinic. I also wanted to study if vitamin D deficiency affected the pregnancy outcome.

I was spurred in my work to continue the studies since the Somali assistant nurses at my clinic reported a clearly positive response in their well-being, stating that they felt stronger after taking vitamin D supplements.

Introduction

The story up to recent days is a terrible but fantastic story of how lack of sunlight was found to be directly responsible for a disease causing abnormal bone structure in children, with severe growth retardation; spontaneous fractures in grown-ups; muscle weakness; cramps and eventually death following seizures [1, 2]. This happened in a time when men went to work in dark places and coal contaminated the air in the cities during industrialisation. Children had now started to play in the shaded narrow streets among high rise buildings in dirty cities or working under slave like conditions as in Charles Dickens' stories. Somewhere in this history, physicians observed and found that sunlight cured the disease; moreover, an oral cure was found, and scientists discovered a new factor in the blood coupled to this [3-5]. The names of the missing factor changed, and the treats were refined and rendered the Nobel Prize in chemistry in 1932 [6]. The factor was the fourth vitamin to be found and therefore named vitamin D.

Vitamin D and deficiency

Long-term deficiency of vitamin D may cause the disorders osteomalacia in adults and rachitis among children [7-9]. Predominant symptoms of these diseases are muscle weakness, aches and pain, disordered bone formation in children as well as fragile bones and risk of spontaneous fractures in adults [10]. The disordered bone formation may also cause a malformed pelvis, which can result in obstructed labour [11]. In very severe cases, such deficiency might lead to hypocalcaemia cramps, seizures and death [11, 12].

Deficient circulating vitamin D might negatively influence progress of birth and also perinatal health [8, 11, 13-15]. A study from the county of Dalecarlia found a higher complication rate among Somalian immigrant women during their pregnancy, delivery and for their newborns [16].

The uterus consists mainly of smooth muscles. The skeletal abdominal muscular system participates in the birth process. Both muscle types have receptors for vitamin D [17, 18].

Low exposure to sun on the skin might lead to deficient levels of vitamin D because UV-light (wavelength 300 nm) is necessary for converting cholesterol (7-dehydrocholesterol) to pre vitamin D in the skin, which is further con-

verted into vitamin D₃ (cholecalciferol) by body heat [19]. Vitamin D is further hydroxylated in the liver to 25(OH)D, which is the substance measured in blood. Serum-25(OH)D is a summation of both vitamin D produced by sun exposure and vitamin D intake in food and the most relevant substance to measure. Protective clothing, dark skin pigment and excessive use of sun protectives, long-term indoor living and low intake of vitamin D from food (for example, vegetarians) might lead to deficient levels of vitamin D [7, 19-21].

Fatty fish, egg yolk, dairy products and mushrooms contain minor amounts of vitamin D. Other alimentary products are those supplemented with vitamin D like margarine and milk [22]. Malabsorption from, for example, coeliac disease, burns, medication (epilepsia) and gastric bypass surgery are other risk factors for deficiency of vitamin D [23, 24].

Skin exposed to the sun on a usual sunny day will produce about 50 times more vitamin D in the skin than through regular daily intake of vitamin D in food. Thus, there is an annual circadian rhythm of the S-25(OH)D levels, with a minimum in late winter/beginning of spring, March to April, and a maximum during the late summer months and a decrease during the autumn months [25]. The value during winter may be half of that seen in summer [26]. A holiday in a sunny climate might increase the S-25(OH)D considerably [27]. In historical times, we saw the death rates increase during spring-winter when weak children with curvy legs would die from cramps affecting their airways.

Circulating vitamin D (S-25(OH)D) has a half-life of two to three weeks [7]. A steady state level of S-25(OH)D will be reached by a period of supplement medication during four months [28]. Also, lifestyle changes can improve the levels of vitamin D. The levels will sink to previous levels when ceased to be followed.

The vitamin is transported from the skin to the liver and hydroxylated to 25-hydroxyvitamin D (25(OH)D) and transported to the kidneys, brain, heart and skeletal muscular cells and smooth muscle cells in organs like intestines, blood vessels and the uterus as well as to immune cells [2]. Existing 25(OH)D in serum is measured as S-25(OH)D in nmol/L, a thousand-fold more than the last effective stage of vitamin D, the hormone calcitriol (1,25(OH)₂D) measured in pmol/L, which is prepared in the kidneys and cells in the target organs by a further enzymatic hydroxylation step. Calcitriol has a half-life of only four to six hours and does not give a good picture of the vitamin D status in the body [2]. Calcitriol effectuates via genes in DNA.

There are enzyme systems that limit excessive levels of vitamin D. These are localised in both the skin and intracellularly in the target organs. Serum 25(OH)D has a direct pathway via enzyme systems on the target organ level, such as muscle, with genetic effects via vitamin D receptors (VDR) and also epigenetic effects [29-31]. This pathway may be called a direct pathway due to a direct transport through the circulation from its hydroxylation in the liver to its target cells, where it is hydroxylated to Calcitriol. Others have also called

it an alternative pathway since it does not respond only to the circulating Calcitriol produced by the kidneys (which has been called the Classic pathway due to the history of knowledge on calcitriol) [32]. The direct effect on muscle cells has been claimed to be limited to development stages, whilst others have found direct effects of VDR on cell respiration and adenosine tri-phosphate (ATP) production from its effects on mitochondria [33-36]. Thousands of gene types are influenced by vitamin D [37]. The most well-known and important effect of vitamin D is the increase of body calcium by increased intestinal absorption of calcium and reabsorption of calcium in the distal tubule of the kidneys [2]. Calcium is vital for all types of muscle cells and for bone strength [38] and needs to be accurately regulated for normal cellular function. Deficient levels affect the brain, heart and airways and may result in hypocalcaemic seizures, arrhythmias and cramps in muscles and air ways [39-41].

Vitamin D and other diseases and causes of death

There are many other diseases than the concern of bone health and bone diseases like rickets and osteomalacia, which have been associated with vitamin D deficiency [42, 43]. Thus, more studies about causality and treatment are needed. Important large cohort studies show an association between levels of S-25(OH)D and risks of dying from different cancers and coronary heart disease but also that an active sun bathing behaviour diminishes the risk of developing diabetes later in life and seems to give a longer life [44-46].

Cohort studies like the Uppsala cohort and a cohort in Copenhagen show that S-25(OH)D between 50-90 nmol/L seems to be optimal [44, 45, 47].

Currently, there is plenty of ongoing research trying to determine what is sufficient, normal and optimal levels of S-25(OH)D, and the scientific debate is sometimes rather “loud”. Conclusive evidence, in randomised controlled studies (RCTs), for vitamin D deficiency causing many so called “soft tissue” diseases (contrary to hard tissue, i.e. bone) is still lacking despite extensive studies showing associations between S-25(OH)D and a multitude of diseases [37]. The method for measuring S-25(OH)D differs, genetics of the vitamin D receptor differ, the hydroxylases and the transport protein vitamin D binding protein (DBP) differ, which also makes it difficult to interpret the results [48]. Another problem is that many RCTs may potentially have studied samples with rather normal vitamin D levels. Due to the circumstances of evidence, we find differences in recommendations.

Recommendations for vitamin D levels

Recommendations for vitamin D levels to avoid deficiency are manifold. A German study on bone marrow biopsies suggested sufficient levels to be > 75

nmol/L [49]. Recommendations on S-25(OH)D levels in the UK and Denmark are based on musculo-skeletal health and suggest levels above 25 nmol/L, whereas American IOM (Institute Of Medicine) considers levels > 50 nmol/L to be sufficient [50]. The Nordic and European recommendations now suggest that levels beyond 50 nmol/L are sufficient, whereas < 25 nmol/L was considered clearly deficient with increased risk of osteomalacia and rickets [51, 52].

Vitamin D and muscle

Serum 25(OH)D impacts on enzyme systems in muscle cells [32, 53, 54]. Myopathy can therefore be a symptom of S-25(OH)D deficiency [55, 56]. Polymorphisms in the vitamin D receptor account for individual differences in muscular strength [57]. Anabolic effects of S-25(OH)D embrace both increased volume and better contraction and relaxation of muscles, while S-25(OH)D deficiency causes atrophy and weakness [58-60].

Muscles respond to mechanical loading and metabolic distress, and vitamin D plays a crucial role in muscle function, calcium absorption and in maintaining homeostasis [2-6]. To note, impaired muscle function may be present before the biochemical signs of bone disease appear [7, 8].

Supplements of vitamin D₃ (Cholecalciferol) for elderly persons as well as among younger people with S-25(OH)D deficiency (< 25 nmol/L) may improve their muscle strength [60].

Skin synthesis of vitamin D₃ decreases with age [54]. Focus of many studies have thus been elderly persons vitamin D status and lower extremity function [53, 61-66]. Little is known regarding hand grip strength or/and physical performance in pregnant women, or their S-25(OH)D status. One reason might be that pregnant and lactating women are often excluded from studies [67]. However, gestational diabetes, pre-eclampsia and birth complications have tentatively been linked to vitamin D insufficiency in US studies [14, 68-73].

Few or no studies have examined adherence to supplemental vitamin D medication in long-term and its effect on muscle strength in pregnant women, focusing on those wearing veiled clothing [9].

A historic case report commented on the waddling gait and weakness in patients with osteomalacia [74]. Also, I have observed waddling gait among my female patients of foreign birth, especially those coming from Somalia (unpublished data).

In Sweden, data regarding vitamin D status in pregnant women are scarce, and only few data on possible consequences of vitamin D deficiency. One Swedish study showed that pregnant Somali women often had severe vitamin D deficiency, but no clinical data were presented [75].

Severe vitamin D deficiency might impair global muscular strength also in younger women and interfere with birth and child care. Immigrant children

have a higher frequency of unintentional injuries according to Scandinavian studies [76-78].

Vitamin D and pregnancy

Low vitamin D levels can impact on labour and perinatal health [13, 14, 39, 68, 79, 80]. Premature delivery, preeclampsia and diabetes in pregnancy are associated with S-25(OH)D levels in earlier studies and also with VDR polymorphisms [15, 79, 81, 82].

Increased frequency of emergency caesarean sections (ECS), perinatal deaths and small for date infants were observed among Somalian women and children in the county of Dalecarlia in central Sweden between 2001 and 2009 [16]. However, levels of S-25(OH)D were not measured.

The uterus consists almost entirely of smooth muscle cells. Skeletal muscle cells in the abdominal muscles are engaged in labour as well as the uterine smooth muscle. Both muscle cell types have vitamin D receptors [17]. Vitamin D has an effect on the muscle cells contraction, relaxation and endurance as mentioned, and vitamin D is also crucial for adequate calcium levels in the body which is necessary for normal muscle cell function [18].

Dystocia, or non-specified prolonged labour, is a frequent cause for caesarean sections [83]. The concept of dystocia includes both obstructed labour for reasons such as malformed pelvis but also uterine muscular dysfunction [14]. So far, there appears to be little evidence for negative effects on the normal progression of labour from low levels of vitamin D as there was no evident seasonal variation of dystocia in need of intervention (ECS, instrumental vaginal birth, oxytocin augmentation) during birth in a Danish study [84]. That study presented no data on S-25(OH)D levels or of supplementation. To compare, a Spanish and an Indian study found caesarean sections to be associated with deficient and insufficient levels of S-25(OH)D [85, 86].

A US study found a doubled frequency of caesarean sections due to prolonged labour for women with S-25(OH)D < 30 nmol/L and slightly elevated PTH levels (mean 5.6 pmol/L) [14]. In contrast, a study of historical material with S-25(OH)D < 30 nmol/L was not associated with prolonged labour or surgical delivery [87]. Likewise, no higher frequency of caesarean sections was found among vitamin D deficient women in Saudi Arabia (mean S-25(OH)D 30.5 nmol/L), Turkey (median S-25(OH)D 27 nmol/L), nor among women in a meta-analysis on pregnancy outcome [79, 88, 89].

I found in a pilot study (unpublished data) that 90% of Somali patients, complaining of pain, weakness and being unwell or depressed, had vitamin D deficiency, in my primary healthcare centre in Borlänge in Dalecarlia in the end of 2009 and beginning of 2010. Many of them had critically low levels (S-25(OH)D < 10 nmol/L) and were very weak with low ability to squat even

once. Indeed, some of them even fell to the floor when trying. Some of the women were pregnant.

It is not known if the higher rate of ECS among Somalis in Dalecarlia was related to prolonged labour and or to low vitamin D levels and has, to our knowledge, not been studied in Sweden before.

Supplement of vitamin D with calcium and lifestyle advice on sun exposure and food to pregnant women could tentatively become a cost-effective simple treatment if vitamin D and calcium deficiency turns out to be a cause for prolonged labour and emergency caesarean section [21, 90, 91].

Association and causality

Most studies on vitamin D only examine the associations with other factors where causality can be questioned. Randomised placebo-controlled treatment studies are limited and also have inherent limitations such as adherence problems. Studies on pregnant women may be even more difficult due to adherence problems; furthermore, there is another ethical situation considering an unborn child growing in the womb.

Randomised placebo-controlled studies (RCTs) in pregnancy for exploring causal inference between S-25(OH)D and caesarean sections (CS) are difficult for practical and ethical reasons. In order to avoid the use of a placebo, or a possible insufficient dosage group of treatment, for comparison, a study may be performed by using a directed acyclic graph (DAG) for systematic representations of causal relationships [92, 93]. A DAG can identify confounders sufficient to close biasing paths to achieve exchangeability between the exposed and non-exposed groups.

Genetical studies by Mendelian randomisation on the vitamin D receptor have been published throughout the years of this project [94, 95]. Mendelian randomisation is a method for studying causality since the genes are inherited and do not change due to, for example, different levels of S-25(OH)D because of the season. For example, an important question has been if low S-25(OH)D causes obesity (high BMI). Recent Mendelian studies have answered this question and point out that high BMI causes a low S-25(OH)D and not vice versa [96].

Many observational studies have been published throughout the years, even during the time of this project, and they mainly show a positive association between S-25(OH)D and strength [35, 97, 98]. Several randomised controlled trials (RCTs) have also been published [99, 100], including even several meta-analyses, with different quality and size. Some were based on rather small studies among athletes with normal or insufficient levels of S-25(OH)D with positive findings, whereas others found no effect [101, 102]. A large meta-analysis including 30 RCTs with 5,615 persons showed a significant positive

effect of vitamin D on strength, with most evident results in people with S-25(OH)D < 30 nmol/L [103].

Genetical studies on single nucleotide polymorphisms (SNPs) across the vitamin D receptor (VDR) have also been published. Results have differed and seem to depend on the SNPs studied [104, 105]. The differing results may also depend on no adjustment for S-25(OH)D levels. For example, the study on a Swedish cohort in a prospective population-based study of 3,014 elderly men found no differences in hand grip strength between participants with the different SNPs studied, but the results were not adjusted for S-25(OH)D [105].

New studies on epigenetics also contribute with interesting information on S-25(OH)D, significantly modulating the human epigenome by enhancing the rate of accessible chromatin and vitamin D receptor binding [31]. Human epigenome is found to respond on the level of histone modifications to calcitriol stimulation. This gives a further understanding of vitamin D target gene regulation. Indeed, a new study on VDR adjusted for S-25(OH)D shows that S-25(OH)D is associated with handgrip strength, and the relationship could be modified with the interaction between S-25(OH)D and gene polymorphisms [106].

Aims

Overall aim

An overall aim of the project was to explore the levels of vitamin D and muscle strength and their tentative association with birth outcome because of prolonged labour as an effect of global muscle weakness. In addition, we wanted to explore if it was possible to improve the vitamin D levels, lifestyle and muscular function through lifestyle intervention and supplementation, with focus on pregnant Somali and Swedish women and new mothers. A long-term overall aim was to find ways to improve antenatal and primary healthcare for all women with deficient vitamin D and promote an improved lifestyle that benefits health in women and their families.

Specific aims

Study I

To examine grip strength, physical performance of the lower limbs and serum concentrations of 25-hydroxy vitamin D S-25(OH)D in a group of pregnant and new mothers with presumably lower (veiled, Somali born) and higher (unveiled, Swedish born) concentrations.

Study II

To explore the adherence to prescribed supplemental vitamin D in pregnant and recently pregnant women with vitamin D insufficiency (S-25(OH)D \leq 50 nmol/L) after ten months treatment and its effect on grip strength and upper leg performance in Somali (target group; TG) and Swedish women (reference group; RG) from spring through winter.

Study III

To estimate the causal effect of critically low serum vitamin D (S-25(OH)D level $<$ 10 nmol/L) on CS and ECS also when connected with prolonged labour (CS-PL and ECS-PL).

Study IV

The aim with this paper was three-fold.

- First, to study if vitamin D related lifestyle differed between women having normal and deficient vitamin D levels within groups of Somali and Swedish pregnant women/new mothers.
- Second, to explore changes in vitamin D related lifestyles within the Somali and Swedish target groups with insufficient vitamin D levels after an intervention by doctors providing brief lifestyle advice on vitamin D and supplementation.
- Third, to explore knowledge, attitudes and behaviour related to vitamin D among the Somali-born participants from baseline and after two years.

Subjects and methods

Designs

- The following designs were used
- Study I: A cross-sectional study
- Study II: A before and after treatment study
- Study III: An observational cohort study
- Study IV: A mixed method study before and after intervention

Setting

The studies were performed in the geographical catchment area of the antenatal clinic of Borlänge. Borlänge is an average sized town in Sweden, with an estimated population of 50,000 inhabitants. It is situated by the river Dalälven in Dalecarlia and is partly surrounded by forestall areas and by the agricultural plains of Stora Tuna in the central part of Sweden, roughly 150 km from the Baltic see.

Borlänge could be a good area for studying vitamin D deficiency and its consequences and treatment since it is situated on the 60:th parallel with long winters and little time in the year for catching the ultraviolet light.

The influx of immigrants in Borlänge has been huge over the previous decades. After the war in the 1950s, there was a great need for workers in this industrial town. Many Finnish immigrants came and worked in the leather-, steel- and paper industry in the area. So did my father from northern Finland, and he married a young Swedish women from Borlänge, my mother. After the Finns came the Yugoslavians, the Turks and in recent years a lot of refugees from Arabia and Africa's horn – Somalia.

At the time of the study, Borlänge had about 2,000 Somalis living in its area due to forced emigration. The Somalis were mostly Muslims and had a strict religious and cultural clothing code and avoided certain foods such as pork. Many were used to eating fresh foods from fruit, vegetable-, fish and meat-markets, and drinking and eating cow milk products was not a common habit like it is in Sweden. The Somali society was built on strong clans, which they preferred not to talk very much about here at the time. The parity of the Somalis was high, and Somali women's health was a natural concern in my

primary healthcare clinic since many of the Somalis lived in the catchment area and came with their children.

The Swedish antenatal care is well organised and well trusted, also by recent immigrants. Therefore, most women contact the antenatal care and sign in for check-ups and information already early in pregnancy.

Subjects

Study I – baseline sample

Low vitamin D and calcium may be negative or even hazardous for the pregnant mother, foetus and lactating child. Due to the authors' observations of low levels of vitamin D in Somalis, we therefore wanted to study a sample of ordinary pregnant or lactating Somali women and to compare them with a reference group of Swedish women. The number of Somalis in the catchment area was limited. Another limitation was the well-known effect of season on the vitamin D levels.

A way to control for seasonal effects would be to take all blood samples and to do all tests under a short period of time. To achieve this with a limited population of women, we decided on finding tentative participants through the local antenatal care. The antenatal clinic was able to provide us with lists of currently pregnant and those having been pregnant recently. Then, using a retrograde inclusion approach could give us the needed numbers of participants to reach power for the study. The study population would include women in all trimesters as well as new mothers.

Our intention was to find our sample of women from the lists of assignment for antenatal care from the time of the study start and backwards, a retrograde inclusion as mentioned. In this way, we could manage to find, contact and include enough women for doing all our tests in a short time period, preferably in late winter/spring or at least before summer to minimise the effects of ultraviolet B radiation.

The focus was on pregnant women but also on lactating women. We received lists of the women assigned to the antenatal clinic in Borlänge. The lists were studied from the study start and nine months backwards to seek inclusion of pregnant women. Since the number of Somali women would not be enough within nine months, we also included Somali women up to one year after the nine months, i.e. altogether up to 21 months. In order to receive as many pregnant women as possible, we included Swedish women from the first nine months.

Somali women were known to have many children. For purposes of comparison, we wanted to get roughly the same amount of primiparous in the two groups.

Our statistician at the Centre for Clinical Research in Falun helped us to randomise the Swedish women and to form new lists with the women categorised as primi- or multipara. The lists were made in a way so that we could contact the women in numerical order from the top and down, until we reached enough power.

Recruitment procedure – baseline

The idea was to recruit a cohort in a baseline study, which could be followed up after intervention (papers II and IV), and also to study the outcome of the pregnancies (paper III). The need for the baseline study population was estimated to roughly 50 Somali and 75 Swedish women. The baseline study was designed as a cross-sectional study of ordinary fertile women.

We found 118 eligible Somali women. Information letters about the study were sent out to all 118 women, in both Swedish and Somali language. The Swedish letters were sent in batches, intending to reach power and then stop inclusion. After a week, the assistant Somali nurse and one research doctor telephoned the women to inform them in Somali and or Swedish and to ask if they would like to participate in the study. When enough women were included, the invitation procedure was stopped.

The study groups were Somali women (target group, TG) and Swedish women (reference group, RG). Women < 18 years were excluded, as were women from other countries, or those who had severe mental or somatic disorders.

Clothing covering the arms, legs and head was considered concealing (veiled clothing). Dates for blood sampling and the written consent were suggested during the phone conversation to those who wanted to participate.

Altogether, 140 women out of 217 contacted came for the blood sampling and the written consent (64.5% or 140/217), (58 Somali, 82 Swedish). They received an appointment ten days later with one of the two research doctors to perform physical tests, receive questionnaires and information. This time interval was required for distribution and analyses of the blood samples. In total, 123 women (56.7% or 123/217) came to the doctors, where they completed the questionnaires and performed the physical tests. These 123 women constituted the study population in the baseline study (52 Somali, 71 Swedish). There were no statistical differences between the participating women and the non-participants.

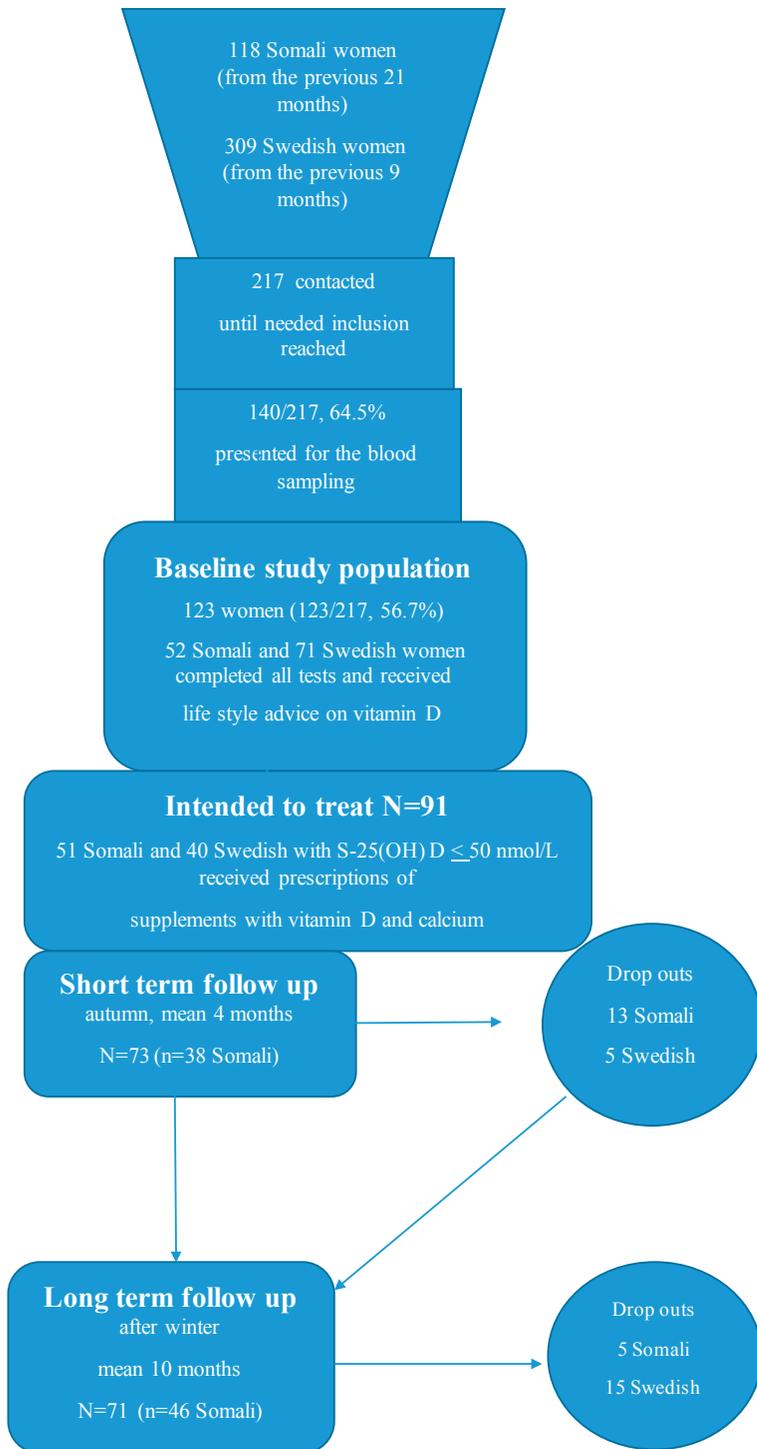


Figure 1. Flow chart for papers I and II

Sample and recruitment study II

All 91 (51 Somali) women with S-25(OH)D levels of 50 nmol/L or less, who had been prescribed supplementation of vitamin D and calcium at baseline, were invited by letters and by phone calls, scheduling appointments for a follow-up. Please see also flow chart above.

Forty-six Somali women in the target group (TG) and 25 Swedish women in the reference group (RG) out of the 91 (78%) participants with low vitamin D at baseline attended the 10-month follow-up measurements (Time II) and were included in the analyses. The participants did not differ significantly from the drop outs.

Sample study III

The cohort of 123 women from study I constituted the study population (71 Swedish, 52 Somali women). Data regarding date of delivery were found in birth protocols for 116 persons (n = 50 Somali, 66 Swedish) out of the 123. Missing consisted of (n = 7) 2 Somali and 5 Swedish women, which were due to spontaneous abortions (n = 3 Swedish), intrauterine death (n = 1 Somali), or move to another county (n = 1 Somali, 2 Swedish). There were no statistical differences between the participating women and the non-participants.

Sample study IV

The study population constitutes the 91 participants (n = 51 Somali, 40 Swedish) from the baseline cohort of 123 women who had S-25(OH)D \leq 50 nmol/L. Due to different drop outs on the two follow-ups, the participants differ, albeit very little (n = 73 and 71), between 4- and 10-month follow-ups as seen in the flow chart, figure 2.

Methods

Studies I and II

The baseline study, paper I, was initiated and data collected over a few weeks in late spring/ beginning of summer 2010. The follow-up study, study II, was initiated in late winter; mean 10 months after the baseline study. A 4-month check-up was performed during autumn (Time I), and the primary outcome data for study II were collected at the 10-month follow-up, after winter (Time II).

Blood sampling

Venous blood samples were collected and centrifuged and measured using Lisason 25 OH Vitamin D total assay, (DiaSorin, Stillwater, USA) at the clinical chemistry laboratory at the University Hospital, Uppsala, Sweden. The method had consistently (10–20%) lower values than the specific LC-MS reference methods used at other laboratories [107]. The other assays (ALP, PTH, Ca, albumin) were measured using Abbott Architect ci8200 (Abbott Laboratories, Illinois, USA) at the Department of Clinical Chemistry, Falun Hospital, Sweden. Haemoglobin and glucose were measured using the Hemocue system (HemoCue Sweden, Ängelholm, Sweden) at the antenatal clinic. Alkaline phosphatase, PTH and calcium in serum as well as albumin corrected calcium levels were analysed to monitor metabolic skeletal activity. Haemoglobin and glucose were measured in baseline blood samples; although they are not presented here, they were screened for non-vitamin D-related reasons for fatigue and muscular weakness. The same laboratories were used to analyse the blood tests at follow-ups. Parathyroid hormone and ALP were not taken at 10-month follow-up due to practical (PTH was taken as a morning sample) and financial reasons.

Measurements and questionnaires

Data collection

Three professional interpreters supported the same two doctors in the data collection from the Somali women.

Anthropometric measurements were performed and questionnaires on lifestyle, pain, medication, disorders and socio-cultural variables, CS, gestational age and lactation were administered by the two doctors. Physical Activity was self-reported and measured as 0, 1, 2 (seldom) or ≥ 3 times weekly (often).

The participants then performed the physical tests.

Measurements and questionnaires were repeated at 4- and 10-month follow-ups. They were slightly adapted to suit the intention of the follow-up, with, for example, questions on how the participants had taken their supplements or not repeating all lifestyle questions such as questions regarding sun exposure of face, neck and arms again after the winter. We also added a question on number of years the participant spent in school at the 4-month follow-up.

Questions about daily consumption of prescribed supplements and compliance with the supplementation dosage were asked at the follow-up appointments, and the total number of supplements consumed for 10 months was calculated based on this information.

Questions about other medication were repeated, and information about any side effects of the supplement were asked during the follow-up meetings. The two research physicians were blinded to the blood test and previous test results during the consultations.

Grip strength and physical performance

Tests for physical function at baseline, of the hand and upper leg muscles, were chosen to reflect everyday practices, and the same tests were repeated at 4- and 10-month follow-ups. The upper leg tests were slightly adapted from regular tests, such as sit-to-stand test, to avoid problems when performed by highly pregnant women, women who had recently given birth or had had a CS.

Hand. Peak grip strength, defined as the highest value of three trials in each hand, was measured in Newton using a hand dynamometer ('GRIPPIT' AB Detektor, Gothenburg, Sweden) [108, 109]. Also, the mean maximum grip strength under ten seconds (M-max grip) was measured for each hand.

Upper leg. Four upper leg tests were performed and rated by the doctors as done without effort (able) or not (unable). *a.* Squatting. The person squatted and rose once. A minimum of 90° angle between thigh and lower leg was demanded. *b.* Standing on one leg. Tested for 30 seconds [110]. *c.* Hip lifting

test (Trendelenburg's sign). The person stood with one hand high up on the wall and lifted the opposite leg for 30 seconds [65]. *d.* Ability to stand up from a chair (sit to stand). The person sat down and stood up five times with hands folded across chest.

Testing and results at follow-ups

Questionnaires, physical testing and information on blood tests results were used at the 4- and 10-month follow-ups. Blood test results were not checked before completing the questionnaires and physical testing for the sake of blinding the doctors. A reminder was given on taking the supplements when the result was presented.

Study III

Data collection

Reasons for caesarean sections and instrument-assisted birth were retrieved from the birth protocols using a triage method. The responsible obstetricians were blinded to this study.

Prolonged labour was identified by three diagnostic codes from the International Statistical Classification of Diseases and Related Health Problems — Tenth Revision (ICD 10): O62.0 (Primary inadequate contraction), O62.1 (Secondary uterine inertia) and O63.1 (prolonged second stage).

Reasons for instrumental intervention were noted, e.g. obstructive labour or severe preeclampsia ($n = 4$ disproportionate pelvis, $n = 3$ twin birth, $n = 5$ breech position, and $n = 1$ severe preeclampsia), and psychological reasons ($n = 4$ Swedish women).

Other data retrieved regarded birth date, gestational age and anthropometric data for the mother and child, pain-reducing measures, stimulatory agents, type of induction, and birth mode. The partographs were studied manually.

Study IV

Data collection

Questionnaires with the same lifestyle questions as in the baseline study were given again after four and ten months with minor adaptations for the seasons (questions not changed but excluded when unnecessary). Questions of follow-up character on supplementation were added. Physical tests were repeated in the same way. Somali-born interpreters were available at all times.

Serum vitamin D was repeatedly measured in the same laboratory at Uppsala Academic Hospital at all occasions using a Liaison 25 OH Vitamin D total assay (DiaSorin, Stillwater, USA).

Lifestyle data

Data on lifestyle were collected at the baseline, 4- and 10-month follow-ups. Lifestyle questions associated with vitamin D were:

Approximately how many decilitres or glasses of milk, sour milk or yoghurt do you drink/eat every day? Answer was noted in decilitres or glasses.

How often do you eat cheese? Respondents ticked the box with the best response alternatives: never (= 0), 1-2 times/w (= 2), 3-5 times/w (= 4), 6-7 times/w (= 6). Figures within brackets were used for the calculations.

How many times per week do you eat salmon or other fatty fish? Respondents ticked the box with the best response alternatives: never (= 0), 1-2 times (= 1), 3 or more times (= 3).

Are you outside during daytime for minimum of 30 minutes every day in summer? Respondents ticked the box with the best response alternatives: no (= 1) or yes (= 0).

Do you expose your face, neck and forearms to the sun during summer? Respondents ticked the box with the best response alternatives: no (= 1) and yes (= 0).

Do you sunbathe in another country for minimum of one week during winter? Respondents ticked the box with the best response alternatives: no (0) and yes (1).

Intervention

Information on vitamin D

Women were informed in the end of the consultation on how vitamin D is synthesised in the skin when the skin is exposed to ultraviolet light from the sun, and by intake of foods rich in vitamin D. Furthermore, they were informed about the efficacy of sun-exposure to the face, neck and forearms and that the greater skin area exposed, the faster and greater amount of vitamin D will be synthesised.

The Somali group of women were informed that dark pigmented skin protects against sun burns and cancer but needs longer time to produce similar amount of vitamin D [111]. All women were informed that preventing sun radiation, including long distances through the atmosphere for sun rays as in winter or evening as well as veiled clothing covering the skin will effectively block synthesising of vitamin D. Participants were also shown a schematic picture of sun radiation through the atmosphere at the equator as compared with Sweden in summer and winter and another picture of common foods rich in vitamin D.

The connection between the need for adequate levels of vitamin D to achieve normal calcium absorption was also briefly explained and that low

blood levels of vitamin D might cause osteomalacia ('bone softening'), and the symptoms were briefly described.

Finally, the sealed envelopes containing the results of S-25(OH)D tests were opened. All the participants were informed of their vitamin D levels. All the women with S-25(OH)D \leq 50 nmol/L were prescribed standard preparations of vitamin D and calcium. Thus, until then the doctors were blinded as to the results of the blood tests.

Supplementation

A vitamin D3 supplement containing calcium (800 IU = 20 μ g vitamin D3 and 500 mg calcium carbonate) was prescribed at baseline for the whole study period. The over-the-counter price was 309 SEK per 180 tablets (approximately 44 USD), and the women paid for these themselves. Two tablets daily were prescribed for the women with S-25(OH)D < 25 nmol/L and once daily for those with 25 – 50 nmol/L of S-25(OH)D. These doses followed the recommendations for pregnant women [13].

Focus group interviews

Two years from baseline, two focus group interviews were held close in time. A female anthropologist held two group interviews with eight and six participating women, respectively. The women invited were all of Somali origin and had all participated at baseline.

Open questions were asked on the subject 'what is appropriate when pregnant', and the questions were chosen to enlighten the emic perspective of the women on lifestyle associated with vitamin D (food, sun exposure, sunbathing and clothing).

The content of the interviews was analysed (inferential analyses).

Power calculations

Study I

The power we needed for the study was calculated based on the difference in S-25(OH)D levels found in Somali (n = 30, all veiled) and Swedish (n = 12) consecutive women in a pilot study in primary care (unpublished data). Somali women had, on average, 17 nmol/L (SD 13) and the Swedish women had, on average, 35 nmol/L (SD 28), resulting in requiring sixty participants (n = 30 Somali; n = 30 Swedish) in order to reach 94% power and α error 0.05.

The power calculation was approved by the regional ethics committee Uppsala (Dnr 2010/40), Sweden.

Study II

Power was calculated based on an upward clinically relevant mean difference in grip strength of 50 N (Std 40). On this basis, at least seven participants were needed in the target group of Somali women and 17 in the reference group of Swedish women (mean change 40, Std 50) for a two-sided *t* test to reach 90% power with an alpha error of 5%.

Study III

To be able to detect a fraction difference of 0.2 of the incidence of emergency caesarean sections between two groups of 25(OH)D (less than 10 nmol/L or more), we needed 35 individuals to reach an alpha error of 5% and a beta error of 20%.

Manuscript IV

Power was not calculated on lifestyle changes.

Statistics

Study I

Mean values with 95% confidence intervals (95% CI) were calculated for interval data. Median values (md) with inter-quartile ranges (IQR) were calculated for ordinal data and small numbers.

Serum-25(OH)D was examined both as a continuous variable, where the undetectable levels of S-25(OH)D were replaced by the figure 9, but also categorised as undetectable or critically low levels (S-25(OH)D < 10 nmol/L), deficient (10-24 nmol/L) and insufficient (25-49 nmol/L) and sufficient (50-75 nmol/L) and optimal (>75-250 nmol/L) [112].

Chi-square statistics, Mann-Whitney U test, ANOVA and t-tests were used to compare median and mean values between two groups. Spearman's rank correlation coefficient (rho) was calculated for grip strength across S-25(OH)D categories. Kruskal-Wallis and Mann-Whitney U test were used to calculate significant differences in distribution of inability to perform the upper leg tests across the S-25(OH)D categories. Pearson correlation statistics were used to explore the uni-level association between S-25(OH)D and grip strength.

A final main effect model was calculated using stepwise linear regression for predicting maximal voluntary grip strength among the independent variables. Two-sided significance tests were used. A p-value of 0.05 or less was considered as statistically significant.

Study II

Levels of S-25(OH)D were examined and categorised as <10, 10-24 or 25-50 nmol/L. Mean values for intervals with Std were calculated. Chi square statistics for nominal data and Mann-Whitney U test were used to test for significant differences in median and mean values between groups. McNemar's test for dichotomous data or Wilcoxon signed-ranks test for ordinal or interval data were used for significant differences between pre- and post-values. Univariate associations were explored using Spearman rho to assess correlations between the number of tablets taken and the difference scores between Baseline and Time II on S-25(OH)D, M-max grip and upper leg performance.

Multiple linear regression with stepwise exclusion was used to estimate the independent variables that were best associated with the change of grip strength from baseline to Time II.

Binary regression was used to calculate odds ratios (OR) with 95% confidence intervals (95% CI) as predicting positive changes in upper leg physical performance in the TG.

Independent variables: age, total tablets taken, height and S-25(OH)D increase were categorised as below and above their mean values.

Study III

Categorising comprised the groups <10 nmol/L and above (10 – 24, 25 – 49, > 50 nmol/L) of S-25(OH)D. Pearson chi-square, Fischer's exact test, Kruskal-Wallis were used to compare group values. Adjusted and unadjusted imputations were used in SPSS version 23.

The estimation of the causal effect of S-25(OH)D on caesarean section was analysed by first building a DAG at Dagitty.net, Version 2.3, to identify a minimal set of confounders to adjust for in the following linear regression calculation [92, 93]. This procedure provided us with a least set of covariates to adjust for in order to minimise confounding bias (figure 2).

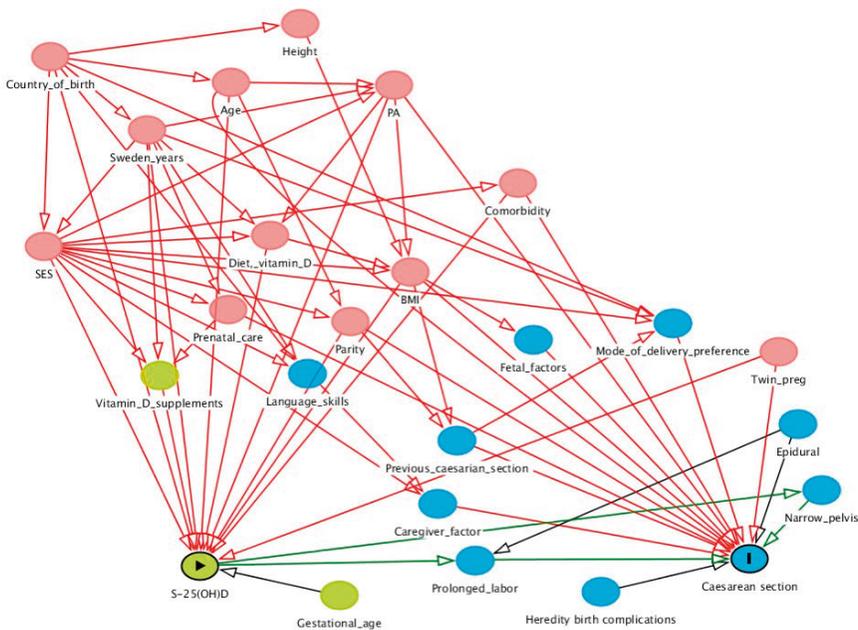


Figure 2. A directed acyclic graph (DAG) for the exposure serum 25-hydroxyvitamin D (S-25(OH)D) and the outcome caesarean section

The resulting minimal sufficient adjustment set for estimating the total effect of exposure to S-25(OH)D on the outcome CS was: age, body mass index (BMI), comorbidity (diseases potentially affecting both exposure and outcome), country of birth, parity (measured as < 1, 1-5, > 5 births), physical activity (PA) expressed in a three-graded scale (seldom or never, 1-2 times/week, > 3 times/week) in the calculations, socioeconomic status (SES) and vitamin D supplement (recorded at baseline, 4-month and 10-month follow-up) and time living in Sweden (years). The outcomes CS, ECS and AB-PL give the same set of covariates.

Time living in Sweden, and not SES, explains unplanned CS among Somali and other immigrants in Sweden [113]. Country of birth was used as a proxy for unknown genetic and lifestyle risk factors including SES.

Outcome variables were CS, with the subgroups CS-PL, ECS, ECS-PL and AB-PL. Independent variables were the same as the minimal set for adjustment according to the DAG.

Analyses were done for all, and separately for the Somali and Swedish women.

First models using multivariable logistic regression calculations were calculated for the outcome variables CS, ECS and AB-PL. Two-sided tests for statistical significance at the level $p < 0.05$ were used.

Study IV

Numbers, percentages, mean and median values with standard deviation and inter quartile ranges were calculated for lifestyle data associated with S-25(OH)D.

The participants were divided into a Swedish reference group with all women with S-25(OH)D > 50 nmol/L at baseline and two target groups (≤ 50 nmol/L). The two target groups were divided into a Somali and a Swedish target group.

T-test and Mann-Whitney Test were used to compare vitamin D lifestyle data before intervention between the Swedish RG and the two target groups, and Wilcoxon signed rank test and Related Samples McNemar test were used to compare before and after intervention data.

Ethical approvals

Study I was approved by Etikprövningsmyndigheten i Uppsala (the regional ethics committee in Uppsala) D 210/140 as well as the amendments for Study II (D 2010/140/1), Study III (210/140/3) and Study IV (210/140/2).

Trial registration

ClinicalTrials.gov Identifier: NCT02922803. Date of registration: 28 September 2016.

Results

Study I – Baseline

The Somali group of women were: younger, shorter, had lower blood pressure, had given birth to more children and had less education. More Somali women were breastfeeding (72% vs 29% Swedish), and some of them were also breastfeeding when pregnant (2%, not in table).

At baseline, 2/3 of the Somali women and 1/3 of the Swedish women had given birth, and no one was using narcotics or anxiolytic analgesics.

Table 1. Descriptive and reported lifestyle data among the 123 pregnant Somali and Swedish women and new mothers

	Somali	Swedish
n	52	71
Age, years, m (Std)	28 (6)	31 (5)
Height, cm, m (Std)	161 (5)	167 (6)
Blood pressure, systolic, mmHg, m (Std)	104 (10)	113 (10)
Children, n, med (IQR)	3 (10)	1 (3)
Education, years, m (Std)	3.3 (3.4)	13.8 (2.5)
Years in Sweden, m (Std)	3.5 (2.6)	
S-25(OH)D, nmol/L, m (Std)	16 (10)	49 (18)
Grip strength, N, m (Std)	199 (58)	318 (66)
<i>Lifestyle data</i>		
Milk, dl/day, m (Std)	1.8 (2.0)	3.6 (1.9)
Cheese times/w, md (IQR)	2.5 (2.5)	4.3 (1.7)
Fish times/w, md (IQR)	0.7 (1.0)	0.8 (0.5)
Exercise times per week, md (IQR)	1.5 (1.5)	1.8 (1.2)
Outdoors, n (%)	31 (61)	66 (96)
Veiled clothing, md (IQR)	1.1 (0.3)	0.0 (0.0)
Sun exposur face, neck, forearms, n (%)	13 (25)	65 (96)
Sun vacation in winter, n (%)	1 (2)	11 (16)

The blood tests showed that the Somali women had much lower S-25(OH)D levels compared to the Swedish women. The Somali median value of S-25(OH)D was 12 nmol/L, and one-third of Somali women had critically low S-25(OH)D (<10 nmol/L) compared to none in the Swedish group; in total, 90% of the Somalis were deficient (< 25 nmol/L) compared to 10% of the Swedes. The mean value for the Swedish women was 50 nmol/L, and only 6% of the Swedish women had > 75 nmol/L.

Parathyroid hormone and ALP levels were increased in the Somali group, and a reverse correlation was found between S-25(OH)D and PTH (corr. -0.39, $p < 0.001$) and ALP (corr. -0.26, $p = 0.003$) in linear calculations (not in table). Meanwhile, the albumin corrected serum calcium levels were normal and the same.

Table 2. Blood test results in the 123 women in the baseline study

Origin, n	Somalia, 52	Sweden, 71
25-OHD nmol/L, median	12	48
PTH pmol/L ¹	12.5	4.6
ALP ukat/L ²	2	1.5
Ca ²⁺ mmol/L	2.3	2.3

¹ Upper normal PTH level was 6.9 pmol/L, ² Upper normal ALP level was 1.8 ukat/L

The grip strength was associated with the S-25(OH)D levels in the Somali group, and the same tendency was found among the Swedish group (not in table).

Three-quarters (73%) of the Somali women were unable to squat, and one-fifth (21%) had a positive Trendelenburg's sign (not in table); however, there were no statistically significant differences in distribution across the 25-(OH)D categories.

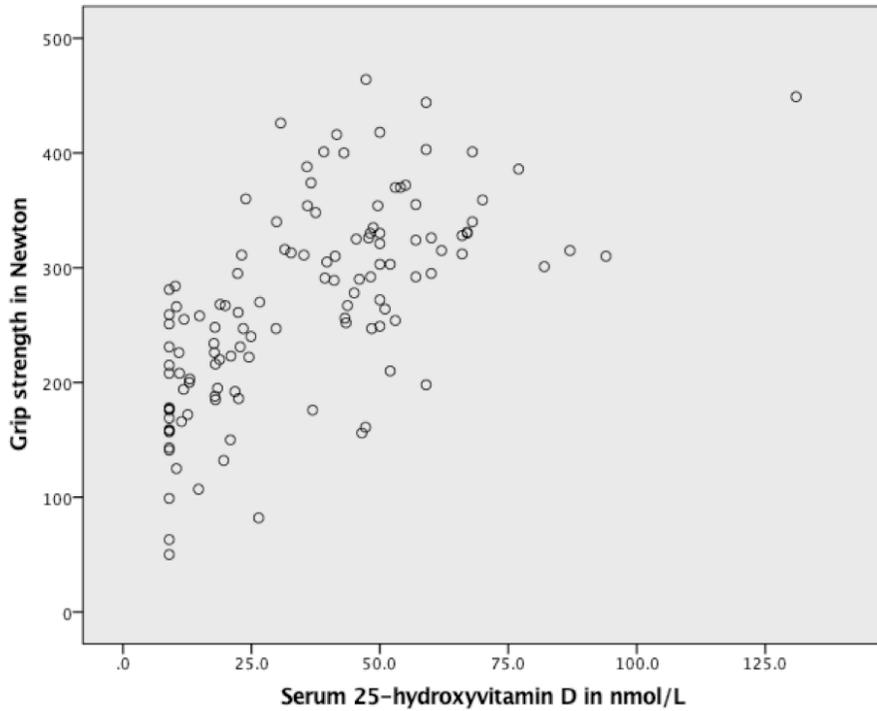


Figure 3. Illustrates that the S-25(OH)D concentrations (nmol/L) and grip strength (N) had a linear correlation.

Pearson correlation coefficient: all together corr. 0.65, $p < 0.001$.

Somali corr. 0.34, $p = 0.015$, Swedish corr. 0.28 $p = 0.02$.

Undetectable (< 10 nmol/L, critically low) levels replaced with the figure 9

Table 3.

Variable	Unstandardised B	95 % CI	
		Lower	Upper
S-25(OH)D	0.94	0.21	1.68
Country	-63.9	-97.0	-29.9
Age	2.5	0.15	4.60
Height	2.6	0.59	4.65

Table 3 shows the final main effect model of multiple linear regression calculation showed that grip strength (N) was predicted by the S-25(OH)D level, adjusted for country of birth, age and height. Physical activity, lactation, parity and gestational age had no significant relationship to grip strength. Adjusted R² was high, 0.54.

Study II

The 4-month follow-up showed improved grip strength (199 to 214 N, $p = 0.037$) and leg strength ($p < 0.001$) in the 38 Somali women. No Somali woman had an immeasurable S-25(OH)D level. Their mean S-25(OH)D had increased from 15.8 to 47 nmol/L ($p < 0.001$), and PTH levels had decreased from 13.0 to 8.3 pmol/L ($p < 0.001$). The Swedish women also had increased their mean S-25(OH)D levels from 39 to 64 nmol/L ($p < 0.001$).

At the 10-month follow-up, the whole group of Somali women had increased their mean levels of S-25(OH)D (15.8 to 49.2 nmol/L, $p = 0.001$) and the mean peak grip strength (153 to 188 N, $p = 0.001$) compared to baseline. Likewise, the Swedish women had increased their S-25(OH)D levels (38.8 to 67.5 nmol/L, $p = 0.001$), as well as their grip strength (mean 257 N to 297 N, $p = 0.003$). This concerned even the women in the higher strata.

Table 4 shows the women who complied with supplementation, categorised into groups according to their S-25(OH)D level at baseline. The larger groups, who adhered to supplementation, had increased their mean grip strength significantly after ten months of treatment, and the number of Somali women unable to squat had decreased. Indeed, the Somali women had increased their grip strength 25% (118 – 148N, $p = 0.039$) in the category < 10 nmol/L and 28% (162 – 208N, $p < 0.001$) in the category 10-24 nmol/L.

Two-thirds (64%) of the Somali women (the categories <10 and 10-24 nmol/L) who adhered to supplementation (compliers) increased their leg strength significantly.

Table 4.

	Somali			Swedish	
n total	46			25	
S-25(OH)D nmol/L categories	< 10	10-24	25-50	10-24	25-50
n complying to supplementation	13	21	4	5	18
S-25(OH)D nmol/L, mean					
Baseline	9	16	39	22	42
10 m	50	58	60	66	67
p	0.001	< 0.001	0.068	0.043	0.001
Mean grip strength, (N)					
Baseline	118	162	193	242	262
10 m	148	208	219	267	310
p	.04	< .001		.08	.01
Inability to squat, n (%)					
Baseline	11(85)	13 (62)	3 (75)	0	0
10 m	0	4(19)	1 (25)	1	0
p	.001	.01	.50		

The tables do not reflect that the increased levels of S-25(OH)D correlated with the total supplement intake in the TG ($\rho = 0.53$, $p < 0.001$). The intake of supplements varied largely among participants. Some took only half the recommended dose of supplement, whereas eight (17%) in the TG vs two (8%) of the RG reported having not taken any supplement. Therefore, the participants became their own controls, as to the effect of the supplementation.

The calculated mean intake at Time II was $n = 322$ (239) supplements in the Somali group and $n = 211$ (136) in the Swedish group. A steady state seemed to have been reached for S-25(OH)D at Time I since the mean 25-OHD levels were stable compared with Time II (47 to 49 nmol/L TG; 64 to 67 nmol/L RG).

Table 5 shows the final effect model for change in grip strength. Total supplement intake predicted improved grip strength adjusted for height. Variables entered at first step: age, country of birth, height, number of total supplement intake, S-25(OH)D change, physical activity change. Adjusted R² 0.100.

Table 5.

	B	95 % CI	
		Lower	Upper
Total supplement intake ¹	0.067	0.008	0.127
Change in S-25(OH)D	-0.338	-0.733	0.056
Height	0.060	0.157	3.966

¹ number of supplements

The regression calculations showed that the ability to squat in the Somali target group showed high odds of 16:1 (1.8 – 144.6) for improved squatting ability if supplement intake exceeded 300 tablets for ten months.

Study III

Table 6 shows obstetric outcome by category of S-25(OH)D nmol/L. Only Somali women had S-25(OH)D levels < 10 nmol/L. Only Swedish women had S-25(OH)D levels > 50 nmol/L, with one exception. Serum 25-(OH)D levels were associated with AB and ECS. Among the Somali women, all women with emergency caesarean section for prolonged labour (ECS-PL) were in the critically low vitamin D category. Fischer's exact test for ECS-PL as associated with S-25(OH)D <10 and above 10 nmol/L was significant (p = 0.010).

Table 6.

	Categories of S-25(OH)D nmol/L				p	Total
	<10	10–24	25–49	>50		
n	17	35	35	29		116
Somali, n (%)	17 (100)	28 (80)	4 (11)	1 (3)		50 (43)
Swedish, n (%)	0	7 (20)	31 (89)	28 (97)		66 (57)
Assisted birth, n (%)	10 (59)	11 (31)	7 (20)	9 (31)	0.047	37 (32)
Caesarean section, n (%)	7 (41)	8 (23)	6 (17)	8 (28)		29 (25)
Emergency caesarean, n (%)	7 (41)	3 (9)	5 (14)	5 (17)	0.031	20 (17)
Prolonged labour (PL), n (%)	6 (35)	5 (14)	4 (11)	6 (21)		21 (18)
AB-PL n (%)	5 (29)	3 (9)	2 (6)	2 (7)	0.047^a	12 (10)
ECS-PL n (%)	4 (24) ^b	0	2 (6)	2 (7)	0.019^a	8 (7)

a > 25% of the cells had an expected count < 5

b p value = 0.010 Fischer's exact test for ECS-PL as associated with S-25(OH)D <10 and above 10 nmol/L

Table 7 shows the results of the first model of multiple binary regression calculation for odds ratio for the outcome variables CS, ECS and prolonged labour causing ECS and AB adjusted for the covariates according to the DAG. The estimated causal effect of critically low vitamin D (< 10 nmol/L) showed high odds for having ECS or AB for prolonged labour, 9.2 (1.9 – 46.0) and 6.0 (1.6 – 22.9), respectively. Critically low vitamin D was also found to have an estimated causal effect with high odds for CS, ECS and AB in total.

Table 7.

	Caesarean, total	Emergency caesarean	Emergency caesarean–prolonged labour	Assisted birth, total	Assisted birth – prolonged labour
< 10 nmol/L	3.6 (1.1-11.3)	4.7 (1.5-14.8)	9.2 (1.9-46.0)	3.9 (1.3-11.3)	6.0 (1.6-22.9)
p	.028	.008	.007	.013	.008
Nagelkerke	0.067	0.098	0.165	0.078	0.119

Study IV

The Somali women had, on average, 3.3 years of school education, compared to 13.8 years for the Swedish women. The Somali women's residence time in Sweden was, on average, 3.5 years. Table 1 above shows that consumption of vitamin D rich foods and sun exposure were lower in the Somali women compared to the Swedish women on all parameters. Sub analyses (not in table) by

categorising the participants into a Swedish reference group (Swedish RG = S-25(OH)D > 50 nmol/L), a Somali target group (Somali TG = S-25(OH)D ≤ 50 nmol/L) and a Swedish target group (Swedish TG = S-25(OH)D ≤ 50 nmol/L) showed even greater differences, where intake of milk and cheese was only half in the Somali TG compared to the Swedish RG, and fish intake was also significantly lower. The Swedish TG drank less milk than the Swedish RG but more than the Somali TG. Significant lower exposure to sun on all sun exposure parameters (outdoors for minimum 30 minutes daily in summer, sun exposure to face, neck and forearms in summer, sun vacation in winter time) were seen for the Somali women compared to the Swedish, including veiled clothing among the Somalis.

Table 8 shows vitamin D lifestyle data for the groups before and after intervention at four and ten months, respectively. Somali women altered their lifestyle by almost doubling their intake of milk and fish, and increased their cheese intake. Cheese intake was still significantly increased after ten months. The mean milk and median fish intake were still reported higher at ten months, though n.s. as to Base. The Somali TG showed a trend towards increased sun exposure after four months but did not alter clothing or outdoor lifestyle. No change in lifestyle was seen in the Swedish TG as reported.

Table 8.

	S-25(OH)D ≤ 50 nmol/L at baseline									
	Somali TG					Swedish TG				
	Base	4 m	p	10 m	p	Base	4 m	p	10 m	p
n	50	43		45		39	30		23	
Milk, dl/day, m (Std)	1.8 (2.0)	3.0 (2.0)	0.001	2.6 (2.1)	0.066	3.1 (1.9)	3.4 (1.9)	0.497	3.5 (3.8)	0.972
Cheese times/w, md (IQR)	2.4 (2.5)	3.7 (2.3)	0.006	3.8 (2.4)	0.009	4.2 (2.0)	4.3 (1.7)	0.129	4.2 (2.1)	0.415
Fish times/w, md (IQR)	0.7 (1.0)	1.3 (1.1)	0.027	1.2 (1.0)	0.095	0.8 (0.6)	0.9 (0.6)	0.317	0.9 (0.6)	0.317
Outdoors, n (%)	30 (60)	33 (77)	0.118			37 (95)	28 (97)	1.000		
Veiled clothing (0-2), md (IQR)	1.1 (0.3)	1.0 (0.5)	0.285			0.0 (0.0)	0.03 (0.2)	0.317		
Sun exposure face,neck,forearms, n (%)	12 (24)	17 (40)	0.092			37 (95)	27 (93)	1.000		
Sun vacation in winter, n (%)	1 (2)			2 (4)	1.00	6 (15)			0 (0)	0.250

Two-year follow-up with focus groups interviews – Somali participants

Results from the two focus groups interviews with Somali-born women showed that they, generally, had obtained an understanding of vitamin D, and that they had introduced some lifestyle changes in order to get more vitamin D and, above all, increased their intake of vitamin D rich foods, not only for themselves but also for their families, especially their children. Overall, the interviewed women displayed an acquired knowledge at completion of the study, compared with the non-existent knowledge of food and benefits of sun exposure or of vitamin D and its importance for the body. This was the main findings, but the interviews revealed diverging attitudes towards vitamin D related behaviour regarding sun exposure but none or at least less with regard to food. Most of them, however, seemed to try hard to change behaviour, both personally and in caring for their children, in receiving vitamin D from exposure to sun and being active outdoors and serving food rich in vitamin D and taking a supplement when necessary.

Additional results in thesis

While working on the thesis, I created an equation for the results from the multiple linear regression seen in table 3 in study I in order to test the results of study I on study II [114]. The results from the multiple regression calculation gives us the resulting linear equation for prediction of the outcome: $y = a + b(x) + c(x) + d(x) + e(x) + f$

if a = y-interception, b = the regression coefficient for S-25(OH)D and f = the residual

$$\text{Grip strength (N)} = a + 0.94(x) - 63.9(x) + 2.5(x) + 2.6(x) + f$$

I tested the equation created with the results from study I by using the mean increase from baseline to ten months of S-25(OH)D (23.7 to 55.7 nmol/L) as well as the mean increase of the grip strength (240 to 273 N) for the whole study sample in study II ($n = 71$).

Putting in the S-25(OH)D increase of $55.7 - 23.7 = 32$ nmol/L in the equation

$$\text{Grip strength (N)} = a + 0.94(x) - 63.9(x) + 2.5(x) + 2.6(x) + f$$

gives us a predicted increase of hand grip strength of $0.94 \times 32 = 30$ N.

As one can see, this predicted value is close to the measured value of $273 - 240 = 33$ N in increase. The predicted value missed with 3 N for the whole group.

Or, we could have put the measured grip strength value into the equation and expected S-25(OH)D to have increased in mean $33/0.94 = 35$ nmol/L, which is only 3 nmol/L from the measured value.

Discussion

Main findings

The thesis presents results observed in a cross-section of supposedly normal, healthy, pregnant Somali and Swedish women and new mothers, recruited in antenatal primary care.

Almost all the Somali women in the baseline study turned out to have a vitamin D deficiency, and one-third of the Somali women had critically low vitamin D levels and increased bone degradation with a very high risk of having or developing osteomalacia. One out of ten Swedish women were deficient but never reached critically low levels.

Study I further presented new data in Sweden showing that the vitamin D levels predicted the grip strength, and that the Somali women with critically low vitamin D levels were very weak in their hands and legs compared to the Swedish reference group as well as other references (Grippit) [108].

Study II presented results from an intervention, among the women in the baseline cohort with S-25(OH)D levels ≤ 50 nmol/L, after ten months of prescribed supplementation with vitamin D3 and calcium. Adherence was studied in a 4- and 10-month follow-up, which showed a varying, but on the whole, good adherence to recommended supplementation in the target group of Somali women. Vitamin D deficiency was efficiently reversed following advice and supplementation of vitamin D3 and calcium, and a decrease of bone degradation markers was seen already after four months.

Improved grip and leg strength was found, and it was predicted by the amount of supplements taken as shown after ten months.

The thesis concludes with the observation that low vitamin D predicted muscle weakness and that low vitamin D was associated with bone degradation, on average, whilst supplementation reversed the deficiency and predicted increased strength. The findings are coherent with current knowledge on the role of vitamin D for absorption of calcium and musculo-skeletal health. Furthermore, the observations showed clinically relevant signs of possible osteomalacia. It therefore identifies pregnant Somali women as a high-risk group for the disease and ends the discussion of whether there is any relevance of the low vitamin D levels observed in this group of immigrant women or not.

In study III, we aimed to find out if this high-risk group for osteomalacia (pregnant Somali women), with risk of muscle dysfunction also had a higher rate of prolonged labour and increased need of assisted births. Higher rate of

emergency caesarean sections had been observed in the Somali group of women in the same county throughout the last decade before the study.

We found an association between critically low vitamin D and emergency caesarean sections for prolonged labour in the cohort.

By building a directed acyclic graph together with several experts in different fields, we were able to identify confounders sufficient to close biasing paths in order to achieve exchangeability between the exposed and non-exposed groups. The results of the following odds regression calculations showed a causal effect of critically low vitamin D on the outcomes caesarean section, emergency caesarean section and assisted birth. The highest odds however, was found for the outcome assisted birth, especially emergency caesarean sections caused by prolonged labour, estimated to six and nine times, respectively.

Study IV shows that a simple intervention, similar to a regular clinical setting in primary care, may result in change of lifestyle associated with vitamin D and in increased knowledge and altered attitudes in the long-term. This is very interesting since it may reduce the birth complications due to low vitamin D, since the complications associated with vitamin D were only found in the group of critically low vitamin D, which seems very possible to treat according to the results of paper two.

What is new?

Study I was the first Swedish study to show that Somali pregnant women and new mothers living in Sweden, not only had very low vitamin D levels but had clinical findings of muscle weakness and degrading bone associated with the low levels of vitamin D.

In study II, we were the first in Sweden to show that prescribed supplementation of vitamin D₃ and calcium was an effective way to treat clinical signs of severe vitamin D deficiency with symptoms of osteomalacia, among newly immigrated and low educated pregnant Somali women and new mothers.

Study III is the first study on birth outcome for women with critically low vitamin D levels. It is one of the first to isolate prolonged labour from all-cause dystocia as a cause of assisted birth due to vitamin D deficiency. It is again the first observational study on the birth outcomes, assisted birth and emergency caesarean section, to estimate a causal effect of vitamin D on the outcome, by presenting a directed acyclic graph and identifying confounders sufficient to close biasing paths in order to achieve exchangeability between the exposed and non-exposed groups and thereby dealing with the ethical dilemma of not treating pregnant women. The paper therefore presents a unique way of estimating the causal effect of vitamin D on the complicated birth outcomes of CS, ECS, and assisted birth for prolonged labour in a difficult ethical situation where an RCT study is not feasible.

Study IV is a unique study of an intervention on lifestyle associated with vitamin D, among Somali pregnant women living in Sweden, due to its mixed method with long-term results on the intervention and on the participants' attitudes after as long as two years. It also validates the results of the questionnaires used.

Strengths and limitations

These observational studies with a large part of retrograde inclusion made it possible to greatly eliminate two very important confounders to the studies. The first is the effect of sunlight, which is the strongest confounder to vitamin D levels known, which may double the levels in summer compared to winter. Secondly, it gave us the opportunity to study women that had not been part of an intervention with deliberate supplementation of vitamin D levels. Considering the very low levels found among the Somali women, this would have been impossible in a prospective study due to ethical reasons.

Furthermore, retrograde inclusion also blinded the obstetricians who decided on the diagnoses since they were not aware of the study coming. Yet, we also wanted to study pregnant women, which is why we decided to include women that were pregnant at the time of the baseline study. This gave us the unique cohort with both pregnant and new mothers where many were lactating, which puts higher demands on the need for vitamin D and calcium.

The design has its flaws and advantages, but is a deliberate choice in order to be able to take all blood samples in a short period of time in an area where the number of pregnant Somali women is limited. This gave us a situation that is similar to real life situations in the clinics in primary healthcare. The material became heterogeneous, which posed a difficulty for the interpretation of the results. However, at the same time, they were similar to the situation in primary care with different ethnicities. Therefore, the results of our study may be transferable to other clinical situations. The reader needs to observe that the heterogeneity and rather few birth outcomes limited the power in our studies and partly explains the wide confidence intervals. The results therefore need to be interpreted with caution. Again, one should remember that the heterogeneity resembles real life in the clinic, and also made it possible to find this high-risk group of Somali women, and to relate the outcomes to a reference group and to see the clinical consequences.

The discussions through the thesis have partly involved the discussion around the mechanisms between vitamin D and muscle weakness. This is, of course, of major interest since it may indicate that we can find vitamin D deficiency even before osteomalacia has presented, if vitamin D has a direct impact on muscle cell function, which current literature and this thesis implicates. Muscle weakness among risk individuals, therefore, needs special attention. Measuring of vitamin D levels among these individuals is strongly recommended as a conclusion of this thesis.

Yet, we know that low calcium levels can cause osteomalacia and major muscle dysfunction, which may explain the positive findings of the treatment in our second study, where we prescribed both vitamin D and calcium supplements. The calcium deficit may be hidden in individuals with normal serum levels of calcium but visible through raised PTH and decreased vitamin D

levels. Actually, the serum calcium levels were equal and normal for both Somalis and Swedish women in the baseline study. We were surprised to not find a linear association between the increase of S-25(OH)D and the increase of strength in study II. While working with the thesis, I created a simple mathematical trial with prediction, by using the adjusted results from the multiple linear regression calculation in study I, which nicely predicts the results for the whole sample in study II. Results regarding the relation between S-25(OH)D and grip strength turned out to be very similar after building a DAG and re-evaluating our findings from study I.

Nonetheless, we did also prescribe calcium in the supplementation process, which may be a very important explanation for the results, since these women probably needed calcium as seen in the raised PTH levels. This may also be the reason why we seem to have succeeded in our treatment compared to, for example, a Norwegian study [115]. However, it seems to have taken quite some time as well, since grip strength had not increased much at four months, which might give support to the hormonal effects of vitamin D. The Norwegian study had supplemented with only vitamin D3 without calcium. The Norwegian study was also shorter (16 weeks). It seems realistic that the results of supplementation with the precursor to the steroid hormone calcitriol would take time, since the potential effect of, for example, doping with anabolic steroids obviously also takes time.

These results of the trial of prediction in the thesis, by using the results from study I to be able to predict the results of study II, may be seen as a validation of the methods and results of the first two studies.

Certainly, there can be many other explanations than vitamin D for the results in such complicated matters as birth outcomes [116]. For example, the Somali women sometimes try to consciously keep the foetus slim by not eating so much, believing that she will have an easier birth [117]. Lower intake of food could decrease her muscle strength and perhaps also her S-25(OH)D. Here again, we found another strength of the study by using the DAG. We can see from the DAG that food intake affects the S-25(OH)D levels, the outcome and BMI and is identified as a confounder. Food intake is the ancestor of BMI in the DAG, and BMI is corrected for in the calculations according to the minimal set of covariates for adjustment.

The DAG was essential for describing and discussing the multitude of tentative reasons for the birth outcomes and their relation to vitamin D, such as twin birth, breech position, induction, comorbidity and more. This was done in order to establish a direction of the effects of the covariates in order to avoid confounding and reversed causality, but also to not adjust for mediators of the effects of S-25(OH)D. Adjusting for mediators would have caused an underestimation of the critically low vitamin Ds effect on the outcome.

Reasons for assisted births are many, and the process of birth is intricate and very complex. Yet, in the end, crude things such as muscle forces, positions and sizes rule out minor reasons. Most women experience how the nature forces of their bodies take over in the moment of giving birth.

This makes us come back to basic questions, such as the ones the author first posed to himself at the clinic. What about if this seemingly great muscle weakness affects the birth outcome? This is the finding in our study, and the estimated effect of critically low vitamin D is measured and expressed in the increased odds and Nagelkerke R² values, which are our best estimates so far. The wide confidence intervals have to be considered for transversability but may, at least partly, be explained by the low number of participants and the few outcomes. Nonetheless, the findings of the birth outcomes are significant and need to be addressed.

Study IV is a mixed methods study on lifestyle intervention. Questionnaires were mostly filled in (with fewer exceptions) before the research doctors saw the patients at the 4- and 10-month follow-ups; thus, the researchers were blinded to the results. The major weakness of the study is the lack of a pure control group and that it may suffer from responder or recall bias and also a selection and observer bias in the focus group interviews. Nevertheless, the participation and responses from the subjects were seemingly trustworthy. Also, the findings in the analysis of the focus group interviews validate the findings of the quantitative part of the results from the questionnaires, which is also a known strength of a mixed method study [118].

Future studies

The findings of the thesis turned out to fit the authors' first clinical observations and concerns about birth outcomes. Yet, it is still intriguing since there is (always) an amount of uncertainty around the results even if it is now on another level of understanding.

We hope to do larger studies in the future with regard to birth outcomes and vitamin D but also include several groups of women to reach higher transversability. It would also be interesting to study the lifestyle associated with vitamin D versus birth outcome.

We also have gathered data on pain from pain drawings and measures in the questionnaires in the cohort from baseline and at follow-ups, which we intend to study further. Another idea is to also do a 10-year follow-up on lifestyle and S-25(OH)D in the cohort, with the same methods used in the baseline study.

Conclusions of the studies

Study I

The Somali women had undetectable or very low S-25(OH)D concentrations and prominent muscular weakness in hands and upper legs, where grip strength was strongly associated with the low S-25(OH)D concentrations.

Study II

Adherence to vitamin D and calcium supplementation should be encouraged, as an even moderate intake was associated with improved grip strength and upper leg performance, which was particularly useful for the women with critically low S-25(OH)D deficiency and poor physical performance at baseline.

Study III

Critically low vitamin D levels had an estimated causal effect on caesarean sections, probably through the mechanism of prolonged labour due to global muscle weakness. Female patients in fertile ages with signs of poor muscle strength and at risk for vitamin D deficiency should be paid special attention in antenatal and primary healthcare.

Study IV

Doctors' simple intervention pertaining to lifestyle seems to have contributed to a positive lifestyle change and change of attitudes associated with vitamin D in pregnant Somali and new mothers living in Sweden. Brief oral information and illustrations about vitamin D given by doctors likely contributed to a change towards healthier food patterns and attitudes towards sun exposure, both on short- and long-term among the Somali women with particularly low vitamin D levels and no prior knowledge. The effect of having a Somali nurse interpreter was not evaluated but could be of considerable importance.

Conclusions of the thesis and clinical and social implications

Deficient levels of vitamin D were very common in a risk group of Somali pregnant women and new mothers with vitamin D deficiency. Blood tests showed a high risk of developing osteomalacia in this group of women. Osteomalacia is known to cause muscle weakness, pain, cramps and even death, following seizures or heart fibrillations.

Many of the women had critically low levels of vitamin D and were not just very weak in their hands and legs but were also found to have a much higher risk of prolonged labour, leading to assisted birth and emergency caesarean sections. The results showed that it was only the group with critically low vitamin D levels that seemed to have higher odds of suffering from this increased risk of birth complications.

Some risk factors for prolonged labour and caesarean sections such as height, age and origin are not possible to alter. Lifestyle, however, can be altered and is obviously very important for the vitamin D levels. We showed that the women in study II could increase their vitamin D levels by following lifestyle advice and by taking supplement. Those who complied with supplementation with vitamin D and calcium were also found to have increased muscle strength.

Furthermore, in study IV, we have documented that the women could change their lifestyle, following a simple lifestyle intervention, in a primary healthcare like setting, and that their attitudes had changed and become more positive towards a vitamin D supporting lifestyle over time.

This thesis therefore implicates that sun exposure, eating vitamin D rich foods and taking vitamin D and calcium supplements may decrease an increased risk for birth complications in a high-risk group of women, since this increased risk was found only among women with critically low vitamin D levels. The pregnant women may also benefit from increased strength and health, which also includes the native Swedish born women. Prevention with vitamin D lifestyle advice is a primary healthcare issue and is supported by this thesis.

The **authors' advice** is that the subject of vitamin D and lifestyle should not only be taught in primary- and antenatal care but also in schools from early childhood. Lifestyle associated with vitamin D is something that may concern all of us, due to low sun exposure, for example, when working long office

hours, attending school during summer in Nordic countries, engaging in excessive computer gaming, using sunscreen, wearing veiled clothes and also when having special diets like vegan food.

Sammanfattning på svenska

Långvarig D-vitaminbrist är en vanlig orsak till osteomalacia på grund av minskad kalciumabsorption från tarmen. Sjukdomen osteomalacia upptäcks ofta på grund av muskelsvaghet, smärta, uppmjukning av skelettet med benbrott, kramper och kan till och med orsaka död på grund av lågt calcium i kroppen genom krampanfall eller hjärtrytmrubbning.

Författaren fann i en pilotstudie att många somaliska kvinnor hade D-vitaminbrist och var mycket svaga. Detta ledde till utformningen av ett projekt för att besvara frågan vilka D-vitaminnivåer gravida somaliskor och nyblivna mödrar hade i vår region samt att jämföra med svenskor och ta reda på om en eventuell svaghet berodde på D-vitaminbrist. Om så, skulle det då vara möjligt att behandla D-vitaminbristen och svagheten med livsstillrådgivning och tillskott av D-vitamin och kalcium? Några deltagare i pilotstudien var gravida. Bristen och svagheten var så uttalad i pilotstudien att frågan också uppkom ifall de somaliska kvinnorna var mer utsatta för allvarliga förlossningskomplikationer på grund av värksvaghet på grund av generell muskelsvaghet? Ett långsiktigt övergripande mål med projektet var att hitta sätt att förbättra primär- och förlossningsvård för alla kvinnor med D-vitaminbrist och sätt att förbättra livsstil som gynnar hälsan hos kvinnor och deras familjer.

Studie 1 syftade till att studera serum 25-hydroxyvitamin D-nivåerna i ett tvärsnitt av annars vanliga kvinnor från Somalia och Sverige som bodde i staden Borlänge i Sverige. Studien syftade också till att ta reda på om D-vitaminnivåerna var relaterade till muskelstyrkan. Resultaten visade att 90% av de gravida och nyblivna mammorna av somalisk härkomst som rekryterats i mödravården hade drabbats av D-vitaminbrist och att en tredjedel hade kritiskt låga nivåer och uttalade tecken på muskelsvaghet och skelettnedbrytning. Även tio procent av de svenska kvinnorna hade D-vitaminbrist men ingen hade kritiskt låga nivåer.

Studie 2 två syftade till att studera resultaten av livsstillrådgivning och tillskott på D-vitamin och kalk till de kvinnor som hade S-25(OH)D-nivåer ≤ 50 nmol/L vid tillfället för basundersökningen, studie 1. Resultaten visade en god följsamhet till intag av tillskott av D-vitamin och kalk bland de somaliska kvinnorna. Intaget av D-vitamin och kalcium hade reverserat D-vitaminbristen redan efter fyra månader och hade minskat skelettnedbrytningen samt ökat styrkan. Styrkan fortsatte öka och var högre efter tio månader. Resultaten visade att intaget av mängden av tillskott predicerade (förutsade) styrkeökningen hos kvinnorna efter tio månader.

Studie 3 syftade till att studera om mycket låga D-vitaminnivåer hade påverkat behovet av kejsarsnitt, akut kejsarsnitt och instrumentellt assisterade förlossningar på grund av värksvaghet. En kohort bestående av kvinnorna från basundersökningen i studie 1 studerades avseende deras förlossningsjournaler som var detaljerade och inkluderade deras förlossningsutfall och orsakerna till dessa. Förlossningsutfallen visade sig vara relaterade till D-vitaminnivåerna.

En ny metod, innefattande en så kallad riktad acyklisk graf, användes för att identifiera faktorer nödvändiga att justera för i de statistiska analyserna, för att undvika resultat orsakade av andra faktorer än låga D-vitaminnivåer. Studien kunde mäta orsakseffekten av kritiskt låga D-vitaminnivåer på utfallet kejsarsnitt totalt samt på instrumentellt assisterad förlossning samt akut kejsarsnitt på grund av värksvaghet, som visade sig ha de ökade oddsen fyra, sex och nio mot ett för att drabbas. Resultaten pekar på att värksvaghet på grund av allvarlig D-vitaminbrist är en viktig riskfaktor för allvarlig komplikation till graviditeten.

Studie 4 syftade till att undersöka om rådgivning angående livsstil associerad till D-vitamin, så som solexponering, klädsel och livsmedel rika på D-vitamin, som gavs vid basundersökningen i studie 1, kunde ha påverkat livsstilen bland deltagarna som sedan undersöktes vid uppföljningarna i studie två. Studien omfattade även fokusgruppintervjuer efter två år. Resultaten visade att de somaliska kvinnorna hade ändrat sin livsstil efter fyra månader mot en livsstil som ger högre D-vitaminnivåer i kroppen genom ökat intag av livsmedel rikt på D-vitamin och också en tendens att öka solexponeringen men ingen förändring av klädseln sågs. Kunskap om livsstil kring D-vitamin och nya attityder i detta avseende dokumenterades i fokusgruppsintervjuerna.

Sammanfattningsvis befanns D-vitaminbrist vara mycket vanligt bland gravida somaliska kvinnor och nyblivna mödrar och också ganska vanligt bland de svenska. Greppstyrkan visade sig prediceras (förutsägas) av D-vitaminnivåerna. Kritiskt låga D-vitaminnivåer och tecken på osteomalacia, såsom muskelsvaghet och bennedbrytning, var mycket vanligt bland de somaliska kvinnorna. De somaliska kvinnorna bör därför betraktas som en högriskgrupp för osteomalacia. De visade sig också ha en hög risk för att få akut kejsarsnitt på grund av värksvaghet vid kritiskt låga D-vitaminnivåer, då ett mått på ett kausalsamband mellan kritiskt låga D-vitamin nivåer och utfallet kejsarsnitt på grund av värksvaghet kunde beräknas, med mycket höga odds för akut kejsarsnitt.

Vi fann glädjande nog att det var möjligt att öka kunskapen om, främja attityder och förbättra en D-vitaminstödande livsstil. Detta uppnåddes i en primärvårdsliknande situation med enkla råd muntligt och visuellt, blodprovtagning samt D-vitamin- och kalktillskott.

Vi fann också att följsamheten till att använda tillskott var bra bland de somaliska kvinnorna. Vidare fann vi att intaget av antalet tillskott (en kombination av D-vitamin och kalk i tablettform) predicerade (förutspådde) ökad styrka.

Interventionerna i projektet påverkade livsstilen positivt, ökade D-vitaminhalterna, styrka och minskade bennedbrytningen hos somaliskorna vilket antyder att det kan vara möjligt att också förhindra svåra förlossningsutfall på grund av kritiskt låga D-vitaminnivåer och värksvaghet. Detta kan troligen uppnås genom att främja D-vitaminsstödjande livsstil och öka D-vitaminnivåerna i gruppen av kvinnor med kritiskt låga D-vitaminnivåer. Vi hoppas kunna testa denna hypotes i framtida studier.

Avhandlingen drar slutsatsen att en livsstil som påverkar D-vitaminnivåerna positivt är något som berör många och kan förbättra hälsa, styrka och kanske även förlossningsutfall vid graviditet. Det är viktigt att undersöka individer i primär- och mödravården med muskelsvaghet och riskfaktorer för D-vitaminbrist och särskilt i högriskgruppen av gravida somaliska kvinnor och nyblivna mödrar.

Livsstil förknippad med D-vitamin är något som kan beröra oss alla på grund av för låg solljusexponering vid långa arbetsdagar på kontoret, skolgång på sommaren, överdrivet datorspelning eller användning av solskyddskrämer, täckande klädsel och utbredda tatueringar men också på grund av specialdieter som vegankost.

Författaren föreslår att förmedling av kunskapen om D-vitamin inte bara bör ske i primär- och mödravården, utan också bör läras ut till de nya generationerna redan i skolan.

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