Vitamin D deficiency in Northern Sweden

A cross-sectional study of an immigrant population at latitude 63°N including an open, partially randomized, controlled clinical trial studying the effect of supplementation with different doses of cholecalciferol

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Sweden 2018
“The more I know, the more I realize I know nothing.”

“Ju mer jag lär mig desto mer inser jag hur lite jag vet”

Sokrates (469-399 BC)
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Abstract

**Background:** Vitamin D is a prohormone that plays a key role in the calcium and phosphate balance and has physiological functions throughout the entire body. Vitamin D is supplied by exposure to ultraviolet light or by food. The prevalence of vitamin D deficiency in immigrants in Northern Sweden was unknown. There was no consensus on how to define or treat vitamin D deficiency and no pure preparations of cholecalciferol available in Sweden.

**Aims:** To study the prevalence and determinants of vitamin D deficiency in immigrants of African and Middle Eastern origin, to examine associations between vitamin D status and muscle strength, anxiety, depression and quality of life, and to determine the effect of supplementation with cholecalciferol on 25-hydroxyvitamin D₃ [25(OH)D] and vitamin D status.

**Methods:** 1. A cross-sectional, population-based study. Immigrants ages 25-65 from Africa and the Middle East (n=1306) living in Umeå, Sweden, were invited to participate. A total of 111 men and 106 women (16.5%) participated. 25(OH)D was measured by LC-MsMs. Anthropometry, medical, socioeconomic and lifestyle data was registered. Examinations: lower limb muscle strength, grip strength, HAD, health-related quality of life (QoL) 2. An open, partially randomized, controlled trial including immigrants from Africa or the Middle East, 192 subjects screened, 160 included and 147 completed the study. Intervention: cholecalciferol 12±2 weeks, 4 parallel groups; Group 1: 25(OH)D <25nmol/L: 10000 IU/d, Groups 2a and 2b: 25(OH)D 25-49 nmol/L: 2000 IU/d or 2000 IU/w, Group 3: 25(OH)D 50-74 nmol/L: 2000 IU/d.

**Results:** Twelve percent of the immigrants showed a vitamin D deficiency (25(OH)D <25 nmol/L) and 73 % showed 25(OH)D <50 nmol/L. Vitamin D deficiency was twice as common in African immigrants as in the Middle Eastern group. Vitamin D deficiency was associated with intake of fatty fish less than once a week, absence of travel abroad and use of long-sleeved clothing in summer. Lower limb muscle strength was associated with 25(OH)D levels and weaker grip strength was associated with vitamin D deficiency. Vitamin D deficiency was not associated with anxiety, depression or QoL in the total immigrant population. In Middle Eastern women, in whom prevalence of anxiety was higher, anxiety was associated with 25(OH)D ≤49 nmol/L. Oral cholecalciferol was effective in increasing 25(OH)D. At study end, 100% in Group 1, 89% in Group 2a, 55% in Group 2b and 96% in Group 3 reached adequate vitamin D status (25(OH)D ≥50 nmol/L). In Group 1; 62 % reached 25(OH)D ≥125 nmol/L.
Conclusions: Vitamin D deficiency and insufficiency was common in the immigrant group and no difference was shown between men and women. A diet including a high intake of fatty fish was most important in avoiding vitamin D deficiency. Vitamin D status was associated with muscle strength in all immigrants. Vitamin D deficiency was not associated with anxiety, depression or QoL in the immigrants. In female immigrants from the Middle East, anxiety was associated with 25(OH)D levels ≤49 nmol/L. Supplementation with cholecalciferol 2000 IU/day for three months was safe in healthy individuals with initial 25(OH)D 25-49 nmol/L, but monitoring is warranted since 11 % did not attain sufficient vitamin D status. The dose 10 000 IU/day in patients with initial 25(OH)D <25 nmol/L was unnecessarily high.
**Sammanfattning på svenska**

**Bakgrund:** D-vitamin är ett prohormon med betydelse för omsättning av kalcium och fosfat samt fysiologiska funktioner i hela kroppen. D-vitamin tillförs vid exposition för ultraviolett ljus eller via föda. Förekomsten av D-vitaminbrist hos immiгранter i Sverige var okänd. Det saknades samstämmighet om hur D-vitaminbrist skulle definieras och behandlas. Inga rena D-vitaminpreparat fanns att föreskriva i Sverige.

**Syfte:** Att studera förekomst av D-vitaminbrist och faktorer med betydelse för uppkomst av D-vitaminbrist hos immiгранter med ursprung i Afrika och Mellanöstern, att undersöka samband mellan D-vitaminstatus och muskelstyrka, depression, ångest och livskvalitet, samt att bestämma effekten av behandling med kolekalciferol på 25-hydroxyvitamin D [25(OH)D] och D-vitaminstatus.

**Metoder:** Två studier genomfördes i Umeå:

**Resultat:** Tolv procent av immiгранterna hade D-vitaminbrist (25(OH)D <25nmol/L) och totalt 73 % hade icke adekvat D-vitaminstatus (25(OH)D <50 nmol/L). Det var ingen skillnad i medelvärdet för 25(OH)D mellan män och kvinnor. D-vitaminbrist var dubbelt så vanligt hos immiгранter från Afrika som hos de från Mellanöstern. Intag av fet fisk mindre än en gång per vecka, frånvaro av utlandsresor och användning av långärmad klädsel utomhus på sommaren var förenat med ökad risk för D-vitaminbrist. Det fanns ett positivt samband mellan D-vitamin och muskelstyrka; muskelstyrkan i benen var högre vid högre 25(OH)D och greppstyrkan var lägre vid D-vitaminbrist. Det fanns inget samband mellan D-vitaminbrist och depression, ångest eller livskvalitet i den totala immiгранtragruppen. Intag av kolekalciferol var effektivt för att höja 25(OH)D, 100% av deltagare i grupp 1, 89% i grupp 2a, 55% in grupp 2b och 96%
in grupp 3 uppnådde adekvat D vitaminnivå. I grupp 1 nådde 62 % 25(OH)D ≥125 nmol/L.

Original papers

I. **Granlund L**, Ramnemark A, Andersson C, Lindkvist M, Fhärm E, Norberg M.
Prevalence of vitamin D deficiency and its association with nutrition, travelling and clothing habits in an immigrant population in Northern Sweden.

Vitamin D is associated with lower limb muscle strength and grip strength in Middle Eastern and African-born immigrants in Sweden.
[Submitted]

Associations between vitamin D status and anxiety, depression and health-related quality of health in an immigrant population. A cross-sectional study from Sweden.
[Manuscript]

IV. **Norberg M**, Granlund L, Ramnemark A, Andersson C, Fhärm E, Lindkvist M.
A randomised trial of vitamin D among immigrants in Sweden: response to treatment - a question of starting point and dose.
[Submitted]
Abbreviations

AE  Adverse events
ALP  Alkaline phosphatase
25(OH)D  25-hydroxyvitamin D3
BMD  Bone mineral density
BMI  Body mass index
CPBA  Competitive protein binding assays
CRF  Case Report Forms
DEQAS  The Vitamin D External Quality Assessment Scheme
EFSA  The European Food Safety Authority
ELISA  Enzyme-linked immunosorbent assays
Eudra-CT  European Clinical Trials Database
EQ-VAS  EuroQoL visual analogue scale
EQ-5D  EuroQoL-5 Dimension Questionnaire 3 Levels
HAD  Hospital Anxiety and Depression Scale
HPLC/UV  High-performance liquid chromatography with UV-detection
IOM  Institute of Medicine (USA)
IU  International units
LC-MsMs  Liquid chromatography-tandem mass spectrometry
MED  Minimum erythemal dose
MPA  Medical Products Agency
MF indices  The standardised muscle function indices of muscle strength
nmol/L  Nanomol/litre
PP  Pharmaceutical product
PTH  Parathyroid hormone
QoL  Health-Related Quality of Life
RCT  Randomized controlled trials
RDI  Recommended daily intake
RIA  Immunoassays (radioimmunoassays)
SACN  the British Scientific Advisory Committee on Nutrition
SD  Standard Deviations
UL  Tolerable Upper Intake Levels
UVB  Ultraviolet B radiation
VDBP  Vitamin D-binding protein
VDSP  Vitamin D Standardization Program
VDR  Vitamin D receptor
VIDI1  Vitamin D Deficiency in Immigrants Survey
Background

The history of vitamin D – a brief summary
Sunrays were considered to have a positive effect on physical strength and energy long before the detection of ultraviolet (UV) radiation and vitamin D. In Ancient Egypt (1550 BC) Amon-Rah (the Sun God) was believed to make men stronger, and in Ancient Greece Herodotus (484 - 425 BC) recommended solaria as a cure for weak and flabby muscles.\(^1\) Associations between depressive disorders and lack of exposure to sun have been noted in literature since the 6\(^{th}\) century.\(^2\) The association between rickets and osteomalacia and muscle weakness has also been observed for many centuries.\(^1\) In 1645 Whistler published a thesis on “De morbo puerili Anglorum” (rickets) and in 1651 Glisson followed with a thesis on rickets being a common disease in children.\(^3\)\(^-\)\(^4\) During the 19th century, rickets developed into an epidemic in children in the industrialized cities of Northern Europe and the United States,\(^5\) most probably due to the effect of less sun exposure for children working in factories.

The importance of endogenous production of vitamin D by the action of sunlight on the skin was described early in the 20th century and developed from the high incidence of osteomalacia in women who, for religious reasons or traditional custom, totally covered themselves in clothes.\(^6\) Dietary deficiency of vitamin D was uncommon as a cause of osteomalacia except in extraordinary circumstances such as during the world wars.\(^7\) In 1919 Sir Edward Mellanby showed that cod liver oil could cure rickets in dogs which had been induced by prolonged stays indoors and, in 1922, McCollum named the new substance vitamin D.\(^8\)\(^,\)\(^9\) Alfred Hess showed that rickets in children could be treated by direct exposure to sunlight in the same year.\(^10\) The chemical structure of cholecalciferol (vitamin D\(_3\)) was identified by Adolf Windaus who was awarded the Nobel prize for his work on sterols and vitamins in 1928.\(^1\)

The positive effects of high dose vitamin D therapy in adults with osteomalacia and weakness affecting lower limb proximal musculature was shown in the 1960s. Chalmers described osteomalacia in elderly women with classic symptoms of progressive proximal muscle weakness, skeletal pain and, in some patients, osteomalacic fractures resembling those in osteoporosis. Correction of diet and supplementation with 200 IU of cholecalciferol per day and calcium was recommended when dietary deficiency alone was the cause of the disease. In cases of malabsorption or resistant rickets, very large doses could be required i.e. 50 000 IU daily orally at start with measurements of phosphate, calcium and urea initially at monthly intervals and then at three monthly intervals.\(^7\) After a clinical cure had been obtained, the dose was reduced to 50 000 IU per week.\(^7\)
**Physiology**

Vitamin D is a prohormone that plays a key role in calcium and phosphate balance and bone structure. Vitamin D is not a true vitamin as these are defined as essential substances that are obtained exclusively from diet. Although small amounts of vitamin D (vitamin D2 or vitamin D3) are supplied by food, more than 90% of human vitamin D supply is synthesized endogenously when the skin is exposed to ultraviolet B (UVB) radiation. (Figure 1) When solar UVB radiation (wavelength 290-315 nm) penetrates the skin, 7-dehydrocholesterol is converted to pre-vitamin D3, which is converted to vitamin D3 (cholecalciferol).

Vitamin D is circulated to the liver where it is hydroxylated to 25-hydroxyvitamin D [25(OH)D] of which the 25(OH)D3 derivate is named calcidiol and the 25(OH)D2 derivate is named ercalcidiol. 25(OH)D is used as a biomarker to determine vitamin D status in populations with low exposure to UVB irradiation. 25(OH)D has a mean half-life of approximately 13–15 days. It is taken up into many tissues, including the adipose tissue, muscle and liver for storage.

In circulation, the vitamin D binding protein (VDBP) is the main carrier of vitamin D metabolites. 85-90% of total circulating 25(OH)D is bound to VDBP, 10-15% is bound to albumin and less than 1% is in the free form.

In the kidneys, 25(OH)D is hydroxylated to the biological active steroid hormone 1,25-dihydroxyvitamin D [1,25(OH)₂D₃ = Calcitriol]. The renal production of 1,25(OH)₂D is tightly regulated by plasma parathyroid hormone (PTH) levels which in turn are regulated by serum calcium and phosphorus levels.

1,25(OH)₂D binds to the vitamin D receptor (VDR) in the target tissue to exert its biological effects. The VDR is a nuclear, ligand-dependent transcription factor that in complex with hormonally active vitamin D as 1,25(OH)₂D, regulates the expression of more than 900 genes involved in a wide array of physiological functions.

The classical target tissues of 1,25(OH)₂D include bone, intestines, kidney and parathyroids. However, the VDR have been identified in the cardiovascular system, in most cell types in the immune system, and also in other tissue such as pancreas, skeletal muscle, lungs, central nervous system and reproductive system. Thus, 1,25(OH)₂D in association with VDR undertakes a biological function not limited to bone, intestine, kidneys and parathyroid glands, but throughout the body, regulating many functions. In muscles, 1,25(OH)₂D increases calcium influx in muscle cells and thus may have both direct and indirect calcium-related effects on muscle. Vitamin D sufficiency enhances calcium absorption by 30–40% and phosphorus absorption by 80%. The level of PTH is shown to be negatively correlated to the 25(OH)D level with a plateau at 25(OH)D 78 nmol/L.
Different genotypes affect synthesis, transport and metabolism of vitamin D, VDBP and VDR, with an impact on bioavailable 25(OH)D, inactivation of degradation resulting in hypercalcemia and nephrolithiasis, increased activation resulting in vitamin D deficiency. Associations have been shown between polymorphism in the gene coding the VDR and different phenotype characteristics such as reduced muscle strength, lower fat free mass, risk of sarcopenia and a greater incidence of falls, although these have not been consistent.\textsuperscript{1, 24, 25}

**Figure 1.** Physiology of vitamin D – the major metabolic pathways of vitamin D. Reproduced with permission from Cambridge University Press: Br J Nutr. 2003;89(5):552-72. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence?\textsuperscript{13}
Dietary intake of vitamin D

Vitamin D from foods is absorbed throughout the small intestine, mostly in the distal small intestine.\textsuperscript{16} Due to the fat-soluble characteristics of vitamin D, the absorption process is more efficient in the presence of biliary salts and when dietary fat is present in the lumen of the small intestine.\textsuperscript{16}

Except from some mushrooms, only animalic foods have a natural content of vitamin D. Table 1. The major food sources for naturally-occurring vitamin D include animal foods such as fatty fish, cod liver oil, egg yolks, offal (particularly liver), meat and meat products.\textsuperscript{12, 26} Further sources of dietary vitamin D are fortified foods (most often milk, margarine and/or butter, and breakfast cereals) and dietary supplements.\textsuperscript{12, 16} In Sweden vitamin D supplementation of milk and margarine is prescribed by law.\textsuperscript{27} The high natural content of vitamin D in fish presumably derives from an accumulation in the food chain originating from microalgae/phytoplankton who, when exposed to sunlight, produce vitamin D and contain both vitamin D\textsubscript{3} and pro-vitamin D\textsubscript{3}.\textsuperscript{5, 28} The vitamin D content in wild salmon is calculated at 600-1000 International units (IU) vitamin D\textsubscript{3}/100 g while farmed salmon is calculated at 100-250 IU vitamin D\textsubscript{3} or D\textsubscript{2}/100 g.\textsuperscript{12} Absence of bioaccumulated vitamin D in fish fodder as well as lack of UVB irradiation in crowded fish farms could explain the difference in vitamin D content in wild salmon compared to farmed salmon. The content of vitamin D in meat products varies and depends, among other things, on the contents of vitamin D in fodder, the fat content of the meat product and the latitude where the animals have grazed.\textsuperscript{16, 29, 30}

Intake of vitamin D via food varies according to eating habits. In a British study of postmenopausal women, median intake of vitamin D by food was 80-100 IU in Caucasian women and 50-65 IU in Asian women.\textsuperscript{31} In a Swedish nutrition study using food questionnaires, mean vitamin D intake was 280 IUs/day (7 micrograms), lower in women (256 IUs/day= 6.4 micrograms) than in men (304 IUs/day =7.6 micrograms) and lower in younger adults compared to elderly. Fish contributed 32\% of vitamin D intake, margarine and dairy products 14\% and 12\% respectively.\textsuperscript{32} However, only a few immigrants participated in this study.
Table 1.

Vitamin D content in food and other sources of vitamin D

<p>| Vitamin D content in food and other sources of vitamin D |</p>
<table>
<thead>
<tr>
<th>Vitamin D content</th>
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<tbody>
<tr>
<td><strong>Food natural content:</strong></td>
<td></td>
</tr>
<tr>
<td>Salmon</td>
<td></td>
</tr>
<tr>
<td>fresh, wild</td>
<td>600-1000</td>
</tr>
<tr>
<td>fresh, farmed</td>
<td>100-250</td>
</tr>
<tr>
<td>Sardines, canned</td>
<td>300</td>
</tr>
<tr>
<td>Mackerel, canned</td>
<td>250</td>
</tr>
<tr>
<td>Tuna, canned</td>
<td>230</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>400-1000 (teaspoon)</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>20 (one egg yolk)</td>
</tr>
<tr>
<td>Chanterelle mushrooms, wild, raw</td>
<td>600</td>
</tr>
<tr>
<td><strong>Fortified foods (guaranteed content in Sweden):</strong></td>
<td></td>
</tr>
<tr>
<td>Skimmed and semi-skimmed milk</td>
<td>15-40</td>
</tr>
<tr>
<td>Margarine and butter/rapeseed oil mix;</td>
<td>300-400</td>
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<tr>
<td><strong>Supplements:</strong></td>
<td></td>
</tr>
<tr>
<td>Multivitamins</td>
<td>300</td>
</tr>
<tr>
<td><strong>Sun light/ UVB exposure:</strong></td>
<td></td>
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<tr>
<td>UV exposure of one-quarter of personal skin area</td>
<td>1000</td>
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Footnote: Recalculated from references using; 1 microgram equals 40 IU, 1 oz = 28.3 grams, 3.5 oz equals approximately 100 grams.

Ultraviolet B radiation-induced vitamin D synthesis in the skin

The synthesis of vitamin D3 in the skin is affected by latitude, season, ozone layer and clouds (absorbing UVB radiation), surface characteristics (reflecting UVB irradiation), altitude, time spent outdoors, use of sunscreen, clothing, skin pigmentation, age and 25(OH)D levels. With increasing latitude vitamin D synthesis in the skin is insufficient in parts of the year, for example in Tromsø, Norway (69.4°N) there is insufficient UVB exposure for vitamin D synthesis between the beginning of October through to mid-March. Using Engelsens calculator tool (https://fastrt.nilu.no/VitD-ez.html) vitamin D synthesis in skin
would be possible in Umeå during the period 11 March – 30 September if the weather is clear and the period would expand to 18 February – 21 October if the ozone layer was thin. Repeated studies show that one full body UV exposure causing a slight pinkness in skin (one minimum erythemal dose, 1 MED) is equivalent to an oral intake of somewhere in the range 250–625 μg (10,000–25,000 IU) of vitamin D₃.³⁶ On the basis of these results, the UV exposure of one-quarter of personal MED on one-quarter of skin area (hands, face and arms) yields a dietary equivalent vitamin D dose of about 1000 IU. The exposure times necessary to obtain this recommended UV dose depend greatly on skin type, time and location as well as ambient conditions and clothing.³⁶ Adequate vitamin D synthesis may require quite long periods of sun exposure even when the sun is high, especially when only parts of the body are exposed.³⁶

**Effects of latitude and season**

The necessity of UVB radiation to enable vitamin D synthesis in the skin implies an effect of season and latitude on vitamin D status. Several studies have shown that the 25(OH)D levels in the population are lowest in winter and spring.³¹, ³⁸⁻⁴⁰ Many population studies also show associations between lower 25(OH)D levels and higher latitude.²³, ³¹, ⁴¹ The effect of latitude seems to be significant only for Caucasians in global ecological studies where a decline in 25(OH)D levels with latitude was calculated to -0.69 nmol/L per degree.⁴² However, in the Nordic countries, 25(OH)D levels are usually higher than in Southern and Middle Europe ⁴³⁻⁴⁵, the explanation for this has been suggested as a combination of lighter skin, sun exposure habits and high levels of fish consumption.⁴⁶ Consequently, latitude is important only when other factors that impact vitamin D status are equal.

**Laboratory analyses of 25(OH)D**

*Total 25(OH)D is used as a biomarker for determining vitamin D status,*¹⁴ *it reflects vitamin D both from UVB exposure and dietary intake.*¹⁶ *One limitation of the use of 25(OH)D is that it has been observed to decrease in response to acute inflammation.*⁴⁷

Common methods used for analysing S-25(OH)D⁴⁸, ⁴⁹ include high-performance liquid chromatography with UV detection (HPLC/UV), liquid chromatography-tandem mass spectrometry (LC–Ms/Ms), and immunoassays; radioimmunoassays (RIA), competitive protein binding assays (CPBA) and enzyme-linked immunosorbent assays (ELISA) that are either manual or automated. LC–Ms/Ms and HPLC are considered as the gold standard methods.⁴⁸, ⁴⁹ Both methods can measure 25(OH)D₃ and 25(OH)D₂ separately.⁵⁰ In the past, there was no international common standard for methods which contributed to the variability of results of 25(OH)D measurements.⁴⁹ In 1989 the
Vitamin D External Quality Assessment Scheme (DEQAS) was introduced with the aim of improving the reliability of 25(OH)D assays. A standard reference material for vitamin D in human serum was introduced by the US National Institute of Standards and Technology to facilitate by providing a reference measurement procedure against which assays could be standardised. The Vitamin D Standardization Program (VDSP) has developed protocols for standardising procedures of 25(OH)D measurement in national health/nutrition surveys to promote 25(OH)D measurements that are accurate and comparable over time, location and laboratory in order to improve public health practices. In the VDSP, LC–Ms/Ms is the reference method.

There is a high level of interassay disagreement between methods for 25(OH)D analyses of which many tend to underestimate 25(OH)D levels compared to LC–Ms/Ms. In a study comparing HPLC atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS), RIA and a chemiluminescent immunoassay (CLIA) mean 25(OH)D levels differed between 85 -70 - 60 nmol/L respectively with the highest level in HPLC-APCI-MS and the lowest levels in CLIA analyses. Using a 50 nmol/L cutoff, 43 % were vitamin D insufficient using CLIA, 22% when using RIA and only 8% were vitamin D insufficient using HPLC-APCI-MS. As 25(OH)D can vary considerably depending on type of assay used, reports on the relationship between 25(OH)D and health outcomes should be interpreted with care, taking into account the type of assay employed, use of automation, year and context of analysis. It has been suggested that cutoff points for vitamin D deficiency should be assay specific.

**Vitamin D and associated medical conditions**

Osteomalacia (and rickets in children) remain the only medical conditions that are unambiguously a consequence of vitamin D deficiency.

**Musculoskeletal health outcomes**

Vitamin D deficiency leads to impaired mineralization of bone due to inefficient absorption of dietary calcium and phosphorus, which is associated with an increase in PTH to prevent hypocalcaemia. Clinically, the impaired mineralization manifest as rickets in children, and osteomalacia in adults.

**Rickets** is mostly observed before 18 months of age. It is characterized by a triad of clinical symptoms: skeletal changes (deformities such as enlargements of the wrists, bowing of the long bone epiphyses and swollen costochondral junctions, craniotabes and growth retardation), radiologic changes (widening of the metaphyseal plates, decreased mineralization, deformities) and increases in bone ALP activity in serum. Depending on the severity and duration of vitamin D deficiency, initial normocalcaemia may progress to hypocalcaemia, tetani and
seizures.\textsuperscript{58} In case reports, 25(OH)D ranges from \(<2.5 \text{ nmol/L}\) - \(<50 \text{ nmol/L}\), however, it is possible that presence of rickets at 25(OH)D levels \(\geq 25 \text{ nmol/L}\) might be explained by calcium deficiency.\textsuperscript{60}

**Osteomalacia** in adults is characterized by increased bone resorption and suppression of new bone mineralization.\textsuperscript{61} S-Calcium levels are often normal.\textsuperscript{59} The osteomalacic patients often complain about skeletal pain and progressive muscle weakness. Muscle weakness produces a typical flat-footed springless gait often characterised as a *penguin walk* and getting up from a chair is difficult for severely-affected patients. The progressive skeletal pain commonly occurred in the thorax, shoulder girdle and thighs, forearms and feet.\textsuperscript{7} Some patients present with fractures, sometimes complete fractures but also stress fractures in ribs, neck of femur or forearm bones or greenstick fractures in adults.\textsuperscript{7} According to the Bingham and Fitzpatrick criteria, osteomalacia diagnosis should be defined by two of the following criteria; low calcium, low phosphatase, elevated total ALP or radiographic findings.\textsuperscript{62, 63}

Evidence on the relationship between 25(OH)D levels and osteomalacia is limited and arises primarily from case reports in which 25(OH)D ranged from 4-20 nmol/L, and two observational studies where osteomalacic patients had 25(OH)D \(<7.5 \text{ nmol/L}\) and 15 nmol/L respectively.\textsuperscript{62, 64} In a post-mortem study, bone undermineralization defined as pathological accumulation of osteoid was assessed and osteomalacia was defined as a ratio of unmineralized osteoid volume to total bone volume \(\geq 2\%\). In this study, no subjects with 25(OH)D \(\geq 75 \text{ nmol/L}\) had osteomalacia, about 1\% of subjects with 25(OH)D \(>50 \text{ nmol/L}\) had osteomalacia and less than half of the subjects with 25(OH)D \(<40 \text{ or even <25 nmol/L}\) had osteomalacia.\textsuperscript{65} The European Food Safety Authority (EFSA) has stated that the risk of vitamin D deficiency osteomalacia is limited with 25(OH)D levels \(\geq 50 \text{ nmol/L}\).\textsuperscript{16}

**Bone mineral density (BMD), osteoporosis and fractures.** Most observational studies of post-menopausal women and older men support an association between 25(OH)D and BMD or change in BMD, especially in the hips where 25(OH)D \(<30 - <80 \text{ nmol/L}\) were associated with an increase of bone loss.\textsuperscript{16, 55, 66} This may provide the preconditions for development of osteoporosis and increase the risk of fracture. Results from randomized controlled trials (RCTs) have been inconsistent whether increasing 25(OH)D by supplementation with vitamin D thereby increasing BMD or whether increasing 25(OH)D affects bone loss.\textsuperscript{67-69} In 2016, EFSA considered that there is some evidence that the risk of increased bone mineral content loss is higher in non-institutionalised adults with 25(OH)D \(<50 \text{ nmol/L}\),\textsuperscript{16} and the British Scientific Advisory Committee on
Nutrition (SACN) have concluded that there is suggestive evidence of a beneficial effect of vitamin D supplementation in adults ≥50 years.\textsuperscript{60}

The majority of observational studies mostly focus on upper middle-aged or elderly individuals and show an association between low 25(OH)D and fractures.\textsuperscript{70-73} In 2014, a Cochrane review stated that vitamin D in combination with calcium may prevent hip fractures, non-vertebral fractures and any fractures.\textsuperscript{74} With respect to studies published later, EFSA in 2016 stated that the majority of studies indicate an increased fracture risk associated with 25(OH)D <18 nmol/L to <50 nmol/L in free-living adults.\textsuperscript{16} Still, RCTs on the effect of vitamin D supplementation have shown inconsistent results.\textsuperscript{75-77}

**Muscle pain and weakness (myopathy).** The clinical symptoms of vitamin D deficiency in adults may include diffuse pain in muscles and bone as well as specific fractures.\textsuperscript{16} Observational studies have shown associations between 25(OH)D <50 nmol/L and muscle strength/physical performance,\textsuperscript{78-82} but RCTs have been inconclusive.\textsuperscript{83-86} However, recent reviews and meta-analyses support that vitamin D supplementation improves limb muscle strength in adults <40 years with mean baseline 25(OH)D of around 30 nmol/L,\textsuperscript{87} and in adults >50 years with 25(OH)D <30 - <66 nmol/L.\textsuperscript{60,88} Muscle pain and weakness that follow the skeletal symptoms in the elderly may contribute to poor physical performance, increased risk of falling and fractures.\textsuperscript{16}

**Non-musculoskeletal health outcomes**

**Pregnancy: Pre-eclampsia;** inverse associations between 25(OH)D and pre-eclampsia have been shown. Smaller-scale intervention studies support the view that treatment with vitamin D during pregnancy may reduce the risk of pre-eclampsia, but there is insufficient data on side effects.\textsuperscript{60,89} **Neonatal hypocalcemia:** vitamin D supplementation during pregnancy has been associated with a reduction of neonatal hypocalcemia.\textsuperscript{60,90-92} A higher incidence of **dental enamel hypoplasia** was also shown in infants who had been hypocalcaemic.\textsuperscript{60,90} Observational studies are inconsistent regarding the association between maternal 25(OH)D and the cognitive and psychological development of the child. RCTs have found no effect of vitamin D supplementation on birth weight, birth length or head circumference.\textsuperscript{60,93}

**Cancer;** observational studies have indicated an inverse association between 25(OH)D and colorectal cancer.\textsuperscript{94-95} Data regarding pancreatic cancer is inconsistent.\textsuperscript{55} There are no RCTs showing an effect of vitamin D supplementation on cancer risk.\textsuperscript{60}
**Cardiovascular disease and hypertension (CVD/HT):** observational studies show associations between 25(OH)D and CVD/HT \(^{96, 97}\) but this is not supported in interventional studies.\(^{60, 98}\)

**All-cause mortality:** a U-shaped relationship between 25(OH)D levels and all-cause mortality has been shown in observational studies.\(^{99, 100}\) In 2011 the IOM concluded that data suggested an increased risk of all-cause mortality within 25(OH)D <30 nmol/L and >75 nmol/L.\(^{55}\) In a meta-analysis, the relative risk for all-cause mortality was shown to decline until 25(OH)D reached 90 nmol/L,\(^{101}\) but reverse causality or confounders could not be excluded. A Cochrane analysis showed that vitamin D3 in combination with calcium appeared to reduce mortality in the elderly\(^{102}\) and later meta-analyses have reinforced that 10-20 microgram per day of vitamin D could reduce both all-cause mortality and cancer mortality in middle-aged and older people.\(^{11, 103}\) However, further placebo-controlled studies are warranted.

**Immune modulation and infectious disease:** An inverse association has been shown between 25(OH)D and Multiple Sclerosis (MS), and it has been suggested that vitamin D is beneficial for the inflammatory component of MS.\(^{104}\) Studies of associations between low 25(OH)D and asthma, atopic conditions and autoimmune diseases are inconsistent.\(^{60}\) Overall, evidence is not consistently supportive of a causal role for vitamin D in reducing risk of asthma or autoimmune disease.\(^{60, 105}\) Observational studies often show an inverse association between 25(OH)D and infectious disease, but reverse causality is possible as RCTs are often inconclusive.\(^{60}\) However, recent meta-analyses have stated that vitamin D supplementation might prevent both upper respiratory tract infections and asthma exacerbations.\(^{103}\)

**Neuropsychiatry and mood disorders:** Cross-sectional data show inverse associations between 25(OH)D and cognitive decline\(^{106-108}\), depression\(^{109-111}\) and anxiety\(^{112, 113}\) However, a reverse causation with change of behaviour or diet is possible.\(^{60}\) RCTs have not supported any effect of vitamin D supplementation on cognition\(^{60}\) and RCTs studying effects on depression yield conflicting or non-significant results.\(^{60, 114-117}\) Longitudinal studies have not confirmed any effect of 25(OH)D on depression\(^{118-120}\) or anxiety.\(^{121}\) However, meta-analyses selecting RCTs without biological flaws demonstrate a significant improvement in depression with vitamin D supplements.\(^{2}\) "Biological flaws" refers to limitations in study design which preclude the study from testing the research hypothesis. The hypothesis can only be tested if the participants are vitamin D deficient at baseline and receive sufficient doses of vitamin D to reach vitamin D sufficiency during the trial.\(^{2}\) Data on autism and schizophrenia is sparse.\(^{60, 122}\)
**Oral health:** the impact of vitamin D on teeth mineralization in patients with rickets has been known for more than a decade.\(^{123}\) The changes in enamel and dentine occur from intrauterine development up to 18 years of age.\(^{60}\) Cross-sectional studies also show associations between 25(OH)D and periodontitis.\(^{60}\)

*In conclusion, 25(OH)D levels have been shown to be reduced in almost every medical or psychiatric disorder studied.*\(^{124}\) Except for osteomalacia and rickets, causality has not been shown for these associations with 25(OH)D and RCTs are often inconclusive or negative. A well-known problem is that the scale of many trials is too small, too short in duration of intervention, use monthly instead of daily dosing and are dominated by vitamin D-replete participants. Large-scale trials are often performed in high-resource countries and do not focus on participants with low 25(OH)D at baseline.\(^{103}\) *In the same way, many overlapping meta-analyses of poor quality fail to identify results of interest to public health thus reducing confidence in results.*\(^{103}\)

**Definitions of vitamin D deficiency and sufficiency**

**European:** The traditional definition of vitamin D deficiency is 25(OH)D < 25 nmol/L based on metabolic bone disease.\(^{60}\) 25(OH)D ≥ 50 nmol/L is considered sufficient.\(^{16}\) This definition is used in this thesis and in the VIDI1 and the Intervention Study. Table 2.

**The Institute of Medicine (IOM)** in the USA consider persons to be at risk for vitamin D deficiency at 25(OH)D < 30 nmol/L. This definition is also based on bone health, 25(OH)D levels of 40 nmol/L should meet the needs of approximately half the population, and 25(OH)D levels of 50 nmol/L should meet the needs of at least 97.5% of the population.\(^{55}\)

**The Endocrine society** (a global organization of endocrinology professionals) suggested in 2011 that 25(OH)D < 50 nmol/litre should be considered as vitamin D deficiency and 25(OH)D 50 – 72.5 nmol/L as vitamin D insufficiency. 25(OH)D should exceed 75 nmol/L to maximize the effect of vitamin D on calcium, bone and muscle metabolism.\(^{125}\) The level 75 nmol/L could be motivated both by the study performed by Priemel, where no subjects with 25(OH)D > 75 nmol/L had osteomalacia, and by the effect of 25(OH)D on PTH which reach a plateau at 24(OH)D ≈ 78 nmol/L.\(^{23, 65}\) According to this definition, approximately 40% of the population of Europe would have vitamin D deficiency.\(^{126}\) The level 75 nmol/L was used for the definition of “optimal vitamin D status in paper 1. Table 2.
Table 2. Overview of definitions, recommended intakes and treatment of vitamin D deficiency

Definitions of vitamin D status according to 25(OH)D levels (nmol/L)

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>IOM</th>
<th>ES</th>
<th>European traditional definition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>&lt;30</td>
<td>&lt;50</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>30-49</td>
<td>&lt;72,5</td>
<td>25-49</td>
</tr>
<tr>
<td>Adequate</td>
<td>≥50</td>
<td></td>
<td>≥50</td>
</tr>
<tr>
<td>Optimal**</td>
<td>-</td>
<td>≥75</td>
<td>-</td>
</tr>
</tbody>
</table>

IOM; Institute of Medicine ES; Endocrine Society, *Definitions used in this thesis. 1 ng/mL equals 2.5 nmol/L. **Definition of “optimal” in Paper 1.

Dietary intake reference values of vitamin D in adults (IU)

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>600</td>
<td>600</td>
<td>400?</td>
<td>600</td>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td>Elderly</td>
<td>≥71y 800</td>
<td>≥70y 800</td>
<td></td>
<td>≥61y 400</td>
<td>≥75y 800</td>
<td></td>
</tr>
</tbody>
</table>

EFSA; The European Food Safety Authority, NNR; The Nordic Nutrition Recommendations, 1 microgram equals 40 IU.

Treatment recommendations of vitamin D deficiency (vitamin D IU/day)*

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>IOM 2011</th>
<th>ES 2011</th>
<th>Swedish guidelines 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency</td>
<td>Tolerable upper level of intake: ≤4000 IU/day. No recommendations regarding treatment of deficiency!</td>
<td>Tolerable upper level of intake: ≤4000 IU/day alternatively 10000 IU/day** Treatment: 6000 IU/day for 8 weeks followed by 1500-2000 IU/day**. 10000 IU/day may be needed initially to correct deficiency.</td>
<td>Treatment: 2000-4000 IU/day for 3-6 months, followed by 800-1600 IU/day. Higher doses may be necessary for symptoms of osteomalacia/myopathy.</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>200-800 IU/day</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Vitamin D should be combined with calcium if tolerated. **For patients at risk of vitamin D deficiency. 1 microgram equals 40 IU.
Dietary intake reference values

**EFSA 2016;** An adequate intake for vitamin D for adults (≥1 year age) including pregnant and breastfeeding women was set at 600 IU/day. At this intake most of the population will achieve a 25(OH)D near or above the target of 50 nmol/L. The adequate intake was based on musculoskeletal health and pregnancy-related health, and set assuming adequate intake of Calcium. Table 2.

**Nordic Nutrition Recommendations (NNR) 2004 & 2012;** In 2004 the daily recommended intake (RDI) of vitamin D for ages 2-60 y was set to 300 IU/day and for those ≥61 year and pregnant or breastfeeding women, 400 IU/day. In 2012, the RDI for vitamin D was increased to 400 IU/day for adults (10-74 years old including pregnant and breastfeeding women) and 800 IU/day in ages ≥75 years. For people with limited sun exposure the RDI was set at 800 IU/day.

**IOM (2011);** The Recommended Daily Allowances of vitamin D exclude vitamin D intake from sun and release of cutaneous produced vitamin D from stores. The recommendations for ages 1-70 year were set to 600 IUs per day and 800 IUs per day for older, corresponding to a 25(OH)D level ≥50 nmol/L. Minimal sun exposure was assumed when establishing the recommendations for vitamin D. The IOM estimated this recommendations to cover requirements of ≥ 97.5 % of the population.

**The Endocrine Society guidelines (2011)** suggested that adults (19–70 years) require at least 600 IU/d of vitamin D and that adults ≥70 years require at least 800 IU/d of vitamin D, to maximize bone health and muscle function. They also stated that whether 600 and 800 IU/d of vitamin D were enough to provide all of the potential non-skeletal health benefits associated with vitamin D was not known. To raise 25(OH)D >75 nmol/L might require at least 1500–2000 IU/d of supplemental vitamin D. Adults ≥ 19 years and older at risk for vitamin D deficiency (defined as 25(OH)D <50 nmol/L) were recommended a daily requirement of 1500-2000 IU. Recently, researchers associated to the Endocrine Society have re-analysed 25(OH)D in earlier studies with the LC-MsMs method and suggested that 400 IU would be enough as recommended daily intake for vitamin D according to bone health in the USA in winter.
Treatment of vitamin D deficiency

Supplementation with vitamin D  Table 2.

The Swedish recommendations (2014); These were published in 2014. In treatment of vitamin D deficiency and insufficiency, vitamin D should be combined with Calcium if tolerated. In subjects with 25(OH)D 25-50 nmol/L, treatment should be initiated with cholecalciferol 200-800 IUs/day based on the 25(OH)D level, and advice on lifestyle including food and sun exposure should be given. The dose of vitamin D in subjects with 25(OH)D <25 nmol/L should be calculated using a formula: target level (nmol/L) - measured level (nmol/L) = treatment dose cholecalciferol (microgram). In symptomatic patients with 25(OH)D <25 nmol/L higher doses were recommended; cholecalciferol 2000-4000 IUs/day for 3-6 months, followed by 800-1600 IUs/day. 25(OH)D should be measured 3-4 months after started treatment to evaluate treatment and enable adjustment of dose. 800 IUs of cholecalciferol are expected to increase 25(OH)D 20 nmol/L. In vitamin D-deficient patients with symptoms of osteomalacia or myopathy, higher initial doses could be indicated.

IOM (2011); The IOM stated that the tolerable upper level of vitamin D intake in adults (≥ 9 years) is 4000 IUs per day, higher intake may increase the risk of harm. The starting point for the upper limit was 10000 IUs/day, as lower intakes have not been linked to hypercalcemia or acute toxicity. The IOM concluded that short-term findings related to toxicity were not considered as the appropriate basis for definition of an upper intake level for the population intended to reflect long-term (essentially lifelong) intake or to be used for public health purposes. Therefore, the tolerable upper limit was corrected for uncertainty regarding chronic disease outcomes, all-cause mortality and concern about risks at 25(OH)D >125 nmol/L.

The Endocrine Society (2011) suggested that adults with 25(OH)D <50 nmol/L should be treated with 50000 IU of vitamin D2 or vitamin D3 once a week for 8 weeks, or its equivalent of 6000 IU of vitamin D2 or vitamin D3 daily, to achieve a target level of 25(OH)D above 75 nmol/L, followed by maintenance therapy of 1500–2000 IU/day. In obese patients, patients with malabsorption syndromes and patients on medications affecting vitamin D metabolism, two to three times higher dose was recommended; at least 6000–10,000 IU/d of vitamin D to treat vitamin D deficiency and to maintain 25(OH)D >75nmol/L followed by maintenance therapy of 3000–6000IU/d. The maintenance tolerable upper limits (UL) of vitamin D, which is not to be exceeded without medical supervision, should be 4000 IU/d for everyone over 8 years of age.
However, higher doses of 10,000 IU/d for adults 19 years and older were thought to be necessary to correct vitamin D deficiency.\textsuperscript{125}

**UVB therapy:** UVB exposure is an effective alternative for raising 25(OH)D levels. Full-body treatment with broadband UVB two to three times a week for 8-12 weeks is proved to raise mean 25(OH)D from 36.8 ±17 ng/ml to 59.6 ±19 ng/ml.\textsuperscript{132} The size of the exposed skin area, UVB dose, skin erythema and BMI were the major determinants for serum levels of skin synthesized cholecalciferol.\textsuperscript{133} The 25(OH)D response in UVB treatment is also inverse correlated to skin pigmentation, the increase in 25(OH)D in extremely dark-skinned individuals may be half of the increase in fair-skinned individuals.\textsuperscript{134} The total synthesis of vitamin D in the skin is regulated by UVB and photodegrading to biological inert isomers, prevents vitamin D toxicity due to prolonged sun exposure.\textsuperscript{135, 136}

**25(OH)D response in vitamin D supplementation**

A classic golden rule in supplementation with vitamin D is that 40 IUs of cholecalciferol daily (1 microgram) will raise 25(OH)D levels 2 nmol/L.\textsuperscript{137, 138} A higher vitamin D dose is shown to raise 25(OH)D more than a lower dose.\textsuperscript{139, 140} However, the dose response varies depending on several factors. There is an inverse relationship with more marked increase of 25(OH)D at the same cholecalciferol dose when given at a lower 25(OH)D baseline level compared to a higher 25(OH)D baseline level.\textsuperscript{141, 142} Supplementation with vitamin D3 is shown to increase 25(OH)D more than supplementation with vitamin D2.\textsuperscript{137} Body mass index (BMI) is associated with dose response, the same cholecalciferol dose will result in a greater increase of 25(OH)D in slim subjects compared to obese subjects.\textsuperscript{141}

**Safety aspects regarding 25(OH)D and high-dose vitamin D treatment**

Toxicity of vitamin D can appear relatively acute within 4 weeks of excess intake and is characterized by hypercalcemia associated with rising 25(OH)D levels. Symptoms include anorexia, weight loss, polyuria and heart arrhythmias. Eventually, tissue and vascular calcification appear with later renal and cardiovascular damage.\textsuperscript{55} Vitamin D doses below 10000 IUs/day are not usually associated with toxicity and most reports suggest that the toxicity threshold is between 10000-40000 IUs/day.\textsuperscript{55} Combined data on 25(OH)D levels from many vitamin D supplementation studies have shown a dose response curve which is surprisingly flat up to 250 microg (10000 IU) vitamin D/day reaching 25(OH)D around 125 nmol/L, at higher doses the increase of 25(OH)D will become steeper.\textsuperscript{143} For comparison, 25(OH)D between 135-163 nmol/L are noticed in subjects with extensive sun exposure not using supplements.\textsuperscript{143} It has been suggested that the flat dose-response curve could be the result of homeostatic control systems maintaining 25(OH)D within the range 75-220 nmol/L across
vitamin D supplementation from 800 IUs/day to a physiological limit of 10000-20000 IUs/day. High 25(OH)D may lead to hypercalcaemia, however hypercalcemia due to vitamin D intoxication was always accompanied by 25(OH)D >220 nmol/L. Still, most reports do not identify vitamin D toxicity until 25(OH)D reaches >500-600 nmol/L. When establishing recommendations on Tolerable Upper Intake Levels (UL) for vitamin D in 2011, the IOM concluded that acute toxicity was not an appropriate basis for an UL that is intended to reflect long-term chronic intake and be used for health purposes. The UL was therefore reduced, taking into concern the uncertainty about chronic diseases and all-cause mortality. 25(OH)D3 >75nmol/L were not consistently associated with increased benefit and there were emerging concerns about risks at 25(OH)D levels above 125 nmol/L. An intervention study giving 5000 IUs cholecalciferol orally for 160 days resulted in 25(OH)D levels 100-150 nmol/L while 10000 IUs/day for 20 weeks resulted in 25(OH)D ≤220 nmol/L. The IOM decided to add a safety margin and reduced the dose 5000 IU by 20% and stated that the highest daily intake of vitamin D that is likely to pose no risk, based upon scientific data, was 4000 IUs vitamin D per day for ages ≥ 9 years.

Later, large population studies have demonstrated that increased risk for both cardiovascular mortality as well as all-cause mortality are associated with both low and high 25(OH)D levels. 25(OH)D around 60-75 nmol/L were associated with the lowest risk of all-cause mortality while 25(OH)D ≤10 nmol/L were associated with doubled risk of mortality and 25(OH)D ≥140 nmol/L were associated with a 40% higher risk. Similarly, 25(OH)D around 70 nmol/L were associated with the lowest risk of cardiovascular mortality while 25(OH)D ≤12.5 nmol/L were associated with doubled risk and 25(OH)D ≥125 nmol/L with a 30% higher risk. However, the cause of these associations is unknown. In 2016 EFSA re-evaluated data on vitamin D toxicity for adults, hypercalcaemia was selected as the indicator of hypervitaminosis D or vitamin D toxicity. Two studies had administered doses between 9360 and 11000 IU/day of vitamin D3 in men without reported hypercalcaemia, and a No Observed Adverse Effect Level of 10000 IUs/day had been suggested. However, EFSA decided to add an uncertainty factor of 2.5 and set the UL for adults at 4000 IUs/day, equal to the UL set by IOM in 2011.

**Risk groups for vitamin D deficiency**

**Medical conditions with impact on absorption or vitamin D synthesis.** Subjects with inflammatory bowel disease, celiac disease and subjects who have undergone gastric bypass surgery have reduced capacity to absorb vitamin D from foods and are at risk of vitamin D deficiency. Subjects with liver failure or kidney failure also have an increased risk of vitamin D deficiency. Obesity
has also been associated with increased risk of vitamin D deficiency however the mechanism for this has not been clarified.\textsuperscript{38, 39, 152, 153}

**Medication:** Subjects using medication with influence on vitamin D metabolism such as antiepileptic drugs, steroids, antifungal drugs, anti-HIV drugs\textsuperscript{130, 154}, medication causing fat malabsorption such as orlistat and cholestyramine and photosensitizers such as amiodarone have an increased risk of vitamin D deficiency.\textsuperscript{131}

**Reduced exposure to UVB radiation.** This is common in in-door living subjects\textsuperscript{152}, nursing home residents\textsuperscript{155}, subjects with frequent use of fully-covering clothes\textsuperscript{40} or use of sunscreen.\textsuperscript{156, 157}

**Subjects with limited ability to vitamin D synthesis in the skin.** The UVB exposure-induced vitamin D synthesis is decreased in subjects with dark skin pigmentation compared to subjects with light skin because of the higher content of melanin in the former group.\textsuperscript{16, 158-160} The ability to synthesize vitamin D in the skin also decreases with age.\textsuperscript{161}

**Dietary habits.** Individuals with poor nutrition, restricted diets avoiding fatty fish, fortified milk etc. are at risk for vitamin D deficiency.\textsuperscript{55, 162}

**Epidemiology**

Many reviews have been performed on the prevalence of vitamin D deficiency worldwide.\textsuperscript{45, 163-165} Global mean 25(OH)D has been calculated to 54 nmol/L (95% CI 52-57 nmol/L).\textsuperscript{42} Mean 25(OH)D <25 nmol/L was reported in 7% of cross-sectional studies, <50 nmol/L in 37% and >75 nmol/L in 12-20% of studies.\textsuperscript{42, 166} Estimated mean 25(OH)D was highest in Northern America (68 nmol/L), the South Asia/Pacific Region including Thailand and Australia (60 nmol/L) and Europe (54 nmol/L) and lowest in the Middle East and Africa (49 nmol/L).\textsuperscript{163, 166} In the USA 13% of adults were found to have 25(OH)D <37.5 nmol/L.\textsuperscript{167} In Europe, 2-30% of adults have been estimated to have 25(OH)D <25nmol/L.\textsuperscript{126, 165} 25(OH)D levels were generally higher in Scandinavia compared to Southern Europe. In Sweden, mean 25(OH)D in the general population has been found to vary between 68 - 95 nmol/L\textsuperscript{43, 82, 168-170} and in Northern Sweden <0.7% had 25(OH)D <25nmol/L.\textsuperscript{168} In the Middle East; Lebanon, Jordan and Iran, 60-80% were found to have 25(OH)D <25nmol/L.\textsuperscript{40, 171-173} Vitamin D deficiency has also been shown to be common in immigrants originating from the Middle East/African region and Asian region settling in Europe, North America and Australia.\textsuperscript{162, 174-176} In Sweden, the prevalence of vitamin D deficiency in immigrant women from Somalia varied between 73 -100%.\textsuperscript{177-179} Data are inconclusive as to whether children and non-institutionalised elderly generally
have lower 25(OH)D levels,\textsuperscript{42} in region-stratified analyses age-related differences in 25(OH)D were significant only in the Asia/Pacific and Middle East/African region,\textsuperscript{166} while in Northern Sweden, 25(OH)D levels were shown to increase with age.\textsuperscript{168} However, vitamin D deficiency was demonstrated to be common in nursing home residents and housebound elderly.\textsuperscript{180, 181} An ecological global study showed that women had a tendency to higher 25(OH)D levels compared to men (56±1.6 nmol/L and 50±2.6 nmol/L respectively)\textsuperscript{42} while cross-sectional studies tend to report lower 25(OH)D levels in women, especially in the Asia/Pacific and Middle East/Africa region.\textsuperscript{40, 166} Globally, Caucasians had on average 21.2 ±5.1 nmol/L higher 25(OH)D compared to non-Caucasians (68±3.2 nmol/L and 47 ±4.0 nmol/L respectively).\textsuperscript{42} In the USA, there were significant differences in mean 25(OH)D levels between Non-Hispanic whites (67 nmol/L), Non-Hispanic blacks (40 nmol/L) and Mexican Americans (54 nmol/L).\textsuperscript{167} However, there was a considerable variation of mean 25(OH)D in different studies within the same country or continent \textsuperscript{164-166} supporting the overriding effect of lifestyle, including sunlight exposure and to some extent the effect of skin pigmentation, on vitamin D status.\textsuperscript{165}

**Depression and anxiety**

**Prevalence and risk factors.**

Depression is one of the most common causes of disability in both low and high-income countries\textsuperscript{182} and is projected to become the second leading cause of disability-adjusted life years by 2030.\textsuperscript{183} There is a high co-morbidity between depression and anxiety.\textsuperscript{184} Mental illness is considered to be one of the current largest public health problems in Sweden\textsuperscript{185} and psychiatric problems are the largest single cause of sick leave for people of working age in Sweden.\textsuperscript{185}

There are several, well-known risk factors for mental illness such as female gender, older age and socio-economic factors including low socioeconomic status, poor financial situation, poor social support and unemployment.\textsuperscript{186} Another independent risk factor for depression is immigrant status. Although both anxiety and depression are common in the population in Sweden, immigrants from non-western countries report symptoms of anxiety and/or depression more frequently than the general population in Sweden.\textsuperscript{187} According to the Swedish National Public Health Survey, 37 % of women and 24 % of men reported slight or severe symptoms of anxiety, younger individuals reported more symptoms than older and women with low educational levels reported more symptoms than those with higher levels of education.\textsuperscript{185, 188} Immigrant women assessed their state of health as poor three times more often than Swedish-born women and immigrant men assessed their state of health as poor twice as often as Swedish-born men.\textsuperscript{185, 189} European surveys have shown that immigrants experience more depressive symptoms than natives in most European countries and the risk was still higher
even after adjusting for socio-economic conditions and the experience of ethnic discrimination. The first generation of immigrants reported more depressive symptoms. Studies in the Netherlands and Finland have shown that immigrants from The Horn of Africa (Somalia) had the lowest prevalence of both depression and anxiety, they had the same or even lower levels of depression and anxiety as natives while Middle Eastern (Kurdish and Iranian) immigrants showed the highest levels of depression and anxiety. In Kurdish immigrants in Finland, men showed clinical symptoms of depression or anxiety two to three times more often and women five times more often than native Finns. Examinations of somatization showed that the difference in symptoms of clinical depression and anxiety between immigrants from the Middle East and Horn of Africa cannot be explained by use of methods that do not measure somatization in depressed or anxious subjects.

**Measurement of depression and anxiety**

There are several different screening instruments for identifying depression and anxiety. Self-rating scales such as the Hospital Anxiety and Depressions Scale (HAD) which measure both symptoms of depression and anxiety and the Patient Health Questionnaire (PHQ-9) which measure symptoms of depression are often used in primary health care. All screening instruments are designed to overestimate the prevalence of morbidity. The HAD screening instrument has a predictive validity of approximately 70%. There are also diagnostic instruments for depression based on interviews, often constructed for grading the severity of depression, for example the Montgomery Åsberg Depression Rating Scale (MADRS) and the Becks Depression Inventory (BDI).

**Biological possibilities for effects of vitamin D on the brain.**

There are biological possibilities as concerns effects of vitamin D on the brain. VDR have been identified on neurons and glia in many areas of the human brain, and animal studies support that active vitamin D may be neuroprotective and contribute to preserve dopamine and serotonin levels. Active vitamin D have been found to antagonize the negative effect of glucocorticoids on cell differentiations in hippocampal cell lines, and it has also been suggested that vitamin D might have neuroprotective effects on diseases such as MS, Parkinson’s disease, chronic stress and depression.

**Muscle strength**

**Classification, measurements and associated factors**

Muscle strength is the extent of the power generated by muscle contraction. Muscle strength can be measured during isometric contraction; static muscular contractions characterized by increase in tension without change in length, or
isokinetic contraction; dynamic concentric (shortening) and eccentric (relaxation) muscle strength.\textsuperscript{203}

There are several different functional tests used for measurements of muscle strength in the legs, usually combining different daily physical activities such as getting up from a chair, walking a specified distance with measurement of time, standing back towards a wall with knees bent, hip lifting, squatting, balance tests etc.\textsuperscript{79, 204-206} Isometric muscle strength can also be measured by dynamometers, for example, in the knee extensors and in the hip flexor muscles.\textsuperscript{207} Muscle strength in the legs measured by walking speed is demonstrated to decrease with age and women generally perform poorer than men in tests of physical performance in the legs.\textsuperscript{78} Muscle strength in the legs is associated with BMI where a higher BMI is associated with reduced upper leg muscle strength.\textsuperscript{208} Muscle strength in the legs is also associated with joint problems in the knees and hips \textsuperscript{204} as well as use of walking devices and comorbidities.\textsuperscript{78} This may be an indicator of the importance of physical activity and training for muscle strength. Some population studies demonstrate ethnic differences in lower leg muscle strength, which makes influences of both lifestyle and genetic differences possible.\textsuperscript{78}

For measurements of grips strength, hand held dynamometers as the JAMAR and the Grippit instrument that measure the amount of static force the hand can squeeze around the dynamometer etc. are often used.\textsuperscript{209-211} Grip strength is demonstrated to be positively associated with length.\textsuperscript{212} Population based studies show that men have higher mean grip strength than women in all age groups \textsuperscript{213} and that grip strength decreases after 40 and 50 years age for women and men respectively.\textsuperscript{213} Hand grip strength is considered to be a highly heritable phenotype of muscle strength, and variations in the individual vitamin D receptor (VDR) supposedly have a direct effect on muscle strength.\textsuperscript{25}

Subjects with severe vitamin D deficiency and rickets, or mutations in the VDR, display generalized muscular and bone atrophy.\textsuperscript{214} The VDR have been found throughout the body.\textsuperscript{1, 215} The presence of VDR in muscle has been reported in animal muscle studies and muscle cell cultures, and the expression of VDR is higher in neonatal muscle and declines with age.\textsuperscript{214} The effects of vitamin D in muscle have been suggested not only to be an effect on calcium balance, but also to include an impact of vitamin D on muscle cell development and differentiation.\textsuperscript{214} The effects of vitamin D could be mediated either by a direct non-genomic action on membrane receptors affecting intra and extracellular calcium concentrations, or by binding to nuclear receptors with an impact on a genomic action, leading to the formation of a calcium-binding protein, resulting in the biological effect.\textsuperscript{216}
The Vitamin D hypothesis – a summary

Figure 2.
Aims of the thesis

RATIONALE
The point of departure of this thesis was the clinical observations of African and Middle Eastern immigrants presenting with musculoskeletal symptoms who were found to have vitamin D deficiency. The prevalence of vitamin D deficiency in immigrants in Northern Sweden was unknown. Some studies indicated that vitamin D deficiency was more common at higher latitudes while other studies demonstrated strong associations with ethnicity and lifestyle. Many studies of vitamin D deficiency focused on immigrant women, pregnant immigrant women, new immigrant mothers or the elderly. Most studies focused on patients. There were few studies including men or people of working age. There was also lack of consensus on the 25(OH)D levels that indicated vitamin D deficiency. There were no established guidelines on how to treat vitamin D deficiency. In Sweden, pure cholecalciferol was not available on general prescription. Vitamin D-deficient patients were difficult to treat as available combinations of vitamin D and calcium often caused gastrointestinal side effects resulting in the patient discontinuing treatment.

The overall aim of this thesis is to estimate the prevalence and determinants of vitamin D deficiency in an immigrant population born in Africa or the Middle East but living in Umeå in Northern Sweden, to examine associations between vitamin D status and health effects in the immigrants and to perform an intervention and examine the effect of pure cholecalciferol treatment on vitamin D status and 25(OH)D levels.

OBJECTIVES OF THE PAPERS INCLUDED
I To estimate the prevalence and determinants of vitamin D deficiency in a population of male and female immigrants from Africa and the Middle East living in Umeå, Northern Sweden.

II To examine whether vitamin D status was associated with muscular strength in these immigrants.

III To examine whether vitamin D status was associated with symptoms of anxiety, depression and health-related quality of life (QoL) in these immigrants.

IV To perform an intervention study and determine the effect of cholecalciferol on vitamin D status and 25(OH)D levels in an immigrant population.
Materials and Methods

The four papers in this study are based on two different studies. An overview of the papers and studies is presented in table 3.

Materials and methods Papers I - III

Study population and recruitment process
The Vitamin D Deficiency in Immigrants Survey (VIDI1) was designed as a population-based cross-sectional study and carried out in Umeå, a university town in Northern Sweden (latitude 63° N) with a population of 114 000 citizens at the time of the study. The study was conducted September 2009 - June 2010 at the Clinical Research Centre at Umeå University Hospital. The target population was men and women originating from nine African and Middle Eastern countries, aged 25–65, living in three districts in Umeå. Recruitment continued for 5 months. Invitations were sent in Swedish, English, and either Persian or Arabic according to the subject’s nationality and reminders were sent to non-responders. According to the population register, 1 544 immigrants were eligible for participation. In total, 1 306 subjects (744 men and 562 women) were invited after exclusion of subjects whose invitations were returned undelivered and one deceased person. Native countries of invited subjects (n=1306) were in the Middle East: Afghanistan 2.4%, Iran 31.4%, Iraq 27.0%, Pakistan 4.0% and Turkey 3.2% and in Africa: Burundi 3.0%, Eritrea 6.6%, Ethiopia 10.1% and Somalia 12.3%. Ultimately, 111 men and 105 women completed the study (Figure 3). Participation rates according to native countries (n=216) were in the Middle East: Afghanistan 1.8%, Iran 39.4%, Iraq 20.4%, Pakistan 0.5% and Turkey 2.3% and in Africa: Burundi 4.6%, Eritrea and Ethiopia 20.4% and Somalia 10.6%. The participation rate was 16.5%. The only known significant difference between the source population and the participants was that the age group 25–35 was underrepresented (P<0.05), see table 4.
### Table 3 Overview of the studies and papers included in the thesis

<table>
<thead>
<tr>
<th>Study</th>
<th>VIDI</th>
<th>VIDI2 Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Paper</strong></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td><strong>Design</strong></td>
<td>Cross-sectional study</td>
<td></td>
</tr>
<tr>
<td><strong>Research question</strong></td>
<td>What is the prevalence and determinants of vitamin D deficiency in immigrants from Africa and the Middle East?</td>
<td>Is vitamin D status associated with muscular strength in these immigrants?</td>
</tr>
<tr>
<td><strong>Setting</strong></td>
<td>Umeå, Sweden</td>
<td></td>
</tr>
<tr>
<td><strong>Period</strong></td>
<td>September 2009 - June 2010</td>
<td></td>
</tr>
<tr>
<td><strong>Study participants</strong></td>
<td>216 immigrants aged 25-65</td>
<td>195 immigrants 25-65, speaking Swedish/English</td>
</tr>
<tr>
<td><strong>Main outcome measurement</strong></td>
<td>Prevalence and determinants of vitamin D deficiency.</td>
<td>Associations between vitamin D deficiency and muscle strength.</td>
</tr>
</tbody>
</table>
**Figure 3.** Recruitment of study participants for the VIDI Study

![Flowchart showing recruitment process]

**Table 4.**

Proportion of age, gender and Middle East or African origin in all invited immigrants and separately for participants and non-participants in the population-based cross-sectional VIDI1 study.

<table>
<thead>
<tr>
<th></th>
<th>Invited n=1306</th>
<th>Participants n=216</th>
<th>Non-participants n=1090</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>57.0</td>
<td>51.4</td>
<td>58.1</td>
<td>ns</td>
</tr>
<tr>
<td>Middle East (%)</td>
<td>68.0</td>
<td>64.4</td>
<td>68.7</td>
<td>ns</td>
</tr>
<tr>
<td>Age group (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>42.0</td>
<td>33.8</td>
<td>43.7</td>
<td>0.037</td>
</tr>
<tr>
<td>35-44</td>
<td>27.6</td>
<td>29.2</td>
<td>27.2</td>
<td>ns</td>
</tr>
<tr>
<td>45-54</td>
<td>20.7</td>
<td>25.4</td>
<td>19.7</td>
<td>ns</td>
</tr>
<tr>
<td>55-65</td>
<td>9.7</td>
<td>11.6</td>
<td>9.4</td>
<td>ns</td>
</tr>
</tbody>
</table>

* p refers to difference between participants and non-participants.
Study population and recruitment process - Paper III

Data from the cross-sectional population-based VID11 Survey were used in the analyses. During the study, the study nurse noticed that immigrants in need of interpreter were likely to misunderstand and had problems with filling in the HAD, EQ-5D and EQ-VAS forms and use of interpreter is not validated when using these forms. Therefore, immigrants in need of interpreter were excluded from the analyses of the Hospital Anxiety and Depression Scale (HAD) and QoL and finally 195 subjects were included in the analyses for Paper III. Native countries of participants in sub-study III were Afghanistan/Pakistan 2.1%. Burundi 4.6%. Eritrea/Ethiopia 20.0%. Iraq 18.5%. Iran 43.1%. Somalia 9.7% and Turkey 2.1%. In total, 128 subjects of Middle Eastern origin (73 men and 55 women) and 67 subjects of African origin (31 men and 36 women) participated.

Data collection
All interviews and examinations were carried out by the same qualified research nurse. Interpreters were used in 7% of the visits, when participants did not speak Swedish or English. Questionnaires were used including age, gender, native country, number of years since immigration to Sweden, socioeconomic and lifestyle variables, medical history including ongoing medication and vitamin D supplement use etc. All the results were collected in case report forms (CRFs) and stored at a central database. For CRF data please see Appendix.

The physical examination
Height and weight were measured in light indoor clothing. Blood samples were drawn, centrifugated, frozen and stored at −80°C.

Lower limb muscle strength measurements
Lower limb muscle strength was measured using the standardised muscle function (MF) indices of muscle strength. The MF indices of muscle strength is a functional test, with relevance for daily activities and it provides a validated progressive scale for measurement of lower limb muscle strength. Studies have shown a high correlation (Spearman) between the MF indices of muscle strength and tested isometric muscle strength of hip extensors, flexors, abductors and knee extensors. The test consists of four parts; a) Getting up from a chair (height 45 cm) to a standing position without hand support, first attempt performed with the right foot in front of the other, the second attempt vice versa. b) Stepping up onto a step 30 cm high without hand support, the first attempt with the right foot first, the second with the left. c) Bending the knees to a maximum of 90° standing with the back against a wall and heels 10 cm from it. d) Lying on the back with knees bent and feet flat, lifting the buttocks and extending one leg for 5 secs, first the right leg and then the left. All parts are scored on a three-point scale as: 0 points = normal function no time delay, 1 point =
slight/moderate impairment and 2 points = cannot complete and summed together (0-14 points) where 14 points corresponded to maximum reduction of lower limb muscle strength. One participant who had undergone a femur amputation could not perform the test and was excluded from the analyses. One participant who had a total knee replacement just before the examination was not allowed to perform the test with his/her operated knee and was therefore registered with the same points for the operated knee as the non-operated knee.

**Grip strength measurements**

Grip strength was measured in kilograms using a hand-held JAMAR Analogue Hand Dynamometer with the handle in the second position, using the testing procedures of positioning and instructions. Participants were seated, their shoulder adducted and in neutral position, their elbow by their side and flexed to right angles, the lower arm in neutral position on the table, their wrist off the table and in a neutral wrist position, the dynamometer handle in position II. This position, followed by calculation of the mean of three trials of grip strength for each hand, has been well-documented as reliable. Each participant’s grip strength was measured three times with each hand, the dominant hand examined first, and mean grip strength of each hand was calculated. The mean value of the strongest hand was used in the analyses. Two participants were only able to perform the grip strength test with one hand, one of them had amputated one hand, the other had recently had surgery in one hand. Both participants grip strength in the unaffected tested hand were used in the analyses.

**The Hospital Anxiety and Depression Scale (HAD)**

The validated questionnaire HAD was used for measuring symptoms of depression and anxiety, for details see p28. The Swedish and English versions of HAD were used.

**Health-related quality of life (QoL)**

The EuroQol-5 Dimension Questionnaire (EQ-5D) was used for measuring QoL. EQ-5D-3L consists of two parts, the first part is the EQ-5D-3L descriptive system comprising the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has three levels: no problems (1 point), some/moderate problems (2 points) or extreme problems (3 points). The health state 11111 indicate no problems in any of the five dimensions while the state 11223 indicate no problems with mobility and self-care, some problems with performing usual activities, moderate pain or discomfort and extreme anxiety or depression. The second part of EQ-5D is the EuroQoL visual analogue scale (EQ-VAS), range 0-100 points where zero points is the worst imaginable health state and 100 points is the best imaginable health state. In the EQ-VAS, the subject is asked to indicate on the scale how good
or bad his/her own health is today in his/her opinion. Use of EQ-5D was approved by the EuroQoL Group.

**Analyses Paper I-III**

**Baseline data**
Education was categorised into <10 years or ≥10 years referring to nine years of schooling being the obligatory elementary school in Sweden. Occupation was categorized as having a paid job (employed/self-employed) or not. The intake of milk products was categorised as at least 4 dl (two glasses) per day or less. Fatty fish intake was categorised into intake of fatty fish at least once a week or less. Physical activity was registered as number of activities/week: each activity with a minimum intensity equivalent to brisk walking for 20-30 minutes and categorised as physical activity at least once a week or not. Travelling abroad within 6 months prior to the examination was categorised as journeys abroad or not. Sick-leave was categorised as <1 month or ≥ 1 months during the last year prior to examination. Medical history was categorized as having a chronic disease of medium-high severity (for example diabetes, hypertension, heart disease, cancer, chronic pain etc.) or not. Supplements were categorised as using vitamin D supplements (all sorts of vitamin D, fish oil and omega-3) or not. In the analyses for Papers I and III, participants were grouped as either of Middle Eastern or African origin.

**Examination data**

**Lower limb muscle strength:** The variable lower limb muscle strength was dichotomized in 0 (full muscle strength, 0 point) and 1 (reduced lower limb muscle strength, 1-14 points).

**Body mass index** (BMI, kg/m²) was calculated and categorised into BMI <25 and BMI ≥25 (normal weight/overweight in Paper I) or BMI ≤ 29.9 and BMI ≥ 30 (according to the definition of obesity in Paper II).

**Anxiety and depressive symptoms:** According to validated interpretation, HAD results were analysed separately as symptoms regarding anxiety (HADA range 0-21 points) or depression (HADD range 0-21 points) and categorised into normal (HADD ≤ 10 points, HADA ≤ 10 points), or indicating significant clinical depression (HADD ≥ 11 points) or significant clinical anxiety (HADA ≥ 11 points) according to standard practice.

**Health-related quality of life**

The EQ-5D was converted to a single summary EQ-5D-index by computing EQ-5D index using the Danish TTO test value sets. There were no available value sets for the EQ-5D regarding the countries included in the VIDI1 study. The EQ-5D-3L-index TTO ranges -0.594 - +1, where 1 point = full health and 0 points =
dead. Negative values indicate that the subject range his/her life quality as worse than dead.

**Analysis of serum-25-hydroxyvitamin D**

All chemical analyses were carried out in the same batch, after all the participants were included. The analytical method used for 25(OH)D was LC-MsM 54 performed at Laboratory Medicine Skåne, Malmö University Hospital. The quality of analyses in this laboratory was ascertained through DEQAS external controls. The inter-assay coefficients of variation (CVs) were 3% at 25(OH)D3 level 88 nmol/L, and 2.6% at 25(OH)D3 level 177 nmol/L. The limit of detection was 6 nmol/L. Both 25(OH)D3 and 25(OH)D2 were quantified separately, but 25(OH)D2 values were only detected in five participants, and it was therefore decided not to include 25(OH)D2 values in the analyses. All results were calculated from 25(OH)D3 levels.

**Definition of vitamin D status**

We used the following definitions; vitamin D deficiency; 25(OH)D3 < 25 nmol/L; vitamin D insufficiency; 25(OH)D3 25-49 nmol/L; adequate vitamin D status; 25(OH)D3 50-74 nmol/L. In Paper 1 we also used the definition optimal vitamin D status; 25(OH)D3 75-125 nmol/L. 55, 125, 222

**Materials and Methods - Paper IV**

**Study design**

The VIDI2 Intervention study was a phase 4 drug study conducted as an open, controlled, partially randomized clinical trial, initiated by the research group and managed according to ICH-GCP (International Conference on Harmonisation- Good Clinical Practice) and regulations from the Medical Products Agency (MPA) in Sweden. For study design see Figure 4. The study was approved by the MPA in 2011-07-20 (151:2011/48597) and registered at the European Clinical Trials Database (Eudra-CT); Eu-nr 2010-024460-18 and at ClinicalTrials.gov (NCT01419119). The study was conducted September 2011–May 2012 at the Clinical Research Centre and monitored by the Clinical Trial Unit, both at Umeå University Hospital, Sweden.
Figure 4.

**Study design of the VIDI2 (Vitamin D deficiency in immigrants Intervention) Study.**

An open, partially randomized, parallel group study of cholecalciferol in the treatment of vitamin D deficiency and insufficiency.

Four parallel groups: treatment:

<table>
<thead>
<tr>
<th>25(OH)D3 &lt;25 nmol/L 10000 IE cholecalciferol/day</th>
<th>Group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D3 25-49 nmol/L 2000 IE cholecalciferol/day</td>
<td>Group 2a</td>
</tr>
<tr>
<td>25(OH)D3 25-49 nmol/L 2000 IE cholecalciferol/w</td>
<td>Group 2b</td>
</tr>
<tr>
<td>25(OH)D3 50-74 nmol/L 2000 IE cholecalciferol/day</td>
<td>Group 3</td>
</tr>
</tbody>
</table>

Visit 1 | Visit 2 | Visit 3 | Visit 4
-4 ww | 0 w | 3-4 ww | 12 ±2 ww

**Study intervention**

All study participants were treated with cholecalciferol in different doses according to baseline 25(OH)D levels. Figure 4. The pharmaceutical product (PP) took the form of a solution, where 1 ml (30 drops) contained 0.5 mg cholecalciferol, equivalent to 20,000 IU (667 IU/drop). Subjects with ongoing supplementation with vitamin D, calcium, fish oil, seal oil, omega3 products and medicines containing aluminium stopped this medication when they began taking the study medication.

**Inclusion and exclusion criteria**

medications to reduce absorption of digested lipids (Orlistat), glucocorticoids. Other medical conditions that preclude participation in the trial as assessed by the principal investigator.

**Study population and recruitment process**
The study population was recruited from the population of immigrant men and women originating from the Middle East or Africa, ages 25-64, who lived in Umeå. Recruitment was initiated by postal invitations to all participants in the VIDI1 study who still lived in the area, followed by invitations based on the population register to additional immigrants from Middle East or Africa, and advertisements on public notice boards. Based on power calculations the recruitment goal was 150 participants, 20 in Group 1, 50 each in Group 2a and 2b and 30 in Group 3. In total, 192 subjects were screened of whom eleven subjects were excluded due to 25(OH)D levels ≥75 nmol/L. 160 subjects were consecutively included until all groups were complete. Subjects with 25(OH)D 25–49 nmol/L were allocated into two groups based on a computer-generated randomization list. Subjects according to groups were: Group 1- 23 subjects, Group 2a- 56 subjects, Group 2b- 50 subjects and Group 3- 31 subjects. Number of discontinuations were: Group 1 - 7 subjects, Group 2a - 2 subjects, Group 2b- 1 subject and Group 3- 3 subjects. Finally, 147 subjects completed the study protocol. Figure 5.

**Figure 5.** Flow chart for participant inclusion in the VIDI2 Intervention study.
**Study protocol**

The protocol included four visits; screening visit with collection of informed consent, base line visit with start of PP within 4 weeks after screening, and end of study visit with end of PP 12±2 weeks after base line. Participants in need of interpreters and women younger than 50 in Group 1 also came for a visit after 3-5 weeks while other participants were followed up by a telephone call to promote compliance and monitor adverse events. Samples of blood were drawn at screening and end of study. Participants filled in a study diary where each dose of PP taken was noted, as well as all kinds of adverse events (AEs). Compliance was assessed at end of study when the PP was returned and counted by the research nurse and Apoteket Farmaci. For details, see Appendices.

**Data collection and analyses**

**Interviews:** See page 26 and Appendices.

**Physical examinations and validated questionnaires:** See page 26-28. Examinations were the same as in the VIDI Study with the addition of the standardised muscle function indices of balance coordination\(^ {204}\), blood pressure and skin type according to Fitzpatrick.\(^ {223}\)

**Blood and urine analyses:** Blood samples for 25(OH)D, PTH, Creatinin and calcium were drawn at screening and Visit 4. Urine analyses (pregnancy test) was performed on women <50 y in Group 1 on Visits 2, 3 and 4.

**Laboratory analyses:** 25(OH)D: see page 29. 25(OH)D before inclusion was analysed regularly once a week, and 25(OH)D after completed study was frozen and analysed in one batch. 25(OH)D2 was only detected in three subjects and not included in the calculations. All results refer to 25(OH)D3. S-PTH was analysed at the same laboratory with a routine clinical method. S-albumin correlated Calcium and S-Creatinine were analysed at Laboratorie-medcin, Umeå University Hospital, using routine methods. U-pregnancy tests were analysed using routine methods.

**Follow up:** After the end of the study when the laboratory results had been obtained, all the participants were informed about the treatment they had received and their 25(OH)D result. If results were 25(OH)D <25 nmol/L, participants would be referred to the Internal Medicine Department at Umeå University Hospital, participants with 25(OH)D 25-49 nmol/L were referred to Primary Health Care and those with 25(OH)D ≥50 nmol/L were recommended vitamin D supplementation during the winter months.
Statistics

All the participants answered all the questions and performed all the examinations except for missing information regarding education level, employment and sick-leave in one individual in Papers I-III (response rate 99.5-100%).

In Papers I, II, III and IV.

Characteristics of the subjects were presented as means ± standard deviations (SD) for continuous variables, and as count and proportions (%) for categorical variables. Assumption of normal distribution was studied using histogram and test of skewness and kurtosis. Differences between groups were tested using T-test for continuous variables, and the Pearson chi-square test for categorical variables. Differences between median values were tested with non-parametric test (Kruskal-Wallis) in Paper 1. P values of less than 0.05 were considered statistically significant. The statistician co-supervisor was consulted in all Papers. All statistical analyses were performed using SPSS Statistics 19.0 – 24.0 software (IBM, Armonk, NY, USA).

Paper I: Both mean and median values of 25(OH)D3 levels were presented in order to facilitate comparison with the results from some other studies. When analysing associations between vitamin D deficiency as a dependent variable and background and lifestyle variables as independent variables, the odds for having 25(OH)D <25nmol/L versus 25(OH)D ≥25nmol/L were analysed using univariate and multivariable logistic models and presented as odds ratios (OR) with 95% confidence intervals (CI). Only variables that were significant in the univariate regression analyses were included in the multivariable regression models. The independent variables were: gender, age groups with 10-year intervals, African or Middle Eastern origin, fatty fish intake, milk intake, use of vitamin D supplements, short-sleeved or long-sleeved clothing in summer, journeys abroad within the 6 months before examination, physical activity, education, having a paid job and normal BMI (< 25). The selection of independent variables was based on univariate statistical analyses of data including correlations, ANOVA tables and Pearson chi-square analyses.

Paper II: Linear regression analyses were used for analysing associations between grip strength as a dependent variable and both categorical variables (BMI (obesity), gender, vitamin D deficiency, physical activity, socioeconomic factors) and continuous variables (age, height, time since immigration, 25(OH)D levels as independent variables). Logistic regression analysis was used for analysing the association between reduced lower limb muscle strength as a dependent variable with both categorical variables (BMI (obesity), gender, vitamin D deficiency, physical activity, socioeconomic factors) and continuous
variables (age, height, time since immigration, 25(OH)D levels) as independent variables. Results from the linear regressions were presented as unstandardised beta (B) with 95% confidence intervals (CI) and p values. Results from the logistic regressions were presented as odds ratios (OR) with 95% confidence intervals (CI) and p values. The multivariable logistic regression analyses were carried out with either vitamin D deficiency or 25(OH)D levels included as independent variables in two separate models. Only variables that were significant in the simple regression analyses were included in the multivariable regression analyses.

**Paper III:**
HAD results were analysed separately regarding symptoms of anxiety (HADA) or depression (HADD). For categorisation of HAD see page 28. The selection of independent variables was based on univariate analyses of associations including correlations and Pearson chi-square analyses between the dependent variables anxiety and depression and the background and lifestyle variables. Subgroup analyses according to African or Middle Eastern origin and gender were performed. In order to reduce the negative effect on power of the small group size of these sub-groups, analyses were also performed with 25(OH)D <50 nmol/L as an independent variable. Logistic regression analysis was used for analysing the association between anxiety (HADA ≥11 points) and depression (HADD ≥11 points) respectively as dependent variables with categorical independent variables including vitamin D deficiency, 25(OH)D levels <50 nmol/L, sick leave, physical activity, paid job, working or studying, education, journeys abroad, age groups, clothing and chronic disease and with the linear variable 25(OH)D. Only variables that were significant in simple regression analyses were included in the multivariable regression analyses.

**Paper IV:** Power calculations based on the VIDI1 study had estimated that a total of 150 participants, including 20 subjects with vitamin D deficiency (Group 1), 100 subjects with vitamin D insufficiency (Group 2a and 2b), and 30 subjects with adequate vitamin D status (Group 3), would be sufficient to allow detection of a change in 25(OH)D from baseline to follow-up of; 2.2 nmol/L in Group 1, 2.4 nmol/L in Group 2a and 2b and 4.2 nmol/L in Group 3, as well as a difference of 3.4 nmol/L between Group 2a and 2b. with a power of 80% and a significance level of 0.05%. Results according to treatment per protocol were reported in Paper IV. Analyses per intention to treat - methodology including an additional 8 initially included subjects where 25(OH)D was analysed at the end of the study, were also performed, substantially with the same results. For each study group, 25(OH)D mean ± SD at baseline and end of study was calculated. Differences within and between groups were tested using t tests, ANOVA and non-parametric tests (Mann-Whitney for analyses between groups and Wilcoxin for differences within groups). Range of 25(OH)D at study end was also presented. PTH results
were analysed in a similar procedure. Linear regression analyses were used for analysing associations between 25(OH)D as a dependent variable with independent variables, both continuous variables including age, BMI, time in Sweden and baseline 25(OH)D and categorical variables including gender, origin and daily dose of cholecalciferol. Only variables that were significant in simple regression analyses were included in the final multiple linear regression model. Results from the final model were presented as unstandardized beta (B) with 95% confidence intervals (CI) and p values. Associations between the dependent variable Change in 25(OH)D per daily microgram cholecalciferol and the continuous variables including age, BMI, time in Sweden, baseline 25(OH)D and daily dose of cholecalciferol and the categorical variables gender and origin were tested using linear regression analyses in a similar procedure. Residuals were used for checking regression assumptions.

**Ethical considerations**

All studies complied with the Helsinki Declaration. Written informed consent was obtained from all participants.

**Papers I-III:**

Ethical approval was obtained from the Regional Ethical Review Board at Umeå University (dnr 08-205M).

The fact that only immigrants were invited to participate in the study and that clothing habits were suspected to influence vitamin D status could be perceived as discriminatory, however this was considered to be outweighed by the advantage of diagnosing vitamin D deficiency and offering treatment to the participants. In order to avoid a doctor-patient relationship between the participants and the researchers, address lists were received from the population register and all study participants were invited by post. All subjects who accepted participation in the study were examined by a research nurse. Subjects with severe depressive or anxiety symptoms according to HAD were offered a referral to their primary health care centre. All analyses of 25(OH)D were performed after all the subjects were included in the VIDI1 study, at most nine months after examination. All subjects were then informed about their 25(OH)D levels and subjects who were found to have 25(OH)D < 25 nmol/L were recommended to contact their primary health care centre for a new test of 25(OH)D. All subjects in the VIDI1 Study were later invited to participate in the Intervention Study.

**Paper IV**

The study was approved by the Regional Ethical Review Board at Umeå University. (2010–338–31M and 2011–217–32M).
There was a risk of allergic reaction against substances in the PP or its packaging (apart from cholecalciferol only coconut, palm kernel oil or rubber), and this was avoided through the exclusion criteria. There is also a risk when vitamin D supplementation is performed of overdose and toxic effects of vitamin D characterised by hypercalcemia. The risk for overdosing and hypercalcemia was managed thorough exclusion criteria, discontinuation of other vitamin D preparations before start of PP, control of calcium and creatinin levels before study start and thorough information about the doses at inclusion/Visit 1 and at follow up/Visit 3 after 3-5 weeks treatment. During pregnancy, treatment of vitamin D deficiency should be handled by a specialist in Endocrinology. In order to ensure this, all women younger than 50 in Group 1 took a pregnancy test at Visits 2, 3 and 4 and pregnant women were excluded from the Intervention Study and referred to the Clinic of Internal Medicine. There was a risk that use of HAD might reveal severe depressive or anxiety symptoms, these subjects would be offered a referral to their health care centre. The participants in the preceding VIDI Study were informed of their vitamin D status and that it would be followed by an Intervention Study, and the Intervention Study enabled both vitamin D substitution and the opportunity to study the effects of the treatment. The fact that only immigrants were invited to the study could be perceived as discriminatory but was considered as motivated by the fact that vitamin D deficiency was very rare in the general population in Northern Sweden. Screening the general population for vitamin D deficiency would generate high costs without adding additional study participants.
Results

An overview of the primary results of all four papers is presented in Table 5.

Demographics Papers I & II
Basic characteristics of all study participants in VIDI1 Papers I and II are presented in Table 6.

Table 6. Basic characteristics of study participants in the VIDI1 study according to Middle East or African origin.

<table>
<thead>
<tr>
<th></th>
<th>Middle East origin (ME)</th>
<th>African origin (A)</th>
<th>ME-A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n=76)</td>
<td>Women (n=63)</td>
<td>p^</td>
</tr>
<tr>
<td>Age</td>
<td>40.4 ±11.7</td>
<td>41.2 ±11.8</td>
<td>0.659</td>
</tr>
<tr>
<td></td>
<td>12.6 ±10.3</td>
<td>13.8 ±8.2</td>
<td>0.697</td>
</tr>
<tr>
<td>Time since</td>
<td>84.2 ±10.8</td>
<td>85.7 ±10.3</td>
<td>0.805</td>
</tr>
<tr>
<td>Education</td>
<td>28.6 ±10.3</td>
<td>48.7 ±8.8</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>47.4 ±10.7</td>
<td>46.0 ±10.3</td>
<td>0.875</td>
</tr>
<tr>
<td>BMI</td>
<td>26.4 ±3.3</td>
<td>26.1 ±4.5</td>
<td>0.613</td>
</tr>
<tr>
<td>Journeys abroad</td>
<td>1.3 ±1.2</td>
<td>19.0 ±2.3</td>
<td>0.044</td>
</tr>
<tr>
<td>Clothing habits</td>
<td>38.7 ±6.8</td>
<td>68.3 ±7.8</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>21.1 ±2.3</td>
<td>28.6 ±4.5</td>
<td>0.305</td>
</tr>
<tr>
<td>Fatty fish intake</td>
<td>≥1x /week</td>
<td>44.7 ±5.4</td>
<td>0.234</td>
</tr>
<tr>
<td>Milk intake</td>
<td>≥4 dl daily</td>
<td>7.9 ±1.5</td>
<td>0.087</td>
</tr>
</tbody>
</table>

Footnote: Significant differences marked with bold p. ^ Difference between men and women in the Middle East and African group, respectively. ^ Difference between immigrants from the Middle East and Africa.
| Study | VIDI1 | | | | VIDI2 Intervention |
|-------|-------|-------|-------|-------|
| Paper | I | II | III | IV |
| Subjects included (n) | 217 | 195 | | 159 |
| Examined/completed | 216 | 195 | | 147 |
| Age mean±SD (y) | 40.4±10.9 | 40.5±10.9 | | 41.6±10.8 |
| Men (%) | 51.4 | 53.3 | | 49.6 |
| Middle East (%) | 64.4 | 65.8 | | 44.2 |
| BMI mean±SD | 26.3±4.1 | 26.1±4.0 | | 26.7±4.2 |
| 25(OH)D mean±SD (nmol/L) | 41.0±16.6 | 41.6±16.4 | | na |
| Vit. D deficient (%) | 12.0 | 10.8 | | na |

**Primary results**

Prevalence of vitamin D deficiency: 12.00%
Vitamin D deficiency was twice as common in immigrants from Africa compared to those from the Middle East.

Determinants of vitamin D deficiency:
- Low fatty fish intake (OR 4.31, 95%CI 1.61-11.55)
- Not travelling abroad (OR 3.76, 95%CI 1.18-11.96)
- Wearing long-sleeved clothes in summer (OR 3.15, 95%CI 1.09-9.12)

25(OH)D levels were associated with reduced lower limb muscle strength (p=0.008).
Vitamin D deficiency was associated with grip strength (p=0.022) after adjustments.

There was no association between vitamin D status and anxiety, depression or QoL in the total immigrant population.
In Middle Eastern women, anxiety was associated with 25(OH)D ≤49nmol/L (p=0.012) after adjustments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Result at study end:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1:</td>
<td>25(OH)D3 ≥50 nmol/L:</td>
</tr>
<tr>
<td>Group 2a:</td>
<td>16/16</td>
</tr>
<tr>
<td>Group 2b:</td>
<td>24/54</td>
</tr>
<tr>
<td>Group 1:</td>
<td>25(OH)D3 ≥75 nmol/L:</td>
</tr>
<tr>
<td>Group 2a:</td>
<td>16/16</td>
</tr>
<tr>
<td>Group 2b:</td>
<td>24/54</td>
</tr>
</tbody>
</table>

Mean 25(OH)D3 increased in all groups.

Change of 25(OH)D (nmol/L) mean (range) was in Group 1: 121.1 (63-178), Group 2a: 38.1 (10-65), Group 2b: 13.6 (-8-64) and Group 3: 22.3 (-15-62).

**Table 5 Primary results of all four papers in this thesis**

na= not applicable
Prevalence of vitamin D deficiency

Twelve % of the immigrants were vitamin D deficient (25(OH)D<25 nmol/L). In total, 73% of the immigrants had 25(OH)D < 50 nmol/L and correspondingly only 27% had adequate vitamin D status. Mean 25(OH)D was 41.0 nmol/L with no difference between genders. 25(OH)D levels were lower (p=0.030) and vitamin D deficiency twice as common in immigrants from Africa compared to those from the Middle East (p=0.039). Table 7. Median 25(OH)D according to African or Middle Eastern origin and genders are presented in Figure 6.

Table 7. S-25(OH)D3 levels (nmol/L) in 216 immigrants from the Middle East and Africa living in Umeå, Northern Sweden, according to gender and geographical origin. Prevalence of Vitamin D deficiency and insufficient, adequate and optimal vitamin D status.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>mean (±SD)</td>
<td>&lt;25 (%)</td>
<td>25-49 (%)</td>
<td>50-74 (%)</td>
</tr>
<tr>
<td>Middle East</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>76</td>
<td>42.6 (±15.6)</td>
<td>5.3</td>
<td>63.2</td>
<td>28.9</td>
</tr>
<tr>
<td>Women</td>
<td>63</td>
<td>42.9 (±16.5)</td>
<td>12.7</td>
<td>52.4</td>
<td>31.7</td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>35</td>
<td>37.4 (±17.8)</td>
<td>22.9</td>
<td>62.9</td>
<td>8.6</td>
</tr>
<tr>
<td>Women</td>
<td>42</td>
<td>37.9 (±17.3)</td>
<td>14.3</td>
<td>66.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>41.0 (±16.6)</td>
<td>12.0</td>
<td>60.6</td>
<td>23.6</td>
</tr>
</tbody>
</table>
Figure 6. S-25(OH)D3 concentrations in 216 immigrants living in Umeå, Sweden, according to Middle Eastern or African origin and gender.

Boxplot. Boxes ranges from 25 percentile to 75 percentile; horizontal line represents median value. Open circles represent outliers (between 1.5 and three times the interquartile range) and asterisks represent extreme outliers (more than three times the interquartile range). In Middle Eastern participants median 25(OH)D was 38 nmol/L in men and 41 nmol/L in women, in African participants median 25(OH)D was 33 nmol/L in both men and women.

Determinants of vitamin D deficiency

Vitamin D deficiency was significantly associated with low fatty fish intake, not travelling abroad and wearing long-sleeved clothes in summer. African or Middle Eastern origin was not significantly associated with vitamin D deficiency. Table 8
Table 8. S-25(OH)D3 levels and prevalence of vitamin D deficiency in 216 immigrants from the Middle East and Africa.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>N</th>
<th>25(OH)D3 mean (nmol/L)</th>
<th>p</th>
<th>Vitamin D deficiency (%)</th>
<th>Vitamin D deficiency Univar.regr.OR (95%CI)</th>
<th>Vitamin D deficiency Multiv.regr.OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>111</td>
<td>41.0</td>
<td>0.973</td>
<td>10.8</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>105</td>
<td>40.9</td>
<td>0.010</td>
<td>13.2</td>
<td>1.27 (0.56-2.89)</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25-34 y</td>
<td>73</td>
<td>39.1</td>
<td></td>
<td>8.2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35-44 y</td>
<td>63</td>
<td>39.5</td>
<td>0.010</td>
<td>14.0</td>
<td>1.86 (0.62-5.55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45-54 y</td>
<td>55</td>
<td>40.2</td>
<td></td>
<td>14.5</td>
<td>1.90 (0.62-5.84)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55-64 y</td>
<td>25</td>
<td>51.4</td>
<td></td>
<td>12.0</td>
<td>1.52 (0.35-6.60)</td>
<td></td>
</tr>
<tr>
<td>Origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle East</td>
<td>139</td>
<td>42.8</td>
<td>0.030</td>
<td>8.6</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Africa</td>
<td>77</td>
<td>37.7</td>
<td></td>
<td>18.2</td>
<td>2.35 (1.03-5.38)</td>
<td>1.83 (0.73-4.60)</td>
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<tr>
<td>Fatty fish intake</td>
<td>≥ once a week</td>
<td>112</td>
<td>44.5</td>
<td>0.001</td>
<td>6.3</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>&lt; once a week</td>
<td>104</td>
<td>37.1</td>
<td></td>
<td>18.3</td>
<td>3.35 (1.35-8.35)</td>
<td>4.31 (1.61-11.55)</td>
</tr>
<tr>
<td>Milk intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 4 dl/day</td>
<td>33</td>
<td>47.8</td>
<td></td>
<td>9.1</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 4 dl/day</td>
<td>183</td>
<td>39.7</td>
<td></td>
<td>12.6</td>
<td>1.44 (0.41-5.09)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D supplements</td>
<td>Yes</td>
<td>53</td>
<td>50.4</td>
<td>&lt; 0.001</td>
<td>5.7</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>163</td>
<td>37.9</td>
<td></td>
<td>14.1</td>
<td>2.74 (0.79-9.52)</td>
<td></td>
</tr>
<tr>
<td>Clothing habits</td>
<td>Short-sleeved</td>
<td>182</td>
<td>41.9</td>
<td>0.056</td>
<td>9.9</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Long-sleeved</td>
<td>34</td>
<td>35.9</td>
<td></td>
<td>23.5</td>
<td>2.80 (1.11-7.10)</td>
<td>3.15 (1.09-9.12)</td>
</tr>
<tr>
<td>Journeys abroad</td>
<td>Yes</td>
<td>85</td>
<td>43.8</td>
<td>0.040</td>
<td>4.7</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>131</td>
<td>39.1</td>
<td></td>
<td>16.8</td>
<td>4.09 (1.36-12.32)</td>
<td>3.76 (1.18-11.96)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>≥ once a week</td>
<td>161</td>
<td>42.2</td>
<td>0.051</td>
<td>9.9</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; once a week</td>
<td>55</td>
<td>37.2</td>
<td></td>
<td>18.2</td>
<td>2.01 (0.85-4.75)</td>
<td></td>
</tr>
<tr>
<td>Education*</td>
<td>≥10 years</td>
<td>161</td>
<td>42.4</td>
<td>0.030</td>
<td>9.9</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;10 years</td>
<td>54</td>
<td>36.8</td>
<td></td>
<td>18.5</td>
<td>2.06 (0.87-4.86)</td>
<td></td>
</tr>
<tr>
<td>Paid job*</td>
<td>Yes</td>
<td>102</td>
<td>43.2</td>
<td>0.060</td>
<td>8.8</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>113</td>
<td>38.9</td>
<td></td>
<td>15.0</td>
<td>1.83 (0.78-4.31)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>&lt;25</td>
<td>85</td>
<td>40.7</td>
<td>0.835</td>
<td>8.0</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥25</td>
<td>131</td>
<td>41.2</td>
<td></td>
<td>18.0</td>
<td>1.55 (0.64-3.70)</td>
<td></td>
</tr>
</tbody>
</table>

Footnote: *Vitamin D deficiency defined as 25(OH)D <25 nmol/L. Odds ratio (OR) for vitamin D deficiency and 95% confidence interval (95% CI). b Univariable logistic regression. c Multivariable logistic regression including origin, fatty fish intake, clothing habits and journeys abroad.
Seasonal data on 25(OH)D levels
The only seasonal variation in 25(OH)D levels noticed was an increase in 25(OH)D levels in the Middle Eastern immigrants in September. Figure 7.

Figure 7. Median 25(OH)D3 concentrations in 216 African and Middle Eastern immigrants in Umeå, Sweden according to month of examination. P-value for difference in median 25(OH)D within immigrants from the Middle East; p=0.007 and within immigrants from Africa p=0.081 (Kruskal-Wallis).

Results of other food and lifestyle on vitamin D status
Adding cheese intake ≤ two slices/day and sick-leave ≥ one month within six months before examination showed significant associations between these factors and vitamin D deficiency (p= 0.019 and p=0.049 respectively). However, these variables were not significant in multivariable logistic regression analyses. Smoking was not associated with 25(OH)D levels or vitamin D deficiency nor being vegetarian or vegan.
Associations between vitamin D status and muscle strength

Lower limb muscle strength
The majority of the participants (55.8%) had full lower limb muscle strength (MF indices 0 points); 61.8% of the men and 49.5% of the women. There was a significant association between reduced lower limb muscle strength and lower 25(OH)D levels in all participants. In the univariate logistic regression, reduced lower limb muscle strength was associated with higher age, higher BMI, lower 25(OH)D levels, vitamin D deficiency, history of sick-leave and low level of education. In the multiple logistic regression including 25(OH)D levels; higher BMI, lower 25(OH)D levels, history of sick-leave and low education continued to be associated with reduced lower limb muscle strength. Table 9. The alternative multiple logistic regression, including the variable vitamin D deficiency, resulted in a model where the p value for reduced lower limb muscle strength in subjects with vitamin D deficiency was 0.052.

Table 9. Multivariable logistic regression for reduced lower limb muscle strength in 216 African and Middle Eastern immigrants.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>OR (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>y</td>
<td>1.03 (0.998,1.06)</td>
<td>0.070</td>
</tr>
<tr>
<td>BMI</td>
<td>kg/m²</td>
<td>216</td>
<td>1.12 (1.04, 1.22)</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>nmol/L</td>
<td>215</td>
<td>0.97 (0.96,0.99)</td>
</tr>
<tr>
<td>Sick-leave</td>
<td>No</td>
<td>166</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>48</td>
<td>2.19 (1.04-4.62)</td>
</tr>
<tr>
<td>Education</td>
<td>≥10 years</td>
<td>165</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>&lt;10 years</td>
<td>50</td>
<td>2.36 (1.16-4.89)</td>
</tr>
</tbody>
</table>

Footnote: *The standardised Muscle Function (MF) indices of muscle strength in lower extremities. Range 0-14 points where 0 points indicate full leg muscle strength and 1-14 points indicate reduced leg muscle strength. Multivariable logistic regression with the variable 25(OH)D included only variables that were significant in the first step linear regression. Relative risk of reduced leg muscle strength specified as 1 point or more. Univariable logistic regression included gender, age, height, BMI, years since immigration, 25(OH)D levels, physical activity, sick-leave, education and paid job. Significant differences denoted by bold p.
Grip strength

Mean grip strength was 33.1±11.8 kg in all participants. Weaker grip strength was associated with vitamin D deficiency in all participants. Other factors associated with weaker grip strength were female gender, higher age, shorter stature, history of sick-leave and low level of education. In the final step linear regression, vitamin D deficiency, female gender, higher age and shorter stature continued to be associated with weaker grip strength. Table 10. Grip strength was not associated with 25(OH)D levels.

Table 10. Final primary effect model using linear regression with stepwise exclusion for maximal voluntary grip strength in 216 African and Middle Eastern immigrants.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>B (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>111</td>
<td>13.5 (10.7,16.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>-0.11 (-0.201,-0.20)</td>
<td>0.017</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td>0.31 (0.15,0.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vit D deficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=25 nmol/L</td>
<td>190</td>
<td>3.4 (0.5,6.3)</td>
<td>0.022</td>
</tr>
<tr>
<td>&lt;25 nmol/L</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sick-leave last year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 month</td>
<td>166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=1 month</td>
<td>49</td>
<td>2.17 (-0.20,4.54)</td>
<td>0.073</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=10 years</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 years</td>
<td>51</td>
<td>-2.11 (0.06,-4.34)</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Footnote: *Grip strength measured in kilograms using a hand-held JAMAR dynamometer. Independent variables included at the first step: gender, age, height, BMI, time since immigration, physical activity, sick-leave, education and paid job. Only variables that were significant in the first step linear regression analyses were included in the final step linear regression. Unstandardised (B) with 95% confidence intervals (95% CI) and p values. Significant p values are in bold. 2.5 nmol/L equals 1 ng/mL.
Associations between vitamin D status and anxiety, depression and health-related quality of life

Possible clinical anxiety occurred in 16.9% of the immigrants, and was more common in women than in men (p=0.004) and in immigrants from the Middle East compared to immigrants from Africa (p=0.032). In subgroup analyses 32.7% of Middle East women had symptoms of clinical anxiety. Possible clinical depression occurred in 4.6% of the immigrants. Middle Eastern participants rated more anxiety and depressive symptoms in EQ5D (p<0.001), and they rated lower at EQVAS as compared to African immigrants (p=0.001).

There was no association between 25(OH)D levels and anxiety, depression or QoL in the total immigrant population. Neither was there any association between vitamin D deficiency and anxiety, depression or QoL in the total immigrant population.

In the Middle Eastern women, there was a negative linear correlation between 25(OH)D levels and HADA points (p=0.022) and between 25(OH)D levels and HADD points (p=0.013). There was also an association between 25(OH)D3 <50 nmol/L and anxiety (p=0.022) in the female immigrants from the Middle East. There was no association between 25(OH)D3 <50 nmol/L and anxiety in the other subgroups. Multivariable logistic regression analyses showed that anxiety was associated with both 25(OH)D <50 nmol/L (p=0.012) and 25(OH)D levels (p=0.015, data not shown) in Middle Eastern women after adjustments. Table 11.

Table 11. Final step multivariable logistic regression with stepwise exclusion for associations between anxiety and 25(OH)D3 <50 nmol/L, physical activity, sick-leave and chronic disease in female immigrants from the Middle East (n=55).

<table>
<thead>
<tr>
<th></th>
<th>OR (Anxiety)</th>
<th>(95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D3 &lt;50 nmol/L</td>
<td>23.16</td>
<td>(1.97-271.87)</td>
<td>0.012</td>
</tr>
<tr>
<td>Sick-leave last year Yes</td>
<td>8.53</td>
<td>(1.33-54.78)</td>
<td>0.024</td>
</tr>
<tr>
<td>Physical activity &lt;1x/week</td>
<td>15.27</td>
<td>(2.34-99.45)</td>
<td>0.004</td>
</tr>
<tr>
<td>Chronic disease Yes</td>
<td>8.84</td>
<td>(1.27-61.22)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Footnote: Final step multivariable logistic regression analyses included only variables that were significant in first step analyses. Anxiety defined on the Hospital Anxiety and Depression Scale (HAD-A) ≥11 indicating possible anxiety disorder. First step univariable analyses included 25(OH)D3<25nmol/L, 25(OH)D3<50nmol/L, sick-leave, physical activity, paid job, working or studying, education, age group, clothing, smoking, journeys abroad and chronic disease. Cox and Snell R Square 0.397, Nagelkerke R Square 0.554.
**Demographics paper IV**

The study protocol was completed by 147 subjects; 73 men and 74 women. There was no difference in gender distribution between groups. The majority of participants (56%) were born in Africa. The participants’ mean age was 42 ± 11 years, they had lived in Sweden for 16 ±10 years and the majority were married or cohabiting. The majority (58%) had a high level of education with at least 13 years of schooling, 61% were working and 21% were studying. Only 13% had maximum 9 years of schooling and 9% were unemployed or on disability pension or social allowance. There were no significant differences between genders regarding, age, BMI and socioeconomic status.

**The effect of cholecalciferol on vitamin D status and 25(OH)D levels**

Results according to treatment per protocol are shown in Table 12. It is notable that 62% in Group 1 attained 25(OH)D >125 nmol/L with a maximum of 197 mmol/L. No-one in the other groups reached 25(OH)D >125 nmol/L. 25(OH)D decreased in 12% of subjects in Group 2b and 7% in Group 3.

**Table 12.** 25(OH)D levels after intervention with different doses of cholecalciferol 12±2 weeks (Treatment per protocol, n= 147).

<table>
<thead>
<tr>
<th>Baseline 25(OH)D</th>
<th>Group 1 &lt;25 nmol/L</th>
<th>Group 2a 25-49 nmol/L</th>
<th>Group 2b 25-49 nmol/L</th>
<th>Group 3 50-74 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (IE)</td>
<td>10000 /d</td>
<td>2000 /d</td>
<td>2000 /w</td>
<td>2000 /d</td>
</tr>
<tr>
<td>n (% )</td>
<td>16 (100)</td>
<td>54 (100)</td>
<td>49 (100)</td>
<td>28 (100)</td>
</tr>
<tr>
<td>End 25(OH)D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25 nmol/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 50 nmol/L</td>
<td>0</td>
<td>6 (11.1)</td>
<td>22 (44.9)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>≥ 50 nmol/L</td>
<td>16 (100)</td>
<td>48 (88.9)</td>
<td>27 (55.1)</td>
<td>27 (96.4)</td>
</tr>
<tr>
<td>≥ 75 nmol/L</td>
<td>16 (100)</td>
<td>24 (44.4)</td>
<td>1 (2.0)</td>
<td>20 (71.4)</td>
</tr>
<tr>
<td>≥ 125 nmol/L</td>
<td>10 (62.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mean±SD</td>
<td>140.8±31.9</td>
<td>72.5±14.6</td>
<td>52.3±9.9</td>
<td>81.0±14.8</td>
</tr>
<tr>
<td>range</td>
<td>87–197</td>
<td>40–101</td>
<td>36–91</td>
<td>46–112</td>
</tr>
</tbody>
</table>
Results according to intention to treat are shown in Figure 8.

Figure 8. S-25(OH)D before and after 12±2 weeks treatment using different doses of cholecalciferol; Group 1 10000 IUs/day, Group 2a 2000 IUs/day, Group 2b 2000 IUs/week and Group 3 2000 IUs/day. Intention to treat, n=155. Boxplot. Boxes ranges from 25 percentile to 75 percentile; horizontal line represents median value. Open circles represent outliers (between 1.5 and three times the interquartile range). Dotted lines represent 25(OH)D levels 25, 50, 75 and 125 nmol/L respectively.

Predictors of change in 25(OH)D and dose response effects
The change in 25(OH)D was positively predicted by the dose of cholecalciferol. The change in 25(OH)D was also inversely related to baseline 25(OH)D and BMI, and positively related to female gender. However, the treatment response (change of 25(OH)D per given dose of cholecalciferol) was only (negatively) associated with the baseline 25(OH)D and given cholecalciferol dose level. For the total study population, 25(OH)D increased by 1.1 nmol/L per daily supplemented microgram cholecalciferol. However, there were considerable differences between groups.
Adverse events, discontinuations etc.

In the study group, 41 adverse events (AEs) were reported; in Group 1: 7, Group 2a: 13, Group 2b: 15 and Group 3: 6 AEs. Of the 13 subjects who did not complete the study, 11 reported that suspected side effects was the reason for stopping medication, in Group 1: 5, Group 2a: 2, Group 2b: 1 and Group 3: 3 subjects. In Group 1, there was one suspected serious adverse event with mental confusion in a subject who ingested several double doses of cholecalciferol in the first ten days, this subject stopped medication after these acute symptoms. The vast majority of the other AEs were miscellaneous and mild without any clear pattern, while four could be due to known causes or conditions that were known before the study. The most frequent symptoms reported were headaches, pain in body and lower extremities, depressive or anxiety symptoms, insomnia and reduced ability to concentration, stitches and numbness in upper extremities and obstipation. Creatinine and calcium levels were within reference levels in all subjects at baseline and at follow-up and changes in PTH were marginal.
Discussion

Discussion Papers I-III

Papers I, II and III in this thesis contribute with knowledge about prevalence and determinants of vitamin D deficiency in immigrants in Northern Sweden and associations between vitamin D status and muscular strength and anxiety.

Primary findings, novelty

In Paper I, the majority of African and Middle Eastern immigrants who live in Northern Sweden were found to have vitamin D deficiency or insufficiency. The prevalence of vitamin D deficiency was 12 % and 73 % of the immigrants had 25(OH)D levels less than the recommended level of 50 nmol/L considered to be sufficient for bone health.\textsuperscript{16} This study also confirms that vitamin D deficiency was not a problem limited to female immigrants although many other studies focus on female subjects.\textsuperscript{177, 224, 225}

The mean 25(OH)D was 41 nmol/L which is higher than in similar studies of immigrants in Northern Europe\textsuperscript{162} despite the fact that this study was performed at latitude 63˚N. Differences in the participants’ native countries may be part of the explanation of the higher 25(OH)D\textsubscript{3} levels in our study. Another explanation could be that the reference method LC-MsMs was used for the analyses of 25(OH)D in this study while 25(OH)D levels were measured by RIA in the Norwegian study, which is known to result in lower 25(OH)D levels compared to LC-MsMs.\textsuperscript{54} However, mean 25(OH)D in the immigrant population was much lower than in reports of mainly native Swedish populations where mean 25(OH)D levels varies between 65-88 nmol/L.\textsuperscript{43, 168, 170}

This study also demonstrates the importance of lifestyle for vitamin D status in the immigrant population. Immigrants are a heterogeneous group and their native countries might reflect differences in both genetics (for example skin pigmentation) and lifestyle with impact on 25(OH)D levels. Although vitamin D deficiency was twice as common in immigrants from Africa compared to those from the Middle East, our results showed that lifestyle factors had the strongest impact on the risk for vitamin D deficiency. The most important determinant of vitamin D deficiency was low fatty fish intake (less than once a week). The strong association between fatty fish intake and 25(OH)D levels is in accordance with other studies.\textsuperscript{38, 162} Other determinants of vitamin D deficiency were absence of journeys abroad and use of long-sleeved clothes during summer indicating reduced sun exposure. Although we did not restrict the journeys abroad to “sun vacations”, all destinations were southern and our results were in line with other studies regarding the positive effects of sun vacations on vitamin D status.\textsuperscript{224, 234, 225} The
risk of having vitamin D deficiency was three times higher in immigrants wearing long-sleeved clothing outside during summer. Studies in the Middle East have shown similar associations between clothing habits and vitamin D deficiency in women, but there are no other studies showing this association between clothing habits and vitamin D deficiency in both immigrant men and women, living in Northern Sweden where the period of possible exposure to UVB radiation is limited compared to southern latitudes.

In this study, mean 25(OH)D levels were higher in the oldest participants (p=0.010), which is in contrast to results in population studies in Africa and the Middle East, but in accordance with findings in the general population in Northern Sweden. However, there was a higher proportion of immigrants from the Middle East in the age group 55-64 (four African and 21 Middle East participants) which may have had impact on the 25(OH)D levels in this age group. The oldest participants also had the highest BMI and this might explain why high BMI was not correlated to low 25(OH)D3 levels in our study, in contrast to other studies.

In Paper II, lower 25(OH)D levels were shown to be significantly associated with reduced lower limb muscle strength and vitamin D deficiency was significantly associated with weaker grip strength in both immigrant men and women in working ages after multiple adjustments.

The positive association between 25(OH)D levels and lower limb muscle strength persisted after adjustments for BMI, in contrast to other studies. There was also a strong association between obesity and reduced lower limb muscle strength, this may be explained by a relatively lower limb muscle weakness in the obese immigrants as we measured functional muscle strength. Most other studies examining associations between vitamin D status and muscular strength focus on elderly or women while few studies examine men and women in working ages. In a Dutch study of elderly, significant associations between physical performance in the lower extremity and 25(OH)D levels were also demonstrated, with the poorest performance in participants with 25(OH)D ≤ 25 nmol/L, and minor reduction of performance in participants with 25(OH)D 25-50 nmol/L compared to those with 25(OH)D >75 nmol/L. Similar findings were shown in American studies of elderly.

In this study, vitamin D deficiency was associated with weaker grip strength in the immigrants, but 25(OH)D levels were not associated with grip strength in contrast to studies of Somali women in Sweden. A possible explanation could be a stronger impact of differences in gender, height and age on grip strength in the VIDI1 Study as the study population consisted of both male and female immigrants in a broad age range, originating from nine different countries.
In the study of Somali women, only 10% had 25(OH)D levels ≥25 nmol/L compared to 88% of immigrants in our study.\textsuperscript{178} For comparison, in a study including both men and women aged 20–76 years, 25(OH)D levels were associated with proximal muscle strength in arms and legs but not with grip strength.\textsuperscript{226} In that study very few of the participants had 25(OH)D levels <50 nmol/L. It has been suggested that there could be different thresholds for the associations between 25(OH)D levels and different outcomes.\textsuperscript{227} Other studies that include few participants with 25(OH)D <50 nmol/L have not found any associations between 25(OH)D levels and muscle strength.\textsuperscript{228, 229}

The primary finding in Paper III was that vitamin D deficiency and 25(OH)D levels were not associated with depression, anxiety or QoL in the total group of immigrants or in any subgroup.

However, in female immigrants from the Middle East, in whom prevalence of anxiety and vitamin D deficiency was higher, there was an association between anxiety and 25(OH)D levels <50 nmol/L after adjustments. As far as we know, there are no other studies showing this significant association between anxiety and 25(OH)D levels after adjustments for several confounders including sick-leaves, physical activity and chronic disease as in the female immigrants from the Middle East in this study.

The prevalence of clinical depression in this study was low, both in the total group of immigrants and in the subgroups. In a larger study regarding associations between depression and 25(OH)D levels, a decrease in depressive symptoms was demonstrated at 25(OH)D levels ≥51 nmol/L.\textsuperscript{230} There are only few studies regarding associations between vitamin D and anxiety, mostly with negative results after adjustments.\textsuperscript{112, 121, 231} However, many studies include subjects with mostly adequate vitamin D status and low scores for anxiety.\textsuperscript{231, 232} In a Canadian study of associations between vitamin D status and QoL, there was a significant association between higher 25(OH)D levels and less reported problems with depression and anxiety after multiple adjustments \textsuperscript{233} and the same association was later demonstrated in a Korean study.\textsuperscript{234} However, in these studies depression and anxiety were measured with EQ-5-D and not possible to analyze separately.

Mood disorders, as anxiety, have a multifactorial aetiology, and vitamin D, with its potential effects on brain functions could have an impact on anxiety.\textsuperscript{109, 200, 201} However, there is a possibility of reverse causality with anxious subjects spending more time indoors, avoiding the sun.\textsuperscript{109}
**Study population, representativeness**

The VIDI1 Survey was designed as a population-based, cross-sectional study; the target population was African and Middle Eastern immigrants of working age living in Umeå. The choice of countries of origin was based on the most frequent origin countries for Middle East and African immigrants living in Umeå. The median 25(OH)D in the VIDI1 population was higher than in other immigrant studies, this may reflect differences in native countries of participants. Participants from Pakistan were underrepresented in the study and Pakistani have been recorded as having a high incidence of vitamin D deficiency. Immigrants are a heterogeneous group and there were differences in ethnicity, genetics, culture, integration etc. within the immigrant group. The study participants had a relatively high education level and had lived in Sweden for many years, which implies an effect of selection and adaption of lifestyle. The youngest age group was underrepresented in the study, which actually led to a more even age distribution in the study population.

In **Paper III** only Swedish and English-speaking subjects were included in the analyses of association between vitamin D status and anxiety, depression and QoL. Consequently, the results might not be representative for immigrants in need of interpreting services.

**Considerations regarding participation rate**

Initially, 1 544 addresses were obtained from the population register, however 15.5% of invitations were returned directly by the post office due to invalid addresses. Totally, 1 306 subjects were invited to the VIDI1 Study of which 790 subjects (60.5%) never answered the invitation. After reminders, 516 subjects answered the invitation and finally 216 were included in the study. See Figure 3. The calculated participation rate was 16.5%. This might appear low but it is in line with other, similar immigrant studies where the same pattern of low response and participation rates is noted. However, there could be several inaccuracies in the address data achieved from the population register, implying that the actual number of immigrants living in the area were considerably fewer than the population register stated. The recruitment area had a young population including many students, as many as seven young immigrants were registered at the same student rooms and none of these answered the invitation. The immigrants travelled a lot and many of the non-responders might be due to invited subjects not staying at their registered address during the recruitment period. One participant had two identities and was invited twice with two different registration numbers. Between three and five subjects who moved between the two initial and the third recruitment area during the 5-month recruitment period were invited twice. These inaccuracies were corrected for among the participants but not among non-participants. Altogether, these factors
indicate that the real participation rate was higher than the calculated. The observed problem of subjects registered as residents without living at the address has recently been noted in the government audit of the national population registration.\(^\text{235}\)

However, in analyses comparing participants with non-participants, there were no differences in gender or in African or Middle Eastern origin between invited and examined populations, the only difference was that there were fewer participants in ages 25-34 years resulting in a more equal age distribution among the participants. Table 4.

**The inconspicuous seasonal effect on 25(OH)D levels**

The VIDI1 Study was conducted from September 2009 to June 2010 and all invitations were sent by mail September – December 2009. All subjects originating from Ethiopia were invited in December and therefore examined January–June 2010, whether this could have had impact on the 25(OH)D levels in the African immigrants is unclear. The only seasonal variations in 25(OH)D levels noticed was an increase in 25(OH)D levels in the Middle Eastern immigrants in September. The absence of seasonal variation in 25(OH)D levels in immigrants of African origin could be an effect of reduced UVB-induced vitamin D synthesis in the skin during summer, related to frequent use of long-sleeved clothing during summer and skin pigmentation.\(^\text{159, 160}\) Seasonal variations with higher 25(OH)D levels in summer and autumn are earlier shown in studies of Caucasians and Middle Eastern populations.\(^\text{38, 40}\) There are few studies reporting seasonal variations in 25(OH)D levels in African populations.\(^\text{236}\)

**The decision to choose 25(OH)D3 for calculations**

25(OH)D2 was detected in only five participants and it was decided early on only to use 25(OH)D3 in the analyses. Later reviewing this decision, recalculating total 25(OH)D in the participants showed that the number of vitamin D deficient participants were unchanged and mean 25(OH)D in the immigrants increased by only 0.3 nmol/L. Associations between total 25(OH)D levels and cofactors examined were unchanged compared to calculations with 25(OH)D3 levels.

**Strengths and weaknesses**

The strengths of the VIDI1 Study are that it is a cross-sectional, population-based survey, performed far north at latitude 63\(^\circ\)N, studying vitamin D status in both male and female immigrants. Anthropometry, physical activity, medical history and socioeconomic variables were examined. 25(OH)D levels were measured with the reference method LC-MsMs performed in a DEQAS-certified laboratory, guaranteeing high-quality analyses. There are no earlier studies of the prevalence of vitamin D deficiency in immigrant populations in Northern Sweden. To the
best of our knowledge, other studies of vitamin D deficiency in immigrants in Sweden only include women.

One limitation is the low participation rate of 16.5%. This could be due to the high level of mobility in the immigrant populations and the recently confirmed inaccuracies in the population registration. However, the participation rate was in line with other similar studies. There is a possibility of social selection bias related to the high educational level and the long period since migration to Sweden in the study population. Travelling abroad and clothing habits during summer could be a proxy for increased sun exposure, however, we did not measure time spent outdoors.

Another limitation was the small size of the VIDI1 Study. In Paper II 25(OH)D levels were significantly associated with lower limb muscle strength but there was no significant association between vitamin D deficiency and reduced lower limb muscle strength (p=0.052) which could be due to lack of power. Grip strength was associated with vitamin D deficiency, but not with 25(OH)D levels. This may also be due to lack of power, with a stronger impact of gender, height and age on grip strength. The lack of association between muscle strength and physical activity implies that the questions for measurement of physical activity may have been inappropriate for measuring physical activities with effect on muscle strength in the hands and lower limb. The positive association between lower limb muscle strength and absence of longer periods of sick-leave indicate that a history of sick-leave could be a better indicator of inactivity and immobilisation relevant to muscle strength as well as an indicator of health problems with impact on muscle strength.

Regarding Paper III, the strengths include the cross sectional study design examining men and women in working ages, while other investigations examining associations between vitamin D status and anxiety or depression often study patients with chronic pain, psychiatric diagnoses or elderly. Other studies regarding associations between anxiety or depression and vitamin D status have shown conflicting results and demonstrated associations have been suggested to be due to poorer lifestyle habits. Therefore, this study including so many possible confounders including medical history, lifestyle and socioeconomic factors is most important.

A limitation is the small size of the study and the low prevalence of anxiety and depression in the total study population, which resulted in low power and precluded detection of any differences in prevalence of anxiety or depression associated with vitamin D deficiency in the total immigrant group or in any subgroup. A retrospective power analyses showed that equal groups sized 49 women would have been required to detect any difference in prevalence of anxiety
associated with vitamin D deficiency in the group of Middle Eastern women. However, there were only seven Middle Eastern women with vitamin D deficiency in this study. Furthermore, there is a possibility of social selection bias as only Swedish and English speaking immigrants were included in the study. There were no reference values regarding EQ-5-D results in immigrant populations, neither in Middle Eastern nor African populations, consequently we used the Danish reference data set in the analyses.221

Discussion Paper 4

Primary findings, novelty
This study contributes new knowledge about the effect on vitamin D status and 25(OH)D levels of treatment with different doses of cholecalciferol depending on baseline 25(OH)D levels for three months.

Supplementation with 10000 IUs cholecalciferol/day for three months in healthy subjects with vitamin D deficiency (initial 25(OH)D <25 nmol/L) was effective in treatment of vitamin D deficiency, all subjects reached adequate vitamin D status (25(OH)D levels ≥50 nmol/L) within 12 weeks. The majority of subjects supplemented with 10000 IU/day attained higher 25(OH)D levels; ≥125 nmol/L and the maximum 25(OH)D level after 12 weeks treatment was 197 nmol/L, however, calcium and creatinin stayed within reference levels. There were large individual variations in 25(OH)D levels after 12 weeks treatment.

In studies performed by Heaney and Barger-Lux, supplementation with cholecalciferol in daily doses of 10000-11000 IU/day to subjects with mostly adequate vitamin D status at baseline, resulted in mean 25(OH)D levels between 213-224 nmol/L after 8-20 weeks treatment and there were no significant changes in calcium.145, 146 Table 13. In the study performed by Heaney, supplementation with 5500 IU cholecalciferol/day for 20 weeks to subjects with mean baseline 25(OH)D 69 nmol/L, resulted in mean 25(OH)D around 160 nmol/L.145 The highest administered dose of cholecalciferol in the study by Barger-Lux; 50000 IU/day, resulted in mean 25(OH)D 710 nmol/L after 8 weeks treatment with no significant change in calcium levels.146 In a study aiming to detain the intake of vitamin D required to attain 25(OH)D levels ≥75 nmol/L, the mean daily dose required was 3440 IU/day, with variations mainly depending on baseline 25(OH)D.238 Another study used a weekly dosage of 20000 IUs cholecalciferol/week (2857 IU/day) for one year, and this resulted in 25(OH)D levels <75 nmol in 12 % of the participants and approximately two percent had 25(OH)D levels < 50 nmol/L.141 In a recent Swedish study, vitamin D deficient and insufficient subjects were treated with 1600 IU of cholecalciferol/day for three months, and mean 25(OH)D was shown to increase with 41 nmol/L.
However, some of those participants were still vitamin D insufficient after treatment.\textsuperscript{239}

In subjects with insufficient vitamin D status (initial 25(OH)D 25-49 nmol/L) 89\% attained adequate vitamin D status after 12 weeks supplementation with 2000 IUs cholecalciferol/day, maximum 25(OH)D was 101 nmol/L and mean 25(OH)D increased with 38 nmol/L. Calcium and creatinine were within reference limits in all participants. However, 11\% of participants with vitamin D insufficiency did not attain adequate vitamin D status after 12 weeks treatment with 2000 IU cholecalciferol/day. For comparison, in the Norwegian study, supplementation of vitamin D insufficient subjects with 20000 IUs cholecalciferol/week in one year resulted in an increase in mean 25(OH)D of 47-58 nmol/L.\textsuperscript{141}

Treatment with 2000 IUs/week in the vitamin D insufficient group resulted in 25(OH)D levels < 50 nmol/L in 45\% of the subjects, demonstrating that the dietary intake recommended by NNR in 2004\textsuperscript{127} was too low to achieve sufficient vitamin D status in the immigrant population.

In subjects with adequate vitamin D status (25(OH)D levels 50-74 nmol/L) supplemented with 2000 IUs/day, mean increase in 25(OH)D was 22 nmol/L, no subjects reached 25(OH)D levels >125 nmol/L and calcium and creatinine were within reference limits. For comparison, in a recent Norwegian study, supplementation with 20000 IUs cholecalciferol/week (2857 IUs/day) for one year resulted in a mean increase in 25(OH)D with 47 nmol/L in subjects with adequate vitamin D status. In that study, 25(OH)D levels were < 131 nmol/L at study end in 97.5\% of subjects.\textsuperscript{141} In other studies supplementing with approximately 1000 IUs cholecalciferol/day to subjects with mean baseline 25(OH)D 67-72 nmol/L, mean 25(OH)D has been shown to increase 12-29 nmol/L after 8-20 weeks treatment.\textsuperscript{145, 146} Another study supplemented subjects with mostly adequate vitamin D status with lower doses of cholecalciferol (400-800 IUs/day) which resulted in a mean increase in 25(OH)D with 8-17 nmol/L.\textsuperscript{240}
Table 13. Results on 25(OH)D (nmol/L in the VIDI2 Intervention study compared to other studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Baseline mean±SD (range)</th>
<th>Dose*</th>
<th>Duration</th>
<th>End mean±SD (range)</th>
<th>Increase mean±SD (range)</th>
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<tr>
<td>VIDI2</td>
<td>20 ±4</td>
<td>10000/d</td>
<td>12 ww</td>
<td>141 ±32</td>
<td>121 (63,178)</td>
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<td>34 ±6</td>
<td>2000/d</td>
<td>12ww</td>
<td>72 ±15</td>
<td>38 (10,65)</td>
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<td>39 ±6</td>
<td>285/d</td>
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<tr>
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<td>400/d</td>
<td>5months</td>
<td>64</td>
<td>8±14</td>
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*Cholecalciferol IUs

Choice of cholecalciferol dose
The supplemental doses used in the VIDI2 Intervention study were based on the most recently-published recommendations that were available at the time of study planning125 222 and the dose was recommended in the Summary of Product Characteristics (SPC) of the PP at the time when the study was planned and conducted. Consequently, we decided to evaluate the dose 10000 IUs/day in treatment of subjects with 25(OH)D levels <25 nmol/L, in a clinical trial where all the participants were thoroughly checked for exclusion criteria and their calcium and creatinin levels were within reference limits. In 2011, additional papers were published regarding the definition of vitamin D deficiency and the recommendations for supplementation regimens.125, 241, 242 However, we decided to not to change the approved study protocol.
Subjects with 25(OH)D 25–49 nmol/L were treated either with 2000 IU/day or with 2000 IU/week. The dose 2000 IU/week was based on the suggestion from the Endocrine Society in 2011 that patients at risk of vitamin D deficiency might have a daily requirement of 1500-2000 IU/day of vitamin D. Earlier studies showed that immigrants from Africa and the Middle East were at risk for vitamin D deficiency. In the two groups with 25(OH)D 25–49 nmol/L, we aimed to compare the supplemental dose of 2000 IU/day with a placebo. However, it was not possible to get a placebo preparation with the PP. Instead, we chose to use the dose of 2000 IU/week (285 IU/day) that was as close as possible to the RDI for adults (300 IU/day) recommended by the National Food Agency in Sweden at the time of planning the Intervention Study in 2010.

In Group 3, subjects with 25(OH)D 50–74 nmol/L were treated with 2000 IU cholecalciferol/day. In 2010, there was no consensus on how to define deficient and insufficient vitamin D status, according to the Endocrine Society, 25(OH)D levels 50–72.5 nmol/L were considered insufficient and the Endocrine Society recommended that 25(OH)D should exceed 75 nmol/L. Subjects with 25(OH)D 50–74 nmol/L were therefore treated with 2000 IU/day according to the Endocrine Society’s suggestion that patients at risk of vitamin D deficiency have a daily requirement of 1500-2000 IU/day of vitamin D. This would also simulate a situation where all immigrants would be recommended preventive supplementation without previous measurements of 25(OH)D and enabled to evaluate the safety of such supplementation in healthy subjects with 25(OH)D 50–74 nmol/L, monitoring results on 25(OH)D, PTH, calcium and creatinin. At the time of the implementation of the Intervention Study, cholecalciferol preparations containing 2000 IUs/capsule were sold without prescription in health food stores with manufacturers prescribed dosage one capsule/day.

**Choice of target 25(OH)D**

In the Intervention Study, the initial target level for 25(OH)D was ≥75 nmol/L according to the Endocrine Society’s definition of vitamin D deficiency and insufficiency at time of planning the study. Taking into account the IOM recommendations published in 2011 stating that 25(OH)D ≥ 50 nmol/L meet the needs for 97.5 % of the population according to bone health, we have also recalculated results according to this definition of adequate vitamin D status.

**The PP**

At the time of study planning there were no pure cholecalciferol preparations available for ordinary prescription to vitamin D-deficient subjects, only combinations of vitamin D in low dose combined with vitamin A or calcium. In clinical practice, tablets containing 400-800 IU cholecalciferol combined with 500 mg calcium or oil containing 80 IU cholecalciferol per drop was prescribed. However, the cholecalciferol solution used in this study was approved for
routine prescription on 26 August 2011. Using a solution meant that different doses were dispensed by counting drops of PP. The bottles dispensed were estimated to suffice for the treatment period, despite this, one subject in Group 1 and seven each in Groups 2a and 3 requested extra bottles of PP. During the study it was observed that the drops fell irregularly and could be difficult to count which might have had impact on the intake of the PP.

**Dose-response in comparison with other studies**

The dose-response variations in the Intervention study were in accordance with other studies. There was an inverse relationship with a higher increase in 25(OH)D at the same dose of cholecalciferol when administered at a lower baseline level compared to a higher baseline level, as earlier demonstrated. There was also an inverse relationship between dose-response and supplemented dose, and dose-response was shown to be similar when a high dose was given at low baseline levels as when a low dose was given at higher baseline levels, which is consistent with findings in other studies. In aggregated data from treatment studies, the relation between dose and 25(OH)D levels has been demonstrated to be non-linear, with a relatively flat dose-response curve up to 10000 IU/day reaching 25(OH)D levels around 125 nmol/L, at higher doses the increase of 25(OH)D will become steeper.

**Safety aspects on 25(OH)D results**

In the vitamin D deficient study group, the dose 10000 IUs/day for 12 weeks was consistent with maintaining calcium and creatinin within reference limits, which is in accordance with other studies using cholecalciferol in doses up to 11000 IUs/day without reporting hypercalcemia. However, later large observational cohort studies have demonstrated the lowest all-cause mortality risks at 25(OH)D around 60-75 nmol/L, with 30-50 % higher risk at 25(OH)D levels exceeding 125-140 nmol/L. Although the cause of these associations is unknown, supplementation with cholecalciferol in doses resulting in 25(OH)D levels exceeding 125 nmol/L could be unnecessary. In the VIDI2 Intervention study, the maximum 25(OH)D result was below levels which may lead to hypercalcemia, however, 62 % of the subjects supplemented with 10000 IUs/day reached 25(OH)D ≥125 nmol/L. This indicate that the dose 10000 IUs/day to the vitamin D deficient subjects could be unnecessarily high. In group 2a and 3, who were supplemented with 2000 IUs/day for 12 weeks, no subjects attained 25(OH)D levels >125 nmol/L and calcium and creatinine were within reference limits indicating that the dose was safe.
**Strengths and weaknesses**

The VIDI2 Intervention Study was thoroughly performed according to ICH-GCP, driven by researchers. Furthermore, a high percentage (92%) completed the trial with good compliance according to the protocol. AEs and causes of discontinuations were thoroughly registered. Additionally, Lc-MsMs, the reference method for analysing 25(OH)D was used in the analyses. Both men and women in a broad range of ages participated.

A limitation is the open study design and the lack of a placebo group. It was also observed that the cholecalciferol solution drops fell irregular and could be difficult to count which might have had impact on the intake of the PP.
Clinical implications

Twelve percent of African and Middle Eastern immigrants in Northern Sweden had vitamin D deficiency and the majority had 25(OH)D levels $<$ 50 nmol/L, which are considered insufficient to meet the needs based on bone health. More active measures regarding food and lifestyle advice among immigrants from Africa and the Middle East may be necessary.

In African and Middle Eastern immigrants, muscle weakness could motivate analyses of 25(OH)D to exclude vitamin D deficiency.

Analyses of 25(OH)D may be considered in Middle Eastern women presenting anxiety symptoms.

25(OH)D should be analysed before starting supplementation with vitamin D. Regular analyses of 25(OH)D is recommended during supplementation to follow the effect on 25(OH)D results and enable dose adjustment.

Supplementation with 10000 IU/day in healthy subjects with initial 25(OH)D$_3$ < 25 nmol/L and 2000 IU/day in healthy subjects with 25(OH)D$_3$ 25-49 nmol/L for three months was consistent with calcium and creatinin remaining within reference levels. However, a large proportion of the subjects supplemented with 10000 IU/day reached high 25(OH)D levels, indicating that the dose was unnecessarily high.

Future research

One direction for future research is to examine whether symptoms such as muscular weakness and anxiety, associated with vitamin D deficiency and insufficiency, are affected by treatment with vitamin D. Intervention studies should focus on populations with high prevalence of vitamin D deficiency and insufficiency. Tentatively, larger randomized trials including participants with high prevalence of vitamin D deficiency would increase the ability to demonstrate any significant effects of treatment when studying muscular, anxiety and depressive symptoms.
Conclusions

I Twelve percent of immigrants from Africa and the Middle East who live in Umeå, Northern Sweden had vitamin D deficiency, with no difference between men and women.

Lifestyle factors were crucial for vitamin D status. A diet with intake of fatty fish less than once a week, absence of journeys abroad and use of long-sleeved clothing outside during summer were determinants of vitamin D deficiency.

II Vitamin D status was associated with muscle strength in Middle Eastern and African-born immigrants.

III There was no association between vitamin D status and anxiety, depression or QoL in the total immigrant population. In Middle Eastern women, 25(OH)D < 50 nmol/L was associated with anxiety.

IV The proportion who reached adequate vitamin D status (25(OH)D≥50 nmol/L) after 12±2 weeks treatment with cholecalciferol was:
  - in Group 1 (baseline 25(OH)D <25nmol/L; dose 10000IUs/day): 100%,
  - Group 2a (baseline 25(OH)D 25-49 nmol/L; dose 2000IUs/day): 89%,
  - Group 2b (baseline 25(OH)D 25-49 nmol/L; dose 2000IUs/week): 55%
  - and in Group 3 (baseline 25(OH)D 50-74 nmol/L; dose 2000IUs/day): 96%. Similarly, 100% in Group 1, 44% in Group 2a, 2 % in Group 2b and 71% in Group 3 reached 25(OH)D≥75 nmol/L. The change of 25(OH)D (nmol/L) mean (range) was in Group 1: 121,1 (63,178), Group 2a: 38.1 (10,65), Group 2b: 13.6 (-8,64) and Group 3: 22.3 (-15,62).
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References


4. Glisson F. *A treatise of the rickets; being a disease common to children.*, 1651.


6. Scott AC. A contribution to the study of osteomalacia in India. *Indian Journal of Medical Research* 1916; 4: 140.


11. Autier P. Vitamin D status and ill health: a systematic review. *Lancet Diabetes Endocrinol* 2014; 2: 76-89. e-pub ahead of print December 6,2013 doi: http://dx.doi.org/10.1016/S2213-8587(13)70165-7


34. Norrmejerier. Näringsvärde Lättmjölk 0,5%. 2018.


77. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 2010; 303(18): 1815-1822. e-pub ahead of print 2010/05/13; doi: 10.1001/jama.2010.594

78. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-
extremity function in both active and inactive persons aged > or =60 y. Am J Clin Nutr 2004; 80(3): 752-758. e-pub ahead of print 2004/08/24; doi: 80/3/752 [pii]


Rabenberg M, Harisch C, Rieckmann N, Buttery AK, Mensink GB, Busch MA. Association between vitamin D and depressive symptoms varies by season: Results from the German Health Interview and Examination Survey for Adults (DEGS1). *J Affect Disord* 2016; 204: 92-98. e-pub ahead of print 2016/06/25; doi: 10.1016/j.jad.2016.06.034

van den Berg KS, Marijnissen RM, van den Brink RH, Naarding P, Comijs HC, Oude Voshaar RC. Vitamin D deficiency, depression course and mortality: Longitudinal results from the Netherlands Study on Depression in Older persons (NESDO). *J Psychosom Res* 2016; 83: 50-56. e-pub ahead of print 2016/03/30; doi: 10.1016/j.jpsychres.2016.03.004


140. Chao YS, Brunel L, Faris P, Veugelers PJ. The importance of dose, frequency and duration of vitamin D supplementation for plasma 25-hydroxyvitamin D.


179. Saaf M, Fernell E, Kristiansson F, Barnevik Olsson M, Gustafsson SA, Bagenholm G. Severe vitamin D deficiency in pregnant women of Somali origin


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van Reenen M OM. EQ-5D-3L User guideBasic information on how to use the EQ-5D-3L instrument. In, 2015. p version 5.1.


Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010; 340: b5664. e-pub ahead of print 2010/01/13;


237. Gowda U, Mutowo MP, Smith BJ, Wluka AE, Renzaho AM. Vitamin D supplementation to reduce depression in adults: meta-analysis of randomized


Appendices
Appendix A

CRF  Data collected in paper I, II and III;

Reg.nr:
Date of examination

Background
Year of birth
Country of birth
Year of arrival in Sweden
Father’s country of birth
Mother’s country of birth
Earlier illnesses
Present illnesses
Knee or joint problems
Medication
Sick-leave more than one month during the previous year

Education
Vocational training
University education

Occupation
Self-employed; in what business?
Student
Unemployed
Sick-leave
Pension
Working at home without salary

Diet and lifestyle
Vegetarian/vegan
Milk/yoghurt/fil
-decilitres/day
Cheese
-slices/day
Fatty fish
-servings/week
Omega-3/fish oil/seal oil
Calcium tablets
-specify
Vitamin tablets
-specify

Use of long-sleeved clothing outside during summer
Smoking: ongoing; previously, have stopped
- years of smoking
Travels abroad within 6 months previously
- specify country
- specify duration in days
Physical activity last week - days
Physical activity a normal week - days

**Symptoms**
Grade: Never=0 1x/month=1 Each week=2 Daily=3
- Pain in muscles
- Numbness in hands /feet
- Muscle cramps
- Difficulties to walk up stairs/get up from chair
- Walk in a different way

**EQ-5D**
Grade: No problems=0 Some/moderate problems=1 Extreme problems=2
- Mobility
- Self-care
- Usual activities
- Pain/discomfort
- Anxiety/depression
EQ-VAS (range 0-100 where 100 is best)

**HAD**
HAD-A (0-21p)
HAD-D (0-21p)
HAD total points (0-42)

**Body measurements**
- Length (cm)
- Weight (kg)

**Calculated BMI**

**JAMAR grip strength**
Test dominant side first, mean of three grips
- Dominant side
  - Right=1 Left=0
- Right hand (kg)
- Left hand (kg)

**Lower limb muscle strength MF-strength**

**Blood sample**

**Others:**
### VIDI2 Intervention Study: STUDY SCHEDULE AND MEDICATION PROCEDURES

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