Vitamin D in Somali women living in Sweden

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Abstract

Introduction: Sunlight is the major source of vitamin D synthesis. Information regarding vitamin D, bone status and general health in Somali women at latitude 0-10° North now living in Sweden at 57° North, is limited. Vitamin D-binding protein (DBP) is the major carrier of most vitamin D metabolites. It is not clear whether the amount of DBP is genetically determined or influenced by external factors.

Aims: To characterize vitamin D status, the effect of vitamin D treatment and possible comorbidity in Somali women living in Sweden.

Methods: Somali women (n = 114), age range 18-56 years, residing in Sweden for at least 2 years (range 2-23) were recruited on a voluntary basis. They were randomized to different treatment arms, vitamin D drops 800 IU, 1600 IU or placebo daily, and UVB light or Woods lamp (placebo light). Blood samples were collected at start and every six weeks during the intervention (three months) and the follow-up period (three months). Bone Mineral Density (BMD) and Health Related Quality of Life (HRQoL) were examined. A random population sample from the WHO MONICA study, Gothenburg, was used as controls.

Results: Vitamin D deficiency; *i.e.,* serum (S)-25(OH)D < 25 nmol/l, was found in 73% of the Somali women. S-25(OH) D increased dose dependently compared with placebo. At least 1600 IU of vitamin D3 daily was needed to raise the levels of S-25(OH)D to a sufficient range ($\geq$ 50 nmol/l). S-DBP was lower in Somali women than in native Swedish women, < 2% of whom had vitamin D deficiency. There was a positive correlation between S-25(OH) D and S-DBP values in Swedish women. S-DBP was not affected by age or sex, and not by vitamin D treatment in the Somali women.

The Somali women had lower lumbar BMD values than white American women and both lumbar and femoral BMD were lower than in African-American women, using the reference provided by the dual energy X-ray absorptiometry manufacturer. Comorbidity, such as hypothyroidism, diabetes mellitus, vitamin B12 deficiency and hypertension, was similar in Somali women and native Swedes. However, the use of allergy medication was higher, fractures and HRQoL, especially the physical component, were lower than in native Swedish women.

Conclusion: Vitamin D deficiency was common, 73%, in Somali women living in Sweden. Vitamin DBP and BMD were lower than in controls, but fractures were rare. S-DBP was unaffected by vitamin D treatment. At least 1600 IU vitamin D per day was needed to reach sufficient levels of S-25(OH)D. It is important to follow the Somali population at northern latitudes in order to prevent osteomalacia.

Sammanfattning på svenska

Bakgrund: Solljus är den viktigaste källan till D-vitamin hos människor. D-vitaminbrist förekommer ofta hos invandrare med mörkt hudpigment som lever på högre breddgrader med mindre solexponering. Informationen om D-vitamin-nivåer och generell hälsa hos somaliska kvinnor i Sverige är begränsad. Vitamin D-bindande protein (DBP) i blod (serum = S) är den främsta bäraren av de flesta vitamin D-metaboliter. Huruvida DBP påverkas av yttre faktorer är inte helt klarlagt.

Syfte: Att studera vitamin D-halten, effekten av vitamin D-behandling och eventuell samsjuklighet hos Somaliska kvinnor i Sverige.

Resultat: Vitamin D-brist (S-25(OH)D < 25 nmol/l) konstaterades hos 73 % av somaliska kvinnor (n = 114) boende i Sverige (Göteborg och Borås). Dessa kvinnor deltog i en placebokontrollerad studie med D-vitamin droppar (800 IU, 1600 IU respektive UVB-ljus och placebo dagligen) D-vitaminhalten ökade dosberoende jämfört med placebogruppen. Minst 1600 IU vitamin D3 behövdes för att höja S-25(OH)D till tillräckliga nivåer (≥ 50 nmol/l).

Somaliska kvinnor hade få frakturer, men lägre bentäthet i rygg och höfter jämfört med vita amerikanska och afroamerikanska kvinnor som utgjorde benmätarens (DXA) referensgrupper.

Både S-DBP och S-25(OH)D var lägre hos somaliska kvinnor än hos infödda svenska kvinnor i ett slumpvist befolkningsurval där < 2% hade vitamin D-brist. S-DBP var positivt relaterat till vitamin D status hos svenska kvinnor. S-DBP påverkades inte av vitamin D behandling hos somaliska kvinnor. De hade inte fler andra sjukdomar än infödda svenskar men de skattade sin fysiska hälsorelaterade livskvalitet lägre och använde mer allergimediciner än kvinnor med svenskt ursprung.

Sammanfattning: D-vitaminbrist var mycket vanligt hos somaliska kvinnor, 73%. Bentätheten var lägre hos somaliska kvinnor än hos både vita och svarta referenspersoner. S-DBP var lågt och påverkades inte av behandling, medan S-25(OH)D steg dosberoende vid vitamin D respektive ljusbehandling. Minst 1600 IU vitamin D dagligen rekommenderas till somaliska kvinnor i Sverige. Långtidseffekterna av vitamin D-brist är okända.

List of papers

I. Osmancevic A, Demeke T, Gillstedt M, Angesjö E, Sinclair H, Abd El-Gawad G, Landin-Wilhelmsen K. Vitamin D Treatment in Somali Women Living in Sweden - Two randomized, placebo-controlled studies. *Clin Endocrinol (Oxf). 2016 May 7. doi: 10.1111/cen.13097. Epub 2016 Jun 2.*

II. Demeke T, El-Gawad GA, Osmancevic A, Gillstedt M, Landin-Wilhelmsen K. Lower bone mineral density in Somali women living in Sweden compared with African–Americans. *Arch Osteoporos. 2015 Dec;10(1):208. doi: 10.1007/s11657-015-0208-5. Epub 2015 Feb 19.*

III. Demeke T, Gillstedt M, Osmancevic A, Krogstad A-L, Sinclair H, Angesjö E, Abd El-Gawad G, Landin-Wilhelmsen K. Vitamin D-binding protein in Somali women living in Sweden was low and unaffected by treatment. *J Prim Care Gen Pract 2017 Vol 1, Issue 1, allied academies; 1-7.*

IV. Demeke T, Osmancevic A, Gillstedt M, Krogstad A-L, Angesjö E, Sinclair H, Abd El-Gawad G, Krantz E, Trimpou P, Landin-Wilhelmsen K. Comorbidity and Health related Quality of Life in Somali women living in Sweden. *Revised and resubmitted Scand J Prim Health Care*

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Abbreviations

BMD = Bone Mineral Density
BMI = Body Mass Index
CI = Confidence Interval
CV = Coefficient of Variation
DBP = Vitamin D-Binding Protein
D2 = Ergocalciferol
D3 = Cholecalciferol
DEQAS = Vitamin D External Quality Assessment Scheme
DXA = Dual-energyX-ray Absorptiometry
EQ-5D = EuroQoL-5 dimensions
7-DHC = 7-dehydrocholestrol
25(OH)D =25-hydroxyvitamin D
1, 25(OH)2D = 1,25-dihydroxyvitamin D
3-epi-25OHD = 3-epi-25-hydroxyvitamin D
LC-MS/MS = Liquid chromatography- tandem mass spectrometry
N = North
PTH = Parathyroid Hormone
RANKL = Receptor-Activated Natural Killer Ligand
S = Serum
SF-36 = Short Form-36
UVA = Ultraviolet radiation A
UVB = Ultraviolet radiation B
UVR = Ultraviolet Radiation
VAS = Visual Analogue Scale
VDR = Vitamin D Receptor
WHO MONICA = World Health Organization, MONItoring of trends and determinants for CArdiovascular disease

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INTRODUCTION

Sun exposure is the major source of vitamin D. It is also called the sunshine vitamin. Few food items contain vitamin D naturally (1-3). Adequate levels of vitamin D are maintained through its cutaneous production (photosynthesis) and through oral ingestion. Vitamin D deficiency is considered a global pandemic (4,5). It is estimated that one billion people worldwide are vitamin D-deficient and insufficient (4). The main cause of vitamin D deficiency is the lack of sunlight exposure.

Vitamin D is a potent hormone and plays an important role in the development and maintenance of bone health throughout life (6,7). It has also been associated with a range of acute and chronic diseases, such as diabetes mellitus, cardiovascular diseases, and autoimmune disorders (8-10).

The aim of this thesis was to assess the prevalence of vitamin D deficiency in a high-risk population from Somalia at latitude 0-10° North (N). Our hypothesis was that Somali women who migrated from lower latitudes around the equator to northern latitudes in Sweden, 57° N, may exhibit a high prevalence of vitamin D deficiency which might be associated with different diseases. Diffuse muscle pain, muscle weakness in proximal muscle groups, and muscle atrophy, causing reduction in speed performance, are some of the manifestations of vitamin D deficiency.

Historical perspective

When a predominantly agrarian population started moving to growing, smog-filled industrial cities to find work, a mysterious disease, known as rickets, began to affect the children of these industrial workers, (11-13). This disease causes bone deformities and painful spasms, followed by difficulty of breathing and nausea (11, 14).

In the mid of 17th century, two physicians, Daniel Whistler and Francis Glisson, (Figure 1 and 2), gave the first complete description of rickets (15). They did not, however, recognize the role of nutrition.
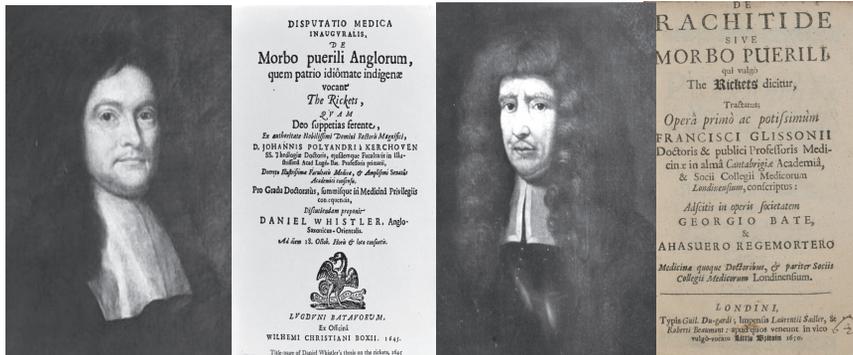

Figure 1 and 2. Daniel Whistler, to the left, wrote his thesis on the disease of English children later known as rickets (1645) and Francis Glisson, to the right, wrote "De rachitide" (1651).

A Polish physician, Jedrzej Sniadecki, observed the geographical distribution of rickets in and around Warsaw (2). Children living in the city were more prone to develop the disease than children living in the outskirts or in the rural areas outside the city. He drew the conclusion that the disease was linked to the lack of exposure to sunlight. Theobald Palm, a medical missionary, discovered the relationship between the geographic distribution of rickets and the amount of sunlight available in the region through correspondences with colleagues and friends in India and China. Children who lived there were free of rickets (16,17). In 1913, based on experiments with lactating goats, a direct link between sunlight and rickets was established by Professor Steenbock (2, 18). Goats kept indoors developed a substantial loss of skeletal calcium whereas the goats exposed to sunlight (outdoors) did not. In 1919, Dr. K Huldschinsky cured children of rickets by using artificial ultraviolet radiation (18). Two years later, Drs. Alfred F.

Hess and L.F Unger, treated children with rickets simply by exposing them to sunlight (19).

Dr. Edward Mellanby explored a nutritional causative factor of rickets. He induced rickets in dogs (by keeping them indoors) and then cured them by feeding them cod-liver oil (20). He credited the vitamin A in the oil with the cure. In 1922, Dr. MacCollum, destroyed the vitamin A in cod-liver and showed that the anti-rachitic effect remained (21). He dubbed the substance vitamin D, according to the designation of vitamins in alphabetical order.

Two scientists, Mildred and Alfred Hess, independently discovered that irradiated foodstuffs possessed anti-rachitic effects (19). The chemical makeup of vitamin D (ergocalciferol or the irradiated form in foods, mainly plants and fungi) was defined by E.A. Askew in 1931 (20). The structure of 7-dehydrocholesterol and vitamin D3 was identified by Dr. Windaus in 1936 (20). The active vitamin produced in the liver was first identified by DeLuca in 1968 (22).

In the 1970s, the final active form of vitamin D was recognized, and the vitamin was reclassified as a hormone regulating the calcium metabolism. A protein receptor that binds vitamin D was confirmed in the nucleus of cells in the intestine by Haussler (23). In the 1980s, the topical administration of vitamin D hormone and its remarkable effect on psoriasis was demonstrated by Professor M.F. Holick and co-workers (24).

Sources of vitamin D

Solar ultraviolet radiation B (UVB) is the major source of vitamin D (12). The zenith angle of the sun and skin pigmentation determine the level of cutaneous vitamin D synthesis. The solar zenith angle, Figure 3 A, in turn depends on the latitude, season and time of day (25). Figure 3B shows the "the shadow rule" (26). When the incident angle is flattened, the skin production of vitamin D is significantly reduced. One minimal erythema

dose leading to pink coloration of the skin 24 hours after exposure, can be compared to oral intake of 10,000-25,000 IU of vitamin D (27,28). Sunlight at latitudes above and below approximately 35°N and South, respectively, during most of the winter do not lead to any vitamin D synthesis (29).
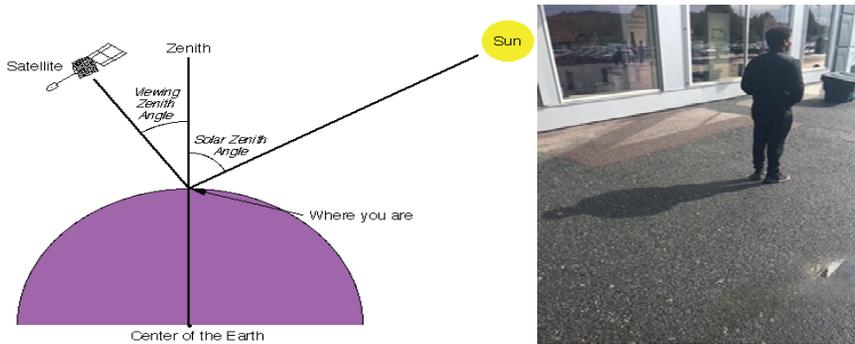

Figure 3 A and B.
The solar zenith angle (time of the day, latitude and seasons affect the zenith angle of the sun) to the left and "the shadow rule" to the right which implies that if the shadow of an individual is longer than her/his height, vitamin D synthesis in the skin will be impossible (26).

The skin pigment in humans, melanin, absorbs UVB photons, thereby inhibiting the synthesis of previtamin D from 7-dehydrocholesterol through photolysis (25). People living at high latitudes become increasingly dependent on dietary vitamin D intake due to the decreased solar intensity. Foods containing vitamin D naturally like oily fish, oils of the liver and sun-exposed mushrooms are very limited. Further irradiation of mushrooms, however, increases the vitamin D2 levels. Fortification of foods (milk, margarine, yoghurt) was introduced to enhance their vitamin D status. Oral supplements are available over the counter in both D2 and D3 forms.

Skin types

Skin types affect the cutaneous production of vitamin D. Blacks need 5 to 10 times more sun exposure than whites to produce the same amount of

vitamin D. This is due to higher melanin pigmentation (30), (Figure 4), as 90-95 % of the vitamin D comes from sunlight exposure (4,31). Only UVB can induce vitamin D synthesis and is available between 10 a.m. and 3 p.m., the same period of the day when it is recommended to avoid exposure to the sun. When UVB rays strike the earth obliquely, due to the increased zenith angle of the sun, more and more of these photons are absorbed by the ozone layer (26). The time of day, latitude and seasons affect the zenith angle of the sun and with that, the availability of UVB. The number of photons that reach the earth at latitudes above 35° N and below 35° South during the winter is limited so that very little if any vitamin D is synthesized (26,29,30). According to "the shadow rule", the UVR intensity is inversely proportional to the length of a person's shadow. If a person's shadow is longer than her/him, vitamin D will not be produced (26).

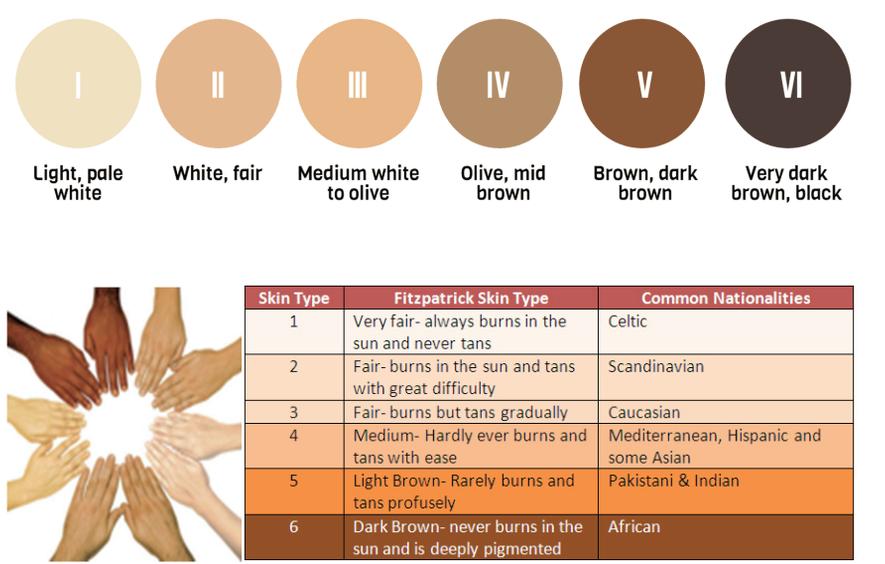| Skin Type	Fitzpatrick Skin Type	Common Nationalities
1	Very fair- always burns in the sun and never tans	Celtic
2	Fair- burns in the sun and tans with great difficulty	Scandinavian
3	Fair- burns but tans gradually	Caucasian
4	Medium- Hardly ever burns and tans with ease	Mediterranean, Hispanic and some Asian
5	Light Brown- Rarely burns and tans profusely	Pakistani & Indian
6	Dark Brown- never burns in the sun and is deeply pigmented	African

Figure 4. Skin phenotypes, erythema and tanning reactions determine skin phenotypes I-VI table.

Poor vitamin D status was observed in some sunny countries (32) too, mostly among women and girls, due to insufficient irradiation of the skin through covering 95% of the body with traditional attire (33).

Sunscreens are widely used to block UVB from reaching the skin, because of concerns related to skin cancer and skin damage caused by exposure to sunlight (34).

Vitamin D synthesis and metabolism

In the skin

The cutaneous production of vitamin D is initiated as solar photons (UVB 290-320 nm, peak at 300 nm), (Figure 5 A and B) penetrate the epithelial layers of the skin and convert 7-dehydrocholecalceferol into previtamin D3 (34,35), (Figure 6), which undergoes thermal isomerization (rearrangement of atoms in the molecule) to form vitamin D3. Ultraviolet light is divided into three bands or ranges of wave length (Figure 5 A and B).

UVA (320-400 nm), known as the "tanning ray" is responsible for skin pigmentation and ageing (36). Exposure to this ray does not result in skin burn; (37). UVB is the primary cause of sunburn, stimulating the production of vitamin D (38). It is also involved in the production of melanin, which protects against UVB (39). UVC (<290 nm), the shortest of the bands, is the most energetic form, which can burn the skin very rapidly (40). It is, however, entirely absorbed by the ozone layer. The quantity of UVB photons and the amount of epidermal cholesterol and skin pigment (28, 34) influence the cutaneous synthesis of pre vitamin D3. The amount of epidermal cholesterol decreases later in life, and exposure to optimal UVB will not result in adequate production of vitamin D. Limited outdoor activity and the decline of the cutaneous precursor, decrease the vitamin D production which is troublesome in elderly patients (41). Institutionalized subjects often exhibit low vitamin D status.
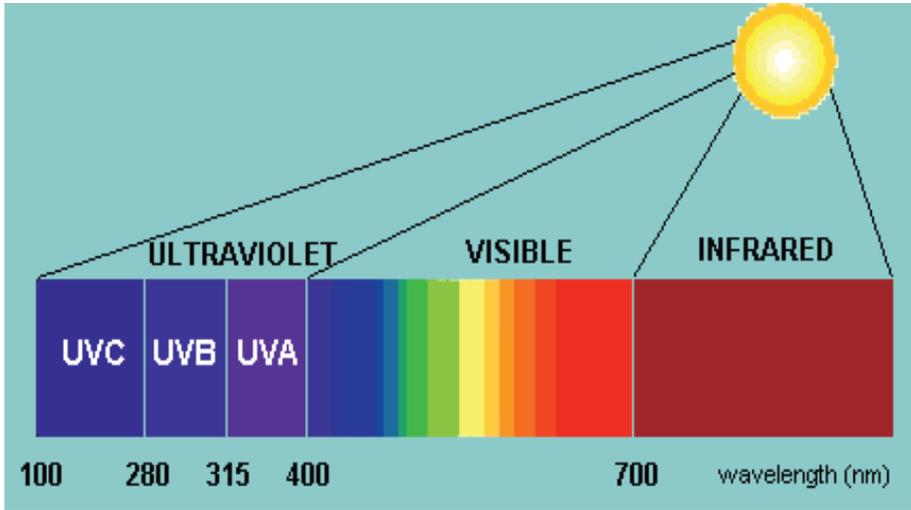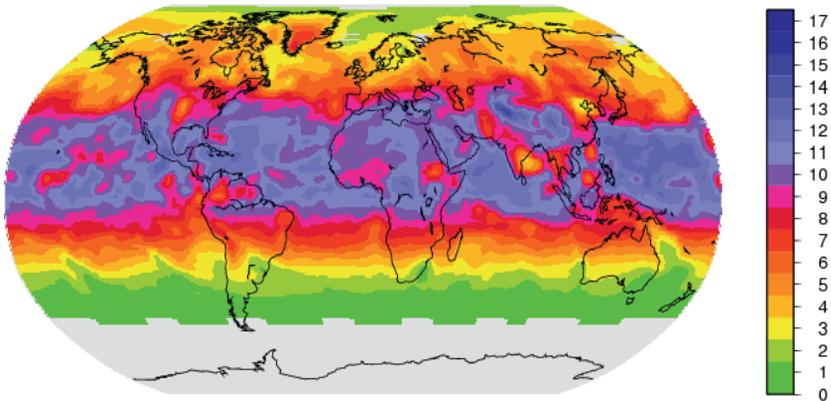
UV Record – Daily Maximum UV Index 2007−06−27

Figure 5 A and B. Solar radiation spectrum of light (5 A) and world UV index, a global distribution index, registered in the month of June 2007 (5 B). A UVI >11 is classified as extremely high and an index between 2 and 3 are referred to as low. The International Satellite Cloud Climatology Project (ISCCP).

Vitamin D can also be obtained through the diet (fish liver oils, egg yolks, liver) and dietary supplements, (Figure 6). The vitamin D obtained from these sources is biologically inert. It has to undergo enzyme-mediated activation, first in the liver and then in the kidney.
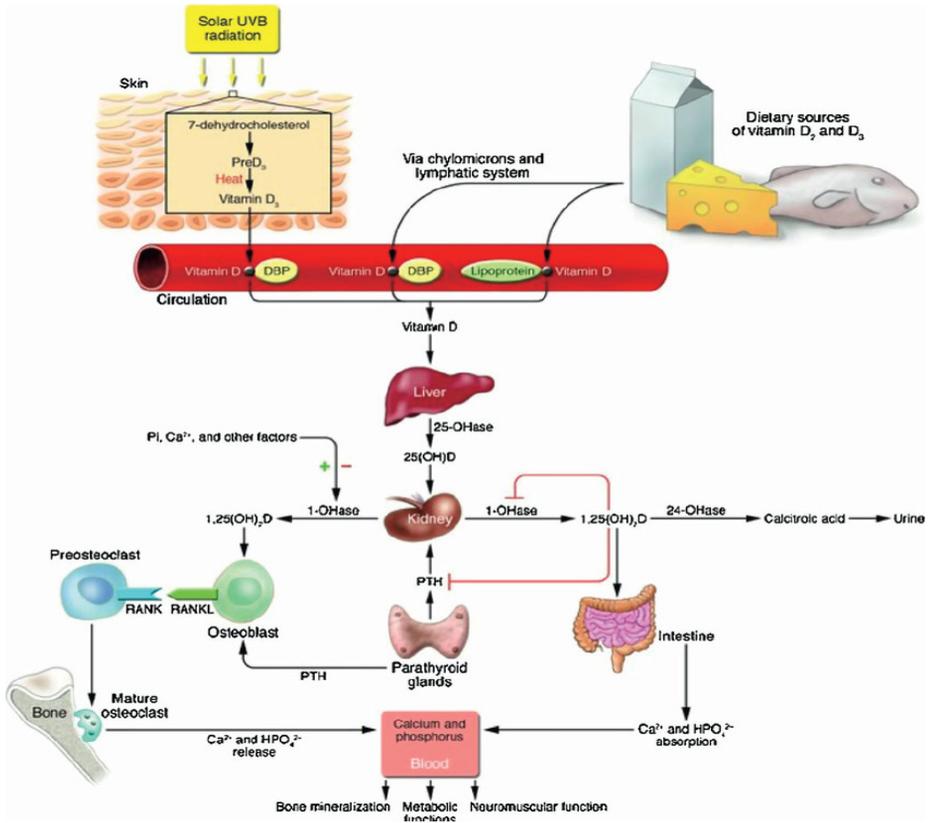

Figure 6.   Diagram showing vitamin D synthesis and metabolism in the skin, the liver, the kidney, the intestine and in the bone (42).

In the liver

Vitamin D produced in the skin or ingested through the diet enters the blood stream and is taken up by vitamin D binding protein (DBP), (Figure 6), which transports it to the liver where it is hydroxylated to calcidiol (25-hydroxy vitamin D2 and 25-hydroxy vitamin D3).

Calcidiol is the major circulating form of vitamin D and is a useful diagnostic marker, in determining the nutritional status of vitamin D (43). The concentration of 25(OH)D in serum (the normal range) is approximately 25-125 nmol/l (10- 50 ng/ml) and its half-life is 2-3 weeks (44). It is stored and released from fat cells (45). Calcidiol is taken up by cells in the body, converted to calcitriol locally and intracellularly and plays a vital role in the normal functioning of other organs. Calcidiol is not biologically active, and must be converted to the hormonally active form, calcitriol, in the kidney.

In the kidney

25-hydroxyvitamin D is converted to the biologically active form, 1,25-dihydroxyvitamin D (calcitriol) by the action of 1-alpha hydroxylase enzyme, (Figure 6). Serum calcium, phosphorous and fibroblast growth factors can influence the renal synthesis of calcitriol.

Calcitriol regulates its own production and reduces the secretion of parathyroid hormone (PTH). By inducing the expression of 24-hydroxylase, 1,25-dihydroxyvitamin D catabolizes into water soluble calcitrionic acid, a major catabolic metabolite (46). It is biologically inactive and is excreted in the bile. 1,25-dihydroxyvitamin D increases the absorption of calcium by enhancing the expression of calcium binding protein and the epithelial calcium channel. Furthermore, through its receptor on the osteoblast, 1,25-hydroxyvitamin D increases the expression of the Receptor-Activated Natural Killer Ligand (RANKL). The RANK, activated by its receptor on the preosteoclast, acts on RANKL leading to the maturity of the osteoclast, (Figure 6), which is important in order to remove calcium and phosphorous from the bone to maintain adequate levels

of both calcium and phosphorous in the blood stream. This system is active in bone turnover or bone remodeling.

Calcidiol produced in the liver is transported by the vitamin D binding protein (DBP) in the circulation (47). The biologically active form of the vitamin D synthesized in the kidney, calcitriol, has a half-life of about 4-6 hours in the circulation. The serum levels of calcitriol are estimated to be 1000 times less than those of calcidiol (42). The analysis of total 25(OH)D is in principle used as a vitamin D status indicator (11,12).

Somali population

Somalia, a brief history

The term Samaale is recognized as a singular ancestry of all Somali clans or paternal lineage groups (48). Somali is derived from two words, soo and maal, meaning "go and milk", giving the impression that pastoralism might be the major occupation of the Somali people. It was also assumed that the name might be derived from the Arabic, meaning "wealthy" which could also indicate that livestock was a major source of income (49,50).

The camel (Figure 7) is widely accepted as a national symbol. It is a source of food (meat and milk), the backbone of the economy and an indicator of social status (51). A large majority of the population is Sunni Muslim, representing a single homogeneous culture and speaking the same language, a single Somali dialect (51).

Figure 7. The camel is the most widely recognized national symbol, providing transportation, milk, meat, income, and status for most Somalis.

Somalia is situated on the Horn of Africa, (Figure 8), very close to the equator, at latitude 0-10° N. Except for its two major rivers (the Shebelle and the Juba), the country is generally considered barren and arid. According to the current World Population Review 2018, Somalia has an estimated population of 15.1 million people (52).

The nation gained independence from former colonial powers, UK and Italy, in 1960. English is spoken in the north and Italian in the south; both were educational and official languages before 1972 (53,54). The Somali language (Afro-Asiatic) was introduced as late as 1972 (48).

An estimated 1.1 million people were internally displaced (Human rights watch) due to the eruption of civil war and the collapse of the government (48,50). There has been no effective government since 1991 and the infrastructure is almost ruined.

Somalis are among the largest group of immigrants in Sweden and currently over 7,000 Somalis live in Gothenburg (according to Gothenburg city census in 2016), most of them in the north-eastern part of the city.
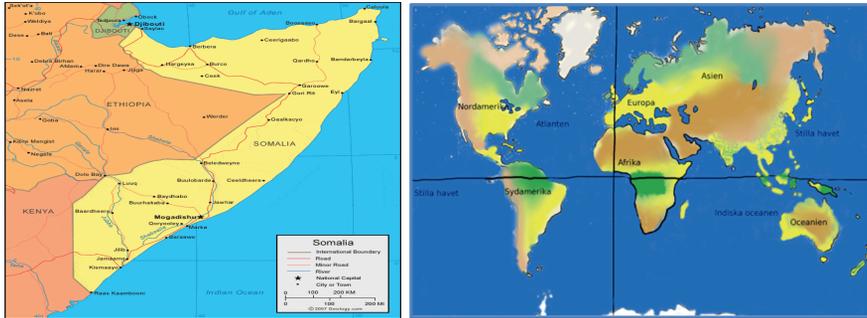
Figure 8. The political map of Somalia and its location in the world (geology.com).

Vitamin D status among Somali women

According to a multi-ethnic study of vitamin D deficiency performed in Finland, a number of Somalis exhibited vitamin D deficiency with secondary hyperparathyroidism in the winter (55).

The intake of foods with a high vitamin D and calcium content has not been sufficient (56) and most Somalis reported symptoms of muscle and bone pain (57,58), obviously a cause for concern regarding bone health and other conditions (10). Vitamin D deficiency is associated with poor muscle strength and performance. In the past, vitamin D deficient mothers died in childbirth as a result of a rachitic pelvis; however, this condition was later reversed by vitamin D treatment (11,59).

Extraskeletal manifestations

A study in Sweden, demonstrated lower vitamin D levels in Somali mothers compared with Swedish mothers (57), and, in addition to this finding, revealed that vitamin D deficiency may be implicated as a predictive risk factor for developing autism (60). Vitamin D deficiency was further implicated as a cause of an increased number of primary cesarean section (61). Somali women in Norway had more prenatal complications than native Norwegian women, but the causative factors for the adverse birth

outcomes were not established (62). One reason for increased infant mortality was ascribed to the mother's fear and avoidance of cesarean section (63).

The same study revealed that Somali women represented a high-risk population in obstetrical units and therefore needed special attention and care (63). Another prenatal study in Sweden demonstrated increased infant death among foreign women from Somalia and sub-Saharan regions of Africa (64). An increased risk of developing allergy, asthma and atopic dermatitis in relation to low levels of vitamin D was investigated (65, 66), but reviews showed conflicting results. Any causal relationship between vitamin D deficiency and comorbidity needs plausible verification. A recent study in Sweden demonstrated the existence of such a relationship between respiratory infections and reduced vitamin D levels (67). Some studies have provided evidence regarding the protective effect of vitamin D against respiratory tract infections (67-69).

Health and migration

A significant number of Somalis abroad living in Western countries have been exposed to a variety of physical and mental traumas, including posttraumatic stress syndrome. There are additional resettlement issues or stressors that contribute to and amplify mental health problems.

There is some degree of evidence supporting the idea that suboptimal vitamin D levels may be implicated in developing depression through the effect of calcitriol on neurotransmitters and neurotrophic factors (70, 71). A recent review study found increased evidence regarding the association between vitamin D deficiency and depression. It was also shown that vitamin D supplementation resulted in beneficial anti-depressive effects (72).

Definition of vitamin D status

Defining low vitamin D level is still controversial; however, there is a reasonable degree of agreement on what constitutes deficiency, based on the role of vitamin D in facilitating the absorption of calcium and phosphorus. Low levels of circulating S-25(OH)D cause rickets and osteomalcia and this relationship has long been recognized and well established (73, 74). Other morbidities (non-skeletal) associated with low vitamin D status have not yet been fully determined (75). Vitamin D deficiency is usually caused by malabsorption as in undiagnosed celiac disease and after bariatric or other gastro-intestinal surgery (76). Cut off values to describe low vitamin D status remain controversial. After assessing available data evidence, the Institute of Medicine (IOM), concluded that bone health was the only outcome whereby causality was determined and a dose-response relationship was established (77). According to the IOM, a S-25(OH)D level < 25 nmol/l reflects deficiency, whereas S-25(OH)D $\geq$25 < 50 nmol/l shows insufficient levels and $\geq$ 50 nmol/l sufficient levels (77). A vitamin D level of 75 nmol/l is considered optimal (8).

Sunlight exposure, skin pigmentation, style of clothing, use of sunscreens, nutrition and supplements determine the vitamin D status (30, 78). Environmental factors play an important role for the vitamin D status through sunlight or food by ensuring the availability of the vitamin D ligand (59, 79). Single nucleotide polymorphism (SNP) may affect the response of S-25(OH)D to supplementation treatment (80).

AIM OF THE THESIS

Hypotheses

The hypotheses of the thesis were the following:

1. Vitamin D deficiency is common in Somali immigrants in Sweden and the suggested recommendations of vitamin D supplementation with 400-800 IU (81) are too low for this group;

2. Somali women living at our northern latitudes have lower bone mineral density (BMD) than the reference values for both black and white women, according to the references used by the BMD measuring device;

3. Serum vitamin D-binding protein (S-DBP) was related to ethnicity rather than vitamin D levels;

4. Vitamin D deficiency in Somali women was related to increased comorbidity and lower health-related quality of life compared with native Swedish women.

Aim

The overall objective of this thesis was to study the prevalence of vitamin D deficiency in Somali women living in Sweden and the effects of treatment with vitamin D. Furthermore, the vitamin D status was studied in relation to bone status, comorbidity and general health in Somali women compared with native Swedish women.

SUBJECTS AND METHODS

Study populations

Somali women

Somali women, n =114, from East Africa (latitude 0-10° N), skin type V, age range 18-56 years, residing since at least two years in Gothenburg and Borås, Sweden (latitude 57º N), were enrolled on a voluntary basis. Flyers and advertisements at health care units were used to reach out to potential study participants (Figure 9). The announcements were translated into Somali language by an authorized translator and both the Somali and the Swedish versions were published.

Du Kvinna 18-50 år, född i Somalia.
Välkommen att delta i en studie med D-vitamin
Brist på D-vitamin är vanligt hos mörkhyade i Sverige och kan ge besvärliga symtom från muskler och skelett.
En grupp läkare från Sahlgrenska universitetssjukhuset och primärvården kommer att undersöka Ditt D-vitamin värde och ge Dig behandling med vitamin-D droppar i 6 månader.

Dumarka soomaaliyeed ee da´doodu uu dhexayso 18-50 sano.
Ku soo dhawaada inaad ka qeyb qaadataan cilmi baaris daaweyn vitamin-D ah, soconaysana mudo 6 bilood ah.
Hadii aad xiseyneyso la soo xiriir telefoonkan 031-747 83 31 (maalmaha shaqada, sacaduna tahay 13-14) ama toos ula soo xiriir xafiskeena (reception).
Vårdcentralen Lärjedalen
Bergsgårdsgärdet 89
424 32 Angered

Figure 9. The text and content of the flyers and advertisement used to recruit Somali women in Hjällbo, Gothenburg.

The advertisements were posted at Somali community shops, a nearby pharmacy and a supermarket. Different Somali associations were contacted and informed about the objective of the study. All subjects willing to participate, n = 114, were referred to our research assistant and were examined by experienced physicians (EA, TD and AO) before enrolment. All but one were premenopausal, (Table 1).

The survey was conducted in Gothenburg, at Hjällbo Primary Health Care Centre, and in Borås, during the autumn and winter of 2010, 2011 and 2012. The eligibility criteria for inclusion were no history of serious diseases, no use of drugs that might interfere or affect the vitamin D metabolism, no pregnancies and no lactations.

Controls

A random population sample of men and women (n = 2400, aged 25-64 years) was invited in 1995 in Gothenburg, Sweden (latitude 57° N), in the third World Health Organization MONItoring of trends and determinants for CArdiovascular disease screening, the WHO MONICA project (82), participation rate 67% (n = 1616). Hormonal sampling was performed on every 4th participating man and woman (randomly selected) aged 25-34, 35-44, 45-54, and 55-64 years, and all women aged 45-54 and 55-64 years, in total n = 662. The n=608 who were alive were invited to a re-investigation in 2008-09. Fifty-four subjects could not be reached or were deceased. Non-attendance was due to travelling, living abroad, difficult family circumstances, or unwillingness to participate giving a participation rate of 67%, n = 410 (315 women and 95 men from all age groups). The age groups 25-34, 35-44 and 45-54 years (n = 95) in 1995, who were re-examined (by PT and KLW) in 2008, n = 69, skin type II and III, were used as controls for the comorbidity study, Paper IV, (Table 1).

Table 1. The study populations and study designs in each of the paper of this thesis.

	Somali women, n	Controls, n	Setting
Paper I	114	Placebo=34, 800 IU=34, 1600 IU=34, UVB upper body=5, face/hand=4, Woods lamp=3	Placebo-controlled, double-blind clinical trial
Paper II	67	Manufacturers´ DXA controls, American white and Afro-American black women	Cross-sectional
Paper III	113 premeno-pausal	228 women, 50 men, the WHO MONICA study	Case control, placebo-controlled and longitudinal
Paper IV	114	69 women, the WHO MONICA study	Case control

Methods

Randomized, placebo-controlled, clinical trials with vitamin D and UVB

A randomized, placebo-controlled double-blind oral vitamin D treatment trial was carried out at Hjällbo Primary Care Centre. At the same time, a single-blind, placebo-controlled UVB treatment trial was performed in Borås.

In Gothenburg, 102 women were randomized to three oral treatment arms, receiving either vitamin D oral drops 800 IU/day or 1600 IU/day, or placebo drops for twelve weeks, respectively, (Figure 10). Sunflower oil drops containing vitamin D (80 IU/drop) was used for treatment and hybrid sunflower oil was used as placebo. Compliance was calculated by counting the remaining content in the bottles. In Borås, Somali women were

randomized to a single-blind study, where UVB (4.3-8.7 J/cm^2) or Woods lamp (placebo) was used to irradiate the upper part of the body or the face and hands, respectively.
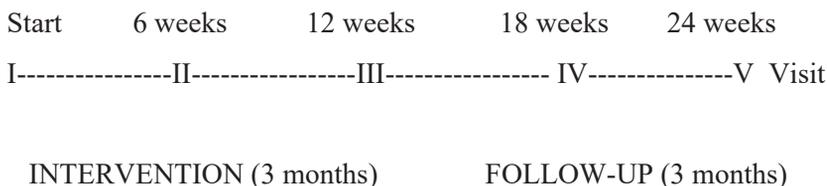

Start   6 weeks   12 weeks   18 weeks   24 weeks

I----------------II-----------------III----------------- IV---------------V Visit

   INTERVENTION (3 months)   FOLLOW-UP (3 months)

Figure 10: Study protocol for the intervention study with vitamin D or UVB light.

With the intention to treat, 40 Somali women from Borås were randomized to two treatment arms to receive treatment with UVB radiation: 20 subjects were to receive UVB radiation of the upper body and the face and hands respectively and the remaining 20 women were to be receive treatment of the corresponding areas with a Woods placebo lamp, according to the same protocol, (Figure 10).

Main outcome measures

Blood levels of serum (S)-25(OH) D, parathyroid hormone (PTH), calcium and ionized calcium were monitored before the start and every six weeks for six months; *i.e.*, three months´ follow-up after the treatment was terminated.

All participants filled in a questionnaire regarding medication, parity, duration of residency in Sweden, smoking habits, previous diseases and fractures. Health-related quality of life (HRQoL) was assessed using questionnaires and a pain evaluation was performed every sixth week. The exclusion criteria were pregnancies, ongoing lactation, intake of drugs and recent travel to sunny countries that could affect the vitamin D levels. The study was performed during the autumn and winter of 2010, 2011 and 2012.

Medical history, medication and life-style factors

Previous diseases, ongoing medications and smoking habits were asked for by the physicians at the examination at the start of the study on Somali women. Medications were coded according to the Anatomical Therapeutical Chemical (ATC) Classification System and were similar in the Somali women and the controls. Smoking was graded as current or ex-smokers.

Anthropometry

Body weight was measured in light underwear without shoes to the nearest 100 g and height was measured using a wall-mounted meter scale to the nearest cm. Body mass index (BMI) was calculated as weight divided by height in meters squared (kg/m^2).

Biochemical analysis

A fasting blood sample was drawn in the morning between 8 and 10 am. The tests were performed on day 7-9 of the menstrual cycle. S-25(OH)D and S-1,25(OH)D were analyzed by automated immunoassay (DiaSorin, Stillwater, MN, USA). The coefficient of variation (CV) for S-25(OH)D

was 6.2%. Vitamin D deficiency was defined as S-25(OH)D < 25 nmol/l, insufficiency as $\geq$ 25 < 50 nmol/l, and sufficiency as $\geq$ 50 nmol/l (77). S-PTH was determined by immunoradiometric assay (Roche Cobas, Rotkreuz, Switzerland), reference (ref.) range 1.6-6.9 pmol/l. The CV was 2.7%, S-calcium (ref. 2.15-2.50) mmol/L, S-ionized calcium (ref. 1.15-1.31 mmol/L), bone-specific alkaline phosphate (ALP) (ref. 5.0-27.0 ug/l), vitamin B12 in serum (ref. $\geq$ 140 pmol/l), S-free thyroxine (T4) (ref. 12-22 pmol/l), S-thyroid stimulating hormone (TSH) (ref. < 4.2 mU/L) and S-thyroid peroxidase antibodies (TPO) (ref. positive > 60 kU/l) were analyzed with routine methods at the accredited laboratory for Clinical Chemistry at the Sahlgrenska University Hospital, Gothenburg, Sweden. Photometry 600 nm was employed to determine concentrations of S-calcium and bone-specific ALP. Ionized calcium in serum was measured using ion-selective electrodes. Vitamin D-binding protein (S-DBP), expressed in ng/ml, was assessed with an enzyme-linked immunosorbent assay (ELISA; Cat. No DVDBP0, R&D Systems, Minneapolis, MN, USA).

Diagnoses of comorbidity

Hypothyroidism was defined as having treatment with levothyroxine or a S-TSH >4.2 mU/l. Vitamin B12-deficiency was defined as having vitamin B12-substitution or a S-B12 < 140 pmol/l. Medications for hypertension, diabetes mellitus, allergy and depression/anxiety were taken as a proxy for the respective diagnosis. Celiac disease was registered if present according to the medical history.

Bone measurements

Bone mineral density (BMD) was measured with Dual-energy X-ray Absorptiometry (DXA) by LUNAR Prodigy enCORE[tm], GE Healthcare,

LU44663, Madison, WI, USA, at the lumbar spine and at the right and left hip within the first eight weeks of the clinical trial in Study I, (Figure 11).

Figure 11. DXA recording using the device from General Electronics (GE), which applies a block phantom and BMD follow-up values to adjust itself to suitable radiation, absorption coefficient and tissue-equivalent materials. Photo by Eva Hällås-Linder of the author.

The CV for the GE/LUNAR device at the lumbar and femoral neck was 1.22 and 1.97, respectively. BMD was recorded as the Z-score (difference in SD from the mean of healthy, age-matched women). The results were adjusted for age, body weight and skin type (ethnicity) for African-American and American women by the DXA device in California, USA, at 35° N, (Figure 12).
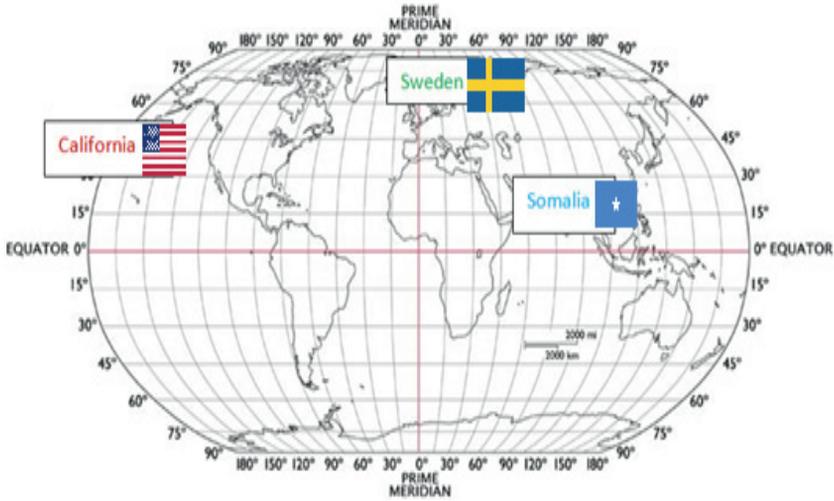
Figure 12. DXA measurement of Somali migrant women (0-10° N) was performed in Sweden (57° N) and compared with African-American black and American white women in California, USA (35° N) according to the referents by LUNAR.

The DXA is considered to be highly accurate and precise in measuring BMD. DXA is commonly used in the diagnosis of osteoporosis, to assess an individual's risk of developing fractures and after treatment to assess drug efficacy. In the present study, all Somali women in Study II were premenopausal, and the Z-score was used instead of the T-score. The quality control of DXA depends on the specific features of the device employed. In general, every device measuring BMD should ensure the use of its own suitable phantom and proper calibration. The radiation corresponds to 1/10 of a chest X-ray and to the average background radiation on earth.

Calcaneal bone measurements were performed with quantitative ultrasound (LUNAR, Achilles Madison, WI, USA) in the men and women of the population (83). Speed of sounds, broadband ultrasound attenuation and a

32

combined stiffness index were recorded. The correlation between quantitative calcaneal ultrasound and DXA at both regions of interest, the lumbar and the femoral neck, is high (84).

Quality of life measures

Two different questionnaires were used to assess Health-Related Quality of Life (HRQoL) in both Somali women and controls. The Short Form-36 (SF-36) is commonly used in clinical trials, in quality control studies and in health-care services, (Figure 13), (85). It has been used effectively to compare different populations (general and specific), in comparing health problems (deficits) and in assessments of treatment efficacy (85).

Thirty-six items are included to generate eight scales; Physical Functioning (PF), Bodily Pain (BP), Role limitations due to Physical health problems (RP), Role limitations due to personal or Emotional problems (RE), general Mental Health (MH), Social Functioning (SF), energy/fatigue or Vitality (VT), and General Health perceptions (GH). These scales are then merged into two summary measures, the Physical Component Score (PCS) and the Mental Component Score (MCS) of health.
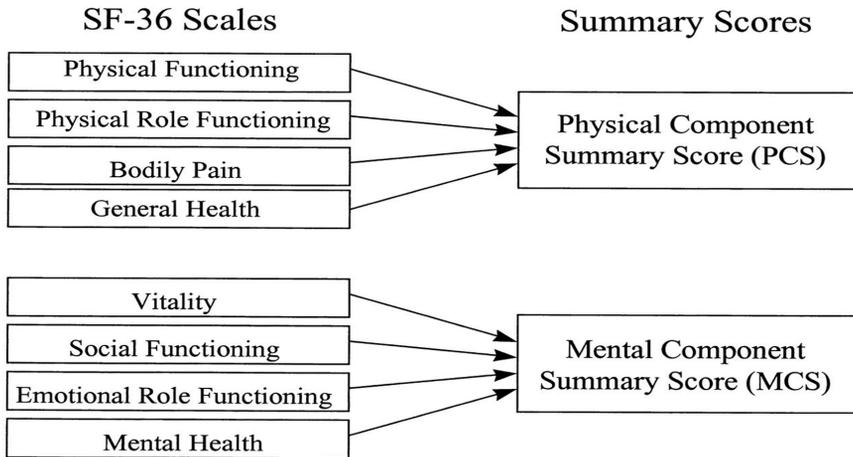
Figure 13. The diagram shows the different items in the Short Form (SF)-36 questionnaire which generate scales, which, in turn, results in summary scores. Eight scales are scored, from 0 (worst health state) to 100 (best health status). The scores combine to make two summary component scores, the physical (PCS) and the mental (MCS) scores.

The other questionnaire used to assess state of health, was question #6 in the EuroQoL-5 Dimensions (EQ-5D) Visual Analogue Scale (VAS). The EQ-5D is based on the individuals´ experience of their general health status. This is recorded on a vertical scale, with two end points, marked 'Best imaginable health state' = 100, and 'Worst imaginable health state' = 0, (Figure 14), (86).

All the questionnaires and scales were translated into Somali language and both the Somali and the Swedish versions were given to the Somali women. The VAS for the estimation of pain, 0-10 cm (no pain to high pain) was also used in the Somali women, (Figure 14).
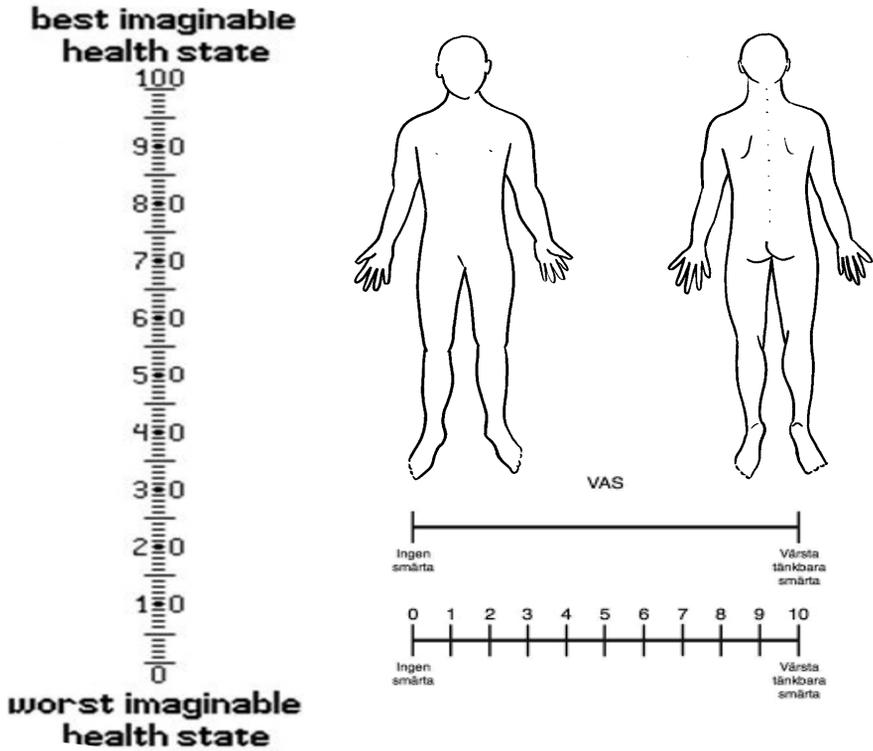
Figure 14.   The EQ-5D-scale (0-100 mm), to the left, was used for measures of the self-rated global health status. The Visual Analogue Scale (VAS), to the right, was used for the estimation of pain, 0-10 cm (no pain to high pain).

Statistical analysis in Papers I-IV

Conventional methods were used to calculate means, medians and standard deviations. All tests were two-sided and $P < 0.05$ was considered as statistically significant.

Statistics Paper I

The Jonckheere test was used for power calculation. This is a non-parametric test for the trend (positive or negative) over three or more independent samples. For a power of 80%, a significance level of 0.05 and a standard deviation of 38 nmol/between three groups with 30 patients in each, a difference of a 25 nmol/l increase in S-25(OH)D between the lowest and the highest levels was needed. If the sample size was reduced to 20 participants in each of the three groups, a difference of 33 nmol/l was needed for a power of 80%, a significance level of 0.05 and a standard deviation of 38 nmol/L. A simulated 7% participant reduction or drop-out rate from each group was considered. In a similar approach, the Jonckheere trend test on the three groups was ordered as: Woods lamp < UVB face and hands < UVB upper body; however, stratification was not performed due to the small number of participants in this UVB trial.

Due to challenges in the recruitment and retention of clinical trial participants, it was decided to include more patients in the oral treatment group, 102 instead of 90 randomized subjects.

Wilcoxons´ signed rank test was used for differences in S-25(OH)D between the start and six and 12 weeks, respectively. Wilcoxons´ rank sum test was used for two-sample tests. Fishers´ exact test was used for the comparison of proportions and Spearmans´ univariate correlation test for analyses between S-25(OH)D levels at baseline and age, BMD, years in Sweden and parity.

Statistics Paper II

Conventional methods were used to calculate means, medians and standard deviations. Differences between subject values and the reference distribution were tested using Wilcoxons´ signed rank test. Spearmans´ test was used for correlations.

Statistics Paper III

For two-sample comparisons, Wilcoxons´ rank sum test was implemented. To test for correlations between two variables, Spearmans´ coefficient and Pearsons´ coefficient were used. A multiple linear regression was employed to correlate S-DBP with S-25(OH)D and BMI for both Somali women and the native women from the control group (WHO population sample). To test if the S-DBP levels were constant in the Somali women throughout the five visits, in the three oral treatment arms, a mixed effects regression analysis was used.

Statistics Paper IV

All data were analyzed using R version 3.0.3 (The R Foundation for Statistical Computing, Vienna, Austria). Wilcoxons´ rank sum test and the t-test were used for two-sample comparisons. Adjustment of confounding factors such as age and BMI was made using a multiple linear regression model.

Ethical considerations

All studies were approved by the Ethics Committee at the University of Gothenburg, following the Helsinki Declaration, 088/06, T282/11, 410/08, T486/10, T063/15, and the National Data Inspection Board. All participants gave their written informed consent.

RESULTS Paper I-IV

Results Paper I

There was a high prevalence of vitamin D deficiency among the Somali women. Vitamin D deficiency, *i.e.*, S-25(OH)D < 25 nmol/l, was found in 73% of the Somali women. S-25(OH)D values increased dose dependently in accordance with the level of treatment doses of oral vitamin D, (Figure 15).
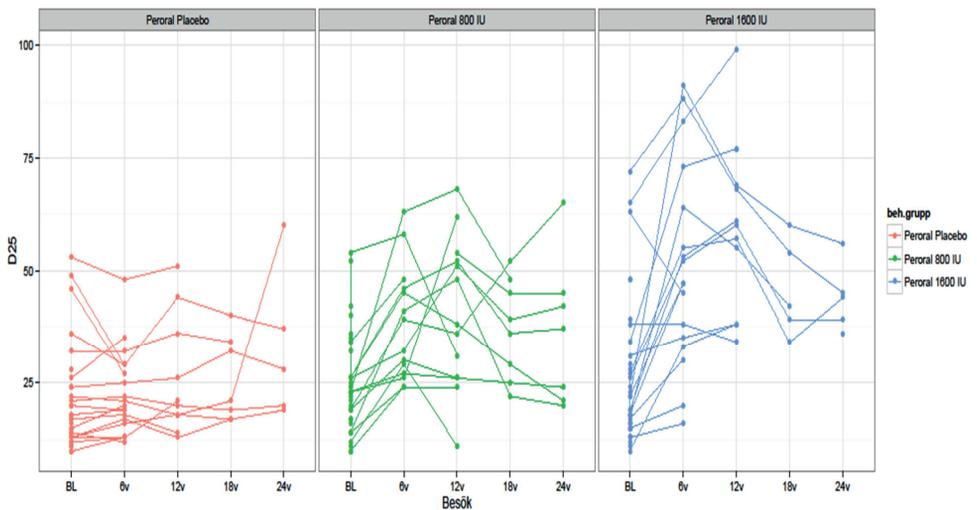

Figure 15. Levels of S-25(OH)D are shown for placebo, daily oral doses of 800 IU and 1600 IU, respectively. At twelve weeks, p = 0.006 for 800 IU/day vs. placebo and p = 0.002 for 1600 IU/day vs. placebo.

The S-25(OH)D levels declined after the treatment was stopped at 12 weeks and during the follow-up time until 24 weeks. However, the increases in S-25(OH)D concentrations remained above baseline throughout the entire study period. The S-25(OH)D levels in the placebo group were unaltered during the entire study period. At least 1600 IU of vitamin D were needed to optimize or to bring the vitamin D concentrations to sufficient levels

($\geq$ 50 nmol/l) of S-25(OH)D in the majority of the women, (Figure 15). The drop-out rate was high.

Only twelve participants signed up and were enrolled for the UVB /Woods lamp treatment. Eight of these subjects were lost to follow-up, further diminishing the number of participants who completed the treatment to four women.

Two women received UVB of the upper body, with total accumulated doses of 4.3 and 8.7 J/cm^2, respectively, whereas one woman received treatment of the face and hands, at an accumulated dose of 5.9 $J/cm.^2$

Three women who received Woods lamp (placebo light) treatment had a total accumulated dose of 0.3 J/cm^2. There was a trend towards a dose-dependent increase also in the UVB arm of the study. Those receiving UVB of the upper body showed the highest S-25(OH)D levels during treatment.

There was a positive association between the increase in S-25(OH)D from baseline and the treatment area in the UVB-treated group after six weeks, when a similar test according to Jonckheere was performed. The women, who received placebo, or Woods lamp treatment, exhibited no changes in vitamin D values.

HRQoL, assessed by the SF-36, was unaltered during the entire treatment and follow-up periods. Nor were there any differences in the VAS scores for pain between subjects randomized to active treatment or placebo, or between the patients who completed the trial or those who dropped out, (Figure 16).
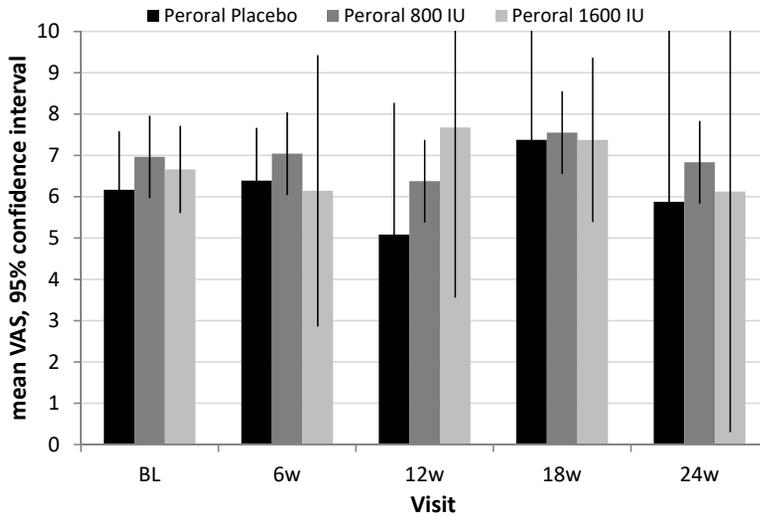
Figure 16. The Visual Analogue Scale (VAS) for the assessment of pain at baseline (BL), after six weeks (6w) and 12 weeks (12w) of treatment with oral vitamin D 800 IU/day, 1600 IU/day or placebo, respectively, and during follow-up until 24 weeks (24w). Means $\pm$ 95% confidence intervals are given. No significant differences between groups were seen.

Results Paper II

BMD was measured in Somali women living in Sweden and compared with the DXA manufacturers´ reference population of different ethnicities. The white American women were from California, USA, and the African-American women from Egypt, Africa, according to the manufacturer LUNAR, (Figure 12). Hence, comparisons were performed as in clinical practice in Sweden, using the Z-score for comparison with age-matched young women, (Figure 17).
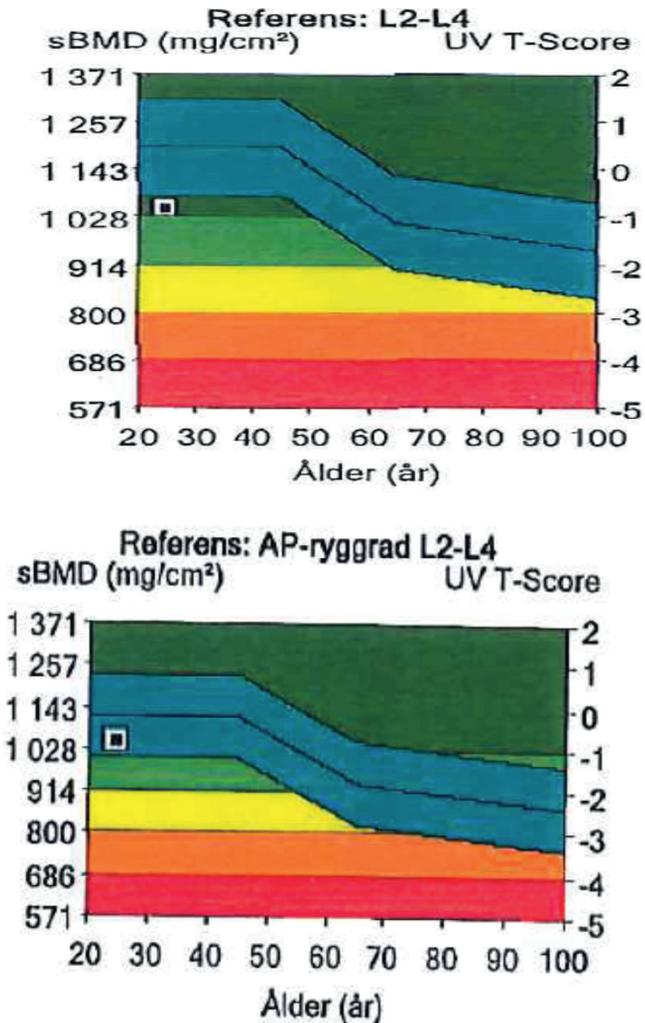
Figure 17. The DXA protocol showing comparisons between a Somali woman and both the black African-American, upper, and white American, lower, reference population according to the manufacturer, LUNAR, California, USA.

The median Z-score at the lumbar spine was lower than both white American women, -0.9 SD, and African-American women, -1.6 SD, $p < 0.00001$ for both, (Figure 18).

The median Z-score at the hips compared with the white American population was 0.1 SD for the left hip and 0.0 SD for the right hip (ns for both). The comparison referring to the hips in relation to the African-American reference were -0.9 SD for the left hip and -0.9 SD for the right hip, p < 0.00001 for both.
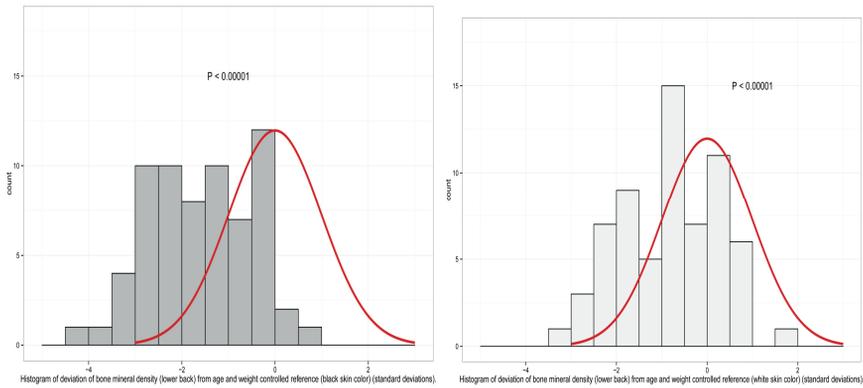
Figure 18. Histogram of bone mineral density (BMD) at the lumbar spine, expressed as the Z-score (SD from the age-matched reference mean) in Somali women compared with black African-American women at the lumbar spine (left), and compared with white American women (right), p < 0.00001 for both (87).

BMD did not correlate to vitamin D levels or to the number of years in Sweden. One wrist fracture was reported in the oldest Somali woman.

Results Paper III

Vitamin D deficiency was prevalent in 73% of the Somali women. No deficiency was recorded in women <50 years of age and 5% was recorded in older men and women in the native control population.

S-DBP was lower in the Somali women than in the native Swedish women in the population. S-DBP was similar among men and women in the control group, (Figure 19). An increase in vitamin D levels on treatment did not affect the S-DBP values. However, there was only a positive correlation

between vitamin D status and S-DBP values in the native Swedish women. Age, sex, body weight, BMD and time elapsed during the 13 years of follow-up did not affect the S-DBP concentrations. Treatment with vitamin D or UVB did not affect S-DBP in this study on Somali women where the majority had vitamin D deficiency, S-25(OH)D < 25 nmol/l. One third of the subjects who received 1600 IU daily reached sufficient levels of S-25(OH)D, but this did not affect S-DBP during the three-month-period of treatment.

DBP conc. ng/ml
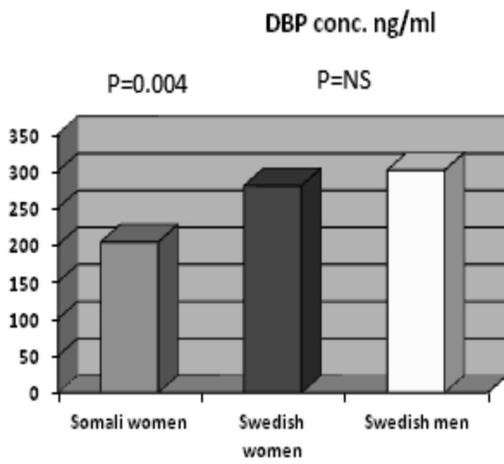
Figure 19. Vitamin D-binding protein (DBP) in Somali women living in Gothenburg, Sweden and in women and men from a random population sample, the WHO MONICA study in Gothenburg, p = 0.004 for Somali women vs. native Swedish women, no significance between the sexes in native women and men.

Results Paper IV

Somali women, n = 114, aged 18-56 years, who had lived in Sweden, for at least two years, were recruited during the autumn-winter of 2010-2011 and compared with a population sample, of native Swedish women, n = 69, age

range 38-56 years, examined during the autumn-winter-spring of 2008-2009, from The WHO MONICA study in Gothenburg.

Vitamin D deficiency, *i.e.,* S-25(OH)D < 25 nmol/l, was found in 73% of the Somali women and in < 2% of the controls, p<0.0001. Elevated S-PTH, > 6.9 pmol/l, was found in 26% and 9%, respectively, p = 0.004. Somali women used less medication in general, 16% vs. 55%, p < 0.0001, but more allergy medication after adjustment for age, p = 0.006.

There were no differences in the prevalence of diabetes mellitus, hypothyroidism, positive thyroid peroxidase antibodies, vitamin B12 deficiency, celiac disease or hypertension. Somali women had fewer fractures, (Figure 20), and consistently lower HRQoL scores, especially for the physical components, than native controls.
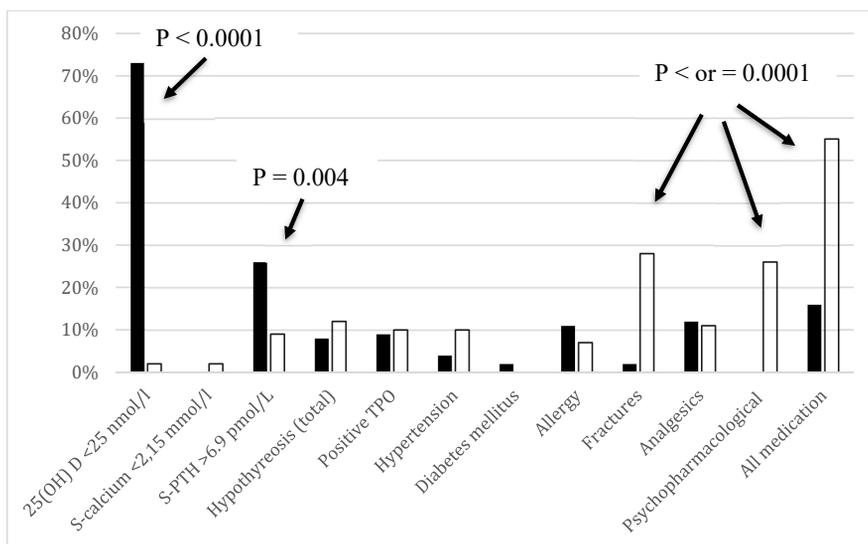

Figure 20. Occurrence of various disease states and medications in the Somali women (black bars), compared with native Swedish women (white bars). P for comparisons corrected for age.

DISCUSSION I-IV

Discussion Paper I

Vitamin D deficiency, *i.e.,* S-25 (OH)D < 25 nmol/l was common, 73%, in Somali women living in Sweden since at least two years, vs. < 5% in native Swedes, depending on age-groups studied, and in the latter mainly due to malabsorption. None of the immigrants had any gastrointestinal surgery or symptoms, respectively, in their medical history.

The figures are in concordance with other Swedish surveys where immigrant women originating from lower latitudes exhibited lower vitamin D levels, whereas native northern women had normal vitamin D values (88, 89) and 78% of pregnant immigrant women living in Sweden had vitamin D deficiency (90). Severe vitamin D deficiency was found in another study on pregnant women from Somalia with median values of S-25(OH)D of 11 nmol/L (range 5-25 nmol/L) compared with 70 nmol/L (range 28-101 nmol/L) in ethnic Swedish women according to Saaf M et al. (57). A study in Gothenburg showed vitamin D deficiency in 51% of pregnant women who were immigrants from Africa (91). A Norwegian study on recently arrived immigrants from Africa showed that the majority had S-25(OH)D <50 nmol/l and 24% had S-25(OH)D < 25 nmol/l (92).

Treatment with vitamin D3 for three months showed a dose-dependent increase in S- 25(OH)D from very low to sufficient serum levels, in the group treated with 1600 IU/day. The increase was maintained for another three months. The oral dose of 800 IU was not sufficiently high to increase the S-25(OH)D to more than within the insufficient range (25-50 nmol/l). Hence, a dose of at least 1600 IU/day is recommended for optimizing the S-25(OH)D level in Somali women living in Sweden. The duration of treatment would probably be life-long.

Interestingly, UVB exposure of the upper body but not smaller areas, was as effective as oral treatment. The size of the exposed skin area is important

for the effect of UVB on S-25(OH)D in subjects with skin type II and III (93). Hence, the dose of UVB adjusted to the skin phototype and the size of the exposed area are important for vitamin D induction in dark as in fair skin color. Furthermore, UVB exposure of the upper body was as effective as the 1600 IU dose of cholecalciferol. Therefore, the treatment with UVB light may be a treatment option for vitamin D deficiency in dark-skinned immigrants. A four-arm clinical trial in native elderly Swedes showed similar effects of UVB and calcium + vitamin D on S-25(OH)D levels (94). The authors suggested that the oral treatment was superior UVB due to the cumbersome and time consuming UVB exposure therapy (94).

The present recommendation of a daily dose of 400-800 IU vitamin D (81) does not seem to be sufficient in this Somali population with skin type V living at our latitude.

The VAS score for the pain evaluation showed high mean levels, around 6 on a scale of 0-10, at start. However, no alleviation was seen after the treatment. The pain may reflect signs of osteomalacia in the Somali women. Unfortunately, no tender points or bone biopsies were specifically checked in order to further elucidate the cause or origin of the pain. Neither was any improvement in HRQoL seen within subjects or between groups, on vitamin D or UVB treatment. One explanation may be the fairly short period of treatment, only three months.

Compliance was poor during the clinical trials. The Somali women were difficult to reach in spite of repeated written and oral information from the principal investigators and the coordinating staff.

Discussion Paper II

The hypothesis was that Somali women may have osteomalacia and that this may compromise bone integrity and quality. Vitamin D is an important

determinant of skeletal development and maintenance of bone mass throughout life. It promotes calcium absorption and bone mineralization and has been shown to be positively associated with BMD (95). However, the DXA device cannot discriminate between quality, *i.e.,* mineralization of the bone, and bone mass, but only estimate the density of the bone, the BMD. Osteomalacia leads to a decrease in the amount of bone with time and lower BMD can be seen if the vitamin D deficiency is not reversed. In the present study, all except one woman were premenopausal and of young age and their bone mass was, to some extent, preserved by the endogenous estradiol. BMD in the Somali women was measured and compared with the reference population (white American women and black African-American women) according to data provided by the DXA manufacturer (LUNAR), as in clinical practice in Sweden. Still, the BMD was lower than the manufacturers´ reference values according to the Z-scores, *i.e.,* in comparison with age-matched reference. This could possibly be explained by a certain degree of osteomalacia and/or due to lower physical loading from physical activity. We did not evaluate the degree of physical activity specifically, but it has been reported in previous study on immigrants from Africa to be low (96).

This is in accordance with Somali immigrants in the USA (97) and Somalis living in Finland (55) who did not exhibit the same robust BMD values as African–American women despite sharing the same African ancestry (55,97). Based on the high prevalence of vitamin D deficiency and the lower BMD values in Somali women, we recommend vitamin D supplementation with at least 1600 IU/day to correct S-25(OH) to sufficient levels, and to prevent the development of osteomalacia, osteoporosis and future fracture risks.

Discussion Paper III

S-DBP is a multifunctional protein, belonging to the albumin super family, synthesized mainly in the liver and released into the blood stream. It has three domains: binding the vitamin D metabolite, binding fatty acids and binding actin. The Gc -gene on chromosome 4Q12-13 encodes DBP. It is found in excess (some 100 times molar excess) in relation for the total vitamin D metabolites and has ten times greater affinity for calcitriol than for calcidiol. Quantification of S-DBP by the monoclonal ELISA method showed lower values for black than white subjects.

The monoclonal analysis was performed differentially using specific isoforms based on race. Polyclonal ELISA showed higher S-DBP values than LC MS/MS; however, yielded similar lower DBP and vitamin D concentrations, regardless of race (80,98). S-DBP measured with polyclonal ELISA and LC-MS/MS showed that S-DBP, free and bioavailable S-25OHD was consistently lower in black subjects than in white participants, indicating that monoclonal ELISA is genotype-biased (80, 98).

S-DBP was lower in Somali women compared with S-DBP in native women < 50 years of age in the population sample. None in the latter group had vitamin D deficiency and S-DBP was similar in men and women in the control group. An increase in vitamin D levels on treatment, from deficient to insufficient and sufficient levels, did not affect the S-DBP values. Nor was there any correlation between S-DBP and BMD in Somali women. S-DBP correlated positively with S-25(OH)D only in the native Swedish women with sufficient S-25(OH)D. Age, sex, body weight, bone mass and time elapsed during the 13 years of follow-up did not affect the S-DBP concentrations.

Powe et al. found a higher BMD in African-American subjects in spite of low S-25(OH)D and S-DBP (98) while others found no association between S-DBP and bone status or body weight, respectively (99). It is not clear whether ethnicity is a major determinant for S-DBP (100). It would have been of interest to have measured S-DBP in Somali women with a longer duration of sufficient S-25(OH)D levels.

Hence, S-DBP was low in vitamin D deficiency and unaffected by treatment in the Somali women. Higher S-25(OH)D levels are probably needed to see any change in S-DBP during treatment. The measurement of S-DBP, in the present study, does not add any new information to the diagnosis of vitamin D deficiency.

Discussion Paper IV

There is a mounting degree of evidence, based on epidemiological study findings, linking vitamin D deficiency to a wide range of chronic and autoimmune diseases (101, 102). Somali women with a high prevalence of vitamin D deficiency may have more chronic diseases and autoimmune-related conditions and use more medication compared with native subjects at northern latitudes. This was also the hypothesis in the present work, where Somali women who had been in Sweden for up to 23 years were compared with native Swedish women.

However, the hypothesis was not verified regarding diabetes mellitus, hypertension, hypothyroidism, thyroid peroxidase antibody levels, signs of secondary deficiencies (malabsorption-related), like B12 deficiency and celiac diseases or fractures.

Despite no increase in comorbidity, the Somali women rated their HRQoL lower in both the psychological, or mental, and especially the physical components than the controls. We know from Paper I and Paper II that Somali women scored fairly high, 6 of 10, on VAS for pain and it is possible

that they have more symptoms related to osteomalacia influencing their overall well-being. The use of psychopharmacological agents was nil.

The Somali women used more allergy medications than the controls. This has also been seen in immigrants in an Italian study (103). The immunomodulatory effects of vitamin D have been described (103, 104). It is not known whether the allergy problems were present already in Somalia or if they were aquired in Sweden. In the Italian study, the allergy started within four years of moving to Europe (103). This supports the link between vitamin D deficiency and allergy. Long-term follow up studies of immigrants in Sweden are important in order to study health and disease in these groups coming from different parts of the world and now living with a variety of allergens in the air, homes and food.

Methodological questions

Vitamin D measurement

S-25(OH)D measurements have been a major challenge due to huge methodological discrepancies worldwide. Such variations in the results were reported from an external data assessment by the International Vitamin D External Quality Assessment Scheme (DEQAS) in 1989 (105).
 In 2011, the Vitamin D Standardization Program (VDSP) was established to solve the problems related to inter-laboratory and inter-assay discrepancies by providing and certifying standard reference materials (SRM 2972 and 972a) for human serum vitamin D metabolites (106). The 3-epi-25(OH) D3 metabolite is considered a major confounding factor in the quantification S-25(OH)D levels. The biological function of this metabolite, the C_3-epimer, is not yet fully established (107). There is, however, some evidences showing that it possesses some, but not all, of the calcemic and non-calcemic regulatory effects (106, 107).

The DEQAS identified assays that detect total vitamin D levels quantitatively (HPLC, LC-MS/MS and the DiaSorin assays). LC-MS/MS measures all vitamin D metabolites simultaneously (107). DiaSorin applies a two-steps procedure: extraction of 25-hydroxyvitamin D from DBP using an antibody-specific binding of 25-hydroxyvitamin D and addition of a vitamin D-isluminal tracer. The DiaSorin liaison and LC-MS/MS demonstrated good agreement according to proficiency testing in 2005 (108). The HPLC method showed discrepancies in results, according to previous tests, causing inaccuracy of the immunoassay methods compared with the reference method (108).

The S-DBP analysis

Monoclonal and polyclonal assays were used to quantify the S-DBP concentrations. The monoclonal ELISA method quantified DBP differently, with 85% of the variability explained by the genotype, in contrast to the polyclonal method which quantified DBP with < 9% genotype variability. The polyclonal ELISA-based analysis demonstrated that S-DBP concentrations did not differ within race. However, the levels of bioavailable and free 25(OH) in blacks were lower than in white subjects; consistent or similar in the LS-MS/MS measurements (80). The Gc1f homozygotes, where 95% are blacks, may indeed account for this measurement discrepancy. Monoclonal ELISA measured DBP lower than both polyclonal ELISA and LC-MS/MS. It would have been of great interest to study S-DBP in a cohort of Somali women with sufficient S-25(OH)D levels, or with longer vitamin D treatment duration. However, in vitamin D sufficient white blood donors, S-DBP was stable over the year while S-25(OH)D followed the seasonal variations (109).

Study limitations

Compliance

Compliance was the major limitation of this study, with a 51% drop-out rate after the first six weeks and reaching 65% at 12 weeks of the clinical trials, despite all practical efforts to facilitate participation. Similar drop-out patterns were observed in other studies and there were indications that the study group is hard to reach and, consequently, difficult to treat (110).

A burgeoning body of literature deals with the traditional majority population-based clinical trials. Ethnic minorities are not well represented in most health care research (111). Mistrust is a universal major factor limiting minority-based research initiatives (112). Many interventional studies with minority groups have encountered difficulties in achieving an adequate sample size due to recruitment and retention problems threatening study validity (112). Any future endeavor to facilitate recruitment and retention of minority/ethnic populations should focus on new strategies. Developing new approaches and new innovative tools may improve and enhance multi-ethnic participation.

Another limitation was that gastroscopy was not performed to diagnose malabsorption or celiac disease or bone biopsies to verify osteomalacia.

Selection bias

Our flyers used to recruit participants may have influenced some people to volunteer for the study. Participants who signed up may be more interested in taking care of their health and be different from others, potentially leading to selection bias. Being aware of this risk, we included as many Somali women as possible and matched controls in our study. A careful power analysis was performed before the clinical trials. We excluded some participants to adjust for factors that might affect the outcome; participants who traveled to sunny countries and pregnancy and lactation during the

study period. The controls were older so the results had to be age-adjusted. The controls were originally randomly selected and re-examined 13 years later, why the latter group was also a selection of those willing to participate. However, we also compared the Somali women with the original WHO MONICA cohort, n = 800 women, 25-64 years of age in 1995, with regard to comorbidity and the results were similar to the comparison with the re-examined, more recent group from 2008, now used in Paper IV.

Strength

The findings are informative and demonstrated a high prevalence of vitamin D deficiency in the Somali immigrant group. Supplemental doses needed to optimize vitamin D treatment to sufficient S-25(OH)D levels were given. We believe that the results close a knowledge gap in the Swedish health care system.

One strength of the study was the homogeneity of the ethnic group of Somali women and that the clinical trials were placebo-controlled. Another strength was that a population sample, which is considered as the optimal reference group, from the native population in Sweden was used as control group.

Future research

In recent years, vitamin D has become an important health issue worldwide and especially in minority ethnic groups including the Somali community. The high prevalence and the severe vitamin D deficiency, which was previously undetected, are now being addressed. As a result of increasing research in the field new dimensions and knowledge are emerging allowing for future explorations and possible new findings in the domain. Our studies

demonstrated a high prevalence of vitamin D deficiency in Somali women in Sweden and recommends treatment doses to reverse the condition.

Future studies could possibly focus not only on Somali women. We need to examine if there is any similar deficiency pattern in Somali men in Sweden. It might also be of interest to study if vitamin D values in both women and men living in Somalia are different from Somalis (men and women) living in Sweden.

Long-term follow-up studies of the present well-categorized Somali cohort will also be of interest regarding their HRQoL, pain, bone health and other comorbidity up to and beyond menopausal ages.

CLINICAL IMPLICATIONS

Vitamin D deficiency was common in immigrants living at higher latitudes. Somali women who have migrated from a location near the equator and now live in Sweden, with less sunlight exposure, constitute a risk population. There is, however limited information regarding vitamin D status and treatment effects on the level of S-25(OH)D in this group in Sweden. This study demonstrated both the high prevalence of vitamin D deficiency and that a vitamin D dose of at least 1600 IU/day is needed to raise and maintain sufficient levels of S-25(OH)D ($\geq$ 50 nmol/l). Serum calcium and renal function were unaltered throughout the clinical trial period.

A third of the participants withdrew from the study for unknown reasons. This challenge indicates the problem of recruitment and retention and that the study group is hard to reach and difficult to treat, as experienced by similar research initiatives in other countries.

CONCLUSIONS

Vitamin D deficiency, S-25(OH)D < 25mmol/l was frequent, 73%, in Somali women living in Sweden. Vitamin D values increased in accordance with the level of the treatment doses with oral vitamin D and UVB, respectively. The BMD of the Somali women was lower than among both the white American and the black African-American women used as reference in the regions of interest. Based on these findings, vitamin D supplementation with at least 1600 IU/day is recommended to correct S-25(OH)D levels to sufficient levels, in order to prevent osteomalacia, osteoporosis and possibly future fractures from developing. The S-DBP concentration was related to the vitamin D status but not related to age, sex, body weight, and bone mass or treatment dose. Comorbidity was not elevated in the Somali women, except for more allergy problems, but their HRQoL, especially the physical component, was lower than in native Swedish women.

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