Introduction
Clinical vitamin D deficiency, manifested as rickets, was common in the early 20th century. It disappeared when it became known that it could be cured by sunshine or the consumption of cod liver oil. However, a resurgence occurred with the arrival of non-western immigrants after 1970, and with the use of macrobiotic diets. In 2001, general practitioner Grootjans-Geerts asked for attention for hypovitaminosis D, “a veiled diagnosis”. She described four patients, one of whom was a 53 year old veiled woman, originally from Somalia, who experienced muscle pain in her upper legs and shoulders, and used high doses of non-steroidal anti-inflammatory drugs (NSAID’s). She could not climb stairs without the use of a handrail, hardly ever spent time outdoors, and rarely used dairy products. The 25-hydroxyvitamin D (25(OH)D) concentration in her blood appeared to be extremely low (<13 nmol/l). She started a daily medication of 1,200 IU of vitamin D (ergocalciferol) and 1,000 mg of calcium. After six weeks, she could climb stairs without any problems and no longer suffered pain in her upper legs and shoulders.

At about the same time (2000-2001), two pilot studies were performed. General practitioners from the ‘De Rubenshoek’ health centre in The Hague assessed the vitamin D status of 240 non-western women who came to the centre with general complaints of pain and fatigue. 82% were diagnosed with vitamin D deficiency (25(OH)D <25 nmol/l). Grootjans-Geerts assessed the vitamin D status of 51 Turkish women who seemed to be healthy and attended a weekly meeting, and 32 indigenous Dutch women as a control group. 82% of the Turkish women were diagnosed with vitamin D deficiency (25(OH)D <20 nmol/l), while only 6% of the Dutch women were vitamin D deficient.

In The Hague, these figures led to political commotion, with newspaper headlines such as: “Veil causes vitamin deficiency”, “Women stay home too much, English disease has its comeback in Schilderswijk” (Schilderswijk is a deprived neighbourhood in The Hague) and “Muslims must expose their wives to sunshine”. The Hague is one of the four largest cities in the Netherlands, with a substantial number of non-western inhabitants (28% in the year 2000). The municipality of The Hague decided that these figures demanded further study.

Vitamin D

Vitamin D is a fat soluble vitamin, and a generic term for two molecules: vitamin D$_3$ (cholecalciferol) and vitamin D$_2$ (ergocalciferol). Vitamin D$_3$ is the more common form of vitamin D in nature, whereas vitamin D$_2$ can be produced industrially through ultraviolet exposure of the plant sterol ergosterol. Their structures are similar, except for a double bond in the side chain in ergocalciferol. Dietary intake recommendations do not distinguish between vitamin D$_2$ and D$_3$.

Houghton and Vieth concluded that the assumption of equivalency between vitamin D$_2$ and D$_3$ – stated by Park in 1940 – was accepted despite Park having noted that studies were confusing and of poor quality. Discussion continues as to whether the forms of vitamin D are equivalent or not. Several studies have observed vitamin D$_3$ being...
more effective compared to vitamin D$_2$ whereas others observed equal effectiveness.$^{10}$ In the studies presented in this thesis, no distinction was made between vitamins D$_2$ and D$_3$. In the Netherlands, where these studies have been performed, most vitamin D in supplements is vitamin D$_3$. Because the vitamin D in food and the vitamin D produced in the skin is also vitamin D$_3$, almost all vitamin D within the Dutch population is expected to be vitamin D$_3$.

In human beings, both vitamin D$_2$ and D$_3$ are hydroxylated into several metabolites. There is rapid hydroxylation of vitamin D into 25-hydroxyvitamin D (25(OH)D), which occurs in the liver.$^{14}$ Serum 25(OH)D is the major circulating form (storage form) of vitamin D in the body. Serum 25(OH)D concentration is presented in nmol/l ($10^{-9}$ mol/l). 25(OH)D has a strong affinity for vitamin D binding protein (DBP).$^{15}$ The active form of vitamin D results from a second hydroxylation into 1,25-dihydroxyvitamin D (1,25(OH)$_2$D); this second hydroxylation mainly occurs in the kidney.$^{15}$ This active form is a steroid hormone, with a typical ring-structure. More specifically, it should be classified as a seco-steroid, one of the rings in the structure being broken.

The serum 1,25(OH)$_2$D concentration is tightly regulated.$^{16}$ The production of 1,25(OH)$_2$D is stimulated by parathyroid hormone (PTH). There is a negative feedback through calcium which decreases PTH and a direct negative feedback from 1,25(OH)$_2$D to PTH.$^{17}$ This feedback classifies 1,25(OH)$_2$D as a hormone. The concentration of serum 1,25(OH)$_2$D is presented in pmol/l ($10^{-12}$ mol/l), thus in the region of one thousandth of the serum 25(OH)D concentration.

Sources of vitamin D

The human body produces vitamin D in the skin from 7-dehydrocholesterol, under exposure to direct sunshine. The radiation necessary for this production is UVB (290-315 nm).$^{18}$ UVB radiation does not pass through glass, and it is blocked by sunscreen and clothing.$^{18-20}$ The intensity of UVB radiation is dependent on the season, time of day and geographical location.$^{21}$ The skin's ability to produce vitamin D decreases with age.$^{22}$ The amount of vitamin D produced upon exposure to UVB radiation is dependent on the type of skin: the darker the skin, the more sunshine is needed to produce a certain amount of vitamin D.$^{23-25}$ Sun exposure does not lead to toxic vitamin D concentrations due to a feed-back mechanism; 7-dehydrocholesterol will instead be transformed into lumisterol and tachysterol: biologically inert isomers.$^{23}$

Vitamin D can also be obtained from food. Fish – mainly fatty varieties such as salmon, mackerel and herring – is a natural source of vitamin D. Smaller amounts of vitamin D are present in eggs and liver.$^{26}$ Several products are fortified with vitamin D, such as margarine in the Netherlands, and milk in the United States and Canada.$^{27}$ Cod liver oil contains large amounts of vitamin D$_3$ and was previously often used in the Netherlands; it is still in common usage in the Nordic countries.$^{28}$ Individuals can also use supplements containing vitamin D. There are several types of supplementation, with variation in administration (e.g. pills, capsules and suspension in oil or water).
Consequences of vitamin D deficiency

Shortness of vitamin D (vitamin D deficiency) leads to decreased calcium absorption, a lower serum calcium and an increase of parathyroid hormone (PTH), which results in a higher bone turnover and increased bone resorption, thereby restoring serum calcium. Severe longstanding vitamin D deficiency leads to osteomalacia (bone weakness) in adults and rickets in children.\(^7\) Osteomalacia is accompanied by deep bone pain.\(^9\) Rickets is accompanied by growth retardation, muscle weakness, skeletal deformities, hypocalcemia, tetany and seizures.\(^{30,31}\) The first symptoms of vitamin D deficiency in the elderly are muscle pain, fatigue, muscular weakness and gait disturbances.\(^9\) The reduced muscle strength, in combination with the lower mineralization of the bone, may lead to an increased risk for falls and fractures in the elderly.\(^{32-34}\)

Several diseases are associated with a low serum 25(OH)D concentration.\(^{35}\) These include diabetes mellitus types 1 and 2, colorectal cancer, breast cancer, prostate cancer, multiple sclerosis, tuberculosis, schizophrenia, high blood pressure, cardiovascular diseases and depression.\(^{36-46}\) Furthermore, an association between vitamin D supplementation and a decreased total mortality rate has been found.\(^{47}\) However, the level of evidence for causal relationships varies.

Criteria for vitamin D deficiency

Vitamin D status is assessed through serum 25(OH)D concentration, which is the sum of vitamin D produced in the skin and intake through foods and supplements.\(^6\) There is no consensus as to the threshold for vitamin D deficiency, nor for the optimal serum 25(OH)D concentration. The Health Council of the Netherlands concluded that the serum 25(OH)D concentration is sufficient above 50 nmol/l for women aged 50 years and older and for men aged 70 years and older, and is sufficient above 30 nmol/l for younger men and women.\(^5\)

To estimate normal serum 25(OH)D concentration, one can assess the serum 25(OH)D concentration in a representative group of healthy individuals, for instance blood donors.\(^{48}\) The range of serum 25(OH)D concentrations within this group can be considered as the normal or reference range. Using this method, the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes from the Food and Nutrition Board of the Institute of Medicine (USA), stated in 1997 that the normal range was 20 to 37.5 nmol/l.\(^5\) Hollis argued in 2005 why he did not assume the concentrations obtained in this way represented a ‘normal’ vitamin D status, but rather a deficient status.\(^49\) The range of serum 25(OH)D concentrations in a population may not express a normal range, as the overall population might have insufficient serum 25(OH)D concentrations due to a prevalence of indoor lifestyles. In the past, mankind used to live predominantly outside. The serum 25(OH)D concentrations amongst groups who live mainly outdoors these days, such as lifeguards or farmers, might therefore also be considered ‘normal.’ Those concentrations have been observed to be higher than the currently recommended norm.\(^49,50\)
Another way to estimate the optimal serum 25(OH)D concentration is to search for a threshold in the association between serum 25(OH)D and negative consequences. A criterion often used for estimating optimal serum 25(OH) is PTH concentration. As serum 25(OH)D concentrations increase, PTH concentrations decrease until a plateau is reached where a further increase in serum 25(OH)D does not result in a decrease of PTH. The serum 25(OH)D concentration where the PTH plateau is reached has been interpreted as the threshold for an optimal 25(OH)D concentration. Aloia et al. published a summary of literature reporting a serum 25(OH)D threshold based on its association with PTH in 2006. The observed thresholds varied between 25 and 122 nmol/l. Bischoff-Ferrari et al. reviewed the literature for a vitamin D threshold according to several health outcomes in 2006. One of their arguments was that although PTH was a useful criterion because it promotes bone loss, it also fluctuates related to diet, time of day, renal function and physical activity. Therefore they used the alternative endpoints of bone mineral density, lower extremity function, dental health, risk of falls, risk of fractures and risk of colorectal cancer. They conclude that the most advantageous thresholds of serum 25(OH)D concentration begin at 75 nmol/l, but are best between 90 and 100 nmol/l. In a study among Dutch elderly, thresholds of 40 to 60 nmol/l were found, depending on the outcome measured. However, a PTH plateau was not reached until 75 nmol/l. The threshold for an optimal serum 25(OH)D concentration might vary by age or ethnic group. Furthermore, normal values of serum 25(OH)D might be influenced by the assay used for its detection, and by the intake of calcium. For instance, DeLucia et al. observed a normal vitamin D status (defined as serum 25(OH)D >37.4 nmol/l) among 21 of 27 (78%) young North American children with nutritional rickets. Rickets in these cases was probably the consequence of a low calcium intake.

Vitamin D intoxication

If the serum 25(OH)D concentration rises to toxic levels, calcium concentration may also rise, leading to hypercalcemia and hypercalciuria. Hypercalcemia is the hazard criterion for vitamin D toxicity. It is not certain what causes the symptoms associated with vitamin D toxicity: whether it is the increase of total 1,25(OH)2D, the increase of free 25(OH)D or the increase of free 1,25(OH)2D.

Individuals with vitamin D intoxication may suffer from symptoms such as abdominal cramps, nausea, vomiting, increased thirst and polyuria. Hypercalcemia may lead to ectopic calcification of tissues and blood vessels, which – for instance – can result in severe nephrocalcinosis.
Criteria for vitamin D intoxication
Due to the feedback mechanism in the production of 25(OH)D in the skin, toxic vitamin D concentrations can only result from the consumption of excessive quantities of vitamin D. The Health Council of the Netherlands considers the maximum tolerable daily intake to be 50 µg (2,000 IU) for individuals older than one year, which is largely in agreement with the Food Safety Authority (50 µg/day for individuals older than ten years). The threshold is in accordance with the dietary reference intakes from the American Institute of Medicine from 1997. However, discussion remains about this upper threshold. A study to assess the upper threshold for the vitamin D intake, or serum 25(OH)D concentration, is ethically unacceptable. Therefore the safe upper level must be derived from animal studies and anecdotal evidence from human toxicity cases. Based on this kind of literature, Jones concluded that hypercalcemia only develops at serum 25(OH)D concentrations over 375-500 nmol/l. Vieth concluded that an intake of 250 µg (10,000 IU) per day should be safe. However, it should be noted that some individuals are more sensitive for vitamin D intoxication; for instance, individuals with primary hyperparathyroidism, and individuals with sarcoidosis, tuberculosis or lymphoma.

Outline of this thesis
The studies presented in this thesis have been performed 1) to estimate the prevalence of vitamin D deficiency among non-western immigrant groups, 2) to estimate the relative contribution of the known determinants of vitamin D deficiency and 3) to estimate the association between vitamin D deficiency and potential consequences, which could be studied well in a general population (muscle strength, muscle pain, functional limitations and diabetes). The prevalence of vitamin D deficiency in adult immigrant groups in the Netherlands, and a control group of indigenous Dutch individuals, is presented in chapter 2. In this chapter, the results of the relative importance of several determinants of vitamin D deficiency are also presented. The prevalence of vitamin D deficiency in pregnant women with various ethnic origins in The Hague is presented in chapter 3. In chapter 4 the results are presented of a literature study on the prevalence of vitamin D deficiency of the earlier studied ethnic groups in their countries of origin. In the study presented in chapter 2 we also measured (potential) consequences of vitamin D deficiency: muscle strength, muscle pain, functional limitations such as rising from a chair, and diabetes mellitus; the association between serum 25(OH)D and muscle related outcomes is presented in chapter 5, the association with diabetes mellitus in chapter 6. Chapter 7 contains the discussion, general conclusion and recommendations.
References

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