

Chapter 1

General Introduction



Vitamin D deficiency is common in older individuals, with prevalence from 45 up to 90% depending on lifestyle, age, gender and the method for the assessment of vitamin D status [1;2]. The classical function of vitamin D is to increase the absorption of calcium from the gut in order to facilitate the mineralization of bone [2]. In the last decades the number of publications regarding vitamin D increased enormously, partly caused by associations of vitamin D status with several other, non-skeletal, outcomes. As a result, the number of laboratory requests for serum 25(OH)D determination in the years 2000-2010 has increased exponentially [3]. This thesis will focus on the determinants and consequences of vitamin D deficiency in order to promote an optimal vitamin D status in the older population.

History of vitamin D

From the mid-1600s, bone-deforming diseases became more common in northern Europe and at the start of the 20th century, these diseases were highly prevalent in children in industrialized cities in Europe and the United States [4]. It was found that 96% of the children who died before the age of 1.5 years had evidence of rickets at autopsy [4]. Because sunlight was blocked due to air pollution in the industrialized cities, the skin was not able to produce vitamin D₃ [5]. However, at that time, it was not known that it was vitamin D deficiency that caused the problems related to rickets. Because rickets was highly prevalent in the Britain, it was called the “English disease” [6]. In the beginning of the 19th century, it was discovered that there was a link between a lack of sunshine and this disease [7]. It was observed that children who lived in the rural areas of Warsaw had no bone deformities, whereas children living in the city did [7]. In 1922, the relationship between vitamin D deficiency and bone deformities was made for the first time by McCollum [8]. In the meantime, it was discovered that sunlight and cod liver oil could partly solve the bone problems [5;6]. Initially, it was thought that vitamin A was responsible for the healing effect of cod liver oil. However, later, researchers found that when the vitamin A part in cod liver oil was destroyed by heating and oxygen, the remaining cod liver oil maintained the healing effect. The new, unknown, vitamin was called vitamin D [7]. However, it was only in 1932 that the chemical structure of vitamin D was discovered [9]. After these important findings, the prevalence of rickets in children declined enormously, because cod-liver oil was given and sun exposure was promoted [5]. However, in the second half of the 20th century, it became clear that osteomalacia (the adult form of rickets) still occurred, especially in older individuals [2]. In the last decades, many papers were published regarding the link between vitamin D and bone. Later, suggestions on associations between vitamin D status and other diseases were made.

Sources and metabolism of vitamin D

Vitamin D refers to two different molecules, i.e. vitamin D₂ or ergocalciferol and vitamin D₃ or cholecalciferol [10]. Only the side chains of the two molecules differ. Vitamin D₂ is found in plants, plant materials and foods after radiation [10], whereas vitamin D₃ is synthesized in the skin under the influence of ultraviolet (UV) radiation, or it is obtained from food, especially fatty fish, such as herring and mackerel [10;11]. Smaller amounts of vitamin D₃ can be found in dairy products, egg yolk, and meat [12]. In the Netherlands, only margarine is fortified with vitamin D, whereas in the United States, and some European countries such as Ireland, also milk is fortified [12].

In the skin, UVB radiation with wavelengths of 280-315 nm is important for the production of vitamin D [11]. The photons are absorbed by 7-dehydrocholesterol in the epidermis, and by using the energy of this process, previtamin D₃ is formed from 7-dehydrocholesterol [13]. Previtamin D₃ has no biological activity and is unstable and therefore, several other steps have to be taken before it can have physiological actions [5;11]. First, a heat reaction occurs, whereby vitamin D₃ is formed. This process takes several hours. Once vitamin D₃ is formed, it enters the blood circulation via the dermal capillary bed and it binds to the vitamin D binding protein (DBP) [11;13]. A prolonged exposure to sunlight will not result in a higher production of previtamin D₃ and vitamin D₃; only around 15% of the total cutaneous 7-dehydrocholesterol will be converted to previtamin D₃ regardless whether the skin is exposed to equatorial sunlight for 30 minutes or 8 hours [7]. When prolonged radiation exists, two inert isomers can also be formed alternatively to vitamin D₃ or it can be photoisomerised back to 7-dehydrocholesterol [11]. Because vitamin D₃ is labile, it will be converted to supersterol I, II or 5,6-trans-cholecalciferol under the influence of sunlight if it is not directly exerted from the basal membrane of the skin into the bloodstream. Therefore, the sun itself may be the best regulator of the cutaneous production and this may also be an explanation why there are no reports on vitamin D intoxication after excessive sunshine exposure [7;11].

Once in the bloodstream and bound to DPB, vitamin D₃ is transported to the liver where it is hydroxylated into 25-hydroxyvitamin D₃ (25(OH)D, also known as calcidiol) by several 25-hydroxylases, such as CYP27A1, CYP3A4, CYP2R1 and CYP2J3 [5]. Although 25(OH)D has no biological activity, it is the major circulating metabolite of vitamin D. 25(OH)D binds to DBP and it is transported to the kidney. In the kidney, a second hydroxylation step takes place, which results in the formation of 1,25-dihydroxyvitamin D (1,25(OH)₂D, also known as calcitriol). The responsible enzyme is 25-hydroxyvitamin D-1 α -hydroxylase, also known as CYP27B1 [5;10]. Alternatively, the enzyme CYP27B1 can also be found in other cell types, such as macrophages, osteoblasts, osteoclasts, myocytes, central nervous system, prostate, and pancreatic cells, in order to facilitate extrarenal

hydroxylation of 25(OH)D into 1,25(OH)₂D [14]. 1,25(OH)₂D is the most active metabolite of vitamin D. When sufficient amounts of 1,25(OH)₂D are available, 24,25-dihydroxyvitamin D is produced in the kidney, which is further catabolized into inactive metabolites [10]. The half-life time of 1,25(OH)₂D is much shorter than of 25(OH)D, namely in the order of 4-6 hours. The half-life of 25(OH)D is relatively long (2-4 weeks), and therefore in clinical practice, serum 25(OH)D is measured instead of 1,25(OH)₂D to determine the individual's vitamin D status [5;15]. 1,25(OH)₂D has to bind the vitamin D receptor (VDR) to be able to perform its actions. The receptor can be found in the nucleus of many cell types throughout the body, such as in the intestine, kidney, muscle and bone [16]. Figure 1 shows the main routes in the metabolism of vitamin D.

Regulation of the production of vitamin D

As mentioned earlier, the sun itself may be the best regulator of the cutaneous production of vitamin D₃ [11]. However, further in the cascade of the production of the biologically active vitamin D metabolite, the parathyroid hormone (PTH) is important as regulator [2]. Together with 1,25-dihydroxyvitamin D, PTH aims to maintain the serum calcium concentrations within the normal ranges [2]. In a low-calcium state, PTH secretion from the parathyroid glands is stimulated, which enhances the hydroxylation of 25(OH)D into 1,25(OH)₂D. The main function of 1,25(OH)₂D is the stimulation of the absorption of calcium from the gut. PTH also has direct effects on the reabsorption of calcium from the renal tubuli and from bone. The resulting rise in calcium concentration in the blood and 1,25(OH)₂D itself provides negative feedback to the parathyroid glands to decrease the secretion of PTH [2;17-19].

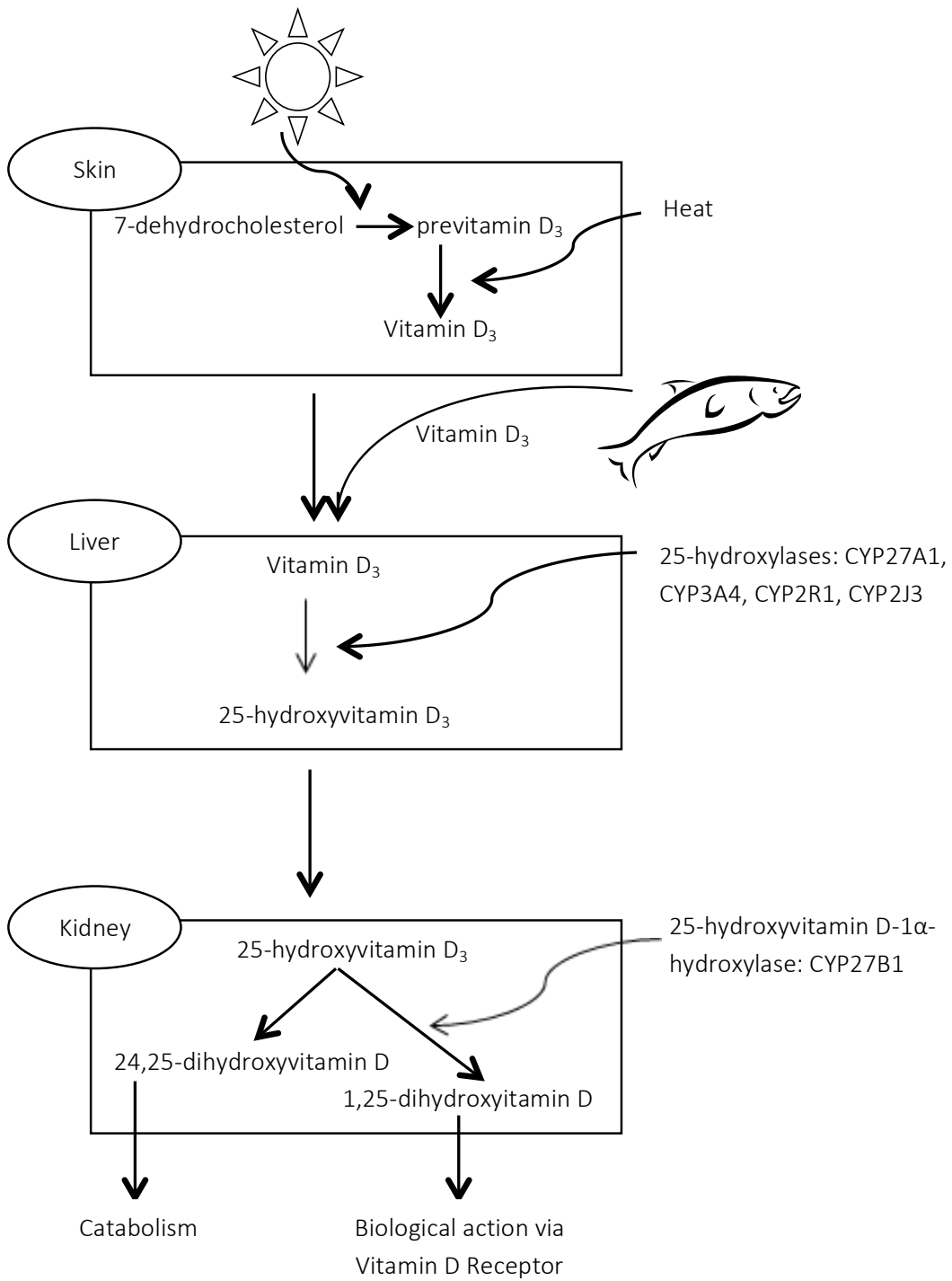


Figure 1. Simplified overview of the main routes in the metabolism of vitamin D

Functions of vitamin D and consequences of vitamin D deficiency

As described earlier, long before the discovery of vitamin D itself, the critical function of an unknown compound under influence of sunlight in the development and maintaining of healthy bone was known. Years later, several other functions of vitamin D have been revealed, which will be described below.

Classical functions: After the binding of $1,25(\text{OH})_2\text{D}$ to the VDR, and heterodimerization of the VDR with the vitamin A receptor RXR, gene transcription and translation occur and different genes are activated, depending on the tissue. In the intestine, several proteins are formed, which support the absorption of calcium from the gut and its transport into the bloodstream [2]. Although the understanding that vitamin D is critical for bone health is known for a long time, the action of $1,25(\text{OH})_2\text{D}$ is not completely understood [2]. On the one hand the effect is anabolic by stimulating the osteoblasts to produce osteocalcin and alkaline phosphatase. On the other hand, there is a catabolic effect by enhancing the bone resorption via the stimulation of the osteoclasts. The positive effect of vitamin D on the mineralization of bone seems to be mainly indirect via the stimulation of calcium absorption from the gut [2;20]. When vitamin D deficiency is severe, rickets can develop in children and osteomalacia in adults. Rickets is characterized by bone pain, bone deformations, and decreased growth of the long bones, caused by a mineralization defect of the growth plate, with subsequently hypertrophies. Osteomalacia is characterized by a mineralization defect of newly synthesized bone matrix during remodeling of the skeleton. The main difference with rickets is that it takes place in a non-growing skeleton [21]. Both rickets and osteomalacia are also associated with muscle weakness [21]. In addition, in a vitamin D deficient state, osteoporosis or a low bone mineral density can be present, which is the loss of mainly cortical bone, caused by higher bone turnover due to secondary hyperparathyroidism [10]. This may play a role in the association of vitamin D deficiency with an increased risk of fractures [22].

Non-classical functions: The VDR and 1α -hydroxylase have been found in many organs, such as the colon, pancreas, parathyroid glands, heart, immune system, brain, and muscle, and therefore it is not surprising that vitamin D deficiency has been associated with (diseases of) these and other systems [16]. However, the exact function of $1,25(\text{OH})_2\text{D}$ in all these systems is not completely understood and the level of evidence is not very high as it is mainly coming from observational studies. Well-designed clinical trials on most of the outcomes are warranted to determine whether there could be any benefit of vitamin D in these systems [23].

In contrast to many other systems, the positive influence of vitamin D on muscle strength and falls has been well studied [24-26]. Both direct and indirect effects of vitamin D on muscles have been proposed to play a role [27]. First vitamin D can affect the calcium

binding, necessary for muscle contraction [28]. Second, muscle weakness can occur indirectly via the phosphate imbalance, which may cause problems with phosphorylation and dephosphorylation of proteins during muscle contraction and relaxation [29]. More direct effects of vitamin D are established through the VDR in muscle cells [27]. Because of the described influence of vitamin D on muscle, it is not surprising that low vitamin D status has been associated with, for example, worse physical performance [30;31]. However, the information on the influence of vitamin D in performing activities of daily life is limited and inconclusive [32-35].

Furthermore, cardiovascular diseases are relatively common in individuals with low levels of serum 25(OH)D [36]. However, most of the previous studies were performed in younger and disease specific populations. Whether preclinical stages of cardiovascular disease in older individuals, such as increased arterial stiffness and atherosclerosis, are associated with serum 25(OH)D level remains to be studied.

Current opinions on optimal levels of serum 25(OH)D and supplementation guidelines

The definition of vitamin D deficiency has been discussed for year and is still a controversial issue. The Institute of Medicine (IOM) and the Endocrine Society have both published their own guidelines with a different scope [37;38]. Although both guidelines are based on the evidence of the effect of vitamin D on skeletal outcomes, the guidelines are different from each other [39]. The IOM focuses on the general population and advocates required levels of 30-50 nmol/l [38], whereas the Endocrine Society focuses on screening and treating patients with vitamin D deficiency. The latter suggests levels of 75 nmol/l to be adequate [37]. The IOM requirements are based on the following: at levels of 30 nmol/l the risk for deficiency will increase, 40 nmol/l represents the estimated average requirement (providing the EAR, corresponding to the median requirement of the population), and 50 nmol/l will meet the requirement of 97.5% of the population (providing the Recommended Dietary Allowance, RDA)[38;40]. However, the Endocrine Society marks “deficiency”, “insufficiency” and “sufficiency” as <50 nmol/l, 50-75 nmol/l and >75 nmol/l [37]. The advice of the Dutch Health Council is in line with the IOM statement that levels of 30-50 nmol/l are desirable. For adults, 30 nmol/l is required, whereas 50 nmol/l is required for women above 50 years and for men above 70 years of age. [41]. The National Osteoporosis Society of the UK also follows the IOM statement [42]. Despite the fact that different guidelines exist, the discussion on the optimal thresholds or levels for serum 25(OH)D still continues [43]. In addition, it can be imagined that the optimal levels vary between different outcomes.

Not surprisingly, the above mentioned guidelines also differ in the advices on vitamin D supplementation. The Endocrine Society advises 1500-2000 IU vitamin D per day in supplements to attain the required level of 75 nmol/l for individuals above 50 years of age [37], whereas the IOM only advises to have a total intake (diet and supplements) of 600-800 IU per day [38]. In the advice of the Dutch Health Council, vitamin D supplements are advised to children < 4 years, pregnant women, persons with insufficient sunshine exposure and a dark skin and women above 50 years of age (all 400 IU per day), and men and women above 70 years of age (800 IU per day).

Prevalence of vitamin D in the aging population

Since the nineteenth century, the life expectancy has increased by almost three months per year for women and by two months for men [44]. Therefore, the proportion of individuals that becomes old and very old will rise. In the Netherlands, the absolute number of persons of 65 years and older will be growing from 2.7 million in 2012 to 4.7 million in 2041, which will be 26% of the total Dutch population. In addition, 25% of the 65+ population will be aged 80 years and over [45].

Because vitamin D deficiency is very common in the older population [2], the total burden of vitamin D deficiency among the population will increase. In the Netherlands, results from the Longitudinal Aging Study Amsterdam revealed that 48% of the individuals of 65 years and older has inadequate levels of serum 25(OH)D (<50 nmol/l; population mean of 54 nmol/l) [31]. Depending on the definition used, studies in other countries reported similar percentages and population mean values [33;46-53]. However, there are remarkable differences between several countries. Mean values in North America are higher than in Europe and the Middle East, partly explained by higher degree of food fortification and clothing habits [54]. In addition, vitamin D deficiency is more prevalent in Asia and Africa. Surprisingly, vitamin D status is much better in the Northern part of Europe compared to Southern part, probably due to different lifestyles, dietary habits and skin types [55;56].

Risk factors for vitamin D deficiency

Older population

Vitamin D deficiency is highly prevalent in the older population. As the main source of vitamin D is the skin, alterations in the skin during aging have an impact on the production of vitamin D [7;11]. First, the skin thickness decreases dramatically [57] and second, the concentration of 7-dehydrocholesterol in the skin also decreases [58]. Older individuals maintain the ability to produce vitamin D₃, but the efficiency is less compared to younger adults [59]. However, it has been shown that the skin of older institutionalized persons is able to produce enough vitamin D to reach sufficient levels after UVB-radiation at an area of 1000 cm² only three times a week for period of 12 weeks [60]. Moreover, it can be imagined that changing lifestyles, like outdoor activities and food consumption, have a negative influence on the vitamin D status and that these factors are of more importance than the reduced capacity of the skin to produce vitamin D₃ [11]. To date it is unclear whether other factors, such as, for example, medication use or specific medicines, influence serum 25(OH)D levels in older people. It is known that some statins elevate serum 25(OH)D [61-63], whereas anti-epileptic drugs lower serum 25(OH)D [64;65]. However, for other groups of medicines and the use of medications in general, this is still unknown. In addition, the chronic diseases, for which the medicines are described, may also influence vitamin D status.

General risk factors

Several other more general, not age-dependent, determinants or risk factors for vitamin D deficiency can be mentioned. Lifestyle factors, like clothing habits, sunscreen use, staying outdoors, physical activity and nutrition are important [12]. For example, people wearing a niqab or even a hijab have much lower vitamin D status compared to people wearing Western clothing [66]. Furthermore, in addition to sunlight, food is a source of vitamin D. The adequate vitamin D status of the Norwegians is partly due to the consumption of fatty fish, cod liver and cod liver oil [67]. Calcium intake may also be important for vitamin D status as the calcium concentration in the blood positively influence the half-life of serum 25(OH)D [68]. Body Mass Index has also been associated with vitamin D status; which may be explained by the fact that vitamin D is a fat-soluble vitamin and that in case of obesity more 25(OH)D is stored in fat cells and not bound to DBP in the blood [69]. Especially in the countries of high latitude, season is an important determinant of vitamin D status. In the winter, sunlight has to pass a longer way through the atmosphere and less UV radiation will reach the earth. Therefore, in the Northern part of Europe, Canada, and the United States the vitamin D production is almost completely absent during the winter months [2;70].

Rationale and aims of this thesis

Determinants of vitamin D status and consequences of vitamin D deficiency have been described previously. However, there are still gaps in the literature, especially regarding the differences in various subgroups, such as different age groups, men and women, and individuals with low-to-normal versus high body mass index. In addition, most of the published studies rely on cross-sectional analyses; longitudinal analyses are relatively scarce.

Therefore this thesis has three different aims:

1. To better define the determinants of vitamin D status in older individuals
2. To better define the consequences of vitamin D deficiency in older individuals
3. To estimate the optimal levels of serum 25(OH)D in older individuals and to describe a strategy for a better implementation of the current Dutch vitamin D supplementation advice.

Outline of this thesis

Chapter 2 describes the association of medication use and vitamin D status. In addition, we examined the impact of different medication groups on serum 25(OH)D concentration. In chapter 3, two prediction models are created, which enables individuals or health care professionals to calculate the risk for being vitamin D deficient, based on simple patient characteristics. In chapter 4 and 5, we focus on the consequences of vitamin D deficiency for muscle function, by studying the association of vitamin D status with physical performance (chapter 4) and functional limitations (chapter 5), both cross-sectionally and longitudinally. Chapter 6 reports the association of vitamin D status with bone health measured by ultrasound and Dual-energy X-ray Absorptiometry. In chapter 7, the results of the study on the influences of vitamin D on preclinical stages of vascular disease are reported. Chapter 8 and 9 are meant to optimize vitamin D status in the population by first determining the optimal levels for serum 25(OH)D with respect to different outcomes (chapter 8) and second, by describing a strategy for a better implementation of the current Dutch vitamin D supplementation advice (chapter 9). In chapter 10 the main findings are summarized and discussed in relation to the results of other studies. Clinical implications and directions for future research are also addressed.

Databases used in this thesis: LASA en B-PROOF

Two existing large studies were used to determine answers on the research questions of this thesis: The Longitudinal Aging Study Amsterdam (LASA) and the baseline data of the B-vitamins for the PRevention Of Osteoporotic Fractures study (B-PROOF).

Longitudinal Aging Study Amsterdam

LASA is an ongoing longitudinal cohort study which focuses on physical, emotional, cognitive and emotional functioning during aging [71]. At the baseline measurement in 1992, 3107 participants of 55-85 years were included. Participants were randomly selected from the population registries from 11 municipalities in three different regions in the Netherlands, thereby creating a representative sample of the Dutch older individuals. Participants were followed up at roughly three years intervals by interviews. In each cycle, participants were interviewed during the main interview and if they agreed, an additional medical interview was held. In some cycles, blood was drawn and serum 25(OH)D was measured. In 2002 (n = 1002) and 2012 (n = 1023) a second and third cohort were added, based on the same sampling frame as the first cohort, both consisting of participants of 55-65 years of age. The sampling and data collection procedures are extensively described elsewhere [72]. A flow chart of the three LASA cohorts is shown in Figure 2. In this thesis, only the first and second cohort were used. The chapters 2, 3, 4, 5, 6 and 8 are based on LASA data.

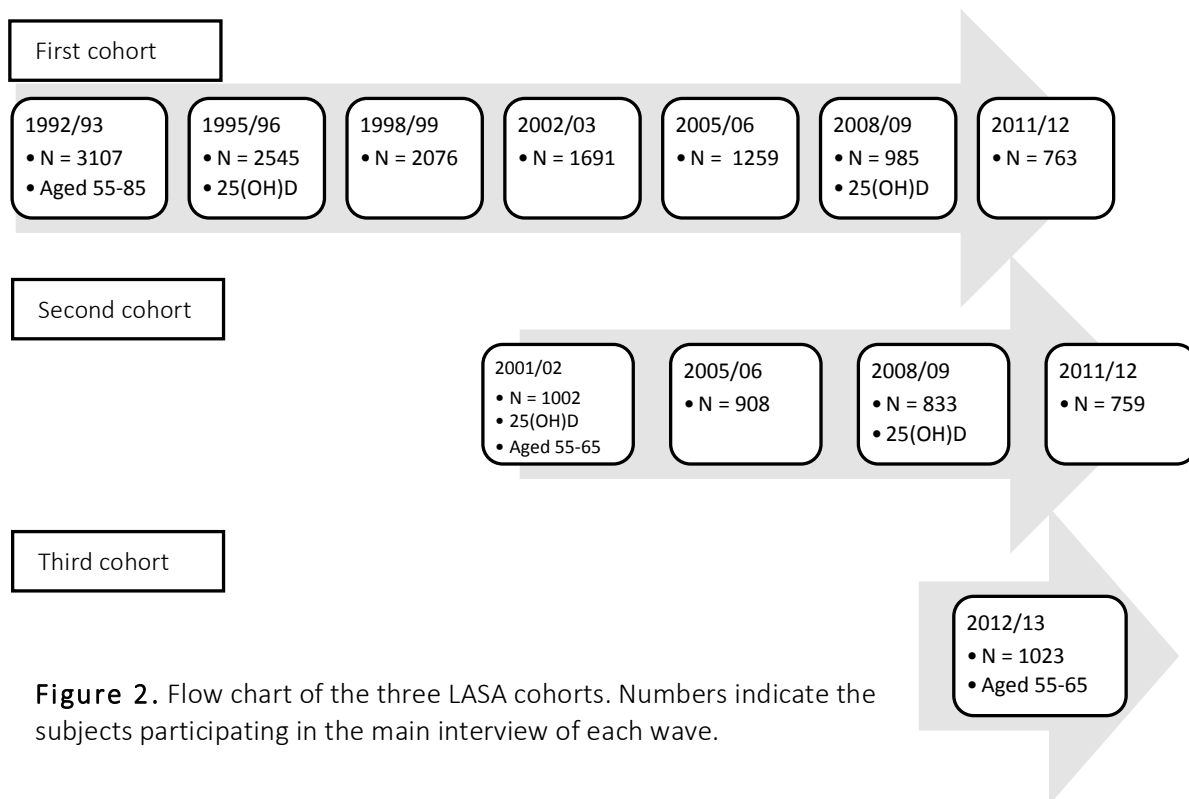


Figure 2. Flow chart of the three LASA cohorts. Numbers indicate the subjects participating in the main interview of each wave.

B-vitamins for the PRevention Of Osteoporotic Fractures

B-PROOF is a randomized double blind placebo-controlled clinical trial on the prevention of osteoporotic fractures by the B-vitamins folic acid and vitamin B12 in older individuals. Participants were mainly recruited via population registries and general practitioners and were all aged 65 years and older. Only participants with elevated homocysteine levels (≥ 12 $\mu\text{mol/l}$) were included. Recruitment took place in 2008-2011; a total of 2919 participants were included of which 2879 individuals had serum 25(OH)D measured. After the baseline interview on physical, cognitive and emotional aspects of functioning and lifestyle, participants were randomized in the intervention group (500 μg vitamin B12, 400 μg folic acid, and 600 IU vitamin D per day) or the control group (600 IU vitamin D per day). B-PROOF is described elsewhere in more detail [73]. For this thesis only the baseline data were used. The chapters 4, 6 and 7 include data from B-PROOF.

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